

# Gas exchange and resource utilization in two alpine oaks at different altitudes in the Hengduan Mountains

Shi-Bao Zhang, Zhe-Kun Zhou, Hong Hu, and Kun Xu

**Abstract:** The evergreen sclerophyllous broadleaf forests composed of alpine oaks in the Hengduan Mountains of south-west China are mainly distributed at altitudes of 1700–4800 m. Gas exchange and related leaf traits of *Quercus guyavifolia* H. Lév. and *Quercus pannosa* Hand.-Mazz. were measured at eight sites along an altitudinal gradient to understand their physiological adaptabilities. Both *Q. guyavifolia* and *Q. pannosa* showed a significant midday depression in the photosynthesis rate ( $P_N$ ) due to a high vapor pressure deficit and high temperature around noon. The optimum temperature for photosynthesis for the two oak species ranged from 17 to 23 °C depending on altitude. When the temperature exceeded 25 °C,  $P_N$  decreased sharply. However,  $P_N$  was not depressed by high irradiance. As altitude increased, the leaf dry mass per unit area of the two oaks increased but the chlorophyll content decreased, while the maximum values of daily mean photosynthesis rate ( $P_d$ ), maximum photosynthesis rate ( $P_{max}$ ),  $P_d/P_{max}$ , water-use efficiency, and photosynthetic nitrogen use efficiency occurred at altitudes of 3240–3610 m. Although the photosynthetic capacities of the two oaks were higher in August than in May, altitudinal trends did not change with season. The altitudinal range from 3240 to 3610 m would be optimal for the growth and development of these two alpine oaks in the Hengduan Mountains.

**Résumé :** Les forêts de feuillus sclérophylles à feuilles persistantes composées de chênes alpins dans les monts Hengduan du sud-ouest de la Chine se retrouvent surtout à des altitudes variant de 1700 à 4800 m. Les échanges gazeux et les caractéristiques foliaires associées ont été mesurés sur des individus de *Quercus guyavifolia* H. Lév. et *Quercus pannosa* Hand.-Mazz. établis sur huit stations le long d'un gradient altitudinal dans le but de comprendre leur capacité d'adaptation physiologique. Une diminution significative du taux de photosynthèse a été observée à mi-journée chez *Q. guyavifolia* et *Q. pannosa* en raison des forts déficits de pression de vapeur et des températures élevées vers midi. La température optimale pour la photosynthèse des deux espèces de chêne variait de 17 à 23 °C selon l'altitude. Lorsque la température dépassait 25 °C, le taux de photosynthèse diminuait abruptement. Toutefois, les fortes valeurs d'irradiation n'ont pas entraîné de diminution du taux de photosynthèse. La masse foliaire anhydre par unité de surface foliaire des deux chênes augmentait avec l'altitude alors que le contenu en chlorophylle diminuait. Les valeurs maximales du taux journalier moyen de photosynthèse ( $P_d$ ), du taux maximal de photosynthèse ( $P_{max}$ ), de  $P_d/P_{max}$ , de l'efficacité d'utilisation de l'eau et de l'efficacité d'utilisation de l'azote pour la photosynthèse ont été mesurées à des altitudes allant de 3240 à 3610 m. Bien que la capacité photosynthétique des deux chênes ait été plus élevée en août qu'en mai, la tendance observée en fonction de l'altitude ne changeait pas selon la saison. Les stations situées à des altitudes allant de 3240 à 3610 m offriraient des conditions optimales pour la croissance et le développement de ces deux chênes alpins dans les monts Hengduan.

[Traduit par la Rédaction]

## Introduction

Every species is limited both geographically and ecologically to a range of habitats. Understanding the patterns and processes governing plant distribution is a central goal of ecology, yet for many species the reasons for distribution

limits are unclear (Angert 2006). Altitudinal gradients provide unique experimental opportunities to study the morphological and physiological responses of plants to environments and describe species richness from low to high elevations, because altitudinal changes in environmental factors have important effects on plant distribution and leaf physiology and

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**Abbreviations:** AQE, apparent quantum efficiency (mol carbon dioxide (CO<sub>2</sub>)-mol<sup>-1</sup> photon); Chl, total chlorophyll content per unit area (mg-dm<sup>-2</sup>); C<sub>i</sub>, intercellular CO<sub>2</sub> concentration (μmol-mol<sup>-1</sup>); δ<sup>13</sup>C ratio, stable carbon isotope ratio; E, transpiration rate; J<sub>max</sub>, light-saturated rate of electron transport (μmol-m<sup>-2</sup>-s<sup>-1</sup>); LMA, leaf dry mass per unit area (g-m<sup>-2</sup>); LNC, leaf N content (g-m<sup>-2</sup>); P<sub>N</sub>, photosynthesis rate (μmol-m<sup>-2</sup>-s<sup>-1</sup>); P<sub>d</sub>, daily mean P<sub>N</sub> (μmol-m<sup>-2</sup>-s<sup>-1</sup>); P<sub>max</sub>, light-saturated P<sub>N</sub> (μmol-m<sup>-2</sup>-s<sup>-1</sup>); PNUE, photosynthetic N use efficiency (μmol-s<sup>-1</sup> CO<sub>2</sub>-g<sup>-1</sup> N); PPFD, photosynthetic photon flux density (μmol-m<sup>-2</sup>-s<sup>-1</sup>); T<sub>a</sub>, air temperature (°C); T<sub>l</sub>, leaf temperature (°C); T<sub>opt</sub>, optimum temperature for photosynthesis (°C); V<sub>cmax</sub>, maximum carboxylation rate by ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco) (μmol-m<sup>-2</sup>-s<sup>-1</sup>); VPD, vapor pressure deficit between leaf and air (kPa); WUE, photosynthetic water-use efficiency (μmol CO<sub>2</sub>-mmol<sup>-1</sup> H<sub>2</sub>O).

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**Table 1.** Locations and air temperatures of eight experimental sites where *Quercus guyavifolia* and *Quercus pannosa* were studied.

	Altitude (m)	Longitude (E)	Latitude (N)	Temp. (°C)	Tree density (no./ha)	Upper canopy	Midcanopy	Understory
<i>Quercus guyavifolia</i>	2720	99°26.69'	28°07.57'	9.6	475	<i>Q. guyavifolia</i> , <i>Pinus armandi</i>	<i>Sabina squamata</i> , <i>Campylotropis polyantha</i>	<i>Leontopodium sinense</i> , <i>Viola delavayi</i>
	3180	99°34.90'	27°57.99'	5.8	673	<i>Q. guyavifolia</i> , <i>Q. longispica</i>	<i>Salix delavayana</i> , <i>Contoneaster franchetii</i>	<i>Potentilla fulgens</i> , <i>Saxifraga filicaulis</i>
	3560	99°36.81'	27°56.03'	2.8	704	<i>Q. guyavifolia</i> , <i>Picea likiangensis</i>	<i>Rhododendron racemosum</i> , <i>Viburnum cylindricum</i>	<i>Sium frigidum</i> , <i>Primula bullata</i>
	3920	99°39.77'	27°53.01'	1.6	598	<i>Q. guyavifolia</i> , <i>Picea likiangensis</i>	<i>Deutzia longifolia</i> , <i>Rhododendron euryssiphon</i>	<i>Potentilla argyrophylla</i> , <i>Saxifraga smithiana</i>
<i>Quercus pannosa</i>	2870	99°43.80'	28°09.23'	8.1	627	<i>Q. pannosa</i> , <i>Sorbus pallescens</i>	<i>Syburnum foeridum</i> , <i>Litsea sericea</i>	<i>Rabdosia oreophila</i> , <i>Festuca leptopogon</i>
	3240	99°38.80'	27°46.10'	5.4	736	<i>Q. pannosa</i> , <i>Acer robusta</i>	<i>Prunus conradinae</i> , <i>Lonicera chlamydata</i>	<i>Viola schultzeana</i> , <i>Rubus lorpetalus</i>
	3650	99°42.46'	28°00.15'	5.5	693	<i>Q. pannosa</i> , <i>Sorbus ruopilosa</i>	<i>Rhododendron atropunicum</i> , <i>Ribes glaciale</i>	<i>Lepisorus contortus</i> , <i>Ainsliaea triflora</i>
	4170	99°49.75'	28°32.57'	-0.2	568	<i>Q. pannosa</i> , <i>P. likiangensis</i>	<i>Spiraea</i> sp., <i>Ilex delavayi</i>	<i>Saxifraga saxatilis</i> , <i>Polygonum amplexicaule</i>

