

Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses

José Luis Quero^{1,2}, Rafael Villar², Teodoro Marañón³ and Regino Zamora¹

¹Grupo de Ecología Terrestre, Departamento de Ecología, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain; ²Área de Ecología, Facultad de Ciencias, Universidad de Córdoba, E-14071 Córdoba, Spain; ³Instituto de Recursos Naturales y Agrobiología, CSIC, PO Box 1052, E-41080 Sevilla, Spain

Summary

Author for correspondence:

José Luis Quero

Tel: +34 958 243242

Fax: +34 958 243238

Email: jlquero@ugr.es

Received: 19 December 2005

Accepted: 6 February 2006

- Here, we investigated the physiological and structural leaf responses of seedlings of two evergreen and two deciduous *Quercus* species, grown in a glasshouse and subjected to contrasted conditions of light (low, medium and high irradiance) and water (continuous watering vs 2-months drought).
- The impact of drought on photosynthetic rate was strongest in high irradiance, while the impact of shade on photosynthetic rate was strongest with high water supply, contradicting the hypothesis of allocation trade-off.
- Multivariate causal models were evaluated using d-sep method. The model that best fitted the dataset proposed that the variation in specific leaf area affects photosynthetic rate and leaf nitrogen concentration, and this trait determines stomatal conductance, which also affects photosynthetic rate.
- Shade conditions seemed to ameliorate, or at least not aggravate, the drought impact on oak seedlings, therefore, the drought response on leaf performance depended on the light environment.

Key words: deciduous, evergreen, leaf traits, Mediterranean oaks, photosynthesis, nitrogen, specific leaf area, water-use efficiency.

New Phytologist (2006) **170**: 819–834

© The Authors (2006). Journal compilation © *New Phytologist* (2006)

doi: 10.1111/j.1469-8137.2006.01713.x

Introduction

Light and water are main resources affecting leaf traits, regulating plant growth and survival, and determining the distribution of plants at global scale. The functional response of seedlings to the combination of shade and drought involves biochemical, physiological, and structural changes at the leaf and whole-plant level (Holmgren, 2000; Sack & Grubb, 2002; Sack, 2004; Aranda *et al.*, 2005). Some hypotheses predict that under limiting light availability (primary limitation), the shortage of another resource such as water should have less impact on plant performance (Canham *et al.*, 1996). In addition, shade by the tree canopy has indirect effects, such as reducing leaf and air temperatures, vapour pressure deficit and oxidative stress, that would alleviate the drought impact on

seedlings in the understorey (Holmgren, 2000). Empirical evidence of facilitation effects of shrubs and trees on seedlings in the understorey in Mediterranean environments has been widely documented (Castro *et al.*, 2004a; Gómez-Aparicio *et al.*, 2004). A contrary hypothesis predicts that deep shade will aggravate the stress imposed by drought, based on the proposed trade-off mechanism that shaded plants allocate more to shoot, and to leaf area, than to root, thereby diminishing the ability to capture water (Smith & Huston, 1989). In fact, some studies have found a higher impact of water stress on shaded plants (Abrams & Mostoller, 1995; Valladares & Pearcy, 2002). A third group of hypotheses posits that the effects of shade and water-shortage are independent, that is, their impacts are orthogonal (Sack & Grubb, 2002; Sack, 2004).

In woody species, there is a suite of leaf traits associated to leaf life span. Deciduous species tend to achieve higher photosynthetic and respiration rates and higher stomatal conductance, and have higher nitrogen (N) concentration in the leaf, compared with related evergreen species (Reich *et al.*, 1992, 1997; Villar *et al.*, 1995; Takashima *et al.*, 2004; Wright *et al.*, 2004). In Mediterranean environments, deciduous species tend to be more abundant in habitats with greater availability of water and nutrients, where the overstorey canopy is denser. Hence, it would be expected that seedlings of deciduous species are more shade-tolerant and water-demanding. By contrast, evergreen species tend to dominate in habitats that are drier and poorer in nutrients, where the overstorey canopy is sparse. We would therefore expect that seedlings of evergreen species are more tolerant to drought but not necessarily to shade.

One way to understand plants function is to explore leaf-trait relationships in different environmental conditions; however, most studies have discussed simple bivariate relationships. In order to develop a quantitative model of plant functioning relating to gas exchange, it would be necessary to move to multivariate relationships to be investigated by causal model (Meziane & Shipley, 2001). These authors proposed a model in which SLA was the forcing variable directly affecting both leaf N and net photosynthetic rate. Leaf N then directly affects photosynthetic rate, which in turn affects stomatal conductance. This model was found to agree with several datasets (Meziane & Shipley, 2001). To date, these models have not been applied to datasets with limiting light and water conditions, as are typical of Mediterranean forest.

We have designed an experiment with controlled conditions of light and water to investigate the physiological and structural leaf traits responses of tree seedlings to six combinations of light (three levels) and water (two levels). Four species of the same genus (*Quercus*) differing in leaf life span, were selected: two evergreens and two deciduous. Thus, we compared deciduous and evergreen species under the same genus, including the phylogeny in the design and data analysis.

There are some specific questions to investigate plant responses to different light–water scenarios: Are shade and drought impacts on seedlings positive, negative or independent? Do species or functional groups (evergreen vs deciduous) respond differently? Which physiological and structural leaf traits are most affected by the combined stress? What are the functional relationships among those variables? The answers to these questions would help to understand the functioning of plants and their implications for the species distribution in nature.

Materials and Methods

Experimental design

Acorns of four oak species, major components of Mediterranean forest – *Quercus suber* L., *Quercus ilex* ssp. *ballota* (Desf.) Samp. (evergreen), *Quercus canariensis* Willd. and *Quercus pyrenaica* Willd. (deciduous) – were collected in the south of Spain. At landscape scale, the evergreen species tend to occupy drier habitats than the deciduous species at each site, although the regional ranges overlap (see Table 1 for more details). Single acorns were weighted individually and sown (in December 2002) in cylindrical pots of 3.9-l volume (50 cm high × 10 cm diameter), thereby avoiding as much as possible interference during root growth. Pots contained a mixed soil of 2 : 3 sand and 1 : 3 peat. Ten grams of a slow-release fertilizer (Plantacote, Pluss NPK: 14 : 9 : 15, Aglukon, Valencia, Spain) were added at the middle of the experiment. The experiment was carried out in a glasshouse of the University of Córdoba (Spain, 37° 51' N, 4° 48' W; at an altitude of 100 m) with an automatic irrigation system and regulation of air temperature.

Oak seedlings were subjected to three light levels: (1) high-irradiance treatment (HI), receiving available radiation inside the glasshouse; (2) medium-irradiance treatment (MI), covered by a light-green screen (27% of available radiation); and (3) deep-shade or low-irradiance treatment (LI), covered by a dense green cloth (3% of available radiation). Each light treatment was imposed using a shade frame (150 × 120 × 200 cm) and

Table 1 Oak species included in the experiment (nomenclature follows Amaral, 1990), their leaf life span, frequency in southern Spain (calculated from 12572 records in the National Forest Inventory) and range of precipitation where they were recorded

Species	Origin of seeds	Functional group	Frequency in southern Spain (%)	Precipitation (mm)	
				Mean	Range
<i>Quercus canariensis</i> Willd.	Sierra del Aljibe (SE Spain)	Deciduous	2.4	1073	628–1338
<i>Quercus ilex</i> ssp. <i>ballota</i> (Desf.) Samp	Sierra Nevada (SW Spain)	Evergreen	50.8	668	268–1366
<i>Quercus pyrenaica</i> Willd.	Sierra de Cardeña (S Spain)	Deciduous	0.4	773	604–990
<i>Quercus suber</i> L.	Sierra del Aljibe (SE Spain)	Evergreen	15.8	839	489–1366

Data from the National Meteorological Institute; Urbietta *et al.* (2004).

Table 2 (a) Soil water content (measured with TDR) at the beginning, middle and end of the experiment (mean \pm SE) in a subsample of pots under the six light and water combinations; (b) results of the three-way ANOVA for the effects of water supply (W), irradiance treatments (I), and species (S), and their interactions at the end of the experiment for all pots where photosynthetic measurements were done

		Combined treatments					
		High water			Low water		
	Time (d)	LI	MI	HI	LI	MI	HI
(a)							
Soil water content (%)	0	13.8 \pm 0.6	12.4 \pm 0.6	12.0 \pm 0.6	13.1 \pm 0.6	10.3 \pm 0.6	11.1 \pm 0.6
	30	11.8 \pm 0.5	11.0 \pm 0.5	11.0 \pm 0.5	6.6 \pm 0.5	5.2 \pm 0.5	3.6 \pm 0.5
	60	12.8 \pm 0.4	13.2 \pm 0.4	13.2 \pm 0.4	3.2 \pm 0.4	2.4 \pm 0.1	2.2 \pm 0.1
(b)							
3-way ANOVA results	Factor	df	Mean squares	<i>P</i>			
	Water (W)	1	3053	< 0.001			
	Irradiance (I)	2	69.02	0.408			
	Species (S)	3	66.14	0.461			
	W \times I	2	16.70	0.804			
	W \times S	3	6.98	0.965			
	I \times S	6	32.96	0.856			
	I \times W \times S	6	7.63	0.996			
	Error	111	76.38				

HI, high irradiance; MI, medium irradiance; LI, low irradiance (see the Materials and Methods section for details).

replicated four times; therefore, there were 12 shade frames in total. Each of the four species and the two levels of watering were set up within each shade frame, each by one plant in a single pot. The experimental light treatments simulated the field conditions in the forest understorey, distinguishing three types of microhabitat: open (HI), under single tree cover (MI), and under shrub and tree cover (LI) (Marañón *et al.*, 2004). The mean \pm SE of the photosynthetic active radiation measured (with EMS7, canopy transmission meter, PP-system, UK) at midday on May 28, 2003, for each light treatment was 760 \pm 150, 187 \pm 27 and 23 \pm 2 μmol of photons $\text{m}^{-2} \text{s}^{-1}$ in HI, MI and LI respectively. Light quality (red:far red (R : FR) ratio, measured with sensor SKR 110; Skye Instruments, Llandrindod Wells, UK) was different from 1 only in LI, but this value (0.25 \pm 0.004) was similar to that for dense forest microhabitat (0.28 \pm 0.03, *t*-test, *P* = 0.31).

