

Foliar photochemical processes and carbon metabolism

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Foliar photochemical processes and carbon metabolism under favourable and adverse winter conditions in a Mediterranean mixed forest, Catalonia (Spain)

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

¹Centre for Ecological Research and Forestry Applications (CREAF), Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

²Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain

³Global Ecology Unit CSIC-CEAB-CREAF, CREAF, Facultat de Ciències, Universitat Autònoma de Barcelona. 08913 Bellaterra. Spain

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Correspondence to: D. Sperlich (dominik@creaf.uab.es)

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Abstract

Evergreen trees in the Mediterranean region must cope with a wide range of environmental stresses from summer drought to winter cold. The mildness of Mediterranean winters can periodically lead to favourable environmental conditions above the threshold for a positive carbon balance, benefitting evergreen woody species more than deciduous ones. The comparatively lower solar energy input in winter decreases the foliar light saturation point. This leads to a higher susceptibility to photoinhibitory stress especially when chilly ($< 12^{\circ}\text{C}$) or freezing temperatures ($< 0^{\circ}\text{C}$) coincide with clear skies and relatively high solar irradiances. Nonetheless, the advantage of evergreen species that are able to photosynthesize all year round where a significant fraction can be attributed to winter months, compensates for the lower carbon uptake during spring and summer in comparison to deciduous species. We investigated the ecophysiological behaviour of three co-occurring mature evergreen tree species (*Quercus ilex* L., *Pinus halepensis* Mill., and *Arbutus unedo* L.) during a period of mild winter conditions and their responses to a sudden cold period. The state of the photosynthetic machinery in both periods was thus tested by estimating the foliar photosynthetic potential with CO_2 response curves in parallel with chlorophyll fluorescence measurements. The studied evergreen tree species benefited strongly from mild winter conditions by exhibiting extraordinarily high photosynthetic potentials similar to those under spring conditions. A sudden period of frost, however, negatively affected the photosynthetic apparatus, leading to significant decreases in key physiological parameters such as the maximum carboxylation velocity ($V_{c, \max}$), the maximum photosynthetic electron transport rate (J_{\max}), and the optimal fluorometric quantum yield of photosystem II (F_v/F_m). This change persisted for several weeks after the cold period despite the recovery of the temperature to the conditions previous to the frost event. The responses of $V_{c, \max}$ and J_{\max} were highly species-specific, where *Q. ilex* exhibited the highest and *P. halepensis* the lowest reductions. In contrast, the optimal fluorometric quantum yield of photosystem II (F_v/F_m) was significantly lower in *A. unedo* after the cold period. The leaf

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position played an important role in *Q. ilex* showing a comparatively stronger winter effect on sunlit leaves. Our results generally agreed with the previous classifications of photoinhibition-avoiding (*P. halepensis*) and photoinhibition-tolerant (*Q. ilex*) species on the basis of their susceptibility to dynamic photoinhibition, whereas *A. unedo* was the least tolerant to photoinhibition, which was chronic in this species. *Q. ilex* and *P. halepensis* seem to follow contrasting photoprotective strategies which are, however, equally successful under the prevailing conditions exhibiting an adaptive advantage over *A. unedo* in our study site. These results show that our understanding of the dynamics of interspecific competition in Mediterranean ecosystems requires consideration of the physiological behaviour during winter which may have important implications for long-term carbon budgets and growth trends.

1 Introduction

Mediterranean-type ecosystems are widely associated with broadleaved evergreen sclerophyllous shrubs and trees, the classic vegetation types in climates where hot and dry summers alternate with cool and wet winters (Aschmann, 1973; Blumler, 1991; Orshan, 1983; Specht, 1969). In summer, water is undoubtedly the most important factor limiting growth and survival in the Mediterranean region, whereas spring and autumn provide better growing conditions (Gracia et al., 1999; Orshan, 1983; Sabaté and Gracia, 2011). In winter, the low temperatures and solar radiation limit the amount of energy available for the vegetation, although soil-water contents and water-pressure deficits are favourable. Mild temperatures in winter can therefore lead to periods of recovery from stressful drought periods, which is particularly essential for evergreen tree species in mixed forests to compensate for their lower photosynthetic rates during the growth period, relative to deciduous species. This highly dynamic seasonality of favourable and unfavourable conditions produces a rich diversity of plants in these regions (Cowling et al., 1996). In turn, this features a highly diverse range of traits and taxa that has produced multiple survival strategies which help to explain the abun-

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dance and distribution of species (Matesanz and Valladares, 2013). Nonetheless, the predicted reductions in annual precipitation, increases in mean temperature, and increases in the variability and occurrence of extreme droughts and heat waves in arid and semi-arid regions are likely to affect species abundance and distribution (Friend, 2010; IPCC, 2013; Somot et al., 2008).

That said, the different adaptive strategies of these taxa can only partly account for the documented shifts in distribution in Mediterranean species (Lenoir et al., 2008; Peñuelas and Boada, 2003; Peñuelas et al., 2007). Phenotypic plasticity and the adaptive value of genotypic evolution in Mediterranean plants can play important roles in immediate, individual responses to environmental change (Baquedano et al., 2008; Matesanz and Valladares, 2013; Peñuelas et al., 2013). We lack information, however, about the adaptive value of plasticity in functionally important traits and its evolutionary potential in Mediterranean plants (Gratani et al., 2013; Jump and Penuelas, 2005; de Luis et al., 2011; Nicotra et al., 2010; Shaw and Etterson, 2012; Valladares and Niinemets, 2008). The battle for survival and dominance in plant communities facing changes in their environments is fought on several levels, such as the adaptive ability of functional traits, adaptation of phenotypic plasticity, and genotypic evolution, which when lost can lead to shifts in distribution or, at worst, local extinction (Matesanz and Valladares, 2013; Peñuelas et al., 2013). Co-occurring tree species of these plant communities sharing the same habitat differ greatly in their ecophysiological traits and in their responses to and tolerance of different stresses (Flexas et al., 2014). Competition is thus a major ecological issue for the dynamics of plant communities and particularly of forests when fighting for scarce resources such as water, light, and nutrients (Valladares and Niinemets, 2008; Vilá and Sardans, 1999). We would then like to know which life-history trait is crucial and possibly superior in the dynamics of forest communities for withstanding increased abiotic stresses. Therefore, we must improve our understanding of the interactions among co-occurring tree species competing for scarce resources and trying to survive and tolerate novel environmental conditions to be able to predict ecosystem responses to global climate change.

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We must also characterise the relative importance of ecophysiological traits of Mediterranean tree species in winter when environmental conditions may be favorable and may determine their ability to withstand these novel environmental conditions in benefitting from periods of potential recovery and growth. In the past decade, leaf gas exchange (GE) studies have been increasingly used together with chlorophyll fluorescence (CF) measurements which when combined can provide a snapshot in the plant's physiology, health status and photosynthetic responses to environmental conditions and to abiotic stress factors (Flexas et al., 2008; Guidi and Calatayud, 2014). The physiological parameters we obtain can be seen as the result of phenotypic and genotypic evolution and of life-history adaptations of functional traits (Zavala et al., 2000). Moreover, mixed forests provide us with an ideal test-bed for investigating the different strategies of ecophysiological growth and their sensitivities to abiotic stresses, because all tree species have to contend equally with the yearly variability of environmental conditions. Most ecophysiological studies have been conducted in spring and summer, and winter has been surprisingly overlooked despite its importance for our understanding of the dominance of certain vegetation-types and of the responses of vegetation to stress, seasonality, and species composition (Oliveira and Peñuelas, 2004; Orshan, 1983; Treitach et al., 1997). Even though efforts have recently been made to elucidate the behaviour of sclerophyllous ecosystems under variable winter conditions) (e.g. García-Plazaola et al., 1999, 1997; Kyparissis et al., 2000; Levizou et al., 2004; Martínez-Ferri et al., 2004; Oliveira and Peñuelas, 2004, 2000), the physiological behaviour of co-occurring species of evergreen trees in the Mediterranean region, including GE and CF, have been insufficiently studied for understanding the dynamics of interspecific competition. This study has thus focused on the ecophysiological behaviour in winter of three species of evergreen trees, *Quercus ilex* L., *Pinus halepensis* Mill., and *Arbutus unedo* L., in a diverse mixed forest in northern Catalonia near Barcelona, Spain.

Our aims were to (i) investigate the foliar physiology of these three species under mild winter conditions, (ii) analyse the effect of sudden changes from favourable to un-

favourable conditions on photochemical and non-photochemical processes associated with electron transport, CO₂ fixation, and heat dissipation, (iii) determine if leaves exhibit distinct locational (sunlit or shaded) responses to winter stress, and (iv) identify the species-specific strategies when coping with stress, induced by low temperatures and frost. These topics are of particular interest due to the recent report of an increased dominance of angiosperm trees and the negative impacts on pines over extensive areas of the Iberian Peninsula (Carnicer et al., 2013).

2 Material and methods

2.1 Field site

Our experiment was conducted at the field station of Can Balasc in Collserola Natural Park, a coastal massif (8500 ha) in the hinterlands of Barcelona, northeastern Spain (41°25' N, 2°04' E, 270 m a.s.l.). The forest stand at the study site has an area of 0.7 ha and is on a northeast-facing slope. The climate is characterised by typical Mediterranean seasonal summer droughts and warm temperatures, with a mean August temperature of 22.8°C. The proximity to the Mediterranean Sea provides mild winters where frosts and snow are rare, as reflected in the 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., 2000). The soils have predominantly developed above lithological strata of shales and granite (Sanchez-Humanes and Espelta, 2011).

2.2 Stand history and composition of tree species

The history of Collserola Natural Park is typical for the area, being characterised by intensive exploitation for charcoal in coppice forests and for agricultural purposes such as olive production. The abandonment of these practices at the beginning of the century led to forest succession and restoration with the early successional and fast growing Aleppo Pine (*P. halepensis* Mill.). As in wide parts of the Mediterranean basin, this tree

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species was favoured by forest management for its rapid growth rates and timber yields (Maestre and Cortina, 2004). The cessation of forest practices in the early 1950s led to a second wave of succession characterised by extensive regeneration of the evergreen Holm Oak (*Q. ilex* L.) and the deciduous Pubescent Oak (*Q. pubescens* Willd.). As a result, many mixed forest stands in Collserola are currently characterised by two-layered canopies consisting of a dense layer from *Quercus* species surmounted by shelter trees of *P. halepensis*. The forest stand at our experimental site has reached the next and final stage of forest succession, where the dense *Quercus* canopy is out-competing the early successional *P. halepensis*, simply by suppressing the growth of the light demanding pine seedlings and saplings. This final stage of succession is typical of many pine-oak forest-type sites in the Iberia Peninsula. *P. halepensis*. is dependent mainly on fire disturbances for natural regeneration (Zavala et al., 2000). The diversity of tree species is enriched by the scattered occurrence of Strawberry trees (*A. unedo*) in the forest canopy being usually more characterised as a shrubby species abundant in the macchia ecosystems (Beyschlag et al., 1986; Reichstein et al., 2002). The forest diversity also encompasses a dense understory mainly consisting of *Pistacia lentiscus* L., *Erica arborea* L., *Phillyrea latifolia* L., *Rhamnus alaternus* L., *Cistus* spp, *Crataegus monogyna* Jacq., *Bupleurum fruticosum* L., and other less abundant species. The stand at our study site has reached a highly diverse stage of forest succession and has provided us with a rare set of some of the most important Mediterranean tree species growing together naturally.

2.3 Sampling

The sampling of the mild winter period took place between 23 January–2 February 2012 and of the cold winter period 14–24 February 2012. Twigs for GE-analysis were cut with a pruning pull in the mornings or afternoon hours after the daily peak of water-pressure deficit. Twigs with sunlit leaves were sampled from the outer part of the upper third of the crowns, and twigs with shaded leaves were sampled from the inner part of the crowns, optimally at similar heights. In the second sampling period,

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however, we were constrained to sample shaded leaves only from *Q. ilex* due to limitation in labour and equipment. The twigs were immediately re-cut under water in buckets in the field and transported to the laboratory retained in plastic bags to minimise transpiration. Five replicates of each species were collected for the analysis of GE.

The twigs were pre-conditioned in the laboratory at a room temperature of 24–28 °C in dim light for 1–3 d and freshly cut the following morning before the measurement of GE (Niinemets et al., 1999, 2005). We assumed that the basic parameters of the photosynthetic machinery were sufficiently stable for measuring excised leaves. We supported this assumption by comparative analyses of attached and excised leaves (see also Epron and Dreyer, 1992; Haldimann and Feller, 2004; Laisk et al., 2002).

2.4 GE and CF analyses

GE and CF were measured with a Li-Cor LI-6400XT Portable Photosynthesis System equipped with a LI-6400-40 Leaf Chamber Fluorometer (Li-Cor, Inc., Lincoln, NE, USA). Response curves for foliar net assimilation vs. CO₂ concentration were recorded from apparently healthy leaves clamped in a leaf cuvette (2 cm²). CF was measured in parallel. *A. unedo* leaves were sufficiently large to cover the leaf cuvette, whereas sunlit leaves of *Q. ilex* were in some cases too small, and the area of the leaves had to be adjusted after the measurements. For the leaves of *P. halepensis*, we positioned a layer of needles on the leaf cuvette, avoiding gaps and overlays. The putty-like adhesive “Blu-tack” (Bostik SA, La Plaine St Denis, France) was also used to seal the gaskets and to keep the needles in position.

2.4.1 Preparation and acclimation

Prior to recording the response curves, the temperature of the clamped leaves (T_{Leaf}) was adjusted to 25 °C, and the flow of ambient CO₂ in the leaf chamber (C_a) was set to 400 μmol CO₂ m⁻² s⁻¹ (controlled with a CO₂ mixer). The leaves were dark-adapted for 15–20 min before the measurements, and the data were logged when the GE-derived

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parameters such as stomatal conductance (g_s), stomatal internal CO_2 concentration (C_i) and mitochondrial respiration in darkness (R_n) had stabilised. For our purposes, dark-adaption did not necessarily mean strict prolonged darkness but referred to a sufficiently low level of ambient background light that did not cause an accumulation of reduced photosystem II (PSII) acceptors, which could be detected as an increase in fluorescence. The leaves were also pre-darkened with special leaf clips or a dark cloth to save time. The chamber light was then turned on at a saturating quantum flux density of $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (20 % blue LED, 80 % red LED). The relatively high percentage of blue light stimulated the stomata to open (Farquhar and Sharkey, 1982; Kang et al., 2009; Niinemets et al., 2005). The relative humidity was maintained at 50 % ($\pm 10\%$), and the air flow was maintained at $500 \mu\text{mol s}^{-1}$. The above conditions were maintained for approximately 20–30 min until the net rate of carbon assimilation (A_{net}), g_s , and C_i of the leaf stabilised.

