Forest acclimation to increasing drought: structural and functional changes.

Ph. D. Thesis

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A la Monica,

als meus pares

i a les meves germanes

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Resum

El canvi climàtic està reduint la precipitació i canviant-ne els patrons temporals en algunes regions com la conca mediterrània, cosa que es preveu que s'intensifiqui en les pròximes dècades. L'increment de temperatura, per altra banda, amplifica els efectes de la sequera ja que augmenta l'evaporació de l'aigua del sòl i la demanda evaporativa de l'atmosfera. En els últims anys, hi hagut un augment en els casos de declivi forestal relacionats amb la sequera. Aquests declivis comporten la defoliació de capçada i la mortalitat d'individus i acostumen a ser provocats per un sol episodi de intensitat i/o durada extrema. Tanmateix, també s'han relacionat canvis a gran escala en la composició i l'estructura dels boscos amb la reducció progressiva de la disponibilitat hídrica. La sensibilitat a la sequera pot variar entre espècies que coexisteixen en un mateix ecosistema, així que un augment de la sequera podria produir canvis en la composició específica i profundes modificacions en l'estructura i composició dels ecosistemes. Per tant, una major comprensió de les estratègies de l'ús de l'aigua de les diferents espècies d'un ecosistema podria ajudar a dilucidar quins són els trets fisiològics i morfològics clau en la resposta a la sequera, i a anticipar futurs canvis en l'estructura i la funció d'aquests ecosistemes. En aquest sentit, els experiments de sequera són una eina útil. Normalment, però, aquests experiments són de curta durada, mentre que les espècies llenyoses són organismes de llarga vida. En conseqüència, els canvis en les comunitats que s'observen a curt termini poden no ser representatius del que pot passar a llarg termini. Per aquesta raó, els experiments a llarg termini ofereixen la oportunitat d'entendre millor la dinàmica dels boscos enfront de l'augment de la sequera. En aquesta tesi, he investigat els efectes de la sequera experimental a llarg termini i de la sequera natural en alzinars (*Quercus ilex*). He estudiat com la sequera afecta el transport de l'aigua des de sota terra fins a l'atmosfera a nivell individual en espècies amb diferent sensibilitat a la sequera. A nivell de bosc, he avaluat si l'efecte de la sequera experimental sobre les taxes de creixement i mortalitat canvia amb el temps i/o després d'episodis de sequera extrema. També he realitzat una síntesis quantitativa sobre l'ús de l'aigua freàtica per part de les plantes estimat amb isòtops estables.

Els resultats d'aquesta tesi confirmen que espècies coexistents presenten diferents estratègies d'ús de l'aigua; les alzines (*Quercus ilex*) podrien ser parcialment substituïdes pel fals aladern (*Phillyrea latifolia*) en els llocs més secs ja que aquest arbust és capaç de transpirar majors quantitats d'aigua durant la sequera. Certament, les taxes de creixement i

mortalitat de *P. latifolia* no es veuen afectades per la sequera experimental mentre que *Q. ilex* presenta una major mortalitat, i tan *Q. ilex* com *Arbutus unedo* creixen menys a les parcel·les de sequera. De tota manera, també s'ha vist com l'efecte de la sequera experimental es va reduint amb el temps. La major mortalitat de peus en les parcel·les de sequera sembla reduir la competència pels recursos hídrics, esmorteint l'efecte del tractament. L'experiment també ha produït canvis en les fonts d'aigua de les plantes, cosa que podria contribuir a l'esmentat esmorteïment de l'efecte de la sequera experimental. A més a més, també he realitzat una revisió bibliogràfica sobre la seqüència de respostes a la sequera dels alzinars. L'alzina és una espècie amb diversos mecanismes i trets que li permeten sobreviure i recuperar-se de les sequeres, però sembla que sequeres extremadament llargues produeixen la pèrdua del fullatge o inclús la mortalitat d'individus sencers. Aquesta espècie té la capacitat de rebrotar vigorosament després d'aquests episodis i s'aclimata a condicions més seques, però no és clar si sequeres més recurrents poden afectar la seva resiliència. Finalment, informo sobre la ubiqüitat de l'ús d'aigües freàtiques o profundes per part de les plantes. Aquest és una font d'aigua crucial, més enllà de la posició topogràfica, el clima, o el tipus de planta. Tot i això, les plantes en ecosistemes riparis i de climes àrids en fan servir una proporció més alta que les plantes d'altres climes o posicions topogràfiques. En resum, els arbres, i els boscos que conformen, tenen diversos mecanismes que permeten que amb el temps, es puguin aclimatar a condicions més seques. Tanmateix, no és gens clar que aquesta aclimatació pugui contrarestar els efectes negatius de l'augment de la freqüència, intensitat i durada de les sequeres.

Abstract

The anthropogenic climate change is producing a reduction of the precipitation and a change in its regime in regions such as the Mediterranean Basin, which will be intensified during the next decades. Warmer temperatures are amplifying the effects of droughts by enhancing soil water evaporation and increasing the atmospheric evaporative demand. In recent years, there has been an increase in reports of forest declines related to drought. They involved widespread crown defoliation and mortality and were usually related to a single extreme drought episode. However, other large-scale changes in forest structure and composition have been linked to the progressive reduction of water availability. Co-occurring woody species may show contrasting drought sensitivity, so increasing drought may produce shifts in species composition and deep modifications of ecosystems' structure and function. Therefore, understanding species-specific water-use strategies may help elucidate which physiological and/or morphological traits play a key role in determining the effects of droughts on forests. In this sense, drought experiments are a useful tool, but they are usually of short duration while woody species are long-lived organisms. Changes in plant communities occurred on the short-term may not be representative of the future performance of individuals and communities. For this reason, long-term experiments can provide better insights on the dynamics of forests. In this thesis, I examined the effect of long-term (13 years) experimental and natural droughts on holm oak (*Quercus ilex*) forests. I studied how drought affects the movement of water from belowground to the atmosphere at the individual level in co-occurring species with different drought sensitivity. At the stand-level, I evaluated whether the effect of experimental drought on growth and mortality rates changes over time and after extreme drought events. I also performed a quantitative synthesis on the use of groundwater by plants as estimated with stable water isotopes.

The results of this thesis confirm that co-occurring species present contrasting water-use strategies; the tree *Quercus ilex* may be out-competed by the tall shrub *Phillyrea latifolia* in the driest sites since the latter transpires larger amounts of water during dry periods. Indeed, the growth and mortality rates of *P. latifolia* were not affected by experimental drought while *Q. ilex* showed higher mortality rates and both *Q. ilex* and *A. unedo* showed lower growth rates. However, I report that the effect of the experimental drought is reduced over time. Relatively higher mortality of stems in the drought plots reduce competition between stems and offsets the effect of treatment. The experiment also produced changes in the plant water sources that

contribute to dampen the effect of the drought treatment. I also reviewed the sequence of drought responses of holm oak forests at different time and organizational scales. *Q. ilex* is a species with mechanisms and traits that allow it to survive and recover from droughts, but extremely long droughts produce on it extensive crown damage. It can still resprout vigorously after such episodes and can acclimate to persistent drier conditions, but it is unclear whether more recurrent extreme droughts can affect its resilience. Finally, I report the widespread occurrence of groundwater uptake by plants. Groundwater is critical regardless of the landscape position, climate and plant anatomy, although plants in riparian ecosystems and dry climates use relatively more groundwater. Summing up, tree individuals and forests have mechanisms and processes related to water use that help them acclimate to drier conditions over time. Nonetheless, it has to be elucidated to which extent this acclimation can offset the negative effects of increasing drought.

Chapter I

General Introduction

WHY SHOULD WE INVESTIGATE THE ECOLOGY OF FORESTS?

Forests cover approximately the 30% of the global land surface, ca. 42 million km^2 distributed among tropical, temperate, and boreal lands, and represent the 45% of terrestrial carbon and the 50% of terrestrial net primary production (Bonan, 2008). Such a large extent and an important relative weight in terms of carbon and ecosystem productivity confer to forests a critical role as regulators of the global carbon cycle. It has been estimated that globally, the forest carbon sink is 4.05±0.67 Pg C year^{-1}, although current land-use changes in the tropics reduce the actual net sink flux to 1.1±0.8 Pg C year^{-1} (Pan *et al.*, 2011). Therefore, forests are a major compartment in the carbon cycle as important as the ocean and consequently influence Earth's climate through the greenhouse effect of carbon dioxide. Forests play a key role in the water cycle as well. It is reported that 64% of the evapotranspiration flux is returned to the atmosphere via plant transpiration and another 27% is intercepted and evaporated from the vegetation cover (Good *et al.*, 2015). These proportions strongly vary as a function of the vegetation type, i.e. the proportion of transpiration and canopy interception is higher in forested ecosystems, as well as the overall contribution to global evapotranspiration (Schlesinger & Jasechko, 2014). By depleting soil water and groundwater through root water uptake and delivering it to the atmosphere through the canopy, forests reduce the amount of water that infiltrates to streams, as it has been shown by the effects of widespread defoliation on watershed-level groundwater discharge on rivers (Bearup *et al.*, 2014). Furthermore, the belowground water movement mediated by roots enhances seasonal transpiration rates, which in turn increase the atmospheric humidity at the regional scale (Lee *et al.*, 2007). In parallel to that, the role of forests in both the water and carbon cycle are tightly linked by photosynthesis. In order to absorb carbon dioxide that will be photosynthesized into sugars, trees (like the other plants) open their stomata and release water due to the different water partial pressure between leaves and the atmosphere (Cochard, 2013). If water availability is limited, the stomata may be partially or totally shut and so carbon dioxide will not be absorbed. Hence, changes in the water cycle will impact the carbon cycle through the hydraulic behavior of plants, among which, trees are most relevant since they have more biomass for photosynthesis and transpiration.

Notwithstanding the paramount role of forests in the carbon and water global cycles, forests have many other ecological functions, and many of them are useful for society. Since

the dawn of humanity, forests were the source of firewood and timber and offered a variety of natural products ready to be collected for alimentary and medicinal uses. Traditional societies associated with forest ecosystems still rely strongly on these products, but modern societies with access to industrial supplies also still use these products even when their necessity is merely recreational (e.g. the mushroom picking fever in Catalonia). Therefore, forests are extremely important not only for their role in global biogeochemical cycles, but also because they harbor biodiversity and commodities that are useful for humans. Research on forest ecology can be thus applied to solve particular problems related to the services they provide to socio-environmental systems. Nevertheless, fundamental ecology, i.e. the work undertaken primarily to acquire new knowledge of the underlying foundations of phenomena and observable facts, is also essential since it is more likely to produce unexpected results, challenge accepted thinking and generate new research fields (Courchamp *et al.*, 2015).

FORESTS IN A DRIER WORLD

In recent years, a warming of the Earth's climate has occurred (IPCC, 2013). The warming trend is linked to an increase of the atmospheric concentration of greenhouse gases such as carbon dioxide, methane and others caused by human activity. The sole increasing trend in temperature without any decrease in precipitation is enough to increase the drought risk (Vicente-Serrano *et al.*, 2014; Diffenbaugh *et al.*, 2015). Indeed, the atmospheric water demand increases nonlinearly with temperatures during drought (Allen *et al.*, 2015). In addition, the alteration of atmospheric circulation is likely to produce longer and more intense droughts with a shorter period of return in regions such as the Mediterranean Basin (IPCC, 2013). In the past, periods with abundant precipitation enhancing pasture production have been the spark that produced the appearance of empires such as Mongolia's Genghis Khan (Pederson *et al.*, 2014). Nowadays, the anthropogenic climatic change is behind the drought in the Eastern Mediterranean that has contributed to the outbreak of the war in Syria (Kelley *et al.*, 2015). This increase in drought has already affected ecosystems and croplands and has echoed dramatically into society. In the case of forests, there are a growing number of observations in almost all of the worlds' biomes reporting drought-induced forest declines, involving extensive crown defoliation and increased mortality rates (Allen *et al.*, 2010). In many cases, forest declines are restricted to a certain area and/or species (Mueller *et al.*, 2005), or show a patchy pattern due to the topographic and edaphic heterogeneity (Lloret *et*

al., 2004). These episodes are likely to affect local fluxes of water, carbon and radiation (Anderegg *et al.*, 2013a).

Large-scale alterations of forest structure and function have been also linked to the amplified effect of drought caused by higher temperature. In southern Europe, forest inventories revealed that defoliation levels have consistently increased in sixteen of the most relevant tree species (Carnicer *et al.*, 2011) and the recruitment of certain species seems to fail in response to increased aridity (Carnicer *et al.*, 2014). At a similar latitude, in California, it has been recently reported that the increase in water deficit experienced during the last seventy years has entailed large-scale changes in forest structure involving higher forest densities, smaller trees and a trend to shifts in dominance (Mcintyre *et al.*, 2014). Although there is compelling evidence that drought is affecting forests all around the globe, there are still significant gaps in our understanding of the mechanisms underlying forest drought responses at the stand- and the tree-level.

TREE WATER USE STRATEGIES UNDER DROUGHT CONDITIONS

As drought progresses, the water located within the root zone is depleted due to a lack of recharge by precipitation coupled to the plant water uptake and evaporation. This decrease in water content within the root zone produces a drop of its water potential. This drop increases tension in the plant's xylem, as a wider water potential gradient between the root zone and the atmosphere is created (Cochard, 2013). There are remarkable differences in how each species copes with such an increase in xylem tension. Some species close their stomata as soon as they perceive a slight decrease in xylem conductivity caused by raised tension, while others maintain their stomata open and let the conductivity drop (Tardieu & Simonneau, 1998). Plants with high stomatal responsiveness to changes in soil water potential will reduce their carbon uptake earlier during drought. On the other hand, plants with a less responsive stomatal behavior will maintain higher carbon uptake rates but the risk of cavitation in xylem will increase (Cochard, 2013). The stomatal behavior and the reduction of conductivity during drought are two key characteristics that have been used to quantitatively define plant hydraulic behavior (Martínez-Vilalta *et al.*, 2014). However, there are many other traits directly or indirectly related to plant hydraulic behavior that would determine the response and sensitivity to drought of plants. Sap flow is an integral measure of

plant hydraulic performance that provides insights into the response of drought at high temporal resolution (Sánchez-Costa *et al.*, 2015). Wood capacitance, i.e. the use of water stored in wood on a daily basis and the wood anatomy seem also to be coordinated and define species-specific solutions under drought (Meinzer *et al.*, 2009). A key trait of plants experiencing drought conditions is the rooting depth, since moisture in deep soil or rock layers lasts longer than in surface soil. Indeed, species-specific differences in rooting depth condition the physiological strategy to respond to drought by coordinating it with the stomatal regulation (West *et al.*, 2012).