Pots were watered weekly during the first stage of the experiment. Once the seedlings emerged (January–February, 2003), a drip-irrigation system was inserted in the pots. Four months after sowing (at the end of April 2003), half of the pots stopped receiving any watering (low-water treatment, LW) while the other half was kept continuously moist (high-water treatment, HW). Low-water treatment simulated a typical Mediterranean-climate situation of seasonal drought, compared with a continuously moist one (HW) with reduced or no drought. During the experiment, we measured soil moisture (in volumetric water content, VWC), measured along the first 20 cm depth (with a TDR mod 100; Spectrum Technologies, Inc., Plainfield, IL, USA) each *c.* 3 d, in a subsample of five pots under different light and water treatments. Pots under LW decrease their water content similarly for the three

light treatments (Table 2a; repeated measures ANOVA, *P* = 0.17). At the same time of photosynthetic measurements (end of July 2003, *c.* 2 months after stopping irrigation), we measured VWC of each pot. For each water treatment, there were no differences in water content between the pots of different species or between the three different light treatments at the end of the experiment (Table 2b). The mean \pm SE values in July 2003, were 13.20 \pm 0.20% (for HW treatment) and 2.96 \pm 0.13% (for LW). The latter value was very similar to those found under field conditions at the end of the drought period (Gómez-Aparicio *et al.*, 2005).

Physiological and structural measurements

Photosynthesis response to irradiance was measured in mid-height fully expanded leaf of, in general, six plants per species and treatment combination. The measurements were done in the four different shade frames (replicates) for each light treatment to avoid pseudoreplication. We used a gas-exchange portable analyser (Ciras-2; PP-System, Hitchin, UK). The instrument was adjusted to have constant conditions of CO₂ concentration (360 p.p.m), flow (150 cm³ min⁻¹), and leaf temperature (25 C) inside the leaf chamber. Photosynthetic rate was measured at 10 light intensities of PAR obtained by using a quartz halogen light unit coupled to leaf chamber following the order 1000, 1300, 1500, 800, 600, 400, 200, 100, 50 and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 1), to reduce the equilibrium time required for stomatal opening and photosynthesis induction (Kubiske & Pregitzer, 1996). Each leaf was kept for 1 min at the same light intensity into the leaf chamber; net assimilation rate, transpiration rate and intercellular CO₂ concentration

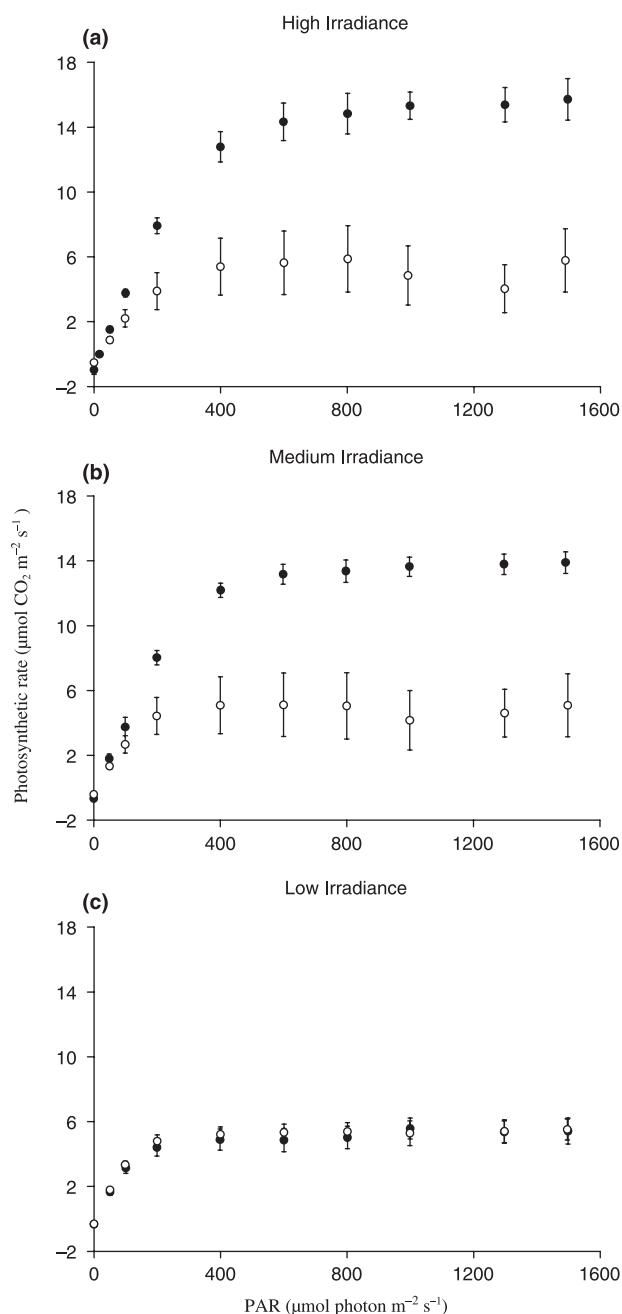


Fig. 1 Evolution of the photosynthetic rate (mean values and SE bars) with increasing irradiance (light curves), for seedlings (e.g. *Quercus pyrenaica*) cultivated in high (100%, a), medium (27%, b), and low (3%, c) irradiance, respectively. In each Figure (of light conditions), seedlings under continuous irrigation treatment (closed circles, high water treatment) are distinguished from seedlings subjected to drought (open circles, low water treatment).

were recorded three times, and the average value at each light intensity was calculated. Net CO₂ assimilation rates (A) were plotted against incident PAR, and the resulting curve was fitted by the nonrectangular hyperbola model of Thornley (1976):

$$A(I) = \frac{\sqrt{(\Phi I + A_{max})^2 - 4\theta I A_{max}} - R_d}{2\theta} \quad (1)$$

(A , photosynthetic rate; I , photosynthetic active radiation (PAR); Φ , apparent quantum yield; A_{max} , maximum light saturated assimilation rate; R_d , dark respiration rate; θ , 'bending degree' or curvature). Parameters of the model were calculated by the nonlinear estimation module (STATISTICA version 6.0, Statsoft, Inc., Tulsa, OK, USA). The variance explained by the model was very high (mean r^2 values of 0.98 ± 0.03). Despite its methodological importance, this value is rarely given, and comparison with other studies is difficult. Using this formula, by definition, the maximum photosynthetic rate is obtained at the infinite light intensity, and then overestimated. Therefore, we recalculated A_{max} (hereafter, A_{area}) assuming a PAR of $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$, the approximate maximum value for that season and latitude (Castro *et al.*, 2004b; Rey-Benayas *et al.*, 2005). The light saturation point (LSP) was calculated as the lowest value of PAR for which photosynthesis reached 90% of A_{area} . Water-use efficiency (WUE) values were calculated as A_{area} : stomatal conductance per area ($g_{s_{area}}$) ratio (Cavender-Bares & Bazzaz, 2000) and photosynthetic N-use efficiency (PNUE) as photosynthetic rate per mass (A_{mass})/N concentration (Field & Mooney, 1986).

In the same leaves, a chlorophyll index was measured using a CCM-200 (Optic Science, Hudson, NH, USA), which works similarly to SPAD (Minolta) and readings are well correlated with chlorophyll content. Leaves were then collected and scanned, and the area was measured with an image analyser (IMAGE PRO-PLUS version 4.5; Media Cybernetic, Inc., Silver Spring, MD, USA). They were oven-dried (at 80 °C for at least 48 h) and weighed. The specific leaf area (SLA) was calculated as the ratio between the leaf area and its dry mass. Leaves were ground with liquid N in an agate mortar, and analysed for N and carbon (C) concentration using an elemental analyser (Eurovector EA 3000; EuroVector SpA, Milan, Italy).

The level of response to the variation of each factor (light and water) was estimated by the indices $\text{Response}_{\text{light}}$ and $\text{Response}_{\text{water}}$, respectively, ranging from 0 to 1. The index of response was calculated as the difference between the maximum and the minimum mean values, divided by the maximum mean value. Although other authors called this the plasticity index (PI) (Valladares *et al.*, 2000a), we have preferred the neutral term 'Response' first, because in the case of water treatment, the seedlings had to adjust to a seasonal drought and were not acclimatized from the beginning of the experiment, and second, because we did not control possible genetic variability.

Statistical analyses

Mean (\pm SE) values of the 20 variables of seedling leaf performance, for each *Quercus* species and irradiance and water treatment, are shown in Appendix 1. To avoid pseudoreplication, we calculate the mean values of the different variables for each light treatment replicates. These mean values were used to test

the differences among species and the effects of light and water treatments on each variable by three-way ANOVAs (species, light, and water as source factors) with Type III sums of squares. Previously, ANCOVA was explored considering the seed mass as covariable; seed mass did not significantly affect leaf traits of 6-month-old seedlings ($P > 0.05$ in all cases); therefore, we present here only the ANOVA results for simplicity. A similar ANOVA procedure was used to explore the differences between deciduous and evergreen species, using leaf habit as factor instead of species. When the difference was significant, a multiple comparison of means test (*post hoc* Unequal *N* Tukey's Honestly Significant Difference test) was carried out. Before ANCOVA and ANOVA, data were square-root-, arcsine-, or log-transformed to satisfy the normality and homoscedasticity assumptions (Zar, 1984). Leaf-trait relationships were studied by Pearson's correlation analyses between pairs of variables, separating watered and drought conditions. The program STATISTICA version 6.0 (Statsoft, Inc., Tulsa, OK, USA) was used for statistical analyses.

In order to explain the empirical patterns of direct and indirect covariation between variables, a multivariate analysis was carried out to test for causal models linking changes in main leaf traits (SLA and N content) with physiological performance (photosynthetic rate and stomatal conductance), following Shipley's d-sep method (Shipley, 2000). Significance was fixed at the 0.05 level throughout the study. In order to control the inflation of type I error derived from repeated testing, the false discovery rate (FDR, the expected proportion of tests erroneously declared as significant) criterion was applied to repeated test tables throughout the paper. The FDR was controlled at the 5% level using a standard step-up procedure (see García, 2004). However, when testing multiple path models, we obtained an estimate for the expected number of erroneously accepted null hypotheses (type II errors), while controlling the FDR at the 5% level (Ventura *et al.*, 2004). This approach allowed us to focus the attention on those accepted models which had a low probability of being type II errors.