The GE-derived parameters A_{net} , g_s , and C_i likely require less time to stabilize, especially in healthy and unstressed leaves, but this minimum time range was necessary for the CF-derived parameters to ensure accurate measurement of the efficiency of harvesting light energy by maximal oxidation and therefore open PSII reaction centres under ambient conditions of CO_2 and saturating light, which can be monitored by observing the stability of steady-state fluorescence (F_s). If this stability is not achieved, the effective quantum yield of PSII (Φ_{PSII}) and subsequent calculations of important parameters such as the rate of electron transport based on the CF measurement (J_{CF}) could be underestimated. After all parameters had stabilised, the steady-state GE-derived parameters and several CF-derived parameters in the light-adapted state were recorded simultaneously. F_s followed shortly afterwards by the maximum fluorescence yield in the light-adapted state (F'_m) were logged by the emission of a pulse of white light at $10\,000 \text{ mmol m}^{-2} \text{ s}^{-1}$ to close all PSII reaction centres, followed by a so-called “dark pulse” for measuring the minimal fluorescence (F'_0) of a light-adapted leaf that has been momentarily darkened. The measurement of CO_2 began after the completion of

the preparation and acclimation, which required approximately 30 min in unstressed leaves and up to 2 h in stressed leaves.

2.4.2 CO₂ experiments

The CO₂-response curves were recorded at a T_{Leaf} of 25°C and a quantum flux density of 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The values of C_a used to generate the response curves were 400 → 300 → 200 → 150 → 100 → 50 → 400 → 400 → 600 → 800 → 1200 → 2000 (in $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$). The minimum and maximum times for stabilising A_{net} , g_s , and C_i for each log were set to 4 and 6 min, respectively.

2.4.3 Light experiments

Light-response curves (A/PPFD) were generated at a C_a of 400 $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ by automatically applying changes in the photosynthetically active radiation with the LI-6400XT light source. To obtain precise responses at the low range of the light gradient for estimating the daily mitochondrial respiration by the Kok effect (Kok, 1948), we used the following PPFD sequence: 2500 → 2000 → 1500 → 1000 → 800 → 600 → 500 → 400 → 300 → 200 → 150 → 125 → 100 → 75 → 50 → 40 → 30 → 20 → 10 → 5 → 0 (in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The minimum and maximum times between each light level for the generation of the A/PPFD curves were set to 1 and 2 min, respectively. The gradient from high to low light during an A/PPFD curve led to a drop in T_{Leaf} as the light decreased. The rapid changes in the light levels prevented the adjustment of T_{Leaf} while guaranteeing stable air and water fluxes and avoiding noisy measurements of C_i and g_s . We thus decided to maintain a stable Peltier-block temperature (T_{block}) in the leaf cuvette. Hence, T_{block} was first adjusted so that T_{leaf} was 25°C at the beginning of the A/PPFD curve and then kept stable throughout the experiment. T_{Leaf} had dropped by approximately 1–3°C by the completion of the A/PPFD curve.

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2.5 Calculation of CF-derived parameters

The maximum efficiency of PSII was calculated by:

$$\frac{F_v}{F_m} = \frac{(F_m - F_o)}{F_m} \quad (1)$$

5 where F_v is the variable fluorescence of a dark-adapted sample, F_m is the maximal fluorescence measured after a saturating light pulse, and F_o is the minimal fluorescence measured under darkness. These parameters were obtained from dark-adapted leaves with closed PSII reaction centres as described in the previous sections. The F_v/F_m ratio describes the fraction of photochemically absorbed photons under dark conditions.
10 Typical values range between 0.75 and 0.85, depending on age, health, and preconditioning (Maxwell and Johnson, 2000). The F_v/F_m ratio provides information about the maximum or optimum quantum yield and serves as an early indicator of stress in photosystems. Ratios below 0.80 are indicative of induced photoprotection and sustained energy dissipation (Maxwell and Johnson, 2000; Verhoeven, 2013), whereas leaves
15 with ratios below 0.74 are considered to be below the recovery threshold (Björkman and Demmig, 1987).

The effective quantum yield of PSII was estimated by:

$$\Phi_{\text{PSII}} = \frac{(F'_m - F_s)}{F'_m} \quad (2)$$

20 where F_s is the steady-state fluorescence in a fully light-adapted sample, and F'_m is the maximal fluorescence yield after a pulse of high light. The Φ_{PSII} is the counterpart of the optimum quantum yield and represents the fraction of photochemically absorbed photons in a light-adapted leaf (Maxwell and Johnson, 2000). The non-photochemical quenching (NPQ) was estimated by both dark- and light-adapted fluorescent signals

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F_m and F'_m by:

$$\text{NPQ} = \frac{(F_m - F'_m)}{F'_m} \quad (3)$$

Photochemical quenching (qP) indicates the proportion of open PSII reaction centres and tends to be highest in low light when leaves use light most efficiently (Maxwell and Johnson, 2000). qP was estimated by:

$$\text{qP} = \frac{F'_m - F_s}{F'_m - F'_o} \quad (4)$$

where F'_o is the minimum fluorescence in a light-adapted leaf after a pulse of darkness. The rate of photosynthetic electron transport, J_{CF} , is calculated by (Genty et al., 1989):

$$J_{CF} = \varepsilon \times \Phi_{\text{PSII}} \times \alpha_L \quad (5)$$

where ε is a scaling factor for the partitioning of intercepted light between photosystems I and II. We assumed that light was equally distributed between both photosystems ($\varepsilon = 0.5$) (Bernacchi et al., 2002; Niinemets et al., 2005). α_L is the foliar absorbance determined in separate measurements of foliar reflectance and transmittance. The following values of α_L were determined: 0.932 for *Q. ilex* and 0.912 for *P. halepensis*, with no differences between sunlit and shaded leaves of these two species, and 0.935 for sunlit leaves of *A. unedo*, and 0.917 for shaded leaves of *A. unedo*. For the determination of these leaf absorptances (α_L), foliar reflectance and transmittance were measured at midday in August 2012 using a spectroradiometer UniSpec Spectral Analysis System (PP Systems, Haverhill, MA, USA). The value of J_{CF} at a CO_2 concentration of $400 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and a PPFD of $1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ is termed ambient photosynthetic electron transport (J_{amb}). Its relationship with the net assimilation rate ($J_{\text{amb}}/A_{\text{net}}$) was used for the analyses of alternative electron sinks beside carbon metabolism.

2.6 Estimation of light respiration and calculation of the effective quantum yield of CO₂ (Φ_{CO_2})

In the literature, the term R_d was sometimes used for dark respiration (Farquhar et al., 1980; Turnbull et al., 2003), but also for day respiration (Flexas et al., 2012; Yin et al., 2011). We will use R_d to represent mitochondrial respiration during the day or under lighted conditions and R_n to represent mitochondrial respiration at night or under dark-adapted conditions. In ecophysiological studies, the latter is often measured at pre-dawn together with water potential or alternatively, we estimated R_n during the day after darkening the leaf for at least 30 min. R_d was estimated from the light-response curve with the combined GE and CF Measurements proposed by Yin et al. (2009), named the CF method. This method is based on that of Kok (1948), whose GE measurements were based on the generally linear response of assimilation to light at low irradiances (0–200 $\mu\text{mol m}^{-2} \text{s}^{-1}$). When light increases within this range, however, light has an inhibitory effect on R_d because respiration interacts with photosynthesis as an integral part of the photosynthetic metabolism in illuminated leaves (Tcherkez and Ribas-Carbó, 2012). As a result, the A/PPFD relationship exhibits a break in the linear light-response curve, with a notably lower slope above this point, described as the Kok effect. The linear A/PPFD relationship above this point is extrapolated, and R_d is estimated by identifying the intersection with the y-axis. The CF method by Yin et al. (2009) also uses CF information for the response of Φ_{PSII} to light that is not constant within the range of limiting light (Yin et al., 2009). The fraction of absorbed photons and hence the effective quantum yield of PSII actually increases with decreasing light, because the few photons reaching the reaction centres of chlorophyll are used as efficiently as possible. Yin et al. (2009) thus amended the Kok method by substituting the A/PPFD relationship with A/PPFD $\times \Phi_{\text{PSII}}$.

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The effective quantum yield of CO₂ (Φ_{CO_2} , unitless) can be calculated using the estimated α_L , R_d , together with A_{net} and PPDF as follows

$$\Phi_{\text{CO}_2} = \frac{(A_{\text{net}} - R_d)}{\text{PPFD} \times \alpha_L} \quad (6)$$

2.7 Estimation of mesophyll conductance

The CO₂ pathway leads from the atmosphere to the intercellular air spaces through the stomata and from there diffuses through the air spaces of the mesophyll, cell walls, cytosol, and chloroplastic envelopes and finally reaches the sites of CO₂ fixation in the chloroplastic stroma where it is fixed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). In this study, we call this pathway the internal mesophyll diffusion conductance (g_m) and estimate it with the variable- J method by Harley et al. (1992):

$$g_m = \frac{A_{\text{net}}}{C_i - \frac{[\Gamma^* \times J_{\text{CF}} + 8(A_{\text{net}} + R_d)]}{J_{\text{CF}} - 4(A_{\text{net}} + R_d)}} \quad (7)$$

where Γ^* is the CO₂ concentration at which the photorespiratory efflux of CO₂ equals the rate of photosynthetic uptake of CO₂ (Table 3). Similarly to g_s , g_m is defined as a unitless molar fraction, rendering the units for conductance the same as those for photosynthesis. Nonetheless, the drawdown of CO₂ from the intercellular airspaces to the sites of carboxylation is thought to be dominated by the liquid phase of the chloroplast and is hence dependent on the partial pressure of the gas according to Henry's law (Harley et al., 1992). The units for conductance ($\text{mol m}^{-2} \text{s}^{-1} \text{bar}^{-1}$) are thus directly comparable to g_s when the atmospheric pressure is 1 bar. We assumed normal pressure (1.01325 bar) in our experiments that were conducted in Barcelona, which is close to sea level. The variable- J method accounts for the variation in g_m with C_i and provides more accurate estimates of photosynthetic parameters than do A/C_c

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curves that assume a constant g_m , especially during episodes of water stress) (Flexas et al., 2007b). The chloroplastic CO_2 concentration can then be determined using C_i , A_{net} , and g_m :

$$C_c = C_i - \frac{A_{\text{net}}}{g_m} \quad (8)$$

where C_c is the chloroplastic CO_2 concentration.

2.8 The Farquhar, von Caemmerer, and Berry (1980) photosynthesis model (FvCB)

The FvCB photosynthesis model was employed on the assumption that foliar carbon assimilation was limited either by Rubisco activity (A_c) or by ribulose-1,5-bisphosphate (RuBP) regeneration (A_j) and was driven by light, temperature, and CO_2 . The model includes a set of kinetic properties which are summarised in Table 3. The model was further complemented with a third limitation: the photosynthetic rate limited by triose-phosphate use (A_p) (Sharkey, 1985). A_{net} can then be determined by the minimum of these three potential rates from an A/C_c curve:

$$A_{\text{net}} = \min \{A_c, A_j, A_p, \} \quad (9)$$

where

$$A_c = V_{c, \text{max}} \times \left[\frac{C_c - \Gamma^*}{C_c + K_c \left(1 + \frac{O}{K_o}\right)} \right] - R_d \quad (10)$$

where $V_{c, \text{max}}$ represents 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 (Table 3). The equation representing photosynthesis limited by RuBP regeneration is:

$$A_j = J \cdot \left[\frac{C_c - \Gamma^*}{4C_c + 8\Gamma^*} \right] - R_d \quad (11)$$

5 where J is the rate of electron transport. The denominator of the above equation represents the stoichiometry of the number of electrons required to regenerate ATP and NADP; we have used four for C_c and eight for Γ^* (Flexas et al., 2012). Other valid stoichiometries, however, are possible (Caemmerer 2000 in Flexas et al., 2012). J becomes J_{\max} under light and CO_2 saturation when the maximum possible rate of electron
10 transport is theoretically achieved.

The photosynthetic rate limited by triose-phosphate use is estimated by:

$$A_p = \frac{3TPU \times C_c}{\Gamma^* \left[C_c - \left(\frac{1+3\alpha_{TPU}}{2} \right) \right]} - R_d \quad (12)$$

15 where TPU is the rate of triose-phosphate use at saturating CO_2 concentrations, and α_{TPU} is the proportion of glycerate not returned to the chloroplasts. This equation fits the A/C_c curve plateau at high concentrations of CO_2 when a further increase in C_c no longer increases A_{net} or, in some cases, decreases A_{net} . A plateau of A_{net} at saturating concentrations of CO_2 is due to the phosphate limitation mentioned above. These three estimated parameters ($V_{c, \max}$, J_{\max} , and TPU) define the biochemical capacity to
20 drive the photosynthetic assimilation of CO_2 but are defined here as the photosynthetic potential (Niinemets et al., 2006). The term photosynthetic capacity is here dismissed, despite its frequent use in the literature, to avoid confusion with studies that have used this term for the maximum rate of assimilation under saturating light conditions (e.g. Bertolli and Souza, 2013).

tested with the CF technique, which should approach zero. This technique allowed us to obtain photosynthetically inactive leaves while minimising the loss of turgor and improving parameterisation relative to the oven-dried leaves used, for example, by Long and Bernacchi (2003) or the manufacturers' equation or no correction at all.

2.12 Statistical analyses

All statistical analyses were performed using the R software package, version 3.0.2 (<http://www.r-project.org/>). Differences in the parameters between the mild and cold winters were determined with Student's *t* tests ($P \leq 0.05$). Shapiro–Wilk tests of normality tested for normality of the data. One-factorial analyses of variance (ANOVAs) with tree species as the main factor tested for differences between tree species of the parameters in the sampling periods. Significant differences were determined at $P \leq 0.05$ with Tukey's HSD tests. Regression analyses were conducted to study the relationship between J_{\max} and $V_{c, \max}$ and between J_{amb} and A_{net} . Analyses of covariance (ANCOVAs) tested for differences in slopes and intercepts.