**Note:** Air temperatures were calculated according to the altitudinal lapse rate of 7.1 °C / 1000 m.

morphology (Hovenden and Brodribb 2000). Species' altitudinal limits may be partly due to metabolic limitations on growth, because both the morphological and the physiological characteristics of plants enable them to survive or reproduce better in a particular environment. Photosynthetic traits governing carbon acquisition have an integral role in vegetative growth, and thus may affect individual fitness (Angert 2006). A good relationship between  $P_N$  and relative growth rate has been observed in seedlings (Pattison et al. 2001; Lajzerowicz et al. 2004), and a correlation between photosynthesis and the percentage of ground covered was found in Chaparral species (Oechel et al. 1981). A large investment in resources leads to a maximum  $P_N$ . Efficient use of resources is also important for the growth, survival, and competition of plants in changing environments (Hirose and Bazzaz 1998).

The sclerophyllous broadleaf evergreen forests composed of alpine oaks in the Hengduan Mountains of southwest China are mainly distributed at altitudes of 1700–4800 m, and are considered to effectively conserve water and soil in the upriver regions of China's major river systems. Usually, sclerophyllous evergreen oaks occur in arid zones of the world, such as the Mediterranean area. However, the oak species belonging to *Quercus* sect. *Heterobalanus* are distributed in cold, moist habitats of the Himalayas (Zhou et al. 2003), but retain obvious xerophytic characters, such as dense hairs, a thick cuticle, and low stomatal density (He et al. 1994). The relationships between the altitudinal distribution of alpine oaks and environmental factors in the Hengduan Mountains are of great interest to plant physiologists and ecologists (He et al. 1994; Zhou et al. 2003; Li et al. 2006). In alpine regions, temperature is thought to be one of the primary determinants of species distribution and growth along altitudinal gradients (Cabrera et al. 1998; Lajzerowicz et al. 2004). Several authors have suggested that alpine oaks adapt to low temperature because of their xerophytic characters, and an altitudinal range from 2400 to 3600 m is their optimum distribution zone (He et al. 1994; Zhou et al. 2003). However, physiological evidence for this hypothesis is lacking. A previous study showed that an altitude near 2800 m could be optimal for growth and develop-

ment of *Quercus aquifolioides* Rehder & E.H. Wilson, and that specific leaf area and stomatal length increased with increasing altitude below 2800 m, but exhibited a converse tendency above 2800 m (Li et al. 2006). Zhang et al. (2005) found that the photosynthetic capacity of *Quercus pannosa* Hand.-Mazz. decreased from 3240 to 4170 m in the Hengduan Mountains. However, the photosynthetic capacity of this oak below 3240 m was not investigated.

To gain further understanding of altitudinal trends in the morphology and physiology of alpine oaks in the Hengduan Mountains, leaf gas exchange and resource-use efficiency in *Quercus guyavifolia* H. Lév. and *Q. pannosa* were investigated at eight sites along an altitudinal gradient. The aims were to determine altitudinal variations in photosynthetic capacity and resource-use efficiency in alpine oaks, and to understand the relationship between their ecophysiological adaptability and geographical distribution. It was hypothesized that the two alpine oak species have greater photosynthetic capacities and resource-use efficiencies in the middle part of altitudinal range than at lower and higher altitudes because the environment is more favorable for their growth and development.

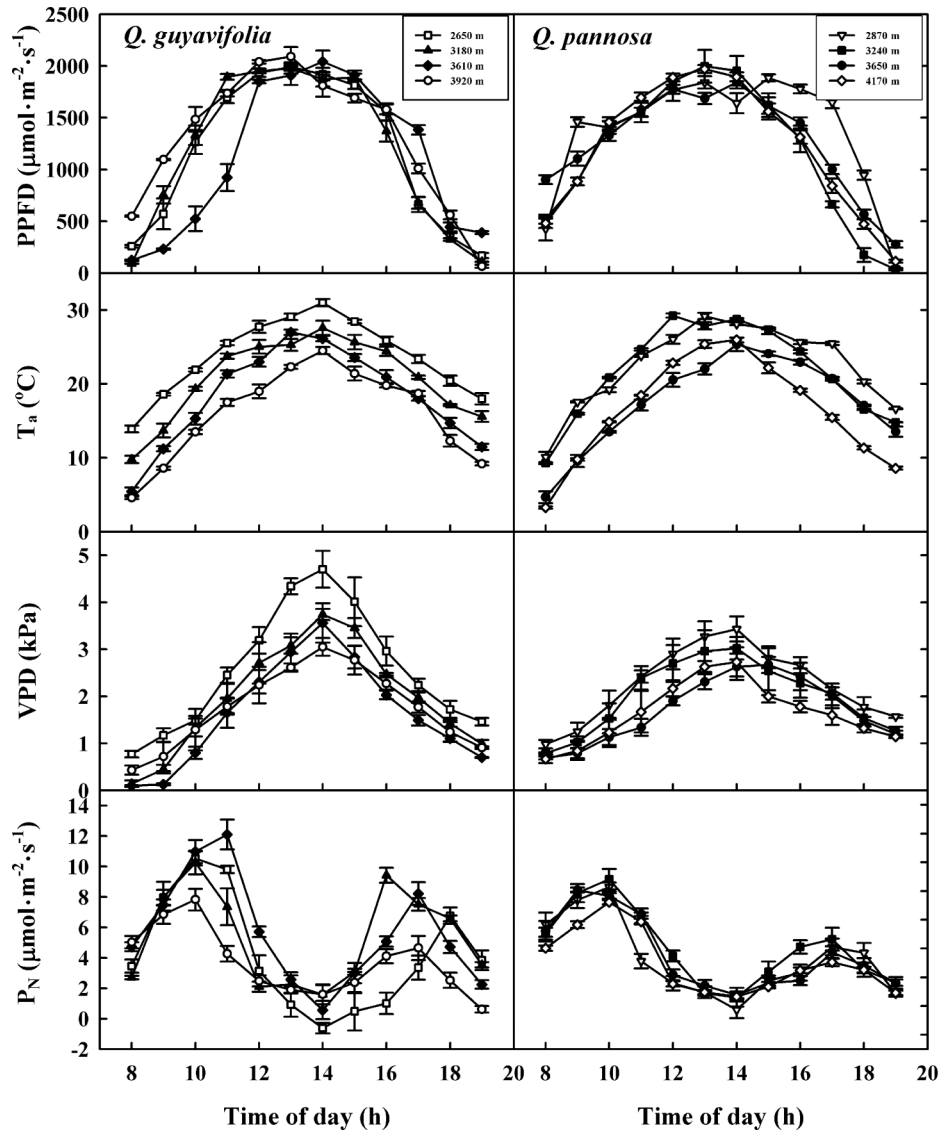
## Materials and methods

### Field sites and species

The study was carried out at eight sites in the Hengduan Mountains. The locations and  $T_a$  of the sites are given in Table 1. The climate is continental, with cold winters and hot, moist summers. Because no long-term climatic data from the sampling sites are available, the  $T_a$  values listed in Table 1 were calculated from the decrease in  $T_a$  with altitude (7.1 °C / 1000 m) in this region (Zhang 1998). May to October is the rainy season (87% of annual rainfall from 1959 to 2000), while November to April is the dry season. The soil type at all sites was brown soil with similar fertility levels and pH 7.5–8.5.

