

Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy?

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SUMMARY

We have explored leaf-level plastic response to light and nutrients of *Quercus ilex* and *Q. coccifera*, two closely related Mediterranean evergreen sclerophylls, in a factorial experiment with seedlings. Leaf phenotypic plasticity, assessed by a relative index ($PI = (\text{maximum value} - \text{minimum})/\text{maximum}$) in combination with the significance of the difference among means, was studied in 37 morphological and physiological variables. Light had significant effects on most variables relating to photosynthetic pigments, chlorophyll fluorescence and gas exchange, whereas nutrient treatment had a significant effect in only 10% of the variables. Chlorophyll content was higher in the shade whereas carotenoid content and nonphotochemical quenching increased with light. Nutrient limitations increased the xanthophyll-cycle pool but only at high light intensities, and the same interaction between light and nutrients was observed for lutein. Predawn photochemical efficiency of PSII was not affected by either light or nutrients, although midday photochemical efficiency of PSII was lower at high light intensities. Photosynthetic light compensation point and dark respiration on an area basis decreased with light, but photosynthetic capacity on a dry mass basis and photochemical quenching were higher in low light, which translated into a higher nitrogen use efficiency in the shade. We expected *Q. ilex*, the species of the widest ecological distribution, to be more plastic than *Q. coccifera*, but differences were minor: *Q. ilex* exhibited a significant response to light in 13% more of the variables than *Q. coccifera*, but mean PI was very similar in the two species. Both species tolerated full sunlight and moderate shade, but exhibited a reduced capacity to enhance photosynthetic utilization of high irradiance. When compared with evergreen shrubs from the tropical rainforest, leaf responsiveness of the two evergreen oaks was low. We suggest that the low leaf-level responsiveness found here is part of a conservative resource use strategy, which seems to be adaptive for evergreen woody plants in Mediterranean-type ecosystems

Key words: phenotypic plasticity, leaf-level traits, photosynthesis, *Quercus coccifera*, *Quercus ilex*, sun and shade, nutrients, xanthophyll cycle.

INTRODUCTION

Productivity of most vascular plants is mediated by leaves and many adaptations of plants to the environment involve leaf-level traits. This is the case with evergreenness and sclerophylly, leaf traits that have been associated with drought tolerance and low resource availability in Mediterranean-type eco-

systems (Turner, 1994; Aerts, 1995; Salleo *et al.*, 1997). Leaves, stems, and roots all respond to environmental stimuli in order to control plant development, but it is the metabolic responses of leaves to different environmental stresses that largely regulate the growth and development of both shoots and roots (Dickson & Isebrands, 1991). Plant performance in Mediterranean-type ecosystems depends not only on leaf adjustment to light, nutrients and moisture but also on its response to the

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multiple stresses that co-occur during summer (Di Castri *et al.*, 1981). Sclerophylls from these ecosystems exhibit remarkable leaf-level morphological and physiological adaptations to cope with high light intensity combined with heat and drought (Valladares & Pearcy, 1997; Martínez-Ferri *et al.*, 2000). Leaf response to multiple stresses can be very complex, since multiple stresses might be additive in their effects, and many interactions might take place. For instance, tolerance of high irradiance, an important constraint in arid environments (Valladares & Pugnaire, 1999), is affected by nutrient availability (Lambers *et al.*, 1998). When light is in excess of that used in photosynthesis, the carotenoids of the xanthophyll-cycle pool dissipate it harmlessly as heat (Demmig-Adams & Adams, 1992; Havaux *et al.*, 1998); their synthesis is affected not only by irradiance itself but also by ambient temperature, nutrient availability and other factors that can restrain the photosynthetic utilization of light (Lambers *et al.*, 1998).

Light is so heterogeneous in nature that different plants of the same species, different leaves of the same plant, and even the same leaf at different ontogenetic stages can be exposed to contrasting light regimes. In Mediterranean ecosystems, the heterogeneous light environment within a plant crown leads to differing stresses for leaves in different positions, and the morphological and physiological responsiveness to light of individual leaves within the crown determines not only the fate of each leaf but also the overall productivity of the crown (Caldwell *et al.*, 1986; Valladares & Pearcy, 1999). Thus, leaf plasticity is crucial not only for shade tolerance of plants (Henry & Aarssen, 1997; Niinemets, 1998a) but also for their tolerance of excessive light and heat (Valladares & Pearcy, 1997, 1998, 1999). Environmental heterogeneity in Mediterranean ecosystems also involves nutrient availability since irregular rainfall and fires create sudden nutrient increases in otherwise oligotrophic soils (Broncano *et al.*, 1998; Serrasolses & Vallejo, 1999). Most ecophysiological studies of plants have focused on mean plant performance but much less is known about plastic response to environmental change, especially in woody plants. As noted by Schlichting (1986), more work is needed on the comparative plasticities of wild plants to document the extent and nature of variation in phenotypic plasticity among taxa. In recent attempts at grouping plant species in functional types, the response to disturbance and the phenotypic plasticity of each taxa were relevant traits for functional classifications of Mediterranean plants (Lavorel *et al.*, 1999). Phenotypic plasticity might play a critical role in the response of natural populations to selective pressures in variable environments and might help explain differences in the ecological and geographical distribution of closely related taxa (Petit *et al.*, 1996).

We have explored the plastic, leaf-level response to light and nutrients of *Quercus ilex* (holm oak) and *Q. coccifera* (Kermes oak), two closely related, Mediterranean evergreen sclerophylls, in a factorial experiment using seedlings. *Quercus ilex* is present over a large area extending 6000 km longitudinally from Portugal to Syria, and 1500 km latitudinally from Morocco to France, whereas *Q. coccifera* is present over a smaller area, absent in the East of the Mediterranean basin and scarce or absent in continental areas experiencing very cold winters (Terradas, 1999). Our objectives were as follows: to study the leaf-level response of these two oak species to nutrients and light, plus the corresponding interactions, with regard to a large number (37) of morphological and physiological variables; to validate a plasticity index ((maximum value - minimum)/maximum) in combination with the significance of the difference among the means, as a simple method to assess both the extent and the significance of plant responsiveness to environment for variables with very different units and ranges of variation; to explore the adaptive value of high versus low responsiveness in relatively harsh environments such as Mediterranean-type ecosystems. On the basis of what could be expected from the abundant literature on plant response to light and nutrients (Lambers *et al.*, 1998; Berendse *et al.*, 1999; Pearcy, 1999), we tested three specific hypotheses: (1) according to the postulate that species from harsh environments are less plastic than those from more favourable environments (Lortie & Aarssen, 1996; Valladares *et al.*, 2000), the responsiveness of the two evergreen oaks should be low compared with that of evergreen woody plants from other more favourable biomes; (2) since the ecological distribution of a species might be partly determined by the plasticity of its individual members (Sultan, 1995), *Q. coccifera*, should be less plastic than *Q. ilex*; (3) as photo-protection via carotenoids interacts with both the light environment and the nutrient supply (Demmig-Adams & Adams, 1996; Skillman & Osmond, 1998), plants at low-nutrient supply should exhibit larger xanthophyll-cycle pools than at high-nutrient supply, especially under conditions of high light intensities.