In general, the traits involved in the plant responses to drought are common across taxa (Johnson *et al.*, 2012; Carnicer *et al.*, 2013a) although significant intra-specific variation also occurs among different provenances (Anderegg, 2015). For this reason, coexisting species may have opposing strategies for coping with drought, which have led to contrasting species-specific effects of recent extreme droughts (Lloret *et al.*, 2004; Breshears *et al.*, 2005; Mueller *et al.*, 2005). Evidence for potential vegetation shifts has been attributed to species-specific sensitivity to drought (Rigling *et al.*, 2013). Therefore, an in-depth understanding of the species-specific water use strategy is required to predict with accuracy the response of the whole community to the ongoing increase in drought severity. Potential vegetation shifts will likely upscale to the ecosystemic services provided by forests, as to the water and carbon cycles. Despite vegetation shifts having been already reported (Peñuelas & Boada, 2003; Mueller *et al.*, 2005) and the fact that changes in demography point to a progressive species substitution as a response to drought episodes (Galiano *et al.*, 2013) or to increasing drought trends (Carnicer *et al.*, 2014), this is not always the norm. Recently, Lloret *et al.*, (2012) reviewed stabilizing processes that operate at the population and community level and that might be delaying or counteracting potential vegetation shifts following extreme events. In this sense, long-term forest monitoring including extreme drought episodes will provide insights on the actual successional trajectory of forests after these episodes.

At the individual- and stem-level, trees are long-lived and sessile organisms that show an array of acclimation mechanisms; many trees living today are likely to be living also in 2100 (Corlett, 2011). The capacity of acclimation has been observed in relation to drought, too. Trees are able to modify growth allocation and tissue functional characteristics and maintain homeostasis in their water relations in response to reduced water availability

(Cinnirella *et al.*, 2002). Furthermore, changes in epigenetic markers have been observed in response to drought stress (González *et al.*, 2013; Rico *et al.*, 2013). In the long-term, it could be hypothesized that the current increase in drought would be partially offset by the acclimation capacity of tree species. Yet, we still have very little information on long-term acclimation mechanisms of trees. At the same time, however, the contrary effect to acclimation has been observed. Consecutive droughts seem to increase the vulnerability in the hydraulic system of *Populus tremuloides* (Anderegg *et al.*, 2013b), demonstrating that there is a legacy effect of droughts. Indeed, a global analysis of dendrochronological series have recently shown that the effects of an extreme drought on growth last for years after the event (Anderegg *et al.*, 2015). As commented above, droughts not only are increasing their intensity due to the amplifying role of temperature, but they are also likely to become more frequent (IPCC, 2013). In this sense, the long-term monitoring of forest plots can help elucidate whether the acclimation mechanisms would offset the deterioration of trees' hydraulic system and growth capacity caused by several consecutive droughts.

THE CASE STUDY OF THIS THESIS

This dissertation is focused on the water use of holm oak forests, including the dominant tree *Quercus ilex* and accompanying species. This is the most widespread evergreen tree of the Western Mediterranean and lies in the transition zone between cool-temperate deciduous forests and Mediterranean shrublands (Terradas, 1999). Its distributional area spans from Portugal to Syria and from the Maghreb to the French Atlantic coast. Holm oaks appeared in the Mediterranean Basin during the Miocene (Barbero *et al.*, 1992) and the species consolidated its presence during the Pliocene as a result of the onset of an irregular precipitation regime (Badal & Roiron, 1995). After the last glacial maximum, *Quercus ilex* began to be dominant in the Iberian Peninsula, and there is evidence of forest exploitation by Neolithic cultures as early as 6000-7000 BP (Badal & Roiron, 1995). For millennia, these forests have been intensively coppiced to provide timber and energy (from charcoal) to the local communities and were culturally central until very recently (Otero *et al.*, 2013). Today, human-induced global change threatens forests all over the world by changes in climate, land use, nutrient supply and pollution and holm oak forests are not an exception. Particularly, these forests are experiencing an abandonment of managing practices and increasingly drier conditions due to global warming. Here we present the results of four inter-related studies

dealing with the effects of decreasing water availability on holm oak forests. This scientific contribution aims to extend previous literature on holm oak forests, such as that compiled in the indispensable book "Ecology of Mediterranean Evergreen Oak Forests" (Rodà *et al.*, 1999).

THESIS STRUCTURE

In this document, I present the results of four investigations focused on different functional, physiological and demographical aspects of the effects of reduced water availability on holm oak forests and of a global analysis on groundwater as a source of water for plants. In Chapter II, I analyzed the growth and mortality rates of a long-term experimental drought system established in 1998 in a holm oak forest. I evaluated the effect of manipulated throughfall and surface runoff on stem growth and mortality rates of the co-occurring *Q. ilex, P. latifolia* and *A. unedo*, and whether species-specific responses to drought observed by previous studies persisted over time in this ecosystem. Additionally, I used the long dataset to assess the response of these variables to the different intensities and durations of natural droughts and whether the structural changes caused by extreme events and long-term experimental drought modify the response of the community to periods of drought. In Chapter III, I used the stable isotopes of water to know what were the sources of water for *Q. ilex*, *P. latifolia* and *A. unedo* and whether they change over time or not. In addition, I tested if the sources of plant water changed after 12 years of experimental drought and during an extreme drought event that entailed stem mortality and crown defoliation. In Chapter IV, I aimed to characterize the sap flow of *Quercus ilex* and the co-occurring evergreen shrub *Phillyrea latifolia* by comparing diurnal, nocturnal and seasonal sap flow patterns and relate them to environmental variables, mainly soil water availability and vapor pressure deficit (atmospheric evaporative demand). In Chapter V, I reviewed the available literature on the responses of holm oak to droughts that operate at different temporal and organizational scales. For this, I put together and linked the results of research on the adaptive traits and mechanisms of holm oaks to summer drought, the effects of extreme drought events and of experimental drought systems. Finally, in Chapter VI, I compiled and analyzed studies reporting the relative contribution of groundwater to plant transpiration using stable water isotopes.

REFERENCES

Allen CD, Macalady AK, Chenchouni H et al. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management, 259, 660–684.

Allen CD, Breshears DD, McDowell NG (2015) On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere, 6, 1–55.

Anderegg WRL (2015) Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. New Phytologist, 205, 1008–1014.

Anderegg WRL, Kane JM, Anderegg LDL (2013a) Consequences of widespread tree mortality triggered by drought and temperature stress. Nature Climate Change, 3, 30–36.

Anderegg WRL, Plavcová L, Anderegg LDL, Hacke UG, Berry JA., Field CB (2013b) Drought's legacy: multiyear hydraulic deterioration underlies widespread aspen forest die-off and portends increased future risk. Global Change Biology, 19, 1188–1196.

Anderegg WRL, Schwalm C, Biondi F et al. (2015) Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models W. Science, 349.

Badal E, Roiron P (1995) La prehistoria de la vegetación en la Península Ibérica. Saguntum: Papeles del Laboratorio de Arqueología de Valencia, 28, 29–48.

Barbero M, Loisel R, Quézel P (1992) Biogeography, ecology and history of Mediterranean Quercus ilex ecosystems. Vegetatio.

Bearup LA, Maxwell RM, Clow DW, Mccray JE (2014) Hydrological e ects of forest transpiration loss in bark beetle-impacted watersheds. Nature Climate Change, 4, 481–486.

Bonan GB (2008) Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science, 320, 1444–1449.

Breshears DD, Cobb NS, Rich PM et al. (2005) Regional vegetation die-off in response to global-change-type drought. Proceedings of the National Academy of Sciences of the United States of America, 102, 15144–8.

Carnicer J, Coll M, Ninyerola M, Pons X, Sánchez G, Peñuelas J (2011) Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proceedings of the National Academy of Sciences of the United States of America, 108, 1474–1478.

Carnicer J, Barbeta A, Sperlich D, Coll M, Peñuelas J (2013) Contrasting trait syndromes in angiosperms and conifers are associated with different responses of tree growth to temperature on a large scale. Frontiers in plant science, 4, 409.

Carnicer J, Coll M, Pons X, Ninyerola M, Vayreda J, Peñuelas J (2014) Large-scale recruitment limitation in Mediterranean pines: The role of Quercus ilex and forest successional advance as key regional drivers. Global Ecology and Biogeography, 23, 371–384.

Cinnirella S, Magnani F, Saracino A, Borghetti M (2002) Response of a mature Pinus laricio plantation to a three-year restriction of water supply: structural and functional acclimation to drought. Tree Physiology, 22, 21–30.

Cochard H (2013) The basics of plant hydraulics. The journal of plant hydraulics, 1, 1–2.

Corlett RT (2011) Impacts of warming on tropical lowland rainforests. Trends in Ecology & Evolution, 26, 606–613.

Courchamp F, Dunne J a., Le Maho Y, May RM, Thébaud C, Hochberg ME (2015) Fundamental ecology is fundamental. Trends in Ecology & Evolution, 30, 9–16.

Diffenbaugh NS, Swain DL, Touma D (2015) Anthropogenic warming has increased drought risk in California. Proceedings of the National Academy of Sciences, 112, 3931–3936.

Galiano L, Martínez-Vilalta J, Eugenio M, Granzow-de la Cerda Í, Lloret F (2013) Seedling emergence and growth of Quercus spp. following severe drought effects on a Pinus sylvestris canopy (ed Ward D). Journal of Vegetation Science, 24, 580–588.

González RM, Ricardi MM, Iusem ND (2013) Epigenetic marks in an adaptive water stress-responsive gene in tomato roots under normal and drought conditions. Epigenetics, 8, 864–872.

Good SP, Noone D, Bowen G (2015) Hydrologic connectivity constrains partitioning of global terrestrial water fluxes. Science, 349, 175–177.

IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (eds Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, V. B, Midgley P.). Cambridge University Press, Cambridge, UK. New York, NY, USA.

Johnson DM, McCulloh K a, Woodruff DR, Meinzer FC (2012) Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different? Plant science an international journal of experimental plant biology, 195, 48–53.

Kelley CP, Mohtadi S, Cane M a, Seager R, Kushnir Y (2015) Climate change in the Fertile Crescent and implications of the recent Syrian drought. Proceedings of the National Academy of Sciences, 112, 3241–3246.

Lee J, Oliveira RS, Dawson TE, Fung I (2007) Root functioning modifies seasonal climate. Proceedings of the National Academy of Sciences, 104.

Lloret F, Siscart D, Dalmases C (2004) Canopy recovery after drought dieback in holm-oak Mediterranean forests of Catalonia (NE Spain). Global Change Biology, 10, 2092–2099.

Lloret F, Escudero A, Iriondo JM, Martínez-Vilalta J, Valladares F (2012) Extreme climatic events and vegetation: the role of stabilizing processes. Global Change Biology, 18, 797–805.

Martínez-Vilalta J, Poyatos R, Aguadé D, Retana J, Mencuccini M (2014) A new look at water transport regulation in plants. New Phytologist, 204, 105–115.

Mcintyre PJ, Thorne JH, Dolanc CR, Flint AL, Flint LE, Kelly M, Ackerly DD (2014) Twentieth-century shifts in forest structure in California: Denser forests , smaller trees , and increased dominance of oaks. 1–6.

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh K a, Woodruff DR (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. Functional Ecology, 23, 922–930.

Mueller RC, Scudder CM, Porter ME, Talbot Trotter R, Gehring C a., Whitham TG (2005) Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. Journal of Ecology, 93, 1085–1093.

Otero I, Boada M, Tàbara JD (2013) Social–ecological heritage and the conservation of Mediterranean landscapes under global change. A case study in Olzinelles (Catalonia). Land Use Policy, 30, 25–37.

Pan Y, Birdsey RA, Fang J et al. (2011) A Large and Persistent Carbon Sink in the World's Forests. Natural Areas Journal, 333, 988–994.

Pederson N, Hessl AE, Baatarbileg N, Anchukaitis KJ, Di Cosmo N (2014) Pluvials, droughts, the Mongol Empire, and modern Mongolia. Proceedings of the National Academy of Sciences of the United States of America, 111, 4375–9.

Peñuelas J, Boada M (2003) A global change-induced biome shift in the Montseny mountains (NE Spain). Global Change Biology, 9, 131–140.

Rico L, Ogaya R, Barbeta A, Peñuelas J (2013) Changes in DNA methylation fingerprint of Quercus ilex trees in response to experimental field drought simulating projected climate change. Plant biology.

Rigling A, Bigler C, Eilmann B et al. (2013) Driving factors of a vegetation shift from Scots pine to pubescent oak in dry Alpine forests. Global change biology, 19, 229–40.

Rodà F, Retana J, Gracia CA, Bellot J (1999) Ecology of Mediterranean Evergreen Oak Forests. Springer, Berlin.

Sánchez-Costa E, Poyatos R, Sabaté S (2015) Contrasting growth and water use strategies in four co-occurring Mediterranean tree species revealed by concurrent measurements of sap flow and stem diameter variations. Agricultural and Forest Meteorology, 207, 24–37.

Schlesinger WH, Jasechko S (2014) Transpiration in the global water cycle. Agricultural and Forest Meteorology, 189-190, 115–117.

Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours Franc. Journal of experimental botany, 49, 419–432.

Terradas J (1999) Holm Oak and Holm Oak Forests: An Introduction. In: Ecology of Mediterranean Evergreen Oak Forests (eds Rodà F, Retana J, Gracia C, Bellot J), pp. 3–14. Springer Berlin / Heidelberg, Berlin.

Vicente-Serrano SM, Lopez-Moreno J-I, Beguería S et al. (2014) Evidence of increasing drought severity caused by temperature rise in southern Europe. Environmental Research Letters, 9, 044001.

West a G, Dawson TE, February EC, Midgley GF, Bond WJ, Aston TL (2012) Diverse functional responses to drought in a Mediterranean-type shrubland in South Africa. The New phytologist, 195, 396–407.

Chapter II

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

Adrià Barbeta, Romà Ogaya and Josep Peñuelas

ABSTRACT

Forests respond to increasing intensities and frequencies of drought by reducing growth and with higher tree mortality rates. Little is known, however, about the long-term consequences of generally drier conditions and more frequent extreme droughts. A Holm oak forest was exposed to experimental rainfall manipulation for 13 years to study the effect of increasing drought on growth and mortality of the dominant species *Quercus ilex*, *Phillyrea latifolia* and *Arbutus unedo*. The drought treatment reduced stem growth of *A. unedo* (-66.5%) and *Q. ilex* (-17.5%), whereas *P. latifolia* remained unaffected. Higher stem mortality rates were noticeable in *Q. ilex* (+42.3%) but not in the other two species. Stem growth was a function of the drought index of early spring in the three species. Stem mortality rates depended on the drought index of winter and spring for *Q. ilex* and in spring and summer for *P. latifolia* but showed no relation to climate in *A. unedo*. Following a long and intense drought (2005-06), stem growth of *Q. ilex* and *P. latifolia* increased, whereas it decreased in *A. unedo*. *Q. ilex* also enhanced its survival after this period. Furthermore, the effect of drought treatment on stem growth in *Q. ilex* and *A. unedo* was attenuated as the study progressed. These results highlight the different vulnerabilities of Mediterranean species to more frequent and intense droughts, which may lead to partial species substitution and changes in forest structure and thus in carbon uptake. The response to drought, however, changed over time. Decreased intra- and interspecific competition after extreme events with high mortality, together with probable morphological and physiological acclimation to drought during the study period, may, at least in the short term, buffer forests against drier conditions. The long-term effects of drought consequently deserve more attention, because the ecosystemic responses are unlikely to be stable over time.

KEYWORDS

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

INTRODUCTION

Forest decline, die-off and vegetational shifts related to drought have been documented in many forest types in recent years (Allen *et al.*, 2010, Anderegg *et al.*, 2012, Breshears *et al.*, 2009, Huang & Anderegg, 2012, Mueller *et al.*, 2005, Peñuelas *et al.*, 2001, Peñuelas *et al.*, 2007a). Although drought is a recurrent disturbance in Mediterranean forests, an increase in intensity and frequency would likely lead to reduced growth and crown condition and to higher mortality rates in these ecosystems (Carnicer *et al.*, 2011, Galiano *et al.*, 2012, Ogaya & Peñuelas, 2007b, Ogaya *et al.*, 2003, Sarris *et al.*, 2011). In Mediterranean regions, General Circulation Models (GCMs) project an average decrease of 15% in soil moisture over the next 50 years and a return period of extreme droughts 10 times shorter than in the twentieth century (Bates *et al.*, 2008). The frequency of heat waves is also expected to increase in the coming years around the Mediterranean area, which would increase evapotranspiration even more during the dry season in this region (Fischer & Schar, 2010).

Experimental manipulations of precipitation are useful for anticipating the future impacts of global climatic scenarios on vegetation (Wu *et al.*, 2011). These experiments, involving long-term treatment and monitoring, are especially needed because the effect size of treatments on ecosystems would not be constant over time (Leuzinger *et al.*, 2011). Several systems of experimental drought have been established in forests across different climatic zones and have demonstrated a general sensitivity of ecosystems to drought. The observed effects included decreased carbon uptake, productivity and aboveground biomass (Wu *et al.*, 2011). For example, drought decreased growth, increased tree mortality and altered carbon cycling after mid-term experimental drought in Mediterranean and Amazonian forests (Brando *et al.*, 2008, da Costa *et al.*, 2010, Ogaya & Peñuelas, 2007b) and modified physiological activity, morphological traits and recruitment patterns of Mediterranean woody species (Limousin *et al.*, 2010, Limousin *et al.*, 2012, Limousin *et al.*, 2009, Lloret *et al.*, 2004a, Ogaya & Peñuelas, 2006, Ogaya *et al.*, 2011, Peñuelas *et al.*, 2007b).

As many studies have reported, short-term experimental drought is enough to induce relatively rapid morphological and functional changes in forests, such as increased mortality of fine roots in a boreal forest after a six-week treatment (Gaul *et al.*, 2008) or the acceleration of CO_2 fluxes to the atmosphere in tropical rainforests after a 12-month treatment (Cleveland *et al.*, 2010). Nonetheless, information on the temporal stability of these drought-induced

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alterations is lacking, because very few experiments have been maintained for more than seven or eight years. In a meta-analysis of global-change experiments, (Leuzinger *et al.*, 2011) provided evidence of a dampened effect size of treatments (i.e. warming, nitrogen fertilization or drought) over time, which may be attributable either to the aforementioned modified plant physiology or morphology (phenotypic plasticity) or even to genetic adaptation (Jump *et al.*, 2006). As recently proposed by (Lloret *et al.*, 2012), demographic stabilizing mechanisms may also be counteracting or minimizing the effects of extreme events and changing climatic trends on vegetation. As suggested by the theoretical framework of Lloret *et al.* (2012), the unprecedented mortality rates expected under drought treatment would subsequently be compensated by a higher survival of the remnant population, because a lower stem or individual density would consequently lower intra- and interspecific competition for water resources. Monitoring experimental drought systems as long as possible is thus indeed desirable to assess these stabilizing processes.

Holm oak (*Quercus ilex* L.) is a widespread sclerophyllous tree that dominates forests distributed in the Mediterranean basin. It is usually accompanied by other Mediterranean woody species with high (mock privet, *Phillyrea latifolia* L.) or low (strawberry tree, *Arbutus unedo* L.) resistance to drought (Asensio *et al.*, 2007, Ogaya & Peñuelas, 2003, Ogaya & Peñuelas, 2006, Ogaya & Peñuelas, 2007a, Ogaya & Peñuelas, 2007b, Ogaya *et al.*, 2011, Ogaya *et al.*, 2003, Peñuelas *et al.*, 2000, Peñuelas *et al.*, 1998, Peñuelas *et al.*, 2001). Experimental drought reduces transpiration and foliar area in *Q. ilex* while increasing foliar mass per area and vulnerability to embolism (Limousin *et al.*, 2010, Limousin *et al.*, 2012, Ogaya & Peñuelas, 2006), which would eventually drive changes in the assimilation of carbon and cause lower growth rates (Ogaya & Peñuelas, 2007b). Also, *A. unedo* reduced stomatal conductance and rates of CO_2 assimilation under drought treatment (Ripullone *et al.*, 2009). Important demographic effects have already been observed in experimental systems of drought, such as a higher mortality of stems and reduced recruitment (Lloret *et al.*, 2004a, Ogaya & Peñuelas, 2007b). In similar studies, *P. latifolia* responded differently when contending with drier conditions, showing no evidence of any effect from drought treatment, probably because this species better dissipates excess radiation, uses water more efficiently and is more resistant to xylem embolism than *Q. ilex* (Martínez-Vilalta *et al.*, 2002, Ogaya *et al.*, 2011, Peñuelas *et al.*, 2000, Peñuelas *et al.*, 1998). Such species-specific differences in

resistance to drought would likely produce a gradual vegetational shift or a partial species substitution in these abundant Mediterranean forest communities.

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

We aimed to evaluate: (a) the effect of manipulating precipitation on stem growth and mortality rates of the co-occurring *A. unedo*, *Q. ilex* and *P. latifolia,* (b) whether species-specific responses to drought observed by previous studies persisted over time in this ecosystem, (c) the response of these variables to the different intensities and durations of natural drought and (d) whether the structural changes caused by extreme events and long-term experimental drought would modify the response of the community to periods of drought. These questions should allow to gain knowledge on the effects of climatic change on Holm oak forests and o determine if stabilizing processes would dampen the future responses of forest communities to both climatic trends and events.

MATERIALS AND METHODS

Experimental site

The experimental site was established in 1999 at the Prades holm oak forest in Southern Catalonia (NE Spain) (41º21'N, 1º2'E), at 930 m a.s.l. and on a south-facing slope (25% slope). The forest has a very dense multi-stem crown (15 433 stems ha^{-1}) dominated by *Q. ilex* (5258

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

The climate is typically Mediterranean. The period of this study (1999-2012) had a mean temperature of 12.2 ºC and a mean annual precipitation of 610 mm. The annual and seasonal distribution of precipitation is irregular, with annual precipitation ranging from 376 to 926 mm in the 13 years of this study. Spring and autumn are the wettest seasons, and summer drought usually lasts 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 (4.4 ºC), and the daily mean temperature is below 0 ºC an average of eight days per winter. The soil is a Dystric Cambisol over Paleozoic schist and ranges from 35 to 90 cm in depth. The climatic, edaphic and physiographic characteristics of this site are fairly representative of Mediterranean forests, which mostly occur in mountainous areas with shallow soils that exacerbate water stress in plants.

The experimental system consisted of eight 150-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 at a height of 0.5-0.8 m above the soil, covering approximately 30% of the plot surfaces. A ditch 0.8 m in depth was excavated along the entire top edge of the plots to intercept runoff water. The water intercepted by the strips and ditches was conducted outside the plots, below their bottom edges. The strips were

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installed below the canopy and thus did not intercept light. Litter falling on the plastic strips was regularly placed below them to ensure that differences in the contents of soil nutrients among treatments and control plots were attributable only to the availability of water for the decomposition of this litter (Ogaya & Peñuelas, 2007b).

Environmental monitoring and drought index

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

The Standardized Precipitation Evapotranspiration Index (SPEI) (Vicente-Serrano *et al.*, 2010) is a multi-scalar drought index that we have calculated using local meteorological data from a 35-year period. The main difference between SPEI and other drought indexes is its use of the difference between precipitation and potential evapotranspiration (P-PET; D). Precipitation is the most important factor in the variability and intensity of drought, but evapotranspiration also plays a major role as a determinant in the variability of soil moisture, a key factor in plant-water relations (Martin-Benito *et al.*, 2013, Vicente-Serrano *et al.*, 2012a, Vicente-Serrano *et al.*, 2012b). The inclusion of potential evapotranspiration (PET) to calculate the SPEI only affects the index when PET differs from average conditions, for example, under scenarios of global change (Vicente-Serrano *et al.*, 2010). The use of PET is especially suitable in our study area because climatic series indicate a warming trend since 1975. Even though our climatic series was not long, we correlated our 13 years of data on precipitation and temperature with the data from a station located 5.6 km northeast of our plots and at 510 m a.s.l. (linear regressions: R^2 = 0.97 for temperature, R^2 = 0.75 for precipitation, N = 48). Using this reconstructed climatic profile (1975-2011), a log-logistic distribution was used to model the values of D, and the resulting cumulative probabilities were transformed into a standardized variable. A SPEI of 0 indicates a value corresponding to 50% of the cumulative probability of D; the SPEI ranges between 3 and -3, and the lower the

value, the more intense the drought. The multi-scalar character of the SPEI distinguishes between short-term and long-term droughts, which would affect the vegetation at different levels. In our study, we selected time scales of three (SPEI-3) and six (SPEI-6) months, because they best fit our annual data on plant growth and population dynamics. For a more detailed description of the methods of calculation, see (Vicente-Serrano *et al.*, 2010). The different SPEI values are provided with month and timescale of calculation (i.e. May SPEI-3 refers to the water balance of March, April and May of a given year).

Growth and mortality

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

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

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

Statistical analyses

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

the formula proposed by (Sheil *et al.*, 1995). We constructed one model for each dependent variable at the species level;

1a)   *A. unedo*: BAI = Treatment + April SPEI-3

         Q. ilex: BAI = Treatment + May SPEI-3

         P. latifolia: BAI = Treatment + May SPEI-3

1b)   *A. unedo*: Stem mortality rate = Treatment + June SPEI-6

         Q. ilex: Stem mortality rate = Treatment + May SPEI-6

         P. latifolia: Stem mortality rate = Treatment + September SPEI-6

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

2a) BAI = Treatment + May-SPEI-3 * species + species * period

2b) Mortality rate = Treatment + September SPEI-6 * species + species * period

where treatment factor (two levels, control and drought), SPEI (best fit for all species together), species and period of the study (before or after the intense droughts of 2005 and 2006) were fixed independent variables, and year and plot were random independent variables, in order to account for temporal and spatial autocorrelation. We included the interactions among fixed independent variables when they improved the model fit (eq. 2a and 2b). Models were selected both by the deviance information criterion (DIC) and by the minimum error.

