
This is the **accepted version** of the article:

Turnbull, Matthew H.; Ogaya Inurriagarro, Romà; Barbeta i Margarit, Adrià; [et al.]. «Light inhibition of foliar respiration in response to soil water availability and seasonal changes in temperature Mediterranean holm oak. (quercus ilex forest)». *Functional plant biology*, Vol. 44, Issue 12 (2017), p. 1178-1193. DOI 10.1071/FP17032

This version is available at <https://ddd.uab.cat/record/218315>

under the terms of the  **CC BY** COPYRIGHT license

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**

4

5 Matthew H. Turnbull¹, Romà Ogaya^{2,3}, Adrià Barbeta^{2,3}, Josep Peñuelas^{2,3}, Joana
6 Zaragoza-Castells⁴, Owen K. Atkin⁵, Fernando Valladares⁶, Teresa E. Gimeno⁷,
7 Beatriz Pías⁸ and Kevin L. Griffin⁹

8

9 ¹Centre for Integrative Ecology, School of Biological Sciences, University of Canterbury,
10 Private Bag 4800, Christchurch 8140, New Zealand

11 ²CREAF, Cerdanyola del Vallès, 08193, Catalonia, Spain

12 ³CSIC, Global Ecology Unit CREAM-CSIC-UAB, Cerdanyola del Vallès, 08193, Catalonia,
13 Spain

14 ⁴Geography, College of Life and Environmental Sciences, University of Exeter, Amory
15 Building, Rennes Drive, Exeter, EX4 4RJ, UK

16 ⁵ARC Centre of Excellence in Plant Energy Biology, Division of Plant Sciences, Research
17 School of Biology, Building 134, The Australian National University, Canberra, ACT, 2601,
18 Australia

19 ⁶Museo Nacional de Ciencias Naturales, CSIC, Serrano 115, E-28006 Madrid, Spain

20 ⁷Hawkesbury Institute for the Environment, University of Western Sydney, Locked bag 1797,
21 Penrith, NSW, 2751, Australia

22 ⁸Departamento de Botánica, Universidad Complutense de Madrid, José Antonio Novais 2,
23 28040, Madrid, Spain.

24 ⁹Lamont-Doherty Earth Observatory of Columbia University, 61 Route 9W, 6 Biology,
25 Palisades, NY 10964, USA

26

27 **Author for correspondence:** Matthew Turnbull +64 (03) 364 2987 x7040, email:
28 matthew.turnbull@canterbury.ac.nz

29 **Number of Figures:** 4 (plus 1 in Supplementary Material)

30 **Number of Tables:** 5

31 **Number of References:** 90

32 **Total Word count:** 7120 (excluding summary, references, figures and tables)

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 ± 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*

62

63

64

65

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 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 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 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 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 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 CO₂ 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 (ECH₂O 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 CO₂ exchange (A_{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_{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 CO₂ 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 [CO₂] 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 CO₂ diffusion through the chamber gasket (Pons
277 and Welschen 2002). Light-saturated photosynthesis (A_{sat}) was measured at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$
278 ¹ 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_{sat} , the irradiance response of net CO₂ 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 CO₂ exchange
283 in darkness were conducted after a further 10 min of darkness – these were not statistical
284 different from values of R_{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_{sat} , R_{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_{L}), we used the Kok (1948) method,
289 where R_{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 ± 0.01 . Using this method, intercellular
294 CO₂ 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_{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 Γ^* is the CO_2 compensation point in the absence of R_L [37 ppm at 25°C (von Caemmerer
303 and Farquhar 1981)], with Γ^* 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_{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_{sat} was
355 strongly reflected in the seasonal response of g_{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 CO₂ 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 ± 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_{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_{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 CO₂ 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 ± 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_{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 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 ~ 6 to >30 °C, mid-morning measured rates of A_{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_{sat} ranged from maximal values in excess of $0.15 \text{ mol m}^{-2} \text{s}^{-1}$ in spring to below 0.05 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°C . As a consequence, variation in R_D/A ratios was relatively
451 constrained $\sim 0.08\text{-}0.18$ 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°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 ± 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 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_{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 ± 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 CO₂
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 CO₂ release – consistent with the Kok effect - and
517 that reduced rates of CO₂ 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 CO₂ 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 [CO₂] 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_{sat} , g_{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 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 ~ 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 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

603 **Acknowledgements**

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

611

612

613 **References**

614

615 Alt, C, Stutzel, H, Kage, H (2000) Optimal nitrogen content and photosynthesis in cauliflower
616 (Brassica oleracea L. botrytis). Scaling up from a leaf to the whole plant. *Annals of*
617 *Botany* **85**, 779-787.

618 Amthor, JS (1989) 'Respiration and Crop Productivity.' (Springer-Verlag: New York)

619 Atkin, OK, Atkinson, LJ, Fisher, RA, Campbell, CD, Zaragoza-Castells, J, Pitchford, J,
620 Woodward, FI, Hurry, V (2008) Using temperature-dependent changes in leaf scaling
621 relationships to quantitatively account for thermal acclimation of respiration in a
622 coupled global climate-vegetation model. *Global Change Biology* **14**, 2709-2726.

623 Atkin, OK, Bruhn, D, Tjoelker, MG (2005) Response of plant respiration to changes in
624 temperature: mechanisms and consequences of variations in the Q_{10} and acclimation.
625 In 'Plant Respiration. From Cell to Ecosystem.' (Eds H Lambers, M Ribas-Carbo.) pp.
626 95-136. (Springer: Dordrecht, the Netherlands.)

627 Atkin, OK, Day, DA (1990) A comparison of the respiratory processes and growth rates of
628 selected Australian alpine and related lowland plant species. *Australian Journal of*
629 *Plant Physiology* **17**, 517-526.

630 Atkin, OK, Edwards, EJ, Loveys, BR (2000a) Response of root respiration to changes in
631 temperature and its relevance to global warming. *New Phytologist* **147**, 141-154.

632 Atkin, OK, Holly, C, Ball, MC (2000b) Acclimation of snow gum (*Eucalyptus pauciflora*) leaf
633 respiration to seasonal and diurnal variations in temperature: the importance of changes
634 in the capacity and temperature sensitivity of respiration. *Plant Cell and Environment*
635 **23**, 15-26.

636 Atkin, OK, Macherel, D (2009) The crucial role of plant mitochondria in orchestrating drought
637 tolerance. *Annals of Botany* **103**, 581-597.

638 Atkin, OK, Scheurwater, I, Pons, TL (2006) High thermal acclimation potential of both
639 photosynthesis and respiration in two lowland *Plantago* species in contrast to an alpine
640 congeneric. *Global Change Biology* **12**, 500-515.

641 Atkin, OK, Scheurwater, I, Pons, TL (2007) Respiration as a percentage of daily photosynthesis
642 in whole plants is homeostatic at moderate, but not high, growth temperatures. *New*
643 *Phytologist* **174**, 367-380.

644 Atkin, OK, Tjoelker, MG (2003) Thermal acclimation and the dynamic response of plant
645 respiration to temperature. *Trends in Plant Science* **8**, 343-351.

646 Atkin, OK, Turnbull, MH, Zaragoza-Castell, J, Fyllas, NM, Lloyd, J, Meir, P, Griffin, KL
647 (2013) Light inhibition of leaf respiration as soil fertility declines along a post-glacial
648 chronosequence in New Zealand: an analysis using the Kok method. *Plant and Soil*
649 **367**, 163-182.

