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The combined effects of a long-term experimental drought and an extreme drought on the use of plant-water sources in a Mediterranean forest

Running head: Effects of drought on plant-water sources

Adrià Barbeta[1, 2*], Monica Mejía-Chang[1, 2*], Romà Ogaya[1, 2], Jordi Voltas[3], Todd E. Dawson[4], Josep Peñuelas[1, 2]

1 CSIC, Global Ecology Unit CREAF-CSIC-UAB, E-08193 Cerdanyola del Vallès (Catalonia), Spain.

2 *CREAF, E-08193 Cerdanyola del Vallès (Catalonia), Spain.*

3 *Department of Crop and Forest Sciences – AGROTECNIO Center, University of Lleida, Spain*

4 *Department of Integrative Biology, University of California at Berkeley, CA, 94720-3140, USA*

* *Adrià Barbeta and Monica Mejía-Chang contributed equally to the manuscript and share first authorship.*

Corresponding Author:

Adrià Barbeta

Global Ecology Unit CREAF-CSIC-UAB,

Center for Ecological Research and Forestry Applications (CREAF) - National Research Council (CSIC)

Building C, Universitat Autònoma de Barcelona, E-08193 Cerdanyola del Vallès, Barcelona

Phone: +34935811312, +34935814221 fax +34935814151

E-mail: a.barbeta@creaf.uab.es

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Abstract

Vegetation in water-limited ecosystems relies strongly on access to deep water reserves to withstand dry periods. Most of these ecosystems have shallow soils over deep groundwater reserves. Understanding the functioning and functional plasticity of species-specific root systems and the patterns of or differences in the use of water sources under more frequent or intense droughts is therefore necessary to properly predict the responses of seasonally dry ecosystems to future climate. We used stable isotopes to investigate the seasonal patterns of water uptake by a sclerophyll forest on sloped terrain with shallow soils. We assessed the effect of a long-term experimental drought (12 years) and the added impact of an extreme natural drought that produced widespread tree mortality and crown defoliation. The dominant species, *Quercus ilex*, *Arbutus unedo* and *Phillyrea latifolia*, all have dimorphic root systems enabling them to access different water sources in space and time. The plants extracted water mainly from the soil in the cold and wet seasons but increased their use of groundwater during the summer drought. Interestingly, the plants subjected to the long-term experimental drought shifted water uptake toward deeper (10-35 cm) soil layers during the wet season and reduced groundwater uptake in summer, indicating plasticity in the functional distribution of fine roots that dampened the effect of our experimental drought over the long term. An extreme drought in 2011, however, further reduced the contribution of deep soil layers and groundwater to transpiration, which resulted in greater crown defoliation in the drought-affected plants. The present study suggests that extreme droughts aggravate moderate but persistent drier conditions (simulated by our manipulation) and may lead to the depletion of water from groundwater reservoirs and weathered bedrock, threatening the preservation of these Mediterranean ecosystems in their current structures and compositions.

Introduction

The consequences of anthropogenic climatic change in the Mediterranean Basin include the ongoing increases in temperature coupled to a very likely notable reduction in precipitation in summer and spring for the coming decades (Christensen *et al.*, 2007). Some Mediterranean forests have already adjusted and in some cases even adapted to seasonal drought and an irregular precipitation regime, but unprecedented duration, intensity and seasonality of future droughts predicted by general circulation models (GCMs) could have strong impacts on the vegetation and therefore the structure and function of ecosystems that are beyond the tolerance of most plants. Indeed, the numbers of documented drought-induced tree mortalities and episodes of forest decline in this region are growing (Peñuelas *et al.*, 2000, 2013; Galiano *et al.*, 2012). These events may lead to community shifts (Mueller *et al.*, 2005) and may cascade to affect nutrient cycling, microclimate and/or hydrology (Anderegg *et al.*, 2013a). The distribution of tree mortality, however, tends to be patchy across landscapes, indicating that certain individuals or populations are more predisposed to death (Suarez *et al.*, 2004). This disparity in the responses to climate is partly driven by the interspecific differences in the ability to cope with water stress and warm temperatures (Breshears *et al.*, 2009; Allen *et al.*, 2010; Carnicer *et al.*, 2013a) but also by site characteristics (Lloret *et al.*, 2004). Detailed knowledge of the diversity of different responses and plant strategies is necessary for understanding the mechanisms behind tree mortality and for improving predictions of future forest declines or community shifts.

The experimental manipulation of precipitation is useful for studying the effects of drought on forest declines (Wu *et al.*, 2011). Such experiments in Mediterranean forests have helped to identify the physiological, morphological, structural (Ogaya & Peñuelas, 2006; Limousin *et al.*, 2010) and temporal (Barbeta *et al.*, 2013; Martin-Stpaul *et al.*, 2013) changes induced by drought. The projected increase in frequency of extreme droughts may imply a carry-over effect of multiple droughts, where plant resilience could be at risk (Anderegg *et al.*, 2012), but more counter-intuitively, structural changes caused by droughts seem to progressively enhance plant resistance (Lloret *et al.*, 2012; Barbeta *et al.*, 2013). Consequently, long-term experiments are desirable both to account for the accumulative effect of multiple droughts or to avoid overestimating the effects of drought on vegetation (Leuzinger *et al.*, 2011).

The use of water by plants has been well studied in temperate ecosystems, but we still have limited knowledge about a wide range of processes, on scales of leaves to entire landscapes, within many water-limited ecosystems (Zeppel, 2013). The effects of increasing drought on the patterns of use of underground water in Mediterranean trees has not been extensively studied, although recent studies have characterized seasonal patterns of water uptake in some *Quercus* species (Kurz-Besson *et al.*, 2014; David *et al.*, 2007; Nadezhdina *et al.*, 2007). The stable-isotope (δ^{18}O and $\delta\,^{2}$H) composition of water is a powerful tool for tracing the movement of water underground (Dawson *et al.*, 2002). Isotopic fractionation does not occur during water absorption by roots (Ehleringer & Dawson, 1992; but see Lin and Sternberg, 1993 for exceptions), so the isotopic signature of xylem water can be used to determine a plant’s source of water at a given moment. Pools of underground water can have different isotopic signatures due to differences in the original water sources (precipitation at different times of the year or from different source areas), and evaporation during and after rains can markedly change the isotopic composition of the soil water (Allison and Barnes 1992). Gradients in the compositions of H or O isotopes of the remaining soil in seasonally dry environments can also develop, with water in the surface layers becoming more enriched (leading to more positive δ values), and water in the deeper layers becoming more depleted, in the heavy isotopes (Allison, 1982). Additionally, groundwater extracted from water tables or bedrock fractures can often have distinct signatures, reflecting the isotopic composition of rainwater during either wet or cold seasons, when these pools are refilled by infiltration with little evaporation (Brooks *et al.*, 2009). Isotopic signatures may also reflect the biased or weighted average of annual inputs of precipitation (Ehleringer & Dawson, 1992), the subsurface fractionation caused by water interacting with charged clays (Oerter *et al.*, 2014) or unique redox chemical evolution (Oshun *et al.*, 2014). These differences in isotopic signatures have been successfully used to determine the sources of water of vegetation in the Mediterranean Basin (David *et al.*, 2007; West *et al.*, 2012) and other biomes (Eggemeyer *et al.*, 2009; Kukowski *et al.*, 2013). Some studies have applied these techniques in short-term experimental droughts or under extreme natural droughts (Schwinning *et al.*, 2005; West *et al.*, 2012; Anderegg *et al.*, 2013b; Kukowski *et al.*, 2013), but little is known about the accumulative effect of long-term experimental drought on the isotopic compositions and sources of the water used by plants.

Some species in seasonally dry climates depend on access to groundwater for withstanding periods without precipitation (Dawson and Pate 1996; Kurz-Besson *et al.*, 2014; David *et al.*, 2007; Eggemeyer *et al.*, 2009; Rossatto *et al.*, 2012; Zeppel, 2013; Oshun *et al*., 2014). Forests commonly occur on mountainsides in Mediterranean climatic zones (Carnicer *et al.*, 2013b) where soils are shallow and roots do not reach the water table but may extract water stored in weathered bedrock (Witty *et al.*, 2003). This situation could be common among many forests in other biomes, because water tables are deeper than 10 m in an estimated 44.8% of terrestrial ecosystems (Fan *et al.*, 2013), while the mean maximum rooting depth is approximately 7 m for trees and 5 m for shrubs (Canadell *et al.*, 1996). Nonetheless, the depth of root systems in sympatric species in Mediterranean ecosystems may differ and sometimes co-vary with other traits such as hydraulic safety margins or photosynthetic activity under water stress (West *et al.*, 2012). These characteristics define a species' water-use strategy as more isohydric or more anisohydric (Tardieu & Simonneau, 1998; Mcdowell *et al.*, 2008). Increasing evaporative demand, together with longer, more intense, more frequent and aseasonal droughts, are likely to reduce groundwater reserves (Eckhardt & Ulbrich, 2003), so the effects on vegetation would highly depend on these water-use strategies; the more isohydric phreatophytic species (West *et al.*, 2012) would be more vulnerable to carbon starvation caused by early stomatal closure, and anisohydric species would have a higher risk of hydraulic failure (Mcdowell *et al.*, 2008). Ecophysiological processes of acclimation (Matesanz & Valladares, 2013) and structural changes forced by previous droughts (Lloret *et al.*, 2012; Barbeta *et al.*, 2013), however, may mitigate the negative effects of drought.

