Dampening effects of long-term experimental drought on growth and mortality rates of a Holm oak forest

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Abstract

Forests respond to increasing intensities and frequencies of drought by reducing growth and with higher tree mortality rates. Little is known, however, about the long-term consequences of generally drier conditions and more frequent extreme droughts. A Holm oak forest was exposed to experimental rainfall manipulation for 13 years to study the effect of increasing drought on growth and mortality of the dominant species *Quercus ilex*, *Phillyrea latifolia*, and *Arbutus unedo*. The drought treatment reduced stem growth of *A. unedo* (−66.5%) and *Q. ilex* (−17.5%), whereas *P. latifolia* remained unaffected. Higher stem mortality rates were noticeable in *Q. ilex* (+42.3%), but not in the other two species. Stem growth was a function of the drought index of early spring in the three species. Stem mortality rates depended on the drought index of winter and spring for *Q. ilex* and in spring and summer for *P. latifolia*, but showed no relation to climate in *A. unedo*. Following a long and intense drought (2005–2006), stem growth of *Q. ilex* and *P. latifolia* increased, whereas it decreased in *A. unedo*. *Q. ilex* also enhanced its survival after this period. Furthermore, the effect of drought treatment on stem growth in *Q. ilex* and *A. unedo* was attenuated as the study progressed. These results highlight the different vulnerabilities of Mediterranean species to more frequent and intense droughts, which may lead to partial species substitution and changes in forest structure and thus in carbon uptake. The response to drought, however, changed over time. Decreased intra- and interspecific competition after extreme events with high mortality, together with probable morphological and physiological acclimation to drought during the study period, may, at least in the short term, buffer forests against drier conditions. The long-term effects of drought consequently deserve more attention, because the ecosystemic responses are unlikely to be stable over time. Nontechnical summary In this study, we evaluate the effect of long-term (13 years) experimental drought on growth and mortality rates of three forest Mediterranean species, and their response to the different intensities and durations of natural drought. We provide evidence for species-specific responses to drought, what may eventually lead to a partial community shift favoring the more drought-resistant species. However, we also report a dampening of the treatment effect on the two drought-sensitive species, which may indicate a potential adaptation to drier conditions at the ecosystem or population level. These results are thus relevant to account for the stabilizing processes that would alter the initial response of ecosystem to drought through changes in plant physiology, morphology, and demography compensation.

Keywords: acclimation, *Arbutus unedo*, experimental drought, global-change-type drought, Holm oak, Mediterranean forest, *Phillyrea latifolia*, *Quercus ilex*, tree growth, tree mortality, vegetation stabilizing processes

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Introduction

Forest decline, die-off, and vegetational shifts related to drought have been documented in many forest types in recent years (Penuelas *et al.*, 2001, 2007a; Mueller *et al.*, 2005; Breshears *et al.*, 2009; Allen *et al.*, 2010; Anderegg *et al.*, 2012; Huang & Anderegg, 2012). Although drought is a recurrent disturbance in Mediterranean forests, an increase in intensity and frequency would likely lead to reduced growth and crown condition and to higher mortality rates in these ecosystems (Ogaya *et al.*, 2003; Ogaya & Penuelas, 2007b; Carnicer *et al.*,

2011; Sarris *et al.*, 2011; Galiano *et al.*, 2012). In Mediterranean regions, General Circulation Models (GCMs) project an average decrease of 15% in soil moisture over the next 50 years and a return period of extreme droughts 10 times shorter than in the twentieth century (Bates *et al.*, 2008). The frequency of heat waves is also expected to increase in the coming years around the Mediterranean area, which would increase evapotranspiration even more during the dry season in this region (Fischer & Schar, 2010).

Experimental manipulations of precipitation are useful for anticipating the future impacts of global climatic scenarios on vegetation (Wu *et al.*, 2011). These experiments, involving long-term treatment and monitoring, are especially needed because the effect size of

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treatments on ecosystems would not be constant over time (Leuzinger *et al.*, 2011). Several systems of experimental drought have been established in forests across different climatic zones and have demonstrated a general sensitivity of ecosystems to drought. The observed effects included decreased carbon uptake, productivity, and aboveground biomass (Wu *et al.*, 2011). For example, drought decreased growth, increased tree mortality, and altered carbon cycling after midterm experimental drought in Mediterranean and Amazonian forests (Ogaya & Penuelas, 2007b; Brando *et al.*, 2008; Da Costa *et al.*, 2010) and modified physiological activity, morphological traits, and recruitment patterns of Mediterranean woody species (Lloret *et al.*, 2004a; Ogaya & Penuelas, 2006; Penuelas *et al.*, 2007b; Limousin *et al.*, 2009, 2010, 2012; Ogaya *et al.*, 2011).

As many studies have reported, short-term experimental drought is enough to induce relatively rapid morphological and functional changes in forests, such as increased mortality of fine roots in a boreal forest after a 6 week treatment (Gaul *et al.*, 2008) or the acceleration of CO_2 fluxes to the atmosphere in tropical rainforests after a 12 month treatment (Cleveland *et al.*, 2010). Nonetheless, information on the temporal stability of these drought-induced alterations is lacking, because very few experiments have been maintained for more than 7 or 8 years. In a meta-analysis of global-change experiments, Leuzinger *et al.* (2011) provided evidence of a dampened effect size of treatments (i.e., warming, nitrogen fertilization, or drought) over time, which may be attributable either to the aforementioned modified plant physiology or morphology (phenotypic plasticity) or even to genetic adaptation (Jump *et al.*, 2006). As recently proposed by Lloret *et al.* (2012), demographic stabilizing mechanisms may also be counteracting or minimizing the effects of extreme events and changing climatic trends on vegetation. As suggested by the theoretical framework of Lloret *et al.* (2012), the unprecedented mortality rates expected under drought treatment would subsequently be compensated by a higher survival of the remnant population, because a lower stem or individual density would consequently lower intra- and interspecific competition for water resources. Monitoring experimental drought systems as long as possible is thus indeed desirable to assess these stabilizing processes.

Holm oak (*Quercus ilex* L.) is a widespread sclerophyllous tree that dominates forests distributed in the Mediterranean basin. It is usually accompanied by other Mediterranean woody species with high (mock privet, *Phillyrea latifolia* L.) or low (strawberry tree, *Arbutus unedo* L.) resistance to drought (Penuelas *et al.*, 1998, 2000, 2001; Ogaya & Penuelas, 2003, 2006, 2007a, b; Ogaya *et al.*, 2003, 2011; Asensio *et al.*, 2007).

