Long-term experimental drought combined with natural extremes accelerate vegetation shift in a Mediterranean holm oak forest

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Abstract

Increasing drought combined with natural extremes are expected to accelerate forest die-off and shifts in vegetation in the Mediterranean Basin. However, fewer studies have explored these climate-driven changes in forest ecosystems.

A long-term (17-year) experimental drought (-30% precipitation) was established in a Mediterranean holm oak forest with high (H) and low (L) canopies to determine the changes in stem mortality, recruitment and composition shifts.

Experimental drought increased annual stem mortality rate at the community level for both H- and L-canopies. Natural drought amplified the effects of experimental drought on stem mortality at the community level and of *Q. ilex* for H- and L-canopies. The timescales of natural drought, however, varied substantially with canopy types and species, with shorter timescales in L- than H-canopy and for *Q. ilex* than *P. latifolia*. Furthermore, experimental drought combined with natural extremes amplified the increases in stem mortality and decreases in growth for L-canopy. Contrasting responses between *Q. ilex* and *P. latifolia* for the relative in abundance and growth were observed in L-canopy and drought treatment reinforced the vegetation shift favoring *P. latifolia*.

These findings suggest continuous drought regimes accelerated a vegetation shift, implying potential consequences for the functions and services for water-limited forest ecosystems.

1. Introduction

Anthropogenic climate change with increasing temperatures and shifting precipitation regimes has contributed to severe water deficits, causing ecological consequences for the Earth's vegetation ecosystems (Allen *et al* 2010; 2015). Water deficits have become common disturbances affecting forest ecosystems globally, and leading to large-scale tree mortality (Phillips *et al.*, 2010; Anderegg *et al.*, 2013; Greenwood *et al.*, 2017), forest canopy die-off or defoliation (Breshears *et al.*, 2005, Allen *et al.*, 2010, 2015) and shifts in composition (Allen & Breshears, 1998; McIntyre *et al.*, 2015). Mediterranean regions are particularly suffering water deficits due to the unprecedented rate of warming and more frequent extreme events (e.g. heat waves and droughts) (Myers *et al.*, 2000; Dai, 2013; BAIC, 2015). Annual mean temperature in Western Mediterranean regions has increased by 0.23°C per decade while annual mean precipitation has decreased by 1.5% per decade between 1950 and 2015, with the changes being more notable in summer (0.36 °C and -4.5% per decade, respectively) (BAIC, 2015). These climatic trends have been associated with episodes of widespread tree mortality (Peñuelas *et al.*, 2000, 2001; Lloret *et al.*, 2004a; Carnicer *et al.*, 2011), changes in the composition and dominance of forest species (Peñuelas & Boada, 2003; Peñuelas *et al.*, 2007; Coll *et al.*, 2013; Saura-Mas *et al.*, 2015) and reversed trajectories of succession (e.g. forests toward open woodlands or shrublands) (Doblas-Miranda *et al.*, 2015; Franklin *et al.*, 2016; Natalini *et al.*, 2016). General circulation models have projected an increase in the intensity and duration of droughts for the coming decades (IPCC, 2014), which would further threaten the conservation of forest biodiversity, carbon budgets and climatic feedbacks (Myers *et al.*, 2000; Peñuelas *et al.*, 2013; Doblas-Miranda *et al.*, 2015, 2017).

Vegetation shifts represent gradual, nonlinear and sometimes abrupt changes in relative abundance or dominance caused by differential mortality rates and recruitment failure between coexisting species (Lloret *et al.*, 2012; Zeppel *et al.*, 2015; Pausas *et al.*, 2016). Martínez-Vilalta & Lloret (2016) recently reported that vegetation shifts in response to drought have clearly occurred in about a quarter of all study cases across terrestrial ecosystems. The scarcity of vegetation shifts has been primarily attributed to the processes of regeneration (e.g. increased survival, enhanced recruitment and regrowth) of dominant species that reinforce their resilience and recovery after droughts (Lloret *et al.*, 2012; Zeppel *et al.*, 2015; Martínez-Vilalta & Lloret, 2016; Pausas *et al.*, 2016). Frequent and intense droughts, however, would weaken these regeneration processes, via more negative effects on seed maturation, seedling survival and growth (Allen & Breshears, 1998; Kéfi *et al.*, 2007; Allen *et al.*, 2015; Reyer *et al.*, 2015). Increased drought would in fact induce differential rates of recruitment among the coexisting species, particularly favoring the re-establishment of drought-resistant species and thus promoting vegetation shifts (Mueller *et al.*, 2005; Suarez & Kitzberger, 2008, 2010; Fauset *et al.*, 2012; Lloret *et al.*, 2012). For example, declines in large trees and increases in seedling establishment of drought-resistant species in Californian forests have been associated with a progressive increase in water deficit (McIntyre *et al.*, 2015). Most field surveys unfortunately cannot deliver the long timescales and precision of field data required for properly tracking changes in forest structure and composition in response to climate change. Forest dynamics (e.g. changes in stem mortality, recruitment and structure) are also complicated by other confounding factors associated with land-use changes, wild fires and insect pests, leading to large uncertainties in the prediction of the effects of future climate change on forest dynamics (Doblas-Miranda *et al.*, 2015, 2017; Franklin *et al.*, 2016; Martínez-Vilalta & Lloret, 2016).

Long-term drought experiment is a practical method for examining the impacts of increasing drought on forest dynamics and can provide unique insights into physiological and demographic processes and their underlying mechanisms (Jentsch *et al.*, 2007; Smith *et al.*, 2009; Martin-Stpaul *et al.*, 2013; Liu *et al.*, 2015; Franklin *et al.*, 2016). Manipulative field experiments in recent decades have identified relevant effects of drought on forests, such as limitations in carbon assimilation (Ogaya & Peñuelas, 2003; Misson *et al.*, 2010; Ogaya *et al.*, 2014), decreases in canopy leaf area (Limousin *et al.*, 2009; Martin-Stpaul *et al.*, 2013) and reductions in biomass accumulation (Ogaya *et al.*, 2003; Ogaya & Peñuelas, 2007a; Barbeta *et al.*, 2013; Liu *et al.*, 2015). These effects may lead to more severe consequences associated with population dynamics and compositional changes by long-term processes (Smith *et al.*, 2009; Martin-Stpaul *et al.*, 2013; Peñuelas *et al.*, 2013). Abrupt changes such as vegetation shifts are thus likely to emerge after long-term drought manipulation, probably in association with the accumulated or legacy effects (Smith *et al.*, 2009; Luo *et al.*, 2011; Allen *et al.*, 2015). In particular, extreme natural droughts could also intensify the forest dynamics that trigger synergistic effects on the changes in forest structure and composition by substantial tree mortality (Lloret *et al.*, 2012). Long-term drought experiments have been relatively rare, especially in forests, leading to a lack of understanding of forest dynamics and vegetation shifts in response to the increasing frequency and severity of droughts (Leuzinger *et al.*, 2011; Luo *et al.*, 2011; Knapp *et al.*, 2015, 2016).

Holm oak (*Quercus ilex* L.) forests are the dominant vegetation type between the temperate forests and shrublands (e.g. maquia) in the Mediterranean Basin (Peñuelas *et al.*, 2000; Lloret *et al.*, 2004a; Ogaya & Peñuelas, 2007a). *Q. ilex* has the typical features of Mediterranean species, with sclerophyllous leaves, high belowground biomass and bimodal growth patterns, but it has been affecting by the increasing length and intensity of droughts with high rates of evapotranspiration and low precipitation (Peñuelas *et al.*, 1998, 2000; Lloret *et al.*, 2004a; Galiano *et al.*, 2012; Barbeta & Peñuelas, 2016). Numerous studies have reported substantial changes in community structure and composition in holm oak forests in response to drought, which were especially severe on hillslopes with shallow soils (Peñuelas *et al.*, 2000; Lloret *et al.*, 2004a; Galiano *et al.*, 2012; Saura-Mas *et al.*, 2015). Recent extreme droughts have triggered large-scale tree mortality and canopy die-off in these forests (Peñuelas *et al.*, 2000, 2001; Carnicer *et al.*, 2011; Saura-Mas *et al.*, 2015). Extreme natural droughts have also reduced the resistance and resilience of holm oak forests; resprouting capacity from above- and belowground buds has decreased (Lloret *et al.*, 2004a; Galiano *et al.*, 2012; Saura-Mas *et al.*, 2015). Some affected forests have therefore degraded into low-canopy type forests due to a greater loss of larger stems that were less resistant to increased drought (Lloret *et al.*, 2004a; Carnicer *et al.*, 2011; Bennett *et al.*, 2015; Natalini *et al*., 2015). Recruitment in these low-canopy forests would be enhanced once competition decreases after drought induced higher mortality, and the forests would be more favorable for drought-resistant seedlings (Lloret *et al.*, 2004b, 2012; Martínez-Vilalta & Lloret, 2016).