MATERIALS AND METHODS

Plant material and experimental design

Acorns of two Mediterranean evergreen oaks, *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. and *Quercus coccifera* L., were planted in February 1996 in a nursery in the vicinity of Torremocha del Jarama (Madrid, Spain). The acorns were collected in autumn 1995; those of *Q. ilex* were collected in Valle del Tietar (Toledo, Spain) and those of *Q. coccifera* in Enguera (Valencia, Spain). The area of

Table 1. *Environmental conditions in the sun and in the shade during the period of in situ measurements (August 1998)*

	Sun	Shade	<i>P</i>
Daily photosynthetic photon flux density (PFD) (mol m ⁻² d ⁻¹)			
Mean	47.4 ± 8.5	9.1 ± 2	<0.001
Maximum	56.9	11.3	
Midday PFD (μmol m ⁻² s ⁻¹)			
Mean	1952 ± 134	506 ± 65	<0.001
Maximum	2320	640	
Maximum daily temperature (°C)			
Mean	37.4 ± 3.7	32.4 ± 2.7	<0.01
Maximum	41.3	35.4	
Minimum daily temperature (°C)			
Mean	11.5 ± 2.7	13.1 ± 2.6	=0.05
Minimum	6.4	7.7	
Mean daily temperature (°C)	23.4 ± 1.7	22.2 ± 1.7	<0.05
Mean relative humidity (%)	14.4 ± 4.9	18.5 ± 5.3	<0.05
Midday vapour pressure deficit (kPa)			
Mean	5.6 ± 1.4	3.9 ± 0.8	<0.05
Maximum	7.8	5.0	

Data are mean ± SD for 21 d of every 2-min recording, except for the absolute maxima and minima. Statistical differences between sun and shade were assessed by a nonparametric Kruskal–Wallis test.

origin of the acorns and the location of the nursery have a Mediterranean-type climate with a dry and hot summer and a cold winter; precipitation is mostly in autumn and spring. Following germination and initial growth, seedlings were placed in 15-l pots filled with washed river sand.

A factorial experiment of three factors (species, light and nutrient availability) at two levels each was designed to test for main effects and interactions on several morphological and physiological variables at the leaf level in these two evergreen oaks. A metal frame with several layers of neutral shade cloth was placed over half of the plants to produce a low-light environment (shade); the other half of the plants were kept outdoors (sun environment). A total of 32 plants, 16 of each species, was chosen at random for the different measurements. Microclimatic data of air temperature (T), photosynthetic photon flux density (PFD) and relative air humidity (rh) were gathered in the two light environments every 2 min throughout the day using a combination of cross-calibrated temperature sensors (thermistor; Grant Instruments, Cambridge, UK), quantum sensors (SKP210; Skye Instruments, UK) and relative humidity probes (HMP 35A; Vaisala, Finland) respectively, connected to a Squirrel 1200 datalogger (Grant Instruments). Air vapour saturation pressure deficit (VPD) was calculated from air temperature and humidity. During the main period of morphological and physiological measurements (Aug 1998), plants in the shade enclosure had a five times lower daily PFD than plants in the sun (Table 1). Both sites exhibited significant diel oscillations in temperature and humidity, which were slightly more

extreme in the sun environment (Fig. 1, Table 1). Even though there were not replicates of each light environment, the differences in other microenvironmental variables between the two light treatments were small, and instantaneous light readings during the middle of the day showed that light heterogeneity within each site was also low (coefficient of variation of 5–10%), especially when compared with the fivefold difference between the treatments. Thus, differences between individual plants exposed to the different light treatments are essentially due to differences in light availability, with little potential interference of within-site heterogeneity.

The influence of nutrient availability was studied by means of slow release nutrient pellets supplied to half of the sun and shade plants (nutrient-rich treatments); the other half was grown on sandy soil (nutrient-poor treatments). Fertilizers were applied in the form of 3.1 kg of Plantacote Mix 4 M (15:17:15, N:P:K) plus 4.4 kg of Guanumus Angibaud (3:35:2, N:P:K) per m³ of sand. At the end of the experiment, twice as much N and K, and three times as much P were available to the plants in the nutrient-rich treatment than in the nutrient-poor treatment (Table 2).

Morphological measurements and nutrient and pigment analyses

Before harvest, leaf angle was measured, using a protractor, in 80–100% of mature leaves in each of the 32 plants. Harvested plants were divided into leaves, stems and roots. A subset of the leaves of each

