Seasonal variability of foliar photosynthetic and morphological traits and drought impacts in a Mediterranean mixed forest

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The Mediterranean region is a hot spot of climate change vulnerable to increased droughts and heat waves. Scaling carbon fluxes from leaf to landscape levels is particularly challenging under drought conditions. We aimed to improve the mechanistic understanding of the seasonal acclimation of photosynthesis and morphology in sunlit and shaded leaves of four Mediterranean trees (Quercus ilex L., Pinus halepensis Mill., Arbutus unedo L. and Quercus pubescens Willd.) under natural conditions. \(V_{c,\text{max}}\) and \(J_{\text{max}}\) were not constant, and mesophyll conductance was not infinite, as assumed in most terrestrial biosphere models, but varied significantly between seasons, tree species and leaf position. Favourable conditions in winter led to photosynthetic recovery and growth in the evergreens. Under moderate drought, adjustments in the photo/biochemistry and stomatal/mesophyllic diffusion behaviour effectively protected the photosynthetic machineries. Severe drought, however, induced early leaf senescence mostly in A. unedo and Q. pubescens, and significantly increased leaf mass per area in Q. ilex and P. halepensis. Shaded leaves had lower photosynthetic potentials but cushioned negative effects during stress periods. Species-specificity, seasonal variations and leaf position are key factors to explain vegetation responses to abiotic stress and hold great potential to reduce uncertainties in terrestrial biosphere models especially under drought conditions.

Keywords: abiotic stress, Arbutus unedo, \(J_{\text{max}}\), leaf position, leaf traits, Pinus halepensis, Quercus ilex, Quercus pubescens, seasonality, \(V_{c,\text{max}}\).

Introduction

The Mediterranean region is dominated by arid or semi-arid ecosystems where high evaporative demand and low soil-water content (SWC) during the summer dry period are the main ecological limitations to plant growth (Specht 1969, Di Castri 1973). The resilience of plants to drought and heat waves is determined by their frequency and duration, which are projected to become much more severe under current climate change scenarios—particularly in the Mediterranean region (Somot et al. 2008, Friend 2010, IPCC 2013). Increased drought-induced defoliation (Poyatos et al. 2013) associated with the depletion of carbon reserves (Galiano et al. 2012) can ultimately lead to catastrophic hydraulic failure and tree mortality (Choat 2013, Url et al. 2013). Drought-induced forest impacts and diebacks in the Mediterranean region have been reported in numerous studies (Peñuelas et al. 2001, Martinez-Vilalta and Piñol 2002, Rafoyannis et al. 2008, Allen et al. 2010, Carnicer et al. 2011, Matusick et al. 2013) and can lead to shifts in vegetation composition (Jump and Penuelas 2005, Anderegg et al. 2013) and to a higher risk of forest fires (Piñol et al. 1998, Pausas et al. 2008). The challenge in the Mediterranean region in the coming years will be to learn how carbon uptake and growth in species and communities will respond to these changes, and how forest management strategies can be adapted to cushion the negative impacts of climate change on forests (Sabaté et al. 2002, Bugmann et al. 2010).
In past decades, ecosystem models on regional or global levels have contributed substantially to our understanding of the implications of climate change on a coarse scale where field experiments are limited (Luo 2007). Much uncertainty, however, remains in the modelled feedback of the global carbon cycle to climatic warming (Friedlingstein et al. 2014) and in the understanding and modelling of species’ responses to climate change (Luo 2007, Beaumont et al. 2008, McDowell et al. 2008). Photosynthesis is generally overestimated in the main Earth system models, with significant regional variations (Anav et al. 2013). Two critical parameters, the maximum rate of carboxylation ($V_{c,max}$) and the maximum rate of electron transport ($J_{max}$), are a prerequisite for scaling foliar photosynthesis to the canopy level at which global dynamic models operate (Friedlingstein et al. 2006, Friedlingstein and Prentice 2010). These two parameters describe the biochemical limitations to carbon assimilation, but are not easily measured, so relatively few data regarding their variability between species or seasons are available. $V_{c,max}$ and $J_{max}$ are thus often used as constants for various plant functional types and seasons or, in some cases, are derived from other parameters such as leaf nitrogen content (Grassi and Magnani 2005, Walker et al. 2014). Moreover, extreme climatic conditions and inter-annual variability in arid and semi-arid regions are challenging for scaling carbon assimilation patterns from one year to another (Reynolds et al. 1996, Morales et al. 2005, Gúlias et al. 2009). Simulations of ecosystem carbon fluxes are consequently limited, first, by underrepresented temporal variability of photosynthetic parameters and soil-water patterns, and second by our limited understanding of the effects of water stress on both carbon uptake and release (Hickler et al. 2009, Niinemets and Keenan 2014). The modelling performance in Mediterranean-type ecosystems is thus particularly poor and stresses the need for a better mechanistic description of photosynthetic processes under water stress (Morales et al. 2005, Keenan et al. 2011, Zheng et al. 2012, Vargas et al. 2013). Mesophyll conductance, $g_m$, might play a future key role in improving model performance of photosynthesis under drought conditions (Keenan et al. 2010).

The photosynthetic limitations of Mediterranean vegetation, especially under drought, have been extensively studied (for a review see Flexas et al. 2014), but fewer studies have thoroughly assessed the seasonal behaviour of photosynthesis and morphology under natural conditions in a mixed mature forest. The information gained from seedlings under controlled conditions can only poorly represent the physiological mechanisms of the long-term acclimation to variable environmental conditions in mature trees (Flexas et al. 2006, Mittler 2006, Niinemets 2010). Seedlings or saplings are characterized by higher metabolism and enzymatic function, lower leaf dry mass per unit area (LMA) and higher photosynthetic potential relative to mature trees (Johnson and Ball 1996, Bond 2000, Niinemets 2015). Responses to short-term stress are related to the mechanisms of prompt reactions (Flexas et al. 2006). Under natural conditions, however, mature trees acclimate to gradually developing water stress through the photosynthetic pathway (biochemical, stomatal or mesophyllic) (e.g., Martin-StPaul et al. 2013), but also through foliar traits such as nitrogen, LMA etc. (Poorter et al. 2009). Less work has evaluated simultaneously the variations of photosynthetic and morphological traits in response to abiotic stress conditions. The variation of these traits is largely species specific (Orshan 1983, Chaves et al. 2002, Gratel and Varone 2004, Krastea et al. 2013), although within-canopy gradients can play an additional overriding role (Valladares and Niinemets 2008, Sperlich et al. 2014). Mixed forests provide ideal test conditions where we can observe distinct species-specific strategies coping equally with the yearly variability of environmental conditions.