3 Results

3.1 Environmental variables

Collserola Natural Park experienced extremely mild winter conditions in November and December 2011 and January 2012, when average minimum temperatures (10.4 °C in November, 5 °C in December, and 3.4 °C in January) remained above 0 °C and no frosts occurred. Average maximum temperatures were 16.3 °C in November, 12.2 °C in December, and 11.4 °C in January. All species had considerable shoot growth of up to 15 cm during this mild period. Sudden low temperatures, however, led to frost on six consecutive days and a minimum average temperature of -2.3 °C (Day of the year (DOY) 21–26) followed by eight days of cool temperatures averaging $+2.6$ °C (DOY 27–35) (Fig. 1). The radiation during the period of frost averaged 58 W m^{-2} .

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3.2 Photosynthetic potentials

Of the three photosynthetic parameters describing the photosynthetic potential, $V_{c, \max}$ and J_{\max} , and TPU, only the first two could be satisfactorily estimated from the A/C_c -response curves. The leaves were only occasionally limited by TPU (6 out of 42), despite the excessive CO_2 concentrations in the higher section of the CO_2 -response curve. TPU was therefore discarded from further analysis. $V_{c, \max}$ and J_{\max} were highest in *Q. ilex* but more importantly also decreased most strongly after the period of frost by nearly 50 % ($P \leq 0.05$; Fig. 2). The photosynthetic potential of *P. halepensis* was affected the least, reflected by moderate decreases in $V_{c, \max}$ and J_{\max} (16 % and 19 %), which were not significant. $V_{c, \max}$ and J_{\max} were lowest in *A. unedo* during the mild winter period and decreased by approximately 33 % after the period of frost. This decrease, however, was not significant due to a large standard error.

3.3 GE-derived parameters under ambient conditions

The period of frost had a strong effect on several GE-derived parameters in *Q. ilex* leaves. The cold temperatures decreased R_n in *Q. ilex* leaves, but the effect was much weaker than for R_d and was not significant (Fig. 3). These parameters responded very weakly to the cold and frost in the leaves of *A. unedo* and *P. halepensis*. A_{net} and Φ_{CO_2} were also reduced in *Q. ilex* leaves by approximately 50 % which was significant for the former (Fig. 4a) and low significant for the latter parameter (Fig. 4b). Further differences were only significant for Φ_{CO_2} in *P. halepensis* leaves being reduced by 12 % ($P \leq 0.05$). The CO_2 conductance was more strongly reduced in g_m than in g_s for *Q. ilex* and *A. unedo* leaves which was only significant for the former whereas these parameters seemed unaffected in *P. halepensis* leaves (Fig. 4a and b). As a consequence, we observed a tendency of an C_i increase in parallel with a C_c decrease in *Q. ilex* and *A. unedo* leaves due to a lower CO_2 uptake in carbon metabolism, but not in *P. halepensis* (Fig. 6a and b). The differences observed were not significant ($P \leq 0.05$). In *Q. ilex*, the lower drawdown of CO_2 to the chloroplasts ($\Delta C_i - C_c$) (Fig. 7b) led to the observed

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decreases in A_{net} (Fig. 4). As a consequence, unused CO_2 accumulated in the stomatal internal air spaces (C_i) (Fig. 6), which led to a lower $\Delta C_a - C_i$ (Fig. 7a). This strong stomatal and mesophyll control did not occur to the same extent in *A. unedo* or *P. halepensis* leaves, which had only marginal differences in $\Delta C_i - C_c$ and $\Delta C_a - C_c$ before and after the cold period.

3.4 CF-derived parameters under ambient conditions

The GE-derived parameters enabled us to study the immediate responses, but several CF-derived parameters allowed us to determine in more depth the physiological changes in parts of the light-harvesting apparatus, namely PSII. F_v/F_m estimated the maximum quantum yield of PSII and representing the health of a leaf (Fig. 8b). *A. unedo* leaves were most strongly affected by the period of frost, followed by *Q. ilex* leaves, whereas *P. halepensis* leaves were only marginally affected. The changes were not statistically significant in the latter two species ($P \leq 0.05$). Φ_{PSII} tended to decrease in all species but most strongly in *Q. ilex* leaves (42%), however insignificantly (Fig. 8a). NPQ responded very differently in the three species. NPQ did not change much between the two sampling periods in the leaves of *P. halepensis* (6%) but decreased significantly by 25% ($0.05 \leq P \leq 0.1$) in *A. unedo* leaves and tended to increase in *Q. ilex* leaves by 31% ($P \geq 0.05$), however insignificantly (Fig. 9a). We found a significant ($P \geq 0.05$) and marginal significant ($0.05 \leq P \leq 0.1$) decrease of qP in the leaves of *P. halepensis* and *Q. ilex*.

3.5 Relationships of foliar photosynthetic variables

The covariance of several relationships of the foliar photosynthetic variables were analysed in an ANCOVA to test for differences in the slopes and intercepts in these relationships. The ANCOVA for the relationship between $V_{c, \text{max}}$ and J_{max} in *Q. ilex* leaves indicated a marginally significant ($P \leq 0.1$) reduction in the slope and a highly significant ($P \leq 0.01$) reduction in the intercept, revealing a comparatively stronger effect on

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J_{\max} than on $V_{c, \max}$ due to the change in weather (Fig. 10 and Table 2). The relationship between the rate of electron transport at ambient conditions derived from CF and the CO_2 assimilation at ambient CO_2 concentrations ($J_{\text{amb}}/A_{\text{net}}$) was similar in all tree species (Figs. 11 and 13 and 15 and Table 2). The slopes were higher in response to the stress imposed by the low temperatures but were not significant. When all species were combined the change of the slope was marginally significant, indicating a possible increased alternative electron sink other than carbon metabolism (Table 2).

3.6 Role of leaf position

Under mild conditions, the leaves of *Q. ilex* showed the most strongly pronounced differences in the leaf position (data of *P. halepensis* and *A. unedo* not shown). Leaves growing under high irradiances had a more active carbon metabolism (A_{net} , R_d , R_n , and Φ_{CO_2}), photochemical efficiency (Φ_{PSII}), and photosynthetic potential (high J_{\max} and $V_{c, \max}$) in all tree species (data only shown for *Q. ilex*).

As described in Sect. 2.3 the effect of the leaf position after the sudden cold period was only studied for *Q. ilex* and is presented in the following. After the sudden frost period, the photosynthetic potential was much higher in sunlit than in shaded leaves of *Q. ilex*, with both J_{\max} and $V_{c, \max}$ being highly significant (Fig. 2 and Table 1). These differences disappeared after the cold period, because J_{\max} and $V_{c, \max}$ in the shaded leaves remained unaffected by the frost. F_v/F_m was generally higher in the shaded leaves, but not significantly ($P \leq 0.05$) (Fig. 8 and Table 1). The photosynthetic parameters under ambient conditions, such as A_{net} , g_s , C_i , C_c , and g_m , were not affected much by the leaf position (Figs. 4, 5 and 6 and Table 1). Although not significant, the effects of the cold period on these parameters were stronger in the sunlit leaves. The leaf position had more pronounced effects on R_n and R_d (Fig. 3 and Table 1). The response of respiration to winter stress, however, differed depending on the location of the leaves. R_n maintained the same balance between sunlit and shaded leaves before and after the cold period, but R_d decreased comparatively more in sunlit leaves due to the period of frost. This pattern was also reflected in Φ_{CO_2} (Fig. 4 and Table 1)

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and in several CF-derived parameters such as Φ_{PSII} , qP , and NPQ , (Figs. 8 and 9 and Table 1) indicating a stronger effect on the photochemical machinery of sunlit leaves than on shaded leaves. Shaded leaves also exhibited a lower J_{amb}/A_{net} ratio, but the ratio increased equally in both leaf positions after the cold period, indicating a similar behaviour of dissipating energy by alternative electron sinks (Fig. 11 and Table 1). As in A_{net} and g_s , both leaf positions acclimated to the mild winter conditions similarly, resulting in a comparable drawdown of CO_2 from C_a to C_i , but also from C_i to C_c , and these deltas remained stable in the shaded leaves but decreased strongly in the sunlit leaves (Fig. 7 and Table 1). All P values are summarized in Table 1.

4 Discussion

Recent efforts have shed light on the behaviour of sclerophyllous ecosystems under variable winter conditions (e.g. García-Plazaola et al., 1999, 1997; Kyriacou et al., 2000; Levizou et al., 2004; Martínez-Ferri et al., 2004; Oliveira and Peñuelas, 2004, 2000), but the physiological behaviour encompassing GE and CF measurements of co-occurring species of evergreen trees in the Mediterranean region have not been sufficiently well studied to determine the dynamics of interspecific competition. This study shows that species of Mediterranean evergreen trees can benefit from mild conditions in winter that provide potential growth periods, but the trees can be particularly vulnerable if the conditions suddenly change from favourable to adverse. Field experiments conducted on trees growing under natural conditions reflect a much more complex and dynamic environment than do controlled laboratory conditions, though field experiments provide a much more realistic assessment of species-specific responses. We thus chose to combine field and laboratory measurements by collecting twigs from naturally grown mature trees and then analysing these twigs in the laboratory after a recovery period, as described in Sect. 2.3. We intended to avoid the problems we had faced in the field, such as the limited ability of the instruments to reach the standard operating temperature of $25^\circ C$, which was hampered by low ambient temperatures

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or unpredictable plant responses such as closed stomata or patchy stomatal conductance (Mott and Buckley, 1998, 2000). The pre-conditioned twigs had a much stabler C_i and a sufficiently stable and high g_s , which are required for conducting a noise-free CO_2 -response curve. The method for cutting twigs enabled the possibly to rehydrate stressed leaves at optimum conditions to allow us to analyse their long-term acclimation to the environmental conditions from which they were derived. This method has been used in other studies (Epron and Dreyer, 1992; Haldimann and Feller, 2004; Laisk et al., 2002; Niinemets et al., 1999, 2005), and we confirmed that the leaves remained fresh and functional for several days controlled by g_s and fluorescent signals (data not shown). Our ambient values of the GE- and CF-derived parameters accordingly represented the “ambient capacity” of pre-conditioned leaves under near-optimal ambient environmental conditions of CO_2 concentrations and saturating light and at a room temperature of 20–25 °C (Reich et al., 1998).

4.1 Winter in the Mediterranean region

Mediterranean-type ecosystems are exposed to stress from summer droughts but also from low temperatures in winter (Mitrakos, 1980). Less attention, however, has been paid to the degree and extent of these stress periods and the wide variation among years and regions, in response to which Mediterranean evergreen species have developed a dynamic photoprotective ability to withstand these stressors (Kyparissis et al., 2000; Martínez-Ferri et al., 2004). In our winter field campaign, we observed very comfortable temperatures during the day, and despite the cool minimum temperatures (minimum average of 3.4 °C in January), the conditions led to considerable growth of shoots and leaves in January 2012. These observations are also reflected in the high sap flow per tree (J_t), ranging on average between 5 and 10 kg d⁻¹ during the mild winter period (Sánchez et al., unpublished results). The carbon balance was thus positive, so that depleted carbon reserves could be refilled, and enough resources were available to invest in an early bud burst. The frost and cold, however, suddenly imposed a period of thermal stress lasting for approximately one week while receiving medium-high ra-

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5 diation ($\sim 60 \text{ W m}^{-2}$) until the temperatures slowly returned to the previous conditions. The change from favourable to adverse conditions has rarely been recognised as an important factor for plant growth and distribution in Mediterranean-type ecosystems (Garcia-Plazaola et al., 2003a), and sudden frosts have been attributed to higher altitudes of the Mediterranean region (Blumler, 1991; Treitach et al., 1997). Our study site, however, was in the proximity of the sea characterised by a sub-humid Mediterranean climate.

4.2 Effects of low temperatures on the foliar photochemical machinery

10 The special challenge photosynthetic systems are facing is that they are designed to absorb large amounts of light energy processing it into chemical energy. At the molecular level, however, the energy in a photon can be damaging, and particularly excessive light intensities can lead to the production of toxic molecules such as superoxides, singlet oxygen, and peroxide (Aroca et al., 2001; Demmig-Adams and Adams, 1992). Such damage occurs in winter, when low temperatures coincide with clear skies and
15 expose leaves to high light intensities, while photosynthetic metabolic processes are inhibited by the low temperatures. This imbalance between light energy absorbed in photochemistry and light energy used in metabolism can inactivate and damage the PSII reaction centres and induce chronic photoinhibition of PSII (Allen and Ort, 2001). These mechanisms are consequently induced during short- and long-term exposure
20 to low temperatures to adjust this imbalance in the photosynthetic apparatus, including changes in energy absorption and photochemical transformation through the partitioning and allocation of energy; changes in chloroplastic carbon metabolism and altered expression of the enzymes of the Calvin cycle; modifications in the thylakoid membrane system, such as changes in foliar protein content, post-transcriptional activation, and increased expression of the enzymes for sucrose synthesis; and the signals that trigger these processes (Ensminger et al., 2012; Taz and Zeiger, 2010). Both cool daytime
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and nighttime temperatures or frosts can affect subsequent daytime photosynthesis and induce the above processes (Flexas et al., 1999).

4.3 High photosynthetic potentials and strong effects of low temperatures

Despite occurring lower temperatures than in spring conditions, photosynthesis can recover once the leaves become acclimated to the new conditions (Hurry et al., 2000). This recovery was demonstrated in a maritime ecotype of a Scots pine forest in the Netherlands that showed a substantial recovery of A_{net} during warm winter days (Dolman et al., 2002). Our study site did not experience a frost during the relatively mild conditions in November and December 2011 as well as January 2012, which lead to notably high photosynthetic potentials and shoot growth in all three tree species. *Q. ilex* had the highest photosynthetic potential, comparable to that under spring conditions, and the photosynthetic potential in *P. halepensis* even exceeded its spring values (Sperlich et al., unpublished data). The photosynthetic exploitation of favourable conditions in winter is crucial for achieving a positive carbon balance (García-Plazaola et al., 1999b; Martínez-Ferri et al., 2004), and photosynthetic potentials and carbon assimilation in winter can be equal to or even exceed those during spring conditions (Martínez-Ferri et al., 2004).