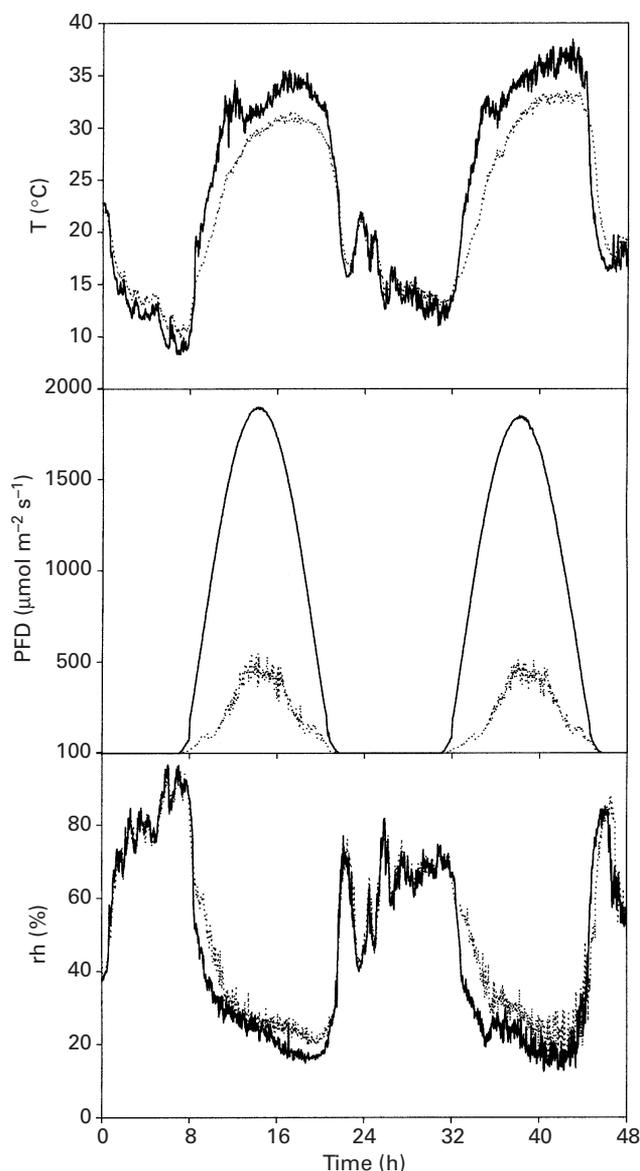


Fig. 1. Diel evolution of photosynthetic photon flux density (PPFD), temperature (T) and relative humidity (rh) in the sun (continuous lines) and in the shade (dotted lines) environments during 5 and 6 August 1998. These two clear days were typical of the period of physiological measurements. See Table 1 for mean values and statistics for the whole period.

plant was scanned with a PC compatible desk scanner at 300 dots per inch resolution, and the area and the perimeter of each individual leaf was estimated using commercially available image analysis software. The perimeter : area ratio was used as an index of lobulation of the leaf. This subset of leaves was weighed to calculate the specific leaf area (SLA). After these measurements, all leaves of each plant were pooled and finely ground. Soil and plant samples for laboratory analyses were dried for 48 h at 65°C. Eight replicates of each species were harvested from each nutrient treatment. Nutrient analyses were carried out at the Unit of Analysis of the Centre of Environmental Sciences (CSIC), Madrid, Spain. For analysis of total P and K, samples were digested in a mixture of HNO_3 and HClO_4 in a warm sand bath at ambient pressure. P and K were determined by emission spectrometry in an inductively coupled plasma (ICP5500; Perkin Elmer, Norwalk, CT, USA). Total N was determined by Kjeldahl analysis with SeSO_4 and K_2SO_4 as catalysers in a Digestion System 20 (Tecator; Stockholm, Sweden). N was automatically determined in a Kjeltec-auto 1030 analyser (Tecator). N, P and K contents are expressed on both a dry mass basis and a leaf area basis.

Before harvest, five leaf samples from five plants per species and treatment were taken before dawn and at noon and immediately stored in liquid N until extraction for pigment composition analysis. Samples were ground in a mortar with cool acetone (20 mg leaf tissue ml^{-1} solvent) and sodium ascorbate. After filtering through a 0.2- μm syringe nylon filter, 30 μl of acetone extract was injected into a Spherisorb ODS2 reverse-phase steel column (25 cm length, 4.6 mm inner diameter). Separation of chlorophylls and carotenoids was carried out in a HPLC system (Waters, Milford, MA, USA) equipped with a Waters 996 photodiode array detector as described by Val *et al.* (1994). Air was removed from solvents for HPLC analyses (LabScan, Ireland) by bubbling helium (Quality U; Air Liquide, Paris, France). Pure commercial

Table 2. Total nutrient content of the soils in the two light and two nutrient treatments at the end of the experiment (August 1998)

	Light treatment		Nutrient treatment	
	Sun	Shade	Nutrient-rich	Nutrient-poor
Soil N (mg g^{-1})	$0.39 \pm 0.22\text{a}$	$0.21 \pm 0.08\text{b}$	$0.41 \pm 0.19\text{A}$	$0.18 \pm 0.09\text{B}$
Soil P (mg g^{-1})	$0.41 \pm 0.21\text{a}$	$0.43 \pm 0.27\text{a}$	$0.63 \pm 0.14\text{A}$	$0.21 \pm 0.03\text{B}$
Soil K (mg g^{-1})	$1.94 \pm 0.76\text{a}$	$1.80 \pm 0.71\text{a}$	$2.41 \pm 0.21\text{A}$	$1.33 \pm 0.60\text{B}$

Data are means \pm SD of six independent samples. No interactions were found between light and nutrient treatments. Significant differences (ANOVA LSD test, $P < 0.01$) between the two levels of each treatment are indicated by a different letter.

standards (VKI, Denmark) were used for the identification and quantification of peaks. Leaf contents of violaxanthin (V), antheraxanthin (A), zeaxanthin (Z) and the xanthophyll-cycle pool (VAZ) were expressed on a total chlorophyll (Chl *a* + *b*) basis and leaf area basis. Leaf content of total carotenoids was expressed on an area, dry mass and chlorophyll basis. Neoxanthin, lutein and β -carotene contents were expressed on a chlorophyll basis only. The de-epoxidation state of the xanthophyll cycle (DPS) was calculated as the ratio of antheraxanthin and zeaxanthin to the total xanthophyll-cycle pool as described previously (Adams & Demmig-Adams, 1995).

Chlorophyll fluorescence measurements

In vivo Chl *a* fluorescence signal of five plants per species per treatment was measured predawn and at midday with a portable fluorometer PAM-2000 (Heinz Walz, Effeltrich, Germany) equipped with a leaf-clip holder, which monitored incident PFD and leaf temperature. Maximal (F_m) and minimal fluorescence (F_o) were measured predawn to calculate maximal photochemical efficiency of PSII (F_v/F_m). These F_m values were also used to calculate diurnal nonphotochemical quenching ($q_N = ((F_m - F_m')/(F_m - F_o'))$; Buschmann, 1995). Daily variation in quantum yield of noncyclic electron transport (Φ_{PSII}), photochemical quenching (q_P) and photochemical efficiency of the open reaction centres of PSII (F_v'/F_m') were assessed according to Genty *et al.* (1989).