The total BAI accumulated over the 13 years was compared between species and treatment for living stems with analysis of variance (ANOVA) and Tukey HSD (honest significant difference) post-hoc tests. Differences in the mean diameters of dead stems were tested with GLMMs using MCMC techniques, as with growth and mortality. We then performed the linear

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

RESULTS

Environmental conditions

During the study period, the annual average temperature was 12.23 ºC, which was slightly warmer than the 1975-2011 average (Fig. 1). Annual precipitation was almost 8% lower (611 mm in 1999-2011 vs. 663 mm in 1975-2011, Fig. 1). The identification of different periods of drought throughout the study was possible using the SPEI. The three-month summer drought was the most consistent, occurring almost every year, with peaks in 2006 and 2009 (Fig. 2). The May SPEI-6 indicated a drought outside the typical season; during these months, precipitation was normally, on average, 55% of the yearly total (Figs. 1 and 2). Two two-year periods (2000-2001 and 2005-2006) were particularly dry. Long-term water deficits were identified with the 12-month and 24-month SPEIs of December in the following periods: 1999-2002, 2005-2007 and 2010-2011. Soil water content was significantly lower in the drought treatment compared to the control plots (-18.06% ± 3.02, pMCMC<0.001), ranging from 25-30% in volume during the rainy seasons to 5-10% during summer droughts (Fig. 1). The period after the 2005-06 droughts was not significantly wetter for April SPEI-3 ($F = 0.63$, $p = 0.44$), May SPEI-3 ($F = 0.80$, $p = 0.39$), May SPEI-6 ($F = 0.99$, $p = 0.35$) or September SPEI-6 ($F = 0.7$, $p = 0.80$), the relevant SPEIs for plant growth and mortality.
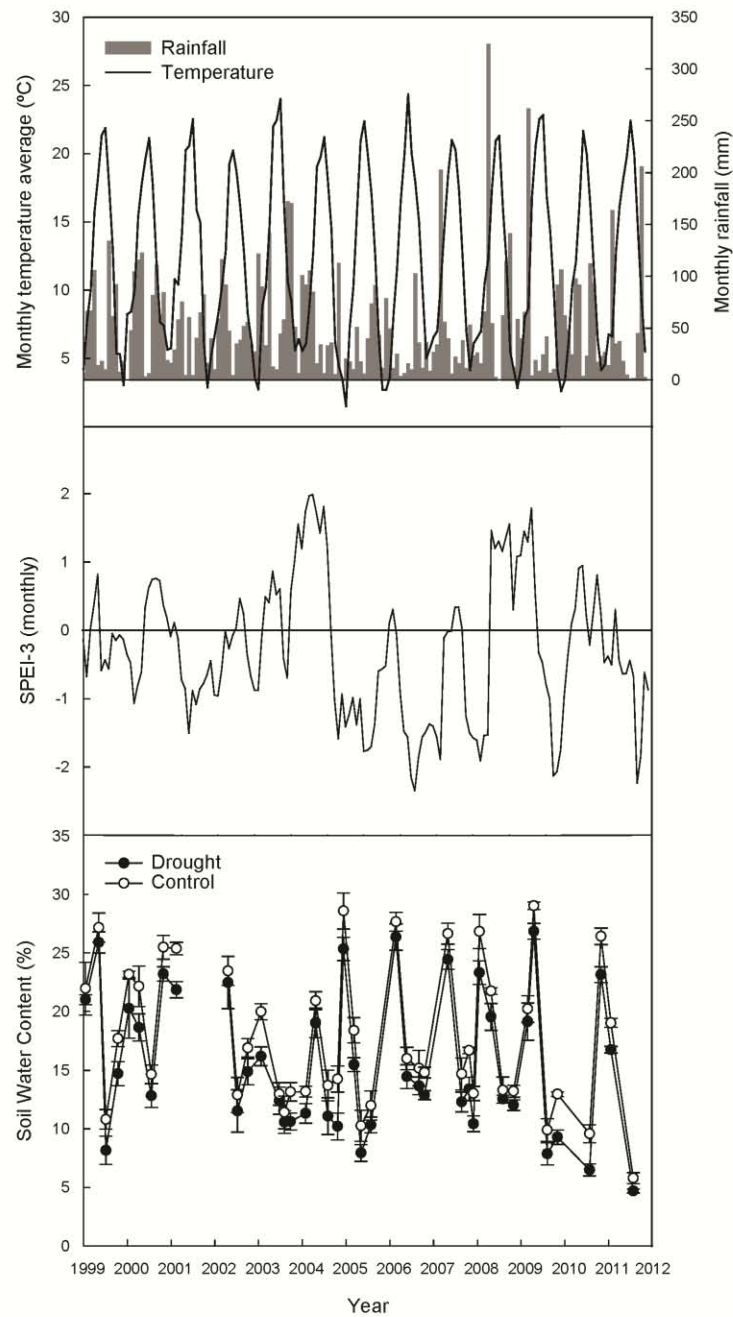
Figure 1. Environmental variables during the period of study (1999-2012). (a) Monthly precipitation and monthly average temperature, (b) monthly SPEI-3 and (c) soil water content in each treatment. Error bars are the standard error of the mean (n = 4).
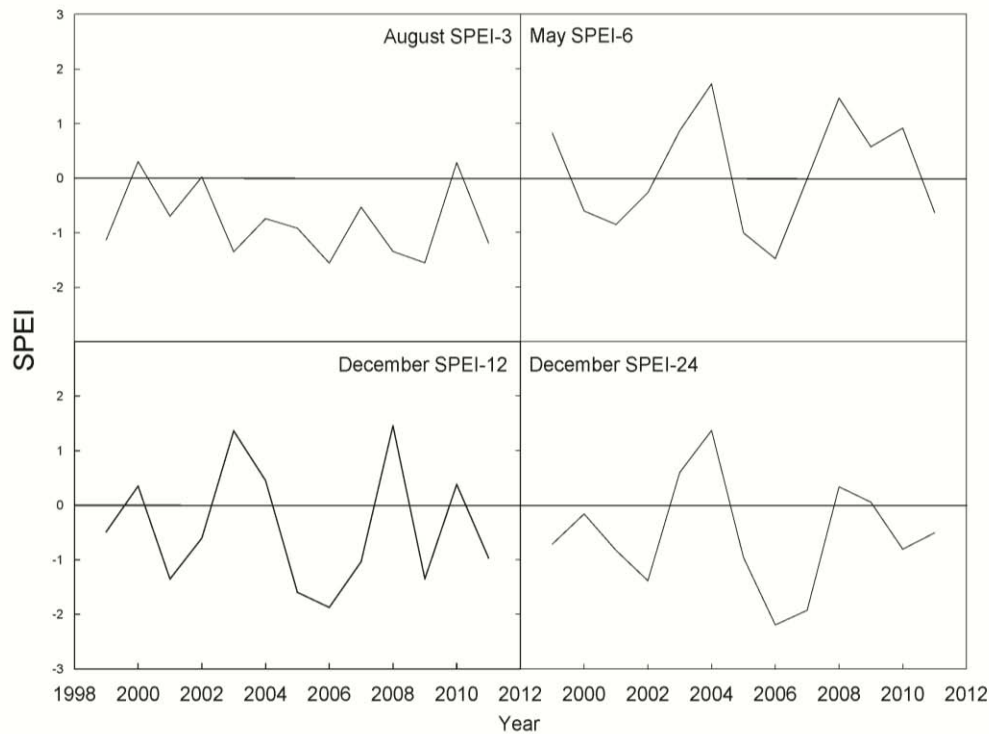
Figure 2. SPEI at different timescales during the study period. Positive values correspond to a difference between precipitation and potential evapotranspiration more positive than the average calculated from 1975 to 2011, for a given month span. Negative values thus represent conditions drier than average.

Stem BAI

Lower soil water contents in the drought plots had an overall negative effect on the stem BAI, but this response was species-specific (Table 1). Stem BAI of *A. unedo* was the most sensitive to drought. It was significantly lower in drought plots, roughly a third of the stem BAI in the control plots (Table 1, Fig. 3). *Q. ilex* also tended to present lower stem BAIs in the drought treatment, while the stem BAI of *P. latifolia* was uninfluenced by drought treatment. The stem BAI of the three species was strongly correlated with the SPEI calculated with a timescale of three months. The stem BAI of *A. unedo* was positively correlated to April SPEI-3, while those of both *Q. ilex* and *P. latifolia* were positively correlated to May SPEI-3 (Table 1, Fig. 3). The effect size of the drought index on stem BAI, however, was higher in *A. unedo* than in *Q. ilex*

(April SPEI-3 *Mean effect* = 0.24, *pMCMC* < 0.01, for *A. unedo*, and May SPEI-3 *Mean effect* = 0.18, *pMCMC* < 0.01, for *Q. ilex*, Table 1), while *P. latifolia* seemed to be relatively less dependent on the drought index that the other two species (May SPEI-3 *Mean effect* = 0.03, *pMCMC* < 0.01 for *P. latifolia*, Table 1) . Our results also indicated that the growth of *A. unedo* was a function of the water balance in late winter and early spring (Februrary, March and April), while the stem BAI of *Q. ilex* and *P. latifolia* depended only on early spring (March, April and May) (Table 1). The stem BAIs of *A. unedo* decreased after the intense droughts of 2005 and 2006, but *Q. ilex* and *P. latifolia* had significantly higher stem BAIs (Table 2a). The negative effect of treatment on the annual stem BAI showed a progressive and significant reduction in *A. unedo* and *Q. ilex* but not in *P. latifolia* (Fig. 4). The total BAI accumulated between 1999 and 2012 differed significantly among species (*F* = 128.89, *p*<0.001, ANOVA) and was lower in the drought treatment (*F* = 9.35, *p*<0.01, ANOVA); a significant interaction between drought treatment and species was observed (*F* = 12.69, *p*<0.001, ANOVA) (Fig. 5). *A. unedo* was the only species with lower stem BAIs in the drought plots for the stems that survived the entire study period (difference = -6.40, *p*<0.001, Tukey-HSD). In both treatments, the stem BAI of *Q. ilex* from 1999 to 2012 did not differ from the stem BAI of *A. unedo* in the drought plots. On the other hand, the BAI of *P. latifolia* for the period 1999-2012 was significantly lower than the BAI in the other two species, in either the drought or control plots (differences = -10.72/-4.32 with *A. unedo* and -4.60/-3.72 with *Q. ilex*, all *p*<0.001, Tukey-HSD), but treatment had no effect in *P. latifolia* (Fig. 5). Overall, the relative change of basal area per plot was lower in the drought treatment (mean effect = -0.01, *pMCMC*<0.05, Fig. 6). Drought treatment tended to reduce basal area in the drought plots in 2002 (mean effect = -0.01, *pMCMC*<0.1) and during the very dry period of 2005-06 (mean effect = -0.03, *pMCMC*<0.1). As shown in Fig. 6, there were losses of basal area in years 2001 and 2006 (control plots) and in years 2000, 2001, 2005, 2006 and 2011 (drought plots).

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

| Species		Fixed effects	Mean effect	Lower 95% CI	Upper 95% CI	pMCMC
A. unedo	BAI	**Intercept**	**0.92**	**0.73**	**1.11**	***
		Drought	**-0.64**	**-0.84**	**-0.43**	***
		April SPEI-3	**0.24**	**0.08**	**0.39**	**
	Stem mortality rate	**Intercept**	**-4.35**	**-7.59**	**-1.76**	***
		Drought	1.81	-1.01	4.52	NS
		June SPEI-6	0.53	-0.79	1.94	NS
Q. ilex	BAI	**Intercept**	**0.35**	**0.25**	**0.5**	***
		Drought	*-0.07*	*-0.16*	*0.01*	(*)
		May SPEI-3	**0.18**	**0.06**	**0.29**	**
	Stem mortality rate	Intercept	0.28	-0.22	0.77	NS
		Drought	*0.36*	*-0.06*	*0.79*	(*)
		May SPEI-6	**-0.67**	**-1.13**	**-0.24**	**
P. latifolia	BAI	**Intercept**	**0.11**	**0.08**	**0.14**	***
		Drought	0.01	-0.02	0.03	NS
		May SPEI-3	**0.03**	**0.01**	**0.06**	**
	Stem mortality rate	**Intercept**	**-1.04**	**-1.69**	**-0.4**	**
		Drought	-0.01	-0.67	0.6	NS
		Sep SPEI-6	**-0.45**	**-0.85**	**-0.04**	*
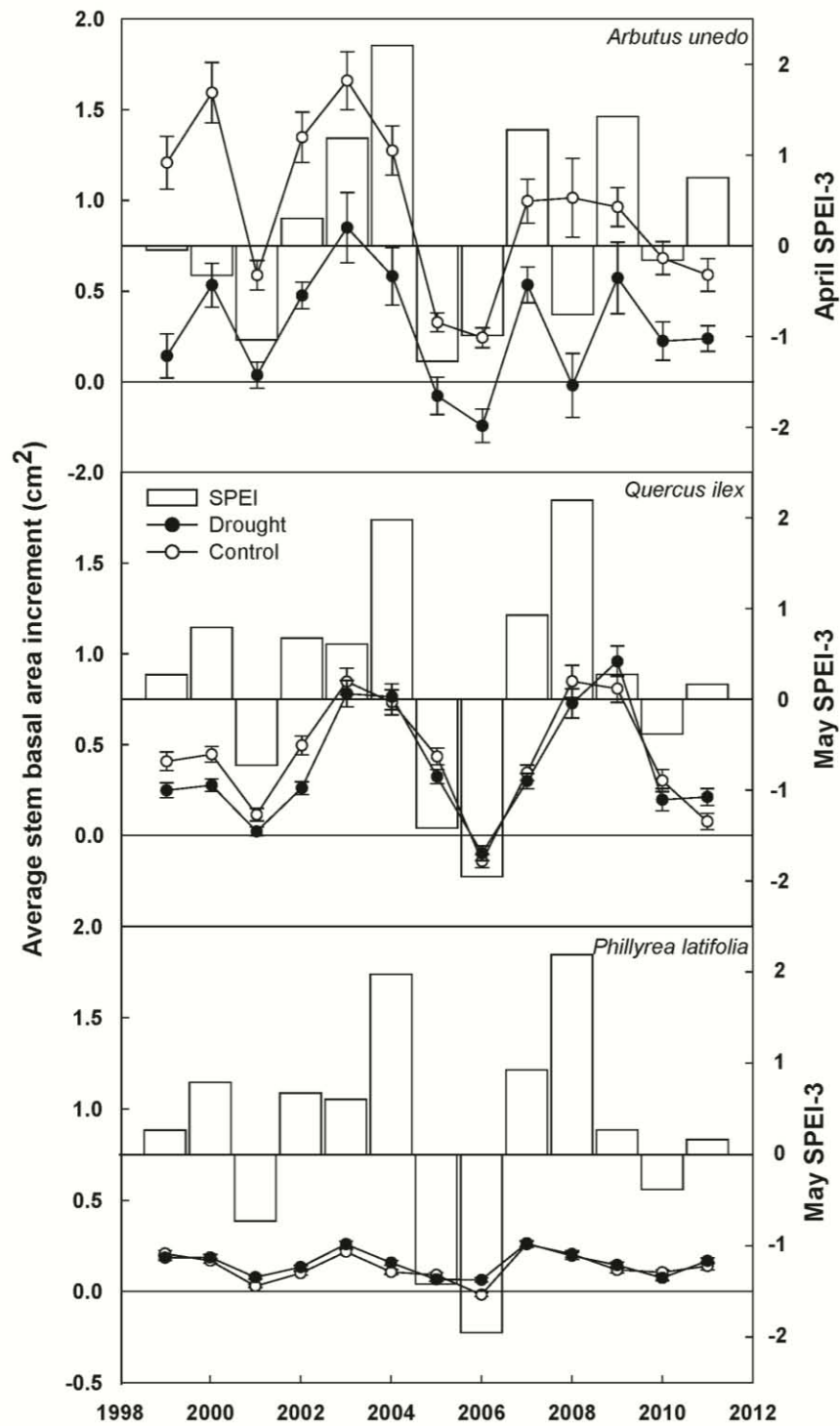
Figure 3. Average stem basal area increments (BAI) (left axis) of the three tree species and in each treatment. Error bars are the standard error of the mean (n = 4). Bars correspond to the annual three-month SPEI of April (*A. unedo*) and May (*Q. ilex* and *P. latifolia*).

