Growth habit and leaf economics determine gas exchange responses to high elevation in an evergreen tree, a deciduous shrub and a herbaceous annual

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Abstract. Plant growth at high elevations necessitates physiological and morphological plasticity to enable photosynthesis (A) under conditions of reduced temperature, increased radiation and the lower partial pressure of atmospheric gases, in particular carbon dioxide (pCO2). Previous studies have observed a wide range of responses to elevation in plant species depending on their adaptation to temperature, elevational range and growth habit. Here, we investigated the effect of an increase in elevation from 2500 to 3500 m above sea level (a.s.l.) on three montane species with contrasting growth habits and leaf economic strategies. While all of the species showed identical increases in foliar d13C, dark respiration and nitrogen concentration with elevation, contrasting leaf gas exchange and photosynthetic responses were observed between species with different leaf economic strategies. The deciduous shrub Salix atopantha and annual herb Rumex dentatus exhibited increased stomatal (Gs) and mesophyll (Gm) conductance and enhanced photosynthetic capacity at the higher elevation. However, evergreen Quercus spinosa displayed reduced conductance to CO2 that coincided with lower levels of photosynthetic carbon fixation at 3500 m a.s.l. The lower Gs and Gm values of evergreen species at higher elevations currently constrains their rates of A. Future rises in the atmospheric concentration of CO2 ([CO2]) will likely predominantly affect evergreen species with lower specific leaf areas (SLAs) and levels of Gm rather than deciduous species with higher SLA and Gs values. We argue that climate change may affect plant species that compose high-elevation ecosystems differently depending on phenotypic plasticity and adaptive traits affecting leaf economics, as rising [CO2] is likely to benefit evergreen species with thick sclerophyllous leaves.

Keywords: Elevation; mesophyll conductance; partial pressure of CO2; photosynthesis; Quercus spinosa; Rumex dentatus; Salix atopantha; stomatal conductance.

Introduction

The adaptational responses of plants to alterations in temperature and the partial pressure of carbon dioxide (pCO2) along elevational gradients have been used to infer the likely responses of vegetation to climatic changes in the past and future (e.g. Körner 2007; Kouwenberg et al. 2007; Bai et al. 2015). The partial pressure of all atmospheric gases declines with elevation, reducing the
availability of CO₂ for photosynthesis and oxygen for respiration, while lower temperatures and higher levels of radiation may decrease the activity of photosynthetic and metabolic enzymes (Gale 1972a; Miroslavov and Kravkina 1991; Allen and Ort 2001). Growth at high elevation, therefore, necessitates physiological and morphological adaptations to enable photosynthesis (e.g. Woodward 1986; Williams and Black 1993; Terashima et al. 1995; Cordell et al. 1998; Körner 2007; Feng et al. 2013). High-elevation ecosystems are often more sensitive to climatic change than those at sea level, as the influence of rising atmospheric temperature and carbon dioxide concentration ([CO₂]) on photosynthesis becomes more pronounced with elevation (Gale 1972a, b, 1973; Terashima et al. 1995). Nonetheless, not all plant species respond in the same manner along elevational gradients, and comparatively little is known regarding the physiological and morphological adaptation of plants growing at high elevations >2500 m above sea level (a.s.l.). An understanding of the physiological and morphological processes that underpin photosynthesis and leaf gas exchange at high elevations may assist in our understanding of the likely impacts of future climate change on these high-elevation ecosystems.

In addition to physical changes to pCO₂ and temperature, an increase in elevation may also be associated with fluctuations in soil type, wind speed, water availability and the quality/quantity of incident radiation (Körner 2007). These factors will all influence plant growth and photosynthesis (e.g. Körner and Cochrane 1985; Woodward 1986). The photosynthetic performance of a plant is determined by its capacity for the uptake and assimilation of CO₂ (Farquhar et al. 1980); this is controlled by stomatal (Gₛ) and mesophyll conductance (Gₘ) to CO₂, the carboxylation capacity of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (RubisCO) (V₉₅₉₅max) and the maximum rate of electron transport for RuBP regeneration (V₉₉₉₉max) (e.g. Shi et al. 2006; Centritto et al. 2009; Haworth et al. 2011; Feng et al. 2013). Stomatal conductance frequently increases with elevation, possibly as a result of the lower availability of CO₂ in the atmosphere necessitating increased rates of conductance to sustain adequate CO₂ uptake (Körner et al. 1986; Woodward 1986). This enhanced Gₛ with elevation is often accompanied by increased stomatal density values and higher rates of transpirative water loss (Körner and Cochrane 1985; Woodward and Bazzaz 1988; Kouwenberg et al. 2007; Wang et al. 2014). The lower partial pressures of gases at high elevations will not only result in more rapid diffusion through the stomatal pore of CO₂ but also water vapour, which when combined with the generally higher irradiances and drier atmosphere at high elevations will enhance the transpirative costs of growth at high elevation (Gale 1972a, b, 1973). However, these patterns of increased Gₛ are not universal, with species such as Quercus aquifolioides (Li et al. 2006; Feng et al. 2013), Q. guayifolia (Hu et al. 2015) and Typha orientalis (Bai et al. 2015) exhibiting reduced Gₛ and stomatal density with elevation. Species with reduced Gₛ at high elevation may exploit the more rapid diffusion of CO₂ at low partial pressures (Gale 1972a) by increasing rates of carboxylation of CO₂ to maintain the diffusion gradient between the internal leaf and external atmosphere (Cordell et al. 1998; Shi et al. 2006) without incurring the additional transpirative costs associated with increased Gₛ and stomatal density (Haworth et al. 2015). However, lower temperatures at high elevation may also minimize the effect of increased diffusion of CO₂ at lower partial pressures during the gaseous phase of CO₂ transport (Körner 2007).