650 Atkin, OK, Westbeek, MHM, Cambridge, ML, Lambers, H, Pons, TL (1997) Leaf respiration
651 in light and darkness. A comparison of slow- and fast-growing *Poa* species. *Plant*
652 *Physiology* **113**, 961-965.

653 Ayub, G, Smith, RA, Tissue, DT, Atkin, OK (2011) Impacts of drought on leaf respiration in
654 darkness and light in *Eucalyptus saligna* exposed to industrial-age atmospheric CO₂
655 and growth temperature. *New Phytologist* **190**, 1003.

656 Ayub, G, Zaragoza-Castells, J, Griffin, KL, Atkin, OK (2014) Leaf respiration in darkness and
657 in the light under pre-industrial, current and elevated atmospheric CO₂ concentrations.
658 *Plant Science* **226**, 120-130.

659 Brooks, A, Farquhar, GD (1985) Effect of temperature on the CO₂ /O₂ specificity of ribulose-
660 1,5-biphosphate carboxylase/oxygenase and the rate of respiration in the light.
661 Estimates from gas exchange measurements on spinach. *Planta* **165**, 397-406.

- 662 Buckley, TN, Adams, MA (2011) An analytical model of non-photorespiratory CO₂ release in
663 the light and dark in leaves of C₃ species based on stoichiometric flux balance. *Plant,*
664 *Cell & Environment* **34**, 89-112.
- 665 Budde, RJA, Randall, DD (1990) Pea leaf mitochondrial pyruvate dehydrogenase complex is
666 inactivated *in vivo* in a light-dependent manner. *Proceedings of the National Academy*
667 *of Science, USA* **87**, 673-676.
- 668 Chaves, MM, Pereira, JS, Maroco, J, Rodrigues, ML, Ricardo, CPP, Osório, ML (2002) How
669 plants cope with water stress in the field: photosynthesis and growth. *Annals of Botany*
670 **89**, 907-916.
- 671 Crous, KY, Zaragoza-Castells, J, Ellsworth, DS, Duursma, RA, Low, M, Tissue, DT, Atkin,
672 OK (2012) Light inhibition of leaf respiration in field-grown *Eucalyptus saligna* in
673 whole-tree chambers under elevated atmospheric CO₂ and summer drought. *Plant Cell*
674 *and Environment* **35**, 966-981.
- 675 Crous, KY, Zaragoza-Castells, J, Low, M, Ellsworth, DS, Tissue, DT, Tjoelker, MG, Barton,
676 CVM, Gimeno, TE, Atkin, OK (2011) Seasonal acclimation of leaf respiration in
677 *Eucalyptus saligna* trees: impacts of elevated atmospheric CO₂ and summer drought.
678 *Global Change Biology* **17**, 1560-1576.
- 679 Farquhar, GD, Busch, FA (2017) Changes in the chloroplastic CO₂ concentration explain much
680 of the observed Kok effect: a model. *New Phytologist* **214**, 570-584.
- 681 Farquhar, GD, von Caemmerer, S (1982) Modelling of photosynthetic response to
682 environmental conditions. In 'Encyclopedia of Plant Physiology. Volume 12B.
683 Physiological Plant Ecology II. Water Relations and Carbon Assimilation.' (Eds OL
684 Lange, PS Nobel, CB Osmond, H Ziegler.) pp. 551-587. (Springer Verlag: Berlin)
- 685 Flexas, J, Bota, J, Galmés, J, Medrano, H, Ribas-Carbó, M (2006) Keeping a positive carbon
686 balance under adverse conditions: responses of photosynthesis and respiration to water
687 stress. *Physiologia Plantarum* **127**, 343-352.
- 688 Flexas, J, Galmes, J, Ribas-Carbo, M, Medrano, H (2005) The effects of water stress on plant
689 respiration. In 'Plant Respiration: From Cell to Ecosystem.' (Eds H Lambers, M Ribas-
690 Carbo.) Vol. 18 pp. 85-94. (Springer: Dordrecht, The Netherlands)
- 691 Gemel, J, Randall, DD (1992) Light regulation of leaf mitochondrial pyruvate dehydrogenase
692 complex. Role of photorespiratory carbon metabolism. *Plant Physiology* **100**, 908-914.
- 693 Ghashghaie, J, Duranceau, M, Badeck, FW, Cornic, G, Adeline, MT, Deleens, E (2001) δ¹³C
694 of CO₂ respired in the dark in relation to δ¹³C of leaf metabolites: comparison between
695 *Nicotiana sylvestris* and *Helianthus annuus* under drought. *Plant, Cell & Environment*
696 **24**, 505-515.
- 697 Gifford, RM (1995) Whole plant respiration and photosynthesis of wheat under increased CO₂
698 concentration and temperature - long-term vs short- term distinctions for modelling.
699 *Global Change Biology* **1**, 385-396.
- 700 Grassi, G, Meir, P, Cromer, R, Tompkins, D, Jarvis, PG (2002) Photosynthetic parameters in
701 seedlings of *Eucalyptus grandis* as affected by rate of nitrogen supply. *Plant, Cell and*
702 *Environment* **25**, 1677-1688.
- 703 Griffin, KL, Anderson, OR, Tissue, DT, Turnbull, MH, Whitehead, D (2004) Variations in
704 dark respiration and mitochondrial numbers within needles of *Pinus radiata* grown in
705 ambient or elevated CO₂ partial pressure *Tree Physiology* **24**, 347-353.
- 706 Griffin, KL, Turnbull, M, Murthy, R (2002a) Canopy position affects the temperature response
707 of leaf respiration in *Populus deltoides*. *New Phytologist* **154**, 609-619.
- 708 Griffin, KL, Turnbull, M, Murthy, R, Lin, GH, Adams, J, Farnsworth, B, Mahato, T, Bazin, G,
709 Potasnak, M, Berry, JA (2002b) Leaf respiration is differentially affected by leaf vs.
710 stand-level night-time warming. *Global Change Biology* **8**, 479-485.

