Foliar CO₂ in a holm oak forest subjected to 15 years of climate change simulation

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A B S T R A C T

A long-term experimental drought to simulate future expected climatic conditions for Mediterranean forests, a 15% decrease in soil moisture for the following decades, was conducted in a holm oak forest since 1999. Net photosynthetic rate, stomatal conductance and leaf water potential were measured from 1999 to 2013 in \textit{Quercus ilex} and \textit{Phillyrea latifolia}, two co-dominant species of this forest. These measurements were performed in four plots, two of them received the drought treatment and the two other plots were control plots. The three studied variables decreased with increases in VPD and decreases in soil moisture in both species, but the decrease of leaf water potential during summer drought was larger in \textit{P. latifolia}, whereas \textit{Q. ilex} reached higher net photosynthetic rates and stomatal conductance values during rainy periods than \textit{P. latifolia}.

The drought treatment decreased ca. 8% the net photosynthetic rates during the overall studied period in both \textit{Q. ilex} and \textit{P. latifolia}, whereas there were just non-significant trends toward a decrease in leaf water potential and stomatal conductance induced by drought treatment. Future drier climate may lead to a decrease in the carbon balance of Mediterranean species, and some shrub species well resistant to drought could gain competitive advantage relative to \textit{Q. ilex}, currently the dominant species of this forest.

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1. Introduction

Summer drought has been recognized as the major stress limiting plant growth and development in all Mediterranean regions of the world [1]. In these Mediterranean regions, a reduction in water availability is expected for the near future as a result of decreases of precipitation and increases of temperatures, and the consequent increases of evapotranspiration rates [2], which are predicted to be greater than those that already occurred in the twentieth century by most General Circulation Models [2]. Lower water availability is also projected for this region by ecophysiological models such as G\textsc{otilha} [3,4].

Holm oak (\textit{Quercus ilex}\textsc{L}) is a widely distributed tree species in Mediterranean basin, that tolerates drought effects by reducing its leaf area and by stomatal closure control [5,6]. Mock privet (\textit{Phillyrea latifolia}\textsc{L}) is a tall shrub species usually associated with holm oak, but with higher capacity to tolerate drought than \textit{Q. ilex} [7–9]. \textit{P. latifolia} showed smaller photosynthetic rates in autumn, winter and spring, and lower photochemical efficiency in winter than \textit{Q. ilex}, but in summer, \textit{P. latifolia} was the species with larger photosynthetic rates and photochemical efficiency [7,9]. Under a decrease in water availability, it has been observed a strong reduction in stem growth and aboveground biomass increment in \textit{Q. ilex}, not observed in \textit{P. latifolia} [10], and higher vulnerability to xylem embolism in \textit{Q. ilex} than in \textit{P. latifolia} [11]. Larger leaf defoliation and stem mortality rates were also higher in \textit{Q. ilex} than in \textit{P. latifolia} under severe drought [10,12,13]. Moreover, these differences could decrease due to a long-term adaptation to new climatic conditions [13]. These two co-occurring woody Mediterranean species may be subjected to a decrease in net photosynthetic rates and to an increasing defoliation and risk of stem mortality under future forecasted climatic conditions, but their different tolerance to hot and dry climate may lead to changes in their capacity to compete between them. \textit{Q. ilex}, the currently dominant species of this Mediterranean forest, could decline in favor of \textit{P. latifolia}, more adapted to new climatic constrains.

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Our aim was to study the effect of a long-term experimental drought on photosynthetic activity in these two co-dominant species of the Mediterranean forest in order to elucidate this possible advantage of *P. latifolia* compared to *Q. ilex* under these drier conditions. The experimental period covered several years and the four seasons of the year, including inter-annual and inter-seasonal variability in the study of the responses of these plants to drought treatment. Climate change experiments in natural ecosystems are scarce, but they are especially valuable because small trees in lab environments could show different responses to climate than mature trees. As far as we know, this is one of the longest climate change experiments in a natural forest of the world, and all possible long-term adaptations to the experimental treatment are included in our long-term measurements.

