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

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33

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

58

59

60 *Keywords: Kok effect, leaf functional traits, leaf dark respiration, leaf light respiration, leaf*  
61 *mass per unit area, nitrogen, photosynthesis, plasticity, season, soil moisture, temperature*

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## 66 Introduction

67

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

78

79 Although leaf  $R$  takes place in both light ( $R_L$ ) and darkness ( $R_D$ ), leaf  $R$  is typically  
80 lower during the day when photosynthesis is concurrently occurring (Brooks and Farquhar  
81 1985), even when re-fixation of respiratory  $\text{CO}_2$  is taken into account (Pärnik *et al.* 2007). The  
82 metabolic basis of the light inhibition of leaf  $R$  is beginning to be better understood (Tcherkez  
83 *et al.* 2017), with factors such as cellular energy status, demand for TCA cycle intermediates,  
84 engagement of the pentose phosphate pathway and/or rates of photorespiration ( $V_o$ ) being  
85 involved in the degree of inhibition (Hurry *et al.* 2005; Tcherkez *et al.* 2005; Tcherkez *et al.*  
86 2008; Buckley and Adams 2011; Tcherkez *et al.* 2012). The extent to which  $R$  is reduced in  
87 the light is, potentially, highly variable among species and environments. For example, when  
88 measured at a common temperature ( $T$ ), the rate of respiration in the light can be as little as 0.2  
89 of the darkened rate (Atkin *et al.* 2000; Ayub *et al.* 2011). On the other hand, light may have  
90 little to no inhibitory effect in some cases, particularly at low measuring  $T$ s (Atkin *et al.* 2000;  
91 Zaragoza-Castells *et al.* 2007). These large potential variations between measured  $R_L$  and  $R_D$   
92 under different experimental conditions are important because the level to which they are  
93 actually expressed in the field will determine the extent to which variations in  $R$  impact on net  
94  $\text{CO}_2$  exchange in individual plants and whole ecosystems. Failure to account for light inhibition  
95 of leaf  $R$  leads to large over- and under-estimates of ecosystem respiration and net primary  
96 productivity, respectively (Lloyd *et al.* 2002; Wohlfahrt *et al.* 2005; Atkin *et al.* 2006; Wingate  
97 *et al.* 2007; McLaughlin *et al.* 2014).

98

99           Establishing the extent to which species differ in rates of  $R_D$  and  $R_L$ , and the impact of  
100 natural environmental gradients on variations of both processes is crucial to successful  
101 incorporation of light inhibition of leaf  $R$  into large-scale models. Furthermore, little is known  
102 about the impact of soil water status or water stress on plant  $R$  (Atkin and Macherel 2009). The  
103 response of  $R_D$  to leaf water content may lag behind that of photosynthesis and may also be  
104 biphasic i.e., it decreases in the initial stages of water stress (lower energy demands for growth)  
105 and may increase with additional stress-related demands e.g. osmoregulation or induced  
106 senescence (Ghashghaie *et al.* 2001; Gulias *et al.* 2002; Flexas *et al.* 2005; Flexas *et al.* 2006).  
107 The environmental response of  $R_D$  will also be complicated by the fact that large shifts in daily  
108 mean  $T$  often co-vary with fluctuations in water availability, with drought often being  
109 associated with high  $T$ s. A number of controlled-environment studies have reported large  
110 variations in leaf  $R_D$  among different species (Atkin and Day 1990; Poorter *et al.* 1990; Loveys  
111 *et al.* 2003; Tjoelker *et al.* 2005; Atkin *et al.* 2008), with a smaller number of lab-based studies  
112 showing that genotype is also a strong determinant of  $R_L$  (Villar *et al.* 1994; Atkin *et al.* 1997).  
113 It is also known that  $R_D$  varies in response to changes in the abiotic environment, both in nature  
114 and under lab-based conditions (Larigauderie and Körner 1995; Ryan 1995; Atkin *et al.* 2000b;  
115 Griffin *et al.* 2002a; Griffin *et al.* 2002b; Griffin *et al.* 2004; Turnbull *et al.* 2005; Wright *et al.*  
116 2006; Xu and Griffin 2006; Tjoelker *et al.* 2009; Searle *et al.* 2011).  $R_D$  often acclimates to  
117 changes in long-term growth  $T$  (Atkin *et al.* 2000a; Atkin and Tjoelker 2003; Atkin *et al.* 2005;  
118 Wythers *et al.* 2005; Ow *et al.* 2008a; Ow *et al.* 2008b; Way and Oren 2010), so that the  $T$   
119 response of respiration to short-term and long-term changes in  $T$  is often different. Likewise,  
120 there is evidence from laboratory-based studies that light inhibition of leaf  $R$  is environmentally  
121 dependent [e.g. as a result of changes in measurement  $T$  (Atkin *et al.* 2000; Zaragoza-Castells  
122 *et al.* 2007; Shapiro *et al.* 2004; Griffin and Turnbull 2013), and/or atmospheric growth  $CO_2$   
123 concentration (Shapiro *et al.* 2004; Wang *et al.* 2001; Wang *et al.* 2004; Tissue *et al.* 2002)].  
124 Although we have previously shown that the balance between  $R_D$  and  $R_L$  changes in response  
125 to field gradients of soil fertility (Atkin *et al.* 2013; Heskell *et al.* 2012; 2013) and imposition  
126 of water stress under controlled conditions (Ayub *et al.* 2011; Crous *et al.* 2012), to date, no  
127 study has assessed how gradients in soil moisture impact on light inhibition of leaf  $R$  under  
128 natural, field conditions. We might predict that drought and/or high  $T$  induced changes in leaf  
129 metabolism, particularly in  $V_o$ , would result in predictable changes in light inhibition of  $R$   
130 (Griffin and Turnbull 2013). Moreover, we lack data on how the balance between  $R_L$  and  $R_D$   
131 varies seasonally (Heskell *et al.* 2013a). It is vital we establish the extent of inhibition of  $R$  in  
132 areas of contrasting water availability if we aim to more accurately predict future rates of C

133 exchange in water-limited environments. Overall, we lack comprehensive field-based studies  
134 that quantify variations in  $R_D$  and  $R_L$  among dominant species growing along natural gradients  
135 of water availability.

136

137 Mediterranean ecosystems offer excellent opportunities to test changes in respiration  
138 under water-limited conditions, such as those that now prevail (Peñuelas and Boada 2003;  
139 Peñuelas *et al.* 2013) and the even drier conditions that are projected for the coming decades  
140 (IPCC 2007). In Mediterranean ecosystems, the availability of water is the greatest  
141 environmental constraint on plant growth, due to the occurrence of high summer  $T$ s and low  
142 rainfall. Here, our aim was to determine if the dominant Mediterranean tree *Quercus ilex*  
143 (Holm oak) and co-occurring trees of Mediterranean forests responded to seasonal changes in,  
144 and natural gradients of, water availability, with changes in  $R$  (as measured by  $\text{CO}_2$  evolution),  
145 and particularly in the inhibition of  $R$  by light. We have previously measured large seasonal  
146 shifts in  $T$  response curves of  $R_D$  in *Q. ilex* subsp. *ballota* in central Spain that were consistent  
147 with thermal acclimation (Zaragoza-Castells *et al.* 2008). However, it is possible that seasonal  
148 variations in water availability may have contributed to such shifts in the daily  $T$  response  
149 curves (Crous *et al.* 2011; Rodríguez-Calcerrada *et al.* 2011). Our current study extends this  
150 research by quantifying seasonal changes in both  $R_D$  and  $R_L$  and associated leaf traits ( $A$ ,  $M_A$ ,  
151 N content) over a 12-month period in the same forest (Villar de Cobeta, central Spain) in *Q.*  
152 *ilex* subsp. *ballota* trees. We also increased the geographical and species scope of our study by  
153 conducting an additional single mid-summer campaign in the Prades Mountains (Catalonia,  
154 NE Spain), with *Q. ilex* subsp. *ilex* forest along a gradient of decreasing water availability from  
155 a stream course in the bottom of a valley to dry sites approaching the ridge-line. In addition, at  
156 the drier end of this gradient, precipitation has been further experimentally restricted for the  
157 last 13 years, reducing soil moisture by 11% with respect to ambient conditions (Ogaya and  
158 Peñuelas 2007). Based on previous findings that conditions which tend to suppress  $V_o$  (e.g. high  
159  $[\text{CO}_2]$  and/or low  $[\text{O}_2]$ ) also decrease  $R_L/R_D$  (Wang *et al.* 2001; Shapiro *et al.* 2004; Crous *et*  
160 *al.* 2012; Griffin and Turnbull 2013), and without considering other processes (e.g. increased  
161 use of stored carbon reserves (e.g. citrate) in the light) which might influence  $R_L$  in the short-  
162 term (Tcherkez *et al.* 2012), we hypothesised that  $R_L/R_D$  would be lowest where water  
163 availability was greatest, and increase at drier sites where stomatal limitations on gas exchange  
164 increase photorespiration. It is worth noting that, considering the influence of water availability  
165 on carbon assimilation, it is possible to interpret a link between photorespiration and the degree  
166 of light inhibition of  $R$  measured in the Kok effect in a number of ways – (i) there may be a

167 direct effect of water availability on internal CO<sub>2</sub> mole fraction, which in turn affects  $V_o$  and  
168 thus  $R_L/R_D$ ; (ii) water availability may have specific photorespiration-independent effects on  
169  $R_L/R_D$ ; or (iii) water availability may affect apparent  $R_L/R_D$  because the Kok-effect  
170 measurement is itself influenced (at least in part) by photorespiration (Tcherkez *et al.* 2012).  
171 Here our focus is to determine patterns of field response rather than attempt to provide a  
172 resolution to this issue.

