

Phenological responses to extreme droughts in a Mediterranean forest

LAURENT MISSON^{*†}, DAVID DEGUELDRÉ^{*}, CHRISTIAN COLLIN^{*}, RAQUEL RODRIGUEZ^{*}, ALAIN ROCHETEAU[†], JEAN-MARC OURCIVAL^{*} and SERGE RAMBAL^{*}

^{*}CEFE, CNRS, UMR5175, 1919 route de Mende, F-34293 Montpellier, Cedex 5, France, [†]IRD, CEFE, UMR5175, 1919 route de Mende, F-34293 Montpellier, Cedex 5, France

Abstract

Mediterranean regions are projected to experience more frequent, prolonged and severe drought as a consequence of climate change. We used a retractable rainfall shelter, to investigate the impact of extreme droughts on the development of *Quercus ilex* leaves, flowers and fruit. In 2008, 97% of rainfall was excluded from a forest plot during the autumn, representing 50% of the 1127 mm of rain that fell during the year. In 2009, 87% of rainfall was excluded during the spring, representing 58% of the 749 mm that fell during the year. The rainfall shelter did not impact neither incident radiation nor air temperature. Autumn rainfall exclusion did not significantly affect leaf, flowers or fruit development. Spring rainfall exclusion resulted in larger and more sustained depression of leaf water potential during the key phases of foliar and floral development. Consequently, only half of the sampled trees (6) reached the shoot lengthening stage which leads to functionally mature leaves (phenophase 4), with one abandoning leaf development at budburst (phenophase 3) and the other two at the bud swelling stage (phenophase 2). All trees of the control plot passed phenophase 4, with most reaching complete leaf development. The impact of extreme droughts on flower development differed between the sexes. The spring exclusion had no effect on male flower, but only one of six trees completed female fruit maturation, compared with four in the control plot. The difference between the male and female drought impacts is likely attributable to the occurrence of male floral development before the period of lowest leaf water potential, and to the lower resource allocation requirements of male flowers. The information provided by our experimental approach may constitute a crucial step to evaluate the impact of increasing drought due to climate change on the most dominant Mediterranean tree species and to help drawing a full picture of the ecological consequences of the decline in water resource on forest dynamics under changing conditions.

Keywords: climate changes, extreme events, flowering, fruiting, leaf-unfolding, rainfall changes

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Introduction

All simulations did with high-resolution coupled atmosphere–ocean regional climate models (RCMs) or with coarser resolution generalized circulation models (GCMs) under scenarios of moderate greenhouse gases emissions give a collective picture of a substantial drying and warming of the regions surrounding the Mediterranean Sea, especially in the warm season (Gao *et al.*, 2006; Giorgi & Lionello, 2008; Somot *et al.*, 2008). The comparative analysis of Giorgi (2006) placed these regions among the most responsive regions to global climate change and one of the main climate change ‘hotspots.’ Such changes have the potential to deeply modify the climate patterns mostly by lower spring soil water availability leading to reduced summer convec-

tion and large land-sea contrast in warming leading to increased air dryness and decreased precipitation over the continent. For precipitations, projections for our study sites yielded decreases of 0.1, 0.4, 0.5 and 0.35 mm day⁻¹ for DJF, MAM, JJA and SON, respectively (Somot *et al.*, 2008). Interannual variability is projected to increase as in the occurrence of extreme heat and drought events (Gao & Giorgi, 2008; Fischer & Schär, 2010). Return periods for drought durations lower than 4–6 months will be multiplied by 3. Those for drought durations longer than 1 year will be multiplied by 7 (Sheffield & Wood, 2008).

Increased temperature and drought severity could impact the carbon sink capacity and the sustainability of forest ecosystems in the Mediterranean region (Lloret *et al.*, 2004; Ciais *et al.*, 2005; Granier *et al.*, 2007; Breda & Badeau, 2008), because of their effects on carbon assimilation (Rambal *et al.*, 2003; Ripullone *et al.*, 2009; Limousin *et al.*, 2010), interspecies competition (Peñuelas & Filella, 2001; Sanchez-Gomez *et al.*, 2008), soil

^{*}Died on March 5, 2010

Correspondence: Serge Rambal, tel. + 33 4 67 61 32 93, fax + 33 4 67 41 21 38, e-mail: serge.rambal@cefe.cnrs.fr

respiration (Joffre *et al.*, 2003), nutrient cycling (Sardans & Penuelas, 2005) and fire frequency (Mouillot *et al.*, 2002). However, to fully understand how abiotic stressors, including drought, may influence ecosystem function, it is also important to determine how the dominant species adapt their growth strategies to drought stress. In this study, we examine the impact of experimentally induced extreme drought conditions on the timing and success of leaf, flower and seed development in a natural *Quercus ilex* L. (holm oak) stand at the Puéchabon Experimental Forest of southern France.

Well-timed sequences of phenological events are crucial for survival, reproduction and successful interspecies competition in higher plants (Mooney, 1983). In the Mediterranean region, phenological events of woody evergreens must be appropriately timed to take advantage of the typically favorable periods of spring and early autumn. Their phenophase progression must proceed through the drought-prone summer period and reach completion before late autumn and winter cold events, with photosynthetic capacity maintained throughout the year. During the past few decades, climate warming and increased drought frequency have caused major shifts in phenophase timing in Mediterranean forest ecosystems (Peñuelas *et al.*, 2002, 2004; Gordo & Sanz, 2009). Owing to the influence of temperature on the timing of phenophase events (Spano *et al.*, 1999), leaves of Mediterranean plants unfold 16 days earlier and fall at an average of 13 days later than in the middle of the 20th century (Peñuelas *et al.*, 2002). Similarly, flowering occurs 6 days earlier, and the timing of fruiting has accelerated by 9 days in only a quarter century.

While warming temperatures can extend the period of maximum carbon uptake, increased drought severity and supraoptimal summer temperature can negatively impact phenophase progression (Peñuelas *et al.*, 2004). The evergreen holm oak *Q. ilex* is intermediate in terms of summer drought tolerance between winter deciduous oaks, such as *Q. pubescens* and *Q. faginea* (Damesin & Rambal, 1995; Montserrat-Martí *et al.*, 2009), and highly drought-tolerant trees such as *Phillyrea latifolia* (Ogaya & Penuelas, 2004). A partial throughfall exclusion experiment had no significant impact on phenophase timing and progression in a *Q. ilex*-dominated forest of northeast Spain (Ogaya & Penuelas, 2004). The lack of drought impacts observed on phenological changes may be related to the fact that maximum relative growth rate of *Q. ilex* occurs in the spring, so that 90% of leaf expansion is often complete before the period of most severe drought stress in July and August (Gratani, 1996). However, it is also possible that the lack of impact was due to the moderate drought severity produced by the below-canopy troughs, which reduced available top soil water content by only 15% relative to a

control plot. To date, no study has examined the phenological impacts of extreme droughts, as expected to occur as a consequence of global climate change later this century (Sheffield & Wood, 2008).

