

# Morphological and phenological shoot plasticity in a Mediterranean evergreen oak facing long-term increased drought

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**Abstract** Mediterranean trees must adjust their canopy leaf area to the unpredictable timing and severity of summer drought. The impact of increased drought on the canopy dynamics of the evergreen *Quercus ilex* was studied by measuring shoot growth, leaf production, litterfall, leafing phenology and leaf demography in a mature forest stand submitted to partial throughfall exclusion for 7 years. The leaf area index rapidly declined in the throughfall-exclusion plot and was 19% lower than in the control plot after 7 years of treatment. Consequently, leaf litterfall was significantly lower in the dry treatment. Such a decline in leaf area occurred through a change in branch allometry with a decreased number of ramifications produced and a reduction of the leaf area supported per unit sapwood area of the shoot (LA/SA). The leafing phenology was slightly delayed and the median leaf life span was slightly longer in the dry treatment. The canopy dynamics in both treatments were driven by water availability with a 1-year lag: leaf shedding and production were reduced following dry years; in contrast, leaf turnover was increased following wet

years. The drought-induced decrease in leaf area, resulting from both plasticity in shoot development and slower leaf turnover, appeared to be a hydraulic adjustment to limit canopy transpiration and maintain leaf-specific hydraulic conductivity under drier conditions.

**Keywords** Allometry · Leaf area · Leaf demography · Litterfall · Shoot growth

## Introduction

Leaf area is a critical factor controlling water and carbon fluxes in plant ecosystems, and its plasticity is a powerful mean for coping with the unpredictability of rainfall in water-limited environments (Poole and Miller 1981). Evidence that leaf area does acclimate to water availability has been shown in ecosystems ranging from sparse shrublands to dense forests (e.g., Specht 1972; Grier and Running 1977; Ghosh 1982; Joffre and Rambal 1993; Martinez-Vilalta et al. 2009), and the relationship between an ecosystem leaf area and its water balance was formalized by Eagleson (1982) as the ecohydrological equilibrium theory. He assumed that in water-limited environments, ecosystems develop a stable canopy density, which both maximizes leaf biomass and minimizes drought stress. More recently, leaf area adjustment to water availability was experimentally verified in several rainfall exclusion experiments applied to water-limited Mediterranean woodlands (Ogaya and Peñuelas 2006; Limousin et al. 2009; Ripullone et al. 2009) and also to Amazonian tropical rain forests (Fisher et al. 2007; Brando et al. 2008).

The mechanisms underlying leaf area adjustment have received less attention than shorter term physiological controls of drought stress, like stomatal closure. Since

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phenotypic plasticity in plants is expressed at sub-individual and nested modular levels (i.e., meristems, leaves, branches), the whole-plant leaf area is an integrative by-product of modular plasticity at several of these levels (de Kroon et al. 2005). This is particularly important and complex in evergreen canopies where photosynthetically active leaves remain on trees all year and generally for several growing seasons. In the Mediterranean evergreen *Quercus ilex*, drought adjustments potentially driving a decrease in transpiring leaf area might involve a change in allocation rules (Pereira and Chaves 1993), modifications of shoot allometry (Villar-Salvador et al. 1997), changes in leaf structure and biochemistry (Castro-Diez et al. 1997), and modifications of the leafing phenology (Castro-Diez and Montserrat-Martí 1998) and the leaf life span (Mediavilla and Escudero 2003; Ogaya and Peñuelas 2006). Some of these drought adjustments have been observed along climatic gradients (Castro-Diez et al. 1997; Villar-Salvador et al. 1997) and others in response to inter-annual climate variations (Montserrat-Martí et al. 2009), but little is known about how they may interact to meet a new ecohydrological equilibrium under predicted climate change.

This question is particularly important for Mediterranean evergreen oak woodlands, which are likely to experience more frequent and intense droughts with the ongoing climate change, and where leaf area is known to be a very effective drought adjustment (Hoff and Rambal 2003; Baldocchi and Xu 2007; Limousin et al. 2009). Recent climate projections for the Mediterranean region forecast a substantial drying and warming, especially in summer (Gao et al. 2006; Giorgi and Lionello 2008; Somot et al. 2008). Rainfalls are projected to decrease by approximately 15% for March–May, 42% for June–August and 10% for September–November (Somot et al. 2008). Concurrently, inter-annual variability is expected to increase (Gao and Giorgi 2008), and the frequency of long drought periods (4–6 months) to be multiplied by 3 at the end of this century (Sheffield and Wood 2008).

In order to study the long-term acclimation to decreasing precipitations in a Mediterranean *Q. ilex* forest, a throughfall exclusion experiment (TEE) that reduces the average water input to the soil by 27% (Limousin et al. 2008) was set up in the south of France in 2003. Previous research in this TEE showed a reduction of transpiration fluxes by 23% (Limousin et al. 2009) and a reduction of the gross primary production by 14% (Misson et al. 2010). Leaf assimilation processes did not acclimate significantly to the experimental drought conditions (Limousin et al. 2010b), whereas the transpiration decline appeared to be mostly driven by a reduction in supported leaf area (Limousin et al. 2009). The lower primary production in the dry treatment of the TEE did not translate into a reduced

secondary growth of the stems (Rodríguez-Calcerrada et al. 2011), but into a lowered allocation to the leaf compartment (Limousin et al. 2009, 2010a) and to seed production (Pérez-Ramos et al. 2010).

In the present study, we analyze the long-term impact of experimentally increased drought on different processes related with aerial primary growth and canopy dynamics, including shoot primary growth, leaf production, litterfall, leafing phenology and leaf demography. Specifically, we tested the following hypotheses: (1) increased drought conditions would reduce primary growth of the aerial woody compartment, (2) decreased canopy leaf area would result from a modified shoot and branch allometry, (3) the decrease in leaf area would be coordinated with changes in leaf demography and turnover rate, and (4) long-term experimental drought conditions would have more impact on the canopy leaf area than the inter-annual climate variations.

## Materials and methods

### Site description

The study site is located 35 km northwest of Montpellier (southern France), on a flat plateau in the Puéchabon State Forest ( $43^{\circ}44'29''\text{N}$ ,  $3^{\circ}35'45''\text{E}$ , 270 m a.s.l.). The site supports a *Q. ilex*-dominated evergreen forest which has been managed as a coppice for centuries, the last clear-cut being performed in 1942. The top canopy height is about 5.5 m and the density of resprouts was 6,070 stems  $\text{ha}^{-1}$  in 2009. At this time, stems with  $\text{DBH} < 4 \text{ cm}$  represented 4% of the total stems, whereas stems with  $\text{DBH} > 10 \text{ cm}$  represented 22.5%. Understorey evergreen species, *Buxus sempervirens*, *Phillyrea latifolia*, *Pistacia terebinthus* and *Juniperus oxycedrus*, compose a sparse shrubby layer with percent cover <25% and a height <2 m.

The area has a Mediterranean-type climate with a mean annual temperature of 13.2°C, the coldest month being January (5.5°C) and the hottest month July (22.9°C). The mean annual precipitation is 901 mm with a range of 556–1,549 mm recorded over the 1984–2009 period. Rainfall mainly occurs during autumn and winter with about 80% between September and April (Allard et al. 2008). The soil is extremely rocky and of 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 fraction of the soil within the 0–50 cm layer is a homogeneous silty clay loam (38.8% clay, 35.2% silt and 26% sand). The soil fills up the space between stones and rocks and 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 from occurring even for high-intensity rain events.

### Experimental design

A throughfall exclusion experiment (TEE) was set up on the study site in March 2003. The TEE was replicated on three plots 200 m from each other, and situated on a flat area with no lateral flow. On each replicate, two 100-m<sup>2</sup> plots were subjected to different precipitation regimes: one was a control treatment facing natural drought conditions, whereas the other was a drier treatment subjected to throughfall exclusion. The throughfall exclusion was achieved using PVC gutters hung under the canopy covering a 33% area and reducing the net input of precipitation to the soil by 27% compared to the control treatment (Limousin et al. 2008). On the control plot, identical gutters were set up upside down so that the albedo and the understorey micro-climate were as close as possible in the two treatments. Each treatment replicate was equipped with litter traps for litterfall collection. One of the replicated plots was further equipped with a scaffolding platform allowing easy access to the top canopy of both treatments. Six trees in the control treatment and seven trees in the dry treatment within a reaching distance from the platform were selected for branch-scale measurements. Forest structure was similar in the two treatments at the start of the experiment and neither stem density, basal area or mean stem diameter, nor stem mortality differed significantly between treatments when all three plots were taken into account (see Supplementary Table 1 in Rodríguez-Calcerrada et al. 2011). In the plot with the scaffolding system, where most of the measurements were made during this study, stem density was 6,500 and 5,300 stems ha<sup>-1</sup>, mean stem diameter was 7.33 and 7.96 cm, and basal area was 27.42 and 26.38 m<sup>2</sup> ha<sup>-1</sup> in the control and the dry treatment, respectively.