The aim of this study was to investigate the impact of seasonal environmental changes (above all drought) on foliar photosynthetic and morphological traits of the winter-deciduous sub-Mediterranean Quercus pubescens Willd., two evergreen sclerophyllous species (Quercus ilex L. and Arbutus unedo L) and an early-successional drought-adapted conifer, Pinus halepensis Mill. Pinus halepensis is characterized as isohydric following a water saving and photoinhibition-tolerant strategy (Martinez-Ferri et al. 2004, Baquedano and Castillo 2006, Sperlich et al. 2014). Quercus ilex is a late-successional, slow growing, water-spending, photoinhibition-avoiding, anisohydric tree species with a plastic hydraulic and morphological behaviour (Villar-Salvador et al. 1997, Fotelli et al. 2000, Corcuer et al. 2005a, Ogaya and Peñuelas 2006, Limousin et al. 2009). The winter-deciduous anisohydric Q. pubescens follows a similar drought-avoiding strategy to Q. ilex, but maximizes gas exchange during a shorter growing season (Baldocchi et al. 2010), resulting in high transpiration rates throughout the summer (Poyatos et al. 2008). Over extensive areas of the Mediterranean region Q. ilex and Q. pubescens form the terminal point of secondary succession (Lookingbill and Zavala 2000). Arbatus unedo—a relict of the humid-subtropical Tertiary tree flora (Gratani and Ghia 2002 and references therein)—typically occurs as shrub or small tree in the macchia ecosystems and holds an intermediate position concerning stomatal (Beysslag et al. 1986, Vitale and Manes 2005, Barbeta et al. 2012) and photoinhibition sensitivity (Sperlich et al. 2014). Prolonged climate stress might disadvantage A. unedo, being more drought sensitive than the companion species (Ogaya and Peñuelas 2004, Barbeta et al. 2012).

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

Materials and methods

Field site

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

Stand structure

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

Sampling method

We conducted eight field campaigns from June 2011 to February 2013. The sampling periods are presented in Figure 1 and Table 2. We avoided difficulties encountered during field measurements such as deviations from the standard temperature (25 °C) or unpredictable plant responses (patchy stomatal conductance) (Mott and Buckley 1998, 2000) by analysing sampled twigs in the laboratory. We cut twigs with a pruning pull from sunlit and shaded leaf positions, optimally at similar heights. The twigs were immediately re-cut under water in the field, wrapped in plastic bags to minimize transpiration, stored in water buckets and transported to the laboratory. Five replicates of each leaf position and tree species were collected for the analysis of gas exchange. The twigs were pre-conditioned in the laboratory at room temperature (24–28 °C) in dim light for 1–3 days and were freshly cut every morning. More details and references can be found in Sperlich et al. (2014).

Analyses of gas exchange and chlorophyll fluorescence

Gas exchange and chlorophyll fluorescence were measured with a Li-Cor LI-6400XT Portable Photosynthesis System equipped with a LI-6400-40 Leaf Chamber Fluorometer (Li-Cor, Inc., Lincoln, NE, USA). Response curves for foliar net assimilation versus CO\(_2\) concentration were recorded in parallel with the chlorophyll fluorescence measurements. In some cases the sunlit leaves of \(Q. \) ilex were too small to fill the leaf cuvette (2 cm\(^2\)) and so the measured parameters were adjusted after the
measurements. For *P. halepensis*, we positioned a layer of needles (∼10–15) on the leaf cuvette, avoiding gaps and overlays and sealed the gaskets with Blu-tack (Bostik SA, La Plaine St Denis, France) to keep the needles in position. The preparation and acclimation of the leaves prior to recording the response curves were conducted as in Sperlich et al. (2014).

**CO₂ experiments**

The CO₂-response curves were recorded at a leaf temperature ($T_{leaf}$) of 25 °C and a quantum flux density of 1000 μmol photons m⁻² s⁻¹. The CO₂ concentrations in the leaf chamber ($C_a$) used to generate the response curves were 400 → 300 → 200 → 150 → 100 → 50 → 400 → 400 → 600 → 800 → 1200 → 2000 μmol CO₂ m⁻² s⁻¹. The minimum and maximum times for stabilizing net assimilation rate ($A_{net}$ in μmol CO₂ m⁻² s⁻¹), stomatal conductance ($g_s$ in mol H₂O m⁻² s⁻¹) and stomatal internal CO₂ concentrations

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**Table 2.** Dates and days of the year (DOY) of seasonal field campaigns.

<table>
<thead>
<tr>
<th>Campaign</th>
<th>Abbreviation</th>
<th>Date</th>
<th>DOY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring 2011</td>
<td>sp11</td>
<td>02.06.11–02.07.11</td>
<td>153–183</td>
</tr>
<tr>
<td>Summer 2011</td>
<td>su11</td>
<td>17.08.11–29.08.11</td>
<td>229–241</td>
</tr>
<tr>
<td>Autumn 2011</td>
<td>au11a</td>
<td>17.10.11–27.10.11</td>
<td>290–300</td>
</tr>
<tr>
<td>Autumn 2011</td>
<td>au11b</td>
<td>28.10.11–11.11.11</td>
<td>301–315</td>
</tr>
<tr>
<td>Winter 2012</td>
<td>wi12</td>
<td>09.01.12–19.01.12</td>
<td>9–19</td>
</tr>
<tr>
<td>Spring 2012</td>
<td>sp12</td>
<td>01.06.12–15.06.12</td>
<td>153–167</td>
</tr>
<tr>
<td>Summer 2012</td>
<td>su12</td>
<td>24.08.12–20.09.12</td>
<td>237–264</td>
</tr>
<tr>
<td>Winter 2013</td>
<td>wi13</td>
<td>11.02.13–21.02.13</td>
<td>42–52</td>
</tr>
</tbody>
</table>

¹The autumn 2011¹ campaign was conducted in a period of prolonged summer drought and the autumn 2011² campaign was conducted after the first rains.
(Ci in μmol CO2 mol air−1) for each log were set to 4 and 6 min, respectively.