Extreme climatic events are episodes in which the acclimatory capacity of an organism or population is substantially crossed (Gutschick & BassiriRad, 2003), triggering nonlinearities of organismal response to abiotic factors (Hamerlynck *et al.*, 2000). Such events exceed the resilience capacity of forest ecosystems, i.e. their ability to return to their former dynamic state (Turner *et al.*, 2003; Lloret *et al.*, 2004; Breda *et al.*, 2006; Granier *et al.*, 2007; Breda & Badeau, 2008). Despite the importance of extreme events on the physiology, ecology and evolution of plants, there still exists critical gaps in our understanding of their effects on vegetation because unambiguously interpretable data are severely missing (Gutschick & BassiriRad, 2003). This situation is due to the difficulty to study *in vivo* time responses because extreme events have large stochastic components and are rare by definition. Often, several abiotic and biotic factors are entangled, which complicate the interpretation of their individual effects (Breda & Badeau, 2008). Effects of extreme events have been more studied for grassland, tundra and shrubland than forests, but they are likely to be different for these different ecosystems (Coret *et al.*, 2005). In consequence, models are unable to simulate and predict the carbon cycle and sustainability of forests under a more extreme climate because they have been developed and parameterized with data under normal conditions (Vetter *et al.*, 2008).

In this study, a mobile rainfall exclusion system was used to simulate extremely severe July–December (Late drought or LD) and February–July (Early drought or ED) droughts in a *Q. ilex* forest. We hypothesized that severe drought would reduce phenophase progression in both treatments, but that ED would have a greater effect than LD because of the co-occurrence of progressive drought intensity with (1) increasing atmospheric demand, (2) the most active period of leaf development and shoot elongation and (3) the critical early phenophases, as defined by Dumerle & Mazet (1983). We further anticipated that female flower and fruit development would be more severely impacted by extreme drought than male floral development. On the basis of our results, we then discuss potential implications of projected climate changes on forest ecosystem structure and function.

Material and methods

Experimental site

The study site is located in the Puéchabon Experimental Forest on a flat plateau, 35 km north-west of Montpellier in southern

France ($3^{\circ}35'45''E$, $43^{\circ}44'29''N$; 270 m asl) (Rambal *et al.*, 2003, 2004; Allard *et al.*, 2008). The forest has been managed as a coppice for centuries and was last clear-cut in 1942. Vegetation is dominated by a dense overstorey of the evergreen *Q. ilex* L. In this tree species, the development of both male and female inflorescences is asynchronous from April to May. Further, acorns grow until September, mature and drop in December (Floret *et al.*, 1989; Siscart *et al.*, 1999).

Understorey coverage is <25% and consists mainly of *Buxus sempervirens*, *Phillyrea latifolia*, *Pistacia terebinthus* and *Juniperus oxycedrus*. In 2005, the canopy height was about 5.5 m (mean is 4.1 m), the density of the resprouted stems was 8170 stems ha^{-1} , the mean diameter at breast height was 7 cm and the leaf area index was $2.8 m^2 m^{-2}$. The area has a Mediterranean-type climate, with 80% of rainfall occurring between September and April. The mean annual precipitation is 908 mm, with a range of 556–1549 mm recorded during the past 22 years. The mean annual temperature over the same period was $13.5^{\circ}C$. Parent material at the site is Jurassic limestone bedrock, which is overlaid by very shallow and homogeneous silt clay loam soil (35% silt and 39% clay). The mean volumetric fractional content of stones is about 0.75 for the top 0–50 cm and 0.90 below. Most of the perennial root system can be found in the first 50 cm, but root water uptake has been observed to a depth of 4.5 m (S. Rambal, unpublished results).

Rainfall exclusion experiment

In 2007, a rainfall exclusion experiment was established on the site and included three plots of $195 m^2$ each ($15 m \times 13 m$) (Table 1). The experiment was designed to exclude 100% of rainfall on two forest plots at different periods of the year, without changes in other meteorological variables such as incident radiation, temperature and vapor pressure deficit. Major constraints for the building of the rainfall shelter were: (1) the 6 m height of the trees, (2) the need to apply the treatments on plots as large as possible, and (3) the high probability of instantaneous wind speed in excess of $30 m s^{-1}$, especially from the north. Two plots were subjected to rainfall exclusion and one plot was used as a control (C). Rainfall exclusion was achieved using a mobile, $15 m \times 13 m$ rainfall shelter, sliding above the canopy on 60 m long rails (Fig. S1). The two exclusion plots were situated at opposite

ends of the rails, and the middle section served as a parking position for the shelter in the absence of rain. The control plot was situated 20 m to the east of the exclusion plots.

The framework of the shelter was made of galvanized steel. This was covered by a transparent greenhouse PVC sheet, flexible enough to absorb kinetic wind forces, but strong enough to resist high winds. The shelter rested on wheels over the rails. Wheels included a special antitearing system that fixed the shelter to the rails in case of high winds. The rails were laid just above the canopy, on 40 adjustable galvanized steel scaffolds, fixed to the ground by cables. The rainfall shelter automatically moved from the parking position to the exclusion plot when it rained. A rain gauge with a tipping bucket of 0.25 mm resolution was installed above the canopy and was connected to a datalogger that drive a generator at the first tip recorded. After 1.5 min of preheating, the generator powered two coupled 400 W engines, mounted on each side of the rainfall shelter. The time required for the shelter to move from the parking position to the exclusion plot after the first tip was 5 min, at a speed of $3 m min^{-1}$. In order to increase exclusion efficiency and avoid repeated movement of the shelter, it remained over the exclusion plots for 4 h after the last tip of the tipping bucket. Vertical plexiglass panels were installed on the side of the rainfall shelter, to prevent lateral penetration of rain and to redirect intercepted rain to gutters that evacuated the water 15 m from the plot.

