

Leaf-level responses to light in two co-occurring *Quercus* (*Quercus ilex* and *Quercus suber*): leaf structure, chemical composition and photosynthesis

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Abstract We studied morphological, biochemical and physiological leaf acclimation to incident Photon-Photosynthetic-Flux-Density (*PPFD*) in *Quercus ilex* (holm oak) and *Quercus suber* (cork oak) at Mediterranean evergreen oak woodlands of southern Portugal. Specific leaf area (*SLA*) decreased exponentially with increasing *PPFD* in both species. *Q. ilex* had lower *SLA* values than *Q. suber*. Leaf nitrogen, cellulose and lignin concentration (leaf area-based) scaled positively with *PPFD*. Maximum rate of carboxylation (V_{cmax}), capacity for maximum photosynthetic electron transport (J_{max}), rate of triose-P utilization (V_{TPU}) and the rate of nonphotorespiratory light respiration (*Rd*) were also positively correlated with *PPFD* in

both *Quercus* species, when expressed in leaf area but not on leaf mass basis. *Q. suber* showed to have higher photosynthetic potential (V_{cmax} , $J_{\text{max}}^{\text{m}}$ and $V_{\text{TPU}}^{\text{m}}$) and a higher nitrogen efficient nitrogen use than *Q. ilex*. Leaf chlorophyll concentration increased with decreasing *PPFD*, improving apparent quantum use efficiency (Φ) in both *Quercus* species. We concluded that, in *Q. ilex* and *Q. suber*, leaf structural plasticity is a stronger determinant for leaf acclimation to *PPFD* than biochemical and physiological plasticity.

Keywords Light · Nitrogen · Photosynthesis · *Quercus ilex* · *Quercus suber* · *SLA*

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Introduction

The *montadoldehesa* agroforestry systems in southern Portugal are characterized by the savanna-type sparse trees (35–45 trees per hectare, Carreiras 2006), mainly composed by the evergreen oak trees *Quercus ilex* spp. *rotundifolia* syn. *Quercus rotundifolia* Lam. (holm oak) and *Q. suber* (cork oak). Although in savanna-type systems large gaps between plant crowns occur, leaves are generally strongly aggregated in shoots and branches (Asner and Wessman 1997; Asner et al. 1998) implying that the gradient in incident light is not necessarily less important than in communities with a continuous vegetation cover

(Caldwell et al. 1986). Hence, the spatial variation of light in the canopy is primarily a function of leaf area index, dispersion and orientation of leaves. Leaf acclimation to light conditions could include morphological, biochemical and physiological responses (Caldwell et al. 1986; Valladares and Pearcy 1999), representing an optimization of photosynthesis at both leaf and canopy levels (Baldocchi and Harley 1995; Niinemets and Tenhunen 1997; Niinemets et al. 1998, 2004). Leaves exposed to high light (sun leaves) usually present higher leaf mass per unit area, usually due to an increase of leaf thickness and/or an increase in biochemical compounds involved in photosynthesis, reflecting a high photosynthetic capacity per unit leaf area (Mendes et al. 2001; Niinemets and Tenhunen 1997); leaves exposed to low light (shade leaves) have an increased assimilate investment in leaf area to improve light interception (Niinemets et al. 1998; Pearcy 1999) and an increase in leaf longevity (Reich et al. 1992). These processes of photosynthesis acclimation include changes in enzymes (e.g. Rubisco), components of the electron transport chain or photosynthetic pigments concentrations (Brooks et al. 1996; Pearcy 1999). Light gradient effects on leaf acclimation are usually described in canopies of closed vegetation stands, in which occurs a vertical light distribution (Evans and Poorter 2001; Niinemets and Tenhunen 1997; Niinemets et al. 1998). In these cases, leaves at the top of the canopy are exposed to higher light intensity as compared to basal leaves (Niinemets, 2007). Studies involving Mediterranean evergreen *Quercus* in a sparse ecosystem are more scarce (Valladares et al. 2000, 2002) and to our knowledge, only a few reports in *Q. suber* have been published (Faria et al. 1996, 1998). However, leaves and canopy response to light exposure in Mediterranean woody species remain unclear (Kull and Kruijt 1999; Valladares et al. 2002). Moreover, globally changing environmental drivers have promoted an enhanced interest in the vegetation acclimation capacity. In addition to temperature and precipitation, there are long term global changes in radiation quality and intensity (Niinemets and Valladares 2004). Thus, the understanding of plant photosynthetic plasticity to light is crucial to support predictions of changes in ecosystems carbon balance in the future. We studied leaf photosynthetic capacity as well as leaf structural and biochemical characteristics in *Quercus ilex* and *Quercus suber*

