

# Long-term transpiration change with rainfall decline in a Mediterranean *Quercus ilex* forest

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## Abstract

In the Mediterranean basin, precipitation is expected to decline as a consequence of climate change. The response of a *Quercus ilex* forest in southern France to such a decline in water availability was studied using a 4-year throughfall exclusion experiment. Seasonal courses of sap flow and leaf water potential were obtained from 2004 to 2007 and used to characterize tree water relations in a control and a dry treatment. The experiment reduced the average precipitation input to the soil by 29%, and resulted in a 23% reduction in annual transpiration. Soil water potential was significantly lower in the dry treatment only during summer drought, but transpiration was reduced all year round even during well-watered periods. Despite a tight stomatal control over transpiration, whole-tree hydraulic conductance was found to be lower in the trees growing in the driest conditions. This reduction in water transport capacity was observed jointly with a reduction in leaf transpiring area. Canopy leaf area decreased by 18% in the dry treatment as a consequence of the throughfall exclusion, which was found to validate the ecohydrological equilibrium theory.

**Keywords:** drought, ecohydrology, hydraulic conductance, leaf area index, leaf water potential, Mediterranean evergreen forest, *Quercus ilex*, throughfall exclusion, transpiration

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## Introduction

Water availability has been shown to have dominant or codominant effects in the productivity of most biomes on Earth (Churkina & Running, 1998) and extreme droughts may severely impact the carbon balance of a wide range of terrestrial ecosystems (Ciais *et al.*, 2005). Drought is particularly crucial for Mediterranean evergreen forests (Reichstein *et al.*, 2002; Rambal *et al.*, 2003), where water availability has been identified as the major factor shaping vegetation and controlling plant functions (Rambal, 2001). These ecosystems are likely to experience more frequent and intense droughts with the on-going climate change. Recent climate projections for the Mediterranean basin forecast a decline in precipitation by >20% during spring and summer, associated with warmer temperatures leading to higher evaporative demand (Giorgi, 2006; Rowel & Jones, 2006).

Plants respond to drought by a variety of mechanisms at different levels associated in a nested hierarchy and phased with the time scale of the response (Rambal, 1993; Maseda & Fernandez, 2006). Stomatal closure is the fastest rate process and has been identified as the main mechanism used by evergreen Mediterranean tree species to regulate transpiration (Sala & Tenhunen, 1994) and to avoid irreversible damage to the plant hydraulic system (Martinez-Vilalta *et al.*, 2002). At a longer time scale, stomatal conductance is controlled by the hydraulic conductance of the soil to leaf pathway (Hubbard *et al.*, 2001) which decrease under drought stress either by the occurrence of xylem embolism (Sperry & Tyree, 1988) or by the decrease of soil hydraulic conductivity due to soil drying (Campbell, 1985). As *Quercus ilex* is a drought-tolerant species (Damesin *et al.*, 1998a), it maintains transpiration during drought at the risk of allowing some embolism (Martinez-Vilalta *et al.*, 2002). High losses of xylem conductivity have been reported in *Q. ilex* species (Tognetti *et al.*, 1998b), which can act as a mechanism to save water

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under severe soil water limitation. However, this mechanism should be co-ordinated with an adjustment of leaf area to reduce transpiration if drought persists (Rambal, 1993; Hoff & Rambal, 2003).

The responses of *Q. ilex* to drought have been intensively studied at leaf, tree or canopy scales (Sala & Tenhunen, 1994; Infante *et al.*, 1997, 2003; Teixeira Filho *et al.*, 1998; Tognetti *et al.*, 1998b; Martinez-Vilalta *et al.*, 2002, 2003; David *et al.*, 2004, 2007), by following time course of ecophysiological parameters during annual drought development in spring and summer and comparing seasonal patterns. This method correctly shows how *Q. ilex* manages its water resource during summer drought but is inappropriate to study longer term adjustment that is expected to occur as a consequence of the trend of decline in water inputs, as predicted by climate models. Moreover, it only yields qualitative information because water use and other physiological parameters cannot be measured simultaneously in co-occurring drought-stressed and well-watered trees. This may be partially overcome by studying trees across environmental gradients (Sala & Tenhunen, 1994; Villar-Salvador *et al.*, 1997) but this approach introduces bias due to genetic variation, management history and soil properties difference among sites. Experimental rainfall manipulations have been proposed as a way to determine how precipitation change affects most ecosystem functions (Weltzin *et al.*, 2003; Heisler & Weltzin, 2006). Such a methodology has been applied with success in a mixed deciduous forest in the United States (Wullschlegel & Hanson, 2006), temperate coniferous forests in France (Lu *et al.*, 1995) and Scotland (Irvine *et al.*, 1998), an Amazonian rain forest (Fischer *et al.*, 2007), a Mediterranean coniferous forest in Italy (Cinnirella *et al.*, 2002) and an evergreen Mediterranean forest in Spain (Martinez-Vilalta *et al.*, 2003).

In this study, the effects of drought on the functioning of a Mediterranean *Q. ilex* forest have been continuously measured since 2003 within a throughfall exclusion experiment by comparing a control and a dry plot in the same environment. This paper describes a 4-year (2004–2007) study of transpiration and related ecophysiological and morphological parameters in response to an experimental reduction in water availability. Our primary objective was to quantitatively evaluate the reduction in daily and seasonal transpiration in response to a decline in water availability consistent with model predictions. We hypothesized that four consecutive years of reduced precipitation regime modify tree water use strategies, so that reduction in transpiration is not only controlled by soil water potential but also by increased hydraulic constraints in the soil to leaf pathway and by reduction in leaf transpiring area.

## Materials and methods

### Site description

The study site is located 35 km northwest of Montpellier (southern France) in the Puéchabon State Forest on a flat plateau (3°35'45"E, 43°44'29"N, 270 m a.s.l.). This forest has been managed as a coppice for centuries and the last clear cut was performed in 1942. Vegetation is largely dominated by a dense overstorey of the evergreen oak *Q. ilex*. The top canopy height is about 5.5 m. In 2007, the density of the resprouted stems was 6393 stems ha<sup>-1</sup>. Stems with DBH < 4 cm represented 12% of the total stems, whereas stems with DBH > 10 cm represented 12.5%. Understorey evergreen species, *Buxus sempervirens*, *Phyllirea latifolia*, *Pistacia terebinthus* and *Juniperus oxycedrus*, compose a sparse shrubby layer with a percent cover < 25% and a height < 2 m.