173

## 174 **Materials and Methods**

### 175 *Study sites*

176 *Villar de Cobeta* - this field site was located 175 km northeast of Madrid in the Iberic System  
177 Mountain Range (950 m a.s.l.) at Los Cerrillos Biological Station, Villar de Cobeta (40°48' N  
178 2°12' W), within the Alto Tajo Natural Park (Guadalajara, Spain). The experimental work was  
179 located in two sites - a steep (20°), south-facing upper slope which was the location for our  
180 previous work (Zaragoza-Castells *et al.* 2008), and an adjacent lower slope, stream-side site.  
181 *Quercus ilex* subsp. *ballota* dominated the vegetation at both sites. The area has a continental  
182 Mediterranean climate, with hot, dry summers and cold winters, and is characterized by large  
183 diurnal and seasonal variations in air  $T$  (diurnal variations in  $T$  in the order of 25 °C are common,  
184 with leaf  $T$ s potentially reaching near 50 °C in summer and -15 °C in winter). Both sites have  
185 shallow, poorly developed soils formed mainly from Cretaceous and Jurassic limestone with a  
186 limited capacity of the soil to retain water (Valladares *et al.* 2008). Meteorological stations  
187 provided environmental data as described in Zaragoza-Castells *et al.* (2008). From December  
188 2006 to November 2007 we monitored microclimatic conditions at each of the study sites. At  
189 the upper slope site, we installed a HOBO ® weather station equipped with a rain gauge (RGA-  
190 M0XX) and a soil moisture sensor (S-SMA-M003) inserted at 30 cm depth. Readings of each  
191 sensor were recorded every 30 minutes with a data logger (HOBO ® H21-001 all components  
192 from HOBO ® Onset Computer Corporation Bourne, MA, USA). At the lower slope site, we  
193 installed a second station with a soil moisture probe (ECH<sub>2</sub>O EC-20 Decagon Devices,  
194 Pullman, WA, USA). Readings were recorded every 30 minutes with a HOBO ® H08-006-04  
195 data logger.

196

197 *Prades* - the second study was undertaken in the Prades Mountains in southern Catalonia (NE  
198 Spain; 41°21' N, 1°2' E), also at 950 m a.s.l. on a south-facing slope (20°). The soil is a Dystric  
199 Cambisol over Paleozoic schist, and its depth ranges from 35 to 90 cm. This forest has been

200 undisturbed for the last 60 years, and the maximum height of the dominant species is about 6–  
201 10 m. Plant community composition is strongly influenced by topographical changes in soil  
202 water availability (Table 1). In this study we identified four community types: (1) Riparian  
203 forest - the moistest sites along stream courses in valleys dominated by a number of deciduous  
204 species (*Tilia platyphyllos*, *Sorbus torminalis* (L.) Crantz and *Acer monspessulanum* L.); (2)  
205 Mid-slope forest - in which Holm Oak (*Q. ilex* subsp. *ilex*) dominates and deciduous species  
206 become much less common, being replaced by evergreen species such as *Phillyrea latifolia*  
207 and *Arbutus unedo* L.; (3) Dry forest - on upper slopes approaching ridge lines with a dense,  
208 multi-stemmed crown and dominated by *Q. ilex*, *Phillyrea latifolia* and *Arbutus unedo* L.; (4)  
209 Shrubland - at the ridge line, these trees continue to dominate but grow in a stunted shrub form  
210 (2-3 m tall) and the canopy opens in places to allow shrub species to establish.

211  
212 In addition to the natural gradient described above, at Prades we sampled in a long-term  
213 rainfall exclusion experiment established in communities 3 and 4 (Ogaya and Peñuelas 2007)  
214 to match the conditions projected in climatic and ecophysiological models for Mediterranean  
215 forests in the coming decades (Sabate *et al.* 2002; IPCC 2007). In each of these communities,  
216 four control and four treatment plots 15 m x 10 m were established at the same altitude along  
217 the mountain face. In the treatment plots, rainfall was partially excluded through the suspension  
218 of PVC strips 0.5–0.8 m above the soil (covering 30% of the soil surface) and the excavation  
219 of a ditch 0.8-m deep at the upper part of the plots to intercept runoff water. Water intercepted  
220 by the strips and ditches was conducted around the plots, below their bottom edges. The other  
221 three plots received no treatment and acted as control plots. The rain exclusion treatment began  
222 in January 1999, 13 years before the sampling for this study. An automated meteorological  
223 station installed between the rainfall exclusion plots monitored *T*, photosynthetically active  
224 radiation, air humidity and precipitation continuously during the experiment. Soil water content  
225 was determined gravimetrically at 30cm depth at all sites at the mid-point of the experimental  
226 campaign (July 2013).

227

#### 228 *Leaf sampling and gas exchange measurements*

229 At the Villar de Cobeta study site, we performed seven measurement campaigns from  
230 December 2006 to November 2007. Four individual trees were identified at both the upper and  
231 lower slope sites, with each tree representing an independent replicate. For each sampling  
232 campaign, leaf physiological measurements were made using a single south-east facing,



233 attached, fully-expanded, mature leaf from each replicate tree. Different leaves were used on  
234 each campaign, with the measured leaves being harvested for determination of chemical–  
235 structural characteristics at the end of each sampling campaign.

236

237         Within the Villar de Cobeta seasonal study, additional measurements of the  
238 instantaneous response of  $R_D$  to  $T$  were made at changing ambient air  $T$  and at ambient relative  
239 humidity (typically 35%–60%) to investigate the extent of thermal acclimation of  $R_D$ .  
240 Measurements of leaf  $R$  took place at regular intervals during the day and night; during the day,  
241 leaves were darkened for 30 min prior to measurement to avoid post-illumination transients.  
242 As Zaragoza-Castells *et al.* (2008) previously found that there were no differences in leaf  $R$   
243 measured during the day and night at equivalent  $T$ ; here,  $T$  response curves were fitted to data  
244 from a combination of night and day measurements. The measurements were carried out at  
245 regular intervals (approximately every 2 h) through the day (interspersed between  
246 measurements of  $R$  on the same leaves), with net CO<sub>2</sub> exchange ( $A_{\text{net}}$ ) being measured at the  
247 prevailing ambient irradiance. On each sampling month, measurements were made on four  
248 replicate leaves.

249

250         The Prades study took place over a 2-week period in late July 2013 (i.e. mid-summer  
251 when the gradient of water availability was most pronounced). At each site, we sampled  
252 detached branches of sun-lit, upper canopy foliage (using a pruning pole) from 6 individuals  
253 of each of 3 of the most abundant species (Table 1) at each site along the gradient. Species  
254 composition changes along the gradient, but it was possible to sample several species on  
255 multiple, consecutive sites (Table 1). Thus, in addition to providing insights into how leaf gas  
256 exchange of the dominant species might vary among sites, our sampling strategy enabled an  
257 assessment of how individual species responded to site-to-site variations in environment  
258 (especially *Q. ilex*, which was sampled at 5 of the 6 sites). Sampling took place in the early  
259 evening; stems were immediately re-cut under water and the branches transported to a nearby  
260 field laboratory and allowed to equilibrate overnight for subsequent gas exchange  
261 measurements through the morning and early afternoon period. Past work has shown that leaf  
262 gas exchange rates can be comparable in cut branches and *in situ* leaves of forest species for  
263 many hours after removal (Mitchell *et al.* 1999; Turnbull *et al.* 2003). In addition, all gas  
264 exchange measurements were corrected for  $C_i$  according to Kirschbaum and Farquhar

265 (1987)(see details below), so any partial stomatal closure that may have occurred was  
266 accounted for.

267

268 For both experimental study sites (i.e. at the Villar de Cobeta and Prades field sites),  
269 light response curves of  $A_{\text{net}}$  were measured on the most recently fully expanded leaves using  
270 Li-Cor 6400 portable photosynthesis systems (Li-Cor Inc., Lincoln, NE, USA) equipped with  
271 a CO<sub>2</sub> controller and a with red-blue light source (6400-02B). The seasonal measurements at  
272 Villar de Cobeta were made at the prevailing day-time  $T$  (see Fig. 1) and the moisture gradient  
273 measurements at Prades were made with the block  $T$  set to the mid-summer prevailing  $T$  of  
274 25°C. In all cases, light-response measurements were conducted under ambient [CO<sub>2</sub>] of 400  
275 ppm controlled using the LI-6400 control system in a large and well ventilated field laboratory;  
276 as such, it was not necessary to correct for CO<sub>2</sub> diffusion through the chamber gasket (Pons  
277 and Welschen 2002). Light-saturated photosynthesis ( $A_{\text{sat}}$ ) was measured at 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$   
278 <sup>1</sup> photosynthetic photon flux density (PPFD) and a relative humidity of 40-70%, and after  
279 leaves had been exposed to saturating irradiance in the cuvette for 10-20 minutes. After  
280 measurement of  $A_{\text{sat}}$ , the irradiance response of net CO<sub>2</sub> exchange was measured, beginning at  
281 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , followed by 500, 250, 150, 100, 90, 80, and then every 5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to 10  
282 and ending at zero  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (i.e. darkness). Additional measurements of net CO<sub>2</sub> exchange  
283 in darkness were conducted after a further 10 min of darkness – these were not statistical  
284 different from values of  $R_{\text{D}}$  measured directly at the end of light response curves. Flow rate  
285 through the chamber was set to 300  $\mu\text{mol s}^{-1}$ . Relationships between key gas exchange  
286 characteristics ( $A_{\text{sat}}$ ,  $R_{\text{D}}$ ) and soil water content have been included for reference in Fig. S2.