Gas-exchange measurements

Photosynthetic response to irradiance was measured in one fully expanded current-year leaf of three plants per species per treatment during August 1998 with a portable open gas exchange system (LCA4, Analytical Development Co., Hoddesdon, UK). The central unit recorded incident PFD and cuvette temperature simultaneously. PFD was supplied by a halogen lamp and different intensities were obtained by using neutral filters. Temperature inside the cuvette was checked; when the internal temperature exceeded the external air temperature by $>4.0^\circ\text{C}$ the measurements were discarded. Net CO_2 assimilation rates (A) were plotted against incident PFD and the curve was fitted using the Photosyn Assistant software version 1.1.1. (Richard Parsons, Dundee, UK). The photosynthetic response of leaf to PFD was modelled by a rectangular hyperbola where the initial slope is the apparent quantum efficiency (Φ), the light compensation point (Γ) and apparent respiration are estimated from axis intercepts, and the photosynthetic capacity (A_{max}) is the upper asymptote. An addition parameter (convexity, Θ) was required to describe the progressive rate of

bending between the linear gradient and the maximum value. All of these parameters can be determined by fitting data to the model function, expressed as a quadratic equation by Chartier & Prioul (1976). Photosynthetic and respiration rates were expressed both on a dry mass basis and on a projected leaf area basis.

Plasticity index and statistics

An index of phenotypic plasticity ranging from 0 to 1 was calculated for each variable and species as the difference between the minimum and the maximum mean values between the two levels of each treatment divided by the maximum mean value, as in a previous study (Valladares *et al.*, 2000). This index was calculated for plant response to PFD and to nutrients independently, and it has the advantage that changes in variables expressed in different units and with contrasting variation ranges can be compared. Three-way ANOVA (LSD test, SYSTAT 6.0 Windows version 1996, SPSS Inc., Chicago IL, USA) was used to test for differences among species, light and nutrient treatments, and interactions. In all cases, the data met the assumptions of normality and homocedasticity. In the text, differences are significant at $P < 0.01$ unless otherwise specified.

RESULTS

Leaf morphology

Leaves of shade plants were larger, had a greater SLA, and were less lobed than their sun counterparts in both oak species (Table 3). In *Q. coccifera* a significant interaction ($P < 0.05$) was found between species and nutrient treatment for the degree of lobulation; leaves from the nutrient-poor treatment were more lobed than those in the nutrient-rich treatment. Leaf angle was also lower in the shade than in the sun in both species. Nutrient availability did not affect SLA or leaf angle, and its effect on the lobulation of the leaves was only significant in *Q. coccifera*; nutrient-poor treatment resulted in very lobed leaves in this species (Table 3).

Leaf-nutrient concentration

Leaves from enriched plants had a higher N content on a dry mass basis but not on a surface area basis in the two oak species (Table 4). Nutrient availability did not affect leaf content of P and K. Leaves of both species had more N and K in the sun than in the shade on an area basis but not on a dry mass basis, whereas the reverse was true (higher content in the shade, and on a dry mass basis but not on an area basis) for P. The N : Chl ratio was higher in the sun than in the shade, and in the nutrient-rich than in the nutrient-poor treatment in both species (Table 4).

Table 3. Mean leaf size, specific leaf area (SLA), degree of lobulation and leaf angle of the two evergreen oaks (*Quercus ilex* and *Q. coccifera*) in the different treatments

Code	Parameter	<i>Q. ilex</i>		<i>Q. coccifera</i>		<i>Q. ilex</i>		<i>Q. coccifera</i>	
		Sun	Shade	Sun	Shade	NR	NP	NR	NP
(1)	Leaf size (cm ²)	2.5a	6.3b	1.6a	3.9c	4.9A	3.9A,B	3.6A,B	1.9B
(2)	SLA (m ² kg ⁻¹)	3.8a	6.7b	4.5a	10b	5.3A	4.8A	7.7A	5.3A
(3)	Lobulation (perimeter/area, cm cm ⁻²)	3.8a	2.6b	5.0c	3.2a,b	3.1A	3.3A	3.4A	4.7B
(4)	Leaf angle (°)	39.1a	27.0b	33.1a	23.2b	33.9A	32.1A	27.6A	28.7A

Data are mean of eight independent samples. Significant differences (ANOVA, LSD test, $P < 0.01$) between the two levels of each treatment are indicated by different letters (lower case, light treatment, upper case, nutrient treatment). Significant interaction ($P < 0.05$) was found between species and nutrient treatment for the degree of lobulation. NP, nutrient poor; NR, nutrient rich. Numbers within brackets are the code used in Fig. 2.

Table 4. Nitrogen, phosphorus and potassium content (expressed on dry-mass and area basis), instantaneous nitrogen-use efficiency (NUE), and N:chlorophylls^{a+b} ratio of the leaves of the two evergreen oaks (*Quercus ilex* and *Q. coccifera*) in the different treatments

Code	Parameter	<i>Q. ilex</i>		<i>Q. coccifera</i>		<i>Q. ilex</i>		<i>Q. coccifera</i>	
		Sun	Shade	Sun	Shade	NR	NP	NR	NP
(5)	N (mg g ⁻¹)	22.3a	21.9a	18.3a	17.3a	24.5A	19.7B	19.9B	15.6C
(6)	N (g m ⁻²)	5.8a	3.3b,c	4.0a,b	1.7c	4.6A	4.1A	2.6A	2.9A
(7)	P (mg g ⁻¹)	1.3a	2.3b	1.7a,b	3.7c	1.4A	1.6A	3.6B	2.5A,B
(8)	P (g m ⁻²)	0.33a	0.34a	0.38a	0.37a	0.26A	0.35A	0.46A	0.47A
(9)	K (mg g ⁻¹)	9.1a	9.1a	8.6a	7.1a	9.2A	9.0A	8.5A	7.2A
(10)	K (g m ⁻²)	2.4a	1.4b,c	1.9a,b	0.7c	1.7A	1.9A	1.1A	1.4A
(11)	NUE ($\mu\text{mol CO}_2 \text{ g}^{-1} \text{ N s}^{-1}$)	1.2a	2.4b	1.1a	2.8b	1.6A	1.9A	1.7A	1.7A
(12)	N:Chl _{a+b} (mol N mol ⁻¹ Chl)	919a	381b	656c	208d	556A	509B	437C	350D

Data are mean of eight independent samples. Significant differences (ANOVA, LSD test, $P < 0.01$) between the two levels of each treatment are indicated by different letters (lower case, light treatment; upper case, nutrient treatment). No significant interactions ($P < 0.05$) were found among the three factors. NP, nutrient poor; NR, nutrient rich. Numbers within brackets are the code used in Fig. 2.

