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Leaf Functional Trait Responses of *Quercus aquifolioides* to High Elevations

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Abstract

Investigations about phenotypic plasticity and adaptive traits of species facing rapidly changing stressful conditions are particularly relevant in context of rapid climate change. Morphological and physiological traits of *Quercus aquifolioides* plants growing at 2600 m and 3500 m above sea level (in the vicinity of the tree line) were studied. Leaf mesophyll conductance (g_m) and stomatal conductance (g_s) decreased, whereas carbon isotope composition ($\delta^{13}C$), nitrogen concentration and dark respiration increased with elevation. The specific leaf area (SLA) did not change, whereas photosynthetic capacity was dramatically inhibited at higher elevation. Differences in decline of photosynthesis (~64%) and g_m (~80%) at elevations were reflected by similar chloroplast (P_c) to atmospheric (P_a) CO_2 partial pressure ratio between the two populations. Therefore, $\delta^{13}C$ changes were not associated to either SLA or P_c/P_a, $\delta^{13}C$ furnished an estimation of long-term P_c/P_a and, in turn, of long-term water use efficiency. Air temperature, which decreased consistently with altitude, significantly affected the long-term P_c/P_a. Plausibly, low temperature is the main determinant affecting $\delta^{13}C$ at high altitude. In conclusion, phenotypic plasticity enabled *Q. aquifolioides* to maintain a positive carbon balance in response to dramatic environmental changes.

Keywords: Carbon isotopic composition; CO_2 transport conductance; Photosynthetic capacity; Mountain plants; Nitrogen concentration; SLA

Introduction

Mountain ecosystems, which comprise about 30% of terrestrial plant species diversity, play important roles in global biogeochemical and biogeophysical cycles as well as environmental sustainability. Recent reports predict a remarkable impact of climate change on the structure and function of high altitude vegetation (Chen *et al*., 2011). Therefore, it is of critical importance to understand adaptive and phenotypic responses of plant species which have a high altitudinal variation. This is because plants have species-specific ecophysiological traits, both purely phenotypic and genetic, significantly influencing carbon balance that are primary drivers of plant responses to global climate change. The ability to maintain a positive carbon balance in response to environmental changes may ultimately determine species survival by altering species distribution and vegetation composition. Hence attitudinal variability manifested by plant species can be used as important feedback mechanism for biogeochemical and biogeochemical cycles.

The partial pressure of all gasses (P) is reduced with elevation. This factor, along with systematic variations of other physical processes with altitude (such as rapid fluctuations in temperature, radiation and moisture regime, superimposed on seasonal changes), causes morphological and physiological adjustments to allow plants to cope with stressful environments associated with high altitudes (Körner, 2007). There is considerable evidence indicating that at higher elevation plants tend to have a more compact growth form associated with reduced specific leaf area (SLA), increased foliar N concentration and respiration rate (R_d) (Friend *et al*., 1989). In addition, early literature has also shown that photosynthetic capacity is increased at high elevation, despite lower atmospheric partial pressure of CO_2 (P_a) and reduced stomatal conductance (g_s) (Körner *et al*., 1988; Cordell *et al*., 1999; Kogami *et al*., 2001; Shi *et al*., 2006). This apparent contradiction can be explained by the increased gas diffusion, including CO_2 diffusion into the leaf, at lower P (Gale, 1972), and by the higher concentration and activity of the enzymes of the photosynthetic machinery with altitude (Körner, 2007). The combination of higher carboxylation efficiency of Rubisco (V_{cmax}) and reduced g_s results in a lower ratio between the CO_2 intercellular P (P_i) and P_a leading, in turn, to higher values of foliar carbon composition ($\delta^{13}C$) and consequently, to increased long-term water use efficiency (WUE) (Farquhar and Richards, 1984) with increasing elevation (Körner *et al*., 1988).

