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

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Running head: SEASONAL PHOTOSYNTHESIS MORPHOLOGY MIXED FOREST

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Abstract

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 *Q. pubescens* Willd.) under natural conditions. $V_{c,max}$ and J_{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*, *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.

Introduction

The Mediterranean region is dominated by arid or semi-arid ecosystems where high evaporative demand and low soil-water content 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 (Urli et al. 2013, Choat 2013). Drought-induced forest impacts and diebacks in the Mediterranean region have been reported in numerous studies (Peñuelas et al. 2001, Martínez-Vilalta and Piñol 2002, Raftoyannis 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 2002, Bugmann et al. 2010).

In past decades, ecosystem models on regional or global level 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, McDowell et al. 2008, Beaumont 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 little data of 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, Gulías 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 characterised by higher metabolism and enzymatic function, lower leaf dry mass per unit area (LMA), and higher photosynthetic potential relative to mature trees (Johnson & Ball, 1996; Bond, 2000; Niinemets, 2014). 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, Gratani and Varone 2004, Krasteva 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 morpholocial traits of the winter-deciduous sub-Mediterranean *Quercus pubescens*, two evergreen sclerophyllous species (*Quercus ilex* and *Arbutus unedo*) and an early-successional drought-adapted conifer, *Pinus halepensis*. *P. halepensis* is characterised as isohydric following a water saving and photoinhibition-tolerant strategy (Martínez-Ferri et al. 2004, Baquedano and Castillo 2006, Sperlich et al. 2014). *Q. ilex* L. 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, Corcuera et al. 2004, Ogaya and Peñuelas 2006, Limousin et al. 2009). The winter-deciduous anisohydric *Q. pubescens* follows a similar drought- avoiding strategy as *Q. ilex*, but maximizes gas exchange during a shorter growing season (Baldocchi et al. 2009), 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). *A. unedo* - relict of the humid-subtropical Tertiary tree flora (Gratani and Ghia, 2002a and references therein) – is typically occurring as shrub or small tree in the macchia ecosystems and holding a intermediate position concerning stomatal- (Beyschlag 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 i) seasonal environmental changes (above all drought) affect the photosynthetic and ii) morphological traits, iii) mesophyllic diffusion conductance (g_m) strongly constrains photosynthesis under drought conditions, iv) the seasonal acclimation varies qualitatively and quantitatively with 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 a.s.l.). Seasonal summer droughts, warm temperatures and mild winters characterise 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, Finland) and solar radiation (SP1110 Skye Instruments Ltd., Powys, UK) were installed at a height of 3 m, in a clearing *ca.* 1 km from the plot.

Stand structure

Our study site is characterised 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 the early-successional and fast growing Aleppo Pine (*P. halepensis* Mill.). The mean heights of each layer are 9.9 m 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.) grows usually 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 (mean height 8.1 m) 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 at 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 Table 2 and Figure 1. 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 minimise 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 d 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, 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 (ca. 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).

CO_2 experiments

The CO_2-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 CO_2 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 CO_2 mol air^{-1}. The minimum and maximum times for stabilising net assimilation rate (A_{net} in μmol $CO_2\ m^{-2}\ s^{-1}$), stomatal conductance (g_s in mol $H_2O\ m^{-2}\ s^{-1}$), and stomatal internal CO_2 concentrations (C_i in μmol CO_2 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 (Φ_{PSII}, unitless) as:

$$\Phi_{\mathrm{PSII}} = \frac{(\mathrm{F_m}' - \mathrm{F_s})}{\mathrm{F_m}'} \qquad (1)$$

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

$$J_{CF} = \varepsilon * \Phi\mathrm{PSII} * \alpha_{\mathrm{L}} \qquad (2)$$

ε 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 (ε = 0.5) (Bernacchi et al. 2002, Niinemets et al. 2005). α_L (unitless) is the foliar absorbance; 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 α_L, foliar reflectance and transmittance were measured at midday in August 2012 using a UniSpec Spectral Analysis System spectroradiometer (PP Systems, Haverhill, USA). The ambient photosynthetic electron transport (J_{amb}) was defined as the value of J_{CF} at a CO_2 concentration of 400 μmol CO_2 mol air^{-1} and a PPFD of 1000 μmol photons m^{-2} s^{-1}. 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_v/F_m and NPQ can be found in the supplementary material (Note S1).

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):

$$g_m = \frac{A_{\mathrm{net}}}{C_{\mathrm{i}} - \frac{[\Gamma^{*} * J_{\mathrm{CF}} + 8(A_{\mathrm{net}} + R_{\mathrm{d}})]}{J_{\mathrm{CF}} - 4(A_{\mathrm{net}} + R_{\mathrm{d}})}} \qquad (6)$$

where Γ^* is the CO_2 concentration at which the photorespiratory efflux of CO_2 equals the rate of photosynthetic CO_2 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 supplementary material for details (Note S2). The chloroplastic CO_2 concentration (C_c in μmol CO_2 mol air^{-1}) was determined as:

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

Photosynthesis model

The photosynthesis model of Farquhar et al. (1980) considers photosynthesis as minimum of the potential rates of Rubisco activity (A_c) and ribulose-1,5-bisphosphate (RuBP) regeneration (A_j). The model was further complemented with a third limitation (A_p) that considers the limitation by triose-phosphate use (TPU) at high CO_2 concentrations when the CO_2 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/C_c curve:

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

where

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

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

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

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 CO$_2$ saturation when the maximum possible rate of electron transport is theoretically achieved, although we may have underestimated the true J_{max} (for further details see Buckley & Diaz-Espejo, 2014). $V_{c,max}$ and J_{max} define the biochemical potential to drive photosynthesis and are summarised 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 2011^a (2011^a 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, cm^2) (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 water content (WC) as [1-(DW/FW)]*100. Leaf mass per area (LMA) (mg cm^{-2}) was calculated as the ratio of DW to LA and leaf tissue density (D, 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 the 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 summarise 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 normalised. One-factorial analyses of variance (ANOVAs) 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 $A_{\mathrm{net}}/g_{\mathrm{s}}$, $A_{\mathrm{net}}/g_{\mathrm{m}}$, $J_{\mathrm{max}}/V_{\mathrm{c,max}}$, $g_{\mathrm{m}}/g_{\mathrm{s}}$, $J_{\mathrm{amb}}/A_{\mathrm{net}}$. 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 characterised by 30% more precipitation than the climatic average of 723 mm (1951-2010) (Ninyerola et al. 2007a, 2007b) (Tab. 3, Fig. 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). *A. unedo* and *Q. pubescens* were strongly defoliated during summer 2012; *Q. ilex* and *P. halepensis* to a lesser extent (Table 4). *Q. 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 Fig. 2a, we present the PCA for the morphological and photosynthetic parameters. No rotation was applied to the space of the PC's. $V_{c,max}$, J_{max}, and g_s were negatively correlated with N_{mass}, C_{mass}, NPQ, and g_m. F_v/F_m, g_s, and water content (WC) were negatively correlated with nitrogen and carbon per unit leaf area (N_{area}, C_{area}), LMA, and density (D). Nitrogen per unit leaf mass (N_{mass}) and g_m correlated well with LT (Fig. 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 Fig. 1b-d exhibited similar behaviours in photosynthetic and morphological traits. Leaf positions, seasons, and species could be separated. Sunlit leaves were characterised 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 and leaf position with ANOVAs for each photosynthetic and morphological parameter.

