Comparative seasonal gas exchange and chlorophyll fluorescence of two dominant woody species in a Holm Oak Forest

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Summary

Diurnal courses of net CO₂ uptake rate, stomatal conductance (gₛ), maximum photochemical efficiency of PSII (Fv/Fm) and apparent photosynthetic electron transport rate (ETR) were measured in Quercus ilex and Phillyrea latifolia in a holm oak forest throughout the seasons of the year. These measurements were complemented with response curves of photosynthetic rates to PPFD and CO₂ concentrations. P. latifolia was better adapted to drought and warm conditions and showed higher net CO₂ uptake, gₛ and Fv/Fm values than Q. ilex in summer. But in autumn and specially in winter P. latifolia was more sensitive to low temperatures and experienced lower net CO₂ uptake, gₛ, ETR and Fv/Fm values than Q. ilex. The maximum net CO₂ uptake values for P. latifolia occurred under summer high temperatures whereas maximum net CO₂ uptake values for Q. ilex occurred under winter low temperatures. However, in summer during midday, both species presented null or slightly negative net CO₂ uptake rates. Since in the summer season both species experienced similar ETR values, the lower net CO₂ uptake values of Q. ilex suggest that Q. ilex presented greater photorespiration rates. During winter, very low Fv/Fm values were found especially for P. latifolia, indicating that maximal photochemical efficiency of PSII is very sensitive to low temperatures. However, they were not accompanied by low net CO₂ uptake rates showing that cold photoinhibition determined a potential but not an actual decrease in photosynthetic performance. Under well watered conditions and with high CO₂ concentration and saturated PPFD, Q. ilex was able to increase its photosynthetic rates whereas P. latifolia had lower plasticity to make a profit of optimal environmental conditions. These results show different strategies between these two dominant co-occurring species. They also indicate that the warmer and drier conditions expected for the Mediterranean region in the near decades as a result of climate change will favour drought resistant species with lower photosynthetic rates such as P. latifolia in detriment of more mesic species such as Q. ilex.

Key words: chlorophyll fluorescence, cold, drought, electron transport rate, Mediterranean trees, photochemical efficiency, photoinhibition, photosynthetic rates, Phillyrea latifolia, Quercus ilex, stomatal conductance, climate change

Introduction

Mediterranean environments are often characterized by a double stress: summer drought and winter cold (Mit- rakos 1980). Summer drought, characterized by low precipitation and high temperature, high irradiance and high water vapour pressure deficit (Terradas & Savé 1992; Di Castri & Mooney 1973; Pereira & Chaves 1995), is frequently considered as a key factor in Mediterranean environments. Many studies have described low photosynthetic rates during summer drought due to a great stomatal control of transpirational water loss (Tenhunen et al. 1981, 1990; Lange et al. 1982; Lösch et al. 1982; Harley et al. 1987; Gratani 1993, 1995; Faria et al. 1998; Filella et al. 1998; Peñuelas et al. 1998; Gratani & Bombelli 1999; Karavatas & Manetas 1999; Llusià & Peñuelas 2000; Crescente et al. 2002; Gratani & Ghia 2002). On the other hand low photosynthetic rates during winter ( García-Plazaola et al. 1997; Tretiach et al. 1997; Lar-...
and partial photoinhibition of PSII under low temperatures (Larcher 2000; Oliveira & Peñuelas 2000, 2001) have also been described.

Co-occurring Mediterranean woody species have often different climatic constraints to photosynthesis under summer drought (Castell et al. 1994; Peñuelas et al. 1998) and low temperatures in winter (Larcher 2000; Oliveira & Peñuelas 2000, 2001). Mediterranean climate is likely to become warmer and with drier summers (IPCC 2001), and the different Mediterranean species will not develop the same physiological responses to the environmental changes. Information of these physiological responses may allow to predict future changes in species development and distribution, community structure and ecosystem functioning.

Holm oak (Quercus ilex L.) is a tree species well adapted to drought and widely distributed in the Mediterranean basin. Phillyrea latifolia L. is a tall shrub species associated with the holm oak forest, that has been described as more drought resistant (Gratani 1993; Lloret & Siscart 1995; Peñuelas et al. 1998, 2000, 2001; Gratani & Bombelli 2000) and less cold tolerant (Tretiach 1993) than Q. ilex. The aim of this work was to characterize photosynthetic performance of Q. ilex and P. latifolia in the different annual seasons, including the dry summer and the relatively cold winter, in order to discuss the adaptive strategies of these two species to a changing climate and the possible consequences in their future distribution.

