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5 traits and drought impacts in a Mediterranean mixed forest

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9 Running head: SEASONAL PHOTOSYNTHESIS MORPHOLOGY MIXED FOREST

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11 D. Sperlich^{1,2*}, C.T. Chang^{1,2}, J. Peñuelas^{2,3}, C. Gracia^{1,2}, S. Sabaté^{1,2}

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13 ¹ Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain,

14 ² CREAM, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain

15 ³ CSIC, Global Ecology Unit CREAM-CSIC-UAB, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain

16 * Author for correspondence

17 *Dominik Sperlich*

18 *Tel.: +34 9358 14850*

19 *E-mail: Dominik@creaf.uab.es*

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30

31 **Abstract**

32 The Mediterranean region is a hot spot of climate change vulnerable to increased
33 droughts and heat waves. Scaling carbon fluxes from leaf to landscape levels is
34 particularly challenging under drought conditions. We aimed to improve the
35 mechanistic understanding of the seasonal acclimation of photosynthesis and
36 morphology in sunlit and shaded leaves of four Mediterranean trees (*Quercus ilex* L.,

37 *Pinus halepensis* Mill., *Arbutus unedo* L., and *Q. pubescens* Willd.) under natural
38 conditions. $V_{c,max}$ and J_{max} were not constant, and mesophyll conductance was not
39 infinite, as assumed in most terrestrial biosphere models, but varied significantly
40 between seasons, tree species, and leaf position. Favourable conditions in winter led to
41 photosynthetic recovery and growth in the evergreens. Under moderate drought,
42 adjustments in the photo/biochemistry and stomatal/mesophyllic diffusion behaviour
43 effectively protected the photosynthetic machineries. Severe drought, however, induced
44 early leaf senescence mostly in *A. unedo*, *Q. pubescens*, and significantly increased leaf
45 mass per area in *Q. ilex* and *P. halepensis*. Shaded leaves had lower photosynthetic
46 potentials but cushioned negative effects during stress periods. Species-specificity,
47 seasonal variations, and leaf position are key factors to explain vegetation responses to
48 abiotic stress and hold great potential to reduce uncertainties in terrestrial biosphere
49 models especially under drought conditions.

50

51 ***Introduction***

52 The Mediterranean region is dominated by arid or semi-arid ecosystems where high evaporative
53 demand and low soil-water content during the summer dry period are the main ecological
54 limitations to plant growth (Specht 1969, Di Castri 1973). The resilience of plants to drought
55 and heat waves is determined by their frequency and duration, which are projected to become
56 much more severe under current climate change scenarios - particularly in the Mediterranean
57 region (Somot et al. 2008, Friend 2010, IPCC 2013). Increased drought-induced defoliation
58 (Poyatos et al. 2013) associated with the depletion of carbon reserves (Galiano et al. 2012) can
59 ultimately lead to catastrophic hydraulic failure and tree mortality (Urli et al. 2013, Choat
60 2013). Drought-induced forest impacts and diebacks in the Mediterranean region have been
61 reported in numerous studies (Peñuelas et al. 2001, Martínez-Vilalta and Piñol 2002,
62 Raftoyannis et al. 2008, Allen et al. 2010, Carnicer et al. 2011, Matusick et al. 2013) and can
63 lead to shifts in vegetation composition (Jump and Penuelas 2005, Anderegg et al. 2013) and to
64 a higher risk of forest fires (Piñol et al. 1998, Pausas et al. 2008). The challenge in the
65 Mediterranean region in the coming years will be to learn how carbon uptake and growth in
66 species and communities will respond to these changes, and how forest management strategies
67 can be adapted to cushion the negative impacts of climate change on forests (Sabaté 2002,
68 Bugmann et al. 2010).

69 In past decades, ecosystem models on regional or global level contributed substantially
70 to our understanding of the implications of climate change on a coarse scale where field
71 experiments are limited (Luo 2007). Much uncertainty, however, remains in the modelled
72 feedback of the global carbon cycle to climatic warming (Friedlingstein et al. 2014) and in the

73 understanding and modelling of species responses to climate change (Luo 2007, McDowell et
74 al. 2008, Beaumont et al. 2008). Photosynthesis is generally overestimated in the main Earth
75 system models, with significant regional variations (Anav et al. 2013). Two critical parameters,
76 the maximum rate of carboxylation ($V_{c,max}$) and the maximum rate of electron transport (J_{max}),
77 are a prerequisite for scaling foliar photosynthesis to the canopy level at which global dynamic
78 models operate (Friedlingstein et al. 2006, Friedlingstein and Prentice 2010). These two
79 parameters describe the biochemical limitations to carbon assimilation, but are not easily
80 measured. So relatively little data of their variability between species or seasons are available.
81 $V_{c,max}$ and J_{max} are thus often used as constants for various plant functional types and seasons or,
82 in some cases, are derived from other parameters such as leaf nitrogen content (Grassi and
83 Magnani 2005, Walker et al. 2014). Moreover, extreme climatic conditions and inter-annual
84 variability in arid and semi-arid regions are challenging for scaling carbon assimilation patterns
85 from one year to another (Reynolds et al. 1996, Morales et al. 2005, Gulías et al. 2009).
86 Simulations of ecosystem carbon fluxes are consequently limited, first, by underrepresented
87 temporal variability of photosynthetic parameters and soil-water patterns, and second by our
88 limited understanding of the effects of water stress on both carbon uptake and release (Hickler
89 et al. 2009, Niinemets and Keenan 2014). The modelling performance in Mediterranean-type
90 ecosystems is thus particularly poor and stresses the need for a better mechanistic description of
91 photosynthetic processes under water stress (Morales et al. 2005, Keenan et al. 2011, Zheng et
92 al. 2012, Vargas et al. 2013). Mesophyll conductance, g_m , might play a future key role in
93 improving model performance of photosynthesis under drought conditions (Keenan et al. 2010).

94 The photosynthetic limitations of Mediterranean vegetation, especially under drought,
95 have been extensively studied (for a review see Flexas *et al.*, 2014), but fewer studies have
96 thoroughly assessed the seasonal behaviour of photosynthesis and morphology under natural
97 conditions in a mixed mature forest. The information gained from seedlings under controlled
98 conditions can only poorly represent the physiological mechanisms of the long-term acclimation
99 to variable environmental conditions in mature trees (Flexas et al., 2006; Mittler, 2006;
100 Niinemets, 2010). Seedlings or saplings are characterised by higher metabolism and enzymatic
101 function, lower leaf dry mass per unit area (LMA), and higher photosynthetic potential relative
102 to mature trees (Johnson & Ball, 1996; Bond, 2000; Niinemets, 2014). Responses to short-term
103 stress are related to the mechanisms of prompt reactions (Flexas et al., 2006). Under natural
104 conditions, however, mature trees acclimate to gradually developing water stress through the
105 photosynthetic pathway (biochemical, stomatal or mesophyllic) (e.g. Martin-StPaul et al. 2013),
106 but also through foliar traits such as nitrogen, LMA etc. (Poorter et al. 2009). Less work has
107 evaluated simultaneously the variations of photosynthetic and morphological traits in response
108 to abiotic stress conditions. The variation of these traits is largely species specific (Orshan 1983,
109 Chaves et al. 2002, Gratani and Varone 2004, Krasteva et al. 2013), although within-canopy

110 gradients can play an additional overriding role (Valladares and Niinemets 2008, Sperlich et al.
111 2014). Mixed forests provide ideal test conditions where we can observe distinct species-
112 specific strategies coping equally with the yearly variability of environmental conditions.

