Universitat Autònoma de Barcelona

Climate change impacts on the mediterranean forest and shrubland, their ecophysiology, demography and community composition

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Tesis doctoral

Centre de Recerca Ecològica i Aplicacions Forestals

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Abstract

Anthropogenic disturbances induce rapid global warming and precipitation changes with a great impact on Mediterranean ecosystems, leading to changes in plant physiological processes (e.g. photosynthesis), demography (e.g. stem growth, mortality and seedling recruitment) and community structure and composition (e.g. species loss, dominance shifts). Moreover, great changes for future climate scenarios are expected to be unabated by most climatic models, and the potential threats of these climatic changes are still dramatic even in the context of under 2 °C global warming target. However, to date, the effective method to accurately assess species growth, community structure and composition shifts in response to future climate change are not well-established. Here, we conducted long-term (≥15 years) climate manipulation experiments in a Mediterranean holm-oak forest and in a Mediterranean shrubland to study the changes in plant ecophysiology, demography and community structure.

In the Mediterranean forest, long-term experimental drought decreased photosynthetic rates of the dominant species of *Q. ilex* and *P. latifolia*, but the latter was more resistant to drought, which could lead it to gain a competitive advantage relative to *Q. ilex* in the future. Moreover, the long-term experimental drought significantly decreased the biomass increment and increased stem mortality, branch and leaf litterfall, and these responses were also strongly correlated with natural droughts. However, these responses were associated with natural droughts, but were also species-specific because *Q. ilex* resulted more vulnerable to drought than *P. latifolia*. Moreover, there was a dampening effect of these changes in aboveground net primary productivity (ANPP) during the long-term experimental drought. The long-term experimental drought also increased annual rates of stem mortality and decreased stem growth at the community level in plots dominated by tall trees (high canopy plots (H-)) and low trees (low canopy

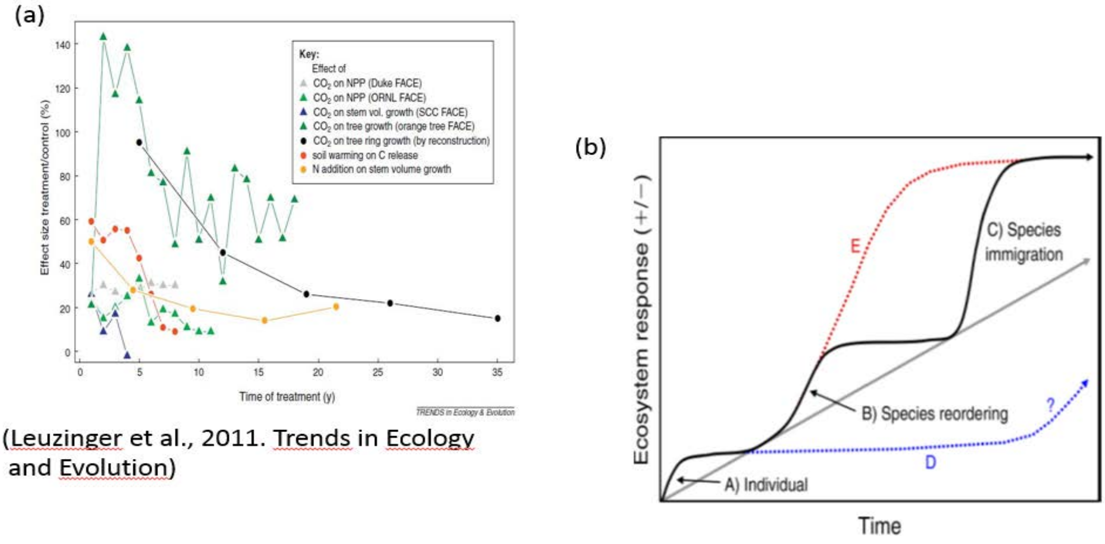
plots (L-)) over the study period. The changes in mean stem mortality, recruitment, stem density and growth indicated that *Q. ilex* was more sensitive to drought than *P. latifolia*. The long-term experimental drought combined with natural droughts accelerated the decreases in percentage of abundance and basal area of *Q. ilex*, but promoted the increases for *P. latifolia* in low canopy plots (L-), indicating a vegetation shift with drier conditions.

In the Mediterranean shrubland, long-term experimental warming moderately influenced photosynthetic rates, shoot water potential and stomatal conductance in *E. multiflora*, whereas drought treatment strongly influenced these variables, especially during summer. However, *E. multiflora* also improved the acclimation to drier and hotter conditions by the adjustments of stomatal conductance and water-use efficiency. And yet, we found that warming and drought imposed the decrease in species abundance associated with the species distributions. Long-term experimental warming caused a progressive decrease in the abundance of those species distributed in cooler regions. Correspondingly, reduced precipitation resulted in abrupt decreases in wet-distributed species, followed by a delayed increase in dry-distributed species. Moreover, we observed that the decreases in species richness in both warming and drought treatments, and the strongest decreases in community diversity and evenness were found under the drought treatment. The responses in community structure and compositional shifts were also strongly correlated with natural droughts. Thus, our results suggest that rapid warming and drought have imposed profound influences on plant physiological processes, population demography and community composition. More attention should also be paid on these changes at regional- and global-scales.

General introduction

Anthropogenic disturbances have accelerated global climate changes with notable warming and changes in the distribution of precipitations (drier conditions in some regions) since the middle of last century, which subsequent effects in plant growth, population demography and community structure and composition (Myers *et al*., 2000; Walther *et al*., 2002; Hector & Bagchi *et al*., 2007; Hooper *et al*., 2012; Peñuelas *et al*., 2013; 2017; Scheffers *et al*., 2016). Mediterranean ecosystems are one of the hotpots suffering profound impacts induced by climate change, including loss of endemic species (Myers *et al.*, 2000; Schröter *et al.*, 2005; Peñuelas *et al.*, 2007; Gottfried *et al.*, 2012), decrease in plant photosynthetic activities (Llorens *et al*., 2003; Prieto *et al*., 2009a; Ogaya *et al*., 2014) and biomass growth (Ogaya & Peñuelas, 2007; Barbeta *et al*., 2013) and shifts in community structure and composition (Prieto *et al*., 2009b; Peñuelas *et al*., 2013; 2017; Doblas-Miranda *et al*., 2015). Moreover, future climate changes are expected to be unabated in the Mediterranean ecosystems, especially combined with climatic extremes as projected by most climatic models, and the impacts of these climate changes are expected to be strong for the immediate coming decades, leading to profound influences for multiple ecosystem functions and climatic feedbacks (Myers *et al*., 2000; Sala *et al*., 2000; Doblas-Miranda *et al*., 2015; 2016).

Effective methods for accurately assessing the impacts of ongoing and future climate change on plant communities, remain poorly established. Over the last two decades, observations of examining biodiversity losses and community changes, are conducted in different time periods, but the responses are usually confused by other covarying factors (e.g. human disturbances) (Schröter *et al.*, 2005; Gottfried *et al.*, 2012). Performing climate manipulation experiments in natural ecosystems offers an opportunity to study plant photosynthetic activities, biomass growth, community structure and functioning changes under manipulated future climatic conditions of the study sites, which can avoid the confounding influences by other covarying factors (Beier *et al.*, 2004; 2012; Leuzinger *et al.*, 2011; Wu *et al.*, 2011; De Boeck *et al.*, 2015). Indeed, numerous experiments have reported strong changes in plant biomass production (Ogaya & Peñuelas, 2007; Prieto *et al.*, 2009c; Barbeta *et al.*, 2013) and species diversity (Prieto *et al.*, 2009b) under manipulated climatic conditions. However, the effect sizes of climatic treatments at short-term are abrupt (Peñuelas *et al.*, 2007, Prieto *et al.*, 2009b; c), but also dampening (Leuzinger *et al.*, 2011; Barbeta *et al.*, 2013) and even none-shifts in long-term experiments can be found (Fig 1a) (Grime *et al.*, 2008; Tielbörger *et al.*, 2014). As predicted by the hierarchical theory, the initial and strong response usually occurr at individual level (such as physiological processes), and also affect the community structure and composition over time, leading to large shifts in functioning and service



(Leuzinger et al., 2011. Trends in Ecology and Evolution)

(Smith et al.,2009. Ecology)

Fig. 1 (a) the effect size is dampening with the experimental years and (b) the hierarchical response of field experiment from the scales of species, population and community.

(Fig 1.b) (Smith *et al*., 2009). However, most results from field experiments are conducted in short-term scales (<10 years), which cannot provide accurate evaluation of the species responses, population dynamics and community structure changes under future climatic conditions.

Long-term climate manipulation experiments are expected to accurately reveal the responses of species adaptions, population demographies and community dynamics under long-term climatic changes (Luo *et al.*, 2011; Wu *et al.*, 2011; Beier *et al.*, 2012; Estiarte *et al.,* 2016). Long-term experiments can also examine the additive and synergistic effects of natural extremes (droughts and heat waves) that are no evaluable in short-term experiments. Conducting long-term manipulation field experiments is thus the best way to comprehensively record transformative changes and to identify the plant physiological, demographical and community dynamics in response to the effects of climatic variability (Smith *et al.*, 2009; Luo *et al.*, 2011; Peñuelas *et al*., 2014; 2017). However, the long-term (>10 years) experiments are relative rare, especially for long-living species of the semi-arid Mediterranean ecosystems.

Here, we conducted a long-term climatic experiments in a Mediterranean holm-oak forest and in an early-successional shrubland.

(a) Holm-oak forest site: the experiment was conducted on a south-facing slope (25%) in the Prades Holm oak forest in southern Catalonia (northeastern Iberian Peninsula) (41°21′N, 1°2′E; 930 m a.s.l.). The drought experiment began in 1999 and consisted in a partial rainfall exclusion that simulated a reduction of a 15% of soil moisture for the Mediterranean holm oak forest (Fig. 2). Four High-canopy plots and four Low-canopy plots (10 × 15 m) were established at the same attitude. Half of the plots (two replicates per canopy type) were randomly selected to receive the drought treatment, and the other four plots remained as controls (natural conditions without treatment). 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 depth) excavated along the upper edge of the plots.

Fig. 2 Experimental drought in the Mediterranean holm-oak forest (started in 1999)

(b) Mediterranean shrubland site: the study site is in a Mediterranean shrubland in the Garraf Natural Park, near Barcelona (northeastern Spain) (41º18´N, 1º49´E; 210 m a.s.l.). This shrubland is the result of an early natural succession after two severe wildfires in 1982 and 1994 in a *Pinus halepensis* forest. The vegetation is composed mainly of short perennial shrubs (<1.5 m height) that dominated the regrowth after the two fires. We conducted a nocturnal-warming experiment from 1999 to 2014 and a drought experiment from 1999 to 2015 on three replicate blocks selected along a south-facing slope (13% slope) (Fig. 3). Each block contained one randomly distributed warming, drought and control plots (4×5 m), for a total of nine plots. The warming experiment was established by passive nocturnal warming covering the vegetation with reflective aluminum curtains (ILS ALU, AB Ludvig Svensson, Kinna, Sweden) at night reducing outgoing infrared radiation. The curtains were activated automatically by preset light conditions (<200 lux). The drought experiment was conducted by excluding precipitation with transparent waterproof plastic covers during the wettest seasons (spring and autumn) to prolong the summer drought. A rain sensor activated the covers to intercept precipitation and retracted them when the rain stopped. The coverings for the warming and drought experiments were removed at wind speeds >10 m s^{-1} to prevent damage. Scaffolding (1.2 m height) was installed in each plot to support the covering systems. Scaffolding was also installed in the control plots, but no curtains or covers were installed.

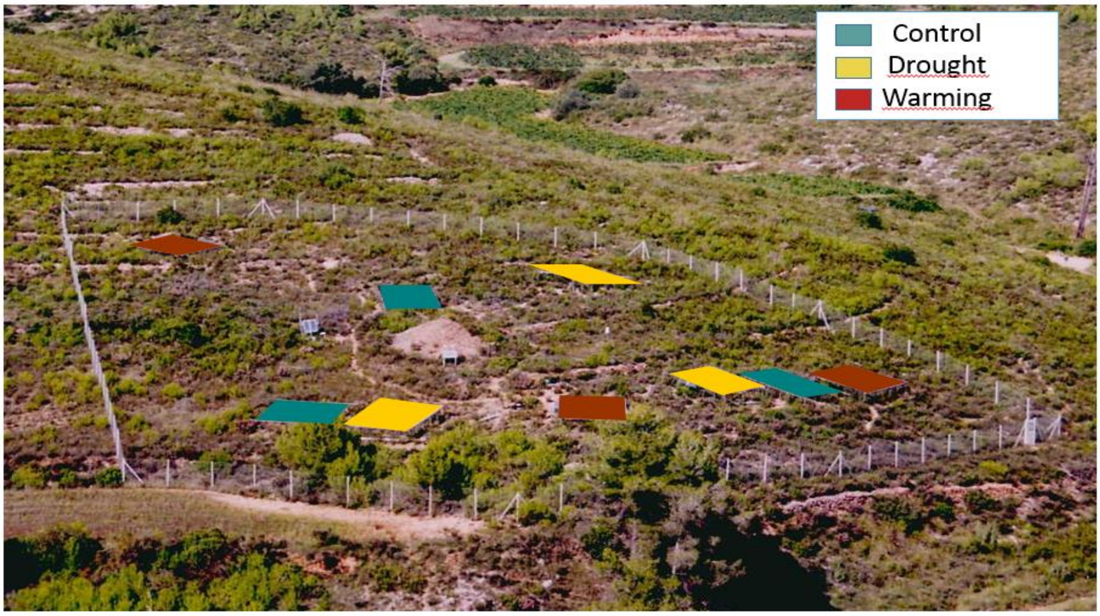


Fig. 3 Experimental warming and drought in the Mediterranean shrubland (started since 1999)

Objectives of the thesis

The main objective of this thesis was to study the impacts of experimental warming and drought and natural extreme droughts on physiological, demographical and community changes of the Mediterranean forest and shrubland. To accomplish that, we divided the thesis into 3 blocks (two chapters per block). The first block is focused on the physiological changes (photosynthesis and water relations) of Mediterranean forest and shrubland in response to long-term experiments. The second block is focused on growth, productivity and species-specific competition of Mediterranean holm-oak forest to long-term experimental drought. Finally, the third block is focused on the community structure and compositional shifts of the Mediterranean shrubland in response to long-term experimental warming and drought. In order to answer all of these questions of the thesis, we further reveal the specific purposes in each chapter of the thesis.

In chapter 1, we aimed to study the impacts of long-term experimental drought on net photosynthetic rate, stomatal conductance and leaf water potential of the two co-existing dominant species, and to elucidate the possible advantages of *P. latifolia* relative to *Q. ilex* under future drier conditions.

In chapter 2, we aimed to investigate the effects of long-term experimental warming and drought on net photosynthetic rate, stomatal conductance, leaf water potential and water-use efficiency of a dominant shrub, *E. multiflora*, and to determine physiological adjustments at long-term.

In chapter 3, we aimed to study the effects of long-term drought and natural extreme drought on biomass accumulation and litterfall in the two dominant species: *Q. ilex* and *P. latifolia*, to study the aboveground net primary productivity (ANPP) of the Mediterranean holm-oak forest, and to reveal the hierarchical responses at long-term.

In chapter 4, we aimed to study the effects of long-term drought and extreme droughts on stem mortality, recruitment, density and growth of the Mediterranean holm-oak forest, and to address the forest degradation and vegetation shifts under the continuous experimental drought and natural droughts.

In chapter 5, we aimed to study the effects of long-term experimental warming and drought on species abundance and community structure changes, and to reveal species responses to temperature and drought correlated with their distribution niches.

In chapter 6, we aimed to study the effects of long-term experimental warming and drought on community diversity (species richness, Shannon index and evenness) and shrub and herb dominance shifts, and to evaluate the impacts of natural extreme droughts on these responses, especially in this early-successional shrubland ecosystem.

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Block 1. Ecophysiological changes (leaf photosynthetic activities) and water relations

Chapter 1 Foliar CO_2 in a holm oak forest subjected to 15 years of climate change simulation

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Abstract

A long-term experimental drought to simulate future expected climatic conditions for Mediterranean forests, a 15% decrease in soil moisture for the following decades, was conducted in a holm oak forest since1999. Net photosynthetic rate, stomatal conductance and leaf water potential were measured from 1999to 2013 in *Quercus ilex* and *Phillyrea latifolia*, two co-dominant species of this forest. These measurements were performed in four plots, two of them received the drought treatment and the two other plots were control plots. The three studied variables decreased with increases in VPD and decreases in soil moisture in both species, but the decrease of leaf water potential during summer drought was larger in *P. latifolia*, whereas *Q. ilex* reached higher net photosynthetic rates and stomatal conductance values during rainy periods than *P. latifolia*. The drought treatment decreased ca. 8% the net photosynthetic rates during the overall studied period in both *Q. ilex* and *P. latifolia*, whereas there were just non-significant trends toward a decrease in leaf water potential and stomatal conductance induced by drought treatment. Future drier climate may lead to a decrease in the carbon balance of Mediterranean species, and some shrub species well resistant to drought could gain competitive advantage relative to Q. ilex, currently the dominant species of this forest.

Keywords: climate change, drought, Mediterranean forest, *Phillyrea latifolia*, photosynthetic rates, *Quercus ilex*, stomatal conductance.

1. Introduction

Summer drought has been recognized as the major stress limiting plant growth and development in all Mediterranean regions of the world [1]. In these Mediterranean regions, a reduction in water availability is expected for the near future as a result of decreases of precipitation and increases of temperatures, and the consequent increases of evapotranspiration rates [2], which are predicted to be greater than those that already occurred in the twentieth century by most General Circulation Models [2]. Lower water availability is also projected for this region by ecophysiological models such as GOTILWA [3,4].

Holm oak (*Quercus ilex* L.) is a widely distributed tree species in Mediterranean basin, that tolerates drought effects by reducing its leaf area and by stomatal closure control [5,6]. Mock privet (*Phillyrea latifolia* L.) is a tall shrub species usually associated with holm oak, but with higher capacity to tolerate drought than *Q. ilex* [7,8,9]. *P. latifolia* showed smaller photosynthetic rates in autumn, winter and spring, and lower photochemical efficiency in winter than *Q. ilex*, but in summer, *P. latifolia* was the species with larger photosynthetic rates and photochemical efficiency [7,9]. Under a decrease in water availability, it has been observed a strong reduction in stem growth and aboveground biomass increment in *Q. ilex*, not observed in *P. latifolia* [10], and higher vulnerability to xylem embolism in *Q. ilex* than in *P. latifolia* [11]. Larger leaf defoliation and stem mortality rates were also higher in *Q. ilex* than in *P. latifolia* under severe drought [10,12,13], moreover, these differences could decrease due to a long-term adaptation to new climatic conditions [13]. These two co-occurring woody Mediterranean species may be subjected to a decrease in net photosynthetic rates and to an increasing defoliation and risk of stem mortality under future forecasted climatic conditions, but their different tolerance to hot and dry climate may lead to changes in their capacity to compete between them. *Q. ilex*, the currently dominant species of this Mediterranean forest, could decline in favor of *P. latifolia*, more adapted to new climatic constrains.

Our aim was to study the effect of a long-term experimental drought on photosynthetic activity in these two co-dominant species of the Mediterranean forest in order to elucidate this possible advantage of *P. latifolia* compared to *Q. ilex* under these drier conditions. The experimental period covered several years and the four seasons of the year, including inter-annual and inter-seasonal variability in the study of the responses of these plants to drought treatment. Climate change experiments in natural ecosystems are scarce, but they are especially valuable because small trees in lab environments could show different responses to climate than mature trees. As far as we know, this is one of the longest climate change experiments in a natural forest of the world, and all possible long-term adaptations to the experimental treatment are included in our long-term measurements.

2. Material and methods

2.1. Study site

The studied area is located in Prades holm oak forest, Catalonia, North-Eastern Spain (41º 21’ N, 1º 2’ E), at an altitude of 950 m a.s.l and on a south-facing slope (25% slope). The soil is a Dystric Cambisol over Paleozoic schist, and its depth ranges from 35 to 100 cm. This forest is dominated by holm oak (*Quercus ilex* L.) (8633 stems ha^{-1}), mock privet (*Phillyrea latifolia* L.) (3600 stems ha^{-1}) and strawberry tree (*Arbutus unedo* L.) (2200 stems ha^{-1}), with abundant presence of other evergreen species well adapted to dry conditions such as *Erica arborea* L., *Juniperus oxycedrus* L., *Cistus albidus* L., and occasional individuals of deciduous species such as *Sorbus torminalis* (L.) Crantz, and *Acer monspessulanum* L. This forest was not perturbed during the last 60 years, and the maximum height of the dominant species is about 6-10 m tall. The average annual temperature is 12.3ºC and the average annual rainfall 603 mm (data from 1999 to 2012, both included). Summer drought is pronounced and usually lasts for 3 months.

2.2. Experimental design

Four 15 m x 10 m plots were delimited at the same altitude along the slope. Two randomly selected plots received the experimental treatment, and the two other plots remained as control plots. The experimental treatment consisted in a partial rain exclusion by suspending plastic strips at a height of 0.5-0.8 m above the soil (covering 30% of soil surface), and the excavation of a 0.8 m deep ditch at the upper part of the plots to intercept runoff water. An automatic meteorological station installed in the study site monitored temperature, photosynthetic active radiation, air humidity, and precipitation during the overall studied period. Soil moisture was measured each annual season using a time domain reflectometer (Tektronix 1502C, Beaverton, OR USA). The reflectometer was connected to the ends of three stainless cylindrical rods, 25 cm long, fully driven into the soil [14], and four sites per plot were randomly selected to install three steel cylindrical rods.

2.3. Gas exchange and leaf water potential measurements

Net photosynthetic rate (A) and stomatal conductance (g_s) were measured in leaves fully exposed to the sun during several annual seasons during the duration of the experimental drought treatment (from 1999 to 2013) (Table 1). Two current-year leaves of two different trees per species were measured in each plot around midday using portable gas exchange systems: an ADC4 system connected to a PLC4 chamber (ADC Inc., Hoddesdon, Hertfordshire, UK) from 1999 to 2001; a CIRAS2 system (PP Systems, Hitchin, Hertfordshire, UK) from 2003 to 2005; and a LI-6400XT system (LI-COR Inc., Lincoln, Nebraska, USA) from 2012 to 2013. Leaf water potential (Ψ) was measured in one terminal twig of two different trees per species in each plot using a Scholander pressure chamber (PMS Instrument Co., Corvallis, Oregon, USA). Leaf water potential (Ψ) was measured during midday at the same date than gas exchange measurements. Midday values of vapor pressure deficit (VPD) were calculated for each day of A, g_s, and Ψ measurements.

Table 1 Date of all gas exchange measurements across the overall duration of the rainfall exclusion experiment (depicted in grey color).

	99	00	01	02	03	04	05	06	07	08	09	10	11	12	13
Wi	■	■	■		■	■	■								■
Sp	■	■			■		■							■	■
Su	■	■			■		■							■	
Au	■	■	■		■	■								■	

2.4. Stem mortality

The percentage of dead stems were calculated in both species counting, in each plot, the number of alive stems at the start of the experiment (1999) and how many of them survived at the end of the experiment (2013).

2.5. Statistical analyses

Simple linear regressions were conducted to examine the relationship of A, g_s, and Ψ values with VPD and soil moisture. Also, other simple linear regressions were conducted to examine the relationships of A with Ψ and g_s values. Later, multiple linear regressions were conducted to test the meteorological influence on A, g_s, and Ψ values. A, g_s, and Ψ were the dependent variables of different multiple linear regressions, whereas VPD and soil moisture were the predictor variables in all multiple linear regressions. In these multiple regressions the forward stepwise regression technique was used. For each day of measurements, analyses of variance (ANOVAs) were conducted with A, g_s and Ψ values as dependent variables, and species and treatment as independent factors. A repeated measurements ANOVA was conducted with soil moisture values in each plot as dependent variable and treatment as independent factor. Other repeated measures ANOVAs were conducted with A, g_s and Ψ values as dependent variables, and species and treatment as independent factors. Finally, a two-way ANOVA was conducted with the percentage of dead stems as dependent variable, and species and treatment as independent factors. Data of percentage of dead stems (p) was transformed to arc sin (p) to reach the normality assumptions of the ANOVA. All analyses were performed with the Statistica 8.0 software package (Statsoft Inc., 2007, Tulsa, Oklahoma, USA).

3. Results

Climate data of the studied site was typically Mediterranean: hot and dry summers, slightly cold winters, and precipitations concentrated in spring and autumn seasons (Fig. 1), excepting autumn 2004 and spring 2005, that were associated to unusual rainfall and soil moisture values (Fig. 1). The drought treatment reduced about 13% soil moisture of drought plots compared to control plots ($P<0.01$), but this reduction was larger during rainy seasons and lower during dry seasons (Fig. 1).

Maximum Ψ, A, and g_s values were reached during rainy seasons (mainly spring and autumn) and during winter, while minimum values were reached during summer drought (Figs. 2 and 3). These three variables were negatively related with VPD and positively related with SM (Fig. 4), although for the later the relationships tended to saturate at high SM (Table 2). Ψ values were more dependent on VPD and SM in *P. latifolia* than in *Q. ilex* (Fig. 4), so *P. latifolia* reached lower Ψ under hot and dry conditions than *Q. ilex* ($P<0.01$). In contrast, A and g_s values were more dependent on VPD and SM in *Q. ilex*

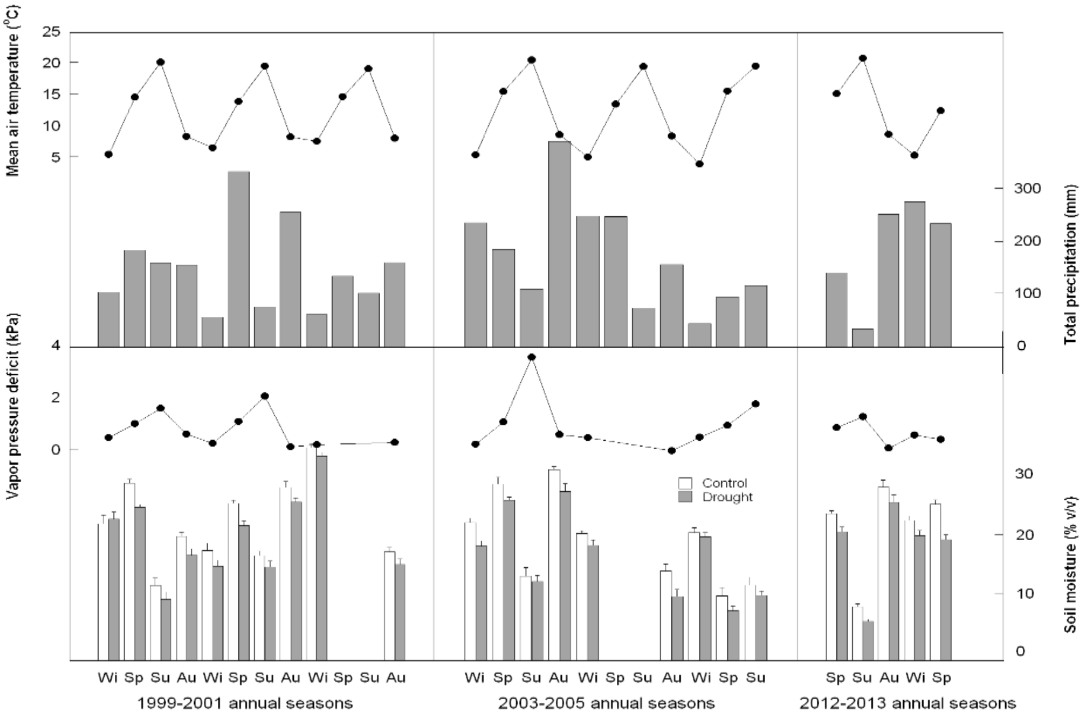

Fig. 1. Monthly values of mean air temperature and total precipitation in upper panels. Instantaneous values of vapor pressure deficit and soil moisture at the same time of leaf water potential and gas exchange measurements. Error bars in soil moisture data indicate the standard error of the mean (n=2 plot means of 4 measurements each).

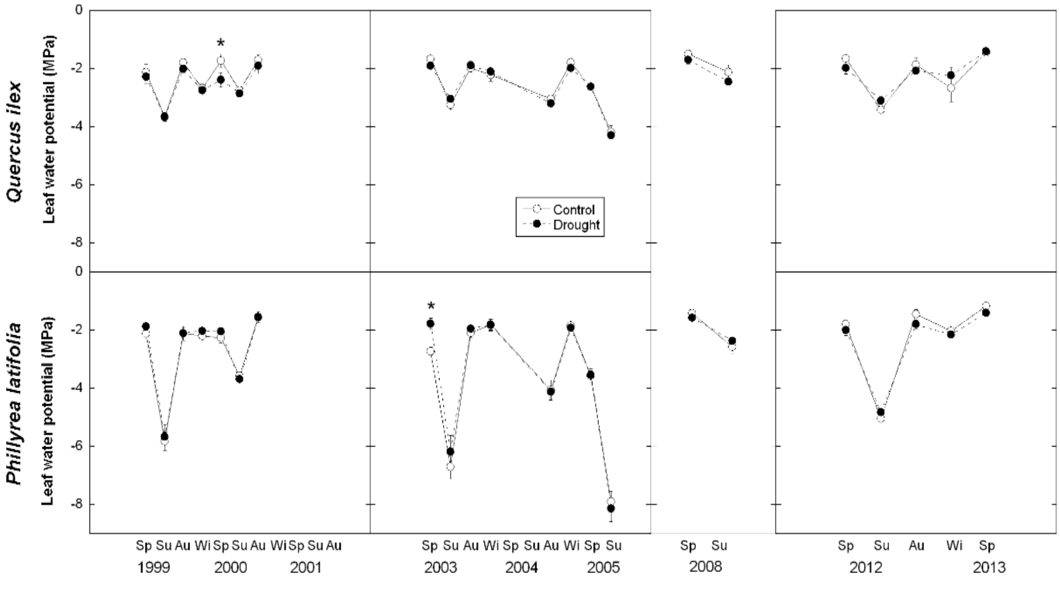

Fig. 2. Seasonal course of leaf water potential (Ψ) in *Q. ilex* and *P. latifolia* during the overall experimental period. Error bars indicate the standard error of the mean (n=2 plot means of 2 measurements each). An asterisk indicates significant difference induced by drought treatment (P<0.05).

than in *P. latifolia* (Fig. 4), so *Q. ilex* reached on average, higher A and g_s values than *P. latifolia* (*P*<0.01), especially during more humid seasons. A was highly correlated with Ψ and g_s in both species (Fig. 5), but the correlation was stronger in *Q. ilex* than in *P. latifolia* (Table 3; Figs. 6 and 7).

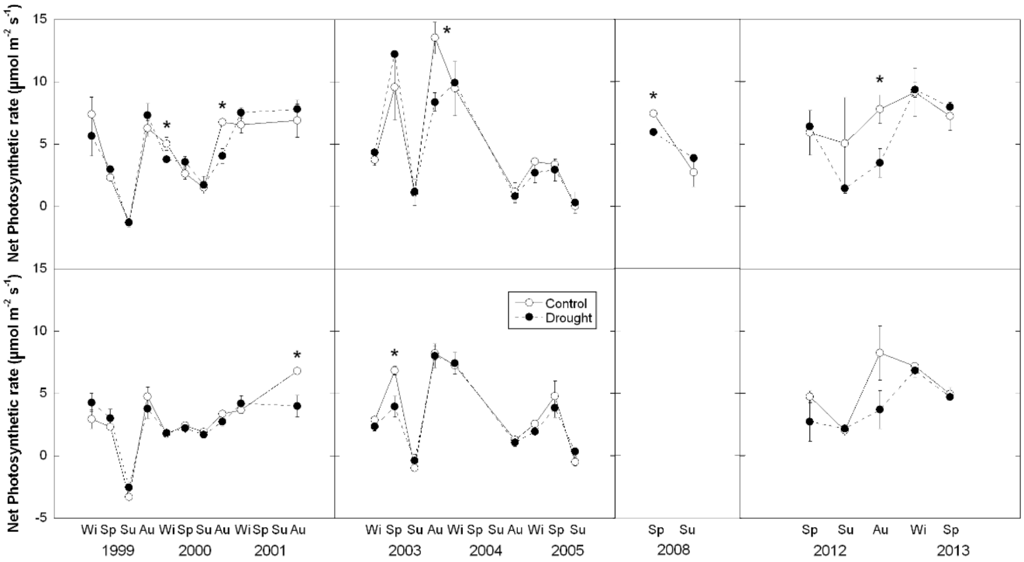

Fig. 3. Seasonal course of net photosynthetic rate (A), in *Q. ilex* and *P. latifolia* during the overall experimental period. Error bars indicate the standard error of the mean (n=2 plot means of 2 measurements each). An asterisk indicates significant difference induced by drought treatment (P<0.05).

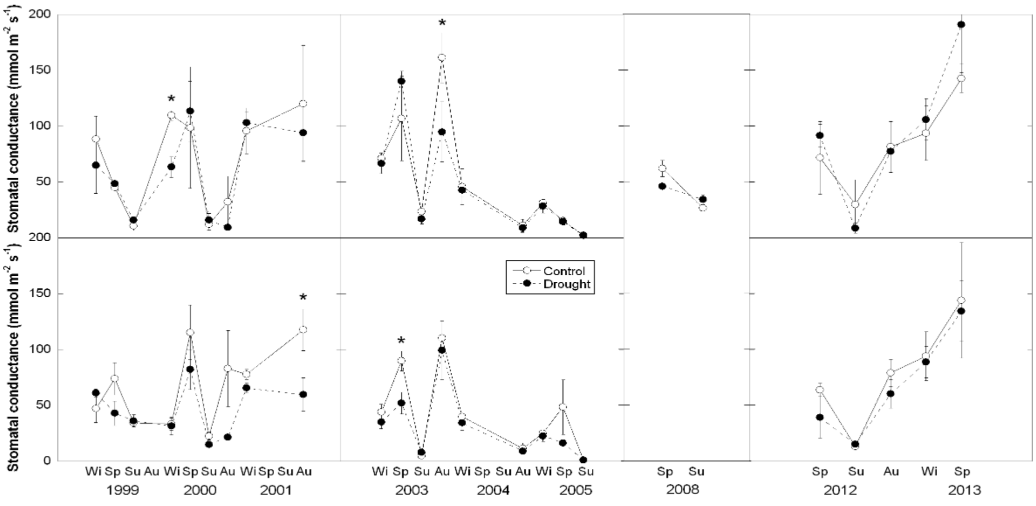

Fig. 4. Seasonal course of stomatal conductance (g_s) in *Q. ilex* and *P. latifolia* during the overall experimental period. Error bars indicate the standard error of the mean (n=2 plot

means of 2 measurements each). An asterisk indicates significant difference induced by drought treatment (P<0.05).

Table 2 Multiple regression equations for leaf water potential (Ψ), net photosynthetic rates (A), and stomatal conductances (g_s) in sunlit leaves of *Q. ilex* and *P. latifolia* as functions of different environmental variables: “VPD” is the vapor pressure deficit (kPa), and “SM” is the soil moisture (% in volume) measured with TDR method.

Species	Relationship	R^2	*P*	N
Q. ilex	$\Psi = -4.91 - 0.21\ \mathrm{VPD} + 0.63\ \mathrm{SM}^{0.5}$	0.66	<0.001	40
P. latifolia	$\Psi = -7.33 - 1.09\ \mathrm{VPD} + 1.25\ \mathrm{SM}^{0.5}$	0.74	<0.001	40
Q. ilex	$A = -2.71 - 1.18\ \mathrm{VPD} + 1.91\ \mathrm{SM}^{0.5}$	0.50	<0.001	48
P. latifolia	$A = -2.06 - 0.70\ \mathrm{VPD} + 1.37\ \mathrm{SM}^{0.5}$	0.38	<0.001	48
Q. ilex	$g_s = -50.9 - 11.8\ \mathrm{VPD} + 30.7\ \mathrm{SM}^{0.5}$	0.32	<0.001	48
P. latifolia	$g_s = -43.8 - 6.45\ \mathrm{VPD} + 24.2\ \mathrm{SM}^{0.5}$	0.34	<0.001	48

Table 3 Polynomial relationships between net photosynthetic rates and leaf water potential (Ψ) and stomatal conductances (g_s). All measurements were performed in sunlit leaves of *Q. ilex* and *P. latifolia*.

Species	Relationship	R^2 value	*P* value	N value
Q. ilex	$A=14.21+5.10\ \Psi+0.38\ \Psi^2$	0.52	<0.001	40
P. latifolia	$A=6.75+1.41\ \Psi+0.06\ \Psi^2$	0.48	<0.001	40
Q. ilex	$A=0.49+0.074\ g_s-0.00016\ g_s^2$	0.64	<0.001	48
P. latifolia	$A=0.17+0.084\ g_s-0.00034\ g_s^2$	0.42	<0.001	48

Table 4 Repeated measures ANOVA of Net Photosynthetic Rate (A) as dependent variable, and species (*Q. ilex* and *P. latifolia*) and treatment (control and drought) as independent factors. All sampling dates (24) were grouped as repeated measures.

	d.f.	Sum of Squares	Mean Square	F value	P value
Treatment	1	10.732	10.372	10.123	0.0335
Species	1	131.277	131.277	128.837	0.0004
Treatment x Species	1	0.082	0.082	0.077	0.7952
Group	4	4.240	1.060		
Category for A	23	1554.714	67.596	37.077	<0.0001
Category for A x Tr.	23	63.942	2.780	1.525	0.0820
Category for A x Sp.	23	99.909	4.344	2.383	0.0019
Category for A x Tr. x Sp.	23	57.288	2.491	1.366	0.1500
Category for A x Group	92	167.727	1.823		

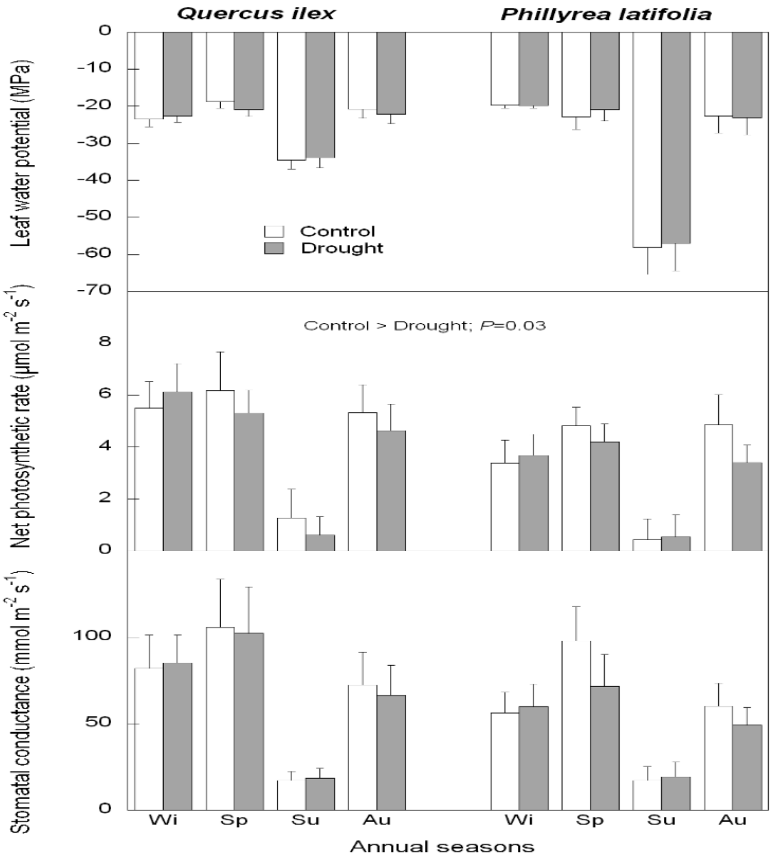

Fig. 5. Mean seasonal values of leaf water potential (Ψ), net photosynthetic rate (A), and stomatal conductance (gs) in *Q. ilex* and *P. latifolia*. Error bars indicate the standard error of the mean (n ranges from 4 to 6 measurements in each annual season).

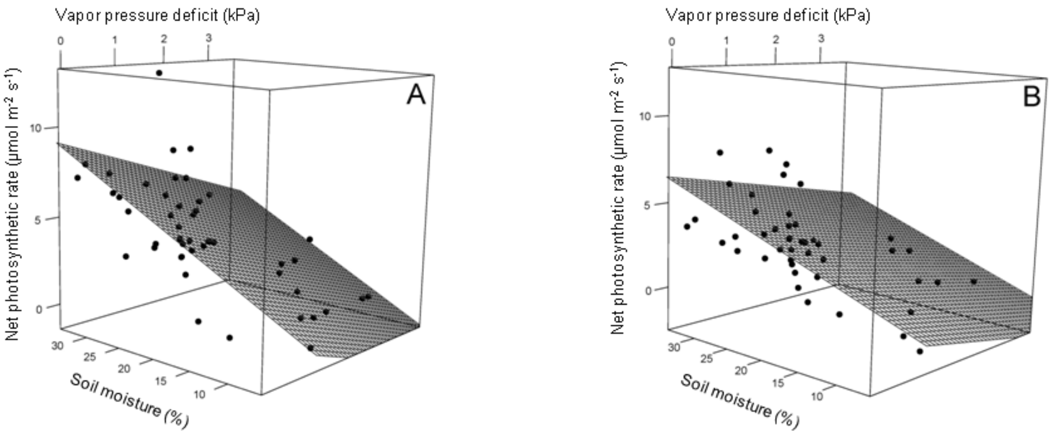

Fig. 6. Net photosynthetic rate response to VPD and soil moisture values in *Q. ilex* (A) and *P. latifolia* (B).

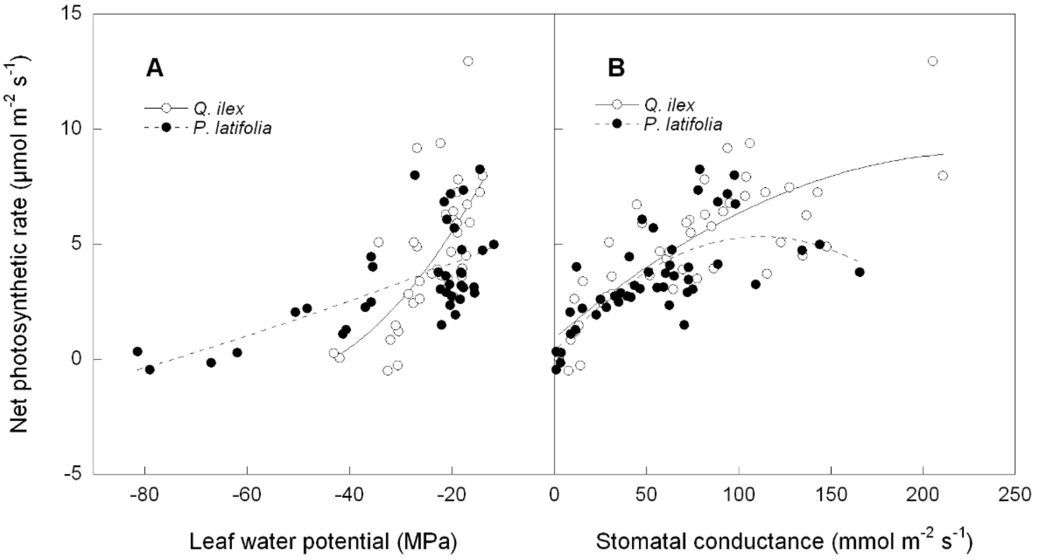

Fig. 7. Polynomial relationships between net photosynthetic rates and leaf water potential (A-Ψ) (panel A), and stomatal conductances (A-g_S) (panel B) in *Quercus ilex* and *Phillyrea latifolia* (n=40 and n=48 for A-Ψ and A-g_S relationships, respectively).

The percentage of *Q. ilex* dead stems during the overall period (1999-2013) was significantly higher in drought plots than in control ones (*P*=0.05) (35.7±8.0 and 27.9±2.0 % for drought and control plots, respectively), whereas no significant differences were found in *P. latifolia* (12.6±4.9 and 10.8±1.3 % for drought and control plots, respectively). Stem mortality was thus clearly higher in *Q. ilex* than in *P. latifolia*.

4. Discussion

Warmer and drier climatic conditions projected for Mediterranean areas [2,3,4] may decrease growth rates of the dominant species of this forest and global ecosystem productivity [10,13] by decreasing their A rates and increasing stem mortality, as observed in the drought treatment during the overall duration of the experiment, and as a result of the dependence of the three studied variables on the climatic conditions (VPD and SM). In this same experimental site, it was observed that a slight 13% decrease in soil water availability produced about 40% decrease of stem growth in *Q. ilex* trees [10], whereas these effects were very small in *P. latifolia*. In addition to the 8 % decrease in A rates, that by itself does not seem enough to explain this heavy drop of stem growth in *Q. ilex* trees subjected to the experimental drought, a major carbon allocation to exudates and roots in the drought plots seem very likely to maximize the uptake of scarcer water and nutrients [15] because, moreover, drought effect on A rate was similar in both species during the overall studied period.

A rates were higher in *Q. ilex* than in *P. latifolia* in all annual seasons except in summer, the hottest and driest season, when these differences between species disappeared. This result alone could induce to conclude that *Q. ilex* has a larger carbon uptake than *P. latifolia*, but A rates are depicted in function of leaf area, and *Q. ilex* leaves have higher LMA than *P. latifolia* ones (the weight of *Q. ilex* leaves is 23 mg cm-2, and the weight of *P. latifolia* leaves 17 mg cm-2) [16]. If carbon uptake is expressed as a function of leaf mass, the difference between species disappears, and even higher A values are observed in *P. latifolia* than in *Q. ilex* during summer season.

The two studied species have a similar response to resist dry conditions: they close stomata under high VPD to avoid excessive water loss throughout transpiration [10, 17], but the architecture of their xylem conduits is a key factor determining the larger capacity to resist dry conditions in *P. latifolia*, and the larger ability to reach high A rates under well-watered conditions in *Q. ilex*. The relationship between A and Ψ is stronger in *Q. ilex* than in *P. latifolia* because when stomata are closed and A rates are continuously very low, Ψ still decreases in *P. latifolia*. The relationship between A and g_s is also stronger in *Q. ilex* than in *P. latifolia* because larger A and g_s values are reached by *Q. ilex* during moist seasons, and the saturation of A rates with high g_s values observed in *P. latifolia*. Narrow xylem conduits of *P. latifolia* are more able to achieve lower Ψ without excessive xylem embolism [18], whereas wider xylem vessels in *Q. ilex* are more susceptible to suffer xylem embolism arising the risk of stem mortality [19], but they provide *Q. ilex* a higher capacity to reach larger photosynthetic rates (due to larger g_s values and transpiration rates) than *P. latifolia* under well-watered conditions. In addition, *P. latifolia* showed higher optimum temperature to reach maximum A rates and an earlier saturation of A rates at high SM than *Q. ilex* [9,17], and *P. latifolia* was more sensitive to photochemical damage during winter cold than *Q. ilex* [19]. So xylem architecture and other physiological adaptations provide more sensitivity to cold and more resistance to heat and drought conditions in *P. latifolia* than in *Q. ilex* [10].

Plant respiration is a very important component of the terrestrial carbon cycle [20], and the increase of temperature is mainly associated to an increase of respiration rates coupled with a smaller increase or even a saturation of A rates, resulting in a global decrease of the net carbon balance [21]. Moreover, another study in this forest revealed very low growth during recent years in *Q. ilex* now-dead stems, while surviving *Q. ilex* trees showed on average a higher stem growth during the same period [13]. After a heavy dry and unusually hot period in the studied forest during summer and autumn 2011, there was a forest decay event with a lot of defoliated and dead trees [12], with a depletion of lignotuber reserves in damaged trees [22], and *Q. ilex* was more affected again than *P.*

latifolia [12]. Higher photorespiration rates as a consequence of drought [23] or high temperatures [24] might have greatly contributed to this decrease in net carbon balance, as shown by the negative A rates obtained during drier summers of the studied period, and the higher mortality rates observed during all experimental period in *Q. ilex* than in *P. latifolia*.

A future increase in VPD and a decrease in soil moisture induced by a raise of air temperatures will lead to a decrease in the carbon balance of Mediterranean species, but some shrub species such as *P. latifolia*, more able to avoid xylem embolism, more resistant to lower leaf water potential and with lower photosynthetic rates during cold and humid periods than *Q. ilex*, could be favored by these new climatic constrains in detriment of the currently dominant tree species of this forest, *Q. ilex*, which could be subjected to an increasing risk of xylem embolism, defoliation, depletion of reserves, smaller carbon balance and tree mortality.

Acknowledgments

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Chapter 2. Physiological adjustments of a Mediterranean shrub to long-term experimental warming and drought treatments

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Abstract

Warmer temperatures and extended drought in the Mediterranean Basin are becoming increasingly important in determining plant physiological processes and affecting the regional carbon budget. The responses of plant physiological variables such as shoot water potential (Ψ), carbon-assimilation rates (A), stomatal conductance (gs) and intrinsic water-use efficiency (iWUE) to these climatic regimes, however, are not well understood. We conducted long-term (16 years) field experiments with mild nocturnal warming (+0.6 ºC) and drought (-20% soil moisture) in a Mediterranean early-successional shrubland. Warming treatment moderately influenced Ψ, A and gs throughout the sampling periods, whereas drought treatment strongly influenced these variables, especially during the summer. The combination of a natural drought in summer 2003 and the treatments significantly decreased A and iWUE. Foliar $\delta^{13}C$ increased in the treatments relative to control, but not significantly. The values of Ψ, A and gs were correlated negatively with vapor-pressure deficit (VPD) and positively with soil moisture and tended to be more dependent on the availability of soil water. The plant, however, also improved the acclimation to drier and hotter conditions by physiological adjustments (gs and iWUE). Understanding these physiological processes in Mediterranean shrubs is crucial for assessing further climate change impacts on ecosystemic functions and services.

Keywords: global warming, frequent droughts, physiological response; long-term experiments; plant acclimation

1. Introduction

Mediterranean-type shrubland ecosystems occupy large areas of the global terrestrial surface and provide important ecosystemic services, such as carbon storage, global biogeochemical cycles and the conservation of biodiversity [1–5]. The impacts of anthropogenic climate change, however, are gradually representing a prominent disturbance, affecting from individuals to ecosystems [3,5–7]. Numerous studies of Mediterranean ecosystems have reported that global warming is advancing plant spring phenology and extending growing periods [3,8–10]. In contrast, rapid changes in the patterns of temperature and precipitation have also negatively affected plant growth and survival by reducing water availability, ultimately leading to catastrophic carbon starvation and widespread mortality [11–15]. Robust climatic models have projected a continuous increase in warming and drought severity in the Mediterranean Basin for the coming decades, which could severely impact carbon sinks in shrubland ecosystems and alter regional carbon budgets [16,17].