The area has a Mediterranean-type climate. Rainfall mainly occurs during autumn and winter with about 80% between September and April. The mean annual precipitation is 907 mm with a range of 556–1549 mm recorded over the 1984–2007 period. Mean annual temperature over the same period was 13.4 °C with a minimum in January (5.5 °C) and a maximum in July (22.9 °C) (Allard *et al.*, 2008). The soil is extremely rocky from hard Jurassic limestone origin; on average the volumetric fractional content of stones and rocks is about 0.75 for the top 0–50 cm and 0.90 below. The stone free fine fraction of the soil is a homogeneous silty clay loam (USDA texture triangle) within the top 0–50 cm layer (38.8% clay, 35.2% silt and 26% sand). The fine fraction fills up the space between stones and rocks and thus provides a source of water throughout the long dry summers for the deep-rooted *Q. ilex* (Rambal *et al.*, 2003). The highly permeable soil prevents any surface runoff to occur even for high-intensity rain events.

### Experimental system

In March 2003, a throughfall exclusion experiment was set up on the site. The throughfall exclusion experiment was replicated on three plots 200 m away one from the other, and situated on a flat area with no lateral flow. On each replication, two throughfall exclusion treatments were set up on two 140 m<sup>2</sup> plots (14 m × 10 m). One was a control (further, control) and the second was subjected to throughfall exclusion (further, dry). On the dry plot, throughfall exclusion was achieved using 14-m-long and 0.19-m-wide PVC gutters covering 33% of the ground area under the tree canopy, with the aim of excluding 33% of throughfall. Taking into account interception losses and stemflow, the throughfall exclusion experiment reduces the net input of precipitation to the

soil by 29% compared with the control treatment (Limousin *et al.*, 2008). On the control plot, identical gutters had been set up upside down so that the albedo and the understorey microclimate were as close as possible in both treatments.

#### Meteorological data

An automatic weather station was installed at the site on the top of a 11 m high scaffolding tower about 5 m higher than the dominant trees. Air temperature and relative humidity (MP100, Rotronic, Bassersdorf, Switzerland), and gross rainfall (tipping bucket rain gauge ARG100, Environmental Measurements Ltd, Sunderland, UK) were recorded every minute and aggregated on half-hourly basis using a data logger (model 21X, Campbell Scientific Ltd, Shepshed, UK). Vapour pressure deficit was calculated every half-hour from relative humidity and temperature.

#### Sap flow

Sap flow was continuously monitored on six trees per treatment within one of the replication plots. Sampled trees were ranging from 7 to 13 cm in diameter (*DBH*), and tree size distribution and mean diameter were similar in both plots. Sap flow was monitored with 20-mm-long constant heat thermal dissipation probes constructed according to Granier (1985, 1987). All sensors were installed the first week of June 2003 on the north side of trees to avoid direct solar heating, and shielded from rain and radiation with aluminium reflectors. Probe pairs were inserted radially into the stem of each tree at breath height after removing the bark to expose the outer surface of sapwood. The vertical separation between probes was 10 cm. The upper probe was heated by a constant current, whereas the lower probe was unheated and remained at trunk temperature. Half-hourly means of temperature difference between the probes were recorded by CR10X data loggers (Campbell Scientific Ltd). Sap flux density was calculated using the empirical equation of Granier (1985, 1987), and taking the absolute maximum temperature difference over running 2-day periods. Sapflow sensors were replaced only in case of disruption, assuming that, in slow growing *Q. ilex* trees, probes are reliable for mid to long-term measurements (David *et al.*, 2004).

Sap flux densities were scaled to the stand level by multiplying by conducting sapwood area per unit ground area. Sapwood conductive thickness was determined by direct measurement of the wet area of sapwood immediately after cutting some sample trees. Measurements were done on 18 trees taken outside the experiment and chosen to cover a *DBH* range

representative of the coppice. An allometric relationship between stem *DBH* (cm) and sapwood area (*SA*; cm<sup>2</sup>) was obtained:

$$SA = 0.363 DBH^{2.149}, r^2 = 0.97, n = 18. \quad (1)$$

Stem diameters for all trees on the experiment were recorded once a year, and used together with Eqn (1) to calculate sapwood area per unit ground area with time. Because the species studied is diffuse-porous, sap flux density was assumed to be uniform over the entire sapwood depth (Martinez-Vilalta *et al.*, 2003; David *et al.*, 2007). However, this assumption is not critical because calculation procedures were the same in both study plots, so any error would tend to be systematic and should not affect our comparative analysis in time and between treatments. Because there is a time lag between transpiration at the leaf level and sap flow at breath height (Lhomme *et al.*, 2001), transpiration (*E*) was considered equal to stem flow only when steady-state conditions prevailed (daily totals and midday values). Transpiration was expressed per unit of ground area (mm day<sup>-1</sup> or mm h<sup>-1</sup>).

#### Leaf water potential

Tree water status was assessed by measuring leaf water potential ( $\Psi_l$ ) with a pressure chamber (PMS Instruments, Corvallis, OR, USA). In each plot, measurements were done about 10 times a year between April and October on four trees supporting sapflow sensors. Two leaves were sampled in each tree just before sunrise (predawn leaf water potential,  $\Psi_{l,pd}$ ) and around midday solar time (midday leaf water potential,  $\Psi_{l,md}$ ). Samples were taken from a scaffold at similar heights and measurements were done within 1 min after leaf excision. A third leaf was sampled if the observed difference in water potential between the two measurements was higher than 0.2 MPa. Predawn leaf water potential was assumed to be a good surrogate of the soil water potential, and the 0.3 MPa disequilibrium (Donovan *et al.*, 2001) observed by Rambal *et al.* (2003) was neglected in this study.

#### Canopy conductance

Given the high degree of coupling between the atmosphere and the canopy in *Q. ilex* trees (Infante *et al.*, 1997), canopy conductance ( $G_c$ ; m s<sup>-1</sup>) can be approximated by

$$G_c = \frac{E \lambda \gamma}{D c_p \rho}, \quad (2)$$

where *E* is the transpiration (mm s<sup>-1</sup>), *D* is the vapour pressure deficit (Pa),  $\lambda$  is the latent heat of evaporation

of water ( $\text{J kg}^{-1}$ ),  $\gamma$  is the psychrometric constant ( $\text{Pa } ^\circ\text{C}^{-1}$ ),  $c_p$  is the heat capacity of water in air ( $\text{J kg}^{-1} ^\circ\text{C}^{-1}$ ), and  $\rho$  is the density of air ( $\text{kg m}^{-3}$ ) (McNaughton & Jarvis, 1983; Jarvis & McNaughton, 1986). Midday canopy conductance ( $G_{\text{c,md}}$ ) was estimated from averaged midday transpiration ( $E_{\text{md}}$ ) and  $D$  for springs and summers from 2004 to 2007.

