

# Climate-dependent variations in leaf respiration in a dry-land, low productivity Mediterranean forest: the importance of acclimation in both high-light and shaded habitats

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## Summary

1. Climate-driven changes in leaf respiration ( $R$ ) in darkness have the potential to determine whether low productivity ecosystems exhibit positive or negative carbon balances.
2. We investigated whether sustained exposure to full sunlight, shade and seasonal drought alters the temperature response of leaf  $R$  of field-grown *Quercus ilex* subsp. *ballota* in a dry-land continental Mediterranean ecosystem. The plants studied, experience large diurnal and seasonal variations in temperature.
3. Whilst growth irradiance impacted on photosynthesis, it had little effect on the short-term temperature dependence of leaf  $R$ . Moreover, although basal rates of leaf  $R$  (i.e. rates of  $R$  at a common measuring temperature) were higher in sun-exposed than shade-exposed leaves, growth irradiance had little impact on the degree of acclimation to seasonal changes in temperature and/or moisture. Basal rates of leaf  $R$  were higher in winter than summer in both sun-exposed and shaded plants. Estimated  $Q_{10}$  values (i.e. proportional increase in  $R$  per 10 °C rise in temperature) for leaf  $R$  were greater in winter than summer; however, no seasonal variation was found in the apparent activation energy ( $E_0$ ) of leaf  $R$ . These observations were used to construct a simple Arrhenius model that fully accounted for both daily and seasonal variations in the temperature dependence of  $R$  in both sun-exposed and shaded plants. Crucial to the model was accounting for the seasonal and irradiance-dependent shifts in the basal rate of leaf  $R$ .
4. Although the balance between daily  $R$  and photosynthesis increased markedly in summer (particularly under full sun), the increase in this ratio was markedly less than would have been the case if leaf  $R$  had not acclimated to the high average day time temperatures in summer.
5. It is concluded that seasonal acclimation of leaf  $R$  plays a crucial role in determining the viability of tree growth in dry-land, low productivity forest ecosystems.

**Key-words:** drought, irradiance, leaf, respiration, temperature

## Introduction

Over half of the Earth's terrestrial surface is covered by ecosystems that exhibit low net primary productivity (NPP – the rate of net carbon gain by vegetation) (Larcher 2004). Under such conditions, plants may be expected to often

operate near the threshold of positive carbon gain. An example of such ecosystems are dry-land forests in Mediterranean regions where rates of carbon loss by plant respiration ( $R$ ) often equal, or exceed, the rate of carbon uptake by photosynthesis ( $P$ ), except at the most favourable times of year (typically spring and autumn; Tenhunen *et al.* 1990; Rambal *et al.* 2003). Exposure to high air temperatures, drought, deep shade and/or nutrient deficiencies would be expected to

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further exacerbate any imbalance between plant  $R$  and  $P$  (Björkman 1981; Dewar, Medlyn & McMurtrie 1999; Walters & Reich 2000; Ciais *et al.* 2005; Bréda *et al.* 2006). Although it is widely accepted that plant  $R$  can play an important role in determining whether low NPP ecosystems exhibit a positive or negative carbon balance (Pereira *et al.* 1986), particularly following drought (Ciais *et al.* 2005; Bréda *et al.* 2006), the quantitative impact of plant  $R$  on the net carbon balance of low NPP ecosystems is unclear.

Under favourable conditions approximately half of whole-plant  $R$  may take place in leaves (Poorter, Remkes & Lambers 1990; Atkin, Scheurwater & Pons 2007), with up to 35% of CO<sub>2</sub> fixed each day by photosynthesis being released back into the atmosphere by leaf  $R$  (Loveys *et al.* 2003). This percentage could be even greater in field-grown plants, where net CO<sub>2</sub> exchange is often limited by reduced stomatal conductance (e.g. due to drought), low irradiance (e.g. in the understorey of mature forests) and high leaf temperatures (where net assimilation is reduced due to high rates of photorespiration). The percentage of daily fixed CO<sub>2</sub> respired by leaves is also dependent on the response of respiratory metabolism in leaves to abiotic factors such as water availability (Flexas *et al.* 2006), growth irradiance (Wright *et al.* 2006), and short- and long-term changes in temperature (Atkin *et al.* 2005). Although initial stages of water stress can inhibit leaf  $R$ , sustained exposure to severe water stress often results in leaf  $R$  increasing, probably in response to an increase in energy demand as leaves cope with drought (Flexas *et al.* 2006). Growth irradiance also impacts on leaf  $R$ , with respiratory rates being lower in shade leaves than in their high-light grown counterparts (Boardman 1977; Sims & Pearcy 1989, 1991, 1994; Turnbull, Doley & Yates 1993; Noguchi & Terashima 1997; Noguchi, Nakajima & Terashima 2001). In their compilation from 20 sites around the world, Wright *et al.* (2006) found that rates of leaf  $R$  were highest at warm sites, reflecting the temperature dependence of leaf  $R$ . Leaf  $R$  was also highest at the high-irradiance, low-rainfall sites (Wright *et al.* 2006).

It has long been recognized that rates of leaf  $R$  are sensitive to short-term changes in temperature (Wager 1941; Forward 1960), with the temperature sensitivity of leaf  $R$  [quantified using the  $Q_{10}$  (i.e. the proportional change in  $R$  per 10 °C rise in temperature)] often assumed to be constant (Ryan 1991; Cox *et al.* 2000; Cramer *et al.* 2001). However, there is growing evidence that  $Q_{10}$  values can be highly variable (Breeze & Elston 1978; Ryan 1991; Azcón-Bieto 1992; Dewar *et al.* 1999; Tjoelker, Oleksyn & Reich 2001). For example, several studies have reported within-canopy variability in the  $Q_{10}$  of leaf  $R$  in some trees (Bolstad, Mitchell & Vose 1999; Griffin, Turnbull & Murthy 2002a; Turnbull *et al.* 2003). Moreover,  $Q_{10}$  values decrease as measuring temperatures increase (Tjoelker *et al.* 2001) and may vary in response to changes in the underlying factors regulating respiratory flux (e.g. maximum enzyme activity, substrate availability and/or the turnover of ATP to ADP; Atkin & Tjoelker 2003). This suggests that the temperature dependence of leaf  $R$  may vary in response to environmentally induced changes in substrate supply that are photosynthetically dependent.

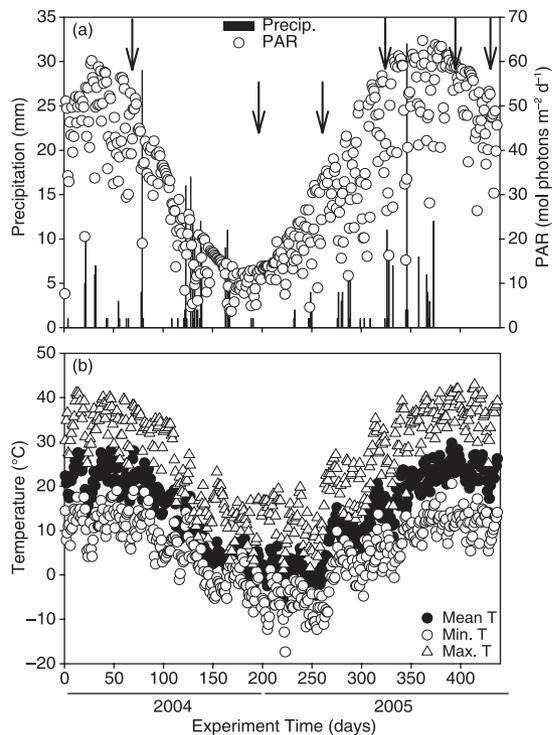
In many global climate models, predictions of future CO<sub>2</sub> exchange are based on the assumption that the temperature dependence of leaf  $R$  remains constant following long-term changes in temperature (i.e. Cox *et al.* 2000). However, in most plant species, leaf  $R$  is able to acclimate to long-term changes in temperature. As a result, rates of  $R$  measured at any given temperature are higher in cold-acclimated plants than their warm-grown counterparts (Atkin & Tjoelker 2003). This acclimation can result in a homeostasis of leaf  $R$  (i.e. constant rates of leaf  $R$  following long-term changes in temperature, when  $R$  is measured at the prevailing growth temperature) (Larigauderie & Körner 1995; Atkin, Holly & Ball 2000). Acclimation can be rapid, with substantial adjustments in leaf  $R$  occurring within 2 days of a change in growth temperature (Rook 1969; Billings *et al.* 1971; Atkin *et al.* 2000; Bolstad, Reich & Lee 2003). Although the degree of acclimation is developmentally dependent in some short-lived herbaceous species (e.g. Armstrong *et al.* 2006a; Armstrong, Logan & Atkin 2006b), substantial acclimation also occurs in mature leaves of long-lived species (Atkin *et al.* 2000; Bolstad *et al.* 2003; Bruhn *et al.* 2007) and irrespective of the growth irradiance (Zaragoza-Castells *et al.* 2007). Thermal acclimation in long-lived evergreen leaves could, therefore, play a pivotal role in allowing the maintenance of positive carbon balances for low NPP forest ecosystems such as in Mediterranean dry-land regions (in full sun and/or in the shaded understorey of large trees). This may especially be the case in summer when extreme leaf temperatures occur and when photosynthesis may simultaneously be limited by severe soil water deficits (Osborne *et al.* 2000).