113 The aim of this study was to investigate the impact of seasonal environmental changes
114 (above all drought) on foliar photosynthetic and morphological traits of the winter-deciduous
115 sub-Mediterranean *Quercus pubescens*, two evergreen sclerophyllous species (*Quercus ilex* and
116 *Arbutus unedo*) and an early-successional drought-adapted conifer, *Pinus halepensis*. *P.*
117 *halepensis* is characterised as isohydric following a water saving and photoinhibition-tolerant
118 strategy (Martínez-Ferri et al. 2004, Baquedano and Castillo 2006, Sperlich et al. 2014). *Q. ilex*
119 L. is a late-successional, slow growing, water-spending, photoinhibition-avoiding, anisohydric
120 tree species with a plastic hydraulic and morphological behaviour (Villar-Salvador et al. 1997,
121 Fotelli et al. 2000, Corcuera et al. 2004, Ogaya and Peñuelas 2006, Limousin et al. 2009). The
122 winter-deciduous anisohydric *Q. pubescens* follows a similar drought-avoiding strategy as *Q.*
123 *ilex*, but maximizes gas exchange during a shorter growing season (Baldochi et al. 2009),
124 resulting in high transpiration rates throughout the summer (Poyatos et al. 2008). Over extensive
125 areas of the Mediterranean region *Q. ilex* and *Q. pubescens* form the terminal point of secondary
126 succession (Lookingbill and Zavala 2000). *A. unedo* - relict of the humid-subtropical Tertiary
127 tree flora (Gratani and Ghia, 2002a and references therein) – is typically occurring as shrub or
128 small tree in the macchia ecosystems and holding an intermediate position concerning stomatal-
129 (Beyschlag et al. 1986, Vitale and Manes 2005, Barbeta et al. 2012) and photoinhibition-
130 sensitivity (Sperlich et al. 2014). Prolonged climate stress might disadvantage *A. unedo* being
131 more drought sensitive than the companion species (Ogaya and Peñuelas 2004, Barbeta et al.
132 2012).

133 Our particular interests were to distinguish the species-specific strategies and to explore
134 the eco-physiological mechanism behind drought responses by examining the fine tuning of
135 foliar photosynthetic potentials/rates and foliar morphological traits. We hypothesized that i)
136 seasonal environmental changes (above all drought) affect the photosynthetic and ii)
137 morphological traits, iii) mesophyll conductance (g_m) strongly constrains
138 photosynthesis under drought conditions, iv) the seasonal acclimation varies qualitatively and
139 quantitatively with species and v) light environment (leaf canopy position). We thus created a
140 matrix of photosynthetic parameters that could be incorporated into process-based ecosystem
141 models to improve estimates of carbon flux in the Mediterranean region.

142

143 **Materials and methods**

144 ***Field site***

145 The experimental site Can Balasc is located in the coastal massif of the Collserola Natural Park
146 (8500 ha), in the province of Barcelona, northeastern Spain (41° 25' N, 2° 04' E, 270 m a.s.l.).
147 Seasonal summer droughts, warm temperatures and mild winters characterise the typical
148 Mediterranean climate with a mean August temperature of 22.8 °C and a mean January
149 temperature of 7.9 °C. Mean annual precipitation and temperature are 723 mm and 15.1 °C
150 (1951-2010), respectively (Ninyerola et al. 2007a, 2007b). Sensors for measuring air
151 temperature (HMP45C, Vaisala Oyj, Finland) and solar radiation (SP1110 Skye Instruments
152 Ltd., Powys, UK) were installed at a height of 3 m, in a clearing *ca.* 1 km from the plot.

153 ***Stand structure***

154 Our study site is characterised by a dense forest stand (1429 stems ha⁻¹) with a two-layered
155 canopy consisting of a dense layer of *Quercus* species surmounted by shelter trees of the early-
156 successional and fast growing Aleppo Pine (*P. halepensis* Mill.). The mean heights of each
157 layer are 9.9 m and 17.1 m, respectively. The *Quercus* species are the late-successional
158 evergreen Holm Oak (*Q. ilex* L.) and the deciduous Pubescent Oak (*Q. pubescens* Willd.). The
159 Strawberry tree (*A. unedo* L.) grows usually as a shrub being widely abundant in the macchia
160 ecosystems of the Iberian peninsula (Beyschlag et al. 1986, Reichstein et al. 2002). In our study
161 site, however, *A. unedo* occurs scattered in the tree canopy (mean height 8.1 m) enriching the
162 forest diversity with its flowering and fruiting habit. The trees with the biggest dimensions are
163 the pines followed by the two *Quercus* species and at last by *A. unedo* (mean DBH of 33.7,
164 12.9, 9.6 cm, respectively). The forest succession has reached the final stage: The dense
165 *Quercus* canopy is out-competing the early-successional *P. halepensis* by suppressing the
166 growth of the light demanding pine seedlings and saplings. More details of stand history and
167 field site are described in Sperlich *et al.* (2014).

168 ***Sampling method***

169 We conducted eight field campaigns from June 2011 to February 2013. The sampling periods
170 are presented in Table 2 and Figure 1. We avoided difficulties encountered during field
171 measurements such as deviations from the standard temperature (25 °C) or unpredictable plant
172 responses (patchy stomatal conductance) (Mott and Buckley 1998, 2000) by analysing sampled
173 twigs in the laboratory. We cut twigs with a pruning pull from sunlit and shaded leaf positions,
174 optimally at similar heights. The twigs were immediately re-cut under water in the field,
175 wrapped in plastic bags to minimise transpiration, stored in water buckets, and transported to
176 the laboratory. Five replicates of each leaf position and tree species were collected for the
177 analysis of gas exchange. The twigs were pre-conditioned in the laboratory at room temperature

178 (24-28 °C) in dim light for 1-3 d and were freshly cut every morning. More details and
179 references can be found in Sperlich *et al.* (2014).

180 ***Analyses of gas exchange and chlorophyll fluorescence***

181 Gas exchange and chlorophyll fluorescence were measured with a Li-Cor LI-6400XT Portable
182 Photosynthesis System equipped with a LI-6400-40 Leaf Chamber Fluorometer (Li-Cor, Inc.,
183 Lincoln, USA). Response curves for foliar net assimilation versus CO₂ concentration were
184 recorded in parallel with the chlorophyll fluorescence measurements. In some cases the sunlit
185 leaves of *Q. ilex* were too small to fill the leaf cuvette (2 cm²) and so the measured parameters
186 were adjusted after the measurements. For *P. halepensis*, we positioned a layer of needles (ca.
187 10-15) on the leaf cuvette, avoiding gaps and overlays, and sealed the gaskets with Blu-tack
188 (Bostik SA, La Plaine St Denis, France) to keep the needles in position. The preparation and
189 acclimation of the leaves prior to recording the response curves were conducted as in Sperlich *et*
190 *al.* (2014).

191 ***CO₂ experiments***

192 The CO₂-response curves were recorded at a leaf temperature (T_{Leaf}) of 25 °C and a quantum
193 flux density of 1000 μmol photons m⁻² s⁻¹. The CO₂ concentrations in the leaf chamber (C_a) used
194 to generate the response curves were 400→300→200→150→100→50→400→400→600→
195 800→1200→2000 μmol CO₂ mol air⁻¹. The minimum and maximum times for stabilising net
196 assimilation rate (A_{net} in μmol CO₂ m⁻² s⁻¹), stomatal conductance (g_s in mol H₂O m⁻² s⁻¹), and
197 stomatal internal CO₂ concentrations (C_i in μmol CO₂ mol air⁻¹) for each log were set to 4 and 6
198 min, respectively.