The presence of drought-resistant species (*Phillyrea latifolia* L.) may promote vegetation shifts in the holm oak forests since they are favored under drought. Holm oak forests usually contain the tall shrub *P. latifolia*, which is more drought-resistant than *Q. ilex*. *P. latifolia* develops physiological plasticity (Peñuelas *et al.*, 1998; Ogaya & Peñuelas, 2003; Ogaya *et al.*, 2014), adjusts phenologically and morphologically (Serrano *et al.*, 2005; Liu *et al.*, 2015) and acclimates its water-use efficiency (Peñuelas *et al.*, 2000; Lloret *et al.*, 2004b; Ogaya & Peñuelas, 2008) in response to drought differently than *Q. ilex*. Experimental drought and/or severe natural droughts that trigger abrupt and nonlinear increases in stem mortality and decreases in aboveground growth in *Q. ilex*, it is expected to affect less *P. latifolia* (Ogaya *et al.*, 2003; Ogaya & Peñuelas, 2007a;

Barbeta *et al.*, 2013, 2015; Liu *et al.*, 2015). Field surveys have demonstrated that *P. latifolia* tended to increase in abundance in some cases, indicating potential shifts in forest structure and composition (Lloret *et al.*, 2004b; Saura-Mas *et al.*, 2015). The claims supporting vegetation shifts, however, are mainly assumptions from the contrasting physiological, phenological and morphological responses to drought between *Q. ilex* and *P. latifolia*, which are insufficiently reliable for predicting long-term shifts in forest composition (Barbeta *et al.*, 2013; Barbeta & Peñuelas, 2016). The absence of supporting data of tree mortality, recruitment and compositional change for *Q. ilex* and *P. latifolia* in response to drought may generate inaccurate assessments of the responses of vegetation shifts to future climate change (Lloret *et al.*, 2012; Martínez-Vilalta & Lloret, 2016).

This study was conducted as part of a long-term drought experiment (-30% precipitation) established in 1999 in a Mediterranean holm oak forest with high (H) and low (L) canopies. H-canopy distributes in the deep soils (30-50 cm) of the study sites, whereas L-canopy distributes in shallow soils (10-30 cm) (more details in Methods). We hypothesized that long-term experimental drought (1999-2015) and natural droughts would imposed severe water deficits, and would trigger differential stem mortality and growth rates for the two canopy types and thus a vegetation shift (*Q. ilex* replaced by *P. latifolia*) in L-canopy. More specifically, we (1) evaluated the combined effects of a long-term (17-year) experimental drought and natural droughts on the changes in stem mortality, new recruitment, density and growth (basal area) for the two canopy types at the community and species levels, (2) assessed the synergistic effects of experimental drought on stem mortality and growth rates during extreme dry years for the two canopy types at the community and species levels and (3) analyzed the net-changes in relative abundance (stem density) and growth for the two species (*Q. ilex* and *P. latifolia*) for the two canopy types.

2. Materials and methods

2.1 Experimental site

The experiment was carried out in the Prades holm oak forest in Catalonia, northeastern Iberian Peninsula (41°21´N, 1°2´E). The vegetation is dominated by the evergreen tree *Q. ilex* and the tall shrub *P. latifolia*, accompanied by other Mediterranean shrub species (e.g. *Arbutus unedo* L., *Erica arborea* L. and *Juniperus oxycedrus* L). The formation and structure of the forest are naturally variable due to the heterogeneity of soil depth and bedrock type. Holm oak forests at sites with deep soils are usually composed by tall, dense stands of *Q. ilex* trees (by 62.9%), *P. latifolia* (20.2%) and other species (16.9%) such as *Arbutus unedo*, reaching a height of 8-10 m (High, H-canopy), but forests at sites with shallow soils are more mixed with *Q. ilex* (27.2%), *P. latifolia* (63.3%) and other species (9.5%) such as *Arbutus unedo*, *Erica arborea* and *Sorbus torminalis*, reaching a height of 4-6 m (Low, L-canopy). The features of the two types of canopies are detailed in Table S1.

The climate is typically Mediterranean with hot and dry summers (mean temperature of 19.4°C and total precipitation of 100 mm from June to August) and rainy springs (mean temperature of 10.4°C and total precipitation of 208.9 mm from March to May) and autumns (average temperature of 12.4°C and total precipitation of 203.1 mm from September to November). The meteorological series for 1975 to 2015 had a mean annual temperature (MAT) of 11.8°C, fluctuating from 10.8°C to 13.2°C and a mean annual precipitation (MAP) of 656.1 mm. MAT has gradually increased (R^2=0.42, p<0.001) (Fig. S1a), more strongly in spring (R^2=0.32, p<0.001) and summer (R^2=0.48, p<0.001) (Fig. S1b, c). The distribution of MAP has been irregular, ranging from 355.4 mm (2015) to 984.2 mm (1996). Summer precipitation for 1975-2015 has progressively decreased (R^2=0.23, p<0.001) (Fig. S1c). The soil is a Dystric Cambisol, and the soil depths ranged differently for the two canopy types, lying over fractured schist that is penetrated by roots. The types of forest canopy and composition are associated with different soil depths and water availabilities (Rivas-Ubach *et al.*, 2016).

2.2 Experimental manipulation

The drought experiment began in 1999 and consisted of a partial rainfall exclusion that simulated a reduction in precipitation of 30% for the Mediterranean holm oak forest. Four H-canopy plots and four L-canopy plots (10 × 15 m) were established at the same altitude (930 m a.s.l.) and slope (25%) along the southern face of a mountain that has the highest temperature and solar irradiation. Half of the plots (two replicates per canopy type) were randomly selected to receive the drought treatment, and the other two plots served as controls (natural conditions). There is 5-m distance between the drought and control plots. Precipitation was partially excluded by the installation of plastic strips 0.5-0.8 m above the ground, covering about 30% of the plot surface. Water runoff in the drought plots was intercepted by ditches (0.8-1 m in depth) excavated along the upper edges of the plots.

The precipitation intercepted by the plastic strips and ditches was conducted outside the plots. All litter falling on the plastic strips was periodically (nearly every month) transferred below them to ensure applying only the drought treatment without nutrient impacts.

2.3 Measurement of indices of experimental drought and extreme dry years

A meteorological station was installed outside the study plots in 1999 for monitoring temperature and precipitation every 30 minutes. Monthly temperature and precipitation data for 1975-2001 were obtained from a nearby meteorological station (Poblet Monastery, 5.6 km northeast of our plots) to determine the history of climate change at the study site. After 2001, the climate data of Poblet Monastery is not available due to the damage. We reconstructed the climate profile of our study site by combining the climate data of 1975-1997 from Poblet Monastery station and the climate data of 1998-2015 from our meteorological station (linear regressions for the period of 1998-2001: R^2=0.97 for temperature; R^2=0.75 for precipitation, n=48). Natural normal and extreme dry years were defined as those occurred between 40th and 60th percentiles and the lowest 10th percentile for MAP respectively during the period of 1975-2015 (Knapp *et al.*, 2015). Soil-water content was measured seasonally by time-domain reflectometry (Tektronix 1502C (Tektronix Company), Oregon, USA) throughout the experimental period from 1999 to 2015 at four randomly selected locations in each plot. Three cylindrical rods were vertically inserted in the upper 25 cm of soil at each location.

We calculated the drought index (Standardized Precipitation-Evapotranspiration Index, SPEI) in our study site based on climate data of 1975-2015. The SPEI has the crucial advantage of including the effects of precipitation (P) and potential evapotranspiration (PET) by temperature fluctuations, which fulfills the requirements of drought because its multi-scalar characteristics enable it to identify drought severity based on intensity and duration (Vicente-Serrano *et al.*, 2010, 2013). We constructed a database of the monthly difference between P and PET (D values) for the 1975-2015, and used it to calculate SPEI at different timescales (from 1-month to 36-month) using SPEI package (version 1.6). SPEI values range from -3 to 3, with the lower values representing a more severe water deficit.