#### Whole-tree hydraulic conductance

Whole-tree hydraulic conductance of the soil to leaf pathway ( $K_{\text{S-L}}$ ;  $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ) can be described according to Darcy's law (Sperry, 2000):

$$K_{\text{S-L}} = F_{\text{md}} / (\Psi_{\text{l,pd}} - \Psi_{\text{l,md}}), \quad (3)$$

where  $F_{\text{md}}$  is midday sap flow per unit of sapwood area ( $\text{mmol m}^{-2} \text{s}^{-1}$ ), and  $\Psi_{\text{l,pd}}$  and  $\Psi_{\text{l,md}}$  are in MPa. The difference between  $\Psi_{\text{l,pd}}$  and  $\Psi_{\text{l,md}}$  is an estimate of the sapflow driving force at midday, because  $\Psi_{\text{l,pd}}$  is considered equal to soil water potential. Calculations were performed for every date when leaf water potentials were available from 2004 to 2007.

#### Leaf area index

*Q. ilex* is an evergreen oak; some leaves can be supported by trees during three consecutive years (Mediavilla & Escudero, 2003) and the leaf area index is fairly constant throughout the year. Leaf area index was assessed from leaf litter fall and leaf life span. On each plot and treatment, 12 litter traps for a total area of  $1.1 \text{ m}^2$  were placed on a grid over the gutters of the throughfall exclusion, at a height of 2 m. Litterfall was collected monthly since 2003, and then separated into leaves, wood, flowers and acorn, oven-dried at  $60 ^\circ\text{C}$  and weighted. The dry mass of leaf litterfall ( $M_{\text{L}}$ ) was expressed in  $\text{g m}^{-2}$  of ground area.  $M_{\text{L}}$  was converted into a leaf area by dividing by the leaf mass area of dead leaves ( $LMA_{\text{lit}}$ ;  $\text{g m}^{-2}$ ).  $LMA_{\text{lit}}$  was measured monthly during 2 years on litterfall sampled outside the throughfall exclusion area. The average  $LMA_{\text{lit}}$  was found to be  $212.8 \text{ g m}^{-2}$ , which is 11% less than the leaf mass area of living leaves, and assumed constant over time.

The leaf area index at a given time was estimated as the sum of areas of leaves falling from this date until all leaves on the tree at the initial date had felt

$$LAI_n = \sum_{i=n}^{n+2} \frac{M_{\text{L}i} P_i}{LMA_{\text{lit}}}, \quad (4)$$

where  $LAI_n$  is the leaf area index ( $\text{m}^2 \text{m}^{-2}$ ) supported during year  $n$ , and  $P_i$  is the proportion of leaves grown during year  $n$  that are still on the tree in year  $i$ . In other

words,  $P_i$  expresses the proportion of leaves falling during year  $i$ , that were already on the tree in year  $n$ .  $P_i$  was determined from leaf demographic analyses conducted on 6 trees  $\text{plot}^{-1}$ . On each sampled tree, three branches were selected at the upper part of the canopy and the number of leaves for each age class was counted twice a year before and after the growing season, in early spring and early summer.

#### Statistical analysis

Leaf water potential and whole-tree hydraulic conductance measured on sampling dates were analysed by multivariate repeated measures analysis of variance (MANOVA) on untransformed data to test for differences among treatments and seasonal trends. Statistical analyses were performed with SIGMASTAT 3.0 (Systat Software Inc., San Jose, CA, USA).

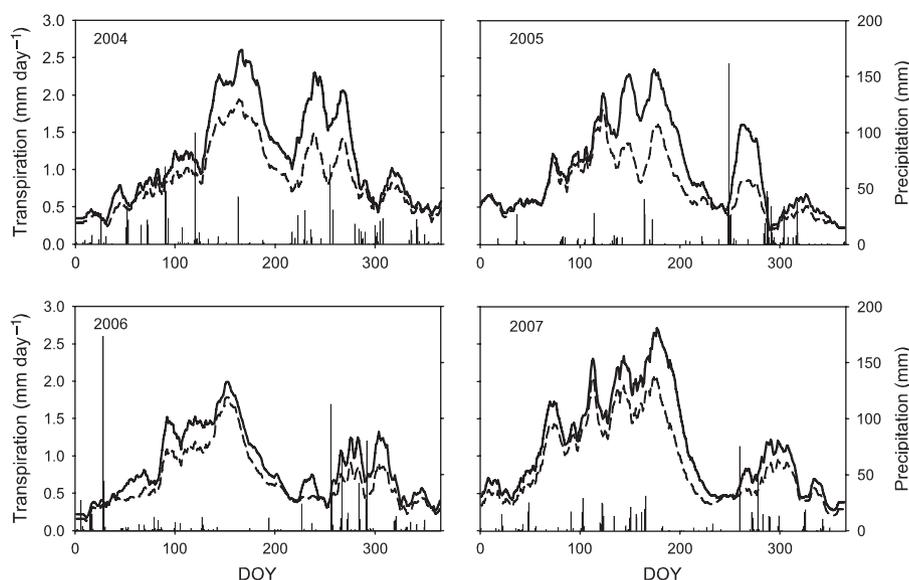
## Results

#### Annual and seasonal patterns of tree transpiration

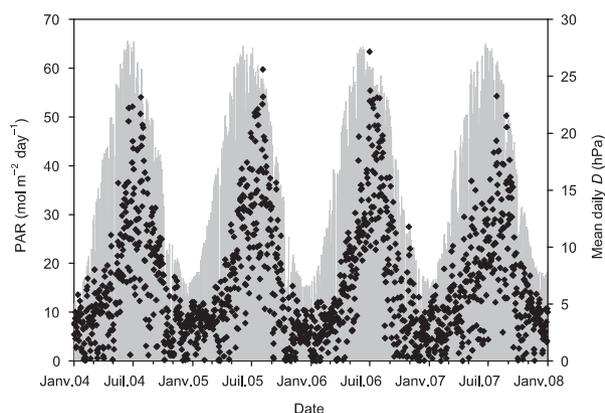
Annual amounts of precipitation and transpiration from 2004 to 2007 are summarized in Table 1. Cumulative rainfall during the study period was similar to the long-term average ( $-5\%$ ). Net precipitation ( $P_{\text{N}}$ ) reaching the soil was in average 79% of gross precipitation in the control plot and 56% in the dry plot, which correspond to a decrease in net precipitation of 29% in the dry plot. Transpiration ( $E$ ) was, on average, decreased by 23% on an annual basis in the dry treatment, this difference of transpiration between treatments being fairly constant among years despite important inter-annual variability in precipitation and transpiration. The proportion of net precipitation which was used by trees ( $E/P_{\text{N}}$ ) varied significantly between years because of uneven and erratic distribution of rainfall throughout the year (Fig. 1).  $E/P_{\text{N}}$  was always higher in