199 ***Calculation of chlorophyll fluorescence parameters***

200 F_m' and F_s were used to estimate the effective quantum yield of photosystem II (Φ_{PSII}, unitless)
201 as:

$$202 \quad \Phi_{\text{PSII}} = \frac{(F_{\text{m}}' - F_{\text{s}})}{F_{\text{m}}'} \quad (1)$$

203 where F_s is the steady-state fluorescence of a fully light-adapted sample, and F_m' is the maximal
204 fluorescence yield reached after a pulse of intense light. The effective quantum yield of PSII
205 represents the fraction of photochemically absorbed photons for a light-adapted leaf. The
206 electron-transport rate based on the effective quantum yield of PSII (J_{CF} in μmol electron m⁻² s⁻¹)
207 was calculated as

$$208 \quad J_{\text{CF}} = \varepsilon * \Phi_{\text{PSII}} * \alpha_{\text{L}} \quad (2)$$

209 ε is a scaling factor accounting for the partitioning of intercepted light between photosystem I
210 (PSI) and PSII. We assumed that light was equally distributed between both photosystems (ε =
211 0.5) (Bernacchi *et al.* 2002, Niinemets *et al.* 2005). α_L (unitless) is the foliar absorbance; we

212 used the following values: 0.932 for *Q. ilex* and 0.912 for *P. halepensis* for both sunlit and
 213 shaded leaves, 0.935 for sunlit leaves of *A. unedo*, 0.917 for shaded leaves of *A. unedo*, 0.939
 214 for sunlit leaves of *Q. pubescens*, and 0.900 for shaded leaves of *Q. pubescens*. For the
 215 determination of α_L , foliar reflectance and transmittance were measured at midday in August
 216 2012 using a UniSpec Spectral Analysis System spectroradiometer (PP Systems, Haverhill,
 217 USA). The ambient photosynthetic electron transport (J_{amb}) was defined as the value of J_{CF} at a
 218 CO₂ concentration of 400 $\mu\text{mol CO}_2 \text{ mol air}^{-1}$ and a PPFD of 1000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The
 219 relationship between J_{amb} and the net assimilation rate (J_{amb}/A_{net}) was used for the analyses of
 220 alternative electron sinks other than carbon metabolism. Calculations of F_v/F_m and NPQ can be
 221 found in the supplementary material (Note S1).

222 ***Estimation of mesophyll conductance***

223 We estimated g_m (in $\text{mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$) using the variable- J method by Harley et al. (1992):

$$224 \quad g_m = \frac{A_{net}}{C_i - \frac{[\Gamma^* J_{CF} + 8(A_{net} + R_d)]}{J_{CF} - 4(A_{net} + R_d)}} \quad (6)$$

225 where Γ^* is the CO₂ concentration at which the photorespiratory efflux of CO₂ equals the rate of
 226 photosynthetic CO₂ uptake, and R_d is the mitochondrial respiration of a leaf in light conditions
 227 and was estimated from the light-response curves combining gas exchange and measurements
 228 with the CF- method proposed by Yin et al. (2009). See supplementary material for details
 229 (Note S2). The chloroplastic CO₂ concentration (C_c in $\mu\text{mol CO}_2 \text{ mol air}^{-1}$) was determined as:

$$230 \quad C_c = C_i - \frac{A_{net}}{g_m} \quad (7)$$

231 ***Photosynthesis model***

232 The photosynthesis model of Farquhar et al. (1980) considers photosynthesis as minimum of the
 233 potential rates of Rubisco activity (A_c) and ribulose-1,5-bisphosphate (RuBP) regeneration (A_j).
 234 The model was further complemented with a third limitation (A_p) that considers the limitation
 235 by triose-phosphate use (TPU) at high CO₂ concentrations when the CO₂ response shows a
 236 plateau or decrease (Sharkey 1985). However, we rarely detected A_p limitations and TPU was
 237 therefore discarded in our analyses. A_{net} was then determined by the minimum of these two
 238 potential rates from an A/C_c curve:

$$239 \quad A_{net} = \min\{A_c, A_j\} \quad (8)$$

240 where

$$241 \quad A_c = V_{cmax} * \left[\frac{C_c - \Gamma^*}{C_c + K_c \left(1 + \frac{\Gamma^*}{K_o}\right)} \right] - R_d \quad (9)$$

242 where $V_{c,max}$ (in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) is the maximum rate of Rubisco carboxylation, K_c is the
243 Michaelis-Menten constant of Rubisco for CO_2 , O is the partial pressure of O_2 at Rubisco, and
244 K_o is the Michaelis-Menten constant of Rubisco for O_2 , taken from Bernacchi et al. (2002). The
245 equation representing photosynthesis limited by RuBP regeneration is:

$$246 \quad A_j = J * \left[\frac{C_c - \Gamma^*}{4C_c + 8\Gamma^*} \right] - R_d \quad (10)$$

247 where J (in $\mu\text{mol electron m}^{-2} \text{ s}^{-1}$) is the rate of electron transport. We assumed that J becomes
248 J_{max} under light and CO_2 saturation when the maximum possible rate of electron transport is
249 theoretically achieved, although we may have underestimated the true J_{max} (for further details
250 see Buckley & Diaz-Espejo, 2014). $V_{c,max}$ and J_{max} define the biochemical potential to drive
251 photosynthesis and are summarised in the term “photosynthetic potential” (Niinemets et al.
252 2006). Curves were fit, and diffusion leakage was corrected, as in Sperlich *et al.* (2014).

253 ***Foliar morphology, chemical analyses, and assessment of crown*** 254 ***condition***

255 Foliar morphological traits were measured on fully expanded leaves ($n = 60$ per leaf position
256 and species) from the excised twigs in five sampling campaigns in spring and autumn 2011^a
257 (2011^a indicates sampling during a drought), and winter, spring, and summer 2012. Immediately
258 after the gas exchange analyses, we measured fresh weight (FW, mg) and projected leaf surface
259 area (LA, cm^2) (including petioles) with Photoshop from scanned leaves at 300 dpi. We oven-
260 dried the leaves at 70°C for 48 h and weighed the leaves for dry weight (DW, mg) and
261 measured leaf thickness (LT, mm) with a portable dial thickness gauge (Baxlo Precisión,
262 Barcelona, Spain). We then calculated the percentage of the leaf water content (WC) as $[1 -$
263 $(\text{DW}/\text{FW})] * 100$. Leaf mass per area (LMA) (mg cm^{-2}) was calculated as the ratio of DW to LA
264 and leaf tissue density (D, mg cm^{-3}) as the ratio of LMA to LT. Foliar Succulence (S) was
265 calculated as $(\text{FW} - \text{DW})/\text{LA}$. We ground the leaves to a fine powder using a MM400 mixer mill
266 (Retsch, Hahn, Germany), encapsulated a sample of 0.7 mg in tin foil and determined carbon
267 and nitrogen contents by EA/IRMS (Elemental Analyzer/Isotope Ratio Mass Spectrometry) and
268 GC/C/IRMS (Gas Chromatography/Combustion/IRMS). The crown condition was assessed
269 using ‘International Co-operative Programme on Assessment and Monitoring of Air
270 Pollution Effects on Forests’ (ICP Forests) standards (Eichhorn et al. 2010).