Our data indicated that sudden changes from favourable to unfavourable winter conditions, with night frosts for several consecutive days, can strongly affect the photosynthetic machinery and carbon metabolism. The physiological responses were highly species-specific. *Q. ilex* leaves responded with significant decreases (approximately 50%) in their photosynthetic potentials (both $V_{\text{c,max}}$ and J_{max}). In contrast, $V_{\text{c,max}}$ and J_{max} decreased in *P. halepensis* leaves by only 16 and 19%, respectively, and in *A. unedo* leaves by approximately 30% (for both parameters). $V_{\text{c,max}}$ and J_{max} are strongly correlated, being regulated in a coordinated manner (Wullschlegler, 1993). Interestingly, the ANCOVAs indicated that J_{max} decreased more strongly than did $V_{\text{c,max}}$ in *Q. ilex* and *P. halepensis* leaves, which we interpreted as a greater stress from frost and cold on the photochemical processes than on the carbon reactions. This interpretation is

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in line with previous findings showing that the limitations of the photosynthetic rate by RuBP regeneration at moderately low temperatures may be stronger than those by RuBP carboxylation and that the differences in the $J_{\max}/V_{c, \max}$ ratio are caused by changes in the relative amounts of photosynthetic proteins (Hikosaka et al., 1999;

Onoda et al., 2005). Moreover, the $J_{\max}/V_{c, \max}$ ratio we derived under mild winter conditions ranged between 1.0 and 1.2, in contrast to typical values previously reported (1.5 to 2.0) (Misson et al., 2006; Wullschlegel, 1993). This difference is probably due to methodological issues when parameterising on A/C_i curves instead of on A/C_c curves, because the former asymmetrically underestimates the two parameters J_{\max} and $V_{c, \max}$ (Sun et al., 2014).

4.4 PSII – primary target of stress induced by low temperatures

The larger decrease of J_{\max} relative to $V_{c, \max}$ indicated that stressful low temperatures became manifest first in a hampered pathway of photochemical energy, because PSII complexes are primarily affected by light-induced damage (Maxwell and Johnson, 2000; Taz and Zeiger, 2010; Vass, 2012). The imbalance between light energy absorbed in photochemistry and light energy used in metabolism can inactivate and damage PSII reaction centres, more precisely, the reaction-centre protein D1 (Aro et al., 1993; Demmig-Adams and Adams, 1992; Mulo et al., 2012). As a response, qP declines because less energy is used in carbon metabolism, and thermally dissipated excess energy increases, as reflected in NPQ. These responses altered the efficiency of PSII (Φ_{PSII}) in our three target species, with *Q. ilex* leaves having the largest declines and *P. halepensis* needles the smallest.

More precise information about the underlying processes that have altered this efficiency is provided by F_v/F_m . Changes in this parameter in our pre-conditioned leaves could indicate chronic photoinhibition and not dynamic photoinhibition, which usually recovers after dawn (Werner et al., 2002). Decreased ratios of F_v/F_m indicated that all species, but most notably *A. unedo*, suffered from photoinhibition induced by cool temperatures and frost. The species-specific responses indicated that *A. unedo*

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Φ_{CO_2} , and R_d , which confirmed this trend and were in accord with the findings by Corcuera et al. (2004). Despite reports of several mechanisms of resistance to drought stress in *A. unedo*, including increased levels of zeaxanthin that indicates an enhanced thermal dissipation of excess excitation energy in periods of summer stress (Munné-Bosch and Peñuelas, 2004), we found that *A. unedo* leaves had a lower capacity of photoprotection in response to induced over-excitation of the photosystems by winter stress.

4.5 Inhibition of carbohydrate metabolism

In addition to adjustments of the energy flow in the antennal systems via thermal dissipation and downregulation of photosynthetic electron transport, regulatory mechanisms include inhibition of Rubisco activity (as shown above by $V_{c, \text{max}}$) and stomatal and mesophyll diffusion behaviour (Ensminger et al., 2012; Taz and Zeiger, 2010). Our data reflected changes in carbon assimilatory parameters such as A_{net} , C_i , g_s , and g_m , where *Q. ilex* leaves had the strongest, *P. halepensis* leaves had no or the weakest, and *A. unedo* leaves had intermediate responses. More precisely, *Q. ilex* leaves had a pronounced stomatal response to low temperatures (Gratani et al., 2000) that imposed a two-fold higher resistance to the foliar exchange of CO_2 and water. The drawdown of CO_2 from C_a to C_i subsequently decreased by approximately 25% (marginally significant at $P \leq 0.1$). Leaves then tended to increase the marginal water-use efficiency, as shown by the C_i/C_a ratio. As a consequence, C_i increased due to an accumulation of unused pool of CO_2 in the stomatal internal air spaces, in contrast to C_c that decreased due to reduced carbon fixation and to a significant increase in foliar internal mesophyll diffusion resistance above that seen in g_s , reflecting a stronger non-stomatal control (Ensminger et al., 2012).

Foliar respiration (day and night) was extraordinarily high in *P. halepensis* leaves, whereas *Q. ilex* leaves had the lowest values. These results demonstrated that R_d and R_n had different respiratory acclimations affecting the efficiency of carbon use in the photosynthetic metabolism and that these foliar respiratory responses were highly

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species dependant (Zaragoza-Castells et al., 2007, 2008). We did not measure photorespiration but could infer some of its characteristics by studying the relationship between J_{amb} and A_{net} . All tree species had a relatively higher proportion of electron flux during the period that can be explained by utilization in the carbon metabolism.

The ANCOVAs did not indicate significant individual differences in the $J_{\text{amb}}/A_{\text{net}}$ relationship but combining all species had a marginally significant effect on the slope. These results indicated a marginal increase in alternative electron sinks, which have been mainly attributed to photorespiration, but also to the Mehler reaction that protects plants from photodamage in bright light (Allen and Ort, 2001; D'Ambrosio et al., 2006; Flexas et al., 1998, 1999; Fryer et al., 1998; Huner et al., 1998).

4.6 Leaf position specific responses to abiotic stress in winter

It is well known that leaves growing under high irradiances have a more active carbon metabolism (A_{net} , R_{d} , R_{n} , and Φ_{CO_2}), photochemical efficiency (Φ_{PSII}), and photosynthetic potential (high J_{max} and $V_{\text{c,max}}$) (Taz and Zeiger, 2010). This is reflected also in our results. Additionally, we showed that leaf position specific responses are species specific: under mild conditions, the leaves of *Q. ilex* showed the most strongly pronounced differences between sunlit and shaded leaves. This is because plants develop leaves with a highly specialised anatomy and morphology for the absorption of the prevailing light in their local environments resulting generally in smaller but also thicker sunlit leaves (Kull and Niinemets, 1993; Terashima and Hikosaka, 1995). Nevertheless, the higher carbon metabolism and photochemical activity photochemical activity in sunlit leaves of *Q. ilex* decreased strongly, partly below the level of shaded leaves, whereas shaded leaves showed little sign of any downregulation but maintained a relatively stable effective quantum yield of CO_2 assimilation in both periods. Furthermore, the photosystems showed no sign of photodamage and generally maintained a higher maximum efficiency than did sunlit leaves. We concluded that foliar-level physiology during winter was better protected in the shaded crown unexposed to the dramatic changes in radiation in the outer canopy, confirming the results by

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Valladares et al. (2008). We also concluded that *Q. ilex* is a highly dynamic species able to rapidly change its metabolism on the antioxidant and photoprotective level in dependence to its leaf position (García-Plazaola et al., 1997, 1999a; Martínez-Ferri et al., 2004). We show that the foliar plasticity in morphology and anatomy of *Q. ilex* (Bussotti et al., 2002; Valladares et al., 2000) can also be attributed to its biochemical metabolism. We stress that the solar environment of the leaves is a crucial factor when assessing tree performance, especially when comparing tree species in a competitive context.

4.7 Ecological context

Q. ilex had the most drastic photoprotective response to frost and cool temperatures, whereas *P. halepensis* exhibited a homeostatic behaviour with a very active carbon metabolism in both periods. *A. unedo* was intermediate, with large decreases in the parameters of carbon metabolism but also a high variation in its response to frost. *A. unedo*, however, also had the lowest photoprotective capability, which might be explained by previous characterisations to be semi-deciduous to drought being at the borderline to evergreen sclerophyllous species (Gratani and Ghia, 2002a, b). Investments in leaves and life cycles were thus lower, and leaves may be more rapidly replaced relative to more sclerophyllous leaves such as those of *Q. ilex*. We postulated that *A. unedo*, considered a relict of the humid-subtropical Tertiary tree flora, was likely to be more sensitive to winter stress, which is consistent with its presence mostly in the western Mediterranean basin and its frequent occurrence in coastal zones where humidity and temperature are the main factors determining its geographical distribution (Gratani and Ghia, 2002a and references therein).

Our results suggested that photosynthetic potential and carbon metabolism in *Q. ilex* could greatly benefit from favourable winter conditions. Moreover, when these relatively favourable conditions changed, *Q. ilex* quickly re-adjusted the photosynthetic machinery to the prevailing conditions, as indicated by the largest decreases in photosynthetic potential and carbon metabolism. Some researchers have proposed the lutein-epoxy

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cycle in photoprotection of *Quercus* as a mechanism to maintain sustained energy dissipation (Garcia-Plazaola et al., 2003b), which could help to account for the higher tolerance to low temperatures in *Q. ilex* relative to other co-occurring Mediterranean trees or shrubs (Ogaya and Peñuelas, 2003, 2007). *P. halepensis* did not suffer a pronounced chronic photoinhibition, confirming the results by Martínez-Ferri et al. (2004). Despite a pronounced downregulation of photosynthetic electron transport and an increase in alternative electron sinks, the light-saturated ambient photosynthesis remained surprisingly high and stable. *P. halepensis* thus exhibited a successful refinement of photosynthetic electron flow and possibly a successful repair of protein D1 in the PSII reaction centre.

The strong downregulation in *Q. ilex* and the homogenous response of *P. halepensis* were possibly due to distinct, previously described strategies. *Q. ilex* has been characterised as a photoinhibition-avoiding species and *P. halepensis* as a photoinhibition-tolerant species (Martinez-Ferri et al., 2000). We have extended this categorisation to a less photoinhibition-tolerant tree species, *A. unedo*, which favoured carbon metabolic processes at the cost of chronic photoinhibition and photodamage. This strategy is similar to those in other semi-deciduous shrubs (Oliveira and Peñuelas, 2001, 2004). The physiological responses of *Q. ilex*, a slowly growing late-successional species, to environmental stressors are highly plastic (Zavala et al., 2000) due to its vegetative activity in a wide range of temperatures and high stomatal control in stressful conditions (Gratani et al., 2000; Savé et al., 1999), high plasticity index and resprouting dynamics (Espelta et al., 1999; Gratani et al., 2000), deep rooting system and large carbohydrate pools (Canadell and Lopez-Soria, 1998; Canadell et al., 1999), and high adaptive variability in foliar phenomorphology (Sabaté et al., 1999). Our findings showed the intra-crown variability in *Q. ilex*, where shaded leaves were widely unaffected by the inhibitory cold stress (Oliveira and Penuelas, 2001). In contrast, *P. halepensis* is a fast growing conifer that quickly occupies open spaces after disturbances such as fires (Zavala et al., 2000). *P. halepensis*, as do all pines, has a low ability to store

carbohydrates and therefore follows a strategy of water conservation and embolism avoidance (Carnicer et al., 2013 and references therein).

High rates of photosynthesis and growth require high concentrations of carboxylation enzymes in the carbon cycle that have high maintenance costs (Valladares and Niinemets, 2008), perhaps accounting for the high respiration rates found in *P. halepensis* leaves. Moreover, differences among the species are also likely to be the result of distinct foliar morphologies and crown architectures. Pine trees are characterised by a relatively low exposure of foliar surface area to direct sunlight due to the cylindrical shape and steep angles of their needles but at the same time are able to exploit a wider range of incident light angles than broadleaved trees. Despite reported flexible adjustments in the orientation of the leaves in several Mediterranean broadleaved sclerophyllic species (Oliveira and Peñuelas, 2000; Vaz et al., 2011; Werner et al., 2002), needle leaves probably still confer some benefits to attain near-saturated photosynthetic rates over a wider range of diurnal and seasonal variation in sun angles (Jordan and Smith, 1993; Lusk et al., 2003), which might account for the good performance of *P. halepensis* under abiotic stresses. Against the background of current issues of climate change and recent large-scale studies of tree growth in the Iberian Peninsula reporting negative growth trends in Mediterranean angiosperms in response to temperature (Carnicer et al., 2013; Coll et al., 2013; Gómez-Aparicio et al., 2011), the winter period may have important implications for long-term carbon budgets. Plants have evolved diverse adaptive mechanisms to cope with the consequences of stress and to acclimate to low temperatures (Blumler, 1991; Öquist and Huner, 2003). Maintaining a long-term photoprotective mechanism could adversely affect the photosynthetic exploitation of relatively favorable winter conditions, which could be crucial in Mediterranean-type ecosystems for achieving a positive annual carbon balance (García-Plazaola et al., 1999b; Martínez-Ferri et al., 2004). The ability of these species to perform rapid metabolic changes in the antioxidant and photoprotective mechanisms could be of adaptive importance (García-Plazaola et al., 1999a).

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References

- Allen, D. J. and Ort, D. R.: Impacts of chilling temperatures on photosynthesis in warm-climate plants, *Trends Plant Sci.*, 6, 36–42, 2001.
- Aro, E. M., Virgin, I., and Andersson, B.: Photoinhibition of photosystem, II. Inactivation, protein damage and turnover, *Biochim. Biophys. Acta–Bioenerg.*, 1143, 113–134, 1993.
- Aroca, R., Jose, J., and Sa, M.: Photosynthetic characteristics and protective mechanisms against oxidative stress during chilling and subsequent recovery in two maize varieties differing in chilling sensitivity, *Plant Sci.*, 161, 719–726, 2001.
- Aschmann, H.: Distribution and Peculiarity of Mediterranean Ecosystems, in: *Mediterranean Type Ecosystems*, vol. 7, edited by: Castri, F. and Mooney, H. A., Springer Berlin Heidelberg, Berlin, Heidelberg, 11–19, 1973.
- Baquedano, F. J., Valladares, F., and Castillo, F. J.: Phenotypic plasticity blurs ecotypic divergence in the response of *Quercus coccifera* and *Pinus halepensis* to water stress, *Eur. J. For. Res.*, 127, 495–506, doi:10.1007/s10342-008-0232-8, 2008.
- Bernacchi, C. J., Portis, A. R., Nakano, H., Caemmerer, S. Von and Long, S. P.: 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, doi:10.1104/pp.008250.water, 2002.
- Bertolli, S. C. and Souza, G. M.: The level of environmental noise affects the physiological performance of *Glycine max* under water deficit, *Theor. Exp. Plant Physiol.*, 25, 36–45, 2013.
- Beyschlag, W., Lange, O. L., and Tenhunen, J. D.: 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, 1986.