**Calculation of chlorophyll fluorescence parameters**

$F'_m$ and $F_s$ were used to estimate the effective quantum yield of photosystem II ($\Phi_{PSII}$, unitless) as:

$$\Phi_{PSII} = \frac{(F'_m - F_s)}{F'_m},$$

(1)

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

$$J_{CF} = \varepsilon \times \Phi_{PSII} \times \alpha_c.$$  

(2)

$\varepsilon$ is a scaling factor accounting for the partitioning of intercepted light between photosystem I (PSI) and PSII. We assumed that light was equally distributed between both photosystems ($\varepsilon = 0.5$) (Bernacchi et al. 2002, Niinemets et al. 2005). $\alpha_c$ (unitless) is the foliar absorband; we used the following values: 0.932 for Q. ilex and 0.912 for P. halepensis for both sunlit and shaded leaves, 0.935 for sunlit leaves of A. unedo, 0.917 for shaded leaves of A. unedo, 0.939 for sunlit leaves of Q. pubescens and 0.900 for shaded leaves of Q. pubescens. For the determination of $\alpha_c$, foliar reflectance and transmittance were measured at midday in August 2012 using a UniSpec Spectral Analysis System spectroradiometer (PP Systems, Amesbury, MA, USA). The ambient photosynthetic electron transport ($J_{amb}$) was defined as the value of $J_{CF}$ at a CO2 concentration of 400 μmol CO2 mol air−1, a PPFD of 1000 μmol photons m$^{-2}$ s$^{-1}$ and at a T$_{leaf}$ of 25 °C. The relationship between $J_{amb}$ and the net assimilation rate ($J_{amb}/A_{net}$) was used for the analyses of alternative electron sinks other than carbon metabolism. Calculations of $F_i/F_m$ and nonphotochemical quenching (NPQ) can be found in the Note S1 available as Supplementary Data at Tree Physiology Online.

**Estimation of mesophyll conductance**

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

$$g_m = \frac{A_{net}}{C_i - \frac{J^* \times J_{CF} + 8(A_{net} + R_d)}{J_{CF} - 4(A_{net} + R_d)}},$$

(3)

where $J^*$ is the CO2 concentration at which the photorespiratory efflux of CO2 equals the rate of photosynthetic CO2 uptake, and $R_d$ is the mitochondrial respiration of a leaf in light conditions and was estimated from the light-response curves combining gas exchange and measurements with the CF-method proposed by Yin et al. (2009) (see Note S2 available as Supplementary Data at Tree Physiology Online). The chloroplastic CO2 concentration ($C_i$ in μmol CO2 mol air$^{-1}$) was determined as:

$$C_i = C_c - \frac{A_{net}}{g_m}.$$  

(4)

**Photosynthesis model**

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

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

(5)

where

$$A_c = V_{c,max} \times \left[\frac{C_c - \Gamma^*}{C_c + K_c(1 + (O_2/K_o))}\right] - R_d,$$

(6)

where $V_{c,max}$ (in μmol CO2 m$^{-2}$ s$^{-1}$) is the maximum rate of Rubisco carboxylation, $K_c$ is the Michaelis–Menten constant of Rubisco for CO2, $O_2$ is the partial pressure of O2 at Rubisco and $K_o$ is the Michaelis–Menten constant of Rubisco for O2, taken from Bernacchi et al. (2002). The equation representing photosynthesis limited by RuBP regeneration is:

$$A_j = J \times \left[\frac{C_c - \Gamma^*}{4C_c + 8\Gamma^*}\right] - R_d,$$

(7)

where $J$ (in μmol electron m$^{-2}$ s$^{-1}$) is the rate of electron transport. We assumed that $J$ becomes $J_{max}$ under light and CO2 saturation when the maximum possible rate of electron transport is theoretically achieved, although we may have underestimated the true $V_{max}$ (for further details see Buckley and Diaz-Espejo 2015). $V_{c,max}$ and $J_{max}$ define the biochemical potential to drive photosynthesis and are summarized in the term ‘photosynthetic potential’ (Niinemets et al. 2006). Curves were fit, and diffusion leakage was corrected, as in Sperlich et al. (2014).

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

Foliar morphological traits were measured on fully expanded leaves ($n = 60$ per leaf position and species) from the excised twigs in five sampling campaigns in spring and autumn 2011a (2011a indicates sampling during a drought), and winter, spring and summer 2012. Immediately after the gas exchange analyses,
we measured fresh weight (FW, mg) and projected leaf surface area (LA, cm²) (including petioles) with Photoshop from scanned leaves at 300 dpi. We oven-dried the leaves at 70 °C for 48 h and weighed the leaves for dry weight (DW, mg) and measured leaf thickness (LT, mm) with a portable dial thickness gauge (Baxlo Precisión, Barcelona, Spain). We then calculated the percentage of the leaf WC as \( [1 - (DW/FW)] \times 100 \). Leaf mass per area (LMA) (mg cm⁻²) was calculated as the ratio of DW to LA and leaf tissue density (\( \rho \), mg cm⁻³) as the ratio of LMA to LT. Foliar succulence (S) was calculated as (FW−DW)/LA. We ground the leaves to a fine powder using a MM400 mixer mill (Retsch, Hahn, Germany), encapsulated a sample of 0.7 mg in tin foil and determined carbon and nitrogen contents by EA/IRMS (Elemental Analyzer/Isotope Ratio Mass Spectrometry) and GC/C/IRMS (Gas Chromatography/Combustion/IRMS). The crown condition was assessed using ‘International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests’ (ICP Forests) standards (Eichhorn et al. 2010).

