

Spatial and age-dependent modifications of photosynthetic capacity in four Mediterranean oak species

Ülo Niinemets^{A,B}, John D. Tenhunen^C and Wolfram Beyschlag^D

^ADepartment of Plant Physiology, University of Tartu, Riia 23, Tartu 51 010, Estonia.

^BCentro di Ecologia Alpina, I-38 040 Viote del Monte Bondone (TN), Italy.

Corresponding author. Email: ulo.niinemets@ut.ee

^CDepartment of Plant Ecology, University of Bayreuth, D-95 440, Bayreuth, Germany.

^DDepartment of Experimental and Systems Ecology, University of Bielefeld, Universitätsstraße 25, 33 615 Bielefeld, Germany.

Abstract. Drought is one of the most important limitations of photosynthesis in Mediterranean climates. However, Mediterranean sclerophyllous species with long-lived leaves also support extensive and dynamic canopies, with potentially large spatial and age-dependent gradients. We studied within-canopy and temporal patterns in foliage structure, chemistry and photosynthesis in the evergreen species *Quercus coccifera* L., *Q. ilex* L. subsp. *ballota* (Desf.) Samp. in Bol. and *Q. suber* L. and in the semi-deciduous marcescent species *Q. faginea* Lam. to determine the role of within-canopy shading and leaf age on foliage functioning. There was a 2.5-fold within-canopy gradient in leaf dry mass per unit area (M_A) that was accompanied by a 3-fold range in area-based leaf nitrogen (N) content, the capacity for photosynthetic electron transport (J_{\max}) and maximum Rubisco carboxylase activity (V_{\max}), while the fractional investments of leaf nitrogen in electron transport (F_B) and in Rubisco (F_R) were relatively constant within the canopy. Leaf aging led to increased M_A , larger or constant mass-based N content, larger phosphorous (P) and structural carbon contents, but decreased movable cation contents. Age-dependent increases in M_A and N per dry mass meant that J_{\max} and V_{\max} per area were weakly related to leaf age, with a trend of decreasing values in older leaves. However, J_{\max} and V_{\max} per unit dry mass decreased 4-fold across the range of leaf age, primarily owing to decreases in apparent N investments in photosynthetic machinery. This decrease in apparent N investments in photosynthetic machinery was possibly the result of a larger fraction of N bound to cell walls, or of an enhanced CO₂ diffusion resistance from the outer surface of cell walls to the chloroplasts in older leaves with thicker and more lignified cell walls. The age-dependent variation in apparent fractional investments of N in photosynthetic machinery reduced the generality of leaf nitrogen v. photosynthesis relationships. Photosynthetic characteristics qualitatively fitted the same patterns with leaf age in all species, but at a common leaf age, area-based leaf photosynthetic potentials depended on species-specific values of M_A . These data collectively demonstrate important canopy and age-dependent controls on leaf structure, chemistry and photosynthetic potentials that should be included in larger-scale photosynthesis simulations in Mediterranean climates.

Keywords: age effects, leaf structure, nitrogen v. photosynthesis relationship, nutrient content, sclerophyll species.

Introduction

Past research has emphasised the role of seasonality, in particular severe summer drought, in modifying the

photosynthetic characteristics in Mediterranean climates (Tenhunen *et al.* 1985, 1990; Beyschlag *et al.* 1986; Sala and Tenhunen 1996; Infante *et al.* 1999; Werner *et al.* 2001a). In

Abbreviations used: A , net assimilation rate; CF, coupling factor; C_i , internal CO₂ concentration; C_M , leaf carbon percentage; $cyt f$, cytochrome f ; F_B , fractional investment of leaf nitrogen in electron transport; F_R , fractional investment of leaf nitrogen in Rubisco; J , photosynthetic electron transport rate; J_{\max} , photosynthetic electron transport capacity; $J_{\max;A}$, photosynthetic electron transport capacity per unit area; $J_{\max;M}$, photosynthetic electron transport capacity per unit dry mass; J_{mc} , capacity for photosynthetic electron transport per unit cytochrome f ; L_{cum} , cumulative leaf area index; M_A , leaf dry mass per unit area; N_M , leaf nitrogen percentage; V_{cr} , specific activity of Rubisco; V_{\max} , maximum Rubisco carboxylase activity; $V_{\max;A}$, maximum Rubisco carboxylase activity per unit leaf area; $V_{\max;M}$, maximum Rubisco carboxylase activity per unit dry mass; Q , quantum flux density; Q_{int} , daily-integrated values of Q ; $Q_{int;0}$, above-canopy seasonal average quantum flux density; Γ^* , hypothetical CO₂ compensation point in the absence of non-photorespiratory respiration.

these environments, lack of precipitation often interacts with excess irradiance, further constraining leaf carbon fixation through photoinhibition (Werner *et al.* 2001a). Apart from seasonality, leaves of Mediterranean evergreen sclerophylls constantly age. In Mediterranean evergreen oak *Q. ilex*, the average fraction of leaves that are more than one year old is 0.4–0.6 (Sala 1992), demonstrating that older leaf age classes play an important part in canopy functioning. In addition, leaf longevity may increase in Mediterranean species with increasing site aridity (Jonasson *et al.* 1997), further underscoring the significance of old leaves in these species.

In evergreen conifers, nitrogen content per dry mass of non-senescent leaves generally decreases continuously with increasing leaf age (e.g. Saur *et al.* 2000; Niinemets and Lukjanova 2003). Given that foliar nitrogen contents are strongly associated with leaf photosynthetic capacity (Evans and Seemann 1989; Wright *et al.* 2004), age-dependent decreases in nitrogen content often leads to concurrent changes in foliar photosynthetic potentials (Schoettle and Smith 1999; Xu and Baldocchi 2003). According to simulation studies, such age-dependent changes significantly alter whole-canopy carbon gain (Bernier *et al.* 2001). Although there is evidence of age-dependent decreases in foliar nutrient contents in non-senescent leaves of Mediterranean sclerophyllous oaks, a series of studies has demonstrated a relative time-independence of leaf nitrogen in these species with long-living foliage (Gratani and Moriconi 1983; de Lillis and Fontanella 1992; Rapp *et al.* 1992; Sabaté *et al.* 1995; Robert *et al.* 1996). Constancy of foliar nutrient contents in aging leaves may be the result of limited sink activity owing to drought-dependent growth cessation. Thus, the time-dependent changes in leaf nutrient contents in Mediterranean evergreens apparently contradict the well-known temporal variation patterns in temperate conifers.

Limited temporal change of foliar nutrient contents suggests that foliage photosynthetic potentials should be relatively constant in time in Mediterranean sclerophylls. However, there is a series of foliage structural and chemical alterations that occur during foliage aging in these species, but this influence on leaf photosynthetic function is poorly characterised. In Mediterranean oaks, there are continuous age-dependent changes in cell wall lignification and total amount of cell walls (Damesin *et al.* 1998) that may modify the diffusion conductance from the outer surface of cell walls to the carboxylation sites in chloroplasts (Evans and Loreto 2000). Hence, changes in mesophyll cell wall thickness and composition potentially have an important effect on leaf photosynthesis rates at any given foliar nitrogen content. Relations between foliar nutrient content and photosynthesis are considered strongly conserved at a global scale (Wright *et al.* 2004), but such age-dependent controls on diffusion conductance may significantly affect their generality.

Long leaf life spans also imply that Mediterranean species may accumulate a large leaf area with significant within canopy shading. In temperate species, there are extensive canopy gradients in leaf photosynthetic potentials (e.g. Niinemets *et al.* 2004) that play a major role in whole-canopy photosynthetic performance, but the within-canopy variation is considered less significant in Mediterranean ecosystems. However, depending on the moisture availability, the canopy leaf area index extends to 2–8 m² m⁻² in Mediterranean macchia ecosystems (Tenhunen *et al.* 1984b; Caldwell *et al.* 1986; Rambal 2001) and up to 5–7 m² m⁻² in sclerophyll evergreen forests dominated by *Q. ilex* (Sala *et al.* 1994; Rambal 2001) with minimum irradiance at the bottom of canopy as low as 1–5% of incident irradiance (Gracia 1984; Caldwell *et al.* 1986). This suggests that the within-canopy variation in light may result in important plastic modifications in leaf photosynthetic potentials in Mediterranean species as well. Simulation studies demonstrate that within-canopy shading may be an important factor that avoids photoinhibition of mid- and lower-canopy leaves during the drought period (Werner *et al.* 2001b). Although the within-canopy gradient in photosynthetic capacity may exert a minor influence on canopy photosynthesis during stress periods when stomata are closed (Werner *et al.* 2001b), whole-canopy carbon gain during late autumn, winter and spring months when the plants are well watered, may be strongly modified by gradients in potential foliage photosynthetic activity. As shown previously for temperate species, canopy gradation in foliage photosynthetic capacities may enhance whole-canopy carbon gain by 10–20% (Baldocchi and Harley 1995).

To test the importance of within-canopy gradients in light and overall variation in leaf age on foliage carbon gain capacity, we studied leaf structural, chemical and photosynthetic characteristics within the canopy of *Q. coccifera* and with leaf aging in *Q. coccifera*, *Q. faginea*, *Q. ilex* subsp. *ballota* and *Q. suber*. *Quercus coccifera*, *Q. ilex* and *Q. suber* are evergreen species, while *Q. faginea* is a semi-deciduous species with marcescent leaves (leaves withering on the plant without abscission). Leaves of *Q. coccifera* and *Q. ilex* are kept on the tree for up to four growing seasons in the driest places. The average longevity of *Q. suber* leaves is 15–18 months. (Escudero *et al.* 1992), but most older leaves fall in May–June before the onset of summer drought (Robert *et al.* 1996). *Quercus coccifera* is the most and *Q. faginea* the least water-stress tolerant species, whilst *Q. ilex* and *Q. suber* exhibit an intermediate tolerance (Acherar and Rambal 1992; Martínez-Ferri *et al.* 2000; Mediavilla and Escudero 2003). All species may co-exist in the Iberian peninsula, but *Q. coccifera* generally dominates the macchia vegetation in more arid locations, *Q. suber* and *Q. ilex* form climax communities in sites with intermediate aridity, while *Q. faginea* grows intermixed with deciduous oak

species such as *Q. pubescens* Willd. in more humid sites (Castro-Díez *et al.* 1997).

Material and methods

Study sites

All measurements in *Q. suber* L. and most measurements in *Q. coccifera* L. were conducted at the Research Station Quinta de São Pedro in Sobreda da Caparica, near Lisbon, Portugal (38°39'N, 9°11'W, elevation 60 m) during 1982–1983 growing seasons. The site supports a natural macchia vegetation on acidic sandy loam. The Mediterranean climate with maritime influences is characterised by hot dry summers and cool rainy winters. Long-term average yearly precipitation is 700 mm (Beyschlag *et al.* 1986), but the total rainfall was only 451 mm for the period 1 September 1982 to 31 August 1983 (Pereira *et al.* 1986). The trees of *Q. coccifera* and *Q. suber* chosen for the experiments were 1.5–2.5 m tall and were fully exposed during most of the day with some shading provided by taller *Eucalyptus globulus* Labill. and *Pinus pinea* L. trees either in the morning or afternoon.