Q. ilex

Q. 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 2011^a. $V_{c,max}$, A_{net} and g_s were significantly ($P < 0.05$), and J_{max} and g_m were marginally significantly lower ($P < 0.10$) (Fig. 3 a1-b1). The means of the majority of the photosynthetic parameters recovered after the first rains in autumn 2011^b (2011^b indicates sampling after the drought), reaching pre-drought values, but accompanied by a high standard error. This recovery was thus only significant for J_{max} and g_m. Surprisingly, $V_{c,max}$ and J_{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 $V_{c,max}$ and J_{max}, F_v/F_m, A_{net}, and g_s peaked in spring 2012 (Figs. 3c1, 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 (Fig. 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 (Figs. 3, 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 $V_{c,max}$ and J_{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 6, Figs. 3, 4).

P. halepensis

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

A. unedo

Similar to *Q. ilex*, the photosynthetic parameters in *A. unedo* varied strongly seasonally but had high standard errors (Figs. 3, 4). A_{net} decreased significantly in winter 2012, in contrast to J_{max} and $V_{c,max}$ that peaked in the same campaign (Figs. 3a1-b1 and 4a1). A decline in g_s and g_m in this campaign suggested that they more strongly regulated A_{net} (Fig. 4b1-c1). A_{net}, g_s, and g_m peaked in spring 2012. These increases were significant for A_{net} and g_s and marginally significant for g_m relative to the other field campaigns (Fig. 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).

Q. pubescens

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

Morphological parameters

The foliar traits of *P. halepensis* and *Q. ilex* acclimated most strongly to drought. LMA was significantly higher in *P. halepensis* and *Q. ilex* in both shaded and sunlit leaves in summer 2012 compared to the previous field campaigns (Fig. 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 response to the drier conditions in 2012 (Figs. S2, S3). N_{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* (Fig. 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 ANCOVAs to test for differences in the slopes between seasons across all species.

The slope the $A_{\mathrm{net}}/g_{\mathrm{s}}$ relationship was significantly steeper in summer and autumn 2011^{a} in all species compared to the other field campaigns (Fig. 6a1, Table S1), 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} (Fig. 6a2). Shallower slopes in autumn 2011^{b} in both leaf positions represent rapid responses (less than one week) to the post-drought rains easing the strict stomatal control.

The $A_{\mathrm{net}}/g_{\mathrm{m}}$ 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} (Fig. 6b1-2, Table S2). In the drier year 2012, g_{m} imposed less resistance on photosynthetic assimilation compared to the wet year 2011. The slope of the $A_{\mathrm{net}}/g_{\mathrm{m}}$ 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 $A_{\mathrm{net}}/g_{\mathrm{m}}$ 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 (Fig. 6c, Table S3). Mesophyllic control was stronger in the dry autumn 2011^a and the two winter periods. In contrast, stomatal control was higher than mesophyllic 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 (Fig. 7a, Table S4) leaves due to a stronger reduction in $V_{c,max}$ compared to 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_{amb}/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 protective energy dissipation and alternative electron pathways under favourable conditions (Fig. 7b, Table S5).

Increased foliar sclerophylly led to higher LMAs and thus to higher diffusion resistances in the mesophyll, as shown by the relationship between g_m and LMA (Fig. 8, Table S6). In spring 2012 and summer 2012, we detected a less negative exponent (hence a gentler curve) (-0.953 and -0.800, respectively) compared to 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 $V_{c,max}$ and J_{max} acclimated strongly to the seasonal changes in temperature and water availability in agreement with previous studies (Vitale and Manes 2005, Corcuera et al. 2005, Misson et al. 2006, Ribeiro et al. 2009, Limousin et al. 2010). High radiation and water stress can have a combinatory 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. $V_{c,max}$ 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 (ROS) 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_{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_{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.*, unpublished results) 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 servere droughts; g_m, in contrast, decreased under moderate, but increased under severe drought. We postulate that altered g_m can ease the leaf internal CO_2 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 Φ_{PSII}, F_v/F_m, and photosynthetic potentials across all species reflected these refinements of the photosynthetic apparatus as responses to chronic water stress in summer 2012.

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

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 to sunlit leaves. Foliar anatomy, morphology, and biochemistry were highly specialised 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 $J_{max}/V_{c,max}$ ratio (see also Le Roux *et al.* 2001). Shaded leaves invest in higher J_{max} relative to $V_{c,max}$ 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 $J_{max}/V_{c,max}$ 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 $J_{\max}/V_{c,\max}$ ratio throughout the crown suggests a higher proportion of sunlit leaves. In contrast, the comparatively higher $J_{\max}/V_{c,\max}$ 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 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 characterised by high leaf-longevities. These changes may be accompanied by increased leaf vein density that may helped to increase the tolerance to foliar hydraulic dysfunction in Mediterranean plants (Nardini et al. 2014). The foliar 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 leaf trait acclimation to functional differences of leaf investment costs and distinct leaf shedding strategies between deciduous / semi-deciduous (*Q. pubescens* and *A. unedo*) to evergreen sclerophyllic species (*Q. ilex* and *P. halepensis*) which we will elaborate further in the following chapter.

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. *Q. 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 (Galiano et al. 2012). *A. 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 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).Though, *A. unedo* might contend with severe climatic stress through its strong capacity to resprout (see also Ogaya & 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). *P. halepensis* had a high growth-based water-use efficiency (WUE_{BAI} = Basal area increment/Tree transpiration) during severe drought (Sánchez-Costa *et al.*, unpublished results), through the combinatory 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*. (unpublished results) observed a higher WUE_{BAI} in *Q. pubescens* compared to *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.