287

288 To estimate rates of leaf respiration in the light ( $R_{\text{L}}$ ), we used the Kok (1948) method,  
289 where  $R_{\text{L}}$  was estimated from the y-axis intercept of a first order linear regression fitted to  $A$ -  
290 irradiance plots to measurements made over the 20-60  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  irradiance range  
291 (Ayub *et al.* 2011); in some species, data was curvilinear at irradiances above 70  $\mu\text{mol m}^{-2} \text{s}^{-1}$   
292 PPFD (data now shown). Averaged across all sites, the  $r^2$  values of the first order regression  
293 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  
294 CO<sub>2</sub> concentrations ( $C_i$ ) tend to increase with decreases in irradiance, resulting in reduced rates  
295 of photorespiration and increased rates of carboxylation; this in turn results in a decrease in the

296 slope of  $A_{\text{net}}$ -irradiance plots in the linear region (Villar *et al.* 1994). Because of this, rates of  
297  $R_L$  were adjusted (by iteration) to ensure that the intercept of plots of photosynthetic electron  
298 transport ( $J$ ) against irradiance are minimized (Kirschbaum and Farquhar 1987).  $J$  was  
299 calculated according to Farquhar and von Caemmerer (1982):

300  
301 
$$J = \frac{[(4 \times (A_{\text{net}} + R_L)) \times (C_i + 2\Gamma^*)]}{(C_i - \Gamma^*)} \quad (\text{Eqn 1})$$

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

306

#### 307 *Leaf structural traits and chemical composition*

308 Leaves used for the light response curves were weighed for fresh mass, photographed [to enable  
309 subsequent calculation of leaf area using *Image J* software (<http://rsbweb.nih.gov/ij/>)] and then  
310 oven dried at 70°C to constant mass. The mass and area data were used to determine ratios of  
311 leaf dry mass to leaf area ( $M_A$ ). Subsequently, leaf samples were ground in a ball mill and  
312 analysed for tissue nitrogen and phosphorus using either a Technicon Auto-analyzer II (Bran  
313 + Luebbe Pty. Ltd, Norderstedt Germany; Villar de Cobeta samples) or a Eurovector 3011  
314 (Milan, Italy) elemental analyser (Prades samples). For the three species in sites 3-6 of the  
315 Prades forest study, we had access to previous measurements of tissue N in July over 4 different  
316 years (Ogaya and Peñuelas 2008). Based on the finding that leaf N was not significantly  
317 influenced by soil moisture content or rain exclusion treatment, we used a 4-year site average  
318 as our measure of leaf N for these species at these sites (reported only in Table 4). Leaf  
319 carbohydrate content was determined during the Villar de Cobeta study (Loveys *et al.* 2003),  
320 but as there was no significant explanatory power of carbohydrate status on respiratory  
321 parameters (see Fig. S1) we did not repeat these measurements in the subsequent Prades  
322 campaign. Leaf tissue relative water content (RWC) was determined according to Koide *et al.*  
323 (1989) for a representative leaf adjacent to the measurement leaf at the same time as gas  
324 exchange measurements.

325

326 *Statistical analyses*

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

334

## 335 **Results**

336

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

338 Volumetric measurements of water content confirmed strong seasonal changes in soil water  
339 content from highest levels in spring (March, ~15%) to lowest levels in late summer (August-  
340 September, ~5%; Fig. 1a). The lower slope site had slightly lower volumetric water contents  
341 than the upper slope site, but there was no significant difference between the sites at the times  
342 measurements were made (Figure 1a). This is a reflection of the fact that rainfall in the region  
343 was unusually high in winter-spring 2006-2007. Leaf mass per unit area ( $M_A$ ) was significantly  
344 greater at the upper slope site and increased significantly in mid-summer, before declining  
345 during autumn (Table 2, 3). Leaf N content did not differ significantly between site nor time  
346 of year, but phosphorus content ([P]) was significantly greater at the lower-slope site (Table 2,  
347 3). Leaf sugar and starch determinations were associated with relatively large error, and thus  
348 displayed only modest significant responses to site and time of year (Table 2, 3). Leaf  $T_s$   
349 reflected seasonal changes in ambient air  $T$ , with lowest values in late winter (February) and  
350 highest values in mid-summer (August, Fig. 1b). Maximum light-saturated photosynthetic rate  
351 ( $A_{\text{sat}}$ ) largely mirrored changes in soil water content, with maximum rates in the spring period  
352 and declining rates in summer-autumn (Fig. 1c, Table 3). Trees in the upper slope site displayed  
353 higher rates during winter-spring (when rainfall was unusually high), but there was no  
354 difference between sites in the summer-autumn period. The pattern of response of  $A_{\text{sat}}$  was  
355 strongly reflected in the seasonal response of  $g_{\text{sat}}$ , with stomatal conductance declining from

356 high values in June to low values in late summer (Fig. 1d, Table 3). This was also reflected in  
357 significant changes in internal CO<sub>2</sub> concentration ( $C_i$ , Table 3).

358

359 Foliar respiration in the dark ( $R_D$ , measured at ambient seasonal  $T_s$  at the end of light  
360 response curves) did not display a significant seasonal response (Table 3), with the exception  
361 of a significant increase at the single time point during a very hot period in August (Fig. 1e).  
362 Inhibition of  $R$  by light (i.e. the Kok effect) was consistently observed in the light-limited phase  
363 of photosynthetic light responses. As a result, we were able to estimate  $R_L$  and thus calculate  
364 the extent of light inhibition of  $R_D$ . Foliar respiration in the light ( $R_L$ ) displayed a very similar  
365 seasonal response to  $R_D$  (Fig. 1f), and so  $R_L/R_D$ , while displaying a range of 0.5 to 0.8 with  
366 large SEM in some months, did not display a significant seasonal response or differ between  
367 the two sites (Fig. 1g, Table 3). Averaged over all seven months,  $R_L/R_D$  was  $0.66 \pm 0.05$  SEM  
368 ( $n=14$ ). The relationship between  $R_L$  and  $R_D$  was a very strong one, and did not differ between  
369 trees at the two sites (Fig. 1h, Table 3). There was no significant relationship between seasonal  
370 changes in  $R_L/R_D$  and soil volumetric water content ( $R_L/R_D = -0.012 * SWC + 0.76$ ,  $P=0.18$ ) or  
371 leaf  $T$  ( $R_L/R_D = 0.007 * T_{leaf} + 0.51$ ,  $P = 0.11$ ). The single-point measurements of  $R_D$  in Figure  
372 1e, when plotted against leaf  $T$ , indicate a relatively muted response in the range of leaf  $T_s$  from  
373 7 to 30°C (Fig. 2a). In August, when daily ambient and leaf  $T_s$  exceeded 30°C during the period  
374 of that field campaign,  $R_D$  values increased (to values in the range 2-5  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). This  
375 response of  $R_D$  was also reflected, in part, by short-term thermal response measurements  
376 generated over daily cycles of ambient  $T$  (Fig. 3). These show shifts in the thermal response  
377 indicative of partial acclimation to increasing temperatures from winter through spring and  
378 summer, most strongly in trees at the upper-slope site (Fig. 3a).  $R_L/R_D$  was not related to  
379 seasonal changes in leaf  $T$  (Fig. 2b;  $R_L/R_D = 0.005 * T_{leaf} + 0.56$ ,  $P = 0.14$ ) or leaf N or sugar  
380 content (Fig. S1). Significant seasonal changes in  $A$  and increases in  $R$  resulted in a significant  
381 seasonal effect on  $R_D/A_{sat}$  ratio (Table 3), and in particular a significant increase in during the  
382 high  $T_s$  in August (data not shown). Because of the lack of systematic response of  $R_L/R_D$ , the  
383 ratio of leaf  $R_L$  to light-saturated photosynthesis (i.e.  $R_L/A_{sat}$ ) did not vary during the year other  
384 than via direct effects on  $A$  and  $R$  (Table 3).

385

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

387 Volumetric measurements confirmed that there was a significant decrease in soil water content  
388 from the riparian to the shrub sites in mid-summer at Prades (Fig. 4a). Although soil moisture  
389 has been, on average, 11% lower in the rainfall exclusion plots than in the control plots during  
390 the entire experimental period (TDR measurements made between 1999 and 2007 reported by  
391 Ogaya and Peñuelas 2008, and unpublished measurements since 2007), differences between  
392 these plots were not evident in the 30cm deep gravimetric measurements made in the present  
393 mid-summer campaign. Leaf mass per unit area and N content differed significantly with  
394 species and site (Table 5). Lowest average  $M_A$  values were found in the species from the  
395 riparian and slope site (*Tilia platyphyllos* and *Acer monspessulanum*) (Table 4), with site-  
396 averaged values increasing along the gradient (Fig. 4b).  $M_A$  did not vary significantly in *Q. ilex*  
397 over the range it occurred. Leaf nitrogen content on a dry mass basis was significantly greater  
398 at the riparian and slope sites (species means in Table 4) and site average values decreased  
399 from the moister to the drier sites (data not shown). Leaf relative water content differed between  
400 species, but did not differ at sites along the soil moisture gradient (Table 4, 5).

401

402 Table 4 shows average rates of light-saturated photosynthesis ( $A_{\text{sat}}$ ) expressed on a leaf  
403 area basis for each species/site combination. Significant differences were found among site  
404 averages (Fig. 4, Table 5), with *Q. ilex* showing a similar trend. Rates of  $A$  were highest at the  
405 riparian and slope sites and lowest at the dry and shrub sites. This trend was also displayed by  
406 light-saturated stomatal conductance ( $g_{\text{sat}}$ ; species averages Table 4, site averages Fig. 4).  
407 **Because the two parameters changed in concert**, there was no significant species or site effect  
408 on internal  $\text{CO}_2$  concentration ( $C_i$ , Table 4, 5). No significant effect of site was found on leaf  
409  $R_D$  (Table 5), although the riparian site did have a lower rate, on average, than the drier sites  
410 (Fig. 4e) - as a result there was a significant species\*site interaction. Irrespective of whether  
411 site-averages were considered (Fig. 4) or whether rates of leaf  $R$  in individual leaves were  
412 compared (Table 4),  $R_L$  was almost always less than  $R_D$  (i.e. light inhibited leaf  $R$ ), although  
413 the degree of inhibition did vary [with  $R_L = R_D$  (i.e. no light inhibition) in a small number of  
414 cases] as a consequence of the technical challenges in the method.  $R_L$  displayed a similar  
415 response to that of  $R_D$  (Fig. 4f), and thus site-averaged  $R_L/R_D$  did not vary significantly across  
416 the gradient (Fig. 4g, Table 5) and  $R_L$  and  $R_D$  were strongly correlated (Fig. 4h). The degree of  
417 light inhibition in individual species ranged from 0.62 to 0.75 (Table 4), and averaged across

418 all species and sites,  $R_L/R_D$  was  $0.69 \pm 0.01$  SEM ( $n=18$ ). Because of the consistency of  $R_L/R_D$ ,  
419 the ratio of leaf  $R_L$  to light-saturated photosynthesis (i.e.  $R_L/A_{\text{sat}}$ ) did not vary along the gradient  
420 other than via direct effects on  $A$ . There was no significant relationship observed between  $R_L/R_D$   
421 and  $A$  or the oxygenation component of  $A$  ( $V_o$ ; data not shown).