Quercus faginea Lam. and *Q. ilex* subsp. *ballota* (Desf.) Samp. in Bol. and some individuals of *Q. coccifera* were measured in Serra da Arrábida, Portugal (38°28'N, 9°02'W, elevation 200–300 m) during May–July 1983. The climate in this site is characterised by higher annual rainfall of 900–1000 mm than the site in Sobreda da Caparica (Catarino *et al.* 1982; Werner *et al.* 2001b). Most of the area is covered with sclerophyllous macchia-type vegetation dominated by *Q. coccifera*, *Arbutus unedo* L. and *Phillyrea latifolia* L. on calcareous shallow rendzinas or brown soils with weakly developed profile (Catarino *et al.* 1982). Plant formations dominated by species with marcescent leaves, *Q. faginea* and *Acer monspessulanum* L., are found on more humid northern slopes (Catarino *et al.* 1982).

According to the phenological observations in the spring 1983, the date of bud-burst was 10 March for *Q. coccifera* and *Q. faginea*, 8 April for *Q. suber* and 19 April for *Q. ilex* (Pereira *et al.* 1987). These dates were used to determine leaf age for the data sampled in all years.

Gas-exchange data

Three types of gas-exchange measurements systems were used to measure leaf net assimilation rates and transpiration rates. Seasonal variation in leaf gas-exchange characteristics in Quinta de São Pedro was assessed with a field laboratory, consisting of controlled environment gas-exchange cuvettes (volume 0.005 m³, H. Walz Meß- und Regeltechnik, Effeltrich, Germany). The complex system provided full control over air humidity and CO₂ and O₂ concentrations and leaf temperature and was operated either to track ambient conditions or by adjusting the gas composition and environmental conditions according to pre-set values. Twigs enclosed in the cuvette were typically measured for periods of 2–7 d. Detailed description of the mobile measurement laboratory is provided by Beyschlag *et al.* (1986) and Tenhunen *et al.* (1984a).

A mobile minicuvette system (cuvette volume 0.0002 m³) of H. Walz (Lange *et al.* 1985) was also employed to study seasonal and within canopy variation in leaf photosynthetic characteristics under controlled conditions (measurements in Serra da Arrábida). In addition, within-canopy variation in gas-exchange rates at different times during the season in specific environmental conditions (measurements in Quinta de São Pedro and Serra da Arrábida) was assessed with a portable steady-state CO₂/H₂O porometer of H. Walz (Schulze *et al.* 1982).

For all types of measurement systems, leaf temperature was measured with a copper-constantan thermocouple and quantum flux density with a quantum sensor (model LI-190SA, Li-Cor, Inc.,

Lincoln, NE) and net CO₂ exchange rate with a Binos infrared CO₂ analyser (Leybold-Heraeus, Hanau, Germany) and H₂O exchange rate by Waltz dew point mirrors (mobile laboratory) or Binos H₂O analyser (minicuvette system CO₂/H₂O porometer). For calibration of the CO₂ analyser, standard calibration gases were used, or precision mixing pumps (Digamix, Wösthoff OHG, Bochum, Germany) were employed to prepare air with varying CO₂ concentrations from pure CO₂ and CO₂-free air. The drift in the sensitivity of Binos CO₂ analyser was less than 0.1–0.3% of full scale per week and, thus, the full calibration was conducted every two to four weeks. Overall, the total error of gas-exchange measurements was less than 5–10% (Beyschlag *et al.* 1986). A comprehensive overview of measurement protocols is provided in Beyschlag *et al.* (1986) and Tenhunen *et al.* (1984a, 1985). Gas-exchange rates were calculated according to von Caemmerer and Farquhar (1981).

Within-canopy variation in foliar assimilation potentials

We measured the light-saturated net assimilation rates from the top to the bottom of *Q. coccifera* canopies in 5-cm steps (Tenhunen *et al.* 1984b) in fully mature non-water-stressed leaves during four campaigns from October 1982 to March 1983 in Quinta de São Pedro to determine the within-canopy variation in foliage photosynthetic capacity. To characterise the light environment during leaf growth, we combined instantaneous measurements of above-canopy quantum flux density (Q) and profiles of leaf area index and relative incident light separately estimated for the same macchia stand. Q was measured continuously in the cuvette of the mobile laboratory and the daily-integrated values of Q (Q_{int}) were calculated (mol m⁻²). These values were corrected for the transmittance of the cuvette cover and averaged for the period of leaf growth and development (1 March–30 June) to obtain the above-canopy seasonal average quantum flux density ($Q_{\text{int},0} = 39.2 \text{ mol m}^{-2} \text{ d}^{-1}$). The values of Q_{int} at any specific leaf location were found from the height profiles of leaf area index and relative incident irradiance measured at the site (Tenhunen *et al.* 1984b; Caldwell *et al.* 1986) using non-linear regression models in the form:

$$Q_{\text{int}} = Q_{\text{int},0} e^{(-aL_{\text{cum}})} \quad (1)$$

where L_{cum} is the cumulative leaf area index and 'a' is the fitted extinction coefficient. For specific sclerophyll oak stands, there are strong relationships between leaf area index, height in the canopy and incident light (Rambal 2001). The canopy extension moderately varied in our study, resulting in 10–20% variation in the amount of light reaching a certain height in the canopy. Although it may be argued that the daily average integrated incident light differs from the intercepted light due to leaf clumping within shoots (Cescatti and Niinemets 2005), Q_{int} generally describes a large fraction of within-canopy variation in foliar structure and photosynthetic potentials (Niinemets *et al.* 2004).

Determination of the initial quantum yield and capacity for photosynthetic electron transport and maximum carboxylase activity of Rubisco

While none of the measurements in Serra da Arrábida have been published, part of the net assimilation (A) data of *Q. coccifera* and *Q. suber* from Quinta de São Pedro has been reported previously (Tenhunen *et al.* 1984a, 1985, 1987). Because net assimilation rates strongly depend on the openness of stomata and also vary with the rate of non-photorespiratory respiration continuing in the light, we determined the Farquhar and von Caemmerer (1982) model parameters — the initial quantum yield for an incident light (α), the capacity for photosynthetic electron transport (J_{max}) and the maximum carboxylase activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco; V_{cmax}) for all measurements in the current study. For the Farquhar and

von Caemmerer version of the model, that uses the Smith equation for photosynthetic electron transport, J ,

$$J = \frac{\alpha Q}{\sqrt{\frac{1 + \alpha^2 Q^2}{J_{\max}^2}}} \quad (2)$$

(Harley *et al.* 1992), these three parameters along with their temperature dependencies and internal CO_2 concentration describe the rate on net leaf carbon assimilation for any combination of environmental drivers.

The parameters of Farquhar and von Caemmerer model are generally derived from the CO_2 response curves of photosynthesis. However, these characteristics may also be derived from the light-response curves as well as daily time-courses at ambient CO_2 concentrations by inverse modelling as detailed in Niinemets (2002). These derivations assume that the light-saturated value of net assimilation at current ambient CO_2 is limited by V_{\max} and that the irradiance at which net assimilation saturates depends on J_{\max} (Niinemets and Tenhunen 1997). The initial slope of net assimilation ($Q < 100 \mu\text{mol m}^{-2} \text{s}^{-1}$) measured at a specific internal CO_2 concentration (ϕ) was used to calculate the value of α (eqn 2) according to Kellomäki and Wang (1997):

$$\phi = \left. \frac{\partial A}{\partial Q} \right|_{Q \rightarrow 0} = \alpha \frac{1 - \Gamma^*/C_i}{4 + 8\Gamma^*/C_i} \Leftrightarrow \alpha = \frac{\phi(4 + 8\Gamma^*/C_i)}{1 - \Gamma^*/C_i}, \quad (3)$$

where C_i is the internal CO_2 concentration and Γ^* is the hypothetical CO_2 compensation point in the absence of non-photorespiratory respiration. Γ^* was calculated for every leaf temperature using the Rubisco temperature responses reviewed by Niinemets and Tenhunen (1997). For the leaves lacking data to determine α values, α was estimated from an empirical function with day of the year (see Results). Although the initial quantum yield is often considered as a relatively invariable characteristic (Niinemets and Tenhunen 1997), it may significantly decrease during drought periods in Mediterranean climates (Werner *et al.* 2001a).

In derivations of J_{\max} and V_{\max} , we used only the data before noon to avoid apparent decreases in leaf photosynthetic capacity because of non-uniform stomatal closure (Tenhunen *et al.* 1984a; Beyschlag *et al.* 1990, 1992). Such a possible non-uniform stomatal closure in water-stressed plants in afternoon hours (Beyschlag *et al.* 1990) would result in overestimations of internal CO_2 concentrations and accordingly underestimations of foliage photosynthetic potentials (Tenhunen *et al.* 1984a).

All values of J_{\max} and V_{\max} were standardised to a common temperature of 25°C using the temperature-responses revised by Niinemets *et al.* (2002) for J_{\max} and Niinemets and Tenhunen (1997) for V_{\max} .

Structural and chemical analyses

Leaf area was measured with a planimeter, lamina dry mass determined after drying at 70°C for at least 48 h and leaf dry mass per unit area (M_A , g m^{-2}) was calculated. Leaf lamina C and N contents were determined with an elemental analyser (CHN-O-Rapid, Foss Heraeus GmbH, Hanau, Germany). In general, foliar N content provides an estimate of leaf protein content and thus, leaf physiological capacity, while C content characterises the structural investments. However, proteins that also contain a significant amount of C and may vary independently of structural leaf C content, complicate interpretation of the variation patterns in foliar C. Therefore, we calculate the protein-free leaf C content (C_S) as

$$C_S = \frac{C_M - 6.25 \frac{53.5 N_M}{100}}{1 - \frac{6.25 N_M}{100}}, \quad (4)$$

where C_M is the leaf carbon and N_M leaf nitrogen percentage, 6.25 converts leaf N content to protein content and 53.5% is the carbon percentage of proteins (Fleck *et al.* 2003). The contents of Ca, K, Mg and P were estimated by ICP (Integra XMP, GBC Scientific Instruments, Melbourne, Vic.) after digestion of pulverised leaves in 65% HNO_3 .

Nitrogen investments in photosynthetic machinery

To distinguish between the structural and biochemical controls on leaf photosynthetic potentials, we express the capacity for photosynthetic electron transport per unit area, $J_{\max;A}$, as (Niinemets and Tenhunen 1997):

$$J_{\max;A} = 8.06 J_{\text{mc}} M_A F_B N_M, \quad (5)$$

where J_{mc} is the capacity for photosynthetic electron transport per unit cytochrome f [$\text{mol e}^- (\text{mol cyt } f)^{-1} \text{s}^{-1}$; $J_{\text{mc}} = 156 \text{ mol e}^- (\text{mol cyt } f)^{-1} \text{s}^{-1}$ at 25°C , Niinemets and Tenhunen 1997], N_M is leaf N content per unit dry mass (g g^{-1}) and F_B is the proportion of leaf N in rate-limiting proteins of photosynthetic electron transport. We assume that the capacity for electron transport is controlled by cyt f , ferredoxin NADP reductase (FNR) and the coupling factor (CF) contents that are present in a constant 1 : 1 : 1.2 (cyt f : FNR: CF) molar ratio (Evans and Seemann 1989). With this stoichiometry, an investment of one g N in bioenergetics is equivalent to $8.06 \mu\text{mol cyt } f$ (Evans and Seemann 1989).

