

## Light acclimation at the end of the growing season in two broadleaved oak species

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### Abstract

The ability of plants to increase their net CO<sub>2</sub> assimilation rate in response to increased irradiance is due to morphological and physiological changes, which might be related to their shade tolerance and leaf ontogeny, but few studies have considered morphology and physiology. Two sympatric oak species (the shade-tolerant *Q. petraea* and the comparatively shade-intolerant *Q. pyrenaica*) were grown in hydroponic solution in low-light (LL) and high-light (HL) conditions. 5 months after leaf expansion under these conditions, half of the LL plants were transferred to high light (TLH). Transfer of *Q. pyrenaica*, from low- to high light led to photoinhibition and after 21 days in higher light there was little acclimation of the maximum rate of carboxylation ( $V_{Cmax}$ ) or the maximum rate of electron transport ( $J_{max}$ ). *Q. pyrenaica* TLH plants showed lower stomatal conductance at all times compared to plants growing in LL. Stomatal closure was the main limitation to photosynthesis after transfer in *Q. pyrenaica*. The increase in evaporative demand upon TLH did not affect hydraulic conductivity of *Q. pyrenaica*. In contrast, the more shade-tolerant *Q. petraea* showed a greater degree of acclimation of gas exchange in TLH than *Q. pyrenaica* and two weeks after transfer gas-exchange rates were as high as in LL plants. In *Q. petraea*, the most important changes occurred at the level of leaf biochemistry with significant increase in  $V_{Cmax}$  that decreased the  $J_{max}/V_{Cmax}$  ratio below values recorded in HL plants. However, this potential increase in photosynthesis was at least partially hamstrung by a decrease in internal conductance, which highlights the importance of internal conductance in acclimation to higher light in mature leaves. Neither oak species reached the photosynthetic rates of HL plants; however a trend towards leaf acclimation was observed in *Q. petraea* while the transfer was harmful to the leaves of *Q. pyrenaica* developed in the shade.

*Additional key words:* hydraulic conductivity; internal conductance; light acclimation; mature leaves; *Quercus petraea*; *Quercus pyrenaica*.

Received 13 December 2010, accepted 25 September 2011.

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**Abbreviations:**  $A_{leaf}$  – plant leaf surface area; Car – total carotenoids pool content;  $C_c$  – [CO<sub>2</sub>] inside chloroplasts; Chl *a* and *b* – chlorophyll *a* and *b* contents;  $C_i$  – [CO<sub>2</sub>] in mesophyll air spaces;  $C_i^*$  – apparent CO<sub>2</sub>-photocompensation point; ETR – rate of noncyclic electron transport through PSII;  $F_v'/F_m'$  – PSII maximum efficiency of the light-adapted state;  $g_i$  – internal conductance to CO<sub>2</sub> diffusion;  $g_{sc}$  – stomatal CO<sub>2</sub> conductance;  $g_{sw}$  – stomatal water conductance; HL – high-light grown conditions;  $J_{max}$  – maximum rate of photosynthetic electron transport; LL – low-light grown conditions; LMA – leaf mass per area;  $l_s$ ,  $l_{mc}$ ,  $l_b$  – relative limitations to photosynthesis due to: stomatal conductance ( $l_s$ ), leaf-internal conductance ( $l_{mc}$ ), and biochemical capacity ( $l_b$ ) of the leaf;  $P_N$  – net assimilation rate under saturating light; PSII – photosystem II;  $q_L$  – fraction of PSII centres which are in open state;  $q_P$  – PSII efficiency factor;  $R_l$  – mitochondrial respiration in light; TLH – transferred plants from low- to high light;  $V_{Cmax}$  – maximum rate of carboxylation;  $\Gamma^*$  – chloroplast CO<sub>2</sub> photo-compensation point;  $\Phi_{PSII}$  – PSII operating efficiency;  $\Psi_{midday}$  – midday water potential.

**Acknowledgements:** This research has been developed in the frame of the Projects “CLIMHAYA” (CGL2007-66066-C04-03/BOS) and SUM2008-00004-C03-01, funded by the Ministry of Science and Innovation of Spain. F.J. Cano is granted by the Regional Government of Madrid with a PhD fellowship; and the postdoctoral activity of D. Sánchez-Gómez and A. Gascó is sponsored by the Ministry of Science and Innovation of Spain through the “Juan de la Cierva” Programme. We thank contributions of three anonymous reviewers. We specially thank Luis Alté for technical assistance and Prof. Fernando Valladares for use of the XYL'EM.

## Introduction

Light is one of the most limiting resources for plant growth (Bazzaz and Wayne 1994, Pearcy and Sims 1994). The germination of many forest species occurs within the forest, but very few seedlings survive in these shady conditions after the first years of establishment (Rodríguez-Calcerrada *et al.* 2010). Gap formation inside the canopy increases light levels on the ground and promotes the growth of pre-established plants in the understory (Sipe and Bazzaz 1995). The degree of response to increased light varies among species as a function of their shade tolerance. Thus the most shade-tolerant species can benefit from very small changes in ambient light, whereas less shade tolerant species are usually less responsive to minor changes (Canham 1988) but have greater capacity for acclimation when the increase in light is greater (Valladares *et al.* 2002b) or gap size is larger (Sipe and Bazzaz 1995, Naidu and DeLucia 1998).

Light-acclimation involves processes from biochemical through to whole plant scales (Givnish 1988). At the leaf level, light acclimation is not only species-specific but also depends on leaf ontogeny. The interaction between shade tolerance and leaf ontogeny was described by Yamashita *et al.* (2002) who showed that young leaves of pioneer tree species had better light acclimation than late successional species, but the opposite was the case for mature leaves. Acclimation to higher light intensities in tree species seems to be reached only by leaves still expanding or not fully mature at the time of transfer (Sims and Pearcy 1992, Terashima and Hikosaka 1995). One of the main constraints to acclimation is the capacity for mature leaves to enlarge palisade mesophyll (Sims and Pearcy 1992, Tognetti *et al.* 1998). Thus leaf anatomy is the ultimate determinant of maximum photosynthetic capacity because once chloroplasts enlarge and fill all the surface area of mesophyll cells facing the intercellular space additional increases in photosynthetic capacity cannot occur (Oguchi *et al.* 2006).

Tree seedlings grown under shade usually have leaves arranged in horizontal position, which is advantageous under low light but can lead to photoinhibition upon transfer to high light (Müller *et al.* 2001, Valladares *et al.* 2002a, Rodríguez-Calcerrada *et al.* 2008b). Increases in light also increase leaf temperature and the vapour pressure gradient between the leaf and the surrounding atmosphere. Transpiration may increase so as to lower leaf temperature, and thereby reduce the risk of overheating and photoinhibition. However plants transferred from low- to high light have a hydraulic system developed in the shade that is sensitive to embolism and has a low capacity to transport water under high evaporative demand (Cochard *et al.* 1999, Barigah *et al.*

2006). So, in the few days after transfer to higher light, stomata behaviour is a trade-off between hydraulic safety and the increase in evaporative demand (Brodribb 2009).