2.4 Stem mortality

All living stems with diameters >2 cm at a height of 50 cm (D_{50}) have been labeled and the species identified in each plot since 1998, and D_{50} has been measured annually in winter, the dormant season for stem growth, using a metric tape for calculating the basal area (BA). Dead stems were also recorded in the annual measurement. The annual stem mortality (m) was calculated as: $m=1-(1-(N_0-N_t)/N_0)^{1/t}$, where N_0 and N_t are the number of living stems at the beginning and end of the year, respectively (described in more detail by Sheil (1995), Ogaya & Peñuelas (2007a) and Barbeta *et al.* (2013)). The mean rate of stem mortality over the study period was calculated from the beginning (1999) to the end (2015, 17 years). The average stem mortality rates in response to treatments (control and drought) during the normal and extreme dry years were also calculated.

2.5 Mean new recruitment

Since the density of this forest is very high, it is difficult to separate the recruitment stems from sprouts and occasional young stems from seedling. New recruitment stems were recorded as the resprouts from dead stems or new seedlings larger than the threshold size (D_{50}>2 cm). The mean recruitment rate (r) was calculated as: $r=100\times n / N_0\times t$, where N_0, n and t are the number of living stems at the beginning year of 1999, the number of new stems emerging until the end year of 2015 and the study period ($t=17$), respectively.

2.6 Mean change in stem density

The mean change in stem density (d) throughout the study period was calculated as: $d=100\times(N_t-N_0)/ N_0\times t$, where N_0, N_t and t are the number of living stems at the beginning year of 1999, at the end year of 2015 and the study period ($t=17$), respectively.

2.7 Response of mean stem growth

The mean stem growth (g) throughout the study period was calculated as: $g= 100\times(BA_t-BA_0)/BA_0\times t$, where BA_0, BA_t and t are the basal areas at the beginning of 1999, at the end of 2015 and the study years ($t=17$), respectively. Moreover, the average stem growth rates in response to experiments (control and drought) during the normal and extreme dry years were also calculated.

2.8 Shift in species composition

We assessed the shift in species composition based on the net-changes in the relative abundance (or stem density) and stem growth for two species *Q. ilex* and *P. latifolia* related to the values at community level (all species). First, we calculated the relative abundance and stem growth for the two species in each plot from the pre-treatment year (1998) to the end of the experiment (2015). Second, we calculated the average values for the two species in the two canopy types (H- and L-canopies) and treatments (drought and control). Third, the net-changes in relative abundance and stem growth for *Q. ilex* and *P. latifolia* during the study period were calculated as the values of the current year minus the values for the beginning of 1998.

2.9 Statistical analyses

To evaluate the effects of experimental drought, SPEI and the interaction of experimental drought and SPEI on annual stem mortality, we used Linear Mixed-Effects Models (*lme4* package, version 1.1-13). The timescales of the SPEI tested from 3-12 months, 15, 18, 21, 24, 27, 30, 33 and 36 months, and months of departure were from January to December. Plot and plot nested with year were selected as random factors to seek the best models. According to the selection, plot nested with year was included as the random factor in the models (Table S2). The best fitted SPEI for annual stem mortality were selected by choosing the models with the lowest values of Akaike's information criterion (AIC). The model was calculated as:

Annual stem mortality (community- or species-level) = treatment × SPEI

where annual stem mortality at the community level (all species in the plot), *Q. ilex* and *P. latifolia*, were calculated separately for the two canopy types. The treatment factor had two levels (drought and control), and the SPEI timescales were considered as independent variables in each model. Based on the values of AIC (shortlisted as Table S3), October SPEI-7 and May SPEI-6 were the best fits for annual stem mortality at the community level for H- and L-canopies, respectively. June SPEI-9 and May SPEI-6 were the best fits for annual stem mortality for *Q. ilex* for H- and L-canopies, respectively. October SPEI-15 was the best fit for annual stem mortality for *P. latifolia* for both H- and L-canopies.

Experimental drought on soil-water content were assessed by analyses of variance (ANOVAS) with Tukey’s HSD (honest significant difference) post-hoc tests after testing normality distribution. Mean summer temperature and precipitation during the study period of 1999-2015 were compared with the values of climate series of 1975-1998 by ANOVA with post-hoc HSD. The variables (e.g. mean stem mortality, mean new recruitment, mean changes in stem density and growth) at the community level (all species in the plot) and the species of *Q. ilex* and *P. latifolia* were calculated for the two canopy types. The effects of experimental drought on these variables at community level were separately analyzed for each canopy type. To compare the differential responses to experimental drought for the two species, the variable of the two species (*Q. ilex* and *P. latifolia*) for each canopy type were analyzed by ANOVA with post-hoc HSD (in terms of drought, species and the interaction of drought and species). Moreover, we also analyzed the correlations between mean stem mortality and mean new recruitment, mean changes in stem density and growth at the community level (all species in the plot) and the species of *Q. ilex* and *P. latifolia*.

In addition, the responses of stem mortality and growth rates to experimental drought during the normal and extreme years were evaluated by ANOVA with post-hoc HSD. The average rates of stem mortality and growth were obtained according to the values in normal and extreme dry years. Thus, the average stem mortality and stem growth for control plots in normal years, control plots in extreme dry years, drought plots in normal years and drought plots in extreme years were compared by ANOVA with post-hoc HSD, respectively. The responses at community level, *Q. ilex* and *P. latifolia* were separately analyzed for each canopy type.

The patterns of net-changes in relative abundance and BA for the two species during the study period of 1998 (pre-treatment) to 2015 were tested by simple linear models (lm function). For each canopy type, the significance for the net-changes in relative abundance and BA during the study period was analyzed in both control and drought plots. In addition, significant differences for the net-changes between control and drought plots for each species were analyzed by Least-Squares Means (*lsmeans* package). All analyses were performed with R version 3.3.2 (R Core Development Team, 2016).

3. Results

3.1 Environmental variables

The mean summer temperature during the period of this study was 20.5±0.3°C, which was 1.8°C higher than the period of 1975-1998 (18.7 ± 0.2°C). Mean summer precipitation during the study period was 61.6±10. 9mm, which was 113% lower than the period of 1975-1998 (131.2±7.0mm). Climatically normal years during our study period were 1999, 2004, 2010 and 2014, whereas the extreme dry years were 2001, 2005, 2006 and 2015 according to the definitions (Table S4).

Soil-water content in winter, spring and autumn were not significantly different for the two canopy types, whereas the values in summer were 23% higher (p<0.05, ANOVA) in H-canopy than L-canopy (Fig. S2). The significant decrease in soil-water by the experimental drought was detected in summer (p<0.05, ANOVA). Summer soil-water content also differed between the canopy types and treatments (p<0.05, ANOVA), with the highest values in H-canopy with control (H-canopy control plots (12.7%) > H-canopy drought plots (10.2%) > L-canopy control plots (9.9%) > L-canopy drought plots (8.6%)).

3.2 Annual stem mortality

For both H- and L-canopies, annual rate of stem mortality at the community level was higher in the drought plots than the control plots (both *pLME*<0.05) (Fig. 1a,b; Table 1). The annual mortality rate of *Q. ilex* and *P. latifolia* were not affected by the drought treatment for H-canopies. The annual mortality rate of *Q. ilex* was significantly higher in drought plots than control plots for L-canopy (*pLME*<0.05). Natural drought (indicated by SPEI) amplified the effects of the experimental drought on the rates for the two canopy types (Table 1). For both H- and L-canopies, the annual rate of stem mortality at the community level increased significantly by the interaction of experimental drought and SPEIs (October SPEI-7 and May SPEI-6, respectively) (*pLME*<0.05 and *pLME*<0.1, respectively). Interestingly, for both H- and L-canopies, the annual mortality rates of *Q. ilex* also increased significantly in the drought plots with the interaction of experimental drought and SPEIs (June SPEI-9 and May SPEI-6, respectively) (both *pLME*<0.05) (Fig. 1c,d). The annual mortality rate of *P. latifolia*, however, did not increase significantly with this interaction in either H- or L-canopy (Fig. 1c,d). But the annual mortality rate of *P. latifolia* response to October SPEI-15 was significantly higher for L-canopy (*pLME*<0.01).