This study quantifies the role of leaf respiratory CO<sub>2</sub> release plays in determining foliar net carbon balances for a Mediterranean dry-land ecosystem in central Spain. We measured diurnal and seasonal variations in leaf  $R$ , and net photosynthesis ( $P_{\text{net}}$ ) of a dominant long-lived evergreen tree species (*Quercus ilex* subsp. *ballota*) over a 12-month period in 2004–2005. Photosynthetic and respiratory rates were measured at the ambient temperatures experienced in the field. Measurements were made at two sites characterized by contrasting growth irradiances [sun-exposed (open forest) and shade (understorey forest)]. The data were then used to model daily and seasonal variations in leaf  $R$ . Three hypotheses were tested: (1) due to climate-mediated changes in photosynthesis and accumulation of sugars, the temperature sensitivity of leaf  $R$  will be lower in plants growing in shade; (2) the degree of acclimation of leaf  $R$  to seasonal changes in the environment is similar in sun-exposed and shaded plants; and (3) the balance between  $R$  and  $P_{\text{net}}$  in field-grown plants is not constant, varying seasonally, and differing between sun-exposed and shaded plants.

## Materials and methods

### SITE DESCRIPTION

The field site was located 175 km east of Madrid in the Iberic System Mountain Range (950 m a.s.l.) at Los Cerrillos Biological Station,



**Fig. 1.** (a) Variations in ambient total daily irradiance ( $\text{mol photons m}^{-2} \text{d}^{-1}$ ) open dots, and precipitation (mm) black bars, throughout the field study (from August 2004 to August 2005). Black arrows represent months upon which measurements of leaf gas exchange took place in *Quercus ilex*. (b) Variations in air temperature ( $^{\circ}\text{C}$ ) throughout the field study (from August 2004 to August 2005). Open triangles represent the maximum temperature, closed dots the mean temperature, while the open dots represent the minimum temperature.

Villar de Cobeta ( $40^{\circ}48' \text{ N } 2^{\circ}12' \text{ W}$ ), within the Alto Tajo Natural Park (Guadalajara, Spain). The experimental area was located on a steep ( $20^{\circ}$ ), south-facing slope, with *Q. ilex* subsp. *ballota* dominating the vegetation. The area has a continental Mediterranean climate, with hot, dry summers and cold winters, and is characterized by large diurnal and seasonal variations in air temperature (diurnal variations in temperature in the order of  $25^{\circ}\text{C}$  are common, with leaf temperatures reaching near  $50^{\circ}\text{C}$  in summer and  $-15^{\circ}\text{C}$  in winter) (Fig. 1). The site was characterized by limestone outcrops and a limited capacity of the soil to retain water. Selected individuals

of *Q. ilex* were small shrubs 20–110 cm height growing under two contrasting growth irradiances [full sun and in the shaded (40%–75% of full sunlight) understorey of mature trees].

Meteorological stations were installed in the full sun and shaded habitats. Each meteorological station was installed in June 2004 and included sensors for air temperature and relative humidity (Hobo H08-032-08, Onset, Pocasset, MA), soil moisture (ECH<sub>2</sub>O EC-20, Decagon Devices, Pullman, WA) and solar irradiance (Apogee quantum sensor QSO-SUN, Logan, UT), cross-calibrated with a Li-Cor SA Li-190 quantum sensor (Li-Cor, Lincoln, NE). Readings of each sensor were recorded every 10 min with a Hobo H08-006-04 data logger. Precipitation was recorded in the open with a Rainomatic small rain gauge (Pronamic Co. Ltd. Silkeborg, Denmark) attached to a Hobo H7 event data logger. Soil volumetric water content was estimated with a portable moisture device using the Time Domain Reflectometry (TDR probes) method, (TRIME-FM, Imko Micromodulentechnik GmbH, Ettlingen, Germany) at 20 cm soil depth, next to where the tree replicates were growing. Figure 1 shows the precipitation events, photosynthetic active radiation (PAR), and mean, maximum and minimum temperatures observed during the period of the study. Table 1 shows soil water content on four of the six visits to the field site over a 12-month period (August 2004 to August 2005).

#### SAMPLING REGIME AND PHYSIOLOGICAL MEASUREMENTS

In August 2004, four individual trees were identified at both the full sun and shaded sites, with each tree representing an independent replicate. For each field campaign, leaf physiological measurements were made using a single south-east facing, attached, fully-expanded, mature leaf from each replicate tree. Unless otherwise stated, measurements were made using single leaves from four replicate trees. Different leaves were used on each sampling month field campaign, with the measured leaves being harvested for determination of chemical–structural characteristics at the end of each sampling month campaign.

Predawn stem water potential was measured with a self-built Scholander chamber using pressurized nitrogen stem water potential and soil moisture content decreased significantly throughout the experiment at both sites (Table 1). Estimates of the predawn, dark-adapted photochemical efficiency of photosystem II ( $F_v/F_m$ ; dark adapted for 30 min) were made using a portable PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany); these measurements were made at the same time as the water potential measurements took place, using leaves adjacent to those used for gas exchange measurements.

**Table 1.** Comparison between full sun and deep shade sites for water potential (MPa), maximal photochemical efficiency of PSII ( $F_v/F_m$ ) and soil water content (%volume) on, or next to, the same trees used for gas exchange measurement over 4 months

Time (month)	Full sun – predawn			Shade – predawn		
	Water potential (MPa)	Soil water content* (% Vol.)	$F_v/F_m$	Water potential (MPa)	Soil water content* (% Vol.)	$F_v/F_m$
March 2005	$-1.33 \pm 0.70^a$	$8.40 \pm 1.23^a$	$0.38 \pm 0.03^a$	$-1.05 \pm 0.69^a$	$9.49 \pm 1.08^a$	$0.66 \pm 0.04^a$
May 2005	$-1.24 \pm 1.44^a$	$3.94 \pm 0.67^b$	$0.82 \pm 0.01^b$	$-1.66 \pm 1.83^b$	$4.28 \pm 0.79^b$	$0.84 \pm 0.00^b$
July 2005	$-3.56 \pm 1.31^b$	$1.33 \pm 0.61^c$	$0.86 \pm 0.01^b$	$-3.75 \pm 0.80^c$	$1.41 \pm 0.55^c$	$0.86 \pm 0.02^b$
August 2005	$-4.60 \pm 2.61^c$	$3.45 \pm 0.86^{b,c}$	$0.75 \pm 0.02^c$	$-5.22 \pm 2.03^d$	$2.00 \pm 0.70^c$	$0.79 \pm 0.03^b$

Measurements took place predawn (0630–0900 h). Mean values  $\pm 1$  SE are shown ( $n = 4$ ). Within each site and measurement variable, months labelled with different letters differ significantly ( $P < 0.05$ ). \*The soil water content was measured around 1000–1100 h.

$F_v/F_m$  ratios range from 0.75 to 0.85 for healthy plants (Corcuera *et al.* 2005).

Measurements of leaf gas exchange were carried out using a Licor 6400 portable photosynthesis system (LI-COR). Leaves were exposed to an atmospheric CO<sub>2</sub> concentration of 400 p.p.m. (using the built-in CO<sub>2</sub> controller), and measurements made at the prevailing ambient air temperature (Atkin *et al.* 2000; Griffin *et al.* 2002b) and at ambient relative humidity (typically 35%–60%).

Measurements of leaf  $R$  took place at regular intervals during the night, starting 1 h after sunset to avoid post-illumination transients (Azcón-Bieto & Osmond 1983; Atkin, Evans & Siebke 1998) with measurements continuing through the night to just before dawn. To expand the temperature range over which leaf  $R$  in the dark was measured, from March 2005 onwards we continued measuring  $R$  on the same leaves during subsequent daylight hours, with leaves darkened for 30 min prior to measurement (due to time constraints, these measurements could only be made using three of the four replicates used in the night-time measurements). As it was found that there were no differences between leaf  $R$  measured during the day and night at the same temperature (see Fig. 3), temperature response curves were fitted to data collected both during night and day. Leaves for which  $R$  in darkness had been determined during the day were subsequently re-exposed to ambient light to allow the plants to photosynthesize and prevent the decline of substrates (Azcón-Bieto 1992). After 1 h exposure to ambient irradiance, measurements of net photosynthesis ( $P_{\text{net}}$ ) were made. The measurements were carried out at regular intervals (approximately every 2 h) through the day (interspersed between measurements of  $R$  on the same leaves), with  $P_{\text{net}}$  being measured at the prevailing ambient irradiance. On each sampling month, measurements were made on three to four replicate leaves.

Following CO<sub>2</sub> exchange measurements on each sampling month, leaves were harvested, fresh mass and area of the leaf sections used for CO<sub>2</sub> exchange measurements quantified, after which they were submerged in liquid N<sub>2</sub>. Once back in the laboratory, leaves were oven-dried for 3 days (65 °C) and then weighed. Leaves were then pooled for each month and each site, and ground to a fine powder using a hammer mill (31–700 Hammer Mill; Glen Creston, Stanmore, UK). Soluble sugars and starch were extracted and measured as described previously (Loveys *et al.* 2003).

## MODELLING RESPIRATION RATES

To account for observed variation in rates of leaf  $R$  at any given temperature ( $T$ ), we compared the applicability of two approaches based on the Arrhenius and  $Q_{10}$  functions.

The following equation provides a simple Arrhenius function standardized to 20 °C:

$$R = R_{20} e^{\frac{-E_0}{r} \left( \frac{1}{T_k} - \frac{1}{293.15} \right)} \quad \text{eqn 1}$$

where  $R_{20}$  is leaf  $R$  at 20 °C,  $E_0$  has the significance of an activation energy,  $r$  is the universal gas constant (8.314 J mol<sup>-1</sup> K<sup>-1</sup>) and  $T_k$  is the leaf temperature expressed in Kelvin. Taking natural logarithms for each side, eqn 1 becomes:

$$\log_e(R) = \log_e(R_{20}) - \frac{E_0}{r} \left( \frac{1}{T_k} - \frac{1}{293.15} \right) \quad \text{eqn 2}$$

which allows  $\log_e(R)$  to be modelled as a simple linear regression function with  $\log_e(R_{20})$  being the intercept, the independent variable being  $(1/T_k - 1/293.15)$  and with  $E_0/r$  being the slope.