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However, the confounding and possibly interacting effects among physical processes, which vary dramatically with altitude, complicate the interpretation of ecophysiological responses to contrasting altitudes, which also appear to differ among species (Zhang *et al*., 2005; Körner, 2007). Moreover, photosynthesis rates (*A*) and the depletion of ^{13}C in leaves are directly related to the chloroplastic *P* of CO_2 (P_c) rather than to P_i (Loreto *et al*., 1992; Centritto *et al*., 2003; Loreto and Centritto, 2008). P_c is determined by the total leaf conductance to CO_2 (g_t) i.e., the inverse of the sum of mesophyll and stomatal resistances. Thus, mesophyll conductance (g_m) is increasingly recognized as being a major transport determinant of *A* (Evans *et al*., 2009; Loreto *et al*., 2009; Centritto *et al*., 2011). Though Terashima *et al.* (1995) hypothesised that *A* would be mostly limited by decreased g_t in high elevation plants; g_m variations with altitude have been reported only in three published studies. In accordance with the hypothesis put forward by Terashima *et al*. (1995), Kogami *et al*. (2001) observed, in a study on two populations of lowland (10 m above sea level - a.s.l.) and highland (2500 m a.s.l.) *Polygonum cuspidatum*, that g_m was strongly reduced with elevation. This decrease in g_m led, in turn, to significantly higher $\delta^{13}C$ and reduced P_c/P_a values in high elevation plants. In contrast, Cordell *et al.* (1999) did not observe significant modification in g_m with increasing altitude in an experiment on *Metrosideros polymorpha* trees grown at five elevations (107, 701, 1280, 1981 and 2469 m a.s.l.). However, because of significantly higher V_{cmax} with increasing elevation, P_c/P_a ratio decreased and $\delta^{13}C$ increased at high elevation. In a more recent study done on two populations of *Buddleja davidii* growing in the south-eastern Tibetan-Qinghai area (China) at 1300 and 3400 m a.s.l., Shi *et al*. (2006) showed that g_m increased with elevation and that the P_c/P_a ratio was similar at two elevations. The authors assumed that the increased $\delta^{13}C$ observed in plant growing at high altitude, resulting from reduced long-term P_c/P_a ratio, was caused by adverse environmental factors affecting both CO_2 diffusion and P_c/P_a at high elevations, rather than physiological modifications brought about by declining P_a.

Quercus aquifolioides is an evergreen sclerophyllous, long-lived species widely distributed in southwestern China, where it grows from 2000 m to 4500 m a.s.l., playing a very important role in maintaining ecosystem services. Previous studies on *Q. aquifolioides* (Li *et al*., 2009) growing along a very high elevation gradient, between 2000 to 3600 m a.s.l., in southwestern China revealed contrasting results. The authors observed that N concentration and $\delta^{13}C$ decreased as elevation increased up to 2800 m a.s.l., but at elevation higher than 2800 m a.s.l. the values of these two parameters increased, whereas SLA showed the opposite trend. However, the photosynthetic characteristics of *Q. aquifolioides* were not analyzed in their study.

In the present work, leaf properties of *Q. aquifolioides* plants growing at the very high elevations of 2600 m (*P* = 73 kPa) and 3500 m a.s.l. (*P* = 68 kPa) i.e., in the vicinity of the tree line, were evaluated to identify if morphological and physiological modifications in foliar traits were associated to carbon metabolism at the minimum limit of temperature at which trees can grow. The major objective of this study was to characterize *in situ*, coordinated changes in photosynthetic capacity, diffusional conductances, $\delta^{13}C$, N concentration and SLA in mountainous environments. Variations in these morphological and physiological functional traits, allowing plants to cope with rapidly changing stressful conditions, have a significant influence on carbon balance and, consequently, on plant fitness. Furthermore, given the strong relationship between g_m and P_c, we aimed to reveal the possible altitude-related variations regulating transport and non-transport limitations to photosynthetic capacity in *Q. aquifolioides*.

Materials and Methods

Well-watered plants of *Q. aquifolioides* Rehder and E.H. Wilson growing at 2600 m and 3500 m a.s.l., respectively, within the Wolong Reserve in south-eastern Tibetan-Qinghai area, Sichuan Province, China (32°25′-32°53′N, 104°20′-104°41′E), were studied during the first 3 weeks of August 2009.