**Table 1** Annual amounts of gross precipitation ( $P_{\text{G}}$ ; mm), net precipitation ( $P_{\text{N}}$ ; mm) estimated following Limousin *et al.* (2008) and transpiration ( $E$ ; mm) measured in the control and dry treatments of the throughfall exclusion experiment from 2004 to 2007

Year	Control				Dry			$E_{\text{dry}}/E_{\text{control}}$
	$P_{\text{G}}$	$P_{\text{N}}$	$E$	$E/P_{\text{N}}$	$P_{\text{N}}$	$E$	$E/P_{\text{N}}$	
2004	989	781	430	0.55	553	322	0.58	0.75
2005	835	671	364	0.54	475	270	0.57	0.74
2006	940	774	308	0.40	550	243	0.44	0.79
2007	681	509	417	0.82	360	330	0.92	0.79
Mean	861	684	380	0.58	485	291	0.63	0.77



**Fig. 1** Seasonal variations of daily precipitation (vertical bar), and daily transpiration in the control treatment (solid line) and the dry treatment (dashed line). Solid and dashed lines represent two weeks running average.



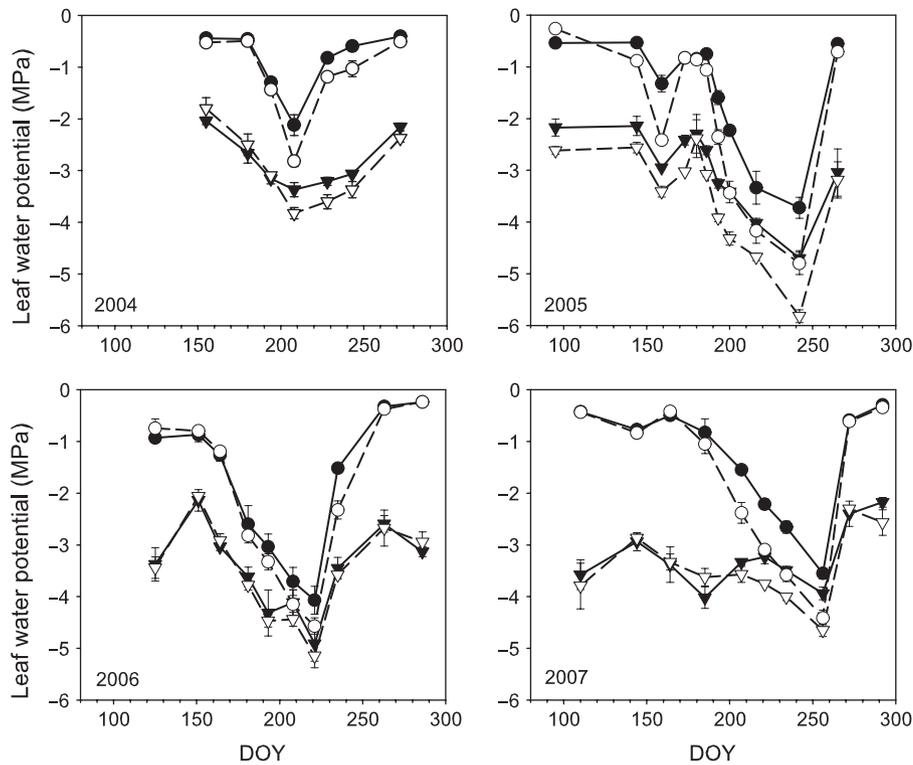
**Fig. 2** Seasonal variations of daily integral of photosynthetically active radiation (PAR; grey area) and mean daily vapour pressure deficit ( $D$ ; black dots) from 2004 to 2007.

the dry treatment (Table 1). Seasonal patterns of transpiration (Fig. 1) were similar every year and in both treatments, as were the seasonal variations of vapour pressure deficit ( $D$ ) and photosynthetically active radiation (Fig. 2). Daily transpiration peaked in late spring when evaporative demand was high and soil water not limiting. Then a rapid decline was observed in summer, as soil water reserve was depleted, until partial recovery with autumn rains (September–October). Depending on years, maximal transpiration was 2.46–3.28  $\text{mm day}^{-1}$  in the control treatment, and 2.17–2.67  $\text{mm day}^{-1}$  in the dry treatment. Daily transpiration was systematically lower in the dry treatment, even during well-watered periods (spring and autumn),

except during short periods when transpiration was strongly limited by low temperature (winter) or severe drought (summer end).

#### *Seasonal patterns in leaf water potential*

Predawn leaf water potential remained high (around  $-0.5$  MPa) and approximately similar in both treatments during the wet periods (Fig. 3). An important decline in  $\Psi_{l,pd}$  was observed during the summer drought, before a full recovery with first autumn rainfalls. During the drought periods,  $\Psi_{l,pd}$  was systematically lower in the dry plot. Differences in  $\Psi_{l,pd}$  between treatments were statistically significant every year, except 2006 (Table 2). Minimum values of  $\Psi_{l,pd}$  reached  $-3.73$  and  $-4.80$  MPa in August 2005 in the control and dry plot, respectively, and  $-4.07$  and  $-4.57$  MPa in August 2006 in the control and dry plot, respectively. Seasonal variations in midday leaf water potential were of lower amplitude, and not always in phase with the variations in  $\Psi_{l,pd}$  because of atmospheric conditions. Minimum values of  $\Psi_{l,md}$  reached  $-4.70$  and  $-5.82$  MPa in August 2005 in the control and dry plot, respectively, and  $-4.91$  and  $-5.14$  MPa in August 2006 in the control and dry plot, respectively. Whatever the atmospheric conditions  $\Psi_{l,md}$  was systematically lower in the dry plot during periods of drought, though the difference between treatments was statistically significant only for year 2005 (Table 2). The sapflow driving force ( $\Psi_{l,pd} - \Psi_{l,md}$ ) was highest during well-watered periods and declined strongly during summer drought (Fig. 4). The sapflow



**Fig. 3** Seasonal variations of predawn (circle) and midday (triangle) leaf water potential in the control (closed symbols) and dry plot (open symbols) during the four growing seasons of the experiment (2004–2007). Error bars are standard errors.