growing under contrasting Mediterranean light environments in natural field conditions in order to test the following hypotheses: (i) do leaf acclimation to light conditions integrate structural, biochemical and physiological adjustments?, (ii) are there differences in the two *Quercus* species concerning their leaf acclimation capacity as related to the light gradient in the canopy?

Materials and methods

The experimental site and plant material

The experiment was conducted in a mixed *montado* located at the estate named Herdade da Alfarrobeira (38°32'26" N, 8°00'01" W, 220–250 m a.s.l.), near Évora (southern Portugal). A more detailed description of the site is given in David et al. (2007) and Pereira et al. (2007). Tree cover consists of sparse holm oak trees with scattered patches of cork oak trees. Average stand density and tree crown cover are about 30 trees ha⁻¹ and 21%, respectively (Carreiras et al. 2006). The understory consists of natural pasture invaded by shrubs, mainly *Cistus salvifolius* L. Climate is Mediterranean with dry, hot summers and rainy, mild winters. Long-term (1951–1980) mean annual rainfall is 665 mm⁻¹, mainly concentrated from autumn to early spring (90%) in less than 75 days of rain per year (INMG 1991). The soil is a dystic cambisol with a maximum soil depth of around 1 m and a low water retention capacity, overlying a granite rock (FAO 1988). Four holm oak (*Q. ilex* spp. *rotundifolia*) and four cork oak (*Q. suber*) trees ca. 80 years old were selected in nearby plots (ca. 150 m apart). In sampled trees, trunk diameter at breast height (DBH), projected area and height of the sampled trees ranged respectively from 0.45 to 0.53 m, 91.13 to 150.19 m² and 8.5 to 9.5 m in *Q. suber* and from 0.33 to 0.43 m, 47.71 to 89.99 m² and 7 to 8 m in *Q. ilex*, respectively. The mean diameter at breast height (DBH) of the stand was 0.42 (± 0.18) m and 0.34 (± 0.17) m, in holm oak and cork, respectively. The measurements were conducted during October–November 2000 after the autumnal rains which follow the regular summer drought. We used leaves developed in the spring of the current-year.

Distribution of Photosynthetic-Photon-Flux-Density in the canopy

Photosynthetic-Photon-Flux-Density (*PPFD*) was measured with *PPFD* sensors (model LI-190SA, LI-COR, USA) in two canopy locations: north (10–30% *PPFD*) and south (60–80% *PPFD*) position in *Q. ilex* and *Q. suber*, during 7 days from sunrise to sunset. A reference *PPFD* sensor was installed outside the crown projected area (100% *PPFD*). All sensors were connected to a storage device (Data-Logger, model DL2, Delta-T Devices Ltd, Cambridge, UK) measuring 30 s intervals and recording 10 min averages. Sensors were calibrated against a sensor with a calibration source (Model LI-190SA, LI-COR, USA) and carefully installed in a horizontal plane in relation to the mid-day sun inclination to ensure that their relative positions was representative of light conditions surrounding the leaves, which were later collected for structural and biochemical analysis. In the results and discussion of this experiment we will use Photosynthetic-Photon-Flux-Density (*PPFD*) to define light conditions.

Predawn leaf water potential

Predawn leaf water potential (Ψ_{IPD}) and mid-day leaf water potential (Ψ_{IMD}) were measured in three to four leaves exposed at 10–30% *PPFD* and 60–80% *PPFD*, with a Scholander pressure chamber (PMS 1000, PMS Instruments, Corvallis, Ore.) (Scholander et al. 1965). Samples were taken at similar heights above ground to avoid variability due to hydrostatic water potential and inserted into a plastic bag immediately after cutting to prevent further transpiration.