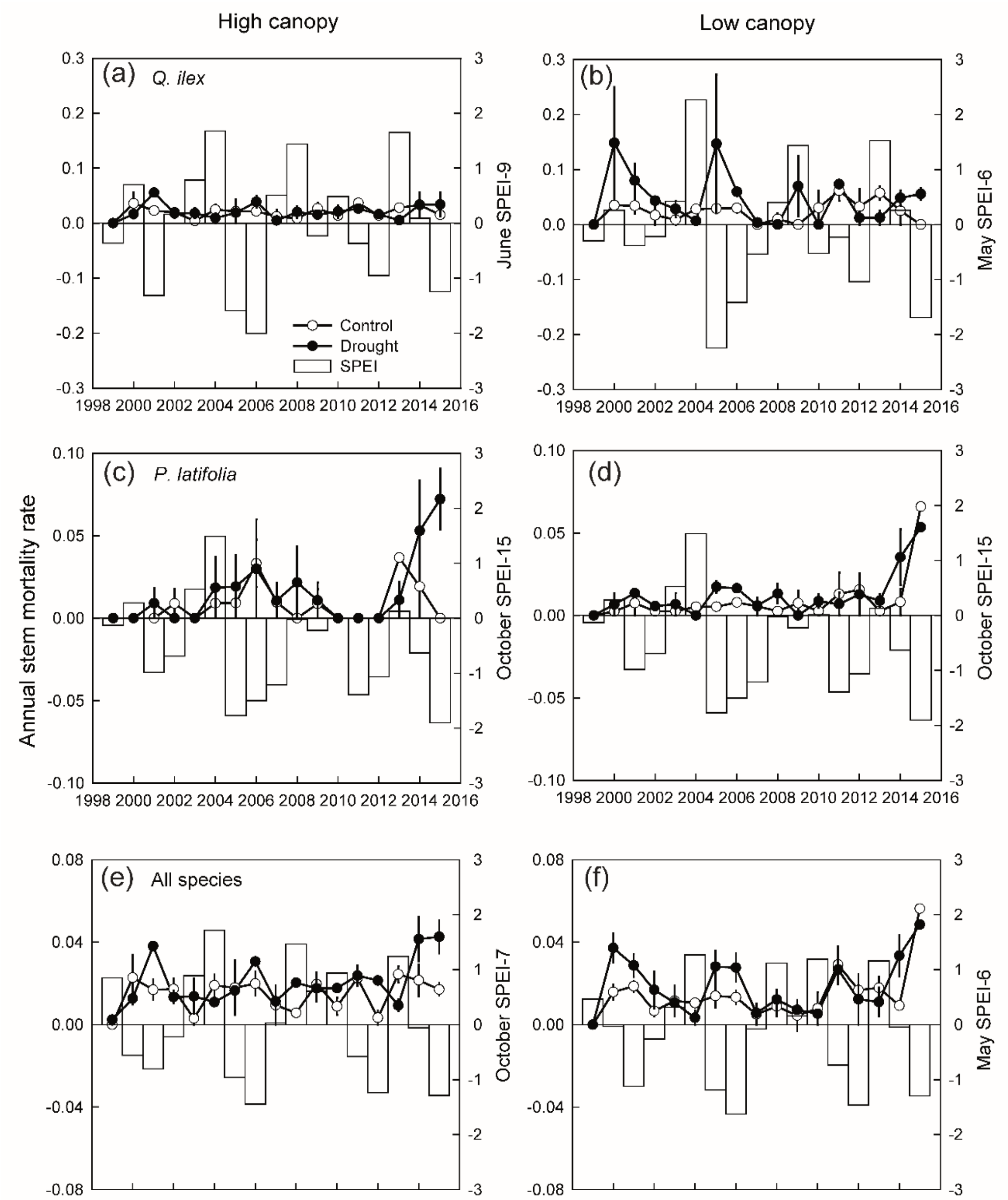

Fig. 1 Responses of annual rate of stem mortality to experimental and natural (SPEI) droughts for both H- and L- canopies. Error bars represent the standard error of the mean (n=2). Vertical bars are the best fit SPEIs at the community level (October SPEI-7 and May SPEI-6 for both H- and L-canopies, respectively) (e, f) and for *Q. ilex* (June SPEI-9 and May SPEI-6 for both H- and L-canopies, respectively) (a, b) and *P. latifolia* (October SPEI-15 for both H- and L-canopies) (c, d).

Table 1 Responses of the annual rates of stem mortality at the community and species (*Q. ilex* and *P. latifolia*) levels to the experimental and natural (SPEI) droughts are analyzed by Linear Mixed Models (LME) (*lme4* package). Estimates of the fixed effects of experimental drought, SPEI and the interaction of experimental drought and SPEIs are listed. The responses at the community, *Q. ilex* and *P. latifolia* were analyzed separately for the two canopy types. October SPEI-7 and May SPEI-6 were the best fits for the rates at the community level for H- and L-canopies, respectively. June SPEI-9 and May SPEI-6 were the best fits for *Q. ilex* rates for H- and L-canopies, respectively. October SPEI-15 was the best fit for *P. latifolia* rates for both H- and L-canopies. +, $p<0.1$; *, $p<0.05$ and **, $p<0.01$.

Canopy types	Fixed effect	*Q. ilex*	*P. latifolia*	All species
High canopy	Experimental drought	0.003	0.003	**0.005***
	SPEI	-0.0003	-0.0001	-0.001
	Experimental drought × SPEI	**-0.007***	-0.007	**-0.005***
Low canopy	Experimental drought	**0.024***	0.003	**0.004***
	SPEI	-0.002	**-0.008****	**-0.006***
	Experimental drought × SPEI	**-0.024***	0.0002	**-0.004+**

3.3 Mean stem mortality

Throughout the study period, the experimental drought significantly increased the mean mortality rate at the community level for H-canopy (both $p<0.01$, ANOVA), but not significantly for L-canopy (Fig. 2a,b; Table S5). For both H- and L-canopies, mean mortality rates of *Q. ilex*, however, were significantly higher than that of *P. latifolia* (both $p<0.05$, ANOVA). The experimental drought significantly increased the mean mortality rate of *Q. ilex* for L-canopy ($p<0.05$, ANOVA).

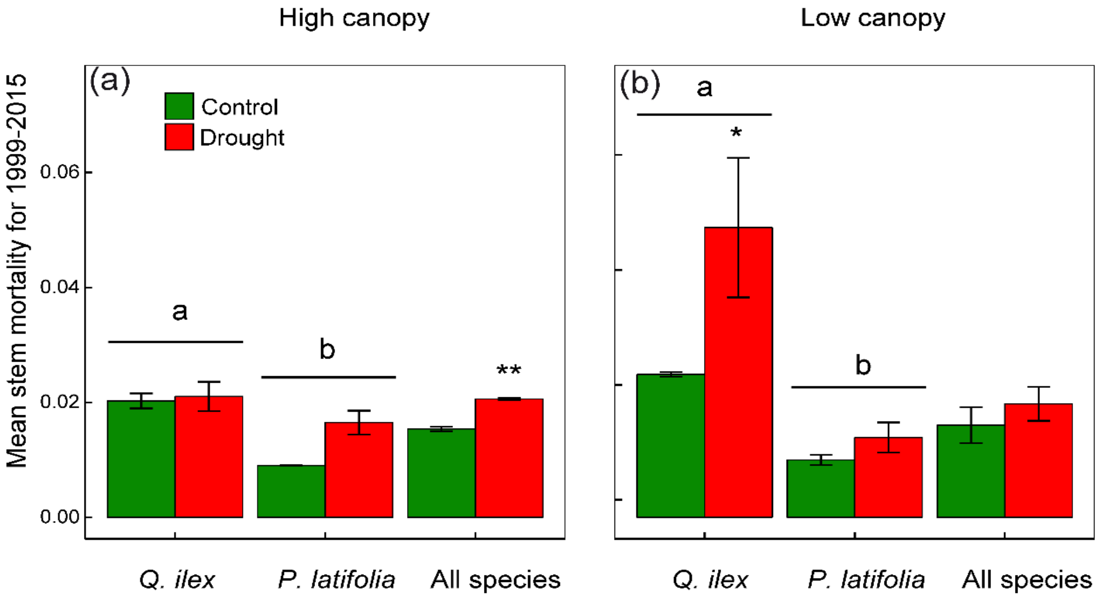

Fig. 2 Mean mortality rates at the community and species (*Q. ilex* and *P. latifolia*) levels over the study period (1999-2015) for H- (a) and L-canopies (b). The green and red bars represent control and experimental drought, respectively. The significant differences between control and drought were marked with the asterisk (*, $p<0.05$; **, $p<0.01$). The significant difference between *Q. ilex* and *P. latifolia* in each canopy type was labeled with different letters in each canopy type (both $p<0.05$).