Simultaneously measurements of gas exchange and fluorescence were made on newly expanded leaves from sunny branches (5 plants per elevation), between 9:00 and 15:00 o'clock, using a LI-6400-40 leaf chamber fluorometer (Li-Cor, Inc., Nebraska, USA) equipped with a 2 cm^2 cuvette. The measurements were made *in situ* in saturating photosynthetic photon flux density (PPFD) of 1200 μmol $m^{-2}s^{-1}$ at the ambient CO_2 concentration of 380 μmol mol^{-1}, with leaf temperature of 25°C and relative humidity ranging between 46-50%. Measurements of R_d (dark respiration) were made at ambient P_a. The variable *J* method was applied to estimate mesophyll conductance (Centritto *et al*., 2009); whereas g_t (total conductance) was computed as: $g_t = g_s\, g_m/(g_s + g_m)$. To measure PPFD-saturated A/P_i response curves, leaves were first pre-conditioned at the [CO_2] of 50 μmol mol^{-1} to force stomatal opening (Centritto *et al*., 2003). These measurements were made on five plants per elevation at the temperature of 30°C and relative humidity of ~50%. Maximum Rubisco carboxylation efficiency (V_{cmax}), CO_2 assimilation rate (A_{max}) and electron transport rate (J_{max}) were estimated from individual A/P_i curves as described by Shi *et al*. (2006).

Two leaves per plant, 12 plants per elevation were cut and stored in sealed plastic bags for subsequent measurements of specific leaf area (SLA), leaf nitrogen (N) concentration and foliar $\delta^{13}C$. SLA was determined as the ratio between leaf area, measured using a leaf area meter (Li-3000, Li-Cor, Lincoln, NE, USA), and leaf dry mass, obtained by weighing leaves dried at 80°C for 48 hours. N concentration (N_{mass}, mg g^{-1}) was assessed on 0.1 g of dried leaves ground to a powder using the standard Kjeldahl technique. N content per unit of leaf area (N_{area}, g m^{-2}) and photosynthetic nitrogen use efficiency (PNUE, µmol mol^{-1} s^{-1}) (Hikosaka *et al*., 2002) were calculated by the formulae:

$$N_{area} = \frac{N_{mass} * SLA}{100}$$

$$PNUE = \frac{A * 14}{N_{area}}$$

Where, 14 is the atomic mass of nitrogen.

Carbon isotope composition ($\delta^{13}C$) was assessed on 1 mg of dried leaves ground to a powder by using a continuous flow isotope ratio mass spectrometer. Samples were quantitatively combusted into an elemental analyzer (Flash-EA 1112, Thermo Electron, Milano, Italy). The CO_2 obtained was injected into the helium stream to the mass spectrometer (DELTAplus XP, Thermo Finnigan, Bremen, Germany). Isotope ratio (R $= {}^{13}C/{}^{12}C$) was measured and used to calculate $\delta^{13}C$ referred to the Pee Dee Belemnite (PDB) standard using the following expression: $\delta^{13}C = R_{sample}/R_{standard} - 1$ (Farquhar and Richards, 1984).

Data were analyzed with one-way ANOVA using 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).

Results

A, g_s and g_m were significantly lower whereas R_d was higher in the plant populations growing at 3500 m a.s.l., with an atmospheric pressure of 68 kPa, as compared to plant populations growing at 2600 m a.s.l., with an atmospheric pressure of 73 kPa (Table 1). Photosynthesis, stomatal and mesophyll conductances were decreased by ~64, 40 and 80%, respectively, whereas R_d was increased by ~126% in higher elevation plants. The different dynamical responses of A and g_s and A and g_m to elevations were mirrored by a significant increase in P_i/P_a in higher elevation plants, and by similar P_c/P_a values between the two populations. Transport resistance to CO_2 was progressively reduced P_c. Data pooled together from both elevations (Fig. 1a) showed a significant ($P < 0.01$) correlation between photosynthesis and g_s, indicating that photosynthesis was limited by reduced g_s. A much better correlation ($P < 0.001$) was also found between A and g_m (Fig. 1b). Moreover, a slightly better linear relationship ($P < 0.001$) between photosynthesis and g_t was found when mesophyll and stomatal conductances (Fig. 1c) were both taken into account.