Net photosynthesis is directly related to stomatal and internal conductances for CO<sub>2</sub> (Lloyd *et al.* 1992, Loreto *et al.* 1994). These resistances are dependent on the light environment in which leaves develop (Piel *et al.* 2002, Warren *et al.* 2007) but could be modified after transfer from low- to high light. Despite the great limitation to carbon fixation in deciduous tree species due to internal conductance ( $g_i$ ) (Epron *et al.* 1995, Grassi and Magnani 2005, Warren *et al.* 2007), little is known about responses of  $g_i$  to a shift in light. Quick changes in light intensity may produce a directly proportional change in  $g_i$  (Gorton *et al.* 2003, Flexas *et al.* 2007 in Fig. 5), or not (Tazoe *et al.* 2009). In contrast, transfer from shade to high light for several days decreased  $g_i$  in mature leaves of the crop species *Brassica carinata*, but this may have been due to advancement of senescence (Monti *et al.* 2009). At the time of writing, we are not aware of any publication for tree seedlings examining if  $g_i$  of shade-acclimated leaves is affected by transfer to high light.

In order to discern the limitations to photosynthesis due to diffusion processes of CO<sub>2</sub> (stomatal and internal limitations) compared to the biochemical component of photosynthesis after transfer to high light we applied a novel method developed by Grassi and Magnani (2005). The study species were the temperate deciduous *Quercus petraea* (Matt.) Liebl. (sessile oak), and the sub-Mediterranean marcescent *Quercus pyrenaica* Willd. (melojo oak). Although both species are traditionally considered as moderately shade-tolerant, recent studies suggest *Q. petraea* is more shade-tolerant, while *Q. pyrenaica* has higher tolerance to drought (Rodríguez-Calcerrada *et al.* 2007, 2010). Previous studies with the same species showed that there could be almost complete acclimation of gas exchange and photochemistry to transfer from low- to high light, even without significant changes in leaf anatomy (Rodríguez-Calcerrada *et al.* 2008a,b). The current study aimed to evaluate light acclimation at the end of the growing season in leaves developed in low light. We focused our analysis towards limitations of CO<sub>2</sub> uptake including internal conductance and possible dysfunctions of water supply after transfer from low- to high light. Understanding the trade-off between carbon gain and hydraulic safety to a sudden increase in irradiance allowed us to investigate the physiological processes underlying the acclimation of mature leaves of tree seedlings and contrasting pre-existing studies on younger leaves upon the plasticity to light regarding to shade tolerance of each species.

## Materials and methods

**Plant material and experimental design:** Acorns of *Q. petraea* and *Q. pyrenaica* were collected in October 2006 in the reserve of the biosphere *Sierra del Rincón* (Madrid Region, central Spain, 41°7'N 3°30'W). This location is characterized as a remnant of central European flora in sub-Mediterranean climate (Nanos *et al.* 2005). Acorns were collected from one tree in each of four pure stands of each species. A subsample of acorns of similar size and mass were stored in the dark at 4°C and then germinated on wet perlite in a germination chamber at 25°C. Just after the epicotyls emerged, seedlings were placed inside a growth chamber with 16 hours of light at  $T = 22^{\circ}\text{C}$ , relative humidity (RH) of 60%, and darkness at  $T = 19^{\circ}\text{C}$  and RH = 70%.  $[\text{CO}_2]$  inside growth chamber ranged between 360 to 420  $\mu\text{mol mol}^{-1}$ . Two contrasting light treatments were applied: low light (LL) was set at a photosynthetic photon flux density (PPFD) of 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (4.6  $\text{mol m}^{-2} \text{day}^{-1}$ ) and high light (HL) at a PPFD of 470  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (27.1  $\text{mol m}^{-2} \text{d}^{-1}$ ). PPFD at HL is in the range previously reported for large gaps in temperate forests (Sipe and Bazzaz 1995), while LL corresponding to field observations (data not shown). The length of day time was chosen to promote a quick response in acclimation in few days. Three weeks later, 18 similar-sized plants per species, representing 4 families from the natural population, were transferred to aerated hydroponic growing culture, which ensures a homogeneous optimum availability of water and nutrients. A modified Hoagland nutritive solution was used at an initial conductivity of 800  $\text{mS cm}^{-1}$  and pH = 5.6, which was frequently corrected by adding 1 N  $\text{H}_2\text{SO}_4$ . Hydroponic growing media was replaced every two weeks.

After 5 months of growth under these conditions, we measured photosynthetic gas-exchange, including  $\text{CO}_2$ -response curves ( $P_N/C_i$  curves), and chlorophyll (Chl) fluorescence. By this time, seedlings of both species had completely consumed the acorn reserves. All measurements were made on mature leaves from the upper third of the shoot of 4 HL and 6 LL plants per species. At this time (day 0, D0), half of LL plants ( $n = 3$ ) were transferred to HL conditions (TLH treatment). The 3 TLH plants were chosen randomly from the initial 6 LL plants. 7 and 14 days after transfer to high light (D7 and D14) we measured instantaneous midday gas exchange and chlorophyll fluorescence in all plants. 21 days after transfer to high light (D21) a new set of measurements was performed in the same leaves of the same plants measured on D0. At D21 we also measured leaf mass per area (LMA), content of photosynthetic pigments, midday leaf water potential ( $\Psi_{\text{midday}}$ ), and hydraulic conductivity of stems and roots.

**Mitochondrial respiration in the light ( $R_i$ ) and apparent  $\text{CO}_2$ -photocompensation point ( $C_i^*$ ):** To estimate  $R_i$  and  $C_i^*$  we measured  $P_N/C_i$  curves at three different

PPFD following the methodology of Laisk (1977). Gas exchange was measured with a *Li-6400 IRGA* (LiCor Inc., Lincoln, NE, USA) with a 6  $\text{cm}^2$  chamber and LED light source. The  $\text{CO}_2$  response of photosynthesis was measured at  $[\text{CO}_2]$  of 300, 250, 200, 150, 100, and 50  $\mu\text{mol mol}^{-1}$  at PPFDs of 400, 150, and 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Preliminary tests showed that these PPFDs were optimal for obtaining large differences in slope among the fitted linear regressions of  $P_N$  vs.  $C_i$ . The point of intersection of these three  $P_N$ - $\text{CO}_2$  linear regressions indicates  $R_i$  on the ordinate axis (y), and  $C_i^*$  on the abscissa axis (x).

**Photosynthesis- $C_e$  curves with Chl fluorescence. Estimation of leaf-internal conductance ( $g_i$ ):** The responses of  $P_N$  and Chl fluorescence to  $C_i$  were measured with a *LiCor 6400* with a 2  $\text{cm}^2$  fluorometer chamber on the same leaves where  $R_i$  and  $C_i^*$  were estimated the previous day. All curves were performed under the same growth conditions ( $T = 22^{\circ}\text{C}$  and RH = 60%) and under light-saturating conditions: PPFD = 1,100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for HL and TLH plants; and 700  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for LL ones with 15% blue light in all treatments to stimulate stomatal opening (values determined in previous assays). Leaves were acclimated to light inside the fluorometer chamber for 20 min at a  $[\text{CO}_2] = 380 \mu\text{mol mol}^{-1}$ . A photosynthesis- $\text{CO}_2$  response curve was then measured by decreasing  $[\text{CO}_2]$  to 50  $\mu\text{mol mol}^{-1}$  in 8 steps as quickly as possible (typically < 120 s per step) to minimize changes in Rubisco activity (Long and Bernacchi 2003).  $[\text{CO}_2]$  was returned to 380  $\mu\text{mol mol}^{-1}$  to check the original  $g_{\text{sw}}$  and  $P_N$  values, and then increased in 8 steps to  $[\text{CO}_2] = 2,000 \mu\text{mol mol}^{-1}$ . Small  $[\text{CO}_2]$  intervals were used to increase accuracy in estimations of parameters such as  $V_{\text{Cmax}}$  (Miao *et al.* 2009). At each  $[\text{CO}_2]$  concentration, gas exchange and Chl fluorescence were measured. Steady-state fluorescence ( $F_s$ ) was recorded under actinic light of measurements from LEDs (red and 15% of blue light) and maximum fluorescence during a light-saturating pulse (0.8 s) of ca. 8,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at wavelength of 630 nm ( $F_m'$ ). Then, far-red light (740 nm) of 5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 6 s was applied without actinic light to get  $F_o'$ , the minimal fluorescence of a light-adapted leaf. Next Chl fluorescence variables related to photosystem II (PSII) operation at the light-adapted state were calculated according to Baker *et al.* (2007): PSII maximum efficiency of the light-adapted state ( $F_v'/F_m'$ ); PSII operating efficiency ( $\Phi_{\text{PSII}}$ ); PSII efficiency factor ( $q_p$ ); fraction of open PSII centres ( $q_L$ ); rate of noncyclic electron transport through PSII (ETR) considering an 84% spectral absorbance of the leaf and a 50% fraction of incident photons that are absorbed by PSII.