Leaf gas exchange measurements

Photosynthetic rate (*A*) and stomatal conductance (*g_s*) were measured under natural conditions in the morning periods (8.00–10.00 h) in four leaves per tree exposed to 10–30% *PPFD* and four leaves exposed to 60–80% *PPFD*. The responses of leaf photosynthesis to CO₂ concentration in the intercellular spaces (*A/C_i*, curves) and to Photosynthetic-Photon-Flux-Density (*A/PPFD*, curves) were performed in twelve leaves exposed to 10–30% *PPFD* and twelve leaves exposed to 60–80% *PPFD*. All Gas exchange measurements were done with a portable

steady-state photosynthetic system (Li-Cor, Li-6400). *A/C_i* curves were produced under light intensity saturation (1500 $\mu\text{mol Photons m}^{-2} \text{s}^{-1}$), leaf temperature around 25°C ($\pm 2^\circ\text{C}$) and relative humidity around 50%. The CO₂ concentration in the leaf chamber was raised from 5, 10, 20, 35, 60, 100 to 120 Pa. *A/PPFD* curves were obtained under ambient CO₂ concentrations (35 Pa), leaf temperature around 25°C ($\pm 2^\circ\text{C}$) and relative humidity around 50%. Light intensity was raised from 50, 100, 200, 500, 1000 and 1500 to 2000 $\mu\text{mol Photons m}^{-2} \text{s}^{-1}$. Leaves were allowed to equilibrate for 8–10 min at each *PPFD* before data logging. All leaf gas exchange measurements (for the *A/C_i* and *A/PPFD* curves) were conducted between morning and mid-day to avoid afternoon stomatal closure (Franks et al. 1997, Pereira et al. 1987).

Leaf structure and biochemical composition

For structural and biochemical composition analysis leaves were collected along a *PPFD* gradient in canopy in four trees per species, using a mobile platform between 7:00 and 9:00 h am. Leaf nitrogen content was determined by the standard Kjeldahl method in a composite leaf sample. Leaves were oven dried at 70°C during 48 h and ground for analysis. Chlorophyll content was measured using a spectrophotometer (Perkin-Elmer 124, USA), as described in Arnon (1949) in leaf discs (0.6 cm in diameter). For non-structural carbohydrate determinations, leaf samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Soluble sugars were extracted in ethanol (70%, v/v), the residue was incubated in HCl (1.1%, v/v) for 30 min at 95°C for starch extraction. Both soluble sugars and starch were determined colorimetrically at 625 nm with anthrone reagent (Robyt and White 1990). For determination of *SLA* (leaf area per unit of dry weight), 30 leaves were collected at each *PPFD* level and transported to the lab in refrigerated bags to avoid weight loss by respiration. Leaf area was measured after petiole removal, in an area meter (LI-3000A, LI-COR). Leaves were then oven-dried at 80°C for at least 48 h, and the dry mass was determined (Mettler, Toledo PB 3002). A commercial Bio-Rad protein assay was used to measure soluble protein content by the Bradford method (Bradford 1976). Cellulose and lignin determination were according Van Soest (1963).

Biochemical parameters

The Farquhar et al. (1980) biochemical photosynthetic model, modified according Sharkey (1985), was used to estimate apparent maximum carboxylation rate (V_{cmax} [Ci]), maximum electron transport rate (J_{max}), respiration in the light rate (Rd) and triose-P utilization rate (V_{TPU}). The Michaelis–Menten constants (Kc and Ko), τ (specificity of Rubisco for CO_2/O_2), α (the fraction of incident-light photons that are converted into electrons) and temperature dependence of the model parameters were corrected as described by Harley et al. (1992).

Analysis of light response curves

The response of leaf photosynthesis to photosynthetic-photon flux-density ($A/PPFD$) can be described by the equation (Harley et al. 1986; Harley and Baldocchi 1995):

$$A = \Phi PPFD / \sqrt{\left(1 + \Phi^2 PPFD^2 / A_{max}^2\right)} - Rd \quad (1)$$