### Plant water status, soil water balance and drought index

Tree water status was assessed by measuring predawn leaf water potential ( $\Psi_{l, \text{pd}}$ ) with a pressure chamber (PMS 1000; PMS Instruments, Corvallis, OR, USA). Measurements were carried out about eight times a year between April and October from 2003 to 2009 on four trees per treatment. Two leaves per tree were taken from the scaffold at similar heights, and the leaf water potential was immediately measured (when the observed difference between the two leaves was greater than 0.2 MPa, a third leaf was sampled). Predawn leaf water potential was assumed to be a surrogate of the soil water potential ( $\Psi_s$ ) given a constant 0.43 MPa disequilibrium taken as the average value of

$\Psi_{l, \text{pd}}$  when the soil water content was at field capacity. Discrete measurements of  $\Psi_{l, \text{pd}}$  were converted to  $\Psi_s$  by adding the 0.43 MPa disequilibrium to all readings. We used the soil water balance model described in Rambal (1993) for simulating soil water storage and water potential over the rooting depth. It simulates on a daily basis the water balance components (transpiration, evaporation and deep drainage) and relates the soil water content to the soil water potential with a Campbell-type equation of the soil retention curve (Campbell 1974). The parameters of the Campbell-type equation depend on soil texture and were taken from Rambal et al. (2003). The model was driven by the same daily climate in the two treatments, and the rainfall interception by the canopy was estimated following Limousin et al. (2008). The water balance of the dry plot was obtained by excluding 33% of the throughfall. We chose to use the water-stress integral (WSI; Myers 1988) as a drought index, because it accounts for both drought intensity and duration. The WSI, expressed in MPa days, is the summation of modeled  $\Psi_s$  on every day during the period of interest. We used the March–June WSI and the annual WSI as spring and annual drought index for this study.

### Litterfall collection and leaf area index estimation

On each treatment replicate, 12 circular plastic traps (35 cm diameter for a total area of 1.1 m<sup>2</sup>) were placed on a grid over the gutters at a height of approximately 1.5 m. Litterfall was collected monthly from 2003 to 2009, oven-dried at 60°C, separated into leaf, woody debris, flower and acorn fractions, and weighed. The dry mass of litterfall was expressed per unit ground area (g m<sup>-2</sup>).

The dry mass of leaf litterfall ( $M_L$ ) was further converted into a leaf area by dividing  $M_L$  by the leaf mass per area of dead leaves ( $LMA_{lit}$ ), taken as constant over time and equal to 212.8 g m<sup>-2</sup> (Limousin et al. 2009). The leaf area index (LAI) at a given time was estimated as the sum of leaf area falling from this time until all leaves on the tree at the initial date had fallen (Limousin et al. 2009):

$$\text{LAI}_n = \sum_{i=n}^{n+2} \frac{M_{Li} P_i}{LMA_{lit}} \quad (1)$$

where  $\text{LAI}_n$  is the leaf area index supported during year  $n$ , and  $P_i$  is the average proportion of leaves grown during year  $n$  that are still on the tree in year  $i$ .  $P_i$  was determined from the leaf demographic census referred to below.  $P_n$  was equal to 1,  $P_{n+1}$  expresses the proportion of leaves remaining on the branches for more than 1 year and  $P_{n+2}$  expresses the proportion of leaves remaining more than 2 years. The proportion of leaves surviving more than 3 years was assumed negligible.

## Phenology, shoot growth and leaf demography

In March 2003, three well-developed branches were selected and marked in the upper canopy layer of each tree (six in the control treatment and seven in the dry treatment) for subsequent annual recording of leaf phenology and shoot growth. Leaf phenology was monitored at 1-week intervals during the growing season (March–July) from 2003 to 2009. Seven phenological phases were defined: phase 1 for closed buds (winter), phase 2 for bud swelling, phase 3 for budburst, phase 4 for shoot lengthening, phase 5 for new leaves opening, phase 6 for fully open leaves, and phase 7 for mature leaves (see Supplementary Material S2 in Misson et al. 2011). A leafing index based on the proportion of branches having reached the phenological phases 4, 5 or 6 in July was defined for each tree as:

$$\begin{aligned} \text{Leafing index} = & [(\text{Nb of branch in phase 4}) \\ & \times 1 + (\text{Nb of branch in phase 5}) \\ & \times 2 + (\text{Nb of branch in phase 6}) \\ & \times 3] / \text{Total Nb of branches} \end{aligned} \quad (2)$$

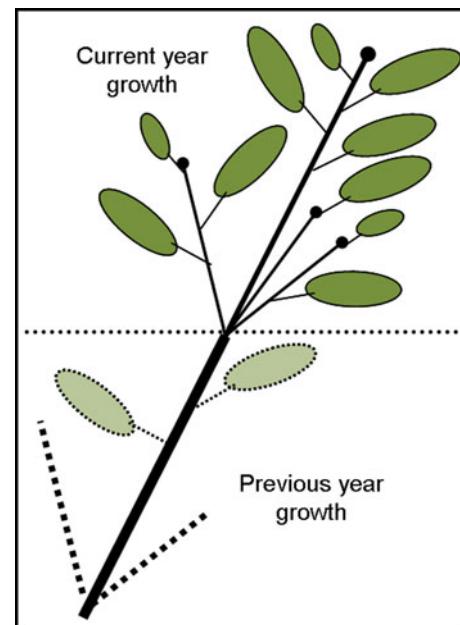
The leafing index was equal to 0 if none of the branches had reached the phenological phase 4 in July, and was equal to 3 if all leaves had reached phase 6.

In late July of every year, when all leaves were fully developed, the length and the base diameter of all the current-year shoots supported by the dominant 1-year-old branch were measured with a digital caliper. The base diameter of the 1-year-old supporting branch was also measured (see Fig. 1 for details). The number of current-year leaves supported by the shoots were counted and then multiplied by the average individual leaf area measured destructively on a nearby branch. The total leaf area per shoot was thus obtained and further expressed per unit cross-sectional area of the shoot after subtracting the bark thickness (the leaf area to sapwood area ratio; LA/SA). LA/SA was calculated for the leading current-year shoot and for the 1-year-old branch located immediately below. In addition, leaf demography was followed from July 2003 to October 2007 by a census of leaves on the leading branch at 20-day intervals.

## Statistical analyses

Differences in primary growth and phenology between the two TEE treatments were assessed by repeated measures analyses of variance (ANOVA), with treatment as the independent factor, and year as the repeated-measures factor. When necessary, data were log-transformed to fulfill the assumption of normality and homoscedasticity.

In addition, differences in primary growth between the two treatments were evaluated using allometric relationships



**Fig. 1** Schematic representation of a *Quercus ilex* branch. For the current-year growth, the base diameter and the length of every new shoot were measured, and the number of leaves on them counted. For the previous-year growth, only the base diameter was measured (*solid line* for organs measured, *dashed line* for organs not considered). The dominant shoot of the current-year growth was considered as the leading axis. The leaf area to sapwood area ratio was calculated for the current-year leading shoot alone, and for the previous-year supporting branch

of (1) leaf area against sapwood area for the current-year leading shoot, (2) current-year sapwood area against previous-year sapwood area, (3) the number of current-year ramifications against previous-year sapwood area and (4) current-year distal leaf area against previous-year sapwood area. Allometric analysis was chosen because it conserves more information than ratios about size dependency of traits (Preston and Ackerly 2003) and avoids statistical problems associated with ratios (Jasienski and Bazzaz 1999). Allometric relationships were derived from standardized major axis (SMA) regressions of log-transformed variables. SMA regressions are more appropriate for allometry than ordinary least square regressions when both variables are subject to error, and when the purpose is not to predict  $Y$  from  $X$  but to estimate the best line describing the scatter of  $X$  and  $Y$  (Warton et al. 2006). To compare allometric relationships between treatments, tests for homogeneity of slope and intercept were performed with the R package ‘smatr’ (v.2.1; D.I. Warton) following the methods described by Warton and Weber (2002). When slopes were homogeneous between treatments, we tested for differences in elevation (intercept) at the mean value of the variable  $X$  for the two treatments pooled (hereafter referred to as the grand mean of  $X$ ).