We present the results of an ecohydrological study applying water stable-isotope techniques in a long-term experimental drought system established in 1998. A forest dominated by Holm oaks (*Quercus ilex* L.) was subjected to a 15% reduction in soil moisture (matching GCM predictions for the Mediterranean Basin (Christensen *et al.*, 2007)) that caused a drastic suppression of growth in the dominant species *Q. ilex* and *Arbutus unedo* L. and an increase in mortality rates in *Q. ilex* but not *Phillyrea latifolia* L. (Ogaya et al 2007). The effect size of the drought treatment, however, was dampened over time (Barbeta et al 2013). The characterization of seasonal changes in plant-water sources is crucial for understanding the mechanisms underlying these species-specific responses to drought. Moreover, an extreme drought during the study period enabled us to investigate the causes of drought-induced mortality in this Holm oak forest. This study asked the following questions: (i) what are/were the sources of water for each plant species, and do they change over time? (ii) did the sources of water change after 12 years of experimental drought? (iii) does constant or excessive use of deeper water sources lead to the progressive depletion of groundwater under drought? (iv) how are water sources related to species-specific drought responses? and (v) is drought-induced mortality linked to changes in usage of particular water sources?

Materials and methods

Experimental site

The experimental site was established in 1998 at the Prades Holm oak forest in southern Catalonia (northeastern Iberian Peninsula) (41°21’N, 1°2’E) at 930 m a.s.l. on a south-facing slope (25% slope). The forest has a very dense multi-stem crown (18 366 stems ha^{-1}) dominated by *Q. ilex* (3850 stems ha^{-1} and 50 Mg ha^{-1}), *P. latifolia* (12 683 stems ha^{-1} and 29 Mg ha^{-1}) and *A. unedo* (667 stems ha^{-1} and 9 Mg ha^{-1}), accompanied by other Mediterranean woody species that do not reach the upper canopy (e.g. *Erica arborea* L., *Juniperus oxycedrus* L. and *Cistus albidus* L.) and the occasional isolated deciduous tree species (e.g. *Sorbus torminalis* L. Crantz and *Acer monspessulanum* L.). The canopy in the study plots did not exceed 4 m. This forest has been managed as a coppice for centuries but has not been significantly disturbed in the last 70 years.

The climate is typically Mediterranean. Since the beginning of the experiment (1998), the mean annual temperature has been 12.2 °C and the mean annual precipitation has been 610 mm. Holm oak forests can occur at sites with a mean annual precipitation as low as 400-450 mm (Terradas, 1999). The annual and seasonal distribution of precipitation is irregular, with annual precipitation ranging from 376 to 926 mm in the 12 years of the experiment. Spring and autumn are the wettest seasons, and summer droughts usually last three months, during which precipitation is ~10% of the annual total and coincides with the highest temperatures. Winters are relatively cold. January is the coldest month (mean temperature of 4.4 °C), and the mean daily temperature is below 0 °C an average of eight days per winter. The soil is a Dystric Cambisol over Paleozoic schist and has a mean depth of ~35 cm. The mean annual precipitation is higher than that in the driest distributional limit of *Q. ilex*, but the topographic characteristics of the study site represent relatively xeric conditions due to the shallow soils and steep terrain.

The experimental system consisted of four 150-m^2 plots delimited at the same altitude along the slope. Half the plots (randomly selected) received the drought treatment, and the other half faced natural conditions. Precipitation was partially excluded from the plots of the drought treatment by PVC strips suspended 0.5-0.8 m above the soil and covering approximately 30% of the plot surfaces. A ditch 0.8 m in depth was excavated along the entire top edge of the plots to intercept runoff water. The water intercepted by the strips and ditches was conducted around the plots, below their bottom edges. The strips were installed below the canopy and thus did not intercept light. Litter falling on the plastic strips was regularly transferred below them to ensure that differences in the content of soil nutrients among treatments and control plots were attributable only to the availability of water for the decomposition of this litter.

Sampling and environmental monitoring

The field work was initially planned for spring 2010 to winter 2011, with one sampling campaign each season. The extreme drought in the summer of 2011 offered the possibility of an extra campaign to monitor plant performance under intense water stress. In each of these campaigns, samples of xylem, bulk-soil and spring water were collected at midday (between 1100 and 1400). For the samples of xylem water, 3-4 sunlit twigs per tree were cut, the bark and phloem were removed to prevent interference from the isotopes in the water of the leaves and the twigs were then transferred to borosilicate glass vials with PTFE/silicone septa tops (National Scientific Company, Rockwood, USA). The vials were sealed with parafilm and stored in a portable cooler to prevent evaporation. In all four plots, the same five dominant individuals of *A. unedo*, *Q. ilex* and *P. latifolia* were sampled in each campaign. The samples of bulk soil were extracted with a soil corer from two layers (0-10 and 10-35 cm). The soil samples were also immediately stored in the same type of glass vials as the xylem samples, sealed with parafilm and stored in a portable cooler. All samples were refrigerated until processing and analysis. Five locations were randomly selected in the control plots for soil sampling. In the drought plots, five locations under the plastics strips and five locations not under the strips were selected to control for potentially different amounts of evaporation. Samples of spring water were collected from a nearby fountain (natural spring); the isotopic signature of this water should be comparable to that of the groundwater. The experimental site is high on a ridge on schist bedrock, so the groundwater may remain in rock fractures for a period of time after infiltration from the surface but without forming a water table.

We also measured the midday foliar water potential in each field campaign with a pressure chamber (PMS Instruments, Corvallis, USA) in the same plots and species where the water samples were collected and in dominant individuals that reached the upper canopy. Ten randomly selected dominant individuals per plot and species were sampled. The selected trees had no significant mechanical damage. Soil moisture was measured each campaign by time-domain reflectometry (Tektronix 1502C, Beaverton, USA) (Zegelin *et al.*, 1989; Gray & Spies, 1995). Three stainless-steel cylindrical rods, 25 cm long, were vertically installed in the upper 25 cm of the soil at four randomly selected locations in each plot. The time-domain reflectometer was manually attached to the ends of the rods for each measurement. An automatic meteorological station installed between the plots monitored temperature, photosynthetically active radiation, air humidity and precipitation every 30 min. Both the Standardized Precipitation and Evapotranspiration Index (SPEI) at different timescales (Vicente-Serrano *et al.*, 2013) and the mortality rates were calculated for the study plots using the same methodology described by Barbeta *et al.* (2013). Additionally, a visual evaluation of crown defoliation estimated the effect of the extreme drought in 2011. Defoliation was defined as the percentage of leaf loss in the assessable crown, using a sliding scale of 10%.

Isotopic analyses

The water in the soil and xylem samples was extracted by cryogenic vacuum distillation following West *et al.* (2006). The extraction system consisted of 10 extraction tubes connected with Ultra-Torr™ fittings (Swagelok Company, Solon, USA) to 10 U-shaped collection tubes specifically designed for this system. The extraction tubes were submerged in a pot containing mineral oil maintained at 110 °C, and the collection tubes were submerged in liquid nitrogen to freeze/capture the extracted water vapor for isotopic analysis. The extraction system was connected to a vacuum pump (model RV3; Edwards, Bolton, UK). The isotopic compositions ($\delta^{18}O$ and $\delta^{2}H$) of the distilled water samples were determined using isotope ratio infrared spectroscopy (IRIS) with a Picarro L2120-i Analyzer (Picarro Inc., Santa Clara, USA). Residual organic compounds in the distilled water can interfere with the analyses of plant and soil samples conducted with IRIS technology (West *et al.*, 2010, 2011). The ChemCorrect™ post-processing software from Picarro, though, can determine the degree of contamination of each sample, and Picarro also offers a post-test correction for the isotopic composition of contaminated samples. To test the reliability of IRIS and therefore our data, we analyzed a subset of plant and soil samples (104, including samples from other studies) using isotope ratio mass spectrometry (IRMS), which is not affected by organic compounds. A detailed description of the methodology of IRMS and IRIS analyses can be found in West *et al.* (2011) and Goldsmith *et al.* (2012) for both $\delta^{18}O$ and $\delta^{2}H$. We then compared the isotopic compositions obtained by IRIS and IRMS and their post-processing corrections and confirmed that IRIS was highly reliable for our samples. The discrepancies between the two methods remained below the instrumental errors. Nonetheless, we discarded those samples with very high concentrations of organic compounds. The isotope ratios in this study are expressed as:

$$\delta^{18}O \text{ or } \delta^{2}H = ((R_{sample} - R_{standard})\text{-}1)$$

where R_{sample} and $R_{standard}$ are the heavy/light isotope ratios ($^{2}H/H$ and $^{18}O/^{16}O$) of the sample and the standard (VSMOW, Vienna Standard Mean Ocean Water), respectively. The water extractions and isotopic analyses were conducted at the Department of Crop and Forest Sciences (University of Lleida, Catalonia, Spain) and at the Center for Stable Isotope Biogeochemistry (University of California, Berkeley, USA).