Instantaneous N-use efficiency (NUE; photosynthetic capacity divided by leaf N content) was higher in the shade than in the sun in both species and was not affected by nutrient availability. No significant differences were found between the two species in leaf N, P or K content, except in the N : Chl ratio, which was higher in *Q. ilex* than in *Q. coccifera*.

Leaf photosynthetic pigments

While differences in light availability had significant effects on photosynthetic-pigment (chlorophylls and carotenoids) variables, nutrient availability had very little effect on pigment composition of leaves in the two oak species (Table 5). Shade plants had a higher total chlorophyll (Chl_{a+b}) than sun plants on both an area basis and dry mass basis in the two oak species. Chl_a:Chl_b was not affected by the PFD

treatment. Total carotenoid content was higher in the sun than in the shade plants on a leaf area basis, but the opposite was true on a dry mass basis (Table 5). Total carotenoids on a chlorophyll basis exhibited a significant light \times nutrient interaction; in the sun, nutrient-poor plants exhibited higher carotenoid : chlorophyll, whereas in the shade carotenoid : chlorophyll was higher in nutrient-rich plants. The VAZ:Chl content was higher in the sun than in the shade, this was also the case for β -carotene, neoxanthin and lutein. The effect of nutrient availability on VAZ depended on light: whereas nutrient availability had no effect in the shade, nutrient-poor plants had higher VAZ than nutrient-rich plants in the sun. Lutein, which was the most abundant carotenoid, had a response to PFD similar to that found in VAZ and the same PFD \times nutrients interaction. DPS was relatively high and did not vary during the day in shade plants of both species,

Table 5. *Photosynthetic pigment composition of the leaves of the two evergreen oaks (Quercus ilex and Q. coccifera) in the different treatments*

Code	Parameter	<i>Q. ilex</i>		<i>Q. coccifera</i>		<i>Q. ilex</i>		<i>Q. coccifera</i>	
		Sun	Shade	Sun	Shade	NR	NP	NR	NP
(13)	Chl (mg m ⁻²)	378.9a	519.6b	366.2a	491.0b	445.5A	452.9A	426.1A	431.1A
(14)	Chl (mg g ⁻¹)	1.96a	3.54b	2.13a	3.86b	2.93A	2.57A	3.02A	2.97A
(15)	Chla:Chlb	2.4a	2.2a	2.3a	2.1a	2.3A	2.3A	2.2A	2.1A
(16)	Carotenoids (mg m ⁻²)	188.2a	163.9b	171.6b	115.5c	187.4A	164.8A,B	143.8B	143.3B
(17)	Carotenoids (mg g ⁻¹)	0.72a	1.09b	0.78a	1.15b	0.95A	0.85A	0.98A	0.97A
(18)	Carotenoids:Chl (mol mol ⁻¹)	0.604a	0.501b	0.596a	0.487b	0.548A	0.558A	0.546A	0.537A
(19)	VAZ:Chl predawn	168.6a	114.9b	173.3a	116.1b	138.4A	145.1A	143.8A	145.5A
(20)	VAZ:Chl midday	140.5a	112.5b	138.7a	105.9b	129.5A	123.4A	128.2A	116.5A
(21)	DPS predawn	0.16a	0.33b	0.15a	0.35b	0.24A	0.25A	0.30A	0.20A
(22)	DPS midday	0.52a	0.38b	0.57a	0.42b	0.46A	0.44A	0.49A	0.50A
(23)	β -carotene:Chl (mmol mol ⁻¹)	175.5a	155.6b	173.8a	139.0c	168.6A	162.4A,B	161.8A,B	151.0B
(24)	Neoxanthin:Chl (mmol mol ⁻¹)	56.3a	52.6b	55.7a	54.7a,b	53.7A	55.2A	54.6A	55.7A
(25)	Lutein:Chl (mmol mol ⁻¹)	218.1a	180.0b	210.3a	182.1b	191.8A	206.4A	193.5A	198.9A

Chlorophyll *a* + *b* (Chl) and total carotenoids are expressed on both area and dry mass bases. Total carotenoids are also expressed on a chlorophyll basis. The only pigments exhibiting diurnal changes were those of the xanthophyll cycle pool (V + A + Z, violaxanthin + antheraxanthin + zeaxanthin), so predawn and midday values are given separately for these pigments. Depoxidation state (DPS = [A + Z]/[V + A + Z]) is also given for predawn and midday separately. Values for individual carotenoids (VAZ, β -carotene, neoxanthin, and lutein) are expressed on a chlorophyll basis (nmol mol⁻¹). Data are mean of 10 independent samples, five measured predawn and five at midday. Significant differences (ANOVA, LSD test, $P < 0.01$) between the two levels of each treatment are indicated by different letters (in lower case for light treatment, in upper case for nutrient treatment). Significant interaction ($P < 0.05$) between light and nutrient treatments were found for Carotenoids:Chl, VAZ:Chl predawn, and Lutein:Chl. NP, nutrient poor; NR, nutrient rich. Numbers within brackets are the code used in Fig. 2.

Table 6. *Chlorophyll fluorescence data of the two evergreen oaks (Quercus ilex and Q. coccifera) in the different treatments*

Code	Variable	<i>Q. ilex</i>		<i>Q. coccifera</i>		<i>Q. ilex</i>		<i>Q. coccifera</i>	
		Sun	Shade	Sun	Shade	NR	NP	NR	NP
(26)	F_v/F_m	0.82a	0.82a	0.80b	0.81a,b	0.82A	0.81A,B	0.81A,B	0.80B
(27)	Φ_{PSII}	0.36a	0.73b	0.29a	0.67b	0.59A	0.51A,B	0.46B	0.49B
(28)	F_v'/F_m'	0.59a	0.81b	0.59a	0.76b	0.68A	0.72A	0.66A	0.68A
(29)	qP	0.62a	0.91b	0.48c	0.88b	0.85A	0.69B	0.66B	0.70B
(30)	qN	0.89a	0.71b	0.89a	0.71b	0.82A	0.78A	0.81A	0.79A

The variables are predawn photochemical efficiency of PSII (F_v/F_m), midday quantum yield of non-cyclic electron transport (Φ_{PSII}), midday photochemical quenching (qP), midday nonphotochemical quenching (qN), and midday photochemical efficiency of the open reaction centres of PSII (F_v'/F_m'). Data are mean of 10 independent samples. Significant differences (ANOVA, LSD test, $P < 0.01$) between the two levels of each treatment are indicated by different letters (lower case, light treatment in upper case, nutrient treatment). No significant interactions ($P < 0.05$) were found among the three factors. NP, nutrient poor; NR, nutrient rich. Numbers within brackets are the code used in Fig. 2.

whereas it exhibited significant diurnal changes in sun plants, being lower predawn and higher at midday than in shade plants (Table 5). Both species exhibited a very similar leaf concentration of photosynthetic pigments, and changes in their relative concentrations in response to PFD were also similar.