Correlation analyses were also performed to evaluate the relationships between morphological variables and the water stress integral by calculating Pearson's correlation coefficients. Statistical analyses were performed using the R software (2007, R Development Core Team) and SigmaStat 3.0 (Systat Software, San Jose, CA, USA).

## Results

### Leaf water potential and water stress integral

Minimum values of  $\Psi_{l,pd}$  over the 2003–2009 period reached  $-3.73 \pm 0.17$  and  $-4.80 \pm 0.19$  MPa in August 2005 in the control and dry treatments, respectively, and  $-4.07 \pm 0.23$  and  $-4.57 \pm 0.14$  MPa in August 2006 in the control and dry treatments, respectively (Table 1).  $\Psi_{l,pd}$  was systematically lower in the dry treatment during drought periods, and the differences between treatments were statistically significant ( $P < 0.05$ ) for every year except 2006 and 2009. During well-watered periods,  $\Psi_{l,pd}$  always remained between  $-0.4$  and  $-0.5$  MPa in the two treatments, and treatment effect on  $\Psi_{l,pd}$  could only be observed in spring, summer and early autumn (Limousin et al. 2009). The model used to interpolate  $\Psi_{l,pd}$  showed very good agreement with measured values: linear regressions of simulated against observed values were, in the control treatment,  $\Psi_{l,pd} \text{ sim} = 1.09 \pm 0.06 \Psi_{l,pd} \text{ obs} - 0.02 \pm 0.11$ , with  $r^2 = 0.84$ ,  $P < 0.001$ ,  $n = 54$ ; in the dry treatment,  $\Psi_{l,pd} \text{ sim} = 0.96 \pm 0.06 \Psi_{l,pd} \text{ obs} - 0.15 \pm 0.12$ , with  $r^2 = 0.83$ ,  $P < 0.001$ ,  $n = 54$ . The two slopes were not significantly different from 1 and the two intercepts were not significantly different from 0. Time courses of monthly water stress integral and rainfall are presented in Fig. 2. The less intense drought occurred in 2004, with an annual WSI of  $-110$  MPa days in the control treatment and  $-145$  MPa days in the dry treatment. The driest year was 2006, with an annual WSI of  $-324$  MPa days in the control and  $-372$  MPa days in the dry treatment (Table 1). Most of the drought stress occurred in summer (Fig. 2) and the March–June WSI were small in the two treatments compared to annual WSI (Table 1). The driest spring occurred in 2006 with a March–June WSI of  $-93.7$  MPa days in the control and  $-110.6$  MPa days in the dry treatment, whereas in 2008 drought did not start before July in any of the plots.

### Shoot growth and ramification

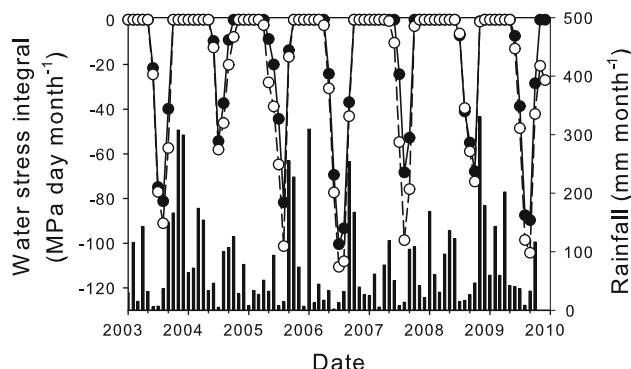
The average leading shoot diameter showed significant inter-annual variations in the two TEE treatments, and it was positively correlated with the March to June WSI ( $r^2 = 0.68$ ;  $P < 0.001$ ;  $n = 14$ ). However, the leading

shoot diameter was not significantly different between treatments, except in 2003 ( $P < 0.001$ ), the first year of the experiment, when shoot diameter was larger in the dry treatment than in the control treatment (Fig. 3a). Leading shoot length in the two treatments was also not significantly different (data not shown). In contrast, the leading shoot leaf area to sapwood area ratio (LA/SA) was on average 16% lower in the dry than in the control treatment over the study period ( $P < 0.001$ ), though no significant difference between treatments could be observed the last 3 years of the study, from 2007 to 2009 (Fig. 3b). The temporal trend in LA/SA was partly explained by the annual WSI, and a positive and significant correlation between the two variables was observed when the year 2006 was excluded ( $r^2 = 0.48$ ;  $P = 0.012$ ;  $n = 12$ ). The year 2006 was an outlier with an unusually high current-year LA/SA, probably because of the conjunction of an early drought that impaired the growth of the new shoots and the need to compensate for a caterpillar defoliation that occurred in 2005. Results observed for the LA/SA ratio were confirmed by the allometric regressions of leaf area against sapwood area for current-year leading shoots (Table 2; Fig. 4a). Slopes for these regressions were considered homogeneous between treatments for every year except 2004, and the adjusted leading shoot leaf area for a common sapwood area ( $Y$  at grand mean  $X$ ) was on average 10% lower in the dry than in the control treatment ( $P = 0.051$ ). Slopes of allometric regressions of the total sapwood area produced in a given year against the sapwood area of the previous-year supporting branch were homogeneous between treatments for all years (Table 2; Fig. 4b). The adjusted sapwood area for a common branch size was, on average, 20% smaller in the dry than in the control treatment ( $P = 0.001$ ), and it was always lower in the dry treatment, even if differences were not significant in every year. This difference was due to a lower number of ramifications, not to smaller lateral shoots, as evidenced by the allometric regressions of the number of ramifications against the sapwood area of the previous-year supporting branch (Table 2; Fig. 4c). Slopes for these regressions were homogeneous between treatments for all years, and the number of ramifications was, on average, 26% lower in the dry than in the control treatment ( $P = 0.001$ ), even if differences were not observed every year. Allometric regressions of the total leaf area produced in a given year against the sapwood area of the previous-year supporting branch are a combination of the previously described allometries (Table 2; Fig. 4d). They showed homogeneous slopes between treatments except for the year 2005. Adjusted total leaf areas (at grand mean  $X$ ) were significantly lower in the dry treatment in 2003 and 2007, and only the year 2009 showed a higher (although not significantly) adjusted leaf area in the dry than in the control

**Table 1** Values of minimum predawn leaf water potential ( $\Psi_{l,pd}$  in MPa  $\pm$  SE), annual water stress integral (WSI in MPa days) and March to June WSI in the two treatments from 2003 to 2009

Year	Minimum $\Psi_{l,pd}$ (MPa)		Annual WSI (MPa days)		March–June WSI (MPa days)	
	Control	Dry	Control	Dry	Control	Dry
2003	-3.42 $\pm$ 0.22 a	-4.82 $\pm$ 0.09 b	-217.6	-250.1	-21.6	-24.5
2004	-2.12 $\pm$ 0.18 a	-2.82 $\pm$ 0.09 b	-110.4	-144.7	-9.6	-12.4
2005	-3.73 $\pm$ 0.17 a	-4.80 $\pm$ 0.19 b	-168.4	-252.0	-28.7	-69.3
2006	-4.07 $\pm$ 0.23 a	-4.57 $\pm$ 0.14 a	-324.4	-372.4	-93.7	-110.6
2007	-3.55 $\pm$ 0.09 a	-4.41 $\pm$ 0.13 b	-145.6	-243.0	0.0	-10.9
2008	-3.17 $\pm$ 0.10 a	-3.89 $\pm$ 0.12 b	-171.6	-178.0	0.0	0.0
2009	-3.33 $\pm$ 0.27 a	-3.72 $\pm$ 0.11 a	-251.6	-354.3	-7.2	-13.0

Different letters within a given year denote that values of  $\Psi_{l,pd}$  were significantly different at  $P < 0.05$

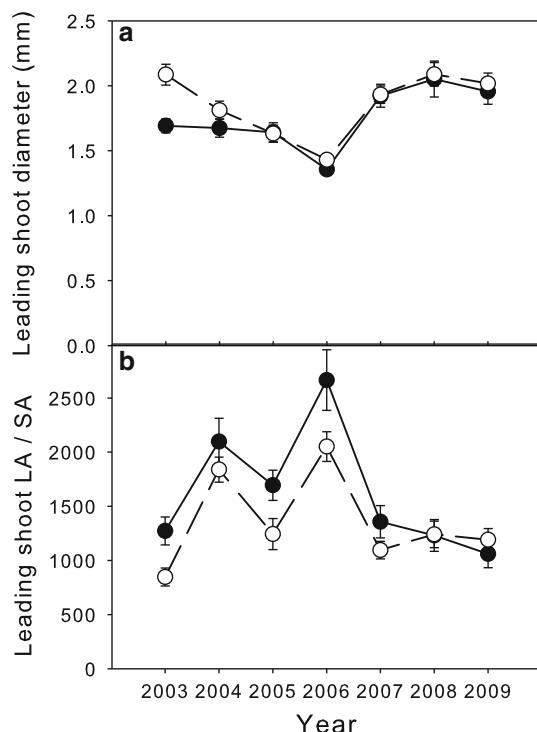


**Fig. 2** Monthly water stress integral (WSI; control: closed symbols; dry treatment: open symbols) and rainfall (vertical bars) from 2003 to 2009

treatment. As a consequence, the average adjusted total leaf area, for all years but 2005, was 18% lower in the dry treatment than in the control treatment ( $P = 0.049$ ). For comparison, the LA/SA ratio for 1-year-old branches was 21% lower in the dry than in the control treatment.