Two rainfall exclusion treatments were carried out. In the first treatment, referred to LD, we simulated an extreme autumn–winter drought, with no rainfall for a 6-month period (July–December 2008). In the second treatment, referred to ED, we simulated an extreme winter–spring drought, with no rainfall for the 6-month period between February and July 2009. The two treatments were distinct from one another, not only because they occurred at different stages of the leaf-life cycle, but also because soil water shortage becomes more severe as atmospheric demand decreases in the autumn exclusion treatment, whereas soil water storage intensifies as atmospheric demand increases in the spring exclusion treatment.

Tree sampling and measurements

A total of 324 growth flushes from 164 branches of 18 trees were monitored at 2–4 week intervals in 2007, 2008 and 2009. Six trees were sampled in C, ED and LD. To account for any within-tree variability (Salmon, 2004), three branches per tree and two growth flushes per branch were randomly sampled and monitored regularly. The sampled trees chosen for phenophase monitoring were located at least 4 m from the boundary of the plot, because our root excavations revealed that lateral roots of *Q. ilex* can extend outward to this distance. The canopy and upper branches of the sampled trees were accessed using a $1 m \times 5 m$ horizontal platform mounted on scaffolds 1.5 m below the top of the trees. On each tree, phenological phases of leaf, male flower or catkin and female flower were monitored according to the Dumerle & Mazet (1983).

Seven phenophases were defined for leaves (Fig. S2): phase 1 is defined as the period of completely closed buds (winter stage), phase 2 corresponds to bud swelling, phase 3 indicates budburst, phase 4 refers to the shoot lengthening period,

Table 1 Structural variables of the three experimental plots

	Control	Autumn drought	Spring drought
Number of trees (ha^{-1})	8125	7641	8744
Diameter at breast height \pm SD (cm)	6.1 ± 3.1	6.4 ± 3.4	6.0 ± 3.2
Basal area ($m^2 ha^{-1}$)	29.8	31.7	31.5
Mean tree height \pm SD (m)	4.0 ± 0.9	4.1 ± 1.0	4.2 ± 1.0
Leaf area index \pm SD ($m^2 m^{-2}$)	2.5 ± 0.22	2.9 ± 0.51	2.6 ± 0.25

phase 5 indicates the opening of new leaves, phase 6 describes the period when leaves are fully open but not fully mature and phase 7 indicates that leaves are fully developed and mature. For female flowers, four phenophases were defined (Fig. S3): phase 1 corresponds to the emergence of the flower bud, phase 2 refers to the flower development period, phase 3 indicates that the flower is pollinated and that the fruit has appeared and phase 4 signals that the fruit has fully matured. Four phenophases were defined for male flowers (Fig. S4): phase 1 corresponds to the emergence of the flower buds, phase 2 is the period of flower development, phase 3 indicates that the flower has matured and phase 4 corresponds to an inactive and drying flower. In the three plots C, ED and LD, we followed the abortion rate by counting the number of acorn on the branches monitored for the phenophase. The sound acorn fraction is also derived.

Predawn leaf water potential was monitored on the same trees at 2–6 week intervals with a pressure chamber (PMS 1000, PMS Instruments, Corvallis, OR, USA).

Environmental measurements

In each plot, soil water content was measured every 5 min in the 0–6 cm soil layer, using three ML2X ThetaProbe Soil Moisture Sensors (Delta-T Devices Ltd., Cambridge, UK) and in the 0–30 cm soil layer, using six CS616-L Water Content Reflectometers (Campbell Scientific, Logan, UT, USA). Soil temperature was measured every 15 min at 0–4 cm, using five thermocouples in each plot. All the soil sensors were located at least 4 m from the border of the plots, to leave a buffer zone where no measurements were performed. Canopy air temperature was measured at a height of 4 m, at the centre of each plot, using thermocouples housed in radiation shields. An automatic rain gauge was installed above the canopy in an unsheltered area, whereas another was installed just above the canopy, at the far end of the shelter exclusion zone. Data from these two rain gauges facilitated calculation of the maximum amount of rainfall input to the exclusion plots. All automatic measurements started at the beginning of 2008. To monitor rainfall entry into the exclusion plots in 2008 and 2009, 80 small manual rain gauges (cylinder of 10 cm height × 8 cm diameter) were placed on soil surface, forming eight transects along the four sides of both exclusion plots.

Experimental design and statistics

To assess treatment effects, we used a Before-After Control-Impact design (BACI) (Stewart-Oaten *et al.*, 1986; Stewart-Oaten & Bence, 2001). The BACI design requires data arranged into two plot pairs. In our case, these included control and exclusion plot pairs, collected both before (2007) and during the 2008 and 2009 treatments (Smith *et al.*, 1993; Carpenter *et al.*, 1996; Cottingham & Carpenter, 1998; Smith, 2002). Since phenological phases were repeatedly recorded on the same trees, a repeated measures analysis of variance test was used, as follows (Green, 1993):

$$\begin{aligned} Y_{ijkl} = & \mu + BA_i + \text{TIMES(BA)}_{ij} + CI_k + \text{TREE(CI)}_{kl} + (\text{BA} \times CI)_{ik} \\ & + [\text{CI} \times \text{TIMES(BA)}]_{ijk} + [\text{BA} \times \text{TREE(CI)}]_{ikl} + \varepsilon_{ijkl} \end{aligned} \quad (1)$$

where Y_{ijkl} is the mean response in the different periods, times, locations and trees, μ is the overall mean, BA_i is the effect of the period (i = before or after), TIMES(BA)_{ij} represents the effect of times within period ($j = 1, 2, \dots, t$), CI_k is the effect of location (k = control or impact), TREE(CI)_{kl} represents the effect of trees within location ($l = 1, 2, \dots, 6$), $(\text{BA} \times CI)_{ik}$ is the interaction between period and location, $[\text{CI} \times \text{TIMES(BA)}]_{ijk}$ is the interaction between times and location, $[\text{BA} \times \text{TREE(CI)}]_{ikl}$ is the interaction between trees and period, and ε_{ijkl} represents the remaining error. The test on the $(\text{BA} \times CI)_{ik}$ interaction is the key because it tells if differences between impact and control locations changed between the before and after periods (Smith, 2002). The test on the interaction of $[\text{CI} \times \text{TIMES(BA)}]_{ijk}$ is also important because this indicates whether the differences observed between control and impact locations changed within times in the before and after periods (Smith, 2002). Only results from these two tests are shown below.