2. Materials and methods

2.1. Study site

The studied area is located in Prades holm oak forest, Catalonia, North-Eastern Spain (41°21′ N, 1°2′ E), at an altitude of 950 m a.s.l and on a south-facing slope (25% slope). The soil is a Dystric Cambisol over Paleozoic schist, and its depth ranges from 35 to 100 cm. This forest is dominated by holm oak (*Q. ilex* L.) (8633 stems ha⁻¹), mock privet (*P. latifolia* L.) (3600 stems ha⁻¹) and strawberry tree (*Arbutus unedo* L.) (2200 stems ha⁻¹), with abundant presence of other evergreen species well adapted to dry conditions such as *Erica arborea* L., *Juniperus oxycedrus* L., *Cistus albidus* L., and occasional individuals of deciduous species such as *Sorbus terminalis* (L.) Crantz, and *Acer monspessulanum* L. This forest was not perturbed during the last 60 years, and the maximum height of the dominant species is about 6–10 m tall. The average annual temperature is 12.3 °C and the average annual rainfall 603 mm (data from 1999 to 2012, both included). Summer drought is pronounced and usually lasts for 3 months.

2.2. Experimental design

Four 15 m × 10 m plots were delimited at the same altitude along the slope. Two randomly selected plots received the experimental treatment, and the other two plots remained as control plots. The experimental treatment consisted in a partial rain exclusion by suspending plastic strips at a height of 0.5–0.8 m above the soil (covering 30% of soil surface, and the excavation of a 0.8 m deep ditch at the upper part of the plots to intercept runoff water. An automatic meteorological station installed in the study site monitored temperature, photosynthetic active radiation, air humidity, and precipitation during the overall studied period. Soil moisture was measured each annual season using a time domain reflectometer (*Tektronix 1502C*, Beaverton, OR, USA). The reflectometer was connected to the ends of three stainless cylindrical rods, 25 cm long, fully driven into the soil [14], and four sites per plot were randomly selected to install three steel cylindrical rods.

2.3. Gas exchange and leaf water potential measurements

Net photosynthetic rate (*A*) and stomatal conductance (*gₛ*) were measured in leaves fully exposed to the sun during several annual seasons during the duration of the experimental drought treatment (from 1999 to 2013) (*Table 1*). Two current-year leaves of two different trees per species were measured in each plot around midday using portable gas exchange systems: an ADC4 system connected to a PLC4 chamber (ADC Inc., Hoddesdon, Hertfordshire, UK) from 1999 to 2001; a CIRAS2 system (PP Systems, Hitchin, Hertfordshire, UK) from 2003 to 2005; and a LI-6400XT system (LI-COR Inc., Lincoln, NE, USA) from 2012 to 2013. Leaf water potential (Ψ) was measured in one terminal twig of two different trees per species in each plot using a Scholander pressure chamber (PMS Instrument Co., Corvallis, OR, USA). Leaf water potential (Ψ) was measured during midday at the same date than gas exchange measurements. Midday values of vapor pressure deficit (VPD) were calculated for each day of *A*, *gₛ*, and Ψ measurements.

2.4. Stem mortality

The percentage of dead stems was calculated in both species counting, in each plot, the number of alive stems at the start of the experiment (1999) and how many of them survived at the end of the experiment (2013).

2.5. Statistical analyses

Simple linear regressions were conducted to examine the relationship of *A*, *gₛ*, and Ψ values with VPD and soil moisture. Also, other simple linear regressions were conducted to examine the relationships of *A* with Ψ and *gₛ* values. Later, multiple linear regressions were conducted to test the meteorological influence on *A*, *gₛ*, and Ψ values. *A*, *gₛ*, and Ψ were the dependent variables of different multiple linear regressions, whereas VPD and soil moisture were the predictor variables in all multiple linear regressions. In these multiple regressions the forward stepwise regression technique was used. For each day of measurements, analyses of variance (ANOVA's) were conducted with *A*, *gₛ*, and Ψ values as dependent variables, and species and treatment as independent factors. A repeated measurements ANOVA was conducted with soil moisture values in each plot as dependent variable and treatment as independent factor. Other repeated measures ANOVAs were conducted with *A*, *gₛ*, and Ψ values as dependent variables, and species and treatment as independent factors. Finally, a two-way ANOVA was conducted with the percentage of dead stems as dependent variable, and species and treatment as independent factors. Data of percentage of dead stems (*p*) was transformed to arc sin (*p*) to reach the normality assumptions of the ANOVA. All analyses were performed with the Statistica 8.0 software package (Statsoft Inc., 2007, Tulsa, OK, USA).