Experimental drought reduces transpiration and foliar area in *Q. ilex* while increasing foliar mass per area and vulnerability to embolism (Ogaya & Penuelas, 2006; Limousin *et al.*, 2010, 2012), which would eventually drive changes in the assimilation of carbon and cause lower growth rates (Ogaya & Penuelas, 2007b). Also, *A. unedo* reduced stomatal conductance and rates of CO_2 assimilation under drought treatment (Ripullone *et al.*, 2009). Important demographic effects have already been observed in experimental systems of drought, such as a higher mortality of stems and reduced recruitment (Lloret *et al.*, 2004a; Ogaya & Penuelas, 2007b). In similar studies, *P. latifolia* responded differently when contending with drier conditions, showing no evidence of any effect from drought treatment, probably because this species better dissipates excess radiation, uses water more efficiently, and is more resistant to xylem embolism than *Q. ilex* (Penuelas *et al.*, 1998, 2000; Martinez-Vilalta *et al.*, 2002; Ogaya *et al.*, 2011). Such species-specific differences in resistance to drought would likely produce a gradual vegetational shift or a partial species substitution in these abundant Mediterranean forest communities.

An experiment of long-term drought was established in 1999 in the Prades Holm oak forest (NE Iberian Peninsula). The interannual and interseasonal climates were highly variable during the study period (1999–2012), mostly due to differences in precipitation; droughts throughout the study period varied in intensity and duration. We have therefore analyzed the relation of stem growth and mortality to different timescales of drought, which would provide clues not only about the effect of climatic trend but also the effects of extreme climatic events, as recommended for studies of global change (Jentsch *et al.*, 2007; Lloret *et al.*, 2012; Reyer *et al.*, 2013). Ecosystemic demography and production have been studied on the same experimental system 2 years (Ogaya *et al.*, 2003) and 5 years (Ogaya & Penuelas, 2007b) after the beginning of the treatment, respectively. We have referred and reasoned our findings based on these previous results and on other studies investigating the effect of drought on physiology, productivity, and demography in the same study system (Lloret *et al.*, 2004a; Ogaya & Penuelas, 2006, 2007a; Ogaya *et al.*, 2011; Barbeta *et al.*, 2012).

We aimed to evaluate: (i) the effect of manipulating precipitation on stem growth and mortality rates of the co-occurring *A. unedo*, *Q. ilex*, and *P. latifolia*; (ii) whether species-specific responses to drought observed by previous studies persisted over time in this ecosystem; (iii) the response of these variables to the different intensities and durations of natural drought; and (iv) whether the structural changes caused by extreme events and long-term experimental drought

would modify the response of the community to periods of drought. These questions should allow to gain knowledge on the effects of climatic change on Holm oak forests and to determine if stabilizing processes would dampen the future responses of forest communities to both climatic trends and events.

Materials and methods

Experimental site

The experimental site was established in 1999 at the Prades Holm oak forest in Southern Catalonia (NE Spain) (41°21′N, 1°2′E), at 930 m a.s.l. and on a south-facing slope (25% slope). The forest has a very dense multistem crown (15 433 stems ha⁻¹) dominated by *Q. ilex* (5258 stems ha⁻¹ and 93 Mg ha⁻¹), *P. latifolia* (7675 stems ha⁻¹ and 17 Mg ha⁻¹), and *A. unedo* (1100 stems ha⁻¹ and 11 Mg ha⁻¹), accompanied by other Mediterranean woody species that usually do not reach the upper canopy (e.g., *Erica arborea* L., *Juniperus oxycedrus* L., and *Cistus albidus* L.) and the occasional isolated deciduous tree (e.g., *Sorbus torminalis* L. Crantz and *Acer monspessulanum* L.). In the Prades Mountains, Holm oak forests grow throughout the altitudinal range (400–1200 m), presenting closed canopies from 3 to 10 m in height depending on site quality. This forest has been managed as a coppice for centuries, but has suffered no significant disturbance for the last 70 years (Ogaya *et al.*, 2011). Selective thinning was the most frequently used coppicing method, but increasing population pressure provoked clear-cuts during the 19th century and into the first half of the 20th century (Rodà *et al.*, 1999). This feature implies that root systems may be much older than stems, but it also impedes the differentiation of genetic individuals because clonal structures in *Q. ilex* may extend to more than 20 m (Ortego *et al.*, 2010).

The climate is typically Mediterranean. The period of this study (1999–2012) had a mean temperature of 12.2 °C and a mean annual precipitation of 610 mm. The annual and seasonal distribution of precipitation is irregular, with annual precipitation ranging from 376 to 926 mm in the 13 years of this study. Spring and autumn are the wettest seasons, and summer drought usually lasts 3 months, during which precipitation is ca. 10% of the annual total and coincides with the highest temperatures. Winters are relatively cold. January is the coldest month (4.4 °C), and the daily mean temperature is below 0 °C an average of 8 days per winter. The soil is a Dystric Cambisol over Paleozoic schist and ranges from 35 to 90 cm in depth. The climatic, edaphic, and physiographic characteristics of this site are fairly representative of Mediterranean forests, which mostly occur in mountainous areas with shallow soils that exacerbate water stress in plants.

The experimental system consisted of eight 150-m² plots delimited at the same altitude along the slope. Half the plots (randomly selected) received the drought treatment, and the other half faced natural conditions. Precipitation was partially excluded from the plots of the drought treatment by PVC strips suspended at a height of 0.5–0.8 m above the soil, covering approximately 30% of the plot surfaces. A ditch 0.8 m in depth was excavated along the entire top edge of the plots to intercept runoff water. The water intercepted by the strips and ditches was conducted outside the plots, below their bottom edges. The strips were installed below the canopy and thus did not intercept light. Litter falling on the plastic strips was regularly placed below them to ensure that differences in the contents of soil nutrients among treatments and control plots were attributable only to the availability of water for the decomposition of this litter (Ogaya & Penuelas, 2007b).

Environmental monitoring and drought index

An automatic meteorological station installed between the plots monitored temperature, photosynthetically active radiation, humidity, and precipitation every 30 min. Soil moisture was measured throughout the experiment each season by time-domain reflectometry (Tektronix 1502C, Beaverton, Oregon, USA) (Zegelin *et al.*, 1989; Gray & Spies, 1995). Three stainless steel cylindrical rods, 25 cm long, were vertically installed in the upper 25 cm of the soil at four randomly selected places in each plot. The time-domain reflectometer was manually attached to the ends of the rods for each measurement (Ogaya & Penuelas, 2007b).

