

# Light inhibition of foliar respiration in response to soil water availability and seasonal changes in temperature in Mediterranean holm oak (*Quercus ilex*) forest

Matthew H. Turnbull<sup>A,K</sup>, Romà Ogaya<sup>B,C</sup>, Adrià Barbeta<sup>B,C,J</sup>, Josep Peñuelas<sup>B,C</sup>, Joana Zaragoza-Castells<sup>D</sup>, Owen K. Atkin<sup>E</sup>, Fernando Valladares<sup>F</sup>, Teresa E. Gimeno<sup>G,I</sup>, Beatriz Pías<sup>H</sup> and Kevin L. Griffin<sup>I</sup>

<sup>A</sup>Centre for Integrative Ecology, School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand.

<sup>B</sup>CREAF, Cerdanyola del Vallès, 08193, Catalonia, Spain.

<sup>C</sup>CSIC, Global Ecology Unit CREAM-CSIC-UAB, Cerdanyola del Vallès, 08193, Catalonia, Spain.

<sup>D</sup>Geography, College of Life and Environmental Sciences, University of Exeter, Amory Building, Rennes Drive, Exeter EX4 4RJ, UK.

<sup>E</sup>ARC Centre of Excellence in Plant Energy Biology, Division of Plant Sciences, Research School of Biology, Building 134, The Australian National University, Canberra, ACT 2601, Australia.

<sup>F</sup>Museo Nacional de Ciencias Naturales, CSIC, Serrano 115, E-28006 Madrid, Spain.

<sup>G</sup>Hawkesbury Institute for the Environment, University of Western Sydney, Locked bag 1797, Penrith, NSW 2751, Australia.

<sup>H</sup>Departamento de Botánica, Universidad Complutense de Madrid, José Antonio Novais 2, 28040, Madrid, Spain.

<sup>I</sup>Lamont-Doherty Earth Observatory of Columbia University, 61 Route 9W, 6 Biology, Palisades, NY 10964, USA.

<sup>J</sup>ISPA, Bordeaux Science Agro, INRA, 33140 Villenave d'Ornon, France.

<sup>K</sup>Corresponding author. Email: matthew.turnbull@canterbury.ac.nz

**Abstract.** In the present study we investigated variations in leaf respiration in darkness ( $R_D$ ) and light ( $R_L$ ), and associated traits in response to season, and along a gradient of soil moisture, in Mediterranean woodland dominated by holm oak (*Quercus ilex* L.) in central and north-eastern Spain respectively. On seven occasions during the year in the central Spain site, and along the soil moisture gradient in north-eastern Spain, we measured rates of leaf  $R_D$ ,  $R_L$  (using the Kok method), light-saturated photosynthesis ( $A$ ) and related light response characteristics, leaf mass per unit area ( $M_A$ ) and leaf nitrogen (N) content. At the central Spain site, significant seasonal changes in soil water content and ambient temperature ( $T$ ) were associated with changes in  $M_A$ , foliar N,  $A$  and stomatal conductance.  $R_D$  measured at the prevailing daily  $T$  and in instantaneous  $R-T$  responses, displayed signs of partial acclimation and was not significantly affected by time of year.  $R_L$  was always less than, and strongly related to,  $R_D$ , and  $R_L/R_D$  did not vary significantly or systematically with seasonal changes in  $T$  or soil water content. Averaged over the year,  $R_L/R_D$  was  $0.66 \pm 0.05$  s.e. ( $n = 14$ ) at the central Spain site. At the north-eastern Spain site, the soil moisture gradient was characterised by increasing  $M_A$  and  $R_D$ , and reduced foliar N,  $A$ , and stomatal conductance as soil water availability decreased. Light inhibition of  $R$  occurred across all sites (mean  $R_L/R_D = 0.69 \pm 0.01$  s.e. ( $n = 18$ )), resulting in ratios of  $R_L/A$  being lower than for  $R_D/A$ . Importantly, the degree of light inhibition was largely insensitive to changes in soil water content. Our findings provide evidence for a relatively constrained degree of light inhibition of  $R$  ( $R_L/R_D \sim 0.7$ , or inhibition of  $\sim 30\%$ ) across gradients of water availability, although the combined impacts of seasonal changes in both  $T$  and soil water content increase the range of values expressed. The findings thus have implications in terms of the assumptions made by predictive models that seek to account for light inhibition of  $R$ , and for our understanding of how environmental gradients impact on leaf trait relationships in Mediterranean plant communities.

**Additional keywords:** Kok effect, leaf functional traits, leaf dark respiration, leaf light respiration, leaf mass per unit area, nitrogen, photosynthesis, plasticity, season, soil moisture, temperature.

Received 27 January 2017, accepted 23 July 2017, published online 17 August 2017

## Introduction

Plant respiration ( $R$ ) is an integral component of the terrestrial global carbon cycle, with between 0.2 and 0.8 of the carbon assimilated by the photosynthesis during the day consumed through autotrophic respiratory processes (Gifford 1995). About half the  $\text{CO}_2$  released in plant respiration comes from leaves (Xu *et al.* 2001; Atkin *et al.* 2007), with this proportion likely to be even greater for plants where  $\text{CO}_2$  assimilation ( $A$ ) is limited by abiotic stresses, including low nutrient availability (Grassi *et al.* 2002; Turnbull *et al.* 2005; Whitehead *et al.* 2005; Heskell *et al.* 2012, 2013) and under drought conditions (Chaves *et al.* 2002). At an individual scale, mitochondrial respiration plays a key role in determining the growth and survival of plants (Hurry *et al.* 1995), as it is associated with the production of energy and carbon skeletons essential for cellular maintenance and biosynthesis.

Although leaf  $R$  takes place in both light ( $R_L$ ) and darkness ( $R_D$ ), leaf  $R$  is typically lower during the day when photosynthesis is concurrently occurring (Brooks and Farquhar 1985), even when re-fixation of respiratory  $\text{CO}_2$  is taken into account (Pärnik *et al.* 2007). The metabolic basis of the light inhibition of leaf  $R$  is beginning to be better understood (Tcherkez *et al.* 2017), with factors such as cellular energy status, demand for tricarboxylic acid (TCA) cycle intermediates, engagement of the pentose phosphate pathway and/or rates of photorespiration ( $V_o$ ) being involved in the degree of inhibition (Hurry *et al.* 2005; Tcherkez *et al.* 2005, 2008, 2012; Buckley and Adams 2011). The extent to which  $R$  is reduced in the light is, potentially, highly variable among species and environments. For example, when measured at a common temperature ( $T$ ), the rate of respiration in the light can be as little as 0.2 of the darkened rate (Atkin *et al.* 2000a; Ayub *et al.* 2011). In contrast, light may have little to no inhibitory effect in some cases, particularly at low measuring temperatures (Atkin *et al.* 2000b; Zaragoza-Castells *et al.* 2007). These large potential variations between measured  $R_L$  and  $R_D$  under different experimental conditions are important because the level to which they are actually expressed in the field will determine the extent to which variations in  $R$  impact on net  $\text{CO}_2$  exchange in individual plants and whole ecosystems. Failure to account for light inhibition of leaf  $R$  leads to large over- and under-estimates of ecosystem respiration and net primary productivity respectively (Lloyd *et al.* 2002; Wohlfahrt *et al.* 2005; Atkin *et al.* 2006; Wingate *et al.* 2007; McLaughlin *et al.* 2014).

Establishing the extent to which species differ in rates of  $R_D$  and  $R_L$ , and the impact of natural environmental gradients on variations of both processes is crucial to successful incorporation of light inhibition of leaf  $R$  into large-scale models. Furthermore, little is known about the impact of soil water status or water stress on plant  $R$  (Atkin and Macherel 2009). The response of  $R_D$  to leaf water content may lag behind that of photosynthesis and may also be biphasic i.e. it decreases in the initial stages of water stress (lower energy demands for growth) and may increase with additional stress-related demands, e.g. osmoregulation or induced senescence (Ghashghaie *et al.* 2001; Gulias *et al.* 2002; Flexas *et al.* 2005, 2006). The environmental response of  $R_D$  will also be complicated by the fact that large shifts in daily mean  $T$  often co-vary with fluctuations in water availability,

with drought often being associated with high temperatures. Several controlled-environment studies have reported large variations in leaf  $R_D$  among different species (Atkin and Day 1990; Poorter *et al.* 1990; Loveys *et al.* 2003; Tjoelker *et al.* 2005; Atkin *et al.* 2008), with a smaller number of laboratory-based studies showing that genotype is also a strong determinant of  $R_L$  (Villar *et al.* 1994; Atkin *et al.* 1997).  $R_D$  also varies in response to changes in the abiotic environment, both in nature and under laboratory-based conditions (Larigauderie and Körner 1995; Ryan 1995; Atkin *et al.* 2000b; Griffin *et al.* 2002a, 2002b, 2004; Turnbull *et al.* 2005; Wright *et al.* 2006; Xu and Griffin 2006; Tjoelker *et al.* 2009; Searle *et al.* 2011).  $R_D$  often acclimates to changes in long-term growth  $T$  (Atkin *et al.* 2000a, 2005; Atkin and Tjoelker 2003; Wythers *et al.* 2005; Ow *et al.* 2008a, Way and Oren 2010), so that the  $T$  response of respiration to short-term and long-term changes in  $T$  is often different. Likewise, there is evidence from laboratory-based studies that light inhibition of leaf  $R$  is environmentally dependent (e.g. as a result of changes in measurement  $T$  (Atkin *et al.* 2000a; Zaragoza-Castells *et al.* 2007; Shapiro *et al.* 2004; Griffin and Turnbull 2013), and/or atmospheric growth  $\text{CO}_2$  concentration (Shapiro *et al.* 2004; Wang *et al.* 2001, 2004; Tissue *et al.* 2002)). Although we have previously shown that the balance between  $R_D$  and  $R_L$  changes in response to field gradients of soil fertility (Atkin *et al.* 2013; Heskell *et al.* 2012, 2013) and imposition of water stress under controlled conditions (Ayub *et al.* 2011; Crous *et al.* 2012), to date, no study has reported how gradients in soil moisture impact on light inhibition of leaf  $R$  under natural, field conditions. We might predict that drought and/or high  $T$  induced changes in leaf metabolism, particularly in  $V_o$ , would result in predictable changes in light inhibition of  $R$  (Griffin and Turnbull 2013). Moreover, we lack data on how the balance between  $R_L$  and  $R_D$  varies seasonally (Heskell *et al.* 2014). It is vital we establish the extent of inhibition of  $R$  in areas of contrasting water availability if we want to more accurately predict future rates of C exchange in water-limited environments. Overall, we lack comprehensive field-based studies that quantify variations in  $R_D$  and  $R_L$  among dominant species growing along natural gradients of water availability.

Mediterranean ecosystems offer excellent opportunities to test changes in respiration under water-limited conditions, such as those that now prevail (Peñuelas and Boada 2003; Peñuelas *et al.* 2013) and the even drier conditions that are projected for the coming decades (IPCC 2007). In Mediterranean ecosystems, the availability of water is the greatest environmental constraint on plant growth, due to the occurrence of high summer temperatures and low rainfall. Here, our aim was to determine if the dominant Mediterranean tree *Quercus ilex* L. (Holm oak) and co-occurring trees of Mediterranean forests responded to seasonal changes in, and natural gradients of, water availability, with changes in  $R$  (as measured by  $\text{CO}_2$  evolution), and particularly in the inhibition of  $R$  by light. We have previously measured large seasonal shifts in  $T$  response curves of  $R_D$  in *Q. ilex* subsp. *ballota* in central Spain that were consistent with thermal acclimation (Zaragoza-Castells *et al.* 2008). However, it is possible that seasonal variations in water availability may have contributed to such shifts in the daily  $T$  response curves (Crous *et al.* 2011; Rodríguez-Calcerrada *et al.*

2011). Our present study extends this research by quantifying seasonal changes in both  $R_D$  and  $R_L$  and associated leaf traits ( $A$ ,  $M_A$  and N content) over a 12 month period in the same forest (Villar de Cobeta, central Spain) in *Q. ilex* subsp. *ballota* trees. We also increased the geographical and species scope of our study by conducting an additional single mid-summer campaign in the Prades Mountains (Catalonia, north-eastern Spain), with *Q. ilex* subsp. *ilex* forest along a gradient of decreasing water availability from a stream course in the bottom of a valley to dry sites approaching the ridge line. In addition, at the drier end of this gradient, precipitation has been further experimentally restricted for the last 13 years, reducing soil moisture by 11% with respect to ambient conditions (Ogaya and Peñuelas 2007). Based on previous findings that conditions which tend to suppress  $V_o$  (e.g. high  $[CO_2]$  and/or low  $[O_2]$ ) also decrease  $R_L/R_D$  (Wang *et al.* 2001; Shapiro *et al.* 2004; Crous *et al.* 2012; Griffin and Turnbull 2013), and without considering other processes (e.g. increased use of stored carbon reserves (e.g. citrate) in the light) which might influence  $R_L$  in the short term (Tcherkez *et al.* 2012), we hypothesised that  $R_L/R_D$  would be lowest where water availability was greatest, and increase at drier sites where increase photorespiration results from stomatal limitations on gas exchange. It is worth noting that in considerations of the influence of water availability on carbon assimilation, it is possible to interpret a link between photorespiration and the degree of light inhibition of  $R$  measured in the Kok effect in several ways: (i) there may be a direct effect of water availability on internal  $CO_2$  mole fraction, which in turn affects  $V_o$  and thus  $R_L/R_D$ ; (ii) water availability may have specific photorespiration-independent effects on  $R_L/R_D$ ; or (iii) water availability may affect apparent  $R_L/R_D$  because the Kok-effect measurement is itself influenced (at least in part) by photorespiration (Tcherkez *et al.* 2012). Here our focus is to determine patterns of field response rather than attempt to provide a resolution to this issue.