The concentration of CO₂ inside the chloroplast envelope is directly related to stomatal and mesophyll conductance to the transport of CO₂ (Loreto et al. 2009; Flexas et al. 2013). As a result, Gₛ and Gₘ often operate synchronously to determine photosynthesis (A) as a function of the total conductance to CO₂ (G₉₉₉₉max) (Centritto et al. 2003; Hu et al. 2010; Lauteri et al. 2014). More rapid diffusion of CO₂ at lower partial pressures may counteract the effect of reduced availability on gaseous transport of CO₂ through the stomata to the spongy mesophyll (Gale 1972a), but this will not influence the transport of CO₂ through the aqueous phase to the chloroplast (Loreto et al. 1994; Flexas et al. 2013). As such, the lower partial pressure of CO₂ will have a significant impact on A, and Gₘ will play a significant role in determining the response of a plant species to growth at high elevation. It may be expected that an increase in elevation would induce a rise in Gₘ, to ensure CO₂ uptake at lower partial pressures; however, Gₘ is determined by a number of physical and biochemical factors (Warren 2007; Niinemets et al. 2009) that are also affected by elevation (Körner et al. 1979, 1986; Kagami et al. 2001; Feng et al. 2013). The specific leaf area (SLA) of temperate species tends to decrease with elevation, resulting in leaves with more closely packed cells (Friend and Pomeroy 1970; Friend et al. 1989; Li et al. 2013; Pan et al. 2013; Haworth and Raschi 2014) and lower Gₘ values (Kagami et al. 2001; Niinemets et al. 2009; Feng et al. 2013). For example, Polygonum cuspidatum exhibited a reduction in SLA and Gₘ over an elevation range of 10–2500 m (Kagami et al. 2001). This reduction in SLA with elevation is likely a result of increased incident radiation inducing a more compact leaf morphology (Niinemets 2001; Poorter et al. 2009). Increased elevation also resulted in a decrease of SLA and Gₘ values, but no change in Gₛ, in the tropical Hawaiian tree species Metrosideros polymorpha (Cordell et al. 1998). In contrast, the highland species Buddleja davidii that grows from 1200 to 3500 m a.s.l. exhibited no
alteration of SLA, but a reduction in $G_s$ alongside an increase in $G_m$ with elevation that corresponded to enhanced $V_{cmax}$ (Shi et al. 2006). This indicates the importance of the temperature to which a species is adapted and leaf economic strategy in determining the response to elevation (Hovenden and Vander Schoor 2004). Photosynthesis rates decline with elevation due to lower partial pressures reducing the availability of CO$_2$ (Gale 1972a; Körner and Diemer 1987) and diminished temperatures resulting in lower activity of photosynthetic enzymes (Fryer et al. 1998). The selective pressures exerted by lower temperatures and pCO$_2$ may induce increased photosynthetic capacity (i.e. enhanced $V_{cmax}$ and $J_{max}$) at high elevation to enable sufficient CO$_2$ uptake (Cordell et al. 1998; Shi et al. 2006; Feng et al. 2013). At higher elevations, greater $V_{cmax}$ and $J_{max}$ are associated with increased leaf nitrogen and chlorophyll concentrations, but lower photosynthetic nitrogen use efficiency (PNUE) (Cordell et al. 1998; Kogami et al. 2001; Feng et al. 2013). Increased leaf nitrogen levels at higher elevations also commonly correspond to reduced SLA (Feng et al. 2013); conversely at sea level, SLA is often positively related to nitrogen concentration (Poorter et al. 1990). At high elevations, many species exhibit a reduction in leaf area (Kouwenberg et al. 2007), possibly as a response to increased levels of incident radiation that would increase leaf temperatures and transpiration rates through increased leaf to air vapour pressure deficit (Körner and Cochrane 1985). This shift in leaf area would affect the calculation of SLA (Poorter et al. 2009), and suggests that the temperature lapse rate and decline in pCO$_2$ with elevation, and the selective pressures they exert induce differential adaptational responses to those observed at low elevation, and may therefore have different effects on plants with contrasting growth habits and leaf economic strategies.

Plants growing at high elevations of 2500–3500 m a.s.l. experience relatively low pCO$_2$ in the range of 27.7–24.6 Pa in comparison with pCO$_2$ of 38 Pa at sea level. The temperature lapse rate equates to an approximate decline in temperature of 5.5 °C with every 1000 m gained in elevation (Barry 2013). In this study, we aimed to characterize the physiological and morphological adaptations of three plant species with different growth habits: an evergreen tree (Quercus spinosa), a deciduous shrub (Salix atopantha) and a herbaceous annual (Rumex dentatus). The responses to growth at high elevation of the three study species were also compared with those of Q. aquifoliodes from the same habitat in an earlier investigation by Feng et al. (2013). To assess the adaptational responses of these plants to high elevations in the range of 2500–3500 m a.s.l., we conducted leaf gas exchange measurements and sampled leaves in the field to (i) analyse photosynthetic physiology at high elevations, (ii) characterize leaf gas exchange through quantification of stomatal and mesophyll conductance responses to CO$_2$ and any diffusional limitation to photosynthesis, (iii) gauge the effect of reduced pCO$_2$ and temperature on leaf economics and nitrogen concentration and (iv) evaluate the likely effect of future climate change in terms of rising pCO$_2$ and temperature on mountainous species and ecosystems.