The Standardized Precipitation Evapotranspiration Index (SPEI) (Vicente-Serrano *et al.*, 2010) is a multiscalar drought index that we have calculated using local meteorological data from a 35 year period. The main difference between SPEI and other drought indexes is its use of the difference between precipitation and potential evapotranspiration (P-PET; D). Precipitation is the most important factor in the variability and intensity of drought, but evapotranspiration also plays a major role as a determinant in the variability in soil moisture, a key factor in plant–water relations (Vicente-Serrano *et al.*, 2012a, b; Martin-Benito *et al.*, 2013). The inclusion of potential evapotranspiration (PET) to calculate the SPEI only affects the index when PET differs from average conditions, for example, under scenarios of global change (Vicente-Serrano *et al.*, 2010). The use of PET is especially suitable in our study area because climatic series indicate a warming trend since 1975. Even though our climatic series was not long, we correlated our 13 years of data on precipitation and temperature with the data from a station located 5.6 km northeast of our plots and at 510 m a.s.l. (linear regressions: $R^2 = 0.97$ for temperature, $R^2 = 0.75$ for precipitation, N = 48). Using this reconstructed climatic profile (1975–2011), a log-logistic distribution was used to model the values of D, and the resulting cumulative probabilities were transformed into a standardized variable. A SPEI of 0 indicates a value corresponding to 50% of the cumulative probability of D; the SPEI ranges between 3 and −3, and the lower the value, the more intense the drought. The multiscalar character of the SPEI distinguishes between short-term and long-term droughts, which would affect the vegetation at different levels. In our study, we selected timescales of 3 (SPEI-3) and 6 (SPEI-6) months, because they best fit our annual data on plant growth and population dynamics. For a more detailed description of the methods of calculation, see (Vicente-Serrano *et al.*, 2010). The different SPEI values are provided with month and timescale of calculation (i.e., May SPEI-3 refers to the water balance of March, April, and May of a given year).

Growth and mortality

All living stems of all species with a diameter larger than 2 cm at a height of 50 cm were measured each winter since 1999. With these data, we then calculated stem basal area increments (BAI). Dead stems were counted each year to obtain the mortality rate (m), calculated according to (Sheil *et al.*, 1995):

$$m = 1 - (1 - ((N_o - N_t)/N_o)^{1/t}$$

where N_o and N_t are the number of living stems at the beginning and the end of a number of years, *t*. From winter 2009, we also began to incorporate the individuals whose diameters were below 2 cm at the beginning of the study, but then reached or exceeded this size. During 2005–2006, extreme meteorological droughts affected the study area. We aimed to study the effects of this event on growth and mortality and were thus able to compare two different pre- and postdrought periods.

Statistical analyses

We evaluated the effect of drought treatment and natural droughts on stem growth and mortality rates of the three dominant species using Generalized Linear Mixed Models (GLMM) fit by Markov Chain Monte Carlo (MCMC) techniques, with the R package MCMCglmm (Hadfield, 2010). We selected this approach instead of methods based on likelihood because it is more appropriate for small sample sizes. Even though our data series was long and large, consisting of thousands of stems, our experimental design allowed us to calculate average stem BAIs per plot and year. Stem mortality rates were also calculated per plot and year by the formula proposed by (Sheil *et al.*, 1995). We constructed one model for each dependent variable at the species level;

$$A.unedo : BAI = Treatment + April\ SPEI\text{-}3$$
$$Q.ilex : BAI = Treatment + May\ SPEI\text{-}3$$
$$P.latifolia : BAI = Treatment + May\ SPEI\text{-}3$$
$$(1a)$$

$$A.unedo : Stem\ mortality\ rate = Treatment + June\ SPEI\text{-}6$$
$$Q.ilex : Stem\ mortality\ rate = Treatment + May\ SPEI\text{-}6$$
$$P.latifolia : Stem\ mortality\ rate = Treatment + September\ SPEI\text{-}6$$
$$(1b)$$

where the treatment factor (two levels, control and drought) and the SPEI calculated at different timescales and for different months were fixed independent variables, and year was the random factor to account for the temporal autocorrelation. We then constructed a model at the community level with the three species for each dependent variable (stem BAI and stem mortality rates);

$$BAI = Treatment + May\text{-}SPEI\text{-}3 * species + species * period$$
$$(2a)$$

$$Mortality\ rate = Treatment + September\ SPEI\text{-}6 * species + species * period$$
$$(2b)$$

where treatment factor (two levels, control and drought), SPEI (best fit for all species together), species and period of

the study (before or after the intense droughts of 2005 and 2006) were fixed independent variables, and year and plot were random independent variables, to account for temporal and spatial autocorrelation. We included the interactions among fixed independent variables when they improved the model fit Eqn (2)a and b. Models were selected both by the deviance information criterion and by the minimum error.

The total BAI accumulated over the 13 years was compared between species and treatment for living stems with analysis of variance (ANOVA) and Tukey HSD (honest significant difference) post hoc tests. Differences in the mean diameters of dead stems were tested with GLMMs using MCMC techniques, as with growth and mortality. We then performed the linear regressions between dead-stem diameters and the SPEI that best fit each species. We excluded *A. unedo* due to its lower abundance, which provided insufficient statistical power for this test. The growth of *Q. ilex* stems that were alive in the last sampling season and the growth of those stems that died during the experiment were also compared using GLMMs fit by MCMC techniques. Differences in environmental conditions, such as the drought index and soil moisture, between drought and control plots were tested with ANOVAS and GLMMs with time as a random factor. Similarly, we evaluated the overall and annual relative changes of basal area in relation to the drought treatment. Finally, linear regressions were used to evaluate the trend of the effect size of treatment. We transformed the data to attain normality when necessary. All analyses were conducted with R version 2.14.1 (R Core Development Team, 2011).