- 711 Griffin, KL, Turnbull, MH (2013) Light saturated RuBP oxygenation by Rubisco is a robust
712 predictor of light inhibition of respiration in *Triticum aestivum* L. and *Zea mays* L.
713 *Plant Biology* 769-775.
- 714 Gulias, J, Flexas, J, Abadia, A, Madrano, H (2002) Photosynthetic responses to water deficit
715 in six Mediterranean sclerophyll species: possible factors explaining the declining
716 distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species. *Tree*
717 *Physiology* **22**, 687-697.
- 718 Heskell, M, Anderson, OR, Atkin, OK, Turnbull, MH, Griffin, KL (2012) Leaf- and cell-level
719 carbon cycling responses to a nitrogen and phosphorus gradient in two Arctic tundra
720 species. *Amer. J. Bot.* **99**, 1702-1714.
- 721 Heskell, M, Bitterman, DS, Atkin, O, Turnbull, MH, Griffin, KL (2013a) Seasonality of foliar
722 respiration in two dominant plant species from the Arctic tundra: response to long-term
723 warming and short-term temperature variability. *Functional Plant Biology* **41**, 287-300.
- 724 Heskell, M, Greaves, H, Kornfeld, A, Gough, L, Atkin, O, Turnbull, M, Shaver, G, Griffin, KL
725 (2013b) Differential physiological responses to environmental change promote woody
726 shrub expansion. *Ecology and Evolution* **3**, 1149-1162.
- 727 Hoefnagel, MHN, Atkin, OK, Wiskich, JT (1998) Interdependence between chloroplasts and
728 mitochondria in the light and the dark. *Biochimica Et Biophysica Acta-Bioenergetics*
729 **1366**, 235-255.
- 730 Hurry, V, Igamberdiev, AU, Keerberg, O, Pärnik, TR, Atkin, OK, Zaragoza-Castells, J,
731 Gardestrom, P, Lambers, H, Ribas-Carbó, M (2005) Respiration in photosynthetic
732 cells: gas exchange components, interactions with photorespiration and the operation
733 of mitochondria in the light. In 'Advances in photosynthesis and respiration: Respiration
734 and the environment.' pp. In Press. (Kluwer Academic Publishers: Dordrecht)
- 735 Hurry, VM, Tobiaeson, M, Kromer, S, Gardestrom, P, Oquist, G (1995) Mitochondria
736 contribute to increased photosynthetic capacity of leaves of winter rye (*Secale cereale*
737 L.) following cold-hardening. *Plant cell and environment* **18**, 69-76.
- 738 Igamberdiev, AU, Romanowska, E, Gardestrom, P (2001) Photorespiratory flux and
739 mitochondrial contribution to energy and redox balance of barley leaf protoplasts in the
740 light and during light-dark transitions. *Journal of Plant Physiology* **158**, 1325-1332.
- 741 IPCC, 2007. Climate Change 2007 - The Physical Science Basis. Contribution of Working
742 Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate
743 Change. Cambridge University Press, United Kingdom and New York, NY, USA. 996
744 pages.
- 745 Kirschbaum, MUF, Farquhar, GD (1987) Investigation of the CO₂ dependence of quantum
746 yield and respiration in *Eucalyptus pauciflora* *Plant Physiology* **83**, 1032-1036.
- 747 Koide, RT, Robichaux, RH, Morse, SR, Smith, CM (1989) Plant water status, hydraulic
748 resistance and capacitance. In 'Plant Physiological Ecology.' (Eds RW Pearcy, J
749 Ehleringer, HA Mooney, PW Rundel.) pp. 161-184. (Chapman and Hall: London)
- 750 Kok, B (1948) A critical consideration of the quantum yield of *Chlorella*-photosynthesis.
751 *Enzymologia* **13**, 1-56.
- 752 Krömer, S (1995) Respiration during photosynthesis. *Annual Review of Plant Physiology &*
753 *Plant Molecular Biology* **46**, 45-70.
- 754 Larigauderie, A, Körner, C (1995) Acclimation of leaf dark respiration to temperature in alpine
755 and lowland plant species. *Annals of Botany* **76**, 245-252.
- 756 Lloyd, J, Shibistova, O, Zolotoukhine, D, Kolle, O, Arneth, A, Wirth, C, Styles, JM,
757 Tchebakova, NM, Schulze, ED (2002) Seasonal and annual variations in the
758 photosynthetic productivity and carbon balance of a central Siberian pine forest. *Tellus*
759 *Series B-Chemical And Physical Meteorology* **54**, 590-610.

- 760 Loomis, RS, Amthor, JS (1999) Yield potential, plant assimilatory capacity, and metabolic
761 efficiencies. *Crop Science* **39**, 1584-1596.
- 762 Loveys, BR, Atkinson, LJ, Sherlock, DJ, Roberts, RL, Fitter, AH, Atkin, OK (2003) Thermal
763 acclimation of leaf and root respiration: an investigation comparing inherently fast- and
764 slow-growing plant species. *Global Change Biology* **9**, 895-910.
- 765 McLaughlin, BC, Xu, C-Y, Rastetter, EB, Griffin, KL (2014) Predicting ecosystem carbon
766 balance in a warming Arctic: the importance of long-term thermal acclimation potential
767 and inhibitory effects of light on respiration. *Global Change Biology* **20**, 1901-1912.
- 768 Mercado, LM, Huntingford, C, Gash, JHC, Cox, PM, Jogireddy, V (2007) Improving the
769 representation of radiation interception and photosynthesis for climate model
770 applications. *Tellus Series B-Chemical And Physical Meteorology* **59**, 553-565.
- 771 Mitchell, KA, Bolstad, PV, Vose, JM (1999) Interspecific and environmentally induced
772 variation in foliar dark respiration among eighteen southeastern deciduous tree species.
773 *Tree Physiology* **19**, 861-870.
- 774 Noguchi, K, Yoshida, K (2008) Interaction between photosynthesis and respiration in
775 illuminated leaves. *Mitochondrion* **8**, 87-99.
- 776 Ogaya, R, Peñuelas, J (2007) Tree growth, mortality and above-ground biomass accumulation
777 in a holm oak forest under a five-year experimental field drought. *Plant Ecology* **189**,
778 291-299.
- 779 Ogaya, R, Peñuelas, J (2008) Changes in leaf $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for three Mediterranean tree
780 species in relation to soil water availability. *Acta Oecologica* **34**, 331-338.
- 781 Ow, LF, Griffin, KL, Whitehead, D, Walcroft, AS, Turnbull, MH (2008a) Thermal acclimation
782 of leaf respiration but not photosynthesis in *Populus deltoides* × *nigra*. *New Phytologist*
783 **178**, 123-134.
- 784 Ow, LF, Whitehead, D, Walcroft, AS, Turnbull, MH (2008b) Thermal acclimation of
785 respiration but not photosynthesis in *Pinus radiata*. *Functional Plant Biology* **35**, 448-
786 461.
- 787 Pärnik, T, Ivanova, H, Keerberg, O (2007) Photorespiratory and respiratory decarboxylations
788 in leaves of C3 plants under different CO2 concentrations and irradiances. *Plant, Cell*
789 *& Environment* **30**, 1535-1544.
- 790 Peñuelas, J, Boada, M (2003) A global change-induced biome shift in the Montseny mountains
791 (NE Spain). *Global Change Biology* **9**, 131-140.
- 792 Peñuelas, J, Sardans, J, Estiarte, M, Ogaya, R, Carnicer, J, Coll, M, Barbeta, A, Rivas-Ubach,
793 A, Llusià, J, Garbulsky, M, Filella, I, Jump, AS (2013) Evidence of current impact of
794 climate change on life: a walk from genes to the biosphere. *Global Change Biology* **19**,
795 2303-2338.
- 796 Pons, TL, Welschen, RAM (2002) Overestimation of respiration rates in commercially
797 available clamp-on leaf chambers. Complications with measurement of net
798 photosynthesis. *Plant Cell and Environment* **25**, 1367-1372.
- 799 Poorter, H, Remkes, C, Lambers, H (1990) Carbon and nitrogen economy of 24 wild species
800 differing in relative growth rate. *Plant Physiology* **94**, 621-627.
- 801 Rodríguez-Calcerrada, J, Jaeger, C, Limousin, JM, Ourcival, JM, Joffre, R, Rambal, S (2011)
802 Leaf CO2 efflux is attenuated by acclimation of respiration to heat and drought in a
803 Mediterranean tree. *Functional Ecology* **25**, 983-995.
- 804 Ryan, MG (1995) Foliar maintenance respiration of subalpine and boreal trees and shrubs in
805 relation to nitrogen content. *Plant, Cell and Environment* **18**, 765-772.
- 806 Sabate, S, Gracia, CA, A., S (2002) Likely effects of climate change on growth of *Quercus*
807 *ilex*, *Pinus halepensis*, *Pinus pinaster*, *Pinus sylvestris* and *Fagus sylvatica* forests in the
808 Mediterranean region. *Forest Ecology and Management* **162**, 23-37.