The maximum Rubisco carboxylase activity per unit leaf area, $V_{\max;A}$, further equals:

$$V_{\max;A} = 6.25 V_{\text{cr}} M_A F_R N_M, \quad (6)$$

where V_{cr} is the specific activity of Rubisco (the maximum rate of ribulose-1,5-bisphosphate carboxylation per unit Rubisco protein, $V_{\text{cr}} = 20.5 \mu\text{mol CO}_2 (\text{g Rubisco})^{-1} \text{s}^{-1}$ at 25°C according to Jordan and Ogren 1984), F_R is the fraction of leaf nitrogen in Rubisco and 6.25 converts nitrogen content to Rubisco protein content.

Data analyses

We used linear and non-linear regression analyses to test for the significant effects of seasonal average quantum flux density (Q_{int}) and leaf age on foliar structural, chemical and photosynthetic characteristics. The form of non-linear regressions was $y = a + b \log(x)$. Species were compared using co-variance analyses. In these analyses, the co-variables were either not transformed, or Q_{int} and leaf age were log-transformed whenever appropriate (Kolmogorov–Smirnov test for the normality of residuals). Separate-slope co-variation analysis was used first to test for the significance of the interaction terms, species $\times Q_{\text{int}}$ and species \times leaf age. When the interaction term was non-significant, the model was refitted without the interaction term (common slope model; Sokal and Rohlf 1995). All statistical tests were considered significant at $P < 0.05$.

Leaf age varied from 230 to 367 d during the measurements of the within-canopy profile of foliage structural and physiological variables in *Q. coccifera*. Multiple linear regressions with $\log(Q_{\text{int}})$ and leaf age as explaining variables demonstrated that for this dataset, leaf age significantly modified M_A ($P < 0.005$) but not foliage photosynthetic potentials (J_{\max} , V_{\max}) either per area or mass ($P > 0.3$). As we were mainly interested in the canopy variation in photosynthetic characteristics for this set of data, all canopy gradient data were pooled in this analysis ($n = 43$). All statistical effects of irradiance were qualitatively identical for this dataset and for a dataset with constrained range of leaf age (317–331 d, $n = 28$), suggesting that the confounding variation in leaf age did not alter qualitatively the conclusions with respect to the canopy effects on foliage functioning.

Because part of the individuals of *Q. coccifera* were sampled from both sites, we also tested for the possible site effect on the relationships

between leaf age and foliar structure, nutrient content and photosynthetic characteristics using a co-variation model (site as the main effect and leaf age as co-variate). In none of the cases, site effect was significant in this analysis ($P > 0.4$ for chemical and structural variables and $P > 0.1$ for photosynthetic characteristics). Thus, the data from both sites were pooled in the final analyses of this species.

Results

Within-canopy variation in leaf structure, chemistry and photosynthetic characteristics

Canopy-variation in foliar characteristics was studied in *Q. coccifera*. Leaf dry mass per unit area (M_A) was strongly related to growth irradiance (Q_{int} ; Fig. 1A), but single leaf area decreased only weakly with Q_{int} ($r^2 = 0.21$, $P < 0.005$). The content of mineral elements per unit dry mass was generally not correlated with Q_{int} (Fig. 1B, $r^2 = 0.01$, $P > 0.5$ for P; $r^2 = 0.00$, $P > 0.7$ for Ca; $r^2 = 0.03$, $P > 0.3$ for Mg) except for K that was negatively correlated with Q_{int} ($r^2 = 0.30$, $P < 0.001$). Total ($r^2 = 0.37$, $P < 0.001$) and structural (Fig. 1C) leaf carbon contents per dry mass scaled positively with Q_{int} .

Although the mass-based foliar nutrient contents were not related to irradiance, the contents per unit area were always positively related to Q_{int} (Fig. 1A for the sample graph of nitrogen content per unit area v. Q_{int}) due to the light effects on M_A (content per area = content per mass $\times M_A$).

The capacity for photosynthetic electron transport per unit area, $J_{\text{max};A}$, (Fig. 2A) and the maximum carboxylase activity of Rubisco per unit area, $V_{\text{cmax};A}$, (Fig. 2B) scaled positively with irradiance. J_{max} per unit dry mass was independent of irradiance ($r^2 = 0.03$, $P > 0.3$) and V_{cmax} per unit dry mass increased weakly with Q_{int} ($r^2 = 0.11$, $P < 0.05$), again suggesting that the capacities per unit area increased mainly because of the light effects on leaf structure (Fig. 1A). The fractional investment of nitrogen in bioenergetics (eqn 5) was independent of irradiance (Fig. 2C), but a weak positive correlation was observed between the fraction of leaf N in Rubisco and Q_{int} (Fig. 2D).

Seasonal modifications in the initial quantum yield of photosynthetic electron transport

We found a strong decrease in the initial quantum yield of photosynthetic electron transport for an incident light (α , eqn 3) in drought-stressed leaves (Fig. 3). The seasonal trend in α was best parameterised with a symmetrical cosine function (Fig. 3) that yields a maximum value of $0.243 \text{ mol mol}^{-1}$ for non-stressed leaves and a minimum value of $0.074 \text{ mol mol}^{-1}$ for most stressed leaves. Given that the quantum yield for absorbed light is $0.292 \text{ mol mol}^{-1}$ and assuming a representative value of 0.85 for leaf absorptance, the theoretical value of α is $0.248 \text{ mol mol}^{-1}$ (Niinemets and Tenhunen 1997). Thus, the maximum values of α of $0.23\text{--}0.25 \text{ mol mol}^{-1}$ observed in our study in non-stressed leaves (Fig. 3) are close to the theoretical maximum.

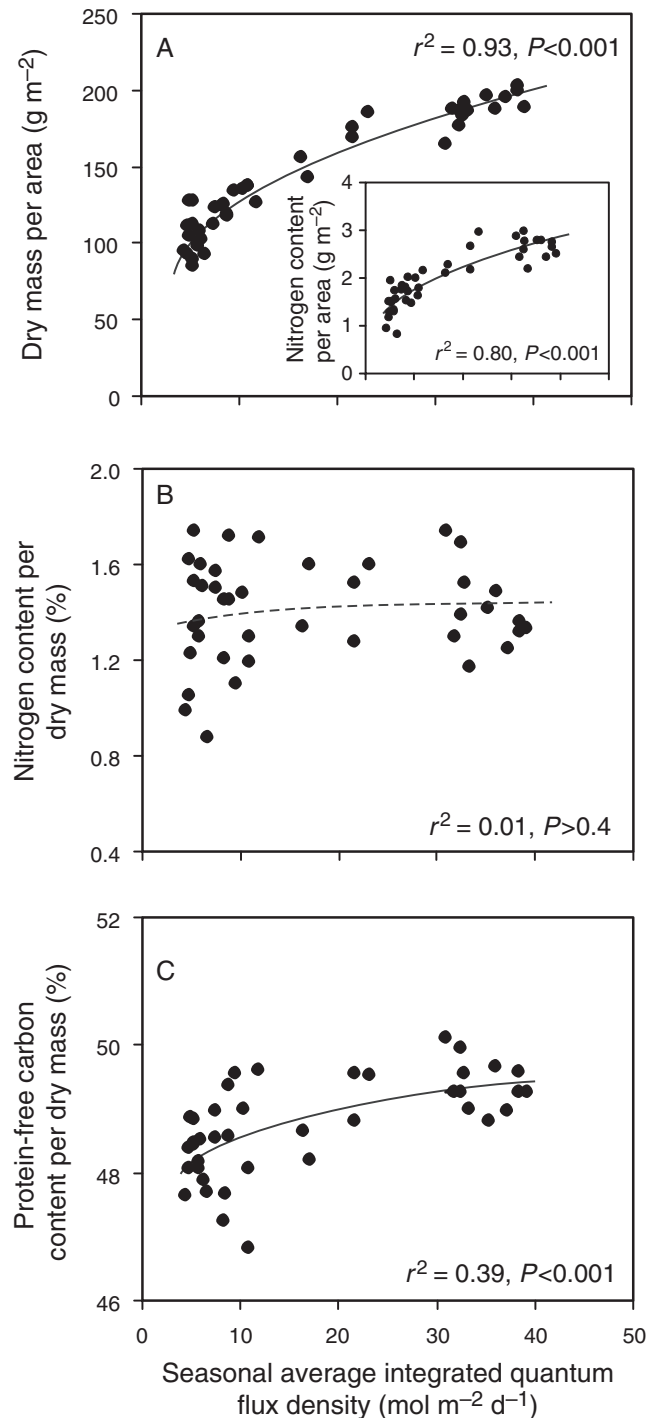


Fig. 1. Effects of growth irradiance (Q_{int}) on (A) leaf dry mass per unit area, (B) nitrogen content per unit dry mass and area (inset in A) and (C) structural leaf carbon content per unit dry mass (eqn 4), in evergreen sclerophyll *Quercus coccifera*. Data were fitted by non-linear regressions in the form of $y = a + b \log(x)$ and the non-significant regression is depicted by a dashed line. The measurements were conducted in one-year-old non-water-stressed leaves during autumn/winter seasons (26 October–12 March).

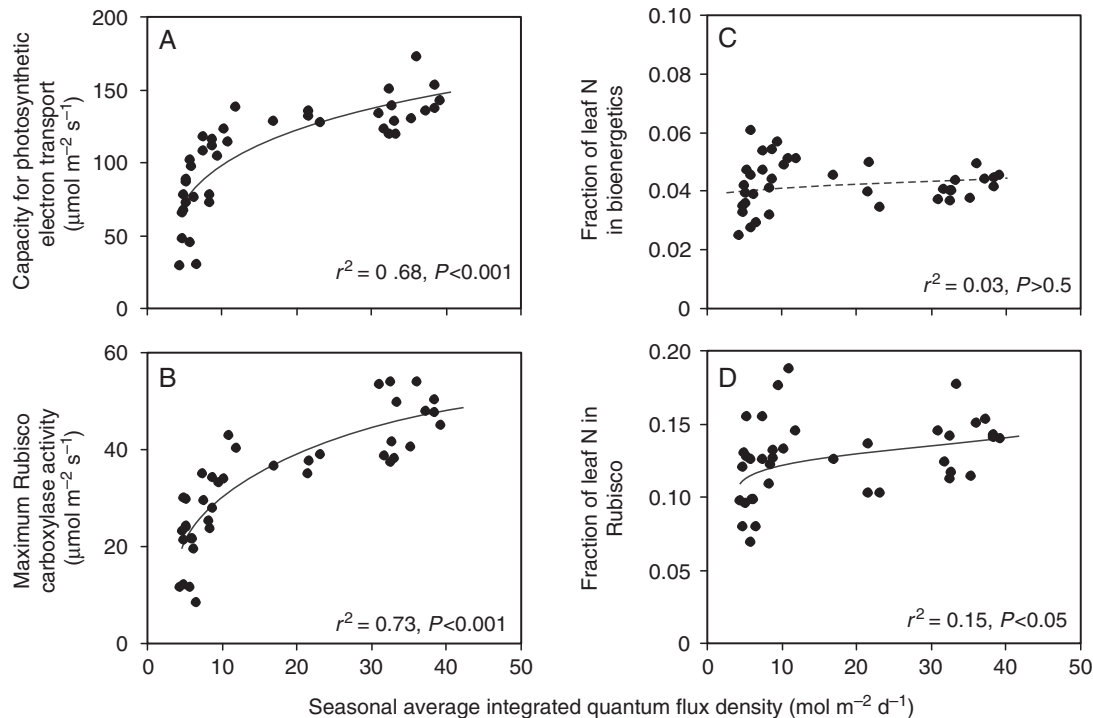


Fig. 2. Dependence of the capacity for photosynthetic electron transport [J_{\max} ; (A)], the maximum carboxylase activity of Rubisco [V_{\max} ; (B)], the fraction of leaf N in rate-limiting components of photosynthetic electron transport chain [F_B , eqn 5, (C)] and in Rubisco [F_R , eqn 6, (D)] on incident integrated irradiance in *Q. coccifera*. Data fitted as in Fig. 1.