Results

Environmental conditions

During the study period, the annual average temperature was 12.23 °C, which was slightly warmer than the 1975–2011 average (Fig. 1). Annual precipitation was almost 8% lower (611 mm in 1999–2011 vs. 663 mm in 1975–2011, Fig. 1). The identification of different periods of drought throughout the study was possible using the SPEI. The 3 month summer drought was the most consistent, occurring almost every year, with peaks in 2006 and 2009 (Fig. 2). The May SPEI-6 indicated a drought outside the typical season; during these months, precipitation was normally, on average, 55% of the yearly total (Figs 1 and 2). Two 2 year periods (2000–2001 and 2005–2006) were particularly dry. Long-term water deficits were identified with the 12 month and 24 month SPEIs of December in the following periods: 1999–2002, 2005–2007, and 2010–2011. Soil water content was significantly lower in the drought treatment compared with the control plots (−18.06% ± 3.02, pMCMC < 0.001), ranging from 25–30% in volume during the rainy seasons to 5–10% during summer droughts (Fig. 1). The period after the 2005–2006 droughts was not significantly wetter for April SPEI-3
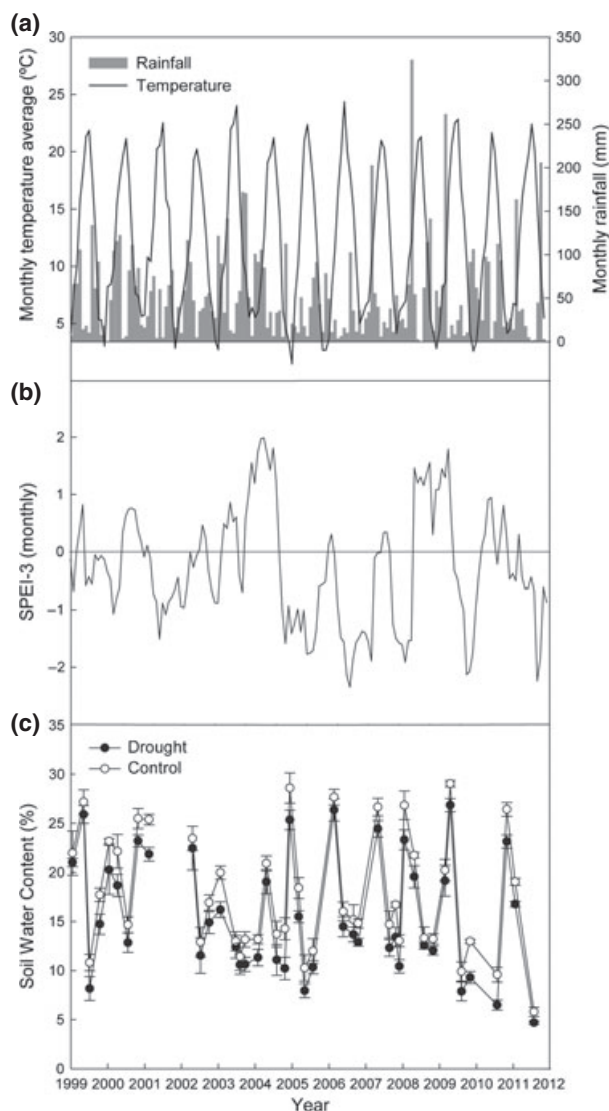
Fig. 1 Environmental variables during the period of study (1999–2012). (a) Monthly precipitation and monthly average temperature, (b) monthly SPEI-3, and (c) soil water content in each treatment. Error bars are the standard error of the mean ($n = 4$).

($F = 0.63$, $P = 0.44$), May SPEI-3 ($F = 0.80$, $P = 0.39$), May SPEI-6 ($F = 0.99$, $P = 0.35$), or September SPEI-6 ($F = 0.7$, $P = 0.80$), the relevant SPEIs for plant growth and mortality.

Stem BAI

Lower soil water contents in the drought plots had an overall negative effect on the stem BAI, but this response was species-specific (Table 1). Stem BAI of *A. unedo* was the most sensitive to drought. It was significantly lower in drought plots, roughly a third of the

stem BAI in the control plots (Table 1; Fig. 3). *Q. ilex* also tended to present lower stem BAIs in the drought treatment, whereas the stem BAI of *P. latifolia* was uninfluenced by drought treatment. The stem BAI of the three species was strongly correlated with the SPEI calculated with a timescale of 3 months. The stem BAI of *A. unedo* was positively correlated with April SPEI-3, whereas those of both *Q. ilex* and *P. latifolia* were positively correlated with May SPEI-3 (Table 1; Fig. 3). The effect size of the drought index on stem BAI, however, was higher in *A. unedo* than in *Q. ilex* (April SPEI-3 *Mean effect* = 0.24, *pMCMC* < 0.01, for *A. unedo*, and May SPEI-3 *Mean effect* = 0.18, *pMCMC* < 0.01, for *Q. ilex*, Table 1), whereas *P. latifolia* seemed to be relatively less dependent on the drought index than the other two species (May SPEI-3 *Mean effect* = 0.03, *pMCMC* < 0.01 for *P. latifolia*, Table 1). Our results also indicated that the growth of *A. unedo* was a function of the water balance in late winter and early spring (February, March, and April), whereas the stem BAI of *Q. ilex* and *P. latifolia* depended only on early spring (March, April, and May) (Table 1). The stem BAIs of *A. unedo* decreased after the intense droughts of 2005 and 2006, but *Q. ilex* and *P. latifolia* had significantly higher stem BAIs (Table 2a). The negative effect of treatment on the annual stem BAI showed a progressive and significant reduction in *A. unedo* and *Q. ilex*, but not in *P. latifolia* (Fig. 4). The total BAI accumulated between 1999 and 2012 differed significantly among species ($F = 128.89$, $P < 0.001$, ANOVA) and was lower in the drought treatment ($F = 9.35$, $P < 0.01$, ANOVA); a significant interaction between drought treatment and species was observed ($F = 12.69$, $P < 0.001$, ANOVA) (Fig. 5). *A. unedo* was the only species with lower stem BAIs in the drought plots for the stems that survived the entire study period (difference = −6.40, $P < 0.001$, Tukey HSD). In both treatments, the stem BAI of *Q. ilex* from 1999 to 2012 did not differ from the stem BAI of *A. unedo* in the drought plots. On the other hand, the BAI of *P. latifolia* for the period 1999–2012 was significantly lower than the BAI in the other two species, in either the drought or control plots (differences = −10.72/−4.32 with *A. unedo* and −4.60/−3.72 with *Q. ilex*, all $P < 0.001$, Tukey HSD), but treatment had no effect in *P. latifolia* (Fig. 5). Overall, the relative change in basal area per plot was lower in the drought treatment (mean effect = −0.01, *pMCMC* < 0.05, Fig. 6). Drought treatment tended to reduce basal area in the drought plots in 2002 (mean effect = −0.01, *pMCMC* < 0.1) and during the very dry period of 2005–2006 (mean effect = −0.03, *pMCMC* < 0.1). As shown in Fig. 6, there were losses of basal area in the years 2001 and 2006 (control plots) and in the years 2000, 2001, 2005, 2006, and 2011 (drought plots).
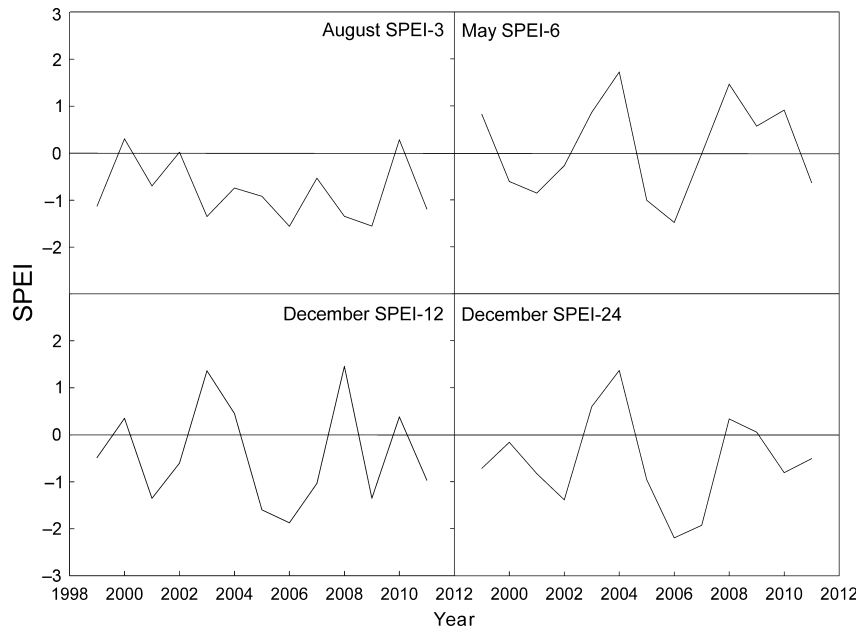
Fig. 2 SPEI at different timescales during the study period. Positive values correspond to a difference between precipitation and potential evapotranspiration more positive than the average calculated from 1975 to 2011, for a given month span. Negative values thus represent conditions drier than average.