Determining the sources of plant water and statistical analyses

The isotopic compositions of the xylem water and its potential sources can be directly compared by plotting both isotopes together (Goldsmith *et al.*, 2012) but also by using the siar (stable isotope analysis in R) package in R (Parnell *et al.*, 2010). These Bayesian mixing models estimate the most likely proportion of plant water taken up from each source, which is a suitable approach in our study because three different monitored sources contributed simultaneously to plant-water use. We applied these models to our data to infer the relative contribution of each water source to the xylem water, producing simulations of plausible contributing values from each source using Markov chain Monte Carlo (MCMC) methods. Stable-isotope mixing models are widely applied to the study of food webs but can also be used for determining plant-water sources. Our model inputs were the isotopic composition ($\delta^{18}O$ and $\delta^{2}H$) and their standard errors for each potential source (shallow (0-10 cm) soil water, deep (10-35 cm) soil water and groundwater) and the isotopic compositions of the xylem water, which were assigned as the target values ("consumers" in Parnell *et al.* (2010)). We set the TEF (trophic enrichment factor) to 0, because of the absence of fractionation during water uptake from soil by roots (Ehleringer & Dawson, 1992), and set concentration dependence to 0. We ran 500 000 iterations and discarded the first 50 000. We ran a model for the isotopic values from each plant in each campaign with the isotopic values from the soil water of the corresponding plot. We thereby obtained the most likely contribution (the mean of the posterior distribution of the MCMC simulation) of each source for every plant measurement. These relative contributions were then compared between seasons and species and between control and droughted individuals using analyses of variance (ANOVAs) with Tukey's HSD (honest significant difference) post-hoc tests. Differences in the midday foliar water potentials and stem mortality rates were also evaluated by ANOVAs and Tukey's HSD post-hoc tests. Soil moisture, soil isotopic signatures and crown defoliation were analyzed with generalized linear mixed models (GLMMs) of the MCMCglmm package in R (Hadfield, 2010) for including plot as a random factor. Furthermore, the MCMCglmm package allows fitting multi-response models, and we assessed the changes in soil-water isotopic composition fitting these multi-response models with $\delta^{18}O$ and $\delta^{2}H$ as dependent variables. We selected the model with the lowest DIC (deviance information criterion) when several combinations of independent factors and interactions were possible. All statistical analyses were conducted using R version 2.14.2 (R Core Development Team, 2012).

Results

Environmental data

The study was carried out between 2010 and 2011. The first year was slightly cooler and wetter than the 1975-2011 average (11.0 vs 11.8 °C mean annual temperature and 687 vs 663 mm annual precipitation), but 2011 was slightly warmer and drier than average (13.1 °C and 549 mm). More importantly, rainfall distribution throughout the year differed between the two years. The seasonality of rainfall was typical for this site in 2010, with a wet spring and autumn and a summer drought that lasted two months. In contrast, 2011 had a wet March but afterward was generally very dry, with little precipitation until the end of October. Total precipitation for 142 consecutive days was only 13 mm, without a single rainfall >3 mm, coinciding with the highest temperatures (Fig. 1). This period from April to September was the driest since 1975, as shown by the lowest September SPEI-6 and SPEI-3 for 1975-2011 (Figs. S1 and S2). The droughted plots during the study period had a significantly lower soil-water content than the control plots (17.32±1.56 vs 14.75±1.59%, $pMCMC<0.05$). Moreover, the droughted plots, which had been subjected to the treatment since 1998, had an average reduction of 14.9±1.1% in total soil-water content ($pMCMC<0.01$, for 1998-2011). Soil moisture ranged between 4.7 and 26.4% (v/v) during the period of study.

Midday foliar water potential

The plants in the drought treatment had significantly lower midday foliar water potentials (Ψ_{md}) than the plants in the control plots (-3.1±0.29 vs -2.8±0.28 MPa, F=5.43, n=6, $p<0.05$). Ψ_{md} differed significantly across seasons (F=144.99, $p<0.001$), becoming more negative in the extreme drought in 2011 (Fig. 2), and species (F=49.94, $p<0.001$). The seasonal variation of Ψ_{md} also differed significantly among species, as shown by the interaction between species and seasonal factors (F=12.04, $p<0.001$), and the effect of the drought treatment also varied across seasons (F=3.52, $p<0.05$). Mean Ψ_{md} was significantly lower in *P. latifolia* than in *Q. ilex* and *A. unedo* (-3.71±0.46, -2.48±0.17 and -2.74±0.28 respectively, $p<0.001$, Tukey's HSD test) but did not differ significantly between the latter two species.

Isotopic composition of plant-water sources

$\delta^{18}O$ and $\delta^{2}H$ in the soil water varied with depth and season. Depth was negatively associated with $\delta^{18}O$ and $\delta^{2}H$: the shallow (0-10 cm) soil layer was significantly more enriched in the heavier isotopes of O and H than the deep (10-35 cm) soil layer (posterior mean of the effect (p.m.e.)=-0.12, *pMCMC*<0.001). The drought treatment did not affect $\delta^{18}O$ and $\delta^{2}H$ (*pMCMC*=0.51). The values of $\delta^{18}O$ and $\delta^{2}H$ indicated the seasonal patterns, being more depleted in autumn and winter than in spring and both summers (winter p.m.e.=-0.84, *pMCMC*<0.01; autumn p.m.e.=-1.19, *pMCMC*<0.001; spring p.m.e.=2.04, *pMCMC*<0.001; summer 2010 p.m.e.=1.68, *pMCMC*<0.001; p.m.e. respect isotopic ratios of summer 2011). Soil-water isotopic levels were significantly more enriched in heavier isotopes under the plastic strips (p.m.e.=0.76, *pMCMC*<0.001). Water collected from a nearby spring, having an isotopic signature representative of the deeper water reserves, remained unchanged throughout the seasons ($\delta^{18}O$=-7.19±0.14 and $\delta^{2}H$=-47.34±1.29 ‰). Springwater samples fell along the local meteoric water line (Neal et al 1992) (Fig. 3), indicating that it did not evaporate during infiltration.

Determination of plant-water sources

The mixing model revealed that the canopy species in Prades forest took up water simultaneously from the three well-defined water pools; shallow soil (0-10 cm), deep soil (10-35 cm) and groundwater. The largest proportion was generally from shallow soil (38.7±1.5%), followed by deep soil (31.23±1.4%) and groundwater (30.10±1.5%). Water uptake, however, strongly varied seasonally, as indicated both graphically (Fig. 3) and in the output of the siar models. The statistical assessment of these seasonal shifts of plant-water sources is summarized in Table S1. The shallow soil layer contributed the most to water uptake in autumn and winter (Table S1, Fig. 4), with significantly higher proportions than in the spring and summer of 2010. The contribution of the shallow soil to water uptake during the abnormally dry summer in 2011, although lower than in the cold seasons, was higher than in the spring and summer of 2010 (Table S1). Deep soil (10-35 cm) was the main source of water in the summer and spring of 2010, with lower relative contributions in cold seasons and in summer 2011 (Fig. 4, Table S2 for statistics). Groundwater was the main water source in the summers of 2010 and 2011 (42.84±8.58 and 39.41±2.66% respectively). The siar mixing models, however, attributed a contribution of approximately 25% of the total extracted water to this water pool, even in spring, autumn and winter when surface-soil water levels were high (Table S1, Fig. 4). The xylem samples to the upper left of the soil samples and near the LMWL in Fig. 3 (autumn and winter panels) indicate that in the cold seasons, the plants absorbed recent rainwater, which was not subject to isotopic enrichment by evaporation from the soil surface. The seasonal patterns of water use did not differ significantly among the three species (Fig. 5, Table S3).