Chlorophyll fluorescence

Photochemical efficiency of PSII (F_v/F_m) predawn was only slightly lower than the theoretical optimum (0.84), and exhibited small differences among species and treatments (Table 6). Midday Φ_{PSII} was lower in sun than in shade leaves of both species, as was

Table 7. Gas exchange data of the two evergreen oaks (*Quercus ilex* and *Q. coccifera*) in the different treatments

Code	Variable	<i>Q. ilex</i>		<i>Q. coccifera</i>		<i>Q. ilex</i>		<i>Q. coccifera</i>	
		Sun	Shade	Sun	Shade	NR	NP	NR	NP
(31)	Photosynthetic capacity (A_{\max}) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	7.19a	8.02a	4.68b	4.89b	7.32A	7.89A	4.53B	5.06B
(32)	Photosynthetic capacity (A_{\max}) ($\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$)	37.1a	55.0b	27.4c	38.6a	48.0A	44.1A,B	31.4B	34.7A,B
(33)	Respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	1.63a	0.67b	1.05a,b	0.55b	1.00A	1.31A	0.90A	0.70A
(34)	Respiration ($\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$)	6.5a	4.6b	5.5a,b	5.9a	5.8A	6.8A	7.9A	5.0A
(35)	Quantum yield (Φ)	0.02a	0.02a	0.03a	0.01a	0.02A	0.02A	0.03A	0.02A
(36)	Curvature factor (Θ)	0.93a	0.88a	0.83a	0.88a	0.92A	0.89A	0.88A	0.83A
(37)	Light compensation point for photosynthesis (Γ)	92a	31b	88a	39b	44A	79A	82A	45A

Data are mean of eight independent samples. Significant differences (ANOVA, LSD test, $P < 0.01$) between the two levels of each treatment are indicated by different letters (lower case, light treatment; upper case, nutrient treatment). No significant interactions ($P < 0.05$) were found among the three factors. NP, nutrient poor; NR, nutrient rich. Numbers within brackets are the code used in Fig. 2.

F_v'/F_m' . At midday qP was also lower in the sun, but exhibited significant differences between the two species (*Q. ilex* had higher qP than *Q. coccifera* in the sun and in the shade). qP was the only chlorophyll fluorescence variable where a significant effect of the nutrient treatment was observed: fertilized plants of *Q. ilex* had higher qP than their nonenriched counterparts, whereas no significant effect was found in *Q. coccifera*. Nonphotochemical quenching at midday was higher in the sun than in the shade plants of both species (Table 6).

Leaf gas exchange

A_{\max} on a leaf area basis was not affected by PFD or nutrient treatments in either species (Table 7). However, A_{\max} on a dry mass basis was higher in the shade than in the sun in both evergreen oaks. *Quercus ilex* had higher A_{\max} than *Q. coccifera* both in the sun and in the shade on area and dry mass bases. Dark respiration was higher in the sun than in the shade in *Q. ilex* (on area and dry mass bases) but not in *Q. coccifera*. Dark respiration was similar in the two species. The light compensation point was lower in the shade plants of both species. Quantum yield and the curvature factor were not affected by the treatments and were similar in the two species. Nutrient treatment had no effect on any of the gas-exchange variables studied.

Leaf plastic response

Leaf responsiveness of the two species to PFD and nutrients was compared for different variables by means of the phenotypic plasticity index ((max - min)/max). A correlation between the plasticity index and the significance of the ANOVA was found for the plastic response to light but not to nutrients, owing to the large number of nonsignificant differences in the latter case (Fig. 2). However, certain variables, such as the quantum yield (slope of the photosynthetic light response curve), exhibited highly nonsignificant values of responsiveness to PFD, whereas moderate but highly significant responsiveness to PFD was obtained for other variables (i.e. leaf lobulation and chlorophyll content on an area basis; Fig. 2). Leaf responsiveness to PFD was greater than to nutrients, indicated by a larger mean responsiveness index (0.3 vs 0.1) and a larger percentage of variables with significant differences among treatments (70–80% vs 10%, Table 8). Responsiveness to PFD was greater and more significant for structural and pigment variables than for gas-exchange variables (Fig. 2, Table 8). Leaf responsiveness of the two species was very similar, although PFD response of *Q. ilex* was significant in 13% more of the variables studied than that of *Q. coccifera* (Table 8).