Table 1 Results of the Generalized Linear Mixed Models of drought for the annual stem basal area increment (BAI) and stem mortality rates in the three tree species (species-level models). Mean effects of the fixed effects are the Bayes estimates, which are the means of the posterior distributions calculated by Monte Carlo Markov Chains (MCMC). l-95% CI and u-95% CI are the confidence intervals of the posterior distribution of each parameter. Significant effects are marked with *** ($P < 0.001$), ** ($P < 0.01$), * ($P < 0.5$), and (*) ($P < 0.1$). Significant effects ($P < 0.05$) are highlighted in bold, and marginally significant effects ($P < 0.1$) are highlighted in italics

Species		Fixed effects	Mean effect	l-95% CI	u-95% CI	pMCMC
A. unedo	BAI	**Intercept**	**0.92**	**0.73**	**1.11**	***
		Drought	**−0.64**	**−0.84**	**−0.43**	***
		April SPEI-3	**0.24**	**0.08**	**0.39**	**
	Stem mortality rate	**Intercept**	**−4.35**	**−7.59**	**−1.76**	***
		Drought	1.81	−1.01	4.52	NS
		June SPEI-6	0.53	−0.79	1.94	NS
Q. ilex	BAI	**Intercept**	**0.35**	**0.25**	**0.5**	***
		Drought	*−0.07*	*−0.16*	*0.01*	(*)
		May SPEI-3	**0.18**	**0.06**	**0.29**	**
	Stem mortality rate	Intercept	0.28	−0.22	0.77	NS
		Drought	*0.36*	*−0.06*	*0.79*	(*)
		May SPEI-6	**−0.67**	**−1.13**	**−0.24**	**
P. latifolia	BAI	**Intercept**	**0.11**	**0.08**	**0.14**	***
		Drought	0.01	−0.02	0.03	NS
		May SPEI-3	**0.03**	**0.01**	**0.06**	**
	Stem mortality rate	**Intercept**	**−1.04**	**−1.69**	**−0.4**	**
		Drought	−0.01	−0.67	0.6	NS
		Sep SPEI-6	**−0.45**	**−0.85**	**−0.04**	*

Stem mortality

The stem mortality rates were higher in the drought treatment throughout the study period. *P. latifolia*, though, tended to be less affected by the lower availability of water than the other two species (Table 2b). We also found other species-specific differences; *Q. ilex* had higher stem mortality rates than the
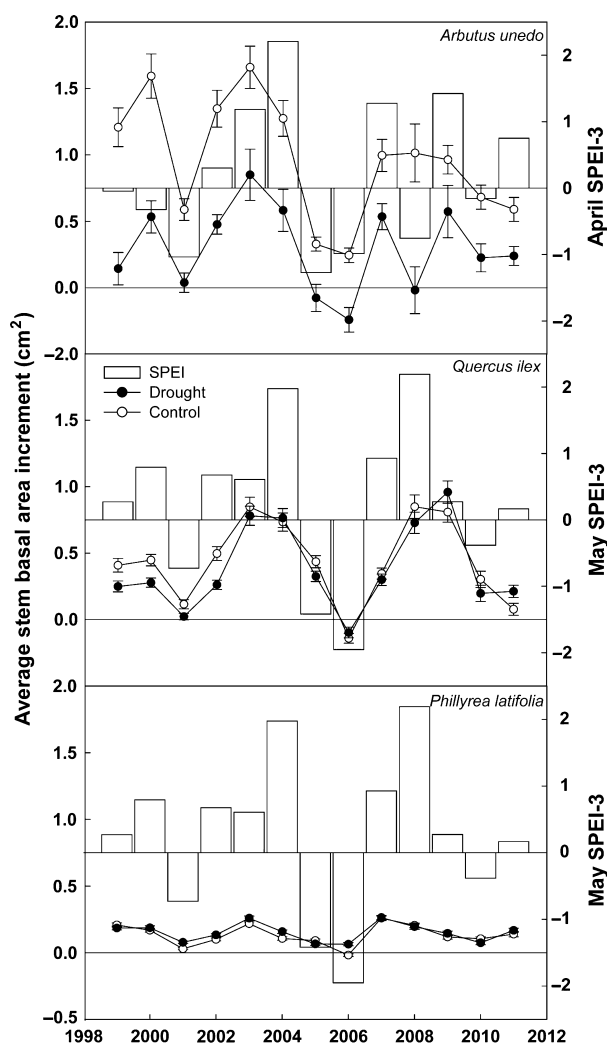
Fig. 3 Average stem basal area increments (BAI) (left axis) of the three tree species and in each treatment. Error bars are the standard error of the mean ($n = 4$). Bars correspond to the annual 3 month SPEI of April (*A. unedo*) and May (*Q. ilex* and *P. latifolia*).

Table 2 Results of the Generalized Linear Mixed Models for the (a) annual stem basal area increment (BAI) and (b) stem mortality rates in the period of study of the three tree species (community-level models). Mean effects of the fixed effects are the Bayes estimates, which are the means of the posterior distributions calculated by Monte Carlo Markov Chains (MCMC). I-95% CI and u-95% CI are the confidence intervals of the posterior distribution of each parameter. The x between two factors indicates interactions among fixed effects. Significant effects are marked with *** ($P < 0.001$), ** ($P < 0.01$), * ($P < 0.5$), and (*) ($P < 0.1$)