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

a) Annual Stem BAI

Fixed effects	Mean effect	l-95% CI	u-95% CI	pMCMC
(Intercept)	0.99	0.84	1.13	***
P. latifolia	-0.89	-1.01	-0.76	***
Q. ilex	-0.64	-0.77	-0.52	***
Drought	-0.65	-0.81	-0.50	***
After 2006	-0.26	-0.46	-0.05	*
May SPEI-3	0.25	0.17	0.34	***
P. latifolia x Drought	0.65	0.49	0.82	***
Q. ilex x Drought	0.58	0.41	0.74	***
P. latifolia x After 2006	0.28	0.11	0.44	**
Q. ilex x After 2006	0.27	0.11	0.45	**
Q. ilex x May SPEI-3	-0.22	-0.29	-0.15	***
P. latifolia x May SPEI-3	-0.07	-0.15	-0.002	*

b) Stem Mortality rates

Fixed effects	Mean effect	l-95% CI	u-95% CI	pMCMC
(Intercept)	-1.81	-2.80	-0.84	***
Drought	1.05	0.04	2.10	*
P. latifolia	0.58	-0.43	1.61	NS
Q. ilex	1.97	1.05	2.89	***
September SPEI-6	0.33	-0.22	0.92	NS
After 2006	0.95	-0.11	2.01	(*)
P. latifolia x Drought	-1.02	-2.19	0.20	(*)
Q. ilex x Drought	-0.65	-1.75	0.44	NS
P. latifolia x September SPEI-6	-0.87	-1.47	-0.31	**
Q. ilex x September SPEI-6	-0.84	-1.37	-0.27	**
P. latifolia x After 2006	-1.07	-2.29	0.07	(*)
Q. ilex x After 2006	-1.62	-2.78	-0.61	**
Drought x September SPEI-6	0.11	-0.27	0.51	NS

Stem mortality

The stem mortality rates were higher in the drought treatment throughout the study period. *P. latifolia* though, tended to be less affected by the lower availability of water than the other two species (Table 2b). We also found other species-specific differences; *Q. ilex* had higher stem mortality rates than the other two species and was the only species to show a trend toward a higher mortality in the drought treatment by the species-level model (Tables 1 and 2b, Fig. 7). Unlike the stem BAI, the timescale of the drought index that better predicted the stem mortality rates of *Q. ilex* and *P. latifolia* was six months. *P. latifolia*, however, appeared to be more dependent on September SPEI-6, while *Q. ilex* did so on May SPEI-6 in the species-level model (Table 1, Fig. 8). In the period after the intense droughts of 2005 and 2006, stem mortality rates in *A. unedo* tended to increase. In contrast, stem mortality rates in *Q. ilex* significantly decreased, and *P. latifolia* also presented a trend toward lower stem mortality (Table 2b). Given the multi-stem structure of this forest, the relevance of stem mortality had to be assessed based on the diameter of dead stems. *Q. ilex* lost significantly larger stems than did *P. latifolia* (mean difference = 0.29 higher in *Q. ilex*, *pMCMC*<0.001), and the drought index had a significant negative effect on the size of dead stems (mean effect = -0.07, *pMCMC*<0.01) when analyzing both species together. Separately, the May SPEI-6 significantly reduced the dead-stem diameter of *Q. ilex* (y = 3.71 – 0.38x, R^2 = 0.59, *p*<0.01) and tended to do so in *P. latifolia* (y = 2.67 – 0.22x, R^2 = 0.29, *p* = 0.07, Fig. 9). Survived *Q. ilex* stems throughout the study period presented significantly higher growth than died stems during the same period (mean difference = 0.55, *pMCMC*<0.001) (Fig. 10).
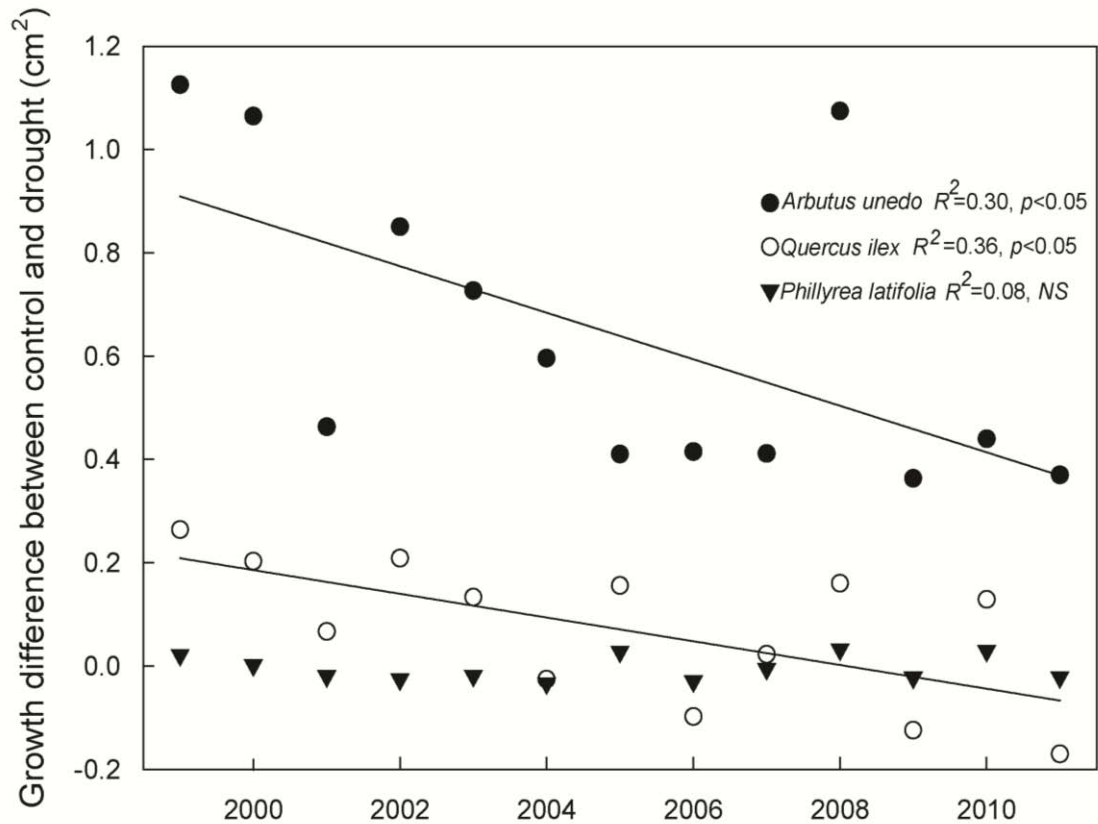
Figure 4. Yearly difference between the mean growth in the control and the drought plots (1999-2011). Regression lines are shown for the species with significant correlations.

DISCUSSION

Effects of experimental drought

The long-term experimental drought significantly reduced global stem growth and caused generally higher stem mortality rates throughout the study period in this precipitation-manipulation experiment, which, as far as we know and according to a recent review (Wu *et al.*, 2011), is the longest field experiment conducted in a forest. These results agree with those from previous studies in the same forest (Ogaya & Peñuelas, 2007b, Ogaya *et al.*, 2003). Long-term experimental drought has also increased stem mortality rates and reduced growth in different ecosystems such as the Amazonian rainforest and North-American deciduous forests (da Costa *et al.*, 2010, Hanson *et al.*, 2001), although with some exceptions; e.g. the growth of *Quercus* species in southeastern USA was unaffected (Wagner *et al.*, 2012). More specifically, stem growth in the drought treatment in our study was 66.5% lower in *A. unedo*, 17.5% lower in *Q. ilex* and remain unaffected in *P. latifolia* compared to control plots (Fig. 3). Stem

mortality rates in *Q. ilex* were 42.3% higher in the drought treatment (Fig. 7). The results suggest a future decrease in carbon sequestration in this Mediterranean forest if soil moisture drops by 15%, as predicted by GCMs (Bates *et al.*, 2008). Also, after 13 years of drought treatment, the species-specific differences in the response of growth and mortality did not differ from what was observed after five years by Ogaya and Peñuelas (2007b), so the more drought-resistant *P. latifolia* could potentially outcompete *Q. ilex* and *A. unedo* along the driest edge of the distribution of Holm oak forests.
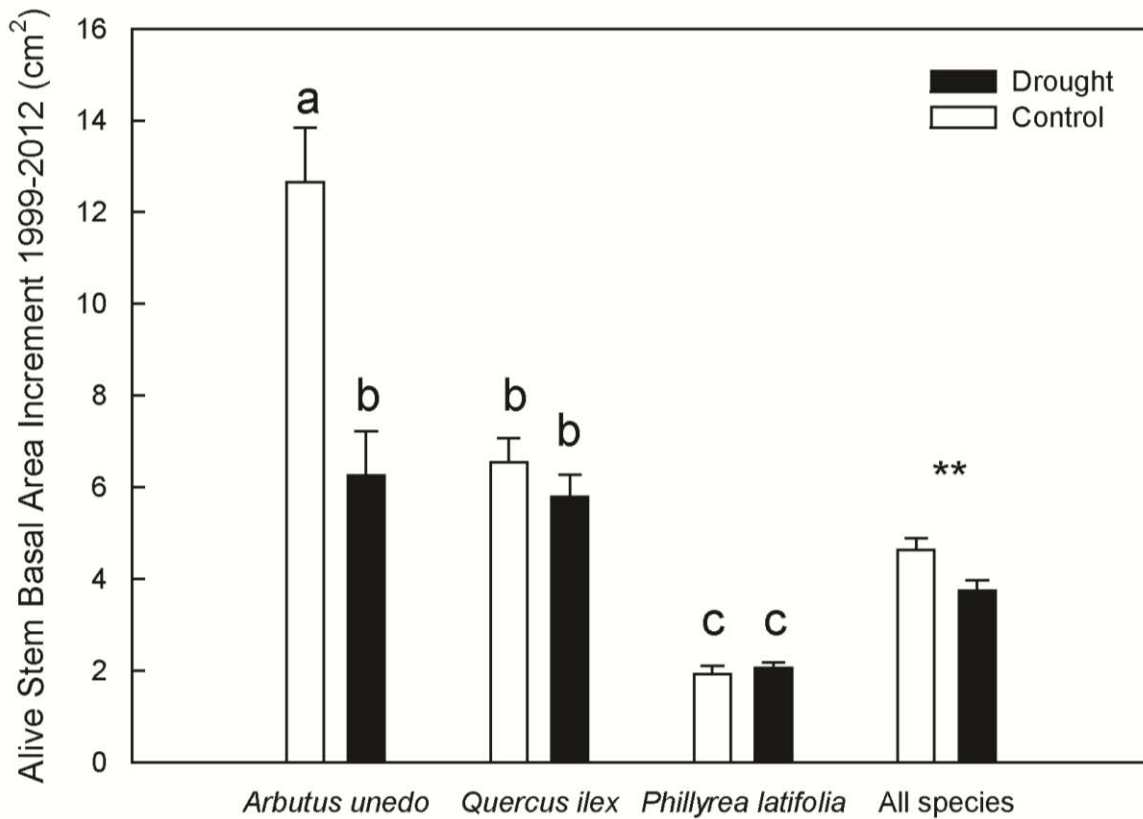
Figure 5. Accumulated stem basal area increments (BAI) for the stems alive after the 13 years of study. Letters indicate significant differences obtained with ANOVA and Tukey HSD post-hoc tests. Significant differences in all species analyzed together are marked with asterisks (**, *p*<0.01). Error bars are the standard error of the mean.