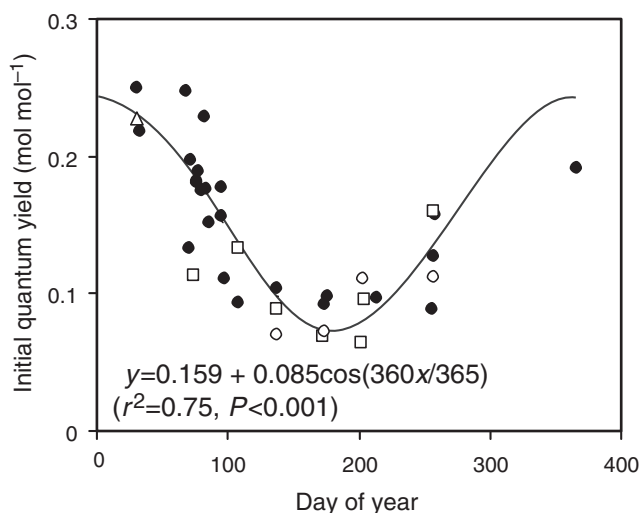


Fig. 3. Seasonal variation in the initial quantum yield for photosynthetic electron transport (eqn 3) in four Mediterranean *Quercus* species. Data were fitted by a symmetrical non-linear regression model. *Quercus coccifera* (●, leaf life span 1–3 years), *Q. ilex* (○, 2–4 years) and *Q. suber* (△, 1.5 years) are evergreen sclerophyllous species with small leaves (on average 2–5 cm²), while *Q. faginea* is a semi-deciduous marcescent (□, leaves withering on the plant) species with larger leaves (on average 5–15 cm²).

Leaf age effects on leaf structural and chemical characteristics in four *Quercus* species

Leaf dry mass per unit area increased with increasing leaf age in all four species (Fig. 4). According to co-variation analyses (separate slope model), leaf age \times species interaction was not significant ($P > 0.6$), but at a common leaf age, *Q. ilex* had consistently larger M_A than the other species ($P < 0.002$, common slope model).

Statistical dependencies between leaf nutrient contents per dry mass and leaf age were examined in *Q. coccifera* and *Q. suber*, for which representative sample sizes were available. The age-dependent changes in nutrient contents were similar in both species (Fig. 5) with the overall trend of N (Fig. 5A), P (Fig. 5C) and Ca (Fig. 5F) accumulation and K (Fig. 5D) and Mg (Fig. 5E) depletion in aging leaves. However, N (Fig. 5A) and Mg (Fig. 5E) contents per dry mass were independent of leaf age and the age-dependence of Ca (Fig. 5F) was marginally significant in *Q. suber*, which has a shorter average leaf-life span (approximately 1 y) than *Q. coccifera* (1–2 y). At a common leaf age, leaf N content (Fig. 5A, $P < 0.005$) and Mg content (Fig. 5E, $P < 0.001$) were larger in *Q. suber* than in *Q. coccifera*, while K content (Fig. 5D, $P < 0.001$) and Ca content (Fig. 5F, $P < 0.001$) were

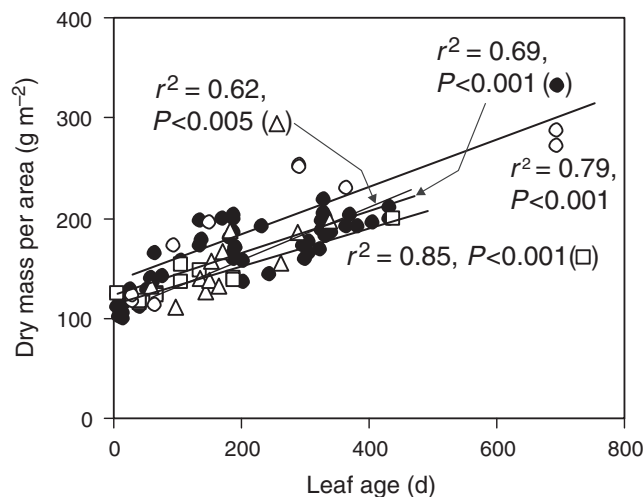


Fig. 4. Lamina dry mass per unit area in relation to leaf age in four co-existing Mediterranean *Quercus* species. ●, *Q. coccifera*; □, *Q. faginea*; ○, *Q. ilex*; △, *Q. suber*. Data were fitted by linear regressions.

larger in *Q. coccifera*. For these dependencies, the interaction, species \times age was never significant ($P > 0.08$).

Both total ($r^2 = 0.46$, $P < 0.001$ for *Q. coccifera* and $r^2 = 0.44$, $P < 0.002$ for *Q. suber*) and structural leaf carbon contents (eqn 2, Fig. 5B) increased with increasing leaf age.

Dependence of leaf photosynthetic characteristics on leaf age

The values of J_{\max} (Fig. 6A) and V_{\max} (Fig. 6B) per unit area were generally not correlated with leaf age. Only *Q. coccifera* exhibited a weak negative relationship between $J_{\max;A}$ and leaf age (Fig. 6A). J_{\max} (Fig. 6C) and V_{\max} (Fig. 6D) per unit dry mass strongly decreased with increasing leaf age in three species, but were independent of leaf age in *Q. faginea* (Fig. 6C, D). Nevertheless, in this species, the relationships of area- and mass-based values of J_{\max} and V_{\max} with leaf age were dependent on the low values of photosynthetic potentials in youngest leaves (leaf age = 5 d; Fig. 6). In older leaves of *Q. faginea*, $J_{\max;A}$ ($r^2 = 0.46$, $P < 0.05$), $J_{\max;M}$ ($r^2 = 0.57$, $P < 0.02$), $V_{\max;A}$ ($r^2 = 0.64$, $P < 0.01$) and $V_{\max;M}$ ($r^2 = 0.71$, $P < 0.005$) decreased significantly with leaf age.

According to co-variation analyses, species \times log(age) interaction was not significant for any of the relationships demonstrated in Fig. 6. However, at a common leaf age, $J_{\max;A}$ ($P < 0.005$) and $V_{\max;A}$ ($P < 0.05$) were larger in *Q. ilex* than in *Q. coccifera* and *Q. suber*. No other species differences were statistically important ($P > 0.2$). For all data pooled, there was a significant age-dependent decline in $J_{\max;A}$ ($r^2 = 0.10$, $P < 0.005$); $J_{\max;M}$ ($r^2 = 0.50$, $P < 0.001$) and in $V_{\max;M}$ ($r^2 = 0.43$, $P < 0.001$) but not in $V_{\max;A}$ ($r^2 = 0.03$, $P > 0.1$).

Nitrogen investments in foliage photosynthetic machinery in relation to leaf age

For fully illuminated leaves with varying age, the capacity for photosynthetic electron transport per unit area (Fig. 7A) and dry mass (Fig. 7C) and maximum carboxylase activity of Rubisco per unit area (Fig. 7B) and dry mass (Fig. 7D) were significantly correlated with foliar nitrogen content per area (Fig. 7A, B) and dry mass (Fig. 7C, D). However, the correlations were scattered, suggesting that for any specific foliar nitrogen content, the apparent fraction of foliar nitrogen invested in photosynthetic machinery (eqns 3, 4) largely varied.

Both the fraction of leaf N in the rate-limiting components of photosynthetic electron transport (F_B , Fig. 8A; $r^2 = 0.40$, $P < 0.001$ for all species pooled) and in Rubisco (F_R , Fig. 8B; $r^2 = 0.31$, $P < 0.001$ for all) scaled negatively with leaf age, explaining a significant part of the scatter in photosynthetic capacity v. N relations (Fig. 7).

Overall, there were weak negative correlations between the nitrogen investments in photosynthetic machinery and leaf structural variables. Both F_B ($r^2 = 0.13$, $P < 0.02$) and F_R ($r^2 = 0.12$, $P < 0.03$) scaled negatively with M_A , and F_B scaled negatively with leaf structural carbon content (eqn 4; $r^2 = 0.13$, $P < 0.02$). These correlations were stronger when the species were considered separately. For instance, for the correlation between F_B and structural carbon, $r^2 = 0.21$, $P < 0.02$ for *Q. coccifera* and $r^2 = 0.38$, $P < 0.05$ for *Q. suber*.

Discussion

Within-canopy variation in foliage structure and photosynthetic potentials

In temperate forests, within-canopy differences in light environment result in a major variation in leaf photosynthetic potentials (Niinemets *et al.* 2004). Crown position is generally considered to play a minor part in photosynthetic responses of Mediterranean sclerophyll species that often grow in relatively open managed savanna-like canopies such as montado (Portugal) and dehesa (Spain) ecosystems or macchia vegetation in the Mediterranean basin (Oliveira *et al.* 1996; Teixeira Filho *et al.* 1998). However, foliage clumping at the shoot and branch scale is generally larger in more open canopies (Asner *et al.* 1998; Rambal 2001; Cescatti and Niinemets 2005). Given that increases in leaf aggregation reduce the mean irradiance on leaf surface, this negative relationship between the degree of foliage clumping at various scales implies that large tree-to-tree spacing is not necessarily always compatible with higher mean irradiance on leaf surface. Furthermore, Mediterranean sclerophyll species with long leaf life span may support large foliar areas that absorb 95–99% of incident light (see Introduction), suggesting severe light limitation of mid- and lower canopy leaves.