**Statistical analyses**

We performed the statistical analyses with R version 3.0.2 (http://www.r-project.org/). The matrix of photosynthetic and morphological traits was subjected to principal component analyses (PCAs) to summarize the principal factors explaining the variation in these parameters. Differences in the parameters between sunlit and shaded leaves were determined with Student’s t-tests (\( P \leq 0.05 \)). The normality of the data was tested with Shapiro–Wilk tests. If the data were not normally distributed, they were normalized. One-factorial analyses of variance (ANOVARs) with season as the main factor were used to test for differences in the parameters in each species and leaf position. Significant differences were determined at \( P \leq 0.05 \) with Fisher’s least significance difference (LSD) tests. Bonferroni correction was used for familywise error rate. Linear regression analyses were conducted to study the relationships among various leaf traits such as \( A_{max}/g_s \), \( A_{sat}/g_m \), \( I_{max}/V_{c,max} \), \( g_m/g_s \) and \( I_{sat}/A_{net} \). With analyses of co-variance (ANCOVAS), we tested for differences in regression slopes and intercepts. We applied a non-linear regression analysis using the nls function in R to study the relationship of \( g_m/LMA \).

**Results**

**Environmental and crown conditions**

The year 2011 was characterized by 30% more precipitation than the climatic average of 573 mm (1951–2010) (Ninyerola et al. 2007a, 2007b) (Table 3, Figure 1), and no drought-induced leaf shedding was observed. The winter from 1 December 2011 to 31 January 2012 was relatively mild with average maximum and minimum temperatures of 11.8 and 4.2 °C, respectively, coinciding with high photosynthetic potentials and shoot growth. The precipitation in 2012 was 20% lower than the climatic average (Table 3). *Arbutus unedo* and *Q. pubescens* were strongly defoliated during summer 2012, *Quercus ilex* and *P. halepensis* to a lesser extent (Table 4). *Quercus ilex* showed some discoloration in the more exposed sites. Only one individual of *P. halepensis* showed discoloration. The defoliated *Q. pubescens* trees recovered completely in 2013. In contrast, heavily affected individuals of *A. unedo* showed an irreversible dieback of the main leading branches but also vigorous re-sprouting in 2013.

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

In Figure 2a, we present the PCA for the morphological and photosynthetic parameters. No rotation was applied to the space of the PCs. \( V_{c,max} \), \( I_{max} \) and \( g_s \) were negatively correlated with \( N_{area} \), \( C_{mass} \), NPQ and \( g_m/F_{v}/F_{m} \). WC and \( g_m \) were negatively correlated with nitrogen and carbon per unit leaf area (\( N_{area} \), \( C_{mass} \)), LMA and density (\( \rho \)). Nitrogen per unit leaf mass (\( N_{mass} \)) and \( g_m \) correlated well with LT (Figure 2). \( A_{net} \) was correlated negatively with succulence (S) and positively with \( g_m \). PC1 and PC2 explained 37.2 and 20.4% of the variation, respectively. The datapoints within the cluster circles in Figure 2b–d exhibited similar behaviours in photosynthetic and morphological traits. Leaf positions, seasons and species could be separated. Sunlit leaves were characterized by higher values on the orthogonal axis. The horizontal axes separated *A. unedo* and *Q. pubescens* from *Q. ilex* and *P. halepensis*. The orthogonal axes separated *Q. ilex* from *P. halepensis* with generally positive values. The seasonality was further investigated for each species

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### Table 3. Environmental conditions of two contrasting years (2011 and 2012). Total precipitation, mean temperature, mean soil-water content (SWC) and mean VPD are listed for each season/year.

<table>
<thead>
<tr>
<th>Season</th>
<th>Precipitation (mm)</th>
<th>Temperature (°C)</th>
<th>SWC (cm³ cm⁻³)</th>
<th>VPD (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>254</td>
<td>8.2</td>
<td>0.17</td>
<td>0.3</td>
</tr>
<tr>
<td>Spring</td>
<td>197</td>
<td>16.6</td>
<td>0.19</td>
<td>0.6</td>
</tr>
<tr>
<td>Summer</td>
<td>81</td>
<td>22.4</td>
<td>0.13</td>
<td>0.9</td>
</tr>
<tr>
<td>Autumn</td>
<td>272</td>
<td>13.4</td>
<td>0.19</td>
<td>0.4</td>
</tr>
<tr>
<td>Total</td>
<td>804</td>
<td>15.3</td>
<td>0.17</td>
<td>0.5</td>
</tr>
</tbody>
</table>

### Table 4. Percentages of crown defoliation of *Quercus ilex*, *P. halepensis*, *A. unedo* and *Q. pubescens* (n= 5, 4, 5 and 5, respectively) assessed during the severe summer 2012 drought, following ICP standards (Eichhorn et al. 2010).

<table>
<thead>
<tr>
<th>Defoliation (%)</th>
<th><em>Q. ilex</em></th>
<th><em>P. halepensis</em></th>
<th><em>A. unedo</em></th>
<th><em>Q. pubescens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>90–95</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>85–90</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>50–55</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>20–25</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10–15</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
and leaf position with ANOVAs for each photosynthetic and morphological parameter.

**Quercus ilex**  *Quercus ilex* had the most plastic response to the environmental conditions. The sunlit leaves of *Q. ilex* exhibited strong declines in several photosynthetic parameters from summer 2011 to autumn 2011a. \( V_{\text{c,max}} \), \( A_{\text{net}} \) and \( g_s \) were significantly \((P < 0.05)\), and \( J_{\text{max}} \) and \( g_m \) were marginally significantly lower \((P < 0.10)\) (Figure 3a1–b1). The means of the majority of the photosynthetic parameters recovered after the first rains in autumn 2011b (2011b indicates sampling after the drought), reaching pre-drought values, but accompanied by a high standard error. This recovery was thus only significant for \( J_{\text{max}} \) and \( g_m \). Surprisingly, \( V_{\text{c,max}} \) and \( J_{\text{max}} \) peaked in winter and not, as expected, in spring. From that peak we observed significant declines from winter to spring to summer 2012. In contrast to the pattern of \( V_{\text{c,max}} \) and \( J_{\text{max}} \), \( F_{\text{v}/F_{\text{m}}} \), \( A_{\text{net}} \) and \( g_s \) peaked in spring 2012 (Figures 3c1 and 4a1–b1). These parameters then also declined significantly in summer 2012. Interestingly, \( g_m \) peaked in summer 2012 in parallel with a reduction in \( g_s \) (Figure 4c1). The photosynthetic parameters of shaded leaves in *Q. ilex* showed a similar trend, declining after the drought in 2011 and recovering after the autumn rains (Figures 3 and 4). The parameter means of shaded leaves remained relatively stable throughout the season, in contrast to the pattern in sunlit leaves, except for a peak of \( V_{\text{c,max}} \) and \( J_{\text{max}} \) in spring 2012. The photosynthetic parameters in *Q. ilex* were significantly lower in shaded leaves. During periods of stress, however, the photosynthetic parameters of sunlit leaves declined and had values similar to those of shaded leaves (Table 5, Figures 3 and 4).