The second function was the more common  $Q_{10}$  equation:

$$R = A e^{BT} \quad \text{eqn 3}$$

where  $A$  is a parameter which describes the overall rate and  $B$  is a constant describing the temperature sensitivity of the reaction rate, which can be expressed in terms of a  $Q_{10}$ , as follows:

$$B = \frac{\log_e(Q_{10})}{10} \quad \text{eqn 4}$$

For example, for a  $Q_{10}$  of 2, then  $B = 0.0693$ . As for the Arrhenius function, eqn 3 can be expressed relative to the modelled respiration rate, in this case 20 °C, as shown:

$$R = R_{20} e^{B(T-20)} \quad \text{eqn 5}$$

which can also be expressed in a linear form:

$$\log_e(R) = \log_e(R_{20}) + B(T - 20) \quad \text{eqn 6}$$

The transformations associated with eqns 2 and 6 also served to stabilize the variances for the data set collected as part of this study.

Estimates of  $R_{20}$  were obtained using multi-level modelling techniques (Snijders & Bosker 1999) allowing us to take into account that measurements were repeatedly made on the same leaves over any 1 day. We also examined potential effects of both growth irradiances and time of year on overall estimates of  $R_{20}$  and also if the temperature sensitivity of respiration also changed according to time of year. That is to say, the fit of the Arrhenius equation the model applied was:

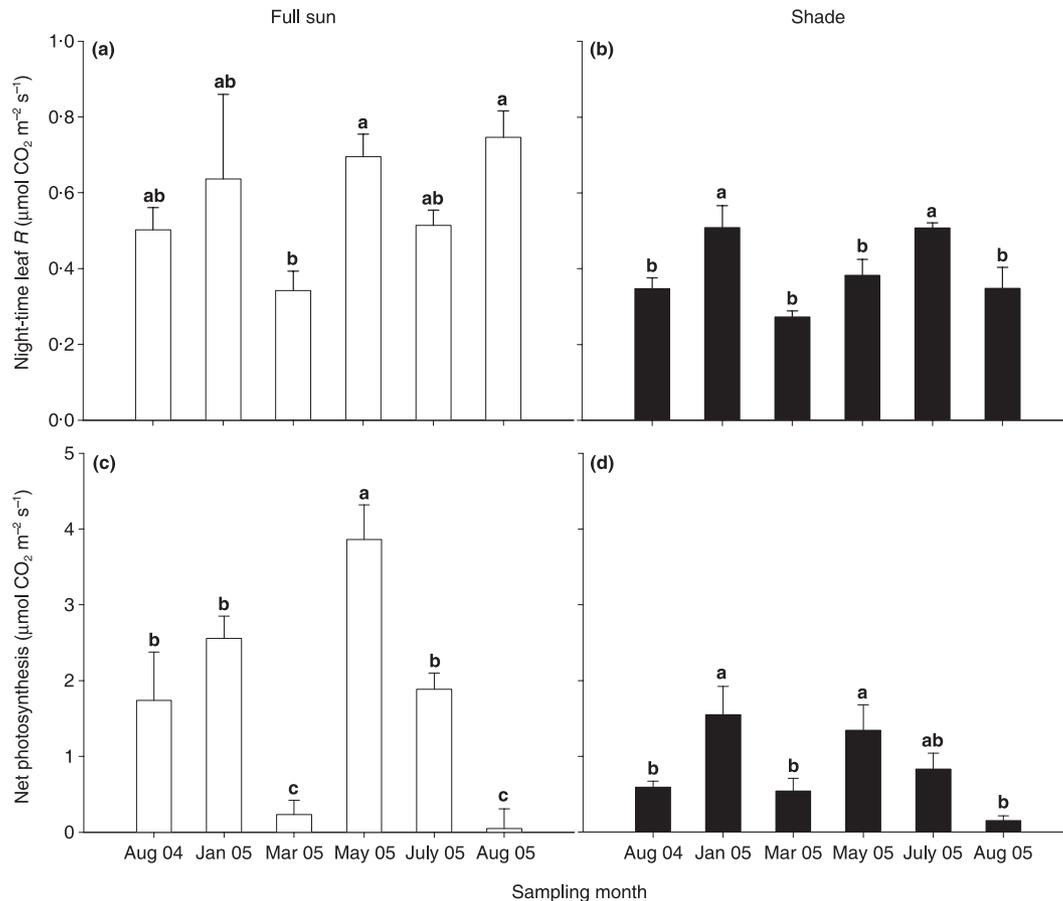
$$\log_e(R_p) = \log_e(R_{20}) - E_0 \left( \frac{1}{T} - \frac{1}{293.15} \right) + b_1 + c_{(2-4)} + d_{(2-4)} \left( \frac{1}{T} - \frac{1}{293.15} \right) + U_{0p} + U_{1p} + R_{ip} \quad \text{eqn 7}$$

where  $R_{ip}$  denoted a respiration measurement made at time  $t$  on plant  $p$ ,  $b_1$  is a categorical variable allowing for respiration rate to differ between sun and shade leaves;  $c_2$ ,  $c_3$  and  $c_4$  are categorical variables which allow for respiration rates to vary according to time of year (May, July and August 2005, respectively) and  $d_2$ ,  $d_3$  and  $d_4$  are categorical variables which allow for the temperature sensitivity of respiration rates to vary according to time of year. All categorical variables (2–4) are expressed relative to the first measurement date in March 2005, and likewise  $b_1$  described the respiration rates of shade leaves relative to those in the sun. Similarly, for the  $Q_{10}$  model:

$$\log_e(R_p) = \log_e(R_{20}) - B(T - 20) + b_1 + c_{(2-4)} + d_{(2-4)} B(T - 20) + U_{0p} + U_{1p} + R_{ip} \quad \text{eqn 8}$$

In both eqns 7 and 8,  $U_{0p}$  represents a random effect which allows  $R_{20}$  to vary from plant to plant and  $U_{1p}$  is a random effect allowing the slope of the temperature–respiration relationships to also vary between plants on any given day. The remaining residual variance is designated  $R_{ip}$ .

Equations 7 and 8 represent a reduced form of an initial model which also included the possibility of differing temperature sensitivities of respiration for sun vs. shade leaves. Nevertheless, as no significant effect of growth irradiance on the temperature sensitivity of leaf  $R$  was found when applying either the Arrhenius or  $Q_{10}$  equations (data



**Fig. 2.** Average rates of night-time leaf respiration in darkness ( $R$ ) and day-time net photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) exhibited by fully-expanded mature *Quercus ilex* leaves each month, both for sun-exposed plants (a and c) and shade-grown plants (b and d) measured at the prevailing temperature experienced by leaves on the day of measurement. Values of respiration represent rates of  $R$  measured at regular intervals during the night-time ( $n = 3-4$ ,  $\pm$  SE). Different letters indicate significant differences between months within each light treatment ( $P < 0.05$ ).

not shown), we employ and show results from the reduced versions only here.

## STATISTICAL ANALYSES

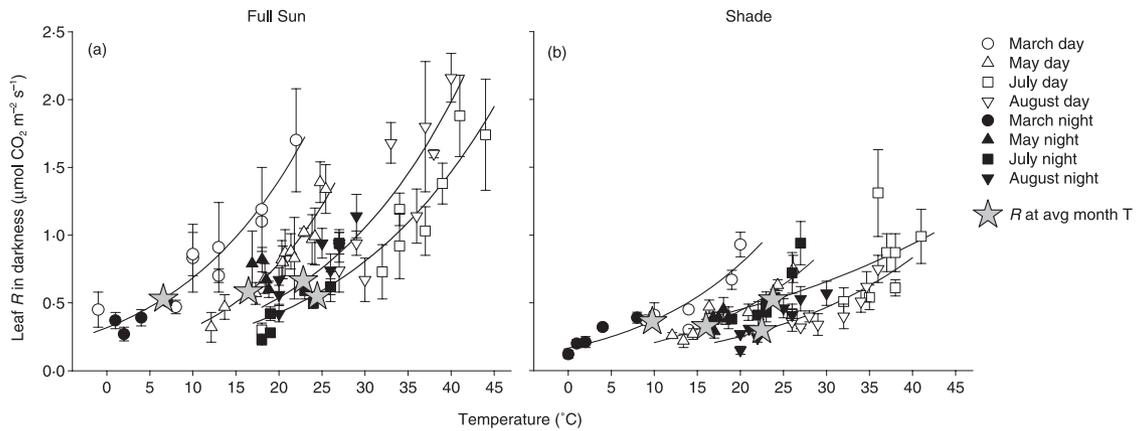
Other than for the statistical modelling of leaf  $R$  described above which were undertaken with MLWIN (Rabash *et al.* 2004), statistical analyses were conducted using SPSS version 11 (SPSS Science, Birmingham, UK). Kolmogorov–Smirnov and Levene’s tests were used to test for normality and homogeneity of variances, respectively. When the variances were found to be not equal,  $\log_{10}$  transformations were performed. If these transformations still failed to produce equal variances then the nonparametric test equivalent was used. Two-way ANOVAs were carried out to investigate the effects of time (month) and site (sun vs. shade) on SLA, leaf thickness, dry matter content (DMC),  $P_{\text{net}}$  and  $R$ . Within a site, one-way ANOVAs were used to investigate how the same variables changed over time (between months). Regression analyses were used to investigate the relationship between stomatal conductance and  $P_{\text{net}}$  using the  $F$ -ratio method (Sokal & Rohlf 1981) to determine whether the relationships differed between sites. For average day-time  $P_{\text{net}}$  and mean night-time  $R$ , significant interactions between month and sun-exposed vs. shade-grown leaves were observed. For this reason,

separate one-way ANOVAs were carried out for sun-exposed or shade-grown leaves to investigate inter-month differences.