Q. 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. 2004). *Q. ilex*, however, was also severely effected in 2012, shedding leaves (Tognetti et al. 1998), reducing radial growth and WUE_{BAI} (Sánchez-Costa *et al.*, unpublished results). 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_i curves are, from a physiological point of view, incorrect. A recent study by Sun *et al.* (2014a) 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_i-based parameters and therefore use apparent values of $V_{c,max}$ and J_{max}. Incorporating values of $V_{c,max}$ and J_{max} parameterised on A/C_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_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)**.** Nonetheless, 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 (Morales 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 ($V_{c,max}$ and J_{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. 2009a). 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.* (2010a) 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 deserves much more attention in terrestrial biosphere modelling because they hold great potential to reduce model uncertainties, especially under Mediterranean climatic conditions.

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References:

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH (Ted) (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manage 259:660–684. http://linkinghub.elsevier.com/retrieve/pii/S037811270900615X (18 July 2011, date last accessed).

Alonso R, Elvira S, Inclán R, Bermejo V, Castillo FJ, Gimeno BS (2003) Responses of Aleppo pine to ozone. In: Karnosky DF, Percy KE, Chappelka AH, Simpson C, Pikkarainen J (eds) Air Pollution, Global Change and Forests in the New Millenium. Elsevier Ltd., pp 211–230.

Anav A, Friedlingstein P, Kidston M, Bopp L, Ciais P, Cox P, Jones C, Jung M, Myneni R, Zhu Z (2013) Evaluating the Land and Ocean Components of the Global Carbon Cycle in the CMIP5 Earth System Models. J Clim 26:6801–6843. http://journals.ametsoc.org/doi/abs/10.1175/JCLI-D-12-00417.1 (27 August 2014, date last accessed).

Anderegg WRL, Kane JM, Anderegg LDL (2013) Consequences of widespread tree mortality triggered by drought and temperature stress. Nat Clim Chang 3:30–36.

Baldocchi DD, Ma S, Rambal S, Misson L, Ourcival J-M, Limousin J-M, Pereira J, Papale D (2009) On the differential advantages of evergreenness and deciduousness in mediterranean oak woodlands: a flux perspective. Ecol Appl 20:1583–1597.

Baquedano FJ, Castillo FJ (2006) Comparative ecophysiological effects of drought on seedlings of the Mediterranean water-saver Pinus halepensis and water-spenders Quercus coccifera and Quercus ilex. Trees 20:689–700. http://link.springer.com/10.1007/s00468-006-0084-0 (17 June 2013, date last accessed).

Barbeta A, Ogaya R, Peñuelas J (2012) Comparative study of diurnal and nocturnal sap flow of Quercus ilex and Phillyrea latifolia in a Mediterranean holm oak forest in Prades (Catalonia, NE Spain). Trees 26:1651–1659. http://link.springer.com/10.1007/s00468-012-0741-4 (8 August 2013, date last accessed).

Barbeta A, Ogaya R, Peñuelas J (2013) Dampening effects of long-term experimental drought on growth and mortality rates of a Holm oak forest. Glob Chang Biol 19:1–12. http://www.ncbi.nlm.nih.gov/pubmed/23712619 (10 August 2013, date last accessed).

Beaumont LJ, Hughes L, Pitman a J (2008) Why is the choice of future climate scenarios for species distribution modelling important? Ecol Lett 11:1135–46. http://www.ncbi.nlm.nih.gov/pubmed/18713269 (23 July 2011, date last accessed).

Bernacchi CJ, Portis AR, Nakano H, Caemmerer S Von, Long SP (2002) Temperature Response of Mesophyll Conductance . Implications for the Determination of Rubisco Enzyme Kinetics and for Limitations to Photosynthesis in Vivo. Plant Physiol 130:1992–1998.

Beyschlag W, Lange OL, Tenhunen JD (1986) Photosynthesis und Wasserhaushalt der immergrünen mediterranen Hartlaubpflanze Arbutus unedo L. im Jahresverlauf am Freilandstandort in Portugal I. Tagesläufe von CO2-Gaswechsel und Transpiration unter natürlichen Bedingungen. Flora 178:409–444.

Booth BBB, Jones CD, Collins M, Totterdell IJ, Cox PM, Sitch S, Huntingford C, Betts R a, Harris GR, Lloyd J (2012) High sensitivity of future global warming to land carbon cycle processes. Environ Res Lett 7:024002. http://stacks.iop.org/1748-

9326/7/i=2/a=024002?key=crossref.0608852a9b4c87899a93d976c7428fa8 (3 November 2014, date last accessed).

Borghetti M, Cinnirella S, Magnani F, Saracino A (1998) Impact of long-term drought on xylem embolism and growth in Pinus halepensis Mill. Trees 12:187–195.

Buckley TN, Diaz-Espejo A (2015) Reporting estimates of maximum potential electron transport rate. New Phytol 205:14–17. http://onlinelibrary.wiley.com/doi/10.1111/nph.13018/abstract (15 September 2014, date last accessed).

Bugmann H, Palahí M, Bontemps J, Tomé M (2010) Trends in modeling to address forest management. For Syst 19:3–7.

Bussotti F, Borghini F, Celesti C, Leonzio C, Bruschi P (2000) Leaf morphology and macronutrients in broadleaved trees in central Italy. Trees 14:361–368. http://link.springer.com/10.1007/s004680000056 (18 September 2014, date last accessed).

Carnicer J, Barbeta A, Sperlich D, Coll M, Peñuelas J (2013) Contrasting trait syndromes in angiosperms and conifers are associated with different responses of tree growth to temperature on a large scale. Front Plant Sci 4:409. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3797994&tool=pmcentrez&rendertype=abstract (12 November 2013, date last accessed).

Carnicer J, Coll M, Ninyerola M, Pons X, Sánchez G, Peñuelas J (2011) Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proc Natl Acad Sci U S A 108:1474–8. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3029725&tool=pmcentrez&rendertype=abstract (13 August 2013, date last accessed).

Castell C, Terradas J, Tenhunen JD (1994) Water relations, gas exchange, and growth of resprouts and mature plant shoots of Arbutus unedo L. and Quercus ilex L. Oecologia 98:201–211.

Di Castri F (1973) Climatographical comparison between Chile and the western coast of North America. In: Di Castri F, Mooney HA (eds) Mediterranean-type ecosystems: origin and structure. Springer, Berlin, pp 21–36.

Chaves MM, Oliveira MM (2004) Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. J Exp Bot 55:2365–84. http://www.ncbi.nlm.nih.gov/pubmed/15475377 (14 August 2013, date last accessed).