**Pinus halepensis**  Mean \( V_{\text{c,max}} \), \( J_{\text{max}} \) and \( F_{\text{v}/F_{\text{m}}} \) were generally higher in sunlit leaves of *P. halepensis* than the other species (Figures 3 and 4). The seasonal variation of the photosynthetic potential was not as strongly pronounced as in *Q. ilex*, and mean \( V_{\text{c,max}} \) and \( J_{\text{max}} \) remained relatively high and stable in 2011 (Figure 3a1–b1). The 2012 drought had comparatively stronger effects on \( V_{\text{c,max}} \) and \( J_{\text{max}} \) than the 2011 drought. Mean \( A_{\text{net}} \), \( g_s \) and \( g_m \), however, were significantly lower in autumn 2011b (Figure 4a1–c1). These values recovered quickly and significantly after the first autumn rains. The relatively high \( V_{\text{c,max}} \), \( J_{\text{max}} \) and \( F_{\text{v}/F_{\text{m}}} \) during this period reflected a stronger limitation of \( g_m \) and \( g_m \) than of the biochemistry imposed on \( A_{\text{net}} \). \( A_{\text{net}} \) recovered in winter 2012 due to the mild conditions (Figure 4a1). The 2012 summer drought significantly reduced the high values of \( A_{\text{net}} \) observed in winter 2012, but not as much as after the 2011 drought (Figure 4a1).
Both \( g_s \) and \( g_m \) remained relatively stable during this period, so the reductions in \( A_{\text{net}} \) were due to biochemical limitations (\( V_{c,\text{max}} \) and \( J_{\text{max}} \)) (Figures 3 and 4). Sunlit and shaded leaves differed the least in \( P. \) halepensis, only \( V_{c,\text{max}} \) and \( J_{\text{max}} \) were significantly different (Table 5). The sunlit and shaded leaves of \( P. \) halepensis had similar patterns of seasonal variation, but changes between seasonal campaigns were not significant (Figure 3a1–b1).

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

**Quercus pubescens**  The photosynthetic potentials were much higher in \( Q. \) pubescens than in the other species but also had high standard errors (Figure 3a1–b1). The 2012 summer drought led to a decline of the photosynthetic potentials by approximately one-third. These decreases were only significant for \( A_{\text{net}} \) and \( g_s \), and marginally significant for \( g_m \), relative to the other field campaigns (Figure 3a2–b2). The photosynthetic parameters were generally lower in the shaded leaves of \( Q. \) pubescens, but with no clear pattern and high variability (Table 5).
peak in spring 2012 being reduced significantly by the 2012 summer drought (Figure 4a1). Stomatal control was more strongly pronounced than mesophyll control (Figure 4b1–c1). Shaded leaves had higher \( A_{\text{net}} \), \( g_m \) and \( g_s \) means throughout the campaigns, in contrast to lower means of \( V_{\text{c,max}} \) and \( J_{\text{max}} \) (Figures 3 and 4a1–b2). Shaded leaves generally showed lower values than sunlit leaves and were less affected by the droughts (Figures 3 and 4).

**Morphological parameters**

The foliar traits of *P. halepensis* and *Q. ilex* acclimated most strongly to drought. Leaf mass per area was significantly higher in *P. halepensis* and *Q. ilex* in both shaded and sunlit leaves in summer 2012 compared with the previous field campaigns (Figure 5a1–a2). This was similar in *A. unedo* but less pronounced. LMA had no clear pattern in *Q. pubescens*. Elevated LMA was accompanied by higher values of leaf density (D), succulence (S) and carbon content, indicating a more sclerophyllic and succulent structure as a response to the drier conditions in 2012 (Figures S2 and S3 available as Supplementary Data at Tree Physiology Online). \( N_{\text{mass}} \) was significantly higher in spring and summer 2012 for *Q. ilex* and *P. halepensis* (shaded and sunlit leaves) and for shaded leaves of *A. unedo*, but not for *Q. pubescens* (Figure 5b1–b2).

**Relationships of photosynthetic and morphological parameters**

In order to analyse the general pattern of several relationships of the photosynthetic parameters and foliar traits, we used...
## Table 5. Means ± standard errors of the differences between sunlit and shaded leaves determined by Student’s t-tests. Significance is indicated with bold text.

<table>
<thead>
<tr>
<th>Species</th>
<th>Leaf Position</th>
<th>Sunlit</th>
<th>Shaded</th>
<th>Sunlit</th>
<th>Shaded</th>
<th>Sunlit</th>
<th>Shaded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q. pubescens</td>
<td>P</td>
<td>1.08 ± 0.13</td>
<td>1.55 ± 0.18</td>
<td>0.47 ± 0.04</td>
<td>0.41 ± 0.06</td>
<td>0.40 ± 0.04</td>
<td>0.76 ± 0.06</td>
</tr>
<tr>
<td>P. halepensis</td>
<td>P</td>
<td>1.02 ± 0.04</td>
<td>1.07 ± 0.05</td>
<td>0.47 ± 0.04</td>
<td>0.60 ± 0.07</td>
<td>0.38 ± 0.04</td>
<td>0.89 ± 0.06</td>
</tr>
<tr>
<td>P. unedo</td>
<td>P</td>
<td>1.04 ± 0.04</td>
<td>1.09 ± 0.05</td>
<td>0.49 ± 0.04</td>
<td>0.62 ± 0.07</td>
<td>0.39 ± 0.04</td>
<td>0.91 ± 0.06</td>
</tr>
</tbody>
</table>

The slope of the $A_{net}/g_s$ relationship was significantly steeper in summer 2011$^a$ in all species compared with the other field campaigns (Figure 6a1, Table S1 available as Supplementary Data at Tree Physiology Online), suggesting an increased intrinsic water-use efficiency during the dry period in 2011. Shaded leaves had a similar conservative water-use strategy in autumn 2011$^a$ (Figure 6a2). Shallower slopes in autumn 2011$^b$ in both leaf positions represent rapid responses (<1 week) to the post-drought rains easing the strict stomatal control.