#### Litterfall

Time courses of cumulated leaf and woody debris litterfall from 2003 to 2009 are shown in Fig. 5. The total amount of leaf litterfall after 7 years of collection was  $1,496 \pm 54$  g m $^{-2}$  and  $1,209 \pm 65$  g m $^{-2}$  (mean  $\pm$  SE) in the control and dry treatments, respectively. In the meantime, the total amount of woody debris litterfall was, respectively,  $502 \pm 52$  g m $^{-2}$  and  $432 \pm 28$  g m $^{-2}$  in the control and dry treatments. With the exception of the first year of the experiment, 2003, leaf litterfall was always lower in the dry treatment of the TEE. A repeated-measures analysis of variance revealed a significant interaction between treatment and date for cumulated leaf litterfall ( $P < 0.001$ ) indicating that the difference between the two treatments was



**Fig. 3** Annual observations of **a** current-year leading shoot diameter and **b** current-year leading shoot leaf area to sapwood area ratio in the control (closed symbols and solid line) and the dry treatment (open symbols and dashed line) from 2003 to 2009. Error bars standard errors

significantly increasing with time. Annual sums of woody debris and leaf litterfall were positively correlated ( $r^2 = 0.62$ ,  $P < 0.001$ ,  $n = 14$ ), but cumulated woody debris litterfall was not significantly different between the two treatments. Inter-annual variations in leaf litterfall amount were explained by the previous-year drought, and a highly significant correlation was found with the previous-year WSI ( $r^2 = 0.74$ ,  $P = 0.001$ ,  $n = 10$ ; Fig. 6a) but not

**Table 2** Allometric relationships represented in Fig. 4

Allometry	Year	Slope				Y at grand mean X		
		Control	Dry	Common	P	Control	Dry	P
Log (leading shoot leaf area) vs. log (leading shoot sapwood area); Fig. 4a	All years	0.753	0.867	0.814	0.184	14.06	12.59	0.051*
	2003	1.278	1.238	1.255	0.919	14.46	8.51	0.007***
	2004	0.438	0.962	0.762	0.001****			
	2005	0.661	1.101	0.817	0.085*	12.73	8.72	0.008***
	2006	0.602	0.73	0.676	0.549	12.84	10.71	0.059*
	2007	0.86	0.85	0.855	0.969	15.31	12.76	0.141
	2008	0.943	1.022	0.976	0.759	15.43	16.05	0.816
	2009	0.859	0.51	0.652	0.091*	12.22	14.80	0.114
Log (total sapwood area for year Y) vs. log (sapwood area for year Y-1); Fig. 4b	All years	1.107	1.285	1.197	0.060*	5.89	4.71	0.001****
	2003	1.543	1.288	1.367	0.501	11.39	8.02	0.067*
	2004	1.021	1.052	1.035	0.895	6.97	5.90	0.257
	2005	1.007	1.436	1.198	0.205	4.11	2.21	0.003***
	2006	0.798	0.864	0.829	0.785	3.65	3.11	0.348
	2007	1.21	1.186	1.203	0.913	5.65	4.61	0.089*
	2008	1.219	1.192	1.207	0.899	5.19	4.94	0.733
	2009	1.307	1.655	1.498	0.23	7.51	6.89	0.64
Log (number of ramifications in year Y) vs. log (sapwood area for year Y-1); Fig. 4c	All years	0.983	1.127	1.061	0.176	3.07	2.27	0.001****
	2003	1.645	1.096	1.291	0.16	5.79	3.47	0.011***
	2004	0.841	0.868	0.855	0.911	4.27	3.24	0.073*
	2005	0.969	1.289	1.115	0.314	2.85	1.26	0.000****
	2006	0.92	0.969	0.94	0.856	3.23	2.29	0.064*
	2007	1.166	1.053	1.108	0.699	2.43	1.72	0.046**
	2008	0.804	1.095	0.938	0.191	1.81	1.78	0.919
	2009	1.018	1.518	1.312	0.068*	2.92	2.92	0.990
Log (total leaf area for year Y) vs. log (sapwood area for year Y-1); Fig. 4d	All years <sup>a</sup>	1.142	1.309	1.228	0.216	33.29	27.34	0.049**
	2003	2.175	1.856	1.98	0.595	57.78	24.52	0.008***
	2004	1.024	1.369	1.169	0.325	57.04	41.29	0.182
	2005	0.93	1.651	1.214	0.039**			
	2006	0.768	1.05	0.88	0.276	30.78	23.31	0.116
	2007	1.442	1.286	1.375	0.568	35.26	20.99	0.001****
	2008	1.184	1.411	1.315	0.426	25.31	24.12	0.818
	2009	1.17	1.6	1.468	0.173	30.83	40.67	0.204

Slopes and adjusted values of leaf area in  $\text{cm}^2$ , sapwood area in  $\text{mm}^2$ , or the number of ramifications ( $Y$  at grand mean  $X$ ) are given.  $Y$  at grand mean  $X$  is the elevation of the allometric relationships at the grand mean of the variable  $X$  for the two treatments pooled

Slopes of the SMA regressions are shown for the control treatment alone, the dry treatment alone and the common slope when pooling the two treatments

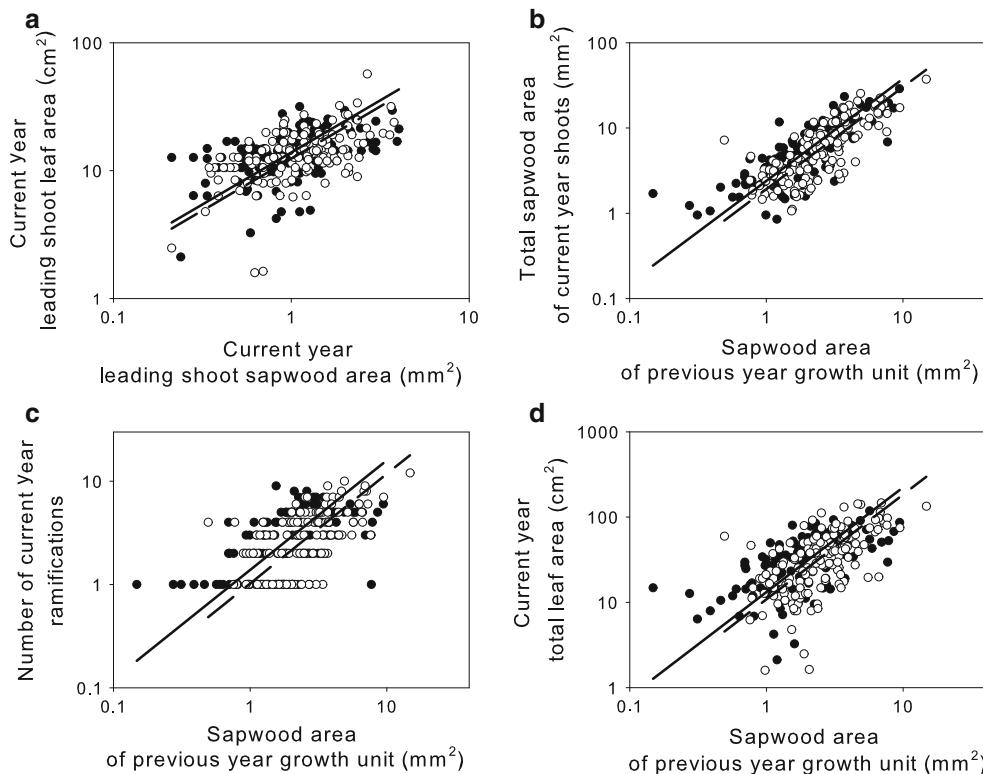
$P$  is the probability of homogeneity between slopes or adjusted  $Y$ , \* $P < 0.1$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ , \*\*\*\* $P < 0.001$ . Adjusted  $Y$  are not given when the hypothesis of homogeneity between slopes is rejected ( $P < 0.05$ )

<sup>a</sup> Year 2005 was not included in the all years allometric relationship because it was an outlier due to partial caterpillar defoliation that prevented the homogeneity of slopes between the two treatments

with the current-year WSI ( $r^2 = 0.002$ ,  $P = 0.904$ ,  $n = 10$ ) nor with the March–June WSI ( $r^2 = 0.03$ ,  $P = 0.631$ ,  $n = 10$ ). The relationship between leaf litterfall and the previous-year WSI was conserved between treatments, after excluding the year 2005 when partial caterpillar defoliation strongly reduced the leaf litterfall.