Leaf-internal conductance ( $g_i$ ) was estimated from the J-constant method (Harley *et al.* 1992) essentially as described previously (Warren *et al.* 2007). This method requires knowledge of chloroplastic photo-compensation

point ( $\Gamma^*$ ).  $\Gamma^*$  is related to  $C_i^*$ ,  $R_i$  and  $g_i$  (von Caemmerer and Evans 1991):

$$\Gamma^* = C_i^* + R_i/g_i \quad (1)$$

Using  $C_i^*$  and  $R_i$  calculated from Laisk method (1977),  $\Gamma^*$  was substituted by Eq. 1 and  $g_i$  was estimated by Harley *et al.* (1992) method using solver option from Excel to find the value of  $g_i$  that minimized variance of J. Then  $[CO_2]$  in the chloroplast ( $C_c$ ) was calculated:

$$C_c = C_i - P_N/g_i \quad (2)$$

**Key variables of photosynthesis model and relative limitation:** From the transformed  $P_N/C_i$  into  $P_N/C_c$  curves using Eq. 2, we determined the maximum carboxylation rate allowed by Rubisco properties ( $V_{Cmax}$ ); and the rate of maximum electron transport at saturating light levels ( $J_{max}$ ) based on the model proposed by Farquhar *et al.* (1980) and using the free software developed by Sharkey *et al.* (2007). We used previously estimated  $g_i$  and  $R_i$  in the model of Sharkey *et al.* (2007).  $V_{Cmax}$  and  $J_{max}$  were calculated from the best fitting that minimized sum of squared model deviations.

We determined the relative limitation of net photosynthesis by stomatal conductance, internal conductance, and biochemistry using the approach of Grassi and Magnani (2005). Functional components limiting assimilation were:  $l_s$ , related to stomatal conductance;  $l_{mc}$ , corresponding to internal conductance; and  $l_b$ , linked to the biochemical characteristics of the leaf. As there were not significant differences in the photosynthesis of plants transferred from low- to high light (Table 1), relative limitations were considered for performing comparisons in place of absolute limitations. Light-saturated photosynthesis was checked to be limited by Rubisco activity at ambient  $CO_2$  levels.

**Photosynthetic pigments and leaf mass per area (LMA):** Two 1-cm (diameter) leaf discs were cut from the lamina of leaves used for gas exchange measurements. In one disc, Chl *a*, Chl *b*, and total carotenoids (Car) were extracted with dimethylsulfoxide (DMSO) and determined with a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan) as described by Wellburn (1994). The other disc was used for LMA determination and was dried at 65°C for 48 h before determining dry

mass. Chl *a*, Chl *b*, and Car contents were expressed on a leaf-area basis.

**Water potential and hydraulic measurements:** Midday leaf water potential ( $\Psi_{midday}$ ) was measured in the leaf nearest to those used for gas exchange and pigments measurements using a pressure chamber (PMS, Corvallis, Oregon, USA).

Hydraulic conductivity ( $K_h$ ) and the percentage loss of conductance (PLC) were determined in stem segments using a *XYL'EM* (Xylem Embolism Meter, Bronkhorst High Tech, Ruurlo, The Netherlands) following a modification of the protocol introduced by Sperry *et al.* (1988), as described in Gascó *et al.* (2007). The length of stem samples was almost the whole length of the shoot (6–40 cm). All hydraulic measurements were performed under laboratory conditions of daily constant temperature (23–25°C), and all  $K_h$  values were corrected for viscosity at 20°C, following the empirical equation provided in the manual of the *XYL'EM* (Cochard *et al.* 2008), which result in about 2.4% per °C. At the end of every experiment, leaves were scanned, and the leaf surface area ( $A_{leaf}$ ) supplied by the measured stem sample was measured using the *WinFOLIA* image analysis system (Régent Instruments Inc., Quebec, Canada). In addition, cross-sectional diameters of the xylem at both ends of every sample were measured with a digital calliper. In order to compensate for possible plant size effects when comparing different species and/or light treatments,  $K_h$  values were scaled by  $A_L$ , that is, leaf-specific hydraulic conductivity ( $K_{h-leaf}$ ) was calculated; or by the xylem cross-sectional area, leading to xylem-specific hydraulic conductivity ( $K_{h-xylem}$ , e.g. Gascó *et al.* 2007).

**Statistical analyses:** Data were analyzed using *STATISTICA 7.0* (StatSoft Inc., Tulsa, Oklahoma, USA). The *Shapiro-Wilk's* test was used to test normality. Homoscedasticity was checked graphically by plotting residuals against predicted values. The Wald statistic was used to test the significance of slopes and intercepts of the regression. Two-way *ANOVAs* were performed to test the effect of light treatment and species on measured parameters. *Duncan* test was used for all pairwise comparisons of means ( $P < 0.05$ ).

## Results

**Overall plant performance:** Total leaf area ( $A_{leaf}$ ) of *Q. petraea* was ten-fold higher under HL than LL, while in *Q. pyrenaica*  $A_{leaf}$  was twice as large under HL than LL (Table 1). LMA of HL plants was larger than LMA of LL plants. There were no differences in  $A_{leaf}$  or LMA between LL and TLH plants of either species (Table 1). Plants transferred from LL to HL conditions had more negative  $\Psi_{midday}$  than plants kept under LL conditions, and this decrease was especially large for *Q. petraea*

(Table 1).  $\Psi_{midday}$  tended to be more negative in TLH plants than HL, but this was not statistically significant.