*Quercus guyavifolia* is an endemic tree that occurs at altitudes between 2500 and 4000 m above sea level, while *Q. pannosa* is distributed at altitudes ranging from 2600 to

**Fig. 1.** Diurnal variations of photosynthesis rates ( $P_N$ ) in *Quercus guyavifolia* and *Quercus pannosa* at different altitudes. Each point represents the mean of three measurements. Error bars represent  $\pm 1$  SE.



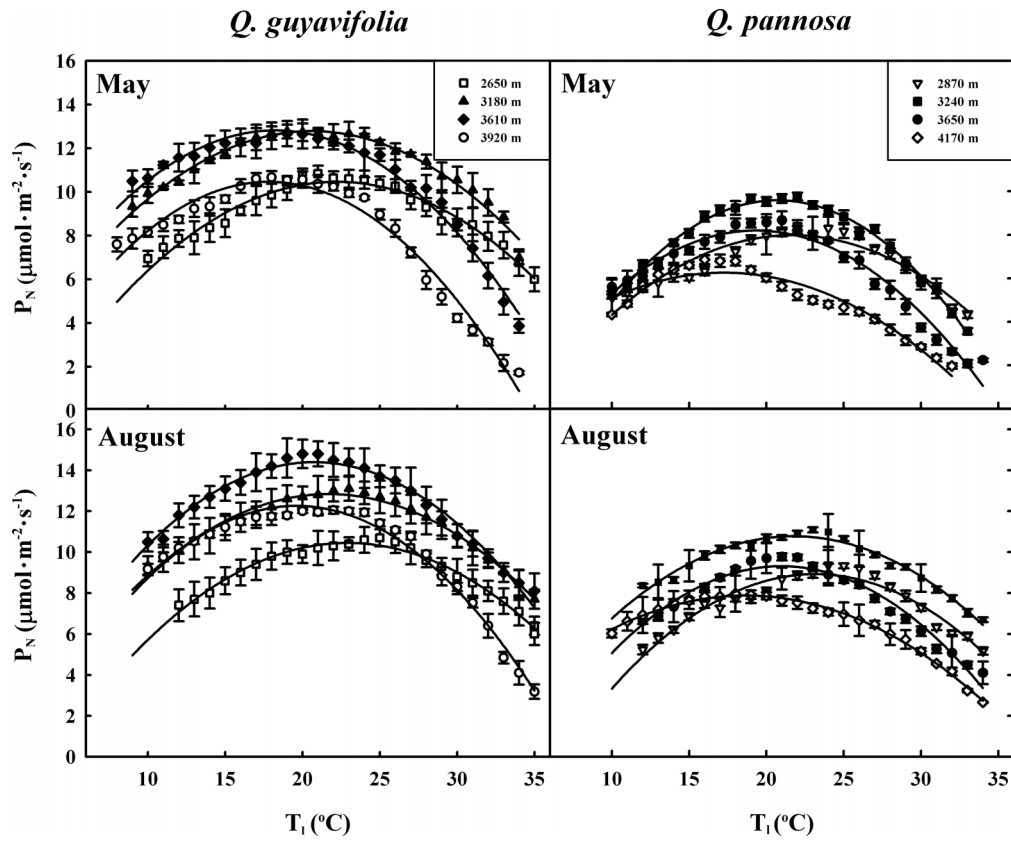
4300 m. Both are important components of the sclerophyllous broadleaf evergreen forests in the southwest of China.

### Photosynthesis measurements

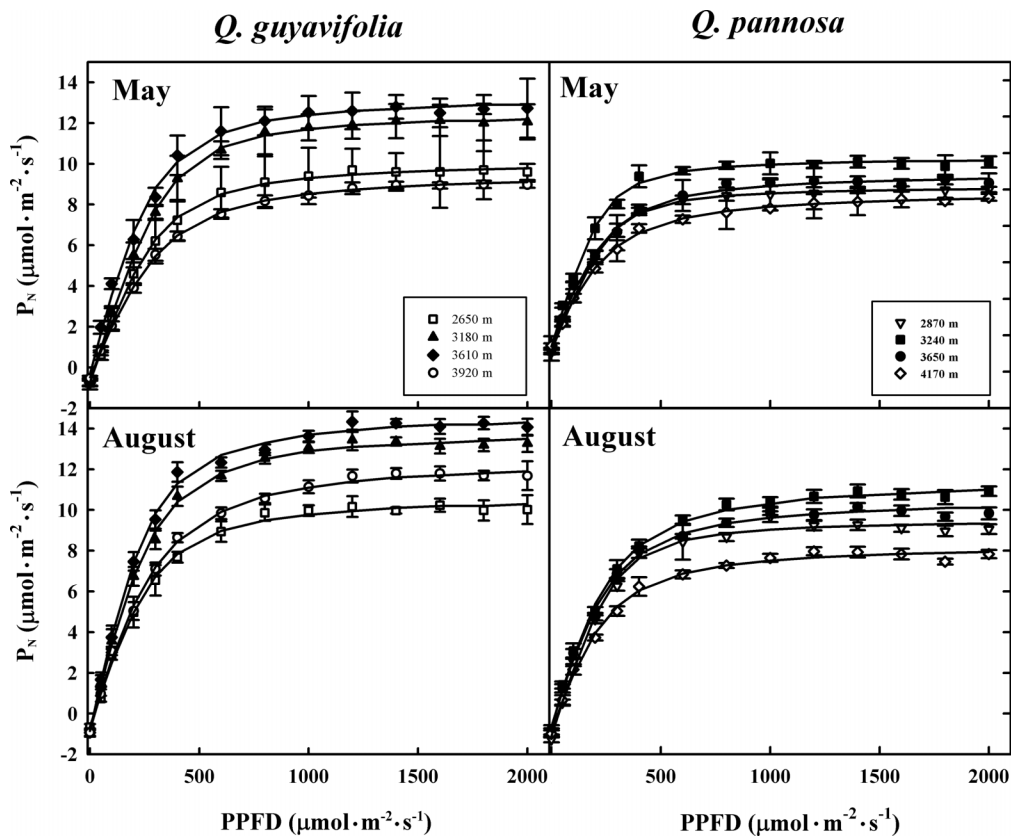
Diurnal variations in gas exchange were measured on three mature, fully expanded leaves from sun-exposed, upper canopies (3–4 m high) of trees sampled at the eight sites at approximately 1 h intervals from 08:00 to 19:00 on the clear days in May 2004. All leaves were carefully selected from branches of the same age to avoid differences in photosynthetic capacity associated with leaf age.  $P_N$ ,  $E$ , stomatal conductance, and  $C_i$  were measured using a portable infrared gas exchange system with a PLC-B leaf chamber (CIRAS-1, PP Systems, Hitchin, UK) in an open-system configuration. Meanwhile, a data logger (Li-1400, Li-Cor, Lincoln, Nebraska) was used to record irradiance (PPFD),  $T_a$ , and relative air humidity hourly.  $T_a$  and relative humidity were used to calculate VPD. WUE was calculated as the ratio between  $P_N$  and  $E$ .