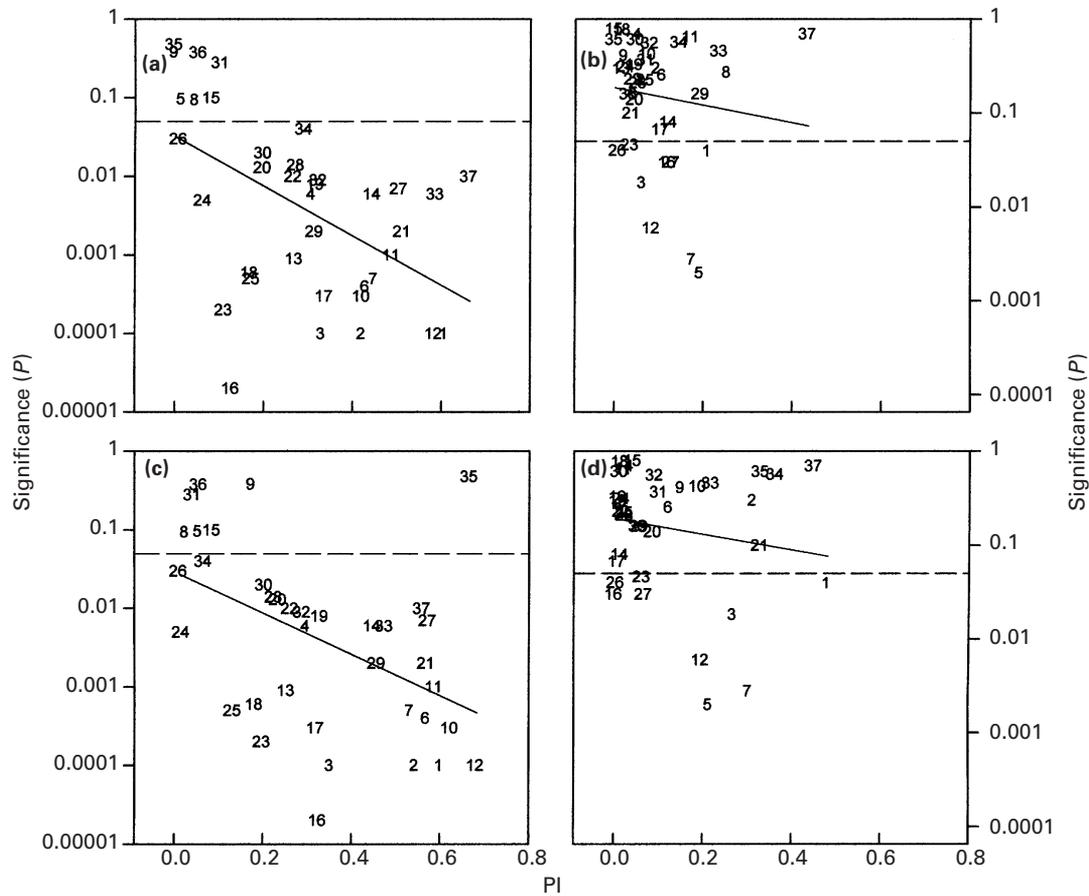


Fig. 2. Phenotypic plasticity index ($PI = (\max - \min)/\max$) against the significance of the difference between the means (P values from ANOVA) for 37 variables measured in *Q. ilex* (a,b) and *Q. coccifera* (c,d) in response to growth photosynthetic photon flux density (PFD) (a,c) and nutrient availability (b,d). The code for the variables is a consecutive numbering of those included in Tables 3–7; the number of each individual variable can be seen in the corresponding Table. Dashed lines indicate the significance threshold ($P = 0.05$). The coefficient of determination (the square of the correlation coefficient) values are as follows: a, $r^2 = 0.39$; b, $r^2 = 0.01$; c, $r^2 = 0.36$; d, $r^2 = 0.03$.

Table 8. Mean phenotypic plasticity index ($PI = (\max - \min)/\max$) of *Quercus ilex* and *Q. coccifera* to photosynthetic photon flux density (PFD) and nutrient treatments, and percentage of the variables with significant differences between the two levels of each treatment (ANOVA, $P < 0.01$) for the 37 variables of Tables 3–7

	<i>Q. ilex</i>		<i>Q. coccifera</i>		<i>Q. ilex</i>		<i>Q. coccifera</i>	
	Response to PFD	Response to nutrients	Response to PFD	Response to nutrients	Response to PFD	Response to nutrients	Response to PFD	Response to nutrients
	PI	Significant (%)	PI	Significant (%)	PI	Significant (%)	PI	Significant (%)
Structural variables (Table 3)	0.42a	100	0.11b	0	0.45a	100	0.28c	25
Nutrient variables (Table 4)	0.31a	75	0.14b	37	0.41c	62	0.15b	25
Pigment variables (Table 5)	0.24a	92	0.05b	0	0.26a	67	0.05b	0
Fluorescence variables (Table 6)	0.26a	80	0.09b	20	0.29a	80	0.04b	0
Gas exchange variables (Table 7)	0.29a	56	0.14b	0	0.31a	29	0.23a,b	0
Total	0.30a	81	0.10b	11	0.34a	68	0.15b	10

DISCUSSION

An evergreen habit has been considered an adaptation to low-nutrient availability (Monk, 1966; Chapin, 1980; Rundel & Vankat, 1989; Joffre *et al.*, 1999). However, the fact that deciduous oaks have leaves with higher nutrient concentrations than those of evergreen oaks in Mediterranean-type ecosystems suggests that evergreenness is more related to the double winter–summer stress than to oligotrophy in these ecosystems (Mitrakos, 1980; Terradas, 1999). Nutrients in Mediterranean-type ecosystems, where evergreen species are characteristic, can be locally and temporally increased owing to the effect of periodic fires and episodic precipitations (DeBano & Conrad, 1978; Rundel, 1983; Serrasolses & Vallejo, 1999). Consequently, generalizations on the eco-physiology of nutrients in evergreen plants have to take account of the dynamic component of plant response to changes in nutrient availability. Aerts (1995) postulated that growth characteristics of evergreens lead to a low responsiveness to environmental change. In the two evergreen oaks studied here, leaves exhibited a very limited morphological and physiological response to nutrient availability, in support of Aerts (1995). Leaf responsiveness to light was greater and more significant than that to nutrients in the two evergreen oaks (Table 8). However, it was remarkably lower than that of 16 evergreen tropical rainforest shrubs compared in a similar study (Valladares *et al.*, 2000). Comparing the data from present study with that of the latter reveals that large differences in responsiveness exist within evergreen woody plants of different biomes, and thus generalizations on evergreens (Aerts, 1995) must be treated with caution. This comparison also supports the hypothesis that species from harsh environments are less plastic than analogous species from more favourable sites (Lortie & Aarssen, 1996; Valladares *et al.*, 2000). Plants growing under adverse conditions tend to show a conservative pattern even when conditions are temporarily favourable, in order to avoid the production of structures too expensive to be sustained once conditions deteriorate (Chapin, 1980, 1991; Waller, 1991; Chapin *et al.*, 1993).

The low responsiveness of our plants to nutrient availability could be due not only to low intrinsic phenotypic plasticity of these two oak species but also to the possibility that the low-nutrient treatment was not sufficiently low to trigger further leaf responses. Nutrient availability was significantly different in the two nutrient treatments (Table 2); N content of the leaves was lower in the nutrient-poor than in the nutrient-rich treatment (Table 4), and leaf nutrient content was within the range of the normal values obtained in previous studies with these species (Oliveira *et al.*, 1996; Timbal & Aussenac, 1996; Sabaté *et al.*, 1999). Thus our

experiment mimicked the normal range of nutrient availability experienced by these plants in the field. Values of leaf N and P lower than those reported here have been reported only for very young (15 d) *Q. ilex* and *Q. coccifera* seedlings by Cornelissen *et al.* (1997).