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osorio ML, Carvalho I, Faria T, Pinheiro C (2002) How Plants Cope with Water Stress in the Field . Photosynthesis and Growth. Ann Bot 89:907–916.

Choat B (2013) Predicting thresholds of drought-induced mortality in woody plant species. Tree Physiol 33:669–671. http://www.ncbi.nlm.nih.gov/pubmed/23878170 (7 August 2013, date last accessed).

Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild TS, Gleason SM, Hacke UG, Jacobsen AL, Lens F, Maherali H, Martínez-Vilalta J, Mayr S, Mencuccini M, Mitchell PJ, Nardini A, Pittermann J, Pratt RB, Sperry JS, Westoby M, Wright IJ, Zanne AE (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–5.

Corcuera L, Morales F, Abadia a., Gil-Pelegrin E (2004) The effect of low temperatures on the photosynthetic apparatus of Quercus ilex subsp. ballota at its lower and upper altitudinal limits in the Iberian peninsula and during a single freezing-thawing cycle. Trees 19:99–108. http://www.springerlink.com/index/10.1007/s00468-004-0368-1 (31 October 2012, date last accessed).

Corcuera L, Morales F, Abadía a, Gil-Pelegrín E (2005) Seasonal changes in photosynthesis and photoprotection in a Quercus ilex subsp. ballota woodland located in its upper altitudinal extreme in the Iberian Peninsula. Tree Physiol 25:599–608. http://www.ncbi.nlm.nih.gov/pubmed/15741152

Eichhorn J, Roskams P, Ferretti M, Mues V, Szepesi A, Durrant D (2010) Visual assessment of crown condition and damaging agents. 49 pp. Manual Part IV. Man methods criteria Harmon sampling, assessment, Monit Anal Eff air Pollut For UNECE ICP For Program Co-ord Centre, Hamburg[http//www icp-forests org/Manual htm]. http://www.icp-forests.org/Manual.htm

Ensminger I, Berninger F, Streb P (2012) Response of photosynthesis to low temperature. In: Flexas J, Loreto F, Medrano H (eds) Terrestrial Photosynthesis in a Changing Environment - A Molecular, Physiological and Ecological Approach. Cambridge University Press, Cambridge, pp 272–289.

Epron D, Dreyer E, Aussenac G (1993) A comparison of photosynthetic responses to water stress in seedlings from 3 oak species: Quercus petraea (Matt) Liebl, Q rubra L and Q cerris L. Ann Des Sci For 50:48–60. http://www.afs-journal.org/10.1051/forest:19930704

Flexas J, Diaz-Espejo A, Gago J, Gallé A, Galmés J, Gulías J, Medrano H (2014) Photosynthetic limitations in Mediterranean plants: A review. Environ Exp Bot 103:12–23. http://linkinghub.elsevier.com/retrieve/pii/S0098847213001238 (6 March 2014, date last accessed).

Fotelli MN, Radoglou KM, Constantinidou HI (2000) Water stress responses of seedlings of four Mediterranean oak species. Tree Physiol 20:1065–75. http://www.ncbi.nlm.nih.gov/pubmed/11269958

Friedlingstein P, Betts R, Bopp L, Bloh W Von, Brovkin V, Doney S, Eby M, Fung I, Govindasamy B, John J, Jones C, Joos F, Kato T, Kawamiya M, Knorr W, Lindsay K, Matthews HD, Raddatz T, Rayner P, Reick C, Roeckner E, Schnitzler KG, Schnurr R, Strassmann K, Thompson S, J Weaver A, Yoshikawa C, Zeng N (2006) Climate –carbon cycle feedback analysis, results from the C4MIP model intercomparison. J Clim In review

Friedlingstein P, Meinshausen M, Arora VK, Jones CD, Anav A, Liddicoat SK, Knutti R (2014) Uncertainties in CMIP5 Climate Projections due to Carbon Cycle Feedbacks. J Clim 27:511–526. http://journals.ametsoc.org/doi/abs/10.1175/JCLI-D-12-00579.1 (8 September 2014, date last accessed).

Friedlingstein P, Prentice I (2010) Carbon–climate feedbacks: a review of model and observation based estimates. Curr Opin Environ Sustain 2:251–257. http://linkinghub.elsevier.com/retrieve/pii/S1877343510000473

Friend AD (2010) Terrestrial plant production and climate change. J Exp Bot 61:1293–309. http://www.ncbi.nlm.nih.gov/pubmed/20202998 (3 March 2012, date last accessed).

Galiano L, Martínez-Vilalta J, Sabaté S, Lloret F (2012) Determinants of drought effects on crown condition and their relationship with depletion of carbon reserves in a Mediterranean holm oak forest. Tree Physiol 32:478–89. http://www.ncbi.nlm.nih.gov/pubmed/22499595 (1 February 2013, date last accessed).

García-Plazaola JI, Artetxe U, Becceril, María J (1999) Diurnal changes in antioxidant and carotenoid composition in the Mediterranean schlerophyll tree Quercus ilex (L.) during winter. Plant Sci 143:125–133.

García-Plazaola JI, Faria T, Abadia J, Abadia A, Chaves MM, Pereira JS (1997) Seasonal changes in xanthophyll composition and photosynthesis of cork oak (Quercus suber L.) leaves under mediterranean climate. J Experimantal Bot 48:1667–1674.

Grassi G, Magnani F (2005) Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant, Cell Environ 28:834–849. http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-3040.2005.01333.x

Gratani L, Ghia E (2002) Adaptive strategy at the leaf level of Arbutus unedo L . to cope with Mediterranean climate. Flora 197:275–284.

Gratani L, Varone L (2004) Adaptive photosynthetic strategies of the Mediterranean maquis species according to their origin. Photosynthetica 42:551–558.

Gratani L, Varone L (2006) Long-time variations in leaf mass and area of Mediterranean evergreen broad-leaf and narrow-leaf maquis species. Photosynthetica 44:161–168.

Grossoni P, Bussotti F, Tani C, Gravano E, Antarelli S, Bottacci A (1998) Morpho-anatomical aterations in leaves of Gagus vatica L. and Quercus ilex L. in different environmental stress condition. Chemosphere 36:919–924. http://www.sciencedirect.com/science/article/pii/S0045653597101485 (22 September 2014, date last accessed).

Gu L, Sun Y (2014) Artifactual responses of mesophyll conductance to CO2 and irradiance estimated with the variable J and online isotope discrimination methods. Plant Cell Environ 37:1231–1249. http://doi.wiley.com/10.1111/pce.12232 (15 November 2013, date last accessed).