The photosynthetic response curves to  $\text{CO}_2$  and light plus temperature were measured two times in May and August 2004, respectively. These measurements were also made on mature, fully expanded leaves from the upper canopy. Sampled leaves were allowed to acclimate to a PPFD of  $1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  about 15 min before measurements. Photosynthetic responses to PPFD were measured on three fully expanded and sun-exposed leaves at 14 light intensities using a CIRAS-1 infrared gas analyzer.  $T_i$  was adjusted to  $20^\circ\text{C}$  using the internal heating/cooling system built into the CIRAS-1. After the initial measurement at  $0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , PPFD was increased to produce the subsequent light intensities. Following a 5–6 min equilibration period at each light intensity,  $P_N$  was recorded when stomatal conductance and  $\text{CO}_2$  uptake stabilized. Data were fit by a nonrectangular hyperbola (Prioul and Chartier 1977). Using this function, AQE and  $P_{\text{max}}$  were estimated by means of Photosyn Assistant<sup>®</sup> software (version 1.1, Dundee Scientific, Dundee, UK).

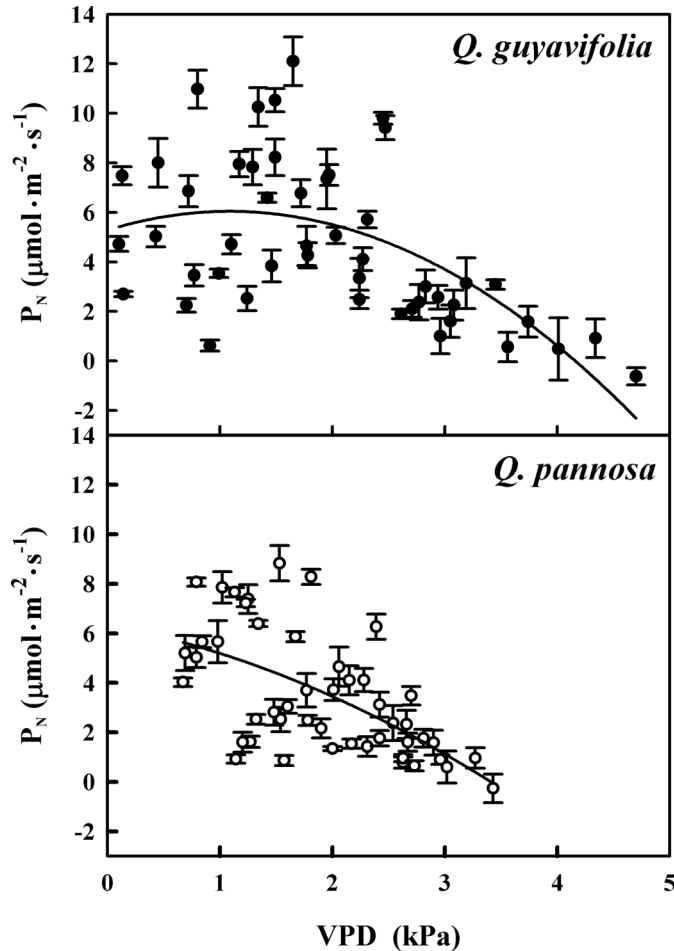
**Fig. 2.** Photosynthesis rates ( $P_N$ ) for *Quercus guyavifolia* and *Quercus pannosa* at different altitudes depending on temperature. Vertical bars indicate the error of the mean for three measurements.



**Fig. 3.** Photosynthetic responses of *Quercus guyavifolia* and *Quercus pannosa* to photosynthetic photon flux density (PPFD). Vertical bars indicate the error of the mean for three measurements.



**Fig. 4.** Effects of vapor pressure deficit (VPD) on photosynthesis rate ( $P_N$ ) for *Quercus guyavifolia* and *Quercus pannosa*. Vertical bars indicate the errors of the mean for three measurements (*Q. guyavifolia*:  $P_N = 5.286 + 1.390VPD - 0.640VPD^2$ ,  $n = 48$ ,  $R^2 = 0.336$ ,  $P < 0.001$ ; *Q. pannosa*:  $P_N = 5.845 - 1.159VPD - 0.292VPD^2$ ,  $n = 48$ ,  $R^2 = 0.367$ ,  $P < 0.001$ ).



Following  $P_N$ -PPFD curves, the  $CO_2$  responses of photosynthesis were determined using a range of  $CO_2$  concentrations at a PPFD of  $1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and  $20^\circ\text{C}$ . Preliminary measurements showed that the  $P_N$  values for the two alpine oak species were light-saturated at an intensity of  $1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .  $CO_2$  was injected into the circuit using the injection system built into the gas analyzer. After the initial measurements at  $2000 \mu\text{mol}\cdot\text{mol}^{-1}$ , the  $CO_2$  concentration was reduced to produce other levels at which  $P_N$  was recorded. Using  $P_N$ - $C_i$  curves,  $V_{c\text{max}}$  and  $J_{\text{max}}$  were calculated using Photosyn Assistant<sup>®</sup> software according to the biochemical model of von Caemmerer and Farquhar (1981).

The dependence of photosynthesis on temperature was examined on fully mature leaves in the morning between 0800 and 1100.  $T_1$  was adjusted using the internal heating and cooling system. During measurements, the  $CO_2$  concentration in the chamber was kept at  $350 \mu\text{mol}\cdot\text{mol}^{-1}$  and PPFD at  $1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The measurements started at  $10^\circ\text{C}$  and increased by  $1^\circ\text{C}$  increments up to  $35^\circ\text{C}$ . A second-order polynomial equation was used to fit  $T_{\text{opt}}$ .

### Measurement of LMA

Following the determination of photosynthetic responses, 100 leaves were harvested from the sampled trees at each site and divided into 10 groups. In the laboratory, leaf areas were measured using a leaf-area meter (LI-3000A, Li-Cor). Dry mass was determined after oven-drying for 48 h at  $70^\circ\text{C}$ . LMA was calculated in grams per square metre.

### Nitrogen analysis

After LMA was measured, the same leaves were analyzed for LNC using a N analyzer (Leco Corporation, St. Joseph, Michigan). PNUE was calculated as the ratio of  $P_{\text{max}}$  to LNC.

### Determination of chlorophyll content

Chlorophyll was extracted from leaf disks from sampled trees with *N,N*-dimethylformamide. Chlorophyll contents were analyzed with a spectrophotometer (UV-2550, Shimadzu, Japan) and calculated using the method of Inskeep and Bloom (1985). Three replicates were made for each measurement.

### Stable carbon isotope analysis

The relationship between  $\delta^{13}\text{C}$  ratio and WUE has led to the widespread use of isotopic analysis in plant physiological ecology. In the present study, the sun-exposed leaves were harvested from the upper part of sampled trees for  $\delta^{13}\text{C}$  analysis. The  $\delta^{13}\text{C}$  ratio of leaf tissues was determined using a mass spectrometer (Finnigan MAT 253, Gainesville, Florida). The  $\delta^{13}\text{C}$  ratio is expressed in delta notation:  $\delta^{13}\text{C}$  (‰) compared with a standard (Pee Dee Belemnite). The precision of the analysis was  $\pm 0.3\%$ .

### Statistical analysis

Statistical analysis was performed using SPSS<sup>®</sup> Version 10.0 (SPSS Inc., Chicago, Illinois). Normality of distribution and homogeneity of variance were checked prior to any further statistical analyses by a one-sample Kolmogorov-Smirnov test. Following standard statistical protocols, differences in leaf morphological and physiological variables among sites were determined using one-way analysis of variance (ANOVA) and a LSD test for multiple comparisons, and between May and August by an independent-samples *t* test. The relationships between photosynthetic parameters and leaf traits were addressed using regression analysis.