**Evolution of leaf gas-exchange parameters and leaf-internal conductance to the diffusion of  $CO_2$  ( $g_i$ ):** Net photosynthesis ( $P_N$ ) was significantly lower in LL than HL, and there was a nonsignificant trend for stomatal conductance ( $g_{sw}$ ) to be lower in LL than HL (Fig. 1, Table 1).  $P_N$  and  $g_{sw}$  did not differ between species

Table 1. Plant growth, water status, gas exchange, and photosynthesis-related parameters 21 days after transferring (TLH) plants from low-light (LL) to high-light (HL) conditions. Plant leaf surface area ( $A_{\text{leaf}}$ ); leaf mass per area (LMA); midday water potential ( $\Psi_{\text{midday}}$ ); net assimilation rate under saturating light ( $P_N$ ); stomatal water conductance ( $g_{\text{sw}}$ ); internal conductance to  $\text{CO}_2$  diffusion ( $g_i$ ); mitochondrial respiration in light ( $R_l$ ); chloroplast  $\text{CO}_2$  photo-compensation point ( $\Gamma^*$ ); maximum rate of carboxylation ( $V_{\text{Cmax}}$ ); maximum rate of photosynthetic electron transport ( $J_{\text{max}}$ ); chloroplast  $\text{CO}_2$  concentration in mesophyll air spaces ( $C_i$ ). Mean values (standard error).  $n = 3$  in LL and TLH and  $n = 4$  in HL. LL =  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; TLH and HL =  $470 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Letters identify homogeneous groups by Duncan test ( $P=0.05$ ) as follows: capital letters indicate homogeneous groups within the significant factor; lowercase letters indicate homogeneous groups when the interaction between factors is significant; capital (species) and lowercase (treatment) letters together are shown when there was no significant interaction. \*Percentage of the explained variance of general linear models, and level of significance:  $*P<0.05$ ;  $**P<0.01$ ;  $***P<0.001$ ; ns – nonsignificant.

	<i>Quercus petraea</i>			<i>Quercus pyrenaica</i>			Factors <sup>+</sup>		Sp × Tre
	LL	TLH	HL	LL	TLH	HL	Species	Treatment	
$A_{\text{leaf}}$ [ $\text{cm}^2$ ]	167 (42) <sup>a</sup>	182 (59) <sup>a</sup>	1093 (145) <sup>b</sup>	173 (81) <sup>a</sup>	181 (41) <sup>a</sup>	414 (99) <sup>a</sup>	6.38 <sup>*</sup>	31.27 <sup>***</sup>	12.74 <sup>**</sup>
LMA [ $\text{g m}^{-2}$ ]	53.5 (3.9) <sup>A</sup>	50.5 (0.6) <sup>A</sup>	83.3 (7.4) <sup>B</sup>	59.3 (4.8) <sup>A</sup>	51.7 (3.9) <sup>A</sup>	75.2 (10.2) <sup>B</sup>	ns	72.17 <sup>**</sup>	ns
$\Psi_{\text{midday}}$ [MPa]	-0.57 (0.27) <sup>B</sup>	-1.63 (0.15) <sup>A</sup>	-1.19 (0.11) <sup>A</sup>	-0.60 (0.10) <sup>B</sup>	-1.27 (0.24) <sup>A</sup>	-1.10 (0.15) <sup>A</sup>	ns	65.02 <sup>**</sup>	ns
$P_N$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	9.3 (2.6) <sup>A</sup>	10.6 (0.7) <sup>A</sup>	14.3 (1.4) <sup>B</sup>	8.8 (0.7) <sup>A</sup>	7.0 (0.3) <sup>A</sup>	13.8 (1.0) <sup>B</sup>	ns	56.99 <sup>**</sup>	ns
$g_{\text{sw}}$ [ $\text{mmol m}^{-2} \text{s}^{-1}$ ]	112 (36)	124 (11)	141 (27)	102 (10)	82 (5)	149 (6)	ns	ns	ns
$g_i$ [ $\text{mol m}^{-2} \text{s}^{-1}$ ]	0.10 (0.02) <sup>A</sup>	0.08 (0.01) <sup>A</sup>	0.15 (0.00) <sup>B</sup>	0.12 (0.02) <sup>A</sup>	0.09 (0.01) <sup>A</sup>	0.14 (0.00) <sup>B</sup>	ns	64.40 <sup>***</sup>	ns
$R_l$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	1.12 (0.22) <sup>b</sup>	0.66 (0.11) <sup>a</sup>	0.76 (0.11) <sup>ab</sup>	1.55 (0.11) <sup>c</sup>	1.69 (0.06) <sup>c</sup>	1.01 (0.16) <sup>ab</sup>	50.70 <sup>***</sup>	3.53 <sup>*</sup>	24.43 <sup>*</sup>
$\Gamma^*$ [ $\mu\text{mol mol}^{-1}$ ]	58.2 (5.4) <sup>B</sup>	48.3 (0.7) <sup>B</sup>	36.3 (0.4) <sup>A</sup>	53.5 (1.5) <sup>B</sup>	58.7 (4.0) <sup>B</sup>	41.1 (1.8) <sup>A</sup>	ns	61.06 <sup>***</sup>	ns
$V_{\text{Cmax}}$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	66 (11) <sup>A</sup>	105 (5) <sup>AB</sup>	126 (8) <sup>B</sup>	55 (5) <sup>A</sup>	62 (15) <sup>A</sup>	119 (27) <sup>B</sup>	ns	54.00 <sup>**</sup>	ns
$J_{\text{max}}$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	105 (6) <sup>A</sup>	120 (5) <sup>A</sup>	156 (11) <sup>B</sup>	96 (7) <sup>A</sup>	104 (8) <sup>A</sup>	157 (26) <sup>B</sup>	ns	62.57 <sup>**</sup>	ns
$V_{\text{max}}/V_{\text{Cmax}}$	1.67 (0.24) <sup>B</sup>	1.14 (0.02) <sup>A</sup>	1.24 (0.01) <sup>A</sup>	1.76 (0.17) <sup>B</sup>	1.68 (0.23) <sup>B</sup>	1.31 (0.10) <sup>A</sup>	ns	42.36 <sup>*</sup>	ns
$V_{\text{Cmax}}/R_l$	67 (20) <sup>Ba</sup>	171 (32) <sup>Bb</sup>	171 (22) <sup>Bb</sup>	36 (6) <sup>Aa</sup>	37 (9) <sup>Aa</sup>	120 (29) <sup>Ab</sup>	34.59 <sup>**</sup>	25.26 <sup>**</sup>	ns
$C_c$ [ $\mu\text{mol mol}^{-1}$ ]	141 (9) <sup>Ab</sup>	100 (6) <sup>Aa</sup>	94 (10) <sup>Aa</sup>	157 (5) <sup>Bb</sup>	148 (7) <sup>Bb</sup>	120 (13) <sup>Ba</sup>	37.78 <sup>**</sup>	34.95 <sup>**</sup>	ns
$C_i - C_c$ [ $\mu\text{mol mol}^{-1}$ ]	97 (11) <sup>a</sup>	132 (9) <sup>b</sup>	95 (11) <sup>a</sup>	76 (9) <sup>a</sup>	83 (5) <sup>a</sup>	99 (8) <sup>a</sup>	19.07 <sup>*</sup>	ns	43.58 <sup>*</sup>