3.4 Mean new recruitment rate

Only for H-canopy, the mean new recruitment rate at the community level was significant lower in drought plots than that of control ($p<0.05$, ANOVA) (Fig. 3a,b; Table S5). Only for H-canopy, the mean recruitment rate was significantly lower for *Q. ilex* than *P. latifolia* ($p<0.05$, ANOVA).

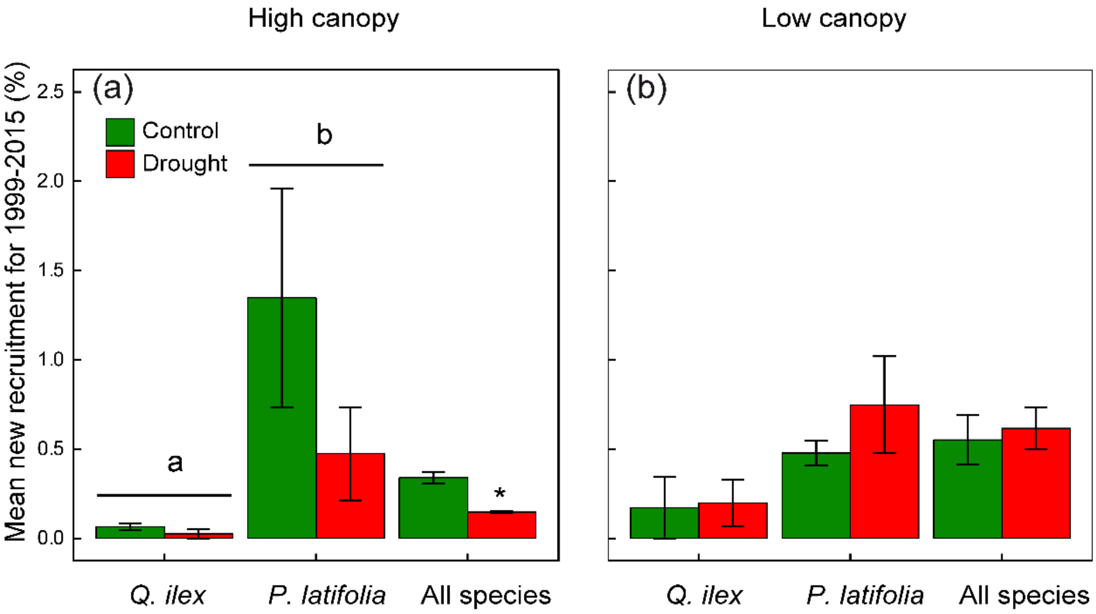

Fig. 3 New recruitment at the community and species (*Q. ilex* and *P. latifolia*) levels over the study period (1999-2015) for H- (a) and L-canopies (b). The green and red bars represent control and experimental drought, respectively. The significant differences between control and drought were marked with the asterisk (*, $p<0.05$). The significant difference between *Q. ilex* and *P. latifolia* in H-canopy was labeled with different letters ($p<0.05$).

3.5 Mean change in stem density

The drought treatment significantly decreased the mean stem density at the community level ($p<0.001$, ANOVA) for H-canopy, but not significantly for L-canopy (Fig. 4a,b; Table S5). For both H- and L-canopies, the changes in mean stem density, however, were significantly larger for *Q. ilex* than *P. latifolia* ($p<0.05$ and $p<0.01$, respectively, ANOVA).

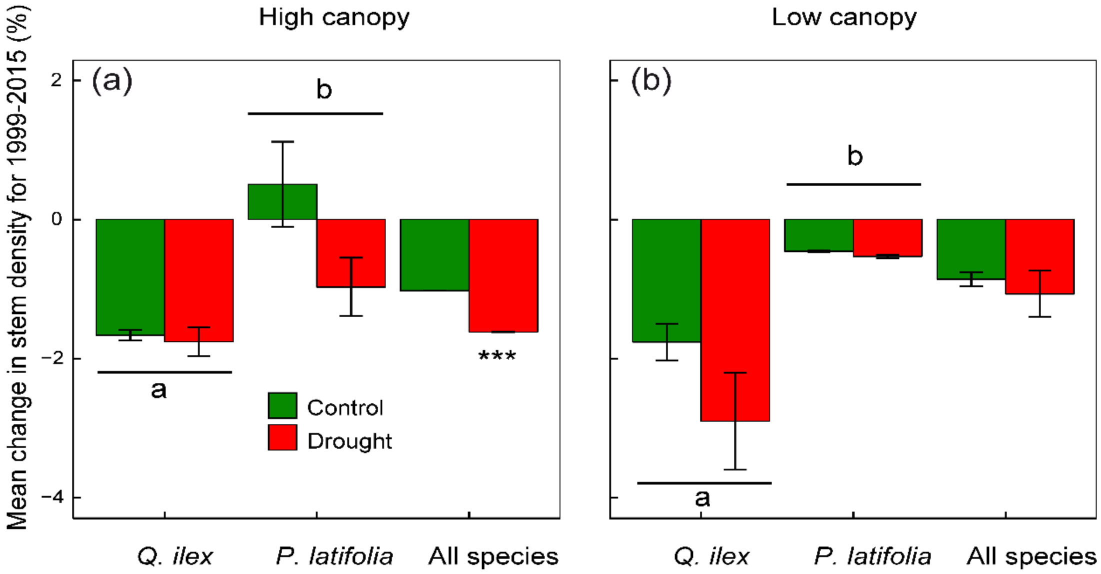

Fig. 4 Mean change in stem density at the community and species (*Q. ilex* and *P. latifolia*) levels over the study period (1999-2015) for H- (a) and L-canopies (b). The green and red bars represent control and experimental drought, respectively. The significant differences between control and drought were marked with the asterisk (***, $p<0.01$). The significant difference between *Q. ilex* and *P. latifolia* in each canopy type was labeled with different letters ($p<0.05$ for H-canopy and $p<0.01$ for L-canopy).

3.6 Mean change in stem growth

Mean change in stem growth at the community level has significantly decreased in the drought plots for both H- and L-canopies (both $p<0.05$, ANOVA) (Fig. 5a,b; Table S5). The experimental drought, however, marginally significantly decreased the mean stem growth of *P. latifolia* for H- canopy ($p<0.1$, ANOVA). The mean change in stem growth for *Q. ilex* was significantly lower than that of *P. latifolia* for L-canopy ($p<0.01$, ANOVA).

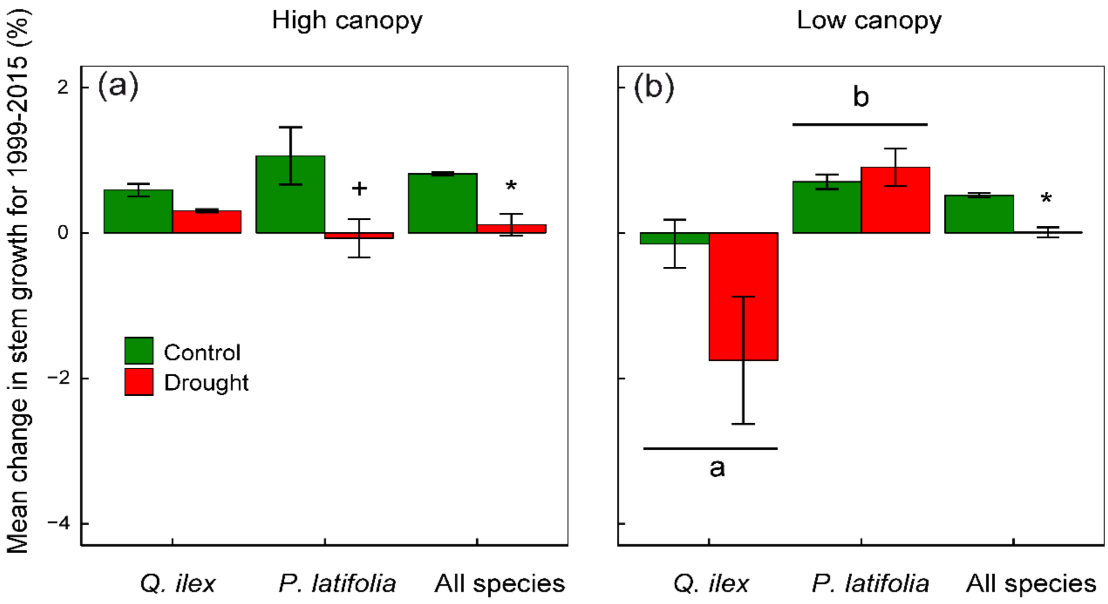

Fig. 5 Mean change in stem growth at the community and species (*Q. ilex* and *P. latifolia*) levels over the study period (1999-2015) for H- (a) and L-canopies (b). The green and red bars represent control and experimental drought, respectively. The significant differences between control and drought were marked with the asterisk (+, $p<0.1$; *, $p<0.05$). The significant difference between *Q. ilex* and *P. latifolia* in L-canopy was labeled with different letters ($p<0.05$).