## Results

At eight sites, maximum PPFD,  $T_a$ , and VPD were observed at midday (Fig. 1). There was no statistical difference in average daily PPFD among altitudes ( $F_{[7,22]} = 0.602$ ,  $p = 0.754$ ). However, both  $T_a$  ( $F_{[7,22]} = 8.466$ ,  $p = 0.000$ ) and VPD ( $F_{[7,22]} = 4.434$ ,  $p = 0.000$ ) decreased as altitude increased.

Across all study sites, the two oaks showed a significantly midday depression in photosynthesis each day (Fig. 1). The average  $P_N$  values for the three leaves sampled at different altitudes peaked rapidly after dawn before subsiding during the middle of the day. Values reached a maximum at about 10:00–11:00, increased gradually again after 14:00, and reached a second peak in the late afternoon. The diurnal var-

**Table 2.** Results of statistical analysis of photosynthetic variables and related leaf traits in *Quercus guyavifolia* and *Quercus pannosa* at different altitudes.

Variable	<i>Quercus guyavifolia</i>				<i>Quercus pannosa</i>				Species	
	Site		Season		Site		Season		t ratio	P
$P_{\max}$	12.323	0.002	2.572	0.017	4.300	0.044	3.155	0.005	5.644	0.000
AQE	8.761	0.007	2.950	0.007	8.454	0.007	1.265	0.219	1.951	0.057
$V_{\text{cmax}}$	7.920	0.009	1.386	0.180	8.682	0.007	2.761	0.011	7.324	0.000
$J_{\max}$	8.341	0.008	3.980	0.001	6.083	0.018	3.546	0.002	5.158	0.000
LNC	0.747	0.554	0.929	0.363	1.771	0.230	0.088	0.930	5.369	0.000
PNUE	4.296	0.044	2.338	0.029	5.294	0.026	3.884	0.001	2.513	0.016
Chl	22.316	0.000	2.304	0.031	38.958	0.000	1.133	0.270	0.192	0.848
LMA	5.514	0.024	1.198	0.244	2.819	0.107	0.311	0.259	1.733	0.090
$\delta^{13}\text{C}$ ratio	15.250	0.001	—	—	5.586	0.023	—	—	0.182	0.857
WUE	10.750	0.004	—	—	5.174	0.028	—	—	1.528	0.141

iations of  $P_N$  showed an inverse relationship to the diurnal trends of  $T_a$  and VPD.

Temperature had an important influence on photosynthesis in the two oaks at all sites.  $T_{\text{opt}}$  for both species decreased as altitude increased (Fig. 2). The two oaks were able to attain higher  $P_N$  values between 17 and 23 °C. At all sites, net photosynthesis decreased sharply when  $T_l$  was above 25 °C. There was no significant variation in  $T_{\text{opt}}$  among species ( $t = 0.102$ ,  $p = 0.919$ ) or between values in May and August ( $t = 2.038$ ,  $p = 0.054$ ).

$P_N$  values were light-saturated at a PPFD of 900–1050  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for *Q. guyavifolia* and at 840–990  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for *Q. pannosa* (Fig. 3). The light-saturated PPFD (at which 95% of  $P_{\max}$  is achieved) was higher for *Q. guyavifolia* than for *Q. pannosa* ( $t = 2.833$ ,  $p = 0.001$ ), but there was no significant difference in photosynthetic light-saturated PPFD among sites (*Q. guyavifolia*:  $F_{[3,10]} = 2.752$ ,  $p = 0.0012$ ; *Q. pannosa*:  $F_{[3,10]} = 1.462$ ,  $p = 0.296$ ) or between May and August ( $t = 0.332$ ,  $p = 0.743$ ).

The relationship between VPD and  $P_N$  was analyzed using measurements of diurnal variation (Fig. 4). VPD had an important effect on photosynthesis in the two oaks (*Q. guyavifolia*:  $R^2 = 0.336$ ,  $p < 0.0001$ ; *Q. pannosa*:  $R^2 = 0.367$ ,  $p < 0.0001$ ). A high VPD significantly reduced  $P_N$ .

Chl values for the two oaks decreased with increasing altitude, while LMA increased. Although LNC values for the two oaks in the middle of their altitudinal range were higher than at lower and higher altitudes, there were no significant differences among sites or between May and August (Table 2). However, *Q. guyavifolia* had a higher LNC than *Q. pannosa* ( $t = 5.369$ ,  $p = 0.000$ ), but Chl and LMA values for the two oaks were not significantly different (LMA:  $t = 1.733$ ,  $p = 0.090$ ; Chl:  $t = 0.192$ ,  $p = 0.848$ ). The  $\delta^{13}\text{C}$  ratio for the two oaks was higher in the middle of their altitudinal range than at lower and higher altitudes, but did not differ significantly between the species (Table 2).

In both May and August,  $P_d$ ,  $P_{\max}$ ,  $P_d/P_{\max}$ , AQE,  $V_{\text{cmax}}$ ,  $J_{\max}$ , WUE, and PNUE for *Q. guyavifolia* were highest at an altitude of 3610 m and decreased towards the upper and lower ends of the altitudinal range, while the maximum values of these physiological parameters for *Q. pannosa* were found at 3240 m (Fig. 5). *Quercus guyavifolia* had higher  $P_{\max}$  and PNUE values than *Q. pannosa* (Table 2); mean-

while, the photosynthetic capacities of the two oaks were higher in August than in May, but the altitudinal trends in photosynthetic capacity did not change with season.