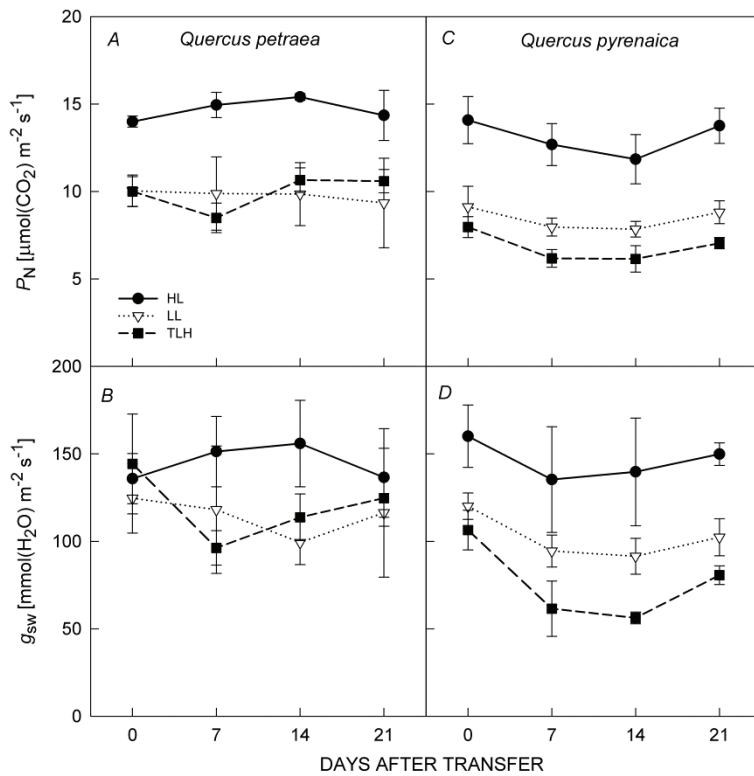


Fig. 1. Light-saturated gas exchange (net photosynthesis,  $P_N$ ; stomatal water conductance,  $g_{sw}$ ) after plant transfer (TLH) from low light (LL) to high light (HL) conditions. Measurements were performed at 0, 7, 14, and 21 days after plant transference. Values represent means  $\pm$  SE ( $n = 3$  in LL and TLH and  $n = 4$  in HL). LL =  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; TLH and HL =  $470 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

Table 2. Repeated Measures Analysis of Variance during 21 days of the trial shown in Fig. 1.  $P_N$  – net assimilation rate under saturating light;  $g_{sw}$  – stomatal water conductance.  $P$  values were given to test differences between light treatments and time.

	<i>Quercus petraea</i>			<i>Quercus pyrenaica</i>		
	Treatment	Time	Time $\times$ treatment	Treatment	Time	Time $\times$ treatment
$P_N$ [ $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ]	0.0564	0.7400	0.7368	0.0034	0.0359	0.9754
$g_{sw}$ [ $\text{mmol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$ ]	0.7427	0.3500	0.3696	0.0405	0.0374	0.9282

(Table 1). Transferring plants from low to high light had only minor effects on  $P_N$  and  $g_{sw}$ , but these effects differed between species (Table 2). In *Q. petraea*, transfer from low to high light led to an initial decrease in  $P_N$  and  $g_{sw}$  the first week after transfer (D7) that was followed by an increase in  $P_N$  and  $g_{sw}$  to values greater than those of LL plants (D14 and D21). By contrast, in *Q. pyrenaica* there was a similar decrease in  $P_N$  and  $g_{sw}$  from transfer to day 7, but little evidence for any subsequent increases in  $P_N$  and  $g_{sw}$  (Table 2).

HL plants had larger  $g_i$  than LL plants, and there was no evidence that transfer from low to high light affected  $g_i$  (i.e. TLH and LL had similar  $g_i$ ; Table 1). The relationship between the positive correlation of  $P_N$  with  $g_i$  had similar slopes in both species, but differences in intercepts meant that *Q. pyrenaica* had lower net assimilation rates than *Q. petraea* at the same  $g_i$  (slope  $P=0.63$ ,

intercept  $P<0.01$ ; Fig. 2A). The linear relationship of  $P_N$  with  $g_{sw}$  was steeper in *Q. pyrenaica* than *Q. petraea* ( $P<0.05$ ; Fig. 2B). The correlation of  $g_i$  with  $g_{sc}$  was weaker than the correlation of  $g_i$  or  $g_{sc}$  with  $P_N$ , especially for *Q. petraea* plants ( $P=0.21$ ; Fig. 2).

Mitochondrial respiration in the light ( $R_l$ ) and chloroplastic- $\text{CO}_2$  photo-compensation point ( $\Gamma^*$ ) were lower under HL conditions than LL (Table 1).  $R_l$  and  $\Gamma^*$  were generally similar in TLH plants and LL plants, with the exception of  $R_l$  in *Q. petraea*, which was the same as in HL plants.

**Key parameters of photosynthesis and relative limitations to carbon uptake:**  $V_{Cmax}$  and  $J_{max}$  were larger in HL plants than LL plants, while plants transferred from low to high light had  $V_{Cmax}$  and  $J_{max}$  similar to LL plants in *Q. pyrenaica* while reached intermediate values in *Q. petraea* (Table 1). In general, there was a strong

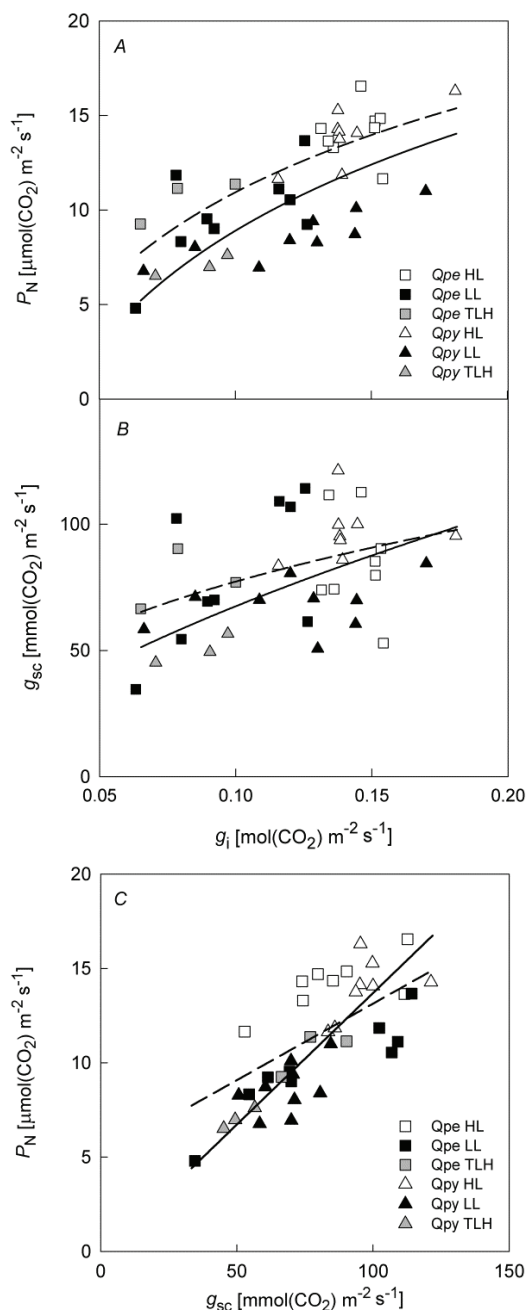


Fig. 2. A: Internal conductance ( $g_i$ ) correlation with net assimilation rate ( $P_N$ ); (*Q. petraea* in squares and dashed line  $P_N = 3.796 + 68.969 \lg(g_i)$ ;  $R = 0.75$ ,  $P < 0.001$  and *Q. pyrenaica* in triangles and solid line  $P_N = 0.3654 + 82.582 \lg(g_i)$ ,  $R = 0.77$ ,  $P < 0.001$ ). B: Internal conductance ( $g_i$ ) correlation with stomatal  $\text{CO}_2$  conductance ( $g_{sc}$ ), symbols and lines legend as in the previous box. Line regressions were for *Q. petraea* ( $g_{sc} = 146 + 67 \lg(g_i)$ ;  $R = 0.34$ ,  $P = 14.56$  and *Q. pyrenaica*  $g_{sc} = 174.4 + 105.7 \lg(g_i)$ ,  $R = 0.60$ ,  $P = 0.0052$ ). C:  $P_N$  vs.  $g_{sc}$  showing good correlation in both species: *Q. petraea*  $P_N = 5.022 + 0.0811 g_{sc}$ ;  $R = 0.65$ ,  $P = 0.0019$  and *Q. pyrenaica*  $P_N = -0.2419 + 0.1393 g_{sc}$ ,  $R = 0.89$ ,  $P < 0.001$ ). Measurements were performed before plants were transferred (TLH) from low light (LL) to high light (HL) conditions, and 21 days afterwards. LL (dark) =  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; TLH (grey) and HL (open symbols) =  $470 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