Return periods

The return periods for the two extreme drought events simulated in this experiment were calculated, using a monthly 233-year precipitation historical dataset we compiled and criticized for Montpellier downtown using different historical data sources recorded over 1762–2008. This dataset was scaled to our experimental site, using overlapping precipitation data from 1984 to 2008. As shown by Rambal & Debussche (1995) and López-Moreno *et al.* (2009), the coefficient of variation for precipitation is regionally conserved and was used to fit theoretical Gaussian distribution functions for extreme precipitation events at our site. Return periods were calculated as $1/p$, where p is the probability of occurrence of the two extreme events simulated in this experiment (Rambal & Debussche, 1995).

Results

Micrometeorology

In 2008, 97% of rainfall was excluded from LD plot from July to December 2008 (559 of 575 mm), representing 50% of the 1127 mm of rain that fell during the year. Although there were no major failures of the rainfall shelter in 2008, 0.25–0.75 mm of rain was usually not excluded by the time the shelter covered the treatment plot. These amounts generally have no effect on top soil water content, because canopy storage capacity before throughfall could occur is 2.6 mm at the site (Limousin *et al.*, 2008). However, up to 4.25 mm of rainfall was not excluded during the most intense rainfall events, as occurred at the end of August 2008.

In 2009, 87% of rainfall was excluded from ED plot from February to July (435 of 491 mm), representing 58% of the 749 mm that fell during the year. The lesser exclusion was the result of two power failures that delayed the movement of the roof. 18.5 mm of rain

was not excluded on April 2–3, and 8.5 mm of rain was not excluded on June 8. Most of the time, rain did not enter the plot laterally and we found a trivial amount of water in the small rain gauges located on the soil surface. However, when rain intensity and wind speed were very strong, like at the beginning of November 2008, lateral entry was more important. In this case, we observed water in the rain gauges up to 2 m inside the plot.

The PVC cover of the rainfall shelter modified the incoming radiation (Table 2). During sunny conditions, 24% of direct light was intercepted by the cover. Under cloudy skies, 20% of direct and diffuse radiation was intercepted. However, the rainfall shelter was over the

Table 2 Photosynthetically active radiation (PAR) above and below the mobile rainfall shelter, under contrasting sky conditions

Meteorological conditions	Radiation type	PAR above ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	PAR under ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Difference (%)
Sunny conditions	Direct light	1930	1470	24
	Diffuse light	150	140	7
Cloudy conditions	Direct light	500	400	20
	Diffuse light	400	320	20

exclusion plot only 4% of the time on an annual basis, and 8% of the time during the 6-month treatments. Furthermore, incoming radiation was low whereas the shelter was over the plot during rainfall events. As a result, the shelter intercepted only 0.5% of total incoming radiation annually and 1% during the two treatment periods. The shelter had no significant effect on the mean diurnal cycle of air temperature measured inside the canopy during dry or rainy conditions, either before or during the application of the spring exclusion treatment in 2009 (Fig. 1). Similarly, no differences in soil, trunk or leaf temperature were observed between the plots (not shown).

Soil water content in the top 0–6 cm soil layer was similar in C, LD and ED during the winter and spring of 2008, before LD treatment (Fig. 2). It decreased in the three plots during a dry period near the end of June. Small summer rainfall events in July, August and September 2008 increased water content in the control and spring exclusion plots, but soil moisture continued to decrease in the autumn exclusion plot. Although substantial rainfall normally occurs during autumn in southern France, the months of September and October were unusually dry in 2008. Consequently, minimum soil water content was similar in C, LD and ED at the end of October 2008. Soils of C and ED were filled to

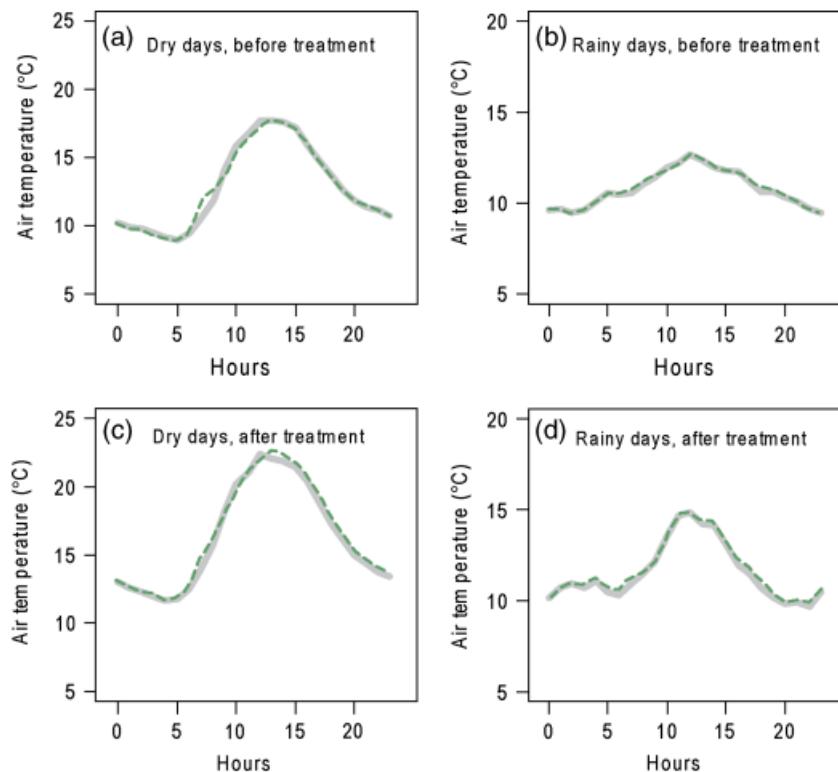


Fig. 1 Mean diurnal variation of air temperature in the control and the spring exclusion treatment in 2009: time course are shown for dry (a, c) and rainy days (b, d), before (a, b) and after (c, d) the start of the exclusion (control, solid line, grey, spring exclusion: dotted line, green).