271 ***Statistical analyses***

272 We performed the statistical analyses with the R version 3.0.2 (<http://www.r-project.org/>). The
273 matrix of photosynthetic and morphological traits was subjected to principal component
274 analyses (PCAs) to summarise the principal factors explaining the variation in these parameters.
275 Differences in the parameters between sunlit and shaded leaves were determined with Student’s

276 t -tests ($P \leq 0.05$). The normality of the data was tested with Shapiro-Wilk tests. If the data were
277 not normally distributed, they were normalised. One-factorial analyses of variance (ANOVAs)
278 with season as the main factor were used to test for differences in the parameters in each species
279 and leaf position. Significant differences were determined at $P \leq 0.05$ with Fisher's least
280 significance difference (LSD) tests. Bonferroni correction was used for familywise error rate.
281 Linear regression analyses were conducted to study the relationships among various leaf traits
282 such as A_{net}/g_s , A_{net}/g_m , $J_{\text{max}}/V_{c,\text{max}}$, g_m/g_s , $J_{\text{amb}}/A_{\text{net}}$. With analyses of co-variance (ANCOVAs),
283 we tested for differences in regression slopes and intercepts. We applied a non-linear regression
284 analysis using the nls function in R to study the relationship of g_m/LMA .
285

286 **Results**

287 ***Environmental and crown conditions***

288 The year 2011 was characterised by 30% more precipitation than the climatic average of 723
289 mm (1951-2010) (Ninyerola et al. 2007a, 2007b) (Tab. 3, Fig. 1), and no drought-induced leaf
290 shedding was observed. The winter from 1 December 2011 to 31 January 2012 was relatively
291 mild with average maximum and minimum temperatures of 11.8 and 4.2 °C, respectively,
292 coinciding with high photosynthetic potentials and shoot growth. The precipitation in 2012 was
293 20% lower than the climatic average (Table 3). *A. unedo* and *Q. pubescens* were strongly
294 defoliated during summer 2012; *Q. ilex* and *P. halepensis* to a lesser extent (Table 4). *Q. ilex*
295 showed some discoloration in the more exposed sites. Only one individual of *P. halepensis*
296 showed discoloration. The defoliated *Q. pubescens* trees recovered completely in 2013. In
297 contrast, heavily affected individuals of *A. unedo* showed an irreversible dieback of the main
298 leading branches but also vigorous re-sprouting in 2013.

299 ***Effect of season, tree species and leaf position on photosynthetic*** 300 ***parameters***

301 In Fig. 2a, we present the PCA for the morphological and photosynthetic parameters. No
302 rotation was applied to the space of the PC's. $V_{c,max}$, J_{max} , and g_s were negatively correlated with
303 N_{mass} , C_{mass} , NPQ, and g_m . F_v/F_m , g_s , and water content (WC) were negatively correlated with
304 nitrogen and carbon per unit leaf area (N_{area} , C_{area}), LMA, and density (D). Nitrogen per unit leaf
305 mass (N_{mass}) and g_m correlated well with LT (Fig. 2). A_{net} was correlated negatively with
306 succulence (S) and positively with g_m . PC1 and PC2 explained 37.2 and 20.4% of the variation,
307 respectively. The datapoints within the cluster circles in Fig. 1b-d exhibited similar behaviours
308 in photosynthetic and morphological traits. Leaf positions, seasons, and species could be
309 separated. Sunlit leaves were characterised by higher values on the orthogonal axis. The
310 horizontal axes separated *A. unedo* and *Q. pubescens* from *Q. ilex* and *P. halepensis*. The
311 orthogonal axes separated *Q. ilex* from *P. halepensis* with generally positive values. The
312 seasonality was further investigated for each species and leaf position with ANOVAs for each
313 photosynthetic and morphological parameter.

314 *Q. ilex*

315 *Q. ilex* had the most plastic response to the environmental conditions. The sunlit leaves of *Q.*
316 *ilex* exhibited strong declines in several photosynthetic parameters from summer 2011 to
317 autumn 2011^a. $V_{c,max}$, A_{net} and g_s were significantly ($P < 0.05$), and J_{max} and g_m were marginally
318 significantly lower ($P < 0.10$) (Fig. 3 a1-b1). The means of the majority of the photosynthetic
319 parameters recovered after the first rains in autumn 2011^b (2011^b indicates sampling after the
320 drought), reaching pre-drought values, but accompanied by a high standard error. This recovery

321 was thus only significant for J_{\max} and g_m . Surprisingly, $V_{c,\max}$ and J_{\max} peaked in winter and not,
322 as expected, in spring. From that peak we observed significant declines from winter to spring to
323 summer 2012. In contrast to the pattern of $V_{c,\max}$ and J_{\max} , F_v/F_m , A_{net} , and g_s peaked in spring
324 2012 (Figs. 3c1, 4a1-b1). These parameters then also declined significantly in summer 2012.
325 Interestingly, g_m peaked in summer 2012 in parallel with a reduction in g_s (Fig. 4c1). The
326 photosynthetic parameters of shaded leaves in *Q. ilex* showed a similar trend, declining after the
327 drought in 2011 and recovering after the autumn rains (Figs. 3, 4). The parameter means of
328 shaded leaves remained relatively stable throughout the season, in contrast to the pattern in
329 sunlit leaves, except for a peak of $V_{c,\max}$ and J_{\max} in spring 2012. The photosynthetic parameters
330 in *Q. ilex* were significantly lower in shaded leaves. During periods of stress, however, the
331 photosynthetic parameters of sunlit leaves declined and had values similar to those of shaded
332 leaves (Table 6, Figs. 3, 4).

333 *P. halepensis*

334 Mean $V_{c,\max}$, J_{\max} , and F_v/F_m were generally higher in sunlit leaves of *P. halepensis* than the
335 other species (Figs. 3, 4). The seasonal variation of the photosynthetic potential was not as
336 strongly pronounced as in *Q. ilex*, and mean $V_{c,\max}$ and J_{\max} remained relatively high and stable
337 in 2011 (Fig. 3a1-b1). The 2012 drought had comparatively stronger effects on $V_{c,\max}$ and J_{\max}
338 than the 2011 drought. Mean A_{net} , g_s , and g_m , however, were significantly lower in autumn 2011^a
339 (Fig. 4a1-c1). These values recovered quickly and significantly after the first autumn rains. The
340 relatively high $V_{c,\max}$, J_{\max} , and F_v/F_m during this period reflected a stronger limitation of g_s and
341 g_m than of the biochemistry imposed on A_{net} . A_{net} recovered in winter 2012 due to the mild
342 conditions (Fig. 4a1). The 2012 summer drought significantly reduced the high values of A_{net}
343 observed in winter 2012, but not as much as after the 2011 drought (Fig. 4a1). Both g_s and g_m
344 remained relatively stable during this period, so the reductions in A_{net} were due to biochemical
345 limitations ($V_{c,\max}$ and J_{\max}) (Figs. 3, 4). Sunlit and shaded leaves differed the least in *P.*
346 *halepensis*; only $V_{c,\max}$ and J_{\max} were significantly different (Table 6). The sunlit and shaded
347 leaves of *P. halepensis* had similar patterns of seasonal variation, but changes between seasonal
348 campaigns were not significant (Fig. 3a1-b1).

349 *A. unedo*

350 Similar to *Q. ilex*, the photosynthetic parameters in *A. unedo* varied strongly seasonally but had
351 high standard errors (Figs. 3, 4). A_{net} decreased significantly in winter 2012, in contrast to J_{\max}
352 and $V_{c,\max}$ that peaked in the same campaign (Figs. 3a1-b1 and 4a1). A decline in g_s and g_m in
353 this campaign suggested that they more strongly regulated A_{net} (Fig. 4b1-c1). A_{net} , g_s , and g_m
354 peaked in spring 2012. These increases were significant for A_{net} and g_s and marginally
355 significant for g_m relative to the other field campaigns (Fig. 4a1-4c1). The photosynthetic
356 parameters were generally lower in the shaded leaves of *A. unedo*, but with no clear pattern and
357 high variability (Table 5).