## Materials and methods

### Study sites

#### Villar de Cobeta

This field site was located 175 km north-east of Madrid in the Iberic System Mountain Range (950 m above sea level) at

Los Cerrillos Biological Station, Villar de Cobeta (40°48'N, 2°12'W), within the Alto Tajo Natural Park (Guadalajara, Spain). The experimental work was located in two sites – a steep (20°), south-facing upper slope which was the location for our previous work (Zaragoza-Castells *et al.* 2008), and an adjacent lower slope, stream-side site. *Quercus ilex* L. subsp. *ballota* dominated the vegetation at both sites. The area has a continental Mediterranean climate, with hot, dry summers and cold winters, and is characterised by large diurnal and seasonal variations in air  $T$  (diurnal variations in  $T$  in the order of 25°C are common, with leaf temperatures potentially reaching near 50°C in summer and –15°C in winter). Both sites have shallow, poorly developed soils formed mainly from Cretaceous and Jurassic limestone with a limited capacity of the soil to retain water (Valladares *et al.* 2008). Meteorological stations provided environmental data as described in Zaragoza-Castells *et al.* (2008). From December 2006 to November 2007 we monitored microclimatic conditions at each of the study sites. At the upper slope site, we installed a HOBO weather station equipped with a rain gauge (RGA-M0XX) and a soil moisture sensor (S-SMA-M003) inserted at 30 cm depth. Readings of each sensor were recorded every 30 min with a data logger (HOBO H21-001 all components from HOBO Onset Computer Corporation Bourne). At the lower slope site, we installed a second station with a soil moisture probe (ECH<sub>2</sub>O EC-20 Decagon Devices). Readings were recorded every 30 min with a HOBO H08-006-04 data logger.

### Prades

The second study was undertaken in the Prades Mountains in southern Catalonia (NE Spain; 41°21'N, 1°2'E), also at 950 m above sea level on a south-facing slope (20°). The soil is a Dystric Cambisol over Paleozoic schist, and its depth ranges from 35 to 90 cm. This forest has been undisturbed for the last 60 years, and the maximum height of the dominant species is ~6–10 m. Plant community composition is strongly influenced by topographical changes in soil water availability (Table 1). In this study we identified four community types: (1) riparian forest – the moistest sites along stream courses in valleys dominated by several deciduous species (*Tilia platyphyllos* Scop., *Sorbus torminalis* (L.) Crantz and *Acer monspessulanum* L.); (2) mid-slope

**Table 1. Site characteristics and species selected for measurements at Prades (Catalunya, north-eastern Spain)**

The elevation is the altitude above sea level. Height refers to the maximum height of the dominant trees, giving an indication of the heights from which the shoots were sampled

Site number	1	2	3	4	5	6
Site name	Riparian forest	Mid-slope forest	(Control)	Dry forest (Exclusion)	(Control)	Dry shrubland (Exclusion)
Elevation (m)	910	950	990	990	995	995
Canopy height (m)	12	7	5	5	3	3
	<i>Species</i>					
<i>Tilia platyphyllos</i>	●					
<i>Acer monspessulanum</i>	●	●				
<i>Quercus ilex</i>		●	●	●	●	●
<i>Phillyrea latifolia</i>		●	●	●	●	●
<i>Arbutus unedo</i>			●	●	●	●

forest – in which Holm oak (*Q. ilex* subsp. *ilex*) dominates and deciduous species become much less common, being replaced by evergreen species such as *Phillyrea latifolia* L. and *Arbutus unedo* L.; (3) dry forest – on upper slopes approaching ridge lines with a dense, multi-stemmed crown and dominated by *Q. ilex*, *P. latifolia* and *A. unedo*; (4) shrubland – at the ridge line, these trees continue to dominate but grow in a stunted shrub form (2–3 m tall) and the canopy opens in places to allow shrub species to establish.

In addition to the natural gradient described above, at Prades we sampled in a long-term rainfall exclusion experiment established in communities 3 and 4 (Ogaya and Peñuelas 2007) to match the conditions projected in climatic and ecophysiological models for Mediterranean forests in the coming decades (Sabaté *et al.* 2002; IPCC 2007). In each of these communities, four control and four treatment plots 15 × 10 m were established at the same altitude along the mountain face. In the treatment plots, rainfall was partially excluded through the suspension of PVC strips 0.5–0.8 m above the soil (covering 30% of the soil surface) and the excavation of a ditch 0.8 m deep at the upper part of the plots to intercept runoff water. Water intercepted by the strips and ditches was conducted around the plots, below their bottom edges. The other three plots received no treatment and acted as control plots. The rain exclusion treatment began in January 1999, 13 years before the sampling for this study. An automated meteorological station installed between the rainfall exclusion plots monitored *T*, photosynthetically active radiation (PAR), air humidity and precipitation continuously during the experiment. Soil water content was determined gravimetrically at 30 cm depth at all sites at the midpoint of the experimental campaign (July 2013).

#### Leaf sampling and gas-exchange measurements

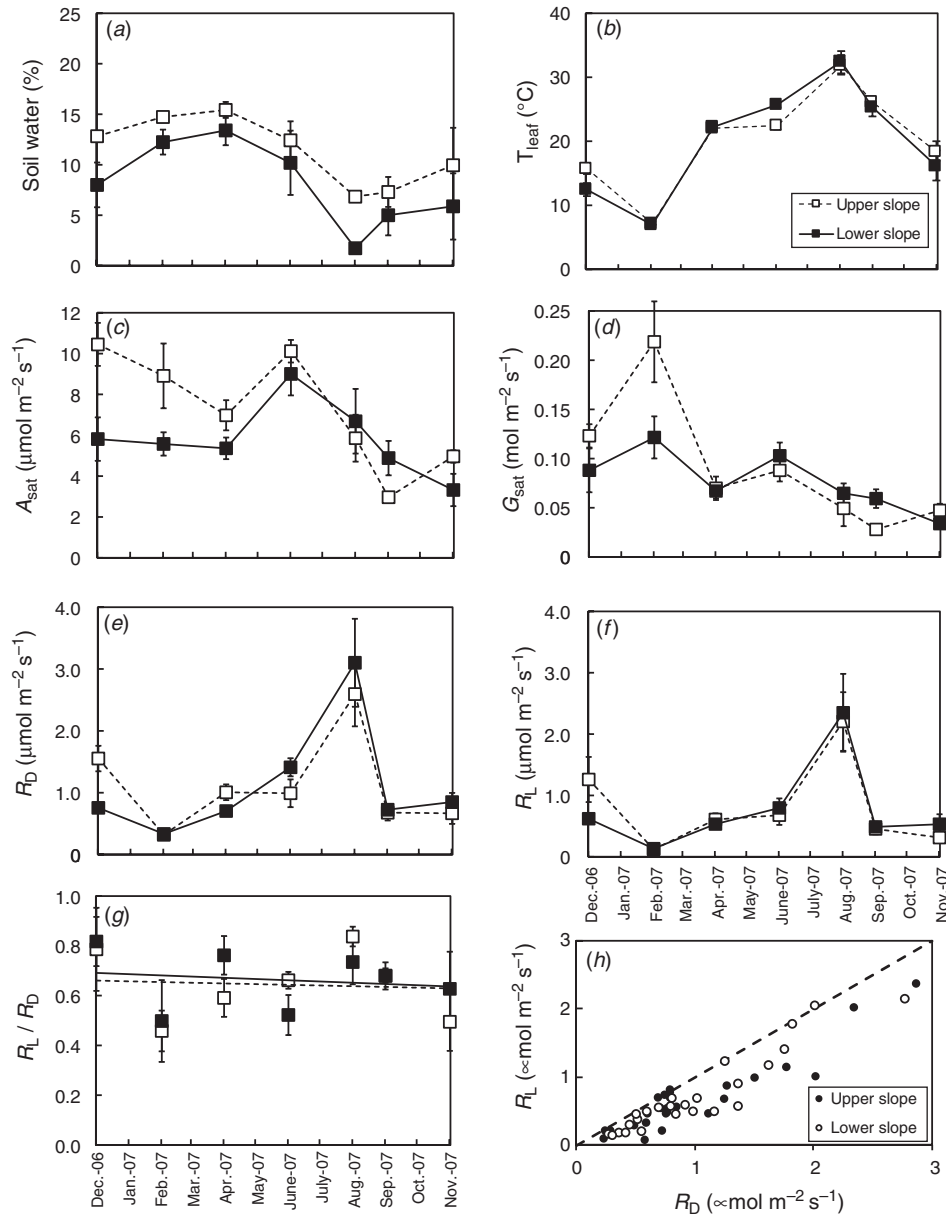
At the Villar de Cobeta study site, we performed seven measurement campaigns from December 2006 to November 2007. Four individual trees were identified at both the upper and lower slope sites, with each tree representing an independent replicate. For each sampling campaign, leaf physiological measurements were made using a single south-east facing, attached, fully-expanded, mature leaf from each replicate tree. Different leaves were used on each campaign, with the measured leaves being harvested for determination of chemical–structural characteristics at the end of each sampling campaign.

Within the Villar de Cobeta seasonal study, additional measurements of the instantaneous response of  $R_D$  to *T* were made at changing ambient air *T* and at ambient relative humidity (typically 35–60%) to investigate the extent of thermal acclimation of  $R_D$ . Measurements of leaf *R* took place at regular intervals during the day and night; during the day, leaves were darkened for 30 min before measurement to avoid post-illumination transients. As Zaragoza-Castells *et al.* (2008) previously found that there were no differences in leaf *R* measured during the day and night at equivalent *T*; here, *T* response curves were fitted to data from a combination of night and day measurements. 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 net CO<sub>2</sub> exchange ( $A_{net}$ ) being measured at the prevailing ambient irradiance. On each sampling month, measurements were made on four replicate leaves.

The Prades study took place over a 2 week period in late July 2013 (i.e. mid-summer when the gradient of water availability was most pronounced). At each site, we sampled detached branches of sun-lit, upper canopy foliage (using a pruning pole) from six individuals of each of three of the most abundant species (Table 1) at each site along the gradient. Species composition changes along the gradient, but it was possible to sample several species on multiple, consecutive sites (Table 1). Thus, in addition to providing insights into how leaf gas exchange of the dominant species might vary among sites, our sampling strategy enabled an assessment of how individual species responded to site-to-site variations in environment (especially *Q. ilex*, which was sampled at five of the six sites). Sampling took place in the early evening; stems were immediately re-cut under water and the branches transported to a nearby field laboratory and allowed to equilibrate overnight for subsequent gas exchange measurements through the morning and early afternoon period. Past work has shown that leaf gas exchange rates can be comparable in cut branches and *in situ* leaves of forest species for many hours after removal (Mitchell *et al.* 1999; Turnbull *et al.* 2003). In addition, all gas exchange measurements were corrected for  $C_i$  according to work by Kirschbaum and Farquhar (1987) (see details below), so any partial stomatal closure that may have occurred was accounted for.