Gulías J, Cifre J, Jonasson S, Medrano H, Flexas J (2009) Seasonal and inter-annual variations of gas exchange in thirteen woody species along a climatic gradient in the Mediterranean island of Mallorca. Flora - Morphol Distrib Funct Ecol Plants 204:169–181. http://linkinghub.elsevier.com/retrieve/pii/S036725300800114X (2 August 2013, date last accessed).

Harley PC, Loreto F, Di Marco G, Sharkey TD (1992) Theoretical Considerations when Estimating the Mesophyll Conductance to CO2 Flux by Analysis of the Response of Photosynthesis to CO2. Plant Physiol 98:1429–1436. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1080368&tool=pmcentrez&rendertype=abstract

Harley PC, Tenhunen JD, Lange OL (1986) Use of an analytical model to study limitations on net photosynthesis in Arbutus unedo under field conditions. Oecologia 70:393–401.

Hickler T, Fronzek S, Araújo MB, Schweiger O, Thuiller W, Sykes MT (2009) An ecosystem model-based estimate of changes in water availability differs from water proxies that are commonly used in species distribution models. Glob Ecol Biogeogr 18:304–313. http://doi.wiley.com/10.1111/j.1466-8238.2009.00455.x (9 August 2013, date last accessed).

Huner NPA, Öquist G, Sarhan F (1998) Energy balance and acclimation to light and cold. Trends Plant Sci 3:224–230. http://linkinghub.elsevier.com/retrieve/pii/S1360138598012485

IPCC (2013) Summary for Policymakers. In: T.F. S, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the physical science basis. Cambridge University Press, Cambridge (UK) and New York (USA)

Jordan DN, Smith WK (1993) Simulated influence of leaf geometry on sunlight interception and photosynthesis in conifer needles. Tree Physiol 13:29–39. http://www.ncbi.nlm.nih.gov/pubmed/14969899

Jump AS, Penuelas J (2005) Running to stand still: adaptation and the response of plants to rapid climate change. Ecol Lett 8:1010–1020. http://doi.wiley.com/10.1111/j.1461-0248.2005.00796.x (3 June 2013, date last accessed).

Keenan T, Maria Serra J, Lloret F, Ninyerola M, Sabate S (2011) Predicting the future of forests in the Mediterranean under climate change, with niche- and process-based models: CO2 matters! Glob Chang Biol 17:565–579. http://doi.wiley.com/10.1111/j.1365-2486.2010.02254.x (23 July 2011, date last accessed).

Keenan T, Sabate S, Gracia C (2010) The importance of mesophyll conductance in regulating forest ecosystem productivity during drought periods. Glob Chang Biol 16:1019–1034. http://doi.wiley.com/10.1111/j.1365-2486.2009.02017.x (19 June 2011, date last accessed).

Kosugi Y, Shibata S, Kobashi S (2003) Parameterization of the CO2 and H2O gas exchange of several temperate deciduous broad-leaved trees at the leaf scale considering seasonal changes. Plant Cell Environ 26:285–301.

Kosugi Y, Takanashi S, Matsuo N, Tanaka K, Tanaka H (2006) Impact of leaf physiology on gas exchange in a Japanese evergreen broad-leaved forest. Agric For Meteorol 139:182–199. http://linkinghub.elsevier.com/retrieve/pii/S0168192306001602 (28 August 2013, date last accessed).

Krasteva V, Alexandrov V, Chepisheva M, Dambov S, Stefanov D, Yordanov I, Goltsev V (2013) Drought induced damages of photosynthesis in bean and plantain plants analyzed in vivo by chlorophyll a fluorescence. Bulg J Plant Physiol 19:39–44.

Kull O, Niinemets U (1993) Variations in leaf morphometry and nitrogen concentration in Betula pendula Roth., Corylus avellana L. and Lonicera xylosteum L. Tree Physiol 12:311–8. http://www.ncbi.nlm.nih.gov/pubmed/14969921

Lawlor DW, Tezara W (2009) Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Ann Bot 103:561–79. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2707350&tool=pmcentrez&rendertype=abstract (14 August 2013, date last accessed).

Limousin J-M, Misson L, Lavoir A-V, Martin NK, Rambal S (2010) Do photosynthetic limitations of evergreen Quercus ilex leaves change with long-term increased drought severity? Plant Cell Environ 33:863–75. http://www.ncbi.nlm.nih.gov/pubmed/20051039 (23 July 2011, date last accessed).

Limousin JM, Rambal S, Ourcival JM, Rocheteau a., Joffre R, Rodriguez-Cortina R (2009) Long-term transpiration change with rainfall decline in a Mediterranean Quercus ilex forest. Glob Chang Biol 15:2163–2175. http://doi.wiley.com/10.1111/j.1365-2486.2009.01852.x (13 August 2013, date last accessed).

Lookingbill TR, Zavala MA (2000) Spatial pattern of Quercus ilex and Quercus pubescens recruitment in Pinus halepensis dominated woodlands. J Veg Sci 11:607–612.

De Luis M, Novak K, Raventós J, Gričar J, Prislan P, Čufar K (2011) Cambial activity, wood formation and sapling survival of Pinus halepensis exposed to different irrigation regimes. For Ecol Manage 262:1630–1638. http://linkinghub.elsevier.com/retrieve/pii/S0378112711004415 (31 March 2014, date last accessed).

Luo Y (2007) Terrestrial Carbon–Cycle Feedback to Climate Warming. Annu Rev Ecol Evol Syst 38:683–712. http://www.annualreviews.org/doi/abs/10.1146/annurev.ecolsys.38.091206.095808

Lusk CH, Wright I, Reich PB (2003) Photosynthetic differences contribute to competitive advantage of evergreen angiosperm trees over evergreen conifers in productive habitats. New Phytol 160:329–336.

Martinez-Ferri E, Balaguer L, Valladares F, Chico JM, Manrique E (2000) Energy dissipation in drought-avoiding and drought-tolerant tree species at midday during the Mediterranean summer. Tree Physiol 20:131–138. http://treephys.oxfordjournals.org/cgi/doi/10.1093/treephys/20.2.131

Martínez-Ferri E, Manrique E, Valladares F, Balaguer L (2004) Winter photoinhibition in the field involves different processes in four co-occurring Mediterranean tree species. Tree Physiol 24:981–90. http://www.ncbi.nlm.nih.gov/pubmed/15234895

Martínez-Vilalta J, Piñol J (2002) Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For Ecol Manage 161:247–256.