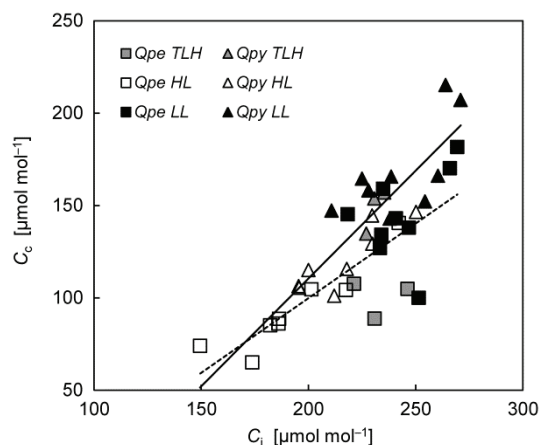


Fig. 3. Relationship between the  $\text{CO}_2$  concentration inside the chloroplasts ( $C_c$ ) and in the air spaces of the mesophyll ( $C_i$ ) along the trial. Measurements were performed at 0 and 21 days after plant transference. *Q. pe* = *Quercus petraea* (dashed line  $C_c = -61.92 + 0.8089 C_i$ ;  $R = 0.79$ ;  $P < 0.001$ ); *Q. py* = *Quercus pyrenaica* (solid line  $C_c = -122.9 + 1.1675 C_i$ ;  $R = 0.84$ ;  $P < 0.001$ ). LL =  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; TLH and HL =  $470 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

positive correlation between  $J_{\max}$  and  $V_{C_{\max}}$  ( $R^2 > 0.9$ , data not shown).  $J_{\max}/V_{C_{\max}}$  was larger in LL plants than HL plants and intermediate in TLH. Furthermore, the ratio of potential carboxylation to  $\text{CO}_2$  release via respiration in light ( $V_{C_{\max}}/R_l$ ) was higher in *Q. petraea* than *Q. pyrenaica* especially in TLH plants (Table 1). The  $\text{CO}_2$  concentration in the chloroplasts ( $C_c$ ) was higher in LL plants than HL plants, while TLH plants had a  $C_c$  similar to HL plants (*Q. petraea*) or intermediate between LL and HL (*Q. pyrenaica*). There was a strong positive correlation between  $C_c$  and  $C_i$  in both species, although the draw down from  $C_i$  to  $C_c$  was higher for *Q. pyrenaica* (slopes  $P = 0.11$ , intercept  $P < 0.001$ ; Fig. 3).  $C_i - C_c$  was unaffected by light treatment in *Q. pyrenaica*, whereas in *Q. petraea* it was significantly larger in TLH plants ( $132 \mu\text{mol mol}^{-1}$ ) than in LL ( $97 \mu\text{mol mol}^{-1}$ ) or HL ( $95 \mu\text{mol mol}^{-1}$ ) (Table 1).

Stomatal limitation ( $l_s$ ) was the largest restriction to  $P_N$  in all species and treatments (Table 3). However, limitation due to internal conductance ( $l_{mc}$ ) was larger in *Q. petraea* TLH than LL or HL plants (reaching same values as  $l_s$ ), whereas in *Q. pyrenaica*  $l_{mc}$  was unaffected by light treatments. The lowest percentage limitation was attributed to biochemical limitations ( $l_b$ ) in HL, and seedlings in LL of *Q. pyrenaica* showed the highest biochemical limitation. It is remarkable that *Q. petraea* showed significant higher  $l_{mc}$  and lower  $l_b$  relative limitations than *Q. pyrenaica* under LL, and especially for TLH (Table 3).

**Chl fluorescence and leaf pigment content:** All Chl fluorescence parameters ( $F_v'/F_m'$ ,  $\Phi_{PSII}$ ,  $q_p$ ,  $q_L$ , and ETR) showed that transferring plants from low light to high light decreases the efficiency of photosystem II relative to

Table 3. Relative limitations to carbon uptake, chlorophyll fluorescence, and photosynthetic pigments contents 21 days after transferring (TLH) plants from low-light (LL) to high-light (HL) conditions. Relative limitations to photosynthesis due to: stomatal conductance ( $I_s$ ), leaf-internal conductance ( $I_{mc}$ ), and biochemical capacity ( $I_b$ ) of the leaf; PSII maximum efficiency ( $F_v/F_m$ ); PSII operating efficiency ( $\Phi_{PSII}$ ); PSII efficiency factor ( $q_p$ ); fraction of PSII centres which are in open state ( $q_L$ ); rate of noncyclic electron transport through PSII (ETR); chlorophyll  $a$  and  $b$  contents (Chl  $a$  and Chl  $b$ ); total carotenoids pool content (Car). Mean values (standard error),  $n = 3$  in LL and TLH and  $n = 4$  in HL. LL = 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; THL and HL = 470  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Letters identify homogeneous groups by *Duncan* test ( $P=0.05$ ) as follows: *capital letters* indicate homogeneous groups within the significant factor; *lowercase letters* indicate homogeneous groups when the interaction between factors is significant; capital (species) and lowercase (treatment) letters together are shown when there was no significant interaction. \*Percentage of the explained variance of general linear models, and level of significance: \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$ ; ns – nonsignificant.