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

also exacerbated by losses of foliar area in the canopies of *Q. ilex* (Limousin *et al.*, 2009, Ogaya & Peñuelas, 2006). The deterioration of the crown in *Q. ilex* following long dry periods with low or negative rates of carbon assimilation has been associated with the depletion of tree carbon reserves (Galiano *et al.*, 2012). The higher stem mortality observed in *Q. ilex* in the drought plots might be a final consequence of this depletion, as further supported by the lower carbon reserves found in defoliated trees at the same study site (Rosas et al., 2013). As recently observed in other species (Anderegg *et al.*, 2012), though, hydraulic failure could also be the ultimate cause of stem death or crown defoliation, because *Q. ilex* is more vulnerable to xylem embolism than are other Mediterranean species (Martínez-Vilalta *et al.*, 2002). *A. unedo* was the only species that showed differences under the drought treatment in the accumulated growth of stems that survived throughout the study (Fig. 5). This result is further supported by the comparison between the annual BAI of newly dead and living stems of *Q. ilex* (Fig. 9). Stems of *Q. ilex* that had died by the end of the study had reduced growth prior to death, as noted by Pedersen (1998) in other *Quercus* species. We can thus suggest that *A. unedo* stems underwent a general reduction in growth induced by drought, while the growth of *Q. ilex* stems had a dual pattern. That is, healthy stems under drought grew at the same level as stems in the control plots, and the stems that died reduced their growth before death, perhaps due to chronic predisposing factors. Dying stems were smaller than living stems, indicating a self-thinning toward the maintenance of stems with better growth (data not shown). More severe droughts, however, kill stems with larger diameters, so we may infer that all size classes are likely to die in response to a certain length and/or intensity of drought.

Effects of meteorological drought

The inter-annual variabilities in stem growth and mortality rates were largely described by the SPEI drought index, more than did the drought treatment, as observed in other *Quercus* species (Wagner *et al.*, 2012) (Table 1). The effect of SPEI-3 on stem growth (Fig. 3, Table 1) revealed that spring was the most active period for plant growth at the study site, as is general for *Q. ilex* (Allard *et al.*, 2008, Corcuera *et al.*, 2004, Gea-Izquierdo *et al.*, 2011, Gutierrez *et al.*, 2011). Stem growth in *A. unedo*, however, matched with April SPEI-3 better than with May SPEI-3, as did the other species, indicating a possible earlier activation of plant growth. The effect of the SPEI-3 on growth was significant in all species; nevertheless, the effect size was much smaller in P. latifolia. This species responds less to increases in soil water content likely because it has lower hydraulic conductivity (Martínez-Vilalta *et al.*, 2002).
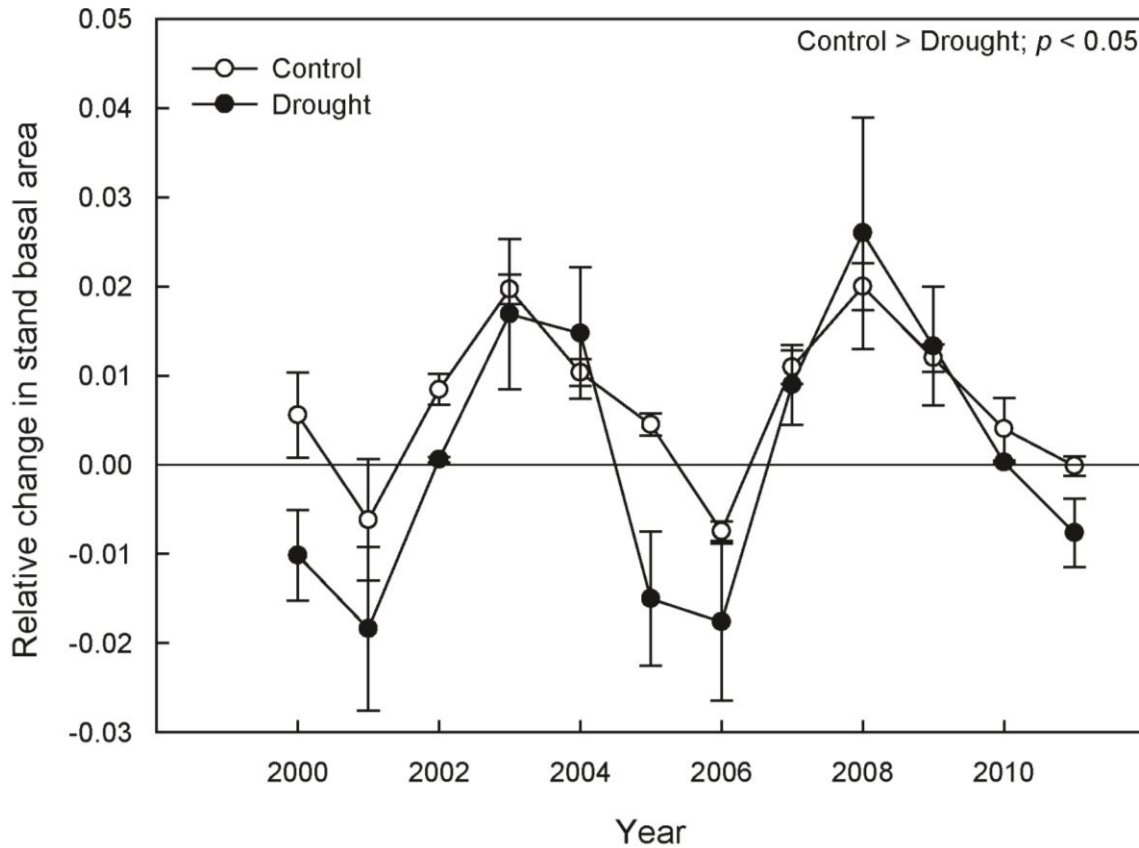
Figure 6. Relative annual change in basal area in control and drought plots for the period 1999–2012, calculated as the varia- tion in the total basal area of a plot for a given year relative to the previous year. It takes into account mortality and the basal area increments of all stems. Error bars are the standard error of the mean (n = 4).

Because stem mortality rates were strongly correlated with SPEI-6 in *Q. ilex* and *P. latifolia*, we may assert that mortality depended more on the water balance over longer periods than did growth. Stem mortality rates in *Q. ilex* and *P. latifolia* appeared to depend on the water balance of the period between April and September, but winter and spring water balance is also important for *Q. ilex*. Precipitation in winter and spring would replenish the water stores in deep soil layers, rock crevices or groundwater that could be accessed by the tap roots of *Q. ilex* to mitigate the summer drought (Baldocchi *et al.*, 2010, Gea-Izquierdo *et al.*, 2011, Gutierrez *et al.*, 2011, Sarris *et al.*, 2007). Stem long-term water storage may also depend on precipitation in winter months. Additionally, winter drought (as well as cold temperatures) may reduce photosynthetic activity in *Q. ilex*, which is higher than in *P. latifolia* during this season (Ogaya & Peñuelas, 2003). The assimilation of carbon in winter may be

used to recover a tree's carbon reserves (Gea-Izquierdo *et al.*, 2011) and may help to minimize drought-induced stem mortality in this species (Galiano *et al.*, 2012, Rosas *et al.*, 2013).
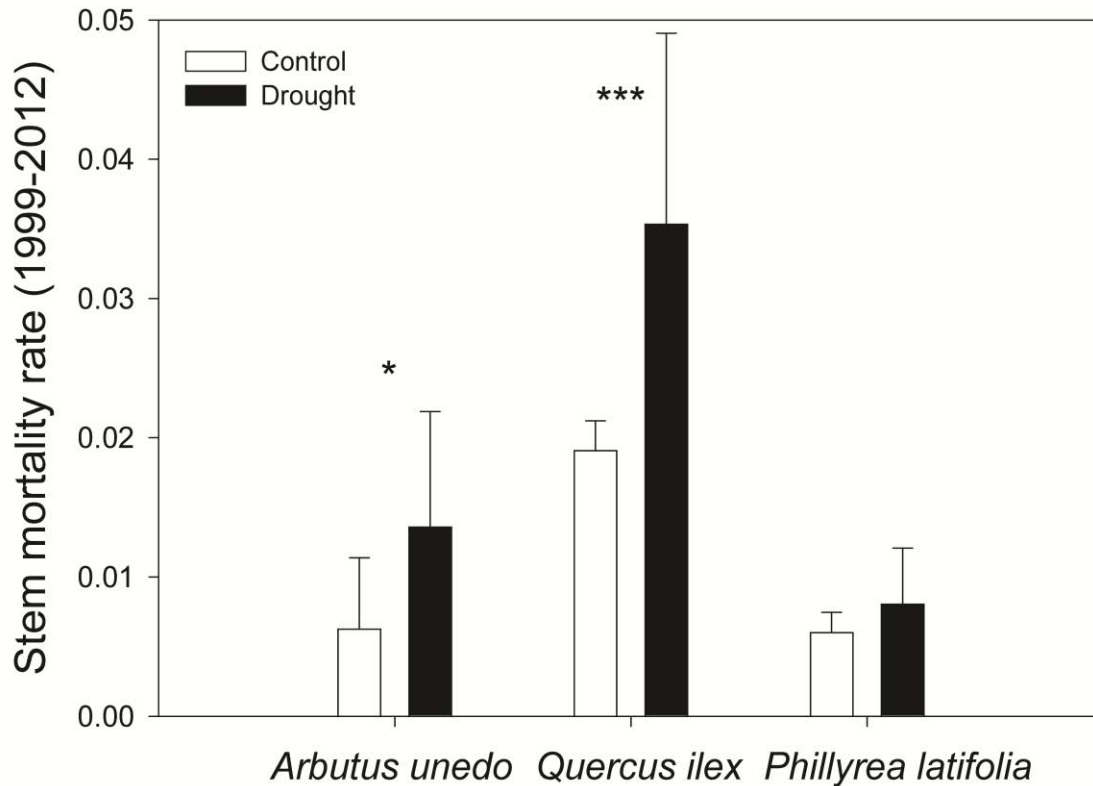

Figure 7. Stem mortality rates per species and treatment for the period 1999-2012. Significant differences are those obtained by the community-level model and are marked with asterisks (***, *p*<0.001) and *, *p*<0.1). Error bars are the standard error of the mean (n = 4).

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

Following the 2005-2006 droughts, *Q. ilex* and *P. latifolia* experienced slightly higher stem growth rates and enhanced stem survival, both in drought and control plots (Fig. 6, Table 2b). Such changes might suggest that climate was more favorable during the second period, but there were no significant differences in the relevant scales and months of the SPEI (Fig. 2). In addition, the effect of treatment on the annual stem BAI of *A. unedo* and *Q. ilex* seemed to consistently decline with time (Fig. 4). The changes in growth and mortality after 2006 could be a consequence of a demographic compensation, as proposed by Lloret *et al.*, (2012).
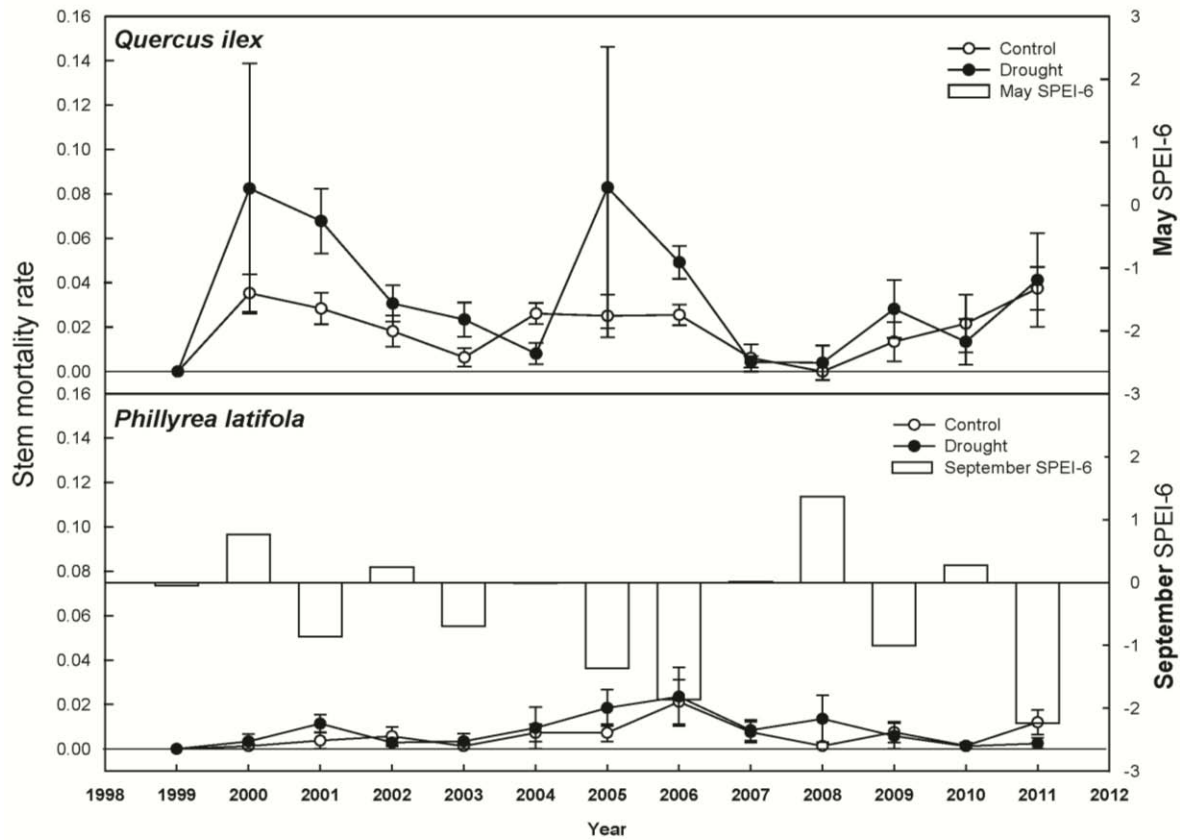
46

Figure 8. Stem mortality rates (left axis) of *Quercus ilex* and *Phillyrea latifolia* in each treatment. Error bars are the standard error of the mean (n = 4). The open boxes are the six-month SPEI that best explained the stem mortality of each species (right axis).