## Results

### AVERAGE NIGHT-TIME RATES OF LEAF $R$

Figure 2 shows average night-time rates of leaf  $R$  on each sampling month over the 12-month period, irrespective of the prevailing temperature. Rates were significantly higher in sun-exposed plants than at the shade site ( $P < 0.001$ ) and a significant interaction term was observed between months and sun-exposed vs. shade environments ( $P = 0.028$ ). This suggests that patterns of seasonal variation differed between the sun-exposed and shade sites for average rates of leaf  $R$ . For this reason, one-way ANOVAs were performed to distinguish between months within both sites. At the sun-exposed site, leaf  $R$  varied between months (Fig. 2a;  $P = 0.04$ ), with  $R$  being significantly lower in March. Similarly, leaf  $R$  varied through time at the shade site (Fig. 2b;  $P = 0.001$ ) with rates in January and July being significantly higher than the other 4 months ( $P < 0.001$ ).



**Fig. 3.** Rates of leaf respiration in darkness ( $R$ ) of fully-expanded, mature *Quercus ilex* leaves plotted against ambient temperature ( $n = 3$ ,  $\pm$  SE), both for plants grown in (a) full sun and (b) deep shade, from March 2005 to August 2005. In both (a) and (b), closed symbols show the rates of leaf  $R$  at night time, whilst open symbols show the subsequent rates of  $R$  exhibited after 30 min in darkness during the day-time. Fitted lines were calculated using eqn 9. Star symbols indicate the  $R$  rate calculated at the mean temperature experienced during each month.

**Table 2.** Estimates for the Arrhenius and  $Q_{10}$  models, using eqns 7 and 8 and data shown in Fig. 3

Fixed effects	Arrhenius model		$Q_{10}$ model	
	Coefficient	SE	Coefficient	SE
Intercept = $\log_e(R_{20})$	<b>-0.3095</b>	<b>0.0931</b>	<b>-0.2947</b>	<b>0.0999</b>
$E_0$ = slope (Arrhenius model)	<b>46.95</b>	<b>3.31</b>		
$B$ = slope ( $Q_{10}$ model)			<b>0.0703</b>	<b>0.0052</b>
$b_1$ = shade effect	<b>0.5228</b>	<b>0.0695</b>	<b>0.5172</b>	<b>0.0723</b>
$c_2$ = date effect (intercept)	<b>-0.5135</b>	<b>0.1076</b>	<b>-0.5288</b>	<b>0.1161</b>
$c_3$ = date effect (intercept)	<b>-0.8798</b>	<b>0.1151</b>	<b>-0.8795</b>	<b>0.1230</b>
$c_4$ = date effect (intercept)	<b>-0.9035</b>	<b>0.1207</b>	<b>-0.9048</b>	<b>0.1280</b>
$d_2$ = date effect (slope)	-7.457	6.967	0.0061	0.0102
$d_3$ = date effect (slope)	5.775	4.732	<b>-0.0167</b>	<b>0.0071</b>
$d_4$ = date effect (slope)	3.537	5.754	-0.0131	0.0083
Random effects	Var. Comp.	SE	Var. Comp.	SE
<i>Level two (plant) random effects</i>				
Variance ( $U_{0p}$ )	<b>0.02649</b>	<b>0.00917</b>	<b>0.03328</b>	<b>0.01109</b>
Variance ( $U_{1p}$ )	3.297	19.86	0.1623	0.2841
Covariance ( $U_{0p}, U_{1p}$ )	0.1623	0.2841	0.0003	0.0004
<i>Level one variance</i>				
Variance ( $R_{0p}$ )	<b>0.09891</b>	<b>0.074437</b>	<b>0.09946</b>	<b>0.007502</b>
Deviance	254.3	—	255.2	—

For the fixed effect terms estimates of the coefficients are shown, along with their standard errors (SE). For the partitioning of the variance for the random (error) terms, the variance component (Var. Comp) is given, along with the standard error. Terms in bold are statistically significant ( $P = 0.05$ ).  $U_{0p}$  refers to the variations in overall respiration rates (i.e. intercept) between the various trees sampled not accounted for by the model, and  $U_{1p}$  refers to the variation in temperature response (i.e. slope) between plants not accounted for by the model.  $R_{0p}$  is a residual term describing the remaining variance after the model fit and systematic plant-to-plant variability have both been considered.

#### MODELLING DAILY AND SEASONAL VARIATIONS IN LEAF $R$

Figure 3 shows leaf  $R$  for both sun-exposed and shade leaves of *Q. ilex*; rates measured during night-time are shown as closed symbols, with rates measured during day-time (after leaves were covered for at least 30 min before measuring) as open symbols. At both sites, leaf  $R$  was temperature sensitive, generally being lower at any given temperature in shade plants than in plants at the sun-exposed site.

We used data in Fig. 3 to model daily and seasonal variations in leaf  $R$  using the Arrhenius and  $Q_{10}$  approaches described in eqns 7 and 8. In preliminary modelling exercises, incorporation of different temperature coefficients (i.e.  $Q_{10}$  or  $E_0$ ) for sun- and shade-grown plants was observed to have no impact on the goodness-of-fit between models and data (data not shown; thereby contradicting Hypothesis 1); because of this, we simply assigned common temperature coefficients to sun- and shade-grown plants in subsequent modelling exercises. Table 2 shows that both models yielded similar

**Table 3.** Estimates for the Arrhenius model with no effect of time of year on the activation energy ( $E_0$ ) using eqn 7 and data shown in Fig. 3

Fixed effects	Arrhenius model	
	Coefficient	SE
Intercept = $\log_e(R_{20})$	<b>-0.335</b>	<b>0.085</b>
$E_0$ = slope	<b>45.13</b>	<b>2.07</b>
$b_1$ = shade effect	<b>0.5237</b>	<b>0.0659</b>
$c_2$ = date effect	<b>-0.4904</b>	<b>0.1000</b>
$c_3$ = date effect	<b>-0.8999</b>	<b>0.1094</b>
$c_4$ = date effect	<b>-0.8975</b>	<b>0.1093</b>
Random effects	Var. Comp.	SE
<i>Level two (tree) random effects</i>		
Variance ( $U_{op}$ )	<b>0.02544</b>	<b>0.009105</b>
Variance ( $U_{1p}$ )	8.392	21.53
Covariance ( $U_{op}, U_{1p}$ )	0.1172	0.2841
<i>Level one variance</i>		
Variance ( $R_{op}$ )	<b>0.09969</b>	<b>0.0750</b>
Deviance	258.2	-

For the fixed effect terms estimates of the coefficients are shown, along with their standard errors (SE). For the partitioning of the variance for the random (error) terms, the variance component (Var. Comp) is given, along with the standard error. Terms in bold are statistically significant ( $P = 0.05$ ).  $U_{op}$  refers to the variations in overall respiration rates (i.e. intercept) between the various plants sampled not accounted for by the model, and  $U_{1p}$  refers to the variation in temperature response (i.e. slope) between plants not accounted for by the model.  $R_{op}$  is a residual term describing the remaining variance after the model fit and systematic plant-to-plant variability have both been considered.

results in terms of model fit (as seen by the similar deviances) but with one important difference – although a single parameterisation of  $E_0$  (45.13 kJ mol<sup>-1</sup>) was adequate to explain the observed daily and seasonal variation in  $R$  using the Arrhenius model, allowing  $Q_{10}$  values to vary between months resulted in a better fit (as shown by the significantly different  $d$  values in  $Q_{10}$  model; Table 2). Modelled  $Q_{10}$  values in March, May, July and August 2005 were: 2.02, 2.15, 1.71 and 1.77, respectively. By contrast, a more parsimonious model could be fitted ignoring the possibility of a changing  $E_0$  with time of year. This is detailed in Table 3, which shows only an insignificant loss in goodness-of-fit model (increase in deviance of 3.9 associated with the loss of three terms). Also worthy of note in the above analysis is that all models suggested significant plant-to-plant variation in the rate of  $R$  at common temperature ( $R_{20}$ ) across the four periods, as evidenced by the significant variance associated with the  $U_{op}$  term (Tables 2 and 3), but with no significant variation in apparent responses to temperatures (as evidenced by the insignificant variances associated with the  $U_{1p}$  terms).

A reduced Arrhenius model was thus applied which did not include the possibility of changes in the temperature sensitivity of  $R$  with time of year (but which did still allow for overall rate

changes with season and differences between sun and shade) namely:

$$\log_e(R_p) = \log_e(R_{20}) - E_0 \left( \frac{1}{T} - \frac{1}{293.15} \right) + b_1 + c_{(2-4)} + U_{op} + U_{1p} + R_p \quad \text{eqn 9}$$

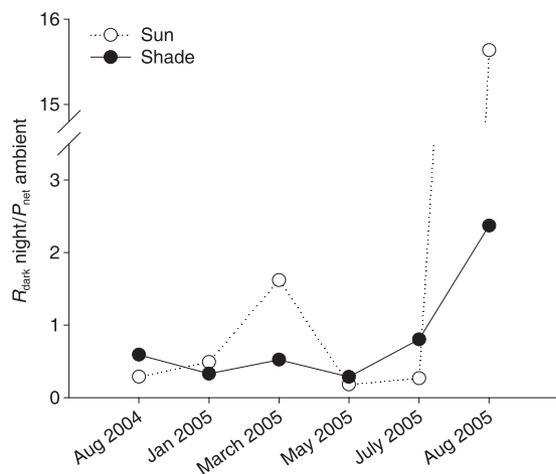
the results of which are shown in Table 3 with the fitted lines (model predictions) being shown in Fig. 3. From Table 3, for the first measurement date used in the model (March 2005),  $R_{20}$  was modelled to be 0.72  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for leaves on plant growing in the shade and 1.69 times greater for leaves on the sun-exposed plants (i.e. 1.21  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). For the subsequent three dates (May, July and August 2005), these rates needed to be modified by the multiplication factors (0.61, 0.41 and 0.41, respectively). In the above, all fitted parameters were highly significant and the coefficients associated with  $c_3$  (July 2005) and  $c_4$  (August 2005) were significantly different to  $c_2$  (May 2005), the parameter estimate for which was, itself, significantly different to the first measurement date (March 2005). That is,  $R_{20}$  (March 2005) >  $R_{20}$  (May 2005; shade and sun leaves = 0.44 and 0.74  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , respectively) >  $R_{20}$  (July 2005; shade and sun leaves = 0.29 and 0.49  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , respectively) =  $R_{20}$  (August 2005). Across all time periods the proportional difference between sun and shade-grown plants did not change significantly:  $R_{20}$  (sun) = 1.69  $R_{20}$  (shade) and, as stated above, there was no evidence of  $E_0$  changing with time of year.