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- Björkman, O. and Demmig, B.: Photon yield of O₂ evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins, *Planta*, 170, 489–504, 1987.
- Blumler, M. A.: Winter-deciduous versus evergreen habit in mediterranean regions?: a model, *USDA For. Serv. Gen. Tech. Rep PSW-126*, 194–197, 1991.
- 5 Bussotti, F., Bettini, D., Grossoni, P., Mansuino, S., Nibbi, R., Soda, C., and Tani, C.: Structural and functional traits of *Quercus ilex* in response to water availability, *Eviron. Exp. Bot.*, 47, 11–23, 2002.
- Canadell, J. and Lopez-Soria, L.: Lignotuber reserves support regrowth following clipping of two Mediterranean shrubs, *Funct. Ecol.*, 12, 31–38, doi:10.1046/j.1365-2435.1998.00154.x, 10 1998.
- Canadell, J., Djema, A., López, B., Lloret, F., Sabaté, S., Siscart, D., and Gracia, C. A.: Structure and dynamics of the root system, in: *Ecology of Mediterranean Evergreen Oak Forests*, edited by: Rodà, F., Retana, J., Gracia, C. A., and Bellot, J., Springer Berlin, Heidelberg, 47–59, 1999.
- 15 Carnicer, J., Barbeta, A., Sperlich, D., Coll, M., and Peñuelas, J.: 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, doi:10.3389/fpls.2013.00409, 2013.
- Coll, M., Peñuelas, J., Ninyerola, M., Pons, X., and Carnicer, J.: Multivariate effect gradients driving forest demographic responses in the Iberian Peninsula, *For. Ecol. Manage.*, 303, 195–209, doi:10.1016/j.foreco.2013.04.010, 20 2013.
- Corcuera, L., Morales, F., Abadia, A., and Gil-Pelegrin, E.: 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, doi:10.1007/s00468-004-0368-1, 2004.
- 25 Cowling, R. M., Rundel, P. W., Lamont, B. B., Arroyo, M. K., and Arianoutsou, M.: Plant diversity in Mediterranean-climate regions, *Trends Ecol. Evol.*, 11, 362–366, 1996.
- D'Ambrosio, N., Arena, C., and De Santo, A. V.: Temperature response of photosynthesis, excitation energy dissipation and alternative electron sinks to carbon assimilation in *Beta vulgaris* L., *Environ. Exp. Bot.*, 55, 248–257, doi:10.1016/j.envexpbot.2004.11.006, 2006.
- 30 De Luis, M., Novak, K., Raventós, J., Gričar, J., Prislán, P., and Čufar, K.: Cambial activity, wood formation and sapling survival of *Pinus halepensis* exposed to different irrigation regimes, *For. Ecol. Manage.*, 262, 1630–1638, doi:10.1016/j.foreco.2011.07.013, 2011.

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- Demmig-Adams, B. and Adams, W. W.: Photoprotection and other responses of plants to high light stress, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 43, 599–626, 1992.
- Demmig-Adams, B. and Adams, W. W.: The role of xanthophyll cycle carotenoids in the protection of photosynthesis, *Trends Plant Sci.*, 1, 21–26, 1996.
- 5 Dolman, a. J., Moors, E. J., and Elbers, J. a.: The carbon uptake of a mid latitude pine forest growing on sandy soil, *Agr. Forest Meteorol.*, 111, 157–170, doi:10.1016/S0168-1923(02)00024-2, 2002.
- Ensminger, I., Berninger, F., and Streb, P.: Response of photosynthesis to low temperature, in: *Terrestrial Photosynthesis in a Changing Environment – A Molecular, Physiological and Ecological Approach*, edited by: Flexas, J., Loreto, F., and Medrano, H., Cambridge University Press, Cambridge, 272–289, 2012.
- 10 Epron, D. and Dreyer, E.: Effects of severe dehydration on leaf photosynthesis in *Quercus petraea* (Matt.) Liebl.: photosystem II efficiency, photochemical and nonphotochemical fluorescence quenching and electrolyte leakage, *Tree Physiol.*, 10, 273–284, 1992.
- 15 Espelta, J. M., Sabaté, S., and Retana, J.: Resprouting dynamics, in: *Ecology of Mediterranean Evergreen Oak Forests*, edited by: Rodà, F., Retana, J., Gracia, C. A., and Bellot, J., 61–71, 1999.
- Farquhar, G. D. and Sharkey, T. D.: Stomatal conductance and photosynthesis, *Annu. Rev. Plant Physiol.*, 33, 317–345, doi:10.1146/annurev.pp.33.060182.001533, 1982.
- 20 Farquhar, G. D., von Caemmerer, S., and Berry, J. A.: A biochemical model of photosynthesis CO₂ assimilation in leaves of C3 species, *Planta*, 149, 78–90, 1980.
- Flexas, J., Escalona, J. M., and Medrano, H.: Down-regulation of photosynthesis by drought under field conditions in grapevine leaves, *Aust. J. Plant Physiol.*, 25, 893, doi:10.1071/PP98054, 1998.
- 25 Flexas, J., Badger, M., Chow, W., Medrano, H., and Osmond, C.: Analysis of the relative increase in photosynthetic O uptake when photosynthesis in grapevine leaves is inhibited following low night temperatures and/or water stress, *Plant Physiol.*, 121, 675–684, 1999.
- Flexas, J., Díaz-Espejo, A., Berry, J. A., Cifre, J., Galmés, J., Kaldenhoff, R., Medrano, H., and Ribas-Carbó, M.: Analysis of leakage in IRGA's leaf chambers of open gas exchange systems: quantification and its effects in photosynthesis parameterization, *J. Exp. Bot.*, 58, 1533–1543, doi:10.1093/jxb/erm027, 2007a.
- 30 Flexas, J., Diaz-Espejo, A., Galmés, J., Kaldenhoff, R., Medrano, H., and Ribas-Carbo, M.: Rapid variations of mesophyll conductance in response to changes in CO₂ concentration

around leaves, *Plant. Cell Environ.*, 30, 1284–1298, doi:10.1111/j.1365-3040.2007.01700.x, 2007b.

Flexas, J., Ribas-Carbó, M., Diaz-Espejo, A., Galmés, J., and Medrano, H.: Mesophyll conductance to CO₂: current knowledge and future prospects, *Plant. Cell Environ.*, 31, 602–621, doi:10.1111/j.1365-3040.2007.01757.x, 2008.

Flexas, J., Loreto, F., and Medrano, H.: *Terrestrial photosynthesis in a changing environment – a molecular, physiological and ecological approach*, edited by: Flexas, J., Loreto, F., and Medrano, H., Cambridge University Press, Cambridge, 2012.

Flexas, J., Diaz-Espejo, A., Gago, J., Gallé, A., Galmés, J., Gulías, J., and Medrano, H.: Photosynthetic limitations in Mediterranean plants: a review, *Environ. Exp. Bot.* 103, 12–23, doi:10.1016/j.envexpbot.2013.09.002, 2014.

Friend, A. D.: Terrestrial plant production and climate change, *J. Exp. Bot.*, 61, 1293–309, doi:10.1093/jxb/erq019, 2010.

Fryer, M. J., Andrews, J. R., Oxborough, K., Blowers, D. A., and Baker, N. R.: Relationship between CO₂ assimilation, photosynthetic electron transport, and active O₂ metabolism in leaves of maize in the field during periods of low temperature, *Plant Physiol.*, 116, 571–580, 1998.

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

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

García-Plazaola, J. I., Artetxe, U., Duñabeitia, M. K., and Becerril, J. M.: Role of photoprotective systems of Holm-Oak (*Quercus ilex*) in the adaptation to winter conditions, *J. Plant Physiol.*, 155, 625–630, doi:10.1016/S0176-1617(99)80064-9, 1999b.

Garcia-Plazaola, J. I., Olano, J. M., Hernandez, A., and Becerril, J. M.: Photoprotection in evergreen Mediterranean plants during sudden periods of intense cold weather, *Trees*, 17, 285–291, doi:10.1007/s00468-002-0234-y, 2003a.

Garcia-Plazaola, J. I., Olano, J. M., Hernandez, A., and Becerril, J. M.: Photoprotection in evergreen Mediterranean plants during sudden periods of intense cold weather, *Trees*, 17, 285–291, doi:10.1007/s00468-002-0234-y, 2003b.

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Genty, B., Briantais, J.-M., and Baker, N. R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, *Biochim. Biophys. Acta*, 990, 87–92, 1989.

Gómez-Aparicio, L., García-Valdés, R., Ruíz-Benito, P., and Zavala, M. A.: Disentangling the relative importance of climate, size and competition on tree growth in Iberian forests: implications for forest management under global change, *Glob. Change Biol.*, 17, 2400–2414, doi:10.1111/j.1365-2486.2011.02421.x, 2011.

Gracia, C. A., Tello, E., Sabat, S., and Bellot, J.: GOTILWA: an integrated model of water dynamics and forest growth. *Ecology of mediterranean evergreen oak forests*, in: *Ecology of Mediterranean Evergreen Oak Forests*, edited by: Rodà, F., Retana, J., Gracia, C. A., and Bellot, J., Springer Berlin Heidelberg, Berlin, 163–178, 1999.

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

Gratani, L. and Ghia, E.: Changes in morphological and physiological traits during leaf expansion of *Arbutus unedo*, *Environ. Exp. Bot.*, 48, 51–60, 2002b.

Gratani, L., Pesoli, P., Crescente, M. F., Aichner, K., and Larcher, W.: Photosynthesis as a temperature indicator in *Quercus ilex* L., *Global Planet. Change*, 24, 153–163, doi:10.1016/S0921-8181(99)00061-2, 2000.

Gratani, L., Catoni, R., and Varone, L.: Morphological, anatomical and physiological leaf traits of *Q. ilex*, *P. latifolia*, *P. lentiscus*, and *M. communis* and their response to Mediterranean climate stress factors, *Bot. Stud.*, 54, 35, doi:10.1186/1999-3110-54-35, 2013.

Guidi, L. and Calatayud, A.: Non-invasive tools to estimate stress-induced changes in photosynthetic performance in plants inhabiting Mediterranean areas, *Environ. Exp. Bot.*, 103, 42–52, doi:10.1016/j.envexpbot.2013.12.007, 2014.

Haldimann, P. and Feller, U.: Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1, 5- bisphosphate carboxylase/oxy, *Plant Cell Environ.*, 27, 1169–1183, 2004.

Harley, P. C., Loreto, F., Di Marco, G., and Sharkey, T. D.: Theoretical Considerations when Estimating the Mesophyll Conductance to CO₂ Flux by Analysis of the Response of Photosynthesis to CO₂, *Plant Physiol.*, 98, 1429–1436, 1992.

Hikosaka, K., Murakami, A., and Hirose, T.: Balancing carboxylation and regeneration of ribulose-1,5- bisphosphate in leaf photosynthesis: temperature acclimation of an ever-

green tree, *Quercus myrsinaefolia*, *Plant Cell Environ.*, 22, 841–849, doi:10.1046/j.1365-3040.1999.00442.x, 1999.

Huner, N. P. A., Öquist, G., and Sarhan, F.: Energy balance and acclimation to light and cold, *Trends Plant Sci.*, 3, 224–230, doi:10.1016/S1360-1385(98)01248-5, 1998.

5 Hurry, V., Strand, A., Furbank, R., and Stitt, M.: The role of inorganic phosphate in the development of freezing tolerance and the acclimatization of photosynthesis to low temperature is revealed by the pho mutants of *Arabidopsis thaliana*, *Plant J.*, 24, 383–396, 2000.

IPCC: IPCC, 2013: Summary for policymakers, in: *Climate Change 2013: The Physical Science Basis*, edited by: S. T. F., D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and Midgley, P. M., Cambridge University Press, Cambridge (UK) and
10 New York (USA), 2013.

Jordan, D. N. and Smith, W. K.: Simulated influence of leaf geometry on sunlight interception and photosynthesis in conifer needles, *Tree Physiol.*, 13, 29–39, 1993.

Jump, A. S. and Penuelas, J.: Running to stand still: adaptation and the response of plants to rapid climate change, *Ecol. Lett.*, 8, 1010–1020, doi:10.1111/j.1461-0248.2005.00796.x, 2005.

Kang, C.-Y., Lian, H.-L., Wang, F.-F., Huang, J.-R., and Yang, H.-Q.: Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in *Arabidopsis*, *Plant Cell*, 21, 2624–2641, doi:10.1105/tpc.109.069765, 2009.

20 Kok, B.: A critical consideration of the quantum yield of *Chlorella*-photosynthesis, *Enzymologia*, 13, 1–56, 1948.

Kull, O. and Niinemets, U.: Variations in leaf morphometry and nitrogen concentration in *Betula pendula* Roth., *Corylus avellana* L., and *Lonicera xylosteum* L., *Tree Physiol.*, 12, 311–318, 1993.

25 Kyparissis, A., Drilias, P., and Manetas, Y.: Seasonal fluctuations in photoprotective xanthophyll cycle) and photoselective (chlorophylls) capacity in eight Mediterranean plant species belonging to two different growth forms, *Aust. J. Plant Physiol.*, 27, 265–272, 2000.

Laisk, A., Oja, V., Rasulov, B., Rämama, H., Eichelmann, H., Kasparova, I., Pettai, H., and Padu, E.: A computer-operated routine of gas exchange and optical measurements to diagnose photosynthetic apparatus, *Plant Cell Environ.*, 25, 923–943, 2002.

30 Lambrev, P. H., Miloslavina, Y., Jahns, P., and Holzwarth, A. R.: On the relationship between non-photochemical quenching and photoprotection of Photosystem II, *Biochim. Biophys. Acta*, 1817, 760–769, doi:10.1016/j.bbabi.2012.02.002, 2012.

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Lenoir, J., Gégout, J. C., Marquet, P. a, de Ruffray, P., and Brisse, H.: A significant upward shift in plant species optimum elevation during the 20th century, *Science*, 320, 1768–1771, doi:10.1126/science.1156831, 2008.

Levizou, E., Drilias, P., and Kyparissis, A.: Exceptional photosynthetic performance of *Capparis spinosa* L. under adverse conditions of Mediterranean summer, *Photosynthetica*, 42, 229–235, 2004.

Long, S. P. and Bernacchi, C. J.: Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error, *J. Exp. Bot.*, 54, 2393–2401, doi:10.1093/jxb/erg262, 2003.

Lusk, C. H., Wright, I., and Reich, P. B.: Photosynthetic differences contribute to competitive advantage of evergreen angiosperm trees over evergreen conifers in productive habitats, *New Phytol.*, 160, 329–336, 2003.