To further assess the elevation influence on photosynthetic capacity and subsequent mesophyll metabolism (as expressed by the PPFD-saturated A/P_i curves), a comparison was made between higher elevation plants, which had intrinsically lower A, and lower elevation ones that had intrinsically higher A. Similar to the instantaneous photosynthetic rates, the photosynthetic capacity was also inhibited with the elevation (Fig. 2a). The initial slope (i.e., V_{cmax}) and the saturating portion (i.e., A_{max}, and J_{max}) of the A/P_i curves were statistically different in the two populations (Table 2). V_{cmax}, A_{max} and J_{max} were decreased by about 43, 57 and 70%, respectively with altitude; whereas the J_{max}/V_{cmax} ratio was significantly reduced by ~44% in higher elevation plants (Table 2). The g_s/P_i curves were strongly influenced by altitude, as g_s resulted consistently reduced at each of the given P_i in higher elevation plants (Fig. 2b).

Long-term WUE was estimated by means of $\delta^{13}C$ analysis of leaf dry mass. Mean $\delta^{13}C$ resulted significantly increased in plants growing at higher elevation (Table 3), and differed by ~1.16‰ between the two populations of plants which implied an average change of +1.29‰ in $\delta^{13}C$ per 1000 m of elevation. There was no significant differences in SLA of the two populations, whereas N_{area} was significant increased with elevation (Table 3). N_{area} was ~21% larger in plants grown at 3500 m a.s.l. than in lower elevation plants. Since an increase in N_{area} with elevation was not matched by similar increase in photosynthetic capacity (Fig. 2a), there were significant inverse relationships between A_{max} ($P < 0.001$), V_{cmax} ($P < 0.05$) and J_{max} ($P < 0.01$) and N_{area} (Fig. 3). Furthermore, because the relative inhibition in A (Table 1) was much higher than the relative increase in N_{area} in higher elevation plants, *PNUE* was significantly reduced by ~72% with elevation (Table 3).

Discussion

Plant species growing along elevation gradients commonly have a high level of morphological and physiological plasticity in leaf functional traits, allowing them to cope with rapid changing environmental conditions and maintain a positive carbon balance (Körner, 2007; Premoli and Brewer, 2007; Bresson *et al*., 2011). Commonly observed traits include increased foliar $\delta^{13}C$, N_{area}, photosynthetic capacity and R_d, and decreased g_s and SLA in higher elevation plants (Körner *et al*., 1988; Cordell *et al*., 1999; Kogami *et al*., 2001; Shi *et al*., 2006). Bresson *et al*. (2011) have recently shown, in a common garden experiment on *Fagus sylvatica* and *Q. petraea*, that only N_{area} and, to less extent, SLA showed a strong genetic pattern with altitude, whereas the other abovementioned functional traits showed strong phenotypic trends suggesting sharp environmental effects on those traits. Our study, regarding the ecophysiological traits of two population of *Q. aquifolioides* growing at elevations of 2600 and 3500 m a.s.l. with atmospheric pressures of 73 and 68 kPa, respectively, confirmed the previous findings that $\delta^{13}C$, N_{area} (Table 3) and R_d (Table 1) increased, whereas g_s decreased with altitude. However, in contrast to these studies, our results revealed inhibition in photosynthesis (Table 1), whilst SLA was unaffected (Table 3) with altitude.

Table 1: Leaf assimilation rate (A), stomatal conductance (g_s), mesophyll conductance (g_m), P_i (CO_2 intercellular partial pressure)/P_a (CO_2 ambient partial pressure), P_c (CO_2 chloroplast partial pressure)/P_a and R_d (dark respiration) of the *Quercus aquifolioides* plants growing at 2600 a.s.l (P = 73 kPa) and 3500 a.s.l. (P = 68 kPa)

Elevation (m a.s.l.)	A (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	g_m (mol m^{-2} s^{-1})	P_i/P_a	P_c/P_a	R_d (μmol m^{-2} s^{-1})
2600	6.01 ± 0.29 b	0.078 ± 0.006 b	0.093 ± 0.008 b	0.57 ± 0.01 a	0.31 ± 0.02 a	0.91 ± 0.06 a
3500	2.18 ± 0.05 a	0.047 ± 0.005 a	0.019 ± 0.002 a	0.75 ± 0.03 b	0.23 ± 0.02 a	2.06 ± 0.18 b

Values are means of five plants per elevation ± 1 SEM. Letters (a, b) indicate significant differences at P < 0.05 in the same column