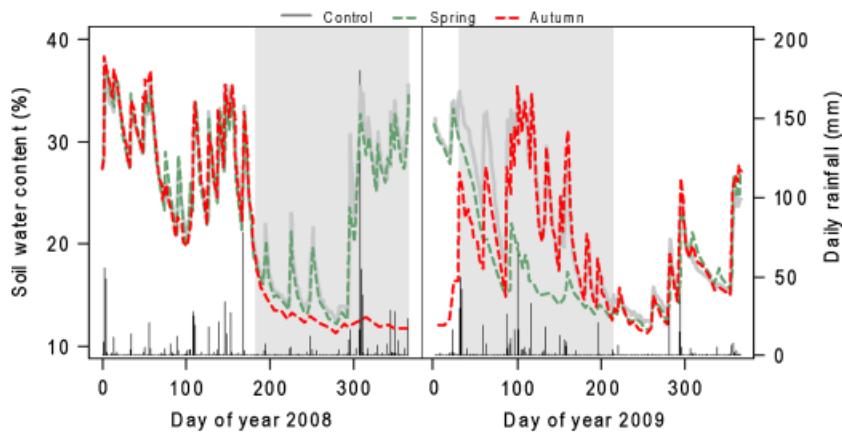


Fig. 2 Seasonal variation of topsoil water content at 0–6 cm (lines, *y*-right axis) in the different treatments, and daily precipitation (mm) (vertical bars, *y*-right axis). The shaded areas show the timing of the autumn exclusion (2008) and of the spring exclusion (2009).

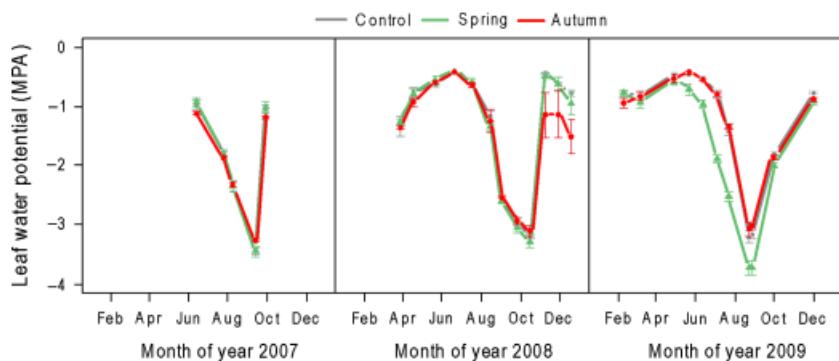


Fig. 3 Seasonal variations of predawn leaf water potential in the different treatments (control: square, solid line, grey; autumn exclusion: circle, solid line, red; spring exclusion: triangle, solid line, green). Standard errors between trees ($n = 5$) are shown.

field capacity by 290 mm of rain from November 1 to 5, but these events did not affect topsoil water content in autumn exclusion plot.

Soil moisture was similar in C and ED early February 2009 (Fig. 2). A 120 mm rainfall event from February 1 to 3 also caused soil moisture to increase again in LD. Soil water content responded to rainfall in C and LD during 2009, but decreased continuously ED, except during two power failures. Minimum soil water content was similar in the three plots in September 2009, and periodic rainfall filled the topsoil layer of all three plots by mid-October.

Leaf water potential

Predawn leaf water potential was similar in C, LD and ED in 2007, before the application of the treatments (Fig. 3). Leaf water potential decreased to values below -1.0 MPa by mid-August 2008 in all three plots. Between-plot differences did not develop until the heavy rainfall event of early November increased leaf water potential to a greater extent in the C and ED than in LD.

After this event, predawn leaf water potential was -0.4 MPa in C and -1.2 MPa in LD. Standard errors between trees increased after this event in LD because one tree did not recover at all. In 2009, substantial differences developed between plots by mid-May and were accentuated on each measurement date until July, when predawn water potential was -1.4 MPa in the control plot, but reached -2.5 MPa in ED. The minimum predawn water potential occurred in August 2009, reaching -3.2 MPa in the C and -3.7 MPa in ED.

Leaf and flower phenology

Leaves reaching phenophase 4 or higher were considered functionally mature (Dumerle & Mazet, 1983). At the end of summer 2007 and 2008, all sampled trees reached this phase (Fig. 4). In 2009, all trees in C reached phenophase 4, but three trees in ED plot failed to reach this phase. One of these trees reached bud-burst (phenophase 3) and the other two only showed signs of bud swelling (phenophase 2). Consequently, the seasonal evolution of average leaf phenophase was

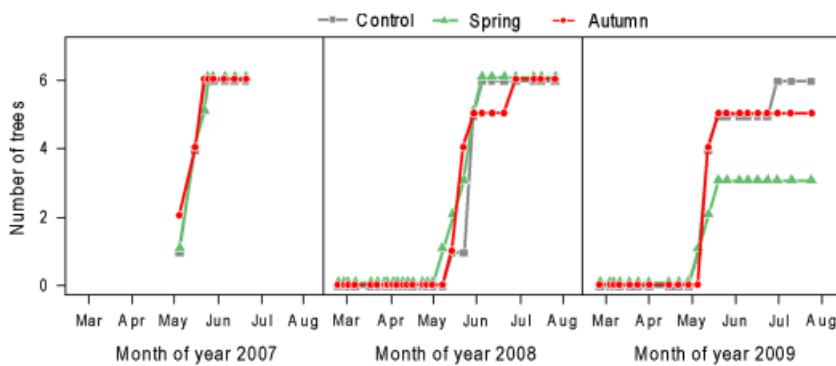


Fig. 4 Number of trees per treatment with leaf phenological stage higher than four (mature leaf), out of a total of six trees per treatment and six branches per tree (control: square, solid line, grey; autumn exclusion: circle, solid line, red; spring exclusion: triangle, solid line, green).

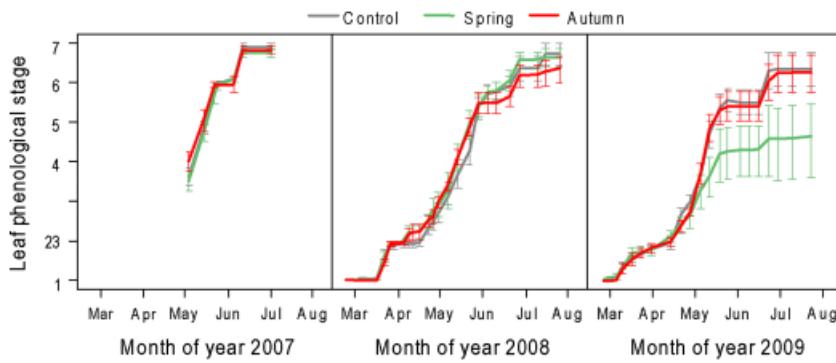


Fig. 5 Mean leaf phenological stage per treatment. Error bars indicate the standard errors (control: solid grey line; autumn exclusion: red solid line; spring exclusion: green solid line).