- 809 Searle, SY, Bitterman, DS, Thomas, S, Griffin, KL, Atkin, OK, Turnbull, MH (2011)
810 Respiratory alternative oxidase responds to both low- and high-temperature stress in
811 *Quercus rubra* leaves along an urban–rural gradient in New York. *Functional Ecology*
812 **25**, 1007-1017.
- 813 Shapiro, JB, Griffin, KL, Lewis, JD, Tissue, DT (2004) Response of *Xanthium strumarium*
814 leaf respiration in the light to elevated CO₂ concentration, nitrogen availability and
815 temperature. *New Phytologist* **162**, 377-386.
- 816 Tcherkez, G, Bligny, R, Gout, E, Mahe, A, Hodges, M, Cornic, G (2008) Respiratory
817 metabolism of illuminated leaves depends on CO₂ and O₂ conditions. *Proceedings of*
818 *the National Academy of Sciences, USA* **105**, 797-802.
- 819 Tcherkez, G, Boex-Fontvieille, E, Mahé, A, Hodges, M (2012) Respiratory carbon fluxes in
820 leaves. *Current Opinion in Plant Biology* **15**, 308-314.
- 821 Tcherkez, G, Cornic, G, Bligny, R, Gout, E, Ghashghaie, J (2005) *In vivo* respiratory
822 metabolism of illuminated leaves. *Plant Physiology* **138**, 1596-1606.
- 823 Tcherkez, G, Gauthier, P, Buckley, TN, Busch, FA, Barbour, MM, Bruhn, D, Heskell, MA,
824 Gong, XY, Crous, K, Griffin, KL, Way, DA, Turnbull, MH, Adams, MA, Atkin, OK,
825 Bender, M, Farquhar, GD, Cornic, G (2017) Tracking the origins of the Kok effect, 70
826 years after its discovery. *New Phytologist* **214**, 506-510.
- 827 Tcherkez, G, Mahe, A, Gauthier, P, Mauve, C, Gout, E, Bligny, R, Cornic, G, Hodges, M
828 (2009) *In folio* respiratory fluxomics revealed by ¹³C isotopic labeling and H/D Isotope
829 effects highlight the noncyclic nature of the tricarboxylic acid "cycle" in illuminated
830 leaves. *Plant Physiology* **151**, 620-630.
- 831 Tissue, DT, Lewis, JD, Wullschlegel, SD, Amthor, JS, Griffin, KL, Anderson, R (2002) Leaf
832 respiration at different canopy positions in sweetgum (*Liquidambar styraciflua*) grown
833 in ambient and elevated concentrations of carbon dioxide in the field. *Tree Physiology*
834 **22**, 1157-1166.
- 835 Tjoelker, MG, Craine, JM, Wedin, D, Reich, PB, Tilman, D (2005) Linking leaf and root trait
836 syndromes among 39 grassland and savannah species. *New Phytologist* **167**, 493-508.
- 837 Tjoelker, MG, Oleksyn, J, Lorenc-Plucinska, G, Reich, PB (2009) Acclimation of respiratory
838 temperature responses in northern and southern populations of *Pinus banksiana*. *New*
839 *Phytologist* **181**, 218-229.
- 840 Turnbull, MH, Tissue, DT, Griffin, KL, Richardson, SJ, Peltzer, DA, Whitehead, D (2005)
841 Respiration characteristics in temperate rainforest tree species differ along a long-term
842 soil-development chronosequence. *Oecologia* **143**, 271-279.
- 843 Turnbull, MH, Whitehead, D, Tissue, DT, Schuster, WSF, Brown, KJ, Griffin, KL (2003)
844 Scaling foliar respiration in two contrasting forest canopies. *Functional Ecology* **17**,
845 101-114.
- 846 Valladares, F, Zaragoza-Castells, J, Sanchez-Gomez, D, Matesanz, S, Alonso, B, Portsmouth,
847 A, Delgado, A, Atkin, OK (2008) Is shade beneficial for mediterranean shrubs
848 experiencing periods of extreme drought and late-winter frosts? *Annals of Botany* **102**,
849 923-933.
- 850 Villar, R, Held, AA, Merino, J (1994) Comparison of methods to estimate dark respiration in
851 the light in leaves of two woody species. *Plant Physiology* **105**, 167-172.
- 852 von Caemmerer, S, Farquhar, GD (1981) Some relationships between the biochemistry of
853 photosynthesis and the gas exchange of leaves. *Planta* **153**, 376-387.
- 854 Wang, XZ, Anderson, OR, Griffin, KL (2004) Chloroplast numbers, mitochondrion numbers
855 and carbon assimilation physiology of *Nicotiana glauca* as affected by CO₂
856 concentration. *Environmental and Experimental Botany* **51**, 21-31.
- 857 Wang, XZ, Lewis, JD, Tissue, DT, Seemann, JR, Griffin, KL (2001) Effects of elevated
858 atmospheric CO₂ concentration on leaf dark respiration of *Xanthium strumarium* in

859 light and in darkness. *Proceedings of the National Academy of Sciences of the United*
860 *States of America* **98**, 2479-2484.

861 Way, DA, Oren, R (2010) Differential responses to changes in growth temperature between
862 trees from different functional groups and biomes: a review and synthesis of data. *Tree*
863 *Physiology* **30**, 669-688.

864 Whitehead, D, Boelman, N, Turnbull, M, Griffin, K, Tissue, D, Barbour, M, Hunt, J,
865 Richardson, S, Peltzer, D (2005) Photosynthesis and reflectance indices for rainforest
866 species in ecosystems undergoing progression and retrogression along a soil fertility
867 chronosequence in New Zealand. *Oecologia* **144**, 233-244.

868 Wingate, L, Seibt, U, Moncrieff, JB, Jarvis, PG, Lloyd, J (2007) Variations in ¹³C
869 discrimination during CO₂ exchange by *Picea sitchensis* branches in the field. *Plant,*
870 *Cell and Environment* **30**, 600-616.

871 Wohlfahrt, G, Bahn, M, Haslwanter, A, Newesely, C, Cernusca, A (2005) Estimation of
872 daytime ecosystem respiration to determine gross primary production of a mountain
873 meadow. *Agricultural and Forest Meteorology* **130**, 13-25.

874 Wright, IJ, Reich, PB, Atkin, OK, Lusk, CH, Tjoelker, MG, Westoby, M (2006) Irradiance,
875 temperature and rainfall influence leaf dark respiration in woody plants: evidence from
876 comparisons across 20 sites. *New Phytologist* **169**, 309-319.

877 Wythers, KR, Reich, PB, Tjoelker, MG, Bolstad, PB (2005) Foliar respiration acclimation to
878 temperature and temperature variable Q_{10} alter ecosystem carbon balance. *Global*
879 *Change Biology* **11**, 435-449.

880 Xu, CY, Griffin, KL (2006) Seasonal variation in the temperature response of leaf respiration
881 in *Quercus rubra*: foliage respiration and leaf properties. *Functional Ecology* **20**, 778-
882 789.

883 Xu, M, Debiase, TA, Qi, Y, Goldstein, A, Liu, Z (2001) Ecosystem respiration in a young
884 ponderosa pine plantation in the Sierra Nevada Mountains, California. *Tree Physiology*
885 **21**, 309-318.

886 Zaragoza-Castells, J, Sanchez-Gomez, D, Hartley, IP, Matesanz, S, Valladares, F, Lloyd, J,
887 Atkin, OK (2008) Climate-dependent variations in leaf respiration in a dry-land, low
888 productivity Mediterranean forest: the importance of acclimation in both high-light and
889 shaded habitats. *Functional Ecology* **22**, 172-184.