Table 2: Best-fit estimates of photosynthetic parameters of the *Quercus aquifolioides* plants growing at 2600 a.s.l and 3500 a.s.l. obtained from the individual A/Ci response curves shown in Figure 1. A_{max} (maximum photosynthetic rate at saturating PPFD per unit leaf area), V_{cmax} (photosynthetic Rubisco capacity per unit leaf area), J_{max} (potential rate of electron transport per unit leaf area), and J_{max} to V_{cmax} ratio

Elevation (m a.s.l.)	A_{max} (μmol m^{-2} s^{-1})	V_{cmax} (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})	J_{max}/V_{cmax}
2600	19.76 ± 1.50 b	58.12 ± 3.02 b	199.04 ± 17.71 b	3.41 ± 0.24 b
3500	8.51 ± 0.84 a	33.26 ± 4.47 a	60.70 ± 4.88 a	1.91 ± 0.17 a

Values are means of five plants per elevation ±1 SEM. Letters (a, b) indicate significant differences at P < 0.05 in the same column

Table 3: Leaf carbon isotope composition ($\delta^{13}C$), specific leaf area (SLA), leaf nitrogen concentration per unit area (N_{area}), and leaf photosynthetic nitrogen use efficiency (PNUE) of the *Quercus aquifolioides* plants growing at 2600 a.s.l. and 3500 a.s.l

Elevation (m a.s.l.)	$\delta^{13}C$ (‰)	SLA (cm^2 g^{-1})	N_{area} (g m^{-2})	PNUE (μmol mol^{-1} s^{-1})
2600	-27.08 ± 0.18 a	66.29 ± 1.56 a	1.93 ± 0.05 a	43.81 ± 1.75 a
3500	-25.92 ± 0.17 b	64.48 ± 0.79 a	2.43 ± 0.06 b	12.15 ± 0.88 b

Values are means of 12 plants per elevation ±1 SEM. Letters (a, b) indicate significant differences at P < 0.05 in the same column

In agreement with Terashima *et al*. (1995), who performed a theoretical study examining the influence of elevation on photosynthesis, and with the observations of Kogami *et al*. (2001) observations made on *P. cuspidatum* populations growing at sea level and at 2500 m a.s.l., we found that g_m decreased consistently with altitude in *Q. aquifolioides* (Table 1). However, this result is in conflict with early findings by Cordell *et al*. (1999) and Shi *et al*. (2006) i.e., the only two other published studies which reported g_m variations with altitude. In fact, Cordell *et al.* (1999) did not find any change in g_m of *M. polymorpha* trees as elevation increased, although Shi *et al.* (2006) showed that g_m of *B. davidii* plants increased with elevation. The latter result was interpreted as a homeostatic response that allowed compensation for a strong decrease in g_s and, thus, in the P_i/P_a ratio with altitude, leading to similar P_c/P_a at both elevations. Interestingly, this latter ratio did not change significantly with elevations also in the *Q. aquifolioides* plants, although the mechanism, which led to similar P_c/P_a ratios at different altitudes was different from that reported by Shi *et al*. (2006). This is because in our study the P_i/P_a ratio was significantly higher at 3500 m a.s.l. than at 2600 m a.s.l. (Table 1). The contrasting P_i/P_a and P_c/P_a values in higher elevation plants were probably due to the differential decreases in g_s (~40%) and g_m (~80%) with respect to the corresponding decrease in A (~64%). It is important to note that studies of Shi *et al*. (2006) and ours were done in the same area. Therefore, species functional diversity, i.e. evergreen sclerophyllous, long-lived *Q. aquifolioides* and mesophyllic deciduous shrub *B. davidii*, may emerge as a noticeable factor that may account for different mechanisms determining the P_c/P_a ratios with increasing elevations.