358 *Q. pubescens*

359 The photosynthetic potentials were much higher in *Q. pubescens* than in the other species but
360 also had high standard errors (Fig. 3a1-b1). The 2012 summer drought led to a decline of the
361 photosynthetic potentials by approximately one third. These decreases were only significant for
362 the average of spring 2011 and spring 2012 relative to the average of summer 2011 and summer
363 2012. A_{net} showed a similar trend, with a peak in spring 2012 being reduced significantly by the
364 2012 summer drought (Fig. 4a1). Stomatal control was more strongly pronounced than
365 mesophyllic control (Fig. 4b1-c1). Shaded leaves had higher A_{net} , g_m , and g_s means throughout
366 the campaigns, in contrast to lower means of $V_{c,\text{max}}$ and J_{max} (Figs. 3a1-c2, 4a1-b2). Shaded
367 leaves generally showed lower values than sunlit leaves and were less affected by the droughts
368 (Figs. 3a1-c2, 4a1-c2).

369 ***Morphological parameters***

370 The foliar traits of *P. halepensis* and *Q. ilex* acclimated most strongly to drought. LMA was
371 significantly higher in *P. halepensis* and *Q. ilex* in both shaded and sunlit leaves in summer
372 2012 compared to the previous field campaigns (Fig. 5a1-a2). This was similar in *A. unedo* but
373 less pronounced. LMA had no clear pattern in *Q. pubescens*. Elevated LMA was accompanied
374 by higher values of leaf density (D), succulence (S), and carbon content, indicating a more
375 sclerophyllic and succulent structure as response to the drier conditions in 2012 (Figs. S2, S3).
376 N_{mass} was significantly higher in spring and summer 2012 for *Q. ilex* and *P. halepensis* (shaded
377 and sunlit leaves) and for shaded leaves of *A. unedo*, but not for *Q. pubescens* (Fig. 5b1-b2).

378 ***Relationships of photosynthetic and morphological parameters***

379 In order to analyse the general pattern of several relationships of the photosynthetic parameters
380 and foliar traits, we used ANCOVAs to test for differences in the slopes between seasons across
381 all species.

382 The slope the A_{net}/g_s relationship was significantly steeper in summer and autumn 2011^a in all
383 species compared to the other field campaigns (Fig. 6a1, Table S1), suggesting an increased
384 intrinsic water-use efficiency during the dry period in 2011. Shaded leaves had a similar
385 conservative water-use strategy in autumn 2011^a (Fig. 6a2). Shallower slopes in autumn 2011^b
386 in both leaf positions represent rapid responses (less than one week) to the post-drought rains
387 easing the strict stomatal control.

388 The A_{net}/g_m relationship in autumn 2011^a also had a significantly steeper slope in both sunlit and
389 shaded leaves recovering after the first rains in autumn 2011^b (Fig. 6b1-2, Table S2). In the drier
390 year 2012, g_m imposed less resistance on photosynthetic assimilation compared to the wet year
391 2011. The slope of the A_{net}/g_m relationship was significantly higher for winter 2012 than spring
392 and summer 2012, suggesting a stronger control of g_m on photosynthesis in winter. The autumn
393 2011^a and summer 2012 droughts had strong effects on the slope of A_{net}/g_m in shaded leaves.

394 With the ANCOVA of the relationship of g_m and g_s , we investigated the proportional diffusion
395 limitation on photosynthesis. We observed seasonal differences across all species (Fig. 6c,
396 Table S3). Mesophyllic control was stronger in the dry autumn 2011^a and the two winter
397 periods. In contrast, stomatal control was higher than mesophyllic control in the mild 2011
398 summer drought. This was most strongly pronounced in *P. halepensis* and *Q. ilex* (data not
399 shown).

400 The slope in the relationship of $V_{c,max}$ and J_{max} was significantly steeper in autumn 2011^a for
401 both sunlit and shaded (Fig. 7a, Table S4) leaves due to a stronger reduction in $V_{c,max}$ compared
402 to J_{max} . The overall $J_{max}/V_{c,max}$ ratios were 1.09 for sunlit and 1.24 for shaded leaves. The slope
403 of the J_{amb}/A_{net} relationship in sunlit and shaded leaves was significantly lower in the more
404 humid periods (autumn 2011^b, winter 2012, and winter 2013), indicating lower protective
405 energy dissipation and alternative electron pathways under favourable conditions (Fig. 7b, Table
406 S5).

407 Increased foliar sclerophylly led to higher LMAs and thus to higher diffusion resistances in the
408 mesophyll, as shown by the relationship between g_m and LMA (Fig. 8, Table S6). In spring
409 2012 and summer 2012, we detected a less negative exponent (hence a gentler curve) (-0.953
410 and -0.800, respectively) compared to winter 2012 and autumn 2011^a) (-1.486 and -1.533,
411 respectively). This shows that, regardless of the drier conditions and higher LMA in 2012, g_m
412 was higher in this period reflecting a regulatory mechanism of g_m in the CO₂ diffusion pathway
413 (in line with the results of the g_m/g_s analyses).

414

415 *Discussion*

416 **Photosynthetic seasonality and effects of drought**

417 We found that $V_{c,max}$ and J_{max} acclimated strongly to the seasonal changes in temperature and
418 water availability in agreement with previous studies (Vitale and Manes 2005, Corcuera et al.
419 2005, Misson et al. 2006, Ribeiro et al. 2009, Limousin et al. 2010). High radiation and water
420 stress can have a combinatory negative effect on the photosynthetic apparatus, especially in
421 sunlit leaves. Stomata close to avoid transpiration loss and hydraulic failure, but stomatal
422 closure impairs the diffusion of the CO_2 needed in the chloroplasts, the site of carboxylation.
423 $V_{c,max}$ is a proxy for the maximum potential rate of carboxylation, which is carried out by
424 Rubisco, a costly nitrogen-rich protein. The temporary unemployment of Rubisco due to limited
425 substrate (CO_2) availability leads to its de-activation and, during chronic water stress, to its
426 decomposition (Parry 2002, Chaves and Oliveira 2004, Lawlor and Tezara 2009). High
427 incoming radiation that cannot efficiently be dissipated in the Calvin cycle over-excites the
428 photoreaction centres (photoinhibition) and produces reactive oxygen species (ROS) that
429 damage the photosystems and the ATP synthase- needed for the carbon reactions (Epron et al.
430 1993). Leaves prevent harmful excess energy with protective actions such as the reorganisation
431 of the thylakoid membrane, closure of reaction centres, and reduced antennal size (Huner et al.
432 1998, Maxwell and Johnson 2000, Ensminger et al. 2012, Verhoeven 2014). These actions
433 reduce PSII efficiency and J_{max} , and enhance alternative energy pathways to prevent damage on
434 the molecular level on the cost of a lower carbon assimilation.

435 The trees in our study site maintained considerable rates of A_{net} during moderate drought
436 through improved water relations via g_s and g_m control. The relatively stable F_v/F_m values
437 indicate that the protective actions against photoinhibitory stress were effective. The trees
438 showed trunk rehydration after the first autumn rain (Sánchez-Costa *et al.*, unpublished results)
439 and quickly recovered their photosynthetic potential, suggesting that the Rubisco content
440 remained unaffected by moderate drought. The drought impacts were much more severe in the
441 dry year 2012, illustrating the vulnerability of tree physiology to the depletion of soil-water
442 reserves during the early growing season. The severity of drought strongly determined the
443 relative limitations of g_s and g_m on photosynthesis, especially in *Q. ilex* and *P. halepensis*.
444 Stomatal closure regulated photosynthesis during both the moderate and severe droughts; g_m , in
445 contrast, decreased under moderate, but increased under severe drought. We postulate that
446 altered g_m can ease the leaf internal CO_2 diffusion needed for photosynthesis, especially under
447 chronic water stress when depleted non-structural carbohydrates (NSCs) make plants
448 particularly reliant on photosynthetic products for refinement, repair, and protective actions
449 (Niinemets et al. 2009). Major changes of Φ_{PSII} , F_v/F_m , and photosynthetic potentials across all

450 species reflected these refinements of the photosynthetic apparatus as responses to chronic water
451 stress in summer 2012.