Fixed effects	Mean effect	l-95% CI	u-95% CI	pMCMC
a) Annual Stem BAI				
(Intercept)	0.99	0.84	1.13	***
P. latifolia	−0.89	−1.01	−0.76	***
Q. ilex	−0.64	−0.77	−0.52	***
Drought	−0.65	−0.81	−0.50	***
After 2006	−0.26	−0.46	−0.05	*
May SPEI-3	0.25	0.17	0.34	***
P. latifolia x Drought	0.65	0.49	0.82	***
Q. ilex x Drought	0.58	0.41	0.74	***
P. latifolia x After 2006	0.28	0.11	0.44	**
Q. ilex x After 2006	0.27	0.11	0.45	**
Q. ilex x May SPEI-3	−0.22	−0.29	−0.15	***
P. latifolia x May SPEI-3	−0.07	−0.15	−0.002	*
b) Stem Mortality rates				
(Intercept)	−1.81	−2.80	−0.84	***
Drought	1.05	0.04	2.10	*
P. latifolia	0.58	−0.43	1.61	NS
Q. ilex	1.97	1.05	2.89	***
September SPEI-6	0.33	−0.22	0.92	NS
After 2006	0.95	−0.11	2.01	(*)
P. latifolia x Drought	−1.02	−2.19	0.20	(*)
Q. ilex x Drought	−0.65	−1.75	0.44	NS
P. latifolia x September SPEI-6	−0.87	−1.47	−0.31	**
Q. ilex x September SPEI-6	−0.84	−1.37	−0.27	**
P. latifolia x After 2006	−1.07	−2.29	0.07	(*)
Q. ilex x After 2006	−1.62	−2.78	−0.61	**
Drought x September SPEI-6	0.11	−0.27	0.51	NS

other two species and was the only species to show a trend toward a higher mortality in the drought treatment by the species-level model (Tables 1 and 2b; Fig. 7). Unlike the stem BAI, the timescale of the drought index that better predicted the stem mortality rates of *Q. ilex* and *P. latifolia* was 6 months. *P. latifolia*, however, appeared to be more dependent on September SPEI-6, whereas *Q. ilex* did so on May SPEI-6 in the species-level model (Table 1; Fig. 8). In the period after the intense droughts of 2005 and 2006, stem mortality rates in *A. unedo* tended to increase. By contrast, stem mortality rates in *Q. ilex* significantly decreased, and *P. latifolia* also presented a trend toward lower stem mortality (Table 2b). Given the multistem structure of this forest, the relevance of stem mortality had to be

assessed based on the diameter of dead stems. *Q. ilex* lost significantly larger stems than did *P. latifolia* (mean difference = 0.29 higher in *Q. ilex*, pMCMC < 0.001), and the drought index had a significant negative effect on the size of dead stems (mean effect = −0.07, pMCMC < 0.01) when analyzing both species together. Separately, the May SPEI-6 significantly reduced the dead-stem diameter of *Q. ilex* ($y = 3.71–0.38x$, $R^2 = 0.59$, $P < 0.01$) and tended to do so in *P. latifolia* ($y = 2.67–0.22x$, $R^2 = 0.29$, $P = 0.07$, Fig. 9). Survived *Q. ilex* stems throughout the study period presented significantly
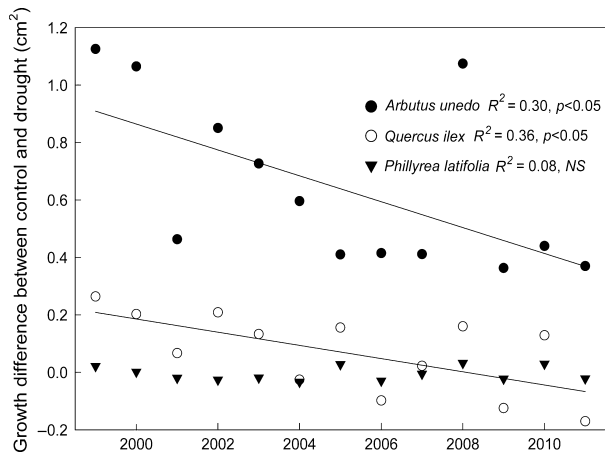
Fig. 4 Yearly difference between the mean growth in the control and the drought plots (1999–2011). Regression lines are shown for the species with significant correlations.
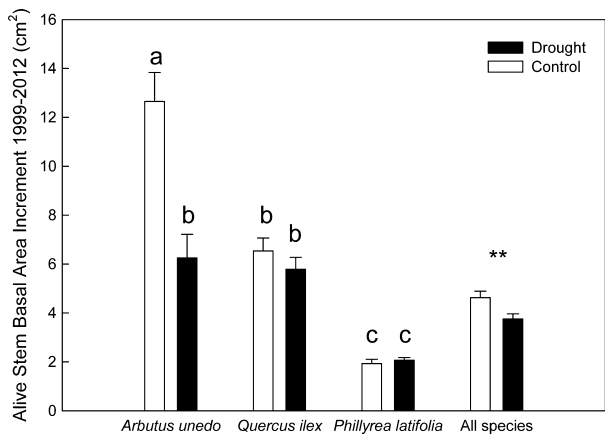

Fig. 5 Accumulated stem basal area increments (BAI) for the stems alive after the 13 years of study. Letters indicate significant differences obtained with ANOVA and Tukey HSD post hoc tests. Significant differences in all species analyzed together are marked with asterisks (**$P < 0.01$). Error bars are the standard error of the mean.

Fig. 6 Relative annual change in basal area in control and drought plots for the period 1999–2012, calculated as the variation in the total basal area of a plot for a given year relative to the previous year. It takes into account mortality and the basal area increments of all stems. Error bars are the standard error of the mean ($n = 4$).

Fig. 7 Stem mortality rates per species and treatment for the period 1999–2012. Significant differences are those obtained by the community-level model and are marked with asterisks (***$P < 0.001$ and *$P < 0.1$). Error bars are the standard error of the mean ($n = 4$).

higher growth than died stems during the same period (mean difference = 0.55, $pMCMC < 0.001$) (Fig. 10).