The long-term experimental drought treatment significantly affected the depth from which water was taken up in all seasons except for spring 2010 (Fig. 4, Table S2). These effects consisted of differences in the relative contribution of the water sources in response to the drought treatment. The shallow (0-10 cm) soil layer contributed relatively more water to the xylems of the droughted individuals during the summer of 2010 (33.83±4.47 vs 5.58±1.94%, F=46.41, p<0.001, ANOVA; Table S2, Fig. 4). This shallow soil layer, though, contributed less water to the droughted individuals in winter (44.91±2.17 vs 59.71±4.06%, F=10.11, p<0.01, ANOVA; Table S2, Fig. 4). In autumn, the deep (10-35 cm) soil layer contributed relatively more water to the droughted individuals than to the control individuals (32.54±1.57 vs 23.30±1.45%, F=17.68, p<0.001, ANOVA; Table 4, Fig. 4). During the extreme drought in the summer of 2011, the droughted individuals had reduced access to the deep water reserves (groundwater) relative to the control individuals (33.95±2.99 vs 44.64±4.13%, F=4.33, p<0.05, ANOVA; Table S2, Fig. 4). The proportion of groundwater uptake remained <30% when the soil-water content was >15%. The soil-water content was <10% in both summers, coinciding with an increase in the proportion of groundwater taken up by the plants. The increase, however, was higher in the control plants (Fig. 6).

Stem mortality rates and crown defoliation

The extreme drought in the summer of 2011 caused a significant increase in stem mortality rates relative to 2010 (F=5.23, p<0.05, ANOVA). Stem mortality rates were significantly higher in *Q. ilex* than in *P. latifolia* (F=7.79, p<0.05, ANOVA; Fig. 7). *Q. ilex* had the second highest annual stem mortality rate in 2011 since the onset of the experiment in 1998, and *P. latifolia* had the third highest rate for the same period. *A. unedo* was not included in these analyses because of its low sample size. The percentages of crown defoliation following the drought in 2011 were generally significantly higher in the drought treatments than in the control plots (p.m.e.=1.20, *pMCMC*<0.01, MCMCglmm; Fig. 8), except for *P. latifolia* (6.0% difference between treatments, *p*=0.84, ANOVA with Tukey's-HSD post-hoc tests). Defoliation percentages for both *Q. ilex* and *A. unedo* analyzed separately, however, were significantly higher in the drought plots (19.5% difference between treatments for *A. unedo*, *p*<0.01; 20.5% difference between treatments for *Q. ilex*, *p*<0.01; ANOVA with Tukey's-HSD post-hoc tests; Fig. 8).

Discussion

This study investigated the combined effects of a long-term (12 years) experimental drought and an extreme natural drought on the patterns of water uptake by a Holm oak forest growing on shallow soils over schist and so lacked access to a water table. The three species of trees studied have dimorphic root systems that enable access to different water sources in space and time but used water primarily from shallow soil layers but also water stored in the fractured schist. These findings are consistent with those of previous studies in other arid and semi-arid communities (Dawson & Pate, 1996; David *et al.*, 2013; Oshun *et al*., 2014). Interestingly, the relative contribution of groundwater decreased in the drought treatment and during the extreme drought in 2011, suggesting that plant access to deeper groundwater pools had declined over time and in the extreme drought in 2011. This response was often coupled with a decrease in Ψ_{md}, hence suggesting that the plants were subjected to high levels of drought-induced water stress. Recent studies have demonstrated the important role of deep water sources in the response to extreme droughts and their links to tree mortality and species-specific water-use strategies (West *et al.*, 2012; Anderegg *et al.*, 2013b; Kukowski *et al.*, 2013), but the long timescale of this study allowed an assessment of the accumulative effect of experimental drought on root functioning and on the zones of water uptake that helped sustain this functioning.

During seasons in which soil-water content was >15%, the soil-water pool (0-10 and 10-35 cm soil layers combined) supported forest transpiration, with a contribution of at least 75% (Fig. 4). David *et al.* (2013) reported a contribution of soil water near 100% in winter in a more mesic savannah containing *Q. suber* oaks. The vegetation on the steep and shallow soils of our study site thus appeared to require a contribution to transpiration from the deeper groundwater, even during wet seasons. Likewise, the highest transpiration rates occurred in summer in the more mesic sites (David *et al.*, 2013), whereas stomatal conductance decreases in spring and summer in the Prades Holm oak forest (Peñuelas *et al.*, 1998; Ogaya & Peñuelas, 2003), suggesting that the groundwater reserves may not be able to meet the high evaporative demand. The use of groundwater, though, increased in both summers (Fig. 4), confirming that the allocation of growth to deep roots is an advantageous strategy for withstanding very dry periods

(Canadell *et al.*, 1996, 1999). The deeper (10-35 cm) soil horizon at our site supplied most of the water that plants used in the relatively wet spring of 2010. The similar isotopic signatures of the xylem waters in the spring and summer of 2010 (Fig. 3) suggest that rainwater from late winter and spring was used throughout the dry season. This finding is further supported by the highest relative contribution of the water from the deep (10-35 cm) soil horizon in the summer of 2010 (Fig. 4) and by correlations between drought indices and stem mortality (Barbeta *et al.*, 2013). The roots of *Q. ilex* can access bedrock fractures seeking moisture, especially in dry areas (Canadell *et al.*, 1999). We also observed this capacity in the tall shrubs *A. unedo* and *P. latifolia*, in agreement with prior observations of woody Mediterranean species (Canadell & Zedler, 1995; West *et al.*, 2012). In addition, the seasonal patterns of water uptake were consistent for the three plant species we studied (Fig. 5). This finding helps us to rule out the possibility of species-specific use of water sources, suggesting that the reported disparity in their physiological, morphological and demographic responses to drought (Martínez-Vilalta *et al.*, 2003; Ogaya & Peñuelas, 2006; Barbeta *et al.*, 2012, 2013) cannot be directly attributed to rooting depth or seasonal patterns of water uptake, as similarly found in South African fynbos (West *et al.*, 2012).

The seasonal patterns of water uptake varied greatly in the three species. The differences we observed in the use of water sources between the drought and control treatments could be a short-term response to the lower availability of water. The effect of the drought treatment on soil moisture, however, was much weaker than that of the seasonal variation. Because we did not detect differences in plant-water sources between autumn and winter despite the different environmental conditions (highlighted by contrasting plant-water status (Fig. 2)), the higher dependence of the droughted plants on water from the deep (10-35 cm) soil horizon (Fig. 4) does not represent a transient response (*sensu* Martin-Stpaul *et al.*, 2013) but a persistent shift in the vertical distribution of fine roots induced by our long-term experiment. Furthermore, short-term experimental drought may not affect the depth of water uptake in trembling aspens (Anderegg *et al.*, 2013b), although the water sources for this species varied little seasonally.

Q. ilex has less fine-root biomass in the top 10 cm of soil than in deeper layers (Canadell *et al.*, 1999; López *et al.*, 2001), arguably because the elevated soil temperatures in summer in Mediterranean ecosystems can dehydrate or even kill fine roots. Consequently, the decrease in soil moisture induced by the drought treatment may have exacerbated this situation, favoring the production of fine roots in deeper soil layers. In contrast, the lower contribution of groundwater in droughted plants in the summer of 2011 relative to the summer of 2010 (Figs. 4 and 6) may be a direct consequence of a decreased recharge of the groundwater or water that resides within the bedrock fractures during rainy seasons. The capacity of plants to redistribute their fine roots within the soil profile, based on our results, is evidence of phenotypic plasticity in a key trait of the plant-water relationship (root functional distribution). Understanding the limits of species-specific plasticity for any trait or suite of traits is crucial for predicting the responses of species to environmental change (Matesanz & Valladares, 2013; Moritz & Agudo, 2013). Together with ecosystem structural changes (Lloret *et al.*, 2012), species-specific responses are likely to help buffer plants against the negative effects of climate change. A dampening of the drought treatment has also been observed in our study system (Barbeta *et al.*, 2013; Rosas *et al.*, 2013); the effect of the drought treatment on tree growth tended to decrease over time. A shift in the distribution of fine roots would thus be another possible factor leading to a dampening pattern, along with other alterations such as reductions in foliar area (Ogaya & Peñuelas, 2006; Limousin *et al.*, 2009) and adjustments of xylem hydraulic properties (Martin-Stpaul *et al.*, 2013).