422

423

## 424 **Discussion**

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

437

### 438 *Responses to seasonal and gradient changes in environment*

439 In the year of our seasonal measurements at Villar de Cobeta (2007), the average rainfall in the  
440 region was significantly greater than average. As a result, the lower slope site was not greatly  
441 different in soil water availability compared to the upper slope site (and we combine the sites  
442 for the purposes of further interpretation); however, significant underlying seasonal variation  
443 in  $T$  and soil water content did provide the driver for significant changes in leaf physiology  
444 which allow us to address questions on environmental influences on  $R_D$  and  $R_L$ . During the  
445 course of the year, leaf  $T$  ranged from  $\sim 6$  to  $>30$  °C, mid-morning measured rates of  $A_{\text{sat}}$  ranged  
446 from around  $3 \mu\text{mol m}^{-2} \text{s}^{-1}$  in late summer/autumn to  $>10 \mu\text{mol m}^{-2} \text{s}^{-1}$  in spring-early summer  
447 and  $g_{\text{sat}}$  ranged from maximal values in excess of  $0.15 \text{ mol m}^{-2} \text{s}^{-1}$  in spring to below  $0.05 \text{ mol}$   
448  $\text{m}^{-2} \text{s}^{-1}$  in late summer. When measured at prevailing mid-morning air  $T$ s,  $R_D$  ranged around 1

449  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for much of the year, but peaked at  $\sim 3 \mu\text{mol m}^{-2} \text{s}^{-1}$  during a very hot August (Fig.  
450 1e), when leaf  $T$ s exceeded  $30^\circ\text{C}$ . As a consequence, variation in  $R_D/A$  ratios was relatively  
451 constrained  $\sim 0.08$ - $0.18$  during spring, early summer and winter, and only increasing  
452 significantly (to  $0.38$ ) during a very hot August. The relatively consistent rate of  $R_D$  for six out  
453 of the seven campaigns is indicative of at least partial thermal acclimation (Atkin and Tjoelker  
454 2003), which is supported by two separate lines of evidence. Firstly, the relationship between  
455 single point measurements of  $R_D$  from light response curves on attached leaves and leaf  $T$  is  
456 fairly muted below  $30^\circ\text{C}$  (Fig. 2). Secondly, short-term  $T$  responses curves (generated by daily  
457 changes in air  $T$  on intact leaves in the field; Fig. 3) indicate shifts in the thermal response  
458 consistent with thermal acclimation, at least in trees at the south-facing, drier site.

459

460         Despite the significant seasonal changes in leaf physiology above (at Villar de Cobeta),  
461  $R_L/R_D$  measured mid-morning did not follow a clear seasonal pattern, and was not significantly  
462 influence by time of year. Averaged over the year, the inhibition of  $R$  was  $\sim 34\%$  (i.e.  $R_L/R_D$   
463 was  $0.66 \pm 0.05$  SEM). The fact that  $R_L/R_D$  did not change seasonally in any systematic way is  
464 somewhat surprising, since we had predicted that light inhibition would increase with  
465 increasing measuring  $T$  and lower soil water content (in summer). That it did not indicates  
466 that, in this forest type under field conditions, soil water availability has no clear impact on the  
467 extent of light inhibition of respiration (discussed further in the next section). It also shows that  
468 both  $R_L$  and  $R_D$  have acclimated to the seasonal shifts in daily average  $T$ . The latter is partially  
469 supported by our data, since we found that there was some seasonal shift in the  $R$ - $T$  curves for  
470 the dry site plants (Fig. 3), but much less evidence for acclimation in the lower slope site  
471 plants. The constant  $R_L/R_D$  found at the lower slope site may suggest that the  $Q_{10}$  is not  
472 different for the two processes, but for the upper slope site the situation is potentially a more  
473 complex mix of acclimation combined with similar  $Q_{10}$  values. Another complication for  
474 interpreting our findings is that the response of  $R$  to leaf water content may also be biphasic  
475 i.e., it may decrease in the initial stages of water stress (lower energy demands for growth) and  
476 may increase with additional stress-related demands e.g. osmoregulation or induced senescence  
477 (Ghashghaie *et al.* 2001; Gulias *et al.* 2002; Flexas *et al.* 2005; Flexas *et al.* 2006). This means  
478 that the timing of investigations during seasonal rainfall cycles is likely to be critical in terms  
479 of the state of the plant response. Further,  $T$  and water availability tend to vary in opposite



480 directions. The syndrome of response to the combined effects of seasonal changes in water  
481 availability and  $T$  clearly requires further investigation to resolve these potentially competing  
482 drivers and responses, and is partially addressed by the companion study using the Prades  
483 gradient, where the mid-summer campaign removes the impact of  $T$ .

484

485 We found changes in leaf structure and nutrient content in species across the Prades  
486 gradient consistent with previous findings (i.e. low  $M_A$  and high N content at the riparian and  
487 slope sites with greater soil water content; Table 4, 5). Rates of photosynthesis and stomatal  
488 conductance varied substantially across the gradient (Table 4; Fig. 4), with species sampled at  
489 the lower sites exhibiting the highest rates of net  $\text{CO}_2$  uptake. We also observed variations in  
490  $R_D/A$  ratios among species, which allows an assessment of the leaf-level balance between the  
491 respiratory cost and photosynthetic capacity in response to soil water content.  $R_D/A$  was highest  
492 (0.14-0.22) in high  $M_A$  species in the drier sites that exhibit low rates of mass-based  
493 photosynthesis, and lower (0.07-0.11) in deciduous species at the riparian site (Table 4). This  
494 is consistent with previous studies that have reported a higher  $R/A_{\text{sat}}$  ratio under drought (Flexas  
495 *et al.* 2006; Atkin and Macherel 2009). The ratio of leaf  $R$  in the light and dark ( $R_L/R_D$ ) was,  
496 by contrast, very consistent despite changes in both  $R_L$  and  $R_D$  (Fig. 4, Table 4).  $R_L/R_D$  across  
497 all sites resolved to an average of  $0.69 \pm 0.01$  SEM, meaning that  $R_L/A$  ratios were consistently  
498 ~30% lower than  $R_D/A$  ratios and were driven by underlying rates of  $A$ . The variation in  $R_L/A$   
499 contrasts with previous findings of greater constancy of  $R_L/A$  (compared to  $R_D/A$ ) in controlled-  
500 environment grown plants exposed to a range of contrasting environments (Atkin *et al.* 2006;  
501 Ayub *et al.* 2011) and in a wide range of temperate rainforest species at contrasting sites  
502 differing in soil age/nutrient availability/species composition/leaf traits (Atkin *et al.* 2013).

503

#### 504 *Lack of variation in the **Kok effect***

505 Past work suggests that light inhibition of leaf  $R$  may be linked to changes in cellular energy  
506 status (due to excess ATP or redox equivalents generated by the light reactions of  
507 photosynthesis, decreasing the demand for respiratory energy compared to leaves in darkness),  
508 photorespiration-dependent inactivation of the pyruvate dehydrogenase (PDH) complex  
509 (Budde and Randall 1990; Gemel and Randall 1992), or transition to a truncated tricarboxylic  
510 acid (TCA) cycle (Igamberdiev *et al.* 2001; Tcherkez *et al.* 2005; Tcherkez *et al.* 2008;  
511 Tcherkez *et al.* 2009). More recently, increased use of stored carbon reserves (e.g. citrate) in

512 the light has been shown to reduce demand for flux through the TCA cycle (Tcherkez *et al.*  
513 2012). Steady-state stoichiometric modelling has also indicated that light suppression of CO<sub>2</sub>  
514 release by the oxidative pentose phosphate pathway (OPPP) has the potential to contribute to  
515 the Kok effect (Buckley and Adams 2011). Collectively, these biochemical and modeling  
516 studies suggest that light inhibits respiratory CO<sub>2</sub> release – consistent with the Kok effect - and  
517 that reduced rates of CO<sub>2</sub> release by the TCA cycle and OPPP could both contribute to cases  
518 where  $R_L < R_D$ . What is less clear, however, is how environmental factors in the field influence  
519 the level of inhibition of  $R$ . More recently, Farquhar and Busch (2017), using a theoretical  
520 modelling approach, have shown it is possible to create a Kok-effect-like response in the  
521 presence of very specific conditions of changing mesophyll conductance ( $g_m$ ) at low light. To  
522 our knowledge, this potential explanation for the Kok-effect is yet to be supported by  
523 experimental evidence documenting the irradiance response of  $g_m$  or the relative importance of  
524 irradiance-mediated changes in respiratory CO<sub>2</sub> release vs  $g_m$ . Nevertheless, the findings of  
525 Farquhar and Busch (2017) highlight the need for cautious interpretation of Kok-effect results.