3. Results

Climate data of the studied site was typically Mediterranean: hot and dry summers, slightly cold winters, and precipitations concentrated in spring and autumn seasons (*Fig. 1*), excepting autumn 2004 and spring 2005, that were associated to unusual rainfall and soil moisture values (*Fig. 1*). The drought treatment reduced about 13% soil moisture of drought plots compared to control plots (*P*<0.01), but this reduction was larger during rainy seasons and lower during dry seasons (*Fig. 1*).

Maximum Ψ, *A*, and *gₛ* values were reached during rainy seasons (mainly spring and autumn) and during winter, while minimum values were reached during summer drought (*Figs. 2 and 3*). These three variables were negatively related with VPD and positively

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Date of all gas exchange measurements across the overall duration of the rainfall exclusion experiment (depicted in gray color).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>99</td>
</tr>
<tr>
<td>Wi</td>
<td></td>
</tr>
<tr>
<td>Sp</td>
<td></td>
</tr>
<tr>
<td>Su</td>
<td></td>
</tr>
<tr>
<td>Au</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Monthly values of mean air temperature and total precipitation in upper panels. Instantaneous values of vapor pressure deficit and soil moisture at the same time of leaf water potential and gas exchange measurements. Error bars in soil moisture data indicate the standard error of the mean (n = 2 plot means of 4 measurements each).

Fig. 2. Seasonal course of leaf water potential (Ψ) in Q. ilex and P. latifolia during the overall experimental period. Error bars indicate the standard error of the mean (n = 2 plot means of 2 measurements each). An asterisk indicates significant difference induced by drought treatment (P < 0.05).
related with SM (Fig. 4), although for the later the relationships tended to saturate at high SM (Table 2). \( \Psi \) values were more dependent on VPD and SM in \( P. \) latifolia than in \( Q. \) ilex (Fig. 4), so \( P. \) latifolia reached lower \( \Psi \) under hot and dry conditions than \( Q. \) ilex (\( P < 0.01 \)). In contrast, \( A \) and \( g_s \) values were more dependent on VPD and SM in \( Q. \) ilex than in \( P. \) latifolia (Fig. 4), so \( Q. \) ilex reached on average, higher \( A \) and \( g_s \) values than \( P. \) latifolia (\( P < 0.01 \)), especially during more humid seasons. \( A \) was highly correlated with \( \Psi \) and \( g_s \) in both species (Fig. 5), but the correlation was stronger in \( Q. \) ilex than in \( P. \) latifolia (Table 3; Figs. 6 and 7).

Analyzing each sampled date separately, \( \Psi \), \( A \), and \( g_s \) values were occasionally lower in drought plots than in control plots (\( P < 0.05 \)), especially in \( Q. \) ilex, but in most sampled dates there were no significant differences between treatments (Fig. 2). On the other hand, repeated-measures ANOVA showed clearly an overall significant decrease about 8% of \( A \) rates induced by drought treatment (\( P = 0.03 \)) (Table 4), both in \( Q. \) ilex and in \( P. \) latifolia (Figs. 2 and 3). Repeated measures ANOVAs for \( \Psi \) and \( g_s \) showed also lower values in drought plots than in control plots, but these differences were statistically non-significant (Figs. 2 and 3).
Table 2

<table>
<thead>
<tr>
<th>Species</th>
<th>Relationship</th>
<th>R² value</th>
<th>P value</th>
<th>N value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q. ilex</td>
<td>$\Psi = -4.91 \times 0.21 \text{VPD} + 0.63 \text{SM}$\textsuperscript{0.5}</td>
<td>0.66</td>
<td>&lt;0.001</td>
<td>40</td>
</tr>
<tr>
<td>P. latifolia</td>
<td>$\Psi = -7.33 \times 0.19 \text{VPD} + 1.25 \text{SM}$\textsuperscript{0.5}</td>
<td>0.74</td>
<td>&lt;0.001</td>
<td>40</td>
</tr>
<tr>
<td>Q. ilex</td>
<td>$A = 2.71 \div 1.18 \text{VPD} + 1.91 \text{SM}$\textsuperscript{0.5}</td>
<td>0.50</td>
<td>&lt;0.001</td>
<td>48</td>
</tr>
<tr>
<td>P. latifolia</td>
<td>$A = 2.06 \div 0.70 \text{VPD} + 1.37 \text{SM}$\textsuperscript{0.5}</td>
<td>0.38</td>
<td>&lt;0.001</td>
<td>48</td>
</tr>
<tr>
<td>Q. ilex</td>
<td>$g_s = -5.09 \div 11.8 \text{VPD} + 30.7 \text{SM}$\textsuperscript{0.5}</td>
<td>0.32</td>
<td>&lt;0.001</td>
<td>48</td>
</tr>
<tr>
<td>P. latifolia</td>
<td>$g_s = -4.38 \div 6.45 \text{VPD} + 24.2 \text{SM}$\textsuperscript{0.5}</td>
<td>0.34</td>
<td>&lt;0.001</td>
<td>48</td>
</tr>
</tbody>
</table>