The vegetation faced average meteorological conditions in the summer of 2010, but April to September 2011 was extraordinarily dry (Poyatos *et al.*, 2013), increasing tree mortality in *Q. ilex* (Fig. 7) and inducing widespread crown defoliation, especially in *A. unedo* and *Q. ilex* (Ogaya *et al.*, 2014) (Fig. 8). The levels of soil moisture in the upper 25 cm, however, were not substantially different between the summers of 2010 and 2011 (Fig. 1), and Ψ_{md} was more negative in the three species in 2011 (Fig. 3). Plants extracted significantly more water from the 10-35 cm soil horizon and less from the 0-10 cm soil horizon during the moderate drought of 2010 than during the drier summer of 2011 (Fig. 4), suggesting that the drought-induced forest decline may have been associated with the lower contributions of deep soil-water reserves to the uptake of water by the trees. The characteristics of the geological substrate (Lloret *et al.*, 2004), soil depth (Galiano *et al.*, 2012) and soil-water storage capacity may thus interact with extreme droughts to determine the patchy landscape of forest declines. Accordingly, the use of deep water reserves are likely required for the maintenance of transpiration and carbon assimilation during droughts in Mediterranean oaks (Canadell *et al.*, 1996; David *et al.*, 2007; 2013). The more anisohydric *P. latifolia*, however, was less affected by the acute drought in 2011 (Fig. 7), and its Ψ_{md} and crown defoliation appeared to be insensitive to the drought treatment despite a similar depth of water uptake. We attribute this response to its higher resistance to xylem embolism (Martínez-Vilalta *et al.*, 2002), which allows this species to maintain carbon assimilation under water stress. The depth of water uptake in this community thus did not seem to co-vary across species with other hydraulic properties, such as xylem anatomy and stomatal regulation. Even though the depth of water uptake did not vary across species, the absolute quantity of water transpired by each species is likely to differ. The species-specific seasonal patterns of transpiration rates should be combined with the depth of water uptake to obtain a complete picture of species-specific water use. Moreover, some of the species studied may be able to move water through roots at different depths (hydraulic lift and downward siphoning), which could mask the impossibility of the roots of the other species to reach deep water reserves.

The lack of hydraulic niche segregation among the co-occurring species in this Holm oak forest contrasts with the findings of other recent studies in other Mediterranean systems (Araya *et al.*, 2011; Peñuelas *et al.*, 2011; West *et al.*, 2012). It implies that the three species could be competing for the same water resources in space. The seasonal resolution of our measurements, however, prevented us from assessing species-specific differences in the timing of water use. The projected increase in the recurrence of extreme droughts, though, could favor the more drought-resistant *P. latifolia* over *Q. ilex* and *A. unedo*. Changes in the distribution of fine roots, as suggested by our data, could buffer the species against environmental change to some extent, but we also found that an extreme drought could cause widespread defoliation and tree mortality in *Q. ilex* and *A. unedo* (Ogaya *et al*., 2014) (Fig. 8), associated with a reduction in groundwater uptake by these species in the drought treatment. Long and intense periods of drought such as occurred during the summer of 2011 will thus likely threaten the preservation of this community in its current structure and composition, and these effects will presumably be amplified by a larger depletion of deep water reserves after several extreme droughts (see Schwinning, 2010).

The impact of recent climatic changes and particularly more acute and prolonged droughts on groundwater reserves is not well understood (Brolsma *et al.*, 2010; Anderegg *et al.*, 2013a; Schäfer *et al.*, 2013). The present study suggests that extreme drought and moderate but persistent drier conditions (simulated by our manipulation) may lead to the depletion of water reservoirs from groundwater and weathered bedrock in this system. Mortality and high defoliation levels may reduce canopy transpiration and interception, which could ultimately trigger an increase in groundwater recharge. Future studies should examine the ability of the impacts of future climate on vegetation to offset the effects of a decline in precipitation and an increase in surface evaporation on groundwater recharge.

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Figure legends

Fig. 1 Daily precipitation and mean temperatures during the study period (2010-2011). Soil moisture in the control and drought plots. The error bars are the standard errors of the means (n=2). Arrows indicate the sampling campaigns.

Fig. 2 Seasonal variation in midday foliar water potentials of the three species for control (open circles) and droughted (closed circles) individuals. The droughted plants had significantly lower midday foliar water potentials (F=5.43, p<0.05, ANOVA). Differences between seasons and species are described in the Results section.

Fig. 3 Water isotopes for all samples of xylem (triangles), soil (circles) and spring (squares) water. All samples are plotted in the upper left panel, with the remaining panels corresponding to single seasons. The line in the panels is the local meteoric water line (LMWL), corresponding to $\delta^{2}H=6.62+7.60*\delta^{18}O$ with R^2=96.03%, obtained by a previous study in the same area (Neal *et al.*, 1992).

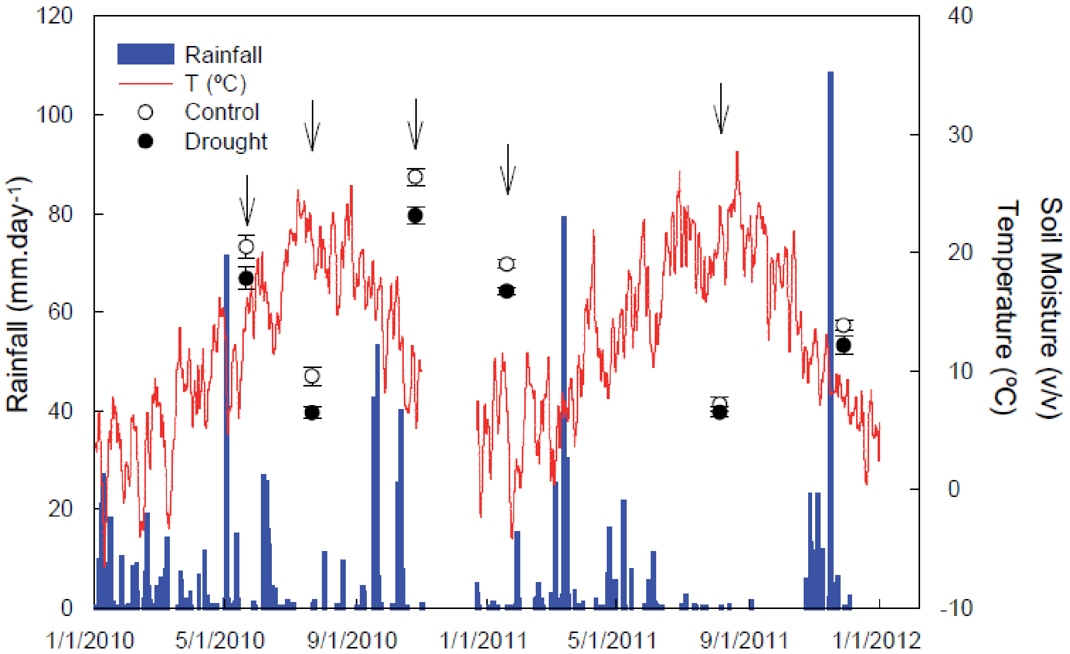
Fig. 4 Mean **c**ontributions of plant-water sources for each season in the control and drought treatments obtained by siar Bayesian mixing models. The error bars are the standard errors of the means. The asterisks denote significance levels for the comparisons between the control and drought treatments performed by ANOVAs and Tukey's HSD post-hoc tests (*** p<0.001, **p<0.01, *p<0.05, (*) p<0.1).

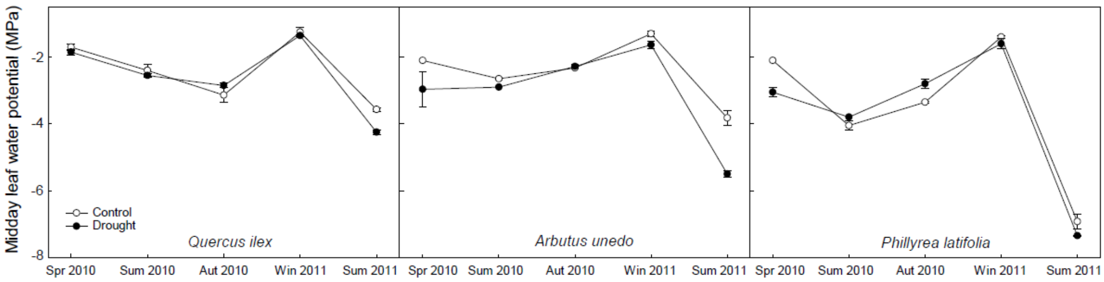
Fig. 5 Seasonal percentages of groundwater uptake in the three species for each season. The errors bars are the standard errors of the means.