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

541

542           What underlying factors might explain the lack of systematic variation in  $R_L/R_D$  found  
543 with season at Villar de Cobeta and along the moisture gradient at Prades? At least three factors  
544 *could have* contributed to variations in  $R_L/R_D$  along the Prades gradient. **First**, a direct effect  
545 of soil water availability on  $C_i$  and the potential rate of photosynthetic oxygenation ( $V_o$ ). Griffin  
546 and Turnbull (2013) found that  $R_L/R_D$  decreased under conditions that suppressed light-  
547 saturated  $V_o$ . Crous *et al.* (2012) also report a positive linear relationship between  $R_L/R_D$  and  
548  $V_{o100}$  (the velocity of RuBP oxygenation at  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD). In terms of our mechanistic  
549 hypothesis (that light inhibition of  $R$  would decrease [and  $R_L/R_D$  increase] at drier sites where  
550 stomatal limitations on gas exchange increase photorespiration),  $C_i$  was significantly  
551 influenced by time of year at Villar de Cobeta (Table 3), but  $R_L/R_D$  was not significantly related  
552 to  $C_i$  at either saturating PPFD (Fig. S1f) or at limiting PPFD ( $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; data not  
553 shown). It is possible that the level of water limitation experienced by trees during the study  
554 was not sufficient to influence the balance between  $R_L$  and  $R_D$ , but the range of  $A_{\text{sat}}$ ,  $g_{\text{sat}}$  and  $C_i$   
555 ( $180\text{-}330 \text{ mmol mol}^{-1}$ ) does not support this. There was no effect of site on  $C_i$  along the Prades  
556 gradient, which clearly reflects some level of balancing between assimilation and conductance  
557 as soil water availability becomes more limited in the dry and shrub sites. **Second**, gradients in  
558 soil nutrient availability might have a secondary effect on foliar N content, and thence on the  
559 degree of light inhibition. Relatively few studies have quantified the effect of nutrient supply  
560 on  $R_L$ . Shapiro *et al.* (2004) found that deficiencies in nitrogen supply resulted in reduced rates  
561 of both  $R_L$  and  $R_D$ , but with the  $R_L/R_D$  ratios being nearly identical in high and low N grown  
562 plants (0.50 and 0.48, respectively for ambient  $\text{CO}_2$  grown *Xanthium strumarium*). We have  
563 previously found that variations in  $R_L/R_D$  in arctic tundra shrubs (Heskel *et al.* 2012) and forest  
564 species along a soil development chronosequence (Atkin *et al.* 2013) may be significantly  
565 related to a number of traits that are strong correlates of metabolic capacity, including leaf [N]  
566 and [P]. In the present study, tissue N was significantly influenced by site (water availability)  
567 in the Prades study, but this had no impact on  $R_L/R_D$ , which supports the findings of Shapiro *et al.*  
568 (2004). **Third**, the degree of light inhibition might differ systematically among species that  
569 exhibit contrasting leaf traits, with highly productive, low  $M_A$  species (which occupy the  
570 riparian and slope sites) exhibiting lower degrees of light inhibition than their high  $M_A$   
571 counterparts. Previous evidence for this is equivocal - we have previously observed weaker  
572 inhibition in high-metabolic rate species growing on young/productive sites (Atkin *et al.* 2013)

573 but similar  $R_L/R_D$  ratios have also been observed in inherently fast- and slow-growing  
574 herbaceous species (Atkin *et al.* 1997; Atkin *et al.* 2006). In the present study, we found that  
575  $M_A$  varied with season at Villar de Cobeta and site at Prades, once again with no impact on  
576  $R_L/R_D$ . Thus, here we add to the existing data that does not support the hypothesis that  $R_L/R_D$   
577 ratios vary systematically among species with inherently different leaf traits.

578

### 579 *Conclusions*

580 Clearly the balance between photosynthetic carbon fixation and respiratory oxidation of  
581 photosynthates is important to tree C balance (Amthor 1989; Loomis and Amthor 1999; Alt *et al.*  
582 *et al.* 2000). Since mitochondrial respiration plays a key role in determining the growth and  
583 survival of plants, and it is associated with the production of energy and carbon skeletons  
584 essential for cellular maintenance and biosynthesis, respiratory responses need to be considered  
585 relative to the effects on carbon gain to elucidate the overall effect on plant performance. Our  
586 estimates based on the Kok method demonstrate that the degree of light inhibition of  $R$  is  
587 relatively constrained seasonally and is fairly consistent across sites differing in soil water  
588 availability and community composition in this Mediterranean forest type (with an average  
589  $R_L/R_D$  of  $\sim 0.7$ ). This level of inhibition is consistent with recent findings (Ayub *et al.* 2014;  
590 McLaughlin *et al.* 2014). This points to  $R_L/A$  ratios being consistent, but around 30% lower  
591 than,  $R_D/A$  ratios, with both being primarily driven by changes in  $A$ . Notwithstanding the  
592 possibility that other methods of determining the degree of light inhibition of  $R$  might provide  
593 slightly different estimates, or that more extreme gradients of soil water availability may elicit  
594 changes not observed here, these findings have important implications for predictive models  
595 that seek to predict rates of leaf  $R_L$  using more commonly measured rates of  $R_D$  and associated  
596 leaf traits such as  $M_A$  and foliar [N] (Mercado *et al.* 2007). Failure to account for light-induced  
597 reduction in leaf  $R$  will clearly lead to errors in predicted rates of ecosystem  $\text{CO}_2$  exchange  
598 (Wohlfahrt *et al.* 2005; Wingate *et al.* 2007; McLaughlin *et al.* 2014). Importantly, while  
599 further work is needed to establish the precise metabolic and environmental drivers of  
600 variations in  $R_L/R_D$ , there is now some evidence for stand and ecosystem models to assume a  
601 relatively constant relationship between  $R_D$  and  $R_L$  along gradients of soil water availability.

602

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611

612

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894

895 **Table 1** Site characteristics and species selected for measurements at Prades (Catalunya, NE Spain). The elevation is the altitude above sea  
 896 level. Height refers to the maximum height of the dominant trees, giving an indication of the heights from which the shoots were sampled.  
 897

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

898  
 899

900 **Table 2.** Seasonal changes in average ( $\pm$  SEM,  $n = 4$ ) values of leaf structural and chemical  
 901 traits for *Q. ilex* subsp. *balotta* at two sites at Villar de Cobeta (central Spain). See Table 3 for  
 902 ANOVA statistics.  
 903

Month	M <sub>A</sub> (g DM m <sup>-2</sup> )	N (mg g <sup>-1</sup> )	P (mg g <sup>-1</sup> )	Sugars (mg g <sup>-1</sup> )	Starch (mg g <sup>-1</sup> )
Upper Slope					
Dec	40 <del>1</del> 51	12.6 $\pm$ 1.7	1.61 $\pm$ 0.11	58.2 $\pm$ 5.3	3.5 $\pm$ 0.2
Feb	35 <del>4</del> 20	12.3 $\pm$ 0.99	1.66 $\pm$ 0.19	56.6 <del>4</del> .1	3.1 $\pm$ 0.2
Apr	37 <del>9</del> 46	11.2 $\pm$ 0.65	1.27 $\pm$ 0.15	55.8 <del>3</del> .5	25.2 <del>6</del> .2
Jun	35 <del>7</del> 38	11.0 $\pm$ 0.82	1.29 $\pm$ 0.11	44.3 <del>1</del> $\pm$ 0	23.6 <del>6</del> .6
Aug	46 <del>3</del> 70	9.8 $\pm$ 0.83	1.08 $\pm$ 0.12	40.8 <del>1</del> $\pm$ 1	2.7 <del>0</del> .3
Sept	22 <del>8</del> 13	9.7 $\pm$ 0.56	1.44 $\pm$ 0.14	43.3 <del>3</del> .0	2.8 <del>0</del> .8
Nov	22 <del>6</del> 13	11.9 $\pm$ 0.45	1.40 $\pm$ 0.0 <del>8</del>	53.1 $\pm$ 0.	1.8 <del>0</del> .3
Lower Slope					
Dec	31 <del>6</del> 13	9.6 $\pm$ 1.4	1.54 $\pm$ 0.2 <del>5</del>	44.9 <del>5</del> .7	1.8 $\pm$ 0.2
Feb	28 <del>1</del> 41	12.2 $\pm$ 0.31	1.80 $\pm$ 0.0 <del>5</del>	56.2 <del>2</del> .4	2.1 $\pm$ 0.
Apr	22 <del>9</del> 28	11.5 $\pm$ 0.37	1.64 $\pm$ 0.2 <del>5</del>	47.3 <del>1</del> .4	14.9 <del>4</del> .7
Jun	25 <del>7</del> 9	9.0 $\pm$ 1.2	1.85 $\pm$ 0.18	53.1 $\pm$ 0.	2.8 $\pm$ 0.
Aug	35 <del>0</del> 28	9.1 $\pm$ 1.2	1.86 $\pm$ 0.19	53.1 <del>3</del> .6	2.7 $\pm$ 2.2
Sept	25 <del>4</del> 20	9.3 $\pm$ 0.78	1.71 <del>0</del> .00	52.7 <del>2</del> .8	5.0 <del>2</del> .3
Nov	27 <del>6</del> 17	11.4 $\pm$ 1.1	1.04 $\pm$ 0.3 <del>2</del>	54.0 $\pm$ 4	1.4 $\pm$ 0.1

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908 **Table 3.** Results of repeated measures two-way ANOVA from the Villar de Cobeta seasonal  
 909 measurements, with time of year (T) and site (S) as the main effects. Abbreviations:  $M_A$ , leaf  
 910 mass per unit area; N and P, foliar nitrogen and phosphorus content, respectively;  $A_{sat}$ , net  
 911 photosynthesis measured at 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $g_{sat}$ , stomatal conductance  
 912 measured at 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $R_D$ , leaf respiration in darkness;  $R_L$ , non-  
 913 photorespiratory mitochondrial leaf respiration in the light;  $V_o$ , estimated rate of  
 914 photorespiration at 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $C_i$ , internal  $\text{CO}_2$  concentration at 1000  
 915  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD. See Table 2 and Fig. 1 for seasonal variation in trait values.  
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Leaf trait category	Variable	Pvalues
918 Leaf structure	919 $M_A$	T <0.001; S <0.05; T*S <0.05
920 Chemical composition	921 N	ns
	922 P	S <0.05
	923 Sugars	T*S <0.01
	924 Starch	S <0.05
925 Areabased gas exchange	926 $A_{sat}$	T <0.001; T*S <0.05
	927 $g_{sat}$	T <0.0001; T*S <0.05
	928 $R_D$	ns
	929 $R_L$	ns
	930 $V_o$	ns
	931 $C_i$	T <0.05
932 Ratios	933 $R_L/R_D$	ns
	934 $R_D/A_{sat}$	T <0.05
	935 $R_L/A_{sat}$	T <0.05