3.7 Stem mortality and growth rates in the extreme dry years

For both H- and L-canopies, the rates of stem mortality at community level was higher in drought plots during extreme dry years compared to control in normal years and also compared to drought plots in normal years for L-canopy (Fig. 6a,b; Table S6). Only for L-canopy, the *Q. ilex* stem mortality was higher in drought plots during extreme dry years than control in normal years, control in extreme dry years and drought in normal years.

The rate of stem growth for all species and *Q. ilex* were severely affected by drought treatment during extreme dry years (Fig. 6c,d; Table S6). The stem growth rates at community level were lower in drought plots during extreme dry years compared to control in normal years both H- and L-canopies and also compared to control in extreme dry years and drought in normal years only for L-canopy. Stem growth rate of *Q. ilex* was lower in drought plots during extreme dry years compared to drought in normal years at both canopies and compared to control in normal years only in L-canopy. Stem growth rate of *P. latifolia* was lower in drought plots during extreme dry years compared to control in normal years and drought in normal years only in L-canopy.

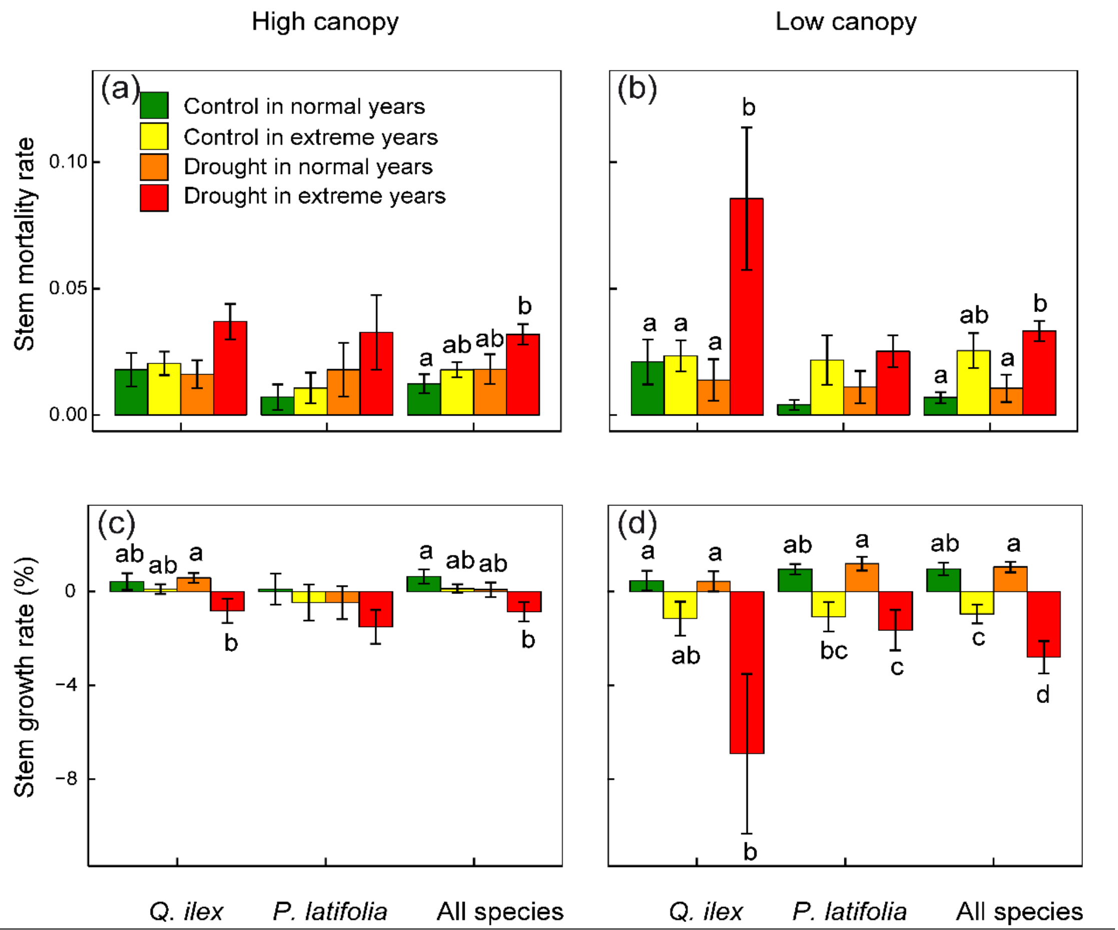

Fig. 6 Changes in stem mortality (a, b) and growth (c, d) rates during the normal (1999, 2004, 2010 and 2014) and extreme dry (2001, 2005, 2006, 2015) years. The stem mortality and growth rates in control and drought plots in normal and extreme dry years were showed in the H- and L- canopies. Significant differences are labeled with different letters (*p*<0.05).

3.8 Net-changes in relative stem abundance and growth

The patterns of net-changes in relative abundance and growth indicated different trajectories for the two species and canopy types for 1998-2015 (Fig. 7; Table S7). In H-canopy, there was a decrease for net-changes in stem abundance of *Q. ilex*, and an increase for that of *P. latifolia* (Fig. 7a). The values of *Q. ilex* in control were significantly lower than that of drought (*pLM*<0.001), as well as the percentage of *P. latifolia* in control was significantly higher than those of drought (*pLM*<0.05). However, we also observed that similar trends (decreasing for *Q. ilex* and increasing for *P. latifolia*) in low canopy (Fig. 7b, d). The net-changes of *Q. ilex* abundance and growth decreased over time in both control and drought plots. Reversely, the relative abundance and growth of *P. latifolia* increased over time for both control and drought plots. More interestingly, the comparative slopes of the net-changes for *Q. ilex* relative abundance and growth were significantly lower in the drought than the control plots (*pLM*<0.001 and *pLM*<0.001 for stem abundance and growth; respectively). Moreover, the comparative slopes of the net-changes for *P. latifolia* relative stem growth were higher in the drought than the control plots (*pLM*<0.001).