For both experimental study sites (i.e. at the Villar de Cobeta and Prades field sites), light response curves of  $A_{net}$  were measured on the most recently fully expanded leaves using LI-6400 portable photosynthesis systems (LI-COR BioSciences) equipped with a CO<sub>2</sub> controller and a red-blue light source (6400-02B). The seasonal measurements at Villar de Cobeta were made at the prevailing daytime *T* (see Fig. 1) and the moisture gradient measurements at Prades were made with the block *T* set to the mid-summer prevailing *T* of 25°C. In all cases, light-response measurements were conducted under ambient [CO<sub>2</sub>] of 400 μmol mol<sup>-1</sup> controlled using the LI-6400 control system in a large and well ventilated field laboratory; as such, it was not necessary to correct for CO<sub>2</sub> diffusion through the chamber gasket (Pons and Welschen 2002). Light-saturated photosynthesis ( $A_{sat}$ ) was measured at 1500 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux density (PPFD) and a RH of 40–70%, and after leaves had been exposed to saturating irradiance in the cuvette for 10–20 min. After measurement of  $A_{sat}$ , the irradiance response of net CO<sub>2</sub> exchange was measured, beginning at 1000 μmol m<sup>-2</sup> s<sup>-1</sup>, followed by 500, 250, 150, 100, 90, 80 and then every 5 μmol m<sup>-2</sup> s<sup>-1</sup> to 10 and ending at zero μmol m<sup>-2</sup> s<sup>-1</sup> (i.e. darkness). Additional measurements of net CO<sub>2</sub> exchange in darkness were conducted after a further 10 min of darkness – these were not statistically different from values of  $R_D$  measured directly at the end of light response curves. Flow rate through the chamber was set to 300 μmol s<sup>-1</sup>. Relationships between key gas exchange characteristics ( $A_{sat}$ ,  $R_D$ ) and soil water content have been included for reference in Fig. S2, available as Supplementary Material to this paper.



**Fig. 1.** Seasonal changes in (a) soil volumetric water content, (b) leaf temperature, (c)  $A_{\text{sat}}$ ; i.e. net  $\text{CO}_2$  uptake measured  $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , (d)  $g_{\text{sat}}$ ; maximum stomatal conductance at  $A_{\text{sat}}$ , (e) foliar respiration rate in darkness ( $R_{\text{D}}$ ) and (f) in the light ( $R_{\text{L}}$ ), (g) the ratio of leaf respiration measured in the light to that in darkness ( $R_{\text{L}}/R_{\text{D}}$ ) in measured in *Quercus ilex* seven times during the course of a year at an upper and lower slope site at Villar de Cobeta (central Spain). (a–g) values shown are averages for each time point ( $\pm$ s.e. of the mean). For (b), measurements were made at the prevailing daytime  $T$  during the active period on any given day (1100 to 1400 hours). See Table 3 for results of two-way ANOVAs testing for differences with time and site. For (h), area-based rates of leaf  $R_{\text{L}}$  plotted against corresponding rates of  $R_{\text{D}}$ . Data shown are for individual leaves sampled during the year. The dashed line shows the 1 : 1 relationship.

To estimate rates of leaf respiration in the light ( $R_{\text{L}}$ ), we used the Kok (1948) method, where  $R_{\text{L}}$  was estimated from the  $y$ -axis intercept of a first order linear regression fitted to  $A$ -irradiance plots to measurements made over the 20–60  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  irradiance range (Ayub *et al.* 2011); in some species, data was curvilinear at irradiances above  $70 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD (data now shown). Averaged across all sites, the  $r^2$  values

of the first order regression fits over the 10–50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  range was  $0.97 \pm 0.01$ . Using this method, intercellular  $\text{CO}_2$  concentrations ( $C_i$ ) tend to increase with decreases in irradiance, resulting in reduced rates of photorespiration and increased rates of carboxylation; this in turn results in a decrease in the slope of  $A_{\text{net}}$ -irradiance plots in the linear region (Villar *et al.* 1994). Because of this, rates of  $R_{\text{L}}$  were

adjusted (by iteration) to ensure that the intercept of plots of photosynthetic electron transport ( $J$ ) against irradiance are minimised (Kirschbaum and Farquhar 1987).  $J$  was calculated according to Farquhar and von Caemmerer (1982):

$$J = \frac{[(4 \times (A_{\text{net}} + R_L)) \times (C_i + 2\Gamma^*)]}{(C_i - \Gamma^*)}, \quad (1)$$

where  $\Gamma^*$  is the  $\text{CO}_2$  compensation point in the absence of  $R_L$  ( $37 \mu\text{mol mol}^{-1}$  at  $25^\circ\text{C}$  (von Caemmerer and Farquhar 1981)], with  $\Gamma^*$  at each measurement  $T$  calculated according to Brooks and Farquhar (1985). Rates of oxygenation and carboxylation by Rubisco ( $V_o$  and  $V_c$  respectively) at any given irradiance were calculated according to Farquhar and von Caemmerer (1982).

#### Leaf structural traits and chemical composition

Leaves used for the light response curves were weighed for fresh mass, photographed (to enable subsequent calculation of leaf area using Image J software (<http://rsbweb.nih.gov/ij/>, accessed December 2013)) and then oven-dried at  $70^\circ\text{C}$  to constant mass. The mass and area data were used to determine ratios of leaf dry mass to leaf area ( $M_A$ ). Subsequently, leaf samples were ground in a ball mill and analysed for tissue nitrogen and phosphorus using either a Technicon Auto-analyser II (Bran + Luebbe Pty Ltd; Villar de Cobeta samples) or a Eurovector 3011 elemental analyser (Prades samples). For the three species in sites 3–6 of the Prades forest study, we had access to previous measurements of tissue N in July over 4 different years (Ogaya and Peñuelas 2008). Based on the finding that leaf N was not significantly influenced by soil moisture content or rain exclusion treatment, we used a 4 year site average as our measure of leaf N for these species at these sites (reported only in Table 4). Leaf carbohydrate content was determined during the Villar de Cobeta study (Loveys *et al.* 2003), but as there was no significant explanatory power of carbohydrate status on respiratory parameters (see Fig. S1) we

did not repeat these measurements in the subsequent Prades campaign. Leaf tissue relative water content (RWC) was determined according to Koide *et al.* (1989) for a representative leaf adjacent to the measurement leaf at the same time as gas-exchange measurements.

#### Statistical analyses

Repeated-measures analysis of variance (ANOVA) was used to test for the effects of time of year and site in the Villar de Cobeta seasonal study and for the effects of plant species and site in the Prades gradient study using SPSS (ver. 16.0; SPSS Inc.) and the R statistical platform (R Development Core Team 2008) with *post hoc* comparisons of sites being made using least significance difference tests ( $P < 0.05$ ). Species was treated as a nominal variable, and site as an ordinal variable (i.e. to account for increasing water availability) for these analyses. Differences were considered significant if probabilities (P) were less than 0.05.

## Results

### Villar de Cobeta seasonal study – *Q. ilex subsp. ballota*

Volumetric measurements of water content confirmed strong seasonal changes in soil water content from highest levels in spring (March, ~15%) to lowest levels in late summer (August–September, ~5%; Fig. 1a). The lower slope site had slightly lower volumetric water contents than the upper slope site, but there was no significant difference between the sites at the times measurements were made (Fig. 1a). This is a reflection of the fact that rainfall in the region was unusually high in winter–spring 2006–2007. Leaf mass per unit area ( $M_A$ ) was significantly greater at the upper slope site and increased significantly in mid-summer, before declining during autumn (Tables 2, 3). Leaf N content did not differ significantly between site nor time of year, but phosphorus content ([P]) was significantly greater at the lower-slope site (Tables 2, 3). Leaf sugar and starch determinations were associated with relatively large

**Table 2.** Seasonal changes in average ( $\pm$ s.e.,  $n=4$ ) values of leaf structural and chemical traits for *Quercus ilex subsp. ballota* at two sites at Villar de Cobeta (central Spain)

See Table 3 for ANOVA statistics

Month	$M_A$ (g DM m <sup>-2</sup> )	N (mg g DM <sup>-1</sup> )	P (mg g DM <sup>-1</sup> )	Sugars (mg g DM <sup>-1</sup> )	Starch (mg g DM <sup>-1</sup> )
<i>Upper slope</i>					
December	401 ± 51	12.6 ± 1.7	1.61 ± 0.11	58.2 ± 5.3	3.5 ± 0.2
February	354 ± 20	12.3 ± 0.99	1.66 ± 0.19	56.6 ± 4.1	3.1 ± 0.2
April	379 ± 46	11.2 ± 0.65	1.27 ± 0.15	55.8 ± 3.5	25.2 ± 6.2
June	357 ± 38	11.0 ± 0.82	1.29 ± 0.11	44.3 ± 1.0	23.6 ± 6.6
August	463 ± 70	9.8 ± 0.83	1.08 ± 0.12	40.8 ± 1.1	2.7 ± 0.3
September	228 ± 13	9.7 ± 0.56	1.44 ± 0.14	43.3 ± 3.0	2.8 ± 0.8
November	226 ± 13	11.9 ± 0.45	1.40 ± 0.08	53.1 ± 1.9	1.8 ± 0.3
<i>Lower slope</i>					
December	316 ± 13	9.6 ± 1.4	1.54 ± 0.25	44.9 ± 5.7	1.8 ± 0.2
February	281 ± 41	12.2 ± 0.31	1.80 ± 0.05	56.2 ± 2.4	2.1 ± 0.1
April	229 ± 28	11.5 ± 0.37	1.64 ± 0.25	47.3 ± 1.4	14.9 ± 4.7
June	257 ± 9	9.0 ± 1.2	1.85 ± 0.18	53.1 ± 3.6	2.8 ± 0.2
August	350 ± 28	9.1 ± 1.2	1.86 ± 0.19	53.1 ± 3.6	2.7 ± 2.2
September	254 ± 20	9.3 ± 0.78	1.71 ± 0.00	52.7 ± 2.8	5.0 ± 2.3
November	276 ± 17	11.4 ± 1.1	1.04 ± 0.32	54.0 ± 1.4	1.4 ± 0.1

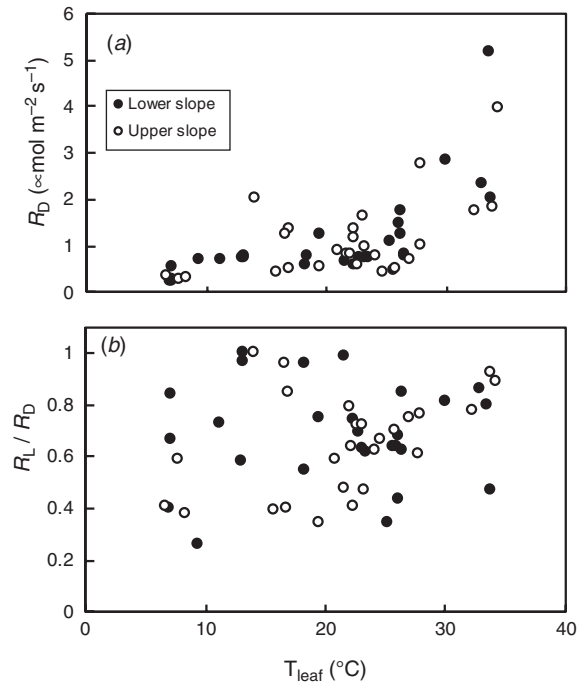
**Table 3. Results of repeated-measures two-way ANOVA from the Villar de Cobeta seasonal measurements, with time of year (T) and site (S) as the main effects**

Abbreviations:  $M_A$ , leaf mass per unit area; N and P, foliar nitrogen and phosphorus content, respectively;  $A_{\text{sat}}$ , net photosynthesis measured at 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $g_{\text{sat}}$ , stomatal conductance measured at 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $R_D$ , leaf respiration in darkness;  $R_L$ , non-photorespiratory mitochondrial leaf respiration in the light;  $V_o$ , estimated rate of photorespiration at 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $C_i$ , internal  $\text{CO}_2$  concentration at 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD. See Table 2 and Fig. 1 for seasonal variation in trait values

Leaf trait category	Variable	P-value
Leaf structure	$M_A$	$t < 0.001$ ; $S < 0.05$ ; $T \times S < 0.05$
Chemical composition	N	ns
	P	$S < 0.05$
	Sugars	$T \times S < 0.01$
	Starch	$S < 0.05$
Area-based gas exchange	$A_{\text{sat}}$	$t < 0.001$ ; $T \times S < 0.05$
	$g_{\text{sat}}$	$t < 0.0001$ ; $T \times S < 0.05$
	$R_D$	ns
	$R_L$	ns
	$V_o$	ns
	$C_i$	$t < 0.05$
Ratios	$R_L/R_D$	ns
	$R_D/A_{\text{sat}}$	$t < 0.05$
	$R_L/A_{\text{sat}}$	$t < 0.05$

error, and thus displayed only modest significant responses to site and time of year (Tables 2, 3). Leaf temperatures reflected seasonal changes in ambient air  $T$ , with lowest values in late winter (February) and highest values in mid-summer (August, Fig. 1b). Maximum light-saturated photosynthetic rate ( $A_{\text{sat}}$ ) largely mirrored changes in soil water content, with maximum rates in the spring period and declining rates in summer–autumn (Fig. 1c; Table 3). Trees in the upper slope site displayed higher rates during winter–spring (when rainfall was unusually high), but there was no difference between sites in the summer–autumn period. The pattern of response of  $A_{\text{sat}}$  was strongly reflected in the seasonal response of  $g_{\text{sat}}$ , with stomatal conductance declining from high values in June to low values in late summer (Fig. 1d; Table 3). This was also reflected in significant changes in internal  $\text{CO}_2$  concentration ( $C_i$ , Table 3).