Fig. 5. Mean seasonal values of leaf water potential ($\Psi$), net photosynthetic rate ($A$), and stomatal conductance ($g_s$) in Q. ilex and P. latifolia. Error bars indicate the standard error of the mean ($n$ ranges from 4 to 6 measurements in each annual season).

Table 3

<table>
<thead>
<tr>
<th>Species</th>
<th>Relationship</th>
<th>R² value</th>
<th>P value</th>
<th>N value</th>
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</thead>
<tbody>
<tr>
<td>Q. ilex</td>
<td>$A = 14.21 \div 5.10 \text{VPD} + 0.38 \text{SM}$\textsuperscript{0.5}</td>
<td>0.52</td>
<td>&lt;0.001</td>
<td>40</td>
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<tr>
<td>P. latifolia</td>
<td>$A = 6.75 \div 1.41 \text{VPD} + 0.06 \text{SM}$\textsuperscript{0.5}</td>
<td>0.48</td>
<td>&lt;0.001</td>
<td>40</td>
</tr>
<tr>
<td>Q. ilex</td>
<td>$A = 0.49 \div 0.074 \div 0.0016 \text{SM}$\textsuperscript{0.5}</td>
<td>0.64</td>
<td>&lt;0.001</td>
<td>48</td>
</tr>
<tr>
<td>P. latifolia</td>
<td>$A = 0.17 \div 0.084 \div 0.0034 \text{SM}$\textsuperscript{0.5}</td>
<td>0.42</td>
<td>&lt;0.001</td>
<td>48</td>
</tr>
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</table>

Table 4

<table>
<thead>
<tr>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F value</th>
<th>P value</th>
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<td>10.372</td>
<td>10.123</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>131.277</td>
<td>131.277</td>
<td>128.837</td>
</tr>
<tr>
<td>Treatment × species</td>
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<td>0.082</td>
<td>0.082</td>
<td>0.077</td>
</tr>
<tr>
<td>Group</td>
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<td>4.240</td>
<td>1.060</td>
<td>0.082</td>
</tr>
<tr>
<td>Category for A</td>
<td>23</td>
<td>1554.714</td>
<td>67.596</td>
<td>37.077</td>
</tr>
<tr>
<td>Category for A × Tr.</td>
<td>23</td>
<td>63.942</td>
<td>2.780</td>
<td>1.525</td>
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<tr>
<td>Category for A × Sp.</td>
<td>23</td>
<td>99.909</td>
<td>4.344</td>
<td>2.383</td>
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<tr>
<td>Category for A × Tr. × Sp.</td>
<td>23</td>
<td>57.288</td>
<td>2.491</td>
<td>1.366</td>
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<tr>
<td>Category for A × group</td>
<td>92</td>
<td>167.727</td>
<td>1.823</td>
<td>1.823</td>
</tr>
</tbody>
</table>

The percentage of Q. ilex dead stems during the overall period (1999–2013) was significantly higher in drought plots than in control plots ($P = 0.05$) (35.7 ± 8.0 and 27.9 ± 2.0% for drought and control plots, respectively), whereas no significant differences were found in P. latifolia (12.6 ± 4.9 and 10.8 ± 1.3% for drought and control plots, respectively). Stem mortality was thus clearly higher in Q. ilex than in P. latifolia.