Discussion

Effects of experimental drought

The long-term experimental drought significantly reduced global stem growth and caused generally higher stem mortality rates throughout the study period in this precipitation-manipulation experiment, which, as far as we know and according to a recent review (Wu *et al.*, 2011), is the longest field experiment conducted in a forest. These results agree with those
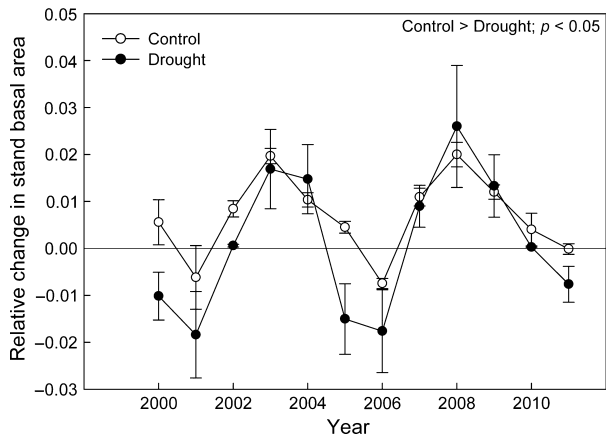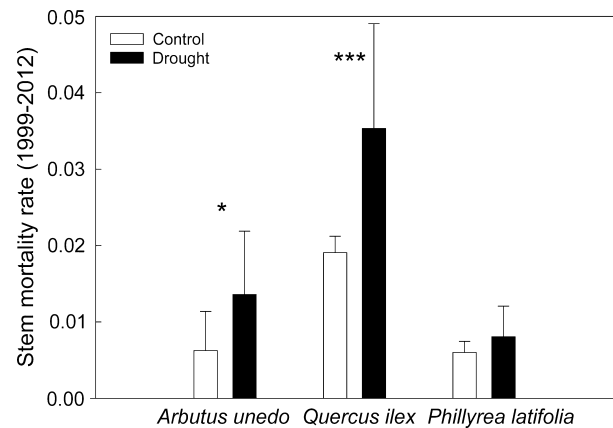
from previous studies in the same forest (Ogaya *et al.*, 2003; Ogaya & Penuelas, 2007b). Long-term experimental drought has also increased stem mortality rates and reduced growth in different ecosystems such as the Amazonian rainforest and North-American deciduous forests (Hanson *et al.*, 2001; Da Costa *et al.*, 2010), although with some exceptions; e.g., the growth of *Quercus* species in southeastern USA was unaffected (Wagner *et al.*, 2012). More specifically, stem growth in the drought treatment in our study was 66.5% lower in *A. unedo*, 17.5% lower in *Q. ilex,* and remain unaffected in *P. latifolia* compared with control plots (Fig. 3). Stem mortality rates in *Q. ilex* were 42.3% higher in the drought treatment (Fig. 7). The results suggest a future
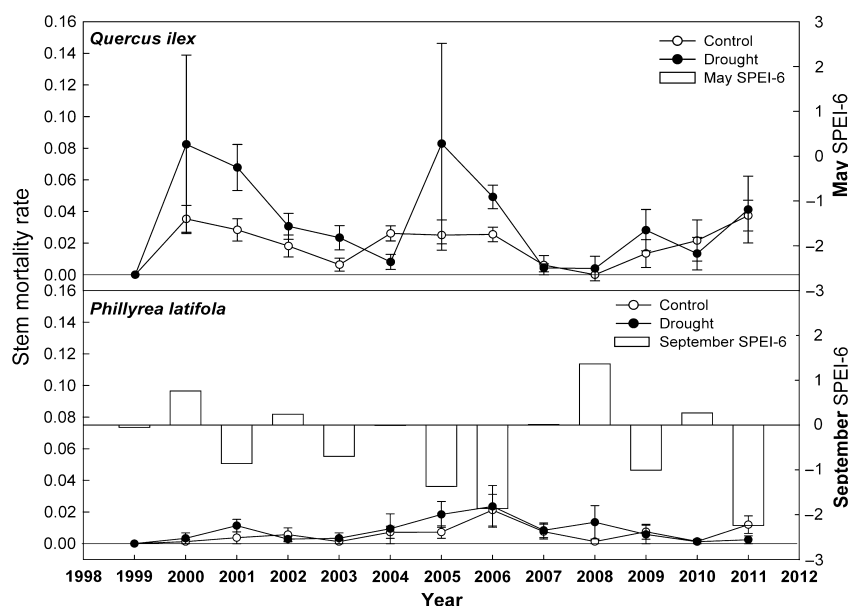
Fig. 8 Stem mortality rates (left axis) of *Quercus ilex* and *Phillyrea latifola* in each treatment. Error bars are the standard error of the mean (n = 4). The open boxes are the 6 month SPEI that best explained the stem mortality of each species (right axis).
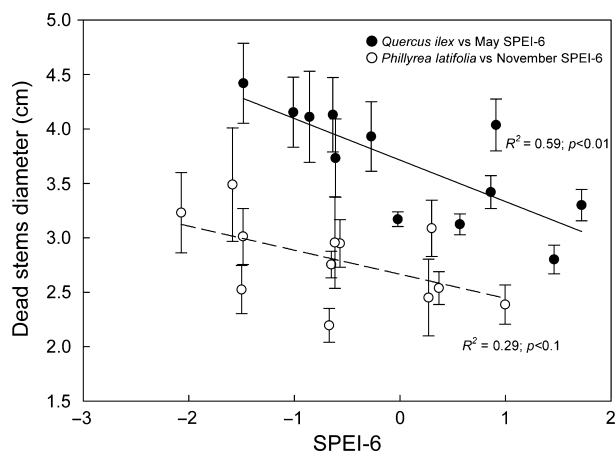

Fig. 9 Linear regressions between the dead-stem diameter and the monthly SPEI that best fit each species.
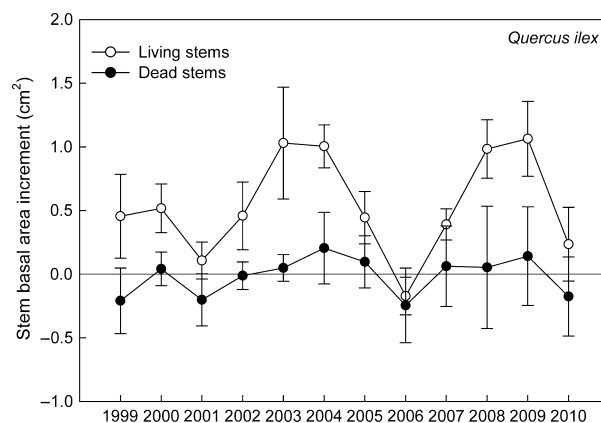

Fig. 10 Annual stem basal area increments (BAI) of the stems that survived throughout the study and those that died during it. Error bars are the standard error of the mean.

decrease in carbon sequestration in this Mediterranean forest if soil moisture drops by 15%, as predicted by GCMs (Bates *et al.*, 2008). Also, after 13 years of drought treatment, the species-specific differences in the response of growth and mortality did not differ from what was observed after 5 years by Ogaya & Penuelas (2007b), so the more drought-resistant *P. latifolia* could potentially outcompete *Q. ilex* and *A. unedo* along the driest edge of the distribution of Holm oak forests.

Droughts lead to reductions in transpiration through stomatal closure in *Q. ilex* (Limousin *et al.*, 2009) and *A. unedo* (Ripullone *et al.*, 2009), which cause lower rates of carbon assimilation. If this reduction persists

over time, it ultimately reduces growth, which is also exacerbated by losses of foliar area in the canopies of *Q. ilex* (Ogaya & Penuelas, 2006; Limousin *et al.*, 2009). The deterioration of the crown in *Q. ilex* following long dry periods with low or negative rates of carbon assimilation has been associated with the depletion of tree carbon reserves (Galiano *et al.*, 2012). The higher stem mortality observed in *Q. ilex* in the drought plots might be a final consequence of this depletion, as further supported by the lower carbon reserves found in defoliated trees at the same study site (T. Rosas, L. Galiano, R. Ogaya, J. Peñuelas, J. Martínez-Vilalta, submitted). As recently observed in other species (Anderegg *et al.*, 2012), though, hydraulic failure could also be the