**Methods**

**Plant material and study area**

Three plant species with contrasting growth habits that grow at high elevations in north-western China were chosen for analysis. Quercus spinosa is a 6–10 m tall evergreen tree occurring in mountain regions of South East Asia over an elevation range of 1000–3500 m and over a range of 2000–3500 m in south-western China (Wu et al. 2011). Salix atopantha is a deciduous shrub 1–2 m in size, specific to western and south-western China occurring in mountainous regions at elevations of 2300–3500 m (Chen-Fu and Skvortsov 1998). Rumex dentatus is a 0.3–0.7 m tall annual herb that grows on moist slopes from sea level to high elevations (>3500 m a.s.l.) in Asia, North Africa and Europe (Kumar et al. 2005). It grows in mountainous regions at elevations of 1200–3600 m in south-western China (Shi and Ming 1987).

Populations of *Q. spinosa*, *S. atopantha* and *R. dentatus* at elevations of 2400 and 3500 m a.s.l. in the Wolong Reserve (south-eastern Tibetan-Qinghai area, Sichuan Province, China) (32°25′–32°53′N, 104°20′–104°41′E) were studied. The populations from the higher and lower elevations did not experience water stress and received full illumination with no shading. The leaves used for gas exchange measurements and collected for leaf economic traits, carbon isotope and nitrogen concentration analysis were at identical developmental stages (i.e. the youngest fully expanded leaf at the end of a branch). Field work was conducted from July to August 2010.

**Gas exchange and fluorescence measurements**

Leaf gas exchange and fluorescence parameters of the central leaf section were simultaneously measured using a LI-6400-40 leaf chamber fluorometer (LI-COR, Inc., Lincoln, NE, USA) equipped with a 2-cm$^2$ cuvette. One leaf was analysed from six plants for each species at each elevation. The concentration of atmospheric gases is constant with elevation; rather, it is the partial pressure of those gases that declines as elevation increases. All measurements were conducted at the same concentration of [CO$_2$] but at the respective partial pressures of 2500 and 3500 m a.s.l. The LiCor Li6400 contains a barometric pressure sensor that allows for compensation of the effects of
changes in partial pressure on measurements over a range of 65–115 kPa with an accuracy of and resolution of 0.002 kPa. Standard atmospheric pressure at sea level is 101.325 kPa, and at 3500 m a.s.l., atmospheric pressure is ~70 kPa, indicating that our measurements were conducted within the operating range of the instrument. The measurements were made in situ between 9:00 and 15:00 at a saturating photosynthetic photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1} for Q. spinosa, 1400 μmol m^{-2} s^{-1} for S. atapantha and 2000 μmol m^{-2} s^{-1} for R. dentatus at a CO2 concentration of 380 μmol mol^{-1}. The saturating PPFD was determined by response curves of A to increasing PAR (Ögren and Sundin 1996). Leaf temperature was set at 25 °C, and the relative humidity in the leaf cuvette ranged between 46 and 50 %. The chlorophyll fluorescence yield (i.e. the quantum yield of photosystem II (PSII) in the light, ΦPSII = ΔF/Φm) was measured using a saturating pulse of white light (10 000 μmol m^{-2} s^{-1}) (Genty et al. 1989). Mesophyll conductance to CO2 diffusion was calculated using the variable J method (Harley et al. 1992). As this work was conducted in the field, it was possible to calibrate electron transport rate under non-photorespiratory conditions; therefore, a standard calibration where α = 0.85 and β = 0.5 was used in the calculation of Gm (Gilbert et al. 2012; Walker and Cousins 2013). The variable J method is sensitive to the estimation of the CO2 compensation point to photorespiration (I*) and leaf respiration (Gilbert et al. 2012). While measurements of dark respiration (Rd) were also made at ambient CO2 concentration in the dark on the same leaves, I* used in the gas exchange algorithm was calculated from the Rubisco-specific factors of Galmeis et al. (2005) using the photosynthetic constants of Von Caemmerer (2000) and formulae of Brooks and Farquhar (1985) (Q. spinosa: I* = 52.513 μmol m^{-1} mol; S. atapantha: I* = 54.145 μmol m^{-1} mol and R. dentatus: I* = 52.512 μmol m^{-1} mol). As I* is a relatively conservative parameter (Harley et al. 1992), we assumed that the I* value used in the gas exchange algorithm did not affect the estimation of Gm. To reduce diffusion leaks through the chamber gasket (Flexas et al. 2007), a supplementary external chamber gasket composed of the same polymer foam was added to create an interspace between the two gaskets (i.e. a double-gasket design with a 5-mm space separating the internal and external gaskets). Then the CO2 and H2O gradients between the in-chamber air and pre-chamber air were minimized by feeding the infrared gas analyse exhaust air into the interspace between the chamber and the pre-chamber gaskets (Rodeghiero et al. 2007). Total conductance to CO2 (Gtot) was calculated from mesophyll and stomatal conductance to CO2 following Loreto et al. (1994) as:

\[ G_{tot} = \frac{G_m G_s}{G_s + G_m} \quad (1) \]

Light saturated A/IP response curves were measured at a leaf temperature of 25 °C and a relative humidity in the leaf cuvette of ~50 % over a range of [CO2] values on a minimum of five plants per species at each elevation. To remove the effect of stomatal limitation on A, the leaves were first pre-conditioned at a [CO2] of 50 μmol mol^{-1} for ~60 min to force stomatal opening as described by Centritto et al. (2003). The concentration of [CO2] within the cuvette was then progressively increased to 2000 μmol mol^{-1}. The photosynthetic parameters Amax (net CO2 assimilation rate under conditions of PPFD and CO2 saturation), Vcmax (RuBP-saturated rate of Rubisco: estimate of the carboxylation efficiency of Rubisco determined from the slope of the A/IP curve at a [CO2] of 40–200 μmol mol^{-1}) and Jmax (maximum rate of electron transport) were estimated by fitting the mechanistic model of Farquhar et al. (1980).