890 Zaragoza-Castells, J, Sanchez-Gomez, D, Valladares, F, Hurrey, V, Atkin, OK (2007) Does
891 growth irradiance affect temperature dependence and thermal acclimation of leaf
892 respiration? Insights from a Mediterranean tree with long-lived leaves. *Plant Cell and*
893 *Environment* **30**, 820-833.

894

Table 1 Site characteristics and species selected for measurements at Prades (Catalunya, NE 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 | 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 _A (g _{DM} m ⁻²) | N (mg g _M ⁻¹) | P (mg g _M ⁻¹) | Sugars (mg g _M ⁻¹) | Starch (mg g _M ⁻¹) |
|-------------|------------------------------------------------------|-----------------------------------------|-----------------------------------------|----------------------------------------------|----------------------------------------------|
| Upper Slope | | | | | |
| Dec | 401 \pm 51 | 12.6 \pm 1.7 | 1.61 \pm 0.11 | 58.2 \pm 5.3 | 3.5 \pm 0.2 |
| Feb | 354 \pm 20 | 12.3 \pm 0.99 | 1.66 \pm 0.19 | 56.6 \pm 4.1 | 3.1 \pm 0.2 |
| Apr | 379 \pm 46 | 11.2 \pm 0.65 | 1.27 \pm 0.15 | 55.8 \pm 3.5 | 25.2 \pm 6.2 |
| Jun | 357 \pm 38 | 11.0 \pm 0.82 | 1.29 \pm 0.11 | 44.3 \pm 1.0 | 23.6 \pm 6.6 |
| Aug | 463 \pm 70 | 9.8 \pm 0.83 | 1.08 \pm 0.12 | 40.8 \pm 1.1 | 2.7 \pm 0.3 |
| Sept | 228 \pm 13 | 9.7 \pm 0.56 | 1.44 \pm 0.14 | 43.3 \pm 3.0 | 2.8 \pm 0.8 |
| Nov | 226 \pm 13 | 11.9 \pm 0.45 | 1.40 \pm 0.08 | 53.1 \pm 1.9 | 1.8 \pm 0.3 |
| Lower Slope | | | | | |
| Dec | 316 \pm 13 | 9.6 \pm 1.4 | 1.54 \pm 0.25 | 44.9 \pm 5.7 | 1.8 \pm 0.2 |
| Feb | 281 \pm 41 | 12.2 \pm 0.31 | 1.80 \pm 0.05 | 56.2 \pm 2.4 | 2.1 \pm 0.1 |
| Apr | 229 \pm 28 | 11.5 \pm 0.37 | 1.64 \pm 0.25 | 47.3 \pm 1.4 | 14.9 \pm 4.7 |
| Jun | 257 \pm 9 | 9.0 \pm 1.2 | 1.85 \pm 0.18 | 53.1 \pm 3.6 | 2.8 \pm 0.2 |
| Aug | 350 \pm 28 | 9.1 \pm 1.2 | 1.86 \pm 0.19 | 53.1 \pm 3.6 | 2.7 \pm 2.2 |
| Sept | 254 \pm 20 | 9.3 \pm 0.78 | 1.71 \pm 0.00 | 52.7 \pm 2.8 | 5.0 \pm 2.3 |
| Nov | 276 \pm 17 | 11.4 \pm 1.1 | 1.04 \pm 0.32 | 54.0 \pm 1.4 | 1.4 \pm 0.1 |

905
 906
 907

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 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.
 916
 917

| Leaf trait category | Variable | P values |
|----------------------------|--------------------------|------------------------------|
| 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 |

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 (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_{sat}), stomatal conductance at 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PPFD (g_{sat}), internal CO_2 concentration at 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PPFD (C_i), estimated rate of photorespiration at 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ 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_{sat} . See Table 1 for sites details and Table 5 for ANOVA results for each trait.