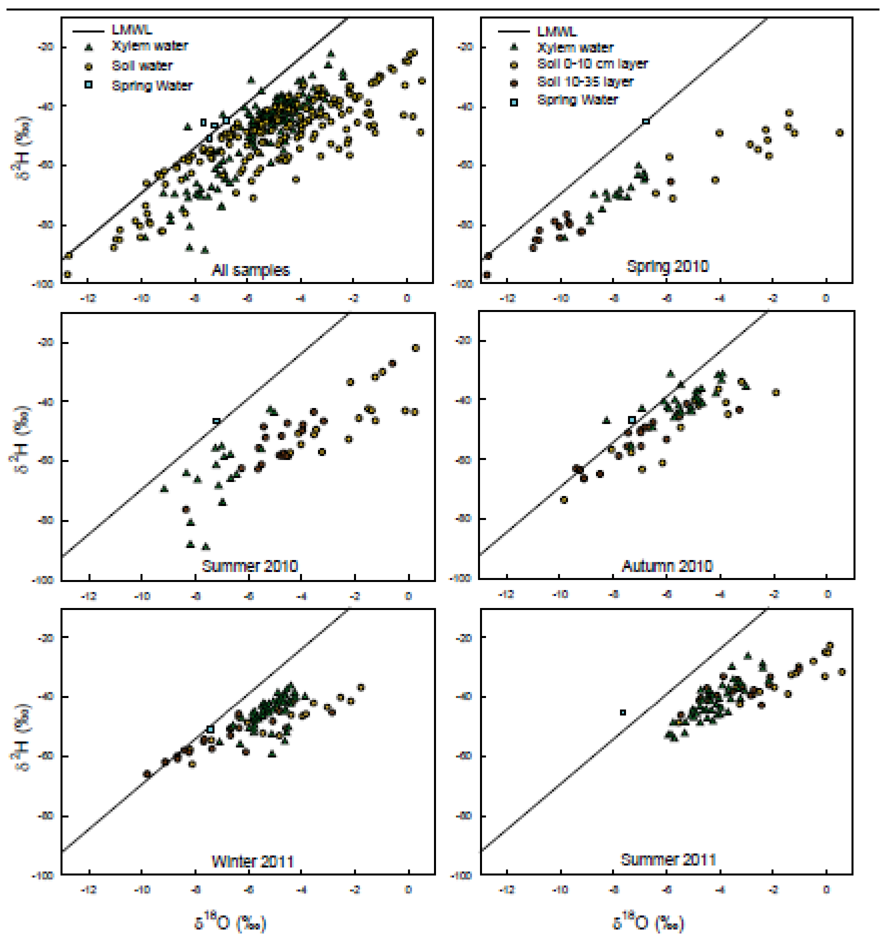
Fig. 6 Relationship between percentage of groundwater uptake and soil moisture in the two treatments. The Y-axis values are the mean seasonal proportions of groundwater uptake for each treatment, and the three species are pooled. The error bars are the standard errors of the means.

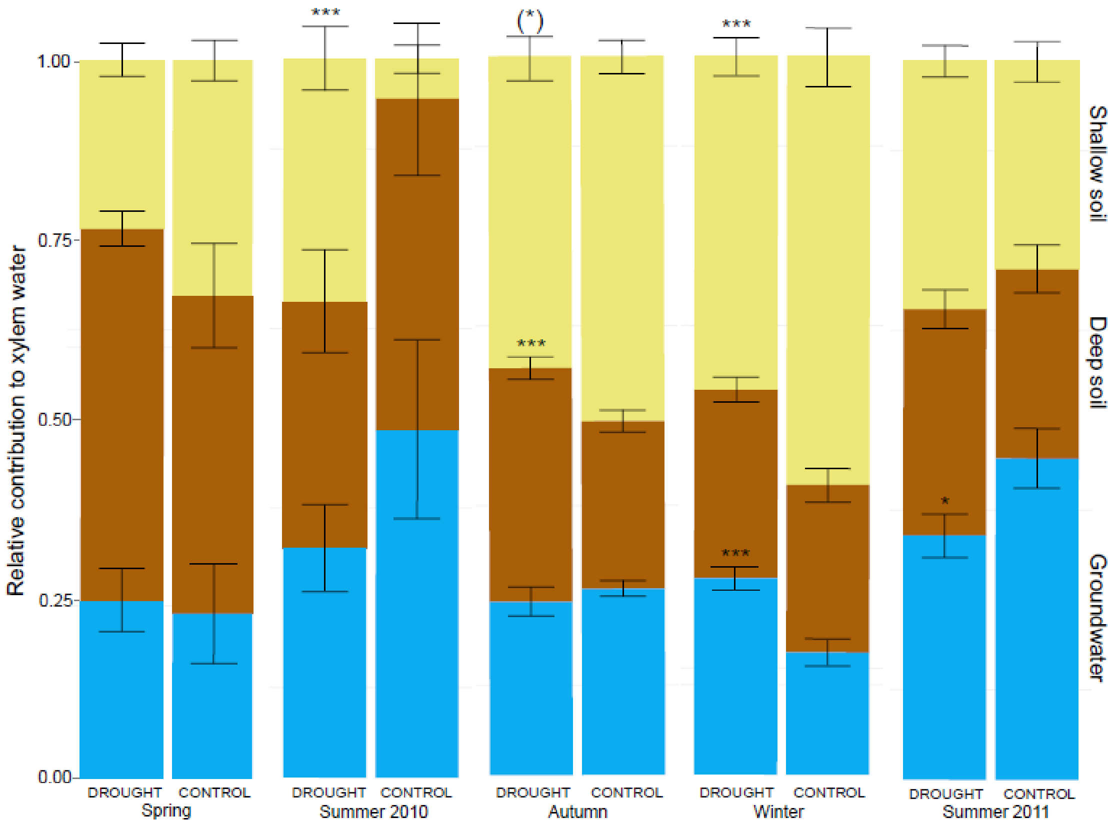
Fig. 7 Stem mortality rates for *Quercus ilex* and *Phillyrea latifolia* (2010 and 2011) calculated for the plots where the isotope samples were collected. Different letters indicate significantly different stem mortality rates, which were assessed by ANOVAs (p<0.05).

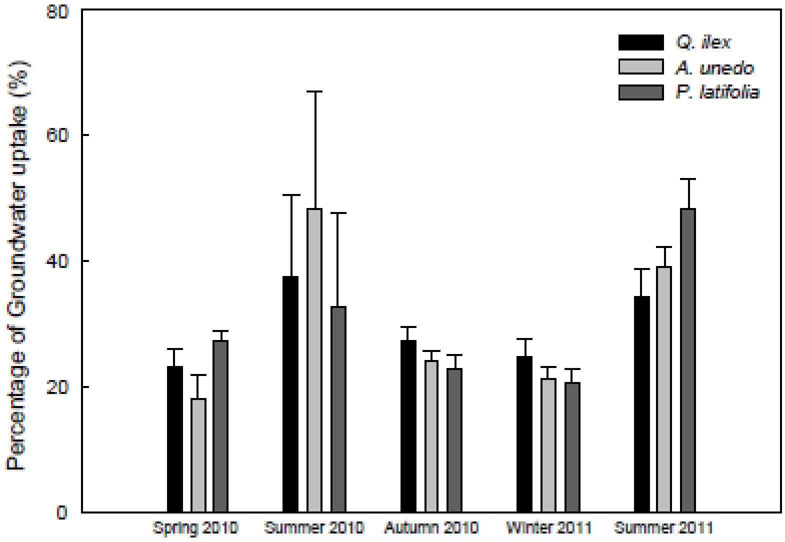
Fig. 8 Crown defoliation (%) following the extreme drought in 2011 for each species and treatment for the plots where the isotope samples were collected. The error bars are the standard errors of the means (n=10). Different letters indicate significant differences between group percentages, assessed by ANOVAs with Tukey's HSD post-hoc tests. The differences between the treatments pooling all species together were assessed using generalized linear mixed models (MCMCglmm) with plot as a random factor.