Our findings suggest a progressive reduction of CO_2 transport to chloroplast due to stomatal and mesophyll resistances (Fig. 1). The significant linear relationships between photosynthesis rates and g_s (Fig. 1a), the better correlations between A and g_m (Fig. 1b), and especially between photosynthesis rates and g_t (Fig. 1c) showed that A was limited by declining CO_2 transfer conductance (Shi *et al*., 2006; Centritto *et al*., 2009). There is also a clear indication that P_c, which is determined by the sum of mesophyll and stomatal resistances (Centritto *et al*., 2003), is one of the main limitation of A in lower as well as in higher elevation plants, as observed in plants with different leaf traits (Aganchich *et al*., 2009; Terashima *et al*., 2011; Velikova *et al*., 2011). However, photosynthesis in higher elevation plants appeared to be limited also by mesophyll metabolism, as shown by comparing photosynthetic capacity (Fig. 2) between trees grown at 2600 m a.s.l., which had intrinsically higher photosynthesis, and those grown at 3500 m a.s.l., which showed intrinsically lower photosynthesis. In fact V_{cmax}, J_{max}, and A_{max} were significantly inhibited in higher elevation plants (Table 2). These findings indicate that there were strong biochemical limitations to photosynthetic capacity in higher elevation *Q. aquifolioides* trees.

Furthermore, the rapid decline in V_{cmax} and J_{max} at higher elevation indicated a lower N allocation in the photosynthetic system, despite N_{area} increased significantly in higher elevation plants (Table 3). Consequently, photosynthetic capacity was inversely correlated with N_{area} with elevation (Fig. 3). Moreover, the strong decrease in the

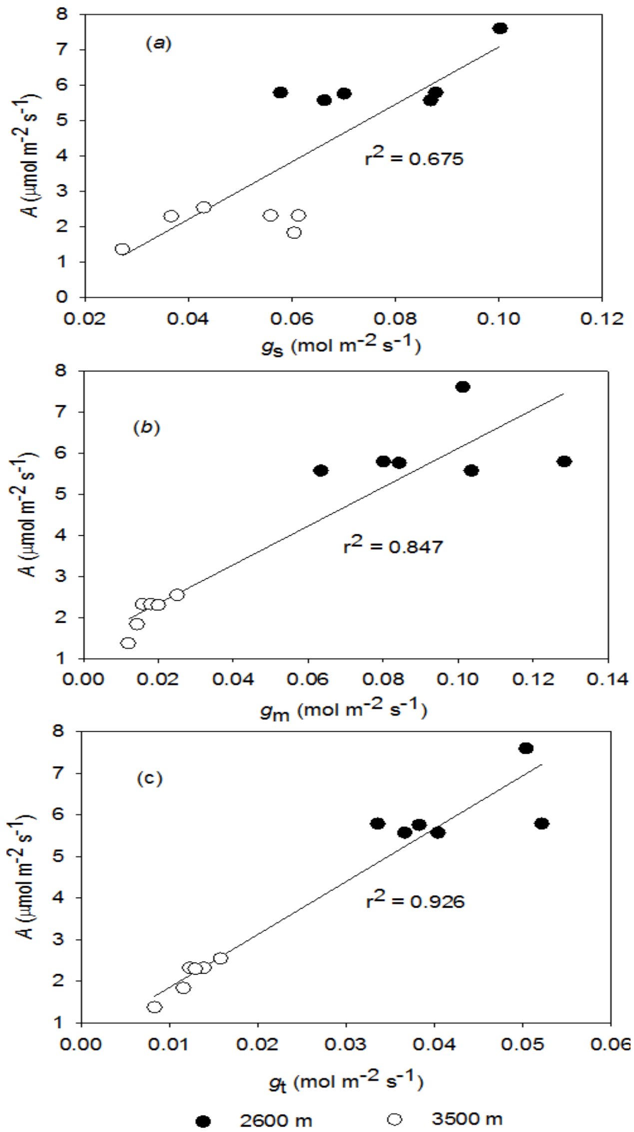

Fig. 1: Relationships between (a) photosynthesis (A) and stomatal conductance (g_s), (b) A and mesophyll conductance (g_m), and (c) A and total conductance (g_t) in *Quercus aquifolioides* plants from the lower and the higher elevations

J_{max}/V_{cmax} ratio in plants grown at 3500 m a.s.l. (Table 2) also indicated that the functional balance between photochemistry and carboxylation capacity in *Q. aquifolioides* trees changed with altitude, because relatively less N was allocated to the photochemical apparatus. The increase in N_{area} is often related to reduced SLA (i.e., increased leaf density and thickness), and both these traits showed a significant genetic pattern with elevation (Bresson *et al*., 2011), with minimal environmental control especially in the case of N_{area}. Thus, the reduced capacity in the biochemistry of photosynthesis (Table 1) can be regarded as an optimization process involving reallocation of N from non-limiting organs into limiting components.