When compared at the prevailing average daily temperature during each measurement period (derived using the model fit of eqn 9), rates of leaf  $R$  were relatively constant in both sun-exposed and shade plants (Fig. 3), despite the large seasonal variation in temperatures (Fig. 1). We interpret this as a near full homeostasis of average daily leaf  $R$  being achieved across the year.

#### PHOTOSYNTHESIS: FLUORESCENCE AND GAS EXCHANGE

When maximal photochemical efficiency of PSII ( $F_v/F_m$ ) was measured predawn there were very low values in winter time, (Table 1; sun site: 0.38, shade site: 0.66). Nevertheless,  $F_v/F_m$  values had recovered by spring (Table 1; sun-exposed: 0.82, shade: 0.84) and did not change significantly throughout the summer, with the exception of a small but significant decrease in the  $F_v/F_m$  values in the sun plants between July and August.

Net photosynthesis ( $P_{\text{net}}$ ), measured at the prevailing ambient irradiance (sun-exposed site, ranging from 610 to 1490  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  from winter to summer, respectively; shaded site: ranging from 120 to 290  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  from winter to summer, respectively).  $P_{\text{net}}$  was significantly higher in sun-exposed plants than in the shade plants (Fig. 2c,d;  $P < 0.001$ ). Further,  $P_{\text{net}}$  changed significantly between months ( $P < 0.001$ ), and a significant interaction term was detected between sampling time and growth irradiance ( $P = 0.005$ ) indicating that the patterns of change through time were not identical at the sun and shade sites. Taken together, these



**Fig. 4.** Ratio of respiration in darkness ( $R$ ) to net photosynthesis ( $P_{\text{net}}$ ) of fully-expanded mature *Quercus ilex* leaves plotted against month, both for full sun (open symbols) and deep shade (closed symbols) grown plants.  $R$  values are the average rates of leaf  $R$  exhibited by leaves during the night-time, whereas  $P_{\text{net}}$  was taken as the average of all net photosynthetic rates exhibited during the day-time (see Fig. 2).

results demonstrate that: (i) there were differences in  $P_{\text{net}}$  between sun-exposed and shade-grown *Q. ilex*; and (ii) that the magnitude of these differences changed through the year, both in an absolute and proportional sense.

In this water-limited ecosystem (see Table 1), it was thought that  $P_{\text{net}}$  may be strongly influenced by stomatal conductance ( $g_s$ ). To determine whether this was the case, linear regressions were carried out between  $P_{\text{net}}$  and  $g_s$  for both sun-exposed and shade-grown plants (see Supplementary Fig. S1) indicating a significant relationships between  $P_{\text{net}}$  and  $g_s$  at the sun ( $P < 0.001$ ) and shade ( $P = 0.020$ ) sites. The  $F$ -ratio method revealed that the two regressions differed significantly ( $F_{2,36} = 17.933$ ,  $P < 0.01$ ).

#### RATIO OF LEAF $R$ TO $P_{\text{NET}}$

To assess whether the balance between  $\text{CO}_2$  release by night-time leaf  $R$  and day-time  $P_{\text{net}}$  varied through the year and/or differed between the sites,  $R/P_{\text{net}}$  ratios were calculated using average rates of  $R$  measured at night, and average rates of  $P_{\text{net}}$  during the day-time (Fig. 4). As  $R/P_{\text{net}}$  values were calculated from average rates, no statistical analysis was possible but the following patterns were observed.  $R/P_{\text{net}}$  at both sites increased substantially in August 2005, but the increase in  $R$  relative to  $P_{\text{net}}$  was particularly high in sun-exposed plants. There was also an increase in  $R/P_{\text{net}}$  during March, again especially at the sun-exposed site. In August 2004 and July 2005, shade plants exhibited higher  $R/P_{\text{net}}$  values than sun-exposed plants, due largely to the large reductions in  $P_{\text{net}}$  that occurred during these months.

#### LEAF BIOMASS ALLOCATION AND CARBOHYDRATE CONCENTRATIONS

Table 4 lists the SLA, leaf thickness (as estimated from the ratio of leaf fresh mass to leaf area), leaf DMC over the 12-month period; from August 2004 through August 2005. Seasonal variation in SLA values was observed by sun-leaves only ( $P = 0.037$ ). To explore what underlying factors were responsible for the differences in SLA values observed, leaf DMC and leaf 'thickness' (as estimated from the ratio of leaf fresh mass to leaf area; Dijkstra 1989) values were calculated. The observed differences in SLA between sun and shade leaves were not due to differences in DMC (Table 4;  $P > 0.05$ ). Rather, the inter-site differences in SLA reflected differences in leaf fresh mass to leaf area, with sun-grown leaves being 'thicker' than their shade-grown counterparts (Table 4;  $P < 0.001$ ). Moreover, the seasonal variations in sun-grown SLA values reflected month-to-month variations in leaf thickness only ( $P < 0.010$ ).

**Table 4.** Effect of growth irradiance (sun- and shade-exposed) on leaf mass-area relationships and carbohydrate concentrations of *Quercus ilex*

Growth irradiance	Leaf trait	Sampling month					
		August 2004	January 2005	March 2005	May 2005	July 2005	August 2005
Full sun	SLA ( $\text{m}^2 \text{kg}^{-1}$ )	$5.2 \pm 0.4$	$3.7 \pm 0.1$	$3.4 \pm 0.2$	$3.5 \pm 0.1$	$4.3 \pm 0.3$	$4.0 \pm 0.2$
	Leaf thickness ( $\text{g FM m}^{-2}$ )	$350 \pm 20$	$503 \pm 36$	$492 \pm 18$	$477 \pm 18$	$361 \pm 39$	$403 \pm 18$
	Leaf DMC (%)	$55.9 \pm 0.9$	$53.9 \pm 2.1$	$59.5 \pm 0.7$	$59.6 \pm 0.5$	$66.4 \pm 5.3$	$62.0 \pm 1.0$
	[Sugar] ( $\text{mg g}^{-1}$ )	33.3	45.7	46.6	37.8	32.7	33.8
	[Starch] ( $\text{mg g}^{-1}$ )	0	0	0	14.6	0	0
Shade	SLA ( $\text{m}^2 \text{kg}^{-1}$ )	$6.4 \pm 0.2$	$5.9 \pm 0.3$	$5.7 \pm 0.1$	$5.7 \pm 0.1$	$5.9 \pm 0.0$	$5.8 \pm 0.2$
	Leaf thickness ( $\text{g FM m}^{-2}$ )	$272 \pm 8$	$299 \pm 14$	$299 \pm 05$	$314 \pm 16$	$270 \pm 04$	$267 \pm 08$
	Leaf DMC (%)	$57.3 \pm 0.6$	$57.3 \pm 1.4$	$59.1 \pm 1.3$	$56.3 \pm 3.5$	$63.2 \pm 1.1$	$64.6 \pm 0.8$
	[Sugar] ( $\text{mg g}^{-1}$ )	28.6	45.5	49.5	32.0	30.8	31.9
	[Starch] ( $\text{mg g}^{-1}$ )	0	0	0	0	0	0

Specific leaf area (SLA, ratio of leaf area to leaf dry mass), leaf thickness [as estimated from the ratio of leaf fresh mass to leaf area (Dijkstra 1989)], leaf dry mass content (DMC, the ratio of leaf dry mass to leaf fresh mass), concentrations of total soluble sugars (i.e. fructose + sucrose + glucose) and starch are shown. Total soluble sugar and starch concentrations were determined using pooled samples of four replicates harvested at each site and time. Biomass allocation parameters are the mean of four replicates ( $\pm$  SE).

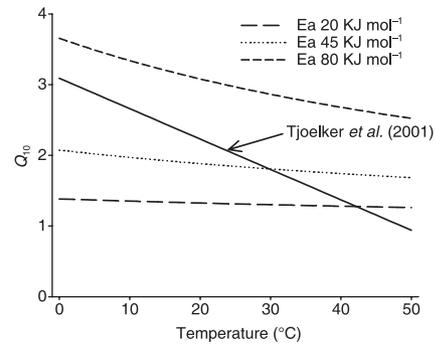
Total soluble sugar concentrations (i.e. sucrose + glucose + fructose) and starch concentrations are also shown in Table 4 (the small sample size necessitated pooling of sugar per starch samples, with the result that statistical analysis was not possible). Nevertheless, the results suggest that sugar concentrations were higher in March 2005 (the coldest month) than the other months sampled (Table 4). No starch was detected in shade-grown leaves at any time of the year. Measurable starch was observed in sun leaves only in May 2005.