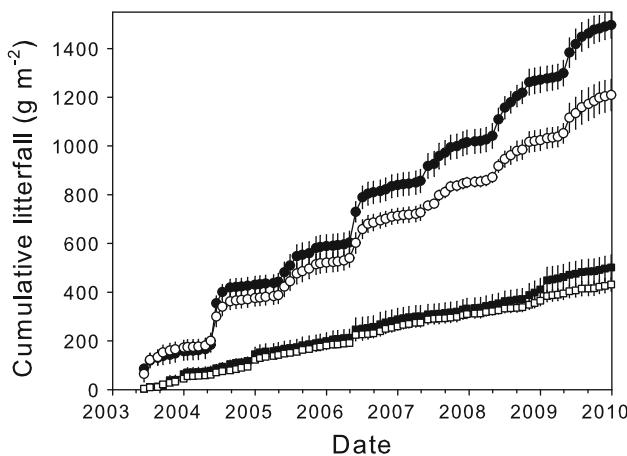
#### Leafing, phenology and leaf life span

As observed for leaf litterfall, inter-annual variations of the leafing index were correlated with the previous year WSI in both treatments ( $r^2 = 0.37$ ,  $P = 0.037$ ,  $n = 12$ ) but not with the current-year WSI ( $r^2 = 0.04$ ,  $P = 0.517$ ,  $n = 12$ )



**Fig. 4** Allometric regressions of **a** current-year leading shoot leaf area against current-year leading shoot sapwood area, **b** total sapwood area of current-year shoots against sapwood area of the 1-year-old supporting branch, **c** number of current-year ramifications against sapwood area of the 1-year-old supporting branch and **d** current-year

total leaf area against sapwood area of the 1-year-old supporting branch for the control treatment (closed circles and solid regression line) and the dry treatment (open circles and dashed regression line). Data points represent branches pooled from years 2003 to 2009



**Fig. 5** Cumulative amount of litterfall in the control (closed symbols) and the dry treatment (open symbols) from 2003 to 2009, separated into leaf litterfall (circles) and woody debris (squares). Error bars standard errors

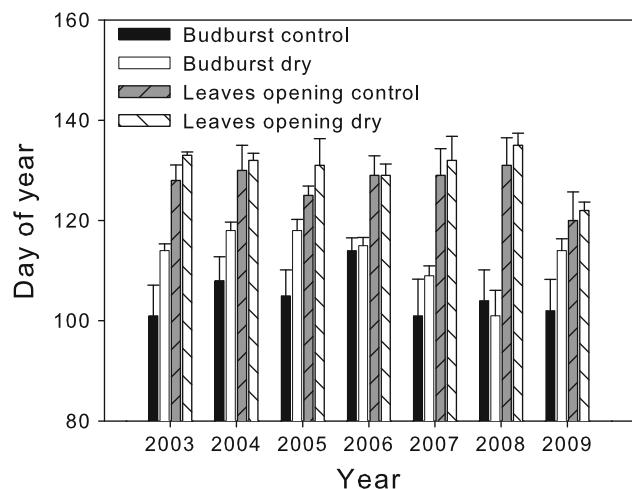
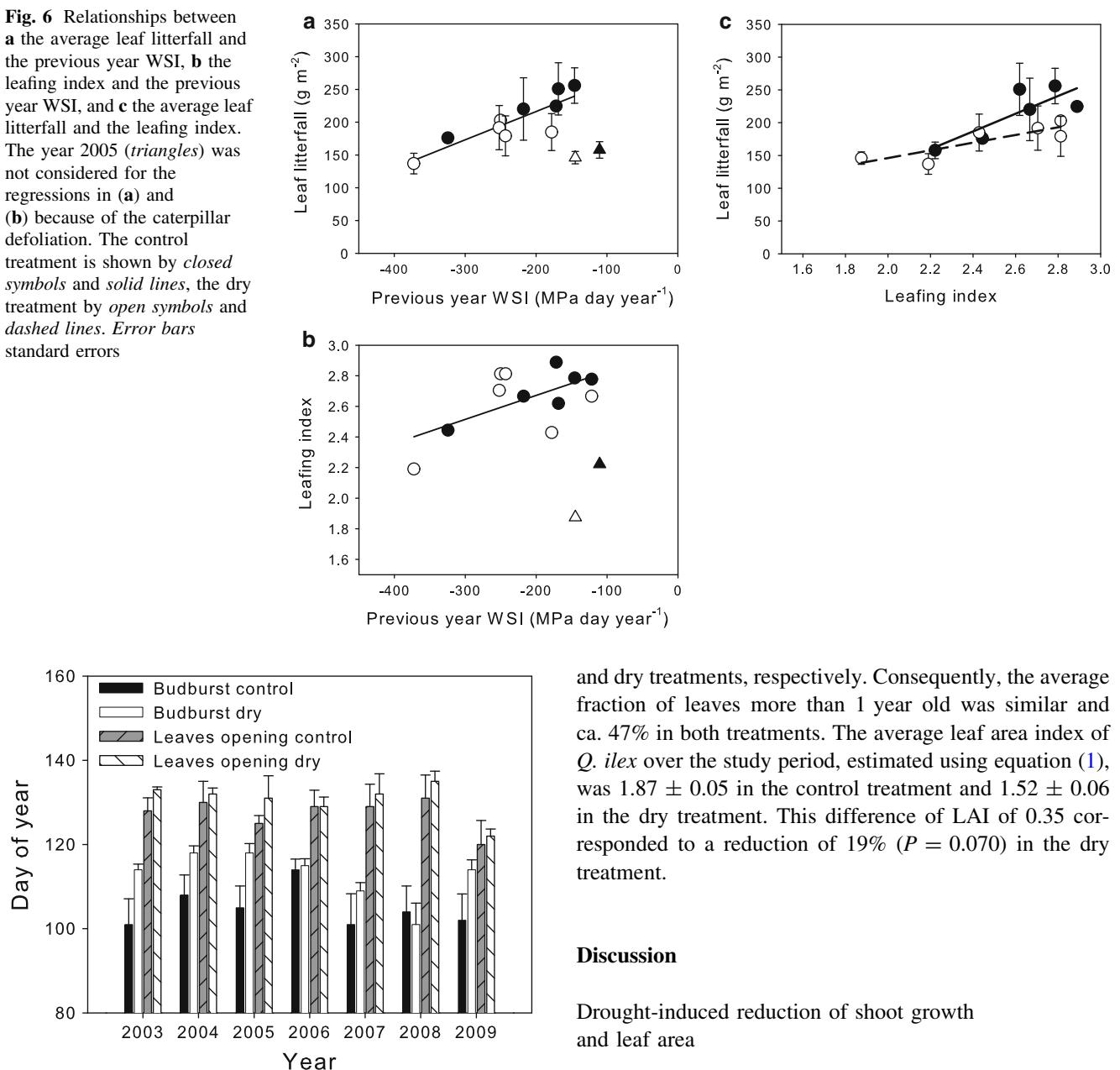
nor with the March–June WSI ( $r^2 = 0.003$ ,  $P = 0.96$ ,  $n = 12$ ). The relationship between the leafing index and the previous-year WSI did not differ significantly between treatments (Fig. 6b). Data for 2005 were outliers caused by a caterpillar attack on new leaves. The leafing index of new

shoots and the leaf shedding of old leaves were correlated to each other in the two treatments, but the relationships were only statistically different at  $P = 0.060$  (Fig. 6c). Linear regressions between leaf litterfall and the leafing index were fitted with  $r^2 = 0.67$ ,  $P = 0.046$  in the control treatment and  $r^2 = 0.72$ ,  $P = 0.033$  in the dry treatment.