Material and methods

Study site

The study was carried out in a natural holm oak forest growing at Prades Mountains in North-Eastern Spain (41°13’ N, 0°35’ E) at 950 m above sea level, on a south-facing slope (25% slope). The soil is a stony xerochrept on a bedrock of metamorphic sandstone, and its depth ranges between 35 and 90 cm. The average annual temperature is 12°C and the annual rainfall 658 mm. Summer drought is pronounced and usually lasts for 3 months. Table 1 shows the average temperature, rainfall and radiation for each season during the period of study. The data were provided by an automatic meteorological station placed in the study site.

Table 1. Mean values of radiation, temperature, and cumulative precipitation during the four annual seasons in the study site.

<table>
<thead>
<tr>
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<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
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<tr>
<td>Radiation</td>
<td>21.2</td>
<td>21.4</td>
<td>7.6</td>
<td>11.4</td>
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<td>(MJ m⁻² day⁻¹)</td>
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<tr>
<td>Temperature</td>
<td>14.4</td>
<td>19.5</td>
<td>9.1</td>
<td>6.6</td>
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<tr>
<td>°C</td>
<td></td>
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<tr>
<td>Precipitation</td>
<td>335.4</td>
<td>76.6</td>
<td>257.8</td>
<td>57.4</td>
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<td>(mm)</td>
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Gas exchange and fluorescence measurements

Gas exchange and chlorophyll fluorescence were measured during 2-6 consecutive days in the four annual seasons: spring, summer, autumn, and winter in current-year leaves (fully developed in spring). Sun leaves (from the upper layer of the canopy, fully exposed to the sun, and southfacing orientation) and shade leaves (from the lower layers of the canopy) were measured under clear-sky conditions.

Net CO₂ uptake rate and stomatal conductance (gₛ) were measured with a portable gas exchange system ADC4, with a PLC4B chamber (ADC Inc., Hoddesdon, Hertfordshire, UK). Four different plants per species were measured three times a day: morning, midday and afternoon. Current-year leaves were measured in each plant and canopy position. Both net CO₂ uptake and gₛ were expressed on a projected leaf area basis measured with a Li-Cor 3100 Area Meter (Li-Cor Inc., Nebraska, USA). The maximum photochemical efficiency of PSII (Fv/Fm) and the apparent photosynthetic electron transport rate (ETR) were measured with a PAM-2000 fluorometer (Walz, Effeltrich, Germany). ETR was estimated as

\[ ETR = \Delta F/F_{m}' \times PPFD \times 0.84 \times 0.5 \]

where \( \Delta F/F_{m}' \) (actual photochemical efficiency of PSII) was calculated according to Genty et al. (1989), 0.84 was the coefficient of absorption of the leaves, and 0.5 was the fraction of electrons involved in the photoexcitation produced by one quantum, as two photosystems are involved. Chlorophyll fluorescence was measured on five current-year leaves of each one of four plants per species and canopy position three times a day: morning, midday and afternoon. In summer the measurements were made on five leaves of each one of three plants per species and canopy position five times a day: dawn, morning, midday, afternoon and evening. The maximum PSII photochemical efficiencies (Fv/Fm) were measured after keeping leaves in the dark for at least 20–25 min.

As complementary measurements, four net CO₂ uptake rate response curves to CO₂ for each one of the two species, and four CO₂ gas exchange response curves to PPFD for each one of the two species and for each one of the two types of leaves (sunlit and shaded ones) were conducted with the portable gas exchange system CIRAS2 (PP Systems, Hitchin, Hertfordshire, UK) in autumn 2001.

Statistical analyses

In each season differences of net CO₂ uptake, gₛ, Fv/Fm and ETR between Quercus ilex and Phillyrea latifolia were tested by analysis of variance (ANOVA) with species as the inde-
pendent factor. Regression analyses were conducted to examine the relationships between net CO$_2$ uptake and PPFD, $g_S$, ETR, and temperature. Analyses of covariance (ANCOVA) were used to test the differences of these relationships between *Q. ilex* and *P. latifolia* and among seasons by using net CO$_2$ uptake as a dependent factor and $g_S$ and ETR as covariates. Non-linear regression analyses were also conducted to examine the CO$_2$ and PPFD response curves. Analyses of covariance (ANCOVA) were used to test the differences of these relationships between *Q. ilex* and *P. latifolia*; net CO$_2$ uptake was the dependent variable and CO$_2$ the covariate. CO$_2$ and PPFD variables were log transformed to reach the normality assumptions of the ANCOVAs. For Michaelis-Menten type relationships, only the linear unsaturated part of the curves was analyzed with ANCOVAs. The saturated values were analyzed with ANOVAs or t-tests. All analyses were performed with the Superanova software package (Abacus Concepts Inc., 1991) and the Statview software package (Abacus Concepts Inc., 1998).