| Site | Species | M_A ($\text{g}_{\text{DM}}\text{m}^{-2}$) | RWC (%) | N ($\text{mg g}_{\text{DM}}^{-1}$) | 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 \pm 2.9 | 86.5 \pm 1.3 | 2.8 \pm 0.05 | 10 \pm 0.8 | 0.13 \pm 0.014 | 235 \pm 21 | 4.2 \pm 0.7 | 0.6 \pm 0.09 | 0.4 \pm 0.08 | 0.6 \pm 0.04 | 0.0 \pm 0.01 | 0.0 \pm 0.01 |
| | <i>Acer monspessulanum</i> | 94.3 \pm 10.4 | 92.6 \pm 2.0 | 1.7 \pm 0.08 | 10.6 \pm 0.8 | 0.09 \pm 0.010 | 231 \pm 22 | 4.9 \pm 0.8 | 0.9 \pm 0.15 | 0.8 \pm 0.14 | 0.7 \pm 0.08 | 0.1 \pm 0.02 | 0.0 \pm 0.02 |
| Slope | <i>Acer monspessulanum</i> | 87.5 \pm 6.1 | 91.5 \pm 0.9 | 1.8 \pm 0.01 | 8.3 \pm 0.9 | 0.10 \pm 0.015 | 223 \pm 12 | 4.7 \pm 0.3 | 0.9 \pm 0.13 | 0.7 \pm 0.14 | 0.7 \pm 0.06 | 0.1 \pm 0.02 | 0.0 \pm 0.02 |
| | <i>Quercus ilex</i> | 169.9 \pm 6.4 | 83.3 \pm 2.4 | 1.4 \pm 0.03 | 10.1 \pm 1.1 | 0.14 \pm 0.029 | 169 \pm 17 | 7.5 \pm 1.4 | 1.2 \pm 0.21 | 1.0 \pm 0.16 | 0.7 \pm 0.03 | 0.1 \pm 0.02 | 0.1 \pm 0.01 |
| | <i>Phillyrea latifolia</i> | 168.3 \pm 7.1 | 91.6 \pm 1.0 | 1.3 \pm 0.05 | 9.2 \pm 1.5 | 0.11 \pm 0.023 | 192 \pm 29 | 6.7 \pm 1.9 | 1.6 \pm 0.30 | 1.1 \pm 0.26 | 0.6 \pm 0.06 | 0.2 \pm 0.04 | 0.1 \pm 0.04 |
| Dry C | <i>Quercus ilex</i> | 204.6 \pm 9.9 | 85.8 \pm 0.7 | 1.2 \pm 0.11 | 8.6 \pm 1.4 | 0.09 \pm 0.021 | 200 \pm 24 | 5.4 \pm 1.4 | 1.2 \pm 0.17 | 0.8 \pm 0.12 | 0.6 \pm 0.03 | 0.1 \pm 0.02 | 0.1 \pm 0.01 |
| | <i>Phillyrea latifolia</i> | 113.1 \pm 9.8 | 93.7 \pm 0.9 | 1.0 \pm 0.12 | 7.6 \pm 0.6 | 0.08 \pm 0.010 | 201 \pm 13 | 5.7 \pm 2.3 | 0.8 \pm 0.07 | 0.6 \pm 0.13 | 0.7 \pm 0.12 | 0.1 \pm 0.01 | 0.0 \pm 0.02 |
| | <i>Arbutus unedo</i> | 134.3 \pm 8.2 | 96.0 \pm 1.1 | 1.0 \pm 0.07 | 11.6 \pm 0.5 | 0.13 \pm 0.015 | 222 \pm 22 | 5.4 \pm 0.9 | 1.1 \pm 0.12 | 0.7 \pm 0.08 | 0.6 \pm 0.06 | 0.1 \pm 0.01 | 0.0 \pm 0.01 |
| Dry E | <i>Quercus ilex</i> | 183.1 \pm 7.4 | 83.8 \pm 2.4 | 1.2 \pm 0.07 | 9.1 \pm 0.8 | 0.12 \pm 0.021 | 199 \pm 20 | 6.0 \pm 0.9 | 0.9 \pm 0.13 | 0.6 \pm 0.12 | 0.6 \pm 0.07 | 0.1 \pm 0.02 | 0.0 \pm 0.02 |
| | <i>Phillyrea latifolia</i> | 141.3 \pm 1.2 | 91.2 \pm 1.3 | 0.9 \pm 0.08 | 8.3 \pm 0.4 | 0.10 \pm 0.019 | 259 \pm 14 | 3.1 \pm 0.4 | 1.1 \pm 0.16 | 0.8 \pm 0.19 | 0.6 \pm 0.07 | 0.1 \pm 0.02 | 0.0 \pm 0.02 |
| | <i>Arbutus unedo</i> | 134.2 \pm 12.3 | 94.9 \pm 1.1 | 1.3 \pm 0.10 | 9.3 \pm 0.4 | 0.11 \pm 0.021 | 200 \pm 25 | 6.5 \pm 1.1 | 0.9 \pm 0.07 | 0.6 \pm 0.06 | 0.6 \pm 0.04 | 0.1 \pm 0.01 | 0.0 \pm 0.01 |
| Shrub C | <i>Quercus ilex</i> | 186.2 \pm 7.5 | 82.6 \pm 1.3 | 1.1 \pm 0.15 | 8.4 \pm 1.1 | 0.07 \pm 0.012 | 241 \pm 15 | 3.8 \pm 0.3 | 1.1 \pm 0.24 | 0.7 \pm 0.21 | 0.6 \pm 0.07 | 0.1 \pm 0.02 | 0.0 \pm 0.02 |
| | <i>Phillyrea latifolia</i> | 167.3 \pm 7.6 | 92.2 \pm 1.7 | 1.1 \pm 0.10 | 8.3 \pm 1.4 | 0.05 \pm 0.015 | 239 \pm 19 | 4.6 \pm 0.7 | 1.4 \pm 0.15 | 1.1 \pm 0.17 | 0.7 \pm 0.09 | 0.2 \pm 0.03 | 0.1 \pm 0.01 |
| | <i>Arbutus unedo</i> | 144.4 \pm 6.5 | 97.3 \pm 0.4 | 1.2 \pm 0.09 | 9.3 \pm 0.6 | 0.07 \pm 0.012 | 232 \pm 21 | 3.9 \pm 0.6 | 0.9 \pm 0.07 | 0.6 \pm 0.10 | 0.6 \pm 0.06 | 0.1 \pm 0.01 | 0.0 \pm 0.01 |
| Shrub E | <i>Quercus ilex</i> | 198.9 \pm 5.5 | 82.6 \pm 1.6 | 1.0 \pm 0.05 | 7.9 \pm 0.6 | 0.09 \pm 0.014 | 249 \pm 8 | 4.7 \pm 0.3 | 1.1 \pm 0.11 | 0.8 \pm 0.12 | 0.7 \pm 0.04 | 0.1 \pm 0.01 | 0.1 \pm 0.01 |
| | <i>Phillyrea latifolia</i> | 179.2 \pm 3.5 | 90.5 \pm 0.9 | 1.1 \pm 0.02 | 8.9 \pm 1.2 | 0.10 \pm 0.022 | 203 \pm 22 | 5.1 \pm 1.4 | 1.8 \pm 0.09 | 1.3 \pm 0.10 | 0.7 \pm 0.05 | 0.2 \pm 0.03 | 0.1 \pm 0.02 |
| | <i>Arbutus unedo</i> | 128.1 \pm 6.0 | 96.7 \pm 0.6 | 1.0 \pm 0.18 | 8.0 \pm 0.6 | 0.07 \pm 0.012 | 189 \pm 26 | 6.3 \pm 1.5 | 0.8 \pm 0.10 | 0.5 \pm 0.10 | 0.6 \pm 0.06 | 0.1 \pm 0.01 | 0.0 \pm 0.01 |

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_{sat} , net photosynthesis measured at 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PPFD; g_{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 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 | P 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_{sat} | Site <0.01 |
| | g_{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_{sat} | Site <0.05; Sp*S <0.01 |
| | R_L/A_{sat} | Sp*S <0.05 |

Figure 1. Seasonal changes in (a) soil volumetric water content, (b) leaf temperature, (c) A_{sat} ; i.e. net CO_2 uptake measured $1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, (d) g_{sat} ; maximum stomatal conductance at A_{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 ($R_{\text{L}}/R_{\text{D}}$) 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_{L} plotted against corresponding rates of R_{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_{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_{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_{sat} ; i.e. net CO_2 uptake measured $1500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, (d) g_{sat} ; maximum stomatal conductance at A_{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 ($R_{\text{L}}/R_{\text{D}}$) 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_{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.