Foliar respiration in the dark ( $R_D$ , measured at ambient seasonal temperatures at the end of light response curves) did not display a significant seasonal response (Table 3), with the exception of a significant increase at the single time point during a very hot period in August (Fig. 1e). Inhibition of  $R$  by light (i.e. the Kok effect) was consistently observed in the light-limited phase of photosynthetic light responses. As a result, we were able to estimate  $R_L$  and thus calculate the extent of light inhibition of  $R_D$ . Foliar respiration in the light ( $R_L$ ) displayed a very similar seasonal response to  $R_D$  (Fig. 1f), and so  $R_L/R_D$ , and displayed a range of 0.5 to 0.8 with large s.e. in some months, did not display a significant seasonal response or differ between the two sites (Fig. 1g; Table 3). Averaged over all seven months,  $R_L/R_D$  was  $0.66 \pm 0.05$  s.e. ( $n = 14$ ). The relationship between  $R_L$  and  $R_D$  was a very strong one, and did not differ between trees at the two sites (Fig. 1h; Table 3). There was no significant relationship between seasonal changes in  $R_L/R_D$  and soil volumetric water content

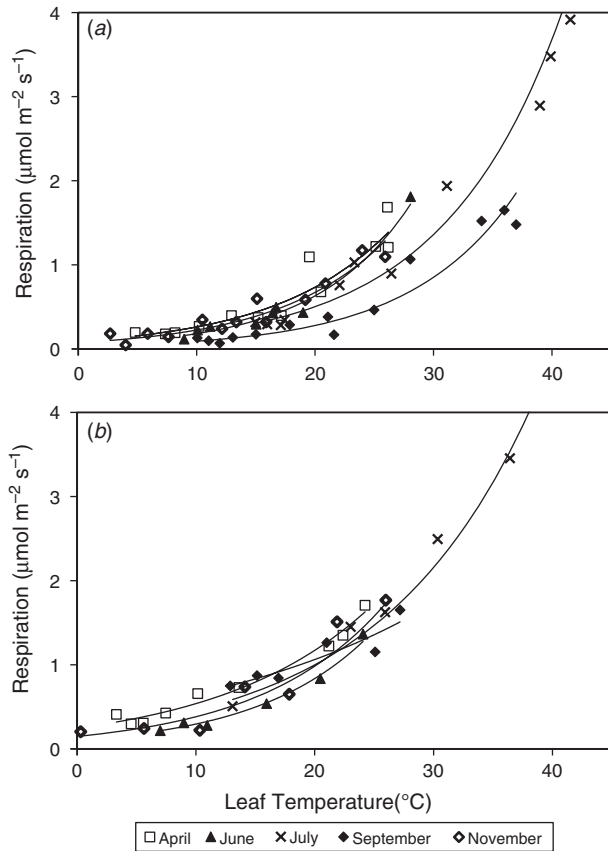


**Fig. 2.** Relationships between (a) foliar respiration rate in darkness ( $R_D$ ) and (b) the ratio of leaf respiration measured in the light to that in darkness ( $R_L/R_D$ ) and leaf temperature in *Quercus ilex* seven times during the course of a year at an upper (closed symbols) and lower slope (open symbols) site at Villar de Cobeta (central Spain).

( $R_L/R_D = -0.012 \times \text{SWC} + 0.76$ ,  $P = 0.18$ ) or leaf  $T$  ( $R_L/R_D = 0.007 \times T_{\text{leaf}} + 0.51$ ,  $P = 0.11$ ). The single-point measurements of  $R_D$  in Fig. 1e, when plotted against leaf  $T$ , indicate a relatively muted response in the range of leaf temperatures from 7 to 30°C (Fig. 2a). In August, when daily ambient and leaf temperatures exceeded 30°C during the period of that field campaign,  $R_D$  values increased (to values in the range 2–5  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). This response of  $R_D$  was also reflected, in part, by short-term thermal response measurements generated over daily cycles of ambient  $T$  (Fig. 3). These show shifts in the thermal response indicative of partial acclimation to increasing temperatures from winter through spring and summer, most strongly in trees at the upper-slope site (Fig. 3a).  $R_L/R_D$  was not related to seasonal changes in leaf  $T$  (Fig. 2b;  $R_L/R_D = 0.005 \times T_{\text{leaf}} + 0.56$ ,  $P = 0.14$ ) or leaf N or sugar content (Fig. S1). Significant seasonal changes in  $A$  and increases in  $R$  resulted in a significant seasonal effect on  $R_D/A_{\text{sat}}$  ratio (Table 3), and in particular a significant increase in during the high temperatures in August (data not shown). Because of the lack of systematic response of  $R_L/R_D$ , the ratio of leaf  $R_L$  to light-saturated photosynthesis (i.e.  $R_L/A_{\text{sat}}$ ) did not vary during the year other than via direct effects on  $A$  and  $R$  (Table 3).

#### Prades gradient study – *Q. ilex* subsp. *ilex* and community

Volumetric measurements confirmed that there was a significant decrease in soil water content from the riparian to the shrub sites in mid-summer at Prades (Fig. 4a). Although soil moisture has been, on average, 11% lower in the rainfall exclusion plots than



**Fig. 3.** Seasonal changes in the instantaneous thermal response of foliar  $R_D$  in *Quercus ilex* at an (a) upper and (b) lower slope site at Villar de Cobeta (central Spain). Responses were determined on intact shoots using ambient changes in air temperature during the course of the day and night in April, June, July, September and November 2007. Each point represents a replicate tree at each time point.

in the control plots during the entire experimental period (TDR measurements made between 1999 and 2007 reported by Ogaya and Peñuelas 2008; and unpublished measurements since 2007), differences between these plots were not evident in the 30 cm deep gravimetric measurements made in the present mid-summer campaign. Leaf mass per unit area and N content differed significantly with species and site (Table 5). Lowest average  $M_A$  values were found in the species from the riparian and slope site (*T. platyphyllos* and *A. monspessulanum*) (Table 4), with site-averaged values increasing along the gradient (Fig. 4b).  $M_A$  did not vary significantly in *Q. ilex* over the range it occurred. Leaf nitrogen content on a dry mass basis was significantly greater at the riparian and slope sites (species means in Table 4) and site average values decreased from the moister to the drier sites (data not shown). Leaf relative water content differed between species, but did not differ at sites along the soil moisture gradient (Tables 4, 5).

Table 4 shows average rates of light-saturated photosynthesis ( $A_{\text{sat}}$ ) expressed on a leaf area basis for each species/site combination. Significant differences were found among site averages (Fig. 4; Table 5), with *Q. ilex* showing a similar trend. Rates of  $A$  were highest at the riparian and slope sites

and lowest at the dry and shrub sites. This trend was also displayed by light-saturated stomatal conductance ( $g_{\text{sat}}$ ; species averages Table 4, site averages Fig. 4). Because the two parameters changed in concert, there was no significant species or site effect on internal  $\text{CO}_2$  concentration ( $C_i$ , Tables 4, 5). No significant effect of site was found on leaf  $R_D$  (Table 5), although the riparian site did have a lower rate, on average, than the drier sites (Fig. 4e) – as a result there was a significant species  $\times$  site interaction. Irrespective of whether site-averages were considered (Fig. 4) or whether rates of leaf  $R$  in individual leaves were compared (Table 4),  $R_L$  was almost always less than  $R_D$  (i.e. light inhibited leaf  $R$ ), although the degree of inhibition did vary (with  $R_L = R_D$  (i.e. no light inhibition) in a small number of cases) as a consequence of the technical challenges in the method.  $R_L$  displayed a similar response to that of  $R_D$  (Fig. 4f), and thus site-averaged  $R_L/R_D$  did not vary significantly across the gradient (Fig. 4g; Table 5) and  $R_L$  and  $R_D$  were strongly correlated (Fig. 4h). The degree of light inhibition in individual species ranged from 0.62 to 0.75 (Table 4), and averaged across all species and sites,  $R_L/R_D$  was  $0.69 \pm 0.01$  s.e. ( $n = 18$ ). Because of the consistency of  $R_L/R_D$ , the ratio of leaf  $R_L$  to light-saturated photosynthesis (i.e.  $R_L/A_{\text{sat}}$ ) did not vary along the gradient other than via direct effects on  $A$ . There was no significant relationship observed between  $R_L/R_D$  and  $A$  or the oxygenation component of  $A$  ( $V_o$ ; data not shown).

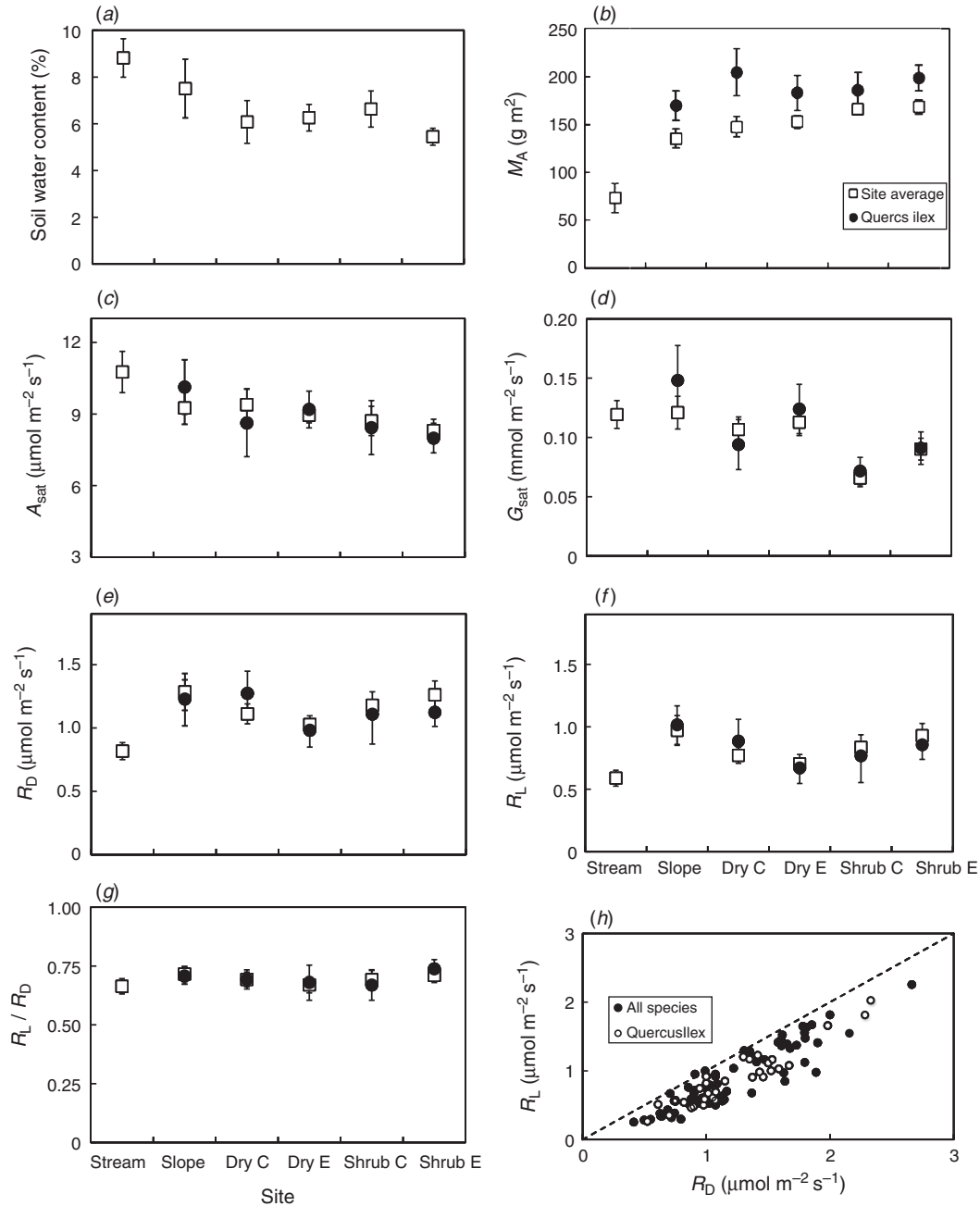
## Discussion

We assessed the importance of (i) seasonal changes in  $T$  and water availability and (ii) a topographically-driven gradient in water availability in determining changes in leaf respiratory  $\text{CO}_2$  evolution (both in darkness and in the light) in a dominant tree of the Mediterranean region (*Q. ilex* subsp. *ballota* and subsp. *ilex*). Our results do not provide support for our hypothesis that lower soil water availability in the field would increase light inhibition of  $R$ .  $R_L$  and  $R_D$  were strongly correlated, with  $R_L$  almost always less than  $R_D$ , but  $R_L/R_D$  did not vary in any way that is systematically explainable by changes in  $T$  or soil moisture in the seasonal study (Villar de Cobeta site), and there was a distinct lack of site-to-site variation in light inhibition with changes in soil water content across the gradient at the Prades site. An important consequence of the lack of site-to-site variation in light inhibition was that the ratio of  $R_L$  to photosynthesis, although clearly lower than that for  $R_D$ , was driven primarily by assimilation rate.