**Results**

While *P. latifolia* presented similar $g_S$ values, and maximum net CO$_2$ uptake rates throughout all seasons (with slightly higher rates in summer), *Q. ilex* had higher maximum net CO$_2$ uptake rates in autumn and winter. On the other hand, both species presented highest $g_S$ values in winter (Fig. 1). During spring both species showed similar maximum net CO$_2$ uptake and $g_S$ values. But in autumn and winter *Q. ilex* experienced larger maximum net CO$_2$ uptake rates, and in winter larger $g_S$ values than *P. latifolia* (Fig. 1). However *P. latifolia* usually showed higher maximum net CO$_2$ uptake and $g_S$ values than *Q. ilex* in summer.

In the summer season, net CO$_2$ uptake declined during midday both in sun and shade leaves, even reaching negative values, and $g_S$ declined only in sun *P. latifolia* leaves because shade *P. latifolia* and *Q. ilex* leaves experienced low values throughout the whole day (Fig. 2). Water use efficiency (WUE), calculated as net CO$_2$ uptake/transpirational rate declined at midday in both species but was overall higher in *P. latifolia* than in *Q. ilex* (Fig. 2). Summer net CO$_2$ uptake rates were higher in sun leaves than in shade leaves in both species except at midday, when all sun and shade leaves showed very low net CO$_2$ uptake rates, often even negative. The $g_S$ values were higher in sun leaves except at midday, and WUE showed similar values in the two types of leaves of the two species except in afternoon for *P. latifolia* which presented slightly higher WUE in shade than in sun leaves (Fig. 2).

In congruence with the different photosynthetic performance of these two woody Mediterranean species, Fv/Fm presented higher values for *P. latifolia* in summer and for *Q. ilex* in winter. In spring Fv/Fm and ETR values were very high compared to the rest of the seasons and very similar in both species, whereas in summer, autumn and especially in winter sun leaves showed low Fv/Fm and ETR values, especially in *P. latifolia* (Fig. 3). In winter even shade leaves showed lower Fv/Fm values than in the other seasons (Fig. 3). Fv/Fm and ETR values experienced a slight decrease around midday in sun leaves during spring and autumn, but not during winter when they were already very low in the morning (Fig. 3). In summer ETR values were low (such as in autumn and winter) and similar in both species, but Fv/Fm of sun leaves decreased around midday and this decrease was higher in *Q. ilex* than in *P. latifolia* (Fig. 3).

Net CO$_2$ uptake and ETR values were correlated in spring and autumn, but not in summer and winter. On the other hand, there were no significant differences in net CO$_2$ uptake-ETR relationships between the two species, but there was a trend towards higher slopes in *Q. ilex* during autumn and winter, and higher slopes in *P. latifolia* during summer (Fig. 4).

The relationship between net CO$_2$ uptake and $g_S$ showed a linear fit in all seasons (Fig. 5). In both species

![Fig. 1. Maximum net photosynthetic rates and stomatal conductance in sun leaves of *Quercus ilex* and *Phillyrea latifolia* in the four annual seasons. Vertical bars indicate standard error of the mean (n = 3 – 4). Two asterisks indicate p < 0.01, and three asterisks p < 0.001.](image-url)
the relationships were very similar during spring and summer, but *Q. ilex* relationships experienced higher slopes than those of *P. latifolia* in autumn and winter (p < 0.001 and p < 0.01, respectively).

In sun leaves net CO₂ uptake, gs and ETR relationships with leaf temperature differed depending on the different seasons (p = 0.025, p = 0.079 and p < 0.0001 for net CO₂ uptake, gs and ETR, respectively). In both species leaf optimal temperature ranged from low values (12 °C for *Q. ilex* and 15 °C for *P. latifolia*) in winter to high values (32 °C for *Q. ilex* and 35 °C for *P. latifolia*) in summer and were higher in *P. latifolia* than in *Q. ilex*. Figure 6 clearly illustrates the contrasting performance of these coexisting dominant species. The maximum net photosynthetic rates for *Q. ilex* occur under winter low temperatures whereas the maximum net photosynthetic rates for *P. latifolia* occur under summer high temperatures. Figure 6 also highlights that the maximum ETR and therefore the maximum capacities for photosynthetic performance of both species occur in spring linked to the highest irradiances and the optimal temperature and water availability conditions.