Leaf phenology was slightly delayed in the dry treatment (Fig. 7). Budburst, the phenological phase 3, occurred on average 8 days later in the dry treatment: on day  $105 \pm 1.8$  in the control and on day  $113 \pm 2.3$  in the dry treatment. This lag between the two treatments was highly significant ( $P < 0.001$ ) and occurred every year except in 2008. However, the phenological lag was much lower for the following phases. The opening of new leaves (phase 5) occurred on average only 3 days later in the dry treatment (control: day  $127 \pm 1.4$ ; dry: day  $130 \pm 1.6$   $P = 0.37$ ). Thus, the time interval between budburst and leaf opening was shorter in the dry treatment.

Leaf demography also differed slightly between the two treatments. The average leaf life span was  $484 \pm 29$  days and  $516 \pm 33$  days in the control and the dry treatments, respectively, but this difference was not statistically significant ( $P = 0.33$ ). However, leaves that survived for a

**Fig. 6** Relationships between **a** the average leaf litterfall and the previous year WSI, **b** the leafing index and the previous year WSI, and **c** the average leaf litterfall and the leafing index. The year 2005 (*triangles*) was not considered for the regressions in **(a)** and **(b)** because of the caterpillar defoliation. The control treatment is shown by *closed symbols* and *solid lines*, the dry treatment by *open symbols* and *dashed lines*. Error bars standard errors



**Fig. 7** Average date of emergence of the phenological phases 3 (budburst) and 5 (opening of new leaves) from 2003 to 2009. For each year, from *left to right*: budburst in the control, budburst in the dry, leaves opening in the control, leaves opening in the dry. *Error bars* standard errors

second year tended to live longer in the dry treatment. As a result, the median leaf life span was longer in the dry treatment,  $555 \pm 39$  days, than in the control treatment,  $489 \pm 32$  days ( $P = 0.038$ ). The proportion of leaves living longer than 1 year ( $P_{n+1}$  in Eq. 1) was  $0.78 \pm 0.03$  and  $0.73 \pm 0.03$  in the control and dry treatments, respectively. The proportion of leaves living longer than 2 years ( $P_{n+2}$  in Eq. 1) was  $0.09 \pm 0.02$  and  $0.16 \pm 0.03$  in the control

and dry treatments, respectively. Consequently, the average fraction of leaves more than 1 year old was similar and ca. 47% in both treatments. The average leaf area index of *Q. ilex* over the study period, estimated using equation (1), was  $1.87 \pm 0.05$  in the control treatment and  $1.52 \pm 0.06$  in the dry treatment. This difference of LAI of 0.35 corresponded to a reduction of 19% ( $P = 0.070$ ) in the dry treatment.

## Discussion

### Drought-induced reduction of shoot growth and leaf area

The diameter and length of new leading shoots were not smaller in the dry treatment of the TEE (Fig. 3a). This observation might be explained by a drought-avoidance strategy in *Q. ilex*, a species characterized by short spring phenophases and performing most of the growth before the onset of drought (Gratani 1996; Castro-Diez and Montserrat-Martí 1998); thus, when water potential was not different in the two treatments or not distinct enough to alter primary growth. As a consequence, the inter-annual variation in rainfall had a greater effect on shoot growth than the 27% decrease imposed by the throughfall exclusion. Since plant growth is mostly dependent on cell expansion, which is a turgor-driven process (Lockhart 1967; Hsiao and Xu 2000), lower xylem water potential,

either due to lower soil water potential or lower hydraulic conductance, usually translates into smaller primary growth (Dale 1988; Koch et al. 2004). We observed evidence for such a drought effect on shoot growth in the correlation between the shoot diameter and the March–June WSI. Furthermore, a total rainfall exclusion conducted from February to July in another experiment at our site showed that more severe drought occurring in spring could completely prevent shoot development in some trees (Misson et al. 2011).

However, while the leading shoot growth was similar in the two treatments, the number of new lateral ramifications was significantly reduced in the dry treatment resulting in an overall decrease in primary growth. The lower number of lateral ramifications suggests that drought stress reinforces the apical dominance on lateral growth, also referred to as correlative inhibition (Cline 1997). This mechanism might contribute to explain how leading shoot growth could be maintained in the dry treatment despite more intense water stress. Besides the lower number of shoots, the leaf area supported by each of the shoots was smaller in the dry treatment (Table 2; Fig. 4). As a result, the reduction of LA/SA was less marked for new shoots (−16%) than for 1-year-old branches (−21%). Ogaya and Peñuelas (2006) also documented a reduction of the leaf area associated to a decreased number of ramifications and leaves per shoot in a *Q. ilex* stand submitted to throughfall exclusion in north-eastern Spain. Interestingly, a similar morphological adjustment was also observed in mature *Q. ilex* trees growing under elevated CO<sub>2</sub> concentration (Hättenschwiler et al. 1997).

Decreased aerial primary growth and leaf area in the dry treatment could come from a carbon limitation due to water stress or from a hydraulic adjustment to drier conditions. In our case, we think it more likely that a hydraulic adjustment occurred, because decreased primary growth translated mainly into a decreased LA/SA ratio. A previous study conducted in the same experimental plots showed that, without any adjustment of xylem vulnerability to embolism, lower water potentials undergone by the trees in the dry treatment were likely to reduce the xylem hydraulic conductivity, thus creating a need for a reduction of the leaf area in order to maintain the leaf specific conductivity (Limousin et al. 2010a). Previous studies have reported strong correlations between shoot hydraulic conductance and shoot and leaf growth in several species (Nardini 2002; Cochard et al. 2005). Cochard et al. (2005) showed that the number of leaf primordia preformed inside beech buds was controlled by the hydraulic conductance of the supporting shoot. A similar hydraulic control of the bud organogenesis can be hypothesized for *Q. ilex*, because growth in oak species is also determined by preformed organs inside the buds (Fontaine et al. 1999).

Interestingly, an adjustment of the leaf area through a reduction in the number of ramifications has morphological and hydraulic advantages over a reduction in the supported leaf area of a conserved number of branches. Decreasing the number of ramifications has cumulative effects over time: a reduced number of ramifications in a single given year translates into a reduced number of shoots and apices in the subsequent years, even if the ramification pattern eventually recovered. This phenomenon would explain the absence of treatment effect on the terminal leaf area for the last years of observation, because our measurements were only focusing on some terminal branches and not on a full census of apices in the crown. Moreover, the slopes of the allometric regressions of leaf area against sapwood area for current-year leading shoots were in most cases lower than 1 (Table 2), which means that small new shoots had comparatively higher LA/SA ratios than large ones. In contrast, slopes of the allometric regressions of total current-year leaf area against sapwood area of the previous-year supporting branch (taking into account the effect of ramifications) were generally higher than 1 (Table 2), which indicates that small 1-year-old branches were proportionally supporting less leaf area than large ones. Favoring the development of the leading shoot instead of the number of ramifications also has hydraulic advantages, firstly because the leading axis generally has higher hydraulic conductivity than lateral branches (Cochard et al. 1997), and secondly because branch ramifications act as hydraulic constrictions, especially when ramifications are unequal (Tyree and Ewers 1991; Tyree and Alexander 1993). Thus we argue that reducing the number of ramifications is probably the most efficient mechanism to lower the total leaf area while maximizing the leaf-specific conductivity.

The decrease in LA/SA that we observed in upper canopy branches matches closely the 19% decrease in leaf area index assessed from leaf litterfall at the canopy scale. A reduction of the whole canopy leaf area is a response often observed in rainfall exclusion experiments (Ogaya and Peñuelas 2006; Fisher et al. 2007; Brando et al. 2008; Ripullone et al. 2009). In our experiment, it was not paralleled by a reduction of stem secondary growth (Rodríguez-Calcerrada et al. 2011), thus the leaf area on a stem basal area basis, which can be viewed as a surrogate of LA/SA at the stand scale, was lower in the dry treatment plot than in the control plot (data not shown).

#### Leaf turnover and temporal adjustments

The onset of shoot development was slightly delayed in the dry treatment, but the lag between the two treatments tended to decrease through the following phenological phases. Such a delaying effect of drought on leaf unfolding has already been reported for several Mediterranean

species (Peñuelas et al. 2004; Misson et al. 2011), but the opposite effect is sometime observed for seedlings or saplings (Pearson and Mansfield 1994; Sanz-Pérez and Castro-Diez 2010). This delayed phenology can affect shoot and leaf development by shortening the window of optimal weather conditions before summer drought arrives (Castro-Diez and Montserrat-Martí 1998; Montserrat-Martí et al. 2009). Moreover, because the photosynthetic potential of *Q. ilex* leaves declines with age and is thus lower in a 1-year-old leaf than in current-year leaves (Niinemets et al. 2005; Limousin et al. 2010b), a delayed leaf phenology has potentially negative consequences on tree growth and carbon assimilation.