### Responses to seasonal and gradient changes in environment

In the year of our seasonal measurements at Villar de Cobeta (2007), the average rainfall in the region was significantly greater than average. As a result, the lower slope site was not greatly different in soil water availability compared with the upper slope site (and we combine the sites for the purposes of further interpretation); however, significant underlying seasonal variation in  $T$  and soil water content did provide the driver for significant changes in leaf physiology which allow us to address questions on environmental influences on  $R_D$  and  $R_L$ . During the course of the year, leaf  $T$  ranged from  $\sim 6$  to  $>30^\circ\text{C}$ , mid-morning measured rates of  $A_{\text{sat}}$  ranged from around  $3 \mu\text{mol m}^{-2} \text{s}^{-1}$  in late





**Fig. 4.** Changes in (a) soil water content, (b) leaf mass per unit area, (c)  $A_{\text{sat}}$ ; i.e. net  $\text{CO}_2$  uptake measured  $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , (d)  $g_{\text{sat}}$ ; maximum stomatal conductance at  $A_{\text{sat}}$ , (e) foliar respiration rate in darkness ( $R_D$ ) and (f) in the light ( $R_L$ ), (g) the ratio of leaf respiration measured in the light to that in darkness ( $R_L/R_D$ ) for the six study sites along the soil moisture gradient at Prades (north-eastern Spain). Values shown (a–g) are averages for all species at the site and values for *Q. ilex* only ( $\pm$ s.e. of the mean). For details of sites, see Table 1. For individual species values see Table 4. See Table 5 for results of two-way ANOVAs testing for differences among species and site averages. (h) Area-based rates of leaf  $R_L$  plotted against corresponding rates of  $R_D$ . Data shown are for individual leaves sampled along soil water availability gradient. The dashed line shows the 1 : 1 relationship.

summer/autumn to  $>10 \mu\text{mol m}^{-2} \text{s}^{-1}$  in spring–early summer and  $g_{\text{sat}}$  ranged from maximal values in excess of  $0.15 \text{ mol m}^{-2} \text{s}^{-1}$  in spring to below  $0.05 \text{ mol m}^{-2} \text{s}^{-1}$  in late summer. When measured at prevailing mid-morning air temperatures,  $R_D$  ranged around  $1 \mu\text{mol m}^{-2} \text{s}^{-1}$  for much of the year, but peaked

at  $\sim 3 \mu\text{mol m}^{-2} \text{s}^{-1}$  during a very hot August (Fig. 1e), when leaf temperatures exceeded  $30^\circ\text{C}$ . As a consequence, variation in  $R_D/A$  ratios was relatively constrained  $\sim 0.08$ – $0.18$  during spring, early summer and winter, and only increasing significantly (to 0.38) during a very hot August. The relatively consistent rate

**Table 4. Average (±s.e., n = 6) values of leaf traits for each species growing at each site along a soil water gradient at Prades**

Abbreviations:  $M_A$ , dry mass per unit area; RWC, relative water content; N, nitrogen content;  $A_{sat}$ , rate of net photosynthesis at 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $g_{sat}$ , stomatal conductance at 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $C_i$ , internal  $\text{CO}_2$  concentration at 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $V_o$ , estimated rate of photorespiration at 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $R_D$ , rate of leaf respiration in darkness;  $R_L$ , rate of leaf respiration in the light;  $R_L/R_D$ , ratio of  $R_L$  to  $R_D$ ;  $R_D/A_{sat}$ , ratio of leaf  $R$  to  $A_{sat}$ . See Table 1 for sites details and Table 5 for ANOVA results for each trait

Site	Species	$M_A$ ( $\text{g}_{DM} \text{m}^{-2}$ )	RWC (%)	N ( $\text{mg g}_{DM}^{-1}$ )	$A_{sat}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$g_{sat}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$C_i$ ( $\mu\text{mol mol}^{-1}$ )	$V_o$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$R_D$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$R_L$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$R_L/R_D$ (ratio)	$R_D/A_{sat}$ (ratio)	$R_L/A_{sat}$ (ratio)
Riparian	<i>Tilia platyphyllos</i>	55.8 ± 2.9	86.5 ± 1.3	2.89 ± 0.05	10.9 ± 0.8	0.137 ± 0.014	235 ± 21	4.24 ± 0.7	0.67 ± 0.09	0.42 ± 0.08	0.61 ± 0.04	0.06 ± 0.01	0.04 ± 0.01
	<i>Acer monspessulanum</i>	94.3 ± 10.4	92.6 ± 2.0	1.76 ± 0.08	10.6 ± 0.8	0.099 ± 0.010	231 ± 22	4.99 ± 0.8	0.99 ± 0.15	0.84 ± 0.14	0.74 ± 0.08	0.10 ± 0.02	0.09 ± 0.02
Slope	<i>Acer monspessulanum</i>	87.5 ± 6.1	91.5 ± 0.9	1.82 ± 0.01	8.3 ± 0.9	0.101 ± 0.015	223 ± 12	4.70 ± 0.3	0.92 ± 0.13	0.73 ± 0.14	0.77 ± 0.06	0.11 ± 0.02	0.09 ± 0.02
	<i>Quercus ilex</i>	169.9 ± 6.4	83.3 ± 2.4	1.46 ± 0.03	10.1 ± 1.1	0.148 ± 0.029	169 ± 17	7.52 ± 1.4	1.22 ± 0.21	1.02 ± 0.16	0.71 ± 0.03	0.12 ± 0.02	0.10 ± 0.01
Dry C	<i>Phillyrea latifolia</i>	168.3 ± 7.1	91.6 ± 1.0	1.31 ± 0.05	9.2 ± 1.5	0.110 ± 0.023	192 ± 29	6.75 ± 1.9	1.64 ± 0.30	1.16 ± 0.26	0.68 ± 0.06	0.20 ± 0.04	0.14 ± 0.04
	<i>Quercus ilex</i>	204.6 ± 9.9	85.8 ± 0.7	1.25 ± 0.11	8.6 ± 1.4	0.094 ± 0.021	200 ± 24	5.46 ± 1.4	1.27 ± 0.17	0.88 ± 0.12	0.69 ± 0.03	0.16 ± 0.02	0.11 ± 0.01
Dry E	<i>Phillyrea latifolia</i>	113.1 ± 9.8	93.7 ± 0.9	1.07 ± 0.12	7.6 ± 0.6	0.083 ± 0.010	201 ± 13	5.73 ± 2.3	0.89 ± 0.07	0.65 ± 0.13	0.71 ± 0.12	0.12 ± 0.01	0.09 ± 0.02
	<i>Arbutus unedo</i>	134.3 ± 8.2	96.0 ± 1.1	1.03 ± 0.07	11.6 ± 0.5	0.139 ± 0.015	222 ± 22	5.40 ± 0.9	1.13 ± 0.12	0.76 ± 0.08	0.68 ± 0.06	0.10 ± 0.01	0.07 ± 0.01
Shrub C	<i>Quercus ilex</i>	183.1 ± 7.4	83.8 ± 2.4	1.20 ± 0.07	9.1 ± 0.8	0.124 ± 0.021	199 ± 20	6.06 ± 0.9	0.98 ± 0.13	0.67 ± 0.12	0.68 ± 0.07	0.11 ± 0.02	0.08 ± 0.02
	<i>Phillyrea latifolia</i>	141.3 ± 1.2	91.2 ± 1.3	0.98 ± 0.08	8.3 ± 0.4	0.102 ± 0.019	259 ± 14	3.16 ± 0.4	1.11 ± 0.16	0.80 ± 0.19	0.68 ± 0.07	0.13 ± 0.02	0.09 ± 0.02
Shrub E	<i>Arbutus unedo</i>	134.2 ± 12.3	94.9 ± 1.1	1.31 ± 0.10	9.3 ± 0.4	0.113 ± 0.021	200 ± 25	6.54 ± 1.1	0.99 ± 0.07	0.64 ± 0.06	0.65 ± 0.04	0.11 ± 0.01	0.07 ± 0.01
	<i>Quercus ilex</i>	186.2 ± 7.5	82.6 ± 1.3	1.12 ± 0.15	8.4 ± 1.1	0.072 ± 0.012	241 ± 15	3.81 ± 0.3	1.11 ± 0.24	0.77 ± 0.21	0.67 ± 0.07	0.14 ± 0.02	0.09 ± 0.02
Shrub E	<i>Phillyrea latifolia</i>	167.3 ± 7.6	92.2 ± 1.7	1.15 ± 0.10	8.3 ± 1.4	0.053 ± 0.015	239 ± 19	4.61 ± 0.7	1.47 ± 0.15	1.11 ± 0.17	0.73 ± 0.09	0.20 ± 0.03	0.14 ± 0.01
	<i>Arbutus unedo</i>	144.4 ± 6.5	97.3 ± 0.4	1.20 ± 0.09	9.3 ± 0.6	0.073 ± 0.012	232 ± 21	3.94 ± 0.6	0.95 ± 0.07	0.67 ± 0.10	0.69 ± 0.06	0.11 ± 0.01	0.08 ± 0.01
Shrub E	<i>Quercus ilex</i>	198.9 ± 5.5	82.6 ± 1.6	1.01 ± 0.05	7.9 ± 0.6	0.091 ± 0.014	249 ± 8	4.79 ± 0.3	1.12 ± 0.11	0.86 ± 0.12	0.74 ± 0.04	0.14 ± 0.01	0.11 ± 0.01
	<i>Phillyrea latifolia</i>	179.2 ± 3.5	90.5 ± 0.9	1.15 ± 0.02	8.9 ± 1.2	0.100 ± 0.022	203 ± 22	5.15 ± 1.4	1.80 ± 0.09	1.35 ± 0.10	0.75 ± 0.05	0.22 ± 0.03	0.16 ± 0.02
	<i>Arbutus unedo</i>	128.1 ± 6.0	96.7 ± 0.6	1.05 ± 0.18	8.0 ± 0.6	0.079 ± 0.012	189 ± 26	6.38 ± 1.5	0.86 ± 0.10	0.57 ± 0.10	0.65 ± 0.06	0.11 ± 0.01	0.07 ± 0.01

**Table 5. Results of two-way ANOVA of leaf traits for species growing along a soil water gradient at Prades, with species (Sp) and site (S) as the main effects**

Abbreviations:  $M_A$ , leaf mass per unit area;  $A_{\text{sat}}$ , net photosynthesis measured at 1500  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  PPFD;  $g_{\text{sat}}$ , stomatal conductance measured at 1500  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  PPFD;  $R_D$ , leaf respiration in darkness;  $R_L$ , non-photorespiratory mitochondrial leaf respiration in the light;  $V_o$ , estimated rate of photorespiration at 1000  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  PPFD;  $C_i$ , internal  $\text{CO}_2$  concentration at 1000  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  PPFD. See Table 2 for trait values

Leaf trait category	Parameter	P-value
Leaf structure	$M_A$	Sp < 0.0001; Site < 0.001; Sp $\times$ S < 0.0001
Chemical composition	RWC	Sp < 0.0001
	N	Sp < 0.0001; Site < 0.0001; Sp $\times$ S < 0.0001
Area-based gas exchange	$A_{\text{sat}}$	Site < 0.01
	$g_{\text{sat}}$	Site < 0.01
	$R_D$	Sp $\times$ S < 0.01
	$R_L$	Sp $\times$ S < 0.05
	$V_o$	ns
Ratios	$C_i$	ns
	$R_L/R_D$	ns
	$R_D/A_{\text{sat}}$	Site < 0.05; Sp $\times$ S < 0.01
	$R_L/A_{\text{sat}}$	Sp $\times$ S < 0.05

of  $R_D$  for six out of the seven campaigns is indicative of at least partial thermal acclimation (Atkin and Tjoelker 2003), which is supported by two separate lines of evidence. First, the relationship between single point measurements of  $R_D$  from light response curves on attached leaves and leaf  $T$  is fairly muted below 30°C (Fig. 2). Second, short-term  $T$  responses curves (generated by daily changes in air temperature on intact leaves in the field; Fig. 3) indicate shifts in the thermal response consistent with thermal acclimation, at least in trees at the south-facing, drier site.