	<i>Quercus petraea</i>			<i>Quercus pyrenaica</i>			Factors <sup>+</sup>	
	LL	TLH	HL	LL	TLH	HL	Species	Sp x Tre
$I_s$ [%]	41 (4)	41 (2)	51 (7)	41 (4)	46 (3)	44 (2)	ns	ns
$I_{mc}$ [%]	30 (2) <sup>a</sup>	40 (3) <sup>b</sup>	27 (3) <sup>a</sup>	23 (3) <sup>a</sup>	27 (3) <sup>a</sup>	30 (3) <sup>a</sup>	14.40 <sup>*</sup>	42.32 <sup>*</sup>
$I_b$ [%]	29 (3) <sup>Ab</sup>	19 (1) <sup>Aa</sup>	22 (4) <sup>Aa</sup>	36 (1) <sup>Bb</sup>	27 (6) <sup>Ba</sup>	26 (5) <sup>Ba</sup>	28.95 <sup>*</sup>	ns
$F_v/F_m$	0.50 (0.03) <sup>B</sup>	0.41 (0.00) <sup>A</sup>	0.50 (0.01) <sup>B</sup>	0.50 (0.01) <sup>B</sup>	0.43 (0.02) <sup>A</sup>	0.49 (0.02) <sup>B</sup>	ns	42.32 <sup>*</sup>
$\Phi_{PSII}$	0.30 (0.03) <sup>B</sup>	0.20 (0.01) <sup>A</sup>	0.31 (0.00) <sup>B</sup>	0.26 (0.04) <sup>B</sup>	0.14 (0.02) <sup>A</sup>	0.29 (0.05) <sup>B</sup>	ns	71.18 <sup>**</sup>
$q_p$	0.59 (0.03) <sup>Bb</sup>	0.49 (0.02) <sup>Bab</sup>	0.61 (0.02) <sup>Bb</sup>	0.51 (0.03) <sup>Aab</sup>	0.30 (0.03) <sup>Aa</sup>	0.58 (0.06) <sup>Ab</sup>	28.49 <sup>*</sup>	41.42 <sup>*</sup>
$q_L$	0.42 (0.01) <sup>Bab</sup>	0.36 (0.02) <sup>Ba</sup>	0.44 (0.03) <sup>Bb</sup>	0.32 (0.02) <sup>Aab</sup>	0.18 (0.02) <sup>Aa</sup>	0.40 (0.05) <sup>Ab</sup>	36.68 <sup>*</sup>	30.46 <sup>*</sup>
ETR [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	88 (10) <sup>A</sup>	93 (4) <sup>A</sup>	143 (2) <sup>B</sup>	78 (13) <sup>A</sup>	65 (8) <sup>A</sup>	1134 (19) <sup>B</sup>	ns	79.68 <sup>***</sup>
Chl $a$ [ $\text{mg m}^{-2}$ ]	282 (20)	197 (23)	254 (45)	247 (28)	225 (36)	153 (35)	ns	ns
Chl $b$ [ $\text{mg m}^{-2}$ ]	82 (17)	78 (18)	83 (13)	104 (12)	100 (16)	74 (3)	ns	ns
Chl ( $a+b$ ) [ $\text{mg m}^{-2}$ ]	364 (34)	275 (33)	337 (58)	351 (40)	326 (64)	227 (38)	ns	ns
ETR/Chl ( $a+b$ )	0.24 (0.02) <sup>abc</sup>	0.34 (0.03) <sup>bc</sup>	0.37 (0.01) <sup>c</sup>	0.22 (0.03) <sup>ab</sup>	0.23 (0.07) <sup>a</sup>	0.69 (0.05) <sup>d</sup>	ns	16.27 <sup>***</sup>
Chl $a/b$	3.72 (0.66) <sup>B</sup>	2.87 (0.87) <sup>B</sup>	3.03 (0.30) <sup>B</sup>	2.36 (0.11) <sup>A</sup>	2.22 (0.13) <sup>A</sup>	2.06 (0.37) <sup>A</sup>	43.54 <sup>*</sup>	ns
Car [ $\text{mg m}^{-2}$ ]	74 (8)	62 (7)	77 (13)	68 (13)	64 (9)	63 (18)	ns	ns
Car/Chl ( $a+b$ )	0.21 (0.03)	0.22 (0.03)	0.23 (0.03)	0.19 (0.01)	0.20 (0.01)	0.27 (0.05)	ns	ns



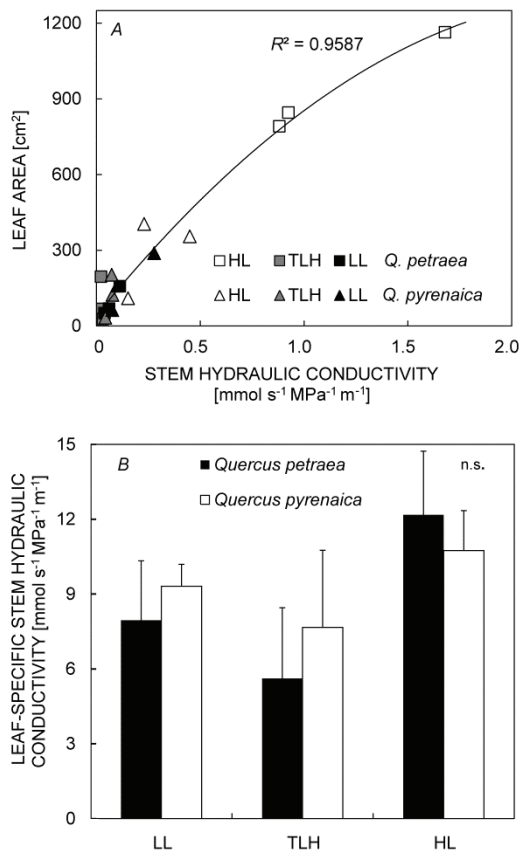


Fig. 4. Leaf-specific hydraulic conductivity ( $K_{h\text{-leaf}}$ ) 21 days after transferring the plants (TLH) from low light (LL) to high light (HL) conditions. Bars are mean values  $\pm$  SE ( $n = 3$  in LL and TLH and  $n = 4$  in HL). Nonsignificant (n.s.) differences among groups were found by Duncan test ( $P < 0.05$ ). LL =  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; TLH and HL =  $470 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

plants maintained under either low or high light (e.g. D21 in Table 3). In both species, TLH plants showed values of  $P_N$  and  $g_{sw}$  similar to LL at the end of the experiment without recovery of  $\Phi_{PSII}$ .

Leaf pigment contents were generally unaffected by light treatments (Table 3). Total Chl content [Chl ( $a+b$ )] was lower in TLH than LL plants. The total carotenoids

## Discussion

Mature leaves of neither species fully acclimated to transfer from low to high light. Even so, we found that species differed in their response to changes in the light regime. Photoinhibition was not overcome in *Q. pyrenaica* TLH and down regulation of  $g_{sw}$  led to smaller  $P_N$  compared to LL plants. In contrast, *Q. petraea* showed an increase in  $V_{Cmax}$  and  $J_{max}$  suggesting partial acclimation of leaf photochemistry that –along with the recovery of  $g_{sw}$ – caused an increase in  $P_N$  for TLH plants relative to LL plants. However, the extent of acclimation to high light in *Q. petraea* was strongly limited by

pool (Car) followed a similar pattern to total Chl. In TLH plants the ETR to total Chl content ratio [ETR/Chl ( $a+b$ )] was similar to HL plants in *Q. petraea*, whereas in *Q. pyrenaica* TLH leaves had lower ETR/Chl ( $a+b$ ) than under LL conditions (Table 3). The ratio of Chl  $a$  to Chl  $b$  (Chl  $a/b$ ) was the same in TLH and HL plants. Chl  $a/b$  was significantly higher in *Q. petraea* than *Q. pyrenaica* in all treatments.

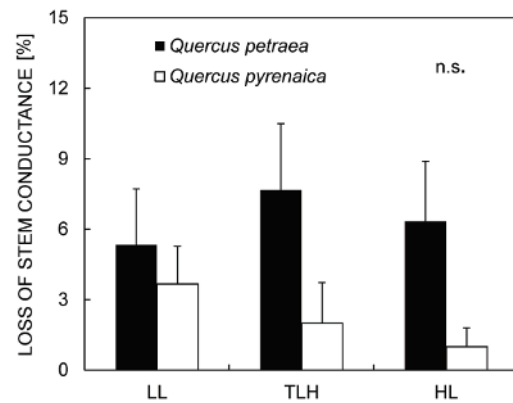


Fig. 5. Percentage loss of conductance (PLC) in stem samples 21 days after transferring the plants (TLH) from low light (LL) to high light (HL) conditions. Bars are mean values  $\pm$  SE ( $n = 3$  in LL and TLH and  $n = 4$  in HL). Nonsignificant (n.s.) differences among groups were found by Duncan test ( $P < 0.05$ ). LL =  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; TLH and HL =  $470 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

**Xylem hydraulics:** *Q. petraea* HL plants had much larger stem hydraulic conductivity ( $K_h$ ) than *Q. petraea* LL or TLH treatments, or any *Q. pyrenaica* treatment (Fig. 4A). The large difference in  $K_h$  is at least partially a function of differences in leaf area because differences were much smaller when data were expressed as leaf-specific stem hydraulic conductivity ( $K_{h\text{-leaf}}$ ).  $K_{h\text{-leaf}}$  tended to be smaller in LL and TLH plants than HL (Fig. 4B), but these differences were not significant probably due to the low number of replicates ( $n = 3\text{--}4$ ). In addition, the native percentage loss of hydraulic conductance (PLC) was very low (treatment average  $< 10\%$ , Fig. 5) in all plants. PLC did not differ among treatments, but was higher in *Q. petraea* than *Q. pyrenaica*.

a decrease of internal conductance.