Many studies over the latest several decades have reported physiological [18–23], morphological [3,24,25] and genetic [3,26–28] changes in Mediterranean plants in response to warming and drying conditions. Among these changes, physiological adjustments are considered the most rapid and effective, because they can increase photosynthetic rates or decrease water loss and improve intrinsic water-use efficiency (iWUE) via stomatal conductance (gs) [3,11,25,29–31]. Recent studies have also demonstrated the resistance and resilience of terrestrial biomes to climatic change by the modulation of the responses to inter-seasonal and inter-annual stresses over time [25,32–34]. Changes in iWUE regulated by gs have been widely studied by the variation of foliar carbon isotope ($\delta^{13}C$) composition [35], so WUE can be evaluated by changes in the amount of foliar $\delta^{13}C$ [36–39]. Our understanding of the effects of future scenarios of warming and drought on these physiological processes in Mediterranean shrubs, however, remains poor due to a lack of long-term data sets.

Climatic experiments have provided effective approaches for studying the potential physiological and phenological changes in plants to future climatic regimes [40–44]. Numerous field experiments manipulating in Mediterranean ecosystems have helped to identify the physiological [18–21,45], morphological [24,25] and structural [11,34,46] adjustments of plants for coping with changes in climatic regimes. Long-term precipitation-manipulation experiments in Mediterranean forests have highlighted dampened effects on carbon assimilation [45], biomass accumulation [47,48] and aboveground net primary production (ANPP) [48]. The physiological adjustments over time in response to new climatic regimes, however, are still unclear because most experimental studies have short terms (<5 years) [18–21,23]. Long-term experiments are consequently desirable for interpreting the cumulative effect of certain climatic regimes and detecting the patterns of plant physiological responses [42,45,48].

We carried out a long-term nocturnal warming and drought experiment in a Mediterranean early-successional shrubland from 1999 to 2014 (16 years), which is one of the longest climate-manipulation experiments ever conducted. Species in early-successional stages are sensitive to rapid climate change but have received little attention [3,18,20,49]. We studied the shrub *Erica multiflora*, which is widely distributed in western and central Mediterranean Basin and is one of the dominant species at the study site [18,20]. Previous experimental studies conducted in this experimental site have observed that the rates of carbon assimilation in *E. multiflora* were not affected by warming but were significantly decreased by drought throughout the first two years of treatment [18]. Prieto et al [20] reported that warming tended to increase the rate of carbon-assimilation of this species in cold seasons, but the response to drought depended on the year and season. Our sampling periods were: 1999-2001 (short-term), 2003-2005 (medium-term) and 2014 (long-term). The specific objectives of our study were to (i) verify if warming and drought exacerbate the loss of shoot water in Mediterranean shrub species, (ii) investigate the effects of experimental warming and drought on A, gs, iWUE (A gs^{-1}) and foliar $\delta^{13}C$ levels at different timescales, and (iii) determine the effect of long-term ecosystem exposure to warmer and drier conditions on plant physiological adjustments. The results will be crucial for identifying the potential physiological responses to climatic changes and will help us to understand further the effects of climate on terrestrial ecosystemic functions and services.

2. Materials and methods

2.1 Study site

We carried out a field experiment on a south-facing hill (13% slope) in Garraf Natural Park near Barcelona (northeastern Spain) (41°18´ N, 1°49´ E; 210 m a.s.l.). The climate at the experimental site is typically Mediterranean, with mild winters, dry summers and rainy springs and autumns. The mean annual air temperature during the study period was 15.8 °C, with the maximum mean summer temperature (June-August) of 23.5 °C. The mean annual precipitation was 537.3 mm, as much as 70% of which falls in spring and autumn. The soil is calcareous and composed of marls and limestone, with depths of 10-40 cm. The site suffered two large fires in summer 1982 and spring 1994, which degraded the vegetation to early-successional shrubland. Most of the current vegetation has sprouted from underground organs after the two fires. The vegetation is co-dominated by *E. multiflora*, a species widely distributed in the Mediterranean Basin. *E. multiflora* grows mainly in the spring but also in the autumn; flowering begins in late summer and ends the following spring.

2.2 Experimental manipulations

Nine plots (5×4 m^2) were randomly organized in three blocks, with each block having one warming, one drought and one control plot. Each treatment thus had three replicates. The experiment was maintained from 1999 to 2014.

The warming treatment consisted of a passive nocturnal warming by covering the plots with aluminum curtains. This covering system reduces the amount of long-wave infrared radiation reflected back to the atmosphere at night [40]. A light scaffold was installed in each warming plot for supporting the covering. The curtains were automatically unfolded at night and retracted during the day and were controlled by light sensors (below and above 200 lux, respectively). This passive nocturnal warming is realistic and effective, because the effects of global warming are predicted to be higher at night than during the day [40,49]. The curtains were automatically retracted during rain to avoid influencing the hydrological cycle.

The drought treatment extended the summer drought to the following spring and autumn by preventing rainwater from entering the plots using transparent waterproof roofs [40]. Scaffolds were also installed in the drought plots, but the curtain material was transparent plastic. Rain sensors activated the curtains to cover the plots whenever it rained during the treatment period and retracted them when the rain stopped. Rain was sensed by a sensitive (>5 mm rainfall) tipping-bucket rain gauge. The rainwater blocked by the waterproof plastic was drained outside the plots. The curtains were also automatically retracted to avoid damage during winds exceeding 10 m·s^{-1}. The drought plots were treated the same as the control plots during the rest of the year. The control plots had similar scaffolds as the warming and drought plots but without curtains.

2.3 Environmental conditions

Air temperature and precipitation have been recorded at the study site since 1998. Soil temperature at -5 cm depth was recorded by temperature sensors distributed in each plot. Precipitation was recorded by a tipping-bucket rain gauge 1.5 m above the ground. Relative humidity was recorded every 30 minutes at a nearby meteorological station. Vapor-pressure deficit (VPD) was calculated every 30 minutes from the relative humidity and air temperature. Soil moisture in the top 15 cm of soil was measured weekly by Time Domain Reflectometry (TDR) using three probes in each plot.

2.4 Measurements of shoot water potential and gas exchange

We monitored the shoot water potential (ψ), foliar carbon-assimilation rates (A) and gas exchange (gs) for 3-6 consecutive days per season during the sampling periods (spring 1999 to winter 2001, winter 2003 to summer 2005 and winter 2014 to autumn 2014). Five current-year shoots of *E. multiflora* with similar growth performance were collected in each plot to measure the changes of ψ at midday (11:00-13:00, solar time) using a Scholander-type pressure chamber (PMS Instruments, Corvallis, USA). Measured Erica plants were always the same ones, permanently marked with labels at the beginning of experiments. Foliar A and gs were measured on three consecutive days in each season with a portable gas-exchange system (an ADC4 system configured with a chamber model PLC4B (Hoddesdon, Hertfordshire, UK) from 1999 to 2001, a CIRAS2 system (Hitchin, Hertfordshire, UK) from 2003 to 2005 and an LI-6400XT system (LI-COR Inc., Lincoln, USA) from 2013 to 2014). Two to six sunny and current-year shoots with similar growth status were collected from each plot, and gas exchange was measured in triplicate. Leaf area was measured using ImageJ 1.46r (NIH, Maryland, USA) from a photocopy of all leaves on the twigs. Intrinsic water-use efficiency (iWUE) was calculated from the gas-exchange measurements as the ratio A gs^{-1}. The medium- (2003-2005) and long-term (2014) periods focused on gs and iWUE.

2.5 Isotopic and elemental analyses

Foliar $\delta^{13}C$ content was measured for current-year leaves in August 2000, 2004 and 2014. Leaves from three *E. multiflora* plants were collected in each plot and analyzed in an EA1108 elemental analyzer (Carlo Erba, Milano, Italy) coupled to a Delta C isotope ratio mass spectrometer with a CONFLO Π interface (Thermo Finnigan MAT, Bremen, Germany). The results were calibrated with interspersed international isotopic carbon standards (IAEA, Vienna, Austria). Values are expressed relative to Pee Dee Belemnite (PDB) standard for $\delta^{13}C$ as:

$$\delta\ ^{13}C = (R_{sample}/R_{standard} - 1) \times 1000\ (‰)$$

where ^{13}C is the heavier carbon isotope and R is the ratio of the heavier to the lighter isotope (^{13}C:^{12}C) for the sample and standard. The accuracy of the measurements was ± 0.15‰ for $\delta\ ^{13}C$.

2.6 Data analyses

For all physiological parameters (Ψ, A, gs, iWUE and $\delta^{13}C$), we calculated one value per plot as the mean of all plants measured per plot. The warming and drought treatments were always separately compared with the controls. The differences between the treatments and controls were analyzed by analyses of variance using R version 3.2.3 (R Core Development Team, 2015). The significance level was $p<0.05$ for the statistical tests within year and season.

3. Results

Climatic parameters

The climatic parameters (rainfall, temperature and VPD) varied greatly throughout the sampling periods (Fig. 1). The highest temperatures were recorded in summer (mean of June, July and August), with the highest summer temperature of 26.4 °C in 2003. The rainy seasons were usually spring and autumn. VPD was correlated with accumulated precipitation and temperature, with extremely severe deficits in summer 2003. The mean annual soil temperature at -5 cm depth was on average 0.6 °C higher in the warming than in the control plots. There were no significant differences between warming and control plots in 2014 (Fig. 2a). Soil moisture in the 0-15 cm layer fluctuated with season and year during the sampling periods, with wet springs and autumns and dry summers (Fig. 2b). Soil moisture averaged 20% lower in the drought treatment than the control plots throughout the sampling periods ($p<0.01$).

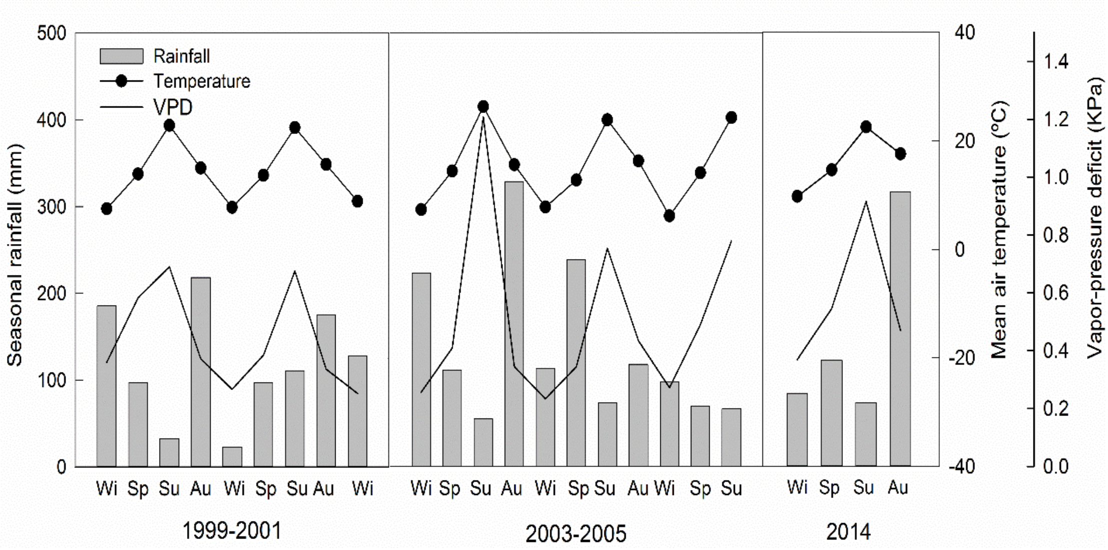

Fig. 1 Seasonal variation of mean air temperature, precipitation and vapor-pressure deficit (VPD) during the sampling periods 1999-2001, 2003-2005 and 2014. Wi, winter; Sp, spring; Su, summer; Au, autumn.

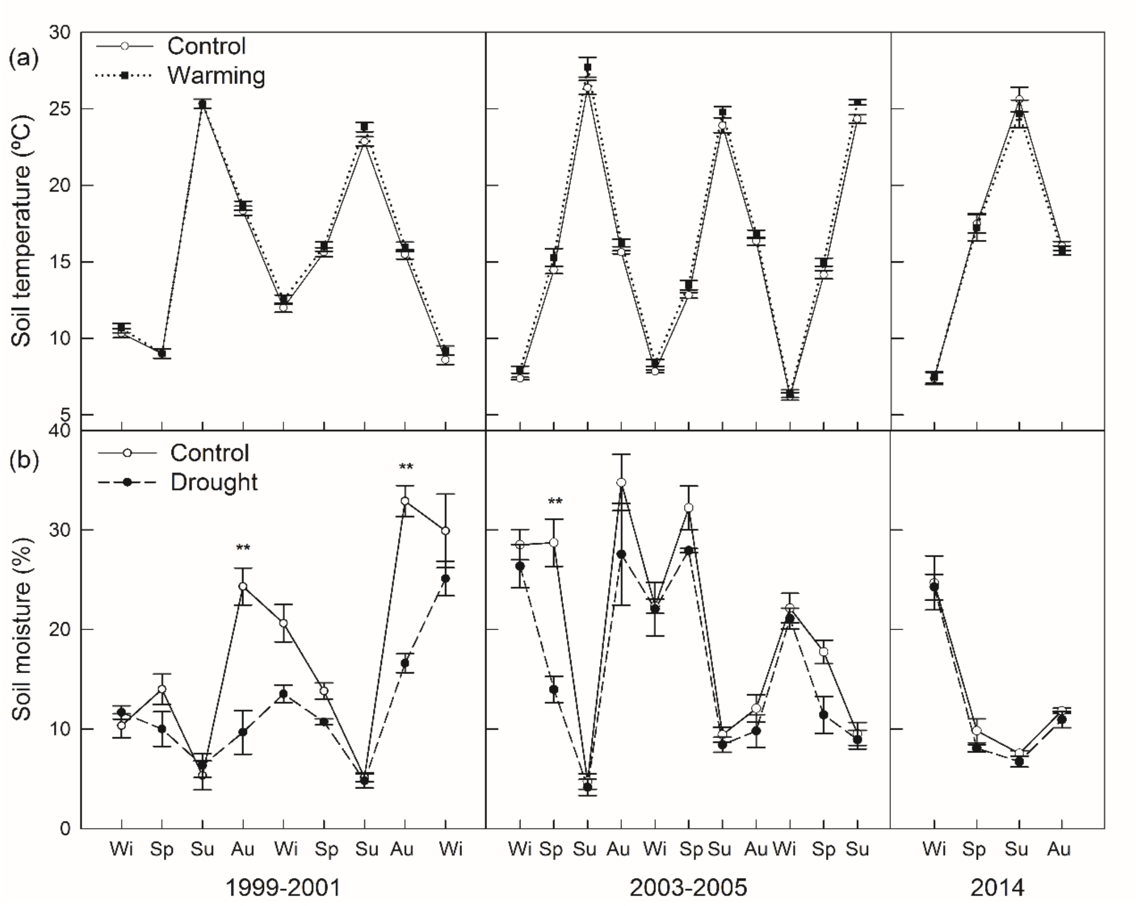

Fig. 2 (a) Changes of seasonal soil temperature in the control and nocturnal warming treatment during the sampling periods. (b) Changes of seasonal soil moisture in the control and drought treatment during the sampling periods. Significant differences between the control and treatment plots are indicated by asterisks (**, $p<0.01$). Each treatment had three replicates (n=3 plots). Wi, winter; Sp, spring; Su, summer; Au, autumn.

Shoot water potential (ψ)

Shoot water potential (ψ) of E. multiflora varied with VPD and was lowest in summer (Fig. 3). The warming treatment did not significantly affect ψ compared to the control, but ψ was significantly lower in the drought treatment throughout the sampling periods ($p<0.01$).

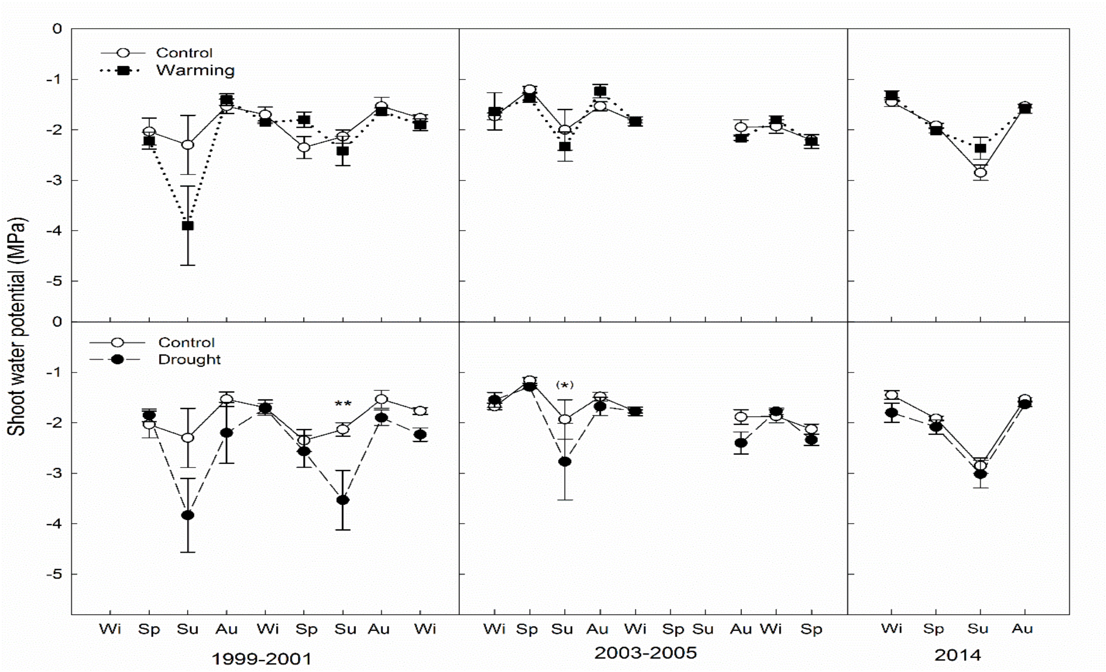

Fig. 3 Changes in shoot water potential in *Erica multiflora* during the sampling periods in the control and warming treatment and drought treatment. Significant differences between the control and treatment plots are indicated by asterisks (*, p<0.05; **, p<0.01). Each treatment had three replicates (n=3 plots). Wi, winter; Sp, spring; Su, summer; Au, autumn.

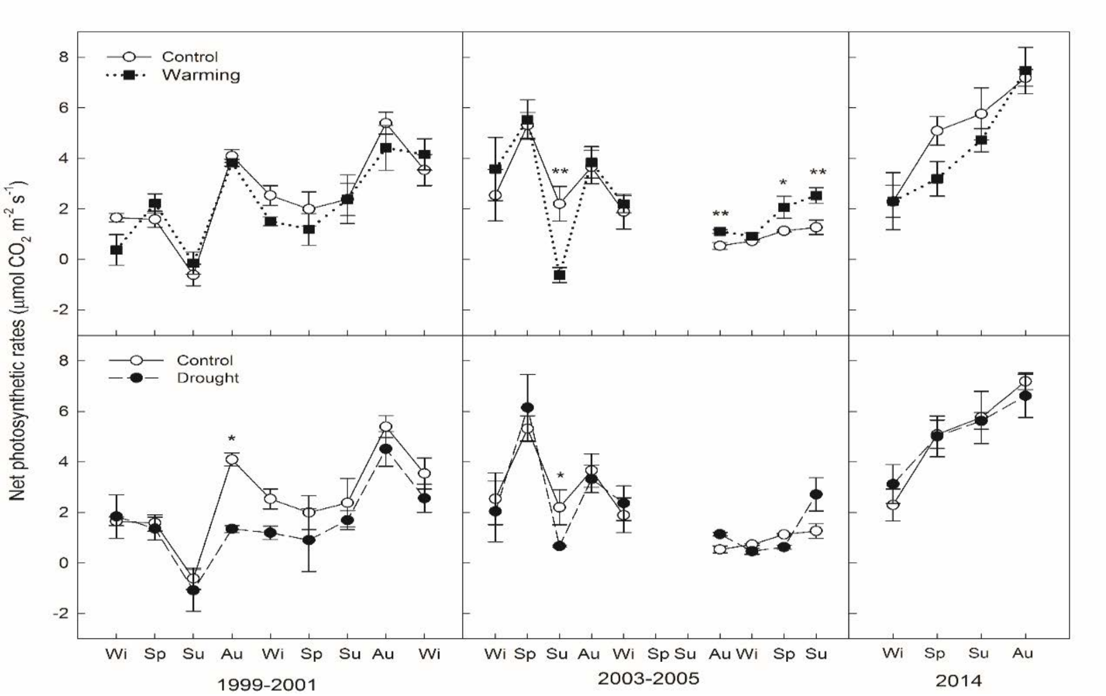

Fig. 4 Seasonal variation of net photosynthetic rates in Erica multiflora in the control and treatment plots during the experimental periods. Error bars indicate the standard errors of the means (n=3 plots). Significant differences between the control and treatment plots are

indicated by asterisks (*, $p<0.05$; **, $p<0.01$). Wi, winter; Sp, spring; Su, summer; Au, autumn.

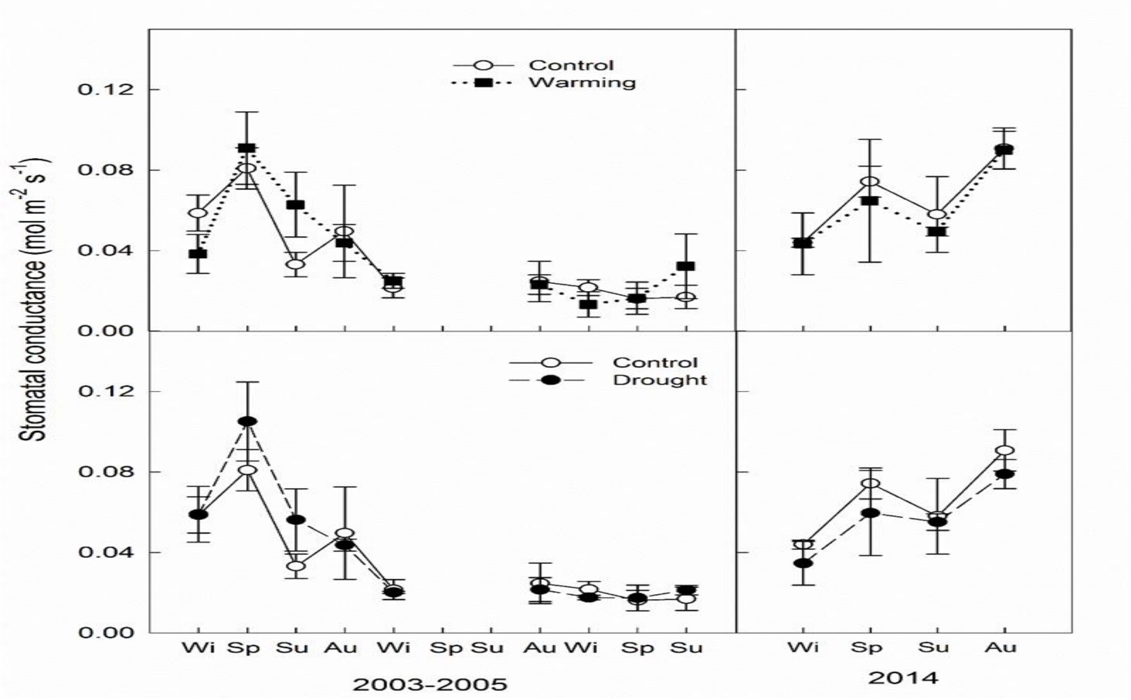

Fig. 5 Seasonal stomatal conductance in Erica multiflora in the control and the warming and drought treatments during the medium- and long-term study periods. Error bars indicate the standard errors of the means (n=3 plots). Wi, winter; Sp, spring; Su, summer; Au, autumn.

Foliar gas exchange

Carbon-assimilation rates (A) was highly variable in the control plants inter-seasonally and inter-annually throughout the sampling periods. Photosynthesis was generally highest during the wet seasons (spring and autumn) and lowest during the hot and dry summers (Fig. 4). Neither warming nor drought treatment significantly decreased the values of A when compared to control, and warming treatment even had some positive effects. The values of A, however, decreased significantly under both warming and drought in the extremely hot and dry summer of 2003 ($p<0.01$ and $p<0.05$, respectively). The values of gs fluctuated greatly with season and year and was lowest in winters and highest in the wet springs and autumns (Fig. 5), but gs in the warming and drought treatments was similar to that in the controls. The iWUE in control was generally highest in summer, except for the extreme summer of 2003 (Fig. 6). The (iWUE) was generally higher in the warming and drought treatments but decreased significantly in summer 2003 due to the combination of the extremely hot summer and the experimental warming ($p<0.05$) and drought ($p<0.05$).

Shoot water potential (ψ), Carbon-assimilation rates (A) and stomatal conductance (gs) were correlated negatively with VPD and positively with soil moisture, indicating a strong dependence on soil moisture. The (iWUE), however, was not affected when soil-water potential and VPD were high (Fig. 7).

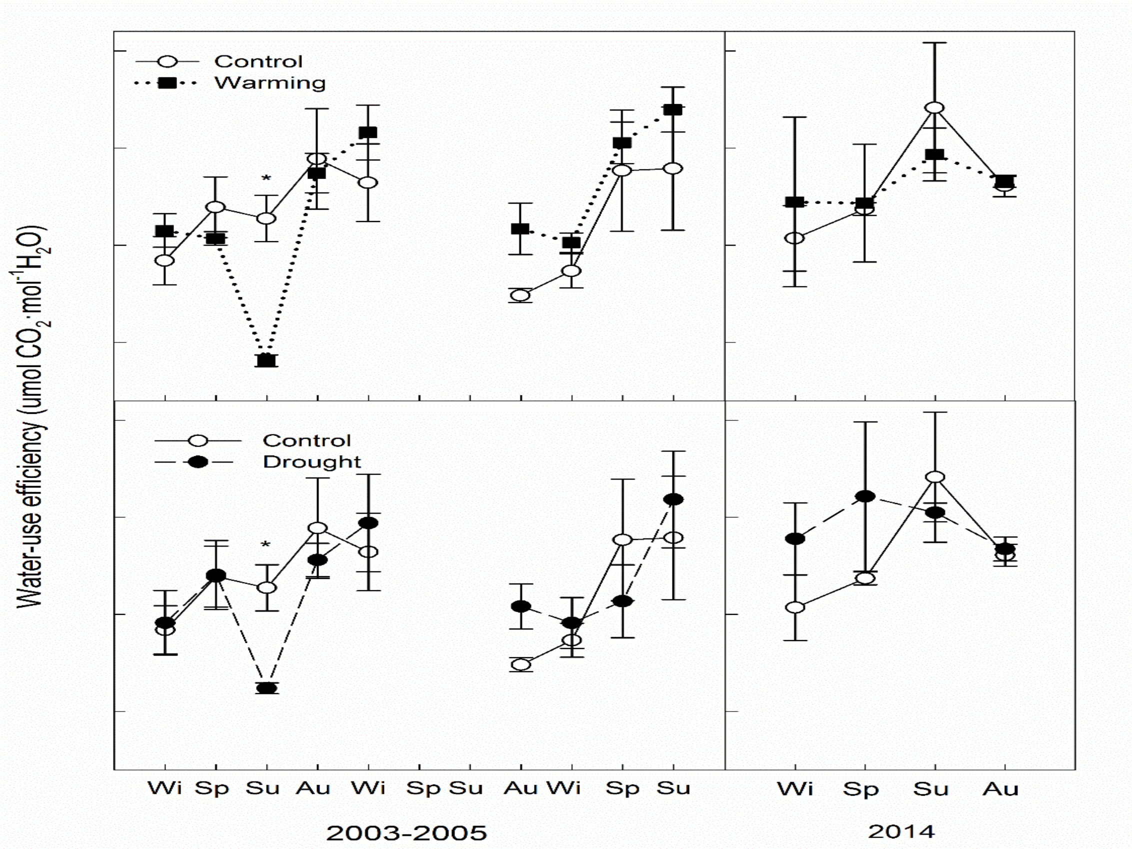

Fig. 6 Intrinsic water-use efficiency (iWUE, A g-1) in E. multiflora leaves in the control and the warming and drought treatments during the medium- and long-term sampling periods. Error bars indicate the standard errors of the means (n=3 plots). Significant differences are indicated by asterisks (*, $p<0.05$). Wi, winter; Sp, spring; Su, summer; Au, autumn.

Variability of isotopic values (^{13}C)

The foliar δ ^{13}C values in E. multiflora varied greatly during the sampling periods (Fig. 8). The δ ^{13}C values after one year of both the warming and drought treatments were similar to those in the controls. After five years of treatment, however, were significantly higher in the warming treatment than the controls. Neither the warming nor the drought treatments had significant effects on δ 13C after 15 years of treatment.

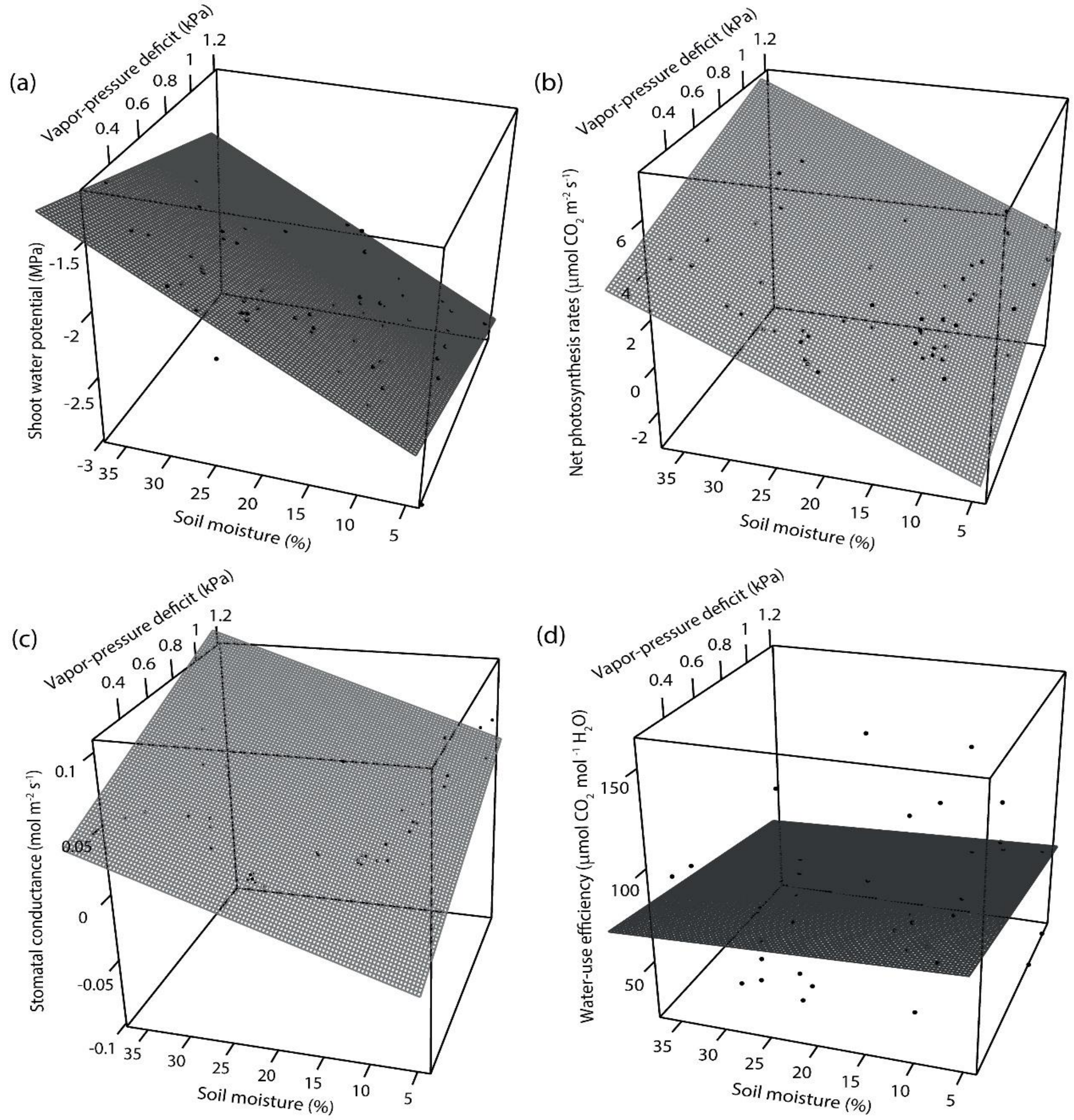

Fig. 7 The relationships of shoot water potential (SWP), net photosynthetic rate, stomatal conductance and intrinsic water-use efficiency (iWUE) with vapor-pressure deficit (VPD) and soil moisture during the sampling periods.

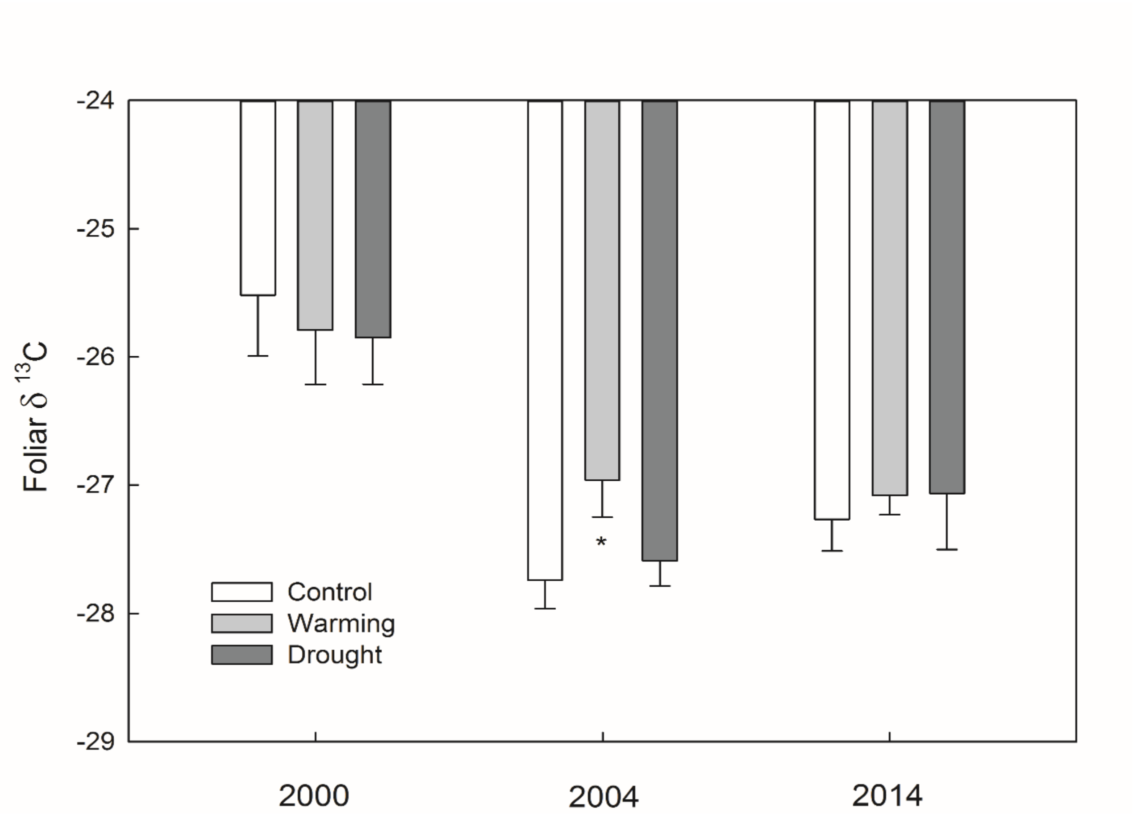

Fig. 8 Foliar δ^{13}C in *E. multiflora* in summer 2000, 2004 and 2014. Error bars indicate the standard errors of the means (n=3 plots). Significant differences with control are indicated by asterisks (*, *p*<0.05).

4. Discussion

Shoot water potential (ψ) in *E. multiflora* was highly variable inter-seasonally and was lowest in hot and dry summers (Fig. 3). The lower ψ in the experimental treatments was likely induced mainly by increased evapotranspiration in the warming treatment and by the decrease in soil moisture due to the exclusion of rainwater in the drought treatment [18,20]. The effects on ψ differed greatly between warming and drought treatment, consistent with the previous studies showing that the impacts of drought treatment on plant water potential were stronger than those of warming treatment [18,20]. Our results indicated that hot and dry summers accelerated the loss of water from plant shoots, which can cause large-scale crown die-off, mortality and vegetation shifts in Mediterranean ecosystems [11,14,15]. Long periods of warmer and drier climate combined with extreme conditions in the future would likely cause more severe shoot-water deficits in Mediterranean shrubland ecosystems [15,17].

Foliar photosynthesis is an important physiological process of plant life that is strongly affected by conditions of temperature and water across various types of ecosystems [23,31,34]. The moderate warming and prolonged drought treatments decreased photosynthetic rates, although the experimental warming had some positive effects in wet seasons (Fig. 4). Photosynthesis in *E. multiflora* was strongly correlated with soil moisture, and it was significantly lower in both the warming and drought treatments during the extreme summer in 2003, probably due to the lower water availability [14,17].

Stomatal adjustment is the primary regulator of foliar hydrological changes, which control the response of WUE to climatic stresses [23,31]. Photosynthesis is highly correlated with gs, although the mechanisms by which water stress limits photosynthesis by stomatal closure or metabolic impairment are controversial [23,30,31]. Most studies accept that stomatal closure is the main determinant of photosynthesis under moderate drought stress [20,23,45,50]. Foliar photosynthetic rates are also well correlated with gs in some studies of Mediterranean species [18–20,45]. Plant photosynthesis, however, may not be limited by stomatal closure but by metabolic impairment [3,20,23,50,51]. Our study indicated that foliar photosynthetic rates were significantly lower in both the warming and drought treatments in the extreme 2003 summer, but this effect was probably associated with the impairment of photosynthetic enzymes, because the treatments did not significantly affect gs [23,31,51].

Intrinsic water-use efficiency (iWUE) is an important indicator of plant physiological response to environmental changes [23,31,39]. In our study, the plants increased iWUE to reduce water loss in summer, except in the extreme 2003 summer (Fig. 6), consistent with the theory that the balance between carbon assimilation and the control of water loss is optimized in hot and dry summers [29,30,39]. Climatic extremes such as during the 2003 summer, however, can cause severe plant-water deficits that can irreversibly damage plant hydraulic systems [11,12,14,15,17]. Changes in foliar $\delta^{13}C$ during warmer and drier conditions can increase plant WUE via photosynthetic or metabolic processes [35–37,39,52]. Our results, however, showed that $\delta^{13}C$ did not change significantly in the warming and drought treatments throughout the study period, except in the warming treatment in 2004 when $\delta^{13}C$ was significantly higher, which was probably due to the higher iWUE after the extremely hot summer in 2003 (Fig. 8). Various studies have reported that ^{13}C discrimination decreases with the concentration of intercellular carbon dioxide due to stomatal closure, and ultimately WUE [3,38,39,52]. Foliar $\delta^{13}C$ was therefore higher under the warming and drought conditions, consistent with the findings of other experiments in terrestrial ecosystems where plants regulate the relationship between WUE and climate change [32,37,39].

The available information on global change indicates that long periods of warming have been impacting terrestrial ecosystems since the mid-20th century [5,16]. We have also concluded that the severe decrease in water availability had substantial effects on physiological processes, such as Ψ, A and gs, whereas the effects of the mild warming treatment were very mild (nearly no influences). These differential treatment effects indicate that low water availability may has a more direct impact on plants [23,31]. These physiological adjustments, however, are essential for adapting to changes in climatic regimes. Our study shows that the physiological responses (A, g_s, iWUE and $\delta^{13}C$) to long-term experimental warming and drought decrease over time, indicating acclimation to the climatic stresses [21,23,45,46]. Robust models, however, predict that global warming will continue to increase, accompanied by other stresses such as extreme droughts and heat waves [5,16,17]. Mediterranean species have shown some acclimation responses and strategies, but these could be potentially overwhelmed by future climate, leading to increased risks of xylem embolism, crown die-off and mortality, which would affect future ecosystemic carbon budgets.

Acknowledgements

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Block 2. The changes in population demography (growth, mortality and recruitment) of Mediterranean holm-oak forest in response to long-term experimental drought

Chapter 3. Contrasting impacts of continuous moderate drought and episodic severe droughts on the aboveground-biomass increment and litterfall of three coexisting Mediterranean woody species

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Abstract

Climate change is predicted to increase the aridity in the Mediterranean Basin and severely affect forest productivity and composition. The responses of forests to different timescales of drought, however, are still poorly understood because extreme and persistent moderate droughts can produce nonlinear responses in plants. We conducted a rainfall-manipulation experiment in a Mediterranean forest dominated by *Quercus ilex*, *Phillyrea latifolia* and *Arbutus unedo* in the Prades Mountains in southern Catalonia from 1999 to 2014. The experimental drought significantly decreased forest aboveground-biomass increment (ABI), tended to increase the litterfall and decreased aboveground net primary production throughout the 15 years of the study. The responses to the experimental drought were highly species-specific. *A. unedo* suffered a significant reduction in ABI, *Q. ilex* experienced a decrease during the early experiment (1999-2003) and in the extreme droughts of 2005-2006 and 2011-2012 and *P. latifolia* was unaffected by the treatment. The drought treatment significantly increased branch litterfall, especially in the extremely dry year of 2011, and also increased overall leaf litterfall. The drought treatment reduced the fruit production of *Q. ilex*, which affected seedling recruitment. The ABIs of all species were highly correlated with SPEI in early spring, whereas the branch litterfalls were better correlated with summer SPEIs and the leaf and fruit litterfalls were better correlated with autumn SPEIs. These species-specific responses indicated that the dominant species (*Q. ilex*) could be partially replaced by the drought-resistant species (*P. latifolia*). However, the results of this long-term study also suggest that the effect of drought treatment has been dampened over time, probably due to a combination of demographic compensation, morphological and physiological acclimation and epigenetic changes. However, the structure of community (e.g. species composition, dominance and stand density) may be reordered when a certain drought threshold is reached.

Keywords: global warming, droughts, extreme episodes, aboveground biomass increment, litterfall, net primary production, forest acclimation

Introduction

The climate changes resulting from anthropogenic activities have already influenced global water cycle, involving changes in precipitation mounts, timing and variability and reductions in water availability since the middle of last century (Beier *et al.*, 2012; Dai, 2012). Water availability is a crucial factor determining global plant distribution and the diversity of terrestrial ecosystems (Lloret *et al.*, 2004; Carnicer *et al.*, 2011; Wu *et al.*, 2011; Vicente-Serrano *et al*, 2013). Climate change in recent decades produced by the continued emission of carbon has included temperature increases, precipitation decreases and more frequent extreme droughts in the Mediterranean Basin (Dai, 2012; Ji *et al.*, 2014; IPCC, 2014). Mediterranean ecosystems have thus already been disturbed by the frequencies and intensities of droughts (Ogaya & Peñuelas, 2007a; Misson *et al.*, 2011; Doblas-Miranda *et al*., 2015). The magnitude of the effects have largely depended on the initial species composition (Lloret *et al*., 2004; Ruiz-Labourdette *et al.*, 2012; Peñuelas *et al.*, 2013a; Doblas-Miranda *et al.*, 2015) and successional trajectories of the ecosystems (Breshears *et al.*, 2005; Mueller *et al.*, 2005; Peñuelas *et al.*, 2013b; Carnicer *et al.*, 2014). Droughts can lead to reductions in primary productivity (Ciais *et al.*, 2005; Boisvenue & Running, 2006; Peñuelas *et al.*, 2007; Wu *et al.*, 2011; Ivits *et al.*, 2014) and the ability of forests to sequester carbon and can thus alter regional carbon budgets (Ogaya & Peñuelas, 2007a; Zhao & Running, 2010; Carnicer *et al.*, 2011; van der Molen *et al.*, 2011;

Peñuelas *et al.*, 2013a). Widespread forest declines, die-offs, species shifts and phenological changes (Peñuelas *et al.*, 2004a, 2013a; Carnicer *et al.*, 2011; Misson *et al.*, 2011; Ivits *et al.*, 2014) have also been observed in more extreme cases (Lloret *et al.*, 2012; Reyer *et al.*, 2013).

Aboveground net primary production (ANPP) is generally assessed as the amount of carbon incorporated into new organic matter produced aboveground over a specified interval (Clark & Brown, 2001; Chapin *et al.*, 2006; Malhi *et al.*, 2011; Clark *et al.*, 2013; Talbot *et al.*, 2014). ANPP consists of several components: aboveground-biomass increment (ABI), fine litterfall, emissions of biogenic volatile organic compounds (BVOCs) and the loss due to consumers. BVOCs and the loss due to consumers are difficult to measure and are of secondary importance, but the assessment of the main components of ANPP, ABI and litterfall (branch, leaf and fruit litterfall), has received considerable attention for many forest ecosystems (Clark & Brown, 2001; Malhi *et al.*, 2011; Talbot *et al.*, 2014). The cumulative evidence suggests that increasing droughts would have negative effects on forest ANPP, reducing stem growth and increasing defoliation (Ogaya & Peñuelas, 2006; Carnicer *et al.*, 2011; Limousin *et al.*, 2012; Reyer *et al.*, 2013). Information for the magnitude of these effects on ANPP, however, is still scarce, because their quantification requires more field data not usually available due to methodological limitations (Boisvenue & Running, 2006; Beier *et al.*, 2012).

Field manipulation experiments can impose continuous and quantitative disturbance in natural ecosystems and shed light on forest changes induced by reductions in water availability (Peñuelas *et al.*, 2004b, 2007; Wu *et al.*, 2011; Beier *et al.*, 2012; Ivits *et al.*, 2014). Compared to observational studies that depend on the occurrence of natural extreme weather events to disturb the forest ecosystem and carbon budget, experiments that manipulate precipitation can simulate rapid and strong drought perturbations and can thus offer an opportunity to study forest ecosystems under water deficits (Leuzinger *et al.*, 2011; Reyer *et al.*, 2013). Many precipitation-exclusion experiments have been conducted in natural or semi-natural ecosystems around the world and have illustrated the effects of altered precipitation regimes on ecosystem functioning (Wu *et al.,* 2011; Beier *et al.*, 2012). Numerous experiments suggest that the variation of effect sizes is unstable, which is a dynamic process followed by periods of potential stability (Leuzinger *et al.*, 2011). The magnitude and stability of the responses for long-lived species to drought, though, may vary at long timescales (Leuzinger *et al.*, 2011; Beier *et al.*, 2012; Barbeta *et al.*, 2013). Recent studies have reported dampening effects on the response to drought over time (Leuzinger *et al.,* 2011; Barbeta *et al.*, 2013). These dampening effects include epigenetic changes (Rico *et al.*, 2014), physiological changes (Martin-Stpaul *et al.*, 2013; Ogaya *et al.*, 2014), morphological adjustments (Misson *et al.,* 2011), functional changes in roots (Barbeta *et al.*, 2015) and reductions in stand density (Lloret *et al.*, 2004; Grimm *et al.*, 2013). These adjustments to drought, however, may not be sufficient to cope with future droughts that may ultimately produce widespread tree mortality and vegetational shifts favoring the most drought-resistant species.

Holm oak (*Quercus ilex* L.) is an intermediate drought-tolerant species widely distributed in the western Mediterranean Basin. This species is usually accompanied by other Mediterranean woody species with more (*Phillyrea latifolia* L.) or less (*Arbutus unedo* L.) drought tolerance (Ogaya & Peñuelas, 2003, 2006, 2007a, 2007b, 2007c; Barbeta *et al.*, 2012, 2013, 2015; Ogaya *et al.*, 2014). The varying ecophysiological responses to drought of these tree species have been reported in previous studies (Ogaya & Peñuelas, 2003, 2007a; Ogaya *et al.*, 2014). For example, drought decreased the photosynthetic rate in *Q. ilex* but not in *P. latifolia* (Ogaya & Peñuelas, 2003) and greatly reduced the increase in biomass in *Q. ilex* and *A. unedo* but not in *P. latifolia* after five years of experimental drought in the same site (Ogaya & Peñuelas, 2007a). Barbeta *et al.* (2013) reported a temporal dampening of the treatment effect on stem growth and mortality during the first 13 years of an experimental drought. Some of these studies have focused on long-term (more than 10 years) growth and mortality rates, but there is still a lack of information on the long-term evolution of aboveground biomass, litterfall and net primary production for forests subjected to moderate but persistent drought and to episodic severe droughts as projected for the Mediterranean regions in the coming decades (IPCC, 2014).

Our general objective was to study the ecological effects of the increasing intensity, frequency and duration of droughts predicted for the coming decades in a Mediterranean holm oak forest. Our initial hypotheses were that forest ANPP could significantly decrease under the moderate and persistent experimental drought and that extreme droughts would exacerbate this effect. We also hypothesized species-specific responses to drought, and different responses of ABI than branches, leaves and fruit litterfall, decreasing and increasing respectively in response to drought, and especially to severe episodes. We also monitored the responses of individuals and the entire community to the drier environmental conditions of the long-term (15 years) experimental drought to identify the detailed hierarchical mechanisms underlying transitions in the ecosystem state and to gain knowledge about the responses of the species and the community when tolerance thresholds (or tipping points) are exceeded by severe droughts. We were thus interested in determining whether long-term experimental and extreme natural droughts would accelerate acclimation and resilience of the Mediterranean forest ecosystem or would increase vegetation shifts toward reverse succession (forest to shrubland).

Materials and methods

Experimental site

The experiment was conducted on a south-facing slope (25%) in the Prades Holm oak forest in southern Catalonia (northeastern Iberian Peninsula) (41°21′N, 1°2′E; 930 m a.s.l.). This forest is widely distributed at 400-1200 m a.s.l., with closed canopies 3-10 m in height, depending on the site microclimate and topography. The vegetation is largely dominated by a dense overstory of the evergreen species *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 (*Erica arborea* L., *Juniperus oxycedrus* L. and *Cistus albidus* L.) and occasional individuals of deciduous species (*Acer monspessulanum* L. and *Quercus* × *cerrioides* Wilk. & Costa). The understory vegetation is very sparse, with abundant litter accumulation. The forest structure consists of a high density of small stems, mostly stump resprouts after a selective thinning, but the forest has not been disturbed for the last 70 years.