The $A_{net}/g_m$ relationship in autumn 2011$^a$ also had a significantly steeper slope in both sunlit and shaded leaves recovering after the first rains in autumn 2011$^b$ (Figure 6b1–b2, Table S2 available as Supplementary Data at Tree Physiology Online). In the drier year 2012, $g_m$ imposed less resistance on photosynthetic assimilation compared with the wet year 2011. The slope of the $A_{net}/g_m$ relationship was significantly higher for winter 2012 than spring and summer 2012, suggesting a stronger control of $g_m$ on photosynthesis in winter. The autumn 2011$^a$ and summer 2012 droughts had strong effects on the slope of $A_{net}/g_m$ in shaded leaves.

With the ANCOVA of the relationship of $g_m$ and $g_s$ we investigated the proportional diffusion limitation on photosynthesis. We observed seasonal differences across all species (Figure 6c1–c2, Table S3 available as Supplementary Data at Tree Physiology Online). Mesophyll control was stronger in the dry autumn 2011$^b$ and the two winter periods. In contrast, stomatal control was higher than mesophyll control in the mild 2011 summer drought. This was most strongly pronounced in *P. halepensis* and *Q. ilex* (data not shown).

The slope in the relationship of $V_{c,max}$ and $J_{max}$ was significantly steeper in autumn 2011$^a$ for both sunlit and shaded (Figure 7a1–a2, Table S4 available as Supplementary Data at Tree Physiology Online) leaves due to a stronger reduction in $V_{c,max}$ compared with $J_{max}$. The overall $J_{max}/V_{c,max}$ ratios were 1.09 for sunlit and 1.24 for shaded leaves. The slope of the $J_{nml}/A_{net}$ relationship in sunlit and shaded leaves was significantly lower in the more humid periods (autumn 2011$^b$, winter 2012 and winter 2013), indicating lower photolytic energy dissipation and alternative electron pathways under favourable conditions (Figure 7b1–b2, Table S5 available as Supplementary Data at Tree Physiology Online).

Increased foliar sclerophyll led to higher LMAs and thus to higher diffusion resistances in the mesophyll, as shown by the relationship between $g_m$ and LMA (Figure 8, Table S6 available as Supplementary Data at Tree Physiology Online). In spring 2012 and summer 2012, we detected a less negative exponent (hence a gentler curve) (~0.953 and ~0.800, respectively) compared with winter 2012 and autumn 2011$^a$ (~1.486 and ~1.533, respectively). This shows that, regardless of the drier conditions and higher LMA in 2012, $g_m$ was higher in this period.
reflecting a regulatory mechanism of \( g_m \) in the CO\(_2\) diffusion pathway (in line with the results of the \( g_m/g_s \) analyses).

**Discussion**

*Photosynthetic seasonality and effects of drought*

We found that \( V_{c_{\text{max}}} \) and \( J_{\text{max}} \) acclimated strongly to the seasonal changes in temperature and water availability in agreement with previous studies (Corcuera et al. 2005b, Vitale and Manes 2005, Misson et al. 2006, Ribeiro et al. 2009, Limousin et al. 2010). High radiation and water stress can have a combined negative effect on the photosynthetic apparatus, especially in sunlit leaves. Stomata close to avoid transpiration loss and hydraulic failure, but stomatal closure impairs the diffusion of the CO\(_2\) needed in the chloroplasts, the site of carboxylation. \( V_{c_{\text{max}}} \) is a proxy for the maximum potential rate of carboxylation, which is carried out by Rubisco, a costly nitrogen-rich protein. The temporary unemployment of Rubisco due to limited substrate (CO\(_2\)) availability leads to its de-activation and, during chronic water stress, to its decomposition (Parry 2002, Chaves and Oliveira 2004, Lawlor and Tezara 2009). High incoming radiation that cannot efficiently be dissipated in the Calvin cycle over-excites the photoreaction centres (photoinhibition) and produces reactive oxygen species that damage the photosystems and the ATP synthase needed for the carbon reactions (Epron et al. 1993). Leaves prevent harmful excess energy with protective actions such as the reorganisation of the thylakoid membrane, closure of reaction centres and reduced antennal size (Huner et al. 1998, Maxwell and Johnson 2000, Ensminger et al. 2012, Verhoeven 2014). These actions reduce PSII efficiency and \( J_{\text{max}} \) and enhance alternative energy pathways to prevent damage on the molecular level on the cost of a lower carbon assimilation.

The trees in our study site maintained considerable rates of \( A_{\text{net}} \) during moderate drought through improved water relations via \( g_s \) and \( g_m \) control. The relatively stable \( F_v/F_m \) values indicate that the protective actions against photoinhibitory stress were effective. The trees showed trunk rehydration after the first autumn rain (Sánchez-Costa et al. 2015) and quickly recovered their photosynthetic potential, suggesting that the Rubisco content remained unaffected by moderate drought. The drought impacts were much more severe in the dry year 2012, illustrating the vulnerability of tree physiology to the depletion of soil-water reserves during the early growing season. The severity of drought strongly determined the relative limitations of \( g_s \) and \( g_m \) on photosynthesis, especially in Q. ilex and P. halepensis. Stomatal closure regulated photosynthesis during both the moderate and severe droughts; \( g_m \), in contrast, decreased under moderate, but increased under severe drought. We postulate that altered \( g_m \) can ease the leaf