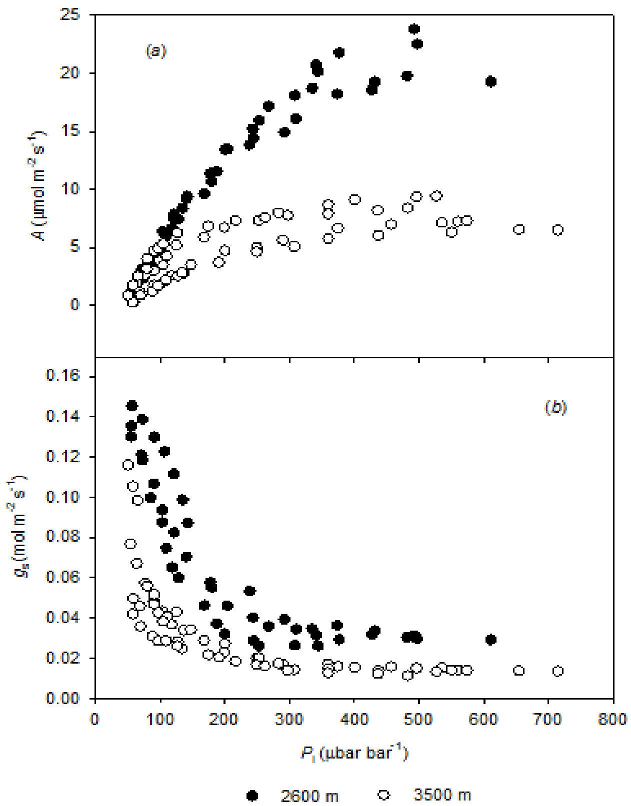

Fig. 2: The relationship between (a) net CO_2 assimilation rate (A) and intercellular partial pressure of CO_2 (P_i), and (b) stomatal conductance (g_s) and P_i in *Quercus aquifolioides* plants from the lower and the higher elevations. The measurements were made on five plants per elevation, in saturating PPFD (1200 μmol $m^{-2}s^{-1}$), with relative humidity ranged between 46-50%, and leaf temperature of 25°C

The genetic control on N_{area} can explain the dramatic decline in *PNUE* in the *Q. aquifolioides* plants growing in the vicinity of the tree line (Table 3), as also found by Hikosaka *et al*. (2002). This has led us to believe that carbon assimilation is generally not constrained among trees growing at their low temperature threshold, but by the capability at which the assimilated carbon can be used for structural growth (Körner, 2007).

Decreased SLA along elevation gradients has been associated to lower long-term P_c/P_a ratio, either resulting from decreased g_m (Kogami *et al*., 2001) or increased V_{cmax} (Cordell *et al*., 1999), and it has been considered tightly coupled to less negative $\delta^{13}C$ in highland plants (Vitousek *et al*., 1990; Cordell *et al*., 1999; Kogami *et al*., 2001; Li *et al*., 2009). In contrast, our results indicated that increases in both foliar N_{area} and $\delta^{13}C$ with altitude did not change the SLA (Table 3). This is similar to observations reported by Shi *et al*. (2006) for *B. davidii* while contrasting results about physiological and morphological features in the populations of *Q. pannosa* (Zhang *et al*., 2005) growing at very high elevations in southwestern China.