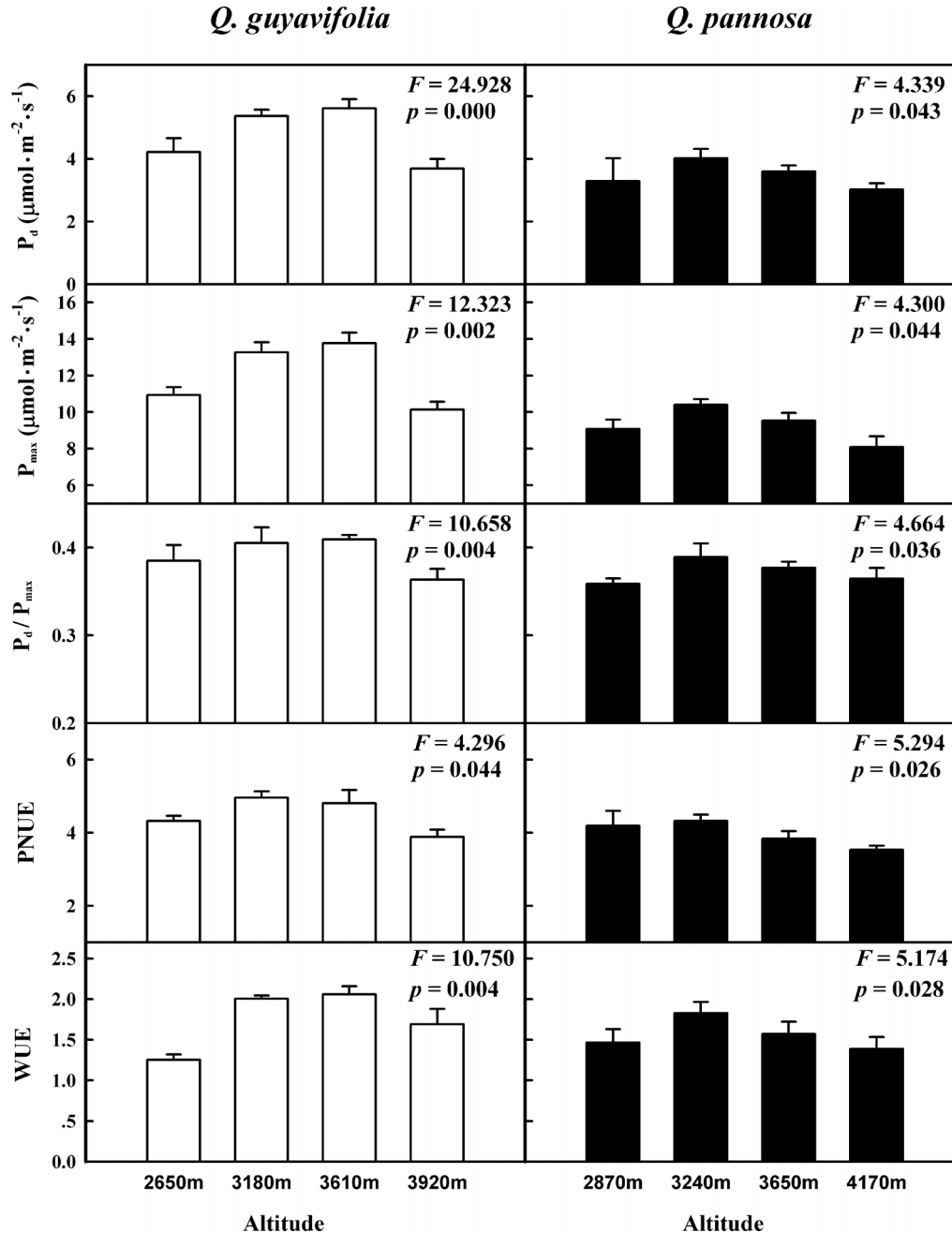
## Discussion

In the Hengduan Mountains, both *Q. guyavifolia* and *Q. pannosa* experienced a pronounced midday depression in photosynthesis ( $P_N$ ), which was similar to the responses of sclerophyllous Mediterranean oaks (*Quercus suber*) (Tenhunen et al. 1984). Although the diurnal variation in photosynthesis exhibited an inverse relationship to that of PPFD,  $T_a$ , and VPD, the two alpine oaks could withstand a high PPFD without photosynthesis decreasing substantially, provided the temperature was favorable. On the other hand, when  $T_l$  went above 25 °C, which typically occurred from 1100 to 1400, photosynthesis in the two oaks decreased sharply. A high  $T_a$  resulted in the increase in VPD ( $R^2 = 0.845$ ,  $p = 0.000$ ), and therefore reduced stomatal conductance (Iio et al. 2004) and  $\text{CO}_2$  supply from the atmosphere to the intercellular spaces. Photosynthesis would also be inactivated by high  $T_a$  at midday (Berry and Björkman 1980). This experiment provided evidence of the important role of high  $T_a$  and high VPD on photosynthetic depression in the two oaks at midday (Fig. 4).

Despite many studies on altitudinal trends in photosynthesis and leaf traits, there was no clear consensus. Most studies in the literature have reported that photosynthetic capacity and carboxylation efficiency decreased with increasing altitude (Cabrera et al. 1998; Rada et al. 1998). It has been considered that the lower  $P_N$  values at higher altitude are caused by significantly thicker leaf pubescence and lower stomatal conductance, photosynthetic-pigment content, and leaf N content (Cabrera et al. 1998; Rada et al. 1998). However, in many studies it has been reported that photosynthetic capacity increased with altitude (Woodward 1986; Körner and Diemer 1987). These results could be explained by the increase in carboxylation efficiency, leaf N content, chlorophyll content, and stomatal conductance with altitude (Körner and Diemer 1987; Friend et al. 1989). This discrepancy would be due to the differences in equipment, plant material, environmental conditions, and altitudinal range (Rada et al. 1998).

The present study showed that individuals of the two oak

Fig. 5. Physiological traits of *Quercus guyavifolia* and *Quercus pannosa* at different altitudes. Error bars represent  $\pm 1$  SE.

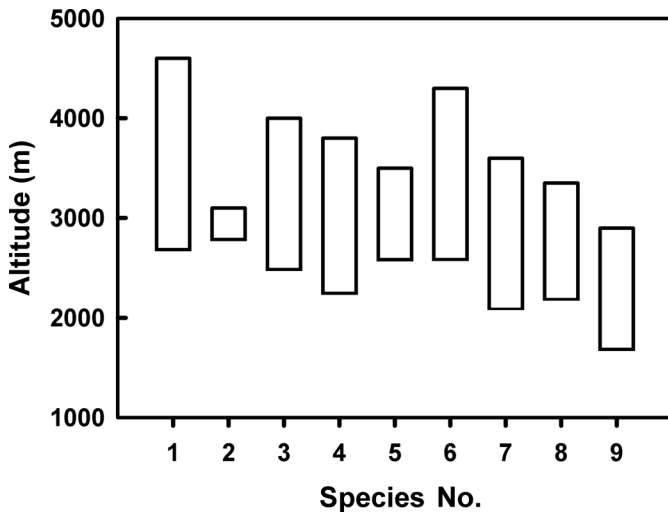


species growing in the middle of their altitudinal range displayed greater photosynthetic capacity ( $P_{\max}$ ) than those growing at the upper and lower ends of the altitudinal range. Angert (2006) suggested that two *Mimulus* species attained the greatest aboveground biomass and  $P_N$  values when grown at a temperature characteristic of the middle of their altitudinal range. The highest  $P_{\max}$  values for *Pinus sylvestris* were found in the middle of the distribution area, and  $P_{\max}$  decreased towards both ends of the transect (Luoma 1997). Although the altitudinal difference in photosynthesis could have been caused by differences in photosynthetic-pigment content, chlorophyll content was not correlated with the  $P_{\max}$  values for the two alpine oaks ( $r^2 = 0.049$ ,  $p = 0.131$ ).

Most studies have shown that LNC increases with altitude (Friend et al. 1989; Cordell et al. 1999). For the two oaks, LNC was higher in the middle of the altitudinal range than at other sites, but there was no significant difference among altitudes. However, PNUE values in the middle of the altitudinal range were higher than at higher and lower altitudes. LNC was significantly correlated with  $P_{\max}$  ( $R^2 = 0.587$ ,  $p = 0.000$ ). This has been confirmed by many studies (Evans 1989; Cordell et al. 1999; Hikosaka et al. 2002). The strong relationship was due to the large proportion of leaf N present in the photosynthetic apparatus.

Photosynthesis depends partly on leaf metabolic capacity, which can be partitioned into the activity of the enzyme ribulose-1,5-bisphosphate (RuBP) carboxylase–oxygenase

**Fig. 6.** Species richness of nine alpine oak species along an altitudinal gradient in the Hengduan Mountains. 1, *Quercus aquifoloides*; 2, *Quercus fimbriata*; 3, *Quercus guyavifolia*; 4, *Quercus longispica*; 5, *Quercus monimotricha*; 6, *Quercus pannosa*; 7, *Quercus smecarpifolia*; 8, *Quercus senescens*; 9, *Quercus spinosa*. The figure was generated from the data of Zhou et al. (2003).