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

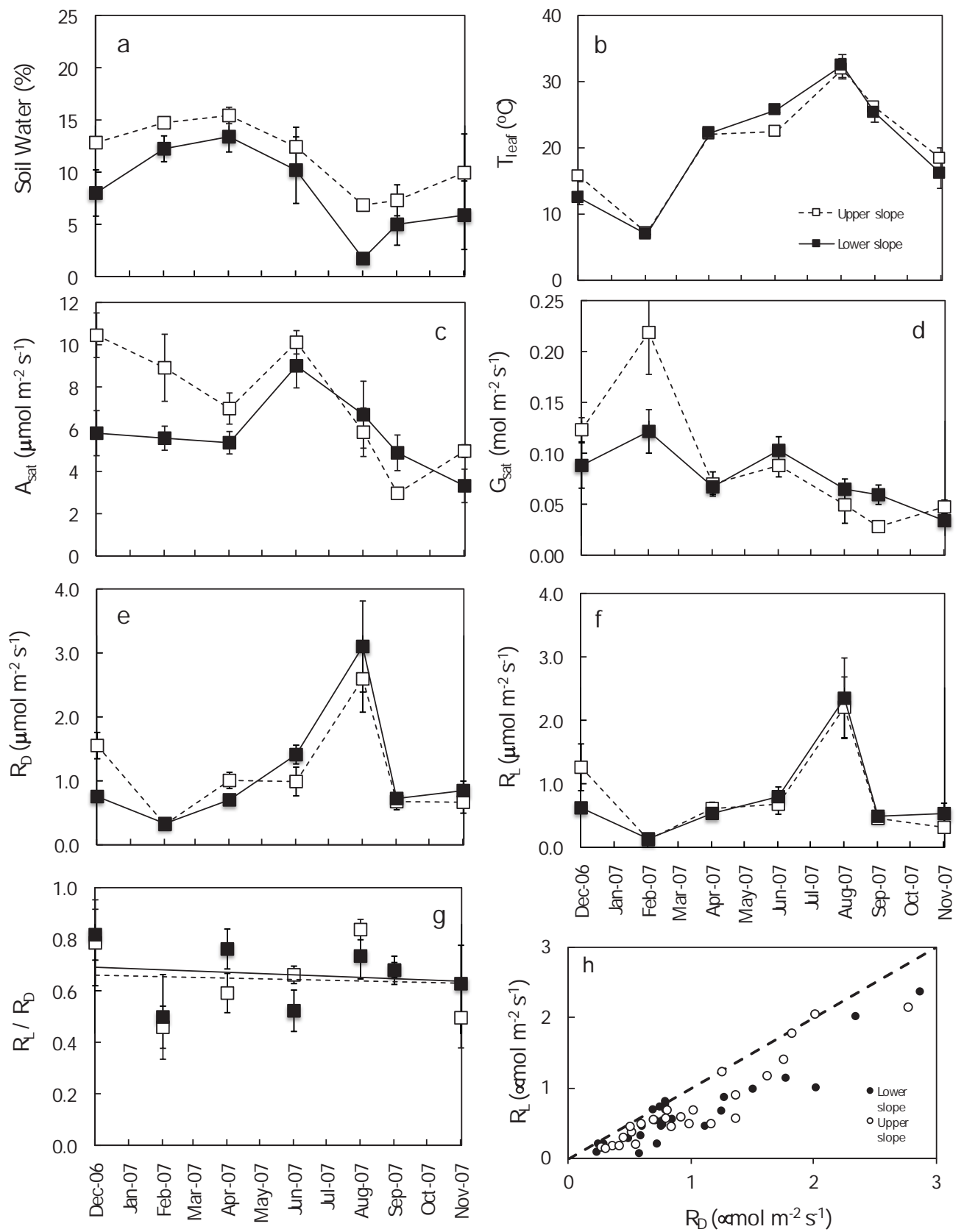


Figure 2. Temperature relationships from seasonal study at Villar de Cobeta

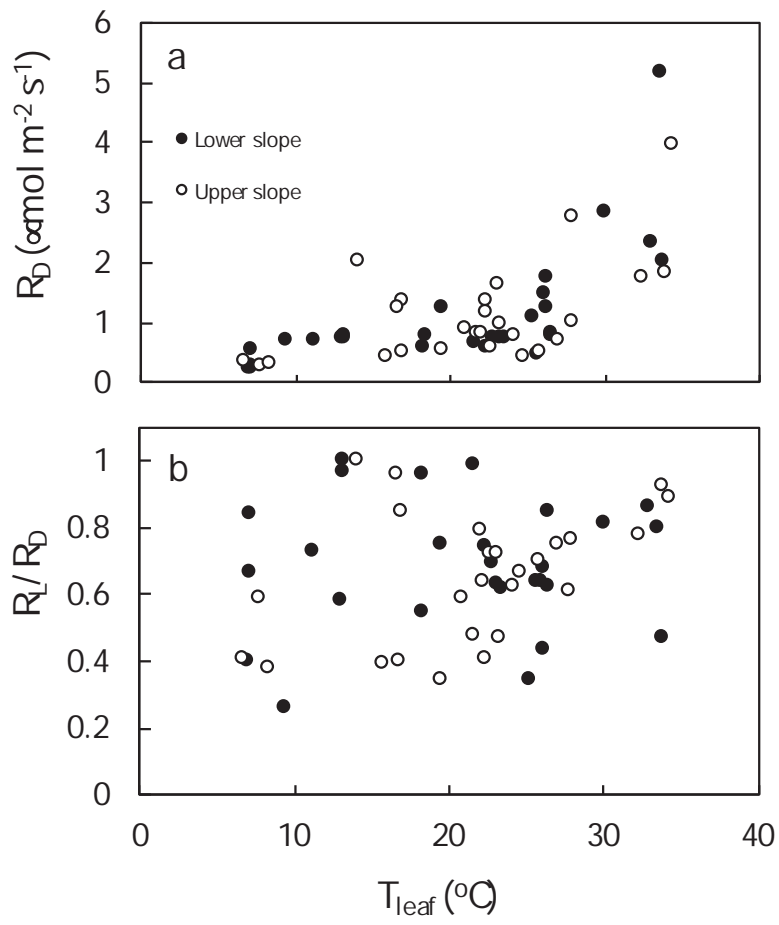


Figure 3. Seasonal changes in the thermal response of R

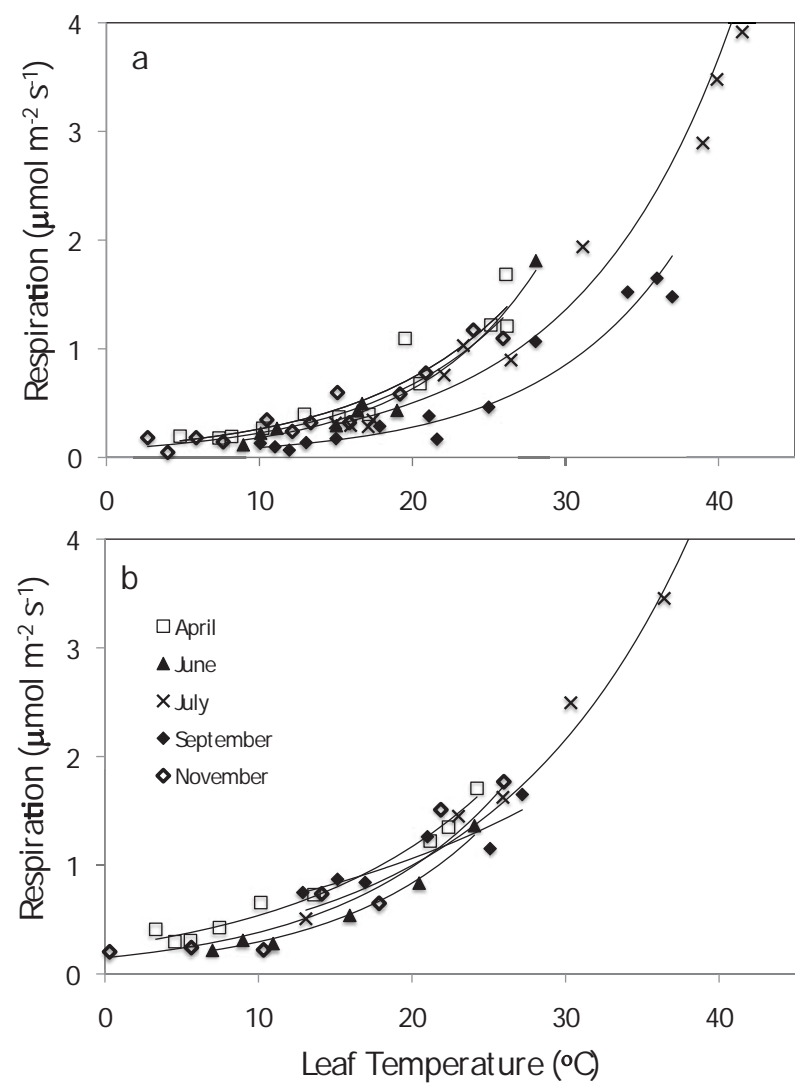
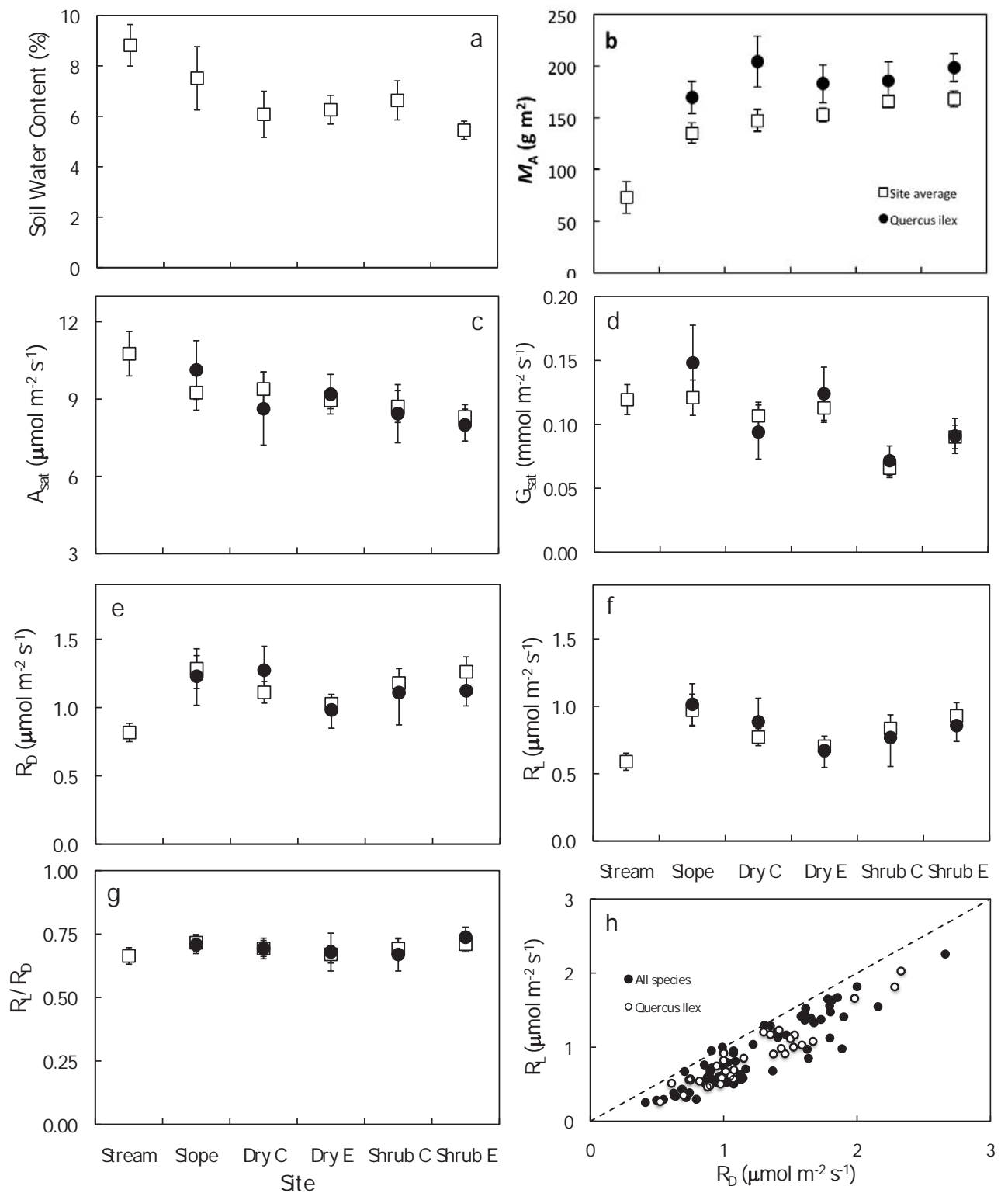