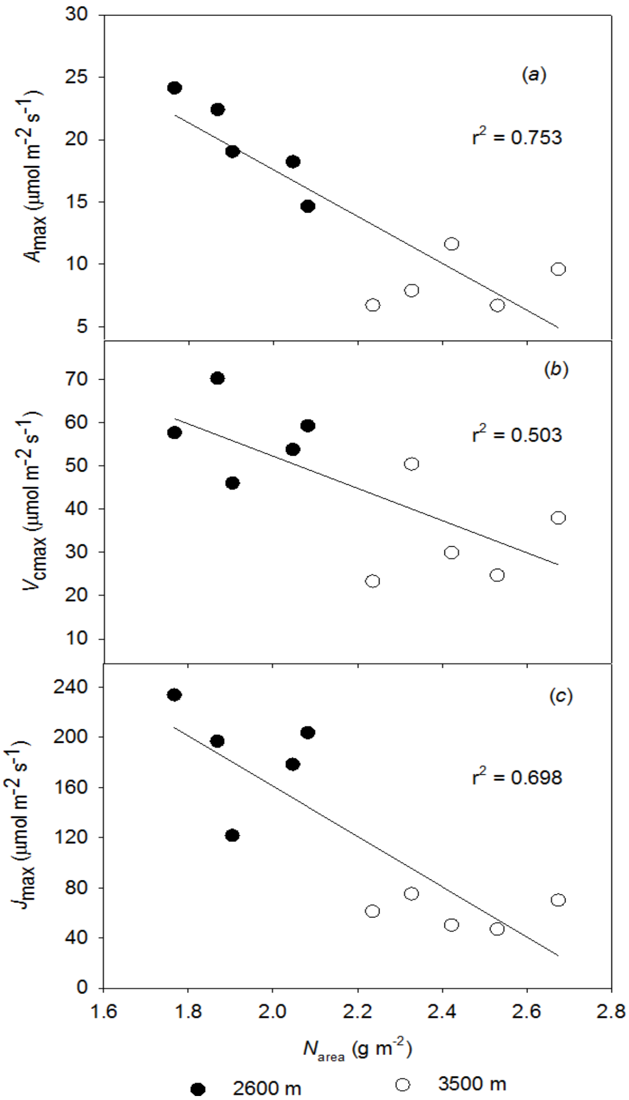

Fig. 3: Linear relationships between best-fit estimates of the photosynthetic capacity parameters and foliar nitrogen concentration (N_{area}) on a leaf area basis in *Quercus aquifolioides* plants from the lower and the higher elevations

Carbon discrimination provides an estimation of both P_c/P_a and WUE integrated over long periods of time (leaf life span) (Ehleringer *et al*., 1993; Farquhar *et al*., 1989). Decreased carbon discrimination implies that photosynthetic system operates at reduced P_c/P_a, and since this ratio is negatively correlated with long-term WUE. Previously published literature (Körner *et al*., 1988; Bresson *et al*., 2011) revealed significant increase in foliar δ^{13}C with elevation, which is in line with the findings of present study (Table 3). Ccontrary to the findings of Kogami *et al.* (2001) and Cordell *et al.* (1999), the dynamics of both V_{cmax} and g_m did not lead to lower instantaneous P_c/P_a ratio (Table 1) in the higher elevation *Q. aquifolioides* plants. It is noteworthy that we studied two populations of *Q. aquifolioides* both growing at very high elevations, rather than comparing population growing approximately at the sea level with those growing at higher elevation. Our elevation gradient was 900 m with an atmospheric pressure difference of only 5 kPa. Thus, it is reasonable to assume that temperature difference was main physical factor between the two sites. Therefore, as also indicated by Friend *et al*. (1989) and Shi *et al.* (2006), the increased δ^{13}C in higher elevation plants, which indicates a reduced long-term ratio between P_c and P_a, may have been caused by adverse environmental factors, and primarily by declining temperature with increasing altitude (Tranquillini, 1964; Körner, 2007). Air temperature, which drops dramatically with altitude and, consequently, strongly influences the diffusion of CO_2 and the activity of Rubisco (Jordan and Ogren, 1984), appears to be the main determinant affecting the long-term P_c/P_a ratio and, thus, ^{13}C discrimination at very high elevation.

In conclusion, this study further provided useful evidence pertaining to environmentally induced short-term ecophysiological responses of phenotypic plastic processes that enabled *Q. aquifolioides* to maintain a positive carbon balance in response to dramatic environmental changes. Furthermore, we assume that higher elevation *Q. aquifolioides* plants will be more responsive to rising atmospheric CO_2 concentrations than plants growing at lower elevation because of the extremely low g_t and higher concentration of N per unit of leaf area that could be better allocated to improve the plant sink-source functional balance. These environmentally and genetically controlled phenotypic traits are the primary drivers of plant responses to stressful conditions and consequently, could determine important functional process in determining the physiological responses of trees to climate change.

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