Despite the significant seasonal changes in leaf physiology above (at Villar de Cobeta),  $R_L/R_D$  measured mid-morning did not follow a clear seasonal pattern, and was not significantly influenced by time of year. Averaged over the year, the inhibition of  $R$  was ~34% (i.e.  $R_L/R_D$  was  $0.66 \pm 0.05$  s.e.). The fact that  $R_L/R_D$  did not change seasonally in any systematic way is somewhat surprising, since we had predicted that light inhibition would increase with increasing measuring  $T$  and lower soil water content (in summer). That it did not indicate that, in this forest type under field conditions, soil water availability has no clear impact on the extent of light inhibition of respiration (discussed further in the next section). It also shows that both  $R_L$  and  $R_D$  have acclimated to the seasonal shifts in daily average  $T$ . The latter is partially supported by our data, since we found that there was some seasonal shift in the  $R$ - $T$  curves for the dry site plants (Fig. 3), but much less evidence for acclimation in the lower slope site plants. The constant  $R_L/R_D$  found at the lower slope site may suggest that the  $Q_{10}$  is not different for the two processes, but for the upper slope site the situation is potentially a more complex mix of acclimation combined with similar  $Q_{10}$  values. Another complication for interpreting our findings is that the response of  $R$  to leaf water content may also be biphasic i.e. it may decrease in the initial stages of water

stress (lower energy demands for growth) and may increase with additional stress-related demands e.g. osmoregulation or induced senescence (Ghashghaie *et al.* 2001; Gulias *et al.* 2002; Flexas *et al.* 2005, 2006). This means that the timing of investigations during seasonal rainfall cycles is likely to be critical in terms of the state of the plant response. Further,  $T$  and water availability tend to vary in opposite directions. The syndrome of response to the combined effects of seasonal changes in water availability and  $T$  clearly requires further investigation to resolve these potentially competing drivers and responses, and is partially addressed by the companion study using the Prades gradient, where the mid-summer campaign removes the impact of  $T$ .

We found changes in leaf structure and nutrient content in species across the Prades gradient consistent with previous findings (i.e. low  $M_A$  and high N content at the riparian and slope sites with greater soil water content; Tables 4, 5). Rates of photosynthesis and stomatal conductance varied substantially across the gradient (Fig. 4; Table 4), with species sampled at the lower sites exhibiting the highest rates of net  $\text{CO}_2$  uptake. We also observed variations in  $R_D/A$  ratios among species, which allows an assessment of the leaf-level balance between the respiratory cost and photosynthetic capacity in response to soil water content.  $R_D/A$  was highest (0.14–0.22) in high  $M_A$  species in the drier sites that exhibit low rates of mass-based photosynthesis, and lower (0.07–0.11) in deciduous species at the riparian site (Table 4). This is consistent with previous studies that have reported a higher  $R/A_{\text{sat}}$  ratio under drought (Flexas *et al.* 2006; Atkin and Macherel 2009). The ratio of leaf  $R$  in the light and dark ( $R_L/R_D$ ) was, by contrast, very consistent despite changes in both  $R_L$  and  $R_D$  (Fig. 4; Table 4).  $R_L/R_D$  across all sites resolved to an average of  $0.69 \pm 0.01$  s.e., meaning that  $R_L/A$  ratios were consistently ~30% lower than  $R_D/A$  ratios and were driven by underlying rates of  $A$ . The variation in  $R_L/A$  contrasts with previous findings of greater constancy of  $R_L/A$  (compared with  $R_D/A$ ) in controlled-environment grown plants exposed to a range of contrasting environments (Atkin *et al.* 2006; Ayub *et al.* 2011) and in a wide range of temperate rainforest species at contrasting sites differing in soil age/nutrient availability/species composition/leaf traits (Atkin *et al.* 2013).

#### Lack of variation in the Kok effect

Past work suggests that light inhibition of leaf  $R$  may be linked to changes in cellular energy status (due to excess ATP or redox equivalents generated by the light reactions of photosynthesis, decreasing the demand for respiratory energy compared with leaves in darkness), photorespiration-dependent inactivation of the pyruvate dehydrogenase (PDH) complex (Budde and Randall 1990; Gemel and Randall 1992), or transition to a truncated TCA cycle (Igamberdiev *et al.* 2001; Tcherkez *et al.* 2005, 2008, 2009). More recently, increased use of stored carbon reserves (e.g. citrate) in the light has been shown to reduce demand for flux through the TCA cycle (Tcherkez *et al.* 2012). Steady-state stoichiometric modelling has also indicated that light suppression of  $\text{CO}_2$  release by the oxidative pentose phosphate pathway (OPPP) has the potential to contribute to the Kok effect (Buckley and Adams 2011). Collectively, these biochemical and modelling studies suggest

that light inhibits respiratory CO<sub>2</sub> release – consistent with the Kok effect – and that reduced rates of CO<sub>2</sub> release by the TCA cycle and OPPP could both contribute to cases where  $R_L < R_D$ . What is less clear, however, is how environmental factors in the field influence the level of inhibition of  $R$ . More recently, Farquhar and Busch (2017), using a theoretical modelling approach, have shown it is possible to create a Kok-effect-like response in the presence of very specific conditions of changing mesophyll conductance ( $g_m$ ) at low light. To our knowledge, this potential explanation for the Kok effect is yet to be supported by experimental evidence documenting the irradiance response of  $g_m$  or the relative importance of irradiance-mediated changes in respiratory CO<sub>2</sub> release vs  $g_m$ . Nevertheless, the findings of Farquhar and Busch (2017) highlight the need for cautious interpretation of Kok effect results.

The tight coupling of leaf mitochondrial metabolism in the light to that of the prevailing rate of photosynthesis (Krömer 1995; Hoefnagel *et al.* 1998; Noguchi and Yoshida 2008) and related processes (e.g. sucrose synthesis, phloem loading, protein turnover) might provide an explanation for variability in  $R_L/R_D$  ratios in the field. Despite recent work (discussed below), uncertainty remains concerning the other main potential driver – that of the linkage between light inhibition of  $R$  and factors which increase the rate of photorespiration ( $V_o$ ). Importantly, Tcherkez *et al.* (2008) found that the degree of light inhibition of  $R$  decreases when *Xanthium strumarium* leaves are exposed to low atmospheric [CO<sub>2</sub>] for short periods (i.e. under conditions which increase the demand for TCA cycle intermediates associated with the recovery of photorespiratory cycle intermediates in the peroxisome). This relationship between  $R_L/R_D$  and carboxylation/oxygenation in the short term has been supported by further recent studies (Ayub *et al.* 2011; Crous *et al.* 2012; Griffin and Turnbull 2013). The literature suggests a putative link between the degree of light inhibition of  $R$  and any factors that would change rates of photorespiration, although the strength of that link under field conditions should be subject of continued investigation.

What underlying factors might explain the lack of systematic variation in  $R_L/R_D$  found with season at Villar de Cobeta and along the moisture gradient at Prades? At least three factors could have contributed to variations in  $R_L/R_D$  along the Prades gradient. First, a direct effect of soil water availability on  $C_i$  and the potential rate of photosynthetic oxygenation ( $V_o$ ). Griffin and Turnbull (2013) found that  $R_L/R_D$  decreased under conditions that suppressed light-saturated  $V_o$ . Crous *et al.* (2012) also report a positive linear relationship between  $R_L/R_D$  and  $V_{o100}$  (the velocity of RuBP oxygenation at 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD). In terms of our mechanistic hypothesis (that light inhibition of  $R$  would decrease (and  $R_L/R_D$  increase) at drier sites where stomatal limitations on gas exchange increase photorespiration),  $C_i$  was significantly influenced by time of year at Villar de Cobeta (Table 3), but  $R_L/R_D$  was not significantly related to  $C_i$  at either saturating PPFD (Fig. S1f) or at limiting PPFD (100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; data not shown). It is possible that the level of water limitation experienced by trees during the study was not sufficient to influence the balance between  $R_L$  and  $R_D$ , but the range of  $A_{\text{sat}}$ ,  $g_{\text{sat}}$  and  $C_i$  (180–330  $\text{mmol mol}^{-1}$ ) does not support this. There was no effect of site on  $C_i$  along the Prades gradient, which clearly reflects some level of balancing between

assimilation and conductance as soil water availability becomes more limited in the dry and shrub sites. Second, gradients in soil nutrient availability might have a secondary effect on foliar N content, and thence on the degree of light inhibition. Relatively few studies have quantified the effect of nutrient supply on  $R_L$ . Shapiro *et al.* (2004) found that deficiencies in nitrogen supply resulted in reduced rates of both  $R_L$  and  $R_D$ , but with the  $R_L/R_D$  ratios being nearly identical in high and low N grown plants (0.50 and 0.48, respectively for ambient CO<sub>2</sub> grown *X. strumarium*). We have previously found that variations in  $R_L/R_D$  in arctic tundra shrubs (Heskel *et al.* 2012) and forest species along a soil development chronosequence (Atkin *et al.* 2013) may be significantly related to several traits that are strong correlates of metabolic capacity, including leaf [N] and [P]. In the present study, tissue N was significantly influenced by site (water availability) in the Prades study, but this had no impact on  $R_L/R_D$ , which supports the findings of Shapiro *et al.* (2004). Third, the degree of light inhibition might differ systematically among species that exhibit contrasting leaf traits, with highly productive, low  $M_A$  species (which occupy the riparian and slope sites) exhibiting lower degrees of light inhibition than their high  $M_A$  counterparts. Previous evidence for this is equivocal – we have previously observed weaker inhibition in high-metabolic rate species growing on young/productive sites (Atkin *et al.* 2013) but similar  $R_L/R_D$  ratios have also been observed in inherently fast- and slow-growing herbaceous species (Atkin *et al.* 1997; Atkin *et al.* 2006). In the present study, we found that  $M_A$  varied with season at Villar de Cobeta and site at Prades, once again with no impact on  $R_L/R_D$ . Thus, here we add to the existing data that does not support the hypothesis that  $R_L/R_D$  ratios vary systematically among species with inherently different leaf traits.

## Conclusions

The balance between photosynthetic carbon fixation and respiratory oxidation of photosynthates is of great important to tree C balance (Amthor 1989; Loomis and Amthor 1999; Alt *et al.* 2000). Since mitochondrial respiration plays a key role in determining the growth and survival of plants, and it is associated with the production of energy and carbon skeletons essential for cellular maintenance and biosynthesis, respiratory responses need to be considered relative to the effects on carbon gain to elucidate the overall effect on plant performance. Our estimates based on the Kok method demonstrate that the degree of light inhibition of  $R$  is relatively constrained seasonally and is fairly consistent across sites differing in soil water availability and community composition in this Mediterranean forest type (with an average  $R_L/R_D$  of  $\sim 0.7$ ). This level of inhibition is consistent with recent findings (Ayub *et al.* 2014; McLaughlin *et al.* 2014). This points to  $R_L/A$  ratios being consistent, but around 30% lower than,  $R_D/A$  ratios, with both being primarily driven by changes in  $A$ . Notwithstanding the possibility that other methods of determining the degree of light inhibition of  $R$  might provide slightly different estimates, or that more extreme gradients of soil water availability may elicit changes not observed here, these findings have important implications for predictive models that seek to predict rates of leaf  $R_L$  using more commonly measured rates of  $R_D$  and associated leaf traits such as  $M_A$  and foliar [N] (Mercado *et al.* 2007). Failure

to account for light-induced reduction in leaf  $R$  will clearly lead to errors in predicted rates of ecosystem  $\text{CO}_2$  exchange (Wohlfahrt *et al.* 2005; Wingate *et al.* 2007; McLaughlin *et al.* 2014). Although further work is needed to establish the precise metabolic and environmental drivers of variations in  $R_L/R_D$ , there is now some evidence for stand and ecosystem models to assume a relatively constant relationship between  $R_D$  and  $R_L$  along gradients of soil water availability.

### Conflicts of interest

The authors declare no conflicts of interest.

### Acknowledgements

This work was funded by grants from the Natural Environment Research Council (NERC) in the UK (NE/DO1168X/1 (OKA)), the European Research Council (ERC-2013-SyG-610028 IMBALANCE-P), the Spanish Government (CGL2013-48074-P and CGL2016-79835-P), and the Catalan Government (SGR 2014–274). We thank to Elena Granda, Silvia Matesanz, Elena Beamonte, Oscar Godoy, Jaime Uria and Maira F Goulart who helped us with the field campaigns and acknowledge logistical support for MHT from the University of Canterbury. The expert technical assistance of David Sherlock is gratefully acknowledged.