452 These acclimatisations occurred not only under dry and hot conditions, but also in winter at high
453 radiation and low temperature. Nevertheless, favourable winter conditions in 2012 resulted in
454 biochemical recovery (peak of $V_{c,max}$ and J_{max}), new shoot growth, and moderate transpiration
455 across species (often exceeding summer values) (Sánchez-Costa *et al.*, unpublished results).
456 Year-round growth patterns with several flushes during the year have also been reported in other
457 studies (Alonso *et al.* 2003). Under novel climatic conditions, favourable conditions in winter
458 may be crucial in the competition between evergreen and deciduous tree species.

459 We observed a highly species-specific pattern. *Q. ilex* and *A. unedo* followed a water-spending,
460 anisohydric strategy that maintained A_{net} and g_s in parallel with lower $V_{c,max}$ and J_{max} . In contrast,
461 *P. halepensis* had significantly decreased g_s , consistent with the conservative water-use strategy
462 and strict stomatal control of isohydric species (Borghetti *et al.* 1998, Martínez-Ferri *et al.*
463 2000). *Q. ilex* generally responded most plastically by rapidly adjusting the photosynthetic
464 machinery to the prevailing conditions (García-Plazaola *et al.* 1997, 1999, Martínez-Ferri *et al.*
465 2004). *P. halepensis* was the most tolerant to photoinhibition and had the most robust
466 photosynthetic machinery to combat abiotic stress (Baquedano and Castillo 2006, Sperlich *et al.*
467 2014). The mesophyll diffusion limitation was lowest in *Q. pubescens* and *A. unedo*, as we
468 claim, due to their deciduous/semi-evergreen foliar habits and lower LMAs (see also Tomás *et*
469 *al.*, 2014). *Q. pubescens* must maximise gas exchange during a shorter growing season, leading
470 to high photosynthetic potentials, A_{net} (Baldocchi *et al.* 2009) and transpiration rates throughout
471 the summer (Sánchez-Costa *et al.*, unpublished results; Poyatos *et al.*, 2008).

472 **Responses specific to leaf position**

473 The seasonality of photosynthetic parameters was qualitatively different between leaf positions
474 (Niinemets *et al.* 2006, Vaz *et al.* 2011) and was mostly pronounced in sunlit leaves. Shaded
475 leaves cushioned the negative climatic effects, maintaining their functionality compared to
476 sunlit leaves. Foliar anatomy, morphology, and biochemistry were highly specialised and
477 dependent on the light regime, leading to smaller but also thicker sunlit leaves and broader and
478 thinner shaded leaves (Kull and Niinemets 1993, Terashima and Hikosaka 1995, Niinemets
479 2001). Shaded leaves had lower N, photosynthetic potentials, carbon metabolisms and higher
480 $J_{max}/V_{c,max}$ ratio (see also Le Roux *et al.* 2001). Shaded leaves invest in higher J_{max} relative to
481 $V_{c,max}$ in order to increase the light-use efficiency. Responses specific to leaf position, however,
482 differed among tree species due to distinct foliar morphologies and crown architectures. The
483 sun-exposed crown position of *P. halepensis*, surmounting the forest canopy resulted in high
484 photosynthetic potentials and a low $J_{max}/V_{c,max}$ ratio throughout the crown. Pine needles attain
485 nearly saturated photosynthetic rates over a wide range of diurnal and seasonal variation in
486 radiation due to their cylindrical shape and steep angles (Jordan and Smith 1993, Lusk *et al.*

487 2003). Similarly, *Q. pubescens* showed a low differentiation between sunlit and shaded leaves.
488 A low $J_{\max}/V_{c,\max}$ ratio throughout the crown suggests a higher proportion of sunlit leaves. In
489 contrast, the comparatively higher $J_{\max}/V_{c,\max}$ ratio of sunlit leaves in *A. unedo* reflects a more
490 shaded growth environment explained by its subordinated position in the forest canopy. The *Q.*
491 *ilex* canopy was dense with a high proportion of shaded leaves, in line with its shade tolerance.
492 Hence, leaf position specific responses were highest in *Q. ilex*. The comparatively higher
493 photosynthetic values in sunlit leaves decreased partly below the level of shaded leaves under
494 stress conditions (see also Sperlich *et al.*, 2014). Shaded leaves are less exposed to the dramatic
495 changes in radiation and temperature in the outer canopy and can be of particular importance for
496 *Q. ilex* to attain a positive net carbon ratio during stress periods (Valladares *et al.* 2008). We
497 stress that the solar environment of the leaves is a crucial factor for assessing tree performance,
498 especially in a competitive environment.

499 **Acclimation of foliar morphology**

500 Mediterranean trees acclimate to water deficits with higher investments in structural
501 compounds, thereby increasing leaf density and succulence (Niinemets 2001, Ogaya and
502 Peñuelas 2006, Poorter *et al.* 2009). Foliar traits are known to be good indicators for the ability
503 of Maquis-species to respond to decreases in rainfall under climate change (Gratani and Varone
504 2006, Ogaya and Peñuelas 2007). We confirm that severe water deficit resulted in increased LT
505 and reduced LA and consequently in higher LMA. It was reported that the plasticity of leaf
506 morphology is generally higher than the plasticity of foliar chemistry and assimilation rates over
507 a wide range of woody species (Niinemets 2001). Under moderate drought, however, foliar
508 morphology was less plastic than foliar chemistry and assimilation rates (Quero *et al.* 2006);
509 severe water stress affected both to a similar extent. Leaf trait acclimation strongly constrained
510 mesophyll conductance under severe drought, especially in *Q. ilex* and *P. halepensis* (see also
511 Tomás *et al.* 2013). We postulate that foliar traits served best as proxies for drought acclimation
512 in *Q. ilex* (Grossoni *et al.* 1998, Bussotti *et al.* 2000) and *P. halepensis* (Alonso *et al.* 2003),
513 both characterised by high leaf-longevities. These changes may be accompanied by increased
514 leaf vein density that may helped to increase the tolerance to foliar hydraulic dysfunction in
515 Mediterranean plants (Nardini *et al.* 2014). The foliar traits of *A. unedo* and *Q. pubescens*
516 acclimated the least, so leaves were susceptible to foliar hydraulic dysfunction and drought-
517 deciduousness. We attribute this species-specificity in leaf trait acclimation to functional
518 differences of leaf investment costs and distinct leaf shedding strategies between deciduous /
519 semi-deciduous (*Q. pubescens* and *A. unedo*) to evergreen sclerophyllic species (*Q. ilex* and *P.*
520 *halepensis*) which we will elaborate further in the following chapter.