## Discussion

### DAILY AND SEASONAL VARIATIONS IN LEAF $R$

At the outset of our study, we hypothesized that the temperature sensitivity of leaf  $R$  would differ among plants growing under contrasting light regimes; this hypothesis was based on the observation that  $Q_{10}$  values can vary with substrate availability depending on the extent to which respiratory flux is limited by ATP turnover and/or enzymatic capacity (Atkin & Tjoelker 2003). To test this hypothesis, we have used two approaches to assess whether site-dependent variations in temperature sensitivity needed to be taken into account when modelling seasonal variations in leaf  $R$ . When using eqn 8, it was necessary to allow for seasonal variations in the  $Q_{10}$ , reflecting the fact that  $Q_{10}$  values were higher in winter than summer, though this was not necessary when modelling the temperature sensitivity of respiration using the Arrhenius function. Nevertheless, neither modelling approach required a difference in the temperature sensitivity of leaf  $R$  according to light regime of growth. In Hartley *et al.* (2006) no correlation between the  $Q_{10}$  of leaf  $R$  and atmospheric  $[\text{CO}_2]$ -induced changes in photosynthesis was observed; however, soluble sugar concentrations remained high in all treatments. Similarly, in Zaragoza-Castells *et al.* (2007) we found no correlation between the  $Q_{10}$  of leaf  $R$  and shade-induced changes in photosynthesis of controlled-environment grown *Q. ilex*, possibly because soluble sugar concentrations were insensitive to transient shade. Sugar concentrations were also similar in sun- and shade-grown plants at the dry-land field site used in our current study (Table 4), which may have been the reason why little difference was observed in the temperature sensitivities of leaf  $R$  for sun and shade plants. While such results do not preclude the possibility that the temperature sensitivity of leaf  $R$  will decline under conditions that severely reduce substrate supply, evidence to date suggests that climate-dependent changes in photosynthesis are unlikely to result in concomitant changes in the temperature sensitivity of leaf  $R$  under field conditions. Nevertheless, further controlled environment experiments that explicitly test the effect of changes in leaf carbohydrate levels on the temperature sensitivity of leaf  $R$  are required.

From the modelling exercise (eqn 9), we found that  $E_0$  remained constant through the year, despite the  $Q_{10}$  of leaf  $R$  being higher in winter than summer. This apparent contradiction can be explained by the fact that constant  $E_0$  values are inevitably associated with a  $Q_{10}$  declining with increasing



**Fig. 5.** Temperature dependence of the  $Q_{10}$  of leaf  $R$  as dependent on the assumed activation energy ( $E_0$ ) value (calculated using eqn 10). The temperature dependence of the  $Q_{10}$  reported as based on data set reported in Tjoelker *et al.* (2001) is shown for comparison.

temperature. This can be shown by the following (see Supplementary Material for a full derivation):

$$Q_{10} = e^{\left(\frac{10E_0}{rT^2}\right)} \quad \text{eqn 10}$$

Importantly, however, a constant  $E_0$  cannot account for scenarios where the temperature dependence of the  $Q_{10}$  is substantial, such as reported for *Eucalyptus pauciflora* growing in SE Australia (Atkin *et al.* 2000), or for diverse plant taxa and biomes sampled around the globe (Tjoelker *et al.* 2001; Atkin & Tjoelker 2003). Rather, changes in  $E_0$  are needed to account for the larger scale temperature-dependent changes in  $Q_{10}$ , as shown in Fig. 5; in this example, a constant  $E_0$  value explains only half of the actual global temperature dependence of  $Q_{10}$  as derived by Tjoelker *et al.* (2001). Thus, while our analysis demonstrates that  $E_0$  of shade- and sun-exposed *Q. ilex* plants at our field site was constant across the year, considerable variability in  $E_0$  must occur in order to account for the large temperature-dependent changes in  $Q_{10}$  reported by Tjoelker *et al.* (2001). Although it is known that the temperature dependence of  $R$  is linked to shifts in the control exerted by maximum enzyme activity at low temperature and substrate limitations at high temperature (Atkin & Tjoelker 2003), little is known about how such factors vary among sites in a way that would explain large variations in  $E_0$ . Establishing a process-based understanding for why  $E_0$  varies within and among sites is necessary, if we are to more accurately incorporate variations in the temperature dependence of leaf  $R$  into large scale models (which at present assume either a constant  $Q_{10}$  or  $E_0$ ).

In contrast to the constant  $E_0$  at our site, basal rates of  $R$  at a reference temperature of 20 °C (i.e.  $R_{20}$ ) varied substantially, decreasing from winter to summer, and being consistently higher in the sun-exposed plants (compared to their shade counterparts). By incorporating additive non-interacting functions of time of year and light regime (in eqn 9) that allowed for variations in  $R_{20}$ , we were able to model seasonal and daily variations in leaf  $R$  in both sun- and shade-exposed plants. Previous studies have also reported shade-induced

reductions in the basal rate of  $R$ , with reductions in respiratory capacity being largely responsible (Noguchi & Terashima 1997; Noguchi *et al.* 2005).

Importantly, both sun- and shade-grown plants exhibited seasonal changes in temperature response curves (Fig. 3) and predicted rates of  $R_{20}$ , suggesting that acclimation occurs independently of the growth irradiance and that acclimation is underpinned by variations in the basal rate of  $R$ . While the season shifts in rates of leaf  $R$  (Fig. 3) could reflect respiratory responses to changes in factors other than temperature [e.g. decreases in water availability and/or foliar N content as the summer progresses (Xu & Griffin 2006)], we have recently observed similar shifts in the daily temperature response of leaf  $R$  in *Q. ilex* under controlled environment conditions, both under high- and low-light (Zaragoza-Castells *et al.* 2007) which suggests that seasonal changes in daily average temperature may have been paramount. Moreover, exposure to severe water stress typically increased rates of leaf  $R$  (Flexas *et al.* 2006; Wright *et al.* 2006), as opposed to the decreased leaf  $R$  that was observed in our study when soil moisture was lowest (Fig. 3, Table 1). Moreover, Bruhn *et al.* (2007) reported that thermal acclimation in an evergreen tree species (in response to artificial heating in the field) was underpinned by variations in the basal rate of  $R$ . Changes in the basal rate of leaf  $R$  likely reflect changes in maximal rates of mitochondrial  $O_2$  uptake per unit protein and alterations in the density of mitochondria per unit cell volume (Armstrong *et al.* 2006a).

#### PHOTOSYNTHESIS IN PLANTS GROWN UNDER FULL SUN AND SHADE

Overall,  $P_{\text{net}}$  was higher at the sun site than at the shade site, especially in May 2005. However, in March and August 2005 (with coolest and hottest temperatures, respectively) the rates dropped sharply at both sites (Fig. 2). This seasonal response is similar to the results of several studies of species growing in Mediterranean ecosystems (i.e. Pereira *et al.* 1986; Faria *et al.* 1998; Peñuelas *et al.* 1998; Gratani *et al.* 2000; Llorens, Peñuelas & Filella 2003; Corcuera *et al.* 2005). The seasonal changes in  $P_{\text{net}}$  also differed between sun and shade sites with the magnitude of the changes in the rate of photosynthesis between months being greater at the sun site (Fig. 2). Rates of  $P_{\text{net}}$  during the summer drought were lower at the sun site than in the shade, suggesting that plants were experiencing greater environmental stress. However, plants at the shade site were experiencing a greater water stress during this period than the sun plants (Table 1). Despite this, shaded plants were capable of re-adjusting their rates of  $P_{\text{net}}$  and  $R$ , which agrees with recent studies assessing the performance of drought-affected Mediterranean plants under shaded conditions (Sack 2004; Sánchez-Gómez, Valladares & Zavala 2006).

In both sun and shade plants, stomatal conductance ( $g_s$ ) values were low throughout the year (see Supplementary Fig. S1) and limitations in  $CO_2$  availability may have limited photosynthesis to some extent, particularly when soil moisture

was lowest and air temperatures highest (Table 1, Fig. 1). The extent to which  $CO_2$  availability limited  $P_{\text{net}}$  was, however, likely to have differed between sun and shade plants, particularly under conditions of increased  $g_s$ . At the low range of observed  $g_s$  values, rates of  $P_{\text{net}}$  were similar at both sun- and shade-exposed sites but at higher  $g_s$  values,  $P_{\text{net}}$  ranges diverged, suggesting that  $P_{\text{net}}$  becomes limited by factors other than  $CO_2$  availability at high  $g_s$  values at the shade site.

#### BALANCE BETWEEN LEAF $R$ AND $P_{\text{NET}}$

Overall, it is expected that there will be a coupling between  $P_{\text{net}}$  and leaf  $R$  given that photosynthesis provides the substrate supply for respiration, and respiration the energy required for the maintenance of photosynthesis (Hoefnagel, Atkin & Wiskich 1998). Moreover, respiration provides the energy for exporting assimilates (Farrar 1985) and oxidizes excess of redox equivalents from photosynthesis (i.e. Saradadevi & Raghavendra 1992). However, the temperature sensitivity of these two processes differs and temperature may, therefore, have an impact on the  $R/P_{\text{net}}$  ratio. Although several studies have reported that  $R/P_{\text{net}}$  remains more or less constant in plants experiencing contrasting growth temperatures (Gifford 2003; Loveys *et al.* 2003), less is known about the extent to which  $R/P_{\text{net}}$  is invariant under the extreme conditions experienced by plants growing in dry-land, low NPP ecosystems such as was the case here. Indeed, our data show that estimates of daily  $R/P_{\text{net}}$  (calculated using the daily average rates of night-time  $R$  and day-time  $P_{\text{net}}$ ) were not constant throughout the year (Fig. 4), with average night-time leaf  $R$  greatly exceeding average day-time  $P_{\text{net}}$  during periods of severe water limitation and high day-time temperatures in summer, and during periods of freezing/photoinhibitory stress in winter (Table 1). The low predawn  $F_v/F_m$  values in the winter (Table 1) have been described as indicative of changes in chlorophyll pigments and associated with the presence of xanthophyll pigments in PSII, resulting in a down-regulation of PSII (Gilmore & Ball 2000) and lower photosynthetic rates (Martínez-Ferri *et al.* 2004; Corcuera *et al.* 2005). In both cases, the imbalance between night-time  $R$  and day-time  $P_{\text{net}}$  was greatest in sun-grown plants, suggesting that leaves maintain a more positive carbon balance at shaded sites at hot, dry sites such as that used in our study. Balanced against this beneficial effect of shade is the fact that maximal rates of  $R$  and  $P_{\text{net}}$  are considerably lower in the shade (Fig. 2), thus potentially reducing growth rates under low irradiance.