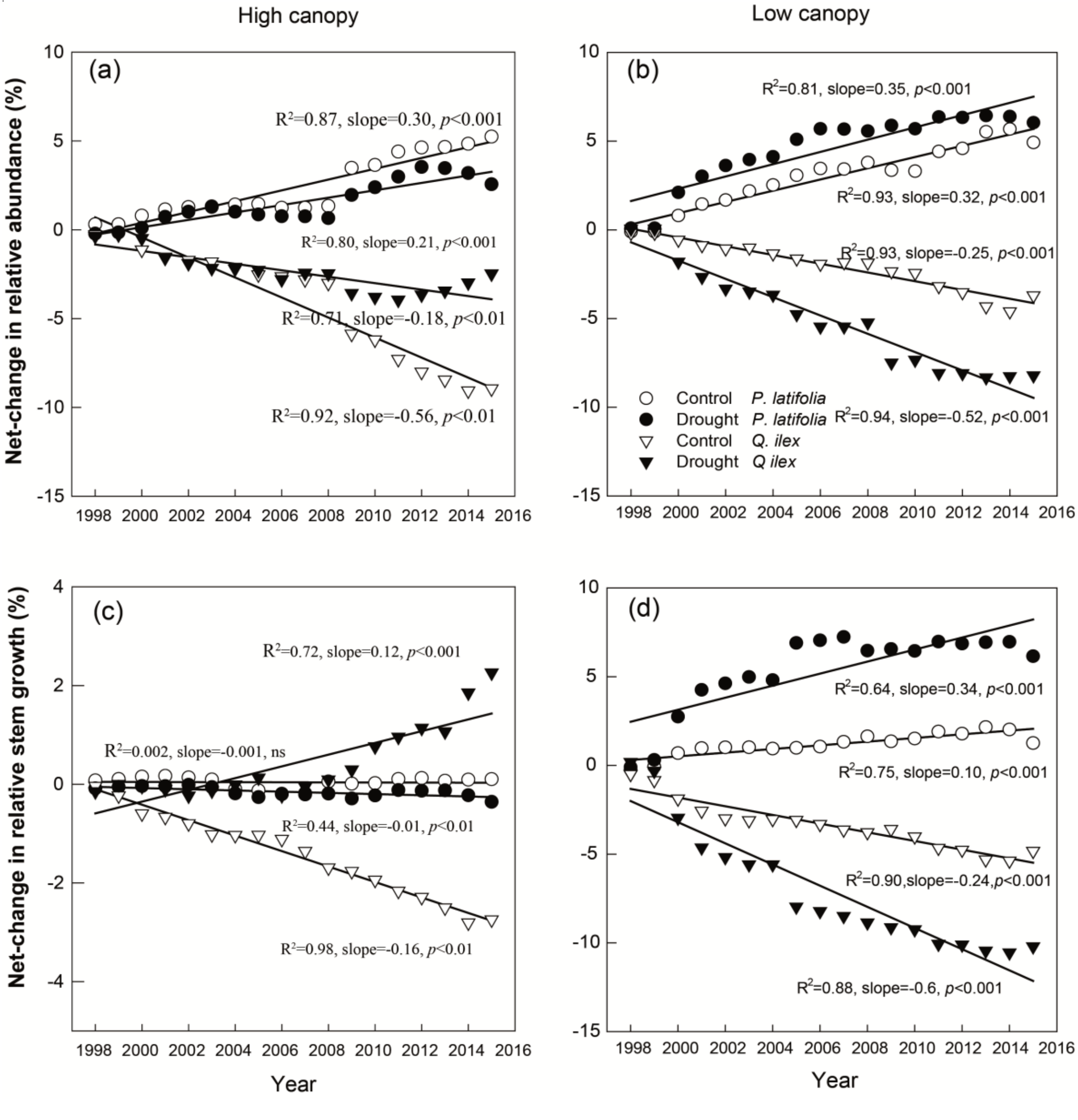

Fig. 7 Net-changes in relative abundance (a, b) and stem growth (c, d) for the two species *Q. ilex* and *P. latifolia* in T- and L-canopies during the study period (1998-2015). The R^2 corresponds to the regression between the net-changes over the study period of 1998-2015. The estimated slopes over the study period were tested by the model and shown in the figures. The net-changes in relative abundance and stem growth were significantly decreased by drought treatment for *Q. ilex* compared to control (both *p*<0.001). The net changes in relative stem growth were significantly increased by drought treatment for *P. latifolia* compared to control (*p*<0.001).

4. Discussion

Increasing drought is triggering the risk of vegetation shift in the holm oak forest

The long-term experimental drought triggered annual stem mortality at the community level for both H- and L-canopies. These results indicated that reduced water inputs in Mediterranean forests would lead to the episodes of tree mortality, which are consistent with the drought experiments in other forest ecosystems (Mueller *et al.*, 2005; Nepstad *et al.*, 2007; Allen *et al*., 2010; 2015). The impacts of experimental drought on annual stem mortality, however, differed between species and forest-canopy types. Our results suggested that annual stem mortality of *Q. ilex* was significantly increased by drought treatment in L-canopy, in agreement with the observation that drought induced dieback of forest canopies particularly over shallow soils (Peñuelas *et al.*, 2000, 2001; Lloret *et al.*, 2004a; Galiano *et al.*, 2012; Rivas-Ubach *et al.*, 2016). The *P. latifolia* rate, though, was not affected by the drought treatment in either H- or L-canopy, was consistent with previous short- and mid-term studies (Ogaya *et al.*, 2003; Ogaya & Peñuelas, 2007a; Barbeta *et al.*, 2013). Mean stem mortality rates also differed between the two species for both H- and L-canopies, with higher mortality in *Q. ilex* than *P. latifolia*. This may be due to differential water-use strategies such as foliar physiological traits (Peñuelas *et al.*, 1998; Ogaya & Peñuelas, 2003; Ogaya *et al.*, 2014), stem hydraulic conductivity (Martínez-Vilalta *et al.*, 2003; Barbeta *et al.*, 2012) and morphological adjustment (Ogaya & Peñuelas, 2007b; Limousin *et al.*, 2012; Liu *et al.*, 2015). Long-term drought would consequently lead to large-scale stem mortality in Mediterranean holm oak forests, especially those with low canopies in shallow soil depth, which could lead to unexpected changes in forest structure and ecosystem dynamics (Allen *et al.*, 2015; Doblas-Miranda *et al.*, 2015, 2017).

Long-term experimental drought affected the new recruitment, but the responses differed with the canopy types and species. Increased recruitment can be stabilized, compensating for the gap openings after drought induced tree mortality involving the loss of large stems (Lloret *et al.*, 2004a, 2012; Saura-Mas *et al.*, 2015). The number of new recruitments did not increase, while the drought treatment induced a recruitment for H-canopy though higher mean stem mortality. Successful recruitment, however, depends on the site characteristics, such as soil-water availability, seed stores and interspecific competition (Suarez & Kitzberger, 2008, 2010; Lloret *et al.*, 2012). Previous studies have reported that drought episodes had more serious impacts on the establishment of *Q. ilex* seedlings, the development of flower sex ratios (Misson *et al.*, 2011), fruit production (Ogaya & Peñuelas, 2007b; Sánchez-Humanes *et al.*, 2011; Liu *et al.*, 2015) and seed maturation and survival (Kéfi *et al.*, 2007; Galiano *et al.*, 2012; Saura-Mas *et al.*, 2015). In our study, we did not observe the treatment effects on recruitment rate of *Q. ilex* in both H- and L-canopies and tended to decrease the *P. latifolia* recruitment rate for H-canopy. This may be due to the self-thinning of small stems under long-term soil desiccation (Lloret *et al.*, 2004a, 2012; Barbeta & Peñuelas, 2016).. However, recruitment rate was lower for *Q. ilex* than *P. latifolia* only in the H-canopy plots, indicating lower seedling establishment of dominant species in the understory (Lloret *et al.*, 2004a, 2004b; Saura-Mas *et al.*, 2015). Across forest canopy types and treatments, recruitment was not significantly correlated with stem mortality for either community level, *Q. ilex* or *P. latifolia* in our study (Fig. S3a), suggesting that recruitment may lag behind mortality, emphasizing the necessity of monitoring recruitment on even longer timescales (Ogaya & Peñuelas, 2007b; Lloret *et al.*, 2012; Liu *et al.*, 2015).

Long-term drought treatment imposed a larger change in stem density, and species responded differently. Mean changes in stem density at the community level were significantly enhanced by experimental drought for H-canopy, which could be attributed to the higher mortality and lower new recruitment rates. Across forest types and treatments, the regressions indicated that mean change in stem density at community level was negatively correlated with mean stem mortality (Fig. S3b) and positively correlated with mean new recruitment (Fig. S3c). Moreover, the mean changes in stem density were significantly larger for *Q. ilex* than *P. latifolia* for both H- and L-canopies, which supported that *Q. ilex* was more drought-sensitive than *P. latifolia*. Linear regressions also indicated that mean change in stem density was positively correlated with mean new recruitment for *P. latifolia*, whereas it was not observed for *Q. ilex* (Fig. S3c). Therefore, these results confirm the sensitivity of *Q. ilex* demography to recent climate, likely leading to a composition shift in the near future.

Drought treatment decreased the mean changes in stem growth at the community level for both H- and L-canopies. Stem growth is an important surrogate of carbon storage in living plants and also an index of plant vigor and competitive ability, which can be associated with reductions in carbon assimilation (Ogaya & Peñuelas, 2003; Ogaya *et al.*, 2014), alive stems (Ogaya & Peñuelas, 2007a; Barbeta *et al.*, 2013, 2015; Ogaya *et al.*, 2014) and limited establishment of new seedlings (Ogaya & Peñuelas, 2007b; Pérez-

Ramos *et al.*, 2010; Liu *et al.*, 2015). Indeed, mean change in stem growth was negatively correlated with mean stem mortality (Fig. S3d) and positively correlated with mean change in stem density (Fig. S3e). Moreover, the rate of stem growth was significantly lower for *Q. ilex* than *P. latifolia* in L-canopy, in accordance with previous studies reporting that *Q. ilex* was more sensitive to drought than *P. latifolia* (Ogaya & Peñuelas, 2007a; Barbeta *et al.*, 2013; Liu *et al.*, 2015). *P. latifolia* stem growth was marginally decreased by drought treatment for H-canopy, but not for L-canopy. The changes in stem growth of *P. latifolia* were also positively correlated with mean change in stem density (Fig. S3e) and mean new recruitment (Fig. S3f). Previous studies have reported that drought modified *Q. ilex* morphological traits such as wood density and vessel size, which may influence water and nutrient transport and thereby limit plant growth (Limousin *et al.*, 2012; Savi *et al.*, 2015). Drier soils would thus limit the allocation of carbon to stem growth in holm oak forests, affecting future forest carbon budgets (McDowell *et al.*, 2008; Allen *et al.*, 2015).