This study site has a Mediterranean climate, with 80% of the rain falling in spring (March, April and May) and autumn (September, October and November). Summer drought is common, usually lasting 3-5 months (Dai, 2012; Lopez-Bustins *et al*., 2013). The annual amount of summer (June, July and August) precipitation averaged 59.2 mm for 1999-2013, less than 10% of the mean annual precipitation (616.1 mm). Summer precipitation was irregular during this period, ranging from 22.0 mm in 2011 to 112.0 mm in 2000. The average summer temperature was 20.5 °C, which was ca. 8.0 °C higher than the mean annual temperature of 12.2 °C. Parent material of the Prades mountain is Jurassic limestone bedrock, which is overlaid by Paleozoic schist and Dystric Cambisol soil and ranges in depth from 30 to 110 cm. The soil is fully covered by litterfall. The climatic, edaphic and physiographic characteristics of this site are archetypical of forests of the Mediterranean Basin, which mostly occur in mountainous areas with shallow soils that exacerbate water deficits in plants.

Experimental design

Eight 15×10 m plots were established in January 1999 at the same altitude (930 m a.s.l.) along the south face of the mountain where the experiment is located. Four randomly selected plots received a drought treatment, and the other four served as control plots. The drought treatment partially excluded throughfall by PVC strips (0.5×18 m^2/strip), which are suspended at a height of 0.5-0.8 m above the ground, and cover approximately 30% of the plot surface. A ditch 0.8-1.0 m deep was excavated across the upper margin of the treatment plot to intercept runoff water. The water intercepted by the strips and ditches was drained outsides of the plots, below their lower margins. The strips were installed under the canopy and did not affect the amount of incident light reaching the leaves. Any litter falling on the PVC strips was regularly (approximately every month) placed below the strips to avoid differences in litter decomposition between the treatment and control plots due to factors other than the availability of water. The forest structure and species composition for all plots was undifferentiated at the start of the experiment.

Measurement of meteorological data and environmental indexes

An automated meteorological station (Campbell Scientific Inc., Logan, USA) was installed at a central point within the experimental system. Air temperature, relative humidity and precipitation were recorded every 30 minutes. Monthly precipitation and temperature data was available from 1975 at a nearby meteorological station (Poblet Monastery). Soil-water content was measured each season throughout the experiment by time-domain reflectometry (Model 1502C, Tektronix, Inc., Beaverton, USA) (Zegelin *et al*., 1989; Whalley, 1993). Three stainless steel cylindrical rods (25 cm long) were permanently installed in the soil at four randomly selected locations in each plot. The time-domain reflectometer was manually connected to the ends of the rods for determining the soil-water content. The Standardized Precipitation-Evapotranspiration Index (SPEI) based on continuous monthly differences between precipitation and potential evapotranspiration (P-PET; D) was used to evaluate drought severity throughout the study period (Vicente-Serrano *et al.*, 2010; 2013). We built a database of the D values (1975-2013) and used the SPEI package (version 1.6) to create different timescales (from 1-month to 36-month) of SPEI. We selected SPEIs at different timescales to best fit the values to forest growth and community dynamics, as described in detail by Barbeta *et al.* (2013). We calculated the SPEI for the drought treatment to evaluate the actual drought conditions in the plots, adjusting for a 13% decrease in precipitation in the drought plots (see Results).

ABI, litterfall and ANPP

All living tree stems with diameters >2 cm at a height of 50 cm (D_{50}) were marked with tags and identified to species in January 1999, and their stem diameters were measured using a metric tape. Stem diameter (D_{50}>2cm) for all trees in the plot was re-measured each year. Aboveground biomass (AB) at the plot level was estimated by allometric relationships from the stem diameters (Ogaya & Peñuelas, 2007a). ABI was calculated annually as the difference in AB between years. Trees that died were not measured or further considered for ABI calculations. New stems were added when their diameters reached the threshold ($D_{50}\geq$2cm). The ABs of the three dominant species were estimated from the allometric relationships between stem diameter and AB (Ogaya & Peñuelas, 2007a).

Aboveground litter production (litterfall) was collected seasonally from January 1999 to December 2013 with twenty traps (27 cm in diameter with 1.5-mm mesh) randomly distributed in each plot. We estimated the annual mass of litterfall as the sum of the litters collected every three months during the study period. Litter was separated in the laboratory into leaves, branches and fruits. Leaves and fruits were characterized to species (*A. unedo*, *Q. ilex* and *P. latifolia*). The dry weights of the samples were then obtained by oven-drying the litter to a constant weight at 70 °C.

ANPP was calculated each year during the study period as the sum of the ABIs (no dead stems) and litterfalls for all species in the plot (Clark & Brown, 2001). The litterfall data for 2009 were lost, so we could not calculate the ANPP for that year. A severe drought in 2011 produced an atypically large litterfall, so this litterfall were not included in the ANPP calculation. Losses to herbivores and the emission of volatile organic compounds (VOCS) were not quantified and were considered negligible relative to the ABI and litterfall.

Statistical analysis

Differences in ABI and litterfall (expressed as a percentage of AB) among the species and the entire forest community were analyzed using repeated-measures analyses of variance (ANOVAs), with treatment as the independent factor and year as the repeated measure. These analyses were performed using Statistica 10.0 (StatSoft Inc., Tulsa, USA). Branch litterfall could not be separated by species, so we calculated the total branch litterfall in each plot. The fruit litterfalls for *A. unedo* and *P. latifolia* were also excluded because of their low abundance. Depending on drought treatments, allometric relationships between SPEI and ABI, litterfall and ANPP were tested by standardized major-axis regression. To compare fitted bivariate slopes of the allometric relationships between treatments, we tested the homogeneity of the slopes and intercepts with the smatr R package (version 3.4-3, Warton) described by Warton, *et al* (2012). The relationship was determined with one regression if the control and treatments did not differ.

Results

Environmental conditions

The mean annual temperature during this 15-year experiment period (12.2 °C) was slightly higher than the average for 1975-2013 (11.8 °C) (Fig. 1). In the study period, there were two hottest years in 2006 (13.0°C) and 2011 (13.1°C). The mean annual precipitation for this period (616.1 mm) was lower than the average for 1975-2013 (662.4 mm), which varied greatly from 379.8 mm in 2006 to 926.7 mm in 2010 (Fig. 1). Soil moisture fluctuated with the evaporative demand and precipitation periodicity (Fig. 1). Mean soil moisture throughout the study period was decreased by 13.0% in drought when compared with control plots. However, both control and drought plots reached the lowest soil-moisture contents in summer (14.9±1.2% and 13.0±1.1%, respectively). In this study period, the amount of soil moisture decreased substantially in the summers of 2006 and 2011. May SPEI-6 demonstrated that the periods of 2000-2001, 2005-2006 and 2011-2012 were the driest records for the growing seasons of the study period.

ABI

The ABI of the three dominant tree species was correlated with the inter-annual fluctuations of precipitation, but the species differed in their sensitivity to the variation (Fig. 2). A. unedo was the most sensitive to water shortages, with a significantly lower ABI in the drought than the control plots for the entire study period (control=0.4±0.1 and drought=0.1±0.1 Mg ha-1, p<0.001). Q. ilex had a marginally lower ABI in the drought than the control plots (control=0.5±0.2 and drought=0.2±0.3 Mg ha-1, p<0. 1); the ABIs differed more during the first five years and the extreme episodes of 2005-2006 and 2011-2012. P. latifolia ABIs were not affected by the drought treatment during the study period.

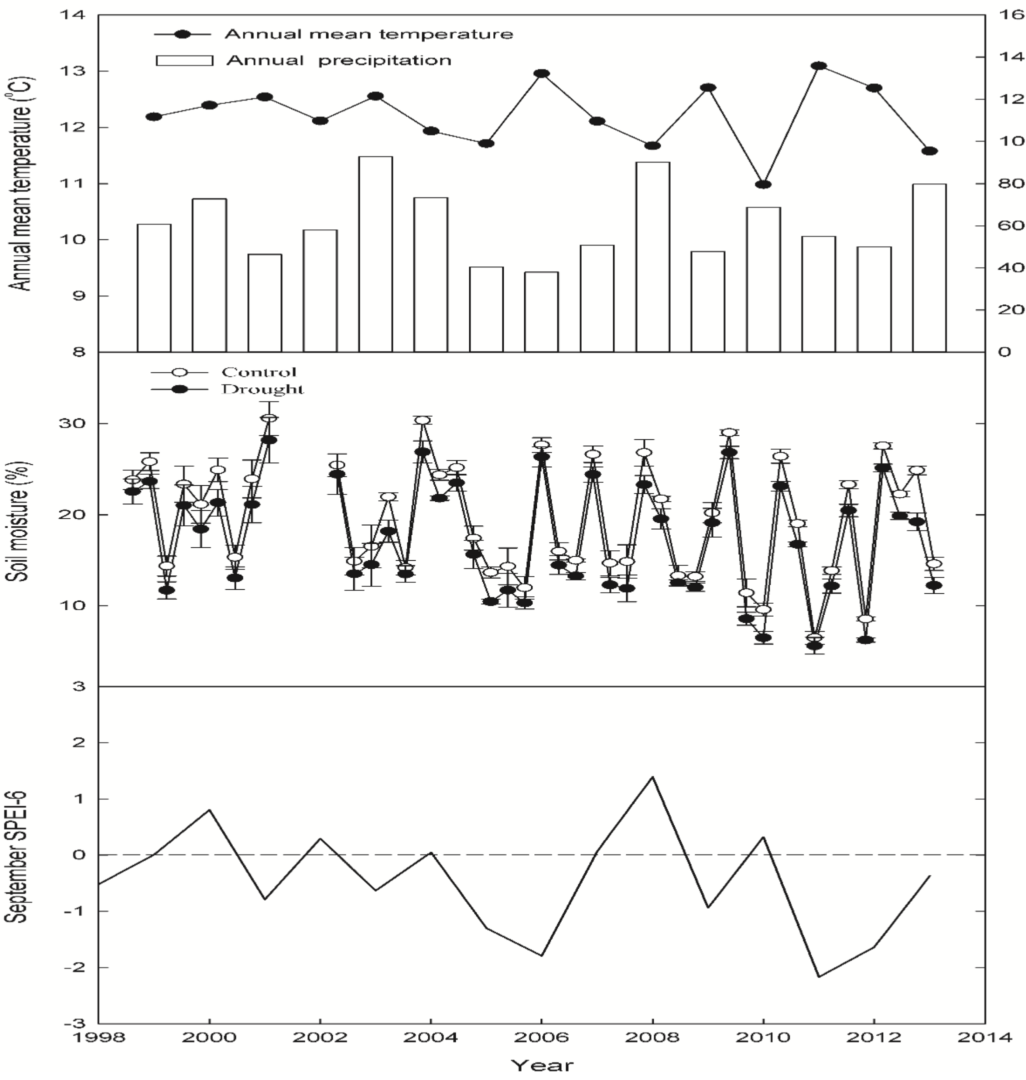

Fig. 1 Mean annual temperature, inter-annual precipitation and May SPEI-6 variables during the study period (1999-2013). The error bars in represent the standard errors of the means (n=4 plots).

ABI was positively correlated with the SPEI drought index in these species (Fig. 3). A. unedo ABI was correlated with April SPEI-1 (R2=0.16), Q. ilex ABI with May SPEI-6 (R2=0.39, p<0.05 and R2=0.61, p<0.001 for the control and drought plots, respectively) and P. latifolia ABI with May SPEI-3 (R2=0.62). Q. ilex was the most sensitive species to natural drought (Fig. 3).

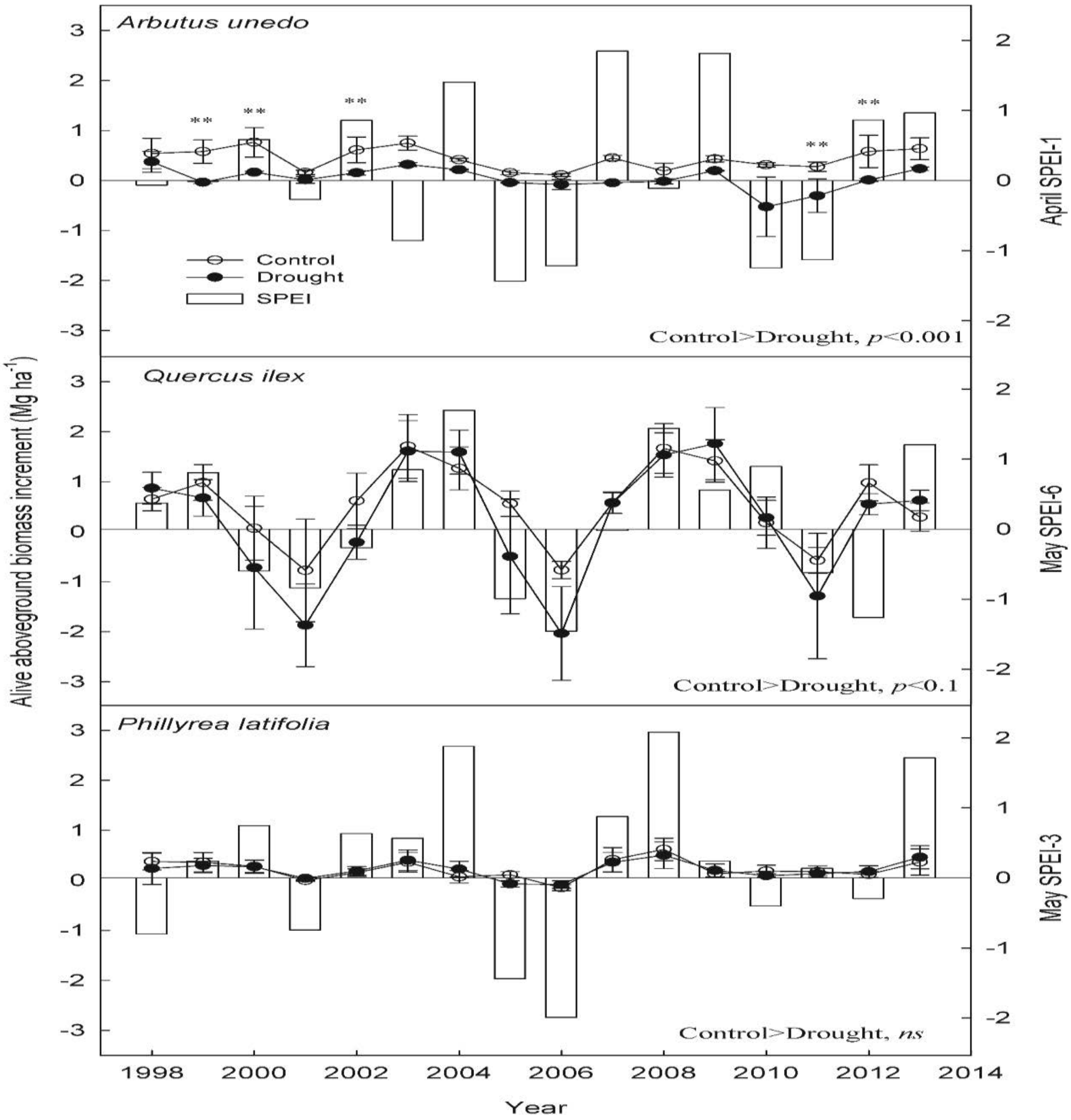

Fig. 2 Aboveground biomass increment (ABI) of the three dominant species in the controls and drought treatments. The bars represent the best-fitted SPEI timescale for *Arbutus unedo* (April SPEI-1), *Quercus ilex* (May SPEI-6) and *Phillyrea latifolia* (May SPEI-3). Significant differences are marked with asterisks (*, p<0.05; **, p<0.01; ns, not significant). The vertical bars represent the standard errors of the means (n=4 plots).

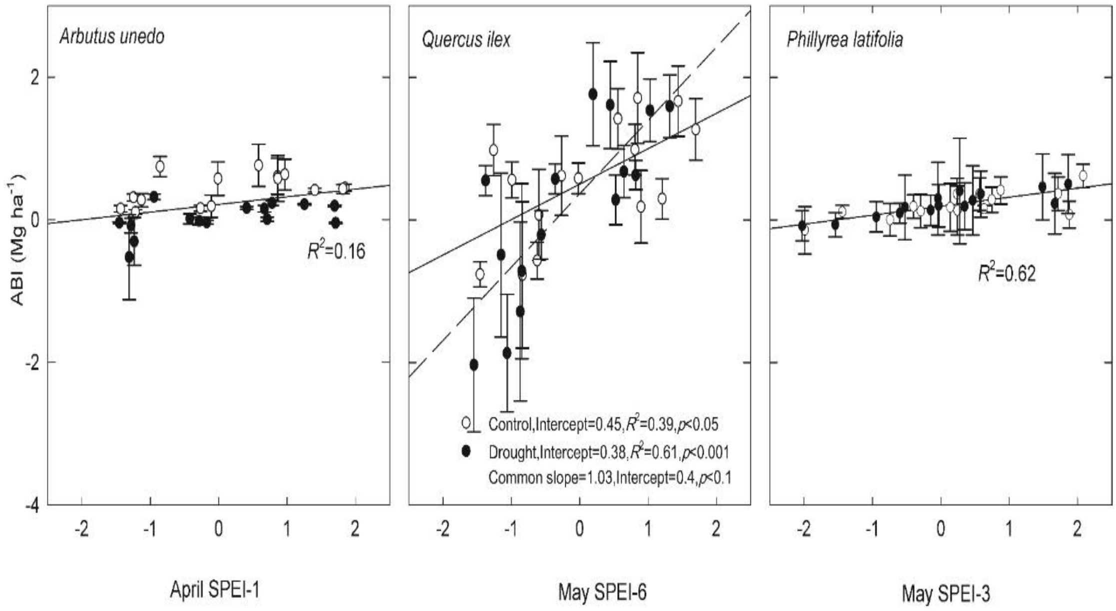

Fig. 3 Multiple-slope comparisons between the controls and drought treatments of the above-ground biomass increment (ABI) for *Arbutus unedo*, *Quercus ilex* and *Phillyrea latifolia* during 1999-2013. The error bars represent the standard errors of the means (n=4 plots).

Litterfall

The experimental drought significantly increased branch litterfall throughout the study period (p<0.01) (Fig. 4a), especially in the severely dry year of 2011 (p<0.001). The relative branch litterfall was also negatively correlated with SPEI; the best fit was with September SPEI-3 (R2=0.28, p<0.05 and R2=0.32, p<0.05 for the control and drought plots, respectively). Branch litterfall was most sensitive to water availability in the dry summer months (July, August and September). The drought treatment significantly increased this sensitivity (SMATR, common slope=-1.96, p<0.05) (Fig. 4b).

The interannual fluctuations of the relative leaf litterfall in these species indicated the different sensitivities to drought (Fig. 5). Leaf litterfall was higher in the drought than the control plots for *A. unedo, Q. ilex* (p<0.05) and *P. latifolia* (p<0.05) during the experimental period. Defoliation rates were significantly higher in the drought than the control plots in 2007 (p<0.001) and 2011 (p<0.001) for *Q. ilex* and in 2004 (p<0.05) and

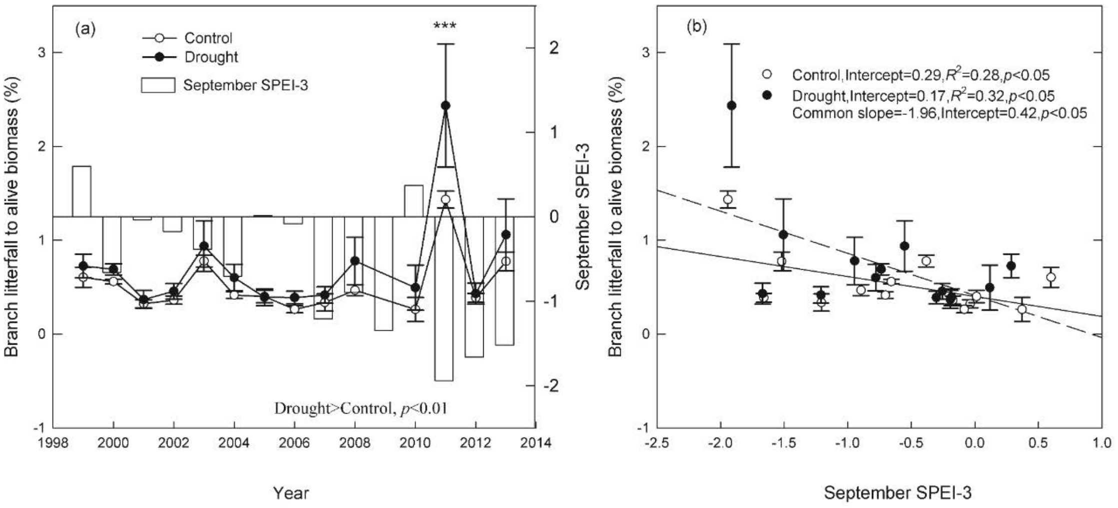

Fig. 4 (a) Relative branch litterfall of all species in the controls and drought treatments (n=4 plots). Significant differences are marked with asterisks (***, p<0.001). (b) The standardized major axis regression between relative branch litterfall and September SPEI-3 in the controls and drought treatments.

2007 (p<0.001), but not 2011, for *P. latifolia*. The relative leaf litterfall for these species was negatively correlated with SPEI; *A. unedo* and *Q. ilex* depended on October SPEI-3 (R2=0.19 and 0.25, respectively), whereas *P. latifolia* was most dependent on November SPEI-3 (R2=0.41) (Fig. 5). Relative leaf litterfall did not differ between the control and drought plots for these species (Fig. 6).

The drought treatment significantly decreased the fruit litterfall for *Q. ilex* (p<0.05), especially in 2000 (p<0.05) and 2003 (p<0.01). The relative fruit litterfall decreased in both the control and drought plots during extreme droughts, indicating less fruit maturation (Fig. 7a). December SPEI-6 was positively correlated with the relative fruit litterfall (R2=0.27).

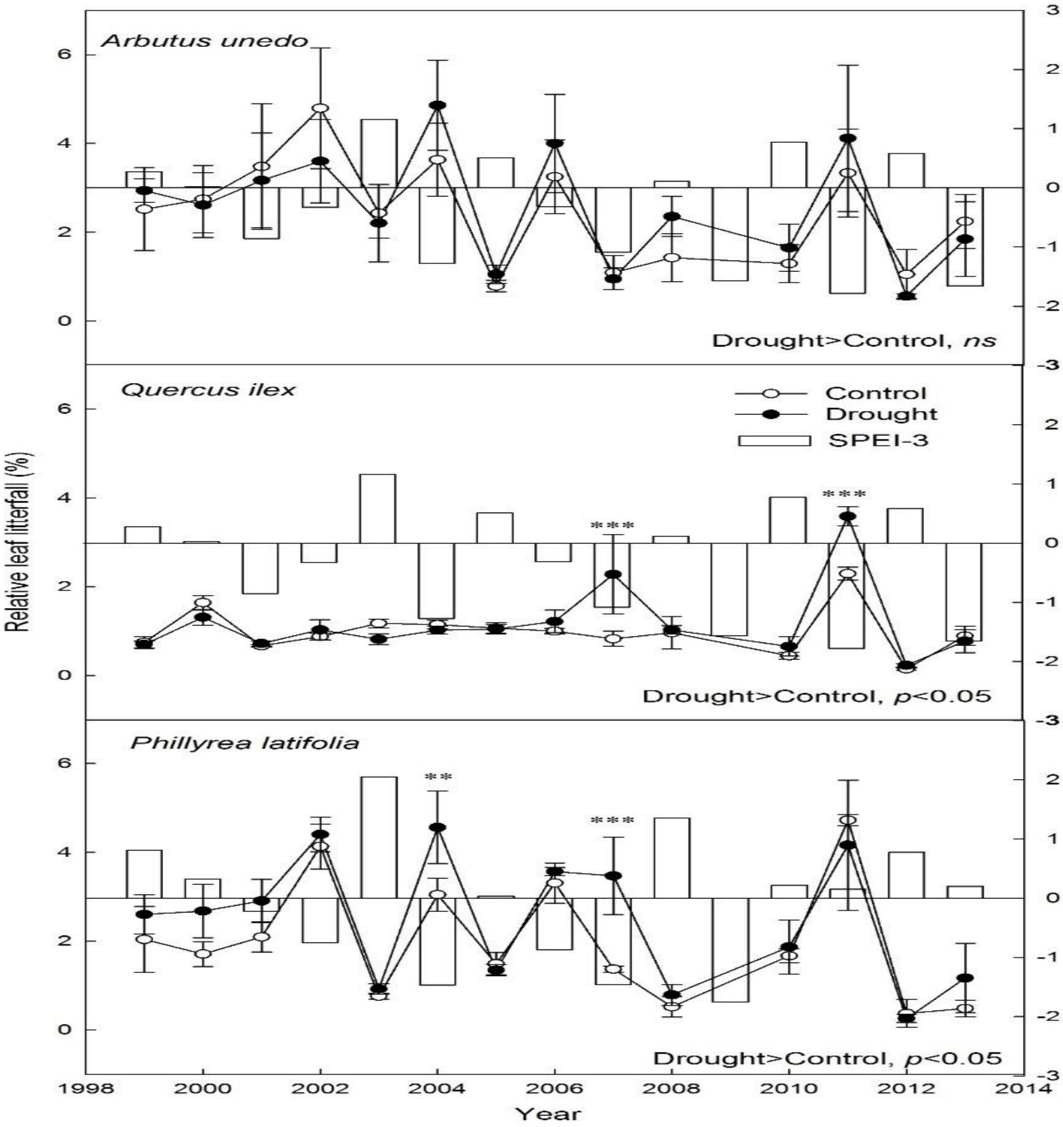

Fig. 5 Relative leaf litterfall to biomass of *Arbutus unedo*, *Quercus ile*x and *Phillyrea latifolia* throughout the study period (1999-2013). Significant differences are marked with asterisks (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$). The error bars represent the standard errors of the means (n=4 plots).

ANPP

ABI of the entire forest community was lower in the drought than the control plots throughout the study period (control=1.1±0.3 and drought=0.4±0.3 Mg ha-1, $p<0.01$) (Fig. 8).

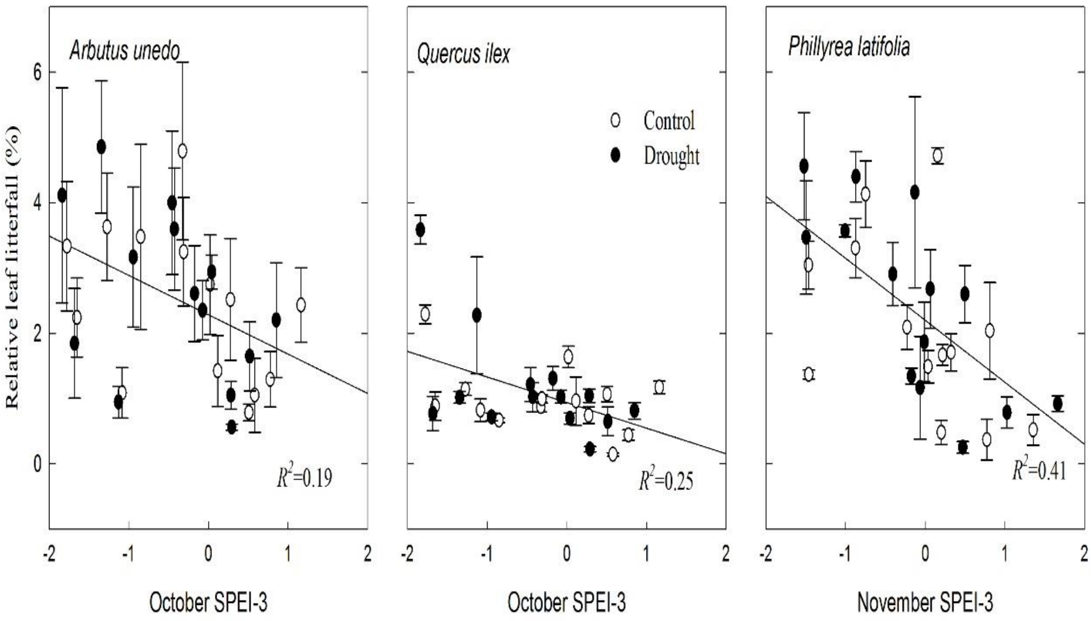

Fig. 6 Allometric relationships between the relative leaf litterfall and October SPEI-3 for *Arbutus unedo*, October SPEI-3 for *Quercus ilex* and November SPEI-3 for *Phillyrea latifolia*. The error bars represent the standard errors of the means (n=4 plots).

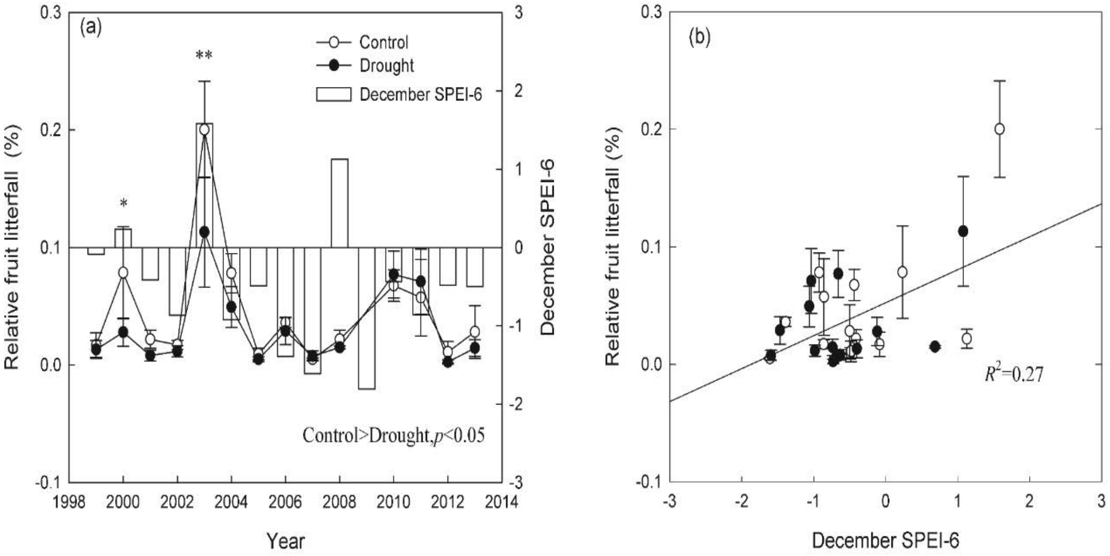

Fig. 7 (a) Relative fruit litterfall to biomass in the control and drought treatments for *Quercus ilex*. The error bars represent the standard errors of the means (n=4 plots). (b) Relationship between the relative fruit litterfall and December SPEI-6 for Q. ilex.

Minimum ABIs occurred during the extreme droughts in 2000-2001, 2005-2006 and 2011-2012. The drought treatment increased forest litterfall (control=2.4±0.3 and drought=2.6±0.4 Mg ha-1, p<0.1), and the extreme drought of 2011 triggered a heavy litterfall (control=5.5±0.5 and drought=7.2±0.7 Mg ha-1, p<0.001). ANPP estimated

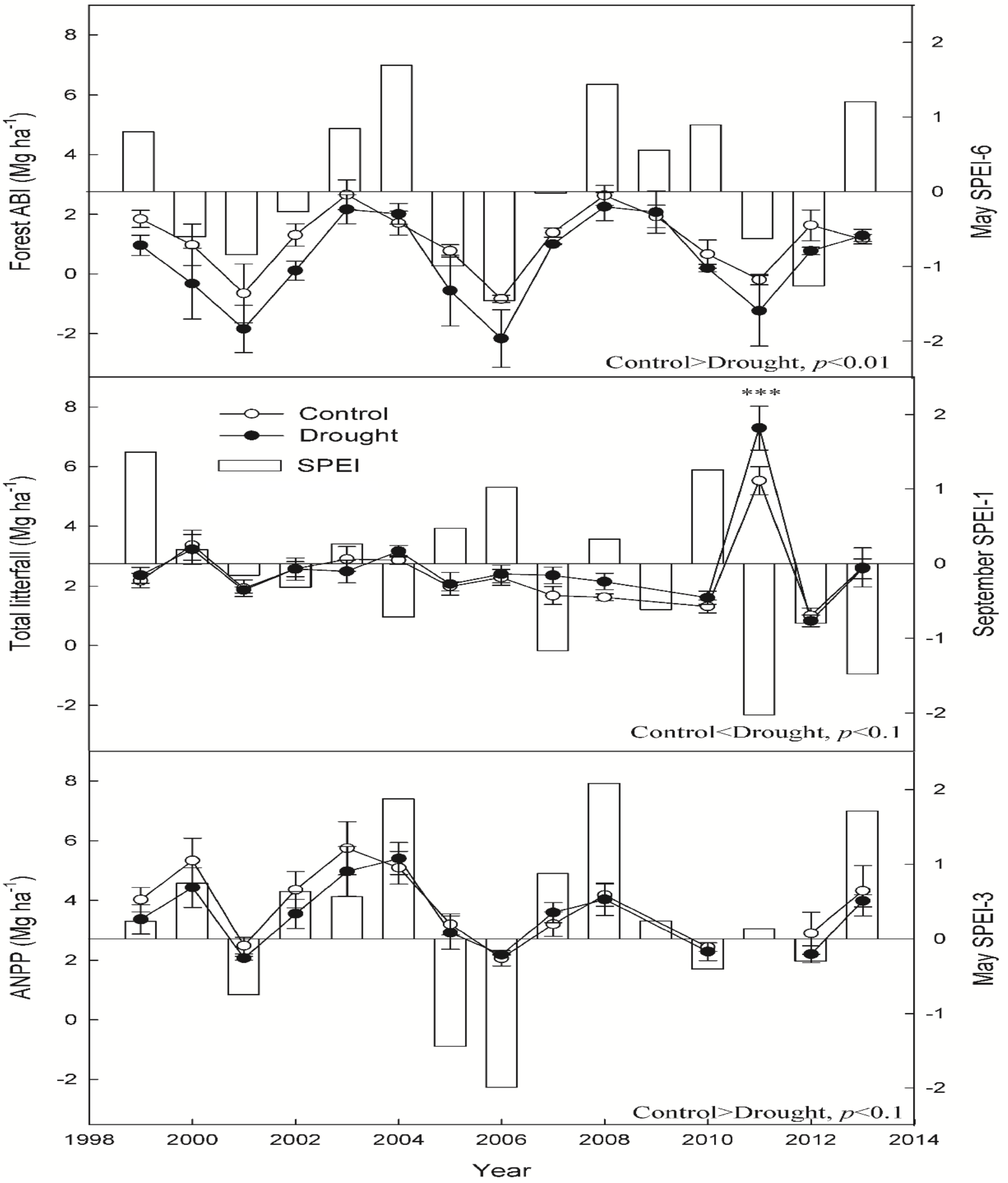

Fig. 8 Mean aboveground biomass increment (ABI) for May SPEI-6, total litterfall for September SPEI-1 and aboveground net primary production (ANPP) for May SPEI-3 of the forest community in the control and drought treatments. Significant differences are marked with asterisks (***, *p*<0.001). The error bars represent the standard errors of the means (n=4 plots). The vertical bars represent the best-fitted SPEI time-scale.

from the sum of ABI and litterfall consequently only tended to be higher in the control plots (control=3.8±0.3 and drought=3.4±0.3 Mg ha-1, p<0.1), despite a clearly higher ABI than in the drought plots. ANPP was even minimal in the dry years of 2001, 2005, 2010 and 2012. Our 15-year drought treatment demonstrated a dampening effect, and ANPP decreased by 10% throughout the experiment.

The best fits with the SPEI indices were with May SPEI-6 (R2=0.52) for ABI, with September SPEI-1 (R2=0.24) for litterfall and with May SPEI-3 (R2=0.55) for ANPP. The drought treatment slightly increased these trends toward lower ABIs and ANPPs and higher litterfalls in the years with lower SPEIs (Fig. 9). ABIs were positive only when May SPEI-6 was >0. ABI reached its maximum of 2.6 Mg ha-1 when May SPEI-6 was >1 and its minimum of -2.0 Mg ha-1 when May SPEI-6 was <-1. Litterfall reached its maximum of 7.0 Mg ha-1 when September SPEI-1 reached its extreme value of -2. ANPP reached its maximum of ca. 5.8 Mg ha-1 at a May SPEI-3 of 2.

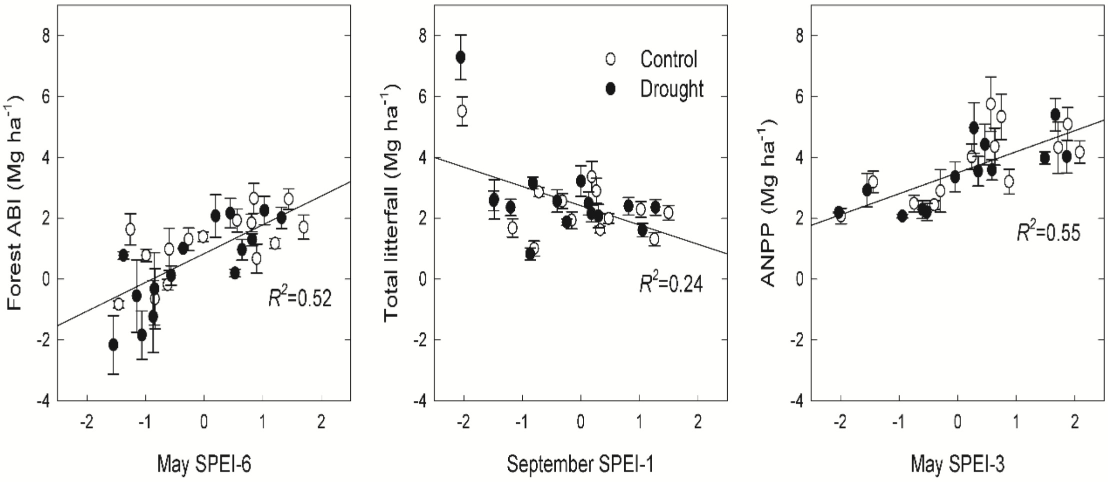

Fig. 9 Relationships between aboveground biomass increment (ABI) and May SPEI-6, total litterfall and September SPEI-1 and aboveground net primary production (ANPP) and May SPEI-3 for the entire forest community. The error bars represent the standard errors of the means (n=4 plots).

Discussion

Effects of experimental and extreme natural droughts on ABI, litterfall and ANPP

Both experimental and extreme natural droughts reduced the forest ABI. Sensitivity to the drought conditions, however, varied among the three dominant species. *A. unedo* was the most sensitive species to the drought treatment, with a significantly lower ABI in the drought than the control plots, but *Q. ilex* was the most sensitive species to extreme natural droughts, with severe reductions in ABI in the three extreme episodes, especially in the drought plots (Fig. 2). The species-specific drought vulnerability is consistent with that of previous studies conducted in the same Mediterranean forest (Ogaya & Peñuelas, 2003, 2007a; Barbeta *et al.*, 2013) and demonstrates that a reduction in soil moisture produces great disparity in the responses depending on species-specific hydraulic conductivity in stems (Barbeta, et al., 2012) or leaf physiological traits (Ogaya & Peñuelas, 2003; Ogaya et al., 2014). *Q. ilex* is more vulnerable to water shortage than *P. latifolia* due to more sensitive foliar photosynthesis (Ogaya & Peñuelas, 2003; Ogaya et al., 2014), aboveground growth (Ogaya & Peñuelas, 2007a; Barbeta et al., 2013) and stem hydraulic conductivity (Martínez-Vilalta et al., 2002; Barbeta et al., 2012). Therefore, this holm oak forest may experience a vegetation shift involving reverse succession (forest to shrubland) under future drier and warmer scenarios, including more frequent extreme droughts.

Litterfall was a 10% higher in the drought treatment plots than in control ones throughout the study period and increased up to 32% more than control following the extreme drought of 2011 (Fig. 4a), a likely consequence of drought-induced xylem cavitation accelerating branch and leaf senescence, which may eventually lead to tree mortality (Ogaya & Peñuelas, 2006; Misson *et al.*, 2011; Choat *et al.*, 2012). Drought-induced litterfall may also result from the reduction in leaf area as a conservative strategy to reduce transpiration and maintain hydraulic conductance under water stress, a response that has been observed under extreme drought conditions (Limousin *et al.*, 2009; Barbeta *et al.*, 2015). Leaf litterfall in all three species was higher in the drought than in the control plots. *P. latifolia,* however, was less affected than the other species, which could be attributed to its hydraulic architecture (the conduit diameter of roots and stems) (Martínez-Vilalta *et al.*, 2003) and its capacity to maintain higher hydraulic conductivity during dry periods (Barbeta *et al.*, 2012). Our results were also consistent with the higher crown defoliation and stem mortality in *Q. ilex* than in *P. latifolia* under severe drought (Ogaya & Peñuelas, 2006, 2007b; Barbeta *et al.*, 2013, 2015)

The drought treatment decreased the long-term relative production of fruit in *Q. ilex*, in agreement with shorter term observations at an earlier stage of the same experiment (Ogaya & Peñuelas, 2007c). Some studies have reported negative effects of drought on flower and fruit development (Pérez-Ramos *et al*., 2010; Sanchez-Humanes & Espelta, 2011), on the phenology of reproduction (Peñuelas *et al.*, 2004a) and on the sex ratio (Misson *et al.*, 2011). It has been reported that Q. ilex can adapt to the limited conditions by adjusting carbon allocation to growth and reproduction (Pulido *et al*., 2014). Nonetheless, in our study, both the ABI and the fruit production were lower under dry condition, which was also found by previous studies conducted in the same type of forest (Pérez-Ramos *et al*., 2010; Limousin *et al*., 2012).

The responses of forest productivity to water limitation are highly dependent on the vegetation types and species interactions (Peñuelas *et al.*, 2007; Kreyling *et al.*, 2008; McDowell *et al.*, 2008; Wu *et al.*, 2011). Most studies indicate that experimental or natural droughts cause lower productivity (Ciais *et al.*, 2005; Peñuelas *et al.*, 2007; Luyssaert *et al.*, 2010; Wu *et al.*, 2011; Tilman *et al.*, 2012; Reyer *et al.*, 2013), but other studies have reported no significant changes (Kreyling *et al.*, 2008; Jentsch *et al.*, 2011).

A review of 34 precipitation experiments ranging in duration from 1 to 11 years reported that decreased precipitation significantly reduced ANPP by an average of 37% (Wu *et al.*, 2011). In our study, forest ANPP decreased by an average of only 10%, substantially lower ($p<0.1$) than expected and a likely consequence of a dampening of the effect of the treatment as the duration of the experiment increased (Barbeta *et al.*, 2013).

Litterfall should not be ignored when estimating ANPP. A large increase in litterfall coincided with water shortage in our study in the dry year of 2011 (Fig. 8). The forest ANPP estimated by the sum of ABI and litterfall would consequently not represent the response of plant production when the water deficit exceeded a determinate threshold. The estimation of ANPP thus remains methodologically difficult in Mediterranean evergreen forests, and caution is recommended when using litterfall to calculate ANPP, because litterfall does not necessarily correspond to annual production.

The duration of natural droughts impacts the increase in forest biomass

The ABIs of the three species were highly correlated with SPEI in spring (April and May) (Fig. 3), indicating that the water balance in spring was particularly important for growth. The relationships with SPEI, however, were species-specific. The ABI of *Q. ilex* was the most sensitive to natural drought (May SPEI-6), and *Q. ilex* was also the only species with different relationships in the control and drought treatments (Fig. 3). The drought treatment thus increased the dependence of *Q. ilex* ABI on water availability. The ABI of *Q. ilex* was best correlated with a longer timescale than the other two species (May SPEI-6), suggesting that water reserves stored during wet and cold seasons may be used later during the growing season (Barbeta et al., 2013; 2015). SPEI is a good predictor of radial tree growth and ANPP for forests across all biomes, and longer SPEI timescales correlate better in seasonally dry environments (Vicente-Serrano *et al.*, 2013). As with *Q. ilex* in our study, accumulated precipitation from the previous autumn and the current spring may be particularly beneficial for tree-ring growth and the features of wood anatomy (Martin-Benito *et al.*, 2012; Drew *et al.*, 2013). Forests in Mediterranean ecosystems may thus undergo significant decreases in ABI if lower precipitation and higher evapotranspiration reduce the autumn, winter and spring water recharge.

This Mediterranean forest suffered frequent natural droughts in summer, which increased branch withering and defoliation (Fig. 4a). Relative branch litterfall was negatively correlated with September SPEI-3 and thus depended on the summer water balance (July, August and September). High intensities of summer droughts could promote hydraulic failure at the branch level and increase branch withering. A previous study at this site emphasized that *Q. ilex* and *P. latifolia* stem mortality depended on the water balance over longer periods (Barbeta *et al.*, 2013). Branch withering would thus represent a response to shorter droughts that do not induce widespread stem mortality. Defoliation may be another short-term adjustment to drought. Indeed, plants adjust to drought by reducing the lifespan of leaves and by increasing turnover rate (Bussotti *et al.*, 2003; Ogaya & Peñuelas, 2006; Limousin *et al.*, 2009, 2012). The accumulated effect of drought in the long-term would substantially reduce crown condition and transpiration, eventually modifying the carbon and water cycles and the ecosystem services.

Fruit production and December SPEI-6 were positively correlated in *Q. ilex* in our 15-year moderate experimental drought, indicating higher fruit production with higher water availability in summer and autumn (Fig. 7b), consistent with a study at this site reporting a strong correlation between fruit production and precipitation in *Q. ilex* 10 months prior to fruit maturation (Ogaya & Peñuelas, 2007c). Drier summer and autumn in the future would thus decrease fruit production. These results also support SPEI as an effective tool to assess the impacts of drought on agricultural and ecological production (Potop, 2011; Vicente-Serrano *et al.*, 2013).

The water balance in March, April and May may be critical for the ANPP of this forest (Fig. 8). The response of ANPP to drought varies among forest types and drought severity and duration, but dry forests, shrublands and steppes with low ANPPs have the highest correlations with the SPEI (Vicente-Serrano *et al*., 2013). The response to drought also depends on the characteristic drought timescale; arid and humid biomes respond to water deficits of shorter duration than do semiarid and subhumid biomes (Vicente-Serrano *et al*., 2012; 2013). Robust observational studies have highlighted that drought is a main driver of reduced forest ANPP and that drought can be amplified by warmer temperatures (Breshears *et al.*, 2005; Mueller *et al.*, 2005; Adams *et al.*, 2009; Wu *et al.*, 2011; Anderegg *et al.*, 2012).

Long-term experimental drought is driving Mediterranean forests to acclimate to extreme conditions

The forest in this study experienced three extreme dry periods during this 15-year study, in 2000-2001, 2005-2006 and 2011-2012. Both the experimental and natural droughts decreased the ABI of the forest. Leaf litterfall was highly variable inter-annually, with higher values in the drought than the control plots throughout the study period and a peak of defoliation in the extreme drought in 2011. Extreme droughts provoked higher tree mortality but also activated adaptive responses linked to forest structure and species composition (Mueller *et al.*, 2005; Ruiz-Labourdette *et al*., 2012; Grimm *et al*., 2013). The effects of drought on forest community were gradually attenuated at our experimental site. ABI and ANPP presented a dampening trend that is likely a result of (i) demographic compensation enhancing recruitment or adult survival due to low competition in the drought plots (Jentsch *et al.*, 2011; Lloret *et al.*, 2012; Barbeta *et al.*, 2013; Peñuelas *et al*., 2013a), (ii) changes in morphology and function at the individual level (Martin-Stpaul *et al*., 2013; Barbeta *et al*., 2015) and (iii) epigenetic modifications (Rico *et al*., 2014).

The results of this study thus show that a partial and gradual acclimation to a drier environment over the long-term is possible if the responses to drought fluctuate below safe thresholds and/or tipping points.

The Mediterranean region is predicted to suffer more rapid and intense climate changes in the coming decades that could severely alter the structure of forests and their capacity to assimilate carbon (IPCC, 2014). Mediterranean forests display tolerance, plasticity and resilience in response to drought, but the progressive increase in aridity combined with extreme droughts will challenge the survival of these forests in their current form. Our 15-year experimental-drought study demonstrated a partial acclimation of a Mediterranean forest to a 13% decrease in soil moisture, but if the effects of chronic and extreme droughts increase beyond a critical threshold, this stabilizing process could be disrupted and produce a shift in the vegetation (a progressive replacement of *Q. ilex* by *P. latifolia*). Future studies should address the complex dynamics (demographical processes and structure and composition changes) underlying the response of ecosystems to extreme droughts and chronically drier conditions.

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Chapter 4. Long-term experimental drought combined with natural extremes accelerate forest degradation and vegetation shifts in a Mediterranean holm oak forest

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Abstract

Increasing drought combined with natural extremes are accelerating forest die-off and shifts in vegetation in the Mediterranean Basin. Our understanding of drought-induced vegetation shifts in forest ecosystems, however, is largely restricted by the lack of practical methods for tracking the underlying process. 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, stem growth and shifts in composition. The experimental drought increased annual rates of stem mortality and decreased stem growth at the community level for both H- and L-canopies over the study period. The changes in mean stem mortality, recruitment, stem density and growth, however, were larger for *Quercus ilex* than *Phillyrea latifolia*. Importantly, natural drought (Standardized Precipitation-Evapotranspiration Index, SPEI) also impacted forest stem mortality and growth. Spring-winter SPEIs amplified the effects of the experimental drought on annual stem mortality at the community level and for H- and L-canopy *Q. ilex*. The relevant timescales of the SPEIs varied with canopy types and species, with shorter timescales for L-canopy and *Q. ilex*. Furthermore, the experimental drought amplified the increases in stem mortality and decreases in stem growth during natural extremes for L-canopy *Q. ilex*. Interestingly, the percentage of stem density decreased for *Q. ilex* and concomitantly increased for *P. latifolia* for both H- and L-canopies, and the experimental drought reinforced a vegetation shift favoring *P. latifolia* by the changes in the percentage of stem density and basal area for L-canopy. These findings suggest that the long-term experimental drought accelerated a vegetation shift due to continuous soil desiccation and frequent natural extremes, implying substantial future alterations in community structure and functioning for water-limited forest ecosystems.