Martin-StPaul NK, Limousin J-M, Vogt-Schilb H, Rodríguez-Calcerrada J, Rambal S, Longepierre D, Misson L (2013) The temporal response to drought in a Mediterranean evergreen tree: comparing a regional precipitation gradient and a throughfall exclusion experiment. Glob Chang Biol 19:2413–26. http://www.ncbi.nlm.nih.gov/pubmed/23553916 (6 August 2013, date last accessed).

Maseyk KS, Lin T, Rotenberg E, Grünzweig JM, Schwartz A, Yakir D (2008) Physiology–phenology interactions in a productive semi-arid pine forest. New Phytol 178:603–616.

Matusick G, Ruthrof KX, Brouwers NC, Dell B, Hardy GSJ (2013) Sudden forest canopy collapse corresponding with extreme drought and heat in a mediterranean-type eucalypt forest in southwestern Australia. Eur J For Res 132:497–510.

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence — a practical guide. J Exp Bot 51:659–668.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez E a (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–39. http://www.ncbi.nlm.nih.gov/pubmed/18422905

Medvigy D, Jeong S-J, Clark KL, Skowronski NS, Schäfer KVR (2013) Effects of seasonal variation of photosynthetic capacity on the carbon fluxes of a temperate deciduous forest. J Geophys Res Biogeosciences 118:1703–1714. http://doi.wiley.com/10.1002/2013JG002421 (26 March 2014, date last accessed).

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh K a., Woodruff DR (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. Funct Ecol 23:922–930. http://doi.wiley.com/10.1111/j.1365-2435.2009.01577.x (25 May 2013, date last accessed).

Misson L, Tu KP, Boniello R a, Goldstein AH (2006) Seasonality of photosynthetic parameters in a multi-specific and vertically complex forest ecosystem in the Sierra Nevada of California. Tree Physiol 26:729–41. http://www.ncbi.nlm.nih.gov/pubmed/16510388

Morales P, Sykes MT, Prentice IC, Smith P, Smith B, Bugmann H, Zierl B, Friedlingstein P, Viovy N, Sabate S, Sanchez A, Pla E, Gracia C a., Sitch S, Arneth A, Ogee J (2005) Comparing and evaluating process-based ecosystem model predictions of carbon and water fluxes in major European forest biomes. Glob Chang Biol 11:2211–2233. http://doi.wiley.com/10.1111/j.1365-2486.2005.01036.x (18 July 2011, date last accessed).

Mott KA, Buckley TN (1998) Stomatal heterogeneity. J Experimantal Bot 49:407–417.

Mott KA, Buckley TN (2000) Patchy stomatal conductance: emergent collective behaviour of stomata. Trends Plant Sci 1385:258–262.

Nardini A, Lo Gullo MA, Trifilò P, Salleo S (2014) The challenge of the Mediterranean climate to plant hydraulics: Responses and adaptations. Environ Exp Bot 103:68–79. http://www.sciencedirect.com/science/article/pii/S0098847213001548 (5 September 2014, date last accessed).

Niinemets Ü (2001) Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. Ecology 82:453–469.

Niinemets Ü, Cescatti A, Rodeghiero M, Tosens T (2005) Leaf internal diffusion conductance limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species. Plant, Cell Environ 28:1552–1566. http://doi.wiley.com/10.1111/j.1365-3040.2005.01392.x

Niinemets Ü, Cescatti A, Rodeghiero M, Tosens T (2006) Complex adjustments of photosynthetic potentials and internal diffusion conductance to current and previous light availabilities and leaf age in Mediterranean evergreen species Quercus ilex. Plant, Cell Environ 29:1159–1178. http://doi.wiley.com/10.1111/j.1365-3040.2006.01499.x (23 July 2011, date last accessed).

Niinemets U, Díaz-Espejo A, Flexas J, Galmés J, Warren CR (2009) Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. J Exp Bot 60:2249–70. http://www.ncbi.nlm.nih.gov/pubmed/19395391 (20 July 2011, date last accessed).

Niinemets Ü, Keenan T (2014) Photosynthetic responses to stress in Mediterranean evergreens: mechanisms and models. Environ Exp Bot 103:24–41. http://linkinghub.elsevier.com/retrieve/pii/S0098847213001925 (26 November 2013, date last accessed).

Ninyerola M, Pons X, Roure JM (2007a) Objective air temperature mapping for the Iberian Peninsula using spatial interpolation and GIS. Int J Climatol 27:1231–1242. http://dx.doi.org/10.1002/joc.1462

Ninyerola M, Pons X, Roure JM (2007b) Monthly precipitation mapping of the Iberian Peninsula using spatial interpolation tools implemented in a Geographic Information System. Theor Appl Climatol 89:195–209. http://link.springer.com/10.1007/s00704-006-0264-2 (14 December 2014, date last accessed).

Ogasa M, Miki NH, Murakami Y, Yoshikawa K (2013) Recovery performance in xylem hydraulic conductivity is correlated with cavitation resistance for temperate deciduous tree species. Tree Physiol 33:335–44. http://www.ncbi.nlm.nih.gov/pubmed/23492871 (22 May 2013, date last accessed).

Ogaya R, Peñuelas J (2004) Phenological patterns of Quercus ilex, Phillyrea latifolia, and Arbutus unedo growing under a field experimental drought. Écosicence 11:263–270.

Ogaya R, Peñuelas J (2006) Contrasting foliar responses to drought in Quercus ilex and Phillyrea latifolia. Biol Plant 50:373–382.

Ogaya R, Peñuelas J (2007) Leaf mass per area ratio in Quercus ilex leaves under a wide range of climatic conditions. The importance of low temperatures. Acta Oecologica 31:168–173. http://linkinghub.elsevier.com/retrieve/pii/S1146609X06001482 (18 February 2014, date last accessed).

Orshan G (1983) Approaches to the Definition of Mediterranean Growth Forms. In: Kruger FJ, Mitchell DT, Jarvis JUM (eds) Mediterranean type-ecosystems - The role of nutrients. Springer-Verlag, Berlin - Heidelberg, pp pp. 86–100.

Parry M a. J (2002) Rubisco Activity: Effects of Drought Stress. Ann Bot 89:833–839.

Pausas JG, Llovet J, Rodrigo A, Ramon Vallejo (2008) Are wildfires a disaster in the Mediterranean basin ? – A review. Int J Wildl Fire 17:713–723.

Peñuelas J, Lloret F, Montoya R (2001) Severe Drought Effects on Mediterranean Woody Flora in Spain. For Sci 47:214–218.

Piñol J, Terradas J, Lloret F (1998) Climate warming, wildfire hazard, and wildfire occurrence in coastal eastern spain. Clim Chance 38:345–357.