521 **Crown defoliation in summer 2012**

522 The lack of rain in early 2012 predisposed the vegetation to leaf senescence observed in summer
523 2012, with high variability across and within species. Leaf senescence was highest in *A. unedo*

524 and *Q. pubescens* – showing partly completely defoliated crowns. *Q. ilex* and mostly *P.*
525 *halepensis* overcame this period with marginal leaf shedding. Stored NSCs strongly determine
526 the recovery of xylem hydraulic conductivity by vessel refilling and the resistance of water
527 transport to drought under prolonged evaporative demand (Ogasa et al. 2013). Depleted NSCs
528 may limit the ability to recover from embolisms (Galiano et al. 2012). *A. unedo* is susceptible to
529 hydraulic dysfunction induced by depleted NSC (e.g. Rosas et al., 2013) which might explain
530 the severe branch dieback of *A. unedo* in our study. As shrubby species characteristic of
531 Maquis-biomes (Beyschlag et al. 1986, Harley et al. 1986), *A. unedo* likely faced a trade-off
532 between growing tall and risking hydraulic dysfunction due to high xylem tension under severe
533 soil-water deficits (Choat et al. 2012). Though, *A. unedo* might contend with severe climatic
534 stress through its strong capacity to resprout (see also Ogaya & Peñuelas, 2004).

535 Pines follow a strategy of water conservation and embolism avoidance, because they have a low
536 capacity to store carbohydrates (Meinzer et al. 2009). *P. halepensis* had a high growth-based
537 water-use efficiency ($WUE_{BAI} = \text{Basal area increment}/\text{Tree transpiration}$) during severe drought
538 (Sánchez-Costa et al., unpublished results), through the combinatory effect of photosynthetic
539 downregulation, foliar-trait acclimation, and improved gas exchange. Thus, this tree species is
540 comparatively the most productive one, especially under drought, confirming its high
541 competitiveness in dry habitats (Zavala and Zea 2004, Maseyk et al. 2008, de Luis et al. 2011).
542 Sánchez-Costa et al. (unpublished results) observed a higher WUE_{BAI} in *Q. pubescens* compared
543 to *Q. ilex* during the soil-moisture deficit in 2012. The “low-cost” leaves of the deciduous *Q.*
544 *pubescens* facilitate drought senescence, so that the reduced transpiratory surface area can
545 effectively avoid damage from hydraulic cavitation and xylem embolism (Ogaya and Peñuelas
546 2006, Barbeta et al. 2013). Fully refoliated crowns in the following growing season was
547 evidence of its success relative to *A. unedo*. The extraordinarily high photosynthetic potentials
548 in the remaining leaves were probably due to a mechanism to compensate for the reduced total
549 leaf area, as indicated by the higher translocation of leaf nitrogen before leaf shedding.

550 *Q. ilex* can effectively tolerate the effects of drought by reducing its LMA and by allowing low
551 water potentials (anisohydric behaviour) (Villar-Salvador et al. 1997, Ogaya and Peñuelas 2006,
552 Limousin et al. 2009). Its hydraulic features are highly plastic, because yearly vessel diameter
553 and recovery are well coupled with annual rainfall (Fotelli et al. 2000, Corcuera et al. 2004). *Q.*
554 *ilex*, however, was also severely effected in 2012, shedding leaves (Tognetti et al. 1998),
555 reducing radial growth and WUE_{BAI} (Sánchez-Costa et al., unpublished results). The positive
556 A_{net} , despite the reduced WUE_{BAI} , suggests that photosynthetic products were used for the
557 maintenance and recovery of xylem hydraulic conductivity instead of growth (Castell et al.
558 1994). In fact, *Quercus* species show generally a good ability in vessel refilling after xylem
559 embolism (Carnicer et al. 2013).

560 **Implications for the global carbon cycle and modelling**

561 There is evidence that the use of seasonally variable photosynthetic potentials reduces
562 uncertainties in modelled ecosystem carbon fluxes relative to the use of constant values (Wilson
563 et al. 2001, Tanaka et al. 2002, Kosugi et al. 2003, 2006, Medvigy et al. 2013). The significant
564 seasonal acclimation of $V_{c,max}$ and J_{max} observed in our study demonstrates that prognostic
565 models should account for seasonal variation, especially in drought-prone areas. Also, the
566 significant role of g_m under abiotic stress periods highlights its importance for estimating the
567 whole-carbon gain. It is now widely accepted that the apparent values of $V_{c,max}$ and J_{max} derived
568 from A/C_i curves are, from a physiological point of view, incorrect. A recent study by Sun *et al.*
569 (2014a) for nearly 130 C_3 species showed that the assumption of infinite g_m in the
570 parameterization of CO_2 -response curves underestimates $V_{c,max}$ and J_{max} by up to 75 and 60%,
571 respectively. Terrestrial biosphere models on regional or global scales are most commonly
572 calibrated on A/C_i -based parameters and therefore use apparent values of $V_{c,max}$ and J_{max} .
573 Incorporating values of $V_{c,max}$ and J_{max} parameterised on A/C_c curves would clearly lead to
574 erroneous results, because their use requires the incorporation of g_m and different Rubisco
575 kinetic parameters into the sub-models of photosynthesis. Therefore, the use of consistent
576 equations and parameters when incorporating parameters from experimental studies into
577 vegetation models is inevitable to correctly estimate photosynthesis (Rogers et al. 2014). From a
578 modelling point of view, it might seem questionable why including g_m and A/C_c - based
579 parameters would improve simulation results and not just increase model complexity.
580 Terrestrial biosphere models are currently well calibrated against observational data despite
581 their use of apparent $V_{c,max}$ and J_{max} . Another criticism often raised is that there are still potential
582 errors in various methods to estimate g_m (and subsequently $V_{c,max}$ and J_{max}) including the
583 variable J - method (used in this study) (Pons et al. 2009, Tholen et al. 2012, Gu and Sun 2014).
584 Nonetheless, large uncertainties remain in the simulations of the future CO_2 fluxes of the global
585 carbon cycle (Anav et al. 2013, Friedlingstein et al. 2014). Patterns of temperature and
586 precipitation are highly uncertain in these models due to both a lack of scientific understanding
587 and model representation (Booth et al. 2012).

588 These uncertainties could partly explain the poor modelling performance for Mediterranean-
589 type ecosystems, because the mechanistic description of the photosynthetic processes under
590 water stress is not very well developed (Morales et al. 2005, Keenan et al. 2011, Zheng et al.
591 2012, Vargas et al. 2013). As we have shown, the limitations imposed by g_m on photosynthetic
592 assimilation can decrease relatively more than the limitations imposed by g_s or biochemistry
593 ($V_{c,max}$ and J_{max}) under drought or winter stress. This distinction has important consequences for
594 the control of water-use efficiency and holds great potential for improving the estimation of
595 ecosystem carbon fluxes under drought conditions (Niinemets et al. 2009a). As already
596 mentioned above, the issue of whether (and how) to include g_m in models is actively debated by
597 physiologists and modellers (see also Rogers et al. 2014). Keenan *et al.* (2010a) showed that g_m

598 was the missing constraint for accurately capturing the response of terrestrial vegetation
599 productivity to drought. Yet relatively little information is available from modelling exercises
600 that have included g_m in their algorithms, and more research in this field is needed.

601 Concluding the above, we underline that we need to consider the seasonality of photosynthetic
602 potentials and mesophyll conductance to explain eco-physiological responses to abiotic stress.
603 These two factors should deserves much more attention in terrestrial biosphere modelling
604 because they hold great potential to reduce model uncertainties, especially under Mediterranean
605 climatic conditions.

606

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617

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619

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- 1062

1063 **Figure captions**

1064 **Fig. 1.** Environmental variables are presented for the day of the year (DOY) from January 2011 until
1065 February 2013; a) atmospheric vapour pressure deficit (VPD), b) rainfall in mm c) soil water content in
1066 $\text{cm}^3 \text{cm}^{-3}$ (gap in data is due to power cut), d) maximum and minimum temperatures in $^{\circ}\text{C}$ on the primary
1067 y-axes (in dark circles) and radiation in W m^{-2} (in light crosses, foreground) on the secondary y-axes.
1068 Field campaigns are indicated (acronyms of seasons are detailed in Tab. 2).