We observed that leafing and leaf shedding were controlled by climate and coordinated one with the other. Leaf shedding occurred mostly in spring, simultaneously with the first phenological phases, and in a lower proportion in autumn, confirming earlier observations by other authors (e.g., Rapp 1969; Bussotti et al. 2003). The amount of leaves shed was controlled by the previous-year water stress: more leaves were shed after wet years while leaves remained on the trees for a longer time following dry years. This phenomenon may validate the cost-benefit hypothesis (Chabot and Hicks 1982; Kikuzawa 1991). A leaf needs to assimilate a given amount of carbon during its lifetime, at least the necessary amount to construct one new leaf in order to maintain the number of leaves in the canopy. Thus, if the leaf carbon gain is reduced by drought stress, leaf life span should be lengthened to pay back the construction costs. The same theory holds for explaining the longer average leaf life span in the dry than in the control treatment, also observed by Ogaya and Peñuelas (2006) in their drought experiment. The strong inter-annual variations in the amount of leaf litterfall were coordinated with the variations of the leafing index to maintain the LAI around a steady state. The alternation of years of high-shoot production and leaf shedding with years of opposite characteristics is a well-described phenomenon for *Q. ilex* (Rapp 1969; Ogaya and Peñuelas 2006; Montserrat-Martí et al. 2009). The interaction between leafing and shedding has been commonly described as the “leaf exchange” pattern among tropical trees growing in seasonally dry environments (Reich and Borchert 1984; Williams et al. 1997). This points to a conservative strategy for maintaining a certain homeostasis of the canopy leaf area under fluctuating soil water availability and ultimately reducing the occurrence of water transport failure. The fact that previous-year WSI was better correlated with leaf litterfall than with the leafing index suggests that the actual leaf area of a tree is controlled by leaf shedding, rather than by leaf growth. The balance between the retention of old leaves and the formation of new ones was assumed by some authors to be the result of a source–sink interaction at shoot

or branch levels (Cherbuy et al. 2001; Hikosaka 2005; Montserrat-Martí et al. 2009). Thus, keeping old leaves for a longer time after dry years would allow a higher leaf lifetime carbon gain, but would come at the cost of impairing shoot growth because old leaves compete for nitrogen and water with meristematic tissues (Milla et al. 2007). This scheme may be further complicated when considering a trade-off for resources between vegetative growth and acorn production (Montserrat-Martí et al. 2009). Although acorn production at our site was found to be driven by water availability, rather than by a switching of resources between growth and reproduction (Pérez-Ramos et al. 2010), growing acorns in masting years will compete for resources with meristematic tissues.

## Conclusion

According to our results, the lower water availability expected in Mediterranean forests under climate change scenarios will result in a decrease in canopy leaf area. This decrease will likely affect the ecosystem energy balance, the water and carbon fluxes between the vegetation and the atmosphere, and the amount of organic matter input to the soil. Our long-term dataset showed that the effects of inter-annual variability in drought severity were somehow damped by a balance between leaf production and shedding, whereas the long-term experimental decrease in precipitation translated into a significant change in leaf area. At the branch scale, the experimentally increased drought reduced primary growth through a modification of the shoot allometry between sapwood area and leaf area and a reduction of the number of ramifications produced. At the tree scale, the growth in height and stem diameter did not vary between the two treatments (Rodríguez-Calcerrada et al. 2011), thus evidencing a change in carbon allocation to the different organs. The decrease in leaf area appeared to be a hydraulic adjustment to maintain leaf-specific hydraulic conductivity under drier conditions. It was also evidenced to be an effective mechanism for reducing canopy transpiration (Limousin et al. 2009), in agreement with the ecohydrological equilibrium theory (Eagleson 1982), and might improve the amortization of foliage and the carbon and nitrogen use efficiency through a reduction of self-shading.

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## References

- Allard V, Ourcival JM, Rambal S, Joffre R, Rocheteau A (2008) Seasonal and annual variation of carbon exchange in an evergreen Mediterranean forest in southern France. *Glob Change Biol* 14:714–725
- Baldocchi DD, Xu L (2007) What limits evaporation from Mediterranean oak woodlands - The supply of moisture in the soil, physiological control by plants or the demand by the atmosphere? *Adv Water Resour* 30:2113–2122
- Brando PM, Nepstad DC, Davidson EA, Trumbore SE, Ray D, Camargo P (2008) Drought effects on litterfall, wood production and belowground carbon cycling in an Amazon forest: results of a throughfall reduction experiment. *Philos Trans R Soc Lond B* 363:1839–1848
- Bussotti F, Borghini F, Celesti C, Leonzio C, Cozzi A, Bettini D, Ferretti M (2003) Leaf shedding, crown condition and element return in two mixed holm oak forests in Tuscany, central Italy. *For Ecol Manag* 176:273–285
- Campbell GS (1974) A simple method for determining unsaturated conductivity from moisture retention data. *Soil Sci* 117:311–314
- Castro-Diez P, Montserrat-Martí G (1998) Phenological pattern of fifteen Mediterranean phanaerophytes from *Quercus ilex* communities of NE-Spain. *Plant Ecol* 139:103–112
- Castro-Diez P, Villar-Salvador P, Pérez-Rontomé C, Maestro-Martínez M, Montserrat-Martí G (1997) Leaf morphology and leaf chemical composition in three *Quercus* (*Fagaceae*) species along a rainfall gradient in NE Spain. *Trees* 11:127–134
- Chabot BF, Hicks DJ (1982) The ecology of leaf life spans. *Annu Rev Ecol Syst* 13:229–259
- Cherbuy B, Joffre R, Gillon D, Rambal S (2001) Internal remobilization of carbohydrates, lipids, nitrogen and phosphorus in the Mediterranean evergreen oak *Quercus ilex*. *Tree Physiol* 21:9–17
- Cline MG (1997) Concepts and terminology of apical dominance. *Am J Bot* 84:1064–1069
- Cochard H, Peiffer M, Le Gall K, Granier A (1997) Developmental control of xylem hydraulic resistances and vulnerability to embolism in *Fraxinus excelsior* L.: impacts on water relations. *J Exp Bot* 48:655–663
- Cochard H, Coste S, Chanson B, Guehl JM, Nicolini E (2005) Hydraulic architecture correlates with bud organogenesis and primary shoot growth in beech (*Fagus sylvatica*). *Tree Physiol* 25:1545–1552
- Dale JE (1988) The control of leaf expansion. *Annu Rev Plant Physiol Plant Mol Biol* 39:267–295
- de Kroon H, Huber H, Stuefer JF, van Groenendael JM (2005) A modular concept of phenotypic plasticity in plants. *New Phytol* 166:73–82
- Eagleson PS (1982) Ecological optimality in water-limited natural soil-vegetation systems. 1. Theory and hypothesis. *Water Resour Res* 18:325–340
- Fisher RA, Williams M, Lola da Costa A, Malhi Y, da Costa RF, Almeida S, Meir P (2007) The response of an Eastern Amazonian rain forest to drought stress: results and modelling analyses from a throughfall exclusion experiment. *Glob Change Biol* 13:2361–2378
- Fontaine F, Chaar H, Colin F, Clement C, Burrus M, Druelle JL (1999) Preformation and neoformation of growth units on 3-year-old seedlings of *Quercus petraea*. *Can J Bot* 77:1623–1631
- Gao XJ, Giorgi F (2008) Increased aridity in the Mediterranean region under greenhouse gas forcing estimated from high resolution simulations with a regional climate model. *Glob Planet Change* 62:195–209
- Gao XJ, Pal JS, Giorgi F (2006) Projected changes in mean and extreme precipitation over the Mediterranean region from a high resolution double nested RCM simulation. *Geophys Res Lett* 33:L03706. doi:10.1029/2005GL024954
- Gholz HL (1982) Environmental limits on aboveground net primary production, leaf area, and biomass in vegetation zones of the Pacific Northwest. *Ecology* 63:469–481
- Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. *Glob Planet Change* 63:90–104
- Gratani L (1996) Leaf and shoot growth dynamics of *Quercus ilex* L. *Acta Oecol* 17:17–27
- Grier CC, Running SW (1977) Leaf area of mature coniferous forests: relation to site water balance. *Ecology* 58:893–899
- Hättenschwiler S, Miglietta F, Raschi A, Körner C (1997) Morphological adjustments of mature *Quercus ilex* trees to elevated CO<sub>2</sub>. *Acta Oecol* 18:361–365
- Hikosaka K (2005) Leaf canopy as a dynamic system: ecophysiology and optimality in leaf turnover. *Ann Bot* 95:521–533
- Hoff C, Rambal S (2003) An examination of the interaction between climate, soil and leaf area index in a *Quercus ilex* ecosystem. *Ann For Sci* 60:153–161
- Hsiao TC, Xu LK (2000) Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *J Exp Bot* 51:1595–1616
- Jasienski M, Bazzaz FA (1999) The fallacy of ratios and the testability of models in biology. *Oikos* 84:321–326
- Joffre R, Rambal S (1993) How tree cover influences the water balance of Mediterranean rangelands. *Ecology* 74:570–582
- Kikuzawa K (1991) A cost-benefit analysis of leaf habit and leaf longevity of trees and their geographical pattern. *Am Nat* 138:1250–1263
- Koch WG, Sillet SC, Jennings GM, Davis SD (2004) The limits to tree height. *Nature* 428:851–854
- Limousin JM, Rambal S, Ourcival JM, Joffre R (2008) Modelling rainfall interception in a Mediterranean *Quercus ilex* ecosystem: lesson from a throughfall exclusion experiment. *J Hydrol* 357:57–66
- Limousin JM, Rambal S, Ourcival JM, Rocheteau A, Joffre R, Rodríguez-Cortina R (2009) Long-term transpiration change with rainfall decline in a Mediterranean *Quercus ilex* forest. *Glob Change Biol* 15:2163–2175
- Limousin JM, Longepierre D, Huc R, Rambal S (2010a) Change in hydraulic traits of Mediterranean *Quercus ilex* submitted to long-term throughfall exclusion. *Tree Physiol* 30:1026–1036
- Limousin JM, Misson L, Lavois AV, Martin NK, Rambal S (2010b) Do photosynthetic limitations of evergreen *Quercus ilex* leaves change with long-term increased drought severity? *Plant Cell Environ* 33:863–875
- Lockhart JA (1967) Physical nature of irreversible deformation of plant cells. *Plant Physiol* 42:1545–1552
- Martinez-Vilalta J, Cochard H, Mencuccini M, Sterck F, Herrero A, Korhonen JFJ, Llorens P, Nikinmaa E, Nolè A, Poyatos R, Ripullone F, Sass-Klaassen U, Zweifel R (2009) Hydraulic adjustment of Scots pine across Europe. *New Phytol* 184:353–364
- Mediavilla S, Escudero A (2003) Photosynthetic capacity, integrated over the lifetime of a leaf, is predicted to be independent of leaf longevity in some tree species. *New Phytol* 159:203–211
- Milla R, Palacio S, Maestro-Martínez M, Montserrat-Martí G (2007) Leaf exchange in a Mediterranean shrub: water, nutrient, non-structural carbohydrate and osmolyte dynamics. *Tree Physiol* 27:951–960
- Misson L, Rocheteau A, Rambal S, Ourcival JM, Limousin JM, Rodríguez-Cortina R (2010) Functional changes in the controls of carbon fluxes after 3 years of increased drought in a Mediterranean evergreen forest? *Glob Change Biol* 16:2461–2575