Results

Combined effects of shade and drought

The reduction in the availability of light and water imposed structural changes in the leaves of oak seedlings and affected their physiological performance (Figs 1 and 2).

Most variables showed strong interactions of light and water effects (as demonstrated by the ANOVAs, Table 3 and Fig. 2), reflecting that the drought impact on the physiological and structural traits of seedlings was highly significant under HI and MI but negligible under LI. Some exceptions were SLA and N concentration (Fig. 2).

Photosynthetic rate and stomatal conductance of the four oak species were similar along the three irradiance levels in HW (Fig. 2a,b). However, these traits decreased with irradiance under the LW. WUE (ratio between these traits) showed

differences in water treatments, being higher in LW. However, PNUE decreased in LW as whole (Table 3).

Leaves of oak seedlings grown under LI had higher SLA (Fig. 2c) and were richer in N (Fig. 2d).

Differences among *Quercus* species

Leaf structural traits were characteristic of each species and showed significant differences in the ANOVAs (see species as factor in Table 3; Appendix 1). For example, leaf area varied across the species (54% of variance explained) and SLA showed statistical differences among each of the four *Quercus* species (30% of variance), with the rank *Q. ilex* < *Q. suber* < *Q. pyrenaica* < *Q. canariensis* (Fig. 2C).

Fewer physiological features varied across the *Quercus* species (only 6 out of 13; Table 3). For example, A_{mass} differed among species (22% of variance; deciduous *Q. pyrenaica* and *Q. canariensis* had higher values than evergreen *Q. ilex* and *Q. suber*) (Fig. 2a). In general, the effects of shade and/or drought on physiological variables were higher than the interspecific variation (for example, LCP was highly affected by light (38% of variance), but varied only slightly across species (1% of variance)) (Table 3).

Differences between functional groups

Leaf traits of seedlings were related to the leaf habit. When the seedlings of deciduous species (*Q. pyrenaica* and *Q. canariensis*) were grouped and compared by ANOVAs with the evergreen species (*Q. ilex* and *Q. suber*), all seven leaf structural traits showed significant differences (Appendix 1). Seedlings of deciduous species had higher leaf area, SLA (Fig. 2c), and N concentration (N_{mass} , Fig. 2d), but lower chlorophyll (Chl) index (Appendix 1).

Differences in life span also predicted some variation in seedling physiological performance (significant ANOVAs for 5 out of 13 variables). Seedlings of deciduous species had higher A_{mass} (19% of variance), respiration rate per mass (R_{mass}) (13% of variance), PNUE (13% of variance), and stomatal conductance (3% of variance) than evergreens. There were no apparent differences between deciduous and evergreen seedlings in WUE.

Responses to variation of light and water

There was a high variation in the degree of response to light vs that to water, for the 20 variables measured (Fig. 3). Results for the four species were averaged to show the general response pattern. The response to light ($\text{Response}_{\text{light}}$) had a mean value of about 0.35 for the 20 variables, with a wide variation among them (Fig. 3). The structural water-induced response of leaf traits was very low (mean $\text{Response}_{\text{water}}$ of 0.07), while the general physiological response was relatively high (mean $\text{Response}_{\text{water}}$ of 0.35) (Fig. 3). Some variables had relatively persistent values even for stressed seedlings (low response traits).

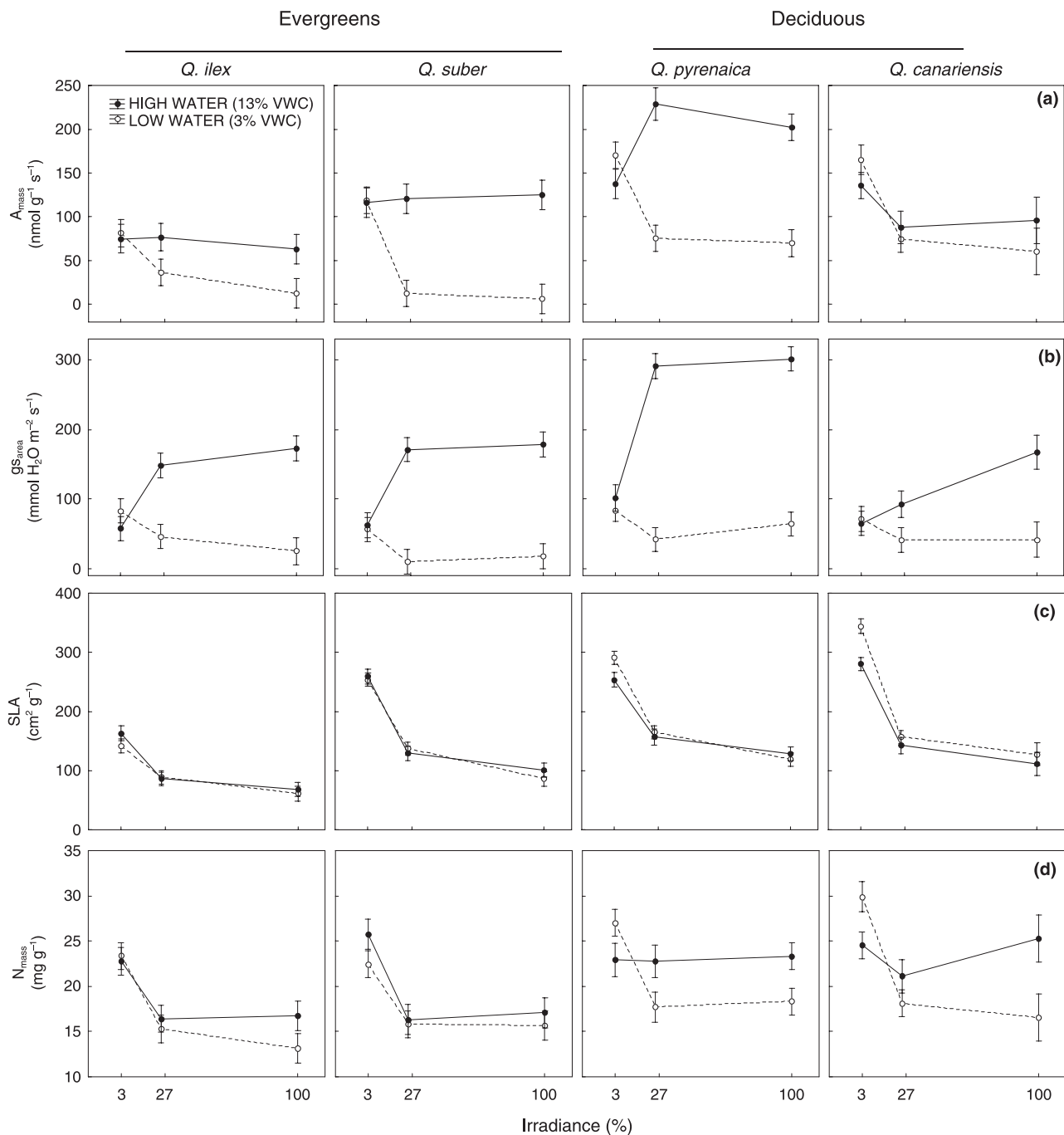


Fig. 2 General variation in leaf traits of oak seedlings (means and SE bars for replicates of the four *Quercus* species) in response to the six combinations of light and water treatments. Light levels are 'Low irradiance' (LI, 3%), 'Moderate irradiance' (MI, 27%), and 'High irradiance' (HI, 100%), and water levels are 'High water' (HW, solid line) and 'Low water' (LW, dashed line). a) A_{mass} , photosynthetic rate per mass ($\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$); b) $g_{\text{s,area}}$, stomatal conductance per mass ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); c) SLA, specific leaf area ($\text{cm}^2 \text{ g}^{-1}$); d) N_{mass} , nitrogen concentration (mg g^{-1}).

Among the variables exhibiting high response, some were highly affected by shade ($\text{Response}_{\text{light}} > 0.5$) but not affected by drought; the most remarkable example is SLA. By contrast, other leaf traits had high response in drought-affected seedlings ($\text{Response}_{\text{water}} > 0.5$), but were more independent of shade stress; the best example here is the $g_{\text{s,area}}$ and A_{area} .

Causal links among leaf structural traits and physiological performance

A diverse correlation patterns were revealed among leaf structural traits and physiological variables. These relationship patterns were similar for the four oak species between different variables

Table 3 Results of the three-way ANOVAs for some structural and physiological leaf traits, according to the factors oak species (S), and light (L) and water (W) treatments

Leaf traits	Species	Factors		Interactions				R ²
		Light	Water	L × W	S × L	S × W	S × L × W	
Structural traits								
Area	53.8***	12.1***	0.0	1.3	2.1	1.4	2.0	72.8
SLA	29.9***	63.7***	0.0	0.1	0.8	0.4	0.5	95.3
N _{mass}	14.5***	39.8***	5.5***	4.9**	3.5	0.4	5.3	73.9
C _{mass}	50.8***	1.0	<u>2.8*</u>	0.3	3.4	6.0*	2.2	66.5
N _{area}	29.0***	44.4***	3.5***	<u>1.8*</u>	2.1	1.7	2.2	84.6
C _{area}	34.1***	59.2***	0.0	0.1	0.9	0.6	0.4	95.3
Chl index	31.9***	5.2*	6.7**	6.5**	3.4	4.3	4.4	62.5
Physiological traits								
Φ	3.2	7.5*	19.6***	7.3*	4.7	2.3	5.9	50.5
θ	4.9	6.6*	1.6	9.3**	14.4*	4.4	9.1	50.3
LCP	0.9	38.2***	0.2	5.4*	2.7	7.7*	5.4	60.5
LSP	0.3	18.1***	10.1***	6.4*	9.1	4.2	5.2	53.5
A _{area}	5.8***	5.9***	37.0***	23.4***	2.3	4.0**	2.3	80.7
R _{area}	3.2	18.2***	16.7***	0.2	5.7	3.0	5.4	47.5
g _{sarea}	7.4***	2.0*	46.0***	24.2***	0.5	3.3**	1.6	84.8
A _{mass}	21.9***	14.2***	21.5***	14.4***	1.9	5.0**	2.9	81.7
R _{mass}	17.9***	3.4	12.5***	0.7	5.6	3.5	5.0	48.6
g _{smass}	19.2***	7.8***	30.8***	15.6***	0.2	4.6**	2.1*	80.3
WUE	2.4	3.0	19.9***	12.6***	7.8	8.6**	5.6	59.9
PNUE	17.5***	6.1**	24.1***	13.7***	1.5	7.6***	<u>6.0*</u>	76.6
Ci/Ca	2.1	<u>5.1*</u>	9.0***	10.7**	13.5***	9.0***	6.3	55.7