That is, the higher mortality rates beyond natural variability caused by extreme droughts during the first period of the study (exacerbated by the experimental drought in the treatment plots) may be partly compensated in the second period by a higher survival of the remaining population, as observed in *Q. ilex*, which was initially most affected. Decreased intra- and interspecific competition might enhance plant survival and growth through an increased availability of water per stem or individual. In drought plots, where stem mortality was formerly higher, decreased competition resulted from stronger reductions of basal area than in control plots (Fig. 6). Furthermore, reductions of stem mortality in the drought plots also may be expected as a result of morphological acclimation after several years of treatment, because, for instance, drought reduces foliar area in *Q. ilex* as an adaptation to scarcer water resources (Limousin *et al.*, 2009, Ogaya & Peñuelas, 2006). Likewise, Martin-StPaul *et al.* (2013) studied the temporal response to drought of *Q. ilex* and suggested that whole tree

water transport and stand leaf area index are the key variables that will acclimate in future drier conditions. Q. ilex stems are more likely to die the higher the number of stems per individual (Galiano *et al.*, 2012), so a previous natural self-thinning caused by extreme droughts could produce a higher resistance to future stresses in the remaining stems. The response of the ecosystem to drought thus did not seem to be accelerated by the extreme events as could be expected (Jentsch *et al.*, 2007). On the contrary, both the duration of the study (Leuzinger *et al.*, 2011) and the extreme events (Lloret *et al.*, 2012) may have dampened or compensated the treatment effects to some extent and promoted non-linear responses of the vegetation to drought. These results should be considered when predicting and modeling the long-term responses of plant communities to climatic change.
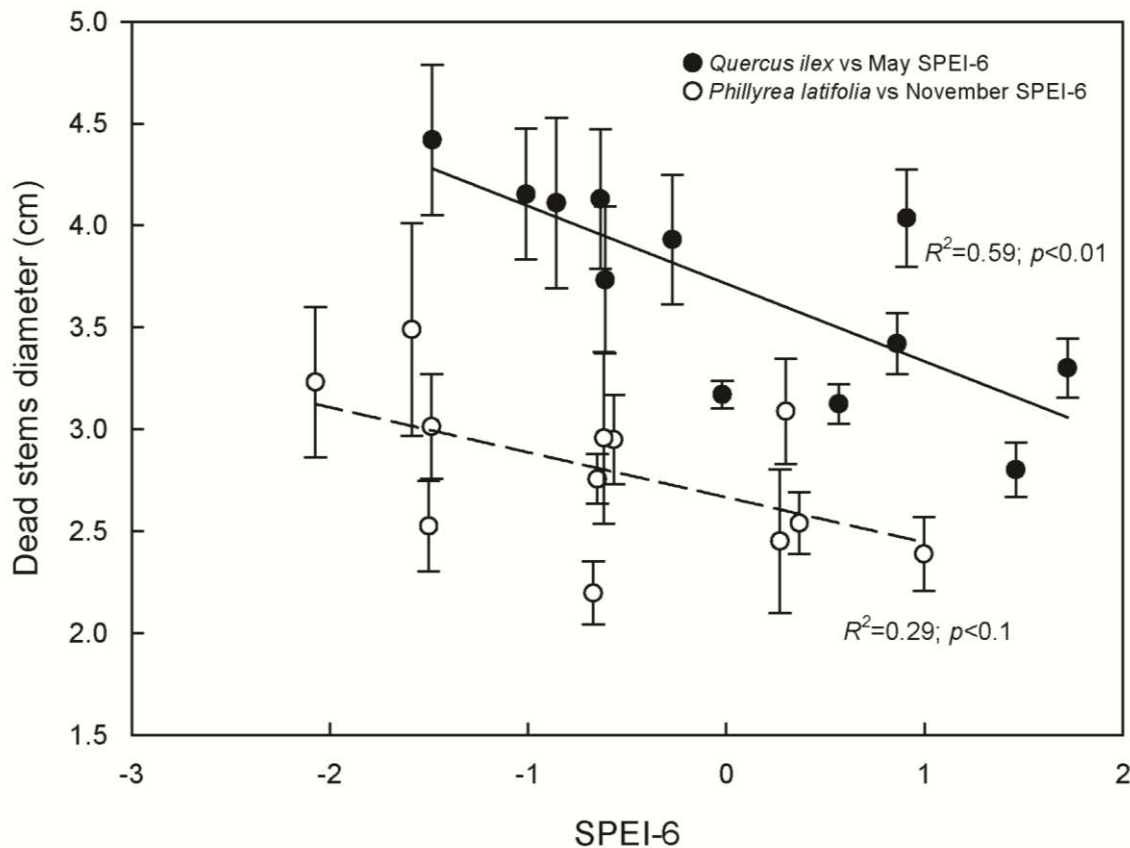

Figure 9. Linear regressions between the dead-stem diameter and the monthly SPEI that best fit each species.
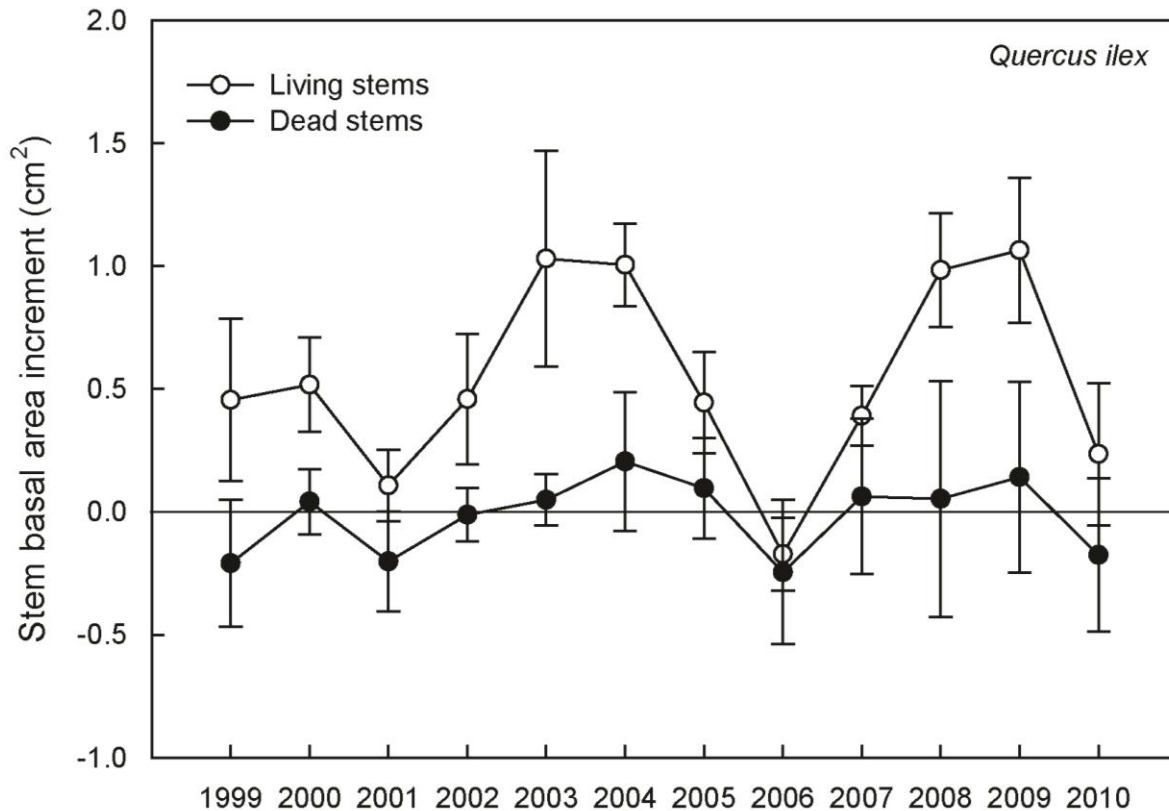
Figure 10. Annual stem basal area increments (BAI) of the stems that survived throughout the study and those that died during it. Error bars are the standard error of the mean.

The results of this study indicate significant decreases in plant growth and accelerated mortality rates in the dominant species of a Holm oak forest subjected to an 18% decrease in soil moisture. The time span of the study provides consistency to these results, which would help to predict more precisely the response of this community to climatic change. The variant species-specific responses to either experimental or natural drought would potentially cause a vegetational shift, as reported in other systems (Mueller *et al.*, 2005), consisting of a partial substitution of the most drought-sensitive species, *Q. ilex* and *A. unedo,* by the most drought-resistant species, *P. latifolia*. This shift may eventually alter the composition of the landscape and the ecosystem's services, although it would present a patchy pattern dependent on site characteristics (Lloret *et al.*, 2004b). On the other hand, changes in forest structure (reductions in density and basal area) and demographic compensation (Lloret *et al.*, 2012), in addition to physiological and morphological acclimation to drought at the individual level (reduced foliar area and growth, greater allocation of carbon to roots or fewer stems per individual), would enhance the persistence of *Q. ilex* and *A. unedo*, presumably maintaining

their dominance in the ecosystem. These processes seem very likely to alter the initial response of vegetation in short-term drought experiments, but they still remain poorly understood. The duration of experimental drought systems should be extended for determining the role of these processes. Also, the possible changes in the seasonal distribution of precipitation and the duration of drought periods should be incorporated into experimental studies to properly predict responses to changes in precipitation.

ACKNOWLEDGEMENTS

REFERENCES

Allard V, Ourcival JM, Rambal S, Joffre R, Rocheteau A (2008) Seasonal and annual variation of carbon exchange in an evergreen Mediterranean forest in southern France. Global Change Biology, 14, 714-725.

Allen CD, Macalady AK, Chenchouni H et al. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management, 259, 660-684.

Anderegg WRL, Berry JA, Smith DD, Sperry JS, Anderegg LDL, Field CB (2012) The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. Proceedings of the National Academy of Sciences of the United States of America, 109, 233-237.

Asensio D, Peñuelas J, Ogaya R, Llusia J (2007) Seasonal soil and leaf CO_2 exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. Atmospheric Environment, 41, 2447-2455.

Baldocchi DD, Ma S, Rambal S et al. (2010) On the differential advantages of evergreenness and deciduousness in mediterranean oak woodlands: a flux perspective. Ecological Applications, 20, 1583-1597.

Barbeta A, Ogaya R, Peñuelas J (2012) Comparative study of diurnal and nocturnal sap flow of Quercus ilex and Phillyrea latifolia in a Mediterranean holm oak forest in Prades (Catalonia, NE Spain). Trees-Structure and Function, 26, 1651–1659.

Bates BC, Kundzewicz ZW, Wu S, Palutikof JP, Eds. (2008) Climate Change and Water. Technical. Paper of the Intergovernmental Panel on Climate Change. 210 pp.

Brando PM, Nepstad DC, Davidson EA, Trumbore SE, Ray D, Camargo P (2008) Drought effects on litterfall, wood production and belowground carbon cycling in an Amazon forest: results of a throughfall reduction experiment. Philosophical Transactions of the Royal Society B-Biological Sciences, 363, 1839-1848.

Breshears DD, Myers OB, Meyer CW et al. (2009) Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. Frontiers in Ecology and the Environment, 7, 185-189.

Carnicer J, Coll M, Ninyerola M, Pons X, Sanchez G, Peñuelas J (2011) Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proceedings of the National Academy of Sciences of the United States of America, 108, 1474-1478.

Cleveland CC, Wieder WR, Reed SC, Townsend AR (2010) Experimental drought in a tropical rain forest increases soil carbon dioxide losses to the atmosphere. Ecology, 91, 2313-2323.

Corcuera L, Camarero JJ, Gil-Pelegrin E (2004) Effects of a severe drought on Quercus ilex radial growth and xylem anatomy. Trees-Structure and Function, 18, 83-92.

Da Costa ACL, Galbraith D, Almeida S et al. (2010) Effect of 7 yr of experimental drought on vegetation dynamics and biomass storage of an eastern Amazonian rainforest. New Phytologist, 187, 579-591.

Fischer EM, Schar C (2010) Consistent geographical patterns of changes in high-impact European heatwaves. Nature Geoscience, 3, 398-403.

Galiano L, Martínez-Vilalta J, Sabate S, Lloret F (2012) Determinants of drought effects on crown condition and their relationship with depletion of carbon reserves in a Mediterranean holm oak forest. Tree Physiology, 32, 478-489.

Gaul D, Hertel D, Borken W, Matzner E, Leuschner C (2008) Effects of experimental drought on the fine root system of mature Norway spruce. Forest Ecology and Management, 256, 1151-1159.

Gea-Izquierdo G, Cherubini P, Canellas I (2011) Tree-rings reflect the impact of climate change on Quercus ilex L. along a temperature gradient in Spain over the last 100 years. Forest Ecology and Management, 262, 1807-1816.

Gray AN, Spies TA (1995) Water-Content Measurement in Forest Soils and Decayed Wood Using Time-Domain Reflectometry. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 25, 376-385.

Gutierrez E, Campelo F, Camarero JJ, Ribas M, Muntan E, Nabais C, Freitas H (2011) Climate controls act at different scales on the seasonal pattern of Quercus ilex L. stem radial increments in NE Spain. Trees-Structure and Function, 25, 637-646.

Hadfield JD (2010) MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software, 33, 1-22.

Hanson PJ, Todd DE, Amthor JS (2001) A six-year study of sapling and large-tree growth and mortality responses to natural and induced variability in precipitation and throughfall. Tree Physiology, 21, 345-358.

Huang CY, Anderegg WRL (2012) Large drought-induced aboveground live biomass losses in southern Rocky Mountain aspen forests. Global Change Biology, 18, 1016-1027.

Jentsch A, Kreyling J, Beierkuhnlein C (2007) A new generation of climate-change experiments: events, not trends. Frontiers in Ecology and the Environment, 5, 365-374.

Jump AS, Hunt JM, Martinez-Izquierdo JA, Peñuelas J (2006) Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in Fagus sylvatica. Molecular Ecology, 15, 3469-3480.

Leuzinger S, Luo Y, Beier C, Dieleman W, Vicca S, Koerner C (2011) Do global change experiments overestimate impacts on terrestrial ecosystems? Trends in Ecology & Evolution, 26, 236-241.