936 **Table 4.** Average ( $\pm$  SEM,  $n = 6$ ) values of leaf traits for each species growing at each site along a soil water gradient at Prades - dry mass per unit area  
937 ( $M_A$ ), relative water content (RWC), nitrogen content, rate of net photosynthesis at 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD ( $A_{\text{sat}}$ ), stomatal conductance at  
938 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD ( $g_{\text{sat}}$ ), internal  $\text{CO}_2$  concentration at 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD ( $C_i$ ), estimated rate of photorespiration at 1500  
939  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD ( $V_o$ ), rates of leaf respiration darkness ( $R_D$ ) and in the light ( $R_L$ ), ratio of  $R_L$  to  $R_D$ , and ratios of leaf  $R$  to  $A_{\text{sat}}$ . See Table 1  
940 for sites details and Table 5 for ANOVA results for each trait.  
941

Site	Species	$M$ ( $\text{g}_M \text{m}^{-2}$ )	RWC (%)	N ( $\text{mg g}^{-1}$ )	$A_{\text{sat}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$g_{\text{sat}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$C_i$ ( $\mu\text{mol mol}^{-1}$ )	$V_o$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$R_D$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$R_L$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$R_L/R_D$ (ratio)	$R_D/A_{\text{sat}}$ (ratio)	$R_L/A_{\text{sat}}$ (ratio)
Riparian	<i>Tilia platyphyllos</i>	55.8 $\pm$ 2.9	86.5 $\pm$ 3	2.8 $\pm$ 0.05	109 $\pm$ 0.8	0.13 $\pm$ 0.014	235 $\pm$ 21	4.24 $\pm$ 0.7	0.67 $\pm$ 0.09	0.42 $\pm$ 0.08	0.61 $\pm$ 0.04	0.06 $\pm$ 0.01	0.04 $\pm$ 0.01
	<i>Acer monspessulanum</i>	94.3 $\pm$ 10.4	92.6 $\pm$ 2.0	1.76 $\pm$ 0.08	10.6 $\pm$ 0.8	0.09 $\pm$ 0.010	231 $\pm$ 22	4.99 $\pm$ 0.8	0.99 $\pm$ 0.15	0.84 $\pm$ 0.14	0.74 $\pm$ 0.08	0.10 $\pm$ 0.02	0.09 $\pm$ 0.02
Slope	<i>Acer monspessulanum</i>	87.5 $\pm$ 6.1	91.5 $\pm$ 0.9	1.82 $\pm$ 0.01	8.3 $\pm$ 0.9	0.10 $\pm$ 0.015	223 $\pm$ 12	4.70 $\pm$ 0.3	0.92 $\pm$ 0.13	0.73 $\pm$ 0.14	0.77 $\pm$ 0.06	0.11 $\pm$ 0.02	0.09 $\pm$ 0.02
	<i>Quercus ilex</i>	169.9 $\pm$ 6.4	83.3 $\pm$ 2.4	1.46 $\pm$ 0.03	10.1 $\pm$ 1.1	0.14 $\pm$ 0.029	169 $\pm$ 17	7.52 $\pm$ 1.4	1.22 $\pm$ 0.21	1.02 $\pm$ 0.16	0.71 $\pm$ 0.03	0.12 $\pm$ 0.02	0.10 $\pm$ 0.01
	<i>Phillyrea latifolia</i>	168.3 $\pm$ 7.1	91.6 $\pm$ 1.0	1.31 $\pm$ 0.05	9.2 $\pm$ 1.5	0.11 $\pm$ 0.023	192 $\pm$ 29	6.75 $\pm$ 1.9	1.64 $\pm$ 0.30	1.16 $\pm$ 0.26	0.68 $\pm$ 0.06	0.20 $\pm$ 0.04	0.14 $\pm$ 0.04
Dry C	<i>Quercus ilex</i>	204.6 $\pm$ 9.9	85.8 $\pm$ 0.7	1.25 $\pm$ 0.11	8.6 $\pm$ 1.4	0.09 $\pm$ 0.021	200 $\pm$ 24	5.46 $\pm$ 1.4	1.27 $\pm$ 0.17	0.88 $\pm$ 0.12	0.69 $\pm$ 0.03	0.16 $\pm$ 0.02	0.11 $\pm$ 0.01
	<i>Phillyrea latifolia</i>	113.1 $\pm$ 9.8	93.7 $\pm$ 0.9	1.07 $\pm$ 0.12	7.6 $\pm$ 0.6	0.08 $\pm$ 0.010	201 $\pm$ 13	5.73 $\pm$ 2.3	0.89 $\pm$ 0.07	0.65 $\pm$ 0.13	0.71 $\pm$ 0.12	0.12 $\pm$ 0.01	0.09 $\pm$ 0.02
	<i>Arbutus unedo</i>	134.3 $\pm$ 8.2	96.0 $\pm$ 1.1	1.03 $\pm$ 0.07	11.6 $\pm$ 0.5	0.13 $\pm$ 0.015	222 $\pm$ 22	5.40 $\pm$ 0.9	1.13 $\pm$ 0.12	0.76 $\pm$ 0.08	0.68 $\pm$ 0.06	0.10 $\pm$ 0.01	0.07 $\pm$ 0.01
Dry E	<i>Quercus ilex</i>	183.1 $\pm$ 7.4	83.8 $\pm$ 2.4	1.20 $\pm$ 0.07	9.1 $\pm$ 0.8	0.12 $\pm$ 0.021	199 $\pm$ 20	6.06 $\pm$ 0.9	0.98 $\pm$ 0.13	0.67 $\pm$ 0.12	0.68 $\pm$ 0.07	0.11 $\pm$ 0.02	0.08 $\pm$ 0.02
	<i>Phillyrea latifolia</i>	141.3 $\pm$ 1.2	91.2 $\pm$ 1.3	0.98 $\pm$ 0.08	8.3 $\pm$ 0.4	0.10 $\pm$ 0.019	259 $\pm$ 14	3.16 $\pm$ 0.4	1.11 $\pm$ 0.16	0.80 $\pm$ 0.19	0.68 $\pm$ 0.07	0.13 $\pm$ 0.02	0.09 $\pm$ 0.02
	<i>Arbutus unedo</i>	134.2 $\pm$ 12.4	94.9 $\pm$ 1.1	1.31 $\pm$ 0.10	9.3 $\pm$ 0.4	0.11 $\pm$ 0.021	200 $\pm$ 25	6.54 $\pm$ 1.1	0.99 $\pm$ 0.07	0.64 $\pm$ 0.06	0.65 $\pm$ 0.04	0.11 $\pm$ 0.01	0.07 $\pm$ 0.01
Shrub C	<i>Quercus ilex</i>	186.2 $\pm$ 7.5	82.6 $\pm$ 1.3	1.12 $\pm$ 0.15	8.4 $\pm$ 1.1	0.07 $\pm$ 0.012	241 $\pm$ 15	3.81 $\pm$ 0.3	1.11 $\pm$ 0.24	0.77 $\pm$ 0.21	0.67 $\pm$ 0.07	0.14 $\pm$ 0.02	0.09 $\pm$ 0.02
	<i>Phillyrea latifolia</i>	167.3 $\pm$ 7.6	92.2 $\pm$ 1.7	1.15 $\pm$ 0.10	8.3 $\pm$ 1.4	0.05 $\pm$ 0.015	239 $\pm$ 19	4.61 $\pm$ 0.7	1.47 $\pm$ 0.15	1.11 $\pm$ 0.17	0.73 $\pm$ 0.09	0.20 $\pm$ 0.03	0.14 $\pm$ 0.01
	<i>Arbutus unedo</i>	144.4 $\pm$ 6.5	97.3 $\pm$ 0.4	1.20 $\pm$ 0.09	9.3 $\pm$ 0.6	0.07 $\pm$ 0.012	232 $\pm$ 21	3.94 $\pm$ 0.6	0.95 $\pm$ 0.07	0.67 $\pm$ 0.10	0.69 $\pm$ 0.06	0.11 $\pm$ 0.01	0.08 $\pm$ 0.01
Shrub E	<i>Quercus ilex</i>	198.9 $\pm$ 5.5	82.6 $\pm$ 1.6	1.01 $\pm$ 0.05	7.9 $\pm$ 0.6	0.09 $\pm$ 0.014	249 $\pm$ 8	4.79 $\pm$ 0.3	1.12 $\pm$ 0.11	0.86 $\pm$ 0.12	0.74 $\pm$ 0.04	0.14 $\pm$ 0.01	0.11 $\pm$ 0.01
	<i>Phillyrea latifolia</i>	179.2 $\pm$ 3.5	90.5 $\pm$ 0.9	1.15 $\pm$ 0.02	8.9 $\pm$ 1.2	0.10 $\pm$ 0.022	203 $\pm$ 22	5.15 $\pm$ 1.4	1.80 $\pm$ 0.09	1.35 $\pm$ 0.10	0.75 $\pm$ 0.05	0.22 $\pm$ 0.03	0.16 $\pm$ 0.02
	<i>Arbutus unedo</i>	128.1 $\pm$ 6.0	96.7 $\pm$ 0.6	1.05 $\pm$ 0.18	8.0 $\pm$ 0.6	0.07 $\pm$ 0.012	189 $\pm$ 26	6.38 $\pm$ 1.5	0.86 $\pm$ 0.10	0.57 $\pm$ 0.10	0.65 $\pm$ 0.06	0.11 $\pm$ 0.01	0.07 $\pm$ 0.01

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943 **Table 5.** Results of two-way ANOVA of leaf traits for species growing along a soil water  
 944 gradient at Prades, with species (Sp) and site (S) as the main effects. Abbreviations:  $M_A$ , leaf  
 945 mass per unit area;  $A_{\text{sat}}$ , net photosynthesis measured at 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $g_{\text{sat}}$ ,  
 946 stomatal conductance measured at 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $R_D$ , leaf respiration in  
 947 darkness;  $R_L$ , non-photorespiratory mitochondrial leaf respiration in the light;  $V_o$ , estimated  
 948 rate of photorespiration at 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD;  $C_i$ , internal  $\text{CO}_2$  concentration at  
 949 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD. See Table 2 for trait values.