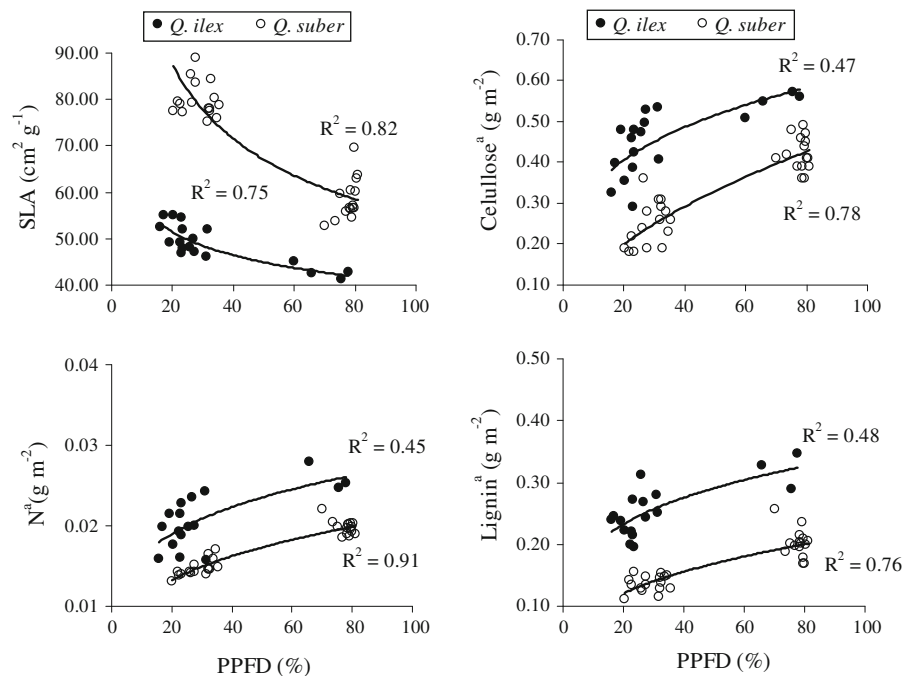
where A is the net photosynthetic rate, I ($PPFD$) is the incident photon flux-density, A_{max} is the light saturated photosynthetic rate, Rd is the rate of

respiration in the light, exclusive of photorespiration, and Φ is the initial slope (apparent quantum yield).

Statistical analysis

Statistical analyses were made using the SPSS v.11 software package. One-way ANOVA was used to test averages on structural, biochemical and physiological leaf parameters. Significant differences between treatments means were evaluated with Tukey's HSD tests with $P < 0.05$. Descriptive statistics and the ANOVA (post hoc Student–Neuman–Keuls test, $P < 0.05$) were performed order to assess the significance of differences observed in the physiological and environmental parameters of each oak species. All measurements shown are the mean \pm standard error of the mean (standard error, s.e.). Before applying the ANOVA test, data were tested for normality by using different tests (Levene and Bartlett) at the significance level of 0.05. When variances across groups were unequal, i.e. the usual ANOVA assumptions were not satisfied, the Welch-ANOVA test was applied.

Fig. 1 Interrelationships between Photosynthetic-Photon Flux-Density ($PPFD$, %), expressed in function of a $PPFD$ reference (100% $PPFD$), and specific leaf area (SLA), leaf nitrogen (N^a), leaf cellulose (cellulose^a) and leaf lignin (lignin^a) in *Q. ilex* and *Q. suber* (area-based)



Results

Leaf structure and biochemical parameters

Specific leaf area (*SLA*) decreased exponentially with increasing relative Photosynthetic-Photon-Flux-Density (*PPFD*) in canopy, in both *Quercus* species (Fig. 1). *Q. ilex* had lower *SLA* (43–55 cm²g⁻¹) than *Q. suber* (53–88 cm²g⁻¹). Leaf biochemical composition in nitrogen (N^a), cellulose (cellulose^a) and lignin (lignin^a) (area-based) increased with increasing *PPFD* (Fig 1). Nitrogen (N^m), cellulose (cellulose^m) and lignin (lignin^m) did not respond significantly to *PPFD* variation, when expressed in leaf mass base (Fig. 2). *Q. ilex* had higher values in biochemical composition concentration, in area-based, than *Q. suber*. Leaf soluble sugar, starch and total protein contents (area-based) were higher in leaves exposed to elevated *PPFD* (60–80% *PPFD*) in relation to leaves exposed to lower *PPFD* (10–30% *PPFD*) in both *Quercus* species (Fig. 3). In contrast, total chlorophyll concentration, on area-based, was higher in both *Quercus* species, in leaves exposed to lower *PPFD* (10–30% *PPFD*) than higher *PPFD* (60–80% *PPFD*).