Although  $R/P_{\text{net}}$  did increase markedly in summer (particularly under full sun; Fig. 4), the increase in this ratio was considerably lower than it would have been if leaf  $R$  had not exhibited large seasonal changes in basal rates (Fig. 3), which we suggest was the result of the thermal acclimation to the high average day time temperatures in summer (see above). If one assumes that the winter (March 2005) temperature response curve of leaf  $R$  was maintained all year round (i.e. assuming no acclimation of leaf  $R$  to seasonal shifts in temperature), then estimated  $R/P_{\text{net}}$  values in summer would have been near double those shown in Fig. 4. Acclimation of

leaf  $R$  thus contributes substantially to help both sun- and shade-grown *Q. ilex* leaves maintain a relatively positive carbon balance than would not be possible in the absence of thermal acclimation.

## Conclusions

We found no difference in the temperature sensitivity of leaf  $R$  between sun- and shade-exposed grown *Q. ilex* growing under field conditions in a dry-land, Mediterranean ecosystem. Thus, while it is theoretically possible for the temperature sensitivity to vary as a function of substrate supply (Atkin & Tjoelker 2003), to date no study has found systematic variations in temperature sensitivity in response to light- or  $[\text{CO}_2]$ -mediated changes in photosynthesis in leaves where substrate concentrations remain relatively high irrespective of the growth treatment (Hartley *et al.* 2006; Zaragoza-Castells *et al.* 2007). Our results add to previous studies showing that the  $Q_{10}$  of leaf  $R$  is greatest at low temperatures (Tjoelker *et al.* 2001); importantly, however, we show moderate temperature-dependent variations in the  $Q_{10}$  can be accounted for via application single  $E_0$  value, with the result that an Arrhenius-based model successfully accounts for daily and seasonal variations in  $R$ . Crucial to the formulation of such a model is accounting for large seasonal shifts in the basal rate of leaf  $R$  (that are consistent with thermal acclimation) that occur in both sun- and shade-grown plants, and ensuring that the irradiance-mediated differences in the basal rate of  $R$  are also taken into account.

Our finding that *Q. ilex* exhibits large seasonal adjustments in the basal rate of  $R$  over the year and in response to shading has important implications for our understanding of the underlying factors controlling net carbon uptake at this dry-land, low NPP ecosystem. Failure to adjust the basal rate of leaf  $R$  would result in respiratory  $\text{CO}_2$  release being excessively high in summer, with concomitant increases in the ratio of leaf  $R$  to photosynthesis and reductions in NPP. By downward adjusting basal rates of leaf  $R$  during hot periods, both sun- and shade-exposed grown plants are more likely to reduce daily  $R/P_{\text{net}}$  ratios and maintain a positive overall annual carbon balance. Acclimation of leaf  $R$  to seasonal changes in the environment may thus play a crucial role in determining the viability of tree growth in dry-land ecosystems.

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## References

- Armstrong, A.F., Logan, D., Tobin, A.K., O'Toole, P. & Atkin, O.K. (2006a) Heterogeneity of plant mitochondrial responses underpinning respiratory acclimation to the cold in *Arabidopsis thaliana* leaves. *Plant, Cell and Environment*, **29**, 940–949.
- Armstrong, A.F., Logan, D.C. & Atkin, O.K. (2006b) On the developmental dependence of leaf respiration: responses to short- and long-term changes in growth temperature. *American Journal of Botany*, **93**, 1633–1639.
- Atkin, O.K. & Tjoelker, M.G. (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science*, **8**, 343–351.
- Atkin, O.K., Bruhn, D., Hurry, V.M. & Tjoelker, M.G. (2005) The hot and the cold: unravelling the variable response of plant respiration to temperature. *Functional Plant Biology*, **32**, 87–105.
- Atkin, O.K., Evans, J.R. & Siebke, K. (1998) Relationship between the inhibition of leaf respiration by light and enhancement of leaf dark respiration following light treatment. *Australian Journal of Plant Physiology*, **25**, 437–443.
- Atkin, O.K., Holly, C. & Ball, M.C. (2000) Acclimation of snow gum (*Eucalyptus pauciflora*) leaf respiration to seasonal and diurnal variations in temperature: the importance of changes in the capacity and temperature sensitivity of respiration. *Plant, Cell and Environment*, **23**, 15–26.
- Atkin, O.K., Scheurwater, I. & Pons, T.L. (2007) The ratio of respiration to photosynthesis in whole-plants is homeostatic across moderate growth temperatures, but not when plants experience unusually high mean daily temperatures. *New Phytologist*, **174**, 367–384.
- Azcón-Bieto, J. (1992) Relationships between photosynthesis and respiration in the dark in plants. *Trends in Photosynthesis Research* (eds J. Barber, M.G. Guerrero & H. Medrano), pp. 241–253. Intercept Ltd, Andover, Hampshire.
- Azcón-Bieto, J. & Osmond, C.B. (1983) Relationship between photosynthesis and respiration. The effect of carbohydrate status on the rate of  $\text{CO}_2$  production by respiration in darkened and illuminated wheat leaves. *Plant Physiology*, **71**, 574–581.
- Billings, W.D., Godfrey, P.J., Chabot, B.F. & Bourque, D.P. (1971) Metabolic acclimation to temperature in Arctic and alpine ecotypes of *Oxyria digyna*. *Arctic and Alpine Research*, **3**, 277–289.
- Björkman, O. (1981) Responses to different quantum flux densities. *Encyclopedia of Plant Physiology I*, vol. 12a (eds O.L. Lange & P.S. Nobel), pp. 57–107. Springer, Berlin, Heidelberg, New York.
- Boardman, N.K. (1977) Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology*, **28**, 355–377.
- Bolstad, P.V., Mitchell, K. & Vose, J.M. (1999) Foliar temperature-respiration response functions for broad-leaved tree species in the southern Appalachians. *Tree Physiology*, **19**, 871–878.
- Bolstad, P.V., Reich, P. & Lee, T. (2003) Rapid temperature acclimation of leaf respiration rates in *Quercus alba* and *Quercus rubra*. *Tree Physiology*, **23**, 969–976.
- Bréda, N., Huc, R., Granier, A. & Dreyer, E. (2006) Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science*, **63**, 625–644.
- Breeze, V. & Elston, J. (1978) Some effects of temperature and substrate content upon respiration and the carbon balance of field beans (*Vicia faba* L.). *Annals of Botany*, **42**, 863–876.
- Bruhn, D., Egerton, J.J.G., Loveys, B.R. & Ball, M.C. (2007) Evergreen leaf respiration acclimates to long-term nocturnal warming under field conditions. *Global Change Biology*, **13**, 1216–1223.
- Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, C., Carrara, A., Chevallier, F., De Noblet, N., Friend, A.D., Friedlingstein, P., Grunwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourival, J.M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D., Vesala, T. & Valentini, R. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature*, **437**, 529–533.
- Corcuera, L., Morales, F., Abadia, A. & Gil-Pelegrin, E. (2005) Seasonal changes in photosynthesis and photoprotection in a *Quercus ilex* subsp. *ballota* woodland located in its upper altitudinal extreme in the Iberian Peninsula. *Tree Physiology*, **25**, 599–608.
- Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A. & Totterdell, I.J. (2000) Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature*, **408**, 184–187.
- Cramer, W., Bondeau, A., Woodward, F.I., Prentice, I.C., Betts, R.A., Brovkin, V., Cox, P.M., Fisher, V., Foley, J.A., Friend, A.D., Kucharik, C., Lomas, M.R.,