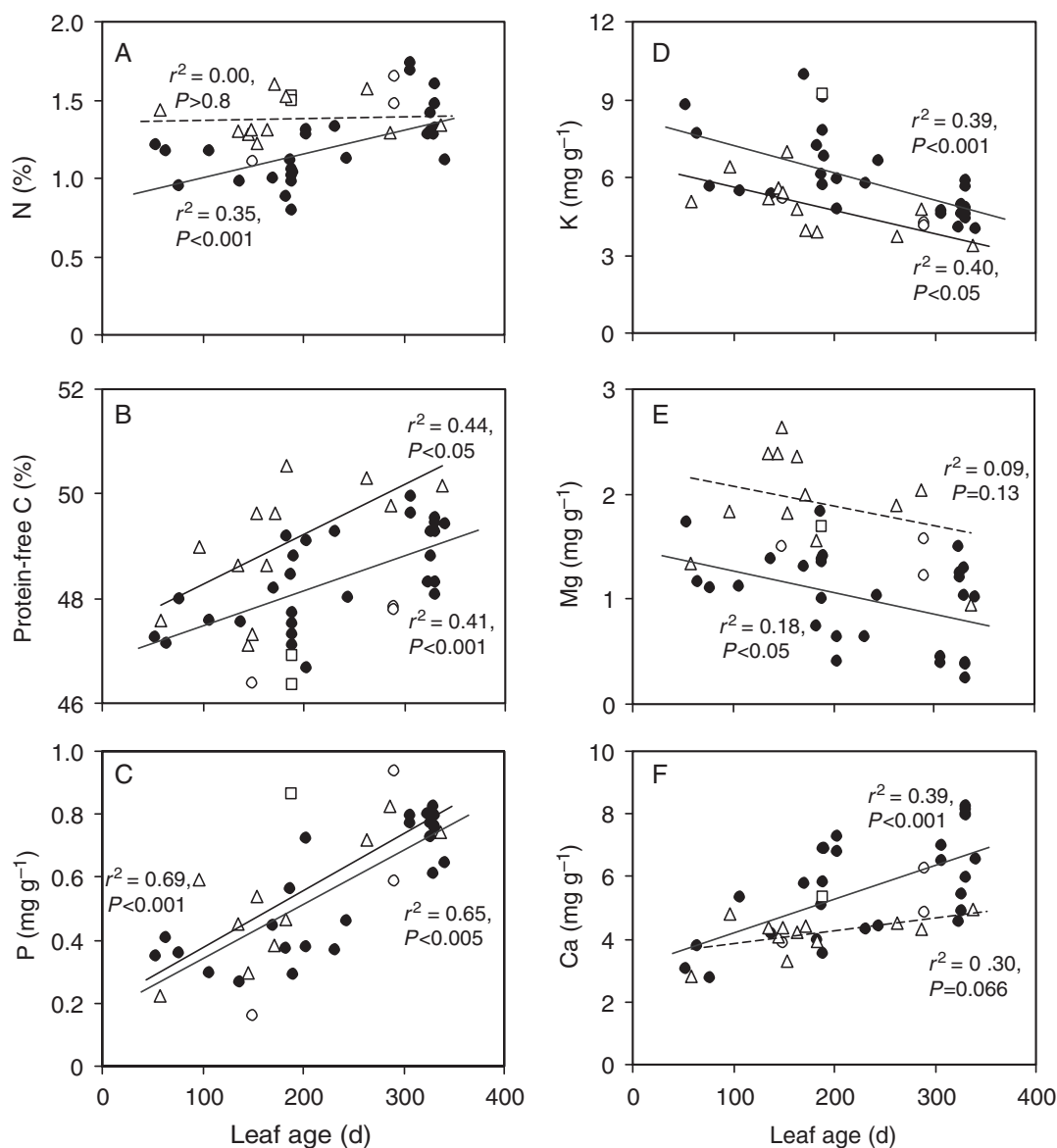


Fig. 5. Changes in leaf mineral nutrient contents (A; C–F) and structural carbon content [eqn 4, (B)] in four Mediterranean *Quercus* species. Data of *Q. coccifera*, ● and *Q. suber*, △ were fitted by linear regressions and non-significant regressions are depicted by ----. Available data for *Q. faginea*, □ and *Q. ilex*, ○ are also shown.

We found 2.4-fold canopy variation in leaf dry mass per unit area (M_A , Fig. 1A) that was accompanied by an approximately 3-fold variation in leaf nitrogen content per unit area (inset in Fig. 1A). This range of variation in M_A in *Q. coccifera* agrees with previous studies demonstrating approximately 1.5–2.2-fold canopy range in M_A in *Q. coccifera* and *Q. ilex* canopies (Gratani *et al.* 1992; Sala *et al.* 1994; Rambal *et al.* 1996) and is also similar to the average canopy gradients in temperate trees (e.g. Niinemets and Kull 1998). Thus, our data along with previous observations suggest that the structural plasticity to light is not necessarily limited in Mediterranean species.

The within-canopy variation in M_A and leaf nitrogen content per unit area was accompanied by a 3-fold variation in leaf photosynthetic potentials per unit area in non-water stressed leaves (Fig. 2A, B). This range is similar to temperate species (e.g. Niinemets *et al.* 2004), demonstrating that the physiological plasticity is also not necessarily low in Mediterranean species. A smaller range, of approximately 1.4-fold, in J_{\max} per area was found in *Q. ilex* by Rambal *et al.* (1996). However, leaves with M_A values larger than 100 g m^{-2} were measured in their study (Rambal *et al.* 1996). Given that leaf photosynthetic potentials appear to scale non-linearly with light (Fig. 2A, B), sampling leaves mostly

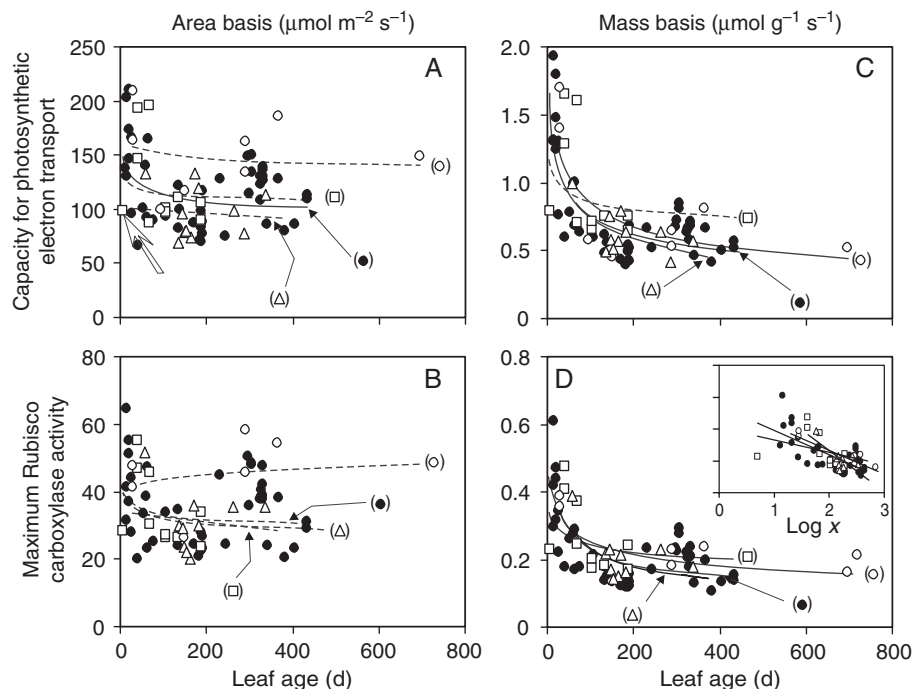


Fig. 6. Age-dependent changes in J_{\max} per unit area (A) and dry mass (C) and V_{\max} per unit area (B) and dry mass (D) in four Mediterranean *Quercus* species. Data were fitted by non-linear regression equations in the form of $y = a + \log(x)$. ●, *Q. coccifera* [$r^2=0.16$, $P<0.01$ for (A); $r^2=0.07$, $P=0.07$ for (B); $r^2=0.55$, $P<0.001$ for (C); $r^2=0.42$, $P<0.001$ for (D)], □, *Q. faginea* [$r^2=0.03$, $P>0.6$ for (A); $r^2=0.06$, $P>0.5$ for (B); $r^2=0.09$, $P>0.4$ for (C); $r^2=0.12$, $P>0.3$ for (D)], ○, *Q. ilex* [$r^2=0.06$, $P>0.5$ for (A); $r^2=0.07$, $P>0.5$ for (B); $r^2=0.65$, $P<0.02$ for (C); $r^2=0.69$, $P<0.02$ for (D)], △, *Q. suber* [$r^2=0.05$, $P>0.5$ for (A); $r^2=0.14$, $P>0.2$ for (B); $r^2=0.42$, $P<0.05$ for (C); $r^2=0.34$, $P<0.05$ for (D)]. Non-significant regressions are depicted by ----. Open arrow in (A) denotes an outlying observation in the youngest leaves of *Q. faginea* that qualitatively affected the significance of the regressions fitted to the data. Inset in (D) demonstrates the correlation between V_{\max} / mass and logarithmic leaf age.

from intermediate and exposed canopy micro-environments in the study of Rambal *et al.* (1996) may contribute to the discrepancy between their study and ours.

As there were only minor changes in dry-mass based nitrogen contents (Fig. 1B) and leaf photosynthetic characteristics and fractional nitrogen investments in leaf photosynthetic machinery (Fig. 2C, D), the light effects on leaf photosynthetic characteristics were mainly driven by light-dependent changes in M_A (capacity per area = capacity per mass $\times M_A$) as is commonly observed in temperate species (Niinemets *et al.* 2004). Nevertheless, light significantly affected leaf structural carbon content (Fig. 1C), possibly reflecting enhanced investment in structural compounds with large carbon concentration such as lignin. Similarly to our study, Rambal *et al.* (1996) reported greater total cell wall percentage in leaves at higher light. Enhanced investment in support may be an important adaptive feature that allows the leaves to balance more negative turgor pressures in leaves intercepting higher irradiances (Niinemets 2001).

Age effects on foliar structure and chemistry in Mediterranean oaks

In addition to important light effects, there are significant time-dependent changes in leaf structure and chemistry. We observed an essentially constant increase in M_A of mature leaves in all species (Fig. 4) with the slope of M_A v. day of the year relationships varying from $0.18 \text{ g m}^{-2} \text{ d}^{-1}$ in *Q. faginea* to $0.27 \text{ g m}^{-2} \text{ d}^{-1}$ in *Q. suber*. Thus, in the evergreen species, the values of M_A increased by 1.6- (*Q. coccifera*, *Q. ilex*) to 1.9-fold (*Q. suber*) during the first growing year and M_A was increased by 1.5-fold at the end of the growing season in the marcescent species *Q. faginea* (Fig. 4). Similar time-dependent increase in M_A has been reported by Gratani *et al.* (1992); Gratani (1996) and Damesin *et al.* (1998) for *Q. ilex* and by Xu and Baldocchi (2003) for Mediterranean deciduous species *Q. douglasii*, further accentuating a strong age-dependent control on the accumulation of foliar biomass per unit area.

In deciduous temperate species, foliar nitrogen contents per unit dry mass strongly decrease during leaf development,

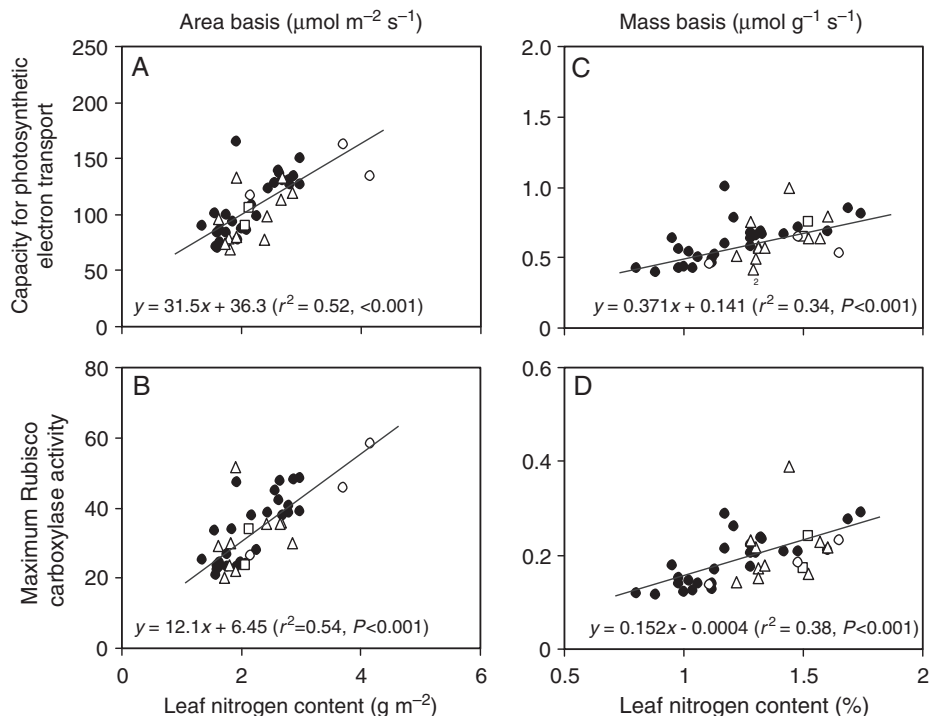


Fig. 7. Correlations between J_{max} per unit area (A) and dry mass (C) and V_{cmax} per unit area (B) and dry mass (D) with leaf nitrogen content per unit area (A, B) and dry mass (C, D) in four Mediterranean *Quercus* species (the same leaves as in Fig. 6). Symbols as in Fig. 4. Data were fitted by linear regressions.

are stable in mature leaves and further decrease in senescing leaves due to remobilisation of leaf nitrogen (Wilson *et al.* 2001; Niinemets *et al.* 2004). Similar time-dependent changes are observed for other mobile elements such as P, K and Mg, while the contents of Ca increase during the season (Insley *et al.* 1981). There is also a strong age-dependent decrease in mobile leaf nutrients in temperate evergreen conifers (Weikert *et al.* 1989; Niinemets and Lukjanova 2003). In addition, older conifer needle age classes are characterised by a significant spring decline in foliar nutrient contents due to nutrient remobilisation that fuels new foliage growth (Weikert *et al.* 1989).