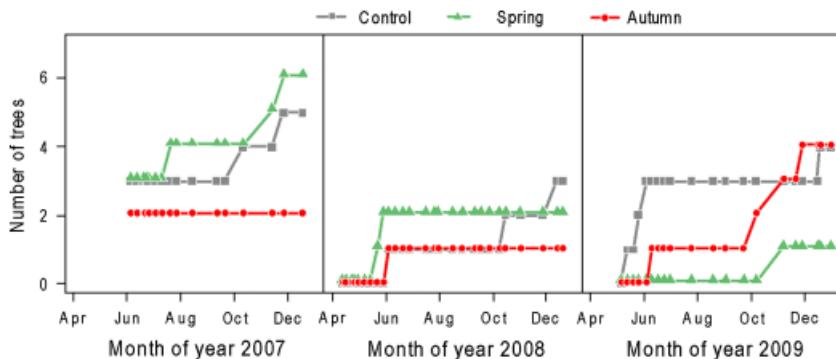


Fig. 6 Number of trees per treatment with female flower phenological stage higher than two (mature fruit), out of a total of six trees per treatment and six branches per tree (control: square, solid line, grey; autumn exclusion: circle, solid line, red; spring exclusion: triangle, solid line, green).

similar in all three plots during 2007 and 2008 (Fig. 5). By contrast, mean leaf phenophase was much lower in ED than in the other two plots starting in mid-May 2009, and this difference grew larger over time (Fig. 5). The $BA \times CI$ interaction showed that differences in leaf phenophase between C and LD plots were similar before and after the start of the treatment in 2008 (Table 4). However, C vs. ED phenophase differences changed significantly before and after the start of the spring

exclusion treatment in 2009 (Table 4). The $CI \times TIMES(BA)$ interaction term also revealed that within-time differences between C and ED plots changed significantly before and after the start of the treatment in 2009 (Table 4).

The number of trees with maturing fruit (female flower phenophase higher than 2) was consistently lower in LD than in C plot, except in late 2009 (Fig. 6). Before the spring exclusion was initiated, the number of

trees with female flower phenophase higher than 2 in ED was six in 2007 and two in 2008. After the 2009 ED exclusion was initiated, only one tree showed fruit development and maturation in ED, whereas four trees reached this phenophase in the other two plots (Fig. 6). The average number of mature fruit per sampled branch was always lower in LD than in C (Table 3). The number of fruit in ED was higher than in C in 2007 and 2008, but lower in 2009, following the initiation of the spring rainfall exclusion (Table 3). The seasonal evolution of average female flower phenophase was similar in all treatments before 2009 and much lower

in ED after the middle of May 2009 (Fig. 7). The $BA \times CI$ and $CI \times TIMES(BA)$ interactions were only significant for ED in 2009 (Table 4).

The number of trees with maturing male flowers (phenophase higher than 2) at the beginning of July was similar in all plots in 2007 (Fig. 8). Fewer trees flowered in the control plot in 2008, and flowering was generally very low in 2009 in all plots. The seasonal evolution of average male flower phenophase was similar in all plots from 2007 to 2009 (Fig. 9). However, because the trees flowered similarly in all plots in 2007, the $BA \times CI$ interaction was significant in the spring exclusion plot in 2008, due to less flowering in the control treatment (Table 4). Other differences were not significant (Table 4).

The sound acorn fractions calculated on the bearing branches were difficult to interpret. Previously the rain exclusion and pooling in the E C, ED and LT plots, we observed in 2007 that the sound acorn fraction is 0.13 ± 0.27 . This fraction is affected by very large between-branch variation. This fraction was similar for

Table 3 Number of fruit per branch \pm SE in the three experimental plots

Year	Control plot	Autumn exclusion	Spring exclusion
2007	2.08 ± 0.49	0.92 ± 0.35	2.17 ± 0.57
2008	0.33 ± 0.21	0.03 ± 0.03	0.64 ± 0.27
2009	0.75 ± 0.27	0.47 ± 0.13	0.36 ± 0.16

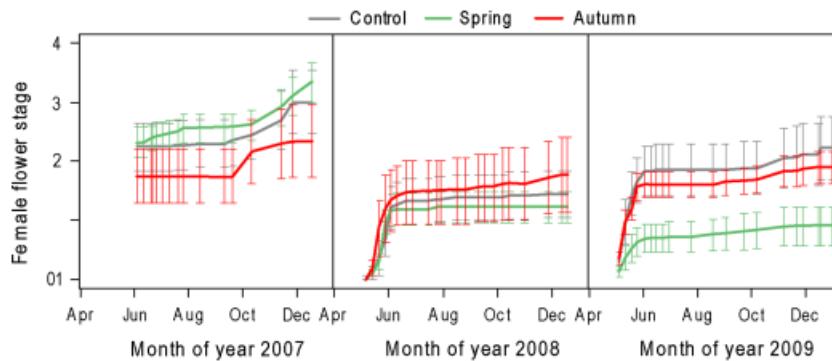


Fig. 7 Mean female flower phenological stage per treatment. Error bars indicate standard errors (control: solid grey line; autumn exclusion: red solid line; spring exclusion: green solid line).

Table 4 Before-After Control-Impact (BACI) design ANOVA results and P -values for control vs. treatment differences in leaf, female flower and male flower phenophase scores

Variable	Year	Treatment	Effect	P
Leaf phenophase	2008	Autumn exclusion	$BA \times CI$	0.49
	2009	Spring exclusion	$CI \times TIMES(BA)$	0.42
Female flower phenophase	2008	Autumn exclusion	$BA \times CI$	<0.01
	2009	Spring exclusion	$CI \times TIMES(BA)$	<0.001
Male flower phenophase	2008	Autumn exclusion	$BA \times CI$	0.70
	2009	Spring exclusion	$BA \times CI$	0.99

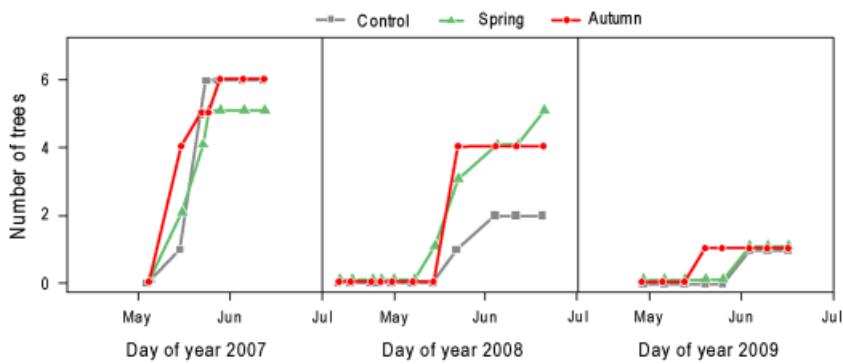


Fig. 8 Number of trees per treatment with male flower phenological stage higher than two (mature flower), out of a total of six trees per treatment and six branches per tree (control: square, solid line, grey; autumn exclusion: circle, solid line, red; spring exclusion: triangle, solid line, green).