Leaf physiology parameters

Ψ IPD values (Table 1) showed that both *Quercus* species were not under drought stress. Leaf water potential measured at midday (Ψ IMD) was lower (more negative) in leaves exposed to high *PPFD* (60–80% *PPFD*) as compared to leaves under low *PPFD* (10–30%), as they experienced a higher evaporative demand indicated by the higher stomatal conductance (*g*s) measured in the morning in both *Quercus* species (Table 1). Photosynthetic rate under ambient conditions (*A*) was higher in sun exposed leaves for *Q.ilex* and *Q.suber* than for shade leaves for both *Quercus* (Table 1). Photosynthetic parameters: V_{cmax}^a , J_{max}^a and V_{TPU}^m (area-based), increased with increasing *PPFD* in both *Quercus* species. However, if the photosynthetic parameters are expressed in leaf mass basis (V_{cmax}^m , J_{max}^m and V_{TPU}^m), no differences between *PPFD* levels were observed. *Q. suber* showed higher V_{cmax} than *Q. ilex*, in both leaf area and mass basis. Rate of non-photorespiratory respiration in light (R_d^a , area-based) increased in leaves exposed to higher *PPFD* which was correlated

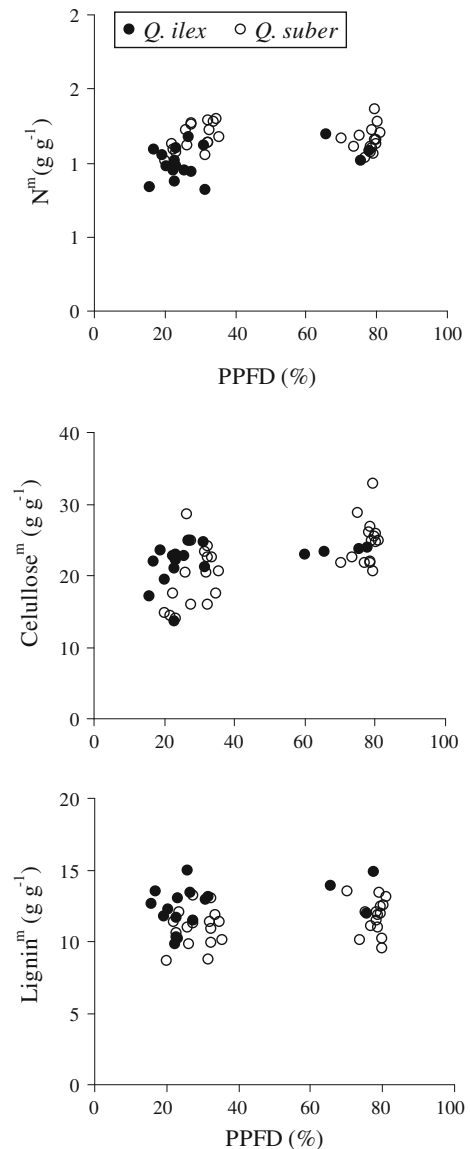


Fig. 2 Interrelationships between Photosynthetic-Photon Flux-Density (*PPFD*, %), expressed in function of a *PPFD* reference (100% *PPFD*), and leaf nitrogen (N^m), leaf cellulose (cellulose^m) and leaf lignin (lignin^m) in *Q. ilex* and *Q. suber* (mass based)

with higher cellulose and lignin contents (Fig. 1) but no differences were observed for the mass-based parameter. Quantum use efficiency (Φ) was higher in leaves exposed to lower *PPFD* (10–30% *PPFD*) than higher *PPFD* (60–80% *PPFD*) (Fig. 3). V_{cmax}^a was related with the leaf nitrogen concentration (N^a) (Fig. 4) in both *Quercus* species, *Q. suber* showing higher nitrogen use efficiency versus V_{cmax}^a .