Keywords: water deficits; Mediterranean forests; Long-term experimental drought; Frequent extreme droughts; Stem-mortality rate; Recruitment; Forest degradation; Vegetation shifts

Introduction

Large-scale tree mortality (Peñuelas *et al.*, 2001; Phillips *et al.*, 2010; Peng *et al.*, 2011; Anderegg *et al.*, 2013; Greenwood *et al.*, 2017), forest canopy die-off or defoliation (Breshears *et al.*, 2005, 2009, Allen *et al.*, 2010, 2015; Carnicer *et al.*, 2011) and shifts in composition (Allen & Breshears, 1998; Peñuelas & Boada, 2003; Mueller *et al.*, 2005; Peñuelas *et al.*, 2007; McIntyre *et al.*, 2015) in recent decades have been attributed to anthropogenic climate change. Water deficits triggered by increasing temperatures and shifting precipitation regimes have become common disturbances affecting forest ecosystems globally (Anderegg *et al.*, 2013; Allen *et al.*, 2015; Franklin *et al.*, 2016). Mediterranean ecosystems have particularly suffered increasing water deficits due to the unprecedented rate of warming and more frequent extreme events (e.g. heat waves and droughts), which have led to dramatic changes in composition such as vegetation shifts (dominance shifts or species replacement) (Myers *et al.*, 2000; Kéfi *et al.*, 2007; Dai, 2013; Doblas-Miranda *et al.*, 2015, 2017; Reyer *et al.*, 2015). A growing number of studies during the last two decades have reported severe effects of drought on Mediterranean forests, such as 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) (Myers *et al.*, 2000; Doblas-Miranda *et al.*, 2015; Franklin *et al.*, 2016; Natalini *et al.*, 2016). General circulation models project an increase in the intensity and duration of droughts for the coming decades, which would further threaten the conservation of forest biodiversity, carbon budgets and climatic feedbacks (Myers *et al.*, 2000; Carnicer *et al.*, 2011; Peñuelas *et al.*, 2013; Doblas-Miranda *et al.*, 2015, 2017).

The evidence for vegetation shifts, however, is sporadic across terrestrial ecosystems, despite extensive episodes of drought-induced tree mortality (Allen & Breshears, 1998; Mueller *et al.*, 2005; Lloret *et al.*, 2012; Franklin *et al.*, 2016). 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 (Gonzalez *et al.*, 2010; 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 only eight of 35 selected case studies (<25%) across terrestrial ecosystems globally. The scarcity of vegetation shifts has been 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). More frequent and intense droughts, though, would also 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 (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, based on field surveys conducted in the 1930s and the 2000s (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 the interactions of other factors such as 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 (Gonzalez *et al.*, 2010; 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; Beier *et al.*, 2012; 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 higher hierarchical levels of biological organization 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 drought effects (Smith *et al.*, 2009; Luo *et al.*, 2011; Beier *et al.*, 2012; Lloret *et al.*, 2012; 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 (Ciais *et al.*, 2005; Lloret *et al.*, 2012; Anderegg *et al.*, 2013; Reichstein *et al.*, 2013). Long-term (decadal) 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; Beier *et al.*, 2012; Knapp *et al.*, 2015, 2016).

Holm oak (*Quercus ilex* L.) forests are the dominant type of vegetation 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 affected by the increasing length and intensity of droughts with high rates of evapotranspiration and low precipitation (Peñuelas *et al.*, 1998, 2000, 2001; 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 also 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; Barbeta & Peñuelas, 2016). 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; Barbeta *et al.*, 2013; Bennett *et al.*, 2015; Natalini *et al.*, 2015). Recruitment in these low-canopy forests would be enhanced by the gap openings after drought-induced mortality, and the forests would be more favorable for highly drought-resistant seedlings (Lloret *et al.*, 2004b, 2012; Martínez-Vilalta & Lloret, 2016).

The presence of drought-resistant species may promote vegetation shifts in the holm oak forests by the high performances of coexisting species under drought (Ogaya *et al.*, 2003; Lloret *et al.*, 2004b, 2012; Ogaya & Peñuelas, 2007a; Barbeta & Peñuelas, 2016). Holm oak forests usually contain the tall shrub *Phillyrea latifolia* L., 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* would thus affect *P. latifolia* less (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 (Leuzinger *et al.*, 2011; Luo *et al.*, 2011; 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). Conducting long-term (decadal) drought experiments in different forest types is thus appropriate for realistically tracking forest dynamics and vegetation shifts at relevant temporal and spatial scales (Jentsch *et al.*, 2007; Leuzinger *et al.*, 2011; Luo *et al.*, 2011; Martin-Stpaul *et al.*, 2013; Franklin *et al.*, 2016; 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. We hypothesized that severe water deficits due to increasing drought would cause forest structural changes (high canopies degrading into low canopies) and a vegetation shift (*Q. ilex* replaced by *P. latifolia*) and that long-term experimental drought (1999-2015) would accelerate these changes. More specifically, we (1) evaluated the effects of a long-term (17-year) experimental drought on two types of canopies by the changes in stem mortality, recruitment, density and growth at the community and species levels, (2) assessed the effects of natural droughts on annual stem mortality at the community and species levels, (3) determined the stem mortality and growth rate during natural extreme years at the community and species levels and (4) analyzed the changes in the percentage of stem density and basal area for the two species (*Q. ilex* and *P. latifolia*) during the study period. Attaining these research goals would provide experimental evidence that forest degradation and vegetation shifts respond to the increasing episodes of long-term and/or extreme droughts in Mediterranean ecosystems, suggesting the importance of applying further effective strategies to monitor forest dynamics at broader scales, especially in arid ecosystems, and to assess future ecosystemic dynamics.

Materials and methods

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., *Cistus albidus* 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 dominated by tall, dense stands of *Q. ilex* trees reaching a height of 8-10 m (H-canopy), but forests at sites with shallow soils are more mixed with *P. latifolia* and reach a height of 4-6 m (L-canopy). The features of the two types of canopies are detailed in Table 1.

Table 1. Characteristics of the two canopy types.

Characteristic	High canopy	Low canopy
Soil depth (cm)	30-50	10-30
Mean tree height (m)	8-10	4-6
Stem density of *Quercus ilex* (per plot)	125.3±7.9	77.8±42.8
Stem density of *Phillyrea latifolia* (per plot)	40.3±11.7	180.8±40.3
Stem density at stand level (per plot)	199.3±17.5	285.8±29.8
Mean basal area of *Quercus ilex* (cm^2 per plot)	5667.4±548.1	2598.5±1146
Mean basal area of *Phillyrea latifolia* (cm^2 per plot)	380±113.5	2099.7±375.5
Mean basal area at stand level (cm^2 per plot)	7072.3±458.8	5330.1±1185.5

The climate is typically Mediterranean with hot and dry summers (June-August) and rainy springs (March-May) and autumns (September-November). The meteorological series for 1975 to 2015 had a mean annual temperature (MAT) of 11.8 °C and a mean annual precipitation (MAP) of 656.1 mm. MAT has gradually increased (R^2=0.42, P<0.001) (Fig. S1), most clearly in spring (R^2=0.32, P<0.001) and summer (R^2=0.48, P<0.001) (Fig. S2). 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). The soil is a Dystric Cambisol, and the soil depths ranged from 30 to 50 cm for H-canopy and from 10 to 30 cm for L-canopy. The types of forest canopy and composition are associated with different soil depths and water availabilities (Rivas-Ubach *et al.*, 2016).

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 attitude (930 m a.s.l.) along the southern face of a mountain (25% slope) that had 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). 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.

Measurement of indices of experimental and natural drought

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) to determine the history of climate change at the study site. Natural extreme years (hot and dry) were defined as those in the highest 10th percentile for MAT and the lowest 10th percentile for MAP during 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 (Ogaya & Peñuelas, 2007a).

A drought index (Standardized Precipitation-Evapotranspiration Index, SPEI) was used to analyze the effect of naturally occurring droughts. The SPEI has the crucial advantage of including the effects of precipitation (P) and potential evapotranspiration (PET) on drought severity. It also 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). The monthly temperatures and precipitation since 1975 were used to calculate the SPEI, and the climatic data before 1999 was estimated from the meteorological data from the nearby station at the Poblet Monastery. A log-logistic distribution was obtained to model the values of difference between precipitation and potential evapotranspiration based on the 1975-2015 profile, and the cumulative probabilities could be translated into standardized variables (Barbeta *et al.*, 2013). SPEI values range from -3 to 3, with the lower values representing a more severe water deficit. SPEIs at different scales (from 3 to 48 months) have been used to study the impacts of natural droughts (evaluated by the SPEIs) on various forest types and species.

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 for obtaining stem mortalities. The annual stem mortality (m) was calculated as:

$m=1-(1-(N_0-N_t)/N_0)$ (1)

where N_0 and N_t are the number of living stems at the beginning and end of the year (beginning in 1999), 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 rates were calculated for the experimental drought and for the natural extreme (hot and dry) years. The rates of stem mortality at the community (all species in the plot) and species (*Q. ilex* and *P. latifolia*) levels were calculated separately for the two canopy types.

Rate of recruitment

Recruitment was defined as new stems resprouting from dead stems or seedlings larger than the threshold size (D_{50} >2 cm). The rate of recruitment was estimated by the percentage of new stems between the beginning of 1999 and the end of 2015. Recruitment at the community and species levels were calculated separately for the two canopy types.

Change in stem density

The differences in the number of living stems were calculated from the beginning of 1999 to the end of 2015. Change in stem density was estimated by the percentage of the differences of living stems to the beginning of 2015. The changes in stem density at the community and species levels were calculated separately for the two canopy types.

Response of stem growth

The annual rate of stem growth (g) was calculated as:

$g= (BA_t-BA_0) \times 100/BA_0$ (2)

where BA_0 and BA_t are the basal areas at the beginning and end of the year, respectively. The rate of stem growth over the study period was calculated for the beginning of 1999 to the end of 2015. The rates of stem growth were calculated for the experimental drought and for the natural extreme years. Stem growth at the community and species levels were calculated separately for the two canopy types.

Shift in species composition

We assessed the shift in species composition based on the changes in the percentage of stem density and BA for *Q. ilex* and *P. latifolia*. First, we calculated the percentage of stem density and BA 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 percentage for the two species in the two canopy types (H- and L-canopies) and treatments (drought and control). Third, we calculated the changes in percentage of stem density and BA for *Q. ilex* and *P. latifolia* for the study period as the percentage of the current year minus the percentage for the beginning of 1998.

Statistical analyses

We evaluated the effects of the experimental and natural droughts on annual stem mortality using nonlinear mixed-effects models. The stem mortality at the community and species (*Q. ilex* and *P. latifolia*) levels were analyzed separately for the two canopy types. The timescales and month of departure of the SPEIs used for stem mortality were selected by choosing the models with the lowest values of Akaike's information criterion:

$$\text{annual stem mortality (community/species)} = \text{treatment} \times \text{SPEI} \qquad (3)$$

where stem mortalities at the community (all species in the plot) and species (*Q. ilex* and *P. latifolia*) levels were calculated separately. The treatment factor had two levels (drought and control), and the SPEI timescales were considered as independent variables in each model. June 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 June SPEI-5 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. Plot and year were included as random factors in the models.

Seasonal and treatment variations in soil-water content in the two canopy types were assessed separately by analyses of variance (ANOVAs). Changes in mean stem mortality, recruitment, stem density and growth due to drought for the two canopy types over the study period were analyzed by ANOVAs with Tukey's HSD (honest significant difference) post hoc tests. The effect of the experimental drought on stem mortality and growth rates during the natural extreme years were evaluated by ANOVAs with Tukey's HSD post hoc tests. All of these changes at the community and species (*Q. ilex* and *P. latifolia*) levels were analyzed separately. The slopes of the lines representing the changes in the percentage of stem density and growth rates for *Q. ilex* and *P. latifolia* in response to drought were analyzed by the R smatr package (Warton *et al.*, 2006). All analyses were performed with R version 3.3.2 (R Core Development Team, 2016).

Results

Environmental variables

MAT was 12.3 °C throughout the study period of 1999-2015, with extreme hot years in 2006 (13.0 °C), 2011 (13.1 °C) and 2015 (13.2 °C) in which average summer temperatures were 21.4, 20.1 and 21.6° C, respectively. MAP was 602.9 mm during the study period, with extreme drought years in 2005 (403.2 mm), 2006 (379.8 mm) and 2015 (355.4 mm) in which winter-spring precipitation was low at 130.5, 200.7 and 167.4 mm, respectively. The extreme years during our study period were 2001, 2005, 2006, 2011 and 2015, based on the definition of natural extremes (hot and dry) (Fig. 1).

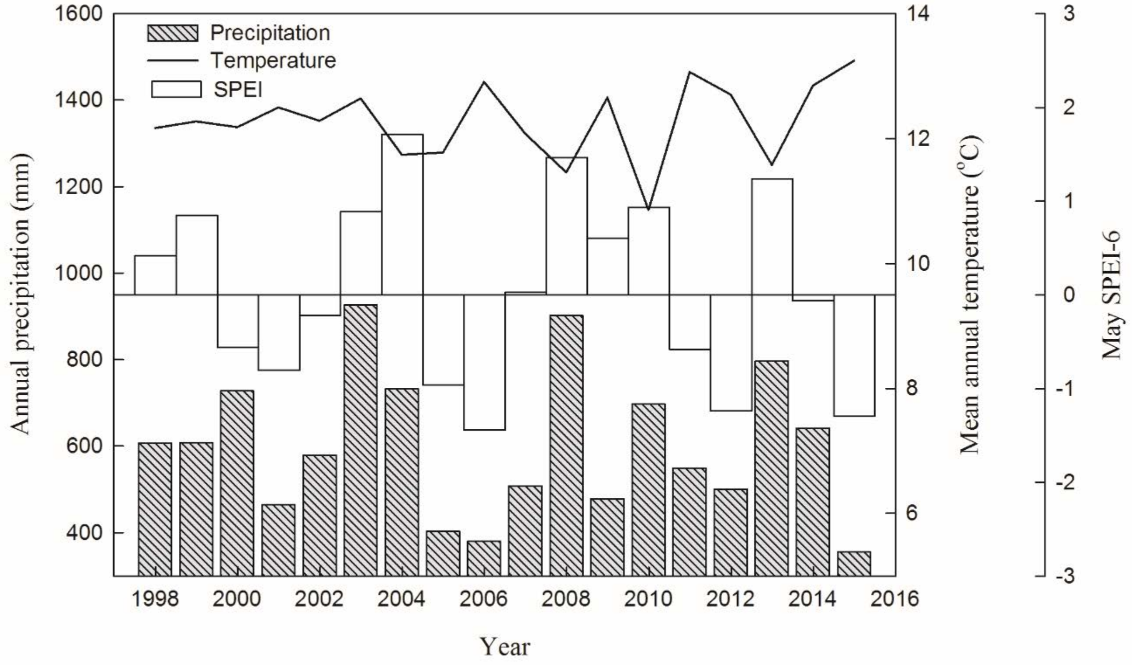

Fig. 1 Environmental variables for 1998-2015.

Soil-water content during the study period was slightly higher (+5.7%) for H- than L-canopies (Fig. S3). The decrease in soil-water due to the experimental drought was similar for H- and L-canopies (by -14.2 and -14.6%, respectively). Soil-water content also differed greatly between the seasons for the two canopy types (both $P<0.001$). Soil-water content was high in winter and spring. And soil-water content was lower in summer than the other seasons for both H- and L-canopies, for which the drought treatment decreased the contents by 19.7 and 13.5%, respectively. Summer soil-water content differed between the canopy types and treatments (H-canopy control plots (12.7%) > H-canopy drought plots (10.2%) > L-canopy control plots (9.9%) > L-canopy drought plots (8.6%)).

Annual stem mortality

The annual rate of stem mortality at the community level was higher in the drought treatment for both H- and L-canopies (both $P<0.05$) (Fig. 2 and Table 2). The rate, however, was not affected by the drought treatment for H-canopies for either *Q. ilex* or *P. latifolia* but was significantly higher for L-canopy *Q. ilex* ($P<0.05$). Natural drought (SPEIs) amplified the effects of the experimental drought on the rates for the two canopy types. The annual rate of stem mortality at the community level increased significantly in the drought plots with June SPEI-7 and May SPEI-6 for both H- and L-canopies ($P<0.05$ and $P<0.1$, respectively). Interestingly, the *Q. ilex* rate increased significantly in the drought plots with June SPEI-9 and June SPEI-5 for both H- and L-canopies (both $P<0.05$). The *P. latifolia* rate, however, did not increase significantly in the drought plots with October SPEI-15 for either H- or L-canopy. But *P. latifolia* rate respond to October SPEI-15 was significantly higher for L-canopy ($P<0.01$).

The experimental drought increased the mean mortality rates at the community level for both H- and L-canopies, but not significantly (Fig. 3). Mean mortality rates, however, differed between species; the rate was significantly higher for *Q. ilex* than *P. latifolia* ($P<0.05$), especially for L-canopy ($P<0.01$). The experimental drought significantly increased the *Q. ilex* rate for L-canopy ($P<0.05$). The *Q. ilex* rate tended to increase in the

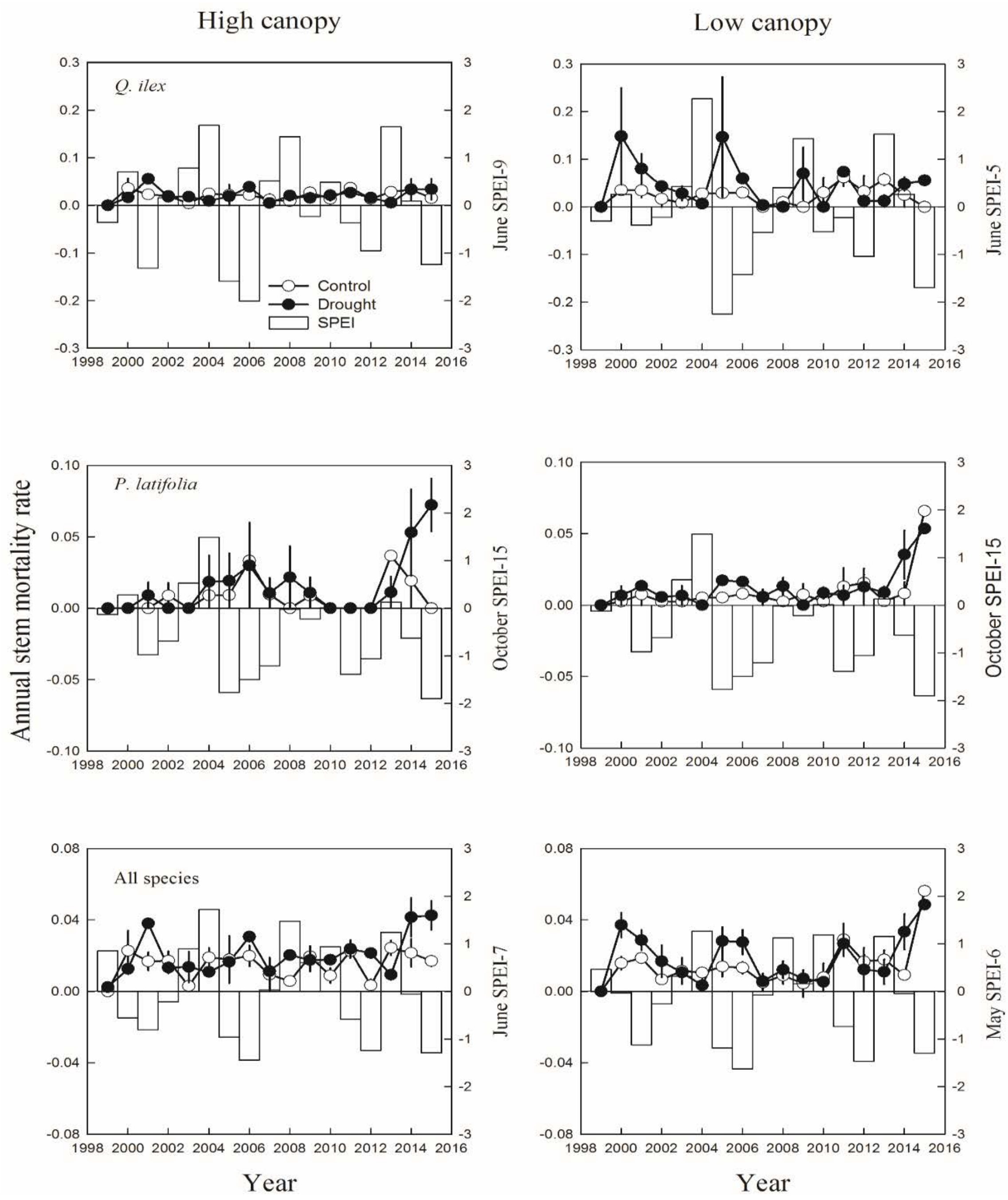

Fig. 2 Responses of annual rate of stem mortality to experimental and natural (SPEI) droughts for both H- and L- canopies. Error bars represent the standard error of the mean (n=2). Vertical bars are the best fit SPEIs at the community level (June SPEI-7 and May SPEI-6 for both H- and L-canopies, respectively) and for *Q. ilex* (June SPEI-9 and June SPEI-5 for both H- and L-canopies, respectively) and *P. latifolia* (October SPEI-15 for both H- and L-canopies).

drought treatment and vary with canopy types (H-canopy control plots (0.02±0.001) < H-canopy drought plots (0.021±0.002) < L-canopy control plots (0.025±0.0004) < L-canopy drought plots (0.05±0.02)).

Table 2 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. Estimates of the fixed effects are listed. The responses to drought were analyzed separately for the two canopy types. June SPEI-7 and May SPEI-6 were the best fits for the rates at the community level for both H- and L-canopies, respectively. June SPEI-9 and June SPEI-5 were the best fits for *Q. ilex* rates for both 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	Experimental drought	0.003	0.003	**0.004***
	SPEI	-0.0003	-0.0001	-0.001
	Experimental drought:SPEI	**-0.007***	-0.007	**-0.005***
Low	Experimental drought	**0.02***	0.003	**0.004***
	SPEI	-0.01	**-0.008****	**-0.006***
	Experimental drought:SPEI	**-0.024***	0.0002	**-0.004(*)**

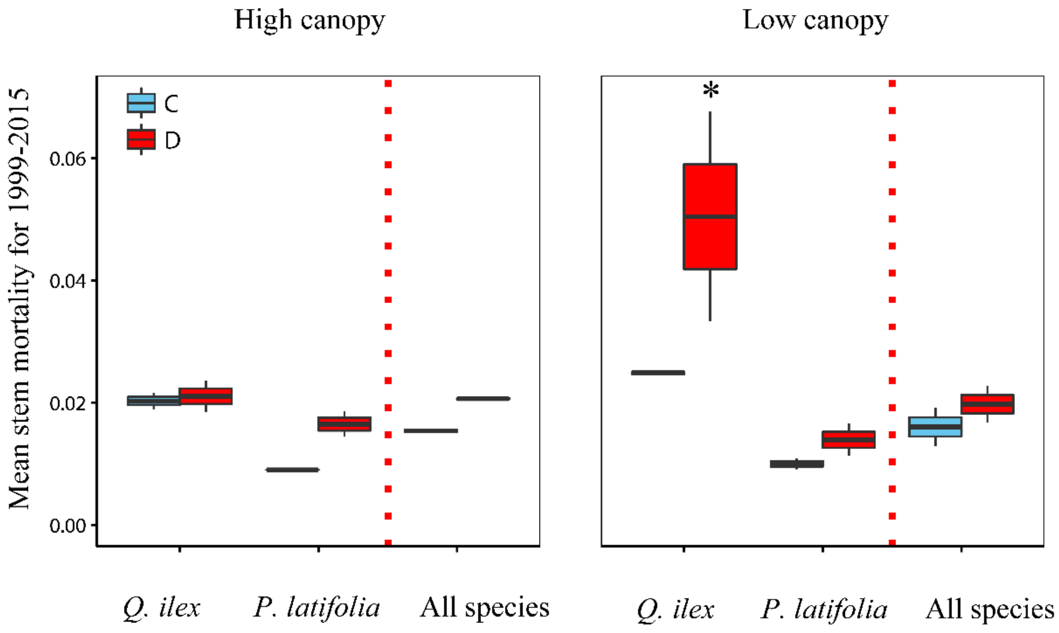

Fig. 3 Mean mortality rates at the community and species (*Q. ilex* and *P. latifolia*) levels over the study period (1999-2015) for H- and L-canopies. The blue and red box plots represent control and experimental drought, respectively. Box plot shows the range of the 25th and 75th percentile of the data. Significant differences ($P<0.05$) between control and experimental drought are marked with an asterisk (*).

Rate of recruitment

Recruitment rate at the community level was lower for H- than L-canopy (*P*<0.05). The experimental drought did not affect the recruitment rate at the community level for either H- or L-canopy (Fig. 4). The recruitment rate was significantly lower for *Q. ilex* than *P. latifolia* (*P*<0.01), especially for H-canopy (*P*<0.05).

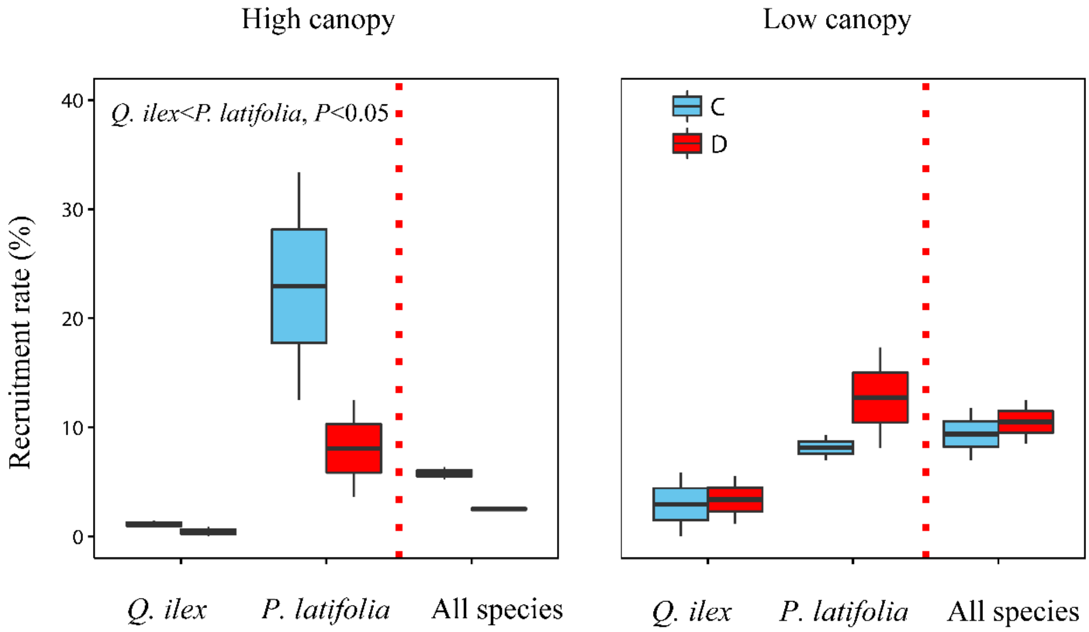

Fig. 4 Recruitment at the community and species (*Q. ilex* and *P. latifolia*) levels over the study period (1999-2015) for H- and L-canopies. The blue and red box plots represent control and experimental drought, respectively. Box plot shows the range of the 25th and 75th percentile of the data.

Change in stem density

The drought treatment decreased stem density at the community level similarly for H- and L-canopies, but not significantly (control *vs* drought: -14.6±1.7 *vs* -18.2±5.7% and -17.4±0.1 *vs* -27.5±0.1%, respectively) (Fig. 5). The changes in stem density of the two species, however, differed substantially between the canopy types (*P*<0.001). The changes in stem density were significantly larger for *Q. ilex* than *P. latifolia* both H- and L-canopies (*P*<0.05 and *P*<0.01, respectively).

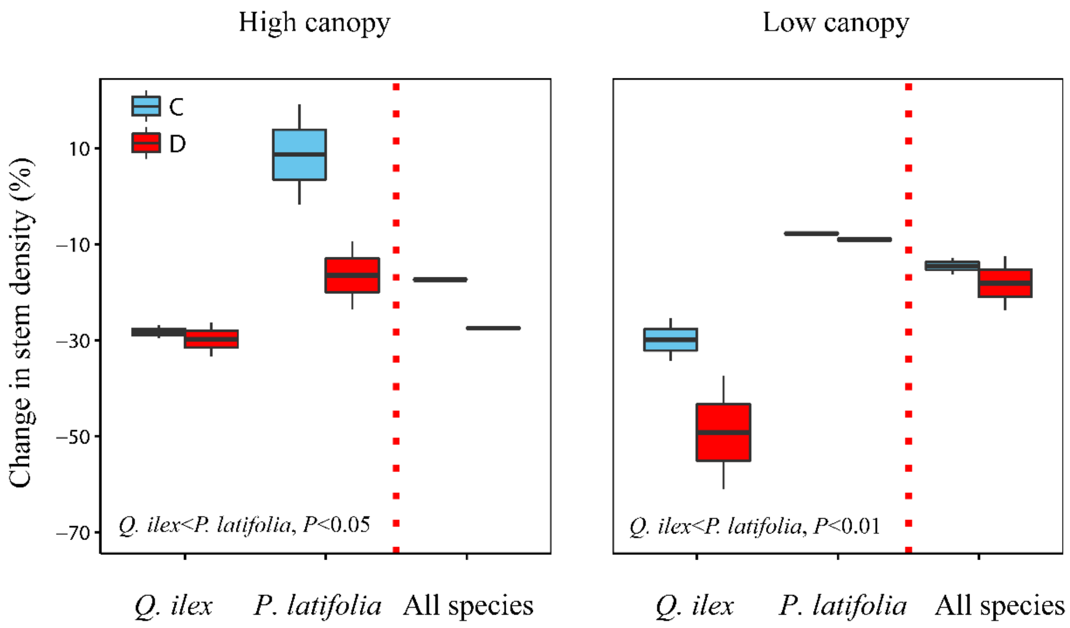

Fig. 5 Changes in stem density at the community and species (*Q. ilex* and *P. latifolia*) levels over the study period (1999-2015) for H- and L-canopies. The blue and red box plots represent control and experimental drought, respectively. Box plot shows the range of the 25th and 75th percentile of the data.

Response of stem growth

The rate of stem growth at the community level was marginally significantly higher for H- than L-canopy (P<0.1). The experimental drought, however, decreased the rates at the community level for both H- and L- canopies (both P<0.01) (Fig. 6). The rate was lower for *Q. ilex* than *P. latifolia* for L-canopy (P<0.01).

Stem mortality and growth rates in the natural extreme years

The experimental drought did not increase the rate of stem mortality at the community level during the natural extreme years in either H- or L-canopy (Fig. 7) but increased the *Q. ilex* rate for L-canopy compared to the control (P<0.05). The experimental drought, however, decreased *Q. ilex* stem growth for L-canopy (P<0.05) and stem growth for L-canopy at the community level (*P*<0.05). The experimental drought did not affect stem mortality or growth at the community or species level for H-canopy.

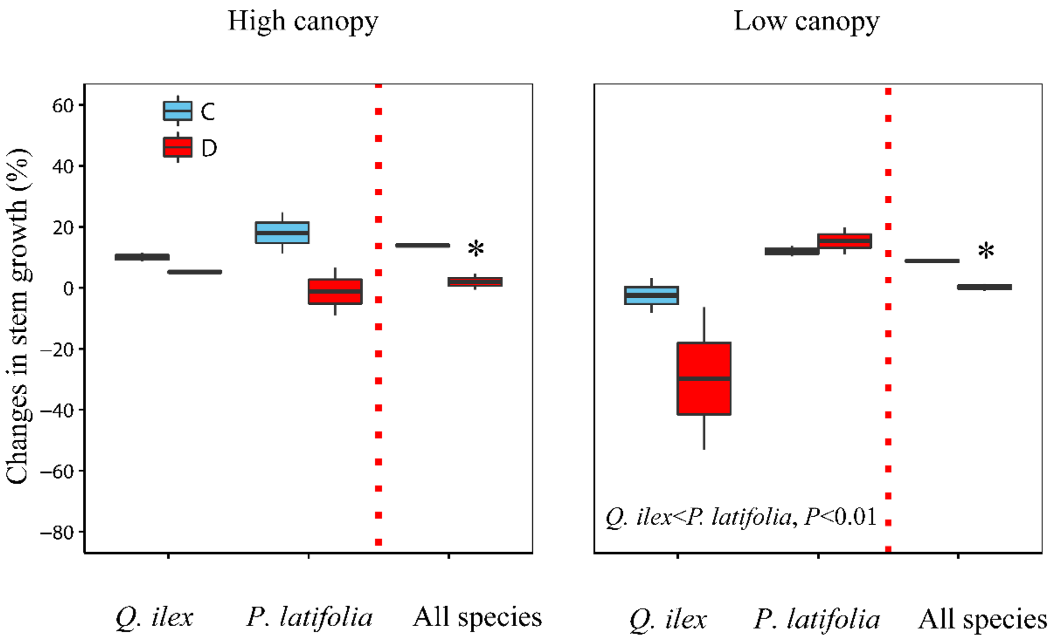

Fig. 6 Stem growth at the community and species (*Q. ilex* and *P. latifolia*) levels over the study period (1999-2015) for H- and L-canopies. The blue and red box plots represent control and experimental drought, respectively. Box plot shows the range of the 25th and 75th percentile of the data. Significant differences (*P*<0.05) between control and treatment are marked with an asterisk (*).

Change in the percentage of stem density and BA

The patterns of changes in the percentage of stem density and BA indicated differential trajectories for the two species for 1998-2015 (Fig. 8). The percentage of *Q. ilex* stem density tended to decrease over time for both H- (both *P*<0.001 for control and drought, respectively) and L-canopies (both *P*<0.001). The *P. latifolia* percentage of stem density increased over time for both H- (both *P*<0.001) and L-canopies (both *P*<0.001). The *Q. ilex* percentage of BA decreased over time for H-canopy (control, *P*<0.001) and L-canopy (*P*<0.01 and *P*<0.001 for control and drought, respectively). *P. latifolia* BA, however, was relatively stable in the control and even decreased in the drought plots (*P*<0.01) for

H-canopy. The *P. latifolia* percentage of BA increased over time for L-canopy (both *P*<0.001 for control and drought). More interestingly, the comparative slopes of the

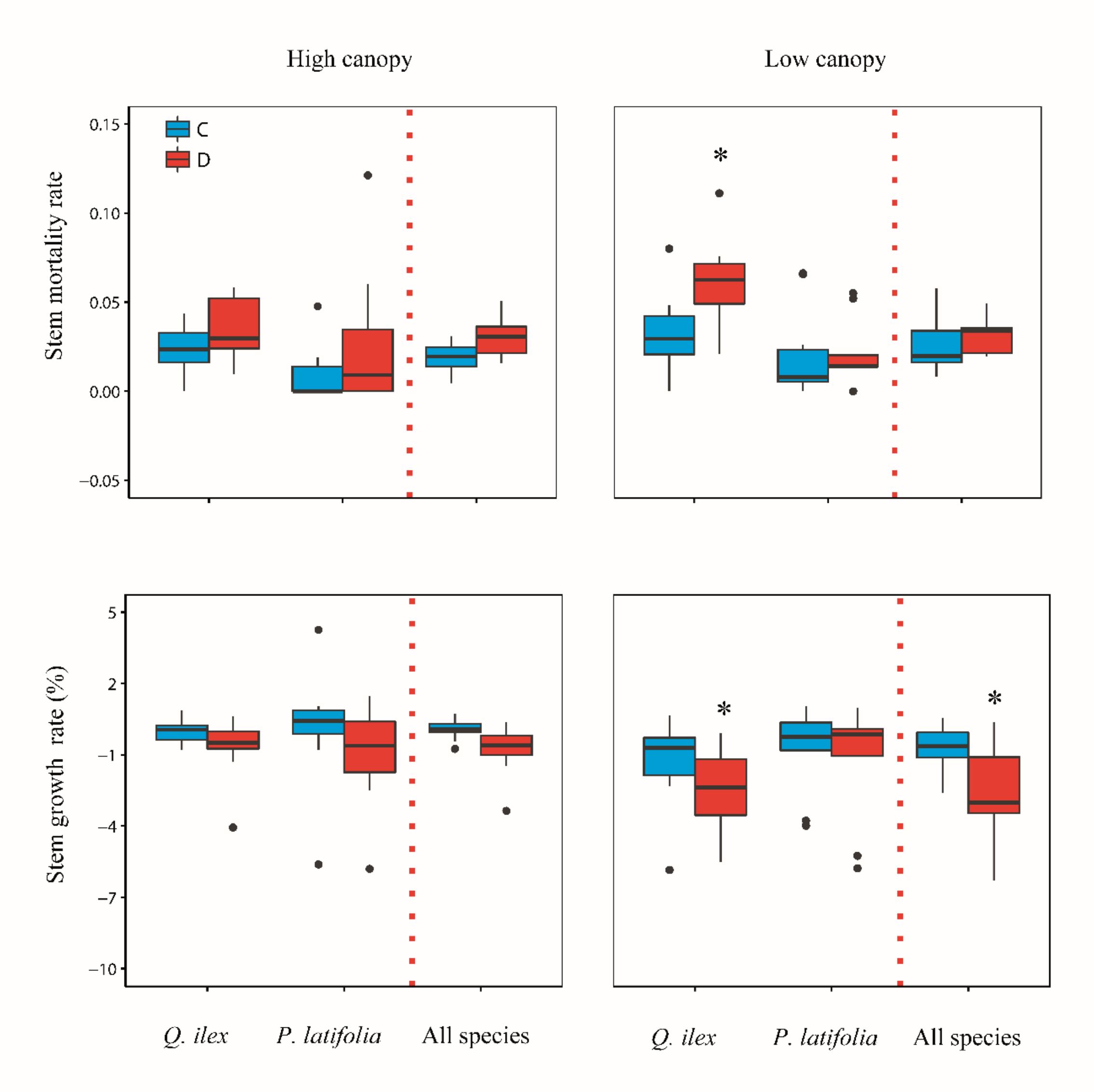

Fig. 7 Effects of the experimental drought on stem mortality and growth during the natural extreme years of 2001, 2005-06, 2011 and 2015. The blue and red box plots represent control and experimental drought, respectively. Box plot shows the range of the 25th and 75th percentile of the data. Significant differences (*P*<0.05) between control and treatment are marked with an asterisk (*).

changes in the percentage of both stem density and BA for *Q. ilex* were lower in the drought than the control plots for L-canopy (both *P*<0.001). In contrast, the slopes of the changes in the percentage for *P. latifolia* were higher in the drought than the control plots (P<0.001 and P<0.1, respectively).

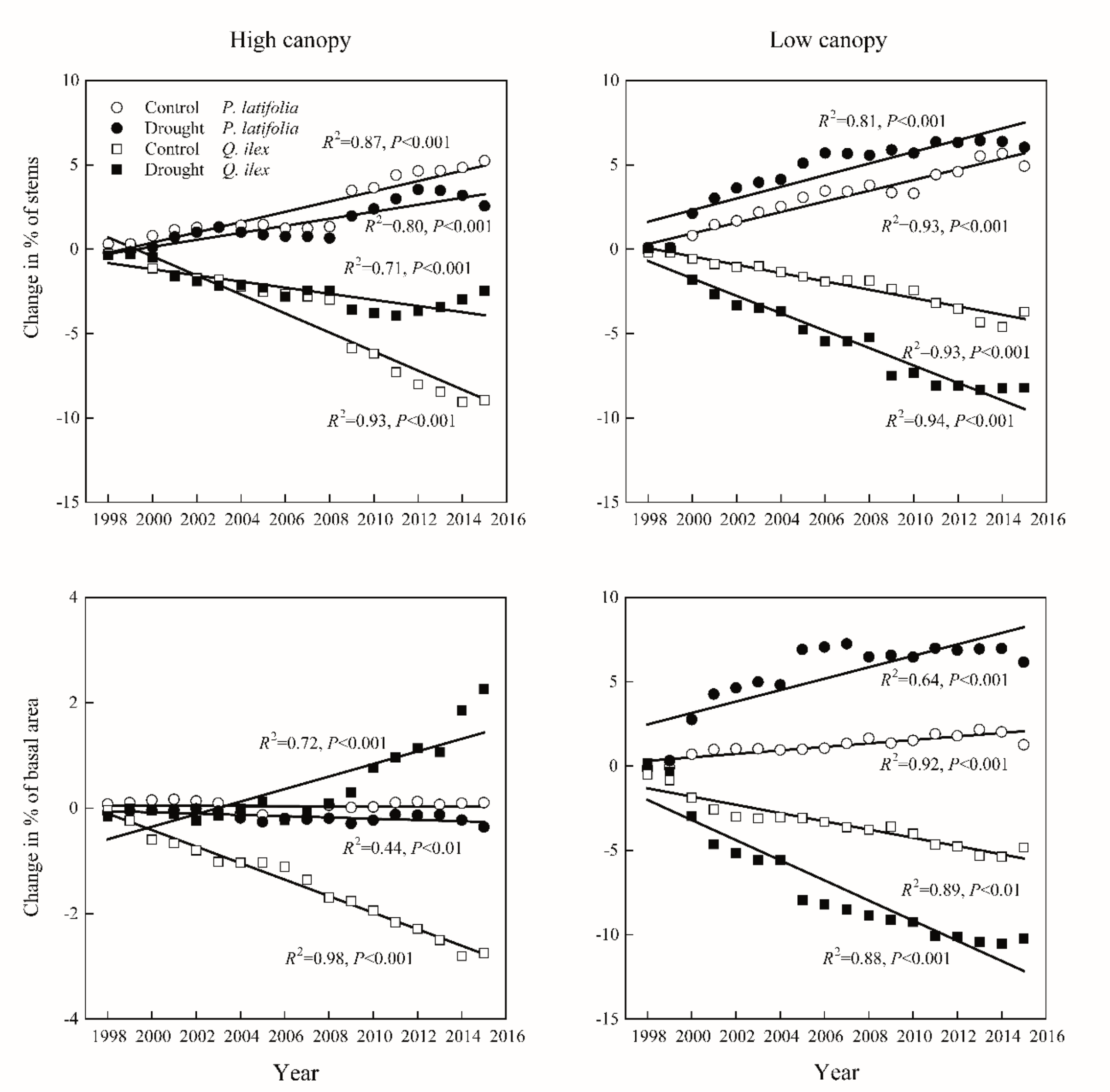

Fig. 8 Changes in the percentage of stem density and basal area for *Q. ilex* and *P. latifolia* during the study period (1998-2015).

Discussion

Increasing drought is triggering forest degradation and vegetation shift in the holm oak forest

The long-term experimental drought triggered large-scale mortality at the community level for both H- and L-canopies. Drought-induced tree mortality has been attributed 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) found that defoliated trees had significantly lower levels of lignotuber NSC at our study site, further supporting the role NSC reservoirs play in reversing the effects of extreme droughts. The annual rates of stem mortality in the present study, however, differed between species and forest-canopy types. The drought treatment increased the *Q. ilex* rate for L- but not H-canopy, in agreement with the observation that drought-induced dieback of forest canopies more severely on the forests growing 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, consistent with previous short- and intermediate-term studies (Ogaya *et al.*, 2003; Ogaya & Peñuelas, 2007a; Barbeta *et al.*, 2013). Mean mortality rates also differed between species, with higher mortality in *Q. ilex* than *P. latifolia*, perhaps 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). Stem mortality was also higher in *Q. ilex* in the drought and L-canopy plots, where water availability was lowest. Long-term drought would consequently lead to large-scale stem mortality in Mediterranean holm oak forests, especially those with low canopies, which could lead to unexpected changes in forest structure and ecosystemic dynamics (Allen *et al.*, 2015; Doblas-Miranda *et al.*, 2015, 2017).

The recruitment rates differed greatly between canopy types and species, suggesting future shifts in species composition. Increased recruitment can be stabilizing, 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 recruitment rates at the community level were lower for H- than L-canopy, which may be associated with the stronger release from competition caused by the higher L-canopy 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). The recruitment rate was significantly lower for *Q. ilex* than *P. latifolia*, probably due to the more seriously 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). Recruitment was particularly significantly lower for *Q. ilex* than *P. latifolia* in the H-canopy plots, indicating lower seedling establishment in the understory (Lloret *et al.*, 2004a, 2004b; Saura-Mas *et al.*, 2015). The experimental drought, however, tended to decrease the *P. latifolia* recruitment rate for H-canopy, perhaps due to the self-thinning of small stems under long-term soil desiccation (Lloret *et al.*, 2004a, 2012; Barbeta & Peñuelas, 2016). Recruitment was not significantly correlated with stem mortality for either *Q. ilex* or *P. latifolia* in our study (Fig. S5), 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). The *P.*

latifolia recruitment rate was nevertheless affected less by drought, which may promote vegetation shifts in holm oak forests in the near future.

The changes in stem density at the community level were similar for the canopy types, but the magnitudes of the reductions were larger in the drought plots, which could be attributed to the higher mortality and lower recruitment (Lloret *et al.*, 2004a, 2004b, 2012). The changes in stem density differed substantially between the two species; they were significantly larger for *Q. ilex* than *P. latifolia* for both H- and L-canopies, further indicating the sensitivity of *Q. ilex* to drought (Ogaya & Peñuelas, 2007a; Barbeta *et al.*, 2013; Liu *et al.*, 2015). The contrasting changes in stem density for the two species differed in the two canopy types. The experimental drought decreased the stem density of *Q. ilex* less for H-canopy (control *vs* drought: -28.3 *vs* -29.8%) than L-canopy (control *vs* drought: -29.9 *vs* -49.3%), likely due to the low water availability (Lloret *et al.*, 2004a, 2012, Barbeta *et al.*, 2013, 2015). In contrast, *P. latifolia* stem density tended to increase in the H-canopy control plots (+8.6%) but decreased slightly in the drought plots (-16.5 %), but not significantly. *P. latifolia* stem density, however, tended to not be affected for L-canopy (control *vs* drought: -7.8 *vs* -9.1%). Stem density therefore decreased over the study period more for *Q. ilex* than *P. latifolia*, which would affect carbon storage and ecosystemic productivity (Barbeta *et al.*, 2013; Liu *et al.*, 2015; Barbeta & Peñuelas, 2016).

The rate of stem growth at the community level was sensitive to the drought treatment for the two canopy types (Fig. 6). Stem growth is an important surrogate of carbon storage in living plants and is an index of plant vigor and competitive ability, which may be associated with reductions in carbon assimilation (Ogaya & Peñuelas, 2003; Ogaya *et al.*, 2014), higher mortality rates (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). The rate of L-canopy stem growth was significantly lower for *Q. ilex* than *P. latifolia*, 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 did not decrease significantly in the drought plots for either H- or L-canopy. *Q. ilex* stem growth was negatively correlated with stem mortality ($P<0.001$) (Fig. S5), further supporting the limitation of biomass accumulation by the loss of stems, especially large stems (Ogaya & Peñuelas, 2007a; Barbeta *et al.*, 2013). Previous studies, though, have reported that drought decreased *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) 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 experimental and natural droughts (SPEIs). SPEIs have not been widely used to represent the effects of experimental drought on forests (Barbeta *et al.*, 2013, 2015; Liu *et al.*, 2015), even though both natural drought (Bennett *et al.*, 2015; Greenwood *et al.*, 2017) and experimental drought (Nepstad *et al.*, 2007; Ogaya & Peñuelas, 2007a; da Costa *et al.*, 2010) can trigger forest mortality. The natural droughts in our study (June SPEI-7 and May SPEI-6) accelerated the effects of the experimental drought on annual stem mortality at the community level for both H- and L-canopies (marginally). The natural droughts also accelerated the effects of the experimental drought on annual *Q. ilex* stem mortality for both H- and L-canopies. More importantly, the rates of *Q. ilex* stem mortality were not correlated with June SPEI-9 and were not affected by the experimental drought for H-canopy but increased significantly due to their synergistic effects. The natural drought, however, did not significantly affect *P. latifolia* stem-mortality rates for either H- or L-canopy. The effect of the combination of soil desiccation by the experimental and natural droughts (SPEIs) on forest dynamics, such as stem mortality and growth, should therefore be considered (Liu *et al.*, 2015; Barbeta *et al*., 2016).

The timescales of SPEI associated with stem-mortality rates differed between the species and forest-canopy types (Fig. 2 and Table 2). Natural droughts (winter-spring SPEIs) amplified the effects of the experimental drought on annual *Q. ilex* stem-mortality rates for both H- and L-canopies, demonstrating the importance of 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, demonstrating the importance of water inputs during the period of summer-autumn. Moreover, the SPEI timescales associated with stem mortality were longer for H- than L-canopy, implying differential sensitivities of the plants to drought. The effects of the natural droughts on stem-mortality rates at the community and species (for *Q. ilex*) levels were correlated with longer SPEI timescales for H-canopy (June SPEI-7 and June SPEI-9, respectively) than L-canopy (May SPEI-6 and June SPEI-5, respectively), which may be due to the differential responses of forests in arid regions to shorter water deficits (Vicente-Serrano *et al.*, 2010, 2013). Recurring natural droughts would also lead to the non-linear (abrupt) impacts on stem mortality of drought-resistant species *P. latifolia*. For example, the long 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 years of 2001, 2005-2006, 2011 and 2015 (Fig. 7). The responses, however, differed between species and canopy types. The experimental drought significantly increased *Q. ilex* stem mortality and growth rates for L-canopy, which may have been associated with severe limitations to radial tree growth (Ogaya & Peñuelas, 2007a; Barbeta *et al.*, 2013; Liu *et al.*, 2015). The frequency of climatically extreme years have recently increased at our study site (five of 18 years for 1998-2015), which could decrease the recovery and/or resilience capacity of holm oak forests, possibly due to the severe effects on root and branch hydraulics (Martínez-Vilalta *et al.*, 2003; McDowell *et al.*, 2008; Barbeta *et al.*, 2012; Limousin *et al.*, 2012), reductions in flower and fruit production (Ogaya & Peñuelas, 2007b; Misson *et al.*, 2011; Liu *et al.*, 2015) and lower seedling establishment (Lloret *et al.*, 2004b). Natural extremes may thus amplify the effects of experimental drought and/or warming manipulations, so more attention should be paid to combined impacts.