1069
1070 **Fig. 2.** Principal component analyses (PCA) for a) all trees species, leaf positions, and seasons, b) with
1071 differentiation between sunlit and shaded leaves, c) with differentiation between seasonal campaigns, and
1072 d) with differentiation between species. We used a subset of all data where both morphological and
1073 photosynthetic information was available. Fifteen parameters were used in the PCA: net assimilation rate
1074 (A_{net}), stomatal conductance (g_s), mesophyll conductance (g_m), maximum carboxylation rate ($V_{c,\text{max}}$),
1075 maximum electron transport rate (J_{max}), nonphotochemical quenching (NPQ), maximum quantum
1076 efficiency of PSII (F_v/F_m), leaf thickness (LT), leaf mass per area (LMA), leaf density (D), water content
1077 (WC), nitrogen content per leaf unit area (N_{area}), nitrogen content per leaf unit mass (N_{mass}), carbon
1078 content per leaf unit area (C_{area}), and carbon content per leaf unit mass (C_{mass}). The directions of the
1079 arrows indicate the higher levels of the parameters. Principal component (PC) 1 explains 37.2% of the
1080 variation, and PC 2 explained 20.4%. The ellipses are normal probability contour lines of 68% for the
1081 factors in b) leaf positions, c) seasons, and d) species.

1082
1083 **Fig. 3.** Line graphs depicting seasonal changes of a) maximum carboxylation rate ($V_{c,\text{max}}$), b) maximum
1084 electron-transport rate (J_{max}), and c) maximum quantum efficiency of PSII (F_v/F_m) for *Q. ilex*, *P.*
1085 *halepensis*, *A. unedo*, and *Q. pubescens* in sunlit (1) and shaded (2) leaves. Seasonal campaigns were
1086 conducted in spring 2011 (sp11), summer 2011 (su11), autumn 2011^a (au11 a), autumn 2011^b (au11b),
1087 winter 2012 (wi12), spring 2012 (sp12), summer 2012 (su12), and winter 2013 (wi13). Missing data
1088 points were due to limitations of labour and equipment. Vertical bars indicate standard errors of the
1089 means ($n = 3-5$).

1090
1091 **Fig. 4.** Line graphs depicting seasonal changes of a) net assimilation (A_{net}), b) stomatal conductance (g_s),
1092 and c) mesophyll conductance (g_m) for *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in sunlit (1) and
1093 shaded (2) leaves. Seasonal campaigns were conducted in spring 2011 (sp11), summer 2011 (su11),
1094 autumn 2011^a (au11 a), autumn 2011^b (au11b), winter 2012 (wi12), spring 2012 (sp12), summer 2012
1095 (su12), and winter 2013 (wi13). Missing data points were due to limitations of labour and equipment.
1096 Vertical bars indicate standard errors of the means ($n = 3-5$).

1097
1098 **Fig 5.** Seasonal changes of the relationships between a) net assimilation (A_{net}) and stomatal conductance
1099 (g_s), b) A_{net} and mesophyll conductance (g_m), and c) g_m and g_s in sunlit (1) and shaded (2) leaves. The
1100 regression lines represent the seasonal changes across species. For regression equations see Table S1-3.
1101 The relationships are shown as a thin solid line for spring 2011, short dashes for summer 2011, dots-

1102 dashes for autumn 2011^a), small dots for autumn 2011^b), dashes for winter 2012, large dots for spring
1103 2012, large dots-dashes for summer 2012, and a thick solid line for winter 2013. Statistical differences in
1104 the slopes between seasonal campaigns were tested by ANCOVAs.

1105

1106 **Fig 6.** Seasonal changes of the relationships between a) the maximum electron-transport rate (J_{\max}) and
1107 the maximum carboxylation rate ($V_{c,\max}$) and b) the electron-transport rate from chlorophyllic
1108 fluorescence (J_{amb}) and net assimilation (A_{net}) at ambient CO_2 concentrations and saturating light in sunlit
1109 (a) and shaded (b) leaves. The regression lines represent the seasonal changes across species. For
1110 regression equations see Table S4-5. The relationships are shown as a thin solid line for spring 2011,
1111 short dashes for summer 2011, dots-dashes for autumn 2011^a), small dots for autumn 2011^b), dashes for
1112 winter 2012, large dots for spring 2012, large dots-dashes for summer 2012, and a thick solid line for
1113 winter 2013.

1114

1115 **Fig 7.** Seasonal changes of the relationship for all species and leaf positions between a) mesophyll
1116 conductance (gm) and leaf mass per area (LMA). We used a subset of morphological and photosynthetic
1117 data. Non-linear regression lines of the form $y = x^{-b}$ were fitted to the data. The upper curve is for summer
1118 2012 ($b = 0.800$), the middle curve is for spring 2012 ($b = 0.953$) and the lower two overlaying curves are
1119 for autumn 2011a) ($b = 1.533$) and winter 2012 ($b = 1.486$).

1120

1121 **Supporting information**

1122 Additional supporting information may be found in the online version of this article.

1123 **Fig. S1.** Line graphs depicting seasonal changes of a) effective quantum efficiency of PSII
1124 (Φ_{PSII}), and b) nonphotochemical quenching (NPQ) for *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q.*
1125 *pubescens* in sunlit (1) and shaded (2) leaves. Missing data points were due to limitations of
1126 labour and equipment. Vertical bars indicate standard errors of the means ($n = 3-5$).

1127 **Fig. S2.** Bar charts depicting seasonal changes of a) succulence (S), b) leaf density (D), c) water
1128 content (WC), and d) leaf thickness (LT) for *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens*
1129 in sunlit (1) and shaded (2) leaves. Error bars indicate standard errors of the means ($n = 3-5$).

1130 **Fig. S3.** Bar charts depicting seasonal changes of a) nitrogen per unit leaf area (N_{area}) and b)
1131 carbon per unit leaf mass (C_{mass}) for *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in sunlit
1132 (1) and shaded (2) leaves. Error bars indicate standard errors of the means ($n = 3-5$).

1133 **Table S1.** Regression equations and coefficients of determination (R^2) for A_{net}/g_s for sunlit and
1134 shaded leaves of *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in eight sampling
1135 campaigns

1136 **Table S2.** Regression equations and coefficients of determination (R^2) for A_{net}/g_m for sunlit and
1137 shaded leaves of *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in eight sampling
1138 campaigns.

1139 **Table S3.** Regression equations and coefficients of determination (R^2) for $J_{\text{max}}/V_{c,\text{max}}$ for sunlit
1140 and shaded leaves of *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in eight sampling
1141 campaigns.

1142 **Table S4.** Regression equations and coefficients of determination (R^2) for $J_{\text{amb}}/A_{\text{net}}$ for sunlit and
1143 shaded leaves of *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in eight sampling
1144 campaigns.

1145 **Table S5.** Regression equations and coefficients of determination (R^2) for g_m/g_s for sunlit and
1146 shaded leaves of *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in eight sampling
1147 campaigns.

1148 **Table S6.** Regression equations and coefficients of determination (R^2) for a) g_m/LMA and b)
1149 $V_{c,\text{max}}/N_{\text{area}}$ in four seasonal campaigns and for sunlit and shaded leaf positions for *Q. ilex*, *P.*
1150 *halepensis*, *A. unedo*, and *Q. pubescens*.

1151 **Note S1** Calculation of maximum quantum yield of PSII and nonphotochemical quenching

1152 **Note S2** Light experiments and estimation of day respiration

1153 *This is a pre-copyedited, author-produced PDF of an article accepted for publication in Tree*
1154 *physiology* following peer review. The version of record Sperlich, D., et al. "Seasonal variability of foliar
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