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Figure 5. Bar charts depicting seasonal changes of (a) leaf mass per area (LMA) and (b) percentage of nitrogen content per unit leaf mass (\( N_{\text{mass}} \)) for Q. ilex, P. halepensis, A. unedo and Q. pubescens in sunlit (1) and shaded (2) leaves. Error bars indicate standard errors of the means (\( n = 3–5 \)).
Figure 6. Seasonal changes of the relationships between (a) net assimilation ($A_{\text{net}}$) and stomatal conductance ($g_s$), (b) $A_{\text{net}}$ and mesophyll conductance ($g_m$) and (c) $g_m$ and $g_s$ in sunlit (1) and shaded (2) leaves. The regression lines represent the seasonal changes across species. For regression equations see Tables S1–S3 available as Supplementary Data at Tree Physiology Online. The relationships are shown as a solid thin line for spring 2011, solid thick line for summer 2011, dots-dash for autumn 2011$^a$, small dots for autumn 2011$^b$, dashes for winter 2012, thick dots for spring 2012, short dashes-large dashes for summer 2012, and short dashes for winter 2013. Statistical differences in the slopes between seasonal campaigns were tested by ANCOVAs.
internal CO₂ diffusion needed for photosynthesis, especially under chronic water stress when depleted non-structural carbohydrates (NSCs) make plants particularly reliant on photosynthetic products for refinement, repair and protective actions (Niinemets et al. 2009). Major changes of ΦPSII, Fv/Fm and photosynthetic potentials across all species reflected these refinements of the photosynthetic apparatus as responses to chronic water stress in summer 2012.

These acclimatizations occurred not only under dry and hot conditions, but also in winter at high radiation and low temperature. Nevertheless, favourable winter conditions in 2012 resulted in biochemical recovery (peak of Vc,max and Jmax), new shoot growth and moderate transpiration across species (often exceeding summer values) (Sánchez-Costa et al. 2015). Year-round growth patterns with several flushes during the year have also been reported in other studies (Alonso et al. 2003). Under novel climatic conditions, favourable conditions in winter may be crucial in the competition between evergreen and deciduous tree species.

We observed a highly species-specific pattern. Quercus ilex and A. unedo followed a water-spending, anisohydric strategy that maintained A_net and gs in parallel with lower Vc,max and Jmax. In contrast, P. halepensis had significantly decreased gs, consistent with the conservative water-use strategy and strict stomatal control of isohydric species (Borghetti et al. 1998, Martinez-Ferri

Figure 7. Seasonal changes of the relationships between (a) the maximum electron-transport rate (Jmax) and the maximum carboxylation rate (Vc,max) and (b) the electron-transport rate from chlorophyll fluorescence (Jamb) and net assimilation (A_net) at ambient CO₂ concentrations and saturating light in sunlit (a) and shaded (b) leaves. The regression lines represent the seasonal changes across species. For regression equations see Tables S4 and S5 available as Supplementary Data at Tree Physiology Online. The relationships are shown as a solid thick line for summer 2011, dots-dash for autumn 2011, small dots for autumn 2011, dashes for winter 2012, thick dots for spring 2012, short dashes-large dashes for summer 2012, and short dashes for winter 2013.
Responses specific to leaf position

The seasonality of photosynthetic parameters was qualitatively different between leaf positions (Niinemets et al. 2006, Vaz et al. 2011) and was mostly pronounced in sunlit leaves. Shaded leaves cushioned the negative climatic effects, maintaining their functionality compared with sunlit leaves. Foliar anatomy, morphology and biochemistry were highly specialized and dependent on the light regime, leading to smaller but also thicker sunlit leaves and broader and thinner shaded leaves (Kull and Niinemets 1993, Terashima and Hikosaka 1995, Niinemets 2001). Shaded leaves had lower N, photosynthetic potentials, carbon metabolisms and higher $J_{\text{max}}/V_{\text{c,max}}$ ratio (see also Le Roux et al. 2001). Shaded leaves invest in higher $J_{\text{max}}$ relative to $V_{\text{c,max}}$ in order to increase the light-use efficiency. Responses specific to leaf position, however, differed among tree species due to distinct foliar morphologies and crown architectures. The sun-exposed crown position of *P. halepensis* surmounting the forest canopy resulted in high photosynthetic potentials and a low $J_{\text{max}}/V_{\text{c,max}}$ ratio throughout the crown. Pine needles attain nearly saturated photosynthetic rates over a wide range of diurnal and seasonal variation in radiation due to their cylindrical shape and steep angles (Jordan and Smith 1993, Lusk et al. 2003). Similarly, *Q. pubescens* showed a low differentiation between sunlit and shaded leaves. A low $J_{\text{max}}/V_{\text{c,max}}$ ratio throughout the crown suggests a higher proportion of sunlit leaves. In contrast, the comparatively higher $J_{\text{max}}/V_{\text{c,max}}$ ratio of sunlit leaves in *A. unedo* reflects a more shaded growth environment explained by its subordinated position in the forest canopy. The *Q. ilex* canopy was dense with a high proportion of shaded leaves, in line with its shade tolerance. Hence, leaf position specific responses were highest in *Q. ilex*. The comparatively higher photosynthetic values in sunlit leaves decreased partly below the level of shaded leaves under stress conditions (see also Sperlich et al. 2014). Shaded leaves are less exposed to the dramatic changes in radiation and temperature in the outer canopy and can be of particular importance for *Q. ilex* to attain a positive net carbon ratio during stress periods (Valladares et al. 2008). We stress that the solar environment of the leaves is a crucial factor for assessing tree performance, especially in a competitive environment.

Acclimation of foliar morphology

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

### Crown defoliation in summer 2012

The lack of rain in early 2012 predisposed the vegetation to leaf senescence observed in summer 2012, with high variability across and within species. Leaf senescence was highest in *A. unedo* and *Q. pubescens*—showing partly completely defoliated crowns. *Quercus ilex* and mostly *P. halepensis* overcame this period with marginal leaf shedding. Stored NSCs strongly determine the recovery of xylem hydraulic conductivity by vessel refilling and the resistance of water transport to drought under prolonged evaporative demand (Ogasa et al. 2013). Depleted NSCs may limit the ability to recover from embolisms (Galano et al. 2012). *Arbutus unedo* is susceptible to hydraulic dysfunction induced by depleted NSC (e.g., Rosas et al. 2013), which might explain the severe branch dieback of *A. unedo* in our study. As a shrubby species characteristic of Maquis biomes (Beyschlag et al. 1986, Harley et al. 1986), *A. unedo* likely faced a trade-off between growing tall and risking hydraulic dysfunction due to high xylem tension under severe soil-water deficits (Choat et al. 2012). However, *A. unedo* might contend with severe climatic stress through its strong capacity to resprout (see also Ogaya and Peñuelas 2004).