Maestre, F. T. and Cortina, J.: Are *Pinus halepensis* plantations useful as a restoration tool in semiarid Mediterranean areas?, *For. Ecol. Manage.*, 198, 303–317, doi:10.1016/j.foreco.2004.05.040, 2004.

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

Martínez-Ferri, E., Manrique, E., Valladares, F., and Balaguer, L.: Winter photoinhibition in the field involves different processes in four co-occurring Mediterranean tree species, *Tree Physiol.*, 24, 981–990, 2004.

Matesanz, S. and Valladares, F.: Ecological and evolutionary responses of Mediterranean plants to global change, *Environ. Exp. Bot.*, doi:10.1016/j.envexpbot.2013.09.004, 2013.

Maxwell, K. and Johnson, G. N.: Chlorophyll fluorescence – a practical guide, *J. Exp. Bot.*, 51, 659–668, 2000.

Misson, L., Tu, K. P., Boniello, R. A., and Goldstein, A. H.: Seasonality of photosynthetic parameters in a multi-specific and vertically complex forest ecosystem in the Sierra Nevada of California, *Tree Physiol.*, 26, 729–741, 2006.

Mitrakos, K.: A theory for Mediterranean plant life, *Acta Oecologica/Oecologia Plant*, 1, 245–252, 1980.

Mott, K. A. and Buckley, T. N.: Stomatal heterogeneity, *J. Experimental Bot.*, 49, 407–417, 1998.

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- Mott, K. A. and Buckley, T. N.: Patchy stomatal conductance: emergent collective behaviour of stomata, *Trends Plant Sci.*, 1385, 258–262, 2000.
- Mulo, P., Sakurai, I., and Aro, E.-M.: Strategies for psbA gene expression in cyanobacteria, green algae and higher plants: from transcription to PSII repair, *Biochim. Biophys. Acta*, 1817, 247–257, doi:10.1016/j.bbabi.2011.04.011, 2012.
- Munné-Bosch, S. and Peñuelas, J.: Drought-induced oxidative stress in strawberry tree (*Arbutus unedo* L.) growing in Mediterranean field conditions, *Plant Sci.*, 166, 1105–1110, doi:10.1016/j.plantsci.2003.12.034, 2004.
- Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, a M., Finnegan, E. J., Mathesius, U., Poot, P., Purugganan, M. D., Richards, C. L., Valladares, F., and van Kleunen, M.: Plant phenotypic plasticity in a changing climate., *Trends Plant Sci.*, 15, 684–692, doi:10.1016/j.tplants.2010.09.008, 2010.
- Niinemets, Ü., Oja, V., and Kull, O.: Shape of leaf photosynthetic electron transport versus temperature response curve is not constant along canopy light gradients in temperate deciduous trees, *Plant. Cell Environ.*, 22, 1497–1513, doi:10.1046/j.1365-3040.1999.00510.x, 1999.
- Niinemets, Ü., Cescatti, A., Rodeghiero, M., and Tosens, T.: Leaf internal diffusion conductance limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species, *Plant Cell Environ.*, 28, 1552–1566, doi:10.1111/j.1365-3040.2005.01392.x, 2005.
- Niinemets, Ü., Cescatti, A., Rodeghiero, M., and Tosens, T.: 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, doi:10.1111/j.1365-3040.2006.01499.x, 2006.
- Ninyerola, M., Pons, X., and Roure, J. M.: A methodological approach of climatological modelling of air temperature and precipitation, *Int. J. Climatol.*, 20, 1823–1841, 2000.
- Ogaya, R. and Peñuelas, J.: Comparative seasonal gas exchange and chlorophyll fluorescence of two dominant woody species in a Holm Oak Forest, *Flora*, 198, 132–141, 2003.
- Ogaya, R. and Peñuelas, J.: Leaf mass per area ratio in *Quercus ilex* leaves under a wide range of climatic conditions. The importance of low temperatures, *Acta Oecol.*, 31, 168–173, doi:10.1016/j.actao.2006.07.004, 2007.
- Oliveira, G. and Peñuelas, J.: Comparative photochemical and phenomorphological responses to winter stress of an evergreen (*Quercus ilex* L.) and a semi-deciduous (*Cistus albidus* L.) Mediterranean woody species, *Acta Oecologica*, 21, 97–107, 2000.

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Oliveira, G. and Peñuelas, J.: Allocation of absorbed light energy into photochemistry and dissipation in a semi-deciduous and an evergreen Mediterranean woody species during winter, *Aust. J. Plant Physiol.*, 28, 471–480, doi:10.1071/Pp00150, 2001.

Oliveira, G. and Peñuelas, J.: Effects of winter cold stress on photosynthesis and photochemical efficiency of PSII of the Mediterranean *Cistus albidus* L., and *Quercus ilex* L., *Plant Ecol.*, 175, 179–191, 2004.

Onoda, Y., Hikosaka, K., and Hirose, T.: Seasonal change in the balance between capacities of RuBP carboxylation and RuBP regeneration affects CO₂ response of photosynthesis in *Polygonum cuspidatum*, *J. Exp. Bot.*, 56(412), 755–63, doi:10.1093/jxb/eri052, 2005.

Öquist, G., and Huner, N. P. A.: Photosynthesis of overwintering evergreen plants, *Annu. Rev. Plant Biol.*, 54, 329–55, doi:10.1146/annurev.arplant.54.072402.115741, 2003.

Orshan, G.: Approaches to the definition of Mediterranean growth forms, in: *Mediterranean Type-Ecosystems – the Role of Nutrients*, edited by: Kruger, F. J., Mitchell, D. T., and Jarvis, J. U. M., 86–100, Springer-Verlag, Berlin, Heidelberg, 1983.

Peñuelas, J. and Boada, M.: A global change-induced biome shift in the Montseny mountains (NE Spain), *Glob. Change Biol.*, 9, 131–140, 2003.

Peñuelas, J., Ogaya, R., Boada, M., and S. Jump, A.: Migration, invasion and decline: changes in recruitment and forest structure in a warming-linked shift of European beech forest in Catalonia (NE Spain), *Ecography*, 30, 829–837, doi:10.1111/j.2007.0906-7590.05247.x, 2007.

Peñuelas, J., Sardans, J., Estiarte, M., Ogaya, R., Carnicer, J., Coll, M., Barbeta, A., Rivas-Ubach, A., Llusà, J., Garbulsy, M., Filella, I., and Jump, A. S.: Evidence of current impact of climate change on life: a walk from genes to the biosphere, *Glob. Change Biol.*, 19, 2303–2338, doi:10.1111/gcb.12143, 2013.

Reich, P. B., Ellsworth, D. S., and Walters, M. B.: Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups, *Funct. Ecol.*, 12, 948–958, doi:10.1046/j.1365-2435.1998.00274.x, 1998.

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

Sabaté, S. and Gracia, C. A.: Water processes in trees: transpiration and photosynthesis, in: *Water for Forests and People in the Mediterranean – What Science Can Tell Us*, edited by:

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Birot, Y., Gracia, C. A., and Palahí, M., European Forest Institut, Joensuu, available at: http://www.efi.int/portal/virtual_library/publications/what_science_can_tell_us/, 72–75, 2011.

Sabaté, S., Sala, A., and Gracia, C. A.: Leaf Traits and Canopy Organisation, in: Ecology of Mediterranean Evergreen Oak Forests, edited by: Rodà, F., Retana, J., Gracia, C. A., and Bellot, J., Springer, Berlin, Heidelberg, 121–131, 1999.

Sanchez-Humanes, B. and Espelta, J. M.: Increased drought reduces acorn production in *Quercus ilex* coppices: thinning mitigates this effect but only in the short term, *Forestry*, 84, 73–82, doi:10.1093/forestry/cpq045, 2011.

Savé, R., Castell, C., and Terrades, J.: Gas Exchange and Water Relations, in Ecology of Mediterranean Evergreen Oak Forests, edited by: Rodà, F., Retana, J., Gracia, C. A., and Bellot, J., Springer, Berlin Heidelberg, 135–144, 1999.

Sharkey, T. D.: Photosynthesis in intact leaves of C3 plants: physics, physiology and rate limitations, *Bot. Rev.*, 51, 53–105, 1985.

Shaw, R. G. and Etersson, J. R.: Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics, *New Phytol.*, 195, 752–765, doi:10.1111/j.1469-8137.2012.04230.x, 2012.

Somot, S., Sevault, F., Déqué, M., and Crépon, M.: 21st century climate change scenario for the Mediterranean using a coupled atmosphere–ocean regional climate model, *Glob. Planet. Change*, 63, 112–126, doi:10.1016/j.gloplacha.2007.10.003, 2008.

Specht, R. L.: 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, 1969.

Sun, Y., Gu, L., Dickinson, R. E., Pallardy, S. G., Baker, J., Cao, Y., Damatta, F. M., Dong, X., Ellsworth, D., van Goethem, D., Jensen, A. M., Law, B. E., Loos, R., Martins, S. C. V., Norby, R. J., Warren, J., Weston, D., and Winter, K.: Asymmetrical effects of mesophyll conductance on fundamental photosynthetic parameters and their relationships estimated from leaf gas exchange measurements, *Plant Cell Environ.*, 37, 978–994, doi:10.1111/pce.12213, 2014.

Taz, L. and Zeiger, E.: *Plant Physiology*, 5th edn., edited by: Taz, L. and Zeiger, E. Sinauer Associates, Inc., Sunderland, 2010.

Tcherkez, G. G. B. and Ribas-Carbó, M.: Interactions between photosynthesis and day respiration, in: *Terrestrial Photosynthesis in a Changing Environment – a Molecular, Physiological*

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- and Ecological Approach, edited by: Flexas, J., Loretto, F., and Medrano, H., Cambridge University Press, Cambridge, 41–53, 2012.
- Terashima, I. and Hikosaka, K.: Comparative ecophysiology of leaf and canopy photosynthesis, *Plant Cell Environ.*, 18, 1111–1128, 1995.
- 5 Treitach, M., Bolognini, G., and Rondi, A.: Photosynthetic activity of *Quercus ilex* at the extremes of a transect between Mediterranean and submediterranean vegetation (Trieste – NE Italy), *Flora*, 192, 369–378, 1997.
- Turnbull, M. H., Whitehead, D., Tissue, D. T., Schuster, W. S. F., Brown, K. J., and Griffin, K. L.: Scaling foliar respiration in two contrasting forest canopies, *Funct. Ecol.*, 17, 101–114, doi:10.1046/j.1365-2435.2003.00713.x, 2003.
- 10 Valladares, F. and Niinemets, Ü.: Shade tolerance, a key plant feature of complex nature and consequences, *Annu. Rev. Ecol. Evol. S.*, 39, 237–257, doi:10.1146/annurev.ecolsys.39.110707.173506, 2008.
- Valladares, F., Martinez-Ferri, E., Balaguer, L., Perez-Corona, E., and Manrique, E.: Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy?, *New Phytol.*, 148, 79–91, 2000.
- 15 Valladares, F., Zaragoza-Castells, J., Sánchez-Gómez, D., Matesanz, S., Alonso, B., Portsmouth, A., Delgado, A., and Atkin, O. K.: Is shade beneficial for mediterranean shrubs experiencing periods of extreme drought and late-winter frosts?, *Ann. Bot.*, 102, 923–933, doi:10.1093/aob/mcn182, 2008.
- Vass, I.: Molecular mechanisms of photodamage in the Photosystem II complex., *Biochim. Biophys. Acta*, 1817, 209–217, doi:10.1016/j.bbabi.2011.04.014, 2012.
- Vaz, M., Maroco, J., Ribeiro, N., Gazarini, L. C., Pereira, J. S., and Chaves, M. M.: Leaf-level responses to light in two co-occurring *Quercus* (*Quercus ilex* and *Quercus suber*): leaf structure, chemical composition and photosynthesis, *Agroforestry Systems*, 82, 173–181, doi:10.1007/s10457-010-9343-6, 2011.
- 25 Verhoeven, A.: Sustained energy dissipation in winter evergreens, *New Phytol.*, 201, 57–65, doi:10.1111/nph.12466, 2014.
- Vilá, M. and Sardans, J.: Plant competition in mediterranean-type vegetation, *J. Veg. Sci.*, 10, 281–294, 1999.
- 30 Werner, C., Correia, O., and Beyschlag, W.: Characteristic patterns of chronic and dynamic photoinhibition of different functional groups in a Mediterranean ecosystem, *Funct. Plant Biol.*, 29, 99–1011, 2002.

- Wullschleger, S. D.: Biochemical limitations to carbon assimilation in C3 plants – a retrospective analysis of the j curves from 109 species, *J. Experimental Bot.*, 44, 907–920, 1993.
- Yin, X., Struik, P. C., Romero, P., Harbinson, J., Evers, J. B., van der Putten, P. E. L., and Vos, J.: 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, 448–464, doi:10.1111/j.1365-3040.2009.01934.x, 2009.
- Yin, X., Sun, Z., Struik, P. C., and Gu, J.: Evaluating a new method to estimate the rate of leaf respiration in the light by analysis of combined gas exchange and chlorophyll fluorescence measurements, *J. Exp. Bot.*, 62, 3489–3499, doi:10.1093/jxb/err038, 2011.
- Zaragoza-Castells, J., Sánchez-Gómez, D., Valladares, F., Hurry, V., and Atkin, O. K.: Does growth irradiance affect temperature dependence and thermal acclimation of leaf respiration? Insights from a Mediterranean tree with long-lived leaves, *Plant. Cell Environ.*, 30, 820–833, doi:10.1111/j.1365-3040.2007.01672.x, 2007.
- Zavala, M. A., Espelta, J. M., and Retana, J.: Constraints and trade-offs in Mediterranean plant communities: the case of Holm Oak–Aleppo Pine forests, *Bot. Rev.*, 66, 119–149, 2000.

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11, 9697–9759, 2014

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Table 1. *P* values of Student's *t* tests for the differences between sunlit and shaded leaves of *Q. ilex* for the measurement periods combined (total) and separated (mild, frost).

	total	mild	frost
$V_{c, \max}$	0.001	0.002	0.172
J_{\max}	0.006	0.002	0.553
J/V	0.279	0.797	0.249
F_v/F_m	0.611	0.533	0.535
A_{net}	0.546	0.594	0.745
g_s	0.156	0.791	0.127
C_i	0.151	0.326	0.154
g_m	0.041	0.066	0.107
C_c	0.138	0.364	0.203
CUE	0.151	0.728	0.439
R_n	0.061	0.470	0.356
R_l	0.016	0.004	0.577
$J_{\text{amb}}/A_{\text{net}}$	0.052	0.014	0.203
Φ_{PSII}	0.290	0.315	0.825
Φ_{CO_2}	0.750	0.886	0.497
qp	0.195	0.045	0.882
NPQ	0.192	0.903	0.126
$\Delta(C_a - C_i)$	0.037	0.321	0.068
$\Delta(C_i - C_c)$	0.043	0.073	0.113
$\Delta(C_a - C_c)$	0.023	0.006	0.122

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Table 2. Regression coefficients and results from ANCOVA analyses of the $J_{\text{amb}}/A_{\text{net}}$ and $J_{\text{max}}/V_{\text{c, max}}$ relationships.