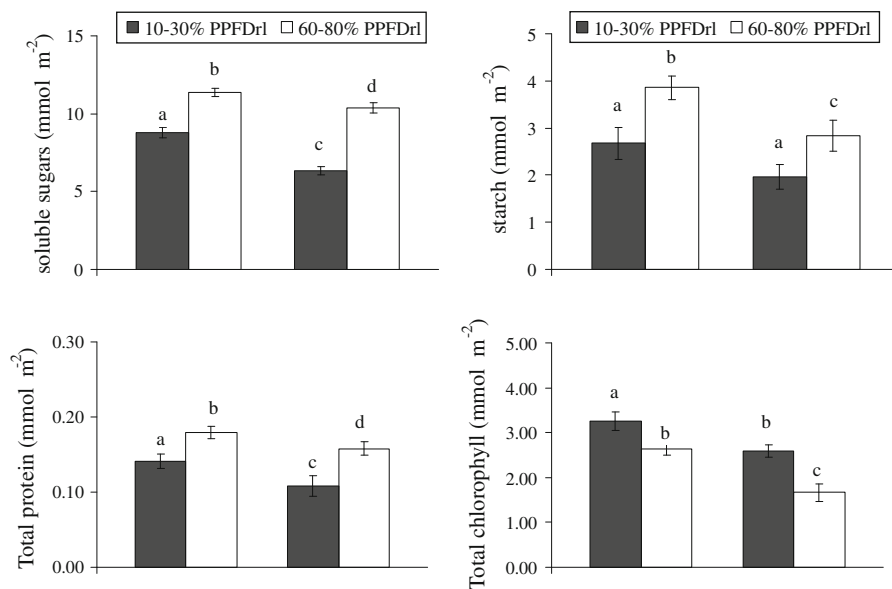


Fig. 3 Soluble sugars, starch, total protein and total chlorophyll, in area-based, in *Quercus suber* and *Quercus ilex* in leaves exposed to 10–30% PPFD and leaves exposed to

60–80% PPFD. Values are means \pm s.e. Means with the same letter are not significantly different ($P > 0.05$)

Discussion

One expression of plant phenotypic plasticity is the modification of leaf morphology in response to light intensity and the major adaptation/acclimation of leaf morphology to changing light is the Specific Leaf Area (SLA) (Gratani 1997). *Q. ilex* and *Q. suber* leaves did acclimate structurally to incident PPFD. SLA was negatively related with the PPFD due to an accumulation of leaf compounds, such as cellulose and lignin. The SLA values obtained, ranging from 42 to 56 gm⁻² in *Q. ilex* and from 52 to 87 g⁻² in *Q. suber*, were of the same order of magnitude of those obtained in adult *Q. ilex* trees (55.49 for sun leaves and 100.02 gm⁻² for shade leaves) by Gratani (1997) and in *Q. suber* (58.5 for sun leaves and 86.0 gm⁻² for shade leaves) by Faria et al. (1996; 1998). In general, leaves exposed to high light may suffer from severe heat, water and photoinhibition stress (Chaves et al. 2002). The lower SLA in sun leaves may be advantageous to reduce leaf transpiration under increasing vapour pressure deficit of the air during the Mediterranean summer drought conditions (Sabaté et al. 1999, Specht and Specht 1989), as transpiring leaf surface is lower as compared to the leaf mass that contributes to the assimilation of carbon. Carbon assimilation rate at ambient

conditions (A, area-based) was higher in sun exposed leaves for *Q. ilex* ($11.85 \pm 2.56 \mu\text{mol m}^{-2}\text{s}^{-1}$) and *Q. suber* ($13.56 \pm 2.12 \mu\text{mol m}^{-2}\text{s}^{-1}$) than for shade leaves (6.35 ± 1.25 and $7.98 \pm 1.35 \mu\text{mol m}^{-2}\text{s}^{-1}$, respectively), confirming earlier data by Faria et al. (1996; 1998) and Garcia-Plazaola et al. (1997) in *Q. suber* in the same ecosystem. *Q. suber* sun leaves were shown to be more protected to excess light by an increase in xanthophyll composition (Garcia-Plazaola et al. 1997). Sun leaves also exhibited higher intrinsic water use efficiency (expressed as the ratio of carbon assimilation per stomatal conductance, A/g_s) than shade ones, demonstrating a high degree of adaptation to this habitat.