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

Increased frequencies of drought and natural extremes in the future are likely to trigger broad-scale vegetation shifts. We observed contrasting trajectories for two species: the changes in the percentage of stem density decreased for *Q. ilex* and increased for *P. latifolia* for both H- and L-canopies. The changes in the L-canopy percentage of BA had similar trajectories. The percentage of 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, which would severely limit stem growth. More importantly, the changes in the percentage of stem density and BA indicated that the drought treatment decreased (*Q.*

ilex) and increased (*P. latifolia*) the magnitudes for L-canopy, suggesting that higher water deficits accelerated the vegetation shift (Fig. 8). The climatic series demonstrated that the water deficits of summer (e.g. August SPEI-3) and summer-autumn (e.g. October SPEI-6) at our study site have increased over the last 40 years, which could shift forest canopies from high to low (Fig. S4). Long-term water deficits have increased vegetation shifts in a 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 forests (Peñuelas & Boada, 2003; Peñuelas *et al.*, 2007). More severe and widespread water deficits caused by warming are becoming global, possibly associated with large-scale forest die-off and tree mortality and broad-scale vegetation shifts (Allen *et al.*, 2010, 2015; Carnicer *et al.*, 2011; Anderegg *et al.*, 2013; Franklin *et al.*, 2016), especially when accompanied by a higher frequency of extreme droughts that may accelerate these processes (Beier *et al.*, 2012; Reichstein *et al.*, 2013; Doblas-Miranda *et al.*, 2015). The combined use of effective methods such as broad-scale field surveys, long-term experimental manipulation and modelling in global forests, and determining the consequences of the negative effects of persistent long-term drying and episodic natural extremes on forest benefits and services, are thus necessary and would provide reliable information for predicting future forest dynamics.

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Block 3. The community structure and compositional shifts of the Mediterranean shrubland in response to long-term experimental warming and drought.

Chapter 5. Species selection under long-term experimental warming and drought explained by distribution range

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Abstract

Global warming and reduced precipitation may trigger large-scale species losses and vegetation shifts in natural ecosystems around the world. However, currently lacking are practical ways to quantify the sensitivity of species and community composition to these often-confounded climatic forces. Here we conducted long-term (16 years) nocturnal-warming (+0.6°C) and reduced precipitation (-20% soil moisture) experiments in a Mediterranean shrubland. Climatic Niche Groups (CNGs) – species ranked or classified by similar temperature or precipitation distributions – successfully described community responses under experimental manipulations. Warming caused progressive abundance decreases of those species distributed in cooler regions. Correspondingly, reduced precipitation resulted in abrupt decreases in wet-distributed species, followed by a delayed increase in dry-distributed species. Notably, while CNG created from temperature or precipitation values were partially correlated, their explanations of community response were stronger for their respective climate parameter, suggesting that some plant species possess specific adaptations to either warming or drought that may lead to independent selection to the two climatic variables. Our findings indicate that when climatic distributions are combined with experiments, the resulting incorporation of the local plant evolutionary strategies and their changing dynamics over time, leads to predictable and informative shifts in community structure under independent climate change scenarios.

Keywords: global warming; frequent drought; Mediterranean ecosystems; long-term manipulation experiments; climatic niche groups; composition shifts; biodiversity loss

Introduction

Climate change has already markedly altered community composition and structure, which has accelerated the loss of biodiversity and degradation of ecosystem functioning in many habitats around the world (Walther *et al.,* 2002; Parmesan & Yohe, 2003; Peñuelas *et al.,* 2013; Pimm *et al.,* 2014; Brose & Hillebrand, 2016). Biodiversity hotspots, such as Mediterranean ecosystems, have experienced drastic losses of biodiversity over the past several decades, attributable in large part to corresponding rapid climate change (Myers *et al.*, 2000; Sala *et al.*, 2000; Peñuelas et al., 2013; Doblas-Miranda *et al.*, 2014). Global warming, accompanied with more frequent droughts projected for Mediterranean regions, will likely add to the vulnerability of these ecosystems and possibly intensify the species and ecosystem function losses in the coming decades (Sala *et al.*, 2000; Walther *et al.*, 2002; Doblas-Miranda *et al.*, 2014; Barros *et al.*, 2014). However, we still lack the mechanistic knowledge that would enable us to predict, and thus attempt to manage, the impacts of climate change on natural ecosystems.

The forecasting of climate change impacts on species diversity and composition may be impeded by the interrelationships among many simultaneously changing climatic factors, and directional shifts in species composition to these changes cannot be easily detected (McMahon *et al.*, 2011; Bellard *et al.*, 2012; Garcia *et al.*, 2014). In addition, climatic factors are often confounded, making it very difficult to make accurate predictions for biodiversity shifts independently for temperature and precipitation change (Loarie *et al.*, 2009; Beaumont *et al.*, 2011). Some studies have reported that increasing temperature and decreasing precipitation could impose similar and additive selection pressures on plant species performance within communities, because both forces decrease soil moisture, while increasing evapotranspiration rates and nutrient mineralization

(Myers *et al.*, 2000; Sardans *et al.*, 2008; Williams *et al.*, 2012). However, the combined effects of temperature and precipitation are highly context dependent. For example, both warming and decreased precipitation may increase the aridity of an already dry and warm habitat, thereby limiting plant growth. However, in cooler habitats not limited by water, warming may have positive effects on the vegetation (e.g. extending the growing season and promoting growth and reproduction) and decreasing precipitation may have little effect on plant growth (Loarie *et al*., 2009; Garcia *et al*., 2014; Andresen et al. 2016). Therefore, approaches are needed that are able to detect the influences of several key climatic factors on the structure and function of ecological communities that are widely applicable and able to disentangle 'climate change' into the separate drivers.

Currently, one of the most mechanistic ways to study plant community responses to climate change is through long-term climate manipulation experiments (Beier *et al.*, 2004; Wu *et al.*, 2011; Elmendorf *et al.* 2015; Kröel-Dulay *et al.* 2015; Andresen *et al.*, 2016). Such studies impose a continuously altered climatic factor onto a local community, providing a known and controlled-for climate change impact, capturing the results of local interactions. This method therefore overcomes some of the confounding effects of long-term observational data (Gottfried *et al.*, 2012), it follows impacts over a continuous time-scale (unlike time-for-time based approaches (Metz & Tielbörger, 2016; Estiarte *et al.*, 2016)), and importantly incorporates the local levels of plasticity and evolutionary adaptations found within a community (unlike gradient space-for-time approaches (Tielbörger *et al.* 2014)). However, for total community parameters (e.g. ANPP, biomass, species richness) climate manipulation experiments often reveal little to no-net change or difficult to explain altering responses over time (Grime *et al.*, 2008; Barbeta *et al.*, 2013; Tielbörger *et al.*, 2014; Andresen *et al.*, 2016; Mueller *et al.*, 2016). Interestingly, often single species and multiple species are shown to change in abundances within these experiments (Harte & Shaw 1995; Lloret *et al.* 2009; Fridley *et al.* 2011; Bilton *et al.*, 2016). But with many species increasing and decreasing in the community, the task is to have a simple, general, and perhaps more informative way to interpret these patterns. By somehow ranking the species based on a factor related to climate, potentially hypotheses can be drawn, and predictions can be tested.

One technique for ranking species in relation to climate is given by their climatic niche distribution e.g. species within the same community that more commonly occur in wetter or drier habitats. In theory, the climatic niche of a species could act as the primary correlate of species sensitivity to climate change (Thuiller *et al.*, 2005; De Frenne *et al.*, 2013). The climatic niche principle has commonly been used in distribution models (Thomas *et al.*, 2004; Thuiller *et al.*, 2005; McMahon *et al.*, 2011; Araújo & Peterson, 2012), but has also shown promise in a process named "thermophilization" for identifying directional changes in whole community composition in observational data under climate warming (Hijmans & Graham, 2006; Gottfried *et al.*, 2012) e.g. the average temperature niche of elevated mountain communities has increased over time due to natural warming. More recently, the thermophilization principle has also proved capable of revealing community shifts in tundra communities under experimentally manipulated warming (Elmendorf *et al.*, 2015). Moreso, when divided into species groups (Climatic Niche Groups CNGs) the climatic niche principle has most recently identified more precise details (i.e. identifying which components of the community increase or decrease) about the compositional shifts and community dynamics for annual species under experimentally manipulated rainfall over time (Bilton *et al.*, 2016).

Therefore, defining species by their climatic niche and monitoring their responses within climate experiments, combines the strengths of both approaches of climatic niche and thermophilization principle, and may be an informative and hypothesis-driven method for examining compositional change within communities. When using the climatic niche principle alongside experiments, response of species can be displayed along the niche axis and some general conclusions about species increasing or decreasing can be drawn. Furthermore, by categorizing the species into groups of similar climatic niche distribution (CNGs), the method overcomes species variance in time and space, and niche definition. CNGs potentially also group together many different traits/adaptations that plants may possess to survive in different climates, thereby strengthening both the power of the test and the interpretation of the result (Lavorel *et al.*, 2016). Certainly a main strength of grouping being its ability to monitor changing responses over time. It seems possible therefore, that aspects of species-specific adaptations to climatic variables can be partly captured by this approach providing further information about the community dynamics and ecology. While promise has been shown, to date, the climatic niche principle has not been applied to separate climatic factors in manipulative field experiments, in order to tease apart the possible responses of species and community composition to the impacts of future temperature and precipitation regimes.

Here, we applied the climatic niche theory into a unique long-term (16 years) experiment to test, for the first time, whether two climatic variables may independently explain species-specific responses to climate change. We conducted nocturnal-warming (+0.6 °C) and sustained-drought (-20% soil moisture) treatments in a Mediterranean early-successional shrubland (1999-2015). Importantly, we assumed that the coexisting species in the community could be ranked or grouped by the similarity of their climatic niches, and that the community responses to climate change would be regulated by the differential response of these climatic niche groups (CNGs). We therefore defined CNGs for the coexisting species based on two climatic variables: temperature and precipitation (see Methods for full details). In doing so, firstly we hypothesized, for this already warm and dry region of southern Europe, that changes in community composition in response to either manipulated warming or drought could be explained by species association with climatic niches. Specifically, those species in the community with core distributions in colder or wetter regions would disproportionately decrease under warming or drought; or even that species distributed in warmer or drier regions may increase under warming or drought, relative to control. Secondly, by incorporating two climate manipulation treatments and two climate niche parameters for our CNGs, due to specific adaptations species possess for temperature and precipitation regimes, we hypothesized that the separate climate change drivers may select for different species within the community which would be related to their climate niche distribution.

Materials and methods

Study site

We conducted the study in a semi-arid Mediterranean shrubland in Garraf Natural Park near Barcelona (southwestern Europe) (41°18′N, 1°49′E; 210 m a.s.l.). The site has a Mediterranean climate with hot and dry summers and precipitation mainly in the spring and autumn. The mean annual temperature during the study period (1998-2014) was 15.5 °C, fluctuating from 14.7 °C to 16.2 °C. The highest temperature often occurs in summer (June, July and August), averaging 23.4 °C throughout the study period. The mean annual precipitation was 560.9 mm during the study period of 1998-2014, ranging from 403.1 mm in 2006 to 956.2 mm in 2002. The thin (10-40 cm) soil is a petrocalcic calcixerept and has a loamy texture and abundant calcareous nodules.

The study site was established on a hill with south-facing aspect (Slope=13%). The area had been cultivated but was abandoned more than a hundred years ago and thereafter colonized by a coniferous forest. This forest experienced two wildfires in summer 1982 and spring 1994, after which succession began. The current vegetation is dominated by the shrubs *Erica multiflora* and *Globularia alypum*, which coexist with small evergreen plants, such as *Dorycnium pentaphyllum*, *Rosmarinus officinalis* and *Ulex parviflorus* (Table S1 for a full list of species).

Experimental design

We carried out moderate nocturnal-warming and drought treatments during the period of 1999 to 2014, with 1998 acting as a pre-treatment year. Triplicate 20 m^2 (5 × 4 m) plots were randomly established for each treatment (warming, drought and control; nine plots in total). All samples were collected from the central 12 m^2 (4 × 3 m) to avoid edge effects.

The warming treatment was achieved by covering the plots at night with reflective curtains to reduce the infrared radiation to the atmosphere, simulating global warming by increasing the minimum temperature at night (Beier *et al.*, 2004; Peñuelas *et al.*, 2007; Prieto *et al.*, 2009). A light scaffolding (1.2 m above the ground) was built to support the reflective curtains. The curtains reflected 97% of the direct and 96% of the diffuse radiation while allowing the transfer of water vapor. The covering was operated automatically by the light level (<200 lux), rainfall (<0.3 mm) and wind (<10 m s^{-1}) throughout the study. The warming treatment increased soil temperatures at -5 cm depth by the average of ca. 0.6 °C, depending on the season and meteorological conditions (Fig. S1 (a)).

The drought treatment was applied in the spring and autumn (main and secondary growing seasons respectively) by covering each plot with a transparent waterproof sheet of plastic (Beier *et al.*, 2004; Peñuelas *et al.*, 2007; Prieto *et al.*, 2009). The facilities were similar in the drought and warming plots except the material of the curtains. The rain sensors during the treatment period activated the waterproof sheet to cover the vegetation during rainfall >1 mm and were removed after the rain had stopped. The water collected by the sheet was drained outside the plots. The curtains were folded automatically if the wind speed exceeded a threshold (>10 m s^{-1}) to avoid damage. The drought plots were treated the same as the control plots during the rest of the year. The drought treatment excluded about 40% of the precipitation and decreased soil moisture by ca. 20% throughout the study period (Fig. S1 (b)).

A control treatment without manipulations was run in parallel to the warming and drought treatments. All of the control plots also had similar scaffolding but without curtains. Meteorological data have been collected at the experimental site at half-hourly intervals since 1999.

Vegetation sampling

Five fixed and parallel transects 3 m long were permanently established in all nine plots at equal distances of 0.8 m. A point-intercept method was applied to record the species composition and abundance at points distributed at 5 cm intervals along the five transects (305 points in each plot). A thin steel pin (3 mm diameter) was vertically dropped at each point, and the parameters (species identity and contacts per pin) were recorded (Prieto *et al.*, 2009). The data were collected annually in the dry season (July and August) from 1998 (pre-treatment) to 2014 (16 years of manipulation). Our experimental plots contained a total of thirty-one species (details in Table S1). Species contacts in each plot were summed to estimate the abundance. Species abundances per plot were log transformed (log+1) to adjust for the large positive skew in distribution, and summed to form groups or total community measures.

Grouping of plant species

To determine community compositional change with respect to the different treatments, we classified the species in the community into climatic niche groups (CNGs) using temperature and precipitation variables. We initially tested a number of climate niche parameters (maximum temperature in hottest month, average and seasonal temperature, and annual and seasonal precipitation) to rank the species to form CNGs (Tables S1, S2, S3, S4). However, we continued the analysis with the most consistent descriptors of community composition response – and those which were assumed to be most valid *a priori* – which were: i) summer temperature (mean temperature from June to August), since this period was most likely to be important for selecting for summer survival of species; and ii) spring precipitation, (the accumulated precipitation from December to May), as this had most effect on early season growth at the site. We created three CNGs for each climatic variable: T1, T2 and T3 (warm to cold) for summer temperature and P1, P2 and P3 (dry to wet) for spring precipitation.

To create these groups, we first obtained the species' geographical distributions in mainland Spain, France and Portugal (southwestern Europe) from the Global Biodiversity Information Facility (GBIF, http://www.gbif.org/), one of the largest universally and detailed biodiversity databases available. Distribution observations were filtered to exclude islands, and reduced to one value per 30 arc cell (~1 km). We then extracted a climatic value from a climatic GIS layer separately for each species for each occurrence. We tested multiple ways of calculating each species niche value from these climate distribution values to test the impact of niche edges on group response: the median (med) of climatic values associated with each observation; the lower 25 percentile of the climatic values (25%); or the upper 25 percentile of climatic values (75%) (see Table S1 for multiple niche values associated with each species). For the remainder of the main article, we display results only from niche values calculated using 25% summer temperature values and median spring precipitation values, because the results for the various approaches were essentially the same. Information for the summer temperatures and spring precipitation was extracted from the WorldClim database (30 arc-seconds (~1 km)) (http://www.worldclim.org/), which contains climatic data for 1950-2000). Species were allocated to three CNGs per climatic variable by distributing plant community abundance equally among groups, i.e. the sum of the (log) species contacts per group was approximately 33% of the total sum of (log) species contacts recorded during the experiment (regardless of year or treatment) (see Tables S2 and S3). Log transformations are commonly applied to reveal community composition change (Šmilauer & Lepš, 2014). Note, however, that our method assigns relatively more weight to rare species in a group. We think this is well justified because, due to the dominance of some species and also the point quadrat method of revealing abundance, normal community percentages could not be used to create arbitrary groups. In addition, species at a boundary with very similar/same niche values were kept together in the same group. These rules for species allocation ensured that each group contained at least six species and that the climatic ranges defined by a group exceeded a certain value (precipitation >20 mm, temperature >1 °C) (details in Table S3).

Statistical analyses of abundances

For the main statistical analyses, the response variables in our models were derived from log-transformed species level contact hits per plot (from 1999-2014)(Eq. 1). Four main response variables were analyzed: total community log-composition (sum of log contact hits of all the species) (Eq. 1), temperature CNG log-composition (sum of log contact hits belonging to species for each temperature CNG group) (Eq. 1), precipitation CNG log-composition (sum of log contact hits belonging to species for each precipitation CNG group) (Eq. 1) and species log-abundance (log contact hits belonging to a single species).

$$\sum_{i=1}^{i=n} \log(hits\ sp\ i)$$

(Eq. 1)

For total community log-composition i=1, ..., total number of sp (species);

for CNG i=1,..., number of sp (species) within the group.

Note here, that the abundances of species (in a given plot and time point) were logged prior to summing to form groups/total. To our knowledge, this appears to be a novel response variable and although testing was carried out, the full properties are partly unknown. However, logging at the species level first is synonymous with log transformations that are commonly applied to reveal community composition change (e.g. in Redundancy Analysis Šmilauer & Lepš, 2014). Similarly, the properties of the log-composition response variable conclude that a doubling or halving of any species within the group/community is given equal weight, which when analysed over time reveal general increases or decreases of multiple species in the group/community rather than absolute changes highly weighted by a single dominant species. We feel this is a more general reflection of composition shifts over time, which may be important for identifying shifts in relation to climate change/manipulations, particularly in a shrub dominated community such as ours.

The effects of the climatic manipulation treatments on log-compositions/abundances were analyzed using linear mixed-effects models (LMEM). Different models were created to analyze either the effect of warming treatment versus the control or the effect of drought treatment versus the control.

A full range of environmental factors (spring, summer and annual temperature and precipitation) of the study site were tested, but the variance (based on the lowest AIC values) was explained best for all analyses by the environmental recordings for spring temperature (ST), spring precipitation (SP) and their interaction. The initial values for log-compositions/abundances in the preliminary year (1998) were included as an underlying covariate (Pre-treat). Finally, the continuous variable Year (16 years: 1999-2014) and the two-level categorical variable Treatments (drought *vs*. control, or warming *vs*. control) were included. For the analysis of CNG log-compositions, the models also included the three-level explanatory categorical variable CNG (either T1, T2, T3 or P1, P2, P3), with the main interaction of interest between Treatment × Year × CNG. The full models for our analyses were therefore:

Total Community log-composition = ST*SP + Pre-treat + Year*Treatment
Species log-abundance (Eq. 2)

CNG log-composition = ST*SP + Pre-treat + Year*Treatment*CNG identity
(Eq. 3)

Plot was included as a random effect term in all models. All analyses were performed in R version 3.2.2 using package lme4 (version 1.1-7 (Bates *et al*., 2007)). Post-hoc analyses of the differences in CNG slopes/responses over time were tested by least-Squares means using the lstrend command of R package lsmeans (Lenth, 2016).

For visual representations of the statistical models (Figs. 1 & 2), treatment effect size was calculated as:

$$\text{Effect size (Year)} = (\mu_{\text{treatmentYear}} - \mu_{\text{treatment1998}}) - (\mu_{\text{controlYear}} - \mu_{\text{control1998}}) \quad \text{(Eq. 4)}$$

where $\mu_{\text{treatment}}$ and μ_{control} are the average log-compositions/abundances in the treatment (warming or drought) and control plots (n=3), respectively in a given year. Effect sizes were adjusted for log-composition/abundance differences in the pre-treatment year (1998) as representative of the LMEMs. Raw log transformed composition plots are shown in Fig. S6.

Group Robustness analysis

In addition to testing CNGs created from multiple species niche values (Table S4), we also performed a formal test of robustness to ensure any emerging patterns from the CNG analyses were not an artefact of the boundaries chosen. To do this we followed the protocol set out in Bilton et al. (2016) and performed Cumulative Group Analyses. This methodology adds one species at a time in niche value rank order to form a group. Unlike the main CNG test described above, which tests for differences in treatment responses over time of the three CNGs simultaneously (3-way interaction), the Cumulative Group Analysis compares multiple single group responses to the treatments, whereby for each species added the LMEM test given in Eq. 2 is performed for each single group (outputting the t-value of the Year x Treatment interaction). The first "group" tested therefore only contains one species, and each species is added in turn (2nd test on two species; 3rd on three species) until the final test is on a group containing all species in the community (31 species) and is synonymous with the total community tests. For each climatic niche parameter, species were accumulated from both the dry/warm end of the gradient (where we predicted a more positive/less negative response of a CNG) and from the wet/cool end of the gradient (where we predicted a more negative response of a CNG). In effect, this analysis was therefore testing treatment responses over time for all possible group boundaries for CNG1 (accumulating most arid species up to total community), and separately for all possible group boundaries for CNG3 (accumulating most humid species up to total community). For further details on the methodology and the interpretation refer to Appendix S1.

Single species response plotted against niche

As a further test of the climatic niche approach, single species responses to the manipulation treatments were plotted against their corresponding climatic niche value. This was formally tested and displayed using Generalized Additive Models (GAMs) using R-package “mgcv” (Wood 2011). The response variable in the GAMs were the t-values of the LMEMs performed on each species (Eq. 2; Table S5) describing the difference in model estimate for the response slope over time between control and treatment (Year x Treatment interaction term). The explanatory variables were the climatic niche values associated with each species (Table S1). GAMs were run for the climate niche values presented in the main article (25% summer temperature; median spring precipitation), and for multiple other climate niche values to check robustness of different niche axes (Figure S5). A simple smoothing parameter was fitted to each GAM set at a value of 0.15 throughout. Each species was also weighted by their corresponding log community percentage value (Table S2).

Results

The total community log-abundance was affected by the moderate warming (0.6 °C) as shown by a significant Year × Treatment interaction (F=4.14, p<0.05, Table 1). Namely, while the communities under the warming and control treatments responded in an apparently similar pattern to within-year conditions across the 16 years of the experiment (Fig. 1a), the analyses of the treatment effects indicated that log-abundances in the warming treatment decreased progressively linearly relative to the control (Fig. 1b). The LMEM also indicated that both temperature and precipitation accounted for the variance in abundances among years, with significant main effects of both natural variables (SP, F=38.2, p<0.0001; ST, F=7.0, p<0.01) and their interaction term (F=4.6, p<0.05).

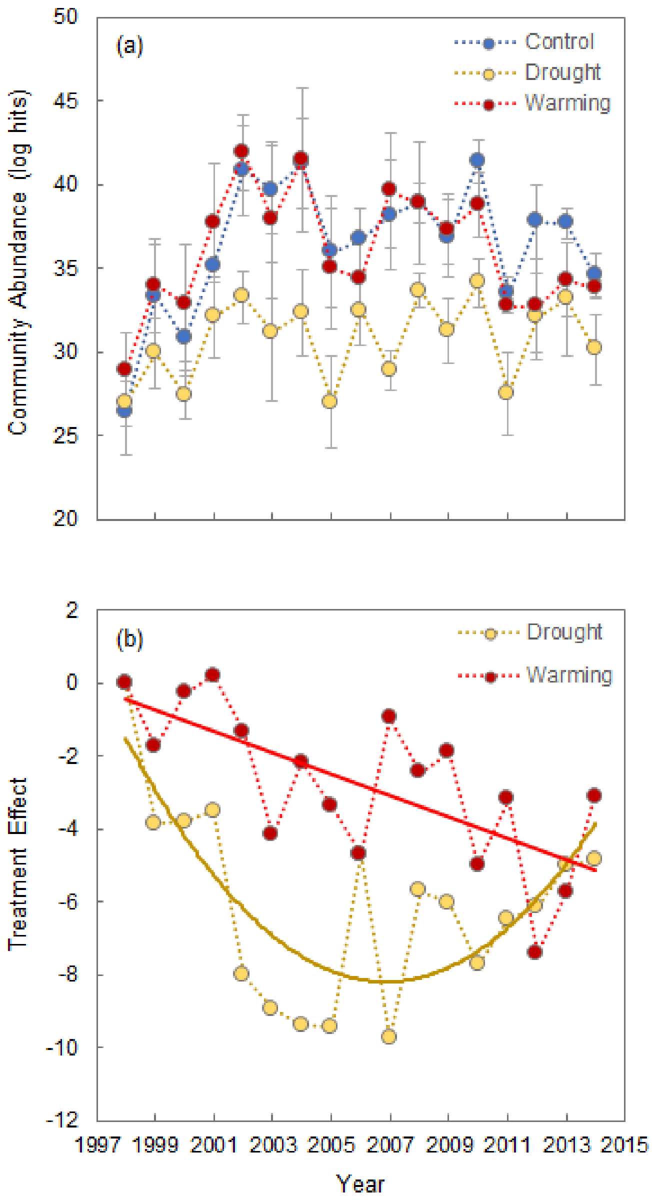

Fig. 1 (a) Total community log-abundance (log contact hits) within plots under control (no manipulation), nocturnal-warming and drought treatments throughout the study period 1999-2014. Warming and drought were not manipulated in 1998. Points represent means and the error bars represent their associated standard errors (n=3 plots). (b) The effect sizes (treatments minus control) for total community log-abundance under warming and drought throughout the study period. Effect sizes are corrected for initial pre-treatment difference in 1998 (see Eq. 4 in main text).

Table 1 Total community abundance responses to 16 years of climatic manipulation. ANOVA type I table for Linear Mixed-Effects Models analyzing the changes in abundance in response to either the warming or drought Treatment compared to the control. Start value indicates the abundance in the initial year of the experiment (1998 before manipulation). P-values in bold are statistically significant to an alpha value of 0.05.

	DF		Warming Treatment		Drought Treatment	
Fixed effect	num	den	*F*	*p*	*F*	*p*
Spring precipitation (SP)	1	85	38.15	**<.0001**	29.62	**<.0001**
Spring temperature (ST)	1	85	7.04	**0.0095**	2.24	0.1384
Start value	1	85	8.68	0.0602	4.50	0.1239
Year	1	85	0.23	0.6307	5.34	**0.0233**
Treatment	1	3	1.91	0.2605	12.38	**0.0390**
SP × ST	2	85	16.62	**<.0001**	17.02	**<.0001**
Year × Treatment	2	85	4.60	**0.0349**	0.03	0.8655

NumDF, numerator degrees of freedom; denDF, denominator degrees of freedom

More visibly, the drought treatment had an immediate impact on total community log-abundance, decreasing it compared to the control (Fig. 1a) which was reflected by a significant main effect of the drought treatment (F= 12.4, p<0.05, Table 1). The Year × Treatment interaction was not significant for drought, with no linear decrease (or increase) in log-abundances in the drought treatment compared to the control over the course of the experiment. However, the effect size of the drought treatment followed a distinct polynomial shape over time (Fig. 1b). Namely, the difference between log-abundances in drought *vs*. control treatments increased for the first nine years of the drought manipulation (until 2007) and then gradually decreased until the end of the study period reminiscent of a dampening effect in a whole-community parameter. The statistical model also indicated that spring precipitation (SP) and the interaction term between SP and spring temperature (ST) significantly affected total community log-abundances (F=29.6, p<0.0001 and F=17.0, p<0.0001; respectively).

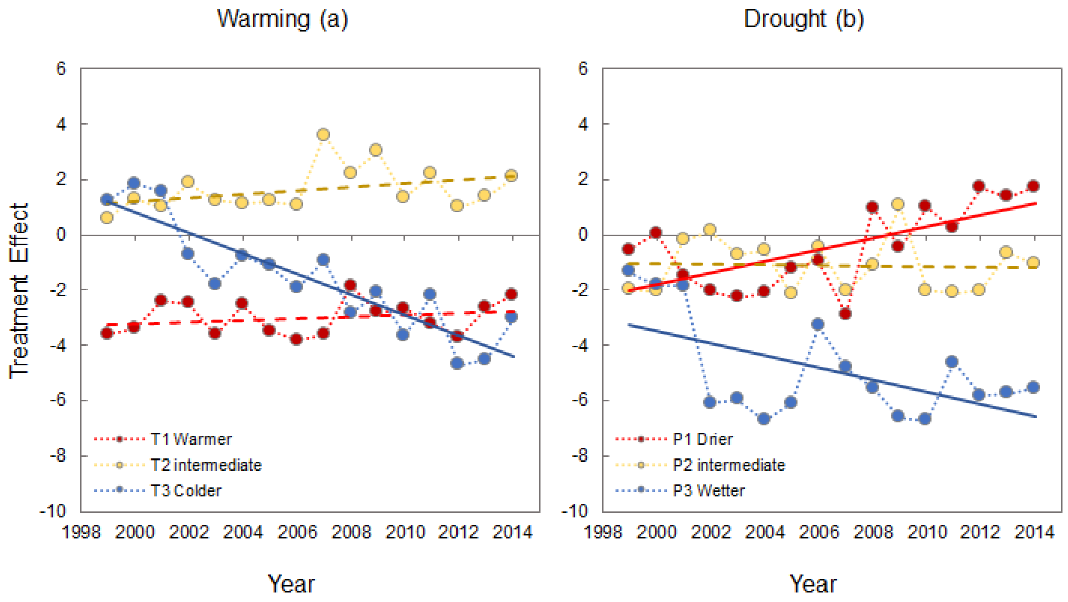

Fig. 2 Responses of (a) summer temperature (25 percentile) CNGs to warming and (b) spring precipitation (median) CNGs to drought throughout the study period 1999 to 2014. Points indicate mean effect sizes (treatments minus control) in a given year corrected for initial pre-treatment difference in 1998 (see Eq. 4 in main text). CNGs are listed in order of aridity: niche values of temperature CNGs from warm to cold (T1>T2>T3), and niche values of precipitation CNGs from dry to wet (P1<P2<P3). Solid lines indicate significant differences between slopes under treatment and control, and dotted lines indicate no significant differences.

Table 2 Climatic Niche Group (CNG) abundance responses to 16 years of climatic manipulation. Shown are results for the Temperature CNG response to warming treatment, and Precipitation CNG response to drought treatment. ANOVA type I table for Linear Mixed-Effects Models. Start value indicates the abundance in the initial year of the experiment (1998 before manipulation). P-values in bold are statistically significant to an alpha value of 0.05.

	DF		Temperature CNG Warming Treatment		Precipitation CNG Drought Treatment	
Fixed effect	num	den	*F*	*p*	*F*	*p*
Spring precipitation (SP)	1	268	24.21	**<.0001**	20.30	**<.0001**
Spring temperature (ST)	1	268	4.47	**0.0354**	1.53	0.2167
Start value	1	268	247.49	**<.0001**	427.57	**<.0001**
Year	1	268	0.15	0.7010	3.66	0.0569
Treatment	1	4	2.41	0.1952	15.56	**0.0169**
CNG	2	268	93.74	**<.0001**	83.72	**<.0001**
SP × ST	1	268	10.55	**<.0001**	11.66	**0.0007**
Year × Treatment	1	268	2.92	0.0888	0.02	0.8883
Year × CNG	2	268	23.75	**<.0001**	0.41	0.6667
Treatment × CNG	2	268	16.62	**<.0001**	36.27	**<.0001**
Year × Treatment × CNG	2	268	6.57	**0.0016**	6.58	0.0016

NumDF, numerator degrees of freedom; denDF, denominator degrees of freedom

Temperature CNGs accounted for the changes in species abundances in the warming treatment (Table 2). The LMEM demonstrated a significant three-way CNG × Treatment × Year interaction for the log-abundance changes of Temperature CNGs (*F*=6.57, *p*<0.01). Among these temperature CNGs, the log-abundances of T1 and T2 (from relatively warmer origins) remained fairly constant in the warming treatment when compared to the control, whereas the log-abundance of T3 (from colder origins) decreased gradually and linearly throughout the study period (estimated difference in slopes = -0.37, p<0.0001) (Fig. 2a, Table S4). In contrast, the three-way interaction was not significant (*p*=0.12) when applying the classification of temperature CNGs to analyze log-abundance changes under the drought manipulations (Table S4). Here, while response slopes comparing drought log-abundances over time to control were ranked in hierarchical order (T1>T2>T3) no temperature-CNG slope differences were significant (Table S4).

As hypothesized, precipitation CNGs also accounted for the contrasting changes in species abundances in the drought treatment (Table 2). The LMEM demonstrated a significant three-way CNG × Treatment × Year interaction for the log-abundance changes of Precipitation CNGs (*F*=6.58, *p*<0.01, Table 2). The response patterns differed greatly among the three CNG-groups. P3 (from wetter origins) initially decreased in log-abundance compared to the control (estimated difference in slopes = -0.22, p<0.01, Table S4), with an abrupt shift after three years of the drought manipulation (after 2001) and fairly constant log-abundances for the remaining years of the study. P2 log-abundances (from moderately dry origins) were similar in the control and drought treatment throughout the study period. However, the log-abundances of P1 (from drier origins) steadily increased in the drought compared to the control (estimated difference in slopes = 0.24, p<0.01, Table S4), particularly after 2007 when they were higher in abundance in the drought treatment than the control. Taken together, the responses of the three groups explain the u-shaped response found in the total community log-abundance data. Similar to the temperature CNGs, the three-way interaction was not significant (*p*=0.188) when applying the classification of Precipitation CNGs to analyze the log-abundance changes

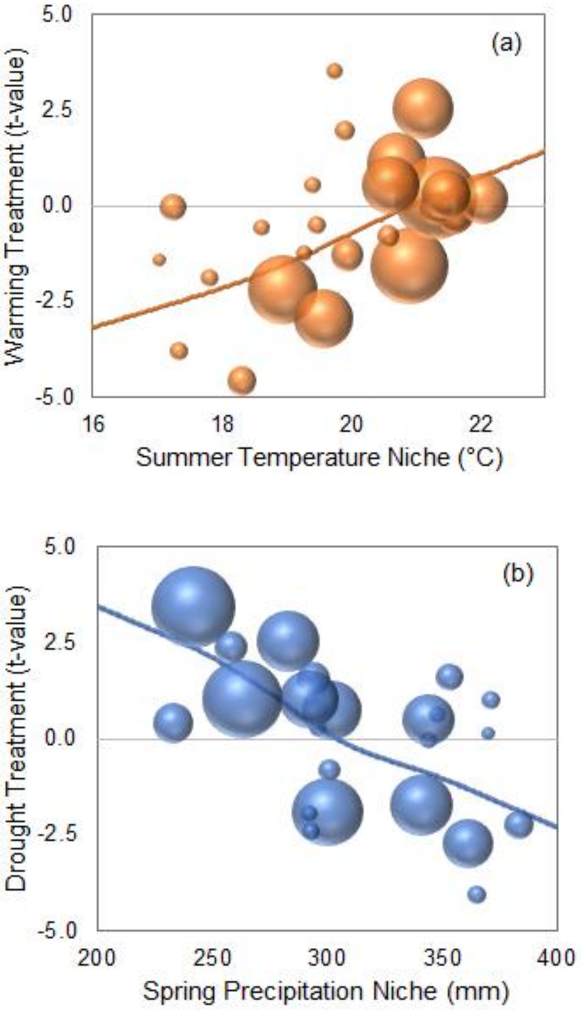

Fig. 3 Single species responses to the (a) warming and (b) drought treatments when plotted along their respective climatic niche axis: Species niche values defined by (a) Summer temperature for lower 25% values of distribution range observations; (b) Spring precipitation for median values of distribution range. Y-axis are the t-values of single species Linear Mixed Model (LMM) tests comparing slope response over time under control to treatments (positive values indicate increase in abundances under treatment, negative values indicate decrease under treatment over time relative to control) . Bubbles are displayed in relative size to the log-community percentage each species occurred throughout the experiment. Fit lines were derived from Generalized Additive Models (GAMs) weighted by species log-community percentage.

in warming treatment (details in Table S4). However, uncorrected post-hoc tests did reveal a significant difference in slope between control and warming for P3 (decrease as predicted for wettest species: slope difference = -0.18, $p<0.05$, Table S4).

Robustness analysis

Multiple CNG analyses were performed in the same manner as the main results presented above, using different niche values to rank species and create groups. Results were generally consistent with the presented findings, and some results of this can be seen in Table S4. In general, most methods highlighted a statistically significant decrease of cool or wet species under warming and that temperature CNGs regularly failed to explain changes in community composition under drought. Annual Precipitation CNGs sometimes managed to capture community composition change under both manipulation treatments (drought and warming), whereas Spring Precipitation CNGs were generally stronger for drought response but did not significantly describe changes under warming, despite revealing hierarchical responses among CNGs as predicted (i.e. drier CNG1 responded less negatively to warming than the wetter CNG3).

A full display of results and discussion of the Cumulative Group Analysis can also be found in Appendix S1 and Figures S2, S3, and S4. In general, it was clear that groups more often than not overcame variance in the single species responses to describe community change as predicted. For groups containing a few species, warm temperature CNGs increased (although not significantly) under warming, cool temperature CNGs significantly decreased under warming, relative to control (Figure S2). Similarly, but more pronounced, dry precipitation CNGs increased under drought, and wet precipitation CNGs decreased under drought (Figure S3). These results were most consistent for the results presented in the main article, when using Summer Temperature (25 percentile) CNGs to describe warming, and Spring Precipitation (median) CNGs to describe drought

(Figures S2 and S3 respectively). In addition, when testing CNG responses in opposing climate manipulations, patterns were generally less clear and more variable (Figure S4). In combination, this confirmed that group boundaries had little qualitative effect on the main results presented.

Single species responses plotted along niche axis

Single species LMEM analyses revealed that under warming, out of the 24 species tested, two species increased over time, and five decreased over time relative to control (Alpha p-value of 0.05 with no multiple test correction, Table S5). For the 23 species tested under drought, three species increased over time, and seven decreased over time relative to control (Table S5). Some care must be taken in interpreting these findings, as due to the large volume of analyses, linearity was rarely confirmed nor unconfirmed.

When single species responses (t-values of difference between control and treatment over time) were plotted against the climatic niche axes, while there is some variation in patterns, as revealed by the CNG analyses, it was generally the cooler species which decreased under warming (Figure 3a). Likewise, also confirming the findings from the CNG analyses, it was generally the drier species which increased under drought, and the wetter species which decreased under drought, relative to control (Figure 3b). Plotting single species drought responses by their temperature niche also regularly failed to reveal any consistent patterns (Figure S5), consistent with the CNG findings.

Discussion

Our overall findings from the 16-year experiment showed a strikingly clear differentiation between plant community response to increasing temperatures and to drought. By classifying species into CNGs we revealed that community changes were highly predictable and based on the species' distribution with respect to climate. In addition, our CNG analyses revealed some interesting explanations for a dampening effect in community-level parameters that without our approach, would have gone unnoticed. In the following, we discuss the effects of the two treatments on the community dynamics within our experiment, when species were defined along two climate niche axes.

The impacts of the warming treatment increased over time, probably because the effects of slightly higher temperatures (+ 0.6 ℃) gradually accumulated. However, previous studies at the same experimental site did not find significant abundance changes after 7-years exposed to the warming treatment (Prieto *et al.*, 2009). The clear but slow effect of warming on community log-abundance and composition in our study, highlights the need for long-term experiments in climate change studies (Smith *et al*., 2009; Leuzinger *et al.*, 2011; De Boeck *et al.*, 2015). More interestingly, the CNG analyses revealed that the gradual decreases in total community log-abundance to experimental warming was limited to decreases only in those species associated with cooler (and sometimes wetter) climates, and therefore presumably those species not well adapted to exposure to higher temperatures. Conversely, species with distribution ranges centered in intermediate and warmer/drier climates were remarkably unaffected by warming, possibly due to inherent adaptations to higher temperatures (Gottfried *et al.*, 2012; Duque *et al.*, 2015; Elmendorf *et al.*, 2015). These patterns thus suggest that under the future warmer temperatures and associated extreme heat waves predicted for the Mediterranean regions (Dai *et al.*, 2013; Barros *et al.*, 2014), abundance of the species from relatively cold origins is likely to decrease.

The CNG analysis was even more enlightening for the drought treatment. Here again, the species hypothesized to possess less adaptations to reduced water levels responded visibly, i.e. the species associated with wetter climates (P3) immediately decreased in log-abundance in the drought treatment. The abundances of these species also abruptly shifted between the third and fourth year, which could be due to the additive impact of the experimental drought and an extreme natural drought in 2001. In addition, the CNG analysis revealed that species from dry climates (P1) showed an increase in log-abundance in the drought treatment after a delay of nine years, and were more present in the drought plots than the control ones after this point. Therefore, for the predicted future scenario of reduced rainfall, the potential losses of species from the system may be partly offset by a gain in drought-adapted species (Sala *et al.*, 2000; Barros *et al.*, 2014, Andresen *et al.* 2016).

In general, while both temperature and precipitation manipulations had a negative impact on the community, the overall effect of the drought treatment in terms of immediate change and community shift was greater. This was likely due to the different severity of warming and drought treatments or differential sensitivity of the community to the two climatic factors (Loarie *et al.*, 2009; Beaumont *et al.*, 2011; Garcia *et al.*, 2014). In our already warm and dry system, elevated temperature can act as a climatic limitation for species establishment and growth, because the associated increased vapor pressure deficit increases the likelihood of high autotrophic respiration, transpiration from plants, and evaporation from the soil (Beier *et al.*, 2004; Williams *et al.*, 2012; Peñuelas *et al.*, 2013). Higher temperatures may therefore accelerate water outputs, greatly increasing the water stress for plants (Beier *et al.*, 2004; Williams *et al.*, 2012; Anderegg *et al.*, 2013). Greater immediate impacts of the drought manipulation in our study were apparent in the responses of total community log-abundance after the first year. Such strong plant responses to reduced precipitation levels are supported by the growing number of studies reporting that water availability is a crucial determinant of species distribution and persistence across global ecosystems (Myers *et al.*, 2000; Peñuelas *et al.*, 2007; Beaumont *et al.*, 2011; Doblas-Miranda *et al.*, 2015; Harrison *et al.*, 2015). Taking together evidence from our study and that from previous findings suggests that reduced precipitation may be a stronger and more immediate selective agent for determining structure and community composition in natural ecosystems, whereas warming could be a progressive and accumulative process (Beier *et al.*, 2004; Prieto *et al.*, 2009; Wu *et al.*, 2011).

Notably, we show evidence that the impacts exerted by temperature and drought alone selected for some different species, with responses determined by their climatic niche distributions. The temperature CNGs only explained the response of log-abundance to warming treatment, regularly failing to explain results under drought. For precipitation CNGs, while there was some correlation in species responses under both treatments, precipitation CNGs generally explained the response to drought treatment far better than to the warming treatment. This suggests, that despite the fact that both climate stressors decrease water availability to plants, they can also select independently for specific adaptations (Mittler, 2006; Beaumont *et al.*, 2011; Garcia *et al.*, 2014). Some care must be taken in fully extracting evidence for independent selection under the different climatic variables, because many of the species in this study were correlated and were indeed either cool-wet distributed (e.g. *Rubia peregrina* which responded negatively under both treatments), or warm-dry distributed species (e.g. *Globularia alypum* which increased under drought and had no response under warming - the general CNG response). However, the few species to express opposing niches, also performed as hypothesized by their niche distribution. For example, *Ulex parviflorus* (a warm-wet distributed species) had no response under warming, and decreased in abundance under drought. This contrasting response under opposing manipulation treatments, not solely in the direction expected by a reduction in water under warming, suggests that it is likely that in some cases the balance between traits related to cooling, capturing water, and preventing water loss (e.g.

stomatal closure, rooting depth, leaf thickness), could be uniquely tailored to the specific needs in response to climatic stress (Mittler, 2006; De Frenne *et al.*, 2013). In our study system, this appears to be reflected by their distribution ranges across climates. Clues for the precise adaptations/mechanisms defining the groups and underlying these changes are revealed by results at the same study site which reported: demographic changes in seedling numbers of a subset of species (Lloret *et al.*, 2009); rapid genetic shifts in the dry (P1) and warm (T1) species *Fumana thymifolia* (Jump *et al.* 2008) which we showed to increase under both manipulations; and potential physiological acclimation – in terms of water-use efficiency, shoot water potential and net photosynthetic rate - of a dry distributed species *Erica multiflora* under the drought treatment (Liu *et al.*¸2016). Indeed, a valuable next step could be a trait screening exercise of species from the distinct niches to confirm precisely which adaptations are similar and different for determining plant responses under the opposing climate drivers. Ultimately, while higher temperatures and lower precipitation are both likely to increase the aridity of the Mediterranean Basin by increasing evapotranspiration (Mittler, 2006; Garcia *et al.*, 2014), some species will not suffer equally from the future temperature and precipitation regimes.

In many climate manipulation studies, including our own, often whole community variables (total density, species richness, community productivity) either show limited to no-net effects (Grime *et al.*, 2008; Tielbörger *et al.*, 2014; Estiarte *et al.*, 2016) or dampening over time (Leuzinger *et al.*, 2011; Barbeta *et al.*, 2013; Liu *et al.*, 2015; Andresen *et al.* 2016). Under the drought manipulation in our study, we also showed a clear polynomial response pattern for total community log-abundance relative to control, which is often interpreted as a dampening of the treatment/climate effect over time (Andresen *et al.* 2016). One of our most interesting findings was that this polynomial pattern could be explained by clear contrasting shifts among the different precipitation

CNGs. This is intriguing, because dampening effects have often been interpreted as an ‘adaptation’ of the system back to its original state, due to acclimation (Leuzinger *et al.*, 2011; Barbeta *et al.*, 2013; Liu *et al.*, 2015; Andresen *et al.* 2016). Without our in-depth community-level analyses we would have probably come to the same -erroneous- conclusion, and community composition changes may help explain contrasting response patterns over time in other long-term manipulation studies (Andersen *et al.* 2016). Importantly, our findings enabled us to identify that the impact of the drought treatment is not dampening, but remains strong, and the effect seen for total community log-abundance was the result of a decrease in abundance of wet-distributed species and a delayed increase in the abundance of dry-distributed species. It seems likely that the reduction in competition exerted from the wet-distributed species may have been responsible for this switch in dominance hierarchy, and that particularly in shrubland systems such as ours, these switches may require long periods of time to emerge (Smith, 2009; Prieto *et al.*, 2009; De Boeck *et al.*, 2015; Andresen *et al.* 2016). Most manipulation studies, however, are conducted on short temporal scales (<10 years), thus limiting our understanding of hierarchical responses among co-existing species (Leuzinger *et al.*, 2011; De Boeck *et al.*, 2015).

The use of CNGs to observe, follow, and predict community dynamics under climate manipulation experiments/ climate change scenarios has proved to be a simple and robust technique to extract general and interpretable trends in community dynamics. The use of many different climate parameters often showed similar qualitative results to the main climate parameters presented here (Summer Temperature and Spring Precipitation). In addition, the Cumulative Group Analyses revealed that the conclusions rarely altered due to where the group boundaries were set. Also, by plotting single species responses along a niche axis we revealed very similar conclusions about which species – in terms of niche distribution - would increase or decrease in abundance in response to the manipulations. A more detailed discussion on some of the different niche parameters used can be viewed in Appendix S2. However, in general we conclude that while niche range would be extremely valuable to consider across large areas, within a community it would seem that an average value (median or mean) captures well both potential increasers and decreasers under manipulations (Elmendorf *et al.*, 2015; Bilton *et al.*, 2016). A valid next step could be to test simple or more complex BEM/SDM predictions of species responses under manipulations to give a different view on the use of the climatic niche principle.

The highly consistent and predictable species responses to the treatments may have an interesting conservation aspect. Namely, our results suggest that with the advent of predicted increases in temperature, decreases in precipitation, along with increases in extreme events in the future (Loarie *et al.*, 2009; Dai, 2013; Reichstein *et al.*, 2013; Barros *et al.*, 2014), there would be an associated decline in species with a lower capability to persist under either warming or drought or both. By applying a CNG approach to manipulation experiments, we show valuable evidence that climatic niche distributions may be able to identify which species may be most vulnerable to shifts in these climate change factors either independently or in conjunction. Therefore, by exploring in more detail the within community dynamics, CNGs may aid in providing predictions for rates of species loss, which at the global scale remains continuing or even sharply increasing. Despite being of great conservation priority, this is especially so in biodiversity hotspots such as Mediterranean ecosystems (Myers *et al*., 2000; Bellard *et al.*, 2012; Kröel-Dulay *et al.*, 2015; Seddon *et al.*, 2016).

However, the decline in the abundance of some climate-sensitive species, may be balanced by an increase in resistant species distributed in warmer or drier niches. This was seen in our study with the delayed increase in species associated with dry climates in our drought treatment (e.g. *G. alypum*). Indeed, growing observational (Gottfried *et al.*, 2012; Duque *et al.*, 2015) and experimental (Harte & Shaw, 1995; Elmendorf *et al.*, 2015) evidence suggests that communities are shifting towards a higher proportion of species associated with warmer climates in response to global warming. Crucially, our findings suggest that similar, and more importantly, independent conclusions can also be drawn for species response to drought. Therefore, our evidence provided here from the CNG approach suggests that it may be possible to depict, on a global scale, how the magnitude of changes of either temperature and/or precipitation may affect those climate-sensitive species. Encouragingly, data bases are becoming more readily available for species distributions (Global Biodiversity Information Facility, GBIF) and are sufficiently precise to define the sensitivity of species by their climatic niche and to group the species in an index of similar niches (Bilton *et al.*, 2016), allowing for the application of the CNG principle to a wide array of habitats for comparison. Our findings provide the first robust evidence that the categorization of species by their climatic niche is suitable, when used in conjunction with manipulation experiments, to be applied to large-scale correlative models in predicting species abundance changes under complex climate scenarios. Thus, our findings add a mechanistic aspect to correlative models and thus address much of the criticism these models have been exposed to (Dormann, 2007). We therefore advocate the combined use of both manipulation experiments and CNGs to improve assessments of community responses to future climate change scenarios.