**Leaf sampling, carbon isotope discrimination and leaf nitrogen analysis**

Immediately after the gas exchange measurements, two leaves per plant from six plants per species at each elevation were detached and stored in sealed plastic bags for the measurement of leaf area, leaf weight, leaf nitrogen concentration and foliar δ13C. Leaf area was measured with a Li-3000 leaf area metre (Li-COR, Inc.). The leaves were then dried at 80 °C for 48 h, the dry mass recorded and then ground into a fine powder using a ceramic grinding container. Specific leaf area (cm² g⁻¹) was determined as the leaf area to leaf dry mass ratio. Nitrogen concentration (Nmass, mg g⁻¹) was measured on 0.1 g of dried, ground tissue by using standard Kjeldahl technique and assayed for ammonium with an ultraviolet visible spectrophotometer (Tu1221, Beijing Purkinje General Instrument Company, Beijing, China). Narea (nitrogen concentration on a leaf area basis, g m⁻²) and PNUE (μmol mol⁻¹ s⁻¹) were calculated using the following formulae:

\[ N_{area} = \frac{10 \times N_{mass}}{SLA} \quad (2) \]

\[ PNUE = A \times \frac{14}{N_{area}} \quad (3) \]

where 14 is the atomic mass of nitrogen.

Carbon isotope composition (δ¹³C) was measured on 0.001 g of ground dried tissue by using a continuous flow isotope ratio mass spectrometer. Samples were quantitatively combusted in an elemental analyser (Flash-EA 1112, Thermo Electron, Milano, Italy). The CO2 obtained was injected into the helium stream of the mass spectrometer (DELTAplus XP, ThermoFinnigan, Bremen, Germany). The ratio of isotopes (R = ¹³C/¹²C) was
measured and used to calculate $\delta^{13}C$ referred to the Pee Dee Belemnite standard according to Farquhar and Richards (1984) as follows:

$$\delta^{13}C = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1$$  (4)

**Statistical analysis**

A one-way analysis of variance (ANOVA) was used to assess differences in the data collected from the plants growing at 2500 and 3500 m a.s.l. using the software package SPSS 13.5 (SPSS, Chicago, IL, USA), and graphs were prepared using SigmaPlot 11.0 software (Systat Software Inc., San Jose, CA, USA).

**Ethics statement**

The field study did not involve endangered or protected species. No specific permissions or permits were required for the analysis and collection of Q. spinosa, S. atopantha and R. dentatus leaves from the Wolong Reserve (32°25′–32°53′N, 104°20′–104°41′E; Tibetan-Qinghai area, Sichuan Province, China). The leaves were collected from public land with the consent of the responsible government agency (the Chinese Academy of Forestry).

**Results**

Photosynthesis was closely related to conductance to CO$_2$ in all of the species at high elevations (Fig. 1). Total conductance ($G_t$) incorporating both $G_s$ and $G_m$ correlated most closely with $A$ (Fig. 1C). However, the species analysed did not display identical responses to increased elevation; Q. spinosa and Q. aquifolioides showed reduced conductance to CO$_2$ with an increase in elevation, while S. atopantha and R. dentatus exhibited higher $G_s$, $G_m$ and $G_{\text{tot}}$ values at higher elevations (Table 1). The annual herb R. dentatus and deciduous shrub S. atopantha exhibited generally higher values of conductance to CO$_2$ and $A$ than the evergreen Quercus species (Fig. 1). Alterations in $G_s$ and $G_m$ with elevation did not significantly affect $P_i/P_a$ or $P_e/P_o$ ratios in any of the species analysed (Table 1), suggesting modification of the photosynthetic physiology alongside adjustment in conductance to CO$_2$ (Figs 1 and 2). Moreover, the similarity in the ratio of $P_i$ to $P_o$ indicates that any variation in or low values of $C_i$ was unlikely to be responsible for any of the observed patterns in $G_m$ reported in this study (Tholen et al. 2012). Respiration in the dark ($R_n$) was greater at the higher elevation in all of the species (Table 1). Leaves of Q. spinosa exhibited the lowest $R_n$ values of $-1.21 \mu$mol m$^{-2}$ s$^{-1}$ at 2500 m a.s.l., but following a 181.8 % increase showed the highest levels of $R_n$ at the greater elevation of 3500 m a.s.l., suggesting that the impact of increased elevation was greatest on the species with sclerophyllous evergreen foliage. The two species with leaf lifespans of $<$9 months,
Table 1. Leaf assimilation rate \((A)\), stomatal conductance \((G_s)\), mesophyll conductance \((G_m)\), \(P_i/\)ambient partial pressure\(P_a\), \(P_c/\)chloroplast partial pressure\(P_a\), and \(R_d\) (dark respiration) values of the three plants growing at higher and lower altitudes. Means of a parameter followed by the same letter were not statistically different using a one-way ANOVA \((P > 0.05)\) with least significant difference (LSD) post hoc test.