The proportion of the explained variance (SS_x/SS_{total}) and the level of significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) for each factor and the interactions are indicated; those values not remaining significant after controlling the false discovery rate are underlined. R² is the proportion of total variance absorbed by the model. Φ, quantum yield (no units); θ, curvature (no units); Area, leaf area (cm²); A_{area}, photosynthetic rate per area (μmol CO₂ m⁻² s⁻¹); A_{mass}, photosynthetic rate per mass (nmol CO₂ g⁻¹ s⁻¹); C_{area}, carbon content per area (g C m⁻²); C_{mass}, carbon concentration (mg g⁻¹); Chl index, chlorophyll index (no units); Ci/Ca, ratio internal vs external CO₂ concentration; g_{sarea}, stomatal conductance per area (mmol H₂O m⁻² s⁻¹); g_{smass}, stomatal conductance per mass (mmol H₂O g⁻¹ s⁻¹); LCP, light compensation point (μmol photons m⁻² s⁻¹); LSP, light saturation point (μmol photons m⁻² s⁻¹); N_{area}, nitrogen content per area (g N m⁻²); N_{mass}, nitrogen concentration (mg g⁻¹); R_{area}, respiration rate per area (μmol CO₂ m⁻² s⁻¹); R_{mass}, respiration rate per mass (nmol CO₂ g⁻¹ s⁻¹); PNUE, photosynthetic nitrogen-use efficiency (μmol CO₂ (mol N)⁻¹ s⁻¹); SLA, specific leaf area (cm² g⁻¹); WUE, water-use efficiency (μmol CO₂ (mmol H₂O)⁻¹).

shown in the four oak species (test of Homogeneity of slopes model, $P > 0.05$ for all cases; data not shown). In many cases, correlations between leaf traits differed depending on the water treatment (44% of bivariate relationships were different, Table 4). A_{mass} and R_{mass} were significantly correlated in both drought and watered conditions (Table 4). A_{mass} was also correlated with g_{sarea}, under drought and water treatments (Fig. 4d).

In some cases, leaf structural traits can be used as predictors of physiological performance. N_{mass} was a good predictor of g_{sarea}; but only for drought-affected seedlings (Fig. 4c). The specific leaf area (SLA) was a good predictor for several physiological activities. Seedlings of higher SLA tended to have higher photosynthetic rate (Fig. 4B), higher N_{mass} (Fig. 4a), and lower LCP and LSP (Table 4). The instantaneous water-use efficiency (WUE) was negatively correlated with the instantaneous photosynthetic N-use efficiency (PNUE) for watered seedlings, but not when affected by drought (Table 4). The SLA of drought-affected seedlings (unlike watered ones) was significantly correlated with PNUE. The WUE was not correlated with SLA for either of the water treatments.

The results of the multivariate analyses (d-sep test) of causal models linking leaf traits (SLA and N_{mass}) and physiological functions (A_{mass} and g_{smass}) are shown in Table 5 and Fig. 5. Model D was accepted by the whole dataset and most of the different light and water treatments. According to this model, the variation in SLA affects A_{mass} and N_{mass}, and this trait determines g_{smass}, which also affects photosynthetic rate. Model E, which best fitted the datasets in the study by Meziane & Shipley (2001), was also accepted by most datasets in this experiment but did not fit the data of LI, and hence it was rejected for the combined dataset (Table 5).

Discussion

Are the impacts of shade and drought on seedlings, positive, negative or independent?

Most leaf traits showed strong interactions in their responses to light and water treatments (Table 3; Fig. 2), and hence their variation was not independent. We did find that oak seedlings

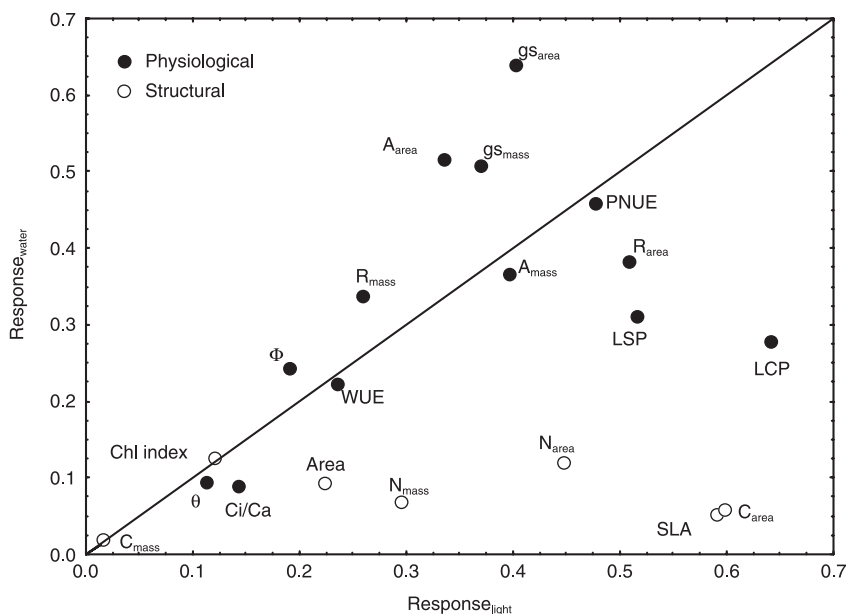


Fig. 3 Bivariate diagram of the comparative response to light ($\text{Response}_{\text{light}}$) vs the response to water ($\text{Response}_{\text{water}}$), for physiological and structural leaf traits, averaged for the four oak species. Response was calculated as (maximum value – minimum value)/maximum value. Φ , quantum yield (no units); θ , curvature (no units); Area, leaf area (cm^2); A_{area} , photosynthetic rate per area ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); A_{mass} , photosynthetic rate per mass ($\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$); C_{area} , carbon content per area (g C m^{-2}); C_{mass} , carbon concentration (mg g^{-1}); Chl index, chlorophyll index (no units); Ci/Ca, ratio internal vs external CO_2 concentration; g_{sarea} , stomatal conductance per area ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); g_{smass} , stomatal conductance per mass ($\text{mmol H}_2\text{O g}^{-1} \text{ s}^{-1}$); LCP, light compensation point ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$); LSP, light saturation point ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$); N_{area} , nitrogen content per area (g N m^{-2}); N_{mass} , nitrogen concentration (mg g^{-1}); R_{area} , respiration rate per area ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); R_{mass} , respiration rate per mass ($\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$); PNUE, photosynthetic nitrogen-use efficiency ($\mu\text{mol CO}_2 (\text{mol N})^{-1} \text{ s}^{-1}$); SLA, specific leaf area ($\text{cm}^2 \text{ g}^{-1}$); WUE, water-use efficiency ($\mu\text{mol CO}_2 (\text{mmol H}_2\text{O})^{-1}$).

Table 4 Pearson's correlation coefficients for some structural and physiological traits. Bold and normal letters represent high- (HW) and low- (LW) water treatments, respectively

		A_{mass}	g_{smass}	PNUE	R_{mass}	C_{mass}	N_{mass}	SLA	WUE	LSP
LCP	HW	-0.16	-0.05	-0.07	0.61***	-0.02	-0.18	-0.53**	<u>-0.36*</u>	0.59***
	LW	-0.76***	-0.75***	-0.75***	0.05	0.24	-0.56***	-0.61***	0.07	0.30
LSP	HW	0.18	0.38*	0.36*	0.41**	-0.15	<u>-0.33*</u>	<u>-0.49**</u>	-0.41*	
	LW	-0.28	-0.17	-0.30*	-0.15	0.20	-0.11	-0.31*	-0.30	
WUE	HW	-0.15	-0.62***	-0.28	-0.19	0.16	0.22	0.28		
	LW	-0.09	-0.27	-0.03	-0.22	-0.17	-0.26	-0.12		
SLA	HW	0.46**	0.24	0.14	0.26	-0.40*	0.71***			
	LW	0.74***	0.74***	0.66***	0.56***	-0.43**	0.80***			
N_{mass}	HW	0.36*	0.15	-0.11	<u>0.34*</u>	-0.21				
	LW	0.74***	0.78***	0.61***	0.49**	-0.25				
C_{mass}	HW	-0.57***	-0.57***	-0.51**	<u>-0.35*</u>					
	LW	-0.43**	-0.39*	-0.44**	-0.30					
R_{mass}	HW	0.38*	0.42**	0.25						
	LW	0.45**	0.43**	0.40**						
PNUE	HW	0.89***	0.83***							
	LW	0.98***	0.94***							
g_{smass}	HW	0.85***								
	LW	0.97***								

The level of significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) is indicated; those values not remaining significant after controlling the false discovery rate are underlined. C_{mass} , carbon concentration (mg g^{-1}); g_{smass} , stomatal conductance per mass ($\text{mmol H}_2\text{O g}^{-1} \text{ s}^{-1}$); LCP, light compensation point ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$); LSP, light saturation point ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$); N_{mass} , nitrogen concentration (mg g^{-1}); R_{area} , respiration rate per area ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); PNUE, photosynthetic nitrogen-use efficiency ($\mu\text{mol CO}_2 (\text{mol N})^{-1} \text{ s}^{-1}$); SLA, specific leaf area ($\text{cm}^2 \text{ g}^{-1}$); WUE, water-use efficiency ($\mu\text{mol CO}_2 (\text{mmol H}_2\text{O})^{-1}$).