Pons TL, Flexas J, von Caemmerer S, Evans JR, Genty B, Ribas-Carbo M, Brugnoli E (2009) Estimating mesophyll conductance to CO2: methodology, potential errors, and recommendations. J Exp Bot 60:2217–34. http://www.ncbi.nlm.nih.gov/pubmed/19357431 (12 March 2012, date last accessed).

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol 182:565–588. http://doi.wiley.com/10.1111/j.1469-8137.2009.02830.x

Poyatos R, Aguadé D, Galiano L, Mencuccini M, Martínez-Vilalta J (2013) Drought-induced defoliation and long periods of near-zero gas exchange play a key role in accentuating metabolic decline of Scots pine. New Phytol. http://www.ncbi.nlm.nih.gov/pubmed/23594415 (31 May 2013, date last accessed).

Poyatos R, Llorens P, Piñol J, Rubio C (2008) Response of Scots pine (Pinus sylvestris L.) and pubescent oak (Quercus pubescens Willd.) to soil and atmospheric water deficits under Mediterranean mountain climate. Ann For Sci 65:306–306.

Quero JL, Villar R, Marañón T, Zamora R (2006) Interactions of drought and shade effects on seedlings of four Quercus species: physiological and structural leaf responses. New Phytol 170:819–33. http://www.ncbi.nlm.nih.gov/pubmed/16684241 (16 September 2014, date last accessed).

Raftoyannis Y, Spanos I, Radoglou K (2008) The decline of Greek fir (Abies cephalonica Loudon): Relationships with root condition. Plant Biosyst 142:386–390. http://www.tandfonline.com/doi/abs/10.1080/11263500802151017 (6 August 2013, date last accessed).

Reichstein M, Tenhunen JD, Roupsard O, Ourcival J-M, Rambal S, Dore S, Valentini R (2002) Ecosystem respiration in two Mediterranean evergreen Holm Oak forests: drought effects and decomposition dynamics. Funct Ecol 16:27–39. http://doi.wiley.com/10.1046/j.0269-8463.2001.00597.x

Reynolds JF, Kemp PR, Acock B, Chen J, Moorhead DL (1996) Progress, Limitations and Challenges in Modeling the Effects of Elevated C02 on Plants and Ecosystems. In: Koch GW, Mooney HA (eds) Carbon Dioxide and Terrestrial Ecosystems. Academic Press, San Diego - New York - Boston - London - Sydney - Tokio - Toronto, pp 347–380.

Ribeiro RV, Machado EC, Santos MG, Oliveira RF (2009) Seasonal and diurnal changes in photosynthetic limitation of young sweet orange trees. Environ Exp Bot 66:203–211. http://linkinghub.elsevier.com/retrieve/pii/S0098847209000653 (23 July 2011, date last accessed).

Rogers A, Medlyn BE, Dukes JS (2014) 9th New Phytologist Workshop, Montauk, New York, USA, April 2014. In: Improving representation of photosynthesis in Earth System Models.pp 12–14. http://onlinelibrary.wiley.com/doi/10.1111/nph.12972/abstract

Rosas T, Galiano L, Ogaya R, Peñuelas J, Martínez-Vilalta J (2013) Dynamics of non-structural carbohydrates in three Mediterranean woody species following long-term experimental drought. Front Plant Sci 4:1–16. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3795346&tool=pmcentrez&rendertype=abstract (6 August 2014, date last accessed).

Le Roux X, Walcroft a S, Sinoquet H, Chaves MM, Rodrigues a, Osorio L (2001) Photosynthetic light acclimation in peach leaves: importance of changes in mass:area ratio, nitrogen concentration, and leaf nitrogen partitioning. Tree Physiol 21:377–86. http://www.ncbi.nlm.nih.gov/pubmed/11282577

Sabaté S (2002) Likely effects of climate change on growth of Quercus ilex, Pinus halepensis, Pinus pinaster, Pinus sylvestris and Fagus sylvatica forests in the Mediterranean region. For Ecol Manage 162:23–37. http://linkinghub.elsevier.com/retrieve/pii/S0378112702000488

Sharkey TD (1985) Photosynthesis in intact leaves of C3 plants: Physics, physiology and rate limitations. Bot Rev 51:53–105.

Somot S, Sevault F, Déqué M, Crépon M (2008) 21st century climate change scenario for the Mediterranean using a coupled atmosphere–ocean regional climate model. Glob Planet Change 63:112–126. http://linkinghub.elsevier.com/retrieve/pii/S0921818107001774 (13 August 2013, date last accessed).

Specht RL (1969) A comparison of the sclerophyllous vegetation characteristics of Mediterranean type climates in France, California, and southern Australia. I. Structure, morphology, and succession. Aust J Bot 17:277–292.

Sperlich D, Chang CT, Peñuelas J, Gracia C, Sabaté S (2014) Foliar photochemical processes and carbon metabolism under favourable and adverse winter conditions in a Mediterranean mixed forest, Catalonia (Spain). Biogeosciences 11:5657–5674. http://www.biogeosciences.net/11/5657/2014/bg-11-5657-2014.html (16 October 2014, date last accessed).

Sun Y, Gu L, Dickinson RE, Pallardy SG, Baker J, Cao Y, Damatta FM, Dong X, Ellsworth D, van Goethem D, Jensen AM, Law BE, Loos R, Martins SCV, Norby RJ, Warren J, Weston D, Winter K (2014) Asymmetrical effects of mesophyll conductance on fundamental photosynthetic parameters and their relationships estimated from leaf gas exchange measurements. Plant Cell Environ 37:978–994. http://www.ncbi.nlm.nih.gov/pubmed/24117476 (18 November 2013, date last accessed).

Tanaka K, Kosugi Y, Nakamura A (2002) Impact of leaf physiological characteristics on seasonal variation in CO2, latent and sensible heat exchanges over a tree plantation. Agric For Meteorol 114:103–122. http://linkinghub.elsevier.com/retrieve/pii/S0168192302001284

Terashima I, Hikosaka K (1995) Comparative ecophysiology of leaf and canopy photosynthesis. Plant Cell Environ 18:1111–1128.

Tholen D, Ethier G, Genty B, Pepin S, Zhu X-G (2012) Variable mesophyll conductance revisited: theoretical background and experimental implications. Plant Cell Environ 35:2087–103. http://www.ncbi.nlm.nih.gov/pubmed/22590996 (6 June 2014, date last accessed).