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Chapter 6. Shift in community structure in an early-successional Mediterranean shrubland driven by long-term experimental warming and drought and natural extreme droughts

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Abstract

Global warming and recurring drought are expected to accelerate water limitation for plant communities in semiarid Mediterranean ecosystems and produce directional shifts in structure and composition that are not easily detected, and supporting evidence is scarce. We conducted a long-term (17 years) nocturnal-warming (+0.6 °C) and drought (-40% rainfall) experiment in an early-successional Mediterranean shrubland to study the changes in community structure and composition, contrasting functional groups and dominant species, and the superimposed effects of natural extreme drought. Species richness decreased in both the warming and drought treatments. Responses to the moderate warming were associated with decreases in herb abundance, and responses to the drought were associated with decreases in both herb and shrub abundance. The drought also significantly decreased community diversity and evenness. Changes in abundance differed between herbs (decreases) and shrubs (increases or no changes). Both warming and drought, especially drought, increased the relative species richness and abundance of shrubs, favoring the establishment of shrubs. Both warming and drought produced significant shifts in plant community composition. Experimental warming shifted the community composition from *Erica multiflora* toward *Rosmarinus officinalis*, and drought consistently shifted the composition toward *Globularia alypum*. The responses in biodiversity (e.g. community biodiversity, changes of functional groups and compositional shifts) were strongly correlated with atmospheric drought (SPEI) in winter-spring and/or summer, indicating sensitivity to water limitation in this early-successional Mediterranean ecosystem, especially during continuous natural severe droughts. Our results suggest that long-term nocturnal warming and drought, combined with natural severe droughts, will accelerate shifts in species assembles and community diversity and composition in early-successional Mediterranean shrublands, highlighting the necessity for assessing the impacts on ecosystemic functioning and services and developing effective measures for conserving biodiversity.

Keywords: global warming; frequent droughts; Mediterranean ecosystems; long-term climatic experiments; species richness; community diversity; functional groups; composition shifts

Introduction

The ecological consequences of the losses of biodiversity and ecosystemic functioning and services have been strongly associated with global climate change (Chapin *et al.*, 2000; Walther *et al.*, 2002; Bellard *et al.*, 2012; Peñuelas *et al.*, 2013; Tilman *et al.*, 2014; Scheffers *et al.*, 2016). Ecosystems in the Mediterranean Basin, one of Earth's foci of the effects of climate change on biodiversity, will likely be affected by rapid warming and drought (Myers *et al.*, 2000; Peñuelas *et al.*, 2007, 2013; Dai, 2013). Climate-induced changes in plant community structure and composition in Mediterranean ecosystems have been documented, such as the losses of endemic species (Myers *et al.*, 2000; Schröter *et al.*, 2005; Peñuelas *et al.*, 2007; Gottfried *et al.*, 2012), changes in patterns of diversity (Chapin *et al.*, 2000; Walther *et al.*, 2002; Prieto *et al.*, 2009a; Kröel-Dulay *et al.*, 2015) and declines in community stability and resilience (Chapin *et al.*, 2000; Prieto *et al.*, 2009a; Doblas-Miranda *et al.*, 2014; Brose & Hillebrand, 2016). The trends in meteorological records and the projections of climatic models suggest future climatic scenarios that would continue to trigger large and irreversible structural and compositional changes in Mediterranean plant communities, which would affect multiple ecosystemic functions and climatic feedback (Myers *et al.*, 2000; Peñuelas *et al.*, 2013; Doblas-Miranda *et al.*, 2014; Tilman *et al.*, 2014).

Strong evidence for the direction and magnitude of the impacts of climate change on plant community structure and composition, however, remains unclear. Ecological modelling (e.g. bioclimatic envelope and dynamic vegetation models) has been commonly used in recent decades for predicting the response of biodiversity to future climate change, such as extinction and shifts in species ranges and abundance (Leuzinger *et al.*, 2011; Araújo & & Peterson, 2012; Bellard *et al.*, 2012). Most of the models, however, have grossly underestimated the importance of species plasticity (e.g. physiological, phenological and morphological plasticity) and biotic interactions (e.g. competition, facilitation and mutualism) (Tylianakis *et al.*, 2008; Leuzinger *et al.*, 2011; Bellard *et al.*, 2012; Tilman *et al.*, 2014). Indeed, many studies have reported physiological (photosynthetic activities) (Llorens *et al.*, 2003; Prieto *et al.*, 2009b; Liu *et al.*, 2016), phenological (earlier or delayed) and morphological (leaf size, number and longevity) (Peñuelas *et al.*, 2004, 2009; Bernal *et al.*, 2011; Scheffers *et al.*, 2016; Thackeray *et al.*, 2016) adjustments associated with climate change as well as evolutionary adaptions (Jump & Peñuelas, 2005; Jump *et al.*, 2008; Hoffmann & Sgrò, 2011; Scheffers *et al.*, 2016) to rapid warming and drought. Species loss or changes in abundance predicted by models do not therefore satisfactorily represent changes in community structure and composition in terrestrial ecosystems under future climatic scenarios. Some models have incorporated species plasticity, acclimation and biotic interactions, but most typically analyze data parameterized on short timescales (Luo *et al.*, 2011; Beier *et al.*, 2012; Estiarte *et al.*, 2016). The responses of community structure and composition to climate change, however, will be driven by slow processes over decadal or even longer timescales (Smith *et al.*, 2009; Luo *et al.*, 2011; Peñuelas *et al.*, 2013; Estiarte *et al.*, 2016). Accurately assessing the changes in community structure and composition caused by long-term processes is thus essential for validating the models and providing realistic supporting information for future climate change.

Long-term experiments of climatic manipulation are among the best methods for studying the responses of species interactions and community dynamics under predicted climatic regimes (Luo *et al.*, 2011; Wu *et al.*, 2011; Beier *et al.*, 2012; Estiarte *et al.*, 2016). A few long-term (>10 years) climatic-manipulation experiments have been established in natural ecosystems in the last three decades (Leuzinger *et al.*, 2011; Luo *et al.*, 2011; Estiarte *et al.*, 2016). These experiments have demonstrated increasing (Walker *et al.*, 2006; Smith *et al.*, 2009; Elmendorf *et al.*, 2015), decreasing (dampening) (Leuzinger *et al.*, 2011; Barbeta *et al.*, 2013; Liu *et al.*, 2015) and unchanged (Grime *et al.*, 2008; Tielbörger *et al.*, 2014; Estiarte *et al.*, 2016) impacts of manipulation on community dynamics. Community responses to manipulative experiments have been reported for tundra (Walker *et al.*, 2006; Elmendorf *et al.*, 2015) and temperate grassland (Yang *et al.*, 2011) ecosystems, whereas no net (or chronic) changes have been reported for semiarid Mediterranean ecosystems (Tielbörger *et al.*, 2014; Estiarte *et al.*, 2016). Large shifts in community structure and composition, as proposed by the hierarchical-response framework, will likely come because of continuous and cumulative climatic disturbances (Smith *et al.*, 2009). Contrasting community responses to long-term climatic-manipulation experiments can be due to differential effects in functional groups or dominant species, indicating alterations in dominance hierarchies and relative abundances (Smith *et al.*, 2009; Luo *et al.*, 2011; Yang *et al.*, 2011; Peñuelas *et al.*, 2013). The shortage of long-term field manipulations has greatly limited our understanding of the alterations in plant community structure and composition and in functional groups and dominance shifts. The cumulative effects of long-term climate change, however, may also be abrupt or non-linear when thresholds (tipping points) are exceeded, exacerbated by climatic extremes (Ciais *et al.*, 2005; Peñuelas *et al.*, 2007; Jentsch *et al*., 2011; Reichstein *et al.*, 2013; Doblas-Miranda *et al.*, 2014). Long-term manipulative field experiments are thus likely to record transformative changes and to identify the mechanisms of community dynamics in response to the overlapping effects of climatic variability and extremes (Smith *et al.*, 2009; Luo *et al.*, 2011; Jentsch *et al*., 2011; Kreyling *et al*., 2011; Estiarte *et al.*, 2016).

Most natural terrestrial ecosystems around the globe are disturbed either by anthropogenic activities or natural climatic events and are either not in equilibrium or are recovering (Scheffer *et al.*, 2009; Seddon *et al.*, 2016). The successional status of an ecosystem is an influential factor that must be considered for accurately forecasting community dynamics under climate change (Prieto *et al.*, 2009a; Kröel-Dulay *et al.*, 2015; Brose & Hillebrand, 2016; Estiarte *et al.*, 2016). The reestablishment of species or structural reordering in these dynamic ecosystems at early successional stages, however, may be strongly affected under the predicted scenarios of climate change (Jump *et al.*, 2008; Prieto *et al.*, 2009a; Peñuelas *et al.*, 2013; Kröel-Dulay *et al.*, 2015). Field experiments have demonstrated that continuous warming and drought manipulations have influenced reproductive outputs and seedling compensation, which could potentially lead to substantial shifts in plant community structure and composition in early-successional shrubland ecosystems (Lloret *et al.*, 2004, 2009; Del Cacho *et al.*, 2012). Some reports, however, have indicated that experimental climatic treatments in early-successional ecosystems have increased physiological adjustments (photosynthetic activities, stomatal conductance and water-use efficiency) (Llorens *et al.*, 2003; Prieto *et al.*, 2009b; Liu *et al.*, 2016), altered phenological activities (Peñuelas *et al.*, 2004; Bernal *et al.*, 2011) and invoked rapid genetic changes toward seedling phenotypes to adapt warming and drought (Jump & Peñuelas, 2005; Jump *et al.*, 2008). Whether physiological, phenological and genetic modifications can increase the occurrence and relative abundance of more resistant species and can compensate for species loss or decreases in the abundance of sensitive species on long-term temporal scales remains unclear. Our understanding of species competition, community dynamics and their mechanisms also remains poor, especially for early-successional ecosystems (Kröel-Dulay *et al.*, 2015; Estiarte *et al.*, 2016). Long-term manipulative experiments in early-successional or recovering ecosystems and monitoring the responses of community structure, functional groups or species dominance to global climate change are therefore urgently needed.

Long-term nocturnal-warming (0.6 °C average temperature increase) and drought (40% decrease in precipitation) experiments have been conducted in an early-successional Mediterranean shrubland since 1999. We hypothesized that long-term manipulations of both nocturnal warming and drought would decrease biodiversity at the community level (species richness (*S*), community diversity (*H*) and evenness (*E*)) and trigger different performances (species richness and abundance) between functional groups, ultimately leading to the decline or loss of sensitive groups (or species) and shifts in community composition to more resistant groups (or species). The specific objectives of this study were to determine: 1) if both warming and drought would significantly decrease community biodiversity (*S*, *H* and *E*) throughout the study period, 2) if contrasting responses of functional groups would decrease *S* and *H*, 3) if long-term experimental warming and drought would shift community composition by altering species dominance and 4) if changes in biodiversity were associated with the impacts of severe droughts (different timescales of the Standardized Precipitation Evapotranspiration Index (SPEI)) The results of this study could provide experimental evidence to help the management and regulation of future biodiversity conservation.

2. Materials and method

2.1 Study site

The study site was in a Mediterranean shrubland on a south-facing slope (13%) in the Garraf Natural Park, near Barcelona (northeastern Spain) (41º18´N, 1º49´E; 210 m a.s.l.). The climate is typically Mediterranean, with hot and dry summers (June to August, average temperature of 22.8 °C) and wet springs (March to May, average rainfall of 147.8 mm) and autumns (September to November, average rainfall of 204.8 mm). The mean annual temperature and mean annual precipitation at the study site are 15.2 °C and 571 mm, respectively. The soil is calcareous, and the substrate is composed of marls and limestone, with rocky outcrops. This shrubland appeared after two severe wildfires in 1982 and 1994 in a *Pinus halepensis* forest. The vegetation is composed mainly of short perennial shrubs (<1.5 m) that dominated the regrowth after the two fires. The dominant species of the shrubland are *Globularia alypum* and *Erica multiflora* (total proportion more than 50%), which are accompanied by other Mediterranean shrub species, such as *Ulex parviflorus*, *Dorycnium pentaphyllum* and *Rosmarinus officinalis*. The undergrowth consists mainly of annual herbaceous plants (mostly Poaceae species) (Species information as described in Table S1).

2.2 Manipulation experiments

We conducted a nocturnal-warming experiment from 1999 to 2014 and a drought experiment from 1999 to 2015 on three replicate blocks selected along a south-facing slope (Llorens *et al.*, 2003; Peñuelas *et al.*, 2007; Prieto *et al.*, 2009a, 2009b; Liu *et al.*, 2016). Each block contained three randomly distributed replicate warming, drought and control plots (4×5 m) (Peñuelas et al., 2004), for a total of nine plots for all treatment. The warming experiment was established by passive nocturnal warming by covering the vegetation with reflective aluminum curtains (ILS ALU, AB Ludvig Svensson, Kinna, Sweden) at night that reduced outgoing infrared radiation. The curtains were activated automatically by preset light conditions (<200 lux). The drought experiment was conducted by excluding precipitation with transparent waterproof plastic covers during the wettest seasons (spring and autumn) to prolong the summer drought. A rain sensor activated the covers to intercept precipitation and retracted them when the rain stopped. The coverings for the warming and drought experiments were removed at wind speeds >10 m s^{-1} to prevent damage. Scaffolding (1.2 m height) was installed in each plot to support the covering systems. Scaffolding was also installed in the control plots, but no curtains or covers were used. The soil temperature at a depth of 5 cm was 0.6 °C higher in the warming treatment than the control, and soil moisture in the 0-15 cm layer was 16.7 % lower annually in the drought treatment than the control, throughout the 17-year experiment.

2.3 Measurements of vegetation and environmental parameters

Vegetation was assessed by pin-pointing once a year at the end of July or beginning of August (after the main growing season) from 1998 (the pre-treatment year) to 2015. The pin-point method describes the vegetation profile, including plant frequencies, that allows the estimation of community structure and composition. Only the central 3×4 m area of each plot was measured to avoid edge effects. Five parallel 3-m transects 0.8 m apart were permanently marked across this central area at the start of the study. Each transect had a sampling point every 5 cm (totaling 61 points per transect and 305 points per plot). We lowered a long steel pin (3 mm diameter) through the vegetation at each sampling point for recording the height, species, organ (leaf, reproductive structure or stem) and state (alive or dead) of all plants the pin touched.

S was obtained for each plot and year as the total number of species recorded. Species were classified by their life forms into two functional groups, herbs (*h*) and shrubs (*s*), and the richness of shrubs relative to the total number of species (*Relative Ss*) was calculated. Community diversity was estimated by the Shannon-Wiener index (*H*) for each plot and year as: $H = -\sum P_i \ln P_i$, where P_i is the number of pin-point contacts for species i divided by the total number of contacts. *E* was estimated by the Pielou index for each plot and year as: $E = (-\sum P_i \ln P_i)/\ln S$, where S is the number of species. The abundance of each species within a plot and year was obtained as the total number of pin-point contacts. The abundances of the two functional groups were calculated using the abundances of species of each life form and were then log-transformed for estimating the abundance of herbs (*Ah*) and shrubs (*As*) and the abundance of shrubs relative to the total abundance of all species (Relative *As*). The abundances of typical species, *G. alypum*, Poaceae species (PO) and *R. officinali*s, were also log-transformed for calculating the changes in abundance due to warming and drought.

Daily air temperature and precipitation were recorded by a meteorological station at the study site. Temperature means were calculated for various periods (annual, AT; winter-spring, WST, from December to May and summer, ST, from June to August), and accumulated precipitation was calculated for the same periods (AP, WSP and SP, respectively). Soil moisture in the plots was measured (bi)weekly by three time-domain reflectometric probes permanently installed in each plot to a depth of 15 cm. Soil temperature in the plots at a depth of 5 cm was measured by temperature RTD Pt100 1/3 DIN probes (Desin Company, Barcelona, Spain) permanently installed in the soil from 2000 to 2015. We used the data from a nearby meteorological station to determine the historical climatic trends for 1951-2015 (Fig. S1). We calculated the SPEI drought index (Vicente-Serrano *et al.*, 2010) using the historical data for 1951-2015 and our data at the site. SPEI incorporates the influences of precipitation and potential evapotranspiration (caused by warming temperatures) and provides information of the water balance at different timescales. We selected the winter-spring (May SPEI-4, from February to May) and summer (August SPEI-3, from June to August) water balances to identify the water deficits during the historical period 1951-2015 (Fig. S2), whereas several timescales were used for modelling the changes in biodiversity.

2.4 Statistical analysis

The effects of the treatments on the parameters of community biodiversity (*S*, *H* and *E*), species richness of functional groups (*Sh*, *Ss* and Relative *Ss*), abundance of functional groups (*Ah*, *As* and Relative *As*) and abundance of the typical species were analyzed, and each parameter was analyzed separately (warming vs control and drought vs control) by linear mixed-effects models. We tested various models, including combinations of the covariates of temperature (AT, WST and ST), precipitation (AP, WSP and SP), SPEIs at different timescales and treatment as fixed variables. We selected a random structure among the random factors block, plot and plot nested within block (block/plot). The best models with the lowest Akaike information criteria included block as a random factor and the covariates:

Biodiversity parameters = SPEI + Treatment   (1)

The SPEI timescales of the best model differed among the biodiversity parameters. May SPEI-4 was applied to analyze the changes in *S*, *H*, *Ss*, Relative *Ss* and the abundance of *G. alypum*; May SPEI-2 was applied to analyze the changes in *Sh*, *As* and Relative *Ss*; April SPEI-3 was applied to analyze the changes in *E*, Relative *As* and the abundance of *R. officinalis* and July SPEI-3 was applied to analyze the changes in *Ah* and the abundance of Poaceae. All models used the lme4 package in R version 3.2.5.

The shifts in community composition were analyzed by a redundancy analysis (RDA) of species abundance, with treatment (control and separately warming or drought) and environmental factors as explanatory variables. Environmental factors included temperature (AT, WST and ST), precipitation (AP, WSP and SP) and SPEIs at different timescales. RDAs were performed separately for various periods to identify the temporal treatment effects on community composition: the first half of the experimental period (1999-2006), the second half (2007-2015) and the entire period (1999-2015). Treatments and SPEIs were selected for each period for analyzing the changes in community composition as:

Community compositions= Treatment + SPEI (2)

Various SPEI timescales (July SPEI-3, May SPEI-2 and May SPEI-4) were selected for analyzing compositional changes during the 1999-2006, 2007-2015 and 1999-2015 periods. We also tested the significance of the changes in community composition for all variables (treatments and SPEIs) by analysis of variance (ANOVA) or each variable (treatments or SPEIs) using a Monte Carlo permutation test. The RDAs were conducted with the vegan package in R version 3.2.5.

3. Results

3.1 Site conditions and abiotic variables of the experimental treatments

The historical climatic series (1951-2015) from the nearby meteorological station exhibited a warming trend of annual temperature (AT) (R^2=0.38, P<0.001) and a moderately stable annual precipitation (AP) (Fig. S1a). The warm and dry summers characteristic of the Mediterranean climate were exacerbated by a trend of increasing ST (R^2=0.24, P<0.001) and decreasing SP (R^2= 0.11, P<0.01) during this period (Fig. S1b).

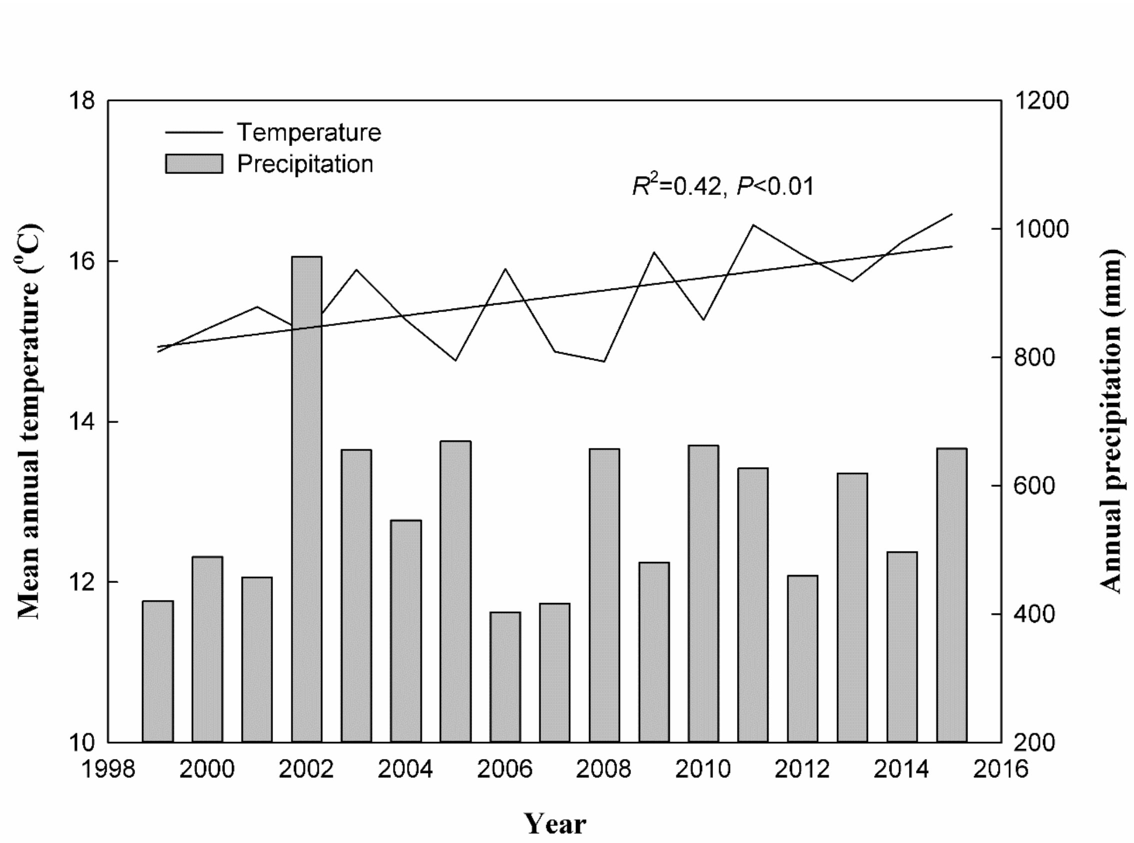

Fig. 1 Mean annual temperature and annual precipitation during the study period 1999-2015.

The trend in August-SPEI (for June, July and August) accordingly indicated a trend of increasing water deficits (R^2=0.13, P<0.01) (Fig. S2b). Average AT increased linearly during 1999-2015 (R^2=0.42, P<0.01) (Fig. 1), but AP fluctuated greatly, from 403.1 mm in 2006 to 956.2 mm in 2002. The study site experienced three extreme spring water deficits in 1999-2001, 2005-2006 and 2014 and four extreme summer water deficits in 1999-2001, 2003-2006, 2009 and 2012-2013 (Fig. S2a, b).

Soil temperature at a depth of 5 cm varied greatly inter-annually during the study period but averaged 0.6 °C (anova, P<0.001) higher in the nocturnal-warming treatment than the control (Fig. S3b). Soil moisture (SM) in the 0-15 cm layer also varied greatly during the study period (Fig. S3a) but decreased significantly by 16.7% in the drought treatment relative to the control (anova, P<0.001). SM was similar in the control and drought treatments in 2006 and 2010 due to damage to the covering systems. SM was not significantly affected in the warming treatment throughout the study period.

3.2 *S*, *H* and *E* at the community level

S, *H* and *E* varied greatly throughout the study period (Fig. 2; Table 1). *S* was lower in both the warming (difference=-0.91, $P<0.05$) and drought (difference=-2.19, $P<0.001$) treatments relative to the control. *H* and *E* were not affected by the experimental warming but both were significantly lower in the drought treatment than the control (difference=-0.22, $P<0.001$; difference=-0.04, $P<0.01$). The changes in *S*, *H* and *E* were associated with winter-spring SPEIs in the models. *S* was positively correlated with May SPEI-4 for warming-control (difference=0.62, $P<0.001$) and drought-control (difference=0.45, $P<0.01$) comparisons. *H*, however, was poorly correlated with May SPEI-4 for warming-control and drought-control comparisons, and *E* was negatively correlated with April SPEI-3 for the warming (difference=-0.12, $P<0.05$) and drought (difference=-0.11, $P<0.05$) models.

Table 1 Results from the best models for the responses of species richness (*S*), community diversity (*H*) and evenness (*E*) of the plant community to experimental warming and drought throughout the study period 1998-2015. The changes in *S* and *H* were associated with May SPEI-4 and the changes in *E* were associated with April SPEI-3 in the models. Warming-control and drought-control differences were analyzed. Significant differences are labeled with asterisks: * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Significant effects are highlighted in bold type.

	S		*H*		*E*	
	Difference	*P*	Difference	*P*	Difference	*P*
Warming-control						
SPEI	**0.62**	***	0.01	ns	**-0.12**	*
Warming	**-0.91**	*	-0.004	ns	0.01	ns
Drought-control						
SPEI	**0.45**	**	0.001	ns	**-0.11**	*
Drought	**-2.19**	***	**-0.22**	***	**-0.04**	**

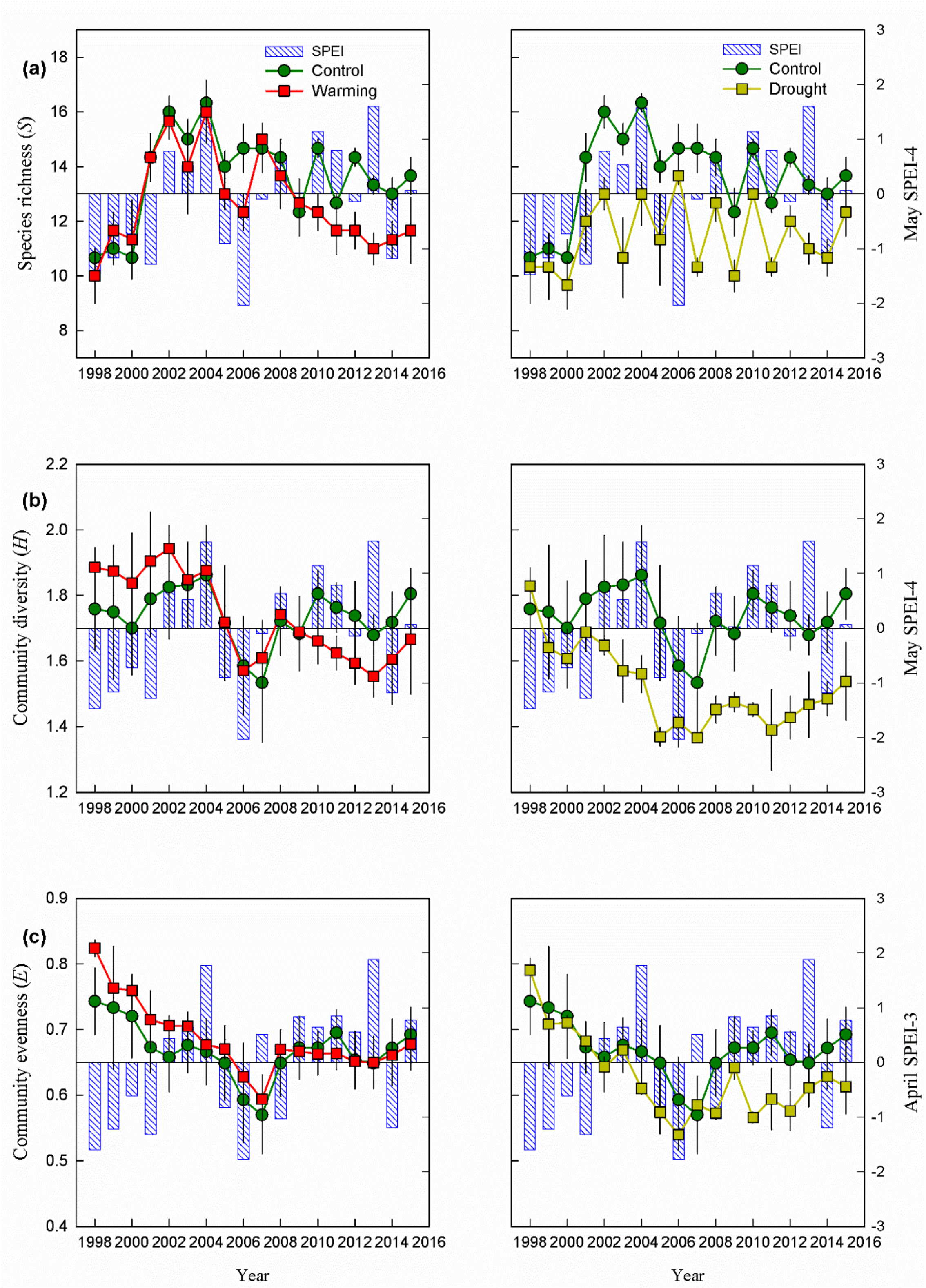

Fig. 2 Changes in (a) species richness (*S*), (b) community diversity (*H*) and (c) community evenness (*E*) in the warming, drought and control treatments during the study period 1998-2015. May SPEI-4 was the covariate factor for the changes in *S* and *H*, and April SPEI-3 was the covariate factor for the changes in *E* in the models. Vertical bars indicate the standard errors of the means (n=3 plots).

3.3 The responses of *S* for the functional groups

S fluctuated differently for the herbs and shrubs during the study period (Fig. 3; Table 2), with *Sh* more stable. In contrast, *Ss* increased gradually, peaked (13.7±0.33) in 2004 and then gradually decreased. *Sh* decreased under both warming (difference=-0.35, $P<0.05$) and drought (difference=-1.11, $P<0.001$) relative to the control. *Ss*, however, did not change under warming but decreased under drought (difference=-1.04, $P<0.01$). Relative *Ss* therefore increased substantially under drought (difference=0.08, $P<0.001$). The changes in *Sh*, *Ss* and Relative *Ss*, however, were associated with the water balance at different SPEI timescales (Table 2). *Sh* was positively correlated with May SPEI-2 in warming-control (difference=0.30, $P<0.001$) and drought-control (difference=0.13, $P<0.1$) comparisons, and *Ss* was positively correlated with May SPEI-4 in warming-control (difference=0.49, $P<0.01$) and drought-control (difference=0.43, $P<0.01$) comparisons. Relative *Ss*, however, was negatively correlated with May SPEI-2 only in the warming-control comparison (difference=0.02, $P<0.05$).

Table 2 Results from the best models for the responses of the species richness of herbs (*Sh*) and shrubs (*Ss*) and of Relative *Ss* to experimental warming and drought throughout the study period 1998-2015. The changes in *Sh* were associated with May SPEI-2 and the changes in *Sh* and Relative *Ss* were associated with May SPEI-4 in the models. Drought-control and warming-control differences were analyzed. Significant differences are labeled with asterisks: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Significant effects are highlighted in bold type.

	Sh		*Ss*		Relative *Ss*	
	Difference	*P*	Difference	*P*	Difference	*P*
Warming-control						
SPEI	**0.3**	***	**0.49**	**	**0.02**	*
Warming	**-0.35**	*	-0.52	ns	0.02	ns
Drought-control						
SPEI	**0.13**	(*)	**0.43**	**	0.002	ns
Drought	**-1.11**	***	**-1.04**	**	**0.08**	***

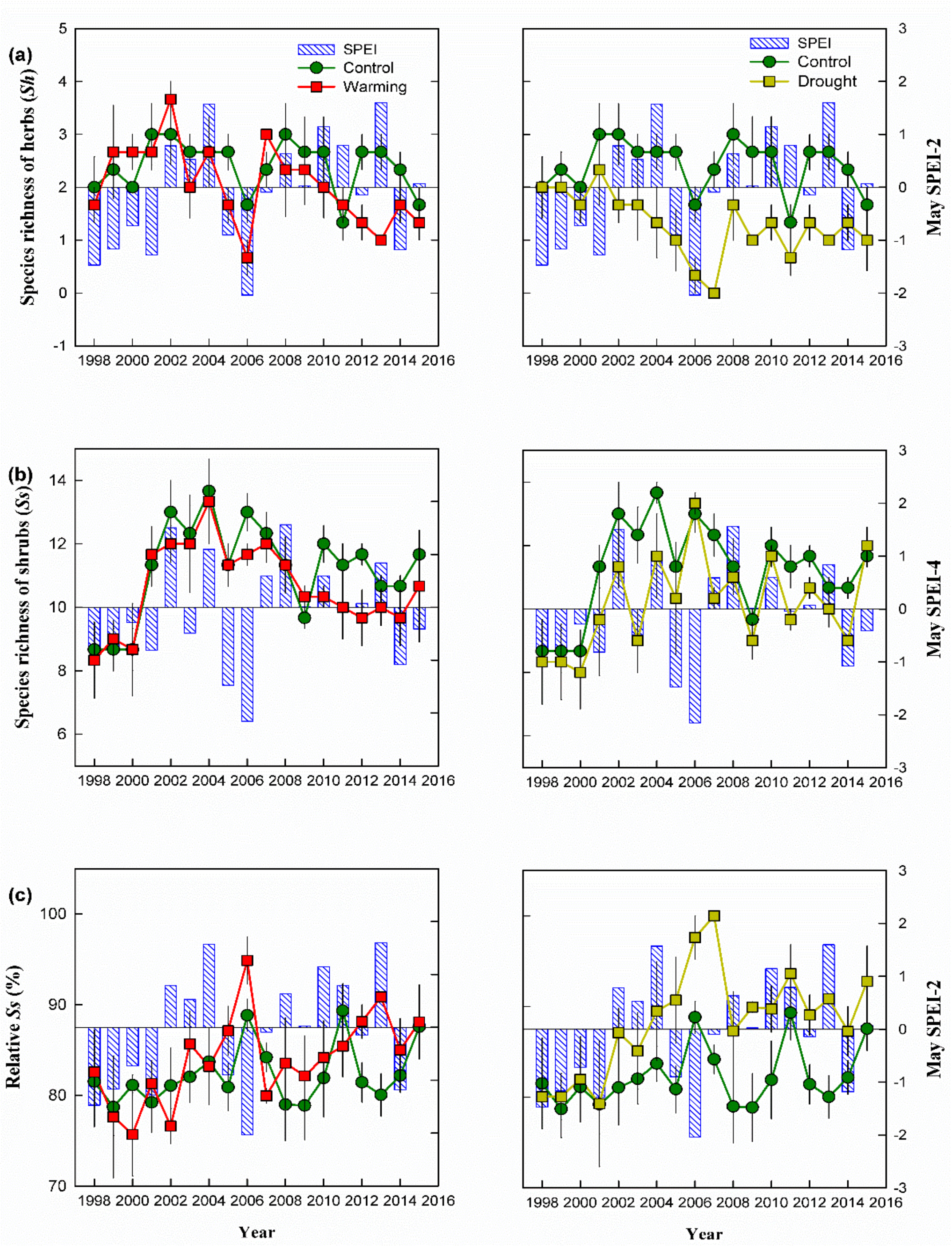

Fig. 3 Changes in the species richness of (a) herbs (Sh), and (b) shrubs (Ss) and (c) in Relative *Ss* in the warming, drought and control treatments during the study period 1998-2015. May SPEI-2 was the covariate factor for the changes in *Sh*, and May SPEI-4 was the covariate factor for the changes in *Sh* and Relative *Ss* in the models. Vertical bars indicate the standard errors of the means (n=3 plots).

3.4 The response of abundance for the functional groups

Ah and *As* fluctuated differently, with a gradual decreasing trend for *Ah* in the first half of the experimental period (1999-2006) and an increasing trend in the second half (2007-2015) and with a gradual increasing trend for *As* during the entire 1999-2015 period (Fig. 4; Table 3). *Ah* decreased both under warming (difference=-0.41, $P<0.01$) and drought (difference=-1.4, $P<0.001$) throughout the experiment relative to the control, but *As* marginally increased with warming (difference=0.13, $P<0.1$) and was not affected by drought. Relative *As* increased under both warming (difference=0.03, $P<0.05$) and drought (difference=0.06, $P<0.01$). *Ah*, *As* and Relative *As* were associated with SPEIs (Table 3). *Ah* was positively correlated with July SPEI-3 in warming-control (difference=0.28, $P<0.001$) and drought-control (difference=0.17, $P<0.1$) comparisons, *As* was positively correlated with May SPEI-2 in warming-control (difference=0.1, $P<0.01$) and drought-control (difference=0.11, $P<0.01$) comparisons and Relative *As*was negatively correlated with April SPEI-3 in the drought-control comparison (difference=-0.02, $P<0.01$).

Table 3 Results from the best models for the responses of the abundance of herbs (*Ah*) and shrubs (*As*) and of Relative *As* to experimental warming and drought throughout the study period 1998-2015. The changes in *Ah*, *As* and Relative *As* were associated with July SPEI-3, May SPEI-2 and April SPEI-3 respectively in the models. Drought-control and warming-control differences were analyzed. Significant differences are labeled with asterisks: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Significant effects are highlighted in bold type.

	Ah		*As*		Relative *As*	
	Difference	*P*	Difference	*P*	Difference	*P*
Warming-control						
SPEI	**0.28**	*******	**0.1**	******	-0.006	ns
Warming	**-0.41**	******	**0.13**	**(*)**	**0.03**	*****
Drought-control						
SPEI	**0.17**	**(*)**	**0.11**	******	**-0.02**	******
Drought	**-1.4**	*******	0.06	ns	**0.06**	******

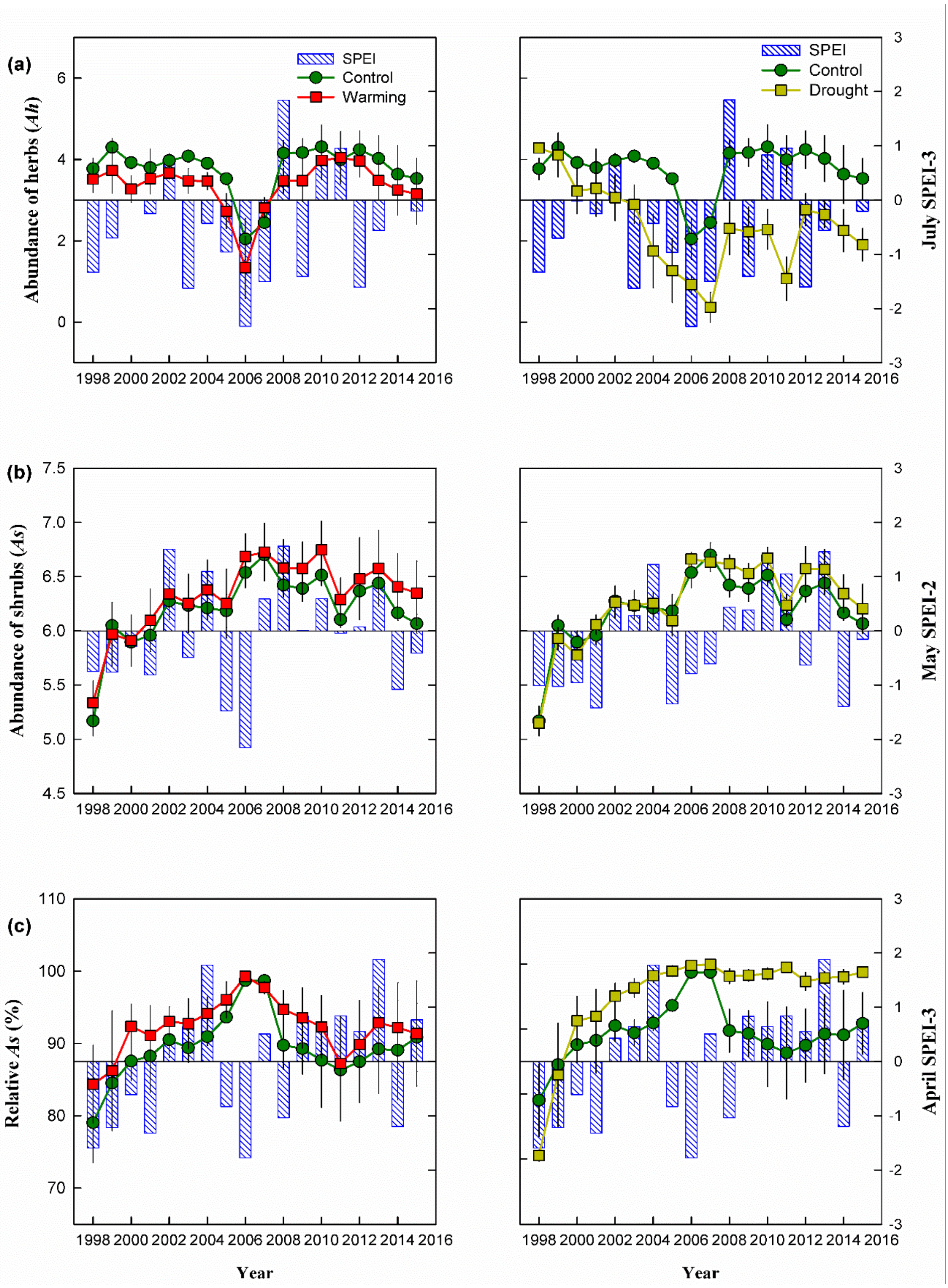

Fig. 4 Changes in the abundance of (a) herbs (*Ah*) and (b) shrubs and in (c) Relative *As* in the warming, drought and control treatments during the study period 1998-2015. July SPEI-3, May SPEI-2 and April SPEI-3 were the covariate factors for the changes in *Ah*, *As* and Relative *As*, respectively. Vertical bars indicate the standard errors of the means (n=3 plots).

3.5 Shifts in community composition in the treatments

Community composition changed significantly in the warming and drought treatments over the first half of the experimental period (1999-2006) ($P<0.001$), the second half (2007-2015) ($P<0.001$) and the entire period (1999-2015) ($P<0.001$) (Fig. 5). The RDA indicated that the climatic treatments and the covariates explained 33% (21 and 12% for axes 1 and 2, respectively), 44% (31 and 13% for axes 1 and 2, respectively) and 45% (29 and 16% for axes 1 and 2, respectively) of the total variability in species composition. The RDA indicated that experimental warming shifted the community composition toward *R. officinalis, E. multiflora* and *U. parviflorus* during 1999-2006 but favored *R. officinalis* when including 2007-2015 and the entire experimental period in the analysis. The drought treatment consistently shifted the community composition, favoring the expansion of *G. alypum* for the first and second half and the entire study period. The RDA also indicated that the changes in community composition were associated with July SPEI-3 during 1999-2006 ($P<0.01$) and May SPEI-2 during 2007-2015 ($P<0.05$). The analysis of species abundance by the statistical models (Table S2) indicated that warming would drive the shifts in composition toward *R. officinalis*, which increased significantly in abundance under experimental warming (difference=0.98, $P<0.001$) but did not change under drought Fig. S4a. Drought consistently drove shifts in community composition toward the dominant species, *G. alypum*, which increased significantly in abundance in the long-term drought treatment (difference=0.29, $P<0.001$) but decreased in the warming treatment (Fig. S4b).

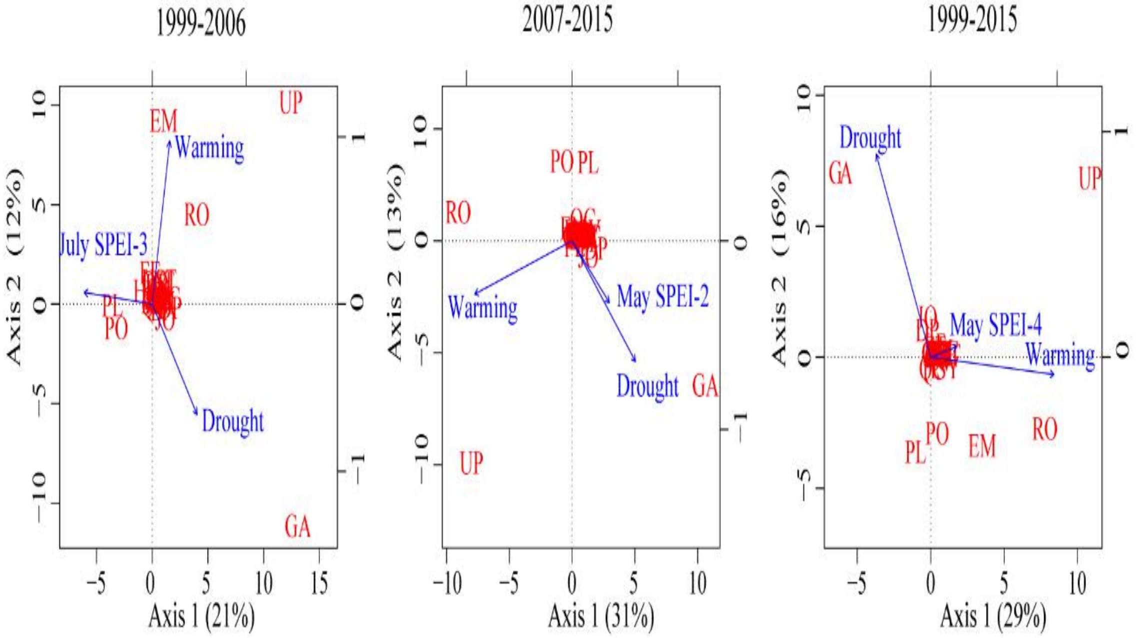

Fig. 5 Shifts of community composition in the first half of the experimental period (1999-2006), the second half (2007-2015) and the entire period (1999-2015). July SPEI-3, May SPEI-2 and May SPEI-4 were the covariate factors for the first half, second half and the entire study period, respectively.

4. Discussion

Community biodiversity

S decreased under long-term experimental warming in the early-successional Mediterranean shrubland. *S* did not decrease at an earlier stage of the same experiment (Peñuelas *et al.*, 2007; Prieto *et al.*, 2009a), indicating a delayed or cumulative effect with long-term warming. The delay may also be due to continuous influences on seedling recruitment and diversity (Lloret *et al.*, 2004, 2009) or to seed-bank density (Del Cacho *et al.*, 2012) under long-term experimental warming. The reductions in *S* with long-term (17 years) experimental warming were in accordance with the decreases in *S* of temperature-sensitive species with natural warming, which have decreased biodiversity in most Mediterranean montane ecosystems (Gottfried *et al.*, 2012). The experimental warming, however, did not affect *H* or *E*, possibly due to the moderate warming in the treatment (0.6 °C average increase). Previous studies reported that experimental warming increased plant growth in spring and autumn and also increased summer physiological adjustments (stomatal conductance and water-use efficiency) (Prieto *et al.*, 2009b; Liu *et al.*, 2016). *S* and plant community structure, however, may likely shift substantially in Mediterranean ecosystems under future warming, because climatic effects are cumulative or even nonlinear, especially if combined with extreme heatwaves and droughts (Smith *et al.*, 2009; Peñuelas *et al.*, 2013).

The drought treatment had early and strong influences on *S*, *H* and *E*, probably due to the intensity of the treatment (decreases in soil-water content of ca. 16.7%). Water is the most limiting factor for plant growth and reproduction in Mediterranean ecosystems, and significant changes in the structure and composition of plant communities at early successional stages have been reported under water deficits (Peñuelas *et al.*, 2007; Prieto *et al.*, 2009a). Manipulative reductions in precipitation have decreased plant growth (biomass accumulation and aboveground net primary productivity (ANPP)) (Peñuelas *et al.*, 2007; Prieto *et al.*, 2009c; Liu *et al.*, 2015), reproductive outputs (Del Cacho *et al.*, 2013) and seedling establishment (Lloret *et al.*, 2004, 2009). These effects will likely influence the dynamics of *S* and the patterns of plant community structure on long timescales. Moreover, large decreases in community biodiversity (*S*, *H* and *E*) under drought may strongly influence species dominance, trophic relationships and ecosystemic functioning (Peñuelas *et al.*, 2007, 2013; Estiarte *et al.*, 2016). Indeed, manipulative droughts (alterations in amounts, patterns and timing of precipitation) around the globe have had stronger impacts on community structure and ecosystemic functioning than other climatic drivers such as warming, because plant growth and reproduction are more sensitive to water stress (Peñuelas *et al.*, 2004, 2013; Wu *et al.*, 2011; Beier *et al.*, 2012). Drier conditions (from both climatic warming and lower precipitation) are likely to emerge in Mediterranean ecosystems in the coming decades, likely leading to loss of biodiversity and decreases in ecosystemic functioning and services (Dai, 2013; Peñuelas *et al.*, 2013).

S and abundance of the functional groups

Experimental warming significantly decreased *Sh* but not *Ss* or Relative *Ss*. The more conspicuous effects of warming on *Sh* than *Ss* are likely due to their different root systems. Deeper root systems may give shrubs an advantage for accessing larger pools of water and nitrogen under moderate warming (Peñuelas *et al.*, 2013). The reductions in *Sh* with experimental warming largely accounted for the decreases in *S* at the community level. Changes in *Ah* and *As*, however, differed under experimental warming, with decreases in *Ah* but increases in *As* that led to an increase in Relative *As*. Previous studies at our experimental site have indicated that moderate warming increased the photosynthetic rates of the shrubs *E. multiflora* and *G. alypum* in cold seasons, in agreement with the increase in growth for shrub species (Prieto *et al.*, 2009b; Liu *et al.*, 2016). Species reorganizations and shifts in community composition are therefore likely at the current magnitude and rate of warming because of severe declines or losses of herbs, whereas shrubs may have a competitive advantage (growth and reproduction), shifting composition toward the establishment of shrub species.

The drought treatment reduced both *Sh* and *Ss*, which could account for the substantial decreases in *S* at the community level. The experimental drought also decreased *Ah* but did not affect *As*. Both Relative *Ss* and *As* consequently increased under the drought treatment, perhaps due to the higher drought resistance of shrubs than herbs. For example, *G. alypum* (the dominant shrub at our site) can persist under dry conditions and can increase by regulating its physiological activities (e.g. stomatal conductance and water-use efficiency) (Llorens *et al.*, 2003; Prieto *et al.*, 2009a; Liu *et al.*, 2016) and altering its phenological periods (Peñuelas *et al.*, 2004). The decline or loss of herbs may indicate a selective sweep in a future drier climate, similar to the effects of warming, especially for lower precipitation during the growing season, which could decrease the presence and abundance of herbs. In contrast, shrubs were more resistant to both the warming and drought treatments, so they may persist with future rapid climate change. Whether the increases in the relative abundance (density) of shrubs in arid Mediterranean ecosystems would increase above- and/or belowground carbon accumulations, which may also influence shrubland productivity and ecosystemic carbon feedback, however, remains unclear.

Shifts in community composition

Experimental warming in our shrubland community led to significant shifts in community composition, consistent with the effects of warming treatments on communities in a montane meadow (Harte & Shaw, 1995), temperate steppe (Yang *et al.*, 2011) and peatland (Dieleman *et al.*, 2015). Shifts in community composition are ascribed to alterations in competitive hierarchies and the relative abundance of dominances or sub-dominances under warming (Harte & Shaw, 1995; Smith *et al.*, 2009; Yang *et al.*, 2011; Dieleman *et al.*, 2015). In our study, experimental warming shifted the community composition toward *R. officinalis*, *E. multiflora* and *U. parviflorus* in the first half of the experimental period (1999-2006) but favoring *R. officinalis* when including the second half (2007-2015) and consequently the entire period (1999-2015) in the analysis. Previous studies have reported that the growth of *E. multiflora* was more limited by summer drought, despite the enhanced growth in cold seasons via higher photosynthetic activities due to experimental warming (Llorens *et al.*, 2003; Prieto *et al.*, 2009a, 2009b; Liu *et al.*, 2016). *R. officinalis* thus tended to increase significantly in abundance in response to warming (Fig. S4a; Table S2), indicating an interspecific competitive advantage over coexisting species under warming. Shifts in dominance under warming in semiarid Mediterranean ecosystems, however, may be slow, so short-term studies would not detect these shifts in community composition. Long-term warming experiments are thus essential for tracking the changes in community composition in response to future climate change.