Q. ilex exhibits large variation in SLA and other leaf traits (Gratani, 1996; Sabaté *et al.*, 1999), which can be considered an indication of its capacity to respond adequately to changing environmental factors through phenotypic plasticity. But large leaf-level phenotypic plasticity was also reported for functional leaf features of *Q. coccifera* (Caldwell *et al.*, 1986). Our factorial experiment has revealed that even though leaf responses to PFD and nutrients were similar in the two oak species (e.g. similar values of the responsiveness indices), *Q. ilex* exhibited a significant response to PFD in 13% more of the variables than *Q. coccifera* (Table 8). Although greater contrast between plasticities could have been expected for these two oak species of rather different ecologies and geographical distributions, our results support the idea that phenotypic plasticity is positively related to ecological distribution.

Carotenoid content and nonphotochemical quenching of excess irradiance increased with PFD in the two oak species, as has been shown in previous comparisons of sun and shade plants (Thayer & Björkman, 1990; Johnson *et al.*, 1993; Logan *et al.*, 1996). However, light environment interacted with nutrient availability in determining carotenoid content and composition. Since N supply affects both the sensitivity to photoinhibition and the production of photoprotective compounds (Ferrar & Osmond, 1986; Demmig-Adams & Adams, 1996; Skillman & Osmond, 1998), we expected that nutrient availability affected photoprotection via the VAZ. But the effect of nutrient availability on VAZ was significant only in the sun, since shade plants of both oak species had similar VAZ contents regardless of the nutrient treatment. This interaction was also observed in the lutein content, but the role of this carotenoid has been far less studied (Johnson *et al.*, 1993). Total carotenoids : chl was higher in nutrient-limited than in nutrient-rich plants grown in the sun, whereas the reverse was true for their shade counterparts. This opposite pattern could be due to the two main but opposing functions of carotenoids in photosynthetic tissues; to increase light harvesting by acting as accessory pigments, and to protect photosynthetic units against high irradiance by dissipating excess energy as heat and quenching triplet excited chlorophyll and singlet oxygen (Codgell, 1988; Havaux *et al.*, 1998). Thus, the increased carotenoid content of nutrient-limited plants in the sun could reflect enhanced photoprotection (Skillman & Osmond, 1998), whereas the increased carotenoid content in enriched plants in the shade could reflect enhanced light harvesting.

At leaf level, N allocation to Rubisco and chlorophylls is manifested as a trade-off between maximizing growth rate at high irradiance and minimizing the light compensation point for growth under low irradiance (Walters & Reich, 1996; Henry & Aarssen, 1997). In agreement with this, N : Chl, which indicates differences in N partitioning among proteins determining light harvesting, electron-transport capacity, and carboxylation efficiency (Kull & Niinemets, 1998), was higher in sun than in shade plants regardless of N availability (Table 4). As hypothesized, photosynthetic acclimation to available irradiance during growth in the two oak species involved a decreased light compensation point and decreased dark respiration in the shade. However, contrary to previous studies (e.g. Valladares & Pearcy, 1998), photosynthetic capacity on a dry mass basis and photochemical quenching were higher in the shade, which translated into a higher NUE in the shade regardless of the N availability. These results suggest that the two oak species perform well in the shade. Germination, seedling survival and growth of *Q. ilex* have been shown to be greater in the shade than in the sun (Broncano *et al.*, 1998; Rey Benayas, 1998). However, our field observations, and leaf traits, such as the low lutein : VAZ ratio obtained here for both *Quercus* species (1.4–1.6, Johnson *et al.*, 1993), suggest a low shade tolerance in these two evergreen oaks. Discrepancies in studies of the shade tolerance of plants are frequently related to the relatively high light intensities used by investigators in low-light treatments (Walters & Reich, 1996; Niinemets, 1998b). The artificial shading in previous studies of *Quercus* seedlings (Broncano *et al.*, 1998; Rey Benayas, 1998) and this study did not simulate deep shade, since they allowed 10–30% of full sunlight, whereas assessing shade tolerance should include plant performance at $\leq 5\%$ full sunlight (Walters & Reich, 1996). The comparatively better photosynthetic performance of shade plants in the present study could, on the one hand, be caused by the moderate shade treatment, or, on the other hand, by the fact that sun plants exhibited a higher nonphotochemical quenching, which correlated with higher carotenoid content and translated into lower diurnal photochemical efficiency (Φ_{PSII}). However, since predawn photochemical efficiency was the same in all plants studied, we found no indication of chronic damage of the PSII associated with the high irradiance experienced by plants of both species in the sun; these results are supported by field data of adult plants of these two species (Martínez-Ferri *et al.*, 2000). In conclusion, *Q. ilex* and *Q. coccifera* can tolerate high irradiance both as seedlings and as adults, and can tolerate moderate shade at least as seedlings.

Although plant response to environmental constraints includes adjustments to maximize functioning, it will necessarily also reflect growth limits

(Sultan, 1995). Thus, not all of the phenotypic change caused by the environment is adaptive. Since the conceptual distinction of functionally adaptive phenotypic responses from those that are not is difficult to make in practice (Sultan, 1995), many studies, including the present one, have taken the approach that plasticity is simply a neutral measure of phenotypic differences in various environments. This approach provides information on the degree and pattern of phenotypic variability, but does not address the selective impact of that variability. However, we agree with Schlichting (1986) that more information is needed on the comparative plasticity of wild plants. To simplify comparisons of the response of different genotypes and to include different variables (expressed in different units and of different ranges of variation) in the comparative studies we suggest the use of a phenotypic plasticity index ($\text{PI} = (\text{maximum} - \text{minimum})/\text{maximum}$; Valladares *et al.*, 2000) together with the significance of the response (e.g. *P* value from the ANOVA; Table 8, Fig. 2).

With a simple method that allows comparisons of the extent and significance of plant response to light and nutrients we have found that leaf responsiveness of *Q. ilex* and *Q. coccifera* assessed for 37 structural and physiological variables was low, especially with regard to nutrient availability. This conforms both with the stress tolerator model (Grime, 1979) and with the low flexibility strategy (Grubb, 1998). Leaf responsiveness of *Q. ilex*, the species with the wider ecological distribution, was only slightly larger than that of *Q. coccifera*. Both species tolerated full sunlight and moderate shade, but exhibited a reduced capacity to maximize photosynthetic utilization of high irradiance. We suggest that the low leaf-level responsiveness found here is part of a conservative resource-use strategy, which seems to be adaptive for evergreen woody plants in the Mediterranean basin.

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