### References

- Alt C, Stutzel H, Kage H (2000) Optimal nitrogen content and photosynthesis in cauliflower (*Brassica oleracea* L. botrytis). Scaling up from a leaf to the whole plant. *Annals of Botany* **85**, 779–787. doi:10.1006/anbo.2000.1139
- Amthor JS (1989) 'Respiration and crop productivity.' (Springer-Verlag: New York).
- Atkin OK, Day DA (1990) A comparison of the respiratory processes and growth rates of selected Australian alpine and related lowland plant species. *Australian Journal of Plant Physiology* **17**, 517–526. doi:10.1071/PP9900517
- Atkin OK, Macherel D (2009) The crucial role of plant mitochondria in orchestrating drought tolerance. *Annals of Botany* **103**, 581–597. doi:10.1093/aob/mcn094
- Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science* **8**, 343–351. doi:10.1016/S1360-1385(03)00136-5
- Atkin OK, Westbeek MHM, Cambridge ML, Lambers H, Pons TL (1997) Leaf respiration in light and darkness. A comparison of slow- and fast-growing *Poa* species. *Plant Physiology* **113**, 961–965. doi:10.1104/pp.113.3.961
- Atkin OK, Edwards EJ, Loveys BR (2000a) Response of root respiration to changes in temperature and its relevance to global warming. *New Phytologist* **147**, 141–154. doi:10.1046/j.1469-8137.2000.00683.x
- Atkin OK, Holly C, Ball MC (2000b) 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 & Environment* **23**, 15–26. doi:10.1046/j.1365-3040.2000.00511.x
- Atkin OK, Bruhn D, Tjoelker MG (2005) Response of plant respiration to changes in temperature: mechanisms and consequences of variations in the  $Q_{10}$  and acclimation. In 'Plant respiration: from cell to ecosystem'. (Eds H Lambers, M Ribas-Carbo) pp. 95–136. (Springer: Dordrecht, The Netherlands).
- Atkin OK, Scheurwater I, Pons TL (2006) High thermal acclimation potential of both photosynthesis and respiration in two lowland *Plantago* species in contrast to an alpine congeneric. *Global Change Biology* **12**, 500–515. doi:10.1111/j.1365-2486.2006.01114.x
- Atkin OK, Scheurwater I, Pons TL (2007) Respiration as a percentage of daily photosynthesis in whole plants is homeostatic at moderate, but not high, growth temperatures. *New Phytologist* **174**, 367–380. doi:10.1111/j.1469-8137.2007.02011.x
- Atkin OK, Atkinson LJ, Fisher RA, Campbell CD, Zaragoza-Castells J, Pitchford J, Woodward FI, Hurry V (2008) Using temperature-dependent changes in leaf scaling relationships to quantitatively account for thermal acclimation of respiration in a coupled global climate-vegetation model. *Global Change Biology* **14**, 2709–2726.
- Atkin OK, Turnbull MH, Zaragoza-Castell J, Fyllas NM, Lloyd J, Meir P, Griffin KL (2013) Light inhibition of leaf respiration as soil fertility declines along a post-glacial chronosequence in New Zealand: an analysis using the Kok method. *Plant and Soil* **367**, 163–182. doi:10.1007/s11104-013-1686-0
- Ayub G, Smith RA, Tissue DT, Atkin OK (2011) Impacts of drought on leaf respiration in darkness and light in *Eucalyptus saligna* exposed to industrial-age atmospheric  $\text{CO}_2$  and growth temperature. *New Phytologist* **190**, 1003–1018. doi:10.1111/j.1469-8137.2011.03673.x
- Ayub G, Zaragoza-Castells J, Griffin KL, Atkin OK (2014) Leaf respiration in darkness and in the light under pre-industrial, current and elevated atmospheric  $\text{CO}_2$  concentrations. *Plant Science* **226**, 120–130. doi:10.1016/j.plantsci.2014.05.001
- Brooks A, Farquhar GD (1985) Effect of temperature on the  $\text{CO}_2/\text{O}_2$  specificity of ribulose-1,5-biphosphate carboxylase/oxygenase and the rate of respiration in the light. Estimates from gas exchange measurements on spinach. *Planta* **165**, 397–406. doi:10.1007/BF00392238
- Buckley TN, Adams MA (2011) An analytical model of non-photorespiratory  $\text{CO}_2$  release in the light and dark in leaves of  $\text{C}_3$  species based on stoichiometric flux balance. *Plant, Cell & Environment* **34**, 89–112. doi:10.1111/j.1365-3040.2010.02228.x
- Budde RJA, Randall DD (1990) Pea leaf mitochondrial pyruvate dehydrogenase complex is inactivated *in vivo* in a light-dependent manner. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 673–676. doi:10.1073/pnas.87.2.673
- Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osório ML (2002) How plants cope with water stress in the field: photosynthesis and growth. *Annals of Botany* **89**, 907–916. doi:10.1093/aob/mcf105
- Crous KY, Zaragoza-Castells J, Low M, Ellsworth DS, Tissue DT, Tjoelker MG, Barton CVM, Gimeno TE, Atkin OK (2011) Seasonal acclimation of leaf respiration in *Eucalyptus saligna* trees: impacts of elevated atmospheric  $\text{CO}_2$  and summer drought. *Global Change Biology* **17**, 1560–1576. doi:10.1111/j.1365-2486.2010.02325.x
- Crous KY, Zaragoza-Castells J, Ellsworth DS, Duursma RA, Low M, Tissue DT, Atkin OK (2012) Light inhibition of leaf respiration in field-grown *Eucalyptus saligna* in whole-tree chambers under elevated atmospheric  $\text{CO}_2$  and summer drought. *Plant, Cell & Environment* **35**, 966–981. doi:10.1111/j.1365-3040.2011.02465.x
- Farquhar GD, Busch FA (2017) Changes in the chloroplastic  $\text{CO}_2$  concentration explain much of the observed Kok effect: a model. *New Phytologist* **214**, 570–584. doi:10.1111/nph.14512
- Farquhar GD, von Caemmerer S (1982) Modelling of photosynthetic response to environmental conditions. In 'Encyclopedia of plant physiology. Vol. 12B. Physiological plant ecology II. Water relations and carbon assimilation'. (Eds OL Lange, PS Nobel, CB Osmond, H Ziegler) pp. 551–587. (Springer Verlag: Berlin).
- Flexas J, Galmes J, Ribas-Carbo M, Medrano H (2005) The effects of water stress on plant respiration. In 'Plant respiration: from cell to ecosystem. Vol. 18'. (Eds H Lambers, M Ribas-Carbo) pp. 85–94. (Springer: Dordrecht, The Netherlands).
- Flexas J, Bota J, Galmes J, Medrano H, Ribas-Carbo M (2006) Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiologia Plantarum* **127**, 343–352. doi:10.1111/j.1399-3054.2006.00621.x

- Gemel J, Randall DD (1992) Light regulation of leaf mitochondrial pyruvate dehydrogenase complex. Role of photorespiratory carbon metabolism. *Plant Physiology* **100**, 908–914. doi:10.1104/pp.100.2.908
- Ghoshghaie J, Duranceau M, Badeck FW, Cornic G, Adeline MT, Deleens E (2001)  $\delta^{13}\text{C}$  of  $\text{CO}_2$  respired in the dark in relation to  $\delta^{13}\text{C}$  of leaf metabolites: comparison between *Nicotiana sylvestris* and *Helianthus annuus* under drought. *Plant, Cell & Environment* **24**, 505–515. doi:10.1046/j.1365-3040.2001.00699.x
- Gifford RM (1995) Whole plant respiration and photosynthesis of wheat under increased  $\text{CO}_2$  concentration and temperature – long-term vs short-term distinctions for modelling. *Global Change Biology* **1**, 385–396. doi:10.1111/j.1365-2486.1995.tb00037.x
- Grassi G, Meir P, Cromer R, Tompkins D, Jarvis PG (2002) Photosynthetic parameters in seedlings of *Eucalyptus grandis* as affected by rate of nitrogen supply. *Plant, Cell & Environment* **25**, 1677–1688. doi:10.1046/j.1365-3040.2002.00946.x
- Griffin KL, Turnbull MH (2013) Light saturated RuBP oxygenation by Rubisco is a robust predictor of light inhibition of respiration in *Triticum aestivum* L. *Plant Biology* **15**, 769–775. doi:10.1111/j.1438-8677.2012.00703.x
- Griffin KL, Turnbull M, Murthy R (2002a) Canopy position affects the temperature response of leaf respiration in *Populus deltoides*. *New Phytologist* **154**, 609–619. doi:10.1046/j.1469-8137.2002.00410.x
- Griffin KL, Turnbull M, Murthy R, Lin GH, Adams J, Farnsworth B, Mahato T, Bazin G, Potasnak M, Berry JA (2002b) Leaf respiration is differentially affected by leaf vs stand-level night-time warming. *Global Change Biology* **8**, 479–485. doi:10.1046/j.1365-2486.2002.00487.x
- Griffin KL, Anderson OR, Tissue DT, Turnbull MH, Whitehead D (2004) Variations in dark respiration and mitochondrial numbers within needles of *Pinus radiata* grown in ambient or elevated  $\text{CO}_2$  partial pressure. *Tree Physiology* **24**, 347–353. doi:10.1093/treephys/24.3.347
- Gulias J, Flexas J, Abadia A, Madrano H (2002) Photosynthetic responses to water deficit in six Mediterranean sclerophyll species: possible factors explaining the declining distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species. *Tree Physiology* **22**, 687–697. doi:10.1093/treephys/22.10.687
- Heskel M, Anderson OR, Atkin OK, Turnbull MH, Griffin KL (2012) Leaf- and cell-level carbon cycling responses to a nitrogen and phosphorus gradient in two Arctic tundra species. *American Journal of Botany* **99**, 1702–1714. doi:10.3732/ajb.1200251
- Heskel M, Greaves H, Kornfeld A, Gough L, Atkin O, Turnbull M, Shaver G, Griffin KL (2013) Differential physiological responses to environmental change promote woody shrub expansion. *Ecology and Evolution* **3**, 1149–1162. doi:10.1002/ecc3.525
- Heskel M, Bitterman DS, Atkin O, Turnbull MH, Griffin KL (2014) Seasonality of foliar respiration in two dominant plant species from the Arctic tundra: response to long-term warming and short-term temperature variability. *Functional Plant Biology* **41**, 287–300. doi:10.1071/FP13137
- Hoefnagel MHN, Atkin OK, Wiskich JT (1998) Interdependence between chloroplasts and mitochondria in the light and the dark. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1366**, 235–255. doi:10.1016/S0005-2728(98)00126-1
- Hurry VM, Tobiaeson M, Kromer S, Gardstrom P, Oquist G (1995) Mitochondria contribute to increased photosynthetic capacity of leaves of winter rye (*Secale cereale* L.) following cold-hardening. *Plant, Cell & Environment* **18**, 69–76. doi:10.1111/j.1365-3040.1995.tb00545.x
- Hurry V, Igamberdiev AU, Keerberg O, Pärnik TR, Atkin OK, Zaragoza-Castells J, Gardeström P (2005) Respiration in photosynthetic cells: gas exchange components, interactions with photorespiration and the operation of mitochondria in the light. In 'Advances in photosynthesis and respiration: respiration and the environment'. (Eds H Lambers and M Ribas-Carbo) pp. 43–61. (Springer: Dordrecht, The Netherlands).
- Igamberdiev AU, Romanowska E, Gardstrom P (2001) Photorespiratory flux and mitochondrial contribution to energy and redox balance of barley leaf protoplasts in the light and during light-dark transitions. *Journal of Plant Physiology* **158**, 1325–1332. doi:10.1078/0176-1617-00551
- IPCC (2007) 'Climate change 2007 – the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.' (Cambridge University Press: Cambridge, UK).
- Kirschbaum MUF, Farquhar GD (1987) Investigation of the  $\text{CO}_2$  dependence of quantum yield and respiration in *Eucalyptus pauciflora*. *Plant Physiology* **83**, 1032–1036. doi:10.1104/pp.83.4.1032
- Koide RT, Robichaux RH, Morse SR, Smith CM (1989) Plant water status, hydraulic resistance and capacitance. In 'Plant physiological ecology'. (Eds RW Pearcy, J Ehleringer, HA Mooney, PW Rundel) pp. 161–184. (Chapman & Hall: London).
- Kok B (1948) A critical consideration of the quantum yield of *Chlorella*-photosynthesis. *Enzymologia* **13**, 1–56.
- Krömer S (1995) Respiration during photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**, 45–70. doi:10.1146/annurev.pp.46.060195.000401
- Larigauderie A, Körner C (1995) Acclimation of leaf dark respiration to temperature in alpine and lowland plant species. *Annals of Botany* **76**, 245–252. doi:10.1006/anbo.1995.1093
- Lloyd J, Shibistova O, Zolotoukhina D, Kolle O, Armeth A, Wirth C, Styles JM, Tchekbakova NM, Schulze ED (2002) Seasonal and annual variations in the photosynthetic productivity and carbon balance of a central Siberian pine forest. *Tellus. Series B, Chemical and Physical Meteorology* **54**, 590–610. doi:10.3402/tellusb.v54i5.16689
- Loomis RS, Amthor JS (1999) Yield potential, plant assimilatory capacity, and metabolic efficiencies. *Crop Science* **39**, 1584–1596. doi:10.2135/cropsci1999.3961584x
- Loveys BR, Atkinson LJ, Sherlock DJ, Roberts RL, Fitter AH, Atkin OK (2003) Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast- and slow-growing plant species. *Global Change Biology* **9**, 895–910. doi:10.1046/j.1365-2486.2003.00611.x
- McLaughlin BC, Xu C-Y, Rastetter EB, Griffin KL (2014) Predicting ecosystem carbon balance in a warming Arctic: the importance of long-term thermal acclimation potential and inhibitory effects of light on respiration. *Global Change Biology* **20**, 1901–1912. doi:10.1111/gcb.12549
- Mercado LM, Huntingford C, Gash JHC, Cox PM, Jogireddy V (2007) Improving the representation of radiation interception and photosynthesis for climate model applications. *Tellus. Series B, Chemical and Physical Meteorology* **59**, 553–565. doi:10.1111/j.1600-0889.2007.00256.x
- Mitchell KA, Bolstad PV, Vose JM (1999) Interspecific and environmentally induced variation in foliar dark respiration among eighteen southeastern deciduous tree species. *Tree Physiology* **19**, 861–870. doi:10.1093/treephys/19.13.861
- Noguchi K, Yoshida K (2008) Interaction between photosynthesis and respiration in illuminated leaves. *Mitochondrion* **8**, 87–99. doi:10.1016/j.mito.2007.09.003
- Ogaya R, Peñuelas J (2007) Tree growth, mortality and above-ground biomass accumulation in a holm oak forest under a five-year experimental field drought. *Plant Ecology* **189**, 291–299. doi:10.1007/s11258-006-9184-6
- Ogaya R, Peñuelas J (2008) Changes in leaf  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for three Mediterranean tree species in relation to soil water availability. *Acta Oecologica* **34**, 331–338. doi:10.1016/j.actao.2008.06.005
- Ow LF, Griffin KL, Whitehead D, Walcroft AS, Turnbull MH (2008a) Thermal acclimation of leaf respiration but not photosynthesis in *Populus deltoides* × *nigra*. *New Phytologist* **178**, 123–134. doi:10.1111/j.1469-8137.2007.02357.x
- Ow LF, Whitehead D, Walcroft AS, Turnbull MH (2008b) Thermal acclimation of respiration but not photosynthesis in *Pinus radiata*. *Functional Plant Biology* **35**, 448–461. doi:10.1071/FP08104