Community composition also shifted significantly under drought in our early-successional Mediterranean shrubland. Similar changes in response to increased drought have been observed in shorter-term studies at the same site (Prieto *et al.*, 2009a; Kröel-Dulay *et al.*, 2015). Moreover, drought consistently increased the expansion of the dominant species, *G. alypum*, when including all periods in the analysis. Other studies at the same experimental site also indicated that *G. alypum* adjusted physiologically (e.g. photosynthesis and stomatal conductance) (Llorens *et al.*, 2003; Prieto *et al.*, 2009b) and phenologically (e.g. delay in flowering time) (Peñuelas *et al.*, 2004) to the dry conditions.

The abundance (assessed as number of contacts) of *G. alypum* was also higher in the drought than the control treatment (Fig. S4b; Table S2). *G. alypum* may therefore maintain its dominant position in response to drought in this early-successional shrubland. Future climate change may shift the community composition, favoring *R. officinalis* under warming and *G. alypum* under drought, implying that shrubland ecosystems may become unstable or transitional.

Biodiversity parameters and meteorological droughts (SPEI)

The significant correlations between winter-spring SPEIs and the community parameters (*S*, *H* and *E*), species richness (*Sh, Ss* and Relative *Ss*) and abundance (*Ah, As* and Relative *As*) of the functional groups, the abundance of the typical species and the shifts in community composition indicated the importance of water balance to changes in structure and composition in this early-successional shrubland ecosystem. These changes in biodiversity were also closely correlated with short-timescale SPEIs (1-4 months), indicating drought sensitivity to water deficits (Vicente-Serrano *et al.*, 2010). In addition, most of these changes in biodiversity were positively correlated with winter-spring (May SPEI-4, May SPEI-2 and April SPEI-3) and summer (July SPEI-3) water balances. As described above, the herb functional group was extremely sensitive to water deficits in summer drought. For example, *Sh* was higher after the wet winter-springs of 2002 and 2004 and lower in the 2005-2007 dry period (Fig. 3a). *Ah* was lowest (mostly for Poaceae at our study site) in the 2005-2007 dry period (Fig. S4c; Table S2), reinforcing the premise of sensitivity to extreme drought. Extreme droughts in spring and summer, however, severely affected *H*. The extreme heat and drought in 2003 that reduced ecosystemic productivity throughout Europe (Ciais *et al.*, 2005) also significantly decreased *S*, plant growth and ANPP at our study site (Peñuelas *et al.*, 2007). We also detected low water availabilities in winter-spring and summer during 2003-2006 that probably caused the severe reductions in *S*, *H*, *E*, *Sh* and *Ah* for that period and in 2007. Natural severe droughts, however, increased Relative *Ss* and *As*, favoring shrubs, especially in the drought treatment (Figs. 3c, 4c). Indeed, the growth and ANPPs of Mediterranean forests have been correlated with the winter-spring water balance, and mortality rates and branch litterfall have been correlated with summer water balance (Barbeta *et al.*, 2013; Liu *et al.*, 2015). The structure and composition of Mediterranean shrubland ecosystems would therefore be substantially degraded if future climate change continues to decrease water reserves in winter-spring and summer.

Sensitivity of successional recovery from climate change

The developmental stage of an ecosystem may affect the responses of the vegetation to climatic disturbances (Peñuelas *et al.*, 2007; Prieto *et al.*, 2009a; Kröel-Dulay *et al.*, 2015; Brose & Hillebrand, 2016). Community structure and composition responded strongly to the climatic treatments, especially the drought treatment, in our early-successional shrubland ecosystem. The effects of climatic treatments, however, are not apparent in some ecosystems, even long-term treatments (Grime *et al.*, 2008; Tielbörger *et al.*, 2014; Estiarte *et al.*, 2016). These climatic treatments may be conducted in moderately mature or stable ecosystems, so the structure of plant communities is not influenced by slow climate change due to the well-developed structure and trophic complexity of the ecosystems (Smith *et al.*, 2009; Tielbörger *et al.*, 2014). Terrestrial ecosystems, however, are likely to experience anthropogenic and/or natural climatic disturbances that could push ecosystems into unstable or earlier successional stages (Chapin *et al.*, 2000; Peñuelas *et al.*, 2013; Seddon *et al.*, 2016). These unstable ecosystems would be strongly influenced by future climate change, leading to large changes in biodiversity and ecosystemic functioning (Kröel-Dulay *et al.*, 2015; Brose & Hillebrand, 2016). For example, Mediterranean ecosystems have historically suffered intense anthropogenic disturbances and are highly vulnerable to the ongoing climate change, because they are near critical ecosystemic tipping points (or thresholds) (Myers *et al.*, 2000; Peñuelas *et al.*, 2007, 2013; Scheffer *et al.*, 2009; Doblas-Miranda *et al.*, 2014). Global warming and drought represent selection pressures on plant species in local communities, which are leading to large losses of species diversity and decreases in ecosystemic functioning (Lloret *et al.*, 2004, 2009; Prieto *et al.*, 2009a; Kröel-Dulay *et al.*, 2015; Scheffers *et al.*, 2016). Future extreme climatic regimes (heat waves and droughts) would especially influence the functioning and services of ecosystems if critical thresholds are surpassed (Ciais *et al.*, 2005; Smith *et al.*, 2009; Jentsch *et al.*, 2011; Kreyling *et al.*, 2011; Reichstein *et al.*, 2013). Changes in community structure and composition could be persistent and even more abrupt as climate change projected by models progresses into the future (Dai, 2013; Doblas-Miranda *et al.*, 2014; Tilman *et al.*, 2014). Long-term climatic manipulations in ecosystems at different successional stages are thus critically necessary for projecting losses of ecosystemic biodiversity, functioning and services in response to future climate.

Acknowledgements

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General conclusions

1. Long-term experimental drought decreased ca. 8% net photosynthetic rates during the overall studied period in both *Q. ilex* and *P. latifolia*, whereas there were just non-significant trends toward a decrease in leaf water potential and stomatal conductance induced by drought treatment. Moreover, net photosynthetic rates, leaf water potential and stomatal conductance decreased with increases in VPD and decreases in soil water content, and the shrub species (e.g. *P. latifolia*) more resistant to drought could gain competitive advantage relative to *Q. ilex*, currently the dominant species of this forest.

2. Warming treatment moderately influenced shoot water potential, photosynthetic rates and stomatal conductance of *E. multiflora* throughout the sampling periods, whereas drought treatment strongly influenced these variables, especially during the summer. The combination of a natural drought in summer 2003 and the treatments significantly decreased the photosynthetic rates and water-use efficiency. However, plants also improved the acclimation to drier and hotter conditions by physiological adjustments (stomatal conductance and water-use efficiency).

3. The impacts of long-term experimental drought were highly species-specific, *A. unedo* suffered a significant reduction in aboveground biomass increment, *Q. ilex* experienced this decrease during the first years of experiment (1999-2003) and in the extreme droughts of 2005-2006 and 2011-2012, and *P. latifolia* was unaffected. The drought treatment significantly increased branch litterfall, especially in the extremely dry year 2011, and also increased overall leaf litterfall. The growth of all species were highly correlated with SPEI in early spring, whereas branch litterfall were better correlated with summer SPEIs, and leaf and fruit litterfall were better correlated with autumn SPEIs. However, the results of this long-term study also suggest that the effect of drought treatment has been

dampened over time, probably due to a combination of demographic compensation, and morphological and physiological acclimation and epigenetic changes.

4. The experimental drought increased annual rates of stem mortality and decreased stem growth at the community level for both high (H-) and low (L-) canopies over the study period. The changes in mean stem mortality, recruitment, stem density and growth, however, were larger in *Quercus ilex* than in *Phillyrea latifolia*. Natural droughts also amplified the effects of experimental drought on stem mortality at community level and for H- and L-canopy *Q. ilex*. Moreover, the decreases in the relative abundance and growth were observed for *Q. ilex* whereas the increases for *P. latifolia* for both H- and L-canopies, and the experimental drought reinforced the magnitude of the increases and decreases in L-canopy.

5. Warming caused a progressive decrease in the abundance of those species distributed in cooler regions. Correspondingly, reduced precipitation resulted in abrupt decreases in wet-distributed species, followed by a delayed increase in dry-distributed species. Notably, while climatic niche groups (CNGs) defined from temperature or precipitation values of the area of distribution were partially correlated, the corresponding explanations of variance in community response were stronger for their respective climate parameter, suggesting that some plant species possess specific adaptations to either warming or drought that may lead to independent selection to the two climatic variables.

6. Species richness decreased in both warming and drought treatments. Responses to the moderate warming were associated with decreases in herb abundance, and responses to the drought were associated with decreases in both herb and shrub abundance. The drought also significantly decreased community diversity and evenness. Both warming and drought produced significant shifts in plant community composition. Experimental warming shifted the community composition from *Erica multiflora* toward *Rosmarinus*

officinalis, and drought consistently shifted the composition toward *Globularia alypum*. The responses in biodiversity (e.g. community biodiversity, changes of functional groups and compositional shifts) were strongly correlated with atmospheric drought (SPEI) in winter-spring and/or summer.

Supplementary material

Chapter 4 Long-term experimental drought combined with natural extremes accelerate forest degradation and vegetation shifts in a Mediterranean holm oak forest

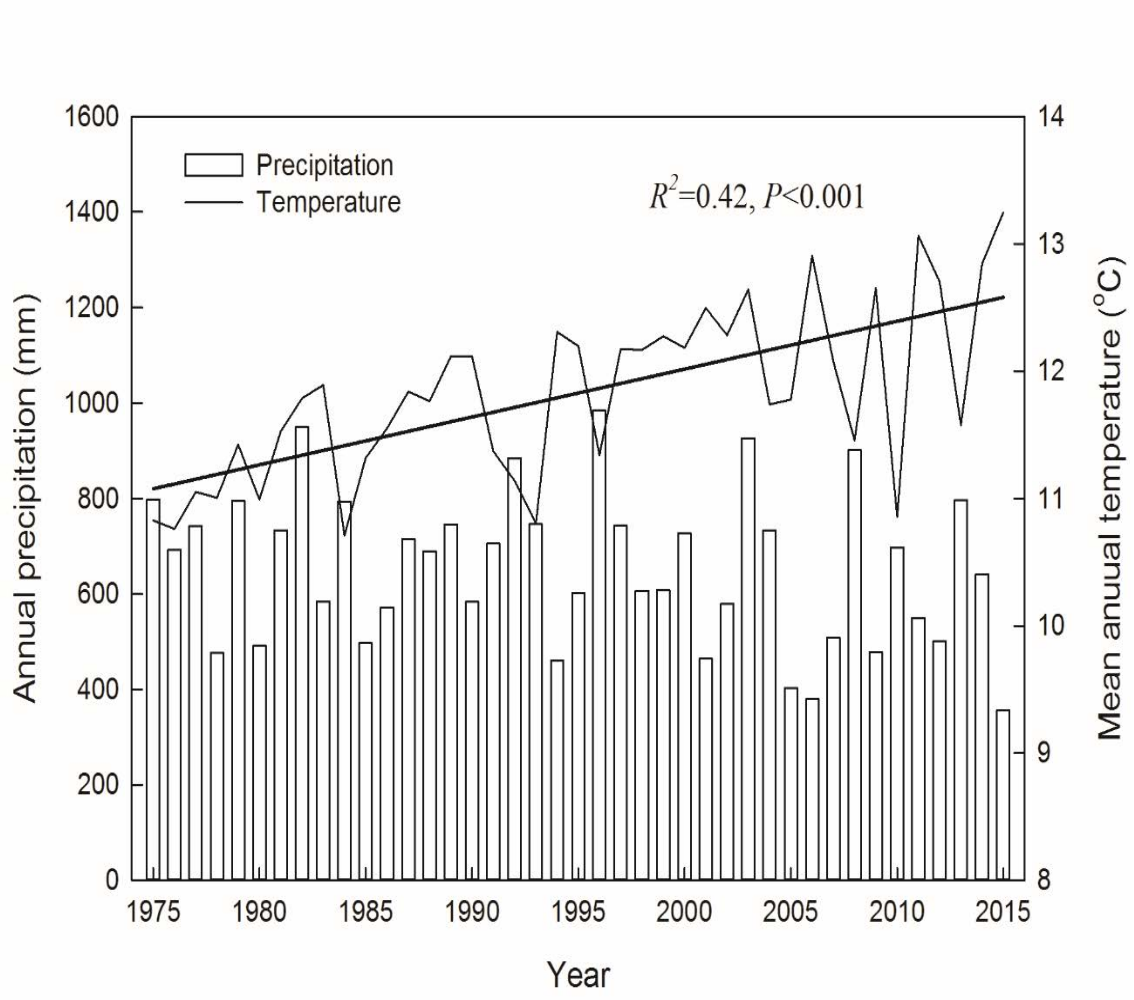


Fig. S1 Annual precipitation and mean annual temperature and during 1975-2015.

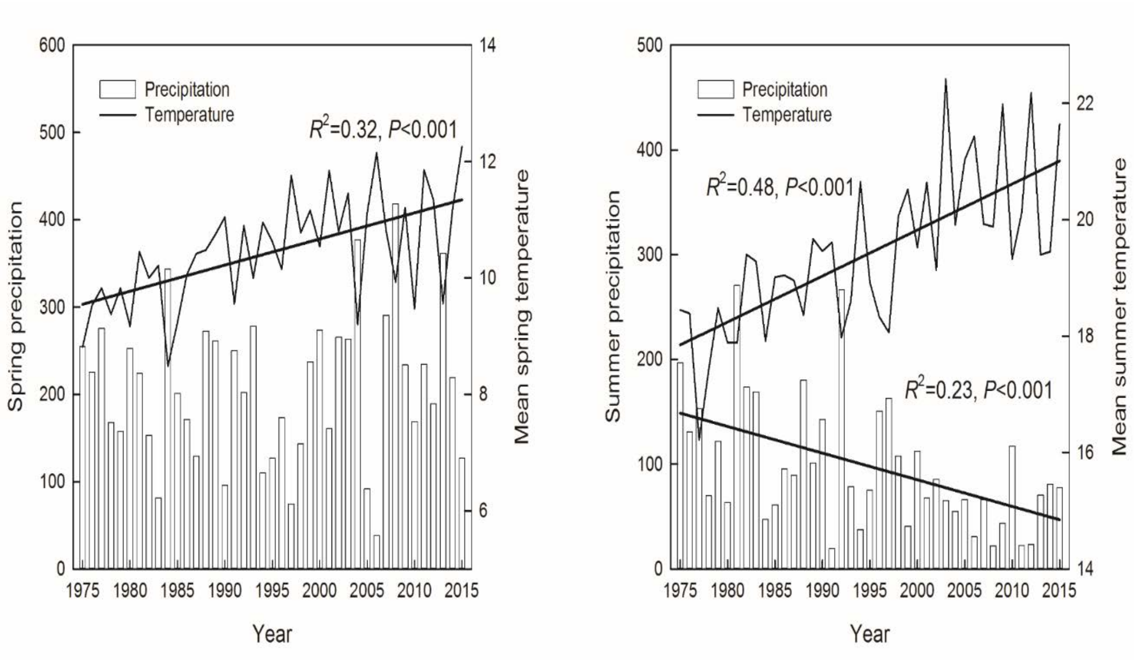


Fig. S2 Average spring and summer precipitation and temperature changes during 1975-2015.

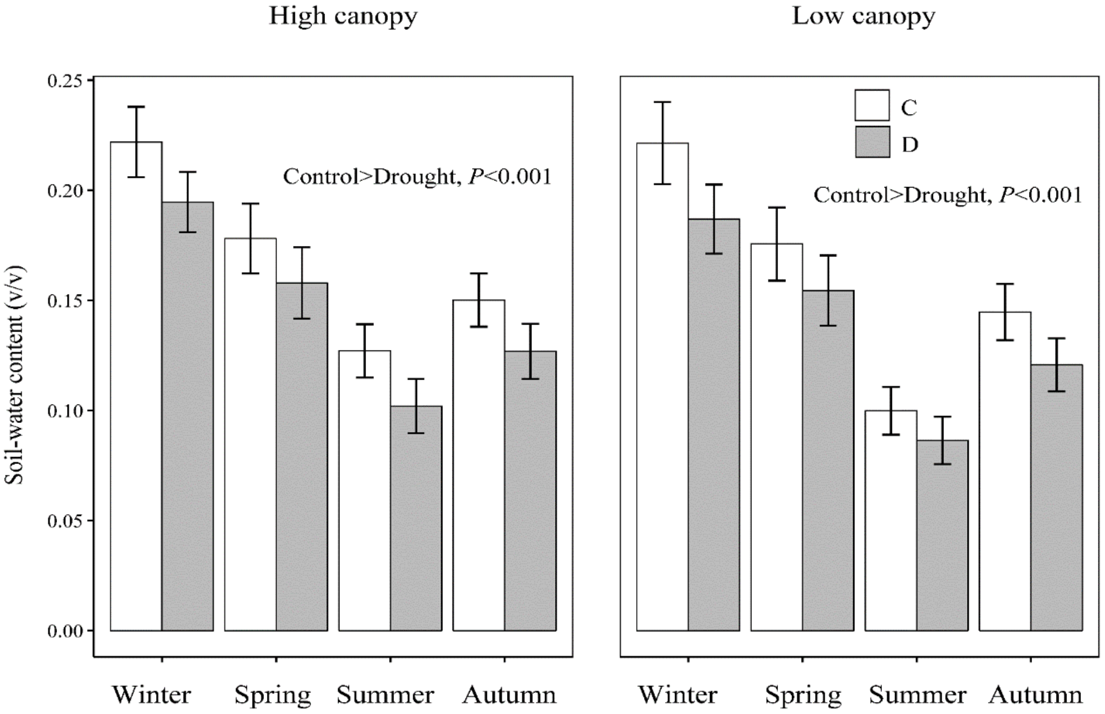


Fig. S3 Seasonal soil-water content responses to drought treatment in H- and L-canopies.

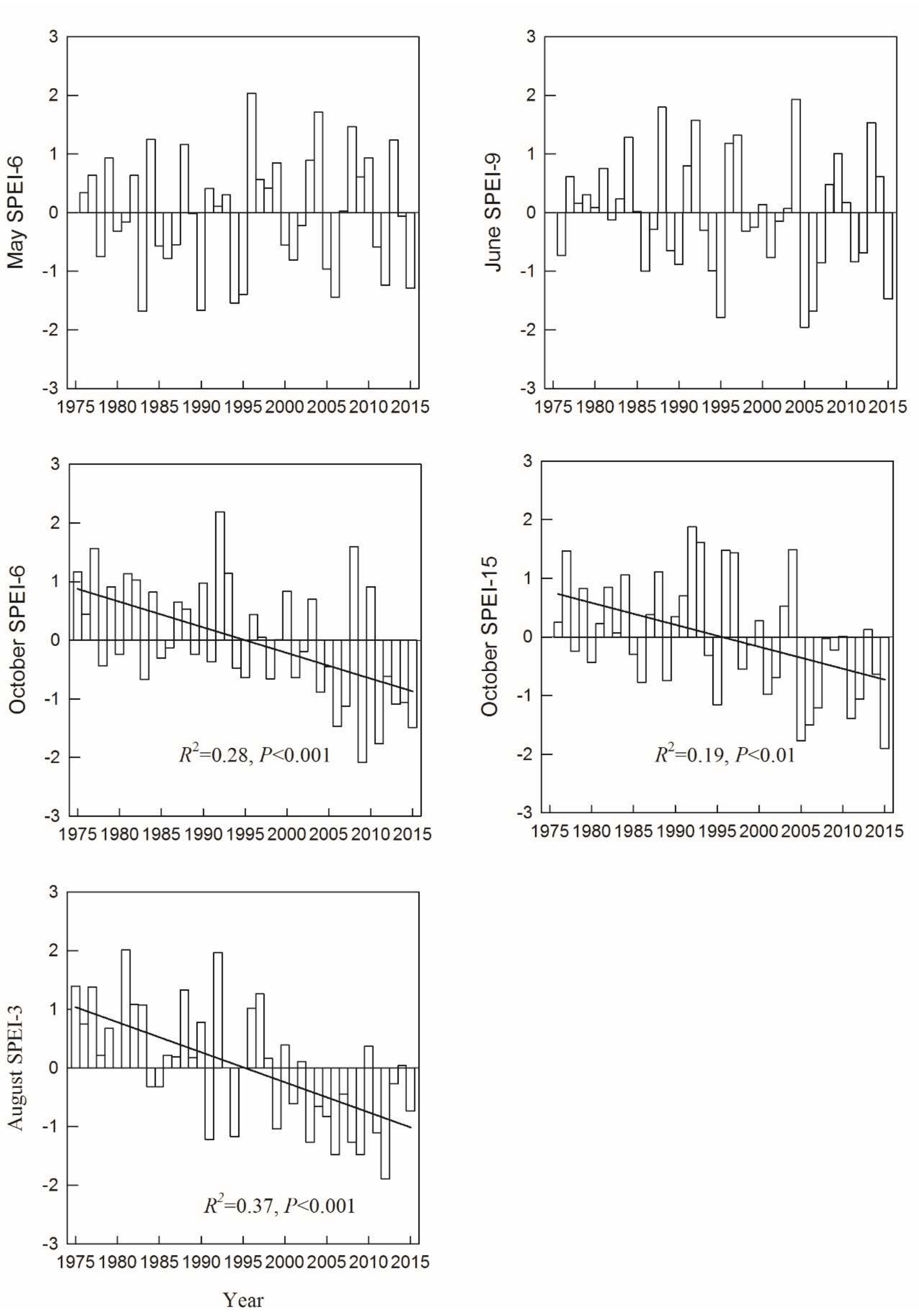


Fig. S4 Water deficits of winter-spring (May SPEI-6 and June SPEI-9), summer (August SPEI-3) and summer-autumn (October SPEI-9 and October SPEI-15) during 1975-2015.

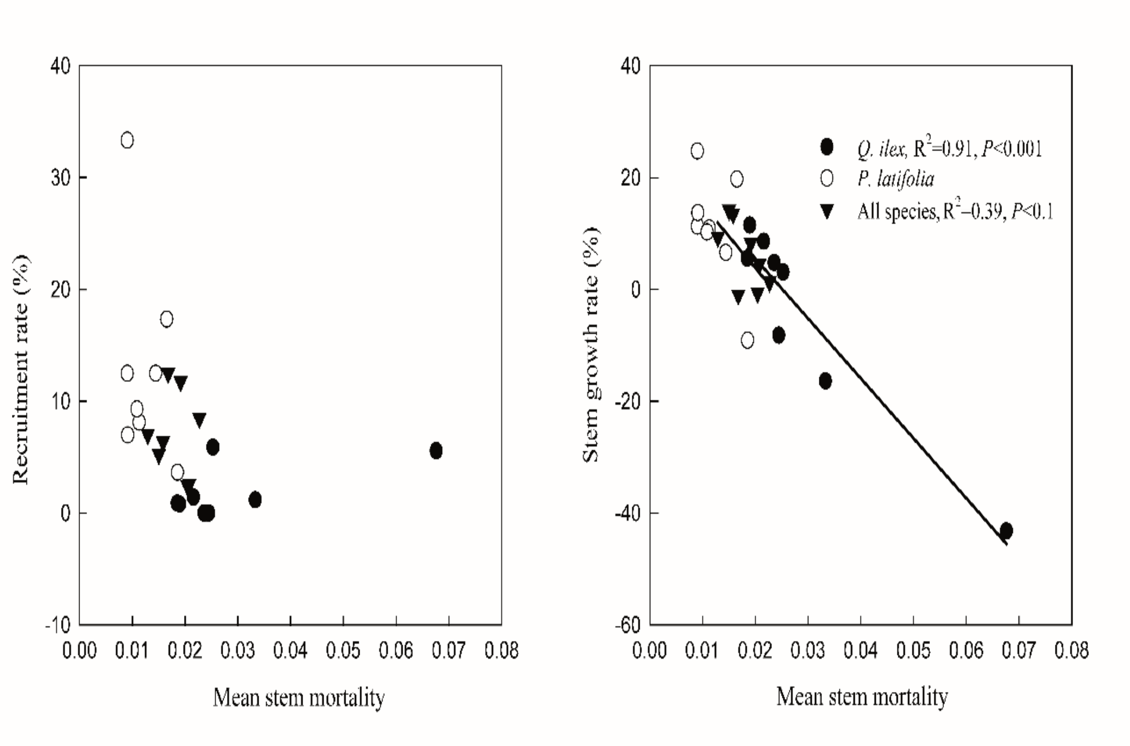


Fig. S5 Relationships between mean mortality rate, recruitment and stem growth over the study period of 1999-2015.

Chapter 5 Species selection under long-term experimental warming and drought explained by climatic distributions

Appendix S1 Cumulative Group Analysis: CNG robustness testing

To test the robustness of the CNGs we followed the protocol set out in Bilton et al. (2016) and performed Cumulative Group Analysis. This methodology adds a species one at a time in niche value rank order to form a group and performs the basic statistical test highlighted in the main article (LMEM to test change in group response slope to treatment over time compared to control). Therefore the first "group" tested only contains one species, and each species is added in turn (2nd test on two species; 3rd on three species) until the final test is on a group containing all species in the community (31 species) and is synonymous with the total community tests described in the main article (Figure 1; Table 1). For each climatic niche parameter, species were accumulated from both the dry/warm end of the gradient (where we predicted a more positive/less negative response of a CNG) and from the wet/cool end of the gradient (where we predicted a more negative response of a CNG).

Interpretation of these robustness tests can be slightly difficult, but a successful grouping approach would be shown by a stronger pattern as more species are added. Patterns should also remain strong for a number of species, and therefore confirm that exact group boundaries do not have a great impact on the qualitative results. If the pattern changes rapidly due to the addition of one species and particularly if it fluctuates over the course of a few species, this would suggest that this particular rank order of species is not ideal for consistently describing community response under the manipulations.

Shown in Supplementary figures S2-S4 are some of the results from the Cumulative Group Analyses (response value shown is the t-value of difference between treatment and control slope over time). 2

Also of interest is the method used to calculate species climatic niche value. When the

25 percentile methodology was applied, this method appears to show clearer patterns for describing accumulated response at the lower end of the gradient (cool/dry first), and less so from the higher end of the gradient (warm/wet first). Whereas when the 75 percentile methodology was applied, this describes patterns better for the higher end of the niche gradient (warm/wet first). Under drought manipulation, some species in the community responded positively to drought compared to control, whereas some responded negatively. Taking the median value of the climatic values associated to the distribution observations therefore seems to be the strongest way to capture the whole community response in such a scenario. The cumulative patterns in Figure S3 agree with this, showing intermediate patterns to the 25 and 75 percentile grouping methods. Under warming, while some species, and therefore groups of species, did increase, patterns were rarely significant. The general pattern was that the warmer/drier species did not increase much over the course of the experiment, resulting in the non-significant responses given for CNGs (Table S4) and in the cumulative analyses (Figure S2). However, the decrease over time noted in the total community analyses (Figure 1; Table 1), can be explained by a decrease in those species with cooler/wetter niches. T-values close lower than -5 (highly significant, and importantly much lower than the total community) were often produced (Figure S2). Therefore, throughout the main article, to describe community change we chose to display the 25 percentile summer temperature groups, as this part of the niche distribution describes the negative response to warming to best effect.

Finally, in figure S4, we highlight that when summer temperature or spring precipitation was used to describe the community composition change under the opposing treatments (precipitation niche under warming; temperature niche under drought), the Cumulative patterns fluctuated to a much greater extent than when describing the treatments directly associated with their niche parameters (Figure S2; S3). While there was clearly a correlation between the niche axes for species in Spain, this suggests that some species exist in wet/warm, or cool/dry locations and therefore possess different adaptations to temperature or precipitation, which were reflected in their response to their abundance change under the two treatments.

Appendix S2 Discussion of niche parameter estimation for forming CNGs.

The use of CNGs to observe, follow, and predict community dynamics under climate manipulation experiments/ climate change scenarios has proved to be a simple and robust technique to extract general and interpretable trends in community dynamics. The use of many different climate parameters often showed similar qualitative results (Table S4) to the main climate parameters presented here (Summer Temperature and Spring Precipitation). In addition, the Cumulative Group Analysis revealed that the conclusions rarely altered due to where the group boundaries were set. Also, by plotting single species responses along a niche axis we revealed very similar conclusions about which species – in terms of niche distribution - would increase or decrease in abundance in response to the manipulations. While extremely valid to confirm trends, care must be taken in extrapolating too far the results simply from the niche axis patterns. As seen for the total abundance responses, single species response patterns are unlikely to always be linear, so some level of variation is to be expected when plotting treatment slope differences. By grouping species by climatic niche – and therefore by categorizing a number of species by similar expected response - patterns also appear to be stronger in general, potentially capturing natural variation in space and time, but also allowing for some error in niche distribution estimation, and have added value over single species and total community responses, by being easy to interpret over the timescale of the experiment. Of interest is perhaps that an average niche value, whether defined by mean or median, has proved to be a valid way to capture the sensitivities of species to climate change factors. In general. we have shown that the lower edge of the climatic range distribution was slightly better to define response of the lower value species to the manipulations (hence the decision to display the results when the niche is defined by the 25% summer temperature range in the main article for warming response, as it was generally only the cooler species that changed in this study). The higher edge of the range (75 percentile – see supplementary material Figures S1, S2, S4) proved to be generally better for observing responses of the higher value species. However, while niche range would be extremely valuable to consider across large areas, within a community it would seem that an average value captures well both potential increasers and decreasers under the manipulations. A valid next step could indeed be to test simple or more complex BEM/SDM predictions of species responses, which potentially has the possibility of defining niche range more clearly, but then also suffers from an inability to define which climate traits are responding (for example whether species are generally from dry or warm regions).

Table S1 List of all species identified throughout the experiment, with their corresponding climatic niche values. Niche values derived from distribution observations acquired from Global Biodiversity Information Facility (GBIF) (#Obser. = number reported in search area) overlaying climate data from the WorldClim database. Four climate parameters were tested: Mean Annual Temperature (°C); Summer Temperature (mean from June to August) (°C); Annual Precipitation (mm); and Spring Precipitation (sum from December to May)(mm). For each climate parameter, niche values were calculated in three different ways: median (med) of climatic values associated with each observation; the lower 25 percentile of the climatic values (25); or the higher 25 percentile of climatic values (75). Species are ranked in order of median Spring Precipitation niche values. Highlighted in green are the two Climatic niche parameters shown throughout the main article (25% Summer Temperature; Median Spring Precipitation).

				Mean Annual Temp.			Summer Temp.			Annual Precip.			Spring Precip.		
Genus	Species	Family	#Obser.	25	med.	75	25	med.	75	25	med.	75	25	med.	75
Helianthemum	*syriacum*	Cistaceae	1868	13.7	14.9	16.4	21.6	22.7	23.6	386	449	537	202	233	284
Globularia	*alypum*	Globulariaceae	3690	13.6	14.7	15.7	21.3	22.4	23.1	440	490	602	212	242	294
Polygala	*rupestris*	Polygalaceae	1767	13.3	14.6	16.2	21.3	22.4	23.4	430	498	577	217	258	318
Bupleurum	*fruticescens*	Umbelliferae	3846	12.1	13.5	14.6	20.1	21.4	22.5	448	501	576	230	262	301
Erica	*multiflora*	Ericaceae	3953	13.3	14.3	15.4	20.9	21.9	22.7	463	537	641	226	263	310
Hyparrhenia	*hirta*	Poaceae	2379	14.8	16.2	17.2	22.3	23.3	24.1	415	519	621	213	283	394
Fumana	*thymifolia*	Cistaceae	3234	13.1	14.6	16.3	21.1	22.4	23.5	429	519	632	224	283	378
Juniperus	*oxycedrus*	Cupressaceae	13224	11.7	13.0	14.4	19.9	21.2	22.4	463	521	623	244	288	368
Euphorbia	*serrata*	Euphorbiaceae	3074	11.9	13.4	14.9	19.7	21.3	22.8	448	553	707	241	292	374
Rosmarinus	*officinalis*	Lamiaceae	25742	12.5	13.8	15.3	20.6	21.9	23.3	456	513	615	244	292	348
Lithodora	*fruticosa*	Boraginaceae	2130	11.5	13.0	14.3	19.4	21.0	22.3	466	550	697	247	293	379
Helichrysum	*stoechas*	Asteraceae	7041	11.6	13.4	15.6	19.9	21.6	23.4	456	519	606	247	294	369
Coris	*monspeliensis*	Primulaceae	3413	11.7	13.3	15.2	19.3	21.2	22.8	454	552	726	240	296	386
Dorycnium	*pentaphyllum*	Fabaceae	10340	11.2	12.5	14.1	18.9	20.4	21.9	473	573	749	250	300	383
Quercus	*coccifera*	Fagaceae	19283	12.6	14.0	15.5	20.6	21.9	23.2	466	548	657	246	301	381
Fumana	*ericoides*	Cistaceae	2900	11.9	13.4	15.4	19.6	21.3	22.9	438	552	755	225	302	395
Ononis	*minutissima*	Fabaceae	2063	11.8	13.3	14.9	19.3	21.0	22.3	507	626	766	251	316	390
Brachypodium	*phoenicoides*	Poaceae	3672	12.6	13.9	15.4	20.7	21.9	23.1	468	571	677	260	341	416
Pistacia	*lentiscus*	Anacardiaceae	16280	14.5	15.7	16.8	22.0	23.1	24.6	484	579	655	271	344	420
Eryngium	*campestre*	Umbelliferae	12138	10.4	11.1	13.4	17.8	18.5	21.4	565	656	749	304	344	393
Argyrolobium	*zanonii*	Fabaceae	3427	11.1	12.6	14.2	18.6	20.4	22.0	513	642	805	267	348	418
Petrosedum	*sediforme*	Crassulaceae	4316	10.5	12.8	14.6	18.0	20.3	22.1	510	652	816	263	350	427
Odontites	*lutea*	Scrophulariaceae	4181	10.0	10.7	11.1	17.2	17.9	18.4	649	712	811	315	353	400
Teucrium	*polium*	Camedris	2199	10.4	12.4	14.0	18.0	20.2	21.8	541	674	783	288	359	415
Aphyllanthes	*monspeliensis*	Asparagaceae	3781	10.6	12.2	13.7	18.0	20.0	21.5	525	683	802	275	359	424
Ulex	*parviflorus*	Fabaceae	7128	13.5	15.2	16.4	21.5	22.8	23.7	500	568	628	279	361	407
Galium	*lucidum*	Rubiaceae	3891	9.7	11.8	13.7	17.3	19.5	21.4	535	719	861	272	365	443
Asperula	*cynanchica*	Rubiaceae	5385	9.6	10.6	11.8	17.0	18.0	19.0	666	758	873	324	370	439
Bituminaria	*bituminosa*	Fabaceae	3411	12.0	13.6	15.5	19.5	21.3	22.9	536	663	782	288	371	419
Cuscuta	*epithymum*	Convolvulaceae	3623	9.6	11.1	13.2	16.7	18.2	20.8	586	739	899	299	371	462
Rubia	*peregrina*	Rubiaceae	9936	11.2	12.9	15.1	18.3	20.3	22.7	563	684	797	320	383	447

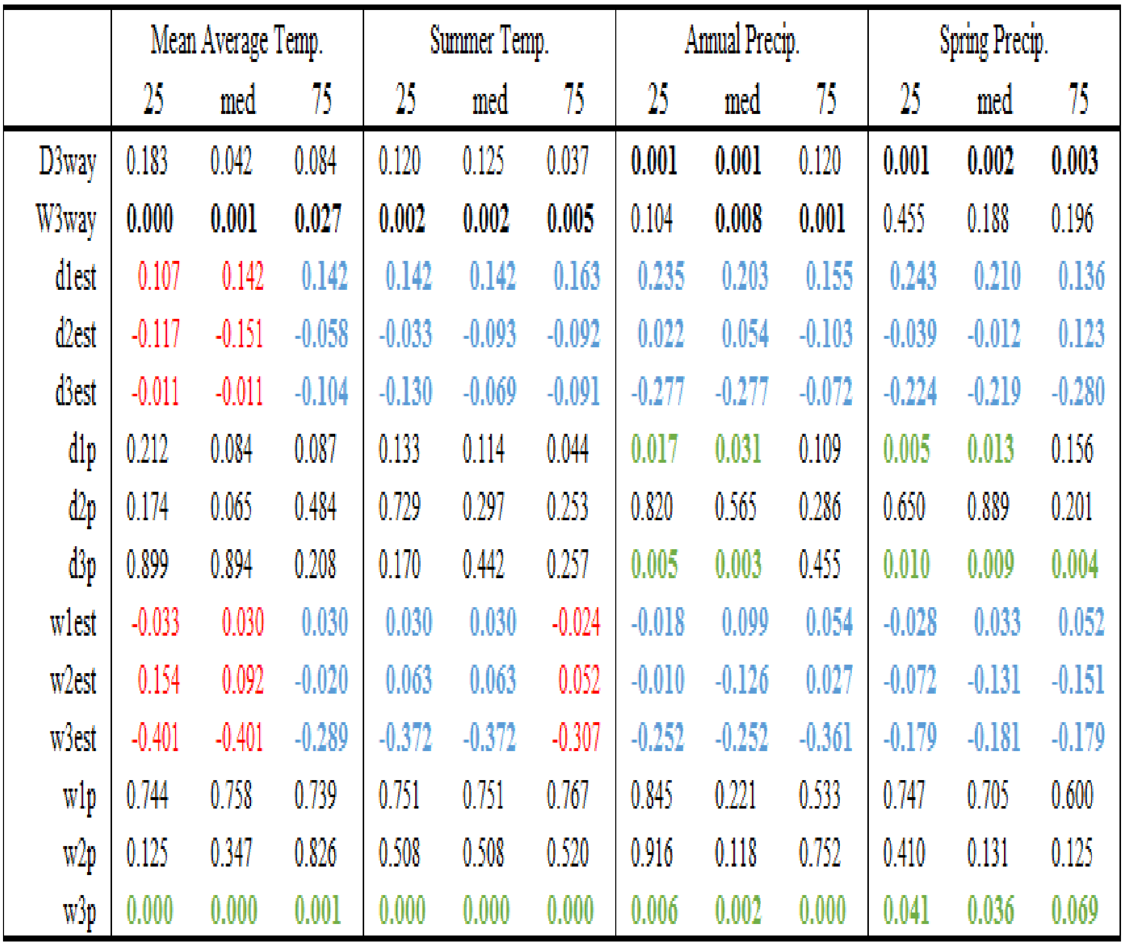
Table S2 Climatic Niche Groups (CNGs) assigned to each species in the experiment for the different climate parameter values tested (Mena Annual Temperature; Summer Temperature (mean from June to August); Annual Precipitation; Spring Precipitation (sum from December to May)), and the three ways pf calculating the niche (25 percentile, Median, 75 percentile). Also displayed are the mean community percentages that the species contributed across the whole experiment: Community Original is the normal percentage of point hits; Percentage Log are the percentage values calculated from the sum of the log hits of each species. In all cases CNG1 represents the more arid group, therefore for temperature CNGs they ranked from CNG1 (warmer) to CNG3 (cooler); and precipitation CNGS are ranked from CNG1 (drier) to CNG3 (wetter). Species are ranked in order of median Spring Precipitation niche values. Highlighted in green are the two Climatic Niche Groups shown throughout the main article (25% Summer Temperature; Median Spring Precipitation).

		Community Percentage		Mean Annual Temp.			Summer Temp.			Annual Precip.			Spring Precip.		
Genus	Species	Original	Log	25	med.	75	25	med.	75	25	med.	75	25	med.	75
Helianthemum	*syriacum*	0.794	3.32	1	1	1	1	1	1	1	1	1	1	1	1
Globularia	*alypum*	32.975	15.15	1	1	1	1	1	2	1	1	1	1	1	1
Polygala	*rupestris*	0.336	2.17	1	1	1	1	1	1	1	1	1	1	1	1
Bupleurum	*fruticescens*	0.015	0.15	2	2	3	2	2	3	1	1	1	2	1	1
Erica	*multiflora*	20.415	13.48	1	2	2	2	2	3	2	2	2	1	1	1
Hyparrhenia	*hirta*	0.076	0.21	1	1	1	1	1	1	1	1	1	1	1	2
Fumana	*thymifolia*	2.812	8.14	2	1	1	1	1	1	1	1	2	1	1	2
Juniperus	*oxycedrus*	0.619	0.88	3	3	3	2	3	3	2	2	2	2	2	1
Euphorbia	*serrata*	0.067	0.54	3	3	3	2	2	3	1	2	3	2	2	2
Rosmarinus	*officinalis*	8.318	7.43	2	2	2	2	2	2	2	1	1	2	2	1
Lithodora	*fruticosa*	0.151	0.62	3	3	3	3	3	3	2	2	2	2	2	2
Helichrysum	*stoechas*	0.466	2.23	3	3	2	2	2	1	2	1	1	2	2	2
Coris	*monspeliensis*	0.062	0.48	3	3	3	3	3	3	2	2	3	2	2	2
Dorycnium	*pentaphyllum*	8.675	10.53	3	3	3	3	3	3	3	3	3	2	2	2
Quercus	*coccifera*	0.359	1.03	2	2	2	2	2	2	2	2	2	2	2	2
Fumana	*ericoides*	3.000	7.55	3	3	2	3	3	2	1	2	3	1	2	2
Ononis	*minutissima*	0.006	0.05	3	3	3	3	3	3	3	3	3	2	3	2
Brachypodium	*phoenicoides*	5.184	8.12	2	2	2	2	2	2	3	3	2	3	3	3
Pistacia	*lentiscus*	3.264	5.81	1	1	1	1	1	1	3	3	2	3	3	3
Eryngium	*campestre*	0.105	0.59	3	3	3	3	3	3	3	3	3	3	3	2
Argyrolobium	*zanonii*	0.058	0.58	3	3	3	3	3	3	3	3	3	3	3	3
Petrosedum	*sediforme*	0.003	0.03	3	3	3	3	3	3	3	3	3	3	3	3
Odontites	*lutea*	0.374	1.56	3	3	3	3	3	3	3	3	3	3	3	2
Teucrium	*polium*	0.023	0.18	3	3	3	3	3	3	3	3	3	3	3	3
Aphyllanthes	*monspeliensis*	0.092	0.25	3	3	3	3	3	3	3	3	3	3	3	3
Ulex	*parviflorus*	11.167	5.32	1	1	1	1	1	1	3	3	2	3	3	3
Galium	*lucidum*	0.101	0.77	3	3	3	3	3	3	3	3	3	3	3	3
Asperula	*cynanchica*	0.068	0.37	3	3	3	3	3	3	3	3	3	3	3	3
Bituminaria	*bituminosa*	0.094	0.68	2	2	2	3	3	2	3	3	3	3	3	3
Cuscuta	*epithymum*	0.002	0.03	3	3	3	3	3	3	3	3	3	3	3	3
Rubia	*peregrina*	0.319	1.76	3	3	3	3	3	3	3	3	3	3	3	3

Table S3 MetaData describing the Climatic Niche Groups (CNGs) tested. Displayed are the number of species assigned to each group, for each climate parameter and niche value method tested. In general, the target was to include a minimum of 6 species per group, of which the sum was approximately 33% of the log community percentage. Displayed are therefore the log community percentage and the normal community percentage values for each CNG calculated from log hits or hits respectively. Displayed also are the boundary values and ranges of the climatic niches captured by the CNGs. N.B. ranges of outer CNGs are often larger due to 1 or 2 outlying species, or particularly when more species are included (CNG3 throughout). Highlighted in green are the two Climatic Niche Groups shown throughout the main article (25% Summer Temperature; Median Spring Precipitation).

		Mean Annual Temp.			Summer Temp.			Annual Precip.			Spring Precip.		
		25	med.	75	25	med.	75	25	med.	75	25	med.	75
Species Number	CNG1	7	7	7	7	7	8	8	8	7	6	7	6
	CNG2	6	6	7	8	7	6	8	8	8	10	9	12
	CNG3	18	18	17	16	17	17	15	15	16	15	15	13
Log Comm. Perc.	CNG1	45.5	40.1	40.1	40.1	40.1	34.6	37.2	38.8	30.7	36.5	42.6	41.7
	CNG2	25.5	30.9	40.5	33.9	33.0	33.5	34.3	29.9	43.4	37.4	31.3	32.8
	CNG3	29.0	29.0	19.4	26.0	26.9	31.8	28.5	31.3	25.9	26.1	26.1	25.4
Comm. Perc.	CNG1	69.03	51.42	51.42	51.42	51.42	27.23	40	46	43	40	57	63
	CNG2	16.78	34.38	37.84	35.44	34.82	41.74	36	36	44	39	22	16
	CNG3	14.19	14.19	10.74	13.13	13.75	31.03	24	18	13	21	21	21
		(°C)			(°C)			(mm)			(mm)		
Group Boundaries	CNG1	14.80	16.20	17.20	22.27	23.30	24.63	386	449	537	202	233	284
	CNG1-2	13.20	14.45	15.65	21.00	22.15	23.35	451	520	622	228	286	369
	CNG2-3	11.95	13.45	15.25	19.65	21.32	22.82	467	561	702	256	309	404
	CNG3	9.60	10.60	11.10	16.70	17.87	18.37	666	758	899	324	383	462
Group Niche Range	CNG1	1.60	1.75	1.55	1.27	1.15	1.28	65	71	85	26	53	85
	CNG2	1.25	1.00	0.40	1.35	0.83	0.53	16	41	80	28	24	35
	CNG3	2.35	2.85	4.15	2.95	3.45	4.45	199	198	197	69	74	59

Table S4 Statistical test results for the different CNGs tested. In each case, the protocol followed that described in the main text, using Linear Mixed Effects Models (LMEM) to test the change in CNG log-abundance over time in the manipulation treatment compared to control. Therefore, displayed in the table are the p-values of 3-way interactions of interest (Year x Treatment x CNG), set-up to test the main hypothesis. Treatments were tested separately, D3way is the CNG response under drought, and W3way is the CNG response under warming compared to control. Also shown are the differences in individual slope estimates (est) and associated p-values (p) for the difference between control and treatment of the different CNGs: d1-d3 (CNG1 to CNG3 response under drought); w1-w3 (CNG1 to CNG3 response under warming). Significant 3-way interactions are highlighted in bold. For estimates, blue indicates a continuous pattern of change across groups, whereas red indicates slope estimate differences are not in a hierarchical order as would be predicted. Significant p-values for group treatment response slope differences are indicated in green, but are not corrected for multiple testing. Note that in general, most methods highlighted a statistically significant decrease of cool or wet species under warming. And that temperature CNGs regularly failed to explain changes in community composition under drought. Annual Precipitation CNGs sometimes managed to capture community composition change under both manipulation treatments, whereas Spring Precipitation CNGs were generally stronger for drought response but did not significantly describe changes under warming, despite revealing hierarchical responses among CNGs as predicted (i.e. drier CNG1 responded less negatively to warming than the wetter CNG3).



	Mean Average Temp.			Summer Temp.			Annual Precip.			Spring Precip.		
	25	med	75	25	med	75	25	med	75	25	med	75
D3way	0.183	0.042	0.084	0.120	0.125	0.037	**0.001**	**0.001**	0.120	**0.001**	**0.002**	**0.003**
W3way	**0.000**	**0.001**	**0.027**	**0.002**	**0.002**	**0.005**	0.104	**0.008**	**0.001**	0.455	0.188	0.196
d1est	0.107	0.142	0.142	0.142	0.142	0.163	0.235	0.203	0.155	0.243	0.210	0.136
d2est	-0.117	-0.151	-0.058	-0.033	-0.093	-0.092	0.022	0.054	-0.103	-0.039	-0.012	0.123
d3est	-0.011	-0.011	-0.104	-0.130	-0.069	-0.091	-0.277	-0.277	-0.072	-0.224	-0.219	-0.280
d1p	0.212	0.084	0.087	0.133	0.114	0.044	0.017	0.031	0.109	0.005	0.013	0.156
d2p	0.174	0.065	0.484	0.729	0.297	0.253	0.820	0.565	0.286	0.650	0.889	0.201
d3p	0.899	0.894	0.208	0.170	0.442	0.257	0.005	0.003	0.455	0.010	0.009	0.004
w1est	-0.033	0.030	0.030	0.030	0.030	-0.024	-0.018	0.099	0.054	-0.028	0.033	0.052
w2est	0.154	0.092	-0.020	0.063	0.063	0.052	-0.010	-0.126	0.027	-0.072	-0.131	-0.151
w3est	-0.401	-0.401	-0.289	-0.372	-0.372	-0.307	-0.252	-0.252	-0.361	-0.179	-0.181	-0.179
w1p	0.744	0.758	0.739	0.751	0.751	0.767	0.845	0.221	0.533	0.747	0.705	0.600
w2p	0.125	0.347	0.826	0.508	0.508	0.520	0.916	0.118	0.752	0.410	0.131	0.125
w3p	0.000	0.000	0.001	0.000	0.000	0.000	0.006	0.002	0.000	0.041	0.036	0.069

Table S5 Statistical test results for single species responses to 16 years of climatic manipulation. Linear Mixed-Effects Models were used where possible to analyse each species recorded within the experiment. Displayed in the table are the coefficient estimates for the difference in slope response over time between control and manipulation treatment (warming or drought), the associated t-value of that hypothesis test, and the statistical significance ("ns" = p>0.1; "." = p<0.1; "*" = p<0.05; "**" = p<0.01; "***" = p<0.01). Species are ranked in order of median Spring Precipitation niche values. N.B. In general, most species that responded significantly positively under the manipulation treatments are near the dry end of the table (top), whereas most species that responded significantly negatively under the manipulation treatments are near the wet end of the table (bottom).