- Misson L, Degueldre D, Collin C, Rodríguez-Cortina R, Rocheteau A, Ourcival JM, Rambal S (2011) Phenological responses to extreme droughts in a Mediterranean forest. *Glob Change Biol* 17:1036–1048
- Montserrat-Martí G, Camarero JJ, Palacio S, Pérez-Rontomé C, Milla R, Albuixech J, Maestro M (2009) Summer-drought constrains the phenology and growth of two coexisting Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. *Trees* 23:787–799
- Myers BJ (1988) Water stress integral—a link between short-term stress and long-term growth. *Tree Physiol* 4:315–323
- Nardini A (2002) Relations between efficiency of water transport and duration of leaf growth in some deciduous and evergreen trees. *Trees* 16:417–422
- Niinemets U, Cescatti A, Rodeghiero M, Tosens T (2005) Leaf internal diffusion limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species. *Plant Cell Environ* 28:1552–1566
- Ogaya R, Peñuelas J (2006) Contrasting foliar responses to drought in *Quercus ilex* and *Phillyrea latifolia*. *Biol Plant* 50:373–382
- Pearson M, Mansfield TA (1994) Effects of exposure to ozone and water stress on the following season's growth of beech (*Fagus sylvatica* L.). *New Phytol* 126:511–515
- Peñuelas J, Filella I, Zhang X, Llorens L, Ogaya R, Lloret F, Comas P, Estiarte M, Terradas J (2004) Complex spatiotemporal phenological shifts as a response to rainfall changes. *New Phytol* 161:837–846
- Pereira JS, Chaves MM (1993) Plant water deficits in Mediterranean ecosystems. In: Smith JAC, Griffiths H (eds) *Water deficits: plant responses from cell to community*. Bios Scientific Publishers, Oxford, pp 235–251
- Pérez-Ramos IM, Ourcival JM, Limousin JM, Rambal S (2010) Mast seeding under increasing drought: results from a long-term dataset and from a rainfall exclusion experiment. *Ecology* 91:3057–3068
- Poole DK, Miller PC (1981) The distribution of plant water stress and vegetation characteristics in Southern California chaparral. *Am Midl Nat* 105:32–43
- Preston KA, Ackerly DD (2003) Hydraulic architecture and the evolution of shoot allometry in contrasting climates. *Am J Bot* 90:1502–1512
- Rambal S (1993) The differential role of mechanisms for drought resistance in a Mediterranean evergreen shrub: a simulation approach. *Plant Cell Environ* 16:35–44
- Rambal S, Ourcival JM, Joffre R, Mouillot F, Nouvellon Y, Reichstein M, Rocheteau A (2003) Drought controls over conductance and assimilation of a Mediterranean evergreen ecosystem: scaling from leaf to canopy. *Glob Change Biol* 9:1813–1824
- Rapp M (1969) Production de litière et apport au sol d'éléments minéraux dans deux écosystèmes méditerranéens: la forêt de *Quercus ilex* L. et la garrigue de *Quercus coccifera* L. *Oecol Plant* 4:377–410
- Reich PB, Borchert R (1984) Water stress and tree phenology in a tropical dry forest in the lowlands of Costa Rica. *J Ecol* 72:61–74
- Ripullone F, Borghetti M, Raddi S, Vicinelli E, Baraldi R, Guerrieri MR, Nolè A, Magnani F (2009) Physiological and structural changes in response to altered precipitation regimes in a Mediterranean macchia ecosystem. *Trees* 23:823–834
- Rodríguez-Calcerrada J, Pérez-Ramos IM, Ourcival JM, Limousin JM, Joffre R, Rambal S (2011) Is selective thinning an adequate practice for adapting *Quercus ilex* coppices to climate change? *Ann For Sci* 68:575–585
- Sanz-Pérez V, Castro-Diez P (2010) Summer water stress and shade alter bud size and budburst date in three Mediterranean *Quercus* species. *Trees* 24:89–97
- Sheffield J, Wood EF (2008) Projected changes in drought occurrence under future global warming from multi-model, multi-scenario, IPCC AR4 simulations. *Clim Dyn* 31:79–105
- Somot S, Sevault F, Deque M, Crepon M (2008) 21st century climate change scenario for the Mediterranean using a couple atmosphere-ocean regional climate model. *Glob Planet Change* 63:112–126
- Specht RL (1972) Water use by perennial evergreen plant communities in Australia and Papua New Guinea. *Aust J Bot* 20:273–299
- Tyree MT, Alexander JD (1993) Hydraulic conductivity of branch junctions in three temperate tree species. *Trees* 7:156–159
- Tyree MT, Ewers FW (1991) Tansley Review No. 34. The hydraulic architecture of trees and other woody plants. *New Phytol* 119:345–360
- Villar-Salvador P, Castro-Diez P, Pérez-Rontomé C, Montserrat-Martí G (1997) Stem xylem features in three *Quercus* (*Fagaceae*) species along a climatic gradient in NE Spain. *Trees* 12:90–96
- Warton DI, Weber NC (2002) Common slope tests for bivariate structural relationships. *Biometrical J* 44:161–174
- Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. *Biol Rev* 81:259–291
- Williams RJ, Myers BA, Muller WJ, Duff GA, Eamus D (1997) Leaf phenology of woody species in a North Australian tropical savanna. *Ecology* 78:2542–2558