- Pärmik T, Ivanova H, Keerberg O (2007) Photorespiratory and respiratory decarboxylations in leaves of  $C_3$  plants under different  $CO_2$  concentrations and irradiances. *Plant, Cell & Environment* **30**, 1535–1544. doi:10.1111/j.1365-3040.2007.01725.x
- Peñuelas J, Boada M (2003) A global change-induced biome shift in the Montseny mountains (NE Spain). *Global Change Biology* **9**, 131–140. doi:10.1046/j.1365-2486.2003.00566.x
- Peñuelas J, Sardans J, Estiarte M, Ogaya R, Carnicer J, Coll M, Barbeta A, Rivas-Ubach A, Llusià J, Garbulsky M, Filella I, Jump AS (2013) Evidence of current impact of climate change on life: a walk from genes to the biosphere. *Global Change Biology* **19**, 2303–2338. doi:10.1111/gcb.12143
- Pons TL, Welschen RAM (2002) Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. *Plant, Cell & Environment* **25**, 1367–1372. doi:10.1046/j.1365-3040.2002.00911.x
- 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. doi:10.1104/pp.94.2.621
- R Development Core Team (2008) 'R: A language and environment for statistical computing.' (R Foundation for Statistical Computing: Vienna) Available at <http://www.R-project.org/> [Verified 6 May 2017].
- Rodríguez-Calcerrada J, Jaeger C, Limousin JM, Ourcival JM, Joffre R, Rambal S (2011) Leaf  $CO_2$  efflux is attenuated by acclimation of respiration to heat and drought in a Mediterranean tree. *Functional Ecology* **25**, 983–995. doi:10.1111/j.1365-2435.2011.01862.x
- Ryan MG (1995) Foliar maintenance respiration of subalpine and boreal trees and shrubs in relation to nitrogen content. *Plant, Cell & Environment* **18**, 765–772. doi:10.1111/j.1365-3040.1995.tb00579.x
- Sabaté S, Gracia CA, Sánchez A (2002) Likely effects of climate change on growth of *Quercus ilex*, *Pinus halepensis*, *Pinus pinaster*, *Pinus sylvestris* and *Fagus sylvatica* forests in the Mediterranean region. *Forest Ecology and Management* **162**, 23–37. doi:10.1016/S0378-1127(02)00048-8
- Searle SY, Bitterman DS, Thomas S, Griffin KL, Atkin OK, Turnbull MH (2011) Respiratory alternative oxidase responds to both low- and high-temperature stress in *Quercus rubra* leaves along an urban–rural gradient in New York. *Functional Ecology* **25**, 1007–1017. doi:10.1111/j.1365-2435.2011.01875.x
- Shapiro JB, Griffin KL, Lewis JD, Tissue DT (2004) Response of *Xanthium strumarium* leaf respiration in the light to elevated  $CO_2$  concentration, nitrogen availability and temperature. *New Phytologist* **162**, 377–386. doi:10.1111/j.1469-8137.2004.01046.x
- Tcherkez G, Cornic G, Bligny R, Gout E, Ghashghaie J (2005) *In vivo* respiratory metabolism of illuminated leaves. *Plant Physiology* **138**, 1596–1606. doi:10.1104/pp.105.062141
- Tcherkez G, Bligny R, Gout E, Mahe A, Hodges M, Cornic G (2008) Respiratory metabolism of illuminated leaves depends on  $CO_2$  and  $O_2$  conditions. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 797–802. doi:10.1073/pnas.0708947105
- Tcherkez G, Mahe A, Gauthier P, Mauve C, Gout E, Bligny R, Cornic G, Hodges M (2009) *In folio* respiratory fluxomics revealed by  $^{13}C$  isotopic labeling and H/D isotope effects highlight the noncyclic nature of the tricarboxylic acid 'cycle' in illuminated leaves. *Plant Physiology* **151**, 620–630. doi:10.1104/pp.109.142976
- Tcherkez G, Boex-Fontvieille E, Mahé A, Hodges M (2012) Respiratory carbon fluxes in leaves. *Current Opinion in Plant Biology* **15**, 308–314. doi:10.1016/j.pbi.2011.12.003
- Tcherkez G, Gauthier P, Buckley TN, Busch FA, Barbour MM, Bruhn D, Heskell MA, Gong XY, Crous K, Griffin KL, Way DA, Turnbull MH, Adams MA, Atkin OK, Bender M, Farquhar GD, Cornic G (2017) Tracking the origins of the Kok effect, 70 years after its discovery. *New Phytologist* **214**, 506–510. doi:10.1111/nph.14527
- Tissue DT, Lewis JD, Wullschlegel SD, Amthor JS, Griffin KL, Anderson R (2002) Leaf respiration at different canopy positions in sweetgum (*Liquidambar styraciflua*) grown in ambient and elevated concentrations of carbon dioxide in the field. *Tree Physiology* **22**, 1157–1166. doi:10.1093/treephys/22.15-16.1157
- Tjoelker MG, Craine JM, Wedin D, Reich PB, Tilman D (2005) Linking leaf and root trait syndromes among 39 grassland and savannah species. *New Phytologist* **167**, 493–508. doi:10.1111/j.1469-8137.2005.01428.x
- Tjoelker MG, Oleksyn J, Lorenc-Plucinska G, Reich PB (2009) Acclimation of respiratory temperature responses in northern and southern populations of *Pinus banksiana*. *New Phytologist* **181**, 218–229. doi:10.1111/j.1469-8137.2008.02624.x
- Turnbull MH, Whitehead D, Tissue DT, Schuster WSF, Brown KJ, Griffin KL (2003) Scaling foliar respiration in two contrasting forest canopies. *Functional Ecology* **17**, 101–114. doi:10.1046/j.1365-2435.2003.00713.x
- Turnbull MH, Tissue DT, Griffin KL, Richardson SJ, Peltzer DA, Whitehead D (2005) Respiration characteristics in temperate rainforest tree species differ along a long-term soil-development chronosequence. *Oecologia* **143**, 271–279. doi:10.1007/s00442-004-1803-0
- Valladares F, Zaragoza-Castells J, Sanchez-Gomez D, Matesanz S, Alonso B, Portsmouth A, Delgado A, Atkin OK (2008) Is shade beneficial for Mediterranean shrubs experiencing periods of extreme drought and late-winter frosts? *Annals of Botany* **102**, 923–933. doi:10.1093/aob/mcn182
- Villar R, Held AA, Merino J (1994) Comparison of methods to estimate dark respiration in the light in leaves of two woody species. *Plant Physiology* **105**, 167–172. doi:10.1104/pp.105.1.167
- von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376–387. doi:10.1007/BF00384257
- Wang XZ, Lewis JD, Tissue DT, Seemann JR, Griffin KL (2001) Effects of elevated atmospheric  $CO_2$  concentration on leaf dark respiration of *Xanthium strumarium* in light and in darkness. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 2479–2484. doi:10.1073/pnas.051622998
- Wang XZ, Anderson OR, Griffin KL (2004) Chloroplast numbers, mitochondrion numbers and carbon assimilation physiology of *Nicotiana sylvestris* as affected by  $CO_2$  concentration. *Environmental and Experimental Botany* **51**, 21–31. doi:10.1016/S0098-8472(03)00057-1
- Way DA, Oren R (2010) Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree Physiology* **30**, 669–688. doi:10.1093/treephys/tpq015
- Whitehead D, Boelman N, Turnbull M, Griffin K, Tissue D, Barbour M, Hunt J, Richardson S, Peltzer D (2005) Photosynthesis and reflectance indices for rainforest species in ecosystems undergoing progression and retrogression along a soil fertility chronosequence in New Zealand. *Oecologia* **144**, 233–244. doi:10.1007/s00442-005-0068-6
- Wingate L, Seibt U, Moncrieff JB, Jarvis PG, Lloyd J (2005) Variations in  $^{13}C$  discrimination during  $CO_2$  exchange by *Picea sitchensis* branches in the field. *Plant, Cell & Environment* **30**, 600–616. doi:10.1111/j.1365-3040.2007.01647.x
- Wohlfahrt G, Bahn M, Haslwanter A, Newsely C, Cernusca A (2005) Estimation of daytime ecosystem respiration to determine gross primary production of a mountain meadow. *Agricultural and Forest Meteorology* **130**, 13–25. doi:10.1016/j.agrformet.2005.02.001
- Wright IJ, Reich PB, Atkin OK, Lusk CH, Tjoelker MG, 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. doi:10.1111/j.1469-8137.2005.01590.x
- Wythers KR, Reich PB, Tjoelker MG, Bolstad PB (2005) Foliar respiration acclimation to temperature and temperature variable  $Q_{10}$  alter ecosystem carbon balance. *Global Change Biology* **11**, 435–449. doi:10.1111/j.1365-2486.2005.00922.x
- Xu CY, Griffin KL (2006) Seasonal variation in the temperature response of leaf respiration in *Quercus rubra*: foliage respiration and leaf

- properties. *Functional Ecology* **20**, 778–789. doi:[10.1111/j.1365-2435.2006.01161.x](https://doi.org/10.1111/j.1365-2435.2006.01161.x)
- Xu M, Debiase TA, Qi Y, Goldstein A, Liu Z (2001) Ecosystem respiration in a young ponderosa pine plantation in the Sierra Nevada Mountains, California. *Tree Physiology* **21**, 309–318. doi:[10.1093/treephys/21.5.309](https://doi.org/10.1093/treephys/21.5.309)
- Zaragoza-Castells J, Sanchez-Gomez D, Valladares F, Hurry V, Atkin OK (2007) Does growth irradiance affect temperature dependence and thermal acclimation of leaf respiration? Insights from a Mediterranean tree with long-lived leaves. *Plant, Cell & Environment* **30**, 820–833. doi:[10.1111/j.1365-3040.2007.01672.x](https://doi.org/10.1111/j.1365-3040.2007.01672.x)
- Zaragoza-Castells J, Sanchez-Gomez D, Hartley IP, Matesanz S, Valladares F, Lloyd J, Atkin OK (2008) 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. *Functional Ecology* **22**, 172–184.