		Warming			Drought			Log
Genus	Species	Est.	t-value	Sig.	Est.	t-value	Sig.	Comm. %
Helianthemum	*syriacum*	-0.010	-0.216	ns	0.017	0.403	ns	3.32
Globularia	*alypum*	0.003	0.211	ns	**0.039**	**3.427**	***	15.15
Polygala	*rupestris*	-0.002	-0.068	ns	**0.071**	**2.378**	*	2.17
Bupleurum	*fruticescens*	*NA*	*NA*		*NA*	*NA*		0.15
Erica	*multiflora*	-0.018	-1.582	ns	0.013	1.024	ns	13.48
Hyparrhenia	*hirta*	NA	NA		*NA*	*NA*		0.21
Fumana	*thymifolia*	**0.045**	**2.545**	*	**0.048**	**2.537**	*	8.14
Juniperus	*oxycedrus*	0.061	1.958	.	*NA*	*NA*		0.88
Rosmarinus	*officinalis*	**0.099**	**3.517**	***	**-0.055**	**-1.952**	.	0.54
Euphorbia	*serrata*	0.013	0.540	ns	0.015	1.042	ns	7.43
Lithodora	*fruticosa*	0.002	0.532	ns	**-0.042**	**-2.415**	*	0.62
Helichrysum	*stoechas*	-0.051	-1.283	ns	0.061	1.560	ns	2.23
Coris	*monspeliensis*	-0.023	-1.239	ns	0.005	0.266	ns	0.48
Dorycnium	*pentaphyllum*	**-0.071**	**-2.205**	*	**-0.058**	**-1.915**	.	10.53
Quercus	*coccifera*	-0.018	-0.793	ns	-0.019	-0.826	ns	1.03
Fumana	*ericoides*	**-0.081**	**-2.954**	**	0.022	0.745	ns	7.55
Ononis	*minutissima*	NA	NA		*NA*	*NA*		0.05
Brachypodium	*phoenicoides*	0.058	1.111	ns	**-0.097**	**-1.744**	.	8.12
Eryngium	*campestre*	0.002	0.191	ns	0.007	0.484	ns	5.81
Pistacia	*lentiscus*	**-0.049**	**-1.887**	.	-0.001	-0.036	ns	0.59
Argyrolobium	*zanonii*	-0.010	-0.582	ns	0.010	0.642	ns	0.58
Petrosedum	*sediforme*	*NA*	*NA*		*NA*	*NA*		0.03
Odontites	*lutea*	-0.002	-0.050	ns	0.049	1.593	ns	1.56
Aphyllanthes	*monspeliensis*	*NA*	*NA*		*NA*	*NA*		0.18
Teucrium	*polium*	*NA*	*NA*		*NA*	*NA*		0.25
Ulex	*parviflorus*	0.008	0.298	ns	**-0.065**	**-2.739**	**	5.32
Galium	*lucidum*	**-0.080**	**-3.796**	***	**-0.083**	**-4.068**	***	0.77
Asperula	*cynanchica*	-0.018	-1.432	ns	0.002	0.141	ns	0.37
Bituminaria	*bituminosa*	-0.009	-0.510	ns	0.018	0.988	ns	0.68
Cuscuta	*epithymum*	*NA*	*NA*		*NA*	*NA*		0.03
Rubia	*peregrina*	**-0.085**	**-4.577**	***	**-0.037**	**-2.253**	*	1.76

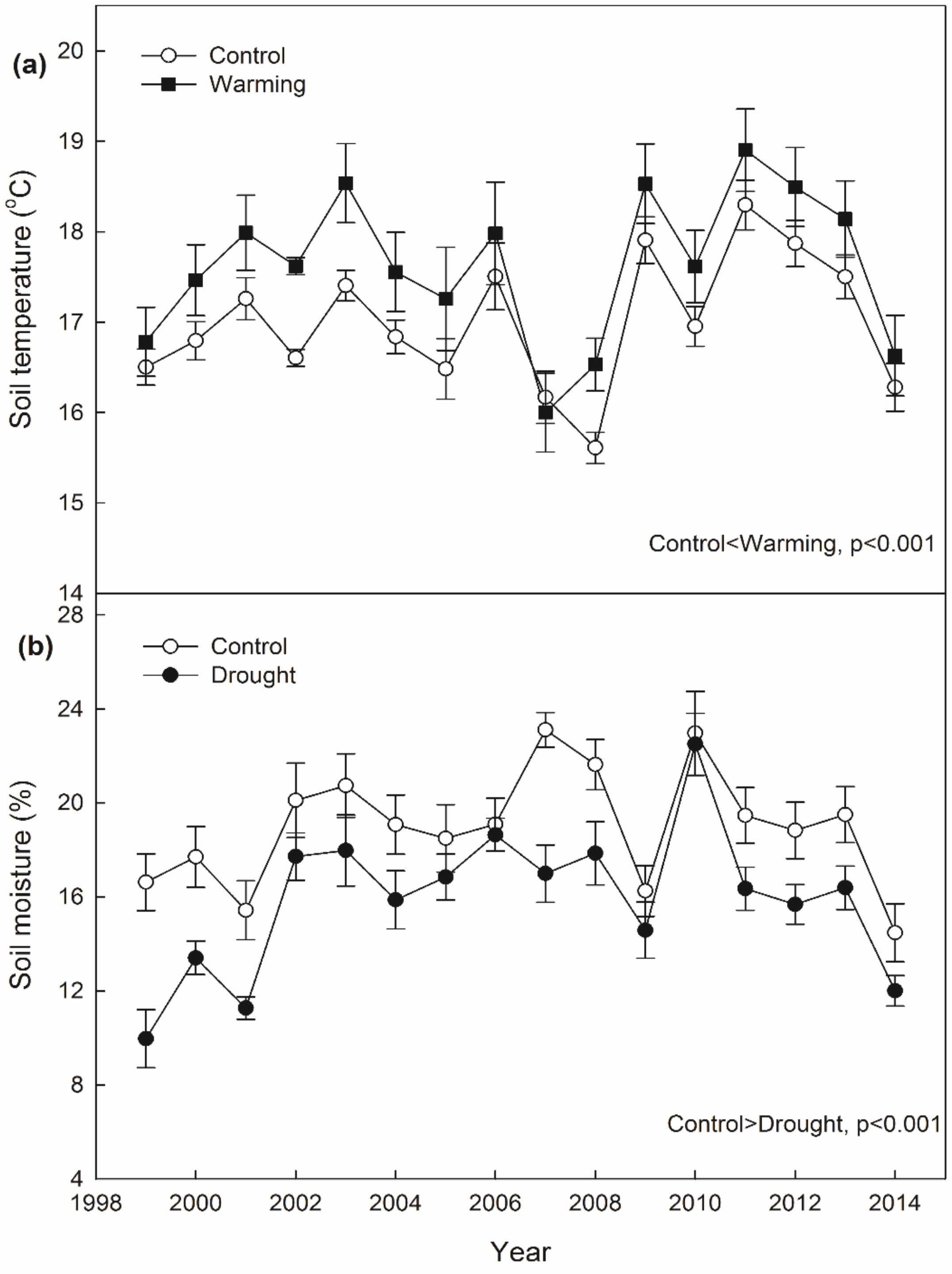


Fig. S1 The soil temperature was significantly increased by warming treatment throughout the study period of 1999-2014 when compared to control (anova, $p<0.001$). Soil moisture was significantly decreased by drought treatment throughout the study period of 1999-2014 (anova, $p<0.001$). During 2011-2013. The vertical bars represent the standard errors of the mean (n=3 plots). Soil temperature and moisture data during 2011-2013 were estimated by the regression of treatment effects in previous year because the sensors were destroyed by lightning strikes.

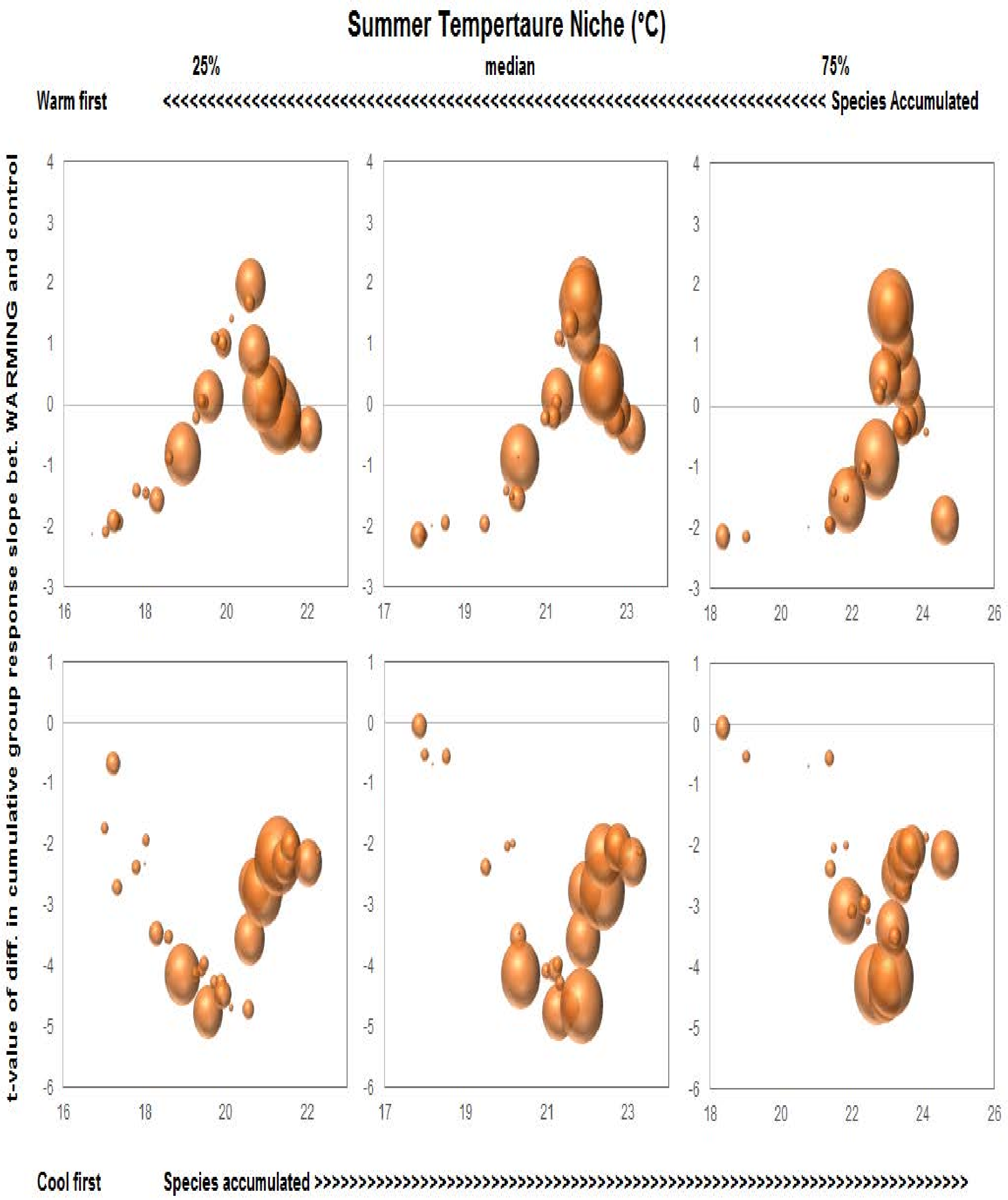


Fig. S2 Warming Cumulative Group Analyses for species groups defined by summer temperature niches in response to manipulated warming. Three methods of niche value were tested: median of climatic values associated with each species observation; the lower 25 percentile of the climatic values (25%); or the upper 25 percentile of climatic values (75%). Upper graphs display groups tested when accumulated from the warm end of the gradient (warm first: right to left); Lower graphs display groups tested when accumulated from the cooler end of the gradient (Cool first: left to right). The x-axis displays the climatic niche value associated to the species added to the cumulative group. The y-axis displays the t-value for the difference in estimated slopes over time for point quadrat hit abundances in control compared to warming (calculated using a Linear Mixed Effect Model). Bubble size is proportional to the log-community percentage of the species added to the group.

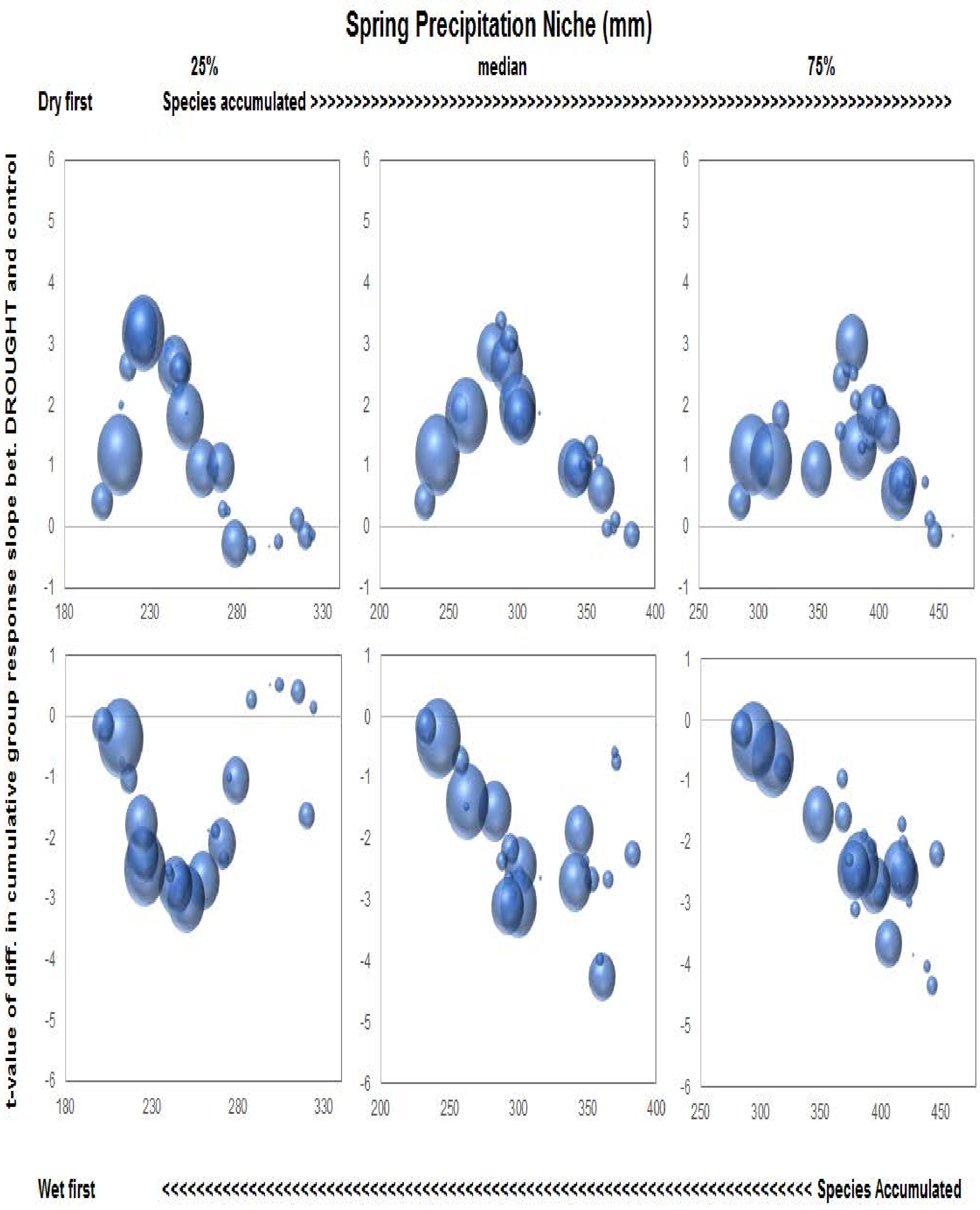


Fig. S3 Drought Cumulative Group Analyses for species groups defined by spring precipitation niches in response to manipulated drought. Three methods of niche value were tested: median of climatic values associated with each species observation; the lower 25 percentile of the climatic values (25%); or the upper 25 percentile of climatic values (75%). Upper graphs display groups tested when accumulated from the dry end of the gradient (dry first: left to right); Lower graphs display groups tested when accumulated from the wetter end of the gradient (Wet first: right to left). The x-axis displays the climatic niche value associated to the species added to the cumulative group. The y-axis displays the t-value for the difference in estimated slopes over time for point quadrat hit abundances in control compared to drought (calculated using a Linear Mixed Effect Model). Bubble size is proportional to the log-community percentage of the species added to the group.

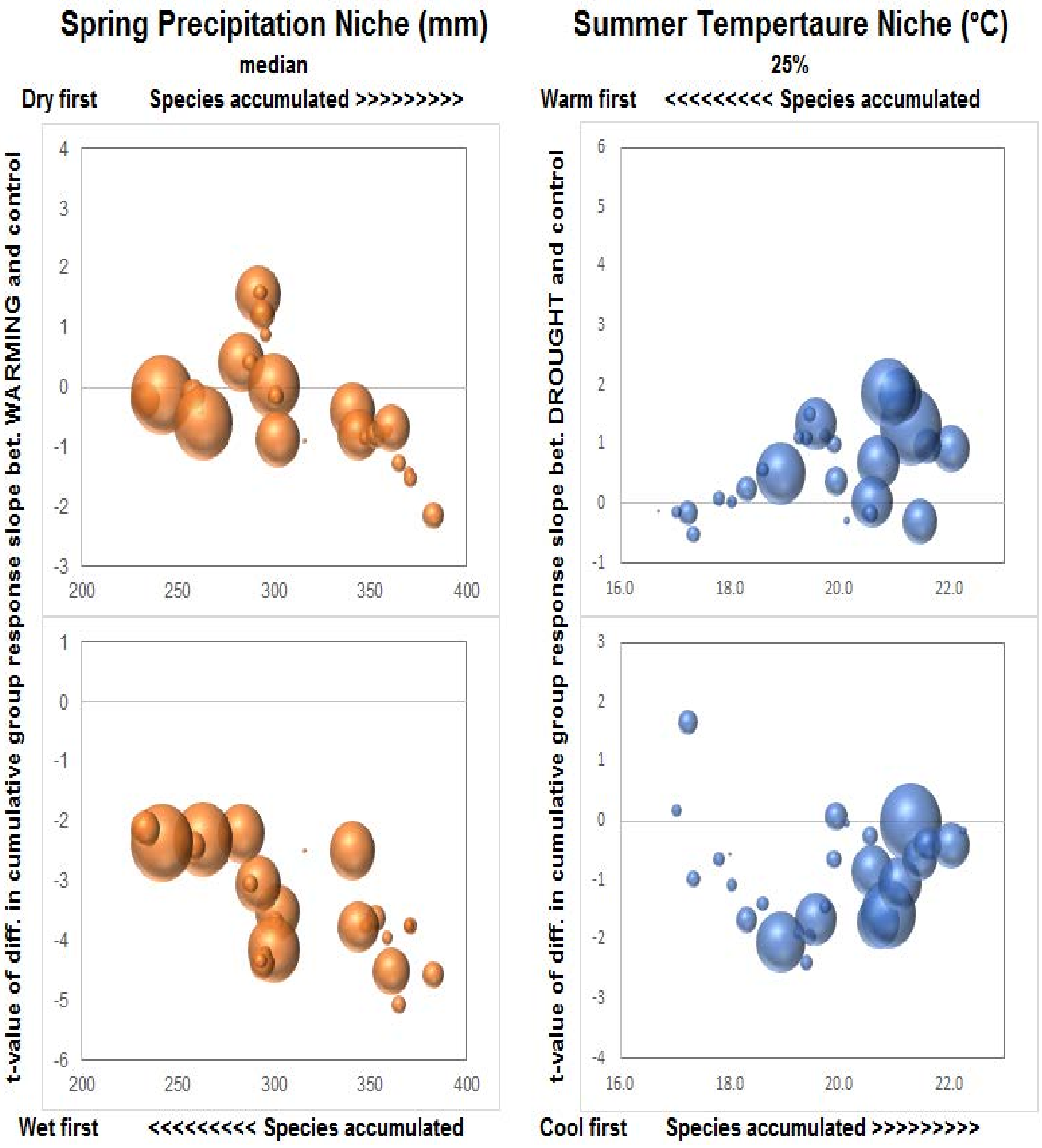


Fig. S4 Opposing Cumulative Group Analyses for species groups defined by median spring precipitation niches in response to manipulated warming (left), and species groups defined by 25 percentile summer temperature niches in response to manipulated drought (right). Upper graphs display groups tested when accumulated from the dry end of the gradient (dry first: left to right) or warm end (warm first: right to left); Lower graphs display groups tested when accumulated from the wetter end of the gradient (Wet first: right to left) or cool end (Cool first: left to right). The x-axis displays the climatic niche value associated to the species added to the cumulative group. The y-axis displays the t-value for the difference in estimated slopes over time for point quadrat hit abundances in control compared to manipulation treatment (calculated using a Linear Mixed Effect Model). Bubble size is proportional to the log-community percentage of the species added to the group. In general, while these patterns suggest a correlation between species' temperature and precipitation niches for those species found in our experiment, the inconsistencies in the patterns in comparison to figures S2 and S3 suggest that some species possess different adaptations to temperature and precipitation.

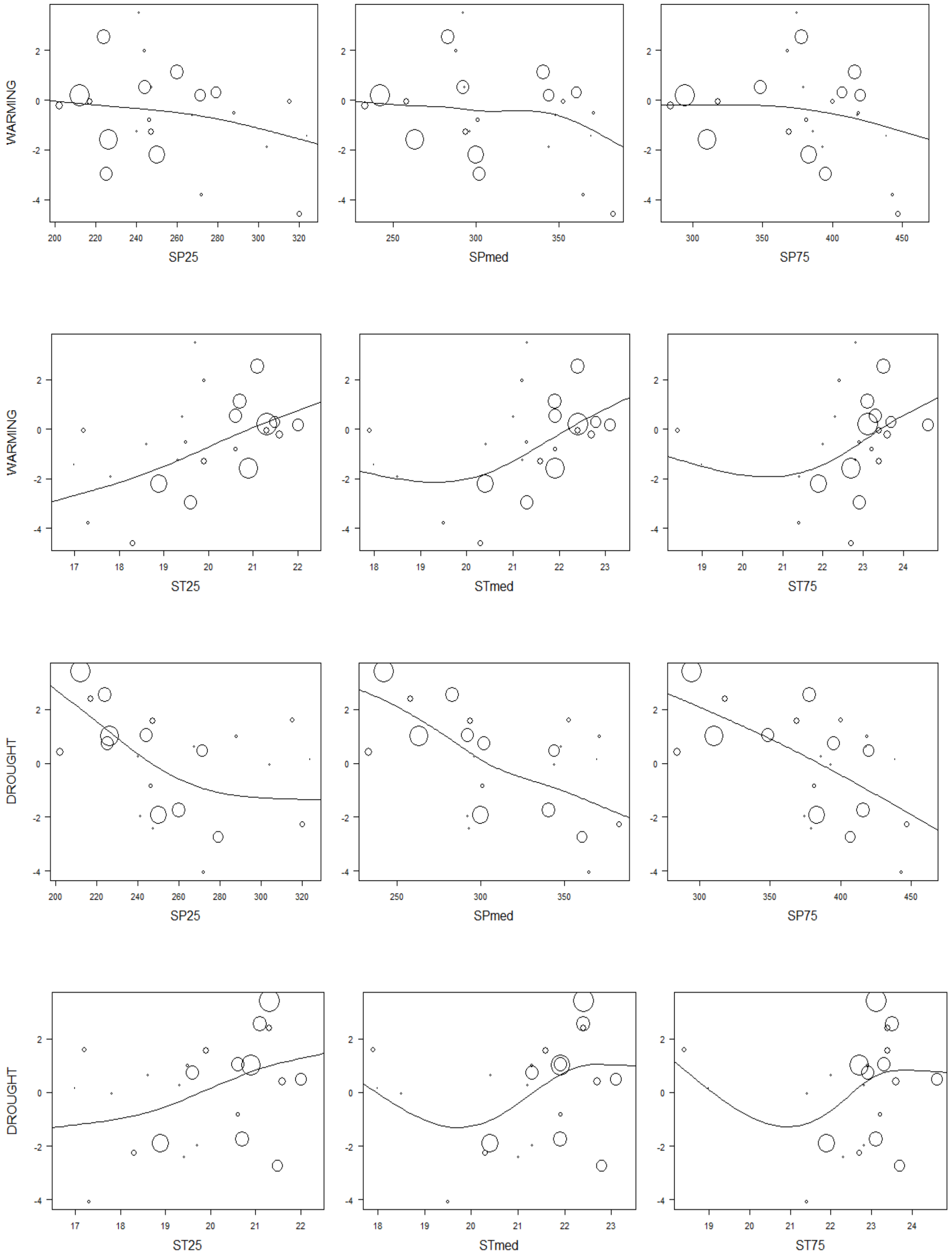


Fig. S5 Single species responses to the warming and drought manipulation treatments when plotted along multiple climatic niche axes. Displayed are species niche values derived from two climatic parameters: Spring precipitation (SP) and Summer temperature (ST); And three methods of calculation: median of climatic values associated with each species observation (med); the lower 25 percentile of the climatic values (25); or the upper 25 percentile of climatic values (75). Y-axis are the t-values of single species Linear Mixed Model (LMM) tests comparing slope response over time under control to treatments (positive values indicate increase in abundances under treatment, negative values indicate decrease under treatment over time relative to control). Bubbles are displayed in relative size to the log-community percentage each species occurred throughout the experiment. Fit lines were derived from Generalized Additive Models (GAMs) weighted by species log-community percentage. N.B. 25 percentile niche values have a tendency to describe/rank the lower value species better (cool temperature decrease, dry precipitation increase), whereas 75 percentile niche values tend to describe/rank the higher value species better (warm temperature increase, wet precipitation decrease). Also note, that summer temperature niche values do not describe species response to drought very well. Finally, while interesting patterns emerge for this analysis, the single species versus niche axis analysis does highlight that some single species may not respond to the manipulations as predicted for various reasons, and that patterns can be difficult to interpret. By using Climatic Niche Groups, there is less reliance on accurate determination of the climatic niche value and/or random variation, resulting in a more general statement that can be made about community composition response to climate manipulations/change.

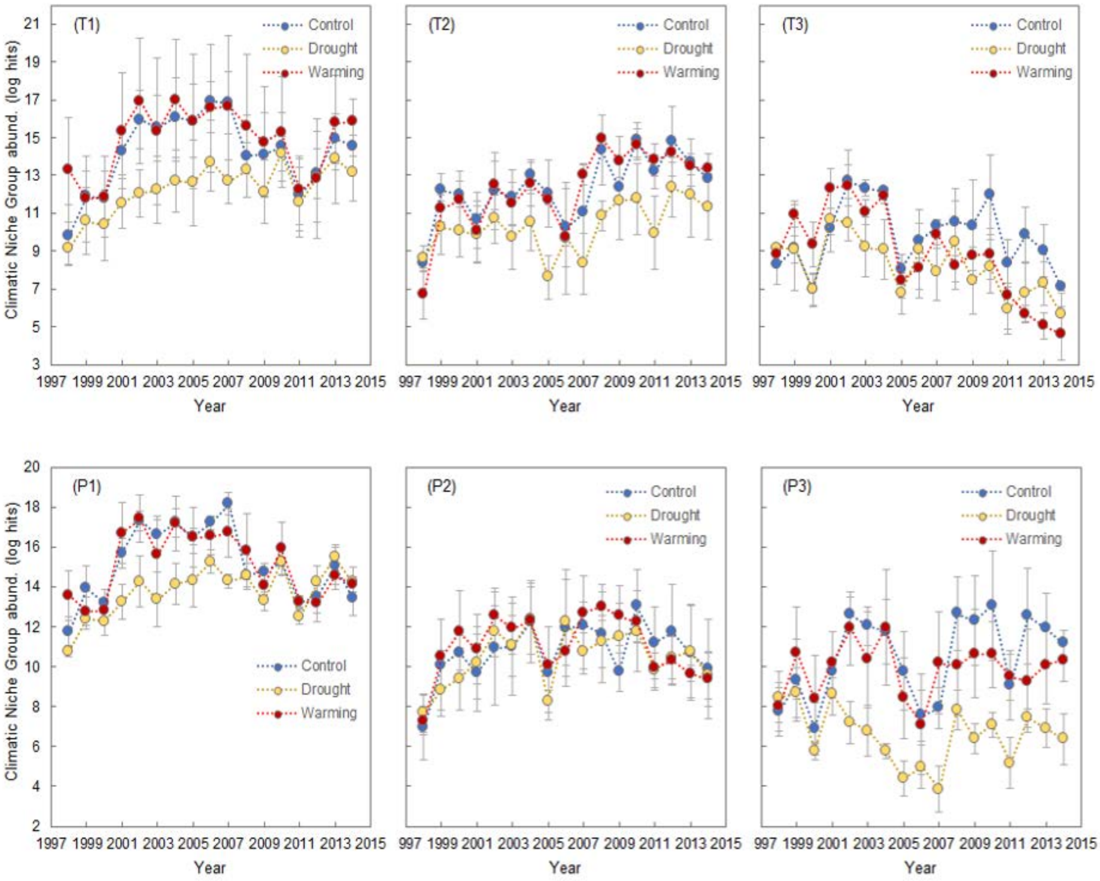


Figure S6 Climatic Niche Group (CNG) raw log-abundance (log contact hits) within plots under control (no manipulation), nocturnal-warming and drought treatments throughout the study period 1999-2014. Warming and drought were not manipulated in 1998. Points represent means and the error bars represent their associated standard errors (n=3 plots). CNGs are shown created from the niche parameters shown in the main article (corresponding to effect sizes in Fig. 2). Top graphs show summer temperature (25 percentile) CNGs ordered from warm to cold (T1>T2>T3). Bottom graphs show spring precipitation (median) CNGs ordered from dry to wet (P1<P2<P3).

Chapter 6

Chapter 6 Shift in community structure mainly by the losses of herbs in long-term warming and drought experiments and natural extreme droughts in an early-successional Mediterranean shrubland

Table S1 Species information for all species of herbs (*H*) and shrubs (*S*) collected in the plots. SP stands for the abbreviation of the species name used in figures.

SP	Species	Family	Life form
AM	*Aphyllanthes monspeliensis*	Asparagaceae	H
ASC	*Asperula cynanchica*	Rubiaceae	H
AZ	*Argyrolobium zanonii*	Leguminosae	S
BB	*Bituminaria bituminosa*	Fabaceae	S
BF	*Bupleurum fruticosum*	Umbelliferae	H
PO	Several species	Poaceae	H
CE	*Cuscuta epithymum*	Convolvulaceae	H
CMO	*Coris monspeliensis*	Primulaceae	S
DP	*Dorycnium pentaphyllum*	Leguminosae	S
EC	*Eryngium campestre*	Umbelliferae	H
EM	*Erica multiflora*	Ericaceae	S
ES	*Euphorbia serrata*	Euphorbiaceae	H
FE	*Fumana ericoides*	Cistaceae	S
FT	*Fumana thymifolia*	Cistaceae	S
GA	*Globularia alypum*	Globulariaceae	S
GL	*Galium lucidum*	Rubiaceae	H
HST	*Helichrysum stoechas*	Sempreviva borda	S
HSY	*Helianthemum syriacum*	Cistaceae	S
JO	*Juniperus oxycedrus*	Cupressaceae	S
LF	*Lithospermum fruticosum*	Boraginaceae	S
OM	*Ononis minutisima*	Fabaceae	S
OV	*Odontites vernus*	Scrophulariaceae	H
PL	*Pistacia lentiscus*	Anarcadiaceae	S
PR	*Polygala rupestris*	Polygalaceae	S
QC	*Quercus coccifera*	Fagaceae	S
RO	*Rosmarinus officinalis*	Labiatae	S
RP	*Rubia peregrina*	Rubiaceae	H
SS	*Sedum sediforme*	Crassulaceae	S
TC	*Teucrium chamaedrys*	Camedris	S
TP	*Teucrium polium*	Labiatae	S
UP	*Ulex parviflorus*	Leguminosae	S

Table S2 Changes in abundance of typical species, *Globularia alypum*, Poaceae species (PO) and *Rosmarinus officinalis*, in response to the treatments during the study period 1998-2015. The changes for *G. alypum*, PO and *R. officinalis* were associated with May SPEI-4, July SPEI-3 and May SPEI-2, respectively, in the models. Drought-control and warming-control differences were analyzed. Significant differences are labeled with asterisks: (*) P<0.1, * P<0.05, ** P<0.01, *** P<0.001. Significant effects are highlighted in bold type.

	G. alypum		PO		*R. officinalis*	
	Difference	*P*	Difference	*P*	Difference	*P*
Warming-control						
SPEI	**0.1**	*	**0.52**	***	**0.51**	***
Warming	**-0.2**	*	**-0.5**	*	**0.98**	***
Drought-control						
SPEI	**0.13**	**	**0.22**	(*)	**0.36**	***
Drought	**0.29**	***	**-1.2**	***	-0.29	ns

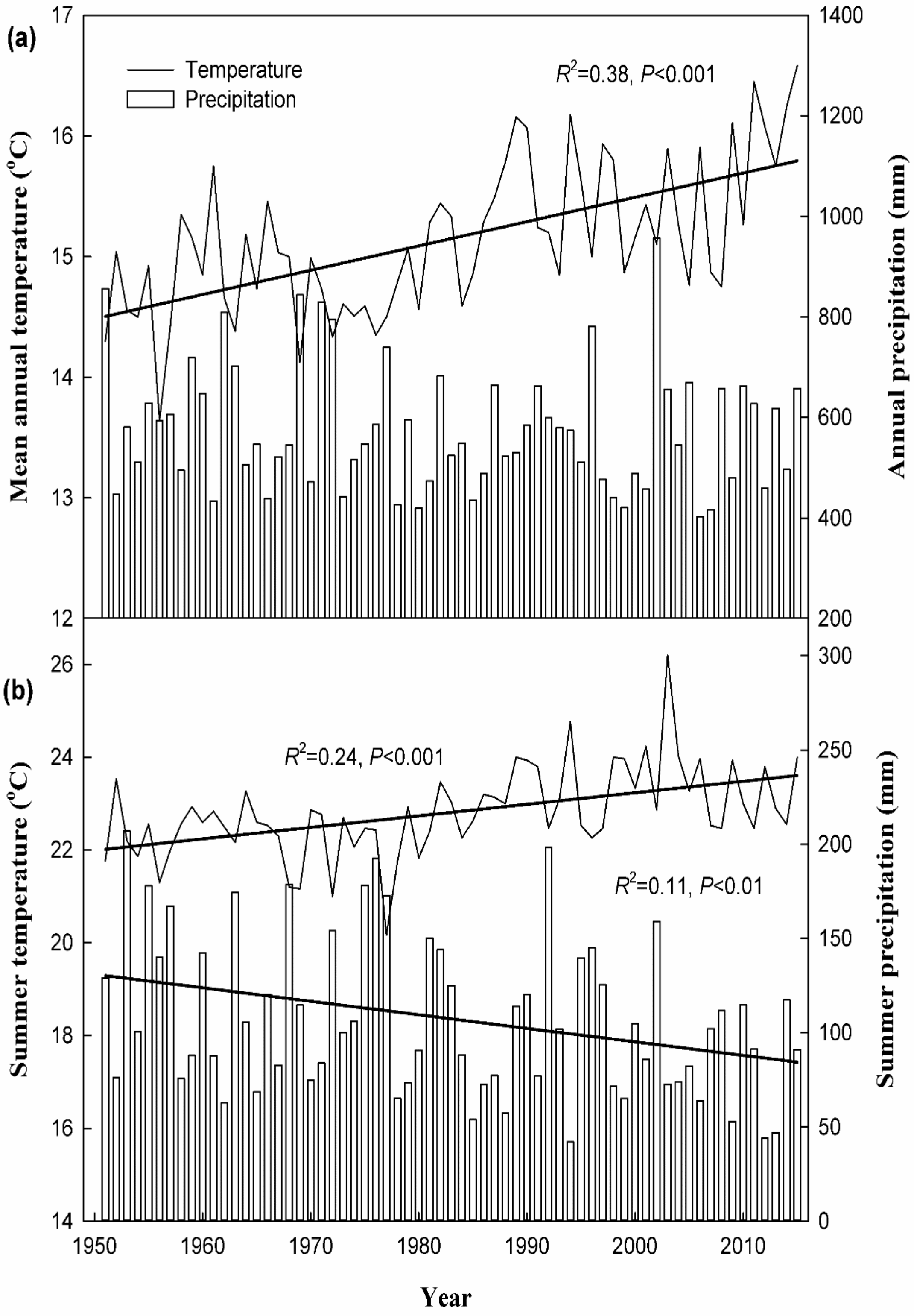


Fig. S1 Historical trends of (a) mean annual temperature and annual precipitation and (b) summer temperature and precipitation for 1951-2015.

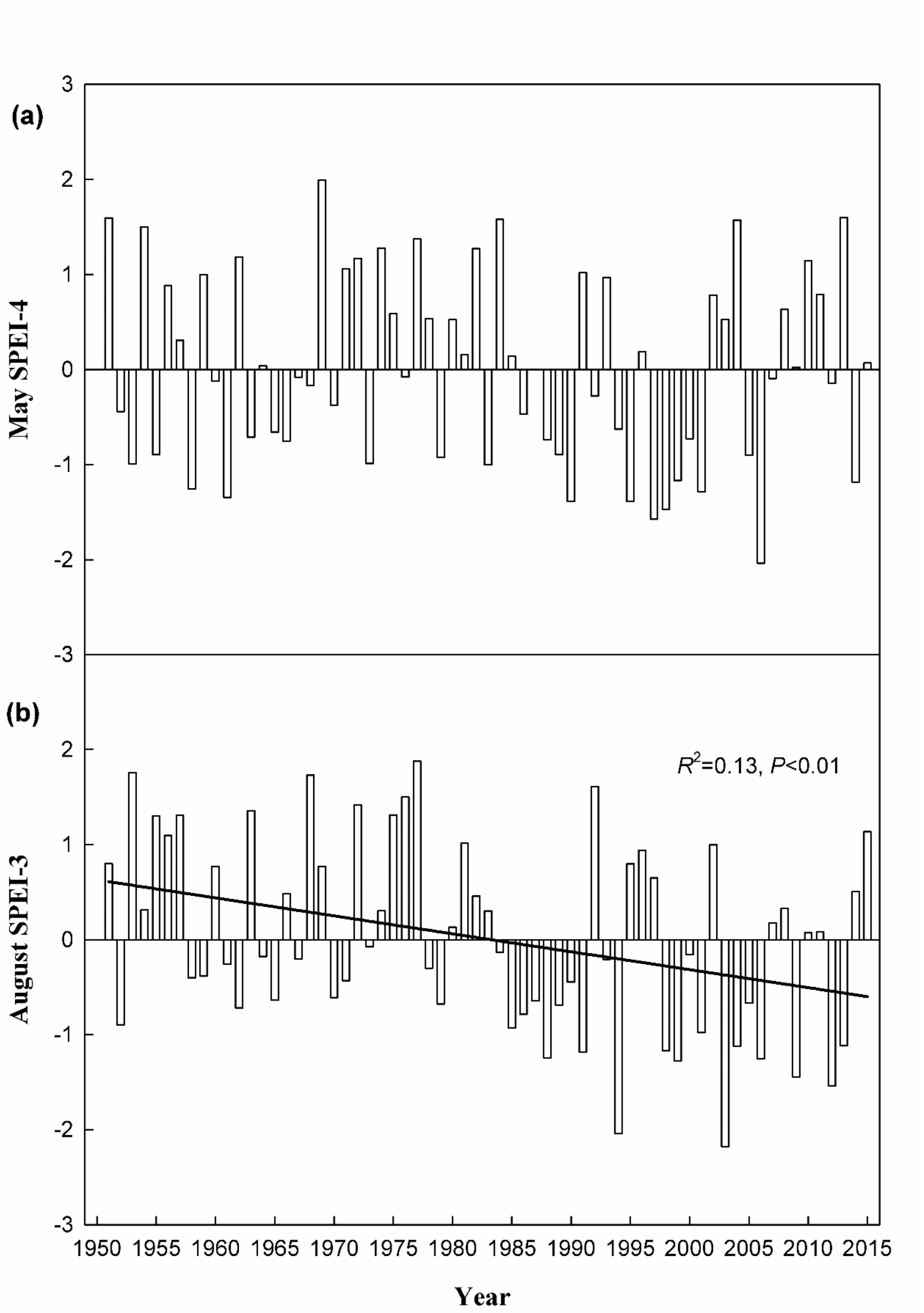


Fig. S2 (a) Spring SPEI-4 and (b) summer SPEI-3 for 1951-2015.

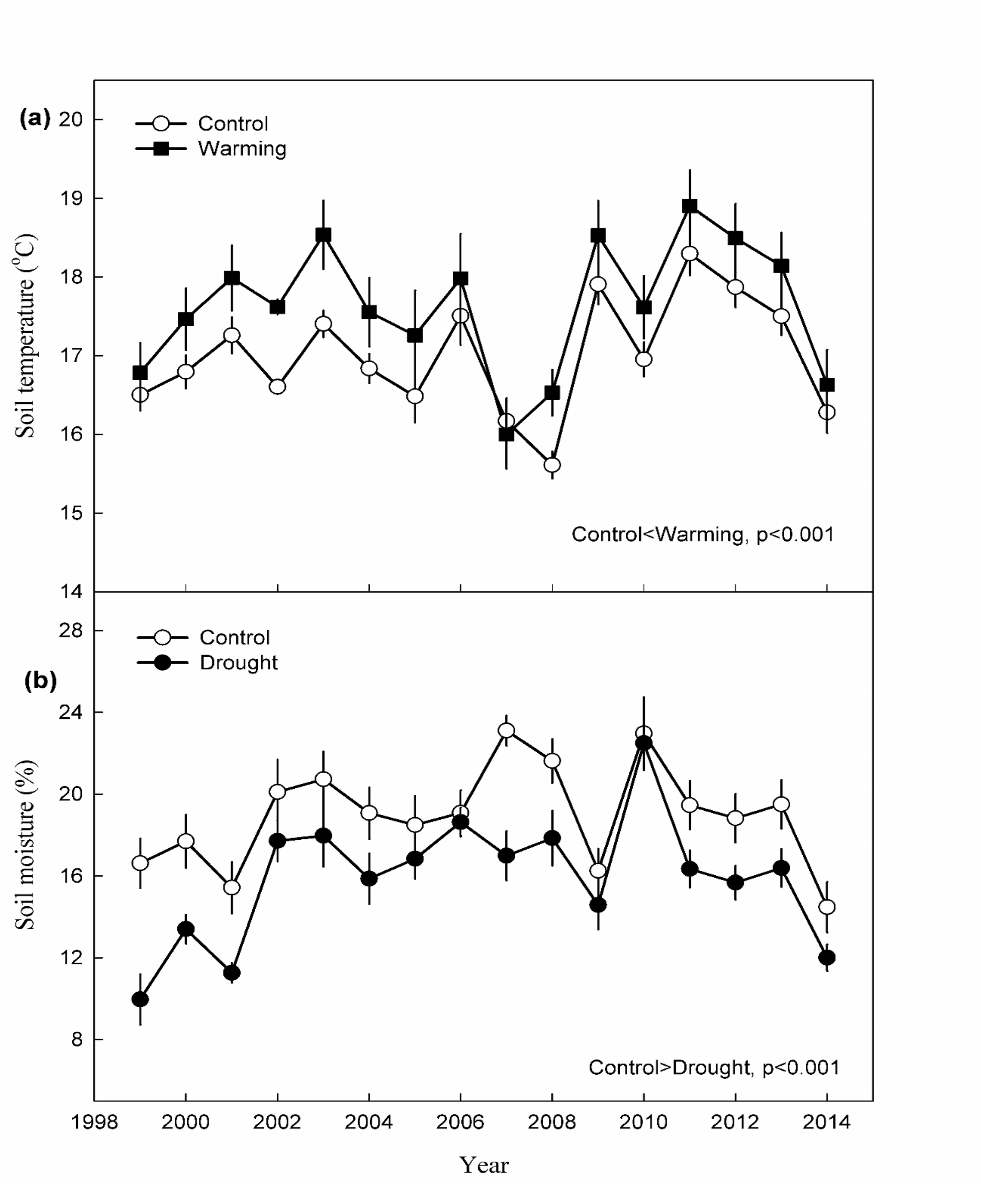


Fig. S3 Average (a) soil moisture and (b) soil temperature in the experimental plots for 1999-2015. Vertical bars indicate the standard errors of the means (n=3 plots).

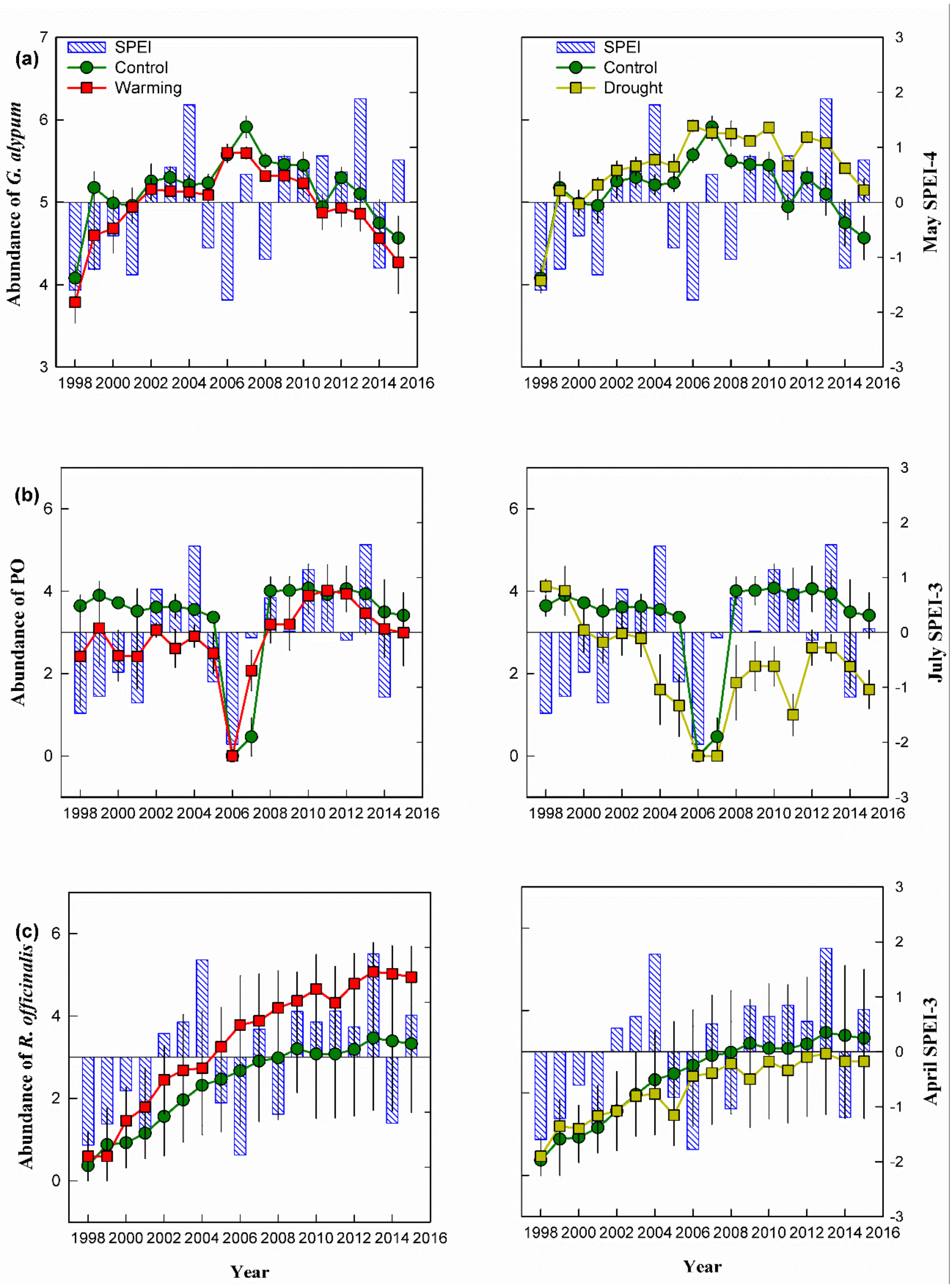


Fig. S4 Changes in abundance of typical species, *Globularia alypum*, Poaceae species (PO) and *Rosmarinus officinalis*, in response to experimental warming and drought during the study period 1998-2015. May SPEI-4, July SPEI-3 and May SPEI-2 were the covariate factor for the changes for *G. alypum*, PO and *R. officinalis* in the models, respectively. Vertical bars indicate the standard errors of the means (n=3 plots).

Published or finished scientific articles during the study period of my PhD

1. Romà Ogaya, Joan Llusià, Adrià Barbeta, Dolores Asensio, **Daijun Liu**, Giorgio Arturo Alessio, Josep Peñuelasa. Foliar CO_2 in a holm oak forest subjected to 15 years of climatechange simulation. ***Plant Science***, 2014, 226, 101-107
2. **Daijun Liu**, Romà Ogaya, Adrià Barbeta, Xiaohong Yang, Josep Peñuelas. Contrasting impacts of continuous moderate drought and episodic severe annual droughts on aboveground biomass increment and litterfall of different species of a Mediterranean holm oak forest. ***Global Change Biology***, 2015, 21, 4196-4209.
3. **Daijun Liu**, Joan Llusia, Romà Ogaya, Marc Estiarte, Laura Llorens; Xiaohong Yang, Josep Peñuelas. Physiological adjustments of a Mediterranean shrub to long-term experimental warming and drought treatments. ***Plant Science***, 2016, 252, 53-61.
4. **Daijun Liu**, Marc Estiarte, Romà Ogaya, Xiaohong Yang, Josep Peñuelas. Shift in community structure in an early-successional Mediterranean shrubland driven by long-term experimental warming and drought and natural extreme droughts, ***Global Change Biology,*** 2017.
5. **Daijun Liu**, Josep Peñuelas, Romà Ogaya, Marc Estiarte, Katja Tielbörger, Fabian Slowik, Xiaohong Yang, Mark C. Bilton. Species selection under long-term experimental warming and drought explained by climatic distributions, ***New Phytologist***, in revision.
6. **Daijun Liu**, Romà Ogaya, Adrià Barbeta, Xiaohong Yang, Josep Peñuelas. Long-term experimental drought combined with natural extremes accelerate forest degradation and vegetation shifts in Mediterranean holm oak forests, ***Global change biology***, under review.
7. Chao Zhang, Iolanda Filella, **Daijun Liu**, Romà Ogaya, Joan Llusià, Dololes

Asensio, Josep Peñuelas. Photochemical reflectance index (PRI) detecting responses of seasonal photosynthetic apparatus to experimental drought and warming in a Mediterranean shrubland, ***Remote Sensing of Environment***, in submission.

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Daijun Liu