Regression analyses of J_{max} and $V_{\text{c, max}}$															
tree species leaf position	<i>Q. ilex</i> sunlit			<i>Q. ilex</i> shaded			<i>P. halepensis</i> sunlit			<i>A. unedo</i> sunlit			all species sunlit		
	reg. line	R^2	P	reg. line	R^2	P	reg. line	R^2	P	reg. line	R^2	P	reg. line	R^2	P
mild	$y = 0.81x + 41.6$	0.97	2E-04	$y = 1.2x + 6.1$	0.48	0.193	$y = 115.9x + 148.8$	0.04	0.32	$y = 0.954x + 31.5$	0.95	0.017	$y = 50.2x + 0.77$	0.94	1.4E-07
frost	$y = 0.94x + 3.6$	0.89	0.035	$y = 1.89x - 9.19$			$y = 971x + 9.9$	0.53	0.1	$y = 0.97x + 13.7$	0.91	0.029	$y = 10.5x + 0.93$	0.90	7.2E-05
ρ (slope)	5.76E-02			0.83			0.022			0.69			0.072		
ρ (intercept)	8.91E-09			0.3			0.058			0.28			0.008		
Regression analyses of J_{amb} and A_{net}															
tree species leaf position	<i>Q. ilex</i> sunlit			<i>Q. ilex</i> shaded			<i>P. halepensis</i> sunlit			<i>A. unedo</i> sunlit			all species sunlit		
	reg. line	R^2	P	reg. line	R^2	P	reg. line	R^2	P	reg. line	R^2	P	reg. line	R^2	P
mild	$y = 10.8 + 56.1$	0.76	0.014	$y = 3.1x + 39$	0.91	0.029	$y = 9.22x + 58.3$	0.51	0.068	$y = 9.7x + 54.9$	0.96	0.005	$y = 10.9x + 51.9$	0.84	7.1E-06
frost	$y = 15.4x + 21.1$	0.73	0.093	$y = 2.7x + 46.8$	-0.13	0.52	$y = 11.9x + 31.9$	0.52	0.105	$y = 14.6x + 14.2$	0.46	0.200	$y = 13.5x + 22.3$	0.76	1.7E-04
ρ (slope)	0.337			0.72			0.59			0.322			0.098		
ρ (intercept)	0.51			0.45			0.31			0.29			0.071		

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Table 3. The scaling constant (c) and energies of activation (ΔH_a) describing the temperature responses for Rubisco enzyme kinetic parameters K_c , K_o and Γ^* . Taken from Bernacchi et al. (2002).

	25 °C	c	ΔH_a	unit
K_c	27.24	35.98	80.99	Pa
K_o	16.58	12.38	23.72	kPa
Γ^*	3.74	11.19	24.46	Pa

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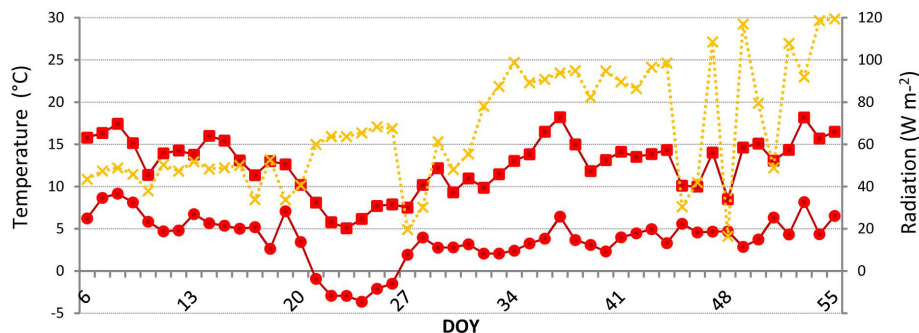


Figure 1. Maximum and minimum temperatures on the primary y-axes (in red squares and circles, respectively) and radiation (in yellow crosses) on the secondary y-axes are presented for the mild and frost winter period for the day of the year (DOY) in January and February 2012.

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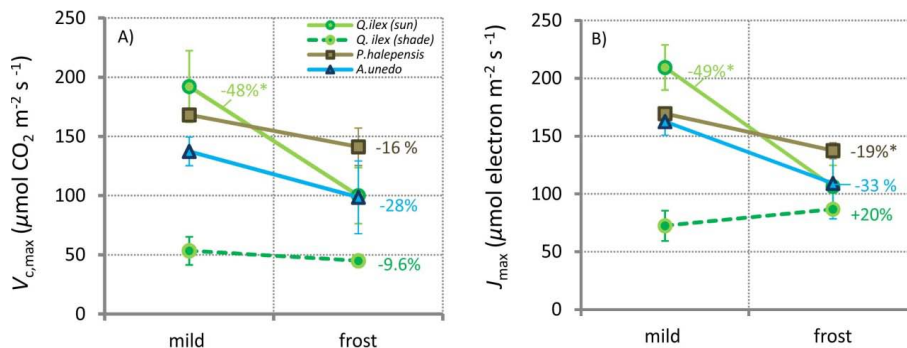


Figure 2. Effect of a sudden period of frost following a mild winter period in 2012 on **(A)** the maximum velocity of carboxylation ($V_{c,max}$) and **(B)** the maximum rate of electron transport (J_{max}) in sunlit leaves of *Q. ilex* (circles), *P. halepensis* (squares), and *A. unedo* (triangles) and in shaded leaves of *Q. ilex* (circles and dashed lines). The error bars represent the standard error, and the percentages indicate the change between periods where significance is indicated with an asterisk ($P \leq 0.05$) and marginal significance with an asterisk in brackets ($0.05 \geq P \leq 0.1$).

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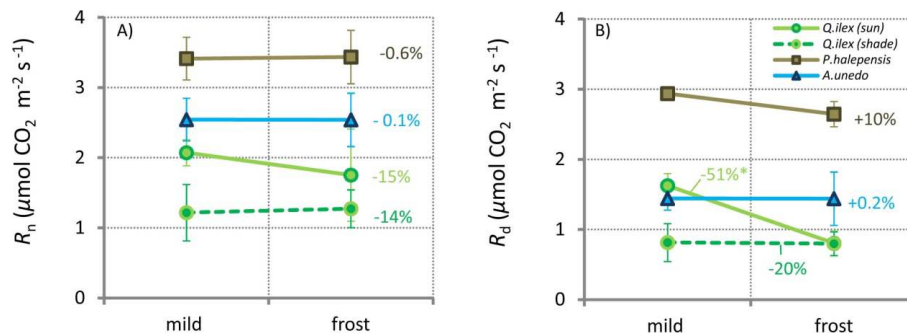


Figure 3. Effect of a sudden period of frost following a mild winter period on (A) nighttime respiration (R_n) and (B) daytime respiration (R_d) in sunlit leaves of *Q. ilex* (circles), *P. halepensis* (squares), and *A. unedo* (triangles) and in shaded leaves of *Q. ilex* (circles and dashed lines). The error bars represent the standard error, and the percentages indicate the change between periods where significance is indicated with an asterisk ($P \leq 0.05$) and marginal significance with an asterisk in brackets ($0.05 \geq P \geq 0.1$).

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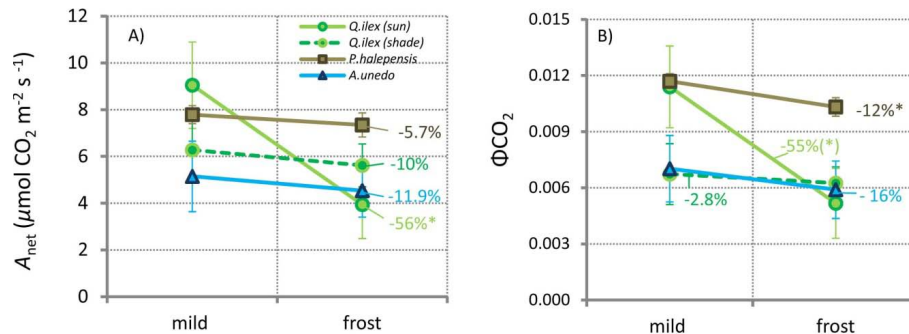


Figure 4. Effect of a sudden period of frost following a mild winter period on **(A)** net assimilation (A_{net}) and **(B)** the effective quantum yield of net CO₂ assimilation (Φ_{CO_2}) in sunlit leaves of *Q. ilex* (circles), *P. halepensis* (squares), and *A. unedo* (triangles) and in shaded leaves of *Q. ilex* (circles and dashed lines). The error bars represent the standard error, and the percentages indicate the change between periods where significance is indicated with an asterisk ($P \leq 0.05$) and marginal significance with an asterisk in brackets ($0.05 \geq P \geq 0.1$).

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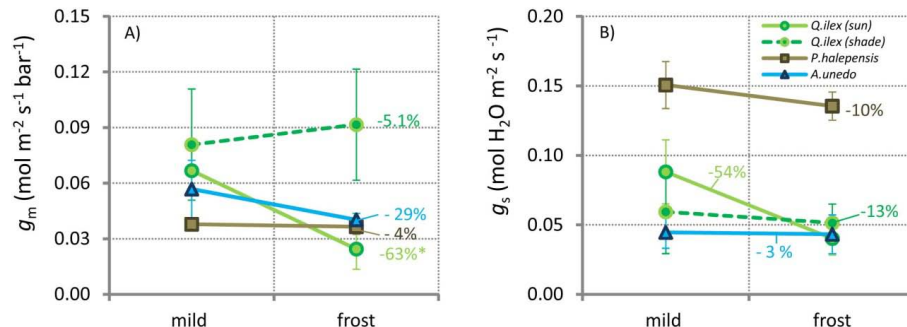


Figure 5. Effect of a sudden period of frost following a mild winter period on **(A)** mesophyll conductance (g_m) and **(B)** stomatal conductance (g_s) in sunlit leaves of *Q. ilex* (circles), *P. halepensis* (squares), and *A. unedo* (triangles) and in shaded leaves of *Q. ilex* (circles and dashed lines). The error bars represent the standard error, and the percentages indicate the change between periods where significance is indicated with an asterisk ($P \leq 0.05$) and marginal significance with an asterisk in brackets ($0.05 \geq P \leq 0.1$).

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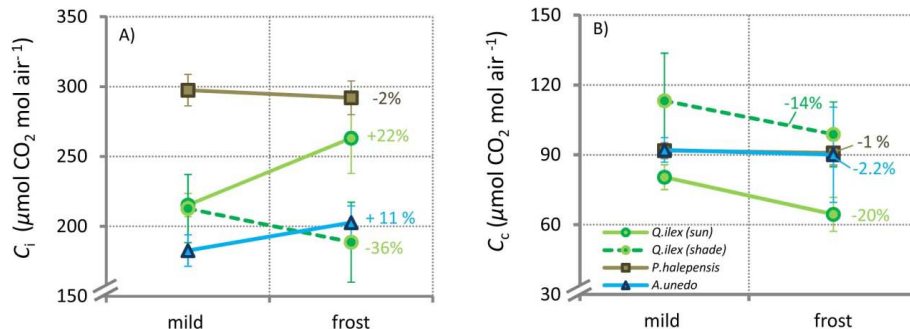


Figure 6. Effect of a sudden period of frost following a mild winter period on **(A)** the stomatal internal CO₂ concentration (C_i) and **(B)** the chloroplastic CO₂ concentration (C_c) in sunlit leaves of *Q. ilex* (circles), *P. halepensis* (squares), and *A. unedo* (triangles) and in shaded leaves of *Q. ilex* (circles and dashed lines). The error bars represent the standard error, and the percentages indicate the change between periods where significance is indicated with an asterisk ($P \leq 0.05$) and marginal significance with an asterisk in brackets ($0.05 \geq P \leq 0.1$).

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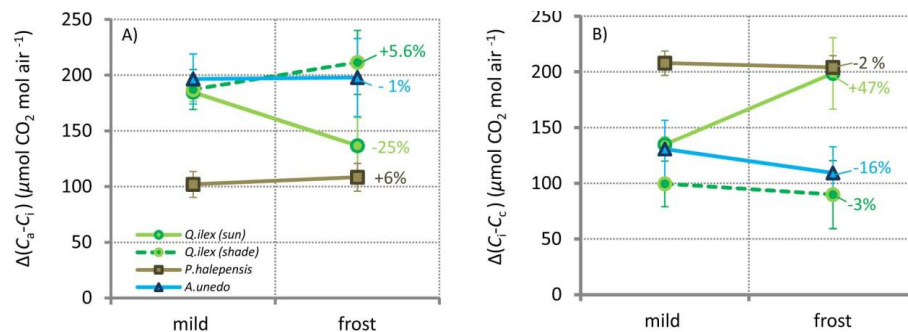


Figure 7. Effect of a sudden period of frost following a mild winter period on **(A)** the drawdown of CO₂ from external air to stomatal internal air spaces ($\Delta C_a - C_i$) and **(B)** the drawdown of CO₂ from stomatal internal air spaces to the chloroplasts ($\Delta C_i - C_c$) in sunlit leaves of *Q. ilex* (circles), *P. halepensis* (squares), and *A. unedo* (triangles) and in shaded leaves of *Q. ilex* (circles and dashed lines). The error bars represent the standard error, and the percentages indicate the change between periods where significance is indicated with an asterisk ($P \leq 0.05$) and marginal significance with an asterisk in brackets ($0.05 \geq P \geq 0.1$).