Figure 4. Prades results by site



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).

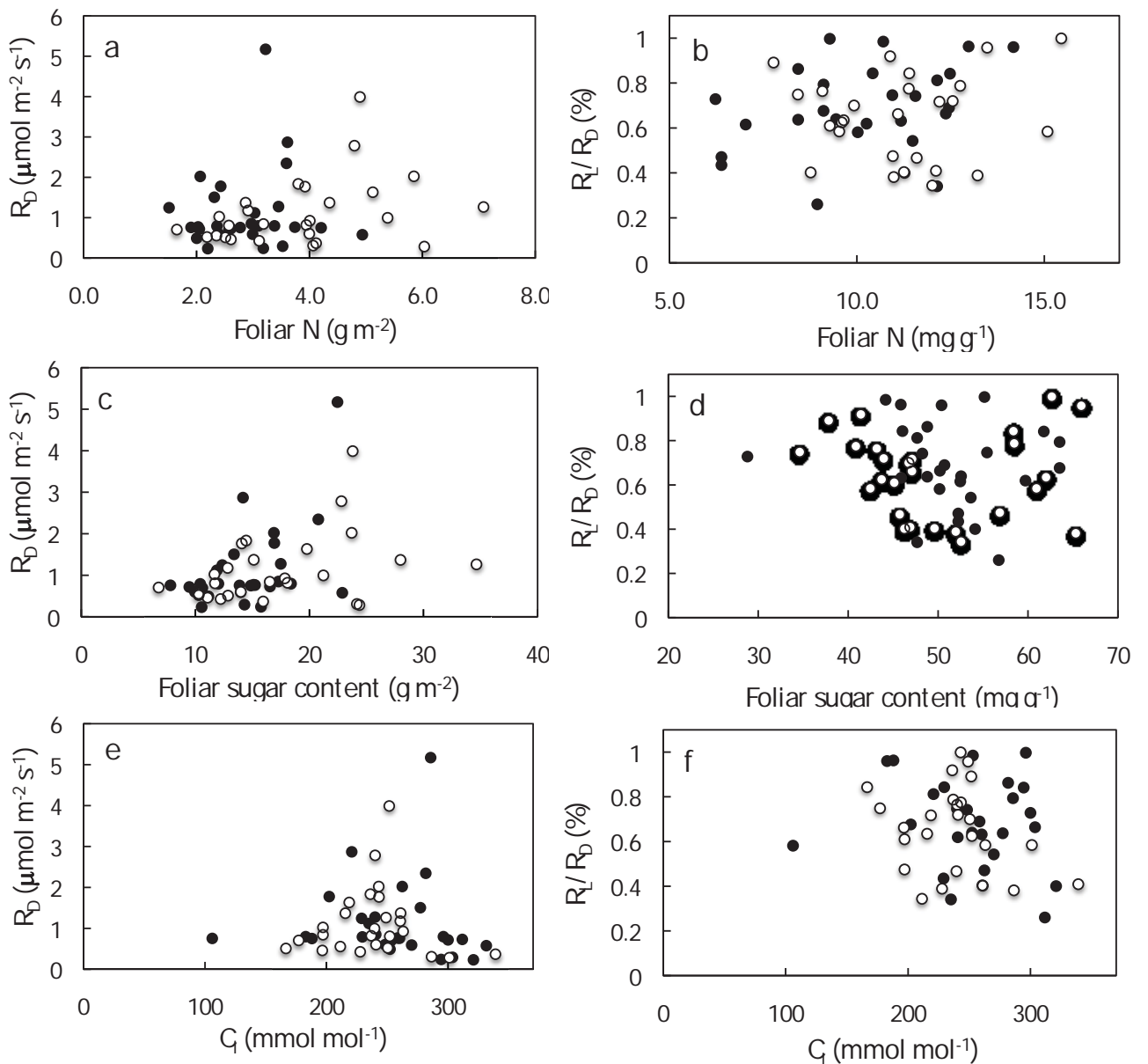


Figure S2. Relationships between soil water content and (a, c) A_{sat} ; CO_2 uptake measured $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and (b,d) foliar respiration rate in darkness (R_D) measured in *Q. ilex* seven times during the course of a year at an upper (closed symbols) and lower (open symbols) 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*).