**Table 2** Repeated measures analysis of variance of predawn leaf water potential ( $\Psi_{l,pd}$ ), midday leaf water potential ( $\Psi_{l,md}$ ), and whole-tree hydraulic conductance ( $K_{S-L}$ ) in the two treatments (control and dry)

	Year	Treatment	F statistic	
			Treatment × Date	Date
$\Psi_{l,pd}$	2004	12.56*	4.91***	175.94***
	2005	26.36**	8.93***	262.45***
	2006	2.04	2.25*	204.25***
	2007	16.98**	9.53***	352.37***
$\Psi_{l,md}$	2004	1.44	3.37*	80.30***
	2005	19.34**	1.28	42.78***
	2006	0.41	0.29	34.16***
	2007	4.54	1.63	21.59***
$K_{S-L}$	2004	3.58	4.72**	5.38***
	2005	15.03**	0.4	2.89**
	2006	1.7	0.39	2.76**
	2007	4.7	1.43	7.84***

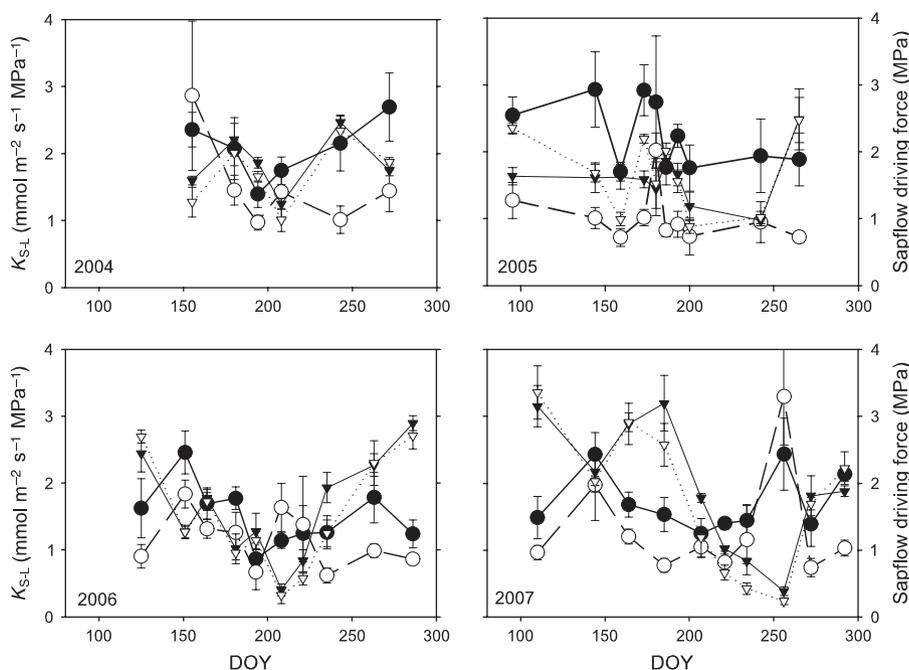
Significance values are indicated as: \* $P < 0.05$ ; \*\* $P < 0.01$ ; and \*\*\* $P < 0.001$ .

driving force did not differ markedly between treatments, neither during well-watered periods nor during drought, except during drought 2007 ( $P < 0.01$ ). The

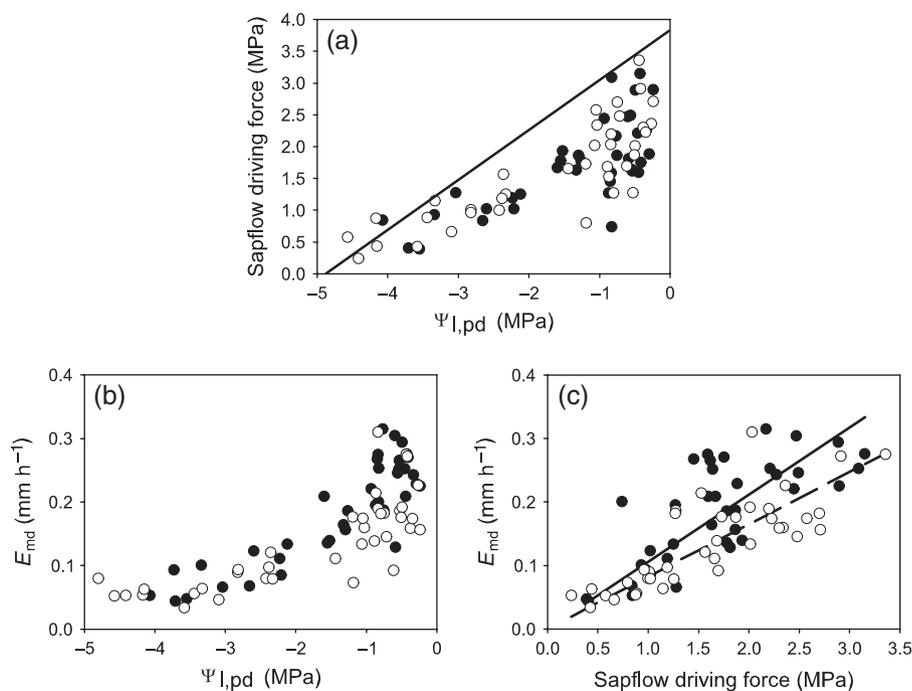
sapflow driving force decreased during summer with reductions in  $\Psi_{l,pd}$ , and the relationship between sapflow driving force and  $\Psi_{l,pd}$  was similar in both treatments (Fig. 5a). The upper boundary line of this scatter plot had a slope of 0.78 and an intersect with  $\Psi_{l,pd}$  axis of  $-4.8$  MPa.

*Seasonal adjustments of canopy conductance*

Figure 6 shows the relationship between  $G_{c,md}$  and  $\Psi_{l,pd}$  for the two treatments of the experiment and the 4 years of observation. It shows a similar hyperbolic decline of  $G_{c,md}$  with decreasing  $\Psi_{l,pd}$  in both treatments, as for the maximum transpiration rate (Fig. 5b). The maximum  $G_{c,md}$  was lower in the dry plot, as were the minimum values of  $\Psi_{l,pd}$  experienced by the trees. Hyperbolic equations of the form  $G_{c,md} = 1/(a-b \Psi_{l,pd})$  were fitted with  $r^2 = 0.78$ ,  $P = 0.03$ ,  $n = 38$  in the control treatment, and  $r^2 = 0.75$ ,  $P = 0.02$ ,  $n = 38$  in the dry treatment, and found to be not significantly different between treatments. The reduction in  $G_{c,md}$  mainly occurs for values of  $\Psi_{l,pd}$  higher than  $-1$  MPa while changes in  $G_{c,md}$  are limited during severe drought.  $G_{c,md}$  decreased by 67% for  $\Psi_{l,pd}$  between  $-0.2$  and  $-0.9$  MPa in the control treatment, and by 73% in the dry treatment. The maximum reduction in  $G_{c,md}$  with



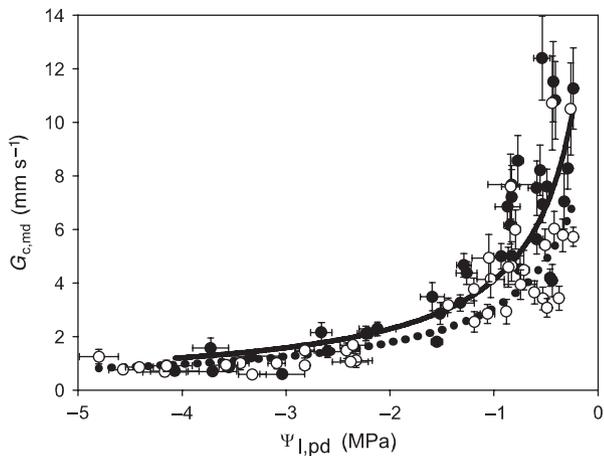
**Fig. 4** Seasonal variations of whole-tree hydraulic conductance (circles) and sapflow driving force (triangles) in the control (closed symbols) and the dry (open symbols) treatments during the four growing seasons of the experiment (2004–2007). Error bars are standard errors.