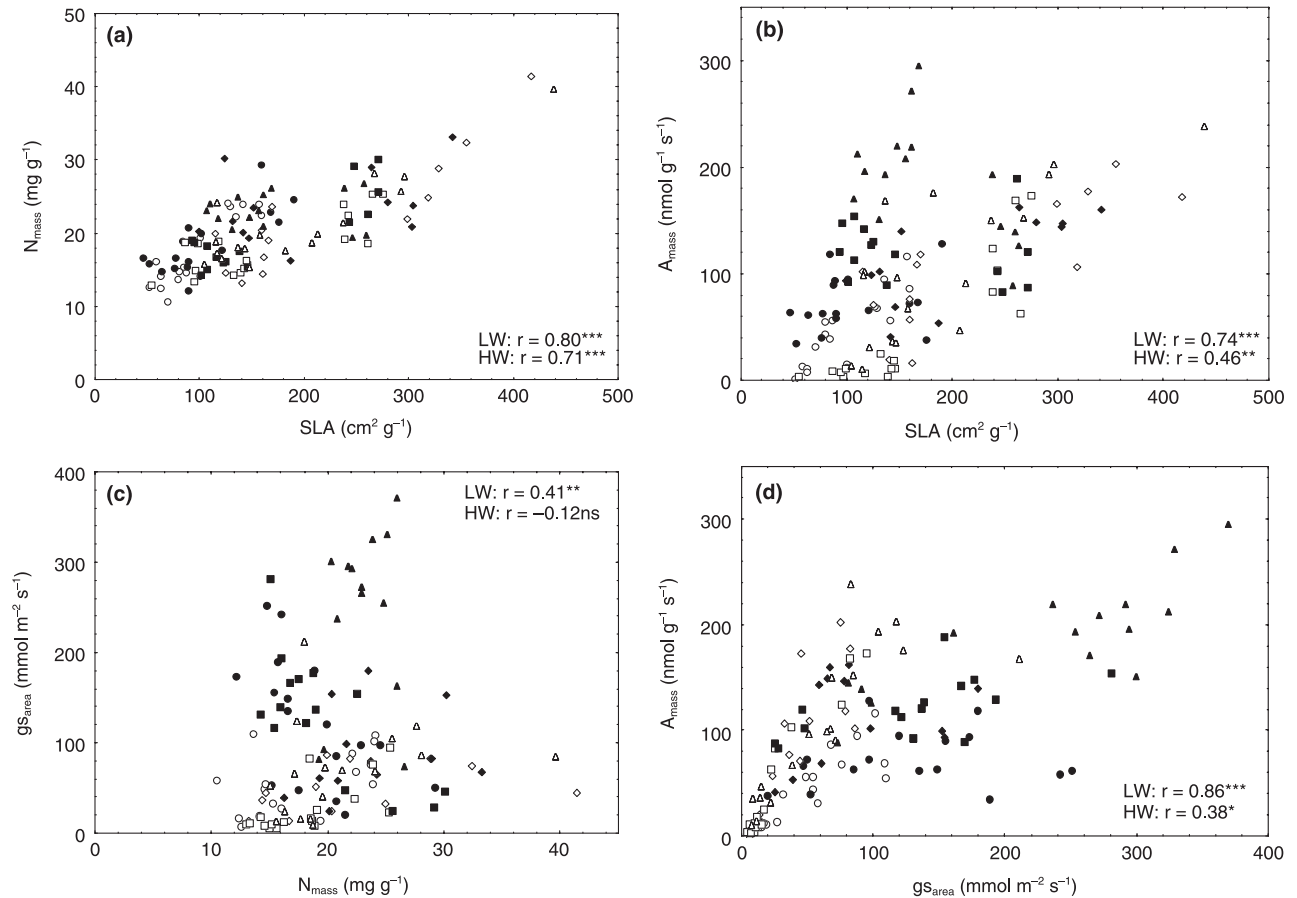


Fig. 4 Correlations between leaf traits. (a) Nitrogen concentration (N_{mass}) and (b) photosynthetic rate on mass basis (A_{mass}) vs specific leaf area (SLA); (c) stomatal conductance on area basis ($g_{s_{area}}$) vs N_{mass} ; (d) A_{mass} vs $g_{s_{area}}$. Circles, *Quercus ilex* ssp. *ballota*; diamonds, *Q. canariensis*; squares, *Q. suber*; triangles, *Q. pyrenaica*. Values for seedlings grown under high-water conditions (closed symbols) are distinguished from those grown under low-water conditions (open symbols).

Table 5 Probabilities under the null hypothesis that the data accord with each of the six proposed models, for each of the treatment conditions and combinations using the d-sep method

Treatment	Model type					
	A	B	C	D	E	F
HI HW	0.02258	0.01406	0.66464	0.01595	0.81801	0.36896
HI LW	0.66877	0.41394	0.74490	0.48724	0.07526	0.50847
MI HW	0.46033	0.52570	0.79599	0.34195	0.72478	0.67319
MI LW	0.45570	0.45980	0.57111	0.66589	0.64803	0.32076
LI HW	0.00745	0.01084	0.09345	0.23975	0.16812	0.11715
LI LW	0.01414	0.00251	0.07707	0.42238	0.06243	0.02955
HI	0.78200	0.14879	0.98447	0.11576	0.00618	0.95464
MI	0.48803	0.47054	0.26774	0.93449	0.01079	0.13347
LI	0.00001	0.00000	0.00623	0.02383	0.01079	0.01376
HW	0.14121	0.05122	0.07750	0.6833	0.08963	0.19061
LW	0.02473	0.00759	0.52332	0.10452	0.01823	0.39736
HI + MI	0.44674	0.09157	0.46729	0.35493	0.00001	0.22299
Combined	0.00044	0.00000	0.00017	0.17411	0.00000	0.00356

HI, high irradiance; MI, medium irradiance; LI, low irradiance or deep shade; HW, high water; LW, nonwatered (drought). Models that would be rejected at the 5% level on a per-test basis are shown in bold type. However those whose values remained significant after controlling the False Discovery Rate (FDR) at the 5% level, following the Ventura *et al.* (2004) criteria, are underlined.

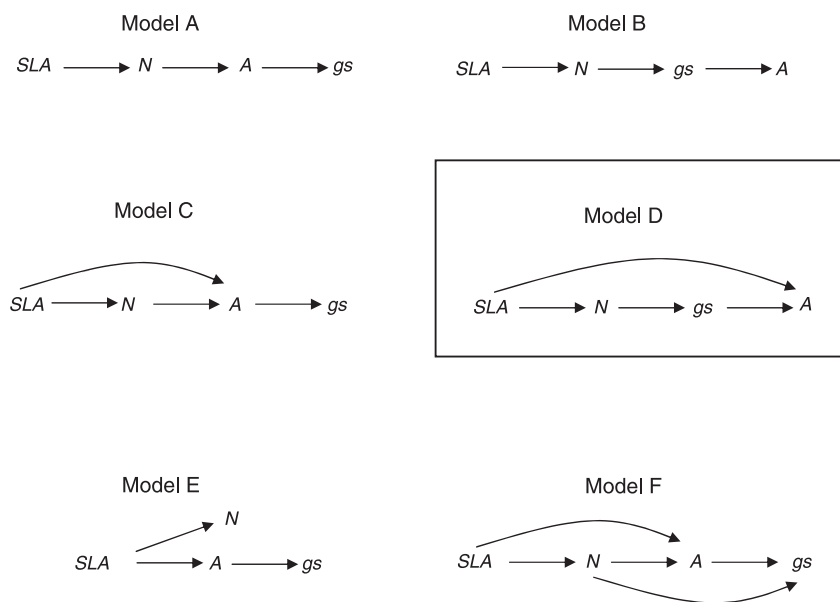


Fig. 5 Alternative multivariate models linking the specific leaf area (SLA), leaf nitrogen content on mass basis (N), net photosynthetic rate on mass basis (A), and stomatal conductance on mass basis (gs). Model D (framed) was the best fitted to the dataset of *Quercus* seedling responses to water and light treatments.

grown under deep shade increased their SLA, but they did not necessarily have a lower physiological performance, in terms of net photosynthetic rate, stomatal conductance or WUE when subjected to drought, as would be expected from the trade-off hypothesis (Smith & Huston, 1989). By contrast, under similar drought conditions, deep-shaded seedlings were able to achieve higher photosynthetic rate, stomatal conductance, and N concentration than seedlings under full light (Fig. 2). Moreover, under drought conditions, seedlings with higher SLA had higher A_{area} while lower R_{area} , indicating a higher positive C balance in these leaves (Table 4). The apparent alleviation of drought impact for seedlings growing in shade, demonstrated here under experimental conditions, could explain the pattern of higher seedling survival under shade of shrubs and trees (facilitation effect), commonly observed in Mediterranean forests (Castro *et al.*, 2004b; Gómez-Aparicio *et al.*, 2004; Marañón *et al.*, 2004).

Other studies have also found structural and physiological evidence supporting the hypothesis of shade as lessening the drought stress on seedlings of woody species (Holmgren, 2000; Prider & Facelli, 2004; Duan *et al.*, 2005). Conversely, plants under high irradiance, when subjected to water stress, suffer a more drastic reduction in net photosynthesis, and can be more predisposed to photo-inhibition, in comparison with plants in the shade (see References in Holmgren, 2000); although sunflecks can cause severe photoinhibition in shaded leaves (Valladares & Pearcy, 2002). However, Sack & Grubb (2002) and Sack (2004) found that the effect of shade and drought showed orthogonal impacts (no interactions) on final dry mass, relative growth rate, and biomass allocation on seedlings of different species. The authors proposed that seedlings are able to tolerate both shade and drought by developing plant features conferring reduced demand for light and/or water (see References in Sack & Grubb, 2002).

By contrast, there are studies showing negative responses to combined shade and drought conditions for *Quercus* species. In a controlled experiment, *Q. suber* seedlings grown in shade were less efficient in developing physiological mechanisms of water tolerance, in particular, osmotic adjustment and effective control of water loss (Aranda *et al.*, 2005). This has been found in field studies with other woody species (Valladares & Pearcy, 2002).

These contrasting results indicate that, physiological and structural mechanisms involved in the integrated responses of the tree seedlings to shade and drought strongly depend on plant functional type.

Do species or functional groups (evergreen vs deciduous) respond differently?

Seedlings of the deciduous species here (*Q. pyrenaica* and *Q. canariensis*) differed in leaf structure (higher values for leaf area, SLA and N, but lower chlorophyll concentrations) and in physiological activities (higher values of photosynthetic and respiration rates, stomatal conductance and PNUE) compared with seedlings of evergreen oaks (*Q. ilex* and *Q. suber*) subjected to the same conditions of light and water. A similar trend in structural and physiological differences between seedlings, associated with the contrasted leaf habit (deciduous vs evergreen) of adults, has been documented for other Mediterranean species (Villar *et al.*, 1995; Villar & Merino, 2001). Within the same genus *Quercus*, Takashima *et al.* (2004) found that the PNUE in evergreen species was lower than in deciduous ones; in evergreen oak seedlings the allocation of N to photosynthesis was smaller, while that to cell walls was greater, in order to acquire leaf toughness.