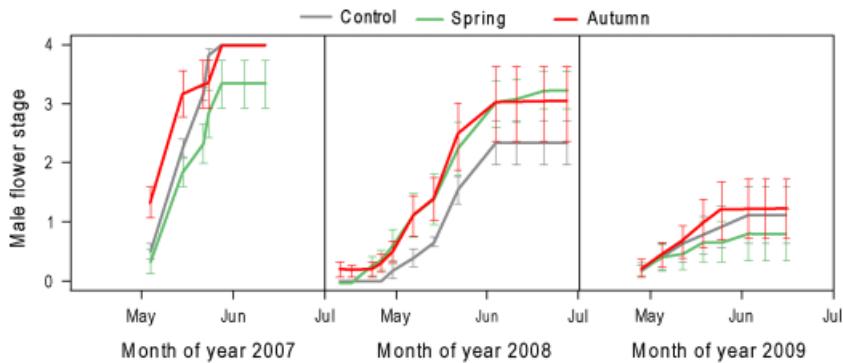


Fig. 9 Mean male flower phenological stage per treatment. Error bars indicate the standard errors (control: solid grey line; autumn exclusion: red solid line; spring exclusion: green solid line).

2008 for the two plots not affected by the rain exclusion C and LT, 0.12 ± 0.28 . In ED, this fraction reached 0.25 ± 0.5 . The last year in 2009 we observed values of 0.22 ± 0.39 and 0.16 ± 0.39 , respectively, for C and LD. This ratio largely declined to 0.05 ± 0.16 in ED.

Discussion

The goal of our rainfall exclusion was to exclude 100% of rainfall, without changing other meteorological variables such as incident radiation and temperature. This procedure appeared more efficient than other exclusion exporting a fixed ratio of throughfall (Borghetti *et al.*, 1998; Irvine *et al.*, 1998; Hanson *et al.*, 2001; Cinnirella *et al.*, 2002; Nepstad *et al.*, 2002; Hanson & O'Hara, 2003a; Martinez-Vilalta *et al.*, 2003; Fisher *et al.*, 2007; Limousin *et al.*, 2009). This was clearly demonstrated in Fig. 2, in which differences in topsoil water content between treatments are shown to be greatest during periods of high rainfall intensity and frequency (e.g. November 2008 and April 2009). Differences were less important when rainfall was infrequent (e.g. July 2008 and July 2009; Fig. 2).

LD treatment had a smaller impact on predawn water potential and phenology than the spring exclusion treatment, even though 144 mm more water was excluded (559 vs. 415 mm). This observation supports the hypothesis that ED exclusion would have a greater impact on phenophase progression than LD, because spring is the most important period of phenological change, and because increasing soil water limitation coincides with increasing atmospheric dryness in spring. Leaf, female flower, fruit development and maturation were strongly affected by this treatment, likely because predawn leaf water potential started to decrease in mid-May, coincident with the first appearance of new leaves and female flowers (Figs 3, 5, 7). ED had much less impact on phenophase progression of male flowers. Catkins develop earlier than leaves and female flowers. As a result, male floral development processes were unaffected by the strong decrease in water potential that occurred in late spring and early summer. In addition, male flowering was generally less abundant in 2009 than in 2007 and 2008.

Only a few other researchers have studied the effects of drought on leaf phenology and development in

ligneous species of the Mediterranean region. Based on a 50-year record in Spain, Peñuelas *et al.* (2004) showed that precipitation was significantly correlated with the timing of leaf development in several species. However, these rainfall responses were strongly species-specific. Most species decreased leaf unfolding after several months of decreased precipitation, but the influence of precipitation was weaker than that of temperature (Peñuelas *et al.*, 2002). Phenological responses are complex because they are controlled by a synergistic interaction of a number of factors including temperature, photoperiod, water availability and carbon reserves (Orshan, 1989; Myking & Heide, 1995; Cherbuy *et al.*, 2001; Sanz-Perez *et al.*, 2009; Körner & Basler, 2010). For chaparral and Chilean matorral Mediterranean species, Kummerow *et al.* (1981) wrote that 'warming hours and a soil moisture threshold value in late fall are the most useful tools to predict initiation of the two key phenological events, i.e. onset of stem elongation and flower initiation'. Some species exhibit distinct interannual cycles of phenophase progression, because of source-sink interactions between internal processes such as growth and reproduction (Montserrat-Martí *et al.*, 2009). In this context, historical studies are often limited because influencing factors are cross-correlated and difficult to separate.

Manipulation experiments provide a tool to isolate the effects of water limitation, but spatial variability between plots and autocorrelation of phenological response through time need to be addressed to provide unambiguous results. Hanson *et al.* (2003b) showed that 33% throughfall exclusion had no discernable effects on leaf emergence and canopy development in a temperate mixed deciduous forest. Results from Ogaya & Penuelas (2006) in a Mediterranean shrubland showed that a 15% experimental decrease in top soil water content slightly delayed ontogeny of sun-exposed leaves of *Q. ilex*. Our results showed a much stronger effect, with half of the measured trees exhibiting no leaf unfolding after the spring rain exclusion, probably because this exclusion was much stronger than the one applied by Ogaya & Penuelas (2006). In addition, this treatment allowed us to advance the start of the drought-stress period in the rain exclusion plot, so that water restriction coincided with the onset of leaf development. Castro-Diez & Montserrat-Martí (1998) showed that *Q. ilex* is part of a phenological group represented by species with short spring phenophases, thereby protecting young tissues from exposure to summer drought and winter frost. Such phenophase – overlappers grow and flower when resources are abundant and maintain low phenological activity during the rest of the year (Milla *et al.*, 2004). However, in case of extreme spring drought simulated in our experiment in 2009, leaf

development was prevented by the decline in cell expansion imposed by drought during leaf growth, the time of greatest leaf sensitivity to resource availability (Dale, 1988). He wrote 'leaf expansion is the developmental behavior that is the most vulnerable to inhibition by water deficit'. Van Volkenburgh (1999) further relayed in quoting 'Water deficit inhibits growth by some more complicated regulating mechanisms of which we know very little'. The reduction of leaf phenological development in response to severe water shortage may be part of a conservative strategy to limit leaf area index and use of water for transpiration (see Limousin *et al.*, 2009 for a substantial account).