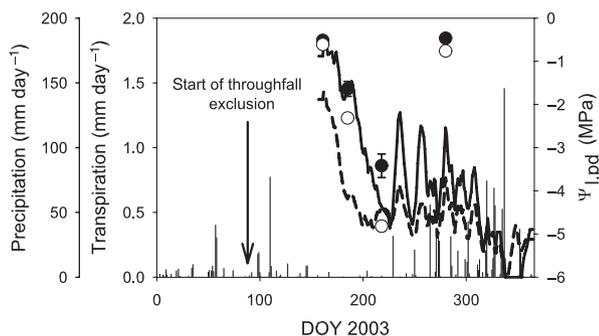
**Fig. 5** Relationships (a) between sapflow driving force and predawn leaf water potential ( $\Psi_{l,pd}$ ), (b) between midday transpiration ( $E_{md}$ ) and  $\Psi_{l,pd}$ , (c) between  $E_{md}$  and sapflow driving force, in the control (closed symbols and solid line) and dry treatment (open symbols and dashed line) for the 4 years of observation (2004–2007).

drought reached 95% in both control and dry treatments. Such important variations in  $G_{c,md}$  before a severe drought stress occurred explain the early difference in transpiration between treatments soon after the

beginning of the exclusion experiment in 2003 (Fig. 7). The 10th of June 2003, a difference in  $\Psi_{l,pd}$  of 0.1 MPa was sufficient to yield the 19% reduction in transpiration we observed.



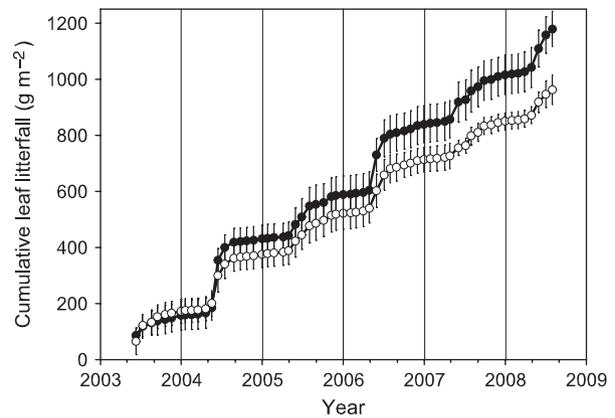
**Fig. 6** Influence of predawn leaf water potential on midday canopy conductance in the control plot (closed symbols and solid line) and the dry plot (open symbols and dashed lines). Each point represent a mean of the six trees for each date when  $\Psi_{l,pd}$  was measured between 2004 and 2007. Error bars are standard errors.



**Fig. 7** Seasonal variations of precipitation (vertical bar), daily transpiration in the control plot (solid line, 14 days running average) and the dry plot (dashed line, 14 days running average), and predawn leaf water potential (closed symbols: control plot; open symbols: dry plot) for the year 2003. Error bars are standard errors.

#### Seasonal adjustments of whole-tree hydraulic conductance

Figure 4 shows the seasonal variations of  $K_{S-L}$  during spring and summer from 2004 to 2007.  $K_{S-L}$  was always higher in the control than in the dry treatment, except for four dates when sapflow driving force was particularly low. The difference in  $K_{S-L}$  between treatments was statistically significant only for year 2005, but varied significantly between measurement dates in both treatments (Table 2). For a given sapflow driving force, the daily maximum transpiration ( $E_{md}$ ) was lower in the dry treatment (Fig. 5c), despite a similar relationship in both treatments between sapflow driving force and  $\Psi_{l,pd}$  (Fig. 5a). This difference is explained by the lower whole-tree hydraulic conductance. The average  $K_{S-L}$



**Fig. 8** Cumulative amount of leaf litter fall in the control (closed symbols) and dry treatment (open symbols) from 2003 to 2008. Error bars are standard deviations.

can also be inferred from the regression between  $E_{md}$  and the sapflow driving force (Fig. 5c). These regressions yield a slope of  $0.11 \text{ mm h}^{-1} \text{ MPa}^{-1}$  ( $r^2 = 0.51$ ;  $n = 38$ ) in the control treatment, and  $0.08 \text{ mm h}^{-1} \text{ MPa}^{-1}$  ( $r^2 = 0.63$ ;  $n = 38$ ) in the dry treatment. The slope difference was statistically significant at  $P = 0.05$ .

#### Adjustment of the leaf area

An allometric relationship between stem *DBH* (cm) and tree leaf area ( $LA$ ,  $\text{m}^2$ ), obtained on 24 trees sampled outside the experiment (Rambal *et al.*, 2004), was used to assess any LAI difference between treatments before the experiment

$$LA = 9.1 \times 10^{-2} DBH^{1.875}, r^2 = 0.92, n = 24. \quad (5)$$

Applying Eqn (5) to all *Q. ilex* stems inside the experimental plots at the beginning of throughfall exclusion, in 2003, yielded no significant difference and a leaf area index of 2.49 and 2.58 in the control and dry treatments, respectively.

The average leaf life span in the control treatment was 683 days, and 690 days in the dry treatment. Leaf litterfall mainly occurs in spring concomitant to the flush of new leaves. The proportion of leaves remaining on tree for two consecutive years was found to be 0.78 and 0.73 in the control and the dry treatments, respectively. The proportion of leaves remaining on tree for a third growing season was found to be 0.09 and 0.16 in the control and the dry treatments, respectively.