In general, leaf traits of seedlings of deciduous species allow them to achieve a higher relative growth rate than that of

seedlings of congeneric, evergreen species (Antunez *et al.*, 2001; Ruiz-Robledo & Villar, 2005).

Which physiological and structural leaf traits are most affected by the combined stress?

Leaf response to irradiance was very variable, in both structural and physiological traits (Fig. 3). For example, shade induced a relatively high variation in the key leaf trait SLA for all four oak species (mean $\text{Response}_{\text{light}}$ of 0.6), similar to the light-induced plasticity values found for evergreen tropical shrubs (16 *Psychotria* species, mean of 0.4; Valladares *et al.*, 2000b). The ability to respond to light by modifying leaf structural traits may confer shade tolerance by increasing light-capture efficiency (Valladares *et al.*, 2002b). At the same time, the relatively high responsiveness of leaf physiology may also indicate a tolerance to high irradiance (Valladares *et al.*, 2002a).

Drought induced a relatively low response in structural leaf traits but a high one in physiological traits (Fig. 3). In this experiment, we have simulated the Mediterranean climate seasonal drought that predictably occurs a few months after seedling emergence. When drought stress becomes more severe, first-year seedlings, grown under varied irradiance conditions, have already completed their growth, and therefore have low ability to modify structural leaf traits, which usually have a large ontogenetic component. However, they show a high physiological responsiveness to optimize photosynthesis : transpiration ratios under drought conditions.

What are the functional relationships among variables?

Because bivariate relationships are unsuccessful for making causal inferences, we tested several causal models of multivariate links among structural (SLA and N_{mass}) and physiological (A_{mass} and g_{mass}) leaf traits (Fig. 5) and accepted one of them (model D) as the best fit to the experiment results. According to this model, there is a direct causal relationship of SLA with dry mass concentration of cytoplasmic constituents, including N, which in turn affects stomatal conductance. Assuming that stomatal behaviour is regulated to maximize WUE, then the passive process of gas exchange across the stomata would result in the net photosynthetic rate (Meziane & Shipley, 2001). In addition, the model proposes a direct causal relationship of SLA with A, not mediated by leaf N. One explanation is that the accumulation of nonstructural carbohydrates will decrease SLA and reduce photosynthesis (Meziane & Shipley, 2001). Another explanation is that self-shading of chloroplasts in the lower part of thicker leaves (with lower SLA) will decrease the net C fixation on a leaf-mass basis (Reich *et al.*, 1999). Thus, there is a complex multivariate link among these three leaf traits: the ratio of leaf area to mass (SLA) is balanced with the amount of organic leaf N per mass (N_{mass}) to maximize photosynthesis rate (A_{mass}) mediated by stomatal conductance

(g_{mass}), hence optimizing loss of water by transpiration, which is so important in Mediterranean environments.

Ecological significance

The four Mediterranean oak species studied here share a general syndrome of leaf traits that can be suited to a 'reduced demand for resources' (Sack *et al.*, 2003), as well as part of a 'conservative resource-use strategy' (Valladares *et al.*, 2000a). Although in the physiological literature these traits are usually considered adaptations to the dry Mediterranean climate, most probably they are ancestral traits of Tertiary subtropical oaks, which allowed them to be sorted in when the climatic change imposing the seasonal drought typical of Mediterranean climate became established *c.* 3.5 million years ago (Herrera, 1992).

Within that general 'Mediterranean oak syndrome', there are interspecific differences in the seedling responses to light and water. The changes in structural leaf traits of leaf area, SLA, and concentrations of N and C, and the physiological performance of photosynthetic and respiration rates, and N efficiency (PNUE), were the most affected by the species factor in this experiment. These leaf traits are associated with the plant's physiological response to the abundance of resources, and determine their growth and survivorship (Lambers & Poorter, 1992; Wright *et al.*, 2004). For example, the seedlings of *Q. pyrenaica* showed the highest values of A_{area} , A_{mass} , g_{area} , g_{mass} , leaf area and PNUE compared with the other three oak species. These leaf traits would favour seedling growth in nutrient-rich and mesic habitats, but they may confer less tolerance to drought (see species distribution in Table 1).

Mediterranean drought, at all levels of light, is a problem for the seedling in terms of avoiding water loss and maintaining C uptake, and therefore of biomass gain. On the other hand, deep shade in the closed forest understorey environment, independently of water availability, can be a limiting factor in maintaining a positive C balance. In this experiment, the shade conditions seemed to ameliorate, or at least not aggravate, the drought impact on oak seedlings therefore drought response on leaf performance depend of light environment.

Acknowledgements

We thank the glasshouse staff of the University of Córdoba for their advice and Miguel Ángel Calero, Carlos Casimiro, Loles Bejarano, Ana Murillo, Juan Rubio, Francisco Conde, Francisco J. Morilla and Miguel A. Nuñez for their help during the experiment. We thank Lawren Sack, Fernando Valladares and Steve Long for their comments on a previous version of the manuscript, Luis V. García for his help with numerical analysis and Esteban Alcántara for his help with chlorophyll determinations. We thank three anonymous referees for comments and improvements on the manuscript. This study was supported by the grant FPI-MEC to J.L.Q. (BES-2003-1716), and by

the coordinated Spanish CICYT project HETEROMED (REN2002-04041). This research is part of the REDBOME network on forest ecology (<http://www.ugr.es/~redbome/>).

References

- Abrams MD, Mostoller SA. 1995. Gas exchange, leaf structure and nitrogen in contrasting successional tree species growing in open and understory sites during a drought. *Tree Physiology* 15: 361–370.
- Amaral J. 1990. *Quercus*. In: Castroviejo S, Lainz M, López González G, Montserrat P, Muñoz Garmendia F, Paiva J, Villar L, eds. *Flora Iberica*, Vol. II. Madrid, Spain: Real Jardín Botánico, CSIC, 15–36.
- Antunez I, Retamosa EC, Villar R. 2001. Relative growth rate in phylogenetically related deciduous and evergreen woody species. *Oecologia* 128: 172–180.
- Aranda I, Castro L, Pardos M, Gil L, Pardos JA. 2005. Effects of the interaction between drought and shade on water relations, gas exchange and morphological traits in cork oak *Quercus suber* L. seedlings. *Forest Ecology and Management* 210: 117–129.
- Canham CD, Berkowitz AR, Kelly VR, Lovett GM, Ollinger SV, Schnurr J. 1996. Biomass allocation and multiple resource limitation in tree seedlings. *Canadian Journal of Forest Research* 26: 1521–1530.
- Castro J, Zamora R, Hódar JA, Gómez JM, Gómez-Aparicio L. 2004a. Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: a 4-year study. *Restoration Ecology* 12: 352–358.
- Castro J, Zamora R, Hódar JA, Gómez JM. 2004b. Seedling establishment of a boreal tree species *Pinus sylvestris* at its southernmost distribution limit: consequences of being in a marginal, Mediterranean habitat. *Journal of Ecology* 92: 266–277.
- Cavender-Bares J, Bazzaz FA. 2000. Changes in drought response strategies with ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees. *Oecologia* 124: 8–18.
- Duan B, Lu Y, Yin C, Junttila O, Li C. 2005. Physiological responses to drought and shade in two contrasting *Picea asperata* populations. *Physiologia Plantarum* 124: 476–484.
- Field C, Mooney HA. 1986. The photosynthesis–nitrogen relationship in wild plants. In: Givnish TJ, ed. *On the economy of plant form and function*. Cambridge, UK: Cambridge University Press, 25–55.
- García LV. 2004. Escaping the Bonferroni iron claw in ecological studies. *Oikos* 105: 657–663.
- Gómez-Aparicio L, Zamora R, Gómez JM, Hódar JA, Castro J, Baraza E. 2004. Applying plant facilitation to forest restoration in Mediterranean ecosystems: a meta-analysis of the use of shrubs as nurse plants. *Ecological Applications* 14: 1128–1138.
- Gómez-Aparicio L, Valladares F, Zamora R, Quero JL. 2005. Response of tree seedlings to the abiotic heterogeneity generated by nurse shrubs: an experimental approach at different scales. *Ecography* 28: 757–768.
- Herrera CM. 1992. Historical effects and sorting processes as explanations for contemporary ecological patterns: character syndromes in Mediterranean woody plants. *American Naturalist* 140: 421–446.
- Holmgren M. 2000. Combined effects of shade and drought on tulip poplar seedlings: trade-off in tolerance or facilitation? *Oikos* 90: 67–78.
- Kubiske ME, Pregitzer KS. 1996. Effects of elevated CO₂ and light availability on the photosynthetic response of trees of contrasting shade tolerance. *Tree Physiology* 16: 351–358.
- Lambers H, Poorter H. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* 23: 187–261.
- Marañón T, Zamora R, Villar R, Zavala MA, Quero JL, Pérez-Ramos I, Mendoza I, Castro J. 2004. Regeneration of tree species and restoration under contrasted Mediterranean habitats: field and glasshouse experiments. *International Journal of Ecology and Environmental Sciences* 30: 187–196.
- Meziane D, Shipley B. 2001. Direct and indirect relation between specific leaf area, leaf nitrogen and leaf gas exchange effects of irradiance and nutrient supply. *Annals of Botany* 88: 915–917.
- Prider JN, Facelli JM. 2004. Interactive effects of drought and shade on three arid zone chenopod shrubs with contrasting distributions in relation to tree canopies. *Functional Ecology* 18: 67–76.
- Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD. 1999. Generality of leaf trait relationships: a test across six biomes. *Ecology* 80: 1955–1969.
- Reich PB, Walters MB, Ellsworth DS. 1992. Leaf lifespan in relation to leaf, plant and stand characteristics among diverse ecosystems. *Ecological Monographs* 62: 365–392.
- Reich PB, Walters MB, Ellsworth DS. 1997. From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences, USA* 94: 13730–13734.
- Rey-Benayas JM, Navarro J, Espigares T, Nicolau JM, Zavala MA. 2005. Effects of artificial shading and weed mowing in reforestation of Mediterranean abandoned cropland with contrasting *Quercus* species. *Forest Ecology and Management* 212: 302–314.
- Ruiz-Robledo J, Villar R. 2005. Relative growth rate and biomass allocation in 10 woody species with different leaf longevity using phylogenetic independent contrasts PICs. *Plant Biology* 7: 484–494.
- Sack L. 2004. Responses of temperate woody seedlings to shade and drought: do trade-offs limit potential niche differentiation? *Oikos* 107: 110–127.
- Sack L, Grubb PJ. 2002. The combined impacts of deep shade and drought on the growth and biomass allocation of shade-tolerant woody seedlings. *Oecologia* 131: 175–185.
- Sack L, Grubb PJ, Marañón T. 2003. The functional morphology of juvenile plants tolerant of strong summer drought in shaded forest understories in southern Spain. *Plant Ecology* 168: 139–163.
- Shipley B. 2000. A new inferential test for path models based on directed acyclic graphs. *Structural Equation Modelling* 7: 206–218.
- Smith T, Huston M. 1989. A theory of the spatial and temporal dynamics of plant communities. *Vegetatio* 83: 49–69.
- Takahshima T, Hikosaka K, Hirose T. 2004. Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. *Plant, Cell & Environment* 27: 1047–1054.
- Thornley JHM. 1976. *Mathematical models in plant physiology*. New York, NY, USA: Academic Press.
- Urbieta IR, Zavala MA, Marañón T. 2004. Distribución y abundancia de alcornoque *Quercus suber* L. y quejigo *Quercus canariensis* Willd. y su relación con factores ambientales en la provincia de Cádiz. *Revista de la Sociedad Gaditana de Historia Natural* 4: 183–189.
- Valladares F, Pearcy RW. 2002. Drought can be more critical in the shade than in the sun: a field study of carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year. *Plant, Cell & Environment* 25: 749–759.
- Valladares F, Martínez-Ferri E, Balaguer L, Pérez-Corona E, Manrique E. 2000a. Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytologist* 148: 79–91.
- Valladares F, Wright SJW, Lasso E, Kitajima K, Pearcy RW. 2000b. Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology* 81: 1925–1936.
- Valladares F, Chico JM, Aranda I, Balaguer L, Dizengremel P, Manrique E, Dreyer E. 2002a. The greater seedling high-light tolerance of *Quercus robur* and over *Fagus sylvatica* is linked to a greater physiological plasticity. *Trees, Structure and Function* 16: 395–403.
- Valladares F, Skillman J, Pearcy RW. 2002b. Convergence in light capture efficiencies among tropical forest understory plants with contrasting crown architectures: a case of morphological compensation. *American Journal of Botany* 89: 1275–1284.