|        | Q. spinosa | S. atopantha | R. dentatus | \(F_{5,20}\) | \(P\)-value |
|--------|------------|--------------|-------------|--------------|-------------|
| \(A\) (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)) |            |              |             |              |             |
| Low elevation | 8.00 ± 0.45 b | 7.02 ± 0.17 b | 11.62 ± 0.54 c | 127.316 | 2.907 × 10\(^{-19}\) |
| High elevation | 2.59 ± 0.30 a | 14.57 ± 0.88 c | 21.33 ± 0.54 d |              |             |
| \(G_s\) (mol m\(^{-2}\) s\(^{-1}\)) |            |              |             |              |             |
| Low elevation | 0.15 ± 0.01 b | 0.10 ± 0.01 c | 0.19 ± 0.01 d | 79.759 | 2.096 × 10\(^{-16}\) |
| High elevation | 0.04 ± 0.01 a | 0.17 ± 0.01 d | 0.33 ± 0.02 e |              |             |
| \(G_m\) (mol m\(^{-2}\) s\(^{-1}\)) |            |              |             |              |             |
| Low elevation | 0.09 ± 0.01 a | 0.12 ± 0.01 a | 0.21 ± 0.02 c | 63.573 | 4.712 × 10\(^{-15}\) |
| High elevation | 0.04 ± 0.01 b | 0.26 ± 0.01 c | 0.44 ± 0.02 d |              |             |
| \(R_d\) (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)) |            |              |             |              |             |
| Low elevation | 1.21 ± 0.04 a | 1.34 ± 0.12 ab | 1.69 ± 0.14 b | 24.353 | 9.959 × 10\(^{-10}\) |
| High elevation | 3.41 ± 0.29 e | 1.83 ± 0.06 c | 2.44 ± 0.12 d |              |             |
| \(P_i/P_a\) |            |              |             |              |             |
| Low elevation | 0.56 ± 0.02 a | 0.58 ± 0.01 a | 0.65 ± 0.02 b | 3.549 | 0.0122 |
| High elevation | 0.58 ± 0.02 a | 0.61 ± 0.01 b | 0.65 ± 0.01 b |              |             |
| \(P_c/P_a\) |            |              |             |              |             |
| Low elevation | 0.31 ± 0.02 a | 0.35 ± 0.02 ab | 0.42 ± 0.03 b | 4.444 | 0.00379 |
| High elevation | 0.31 ± 0.03 a | 0.36 ± 0.01 a | 0.44 ± 0.01 b |              |             |

Figure 2. Photosynthetic response curves to internal [CO\(_2\)] (\(A/P_i\)) and stomatal response to [CO\(_2\)] of plants growing at high elevations of 2500 m (open symbols) and 3500 m (filled symbols) a.s.l. of Q. spinosa (A and D), S. atopantha (B and E) and R. dentatus (C and F). Symbols as in Fig. 1.

S. atopantha and R. dentatus, exhibited respective increases of 36.6 and 44.4 % in \(R_d\) between 2500 and 3500 m a.s.l. Photosynthetic response curves to increased [CO\(_2\)] (Fig. 2) performed \textit{in situ} suggest that modification of the photosynthetic physiology with elevation occurred.
Table 2. Photosynthetic parameters of the three species growing at lower and higher elevations (detailed in Table 1). Values of $A_{\text{max}}, V_{\text{cmax}}$ and $J_{\text{max}}$ were obtained by fitting the Farquhar et al. (1980) model of leaf photosynthesis to five $A/J_P$ response curves for each species at each elevation. Means of a parameter followed by the same letter were not statistically different using a one-way ANOVA ($P > 0.05$) with LSD post hoc test.

| Species      | Low elevation | High elevation | $F_{5,30}$ | $P$-value |
|--------------|---------------|----------------|------------|-----------|
| $A_{\text{max}}$ ($\mu$mol m$^{-2}$ s$^{-1}$) |               |                |            |           |
| Q. spinosa   | 23.31 ± 1.33 b | 28.52 ± 1.18 c | 32.50 ± 0.95 d | 171.800    | 3.934 × 10$^{-21}$ |
| S. atapantha | 7.75 ± 0.55 a  | 46.02 ± 1.23 d | 55.45 ± 1.47 e |          |            |
| R. dentatus  | 58.26 ± 2.42 b | 58.56 ± 2.22 b | 81.25 ± 2.87 c | 72.301     | 8.123 × 10$^{-6}$ |
| $V_{\text{cmax}}$ ($\mu$mol m$^{-2}$ s$^{-1}$) |               |                |            |           |
| Q. spinosa   | 31.73 ± 1.66 a | 80.85 ± 2.49 c | 101.90 ± 3.86 c |          |            |
| S. atapantha | 236.70 ± 13.06 b | 249.49 ± 13.40 b | 334.37 ± 19.66 c | 79.564     | 2.168 × 10$^{-16}$ |
| R. dentatus  | 56.65 ± 4.36 a | 381.31 ± 6.49 c | 450.36 ± 21.61 d |          |            |
| $J_{\text{max}}$ ($\mu$mol m$^{-2}$ s$^{-1}$) |               |                |            |           |
| Q. spinosa   | 4.09 ± 0.24 b  | 4.25 ± 0.10 b  | 4.10 ± 0.15 b  | 41.107     | 1.533 × 10$^{-12}$ |
| S. atapantha | 1.80 ± 0.13 a  | 4.73 ± 0.09 b  | 4.42 ± 0.16 b  |          |            |
| R. dentatus  | 0.95 d 171.800 | 3.934 × 10$^{-21}$ | 0.05) with LSD post hoc test. | 3.934 × 10$^{-21}$ | 0.05) with LSD post hoc test. |