Time courses of cumulated leaf litter fall from 2003 to 2008 are shown in Fig. 8. Leaf litter fall was slightly higher in the dry treatment in 2003, but lower for all following years. The low and similar amount of leaf litter fall in both plots for the year 2005 was due to partial caterpillar defoliation. Average leaf area index of

*Q. ilex* over the study period, estimated from both leaf litterfall and life span, was found to be  $1.89 \text{ m}^2 \text{ m}^{-2}$  in the control treatment and  $1.55 \text{ m}^2 \text{ m}^{-2}$  in the dry treatment. This difference of LAI of  $0.33 \text{ m}^2 \text{ m}^{-2}$  between the two treatments corresponded to a reduction of 18% of the leaf area and was statistically significant at  $P = 0.06$ .

## Discussion

### *Annual and seasonal patterns of transpiration*

Seasonal patterns of transpiration, peaking in late spring and strongly decreasing with summer drought, were typical of Mediterranean evergreen forests (Tognetti *et al.*, 1998b; Infante *et al.*, 2001; Martinez-Vilalta *et al.*, 2003). The decrease in transpiration during drought is synchronized with the sharp decline in predawn leaf water potential. Except for the year 2004, transpiration during late summer was similar in both treatments and as low as during winter time. On an annual basis, transpiration in the control plot was in average 44% of gross precipitation (Table 1) with important variations between years due to the time distribution of rainfall across seasons. Allard *et al.* (2008) showed, on the same study site, that the rainfall amount occurring between March and June was the main factor determining net ecosystem exchange. As for net ecosystem exchange, rainfall occurring during the active growth period is a major determinant of annual transpiration. Despite this interannual variability, the ratio of transpiration between the two treatments remained similar during the 4 years of the study, and was exactly the same for the two contrasted years 2006, as a dry year, and 2007, as a wet year. Such an observation is explained by the systematic reduction of transpiration in the dry treatment, even during well-watered periods of springs and autumns. The difference in daily transpiration between both treatments was not only controlled by the instantaneous difference in soil water potential, but also by longer term adjustments such as loss of hydraulic conductance or reduced transpiring leaf area. However, it can be seen on Fig. 1 that long-term adjustments of the transpiration capacity in the dry treatment applied from 2004, the second year of the experiment. Similar observations were made in wet-dry tropical savannas, where dry season conditions were shown to determine the transpiration rate during the well-watered wet season (Eamus *et al.*, 2000).

### *Strategies of hydraulic regulation*

During the summer drought,  $\Psi_{l,pd}$ , transpiration and  $G_{c,md}$  were lower in the dry treatment. However, the

response of  $G_{c,md}$  to  $\Psi_{l,pd}$  was similar in both treatments, despite lower maximum values of  $G_{c,md}$  in the dry treatment. Our results support earlier finding that *Q. ilex* close stomata at relatively high water potentials (Castell *et al.*, 1994; Sala & Tenhunen, 1994; Damesin *et al.*, 1998b; Teixeira Filho *et al.*, 1998; Tognetti *et al.*, 1998a), stomatal closure was even observed without any change in  $\Psi_{l,pd}$  (Gulias *et al.*, 2002). Such a stomatal regulation of transpiration, described as a pre-emptive response to drought stress (Buckley, 2005), cannot be explained by the only hydraulic control (Sperry, 2000). Given the nonlinearity of the relationship between soil water content and  $\Psi_{l,pd}$  (Rambal *et al.*, 2003) and the shape of the relationship between  $G_{c,md}$  and  $\Psi_{l,pd}$ , it can be hypothesized that  $G_{c,md}$  respond to the soil water content before the change in leaf water potential that occurs during the drought. The mechanism by which soil water content has been sensed remains unclear: the ABA-mediated control of stomatal closure (Zhang & Davies, 1990) cannot be excluded but seems unlikely in mature trees due to the length of the pathway between roots and leaves (Triboulot *et al.*, 1996).

As a consequence of the similar stomatal closure (Fig. 6), the midday leaf water potential, and thus the sapflow driving force followed similar relationships with  $\Psi_{l,pd}$  in the two treatments (Fig. 5a). Variations of  $\Psi_{l,md}$  with  $\Psi_{l,pd}$  define two types of plant response facing water limitation (Tardieu & Simonneau, 1998; Maseda & Fernandez, 2006): isohydric plants exhibit strong stomatal control to maintain the predrought  $\Psi_{l,md}$  in a narrow range during stress, while anisohydric plants tolerate a drop in  $\Psi_{l,md}$  during stress to sustain transpiration. The stomatal regulation of leaf water potential observed in this study suggests an intermediate pattern for *Q. ilex*. Transpiration and sapflow driving force were strongly reduced by drought (Fig. 5a and b), and strong stomatal closure was observed even for moderate stress (Fig. 6). Evidence for the ability of *Q. ilex* trees to regulate  $\Psi_{l,md}$  with decreasing  $\Psi_{l,pd}$  is shown on Fig. 5a. However, perfect isohydric behaviour would yield a slope of 1 for the upper boundary line of the scatter plot, while we observed a slope of 0.78. Comparing midday leaf water potential between predrought and drought conditions or between treatments (Fig. 3 and Table 2) shows that stomatal regulation is not able to maintain homeostasis in  $\Psi_{l,md}$  during drought.

Stomatal control of leaf water potential is usually considered to maintain  $\Psi_{l,md}$  above a cavitation threshold (Cochard *et al.*, 1996; Salleo *et al.*, 2000) and such a mechanism was verified for *Q. ilex* (Martinez-Vilalta *et al.*, 2003; David *et al.*, 2007). However, minimum leaf water potentials observed in this study are largely below the 50% cavitation potential threshold reported

in most studies (Tognetti *et al.*, 1998b; Martinez-Vilalta *et al.*, 2002; Gartner *et al.*, 2003) and contradict this hypothesis. Only Tyree & Cochard (1996) report a 50% cavitation threshold of  $-6.0$  MPa for this species. Given the very low values of leaf water potentials observed at our site, it can be hypothesized that stomatal regulation was not strong enough to fully prevent xylem embolism and that a controlled loss of hydraulic conductance happened (Sperry, 2000).