When comparing the two species, *Q. ilex* leaves had lower SLA values than *Q. suber* leaves leading to a higher degree of sclerophylly that may be useful under drought summer conditions. Additionally, other physiological parameters (e.g. leaf water potential and stomatal conductance) indicate that *Q. ilex* has higher water use efficiency than *Q. suber* (David et al. 2007; Vaz et al. 2010). The photosynthetic parameters V_{cmax}^a , and J_{max}^m (area-based) and non-photorespiratory respiration rates in light (R_d^a) were strongly associated with leaf light environment in both *Quercus* species. However, PPFD environment did not affect V_{cmax}^a and J_{max}^m (mass-based). We found a

Table 1 Leaf physiological characteristics in *Quercus ilex* and *Quercus suber* leaves (averages \pm se)^a

	<i>Q. ilex</i> (10–30% PPFD)	<i>Q. ilex</i> (60–80% PPFD)	<i>Q. suber</i> (10–30% PPFD)	<i>Q. suber</i> (60–80% PPFD)
Ψ_{IPD} , bar	-0.086 (\pm 0.005) a	-0.094 (\pm 0.003) a	-0.101 (\pm 0.003) a	-0.104 (\pm 0.002) a
Ψ_{IMD} , bar	-0.965 (\pm 0.187) b	-1.988 (\pm 0.254) c	-0.923 (\pm 0.192) b	-1.718 (\pm 0.202) c
A, $\mu\text{mol m}^{-2} \text{s}^{-1}$	6.35 (\pm 1.25) a	11.85 (\pm 2.56) b	7.98 (\pm 1.35) a	13.56 (\pm 2.12) b
gs, $\text{mol m}^{-2} \text{s}^{-1}$	0.078 (\pm 0.012) a	0.096 (\pm 0.015) b	0.102 (\pm 0.068) c	0.158 (\pm 0.045) c
A/gs	81.41 (\pm 7.86) a	123.43 (\pm 9.82) b	78.23 (\pm 8.23) a	85.39 (\pm 0.012) a
$V_{\text{cmax}}^{\text{a}}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$	29.60 (\pm 1.80) a	47.91 (\pm 1.27) b	38.69 (\pm 2.45) c	54.64 \pm 3.93 d
$V_{\text{cmax}}^{\text{m}}$, $\mu\text{mol g}^{-1} \text{s}^{-1}$	0.163 (\pm 0.027) a	0.201 (\pm 0.035) a	0.310 (\pm 0.016) b	0.339 (\pm 0.020) b
$J_{\text{max}}^{\text{a}}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$	68.08 (\pm 3.63) a	125.20 (\pm 7.61) b	75.96 (\pm 4.65) c	125.82 \pm 8.65 b
$J_{\text{max}}^{\text{m}}$, $\mu\text{mol g}^{-1} \text{s}^{-1}$	0.374 (\pm 0.033) a	0.526 (\pm 0.062) a	0.608 (\pm 0.098) b	0.780 (\pm 0.085) b
$V_{\text{TPU}}^{\text{a}}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$	2.35 (\pm 0.22) a	6.41 (\pm 0.11) b	3.25 (\pm 0.69) a	6.20 \pm 0.30 b
$V_{\text{TPU}}^{\text{m}}$, $\mu\text{mol g}^{-1} \text{s}^{-1}$	0.013 (\pm 0.002) a	0.027 (\pm 0.006) b	0.026 (\pm 0.009) b	0.038 (\pm 0.002) c
R_{d}^{a} , $\mu\text{mol m}^{-2} \text{s}^{-1}$	1.35 (\pm 0.10) a	2.34 (\pm 0.36) b	0.89 (\pm 0.09) c	1.26 \pm 0.34 a
R_{d}^{m} , $\mu\text{mol g}^{-1} \text{s}^{-1}$	0.007 (\pm 0.001) a	0.010 (\pm 0.00) a	0.007 (\pm 0.000) a	0.008 (\pm 0.001) a
$A_{\text{max}}^{\text{a}}$, $\mu\text{mol m}^{-2} \text{s}$	18.48 (\pm 2.55) a	21.66 (\pm 1.78) b	20.63 (\pm 3.98) a	25.69 (\pm 2.76) b
$A_{\text{max}}^{\text{m}}$, $\mu\text{mol g}^{-1} \text{s}$	0.102 (\pm 0.01) a	0.091 (\pm 0.03) a	0.165 (\pm 0.026) b	0.159 (\pm 0.013) b
Φ , $\mu\text{mol CO}_2/\mu\text{mol photons}$	0.069 (\pm 0.002) a	0.056 (\pm 0.003) b	0.089 (\pm 0.009) c	0.051 (\pm 0.006) b