in concert with shifts in $G_{\text{tot}}$. Quercus spinosa exhibited 46.5 and 76.1 % reductions in $V_{\text{cmax}}$ and $J_{\text{max}}$, respectively, that translated into 56 % reduction in the ratio of $J_{\text{max}}$ to $V_{\text{cmax}}$ (Table 2). In contrast, both S. atapantha and R. dentatus exhibited increased values of conductance to CO$_2$ (Table 1) and corresponding increases in the physiological capacity to assimilate CO$_2$ (Table 2). Salix atapantha and R. dentatus, respectively, showed 38.1 and 25.4 % increases in $V_{\text{cmax}}$, alongside 52.8 and 34.7 % rises in $J_{\text{max}}$ that did not significantly alter the $J_{\text{max}}$ to $V_{\text{cmax}}$ ratio of either species. The maximum rate of photosynthesis ($A_{\text{max}}$, $V_{\text{cmax}}$ and $J_{\text{max}}$ all correlated to $G_s$, $G_m$ and $G_{\text{tot}}$ (Fig. 3). However, at higher $G_s$, $G_m$ and $G_{\text{tot}}$ values, photosynthetic capacity stabilizes and no longer increases, possibly due to physiological limitations to the rate of photosynthesis. Individuals of the annual herb R. dentatus at the higher elevation displayed the greatest values of $A_{\text{max}}$, $V_{\text{cmax}}$ and $J_{\text{max}}$ while at the higher elevation, Quercus species exhibited the lowest levels of conductance and photosynthetic capacity to assimilate CO$_2$ (Fig. 3).

The photosynthetic capacity of a leaf is generally related to the concentration of nitrogen within the foliage (Evans 1989). Both S. atapantha and R. dentatus showed increases in leaf nitrogen alongside $A_{\text{max}}$, $V_{\text{cmax}}$ and $J_{\text{max}}$ with elevation (Fig. 4). Despite exhibiting reduced photosynthetic capacity and a 57 % increase in nitrogen concentration at higher elevations, Q. spinosa showed no significant relationship between leaf nitrogen and $A_{\text{max}}$, $V_{\text{cmax}}$ and $J_{\text{max}}$. The PNUE values of S. atapantha and R. dentatus increased by 79.4 and 16.7 % at the higher elevation, while Q. spinosa showed a 69.2 % reduction of PNUE at 3500 m a.s.l. All three species showed increased foliar nitrogen concentration at the higher elevation, but this did not correspond to an increase in SLA (Table 3). While the evergreen Q. spinosa and annual herb R. dentatus showed respective reductions of 13.0 and 31.5 % in SLA at higher elevation, the SLA of S. atapantha was relatively unchanged. Growth at the higher elevation of 3500 m resulted in significant increases of 5.3–10.2 % in the foliar $\delta^{13}C$ of all three species. The $\delta^{13}C$ values of the plants from 2500 m a.s.l. were significantly enriched in the heavier $^{13}C$ isotope relative to C3 plants growing at sea level (approximately $+1–2\%$) (Körner et al. 1988), and this enrichment in $^{13}C$ became more pronounced at 3500 m a.s.l. (approximately $+3–4\%$).

The results of this study have indicated the coordination of photosynthetic, gas exchange and morphological foliar responses to growth at high elevations of 2500 and 3500 m a.s.l. Nonetheless, two generally divergent responses to increased elevation become apparent from the study between the evergreen Quercus species with leaf lifespans of 1–3 years, and S. atapantha and R. dentatus that possess foliage with a leaf lifespan of <9 months. To illustrate these contrasting leaf responses to increased elevation, the relative changes of the physiological, morphological and compositional responses were plotted in Fig. 5. These suggest that the sclerophyllous Quercus species generally reduce conductance to CO$_2$ and photosynthetic capacity with an increase in elevation from 2500 to 3500 m a.s.l., while the shorter-lived foliage of S. atapantha and R. dentatus showed the opposite response.
Discussion

The results of our study have demonstrated contrasting physiological and morphological plasticity responses to an increase in elevation from 2500 to 3500 m a.s.l. in three Chinese montane species. *Quercus spinosa* and *Q. aquifolioides* possess robust sclerophyllous evergreen foliage with an average leaf lifespan > 1 year. These *Quercus* species generally showed reductions in leaf gas exchange and photosynthetic capacity at the higher elevation. In contrast, the shorter-lived mesophytic foliage of *S. atopantha* and *R. dentatus* exhibited increases in conductance to CO₂ and the capacity of photosynthetic physiology at the higher elevation (Fig. 5). This may suggest that the response of the plant species analysed to increased elevation was associated with growth habit and leaf economic traits (Milla and Reich 2011).

A rise in elevation confers a number of challenges to photosynthesis such as reduced pCO₂ and temperature (Körner 2007). Despite the contrasting responses in terms of conductance to CO₂ and photosynthetic physiology observed among different species in this study, increased elevation did result in more positive foliar δ¹³C, greater nitrogen concentration, enhanced Rn and reduced SLA in all species. At the same latitude, the δ¹³C within the atmosphere is generally lower at high elevations (Trolier et al. 1996). The increased δ¹³C observed in all four species growing at 3500 m reflects the lower availability of CO₂ reducing discrimination against ¹³C (Farquhar et al. 1989), despite the heavier isotope forming a reduced proportion of CO₂ at the higher elevation (Trolier et al. 1996). The δ¹³C of a leaf is frequently affected by Gs and is indicative of the water use efficiency of a plant (Farquhar and Richards 1984;
However, δ13C increases in all three species, despite S. atopantha and R. dentatus exhibiting increased $G_s$ at the higher elevation (Fig. 1), suggest that at elevations > 2500 m, factors other than $G_s$ may influence carbon isotope discrimination. The increase in δ13C values of plants with elevation is commonly considered to reflect reduced $P_i/P_a$ ratios. However, due to modification of $G_{tot}$, $V_{cmax}$ and $J_{max}$ with elevation, none of the species analysed in this study exhibited reduced $P_i/P_a$ ratios at the higher elevation (Table 1). Nonetheless, increased foliar δ13C observed in this study may indicate longer-term reductions in the $P_i/P_a$ ratio of the three species that was not apparent during the comparatively short duration of the gas exchange measurements. However, the δ13C values of the species analysed in this study are at the lower end of the range of values exhibited by plant species adapted to growth at high elevations (i.e. >2500 m) (Körner et al. 1988), possibly indicating that the combination of reduced $P_i/P_a$ ratios alongside the decreased availability of CO2 may induce lower δ13C values in other species.