As in temperate species, previous work has demonstrated a significant drop in leaf N contents during leaf expansion in *Q. coccifera* (Meletiou-Christou *et al.* 1994) and N and P contents in *Q. ilex* (Rapp *et al.* 1992). Given that leaf nutrient contents were available for the leaves older than 50 days in our study (Fig. 5), this early-seasonal peak was probably not captured in our study. In fully-expanded non-senescent leaves, only changes in the cation contents per dry mass (Fig. 5D–F) agreed with the previous observations on age-dependent nutrient changes in temperate deciduous and evergreen trees, while N content was independent of age in *Q. suber* and it increased with age in *Q. coccifera*

(Fig. 5A) and P contents increased in both species (Fig. 5C). Nevertheless, at any specific date, the scatter was large (Fig. 5).

Although such changes observed in our study contrast the responses in temperate species, further studies demonstrate that there is an overall significant study-to-study variation in temporal time-courses in foliar N and P contents in fully-developed non-senescent leaves of Mediterranean *Quercus* species. While N content per dry mass decreased with increasing age in *Q. coccifera* (Meletiou-Christou *et al.* 1994), relative age-independence (Gratani and Moriconi 1983; de Lillis and Fontanella 1992; Rapp *et al.* 1992; Sabate *et al.* 1995; Robert *et al.* 1996) or increase (Castell *et al.* 1994; Sabaté *et al.* 1995; Damesin *et al.* 1998) of foliar N and P in mature non-senescent leaves was reported for *Q. ilex* and *Q. suber*. Part of this study-to-study variation is apparently associated with species- and study-specific accumulation patterns in non-structural carbohydrates (Drossopoulos and Niavis 1988; Meletiou-Christou *et al.* 1994; Fleck *et al.* 1995) that may be more strongly related to seasonality than to leaf age. In addition, there are also important species variations in age-dependent change patterns of structural carbon (de Lillis and Fontanella 1992; Fleck *et al.* 1995; Fig. 5B).

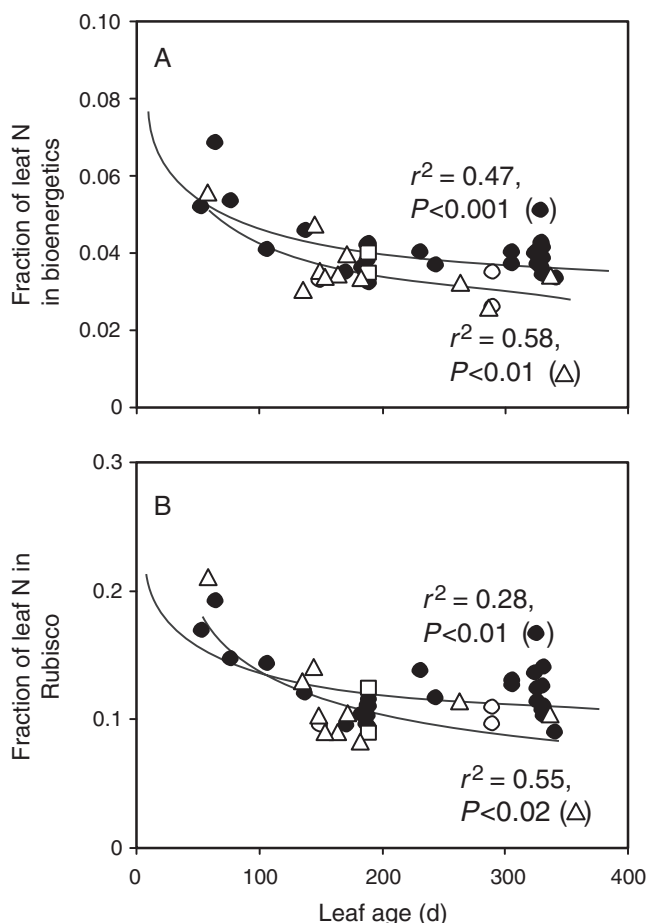


Fig. 8. Leaf age effects on the apparent fractions of leaf nitrogen invested in photosynthetic electron transport (eqn 5; A) and in Rubisco (eqn 6; B) in Mediterranean *Quercus* species. Symbols as in Fig. 4. Data of *Q. coccifera*, ● and *Q. suber*, △ were fitted by non-linear regressions as in Fig. 6. Data available for *Q. faginea*, □ and *Q. ilex*, ○ are also depicted.

These data collectively suggest that age-dependent modifications in nutrient concentrations are less significant in Mediterranean evergreens than in temperate species. This conclusion is further reinforced by the evidence demonstrating that leaf N content of older leaves decreases by only 10–15% in the spring due to remobilisation of foliar nutrients (Rapp *et al.* 1992; Cherbuy *et al.* 2001) in Mediterranean *Quercus* species. This is low compared with 30–60% N retranslocation in temperate conifers (Weikert *et al.* 1989; Millard 1994) and suggests that old leaves play a minor role as the nutrient deposit for the growth of new foliage in Mediterranean *Quercus* species. Rather, it seems that high nitrogen contents of older leaves serve to maintain high foliage assimilation potential during favourable soil water conditions in spring. In more arid sites that support deciduous species, foliage assimilation capacities and nitrogen contents are extremely high in the spring

(Smith and Nobel 1986; Xu and Baldocchi 2003) further strengthening the argument of the role of high N in maximisation of the carbon gain during periods of ample water availability.

Contrary to the variable age-dependent patterns in N contents per dry mass, N contents per area consistently increase in Mediterranean evergreen species (this study, Castell *et al.* 1994; Sabaté *et al.* 1995) demonstrating the overwhelming effect of age-dependent changes in M_A (Fig. 4).

Changes in leaf photosynthetic activity with seasonality and leaf age

Previous research has emphasised the role of seasonality, in particular summer drought in modifying the photosynthetic characteristics in Mediterranean climates (Tenhunen *et al.* 1985, 1990; Beyschlag *et al.* 1986; Werner *et al.* 2001a). Although there may be large decreases in realised net assimilation rates in droughted plants, biochemical capacities for photosynthesis are relatively insensitive to diurnal and seasonal water stress in Mediterranean species (Demmig-Adams *et al.* 1989; Methy *et al.* 1996; Xu and Baldocchi 2003). According to detailed studies, the initial quantum yield of photosynthesis is the characteristic most sensitive to water stress in Mediterranean species (Demmig-Adams *et al.* 1989; Faria *et al.* 1996), as was confirmed by our study (Fig. 3).

Apart from seasonality, our results demonstrate a significant age-dependent decline in leaf mass-based photosynthesis potentials (Fig. 6C, D), but also show that on the area basis, this decline was somewhat compensated for by increases in M_A (cf. Fig. 4 and Fig. 6) such that the age-dependent decrease of leaf photosynthesis potentials per unit area was significant only in *Q. coccifera* (Fig. 6A, B). Some scatter in the age *v.* photosynthesis capacity relations may also result from low fractional investments in leaf nitrogen in photosynthetic machinery as is common in young leaves (Niinemets *et al.* 2004).

The overall decrease in foliage assimilation potentials per unit dry mass in Mediterranean oaks agrees with a previous report for evergreen *Q. ilex* (Leßner 1994) and deciduous *Q. douglasii* (Xu and Baldocchi 2003) as well as with the general age-dependent decrease in temperate evergreen conifers (Niinemets 2002). Our data also confirm that the decline in assimilation potentials is mainly linked to decreases in mass-based foliar nitrogen contents (Niinemets 2002; Xu and Baldocchi 2003). However, Flexas *et al.* (2001) observed an age-dependent increase in leaf photosynthetic capacity per unit area in Mediterranean evergreen species *Pistacia lentiscus* L. Their study, as well as our finding of essentially constant leaf photosynthetic potentials per unit area (Fig. 6A, B) underscore the importance of concomitant modifications in M_A (Fig. 4) and leaf nitrogen content

per dry mass (Fig. 5A) on age-related changes in leaf photosynthetic rates.

Does the accumulation of structural compounds lead to decreases in photosynthetic capacity?

The relationships between leaf nitrogen content and leaf photosynthetic potentials in leaves of Mediterranean oak species of varying age were scattered (Fig. 7). Reich *et al.* (1995) and Ripullone *et al.* (2003) have demonstrated that leaf nitrogen *v.* photosynthesis relationships may differ among different plant functional groups. These differences are mainly associated with plant group differences in nitrogen investments in photosynthetic machinery (Ripullone *et al.* 2003). In particular, conifers have relatively low apparent fractional investments of foliar nitrogen in bioenergetics (F_B , eqn 5) and Rubisco (F_R ; eqn 4), e.g. $F_R = 0.1\text{--}0.15$ in conifers *v.* $F_R = 0.2\text{--}0.3$ in deciduous temperate trees (Ripullone *et al.* 2003). Thus, compared with deciduous temperate species, the overall investment of leaf N in photosynthetic machinery is also low in evergreen Mediterranean oaks (Fig. 8).

The initial relative low values of F_B and F_R further decreased with increasing leaf age (Fig. 8). Increases in the fraction of non-photosynthetic nitrogen with increasing leaf age may provide an explanation for the age-dependent decreases in F_B and F_R (Fig. 8). There is evidence that the fraction of leaf N in cell wall materials increases with increasing leaf age in *Fagus sylvatica* L. (Dyckmans *et al.* 2002). This suggests that the fraction of photosynthetic N may indeed decrease in aging leaves. In addition, such an enhancement of bound N pool size in aging leaves may partly explain the puzzling increase in leaf N with increasing leaf age (Fig. 5A).

However, in interpreting the low values of F_R and F_B and their decline, it is further important that both F_B and F_R are the apparent nitrogen investments in photosynthetic machinery that are calculated on the basis of the assumption that the CO₂ transfer conductance from the sub-stomatal cavities to the carboxylation sites in the chloroplasts does not vary among the leaves with differing age. This should not necessarily be the case, especially given that the decrease in F_B and F_R coincided with increases in leaf total and structural carbon contents per dry mass (Fig. 5B). Similarly to these data, large age-dependent changes in carbon (Fleck *et al.* 1995) and total cell wall content have also been reported for the other Mediterranean evergreens (Diamantoglou and Kull 1988; Damesin *et al.* 1998) as well as for the temperate conifers (Niinemets and Lukjanova 2003). Enhanced cell wall accumulation and lignification should significantly affect the liquid-phase diffusion conductance from the outer surface of cell walls to chloroplasts. Such a decrease in diffusion conductance would result in significant changes in the apparent nitrogen investments in

photosynthetic machinery. Taking a representative value of $0.15\text{ mol m}^{-2}\text{ s}^{-1}$ for the mesophyll diffusion conductance in young sclerophyll leaves (Loreto *et al.* 1992) with a fractional N investment of Rubisco of 0.20 (Fig. 8B), we calculated that an apparent change in F_R from 0.20 to 0.09 (Fig. 8) would correspond to 1.4–1.5-fold decrease in the internal diffusion conductance. Although these calculations should be interpreted as only approximations, they still underscore the potential importance of age-dependent changes in leaf structure and chemistry on the use efficiency of limiting resources in photosynthesis.