(Rubisco), which catalyzes the first step in carbon fixation ( $V_{cmax}$ ), determines the rate of RuBP regeneration ( $J_{max}$ ) via electron transport and influences other minor components. In the present study, LNC was also related to  $V_{cmax}$  ( $R^2 = 0.574$ ,  $p = 0.000$ ) and  $J_{max}$  ( $R^2 = 0.478$ ,  $p = 0.000$ ). This indicated that a higher LNC for the two oaks in the middle part of the altitudinal range would result in a higher Rubisco content and therefore a higher  $P_{max}$ . In addition, LMA for the two oaks increased with altitude. The plants with a higher LMA limited the supply of  $CO_2$  to the chloroplasts because the path of diffusion became longer in a thicker leaf (Kao and Chang 2001). The lower  $P_N$  would be also related to the higher LMA at higher altitudes.

Stomatal conductance in the two oaks in the middle of the altitudinal range was greater than at higher and lower altitudes, but there were no statistical differences in stomatal conductance with altitude. Kumar et al. (2005) found that stomatal conductance increased with altitude, and suggested that the insensitivity of stomatal conductance to PPFD could be one of the adaptive features that allows a wider altitudinal distribution of plants. In our study, there was no significant difference in PPFD at different altitudes ( $F_{[7,22]} = 0.503$ ,  $p = 0.681$ ).

Because stable carbon isotope ( $\delta^{13}C$ ) composition of leaf tissue reflects the balance between mesophyll demand for  $CO_2$  and diffusion through the stomata, it can give a relative index of long-term WUE and carboxylation efficiency of plants (Patterson et al. 1997; Cordell et al. 1999; Hultine and Marshall 2000). In the present study, the altitudinal trend in foliar  $\delta^{13}C$  in the two oaks was similar to that in *Q. aquifolioides* (Li et al. 2006) but not *Metrosideros polymorpha* (Cordell et al. 1999). Foliar  $\delta^{13}C$  of the two oaks was positively correlated with WUE ( $R^2 = 0.490$ ,  $p = 0.000$ ) and  $V_{cmax}$  ( $R^2 = 0.202$ ,  $p = 0.008$ ). These values also confirmed that the two alpine oaks in the middle part of the altitudinal range had higher WUE values than those at lower and higher altitudes.

In the Hengduan Mountains, the photosynthetic capacities and WUE values of the two oaks were similar to those of Mediterranean oaks (Mediavilla et al. 2001). The LMA values of the two alpine oaks were higher than those of Mediterranean oaks (203–253 versus 86–242  $g \cdot m^{-2}$ ) (Damesin et al. 1997; Mediavilla et al. 2001) and similar to that of *Quercus semecarpifolia* from the Himalayas (Delillis et al. 2004). Lower temperatures could have reduced leaf extension in the alpine oaks in the Hengduan Mountains, resulting in thicker leaves (Kao and Chang 2001).

Temperature determines the altitudinal distribution of trees in at least two ways: it reduces survival through freezing or by a negative carbon balance through a lower  $P_N$  (Cavieres et al. 2000). Diminished growth at low temperatures in subalpine forests was associated with less photosynthesis (Lajzerowicz et al. 2004). Usually, sclerophyllous oaks were distributed in xerothermic regions, but *Q. guyavifolia* occurred in the relatively cold, moist habitats in the Hengduan Mountains.  $T_{opt}$  values for the two alpine oaks were significantly lower than that for the Mediterranean sclerophyllous oak (18–23 versus 25–30 °C) (Gratani et al. 2000). Sakai (1981) reported that *Q. semecarpifolia* could resist temperatures down to  $-15$  °C.

The species richness of sclerophyllous oaks in the Hengduan Mountains peaked in the altitudinal range from 2400 and 3600 m (Fig. 6). Li et al. (2006) further confirmed that an altitude near 2800 m could be the optimum zone for growth and development of *Q. aquifolioides*. This species had higher genetic variability at altitudes between 2400 and 3300 m (Li et al. 1998). In this study, individuals of the two oak species in the middle of the altitudinal range (3240–3610 m) exhibited greater photosynthetic capacity and resource-use efficiency. The degree to which plants adapt to their environment can affect their utilization of environmental resources. Efficient use of resources could lead to maximum  $P_N$ . Photosynthetic capacity would affect competition and survival of species (Nagel and Griffin 2004). The unfavorable environments at lower or higher altitudes would limit carbon assimilation, growth, and competition by plants (Zu et al. 1998), while a high  $P_N$  achieved by maximizing the biochemical capacity for photosynthesis could contribute to the success of certain genotypes under stressful conditions (Wu and Campbell 2006). These results indicate that the two alpine oaks were able to fulfill their photosynthetic and competitive potential at altitudes of 3240–3610 m. This is also confirmed by the  $P_d/P_{max}$  ratio, which expresses the effect of the currently prevailing environment on photosynthesis.

In conclusion, altitude has a major effect on LMA and the physiology of the two oak species studied. LMA increased with altitude, while chlorophyll content decreased.  $P_{max}$ , PNUE, and WUE values were highest in the middle of their altitudinal ranges and decreased towards both ends of their altitudinal ranges. The altitudinal trend in photosynthesis was linked to biochemical efficiency and LNC. Altitudes from 3240 to 3610 m would be optimal for the growth and development of these two oak species.