Whole-tree hydraulic conductance was significantly lower in the dry treatment than in the control, but did not vary much over time in either treatment (Fig. 4). Whole-tree hydraulic conductance is the resultant of the soil-to-root (Newman, 1969) and the root-to-leaf serial hydraulic conductances. Lower  $K_{S-L}$  values in the dry treatment could result from two mechanisms: a decline in the hydraulic conductance of the soil-to-root component as soil dries (Campbell, 1985), and a lower hydraulic conductance of the root-to-leaf pathway due to hydraulic architecture or cavitation. Decline in soil hydraulic conductance occurs when air displaces water from the pore space and is totally and rapidly reversible with refilled soil water content. Given the soil properties at our site (see Rambal *et al.*, 2003), the soil hydraulic conductance, following Campbell (1985), shows a 100-fold decrease for a decrease in soil water potential from  $-0.5$  to  $-4.5$  MPa. If soil hydraulic conductance was the only determinant of  $K_{S-L}$ , as for perfect isohydric control (Duursma *et al.*, 2008), large variations would be observed between predrought and drought conditions and  $K_{S-L}$  values would be equivalent in both treatments during well-watered periods. It is likely that most of the decline in  $K_{S-L}$  in the dry treatment occurred in the root-to-leaf pathway, so that the root-to-leaf conductance remained the most limiting whatever the soil water potential (Blizzard & Boyer, 1980; Sperry, 2000). This is especially the case for obligate sprouters like *Q. ilex*, for which the root xylem component was found to limit hydraulic conductance more strongly than the rhizospheric component in the Californian chaparral (Pratt *et al.*, 2007). Two hypotheses can explain a lower root-to-leaf hydraulic conductance in the dry treatment. First, lower minimum leaf water potentials experienced in the dry treatment resulted in more xylem cavitation (Sperry & Tyree, 1988). Such a reduction of  $K_{S-L}$  sacrifices xylem conducting elements but can in turn be advantageous by promoting more efficient stomatal regulation (Sperry, 2000) and more conservative water use (Maseda & Fernandez, 2006). It can also be hypothesized that, because a trade-off exists between hydraulic efficiency and resistance to cavitation (Martinez-Vilalta *et al.*, 2002), lower  $K_{S-L}$  values in the dry treatment were a consequence of a lower vulnerability to embolism. In that case, trees in the dry

treatment would have adjusted their transpiration rate through a more resistant hydraulic pathway, and the advantages of a lower hydraulic conductance would come together with a better ability to face severe drought.

#### *Change in standing leaf area*

Leaf area index were similar in both treatments at the beginning of the experiment in 2003. After only 1 year of throughfall exclusion, leaf litter and leaf area index were lower in the dry treatment. The higher leaf litter fall in the dry treatment in 2003, some month after the start of rain exclusion, can be due either to the slightly higher leaf area in this plot previous to the experiment or to an increased mortality of leaves as a consequence of drought, or both. Leaf area index assessed from leaf litterfall and leaf life span are lower than values obtained from allometric relationship or the commonly reported value of LAI of 2.8 evaluated at our site from optical methods (Allard *et al.*, 2008). Such a discrepancy can be explained from the height of litter traps (2 m above ground) and the fact that contrarily to optical methods, wood area and understorey species are not taken into account.

The reduction of tree transpiration through adjustment of the leaf area can be considered as a conservative strategy associated with anisohydric behaviour because it balances the low stomatal control at the leaf level (Maseda & Fernandez, 2006). The reduction in leaf area also balances the decrease in hydraulic conductance and allows the trees to keep a constant or even increased leaf-specific hydraulic conductance when submitted to drought stress. In this study, the 18% decrease in leaf area observed in the dry treatment balanced almost completely the reduction of the whole-tree hydraulic conductance. The water transport capacity per unit leaf area was thus little affected in the dry treatment, despite a lower hydraulic conductance. Such maintenance of leaf-specific conductivity during drought has already been observed in *Quercus coccifera* seedlings (Vilagrosa *et al.*, 2003). A rapid decrease of leaf area following drought stress can result from cavitation in leaf petioles (Vilagrosa *et al.*, 2003; Kelley *et al.*, 2007), and is called the drought-deciduous syndrome. However, this syndrome is unlikely in our experiment because the average leaf life span was similar in the dry and the control treatments, indicating that lower leaf water potentials had little effect on leaf mortality.

The leaf area index of a plant ecosystem has been shown to meet an ecohydrological equilibrium with its water balance (Eagleson, 1982), and such a finding was supported for Mediterranean evergreen oak woodlands or forests (Joffre & Rambal, 1993; Hoff & Rambal, 2003;

Baldocchi & Xu, 2007). Poole & Miller (1981) first suggested the key-role of the leaf area index in anticipating the unpredictability of water input in Mediterranean-type ecosystems and summarized their results in a very interesting proposal that could be considered as a determining contribution to the idea of ecohydrological optimality: 'The main response of the shrubs to different precipitation regimes in the chaparral range is to change leaf area index, not physiological parameters'.

Specht (1972), Grier & Running (1977) and Gholz (1982) related leaf area index or above ground biomass of water-limited ecosystems ranging from sparse desert shrublands to dense forests with their site water balances. Eagleson (1982) was the first author to derive a one-dimensional, statistical-dynamic model for the equilibrium between the hydrological and the biological components of an ecosystem. He assumed that in water-limited environments, ecosystems develop a stable canopy density, which both maximize biomass and minimize drought stress.

The ecohydrological equilibrium idea was mostly supported by results obtained by model simulations assuming that ecosystems both maximize water use efficiency and limit drought as Nemani & Running (1989) did with Forest-BGC, or maintain concurrently transpiration by unit leaf area within a limited target range while minimizing plant water stress (Rambal, 1993). By applying the same idea and model as Nemani & Running (1989), Hoff & Rambal (2003) derived a sensitivity analysis for *Q. ilex* ecosystem in southern France to change in climate, water input and soil retention properties. For a low soil water storage capacity such as the one in our site, ca. 150 mm, reducing rain amounts by climate change or experimental drought induced a rather low change in equilibrium LAI. The predicted response to the 29% decline in rainfall caused by the throughfall exclusion experiment is a reduction of 0.23 in equilibrium LAI, a value in close agreement with the 0.33 LAI decline that we observed from leaf litter fall.

## Conclusion

A reduction of 29% of the net precipitation input to the soil reduced the annual transpiration of the *Q. ilex* forest by 23%. Such a reduction of tree water use was achieved through three nested regulations: (1) control of stomatal conductance, (2) change in hydraulic conductance of the soil-to-leaf pathway, and (3) decline in the leaf area. *Q. ilex* trees exhibit a tight stomatal control over transpiration but insufficient to prevent loss of hydraulic conductance under severe drought stress. As a consequence, whole-tree hydraulic conductance was lower in trees facing dryer conditions. However, the reduction in

transpiration was mainly driven by a reduction of the leaf area, resulting in an almost unaffected leaf-specific conductivity. Because tree transpiration in the dry treatment was limited by long-term adjustments such as hydraulic efficiency and leaf transpiring area, it was reduced all year round even during well-watered periods. This result stresses the importance of multiyear studies for observing the adjustments of water consumption in mature forests. Taking into account some adjustments of the water use efficiency for photosynthesis, it can be hypothesized that such a limitation upon transpiration will have important consequences for the ecosystem carbon budget by decreasing the gross primary production and changing ecosystem respiration through a reduced input of carbon to soil.

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