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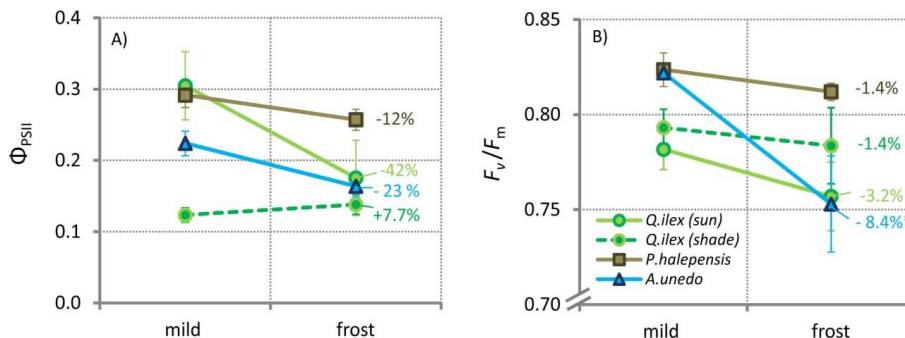


Figure 8. Effect of a sudden period of frost following a mild winter period on (A) the effective quantum yield of photosystem II (Φ_{PSII}) and (B) the maximum efficiency of photosystem II (F_v/F_m) in sunlit leaves of *Q. ilex* (circles), *P. halepensis* (squares), and *A. unedo* (triangles) and in shaded leaves of *Q. ilex* (circles and dashed lines). The error bars represent the standard error, and the percentages indicate the change between periods where significance is indicated with an asterisk ($P \leq 0.05$) and marginal significance with an asterisk in brackets ($0.05 \geq P \leq 0.1$).

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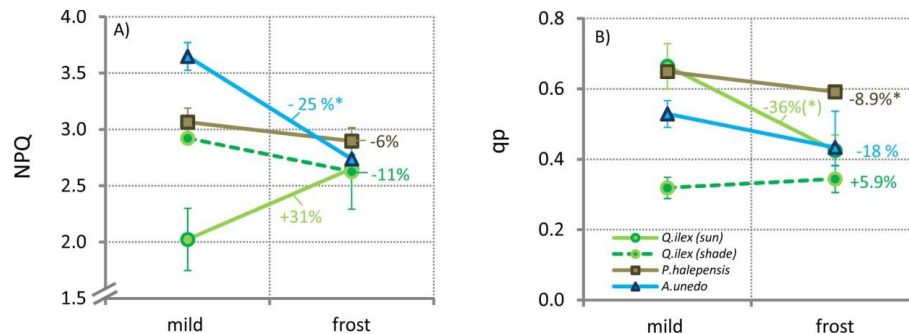


Figure 9. Effect of a sudden period of frost following a mild winter period on **(A)** non-photochemical quenching (NPQ) and **(B)** photochemical quenching (qP) in sunlit leaves of *Q. ilex* (circles), *P. halepensis* (squares), and *A. unedo* (triangles) and in shaded leaves of *Q. ilex* (circles and dashed lines). The error bars represent the standard error, and the percentages indicate the change between periods where significance is indicated with an asterisk ($P \leq 0.05$) and marginal significance with an asterisk in brackets ($0.05 \geq P \leq 0.1$).

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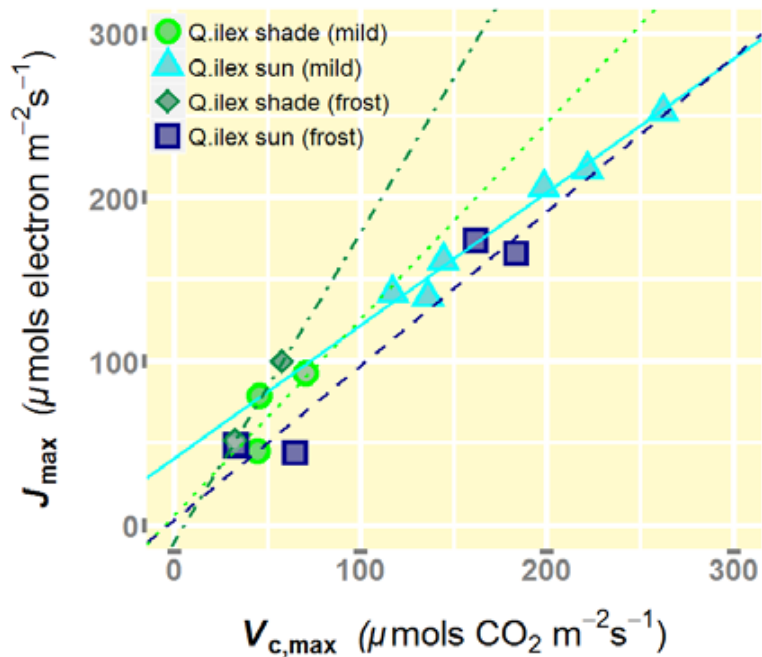


Figure 10. Relationship between the maximum velocity of carboxylation ($V_{c,max}$) and the maximum rate of electron transport (J_{max}) in *Q. ilex* leaves. Leaves measured under mild conditions are indicated by green circles and cyan triangles in shaded and sunlit locations, respectively. Leaves measured after the period of frost are indicated by green diamonds and blue squares in shaded and sunlit locations, respectively.

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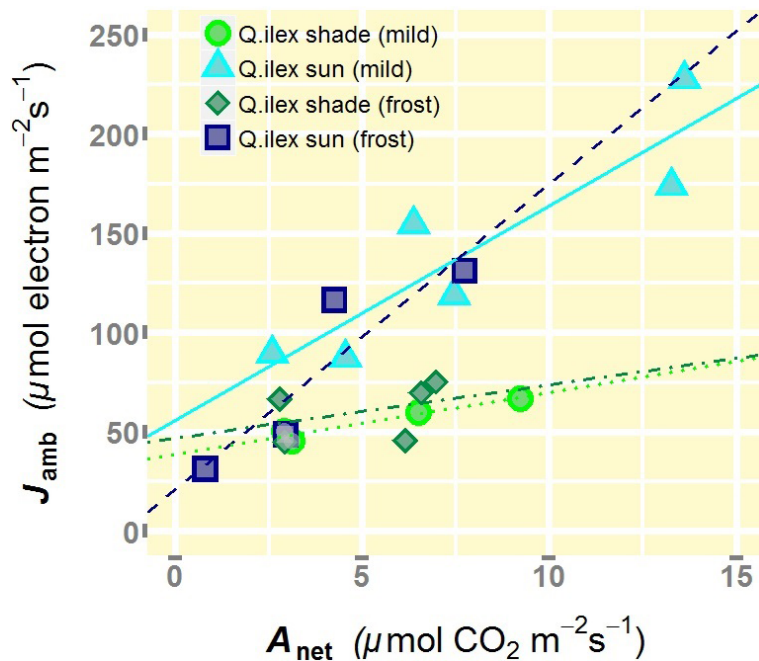


Figure 11. Relationship between the rate of electron transport from chlorophyllic fluorescence (J_{amb}) and net assimilation (A_{net}) at ambient CO_2 concentrations and saturating light (A_{net}) in *Q. ilex* leaves. Leaves measured under mild conditions are indicated by green circles and cyan triangles in shaded and sunlit locations, respectively. Leaves measured after the period of frost are indicated by green diamonds and blue squares in shaded and sunlit locations, respectively.

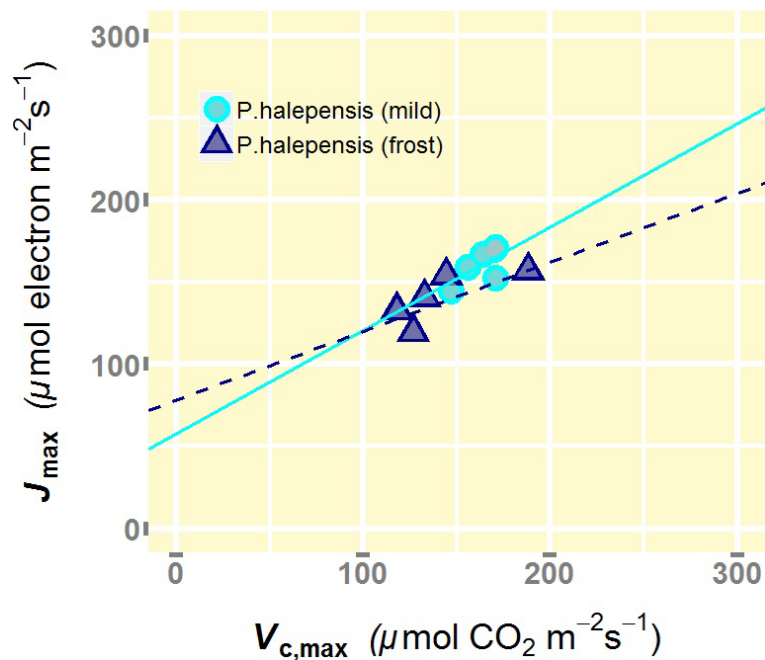


Figure 12. Relationship between the maximum velocity of carboxylation ($V_{c,max}$) and the maximum rate of electron transport (J_{max}) in sunlit *P. halepensis* leaves. Leaves measured under mild conditions are indicated by cyan circles, and leaves measured after the period of frost are indicated by blue triangles.

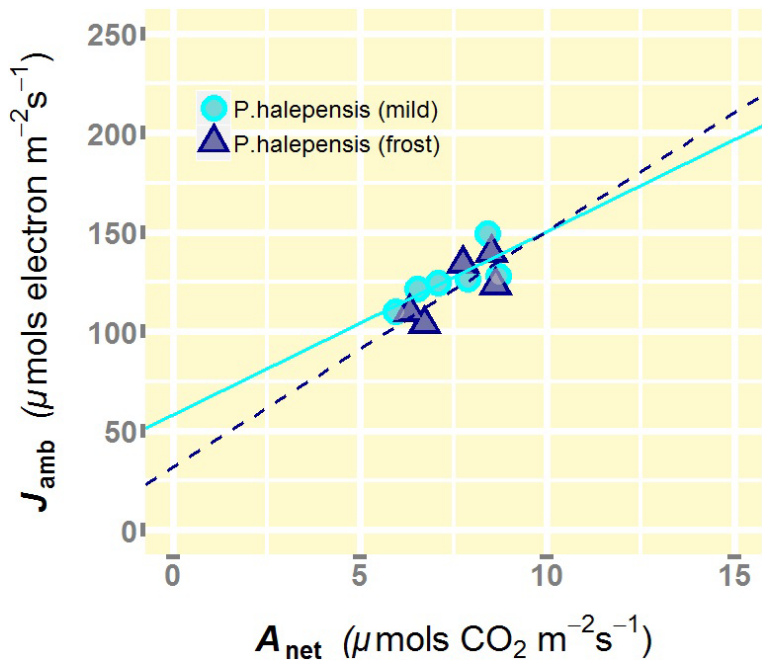


Figure 13. Relationship between the rate of electron transport from chlorophyllic fluorescence (J_{amb}) and net assimilation (A_{net}) at ambient CO_2 concentrations and saturating light (A_{net}) in sunlit *P. halepensis* leaves. Leaves measured under mild conditions are indicated by cyan circles, and leaves measured after the period of frost are indicated by blue triangles.

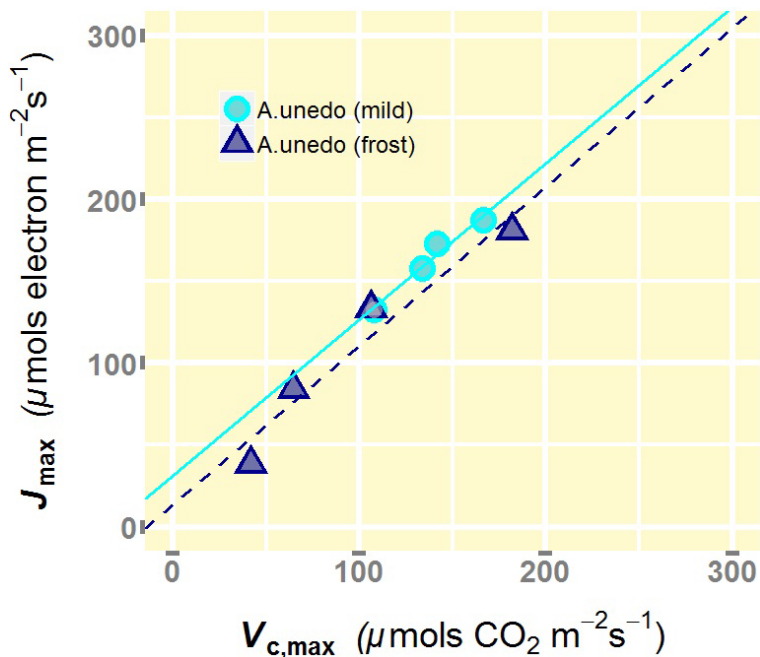


Figure 14. Relationship between the maximum velocity of carboxylation ($V_{c,max}$) and the maximum rate of electron transport (J_{max}) in sunlit *A. unedo* leaves. Leaves measured under mild conditions are indicated by cyan circles, and leaves measured after the period of frost are indicated by blue triangles.

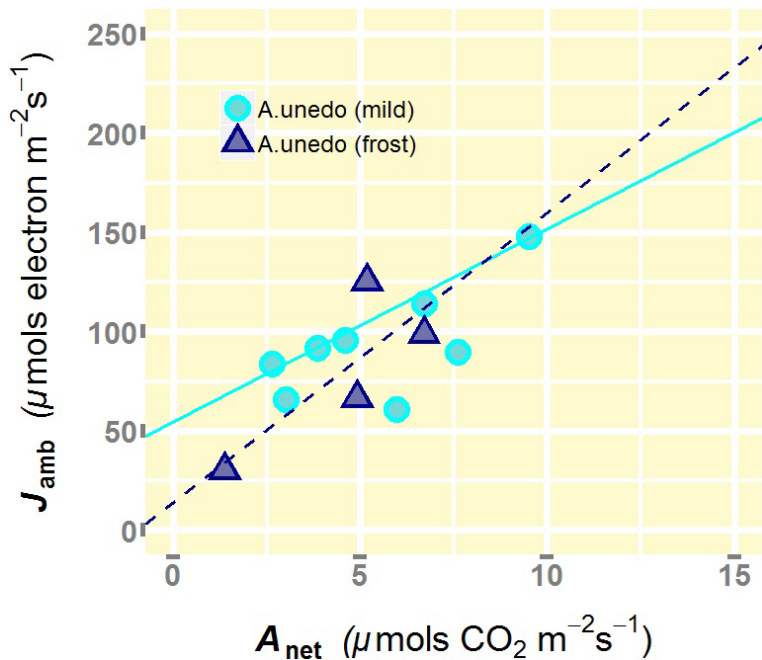


Figure 15. Relationship between the rate of electron transport from chlorophyllic fluorescence (J_{amb}) and net assimilation (A_{net}) at ambient CO_2 concentrations and saturating light (J_{amb}/A_{net}) in sunlit *A. unedo* leaves. Leaves measured under mild conditions are indicated by cyan circles, and leaves measured after the period of frost are indicated by blue triangles.