Ψ_{IPD} , leaf water potential at predawn; Ψ_{IMD} , leaf water potential at midday; A, photosynthetic rate at ambient conditions; gs, stomatal conductance at ambient conditions; $V_{\text{cmax}}^{\text{a}}$, area-based maximum ribulose 1,5-bisphosphate carboxylase/oxygenase, Rubisco) carboxylase activity; $V_{\text{cmax}}^{\text{m}}$, mass-based maximum ribulose 1,5-bisphosphate carboxylase/oxygenase, Rubisco) carboxylase activity; $J_{\text{max}}^{\text{a}}$, area-based capacity for photosynthetic electron transport; $J_{\text{max}}^{\text{m}}$, mass-based capacity for photosynthetic electron transport; R_{d}^{a} , area-based rate of non-photorespiratory respiration in light; R_{d}^{m} , mass-based rate of non-photorespiratory respiration in light; $V_{\text{TPU}}^{\text{a}}$, area-based rate of triose-P utilization; $V_{\text{TPU}}^{\text{m}}$, mass-based rate of triose-P utilization; $A_{\text{max}}^{\text{a}}$, area-based photosynthetic rate at light saturation and ambient CO_2 ; $A_{\text{max}}^{\text{m}}$, mass-based photosynthetic rate at light saturation and ambient CO_2 ; Φ , apparent quantum use efficiency; average \pm se. The means were compared by analysis of variance (ANOVA). After conduction of ANOVA, the means were separated between the age classes by Bonferroni test. Means with the same letter are not significantly different ($P > 0.05$)

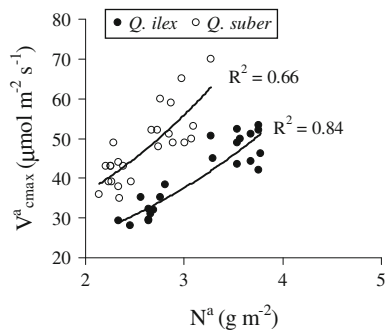


Fig. 4 Relationship between maximum rate of Rubisco carboxylation ($V_{\text{cmax}}^{\text{a}}$) and leaf nitrogen concentration (N^{a}) in *Q. ilex* and *Q. suber*

positive relation between leaf nitrogen concentration and $V_{\text{cmax}}^{\text{a}}$ suggesting that a significant fraction of foliar nitrogen is invested in photosynthetic proteins as shown elsewhere in different *Quercus* species: *Q. ilex* (Sabaté et al. 1995; Valladares et al. 2000),

Q. coccifera (Valladares et al. 2000), *Q. faginea* and *Q. suber* (Niinemets et al. 2004). As compared with temperate deciduous trees species, Mediterranean evergreen species might show lower light responses in face of leaf nitrogen concentration invested, reflecting a conservative strategy for resources usage (water and nutrients) (Robinson and Robinson 1988; Schlichting 1986; Valladares et al. 2000). Takashima et al. (2004) found that evergreen species allocate more nitrogen to cell wall proteins at the expense of reduced nitrogen allocation to the photosynthetic apparatus. At low PPFD, nitrogen is partitioned into chlorophyll and light-harvesting proteins improving light harvesting in relation to leaves exposed to high PPFD (Hollinger 1996) as observed in *Q. ilex* (our study, Gratini 1997) and *Q. suber* (our study). Thus, increasing the apparent quantum efficiency (Φ , area-based) in leaves exposed to low PPFD. *Q. suber* presented a higher photosynthetic potential ($V_{\text{cmax}}^{\text{a}}$,

J_{\max}^m and V_{TPU}^m) than *Q. ilex*. Assuming that photosynthetic acclimation results in an optimal distribution of nitrogen in the photosynthetic machinery, *Q. suber* leaves showed higher nitrogen investments in photosynthetic apparatus, such as in Rubisco, than *Q. ilex* as observed previously in the same trees (see Vaz et al. 2010). The ability to adjust the allocation of total leaf nitrogen to the different pools of the photosynthetic machinery to carboxylation, electron carriers and chlorophyll partly explains these different responses to irradiance (Le Roux et al. 2001).

Overall, leaf acclimation to *PPFD* gradient in *Quercus* species seems to be mainly controlled by changes in *SLA* that, in turn, will control the amount of photosynthetic machinery available per unit of leaf area. Thus, these results have important implications for canopy-level oak photosynthesis model parameterization and scaling up to carbon balance at *montadoldehesa* agroforestry systems.

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