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## References

- Angert, A.L. 2006. Growth and leaf physiology of monkey flowers with different altitude ranges. *Oecologia*, **148**: 183–194. doi:10.1007/s00442-006-0361-z. PMID:16468056.
- Berry, J.A., and Björkman, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol.* **31**: 491–543. doi:10.1146/annurev.pp.31.060180.002423.
- Cabrera, H.M., Rada, F., and Cavieres, L. 1998. Effects of temperature on photosynthesis of two morphologically contrasting plant species along an altitudinal gradient in the tropical high Andes. *Oecologia*, **114**: 145–152. doi:10.1007/s004420050430.
- Cavieres, L.A., Rada, F., Azocar, A., García-Núñez, C., and Cabrera, H.M. 2000. Gas exchange and low temperature resistance in two tropical high mountain tree species from Venezuelan Andes. *Acta Oecol.* **21**: 203–211. doi:10.1016/S1146-609X(00)01077-8.
- Cordell, S., Goldstein, G., Meinzer, F.C., and Handley, L.L. 1999. Allocation of nitrogen and carbon in leaves of *Metrosideros polymorpha* regulates carboxylation capacity and  $\delta^{13}\text{C}$  along an altitudinal gradient. *Funct. Ecol.* **13**: 811–818. doi:10.1046/j.1365-2435.1999.00381.x.
- Damesin, C., Rambal, S., and Joffre, R. 1997. Between-tree variations in leaf  $\delta^{13}\text{C}$  of *Quercus pubescens* and *Quercus ilex* among Mediterranean habitats with different water availability. *Oecologia*, **111**: 26–35. doi:10.1007/s004420050204.
- Delillis, M., Matteucci, G., and Valentini, R. 2004. Carbon assimilation, nitrogen, and photochemical efficiency of different Himalayan tree species along an altitudinal gradient. *Photosynthetica*, **42**: 597–605. doi:10.1007/S11099-005-0019-9.
- Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of  $\text{C}_3$  plants. *Oecologia*, **78**: 9–19. doi:10.1007/BF00377192.
- Friend, A.D., Woodward, F.I., and Switsur, V.R. 1989. Field measurements of photosynthesis, stomatal conductance, leaf nitrogen and  $\delta^{13}\text{C}$  along altitudinal gradients in Scotland. *Funct. Ecol.* **3**: 117–122. doi:10.2307/2389682.
- Gratani, L., Pesoli, P., Crescente, M.F., Aichner, K., and Larcher, W. 2000. Photosynthesis as a temperature indicator in *Quercus ilex* L. *Global Planet. Change*, **24**: 153–163. doi:10.1016/S0921-8181(99)00061-2.
- He, J.S., Chen, W.L., and Wang, X.L. 1994. Morphological and anatomical features of *Quercus* section *suber* and its adaptation to the ecological environment. *Acta Phytoecol. Sin.* **18**: 219–227.
- Hikosaka, K., Nagamatsu, D., Ishii, H.S., and Hirose, T. 2002. Photosynthesis–nitrogen relationships in species at different altitudes on Mount Kinabalu, Malaysia. *Ecol. Res.* **17**: 305–313. doi:10.1046/j.1440-1703.2002.00490.x.
- Hirose, T., and Bazzaz, F.A. 1998. Trade-off light- and nitrogen-use efficiency in canopy photosynthesis. *Ann. Bot. (Lond.)*, **82**: 195–202. doi:10.1006/anbo.1998.0668.
- Hovenden, J.M., and Brodribb, T. 2000. Altitude of origin influences stomatal conductance and therefore maximum assimilation rate in southern Beech, *Nothofagus cunninghamii*. *Aust. J. Plant Physiol.* **27**: 451–456.
- Hultine, K.R., and Marshall, J.D. 2000. Altitude trends in conifer leaf morphology and stable carbon isotope composition. *Oecologia*, **123**: 32–40. doi:10.1007/s004420050986.
- Iio, A., Fukasawa, H., Nose, Y., and Kakubari, Y. 2004. Stomatal closure induced by high vapor pressure deficit limited midday photosynthesis at the canopy top of *Fagus crenata* Blume on Naeba Mountain in Japan. *Trees (Berl.)*, **18**: 510–517. doi:10.1007/s00468-004-0327-x.
- Inskeep, W.R., and Bloom, P.R. 1985. Extinction coefficients of chlorophyll *a* and *b* in *N,N*-dimethylformamide and 80% acetone. *Plant Physiol.* **77**: 483–485. PMID:16664080.
- Kao, W.Y., and Chang, K.W. 2001. Altitudinal trends in photosynthetic rate and leaf characteristics of *Miscanthus* populations from central Taiwan. *Aust. J. Bot.* **49**: 509–514. doi:10.1071/BT00028.
- Körner, C., and Diemer, M. 1987. In situ photosynthetic responses to light, temperature and carbon dioxide in herbaceous plants from low and high altitude. *Funct. Ecol.* **1**: 179–184. doi:10.2307/2389420.
- Kumar, N., Kumar, S., and Ahuja, P.S. 2005. Photosynthetic characteristics of *Hordeum*, *Rumex*, and *Trifolium* species at contrasting altitudes. *Photosynthetica*, **43**: 195–201. doi:10.1007/s11099-005-0033-y.
- Lajzerowicz, C.C., Walters, M.B., Krasowski, M., and Massicotte, H.B. 2004. Light and temperature differentially colimit subalpine fir and Engelmann spruce seedling growth in partial-cut subalpine forests. *Can. J. For. Res.* **34**: 249–260. doi:10.1139/x03-198.
- Li, C., Zhang, X.J., Liu, X.L., Luukkanen, O., and Berninger, F. 2006. Leaf morphological and physiological responses of *Quercus aquifolioides* along an altitudinal gradient. *Silva Fenn.* **40**: 5–13.
- Li, J., Chen, K.Y., and Li, B.S. 1998. The variation of genetic diversity of *Quercus aquifolioides* in different elevations. *Acta Bot. Sin.* **40**: 761–767.
- Luoma, S. 1997. Geographical pattern in photosynthetic light response of *Pinus sylvestris* in Europe. *Funct. Ecol.* **11**: 273–281. doi:10.1046/j.1365-2435.1997.00089.x.
- Mediavilla, S., Escudero, A., and Heilmeyer, H. 2001. Internal leaf anatomy and photosynthetic resource-use efficiency: interspecific and intraspecific comparisons. *Tree Physiol.* **21**: 215–259.
- Nagel, J.M., and Griffin, K.L. 2004. Can gas-exchange characteristics help explain the invasive success of *Lythrum salicaria*? *Biol. Invasions*, **6**: 101–111. doi:10.1023/B:BINV.0000010125.93370.32.
- Oechel, W.C., Lawrence, W., Mustafa, J., and Martinez, J. 1981. Energy and carbon acquisition. In *Resource use by chaparral and matorral: a comparison of vegetation function in two Mediterranean type ecosystems*. Edited by P.C. Miller. Springer-Verlag, New York. pp. 151–183.
- Patterson, T.B., Guy, R.D., and Dang, Q.L. 1997. Whole-plant nitrogen- and water-relations traits, and their associated trade-offs, in adjacent muskeg and upland boreal spruce species. *Oecologia*, **110**: 160–168. doi:10.1007/s004420050145.
- Pattison, R.R., Goldstein, G., and Ares, A. 2001. Growth, biomass allocation and photosynthesis of invasive and native Hawaiian species. *Oecologia*, **117**: 449–459. doi:10.1007/s004420050680.
- Prioul, J.L., and Chartier, P. 1977. Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic  $\text{CO}_2$  fixation: a critical analysis of the methods used. *Ann. Bot. (Lond.)*, **41**: 789–800.
- Rada, F., Azocar, A., Gonzalez, J., and Briceno, B. 1998. Leaf gas exchange in *Espeletia schultzei* Wedd, a giant caulescent rosette species, along an altitudinal gradient in the Venezuelan Andes. *Acta Oecol.* **19**: 73–79. doi:10.1016/S1146-609X(98)80010-6.
- Sakai, A. 1981. Winter hardiness of tree species at high altitudes in the east Himalaya, Nepal. *Ecology*, **62**: 1288–1298. doi:10.2307/1937293.

- Tenhunen, J.D., Lange, O.L., Gebel, J., Beyschlag, W., and Weber, J.A. 1984. Changes in photosynthetic capacity, carboxylation efficiency, and CO<sub>2</sub> compensation point associated with midday stomatal closure and midday depression of net CO<sub>2</sub> exchange of leaves of *Quercus suber*. *Planta (Berl.)*, **162**: 193–203. doi:10.1007/BF00397440.
- von Caemmerer, S., and Farquhar, G.D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange rates of leaves. *Planta (Berl.)*, **153**: 376–387. doi:10.1007/BF00384257.
- Woodward, F.I. 1986. Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. *Oecologia*, **70**: 580–586. doi:10.1007/BF00379908.
- Wu, C.A., and Campbell, D.R. 2006. Environmental stressor differentially affect leaf ecophysiological response in two *Ipomopsis* species and their hybrids. *Oecologia*, **148**: 202–212. doi:10.1007/s00442-006-0363-x. PMID:16496183.
- Zhang, S.B., Zhou, Z.K., Hu, H., Xu, K., Yan, N., and Li, S.Y. 2005. Photosynthetic performances of *Quercus pannosa* vary with altitude in the Hengduan Mountains, southwest China. *For. Ecol. Manage.* **212**: 291–301. doi:10.1016/j.foreco.2005.03.031.
- Zhang, Y.G. 1998. Several issues concerning vertical climate of the Hengduan Mountains. *Resour. Sci.* **20**: 12–14.
- Zhou, Z.K., Pu, C.X., and Chen, W.Y. 2003. Relationships between the distributions of *Quercus* sect *Heterobalanus* (Fagaceae) and uplift of Himalayas. *Adv. Earth Sci.* **18**: 884–890.
- Zu, Y.G., Yan, X.F., Zhang, W.H., Wu, S.X., Zhou, F.J., and Sun, H.Q. 1998. Gas exchange and water use efficiency of *Adenophora lobophylla* at different altitudes on the east boundary of Qing-Zang plateau. *Acta Bot. Sin.* **40**: 947–954.