CO₂ and PPFD response curves, conducted in autumn, when plants are under moderate temperatures and high water availability, showed good fits to logarithmic and Michaelis-Menten relationships, respectively. CO₂ and PPFD response curves showed earlier saturation and lower net CO₂ uptake values at saturating PPFD in *P. latifolia* than in *Q. ilex* (p < 0.0001) (Fig. 7, Fig. 8). For both species, there was a trend to earlier saturation of net photosynthetic rates at lower PPFD.
Fig. 3. Daily course of maximum photochemical efficiency (Fv/Fm) in sun and shade leaves, and apparent photosynthetic electron transport rate (ETR) in sun leaves of *Quercus ilex* and *Phillyrea latifolia* during the four annual seasons. Vertical bars indicate standard error of the mean (n = 15 – 20). One square indicate p < 0.1, one asterisk p < 0.05, two asterisks p < 0.01, and three asterisks p < 0.001.

Fig. 4. Relationship between net CO₂ uptake rates and apparent photosynthetic electron transport rate (ETR) in *Quercus ilex* and *Phillyrea latifolia* during the four annual seasons. Data include sun and shade leaves. Bars indicate standard error of the mean (n = 3 – 4 for net photosynthetic rates and n = 15 – 20 for ETR). Values correspond to measurements in morning, midday and afternoon for shade and sun leaves.
levels in shade than in sun leaves. However, sun and shade leaves of both species showed similar saturation net CO₂ uptake rates (Fig. 8).

**Discussion**

Equinoctial periods, without drought and cold stresses, are widely described as the most favourable seasons to photosynthetic activity of Mediterranean vegetation (Savé et al. 1999). In this work, high net CO₂ uptake and gₛ values were also reached in winter in *Q. ilex* despite the low temperatures. As this was not the case for *P. latifolia*, *Q. ilex* showed higher photosynthetic rates than *P. latifolia*, suggesting a higher tolerance of photosynthesis of *Q. ilex* to low temperatures in agreement with a previous report of Tretiach (1993). During hot and dry summer both species experienced low net CO₂ uptake rates during midday due to stomatal closure, avoiding excessive water loss during the central hours of the day (Tenhunen et al. 1981, 1990; Lange et al. 1982; Lösch et al. 1982; Harley et al. 1987; Gratani 1993, 1995; Faría et al. 1998; Karavatas & Manetas 1999; Llusia & Peñuelas 2000). However, *P. latifolia* reached higher net CO₂ uptake and WUE values than *Q. ilex* in the morning and in the afternoon showing a better adaptation to drought and warm conditions (Filella et al. 1998; Peñuelas et al. 1998; Gratani & Bombelli 2000).

Maximal photochemical efficiency of PSII was very sensitive to low temperatures (Larcher 2000; Oliveira & Peñuelas 2000, 2001), and in second term to summer low water availability. Cold stress reduced Fv/Fm in both sun and shade leaves, but Fv/Fm had a stronger decrease in sun leaves due to the combined cold and high irradiance stress, while low water availability also reduced Fv/Fm values only when combined with high irradiance. When no cold or drought stress occurred Fv/Fm values remained high all the day in sun and shade leaves.

Actual photochemical efficiency of PSII was high in spring and lower in the other periods, probably because spring was the only season where irradiance was high and no cold or drought stress occurred. In the coldest days *Q. ilex* had higher ETR and Fv/Fm values than *P. latifolia*, but in summer, despite of the great Fv/Fm in *P. latifolia*, both species showed similar ETR values or they were even higher in *Q. ilex*. Other reports have also shown higher net CO₂ uptake and gₛ in *P. latifolia* than in *Q. ilex* during summer drought, and also similar low
Fig. 6. Relationship between net photosynthetic rates, stomatal conductance, apparent photosynthetic electron transport rate (ETR) and leaf temperature in sun and shade leaves of *Quercus ilex* and *Phillyrea latifolia* during the four annual seasons. Each point is an individual measurement.

Fig. 7. Responses of PPFD-saturated photosynthetic rates to different CO₂ concentrations in sun leaves of *Quercus ilex* and *Phillyrea latifolia*. Each point is an individual measurement. (These response curves were measured in autumn.)
ETR values (Filella et al. 1998; Peñuelas et al. 1998). Those authors have hypothesized higher photorespiration rates in Q. ilex to explain this mismatch between net CO\textsubscript{2} uptake and ETR.