Leaf trait category	Parameter	<i>P</i> values
Leaf structure	$M_A$	Sp <0.0001; Site <0.001; Sp*S <0.0001
Chemical composition	RWC	Sp <0.0001
	N	Sp <0.0001; Site <0.0001; Sp*S <0.0001
Areabased gas exchange	$A_{\text{sat}}$	Site <0.01
	$g_{\text{sat}}$	Site <0.01
	$R_D$	Sp*S <0.01
	$R_L$	Sp*S <0.05
	$V_o$	ns
	$C_i$	ns
Ratios	$R_L/R_D$	ns
	$R_D/A_{\text{sat}}$	Site <0.05 Sp*S <0.01
	$R_L/A_{\text{sat}}$	Sp*S <0.05



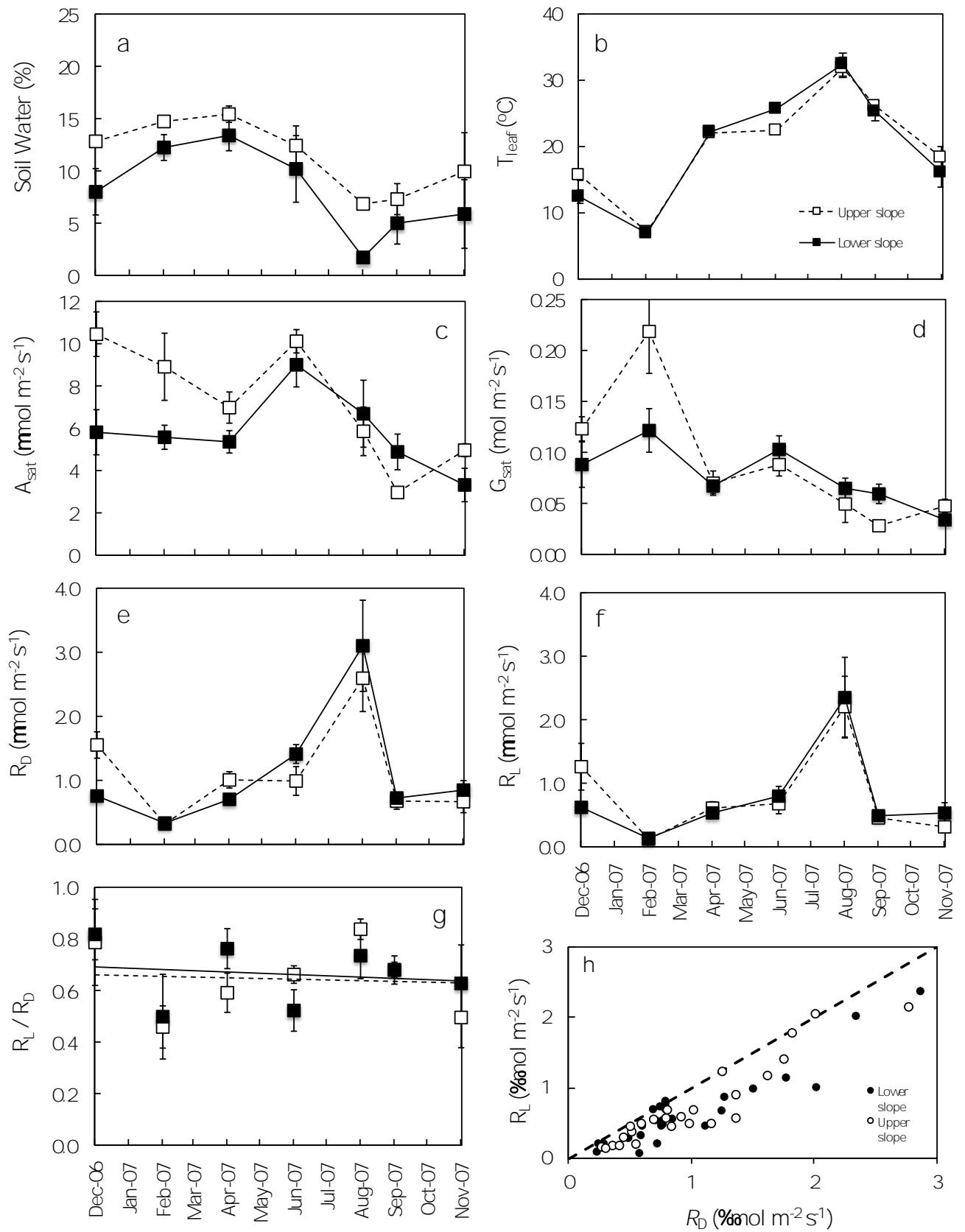
**Figure 1.** Seasonal changes in (a) soil volumetric water content, (b) leaf temperature, (c)  $A_{\text{sat}}$ ; i.e. net  $\text{CO}_2$  uptake measured  $1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , (d)  $g_{\text{sat}}$ ; maximum stomatal conductance at  $A_{\text{sat}}$ , (e) foliar respiration 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 *Q. 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 day-time  $T$  during the active period on any given day (1100 to 1400 hrs).** See Table 3 for results of two-way ANOVAs testing for differences with time and site. (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.

**Figure 2.** Relationships between (a) foliar respiration rate in darkness ( $R_{\text{D}}$ ) and (b) the ratio of leaf respiration measured in the light to that in darkness ( $R_{\text{L}}/R_{\text{D}}$ ) and leaf temperature in *Q. 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).

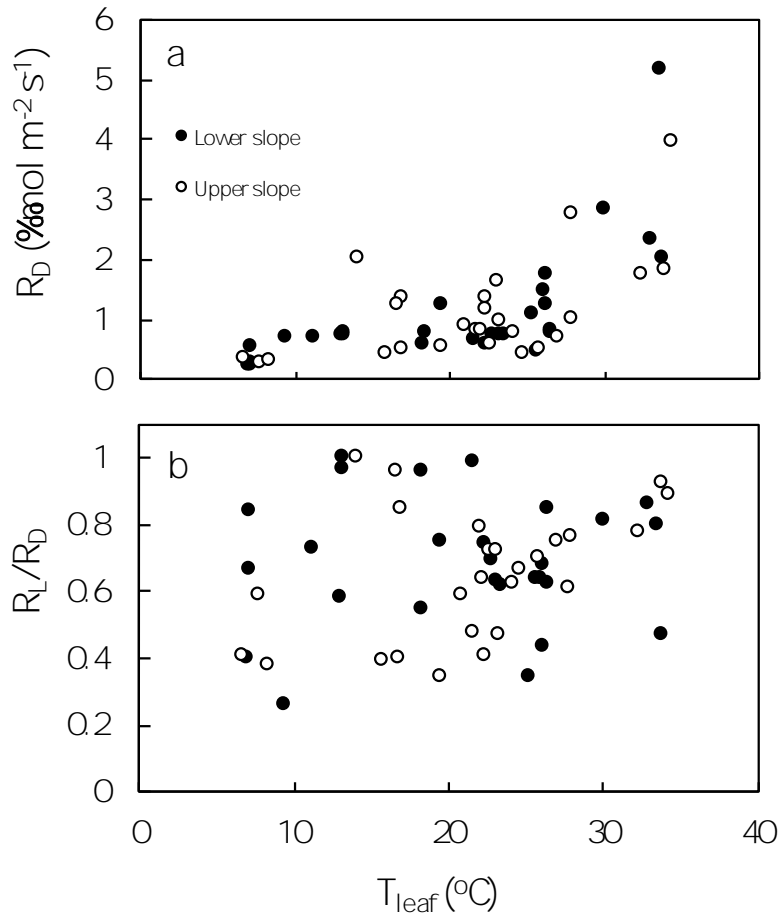
**Figure 3.** Seasonal changes in the instantaneous thermal response of foliar  $R_{\text{D}}$  in *Q. ilex* at an (a) upper and (b) lower slope site at Villar de Cobeta (central Spain). Responses were determined on intact shoots using ambient changes in air temperature during the course of the day and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

**Figure 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 ( $R_{\text{D}}$ ) and (f) in the light ( $R_{\text{L}}$ ), (g) the ratio of leaf respiration measured in the light to that in darkness ( $R_{\text{L}}/R_{\text{D}}$ ) for the six study sites along the soil moisture gradient at Prades (NE Spain). (a-g) values shown 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  $R_{\text{L}}$  plotted against corresponding rates of  $R_{\text{D}}$ . Data shown are for individual leaves sampled along soil water availability gradient. The dashed line shows the 1:1 relationship.

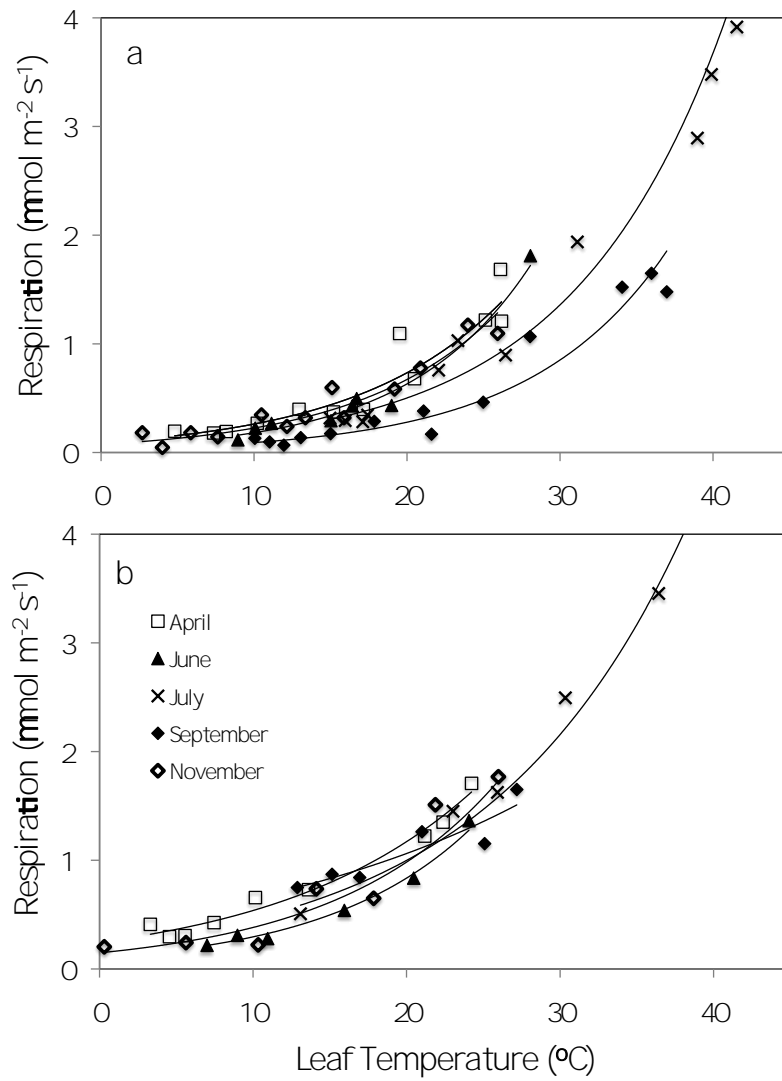
**Figure 1.** Environment and leaf traits through year at Villar de Cobeta