Conclusions

Our data collectively demonstrate important canopy and temporal gradients in foliar structure, chemistry and physiology in four wide-spread Mediterranean species. Simulation studies in temperate deciduous species suggest that within-canopy variation in foliage photosynthetic characteristics may alter whole canopy carbon gain by 10–20% (Baldocchi and Harley 1995), while consideration of phenology and leaf age can further modify the simulated carbon gain by 20–30% (Morecroft *et al.* 2003). So far, the spatial and age-dependent variations in leaf photosynthetic characteristics are widely considered to play a minor role in whole canopy functioning in Mediterranean species and are generally not included in whole-canopy photosynthesis simulations (e.g. Tenhunen *et al.* 1990; Infante *et al.* 1999; Werner *et al.* 2001b) with a few exceptions (Sala and Tenhunen 1996). However, most of the foliage of Mediterranean species experiences shade at least during certain periods of the day, bringing about acclimation responses to the light gradient. In addition, important changes in leaf photosynthetic potentials occur continuously throughout the life span of the leaves. We suggest that the ranges of spatial and age-dependent modifications in foliage functioning observed in our study are large enough to significantly modify the whole canopy carbon balance, especially during periods of non-limiting or moderately limiting soil water availability when the daily photosynthesis integral is more strongly controlled by potential leaf photosynthetic capacities than during drought periods. Taken together, seasonal carbon gain of Mediterranean species is significantly affected by within-canopy and age-dependent variations in leaf photosynthesis potentials and we strongly suggest that these controls be included in large-scale carbon balance estimations in these communities.

Acknowledgments

We thank Annette Suske for the help with chemical analyses. Financial support was provided by the Estonian Science Foundation (grant 5702), the Estonian Ministry of Education and Science (grant 0182468As03) and the Deutsche Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (Grant PT BEO 51–0339476B).

References

- Acherar M, Rambal S (1992) Comparative water relations of four Mediterranean oak species. *Vegetatio* **99–100**, 177–184.
- Asner GP, Wessman CA, Archer S (1998) Scale dependence of absorption of photosynthetically active radiation in terrestrial ecosystems. *Ecological Applications* **8**, 1003–1021.
- Baldocchi DD, Harley PC (1995) Scaling carbon dioxide and water vapour exchange from leaf to canopy in a deciduous forest. II. Model testing and application. *Plant, Cell and Environment* **18**, 1157–1173.
- Bernier PY, Raulier F, Stenberg P, Ung C-H (2001) Importance of needle age and shoot structure on canopy net photosynthesis of balsam fir (*Abies balsamea*): a spatially inexplicit modeling analysis. *Tree Physiology* **21**, 815–830.
- Beyschlag W, Lange OL, Tenhunen JD (1986) Photosynthese und Wasserhaushalt der immergrünen mediterranen Hartlaubpflanze *Arbutus unedo* L. im Jahreslauf am Freilandstandort in Portugal. I. Tagesläufe von CO₂-Gaswechsel und Transpiration unter natürlichen Bedingungen. *Flora* **178**, 409–444.
- Beyschlag W, Phibbs A, Pfanz H (1990) The role of temperature and humidity in controlling the diurnal stomatal behaviour of *Arbutus unedo* L. during the dry season. *Biochemie und Physiologie der Pflanzen* **186**, 265–271.
- Beyschlag W, Pfanz H, Ryel RJ (1992) Stomatal patchiness in Mediterranean evergreen sclerophylls. Phenomenology and consequences for the interpretation of the midday depression in photosynthesis and transpiration. *Planta* **187**, 546–553. doi: 10.1007/BF00199976fs
- Caldwell MM, Meister H-P, Tenhunen JD, Lange OL (1986) Canopy structure, light microclimate and leaf gas exchange of *Quercus coccifera* L. in a Portuguese macchia: measurements in different canopy layers and simulations with a canopy model. *Trees: Structure and Function* **1**, 25–41.
- Castell C, Terradas J, Tenhunen JD (1994) Water relations, gas exchange and growth of resprouts and mature plant shoots of *Arbutus unedo* L. and *Quercus ilex* L. *Oecologia* **98**, 201–211.
- Castro-Diez P, Villar-Salvador P, Pérez-Rontomé C, Maestro-Martínez M, Montserrat-Martí G (1997) Leaf morphology and leaf chemical composition in three *Quercus* (Fagaceae) species along a rainfall gradient in NE Spain. *Trees: Structure and Function* **11**, 127–134.
- Catarino FM, Correia OCA, Correia AIVD (1982) Structure and dynamics of Serra da Arrábida Mediterranean vegetation. *Ecologia Mediterranea* **8**, 203–222.
- Cescatti A, Niinemets Ü (2005) Light harvesting: from leaf to landscape. In 'Evaluating photosynthetic adaptation from the chloroplast to the landscape. Ecological studies, 178'. (Eds WK Smith, C Crichtley, T Vogelmann) In press. (Springer-Verlag: Berlin)
- Cherbuy B, Joffre R, Gillon D, Rambal S (2001) Internal remobilization of carbohydrates, lipids, nitrogen and phosphorus in the Mediterranean evergreen oak *Quercus ilex*. *Tree Physiology* **21**, 9–17.
- Damesin C, Rambal S, Joffre R (1998) Seasonal and annual changes in leaf $\delta^{13}\text{C}$ in two co-occurring Mediterranean oaks: relations to leaf growth and drought progression. *Functional Ecology* **12**, 778–785. doi: 10.1046/J.1365-2435.1998.00259.X
- de Lillis M, Fontanella A (1992) Comparative phenology and growth in different species of the Mediterranean maquis of central Italy. *Vegetatio* **99–100**, 83–96.
- Demmig-Adams B, Adams WW III, Winter K, Meyer A, Schreiber U, Pereira JS, Krüger A, Czygan F-C, Lange OL (1989) Photochemical efficiency of photosystem II, photon yield of O₂ evolution, photosynthetic capacity and carotenoid composition during the midday depression of net CO₂ uptake in *Arbutus unedo* growing in Portugal. *Planta* **177**, 377–387.
- Diamantoglou S, Kull U (1988) Der Stickstoffhaushalt immergrüner mediterraner Hartlaubblätter. *Flora* **180**, 377–390.
- Drossopoulos JB, Nivais CA (1988) Seasonal changes of the metabolites in the leaves, bark and xylem tissues of olive tree (*Olea europaea* L.). II. Carbohydrates. *Annals of Botany* **62**, 321–327.
- Dyckmans J, Flessa H, Brinkmann K, Mai C, Polle A (2002) Carbon and nitrogen dynamics in acid detergent fibre lignins of beech (*Fagus sylvatica* L.) during the growth phase. *Plant, Cell and Environment* **25**, 469–478. doi: 10.1046/J.1365-3040.2002.00826.X
- Escudero A, del Arco JM, Sanz IC, Ayala J (1992) Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different woody species. *Oecologia* **90**, 80–87.
- Evans JR, Loreto F (2000) Acquisition and diffusion of CO₂ in higher plant leaves. In 'Photosynthesis: physiology and metabolism'. (Eds RC Leegood, TD Sharkey, S von Caemmerer) pp. 321–351. (Kluwer Academic Publishers: Dordrecht)
- Evans JR, Seemann JR (1989) The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences and control. In 'Plant biology'. pp. 183–205. (AR Liss Inc.: New York)
- Faria T, Garcia-Plazaola JI, Abadía, Cerasoli S, Pereira JS, Chaves MM (1996) Diurnal changes in photoprotective mechanisms in leaves of cork oak (*Quercus suber*) during summer. *Tree Physiology* **16**, 115–123.
- Farquhar GD, von Caemmerer S (1982) Modeling of photosynthetic response to environmental conditions. In 'Physiological plant ecology'. (Eds OL Lange, PS Nobel, CB Osmond, H Ziegler) pp. 549–588. (Springer-Verlag: Berlin)
- Fleck I, Diaz C, Pascual M, Iniguez FJ (1995) Ecophysiological differences between first-year resprouts after wildfire and unburned vegetation of *Arbutus unedo* and *Coriaria myrtifolia*. *Acta Oecologica* **16**, 55–69.
- Fleck S, Niinemets Ü, Cescatti A, Tenhunen JD (2003) Three-dimensional lamina architecture alters light harvesting efficiency in *Fagus*: a leaf-scale analysis. *Tree Physiology* **23**, 577–589.
- Flexas J, Gulías J, Jonasson S, Medrano H, Mus M (2001) Seasonal patterns and control of gas exchange in local populations of the Mediterranean evergreen shrub *Pistacia lentiscus* L. *Acta Oecologica* **22**, 33–43. doi: 10.1016/S1146-609X(00)01099-7
- Gracia CA (1984) Response of the evergreen oak to the incident radiation at the Montseny (Barcelona, Spain). *Bulletin de la Société Botanique de France. Actualités botaniques* **131**, 595–597.
- Gratani L (1996) Leaf and shoot growth dynamics of *Quercus ilex* L. *Acta Oecologica* **17**, 17–27.
- Gratani L, Moriconi M (1983) Seasonal changes in nutrient concentration in *Quercus ilex*. *Annals of Botany* **41**, 185–195.
- Gratani L, Marzi P, Crescente MF (1992) Morphological adaptations of *Quercus ilex* leaves in the Castelporziano forest. *Vegetatio* **100**, 155–161.
- Harley PC, Thomas RB, Reynolds JF, Strain BR (1992) Modelling photosynthesis of cotton grown in elevated CO₂. *Plant, Cell and Environment* **15**, 271–282.
- Infante JM, Damesin C, Rambal S, Fernández-Alés R (1999) Modelling leaf gas exchange in holm-oak trees in southern Spain. *Agricultural and Forest Meteorology* **95**, 203–223. doi: 10.1016/S0168-1923(99)00033-7
- Insley H, Boswell RC, Gardiner JBH (1981) Foliar macronutrients (N, P, K, Ca and Mg) in lime (*Tilia* spp.). II. Seasonal variation. *Plant and Soil* **61**, 391–401.
- Jonasson S, Medrano H, Flexas J (1997) Variation in leaf longevity of *Pistacia lentiscus* and its relationship to sex and drought stress inferred from leaf $\delta^{13}\text{C}$. *Functional Ecology* **11**, 282–289. doi: 10.1046/J.1365-2435.1997.00090.X