Strong irradiation combined with cold or drought result in an activation of some photoprotective mechanisms such as the de-epoxidation of the xanthophyll cycle that lowers the photochemical efficiency of PSII (Demmig-Adams & Adams 1992; Long et al. 1994). During the coldest seasons Fv/Fm values were low and no recovery took place during the night, probably because re-epoxidation did not occur during the night if there was frost (Adams & Demmig-Adams 1995). In summer a high difference between predawn and midday de-epoxidation values have been described (Martínez-Ferri et al. 2000), which is in agreement with the recovery of Fv/Fm values found here during the night. During the summer drought the low Fv/Fm and ETR values coincided with low net CO\textsubscript{2} uptake rates, but in the coldest season the low photochemical efficiencies did not imply low net CO\textsubscript{2} uptake rates. Thus winter photoinhibition only determined a potential decrease in net CO\textsubscript{2} uptake rates, but it was summer stomatal closure that produced a strong actual decrease on net assimilation rates.

Likely, higher photorespiration rates in Q. ilex (Heber et al. 1990; Scarascia-Mugnozza et al. 1996) may explain the trend towards a lower slope in the net CO\textsubscript{2} uptake-ETR relationship in Q. ilex than in P. latifolia observed during summer drought (Filella et al. 1998; Peñuelas et al. 1998). On the other hand, the trend to higher net CO\textsubscript{2} uptake-ETR relationship slope in Q. ilex than in P. latifolia during the colder seasons can also be explained by the lower photorespiration rates in Q. ilex in cold seasons than in summer. Photorespiration is suggested as a possible mechanism of dissipating excess photochemical energy in water-stressed and heat-stressed plants (Peñuelas & Llusia 2002) and it decreases exponentially when temperatures decrease (Cornic & Briantais 1991; Kozaki & Takeba 1996). In any case, in winter P. latifolia experienced higher photoinhibition rates (lower ETR values) than Q. ilex. But the net CO\textsubscript{2} uptake-ETR relationship slope was still lower because of the stronger decrease in net CO\textsubscript{2} uptake rates.

Linear regressions between net CO\textsubscript{2} uptake and gs measured throughout the whole day showed a good linear fit in all seasons, even though in other studies of Quercus species with data only from the morning, it has been observed a better fit with a rectangular hyperbola (Mediavilla et al. 2001). The proportionality between gs and net CO\textsubscript{2} uptake indicates again that photosynthesis of the two species is strictly controlled by stomatal apertures. P. latifolia seemed to be able to cope better with summer heat and drought than Q. ilex because P. latifolia had higher net CO\textsubscript{2} uptake and also gs values despite of similar net CO\textsubscript{2} uptake-gs rates in both species. On the other hand, P. latifolia showed lower slopes in net CO\textsubscript{2} uptake-gs relationships than Q. ilex during the colder seasons. The lower gs values in P. latifolia...
than in *Q. ilex* implied lower net CO$_2$ uptake rates, but *P. latifolia* also presented a higher photoinhibition as another factor involved in its low net CO$_2$ uptake rates in colder seasons without a direct effect on g$_S$ values. Thus, *Q. ilex* seemed to cope better with winter cold.

Temperatures were very different between seasons (Table 1), and photosynthetic parameters such as net CO$_2$ uptake, g$_S$ and ETR were strongly influenced by these seasonal changes in temperature. In both species, it seems that optimum temperature for net CO$_2$ uptake and g$_S$ is seasonally adjusted depending on the range of temperatures that occurred in each season. *P. latifolia* showed again higher tolerance to high temperatures and lower tolerance to winter cold than *Q. ilex*; it had higher optimum temperature for net CO$_2$ uptake rates both in summer and winter. This higher tolerance is further indicated by the maximum response curves (Fig. 6) that show decreasing net CO$_2$ uptake values with high temperatures in *Q. ilex* and, on the contrary, increasing net CO$_2$ uptake values with high temperatures in *P. latifolia*.

CO$_2$ and PPFD response curves were measured in autumn, a season with well watered conditions and moderate temperatures. *Q. ilex* was able to reach higher net CO$_2$ uptake rates under PPFD and CO$_2$ saturating conditions. *P. latifolia*, which was more drought resistant than *Q. ilex*, presented, however, lower plasticity to obtain high photosynthetic rates under non stressed environmental conditions.

The two dominant species of this Mediterranean forest, *Q. ilex* and *P. latifolia*, presented different photosynthetic performances in response to the variable temperature and water conditions throughout the year. The increase of temperatures and summer drought predicted by global circulation models (IPCC 2001) could favour more drought and heat resistant species such as *P. latifolia* compared to more mesic ones such as *Q. ilex*.

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**References**