Phenological processes such as flowering and fruiting have attracted a lot of recent interest, because of their implications for interspecific competitive relationships influencing ecosystem structure and functions. Peñuelas *et al.* (2004) showed that the response of flowering and fruiting to precipitations was species-dependent, but most species delayed flowering and fruit maturation during dry years. In the case of fruiting, the relative influence of precipitation can be stronger than that of temperature (Peñuelas *et al.*, 2002, 2004), though Espelta *et al.* (2008) showed that the acorn production in some Mediterranean oak species was negatively correlated with summer water stress. Montserrat-Martí *et al.* (2009) studied summer drought effects on the phenology of *Quercus* species during 2 years with contrasting water conditions and showed delayed flower bud formation, flowering, fruit setting and lower acorn dry mass for *Q. ilex* during the drier years. In Mediterranean *Quercus* species, acorn production has been related to both pollen availability and weather-related factors, including water availability (Koenig *et al.*, 1994; Cecich & Sullivan, 1999; Garcia-Mozo *et al.*, 2007). Our results showed that fruit maturation was directly linked to water availability, in the presence of mature male flowers (Figs 6 and 7; Table 3). Contrarily, data from a rainfall exclusion experiment showed no significant differences in flowering and fruiting in *Q. ilex*, in response to limitations in top soil water over a 2-year period (Ogaya & Penuelas, 2004). In a study did on the same site (Perez-Ramos *et al.*, 2010), we observed that annual yield of acorns was always influenced by environmental factors related to water resource that disrupted the fruiting process at different periods of time. Sound acorn production was strongly dependent on the water availability for the plant at spring and advanced or summer stages of the maturation cycle, whereas the final step of the seed development was negatively affected by the frequency of intense rain events. The high interannual variation in acorn production was mainly determined by seed survival rather than by the flowering effort.

Table 5 Characteristic of Montpellier historical rainfall series (1762–2008) and return period for simulated droughts

	N	Mean (mm)	SD (mm)	CV (%)	Simulated droughts (mm)	Return periods (years)
Annual rainfall	232	770.4	226.2	29.4		
July–December rainfall (autumn exclusion period)	233	422.5	194.4	45.5	16	60.3
February–July rainfall (spring exclusion period)	233	299.9	116.7	38.9	56	56.0

Although the higher impact of the spring exclusion was related to the timing of important leaf phenophase progressions and the concurrence of increasing soil moisture stress and atmospheric demand, the low impact of the autumn exclusion treatment may also have been affected by a lack of rainfall in summer and early autumn 2008. Summer storms usually occur in late August and September in south-east France, replenishing soil moisture in the form of heavy rainfall. In 2008, almost no rainfall occurred in August, September and October. Cumulative precipitation during this 3-month period was the lowest recorded in the last 25 years. Rainfall exclusion was, thus, not possible before 290 mm of rain fell during the first 5 days of November. This intense rainfall was associated with strong winds, resulting in lateral rainfall entry up to 2 m inside the autumn exclusion treatment. This explains the increase in predawn leaf water potential observed in the autumn exclusion plot in November 2008. Some trees in this plot retained low predawn water potential, probably because their roots did not extend laterally enough to access this moisture source. In most water exclusion experiments, the plots are excluded from lateral water supply by a ditch (Irvine *et al.*, 1998; Cinnirella *et al.*, 2002; Fisher *et al.*, 2007), but not in all cases (Hanson *et al.*, 2001). At the Puéchabon Experimental Site, the construction of a ditch was not possible, because of the high stone content of the soils. Increasing the size of the plots would help to resolve this issue, but would be technically difficult.

How extreme were the simulated droughts? Based on continuous measurements of soil water content at the site, our 6-month rainfall exclusion treatments had stronger and longer-lasting effects on topsoil water content than any of the natural droughts recorded since 1998 (Reichstein *et al.*, 2002; Joffre *et al.*, 2003; Misson *et al.*, 2010). Plant water potential data from the 1998–2009 period shows that predawn leaf water potential usually starts to decrease under -1 MPa around the beginning of July (Rambal *et al.*, 2003; Limousin *et al.*, 2009). Trees in our spring exclusion treatment reached this threshold 1 month earlier, at the beginning of June. Only in 2006, the driest year recorded at the site since

1998 (Allard *et al.*, 2008), was this threshold reached in June. The lowest predawn water potential reached by the trees in the spring exclusion plot in 2009 was -3.7 MPa at the end of August, whereas potential down to -4.0 MPa was recorded at the site in mid-August 2006. The spring exclusion, therefore, simulated drought intensity close to that of summer 2006. Rainfall during the February–July 2006 was 64 mm, which is very similar to the amount of rainfall that was not excluded during the spring rainfall exclusion treatment in 2009 (56 mm). A long-term precipitation dataset (233 years) was used to calculate return periods for droughts of this magnitude (Table 5). The autumn exclusion treatment was found to have simulated a 60.3-year drought event (16 mm of rainfall in July–December), whereas the spring exclusion treatment simulated a drought with a 56.0-year return period (56 mm of rainfall for February–July). Model simulations indicate that drought events of these intensities may have return periods of 25–30 years by the end of the 21st century (Sheffield & Wood, 2008).

The absence of new leaves in several trees of the spring exclusion treatment will likely result in higher leaf retention and lifespan, given that new leaf production is known to be related to litterfall in *Q. ilex* (Bellot *et al.*, 1992; Bussotti *et al.*, 2003). The phenological changes resulting from increased drought frequency and severity may, therefore, reduce the amount and alter the quality of organic matter input to the soil. In addition, canopy physiology and leaf level gas-exchange will be affected by increased drought exposure, as a result of lower leaf water potential and higher mean leaf age (Niinemets *et al.*, 2005). In the context of global and regional climate change, there exists a need for further investigation of functional implications of extreme drought impacts on phenophase progression, especially in the Mediterranean region. The information provided by our experimental approach may constitute a crucial step to evaluate the impact of increasing drought due to climate change on the most dominant Mediterranean tree species and to help drawing a full picture of the ecological consequences of the decline in water resource on forest dynamics under changing conditions.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Rainfall shelter and experimental design.

Figure S2. Leaf phenophases for *Quercus ilex*.

Figure S3. Female flower phenophases for *Quercus ilex*.

Figure S4. Male flower phenophases for *Quercus ilex*.

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