Pines follow a strategy of water conservation and embolism avoidance, because they have a low capacity to store carbohydrates (Meinzer et al. 2009). *Pinus halepensis* had a high growth-based water-use efficiency (WUE$_{BAI}$ = Basal area increment/Tree transpiration) during severe drought (Sánchez-Costa et al. 2015), through the combined effect of photosynthetic downregulation, foliar-trait acclimation and improved gas exchange. Thus, this tree species is comparatively the most productive one, especially under drought, confirming its high competitiveness in dry habitats (Zavala and Zea 2004, Maseyk et al. 2008, de Luis et al. 2011).

Sánchez-Costa et al. (2015) observed a higher WUE$_{BAI}$ in *Q. pubescens* compared with *Q. ilex* during the soil-moisture deficit in 2012. The ‘low-cost’ leaves of the deciduous *Q. pubescens* facilitate drought senescence, so that the reduced transpiratory surface area can effectively avoid damage from hydraulic cavitation and xylem embolism (Ogaya and Peñuelas 2006, Barbeta et al. 2013). Fully refoliated crowns in the following growing season was evidence of its success relative to *A. unedo*. The extraordinarily high photosynthetic potentials in the remaining leaves were probably due to a mechanism to compensate for the reduced total leaf area, as indicated by the higher translocation of leaf nitrogen before leaf shedding.

*Quercus ilex* can effectively tolerate the effects of drought by reducing its LMA and by allowing low water potentials (anisohydric behaviour) (Villar-Salvador et al. 1997, Ogaya and Peñuelas 2006, Limousin et al. 2009). Its hydraulic features are highly plastic, because yearly vessel diameter and recovery are well coupled with annual rainfall (Fotelli et al. 2000, Corcuera et al. 2005a). *Quercus ilex*, however, was also severely effected in 2012, shedding leaves (Tognetti et al. 1998), reducing radial growth and WUE$_{BAI}$ (Sánchez-Costa et al. 2015). The positive $A_{net}$, despite the reduced WUE$_{BAI}$ suggests that photosynthetic products were used for the maintenance and recovery of xylem hydraulic conductivity instead of growth (Castell et al. 1994). In fact, *Quercus* species show generally a good ability in vessel refilling after xylem embolism (Carnicer et al. 2013).

### Implications for the global carbon cycle and modelling

There is evidence that the use of seasonally variable photosynthetic potentials reduces uncertainties in modelled ecosystem carbon fluxes relative to the use of constant values (Wilson et al. 2001, Tanaka et al. 2002, Kosugi et al. 2003, 2006, Medvigy et al. 2013). The significant seasonal acclimation of $V_{c,max}$ and $J_{max}$ observed in our study demonstrates that prognostic models should account for seasonal variation, especially in drought-prone areas. Also, the significant role of $g_{m}$ under abiotic stress periods highlights its importance for estimating the whole-carbon gain. It is now widely accepted that the apparent values of $V_{c,max}$ and $J_{max}$ derived from $A/C$ curves are, from a physiological point of view, incorrect. A recent study by Sun et al. (2014) for nearly 130 C$_3$ species showed that the assumption of infinite $g_{m}$ in the parameterization of CO$_2$-response curves underestimates $V_{c,max}$ and $J_{max}$ by up to 75 and 60%, respectively. Terrestrial biosphere models on regional or global scales are most commonly calibrated on $A/C$-based parameters and therefore use apparent values of $V_{c,max}$ and $J_{max}$. Incorporating values of $V_{c,max}$ and $J_{max}$ parameterized on $A/C$ curves would clearly lead to erroneous results, because their use requires the incorporation of $g_{m}$ and different Rubisco kinetic parameters into the sub-models of photosynthesis. Therefore, the use of consistent equations and parameters when incorporating parameters from experimental studies into vegetation models is inevitable to correctly estimate photosynthesis (Rogers et al. 2014). From a modelling point of view, it might seem questionable why including $g_{m}$ and $A/C$-based parameters would improve simulation results and not just increase model complexity. Terrestrial biosphere models are currently well calibrated against observational data despite their use of apparent $V_{c,max}$ and $J_{max}$. Another criticism often raised is that there are still potential errors in various methods to estimate $g_{m}$ (and subsequently $V_{c,max}$ and $J_{max}$) including the variable $J$-method (used in this study) (Pons et al. 2009, Tholen et al. 2012, Gu and Sun 2014). On the other hand, although this may not represent a perfectly accurate approach, we claim that representing $g_{m}$ is preferable to neglecting $g_{m}$ completely (Parkhurst 1994) as large uncertainties remain in the simulations of the future CO$_2$ fluxes of the global carbon cycle (Anav et al. 2013, Friedlingstein et al. 2014).
Patterns of temperature and precipitation are highly uncertain in these models due to both a lack of scientific understanding and model representation (Booth et al. 2012).

These uncertainties could partly explain the poor modelling performance for Mediterranean-type ecosystems, because the mechanistic description of the photosynthetic processes under water stress is not very well developed (Moraes et al. 2005, Keenan et al. 2011, Zheng et al. 2012, Vargas et al. 2013). As we have shown, the limitations imposed by \( g_m \) on photosynthetic assimilation can decrease relatively more than the limitations imposed by \( g_s \) or biochemistry \((V_c, \text{max} \text{ and } J_{\text{max}})\) under drought or winter stress. This distinction has important consequences for the control of water-use efficiency and holds great potential for improving the estimation of ecosystem carbon fluxes under drought conditions (Niinemets et al. 2009). As already mentioned above, the issue of whether (and how) to include \( g_m \) in models is actively debated by physiologists and modellers (see also Rogers et al. 2014). Keenan et al. (2010) showed that \( g_m \) was the missing constraint for accurately capturing the response of terrestrial vegetation productivity to drought. Yet relatively little information is available from modelling exercises that have included \( g_m \) in their algorithms, and more research in this field is needed.

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

**Supplementary Data**

Supplementary data for this article are available at *Tree Physiology* Online.

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**Conflict of interest**

None declared.

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