Leaf ontogeny may limit and modulate acclimation to increased light because younger leaves are more plastic than mature leaves (Prioul *et al.* 1980, Sims and Pearcy 1992, Ruuska *et al.* 1994). Indeed a previous study with the same species found complete acclimation of gas exchange after transfer from low- to high light in young leaves (Rodríguez-Calcerrada *et al.* 2008a,b). Monti *et al.* (2009) observed a decrease in  $V_{Cmax}$  and  $J_{max}$  of mature shaded leaves of *Brassica carinata* 21 days after transfer from low to high light that was attributed to accelerated

leaf senescence. In our study we have no evidence of accelerated senescence in either species, although the leaves of the plants were probably rather old and nearing senescence given that they had low concentrations of Chls (Table 3) and low  $g_{sw}$  (Table 1).

Transfer of mature leaves of *Q. pyrenaica* from low- to high light led to photoinhibition that was maintained for at least 21 days and possibly limited the expected increase in photosynthetic capacity. This limited acclimation was suggested by some of the Chl fluorescence parameters.  $J_{max}$  and ETR were unaffected by transfer from low to high light (Table 1), so there was an excess of light that did not follow photosynthesis pathway, as is represented by low values of  $q_p$  especially in *Q. pyrenaica* (Table 3). This excess of energy might decrease the fraction of open PSII reaction centers and the yield of open centers (Müller *et al.* 2001). In turn, stomatal closure would prompt an increase in leaf temperature (data not measured) therefore decreasing the ability of *Q. pyrenaica* leaves to dissipate excess excitation energy as latent heat (Wong *et al.* 1985, Baker *et al.* 2007). In fact, the lower PSII efficiency (expressed as  $F_v'/F_m'$  at open reaction centres and  $\Phi_{PSII}$ ) observed in TLH plants at the end of the study could be a result of strong nonphotochemical quenching. However fluorescence in dark-adapted leaves was not measured and we can only hypothesize an increase in nonphotochemical quenching in *Q. pyrenaica* over *Q. petraea*.

More negative leaf water potential of TLH plants than HL plants (Table 1) suggested the occurrence of mild water stress, probably due to an increase in vapour pressure deficit and absence of change in  $K_h$  when changing from LL to HL conditions (Cochard *et al.* 1999, Barigah *et al.* 2006). The resistances to water flow and xylem vulnerability to cavitation are a function of xylem anatomy. Large vessels are more conductive, but they may also be more susceptible to dysfunctions by embolism (Sperry *et al.* 1994). In fact, the ring porous *Q. petraea* has larger vessels than *Q. pyrenaica* (Igboanugo 1990, Corcuera *et al.* 2006). However, *Q. pyrenaica* plants under the high light environment (HL and TLH) had smaller losses of hydraulic conductivity than under LL conditions (Fig. 5). Therefore it can not be ruled out that *Q. pyrenaica* keeps its hydraulic system intact by stomatal regulation and limited light acclimation (Rodríguez- Calcerrada *et al.* 2007, 2008a). On the other hand, although the percentage loss of conductivity (PLC) was very low due to optimal water availability, it was higher in *Q. petraea* in all cases, especially in TLH plants that reached the lowest  $\Psi_{midday}$ .

Limitations to the diffusion of  $CO_2$  were important for light acclimation of both oak species (Table 3). Some studies suggest that photosynthesis is limited by stomatal closure in plants transferred from low- to high light, but these earlier studies did not take into account  $g_i$  (Ellsworth and Reich 1992, Naidu and Delucia 1998).

Although low  $g_{sw}$  was observed throughout the experiment and was the main limitation in *Q. pyrenaica*,  $g_i$  was of equal importance in limiting photosynthetic acclimation to high light in *Q. petraea* at the end of growing season. These results confirm the importance of mesophyll conductance as limiting factor to photosynthesis in broadleaves species (Warren *et al.* 2007). In addition, a higher  $V_{Cmax}$  but a lower (although not significant)  $g_i$  than in LL treatment led to even lower  $C_c$  and higher  $C_i-C_c$  in *Q. petraea* TLH leaves in comparison to HL ones. Therefore, low  $C_c$  in *Q. petraea* TLH plants may have minimized the expected increase in  $P_N$  (Evans 1989, Hanba *et al.* 2002). It is generally assumed that  $g_i$  is mainly limited by the diffusion of  $CO_2$  in liquid phase, at least in deciduous broadleaved species (Gorton *et al.* 2003, Flexas *et al.* 2008). HL leaves are thicker than LL leaves and might be expected to have higher  $g_i$  due to greater chloroplast surface area exposed to intercellular airspaces (Evans *et al.* 1994, Piel *et al.* 2002, Warren *et al.* 2007). Several woody species increase the surface area of the chloroplasts bordering intercellular spaces upon transfer from low- to high light (Oguchi *et al.* 2006), thereby increasing  $g_i$  (Hanba *et al.* 2002). One logical reason  $g_i$  did not increase as expected may be because the leaves developed under shade were unable to change their morphology (Rodríguez- Calcerrada *et al.* 2008a). In addition, an increase in the effective water stress endured by the plants transferred to high light may have induced a decrease in  $g_i$  (Warren 2008, Centritto *et al.* 2009). In fact, *Q. petraea* TLH plants showed a  $\Psi_{midday}$  even lower than in HL plants (Table 1). Finally,  $g_i$  may also decrease with leaf ageing (Miyazawa and Terashima 2001) which may be accelerated under water stress (Galle *et al.* 2009) and/or increased irradiance (Monti *et al.* 2009). The uncoupling of relationships between photosynthetic capacity,  $g_{sc}$ ,  $V_{Cmax}$ , and  $g_i$  (Table 1, Fig. 2B) provides additional evidence that transfer from low- to high light disrupts normal relationships among photosynthetic parameters.

This study highlights different capacities and mechanisms of acclimation to an increase in light availability late in the growing season. *Q. petraea* benefitted from increased light by increasing  $V_{Cmax}$  that led the ratio  $V_{Cmax}/R_i$  reached HL levels (Table 1). In contrast, *Q. pyrenaica* was unable to acclimate their photosynthetic system and decreased stomatal conductance to minimize hydraulic failure when light intensity and evaporative demand increased. The conservative strategy of *Q. pyrenaica* would allow optimum water flow during flushing of the new leaves in the next growing season which would play a major role in interspecific competition at the new environment (Canham 1988, Naidu and Delucia 1998). Overall these findings are consistent with the hypothesis that the more shade-tolerant species, in our case *Q. petraea*, is more plastic to a transfer from low- to high light at the end of growing season (Yamashita *et al.* 2002).

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