Tognetti R, Longobucco A, Raschi A (1998) Vulnerability of xylem to embolism in relation to plant hydraulic resistance in Quercus pubescens and Quercus ilex co-occurring in a Mediterranean coppice stand in central Italy. New Phytol 139:437–447. http://doi.wiley.com/10.1046/j.1469-8137.1998.00207.x (17 September 2014, date last accessed).

Tomás M, Flexas J, Copolovici L, Galmés J, Hallik L, Medrano H, Ribas-Carbó M, Tosens T, Vislap V, Niinemets Ü (2013) Importance of leaf anatomy in determining mesophyll diffusion conductance to CO2 across species: quantitative limitations and scaling up by models. J Exp Bot 64:2269–81. http://www.ncbi.nlm.nih.gov/pubmed/23564954 (19 March 2014, date last accessed).

Tomás M, Medrano H, Brugnoli E, Escalona JM, Martorell S, Pou A, Ribas-Carbó M, Flexas J (2014) Variability of mesophyll conductance in grapevine cultivars under water stress conditions in relation to leaf anatomy and water use efficiency. Aust J Grape Wine Res:n/a–n/a. http://doi.wiley.com/10.1111/ajgw.12069 (10 April 2014, date last accessed).

Urli M, Porté AJ, Cochard H, Guengant Y, Burlett R, Delzon S (2013) Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree Physiol 33:672–683. http://www.ncbi.nlm.nih.gov/pubmed/23658197 (6 August 2013, date last accessed).

Valladares F, Niinemets Ü (2008) Shade Tolerance, a Key Plant Feature of Complex Nature and Consequences. Annu Rev Ecol Evol Syst 39:237–257. http://www.annualreviews.org/doi/abs/10.1146/annurev.ecolsys.39.110707.173506 (21 March 2014, date last accessed).

Valladares F, Zaragoza-Castells J, Sánchez-Gómez D, Matesanz S, Alonso B, Portsmuth A, Delgado A, Atkin OK (2008) Is shade beneficial for mediterranean shrubs experiencing periods of extreme drought and late-winter frosts? Ann Bot 102:923–33. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2712400&tool=pmcentrez&rendertype=abstract (28 March 2014, date last accessed).

Vargas R, Sonnentag O, Abramowitz G, Carrara A, Chen JM, Ciais P, Correia A, Keenan TF, Kobayashi H, Ourcival J, Papale D, Pearson D, Pereira JS, Piao S, Rambal S, Baldocchi DD (2013) Drought Influences the Accuracy of Simulated Ecosystem Fluxes : A Model-Data Meta-analysis for Mediterranean Oak Woodlands. Ecosystems 16:749–764.

Vaz M, Maroco J, Ribeiro N, Gazarini LC, Pereira JS, Chaves MM (2011) Leaf-level responses to light in two co-occurring Quercus (Quercus ilex and Quercus suber): leaf structure, chemical composition and photosynthesis. Agrofor Syst 82:173–181. http://link.springer.com/10.1007/s10457-010-9343-6 (28 March 2014, date last accessed).

Verhoeven A (2014) Sustained energy dissipation in winter evergreens. New Phytol 201:57–65. http://doi.wiley.com/10.1111/nph.12466

Villar-Salvador P, Castro-Díez P, Pérez-Rontomé C, Montserrat-Martí G (1997) Stem xylem features in three Quercus (Fagaceae) species along a climatic gradient in NE Spain. Trees 12:90–96. http://link.springer.com/10.1007/PL00009701 (30 August 2014, date last accessed).

Vitale M, Manes F (2005) Role of changing environmental parameters in leaf gas exchange of Arbutus unedo L . assessed by field and laboratory measurements. Photosynthetica 43:99–106.

Walker AP, Beckerman AP, Gu L, Kattge J, Cernusak L a., Domingues TF, Scales JC, Wohlfahrt G, Wullschleger SD, Woodward FI (2014) The relationship of leaf photosynthetic traits - Vcmax and Jmax - to leaf nitrogen, leaf phosphorus, and specific leaf area: a meta-analysis and modeling study. Ecol Evol 4:3218–3235. http://doi.wiley.com/10.1002/ece3.1173 (28 July 2014, date last accessed).

Wilson KB, Baldocchi DD, Hanson PJ, Ridge O (2001) Leaf age affects the seasonal pattern of photosynthetic capacity and net ecosystem exchange of carbon in a deciduous forest. Plant, Cell Environ 24:571–583.

Yin X, Struik PC, Romero P, Harbinson J, Evers JB, Van Der Putten PEL, Vos J (2009) Using combined measurements of gas exchange and chlorophyll fluorescence to estimate parameters of a biochemical C photosynthesis model: a critical appraisal and a new integrated approach applied to leaves in a wheat (Triticum aestivum) canopy. Plant Cell Environ 32:448–64. http://www.ncbi.nlm.nih.gov/pubmed/19183300 (9 November 2012, date last accessed).

Zavala M a., Zea E (2004) Mechanisms maintaining biodiversity in Mediterranean pine-oak forests: insights from a spatial simulation model. Plant Ecol (formerly Veg 171:197–207. http://link.springer.com/10.1023/B:VEGE.0000029387.15947.b7

Zheng Y, Zhong Z, Zhou JJ, Zhou H (2012) Evaluations of different leaf and canopy photosynthesis models: a case study with black locust (Robinia pseudoacacia) plantations on a loess plateau. Pakistan J Bot 44:531–539.

Figure captions

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

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

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

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

Fig 5. Seasonal changes of the relationships between a) net assimilation (A_{net}) and stomatal conductance (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 regression lines represent the seasonal changes across species. For regression equations see Table S1-3. The relationships are shown as a thin solid line for spring 2011, short dashes for summer 2011, dots-dashes for autumn 2011$^{a)}$, small dots for autumn 2011$^{b)}$, dashes for winter 2012, large dots for spring 2012, large dots-dashes for summer 2012, and a thick solid line for winter 2013. Statistical differences in the slopes between seasonal campaigns were tested by ANCOVAs.

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

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

Supporting information

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

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

Fig. S2. Bar charts depicting seasonal changes of a) succulence (S), b) leaf density (D), c) water content (WC), and d) leaf thickness (LT) 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).

Fig. S3. Bar charts depicting seasonal changes of a) nitrogen per unit leaf area (N_{area}) and b) carbon per unit leaf mass (C_{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).

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

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

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

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

Table S5. Regression equations and coefficients of determination (R^2) for $g_{\mathrm{m}}/g_{\mathrm{s}}$ for sunlit and shaded leaves of *Q. ilex, P. halepensis, A. unedo,* and *Q. pubescens* in eight sampling campaigns.

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

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

Note S2 Light experiments and estimation of day respiration