The concentration of nitrogen per unit leaf area of the four species increased on average 29.4% with a rise in elevation from 2500 to 3500 m a.s.l. (Fig. 5). Foliar nitrogen content and SLA are positively related to $A_{max}$ (Poorter et al. 1990; Terashima et al. 2011). Point gas exchange measurements of $A$ were taken under identical conditions of temperature, light and [CO2] at both 2500 and 3500 m a.s.l. This showed increased $A$ at the higher elevation in the two species with short-lived foliage and lower $A$ in the evergreen Quercus leaves. The greater values of $A$ at the higher elevation in the two species where $G_s$ and $G_m$ rose with elevation may suggest that temperature plays a major role in limiting photosynthesis, thus affecting δ13C and determining the response of plants to elevation. In plants adapted to cool climates, leaves developed...
under low temperatures exhibited higher $V_{\text{cmax}}$ and $J_{\text{max}}$ values (Bunce 2000). However, despite exhibiting an increase in $N_{\text{area}}$ at the higher elevation, the two Quercus species showed reductions in $A$, $V_{\text{cmax}}$ and $J_{\text{max}}$ at the greater elevation; this suggests that the greater $N_{\text{area}}$ observed in Q. spinosa and Q. aquifolioides at the higher elevation was not associated with increased allocation of nitrogen to photosynthetic physiology (cf. Shi et al. 2006), or that diffusive limitations imposed by reduced $G_s$ and $G_m$ constrain any effect of increased allocation of nitrogen to RubisCO on $A$ (e.g. Centritto et al. 2003; Loreto and Centritto 2008). The decrease in the $J_{\text{max}}$ to $V_{\text{cmax}}$ ratio observed in Q. spinosa is indicative of reduced allocation of nitrogen into light harvesting activities (Wullschleger

Table 3. Leaf carbon isotopic composition ($\delta^{13}$C), SLA, leaf nitrogen concentration per unit mass ($N_{\text{mass}}$), leaf nitrogen concentration per unit area ($N_{\text{area}}$) and leaf PNUe of the three plants growing at lower and higher elevations (elevations detailed in Table 1). Means of a parameter followed by the same letter were not statistically different using a one-way ANOVA ($P > 0.05$) with LSD post hoc test.

|                      | Q. spinosa | S. ataplanha | R. dentatus | F$_{3,30}$ | P-value     |
|----------------------|------------|--------------|-------------|------------|-------------|
| $\delta^{13}$C (‰)  |            |              |             |            |             |
| Low elevation        | $-28.04 \pm 0.03$ c | $-29.28 \pm 0.19$ b | $-30.63 \pm 0.09$ a | 35.525     | $9.894 \times 10^{-12}$ |
| High elevation       | $-26.23 \pm 0.34$ d | $-27.74 \pm 0.06$ c | $-27.52 \pm 0.18$ c |            |             |
| SLA (cm$^2$ g$^{-1}$)|            |              |             |            |             |
| Low elevation        | $92.82 \pm 2.01$ b | $145.95 \pm 5.68$ c | $288.31 \pm 11.34$ d | 29.869     | $2.109 \times 10^{-10}$ |
| High elevation       | $80.71 \pm 3.22$ a | $145.68 \pm 2.68$ b | $197.59 \pm 2.24$ e |            |             |
| $N_{\text{area}}$ (g m$^{-2}$) |            |              |             |            |             |
| Low elevation        | $1.87 \pm 0.09$ c | $1.51 \pm 0.04$ b | $1.21 \pm 0.05$ a | 147.774    | $3.182 \times 10^{-19}$ |
| High elevation       | $2.23 \pm 0.11$ d | $1.74 \pm 0.02$ c | $1.90 \pm 0.02$ cd |            |             |
| PNUe (µmol mol$^{-1}$ s$^{-1}$) |            |              |             |            |             |
| Low elevation        | $63.65 \pm 4.64$ b | $65.56 \pm 2.44$ b | $134.82 \pm 1.71$ d | 210.276    | $2.101 \times 10^{-22}$ |
| High elevation       | $19.61 \pm 1.65$ a | $117.64 \pm 8.36$ c | $157.38 \pm 4.25$ d |            |             |

Figure 5. Relative effect of an increase in elevation from 2500 to 3500 m a.s.l. on photosynthetic, morphological and compositional characteristics of Q. spinosa, S. ataplanha and R. dentatus from this study and Q. aquifolioides from the study of Feng et al. (2013). Error bars indicate 1 SE.
1993), possibly due to increased levels of radiation at the higher elevation. The greater values of A evident at higher elevations from the point measurements of leaf gas exchange at identical light and temperature may reflect the enhanced photosynthetic capacity and $G_{\text{tot}}$ of *S. atopantha* and *R. dentatus*. This enhancement of A at higher elevations would likely not be evident under ambient growth conditions when temperatures and high incident radiation would not be conducive to photosynthesis (Fujimura et al. 2010). Woody species with thick sclerophyllous leaves generally have lower levels of $G_{\text{tot}}$ than deciduous species that exhibit higher SLA values (Centritto et al. 2011). This lower conductance to CO$_2$ reduces $P_e$ levels and as a result constrains photosynthetic rates (Flexas et al. 2013; Lauteri et al. 2014). Any increase in the availability of CO$_2$ will disproportionately affect evergreen species with low SLA values due to this limitation on transport of CO$_2$ to the chloroplast envelope (Centritto et al. 2011; Niinemets et al. 2011). Future rises in atmospheric [CO$_2$] would, therefore, most likely favour evergreen species growing at high elevations such as *Q. spinosa* rather than those plants with short leaf life-spans such as *S. atopantha* that exhibit increased $G_{\text{tot}}$ with elevation (Table 1).