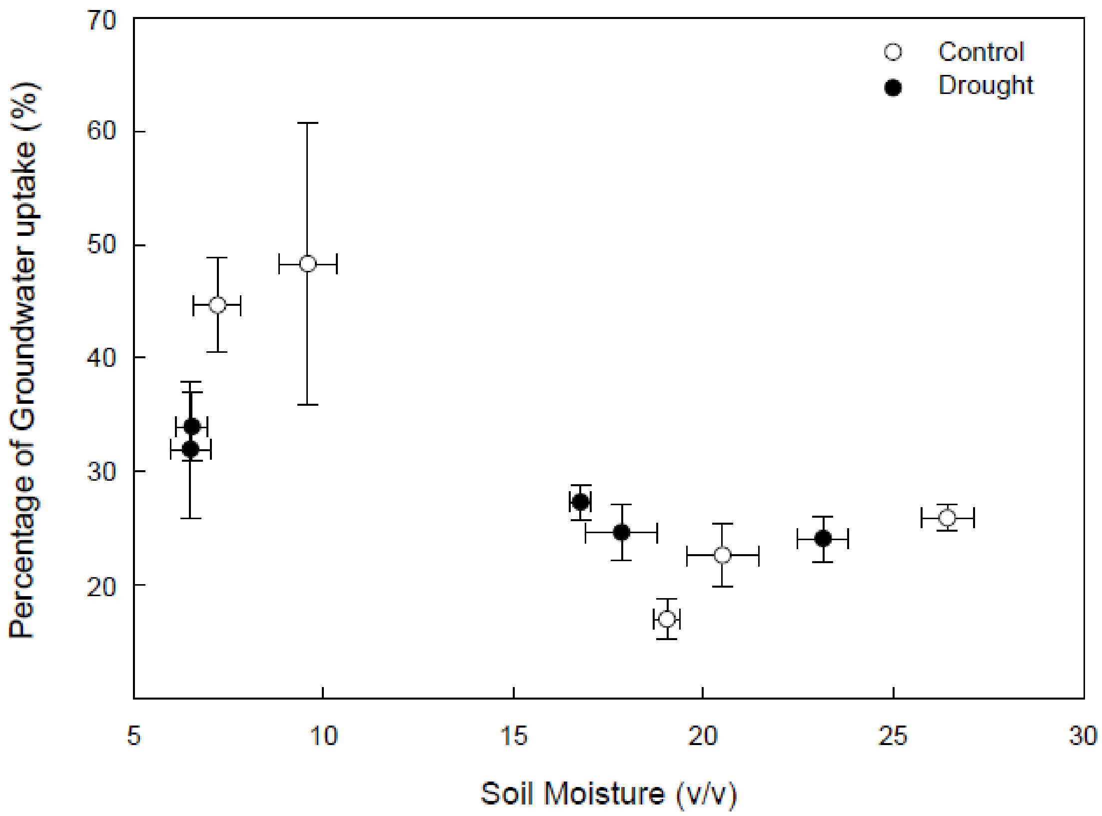

Rainfall
T (ºC)
Control
Drought
Rainfall (mm.day-1)
Soil Moisture (v/v)
Temperature (ºC)
1/1/2010
5/1/2010
9/1/2010
1/1/2011
5/1/2011
9/1/2011
1/1/2012

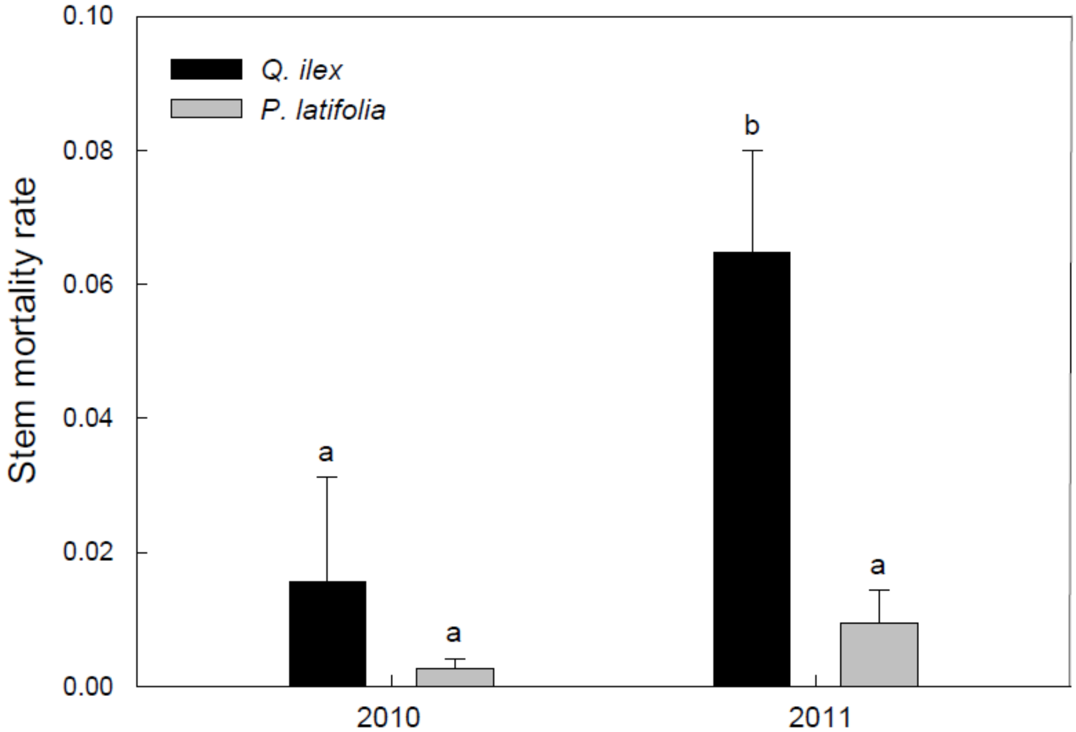

Midday leaf water potential (MPa)
-2
-4
-6
-8
Control
Drought
Quercus ilex
Arbutus unedo
Phillyrea latifolia
Spr 2010
Sum 2010
Aut 2010
Win 2011
Sum 2011


LMWL
Xylem water
Soil water
Spring Water
All samples
LMWL
Xylem water
Soil 0-10 cm layer
Soil 10-35 layer
Spring Water
Spring 2010
Summer 2010
Autumn 2010
Winter 2011
Summer 2011
δ^{2}H (‰)
δ^{18}O (‰)

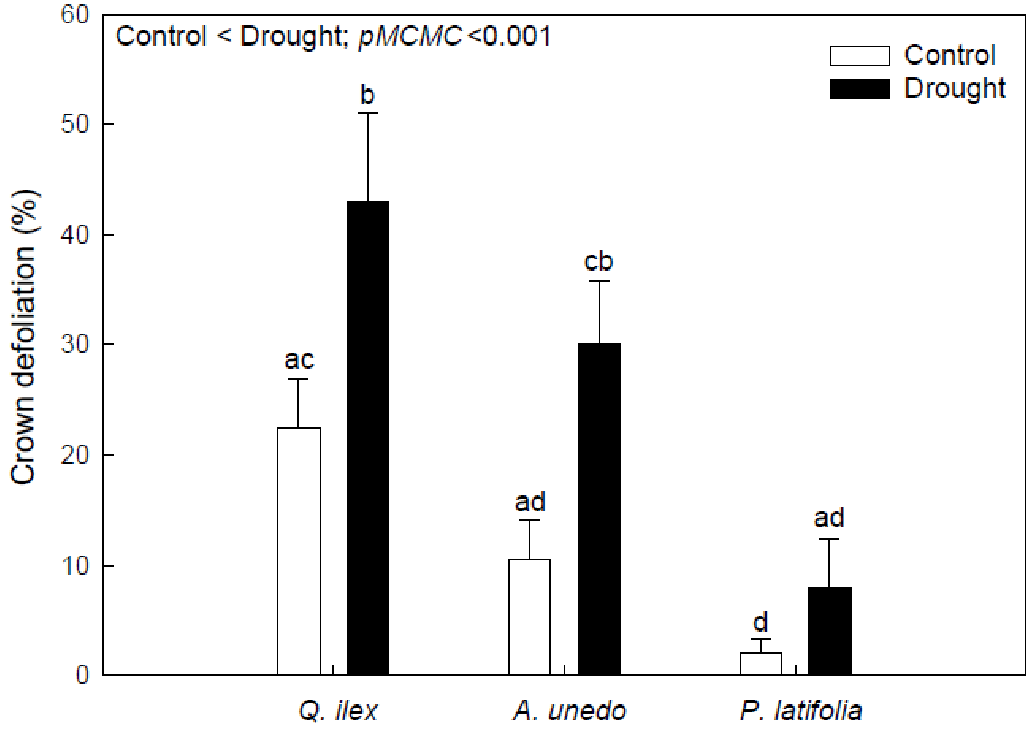

Relative contribution to xylem water
1.00
0.75
0.50
0.25
0.00
***
(*)
***
***
***
*
DROUGHT
CONTROL
Spring
Summer 2010
Autumn
Winter
Summer 2011
Shallow soil
Deep soil
Groundwater


Percentage of Groundwater uptake (%)
0
20
40
60
80
Q. ilex
A. unedo
P. latifolia
Spring 2010
Summer 2010
Autumn 2010
Winter 2011
Summer 2011


Control
Drought
Percentage of Groundwater uptake (%)
70
60
50
40
30
20
5
10
15
20
25
30
Soil Moisture (v/v)



Stem mortality rate
0.10
0.08
0.06
0.04
0.02
0.00
Q. ilex
P. latifolia
a
a
b
a
2010
2011



Control < Drought; pMCMC <0.001
Control
Drought
Crown defoliation (%)
0
10
20
30
40
50
60
ac
b
ad
cb
d
ad
Q. ilex
A. unedo
P. latifolia

Supplementary Materials

Table S1. Pairwise comparison between the relative seasonal contributions of water sources using Tukey's HSD post-hoc tests. The mean difference is between pairs of seasons, and the range is the 95% confidence interval. The asterisks denote significance levels (*** p<0.001, **p<0.01, *p<0.05, (*) p<0.1).