- Jordan DB, Ogren WL (1984) The CO_2/O_2 specificity of ribulose 1,5-bisphosphate carboxylase/oxygenase. Dependence on ribulose bisphosphate concentration, pH and temperature. *Planta* **161**, 308–313.
- Kellomäki S, Wang K-Y (1997) Effects of elevated O_3 and CO_2 concentrations on photosynthesis and stomatal conductance in Scots pine. *Plant, Cell and Environment* **20**, 995–1006.
- Lange OL, Tenhunen JD, Harley P, Walz H (1985) Method for field measurements of O_2 -exchange. The diurnal changes in net photosynthesis and photosynthetic capacity of lichens under Mediterranean climatic conditions. In 'Lichen physiology and cell biology'. (Ed DH Brown) pp. 23–39. (Plenum Publishing Corporation: New York)
- Leßner A (1994) 'Die Beziehung zwischen Gaswechsel, Blattdemographie und Stickstoffhaushalt an immer- und wechselgrünen mediterranen Holzgewächsen.' Diploma Dissertation, Fakultät für Biologie, Chemie und Geowissenschaften der Universität Bayreuth, Germany.
- Loreto F, Harley PC, di Marco G, Sharkey TD (1992) Estimation of mesophyll conductance to CO_2 flux by three different methods. *Plant Physiology* **98**, 1437–1443.
- Martínez-Ferri E, Balaguer L, Valladares F, Chico JM, Manrique E (2000) Energy dissipation in drought-avoiding and drought-tolerant tree species at midday during the Mediterranean summer. *Tree Physiology* **20**, 131–138.
- Mediavilla S, Escudero A (2003) Stomatal response to drought at a Mediterranean site: a comparative study of co-occurring woody species differing in leaf longevity. *Tree Physiology* **23**, 987–996.
- Meletioux-Christou MS, Rhizopoulou S, Diamantoglou S (1994) Seasonal changes of carbohydrates, lipids and nitrogen content in sun and shade leaves from four Mediterranean evergreen sclerophylls. *Environmental and Experimental Botany* **34**, 129–140. doi: 10.1016/0098-8472(94)90032-9
- Méthy M, Damesin C, Rambal S (1996) Drought and photosystem II activity in two Mediterranean oaks. *Annales Des Sciences Forestières* **53**, 255–262.
- Millard P (1994) Measurement of the remobilisation of nitrogen for spring leaf growth of trees under field conditions. *Tree Physiology* **14**, 1049–1054.
- Morecroft MD, Stokes VJ, Morison JIL (2003) Seasonal changes in the photosynthetic capacity of canopy oak (*Quercus robur*) leaves: the impact of slow development on annual carbon uptake. *International Journal of Biometeorology* **47**, 221–226. doi: 10.1007/S00484-003-0173-3
- Niinemets Ü (2001) Climatic controls of leaf dry mass per area, density and thickness in trees and shrubs at the global scale. *Ecology* **82**, 453–469.
- Niinemets Ü (2002) Stomatal conductance alone does not explain the decline in foliar photosynthetic rates with increasing tree age and size in *Picea abies* and *Pinus sylvestris*. *Tree Physiology* **22**, 515–535.
- Niinemets Ü, Kull O (1998) Stoichiometry of foliar carbon constituents varies along light gradients in temperate woody canopies: implications for foliage morphological plasticity. *Tree Physiology* **18**, 467–479.
- Niinemets Ü, Lukjanova A (2003) Needle longevity, shoot growth and branching frequency in relation to site fertility and within-canopy light conditions in *Pinus sylvestris*. *Annals of Forest Science* **60**, 195–208. doi: 10.1051/FOREST:2003012
- Niinemets Ü, Tenhunen JD (1997) A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant, Cell and Environment* **20**, 845–866. doi: 10.1046/J.1365-3040.1997.D01-133.X
- Niinemets Ü, Seufert G, Steinbrecher R, Tenhunen JD (2002) A model coupling foliar monoterpene emissions to leaf photosynthetic characteristics in Mediterranean evergreen *Quercus* species. *New Phytologist* **153**, 257–276. doi: 10.1046/J.0028-646X.2001.00324.X
- Niinemets Ü, Kull O, Tenhunen JD (2004) Within canopy variation in the rate of development of photosynthetic capacity is proportional to integrated quantum flux density in temperate deciduous trees. *Plant, Cell and Environment* **27**, 293–313.
- Oliveira G, Werner C, Correia O (1996) Are ecophysiological responses influenced by crown position in cork-oak? *Annales des Sciences Forestières* **53**, 235–241.
- Pereira JS, Tenhunen JD, Lange OL, Beyschlag W, Meyer A, David MM (1986) Seasonal and diurnal patterns in leaf gas exchange of *Eucalyptus globulus* trees growing in Portugal. *Canadian Journal of Forest Research* **16**, 177–184.
- Pereira JS, Beyschlag G, Lange OL, Beyschlag W, Tenhunen JD (1987) Comparative phenology of four mediterranean shrub species growing in Portugal. In 'Plant response to stress. Functional analysis in Mediterranean ecosystems'. (Eds JD Tenhunen, F Caterino, OL Lange, WC Oechel) pp. 503–513. (Springer-Verlag: Berlin)
- Rambal S (2001) Productivity of Mediterranean-type ecosystems. In 'Terrestrial global productivity: past, present and future'. (Eds HA Mooney, B Saugier, J Roy) pp. 315–344. (Academic Press Inc.: San Diego)
- Rambal S, Damesin C, Joffre R, Méthy M, Lo Seen D (1996) Optimisation of carbon gain in canopies of Mediterranean evergreen oaks. *Annales des Sciences Forestières* **53**, 547–560.
- Rapp M, Ed Derfoufi F, Blanchard A (1992) Productivity and nutrient uptake in a holm oak (*Quercus ilex* L.) stand and during regeneration after clearcut. *Vegetatio* **99–100**, 263–272.
- Reich PB, Kloeppel BD, Ellsworth DS, Walters MB (1995) Different photosynthesis-nitrogen relations in deciduous hardwood and evergreen coniferous tree species. *Oecologia* **104**, 24–30.
- Ripullone F, Grassi G, Lauteri M, Borghetti M (2003) Photosynthesis-nitrogen relationships: interpretation of different patterns between *Pseudotsuga menziesii* and *Populus × euramericana* in a mini-stand experiment. *Tree Physiology* **23**, 137–144.
- Robert B, Berton G, Sayag D, Masson P (1996) Assessment of mineral nutrition of cork oak through foliar analysis. *Communications in Soil Science and Plant Analysis* **27**, 2091–2109.
- Sabaté S, Sala A, Gracia CA (1995) Nutrient content in *Quercus ilex* canopies: seasonal and spatial variation within a catchment. *Plant and Soil* **168–169**, 297–304.
- Sala A, Tenhunen JD (1996) Simulations of canopy net photosynthesis and transpiration in *Quercus ilex* L. under the influence of seasonal drought. *Agricultural and Forest Meteorology* **78**, 203–222. doi: 10.1016/0168-1923(95)02250-3
- Sala A, Sabaté S, Gracia C, Tenhunen JD (1994) Canopy structure within a *Quercus ilex* forested watershed: variations due to location, phenological development and water availability. *Trees: Structure and Function* **8**, 254–261.
- Sala i Serra A (1992) 'Water relations, canopy structure and canopy gas exchange in a *Quercus ilex* forest: variation in time and space.' PhD thesis. Universitat de Barcelona, Facultat de Biologia, Departament d'ecologia, Spain.
- Saur E, Nambiar EKS, Fife DN (2000) Foliar nutrient retranslocation in *Eucalyptus globulus*. *Tree Physiology* **20**, 1105–1112.
- Schoettle AW, Smith WK (1999) Interrelationships among light, photosynthesis and nitrogen in the crown of mature *Pinus contorta* ssp. *latifolia*. *Tree Physiology* **19**, 13–22.
- Schulze E-D, Hall AE, Lange OL, Walz H (1982) A portable steady-state porometer for measuring the carbon dioxide and water vapour exchanges of leaves under natural conditions. *Oecologia* **53**, 141–145.

- Smith SD, Nobel PS (1986) Deserts. In 'Photosynthesis in contrasting environments. Topics in photosynthesis, 7'. (Eds NR Baker, SP Long) pp. 13–62. (Elsevier Science Publishers: Amsterdam)
- Sokal RR, Rohlf FJ (1995) 'Biometry. The principles and practice of statistics in biological research.' 3rd edn. (WH Freeman and Co.: New York)
- Teixeira Filho J, Damesin C, Rambal S, Joffre R (1998) Retrieving leaf conductances from sap flows in a mixed Mediterranean woodland: a scaling exercise. *Annales des Sciences Forestieres* **55**, 173–190.
- Tenhunen JD, Lange OL, Gebel J, Beyschlag W, Weber JA (1984a) Changes in photosynthetic capacity, carboxylation efficiency and CO₂ compensation point associated with midday stomatal closure and midday depression of net CO₂ exchange of leaves of *Quercus suber*. *Planta* **162**, 193–203.
- Tenhunen JD, Meister HP, Caldwell MM, Lange OL (1984b) Environmental constraints on productivity of the Mediterranean sclerophyll shrub *Quercus coccifera*. In 'Proceedings of INTECOL workshop — rates of natural primary productivity and agricultural production'. pp. 33–53. (Institutio Agronomico Mediterraneo de Zaragoza: Zaragoza, Spain)
- Tenhunen JD, Lange OL, Harley PC, Beyschlag W, Meyer A (1985) Limitations due to water stress on leaf net photosynthesis of *Quercus coccifera* in the Portuguese evergreen shrub. *Oecologia* **67**, 23–30.
- Tenhunen JD, Beyschlag W, Lange OL, Harley PC (1987) Changes during summer drought in leaf CO₂ uptake rates of macchia shrubs growing in Portugal: limitations due to photosynthetic capacity, carboxylation efficiency and stomatal conductance. In 'Plant response to stress. Functional analysis in Mediterranean ecosystems'. (Eds JD Tenhunen, FM Catarino, OL Lange, WC Oechel) pp. 305–327. (Springer-Verlag: Berlin)
- Tenhunen JD, Sala Serra A, Harley PC, Dougherty RL, Reynolds JF (1990) Factors influencing carbon fixation and water use by mediterranean sclerophyll shrubs during summer drought. *Oecologia* **82**, 381–393.
- von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376–387.
- Weikert RM, Wedler M, Lippert M, Schramel P, Lange OL (1989) Photosynthetic performance, chloroplast pigments and mineral content of various needle age classes of spruce (*Picea abies*) with and without the new flush: an experimental approach for analysing forest decline phenomena. *Trees: Structure and Function* **3**, 161–172.
- Werner C, Ryel RJ, Correia O, Beyschlag W (2001a) Effects of photoinhibition on whole-plant carbon gain assessed with a photosynthesis model. *Plant, Cell and Environment* **24**, 27–40. doi: 10.1046/J.1365-3040.2001.00651.X
- Werner C, Ryel RJ, Correia O, Beyschlag W (2001b) Structural and functional variability within the canopy and its relevance for carbon gain and stress avoidance. *Acta Oecologica* **22**, 129–138. doi: 10.1016/S1146-609X(01)01106-7
- Wilson KB, Baldocchi DD, Hanson PJ (2001) Leaf age affects the seasonal pattern of photosynthetic capacity and net ecosystem exchange of carbon in a deciduous forest. *Plant, Cell and Environment* **24**, 571–583. doi: 10.1046/J.0016-8025.2001.00706.X
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, *et al.* (2004) The world-wide leaf economics spectrum. *Nature* **428**, 821–827. doi: 10.1038/NATURE02403
- Xu L, Baldocchi DD (2003) Seasonal trends in photosynthetic parameters and stomatal conductance in blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. *Tree Physiology* **23**, 865–877.

Manuscript received 15 July 2004, accepted 21 September 2004