4. Discussion

Warmer and drier climatic conditions projected for Mediterranean areas [2–4] may decrease growth rates of the dominant species of this forest and global ecosystem productivity [10,13] by decreasing their $A$ rates and increasing stem mortality, as observed in the drought treatment during the overall duration of the experiment, and as a result of the dependence of the three studied variables on the climatic conditions (VPD and SM). In this same experimental site, it was observed that a slight 13% decrease in soil water availability produced about 40% decrease of stem growth in Q. ilex trees [10], whereas these effects were very small in P. latifolia. In addition to the 8% decrease in $A$ rates, that by itself does not seem enough to explain this heavy drop of stem growth in Q. ilex trees subjected to the experimental drought, a major carbon allocation to exudates and roots in the drought plots seem very likely to maximize the uptake of scarcer water and nutrients [15] because, moreover, drought effect on $A$ rate was similar in both species during the overall studied period.

$A$ rates were higher in Q. ilex than in P. latifolia in all annual seasons except in summer, the hottest and driest season, when these differences between species disappeared. This result alone could induce to conclude that Q. ilex has a larger carbon uptake than P. latifolia, but $A$ rates are depicted in function of leaf area, and Q. ilex leaves have higher LMA than P. latifolia ones (the weight of Q. ilex leaves is 23 mg cm$^{-2}$, and the weight of P. latifolia leaves is 17 mg cm$^{-2}$) [16]. If carbon uptake is expressed as a function of leaf mass, the difference between species disappears, and even higher $A$ values are observed in P. latifolia than in Q. ilex during summer season.
The two studied species have a similar response to resist dry conditions: they close stomata under high VPD to avoid excessive water loss throughout transpiration [10,17], but the architecture of their xylem conduits is a key factor determining the larger capacity to resist dry conditions in *P. latifolia*, and the larger ability to reach high *A* rates under well-watered conditions in *Q. ilex*. The relationship between *A* and *Ψ* is stronger in *Q. ilex* than in *P. latifolia* because when stomata are closed and *A* rates are continuously very low, *Ψ* still decreases in *P. latifolia*. The relationship between *A* and *g* is also stronger in *Q. ilex* than in *P. latifolia* because larger *A* and *g* values are reached by *Q. ilex* during moist seasons, and the saturation of *A* rates with high *g*, values observed in *P. latifolia*. Narrow xylem conduits of *P. latifolia* are more able to achieve lower *Ψ* without excessive xylem embolism [18], whereas wider xylem vessels in *Q. ilex* are more susceptible to suffer xylem embolism arising the risk of stem mortality [19], but they provide *Q. ilex* a higher capacity to reach larger photosynthetic rates (due to larger *g*, values and transpiration rates) than *P. latifolia* under well-watered conditions. In addition, *P. latifolia* showed higher optimum temperature to reach maximum *A* rates and an earlier saturation of *A* rates at high SM than *Q. ilex* [9,17], and *P. latifolia* was more sensitive to photochemical damage during winter cold than *Q. ilex* [19]. So xylem architecture and other physiological adaptations provide more sensitivity to cold and more resistance to heat and drought conditions in *P. latifolia* than in *Q. ilex* [10].

Plant respiration is a very important component of the terrestrial carbon cycle [20], and the increase of temperature is mainly associated to an increase of respiration rates coupled with a smaller increase or even a saturation of *A* rates, resulting in a global decrease of the net carbon balance [21]. Moreover, another study in this forest revealed very low growth during recent years in *Q. ilex* now-dead stems, while surviving *Q. ilex* trees showed on average a higher stem growth during the same period [13]. After a heavy dry and unusually hot period in the studied forest during summer and autumn 2011, there was a forest decay event with a lot of defoliated and dead trees [12], with a depletion of lignotuber reserves in damaged trees [22], and *Q. ilex* was more affected again than *P. latifolia* [12]. Higher photorespiration rates as a consequence of drought [23] or high temperatures [24] might have greatly contributed to this decrease in net carbon balance, as shown by the negative *A* rates obtained during drier summers of the studied period, and the higher mortality rates observed during all experimental period in *Q. ilex* than in *P. latifolia*.

A future increase in VPD and a decrease in soil moisture induced by a raise of air temperatures will lead to a decrease in the carbon balance of Mediterranean species, but some shrub species such
as *P. latifolia*, more able to avoid xylem embolism, more resistant to lower leaf water potential and with lower photosynthetic rates during cold and humid periods than *Q. ilex*, could be favored by these new climatic constrains in detriment of the currently dominant tree species of this forest, *Q. ilex*, which could be subjected to an increasing risk of xylem embolism, defoliation, depletion of reserves, smaller carbon balance and tree mortality.

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