- Ventura V, Paciorek CJ, Risbey JS. 2004. Controlling the proportion of falsely rejected hypotheses when conducting multiple tests with climatological data. *Journal of Climate* 17: 4343–4356.
- Villar R, Held AA, Merino J. 1995. Dark leaf respiration in light and darkness of an evergreen and a deciduous plant-species. *Plant Physiology* 107: 421–427.
- Villar R, Merino J. 2001. Comparison of leaf construction costs in woody species with differing leaf life-spans in contrasting ecosystems. *New Phytologist* 151: 213–226.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas ML, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.
- Zar JH. 1984. *Biostatistical analysis*, 2nd edn. Englewood Cliffs, NJ, USA: Prentice Hall.

Appendix 1

Mean \pm SE values of structural and physiological leaf traits analysed for *Quercus* seedlings in different light and water treatments. In general, there were four replicates per treatments, exceptions are indicated in parentheses

Trait	HI		MI		LI	
	LW	HW	LW	HW (<i>n</i> = 3)	LW	HW
<i>Quercus suber</i> (evergreen)						
Structural traits						
Area	9.2 \pm 0.6	10.8 \pm 0.6	12.6 \pm 0.6	12.1 \pm 0.8	8.3 \pm 0.5	9.9 \pm 0.8
SLA	90.32 \pm 6.61	101.62 \pm 3.38	137.59 \pm 3.17	131.01 \pm 5.71	246.85 \pm 6.84	259.54 \pm 4.81
N _{mass}	15.66 \pm 1.09	17.21 \pm 0.88	15.68 \pm 0.67	16.54 \pm 0.54	22.13 \pm 1.05	26.34 \pm 1.92
C _{mass}	480.3 \pm 4.2	446.1 \pm 5.7	468.9 \pm 4.4	455.9 \pm 6.7	470.1 \pm 3.3	456.7 \pm 13.1
N _{area}	1.77 \pm 0.19	1.71 \pm 0.13	1.15 \pm 0.06	1.27 \pm 0.06	0.90 \pm 0.04	1.02 \pm 0.08
C _{area}	5.52 \pm 0.58	4.40 \pm 0.11	3.43 \pm 0.12	3.50 \pm 0.11	1.91 \pm 0.06	1.76 \pm 0.06
Chl index	19.2 \pm 2.1	26.5 \pm 2.9	24.4 \pm 0.5	29.1 \pm 1.8	24.4 \pm 1.9	29.4 \pm 2.5
Physiological traits						
Φ	0.0274 \pm 0.0118	0.0470 \pm 0.0029	0.0312 \pm 0.009	0.0531 \pm 0.003	0.0438 \pm 0.0044	0.0450 \pm 0.004
θ	0.545 \pm 0.273	0.931 \pm 0.023	0.766 \pm 0.124	0.727 \pm 0.107	0.927 \pm 0.034	0.827 \pm 0.089
LCP	45.2 \pm 1.2	15.1 \pm 3.7	27.0 \pm 8.0	13.4 \pm 1.5	6.5 \pm 1.3	10.0 \pm 1.7
LSP	735.0 \pm 155.8	395.2 \pm 74.4	337.2 \pm 100.4	517.3 \pm 129.3	155.1 \pm 27.1	227.7 \pm 74.0
A _{area}	0.94 \pm 0.25	12.72 \pm 0.97	1.08 \pm 0.32	10.5 \pm 1.80	4.48 \pm 0.40	4.29 \pm 0.51
R _{area}	0.56 \pm 0.10	0.73 \pm 0.19	0.37 \pm 0.06	0.69 \pm 0.06	0.26 \pm 0.04	0.42 \pm 0.05
g _s _{area}	14.3 \pm 4.3	166.0 \pm 29.4	11.4 \pm 2.3	179.7 \pm 20.9	51.6 \pm 11.9	56.1 \pm 19.6
A _{mass}	6.9 \pm 1.5	126.8 \pm 9.2	14.8 \pm 4.2	115.7 \pm 13.7	111.1 \pm 11.4	121.6 \pm 23.7
R _{mass}	4.6 \pm 1.1	7.7 \pm 2.2	5.1 \pm 0.9	8.5 \pm 0.6	6.6 \pm 1.1	10.7 \pm 1.0
g _s _{mass}	0.10 \pm 0.02	1.78 \pm 0.42	0.16 \pm 0.03	2.07 \pm 0.15	1.28 \pm 0.31	1.72 \pm 0.78
WUE	76.6 \pm 8.2	80.1 \pm 7.0	10.6 \pm 2.3	57.8 \pm 7.0	96.5 \pm 11.8	93.7 \pm 13.9
PNUE	5.97 \pm 1.26	104.68 \pm 13.04	13.58 \pm 4.20	98.72 \pm 13.87	70.89 \pm 5.52	67.50 \pm 17.00
Ci/Ca	0.62 \pm 0.03	0.52 \pm 0.02	0.49 \pm 0.01	0.63 \pm 0.04	0.51 \pm 0.05	0.53 \pm 0.05

Trait	HI		MI		LI	
	LW (<i>n</i> = 3)	HW	LW	HW	LW	HW
<i>Quercus ilex</i> ssp. <i>ballota</i> (evergreen)						
Structural traits						
Area	7.9 \pm 0.8	8.4 \pm 0.3	8.6 \pm 0.9	8.0 \pm 0.3	7.3 \pm 1.1	5.5 \pm 0.4
SLA	61.00 \pm 1.27	64.64 \pm 8.98	91.68 \pm 5.01	86.91 \pm 1.35	142.74 \pm 6.64	166.74 \pm 8.86
N _{mass}	13.65 \pm 1.31	16.57 \pm 0.33	15.68 \pm 1.25	15.74 \pm 1.58	23.31 \pm 0.42	23.60 \pm 2.04
C _{mass}	481.8 \pm 1.9	473.8 \pm 5.5	477.6 \pm 2.1	469.6 \pm 5.3	460.4 \pm 3.4	462.8 \pm 5.3
N _{area}	2.27 \pm 0.24	2.72 \pm 0.35	1.71 \pm 0.11	1.82 \pm 0.19	1.65 \pm 0.08	1.45 \pm 0.13
C _{area}	7.98 \pm 0.16	7.88 \pm 1.13	5.26 \pm 0.29	5.42 \pm 0.07	3.25 \pm 0.17	2.84 \pm 0.17
Chl index	35.6 \pm 3.0	34.5 \pm 2.7	32.2 \pm 1.9	36.2 \pm 4.2	39.7 \pm 3.8	32.5 \pm 2.6
Physiological traits						
Φ	0.0163 \pm 0.0051	0.0470 \pm 0.0081	0.0413 \pm 0.0033	0.0524 \pm 0.0054	0.0502 \pm 0.0025	0.0404 \pm 0.0018
θ	0.805 \pm 0.157	0.892 \pm 0.065	0.906 \pm 0.053	0.874 \pm 0.053	0.801 \pm 0.049	0.896 \pm 0.025
LCP	24.3 \pm 1.90	27.2 \pm 2.9	19.0 \pm 4.5	15.1 \pm 4.1	6.4 \pm 1.2	14.6 \pm 4.0
LSP	325.0 \pm 65.1	508.0 \pm 122.0	158.8 \pm 41.4	497.3 \pm 99.0	294.7 \pm 36.2	233.3 \pm 33
A _{area}	1.98 \pm 0.25	9.39 \pm 1.50	3.56 \pm 1.33	9.23 \pm 1.15	6.10 \pm 0.65	4.43 \pm 0.27
R _{area}	0.46 \pm 0.18	1.20 \pm 0.26	0.76 \pm 0.17	0.79 \pm 0.19	0.31 \pm 0.05	0.57 \pm 0.15
g _s _{area}	25.7 \pm 4.3	170.1 \pm 12.3	38.4 \pm 15.5	152.2 \pm 8.4	85.8 \pm 8.8	61.8 \pm 13.5
A _{mass}	12.7 \pm 1.9	59.4 \pm 9.2	30.7 \pm 10.4	80.5 \pm 10.7	87.5 \pm 11.2	81.0 \pm 16.2
R _{mass}	2.9 \pm 1.2	8.1 \pm 2.8	7.1 \pm 1.9	6.8 \pm 1.6	4.5 \pm 0.7	8.9 \pm 2.6
g _s _{mass}	0.16 \pm 0.03	1.03 \pm 0.15	0.33 \pm 0.12	1.3 \pm 0.08	1.22 \pm 0.14	1.19 \pm 0.33
WUE	86.8 \pm 18.1	58.2 \pm 10.0	89.6 \pm 7.7	67.1 \pm 3.9	71.9 \pm 4.6	83.7 \pm 14.0
PNUE	14.01 \pm 3.55	49.63 \pm 6.80	28.83 \pm 10.71	74.49 \pm 13.75	52.70 \pm 6.62	47.53 \pm 8.7
Ci/Ca	0.56 \pm 0.08	0.64 \pm 0.05	0.54 \pm 0.04	0.60 \pm 0.02	0.60 \pm 0.02	0.56 \pm 0.06