Shallow soil (0-10 cm)	Mean difference	Range	*p*	
Winter 2011 - Summer 2011	0.21	0.08	<0.001	***
Summer 2010 - Summer 2011	-0.17	0.11	<0.001	***
Spring 2010 - Summer 2011	-0.05	0.12	0.767	
Autumn 2010 - Summer 2011	0.15	0.10	<0.001	***
Summer 2010 - Winter 2011	-0.37	0.11	<0.001	***
Spring 2010 - Winter 2011	-0.26	0.11	<0.001	***
Autumn 2010 - Winter 2011	-0.06	0.09	0.414	
Spring 2010 - Summer 2010	0.12	0.14	0.124	
Autumn 2010 - Summer 2010	0.32	0.12	<0.001	***
Autumn 2010 - Spring 2010	0.20	0.12	<0.001	***
Deep soil (10-35 cm)				
Winter 2011 - Summer 2011	-0.04	0.09	0.804	
Summer 2010 - Summer 2011	0.13	0.13	0.041	*
Spring 2010 - Summer 2011	0.20	0.13	<0.001	***
Autumn 2010 - Summer 2011	0.00	0.11	1.000	
Summer 2010 - Winter 2011	0.17	0.13	0.003	**
Spring 2010 - Winter 2011	0.24	0.13	<0.001	***
Autumn 2010 - Winter 2011	0.03	0.11	0.896	
Spring 2010 - Summer 2010	0.07	0.16	0.724	
Autumn 2010 - Summer 2010	-0.14	0.14	0.056	(*)
Autumn 2010 - Spring 2010	-0.21	0.14	<0.001	***
Groundwater				
Winter 2011 - Summer 2011	-0.17	0.09	<0.001	***
Summer 2010 - Summer 2011	0.03	0.13	0.947	
Spring 2010 - Summer 2011	-0.16	0.13	<0.05	*
Autumn 2010 - Summer 2011	-0.15	0.11	0.003	**
Summer 2010 - Winter 2011	0.20	0.13	<0.001	***
Spring 2010 - Winter 2011	0.01	0.13	0.998	
Autumn 2010 - Winter 2011	0.02	0.11	0.972	
Spring 2010 - Summer 2010	-0.19	0.16	0.009	**
Autumn 2010 - Summer 2010	-0.18	0.14	0.004	**
Autumn 2010 - Spring 2010	0.01	0.14	1.000	

Table S2. Evaluation of the effect of the drought treatment and the species factor plus their interaction on the relative seasonal contribution of water sources by ANOVAs. The asterisks denote significance levels (*** *p*<0.001, ***p*<0.01, **p*<0.05, (*) *p*<0.1).

Shallow soil (0-10 cm)	Df	SS	Mean Sq	*F*	*p*	
Drought	1	0.034	0.034	1.967	0.188	
Species	2	0.000	0.000	0.01	0.990	
Drought x Species	2	0.024	0.012	0.695	0.520	
Residuals	11	0.192	0.017			
Drought	1	0.319	0.319	81.876	<0.001	***
Species	2	0.003	0.001	0.32	0.732	
Drought x Species	2	0.061	0.030	7.795	0.007	
Residuals	12	0.047	0.004			
Drought	1	0.041	0.041	3.123	0.090	(*)
Species	2	0.028	0.014	1.064	0.361	
Drought x Species	2	0.002	0.001	0.076	0.927	
Residuals	24	0.312	0.013			
Drought	1	0.279	0.279	10.942	0.002	**
Species	2	0.126	0.063	2.461	0.097	
Drought x Species	2	0.079	0.040	1.551	0.223	
Residuals	45	1.147	0.026			
Drought	1	0.035	0.035	2.476	0.124	
Species	2	0.022	0.011	0.763	0.473	
Drought x Species	2	0.055	0.028	1.965	0.154	
Residuals	39	0.550	0.014			
Deep soil (10-35 cm)						
Drought	1	0.021	0.021	0.765	0.400	
Species	2	0.013	0.007	0.237	0.793	
Drought x Species	2	0.051	0.026	0.929	0.424	
Residuals	11	0.304	0.028			
Drought	1	0.057	0.057	0.308	0.589	
Species	2	0.024	0.012	0.064	0.939	
Drought x Species	2	0.105	0.052	0.283	0.758	
Residuals	12	2.219	0.185			
Drought	1	0.063	0.063	15.987	<0.001	***
Species	2	0.005	0.002	0.579	0.568	
Drought x Species	2	0.001	0.000	0.083	0.920	
Residuals	24	0.094	0.004			
Drought	1	0.016	0.016	1.597	0.213	
Species	2	0.037	0.019	1.883	0.164	
Drought x Species	2	0.041	0.021	2.085	0.136	
Residuals	45	0.447	0.010			
Drought	1	0.029	0.029	1.387	0.246	
Species	2	0.053	0.026	1.238	0.301	
Drought x Species	2	0.011	0.005	0.256	0.775	
Residuals	39	0.827	0.021			

Groundwater						
Drought	1	0.002	0.002	0.33	0.577	
Species	2	0.014	0.007	1.507	0.264	
Drought x Species	2	0.021	0.010	2.166	0.161	
Residuals	11	0.053	0.005			
Drought	1	0.107	0.107	0.627	0.444	
Species	2	0.033	0.017	0.098	0.907	
Drought x Species	2	0.074	0.037	0.218	0.808	
Residuals	12	2.038	0.170			
Drought	1	0.002	0.002	0.5	0.486	
Species	2	0.010	0.005	1.025	0.374	
Drought x Species	2	0.002	0.001	0.206	0.815	
Residuals	24	0.117	0.005			
Drought	1	0.162	0.162	26.477	<0.001	***
Species	2	0.026	0.013	2.142	0.129	
Drought x Species	2	0.006	0.003	0.508	0.605	
Residuals	45	0.275	0.006			
Drought	1	0.128	0.128	4.485	0.041	*
Species	2	0.135	0.067	2.351	0.109	
Drought x Species	2	0.025	0.013	0.441	0.647	
Residuals	39	1.117	0.029			

Fig. S1 Temporal series (1975-2011) of the Standardized Precipitation and Evapotranspiration Index (SPEI) for September calculated for two timescales: 3 months (black line), which integrates the water balances of July, August and September, and 6 months (red line), which integrates the water balances of April, May, June, July, August and September. Note that both indices reached the period's minimum in 2011.

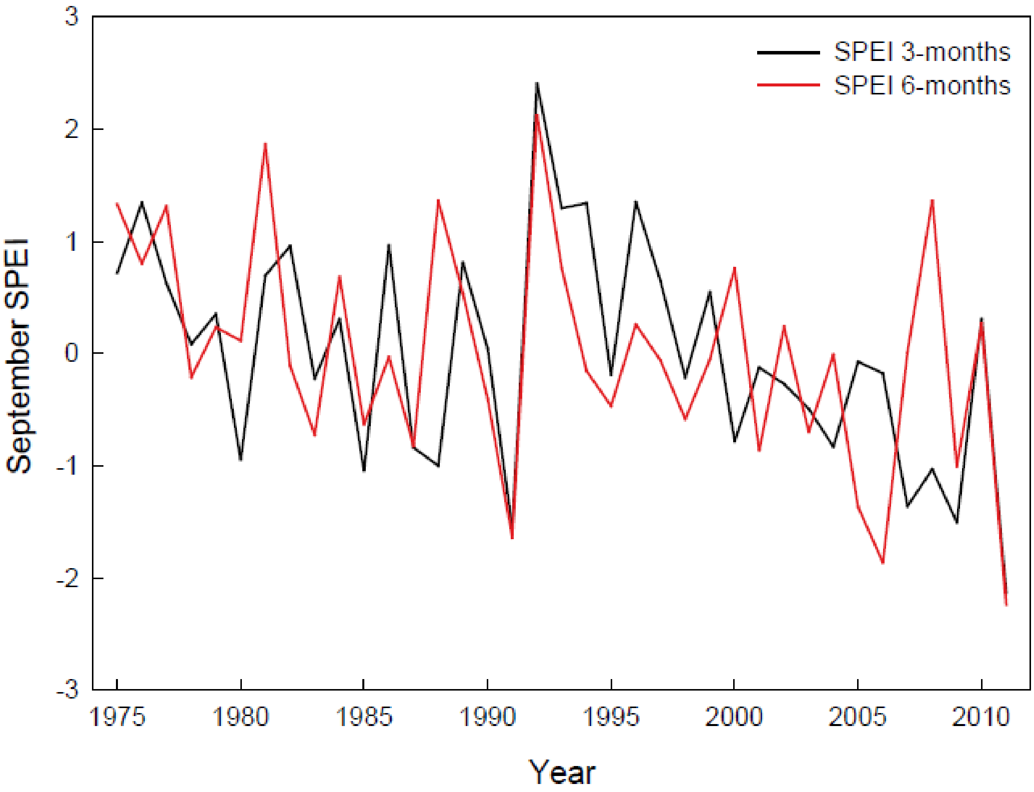


Fig. S2 Monthly values of the Standardized Precipitation and Evapotranspiration Index (SPEI) during the study period (2010, black line; 2011, red line). Each panel corresponds to the timescale at which the index was calculated (1 month, 3 months, 6 months and 12 months).