The natural drought (SPEIs) and extreme dry episodes amplified the effect of the experimental drought on stem mortality and growth

Our results suggested that the forest suffered two dimensions of water limitations: increased soil desiccation caused by the experiment and natural droughts (SPEI). Global warming and the decreases in precipitation amounts have increasingly generated the water deficits of natural ecosystems, which may lead to the increases in soil desiccation and water limitation for plants in future. The experimental drought was simulated the future conditions of continuous moderate water stress. Extreme episodes such as severe drought and heat waves may exacerbate the severity of water-shortage, which are projected to become more frequent and destructive for natural ecosystems (Carnicer *et al.*, 2011). Even though either experimental drought (Nepstad *et al.*, 2007; da Costa *et al.*, 2010) or natural drought (Bennett *et al.*, 2015; Greenwood *et al.*, 2017) can trigger forest mortality, the combined effects of experimental drought and SPEI on forest dynamics have not been widely reported (Barbeta *et al.*, 2013, 2015; Liu *et al.*, 2015). The natural droughts in our study (quantified by October SPEI-7 and May SPEI-6) tended to accelerate the effects of the experimental drought on annual stem mortality at the community level for both H- and L-canopies. The natural droughts also accelerated the effects of experimental drought on annual *Q. ilex* stem mortality for both H- and L-canopies. Importantly, the rates of *Q. ilex* stem mortality were not correlated with June

SPEI-9 and were neither affected by the experimental drought alone in H-canopy, but increased significantly due to their synergistic effects. The synergistic effects, however, were observed for *P. latifolia* stem-mortality rates for either H- or L-canopy.

The timescales of SPEI associated with stem-mortality rates differed between the forest-canopy types and species. The effects of the natural droughts on stem-mortality rates at the community and species (*Q. ilex*) levels were correlated with longer SPEI timescales for H-canopy than L-canopy (community level: October SPEI-7 *vs* May SPEI-6 and *Q. ilex*: June SPEI-9 *vs* May SPEI-6). The longer timescales of SPEI associated with stem mortality in H-canopy, which may be explained by a higher water retention capacity of the deeper soils in H-canopy. Winter and spring drought amplified the effects of the experimental drought on annual *Q. ilex* stem-mortality rates for both H- and L-canopies, suggesting an importance role of the replenishment of belowground water reserves during these seasons for this Mediterranean species (Ogaya & Peñuelas, 2007a, 2007b, Barbeta *et al.*, 2013, 2015; Liu *et al.*, 2015). A longer SPEI timescale (October SPEI-15), however, was significantly correlated with annual *P. latifolia* stem mortality for L-canopy, showing that water inputs during summer and autumn are also critical for this species. Recurring natural droughts would also lead to the non-linear (abrupt) impacts on stem mortality of relatively more drought-resistant *P. latifolia*. For example, the long-lasting water deficits during 2011-2015 could also have induced the abrupt episode of mortality in 2015 for both H- (drought plots) and L-canopies (both control and drought plots) after exceeding the thresholds of drought resistance.

The effects of the experimental drought on stem mortality and growth were more severe during the climatically extreme dry years. We observed that stem mortality and growth at community level were significantly larger in the drought plots during the extreme dry years than the values in control during normal years, control during extreme dry years and drought during normal years both in H- and L-canopies. Particularly, stem mortality and growth of *Q. ilex* responded more strongly in drought plots during extreme dry years for L-canopy. For the first time, we report increased stem mortality and reduced growth also for *P. latifolia* in the L- canopy plots. The extremely dry years (lowest 10th percentile for MAP) from the period of 1975-2015 distributed during our study period (2001, 2005, 2006 and 2015). This frequency (per four years: 16/4) is high enough to threat the resilience capacity of *Q. ilex*, through severe effects on root and branch hydraulics (Martínez-Vilalta *et al.*, 2003; McDowell *et al.*, 2008; Barbeta *et al.*, 2012;

Limousin *et al.*, 2012), lead to hydraulic failure (McDowell *et al.*, 2008, 2016; Allen *et al.*, 2015) and/or the depletion of carbon reserves, such as non-structural carbohydrates (NSC) (Galiano *et al.*, 2012; Sala *et al.*, 2012; Rosas *et al.*, 2013). Thus, it is crucial important to study the combined effects of moderate drought stress and natural severe drought on forest structure and functioning changes.

Implications of broad-scale vegetation shifts in water-limited forests

Our results confirm that increased drought frequency are likely to trigger broad-scale vegetation shifts in water-limited ecosystems. We observed contrasting trajectories of the two species in L-canopy: the net-changes in relative abundance and growth decreased for *Q. ilex* and increased for *P. latifolia*. For H-canopy, the net-change in relative abundance also decreased for *Q. ilex* and increased for *P. latifolia* (Fig. 7a). The net-change in BA for *P. latifolia*, however, did not change for H-canopy, which may have been due to the strong self-thinning of small stems and growth limitations (Fig. 7c). More importantly, the net-changes in relative stem abundance and BA indicated that the drought treatment intensified the magnitudes of decreasing (*Q. ilex*) and increasing (*P. latifolia*) for L-canopy, suggesting that higher water deficits by experimental drought accelerated the vegetation shift (Fig. 7b, d). The climatic series demonstrated that water deficits in summer (e.g. August SPEI-3) and the whole spring and summer period (e.g. October SPEI-6) increased over the last 40 years at this site (Fig. S4c, e). Low canopy forests (lying over shallow soils) are experiencing a vegetation shift favoring the drought-resistant shrub. Long-term water deficits have been shown to accelerate vegetation shifts in forest–woodland ecotone (Allen & Breshears, 1998), evergreen and deciduous tropical forests in Ghana (Fauset *et al.*, 2012), coniferous forests in the United States (McIntyre *et al.*, 2015) and Mediterranean evergreen forests (Peñuelas & Boada, 2003; Peñuelas *et al.*, 2007). Our results by being experimental were obtained from a limited forest area. Future work should pay more attention on the responses of forest ecosystems at larger scales. The use of long-term experimental manipulations like the one of this study combined with broad-scale field surveys, long-term experimental manipulation and large-scale modelling to evaluate the consequences of persistent drying and episodic natural extremes on forest structure, functioning and services, are thus necessary and would provide reliable information for assessing broader scales of forest dynamics to ongoing and future climate regimes.

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Author contribution

D.L analyzed the data and wrote the paper and J.P. designed the experiment, performed the research and revised the manuscript. R.O. and A.B. performed the research and provided the experimental data and extensive editing and conceptual advice. X.Y. helped in manuscript revision.

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Supporting Information

Fig. S1 Climate data of temperature and precipitation during the period of 1975-2015.

Fig. S2 Seasonal soil-water content responses to drought treatment in H- and L-canopies.

Fig. S3 Relationships between mean stem mortality and mean new recruitment (a), between mean stem mortality and mean change in stem growth (b), between mean stem mortality and mean change in stem growth (c), between mean change in stem density and mean new recruitment (d), between mean change in stem density and mean change in stem growth (e) and between mean recruitment and mean change in stem growth.

Fig. S4 SPEIs of winter-spring (May SPEI-6 and June SPEI-9) (a, b), Autumn-summer-spring (October SPEI-9 and October SPEI-15) (c, d) and summer (August SPEI-3) (e) during the period of 1975-2015for our study site.

Table S1 Characteristics of the two canopy types in our study site.

Table S2 The results for random factors selected for the models for the annual stem mortality at community level, *Q. ilex* and *P. latifolia* for the two canopy types.

Table S3 The selected SPEIs for the interaction model (treatment x SPEI) for annual stem mortality rates during the study period of 1999-2015.

Table S4 The episodes of climate normal and extreme dry years during the period of 1975-2015.

Table S5 The analysis of drought treatment on mean stem mortality, mean new recruitment, mean change in stem density and mean change in stem growth for 1999-2015.

Table S6 The results of Tukey's HSD post-hoc tests for the stem mortality and growth rates during the normal and extreme drought years.

Table S7 The comparisons between drought and control for the net-changes in relative abundance and stem growth of the two species *Q. ilex* and *P. latifolia*.