- Ramankutty, N., Sitch, S., Smith, B., White, A. & Young-Molling, C. (2001) Global response of terrestrial ecosystem structure and function to CO<sub>2</sub> and climate change: results from six dynamic global vegetation models. *Global Change Biology*, **7**, 357–373.
- Dewar, R.C., Medlyn, B.E. & Mcmurtrie, R.E. (1999) Acclimation of the respiration/photosynthesis ratio to temperature: insights from a model. *Global Change Biology*, **5**, 615–622.
- Dijkstra, P. (1989) Cause and effect of differences in SLA. *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants* (eds H. Lambers, M.L. Cambridge, H. Konings & T.L. Pons), pp. 125–140. SPB Academic Publishers, The Hague.
- Faria, T., Silverio, D., Breia, E., Cabral, R., Abadia, A., Abadia, J., Pereira, J.S. & Chaves, M.M. (1998) Differences in the response of carbon assimilation to summer stress (water deficits, high light and temperature) in four Mediterranean tree species. *Physiologia Plantarum*, **102**, 419–428.
- Farrar, J.F. (1985) Fluxes of carbon in roots of barley roots. *New Phytologist*, **99**, 57–69.
- Flexas, J., Bota, J., Galmes, J., Medrano, H. & Ribas-Carbó, M. (2006) Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiologia Plantarum*, **127**, 343–352.
- Forward, D.F. (1960) Effect of temperature on respiration. *Encyclopedia of Plant Physiology* (vol. 12) (ed. W. Ruhland), pp. 234–258. Springer-Verlag, New York.
- Gifford, R.M. (2003) Plant respiration in productivity models: conceptualisation, representation and issues for global terrestrial carbon-cycle research. *Functional Plant Biology*, **30**, 171–186.
- Gilmore, A.M. & Ball, M.C. (2000) Protection and storage of chlorophyll in overwintering evergreens. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 11098–11101.
- Gratani, L., Pesoli, P., Crescente, M.F., Aichner, K. & Larcher, W. (2000) Photosynthesis as a temperature indicator in *Quercus ilex* L. *Global and Planetary Change*, **24**, 153–163.
- Griffin, K.L., Turnbull, M.H. & Murthy, R. (2002a) Canopy position affects the temperature response of leaf respiration in *Populus deltoides*. *New Phytologist*, **154**, 609–619.
- Griffin, K.L., Turnbull, M.H., Murthy, R., Lin, G.H., Adams, J., Farnsworth, B., Mahato, T., Bazin, G., Potasnak, M. & Berry, J.A. (2002b) Leaf respiration is differentially affected by leaf vs. stand-level night-time warming. *Global Change Biology*, **8**, 479–485.
- Hartley, I.P., Armstrong, A.F., Murthy, R., Barron-Gafford, G., Ineson, P. & Atkin, O.K. (2006) The dependence of respiration on photosynthetic substrate supply and temperature: integrating leaf, soil and ecosystem measurements. *Global Change Biology*, **12**, 1954–1968.
- Hoefnagel, M.H.N., Atkin, O.K. & Wiskich, J.T. (1998) Interdependence between chloroplasts and mitochondria in the light and the dark. *Biochimica et Biophysica Acta-Bioenergetics*, **1366**, 235–255.
- Larcher, W. (2004) *Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups*, 4th edn. Springer-Verlag, Berlin.
- Larigauderie, A. & Körner, Ch. (1995) Acclimation of leaf dark respiration to temperature in alpine and lowland plant species. *Annals of Botany*, **76**, 245–252.
- Llorens, L., Peñuelas, J. & Filella, I. (2003) Diurnal and seasonal variations in the photosynthetic performance and water relations of two co-occurring Mediterranean shrubs, *Erica multiflora* and *Globularia alypum*. *Physiologia Plantarum*, **118**, 84–95.
- Loveys, B.R., Atkinson, L.J., Sherlock, D.J., Roberts, R.L., Fitter, A.H. & Atkin, O.K. (2003) Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast- and slow-growing plant species. *Global Change Biology*, **9**, 895–910.
- Martínez-Ferri, E., Manrique, E., Valladares, F. & Balaguer, L. (2004) Winter photoinhibition in the field involves different processes in four co-occurring Mediterranean tree species. *Tree Physiology*, **24**, 981–990.
- Noguchi, K. & Terashima, I. (1997) Different regulation of leaf respiration between *Spinacia oleracea*, a sun species, and *Alocasia odora*, a shade species. *Physiologia Plantarum*, **101**, 1–7.
- Noguchi, K., Nakajima, N. & Terashima, I. (2001) Acclimation of leaf respiratory properties in *Alocasia odora* following reciprocal transfers of plants between high- and low-light environments. *Plant, Cell and Environment*, **24**, 831–839.
- Noguchi, K., Taylor, N.L., Millar, A.H., Lambers, H. & Day, D.A. (2005) Response of mitochondria to light intensity in the leaves of sun and shade species. *Plant Cell and Environment*, **28**, 760–771.
- Osborne, C.P., Mitchell, P.L., Sheehy, J.E. & Woodward, F.I. (2000) Modelling the recent historical impacts of atmospheric CO<sub>2</sub> and climate change on Mediterranean vegetation. *Global Change Biology*, **6**, 445–458.
- Peñuelas, J., Filella, I., Llusà, J., Siscart, D. & Piñol, J. (1998) Comparative field study of spring and summer leaf gas exchange and photobiology of the Mediterranean trees *Quercus ilex* and *Phillyrea latifolia*. *Journal of Experimental Botany*, **49**, 229–238.
- Pereira, J.S., Tenhunen, J.D., Lange, O.L., Beyschlag, W., Meyer, A. & David, M.M. (1986) Seasonal and diurnal patterns in leaf gas exchange of *Eucalyptus globulus* trees growing in Portugal. *Canadian Journal of Forest Research*, **16**, 177–184.
- Poorter, H., Remkes, C. & Lambers, H. (1990) Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiology*, **94**, 621–627.
- Rabash, J., Steele, F., Browne, W. & Prosser, B. (2004) *A User's Guide to MLWiN Version 2.0*. (Centre for Multilevel Modelling, Institute of Education, University of London, London, UK).
- Rambal, S., Ourcival, J.M., Joffre, R., Mouillot, F., Nouvellon, Y., Reichstein, M. & Rocheteau, A. (2003) Drought controls over conductance and assimilation of a Mediterranean evergreen ecosystem: scaling from leaf to canopy. *Global Change Biology*, **9**, 1813–1824.
- Rook, D.A. (1969) The influence of growing temperature on photosynthesis and respiration of *Pinus radiata* seedlings. *New Zealand Journal of Botany*, **7**, 43–55.
- Ryan, M.G. (1991) Effects of climate change on plant respiration. *Ecological Applications*, **1**, 157–167.
- Sack, L. (2004) Responses of temperate woody seedlings to shade and drought: do trade-offs limit potential niche differentiation? *Oikos*, **107**, 110–127.
- Sánchez-Gómez, D., Valladares, F. & Zavala, M.A. (2006) Performance of seedlings of Mediterranean woody species under experimental gradients of irradiance and water availability: trade-offs and evidence for niche differentiation. *New Phytologist*, **170**, 795–806.
- Saradadevi, K. & Raghavendra, A.S. (1992) Dark respiration protects photosynthesis against photoinhibition in mesophyll protoplasts of pea (*Pisum sativum*). *Plant Physiology*, **99**, 1232–1237.
- Sims, D.A. & Pearcy, R.W. (1989) Photosynthetic characteristics of a tropical forest understorey herb, *Alocasia macrorrhiza*, and a related crop species, *Colocasia esculenta* grown in contrasting light environments. *Oecologia*, **79**, 53–59.
- Sims, D.A. & Pearcy, R.W. (1991) Photosynthesis and respiration in *Alocasia macrorrhiza* following transfers to high and low light. *Oecologia*, **86**, 447–453.
- Sims, D.A. & Pearcy, R.W. (1994) Scaling sun and shade photosynthetic acclimation of *Alocasia macrorrhiza* to whole-plant performance. I. Carbon balance and allocation at different daily photon flux densities. *Plant, Cell and Environment*, **17**, 881–887.
- Snijders, T.A.B. & Bosker, R.J. (1999) *Multilevel Analysis*. Sage Publications, London UK.
- Sokal, R.R. & Rohlf, F.J. (1981) *Biometry*, 3rd edn. Freeman, San Francisco.
- Tenhunen, J.D., Serra, A.S., Harley, P.C., Dougherty, R.L. & Reynolds, J.F. (1990) Factors influencing carbon fixation and water-use by Mediterranean sclerophyll shrubs during summer drought. *Oecologia*, **82**, 381–393.
- Tjoelker, M.G., Oleksyn, J. & Reich, P.B. (2001) Modelling respiration of vegetation: evidence for a general temperature-dependent  $Q_{10}$ . *Global Change Biology*, **7**, 223–230.
- Turnbull, M.H., Doley, D. & Yates, D.J. (1993) The dynamics of photosynthetic acclimation to changes in light quantity and quality in 3 Australian rain-forest tree species. *Oecologia*, **94**, 218–228.
- Turnbull, M.H., Whitehead, D., Tissue, D.T., Schuster, W.S.F., Brown, K.J. & Griffin, K.L. (2003) Scaling foliar respiration in two contrasting forest canopies. *Functional Ecology*, **17**, 101–114.
- Wager, H.G. (1941) On the respiration and carbon assimilation rates of some arctic plants as related to temperature. *New Phytologist*, **40**, 1–19.
- Walters, M.B. & Reich, P.B. (2000) Trade-offs in low-light CO<sub>2</sub> exchange: a component of variation in shade tolerance among cold temperate tree seedlings. *Functional Ecology*, **14**, 155–165.
- Wright, I.J., Reich, P.B., Atkin, O.K., Lusk, C.H., Tjoelker, M.G. & Westoby, M. (2006) Irradiance, temperature and rainfall influence leaf dark respiration in woody plants: evidence from comparisons across 20 sites. *New Phytologist*, **169**, 309–319.
- Xu, C.Y. & Griffin, K.L. (2006) Seasonal variation in the temperature response of leaf respiration in *Quercus rubra*: foliage respiration and leaf properties. *Functional Ecology*, **20**, 778–789.
- Zaragoza-Castells, J., Sánchez-Gómez, D., Valladares, F., Hurry, V. & Atkin, O. (2007) Does growth irradiance affect temperature-dependence and thermal acclimation of leaf respiration? Insights from a Mediterranean tree with long-lived leaves. *Plant, Cell and Environment*, **30**, 820–833.

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### Supplementary material

The following supplementary material is available for this article:

**Appendix S1.** Derivation of eqn 10 in main text; that is,  $Q_{10} = \exp[10 E_0/(rT^2)]$

**Fig. S1.** The relationship between rates of net photosynthesis ( $P_{\text{net}}$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and stomatal conductance ( $g_s$ ) in the sun (○) and shade (●) sites. All data collected over the six measurement months are presented. Values are the mean of three replicates for March, May, July and August 2005, and

four replicates for August 2004 and January 2005 ( $\pm$  SE). First order linear regressions plotted for sun (dotted line) and shade sites (solid line) are shown.

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