Appendix 1 Continued

Trait	HI		MI		LI	
	LW (n = 3)	HW (n = 3)	LW	HW (n = 3)	LW (n = 3)	HW
<i>Quercus canariensis</i> (deciduous)						
Structural traits						
Area	11.2 ± 0.6	9.5 ± 0.8	12.6 ± 1.8	13.6 ± 5.4	11.7 ± 0.5	9.1 ± 1.2
SLA	127.81 ± 13.07	111.57 ± 11.98	154.83 ± 9.98	145.76 ± 5.69	339.72 ± 23.48	281.32 ± 8.23
N _{mass}	16.57 ± 3.37	25.26 ± 4.95	18.38 ± 1.90	21.91 ± 1.56	29.08 ± 3.92	24.60 ± 1.66
C _{mass}	451.4 ± 7.6	445.9 ± 11.4	451.4 ± 2.4	443.12 ± 7.14	445.1 ± 5.3	436.8 ± 9.1
N _{area}	1.34 ± 0.4	2.24 ± 0.2	1.18 ± 0.07	1.50 ± 0.05	0.85 ± 0.05	0.88 ± 0.08
C _{area}	3.58 ± 0.43	4.03 ± 0.33	2.9 ± 0.21	3.04 ± 0.07	1.32 ± 0.07	1.59 ± 0.09
Chl index	16.5 ± 0.9	30.1 ± 2.2	25.6 ± 1.4	28.0 ± 1.4	24.1 ± 1.4	24.2 ± 3.9
Physiological traits						
Φ	0.0342 ± 0.0104	0.0436 ± 0.0025	0.0325 ± 0.0048	0.0466 ± 0.032	0.0478 ± 0.0020	0.0448 ± 0.0062
θ	0.308 ± 0.308	0.744 ± 0.144	0.927 ± 0.029	0.760 ± 0.105	0.929 ± 0.036	0.896 ± 0.027
LCP	29.7 ± 4.6	20.5 ± 4.2	16.4 ± 4.3	16.0 ± 1.8	6.6 ± 1.5	9.1 ± 4.1
LSP	651.8 ± 256.3	619.7 ± 129.5	240.5 ± 49.2	430.4 ± 0.1	167.03 ± 47.7	222.9 ± 38.3
A _{area}	3.71 ± 2.58	10.54 ± 1.86	5.14 ± 0.74	7.45 ± 1.75	4.85 ± 0.77	5.08 ± 0.43
R _{area}	0.77 ± 0.28	0.84 ± 0.10	0.43 ± 0.06	0.63 ± 0.02	0.31 ± 0.07	0.33 ± 0.09
g _{sarea}	41.7 ± 22.4	174.3 ± 21.0	46.5 ± 11.4	125.4 ± 54.3	64.1 ± 16.0	66.8 ± 5.2
A _{mass}	60.8 ± 40.9	96.1 ± 3.1	79.6 ± 13.0	105.0 ± 34.4	155.0 ± 25.0	143.7 ± 8.8
R _{mass}	11.1 ± 4.2	10.7 ± 2.9	6.8 ± 1.3	9.0 ± 0.2	11.8 ± 3.7	9.1 ± 2.9
g _s ^{mass}	0.59 ± 0.39	1.71 ± 0.18	0.73 ± 0.21	1.78 ± 0.94	1.97 ± 0.48	1.89 ± 0.09
WUE	68.8 ± 16.2	60.6 ± 2.8	105.5 ± 13.9	69.3 ± 13.2	78.8 ± 7.0	78.4 ± 3.0
PNUE	46.22 ± 25.18	55.08 ± 9.05	60.35 ± 5.43	65.66 ± 17.49	76.17 ± 10.65	81.93 ± 6.22
Ci/Ca	0.64 ± 0.09	0.62 ± 0.02	0.46 ± 0.06	0.60 ± 0.04	0.59 ± 0.03	0.59 ± 0.01

Trait	HI		MI		LI	
	LW	HW	LW	HW	LW	HW (n = 3)
<i>Quercus pyrenaica</i> (deciduous)						
Structural traits						
Area	13.0 ± 2.2	16.5 ± 2.4	20.1 ± 2.2	18.3 ± 1.8	12.1 ± 1.5	14.0 ± 2.5
SLA	117.54 ± 4.69	123.63 ± 7.04	160.11 ± 8.96	158.63 ± 3.10	281.52 ± 29.14	255.15 ± 6.29
N _{mass}	17.75 ± 1.20	22.54 ± 0.96	17.71 ± 0.81	22.85 ± 1.25	25.95 ± 2.93	22.92 ± 0.25
C _{mass}	429.2 ± 11.4	439.7 ± 6.3	433.8 ± 0.9	437.2 ± 11.1	438.2 ± 6.8	440.6 ± 9.8
N _{area}	1.52 ± 0.07	1.85 ± 0.12	1.10 ± 0.07	1.44 ± 0.08	0.92 ± 0.02	0.92 ± 0.02
C _{area}	3.68 ± 0.19	3.61 ± 0.20	2.69 ± 0.17	2.76 ± 0.03	1.63 ± 0.14	1.76 ± 0.10
Chl index	19.2 ± 2.9	24.3 ± 1.7	23.0 ± 1.6	31.4 ± 1.5	29.0 ± 3.5	25.4 ± 3.4
Physiological traits						
Φ	0.0372 ± 0.008	0.0523 ± 0.0007	0.0310 ± 0.0047	0.0529 ± 0.0039	0.0451 ± 0.0059	0.0643 ± 0.0092
θ	0.980 ± 0.011	0.824 ± 0.057	0.974 ± 0.023	0.906 ± 0.026	0.797 ± 0.097	0.615 ± 0.069
LCP	19.7 ± 1.9	23.4 ± 3.6	11.6 ± 4.3	17.9 ± 6.7	9.6 ± 3.1	14.5 ± 7.0
LSP	293.8 ± 93.3	621.8 ± 54.7	153.2 ± 53.2	434.1 ± 21.8	294.1 ± 53.7	378.4 ± 46.6
A _{area}	4.69 ± 2.04	15.22 ± 1.27	4.00 ± 1.35	13.76 ± 0.22	5.51 ± 0.62	5.36 ± 0.80
R _{area}	0.55 ± 0.03	1.19 ± 0.17	0.32 ± 0.10	0.85 ± 0.24	0.38 ± 0.06	0.85 ± 0.38
g _{sarea}	52.7 ± 29.3	293.9 ± 10.7	34.6 ± 18.0	274.8 ± 33.4	86.1 ± 7.7	101.2 ± 11.2
A _{mass}	57.8 ± 26.8	187.2 ± 17.8	65.3 ± 23.8	234.1 ± 18.2	163.2 ± 24.8	135.6 ± 16.6
R _{mass}	6.6 ± 0.6	14.7 ± 2.1	5.3 ± 2.0	14.1 ± 4.5	11.3 ± 1.4	22.0 ± 10.3
g _s ^{mass}	0.67 ± 0.40	3.67 ± 0.34	0.58 ± 0.32	4.49 ± 0.44	2.53 ± 0.35	2.56 ± 0.23
WUE	108.0 ± 8.6	52.0 ± 5.0	147.2 ± 17.7	52.2 ± 4.7	63.8 ± 3.8	56.4 ± 4.8
PNUE	43.87 ± 18.12	115.63 ± 7.07	61.15 ± 26.96	143.31 ± 5.42	87.20 ± 9.58	87.99 ± 15.83
Ci/Ca	0.45 ± 0.04	0.62 ± 0.03	0.31 ± 0.06	0.63 ± 0.02	0.65 ± 0.02	0.68 ± 0.03

Light treatments: HI, high irradiance, MI, medium irradiance; LI, low irradiance. Water treatments: LW, low water; HW, high water. Φ, Quantum yield (no units); θ, curvature (no units); Area, leaf area (cm²); A_{arear}, photosynthetic rate per area (μmol CO₂ m⁻² s⁻¹); A_{mass}, photosynthetic rate per mass (nmol CO₂ g⁻¹ s⁻¹); C_{arear}, carbon content per area (g C m⁻²); C_{mass}, carbon concentration (mg g⁻¹); Chl index, chlorophyll index (no units); Ci/Ca, ratio internal vs external CO₂ concentration; g_{sarear}, stomatal conductance per area (mmol H₂O m⁻² s⁻¹); g_s^{mass}, stomatal conductance per mass (mmol H₂O g⁻¹ s⁻¹); LCP, light compensation point (μmol photons m⁻² s⁻¹); LSP, light saturation point (μmol photons m⁻² s⁻¹); N_{arear}, nitrogen content per area (g N m⁻²); N_{mass}, nitrogen concentration (mg g⁻¹); R_{arear}, respiration rate per area (μmol CO₂ m⁻² s⁻¹); R_{mass}, respiration rate per mass (nmol CO₂ g⁻¹ s⁻¹); PNUE, photosynthetic nitrogen-use efficiency (μmol CO₂ (mol N)⁻¹ s⁻¹); SLA, specific leaf area (cm² g⁻¹); WUE, water-use efficiency (μmol CO₂ (mmol H₂O)⁻¹).