ultimate cause of stem death or crown defoliation, because *Q. ilex* is more vulnerable to xylem embolism than are other Mediterranean species (Martinez-Vilalta *et al.*, 2002). *A. unedo* was the only species that showed differences under the drought treatment in the accumulated growth of stems that survived throughout the study (Fig. 5). This result is further supported by the comparison between the annual BAI of newly dead and living stems of *Q. ilex* (Fig. 9). Stems of *Q. ilex* that had died by the end of the study had reduced growth prior to death, as noted by Pedersen (1998) in other *Quercus* species. We can thus suggest that *A. unedo* stems underwent a general reduction in growth induced by drought, whereas the growth of *Q. ilex* stems had a dual pattern. That is, healthy stems under drought grew at the same level as stems in the control plots, and the stems that died reduced their growth before death, perhaps due to chronic predisposing factors. Dying stems were smaller than living stems, indicating a self-thinning toward the maintenance of stems with better growth (data not shown). More severe droughts, however, kill stems with larger diameters, so we may infer that all size classes are likely to die in response to a certain length and/or intensity of drought.

Effects of meteorological drought

The interannual variabilities in stem growth and mortality rates were largely described by the SPEI drought index, more than did the drought treatment, as observed in other *Quercus* species (Wagner *et al.*, 2012) (Table 1). The effect of SPEI-3 on stem growth (Fig. 3; Table 1) revealed that spring was the most active period for plant growth at the study site, as is general for *Q. ilex* (Corcuera *et al.*, 2004; Allard *et al.*, 2008; Gea-Izquierdo *et al.*, 2011; Gutierrez *et al.*, 2011). Stem growth in *A. unedo*, however, matched with April SPEI-3 better than with May SPEI-3, as did the other species, indicating a possible earlier activation of plant growth. The effect of the SPEI-3 on growth was significant in all species; nevertheless, the effect size was much smaller in P. latifolia. This species responds less to increases in soil water content likely because it has lower hydraulic conductivity (Martinez-Vilalta *et al.*, 2002).

Because stem mortality rates were strongly correlated with SPEI-6 in *Q. ilex* and *P. latifolia*, we may assert that mortality depended more on the water balance over longer periods than did growth. Stem mortality rates in *Q. ilex* and *P. latifolia* appeared to depend on the water balance of the period between April and September, but winter and spring water balance is also important for *Q. ilex*. Precipitation in winter and spring would replenish the water stores in deep soil layers, rock crevices, or groundwater that could be accessed by the tap roots of *Q. ilex* to mitigate the summer drought (Sarris *et al.*, 2007; Baldocchi *et al.*, 2010; Gea-Izquierdo *et al.*, 2011; Gutierrez *et al.*, 2011). Stem long-term water storage may also depend on precipitation in winter months. In addition, winter drought (as well as cold temperatures) may reduce photosynthetic activity in *Q. ilex*, which is higher than in *P. latifolia* during this season (Ogaya & Penuelas, 2003). The assimilation of carbon in winter may be used to recover a tree's carbon reserves (Gea-Izquierdo *et al.*, 2011) and may help to minimize drought-induced stem mortality in this species (Galiano *et al.*, 2012; T. Rosas, L. Galiano, R. Ogaya, J. Peñuelas, J. Martínez-Vilalta, submitted).

Potential dampening of the response to drought after long-term drought

Following the 2005–2006 droughts, *Q. ilex* and *P. latifolia* experienced slightly higher stem growth rates and enhanced stem survival, both in drought and control plots (Fig. 6; Table 2b). Such changes might suggest that climate was more favorable during the second period, but there were no significant differences in the relevant scales and months of the SPEI (Fig. 2). In addition, the effect of treatment on the annual stem BAI of *A. unedo* and *Q. ilex* seemed to consistently decline with time (Fig. 4). The changes in growth and mortality after 2006 could be a consequence of a demographic compensation, as proposed by Lloret *et al.*, (2012). That is, the higher mortality rates beyond natural variability caused by extreme droughts during the first period of the study (exacerbated by the experimental drought in the treatment plots) may be partly compensated in the second period by a higher survival of the remaining population, as observed in *Q. ilex*, which was initially most affected. Decreased intra- and interspecific competition might enhance plant survival and growth through an increased availability of water per stem or individual. In drought plots, where stem mortality was formerly higher, decreased competition resulted from stronger reductions of basal area than in control plots (Fig. 6). Furthermore, reductions of stem mortality in the drought plots also may be expected as a result of morphological acclimation after several years of treatment, because, for instance, drought reduces foliar area in *Q. ilex* as an adaptation to scarcer water resources (Ogaya & Penuelas, 2006; Limousin *et al.*, 2009). Likewise, Martin-StPaul *et al.* (2013) studied the temporal response to drought of *Q. ilex* and suggested that whole tree water transport and stand leaf area index are the key variables that will acclimate in future drier conditions. *Q. ilex* stems are more likely to die the higher the number of stems per individual (Galiano *et al.*, 2012),

so a previous natural self-thinning caused by extreme droughts could produce a higher resistance to future stresses in the remaining stems. The response of the ecosystem to drought thus did not seem to be accelerated by the extreme events as could be expected (Jentsch *et al.*, 2007). On the contrary, both the duration of the study (Leuzinger *et al.*, 2011) and the extreme events (Lloret *et al.*, 2012) may have dampened or compensated the treatment effects to some extent and promoted nonlinear responses of the vegetation to drought. These results should be considered when predicting and modeling the long-term responses of plant communities to climatic change.

The results of this study indicate significant decreases in plant growth and accelerated mortality rates in the dominant species of a Holm oak forest subjected to an 18% decrease in soil moisture. The time span of the study provides consistency to these results, which would help to predict more precisely the response of this community to climatic change. The variant species-specific responses to either experimental or natural drought would potentially cause a vegetational shift, as reported in other systems (Mueller *et al.*, 2005), consisting of a partial substitution of the most drought-sensitive species, *Q. ilex* and *A. unedo*, by the most drought-resistant species, *P. latifolia*. This shift may eventually alter the composition of the landscape and the ecosystem's services, although it would present a patchy pattern dependent on site characteristics (Lloret *et al.*, 2004b). On the other hand, changes in forest structure (reductions in density and basal area) and demographic compensation (Lloret *et al.*, 2012), in addition to physiological and morphological acclimation to drought at the individual level (reduced foliar area and growth, greater allocation of carbon to roots, or fewer stems per individual), would enhance the persistence of *Q. ilex* and *A. unedo*, presumably maintaining their dominance in the ecosystem. These processes seem very likely to alter the initial response of vegetation in short-term drought experiments, but they still remain poorly understood. The duration of experimental drought systems should be extended for determining the role of these processes. Also, the possible changes in the seasonal distribution of precipitation and the duration of drought periods should be incorporated into experimental studies to properly predict responses to changes in precipitation.

Acknowledgements

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