The divergent responses between the species may be due to the selective pressures associated with long- and short-lived foliage (Reich et al. 1991). Higher elevations experience factors such as increased incident radiation, drier air and stronger winds that increase the leaf to air vapour pressure deficit and the transpirative demand per unit leaf area (Körner and Diemer 1987; Körner 2007; Kouwenberg et al. 2007). Evergreen species such as *Q. spinosa* and *Q. aquifoliiodes* maintain leaves during periods that are not conducive to photosynthesis; this may necessitate a reduction in levels of $G_e$ to prevent desiccation. In contrast, at higher elevations, the species with short-lived foliage can more fully exploit episodes where conditions are favourable to photosynthesis through enhanced levels of $G_c$ and $G_m$ at higher elevations before dispensing with leaves when growth conditions deteriorate and excessive transpirative and carbon balance costs are incurred. This behaviour does, however, incur costs in terms of the replacement of foliage each year, accounting for the lower SLA values of *S. atopantha* and *R. dentatus* in comparison with the two species of *Quercus* (Table 3). However, the construction costs per leaf are lower as investment in robust foliage capable of tolerating physical abrasion in a high energy windy environment (Wilson 1984) or physiological protection from high levels of harmful radiation over long periods of time is not required for leaves with a short leaf lifespan (Venema et al. 2000).

The number of mitochondria per unit leaf area increases with elevation (Miroslavov and Kravkina 1991), accounting for the increased $R_n$ values at higher elevations observed in this (Table 2) and other studies (Ledig and Korbobo 1983; Shi et al. 2006; Feng et al. 2013). This increased $R_n$ may be associated with lower partial pressures reducing the availability of oxygen (Crawford 1992), the increased respiratory demand required to support an enhanced photosynthetic physiology (Shi et al. 2006) or reduced ambient growth temperatures that result in higher $R_n$ values when $R_n$ is determined at the same leaf temperature (Atkin and Tjoelker 2003). When grown in a common garden study, 11 plant species collected from high elevations exhibited enhanced respiration at high temperatures relative to individuals of the same species that originated at low elevations. However, when temperature was reduced, $R_n$ values were identical between individuals from low and high elevations (Larigauderie and Körner 1995). This may suggest that respiratory adaptation to growth at high elevations is due to the lower temperatures experienced at elevation. Temperature is likely a major factor in shaping plant photosynthetic responses to growth at high elevations. The reduction in temperature associated with an increase in elevation from 2500 to 3500 m a.s.l. will result in lower photosynthetic activity (Brooks and Farquhar 1985). The two species with short leaf lifespans will be able to shed their foliage during winter, whereas the foliage of the evergreen *Quercus* species will have to withstand the months with the lowest temperatures. This will necessitate a degree of morphological (Cordell et al. 1998) and physiological (Körner and Diemer 1987) tolerance to low temperatures in the evergreen species (Öquist and Huner 2003) and may account for the generally higher values of $R_n$ observed in the leaves of *Q. spinosa* at 3500 m a.s.l. Furthermore, the effect of the decline in temperature associated with increased elevation is more apparent in trees that are directly affected by atmospheric circulation than shrub or herb layer plants that are smaller and generally sheltered, and as a result under radiation can maintain a significantly higher temperature than the surrounding trees (Körner 2007). This differential effect of temperature changes at high elevation may contribute to the increase in $G_{\text{tot}}$ and photosynthetic capacity observed in *S. atopantha* and *R. dentatus*.

**Conclusions**

The three plant species analysed in this study exhibited significant physiological and morphological plasticity to enable growth at 2500 and 3500 m a.s.l., an elevational gradient equivalent to a 5.5 °C decline in temperature and 11.2 % reduction in pCO$_2$. The results of this study suggest that the physiological and morphological adaptations required for growth at high elevations may be associated with plant growth habit and leaf economics.
Consistent with previous studies, all three species showed increased $\delta^{13}C$, $R_n$, and leaf nitrogen at the higher elevation. However, critical differences in the photosynthetic and leaf gas exchange response to elevation were observed between the plants. The evergreen, *Q. spinosa*, showed reduced conductance to $CO_2$ and diminished levels of $V_{c,max}$ and $J_{max}$ at the higher elevation. In contrast, *S. atapalapa* and *R. dentatus* with a leaf lifespan of $<9$ months exhibited increased $G_{tot}$ and enhanced photosynthetic capacity to fix $CO_2$ at 3500 m. The selective pressures exerted by an increase in elevation may act differently dependent on the leaf lifespan of a species. Those species with short leaf lifespans can more fully exploit favourable growth conditions through increased conductance to $CO_2$ and $A$ before shedding foliage as conditions become less conducive to photosynthesis. Whereas evergreen species need to invest in physically robust leaves and physiological protective mechanisms to endure unfavourable conditions, this may necessitate a decrease in $G_s$ to reduce water loss associated with the higher transpirative demands at higher elevations due to increased wind and radiation. Climate change may affect the plant species that compose high-elevation ecosystems differently depending on leaf economic traits as increased $pCO_2$ is likely to benefit evergreen species with thick sclerophyllous leaves to a greater extent than deciduous species.

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**Contributions by the Authors**

Z.S. and M.C. designed the experiment. Z.S., Q.F. and R.C. conducted the measurements. M.H. and M.C. analysed the data and wrote the manuscript.

**Conflict of Interest Statement**

None declared.

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