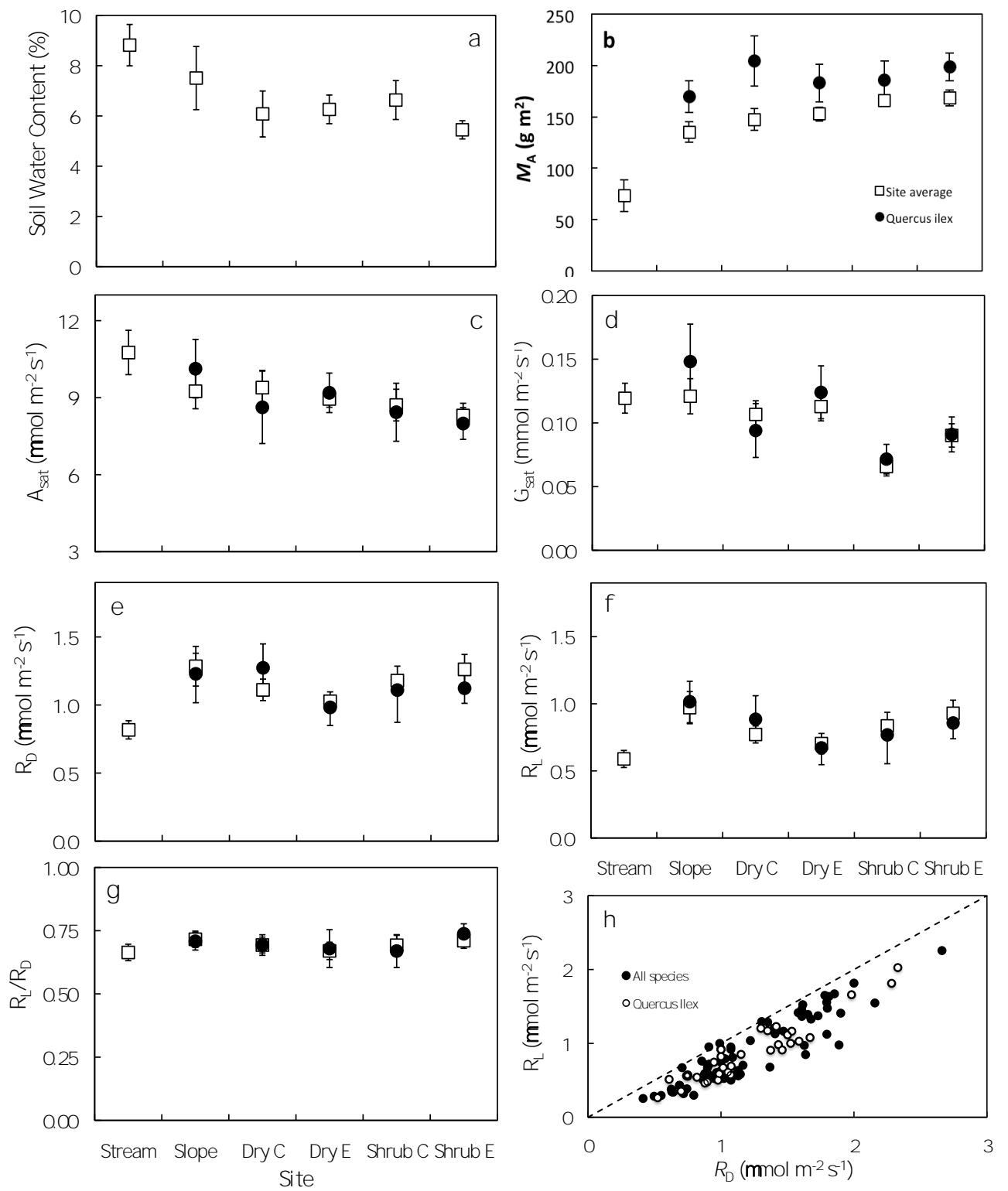
**Figure 2.** Temperature relationships from seasonal study at Villar de Cobeta



**Figure 3.** Seasonal changes in the thermal response of *R*



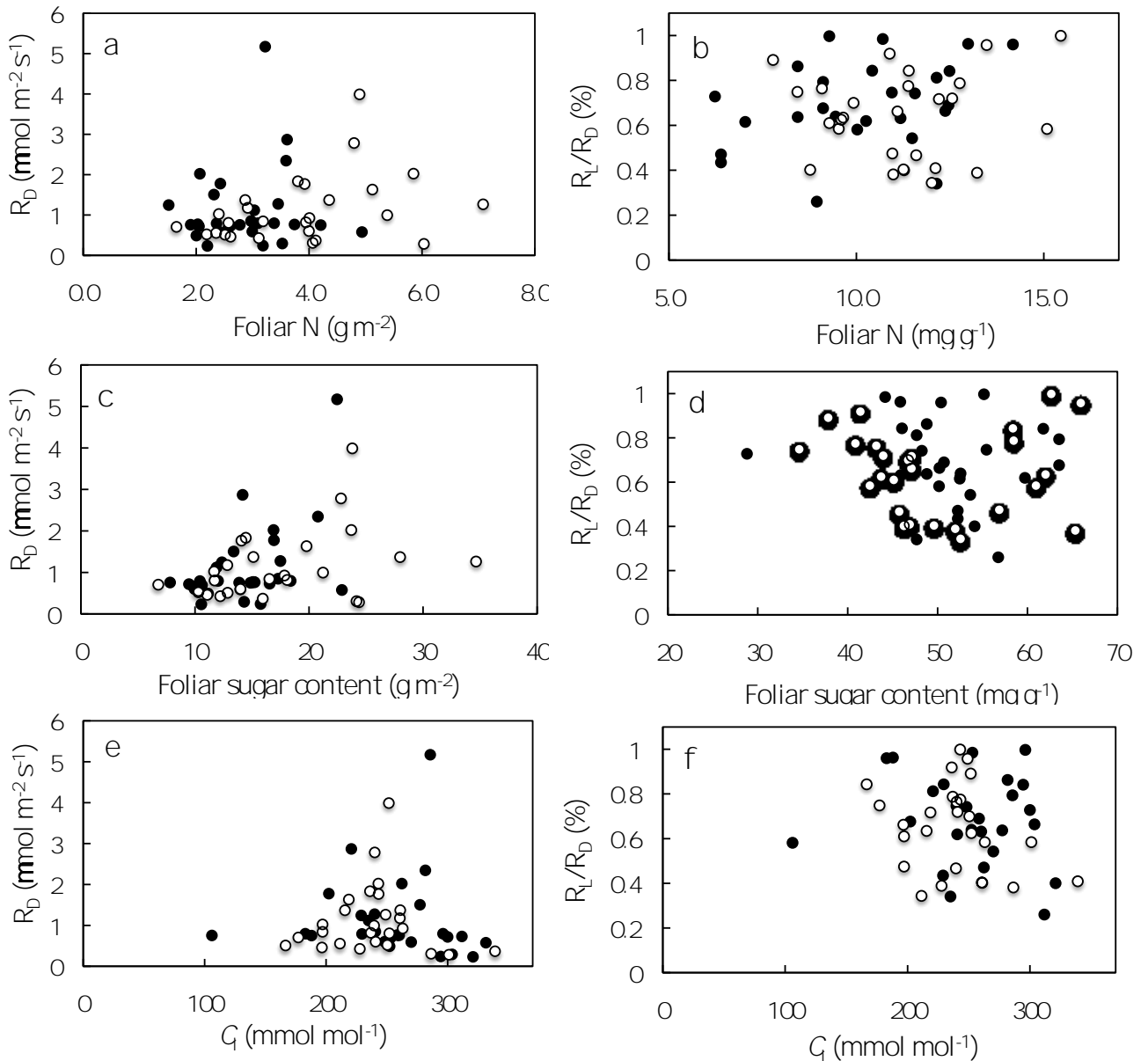
**Figure 4.** Prades results by site



## Supplementary Material

The following supplementary material is available for this article online:

**Figure S1.** Relationships between (a, c) foliar respiration rate in darkness ( $R_D$ ) and (b, d) the ratio of leaf respiration measured in the light to that in darkness ( $R_L/R_D$ ) and foliar N content and foliar soluble sugar content measured in *Q. ilex* seven times during the course of a year at an upper (closed symbols) and lower (open symbols) slope site at Villar de Cobeta (central Spain).



**Figure S2.** Relationships between soil water content and (a, c)  $A_{\text{sat}}$ ;  $\text{CO}_2$  uptake measured  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  and (b,d) foliar respiration rate in darkness ( $R_D$ ) measured in *Q. ilex* seven times during the course of a year at an upper (closed symbols) and lower (open symbols) slope site at Villar de Cobeta (central Spain, upper panels), and along a soil water gradient at Prades in NE Spain (lower panels, open symbols denote community average, closed symbols for *Q. ilex*).