Limousin JM, Longepierre D, Huc R, Rambal S (2010) Change in hydraulic traits of Mediterranean Quercus ilex subjected to long-term throughfall exclusion. Tree Physiology, 30, 1026-1036.

Limousin JM, Rambal S, Ourcival JM et al. (2012) Morphological and phenological shoot plasticity in a Mediterranean evergreen oak facing long-term increased drought. Oecologia, 169, 565-577.

Limousin JM, Rambal S, Ourcival JM, Rocheteau A, Joffre R, Rodriguez-Cortina R (2009) Long-term transpiration change with rainfall decline in a Mediterranean Quercus ilex forest. Global Change Biology, 15, 2163-2175.

Lloret F, Escudero A, Maria Iriondo J, Martínez-Vilalta J, Valladares F (2012) Extreme climatic events and vegetation: the role of stabilizing processes. Global Change Biology, 18, 797-805.

Lloret F, Peñuelas J, Ogaya R (2004a) Establishment of co-existing Mediterranean tree species under a varying soil moisture regime. Journal of Vegetation Science, 15, 237-244.

Lloret F, Siscart D, Dalmases C (2004b) Canopy recovery after drought dieback in holm-oak Mediterranean forests of Catalonia (NE Spain). Global Change Biology, 10, 2092-2099.

Martin–StPaul NK, Limousin JM, Vogt-Schilb H, Rodríguez-Calcerrada J, Rambal S, Longepierre D, Misson L (2013) The temporal response to drought in a Mediterranean evergreen tree: comparing a regional precipitation gradient and a throughfall exclusion experiment. Global Change Biology. doi: 10.1111/gcb.12215.

Martin-Benito D, Beeckman H, Canellas I (2013) Influence of drought on tree rings and tracheid features of Pinus nigra and Pinus sylvestris in a mesic Mediterranean forest. European Journal of Forest Research, 132, 33-45.

Martínez-Vilalta J, Prat E, Oliveras I, Pinol J (2002) Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. Oecologia, 133, 19-29.

Mueller RC, Scudder CM, Porter ME, Trotter RT, Gehring CA, Whitham TG (2005) Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. Journal of Ecology, 93, 1085-1093.

Ogaya R, Peñuelas J (2003) Comparative field study of Quercus ilex and Phillyrea latifolia: photosynthetic response to experimental drought conditions. Environmental and Experimental Botany, 50, 137-148.

Ogaya R, Peñuelas J (2006) Contrasting foliar responses to drought in Quercus ilex and Phillyrea latifolia. Biologia Plantarum, 50, 373-382.

Ogaya R, Peñuelas J (2007a) Species-specific drought effects on flower and fruit production in a Mediterranean holm oak forest. Forestry, 80, 351-357.

Ogaya R, Peñuelas J (2007b) Tree growth, mortality, and above-ground biomass accumulation in a holm oak forest under a five-year experimental field drought. Plant Ecology, 189, 291-299.

Ogaya R, Peñuelas J, Asensio D, Llusia J (2011) Chlorophyll fluorescence responses to temperature and water availability in two co-dominant Mediterranean shrub and tree species in a long-term field experiment simulating climate change. Environmental and Experimental Botany, 71, 123-127.

Ogaya R, Peñuelas J, Martínez-Vilalta J, Mangiron M (2003) Effect of drought on diameter increment of Quercus ilex, Phillyrea latifolia, and Arbutus unedo in a holm oak forest of NE Spain. Forest Ecology and Management, 180, 175-184.

Ortego J, Bonal R, Munoz A (2010) Genetic Consequences of Habitat Fragmentation in Long-Lived Tree Species: The Case of the Mediterranean Holm Oak (Quercus ilex, L.). Journal of Heredity, 101, 717-726.

Pedersen BS (1998) The role of stress in the mortality of midwestern oaks as indicated by growth prior to death. Ecology, 79, 79-93.

Peñuelas J, Filella I, Lloret F, Pinol J, Siscart D (2000) Effects of a severe drought on water and nitrogen use by Quercus ilex and Phillyrea latifolia. Biologia Plantarum, 43, 47-53.

Peñuelas J, Filella I, Llusia J, Siscart D, Pinol J (1998) Comparative field study of spring and summer leaf gas exchange and photobiology of the Mediterranean trees Quercus ilex and Phillyrea latifolia. Journal of Experimental Botany, 49, 229-238.

Peñuelas J, Lloret F, Montoya R (2001) Severe drought effects on Mediterranean woody flora in Spain. Forest Science, 47, 214-218.

Peñuelas J, Ogaya R, Boada M, Jump AS (2007a) Migration, invasion and decline: changes in recruitment and forest structure in a warming-linked shift of European beech forest in Catalonia (NE Spain). Ecography, 30, 829-837.

Peñuelas J, Prieto P, Beier C et al. (2007b) Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought: reductions in primary productivity in the heat and drought year of 2003. Global Change Biology, 13, 2563-2581.

R Core Development Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reyer CPO, Leuzinger S, Rammig A et al. (2013) A plant's perspective of extremes: terrestrial plant responses to changing climatic variability. Global Change Biology, 19, 75-89.

Ripullone F, Borghetti M, Raddi S et al. (2009) Physiological and structural changes in response to altered precipitation regimes in a Mediterranean macchia ecosystem. Trees-Structure and Function, 23, 823-834.

Rodà F, Retana J, Gracia CA, Bellot J (1999) Ecology of Mediterranean evergreen oak forests, Berlin, Springer.

Rosas T, Galiano L, Ogaya R, Peñuelas J, Martínez-Vilalta J (2013) Dynamics of non-structural carbohydrates in three Mediterranean woody species under long-term experimental drought. Frontiers in Plant Science (submitted).

Sarris D, Christodoulakis D, Koerner C (2007) Recent decline in precipitation and tree growth in the eastern Mediterranean. Global Change Biology, 13, 1187-1200.

Sarris D, Christodoulakis D, Koerner C (2011) Impact of recent climatic change on growth of low elevation eastern Mediterranean forest trees. Climatic Change, 106, 203-223.

Sheil D, Burslem D, Alder D (1995) The Interpretation and Misinterpretation of Mortality-Rate Measures. Journal of Ecology, 83, 331-333.

Vicente-Serrano SM, Begueria S, Lopez-Moreno JI (2010) A Multiscalar Drought Index Sensitive to Global Warming: The Standardized Precipitation Evapotranspiration Index. Journal of Climate, 23, 1696-1718.

Vicente-Serrano SM, Begueria S, Lorenzo-Lacruz J et al. (2012a) Performance of Drought Indices for Ecological, Agricultural, and Hydrological Applications. Earth Interactions, 16.

Vicente-Serrano SM, Gouveia C, Julio Camarero J et al. (2012b) Response of vegetation to drought time-scales across global land biomes. Proceedings of the National Academy of Sciences of the United States of America, 110, 52-57.

Wagner RJ, Kaye MW, Abrams MD, Hanson PJ, Martin M (2012) Tree-Ring Growth and Wood Chemistry Response to Manipulated Precipitation Variation for Two Temperate Quercus Species. Tree-Ring Research, 68, 17-29.

Wu Z, Dijkstra P, Koch GW, Peñuelas J, Hungate BA (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Global Change Biology, 17, 927-942.

Zegelin SJ, White I, Jenkins DR (1989) Improved Field Probes for Soil-Water Content and Electrical-Conductivity Measurement Using Time Domain Reflectometry. Water Resources Research, 25, 2367-2376.

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

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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.

KEYWORDS

Holm oak, experimental drought, stable isotopes, water uptake, Mediterranean forest, climate change, extreme drought, water-use strategies, *Arbutus unedo*, *Quercus ilex*, *Phillyrea latifolia*.

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; Sarris *et al.*, 2007; 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, 2007a; Limousin *et al.*, 2010a) 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 ($\delta^{18}O$ and $\delta^{2}H$) composition of water is a powerful tool for tracing the movement of water underground (Dawson *et al.*, 2002; Werner *et al.*, 2012). Isotopic fractionation does not occur during water absorption by roots (Ehleringer & Dawson, 1992), 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; Moreno-Gutiérrez *et al.*, 2012). 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; Rossatto *et al.*, 2012; 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 (Moreno-Gutiérrez *et al.*, 2012; 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; Rico *et al.*, 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?

MATERIAL 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(Ogaya & Peñuelas, 2007a). 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 (δ ^{18}O and δ ^{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 δ^2H 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
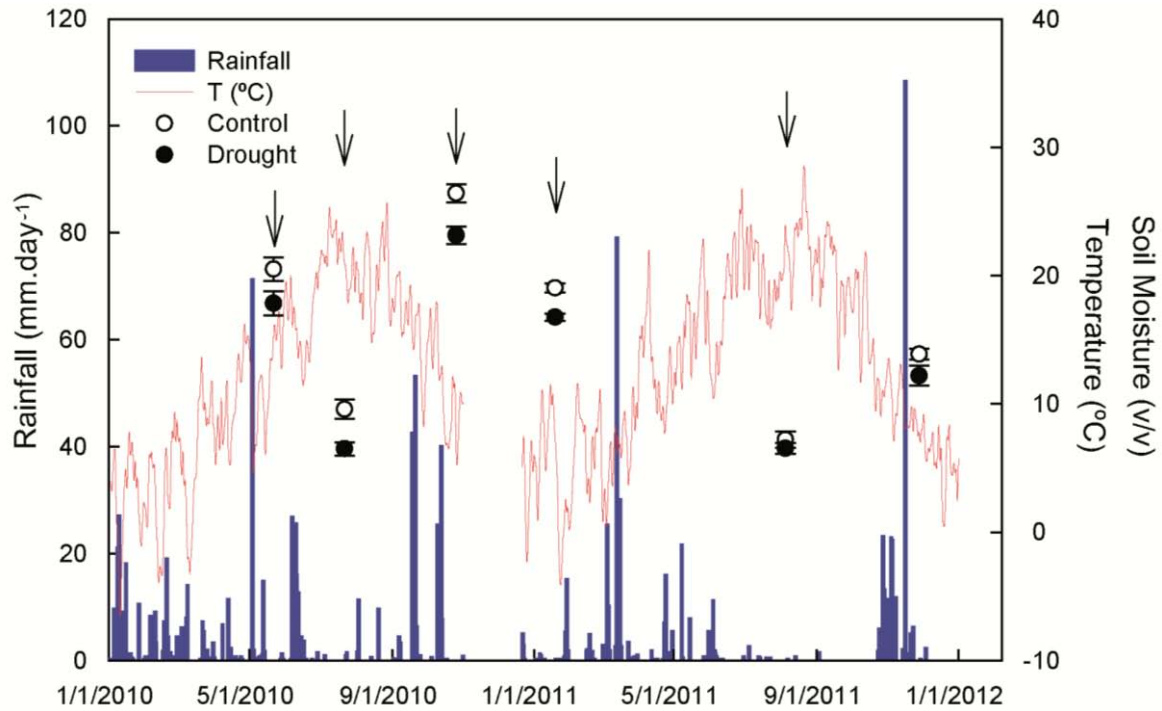
Figure 1. Daily precipitation and mean temperatures during the study period (2010-2011). Soil moisture in the control and drought plots. The error bars are the standard errors of the means (n=2). Arrows indicate the sampling campaigns.

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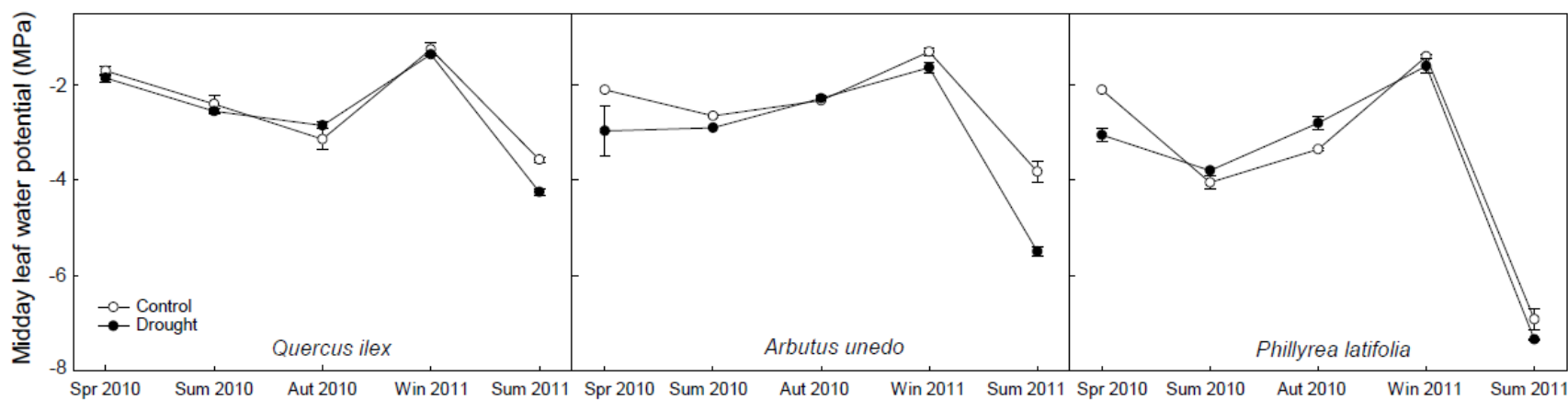
Figure 2. Seasonal variation in midday foliar water potentials of the three species for control (open circles) and droughted (closed circles) individuals. The droughted plants had significantly lower midday foliar water potentials (F=5.43, $p<0.05$, ANOVA). Differences between seasons and species are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

Isotopic composition of plant-water sources

δ^{18}O and δ^{2}H in the soil water varied with depth and season. Depth was negatively associated with δ^{18}O and δ^{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 δ^{18}O and δ^{2}H (*pMCMC*=0.51). The values of δ^{18}O and δ^{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 (δ^{18}O=-7.19±0.14 and δ^{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.

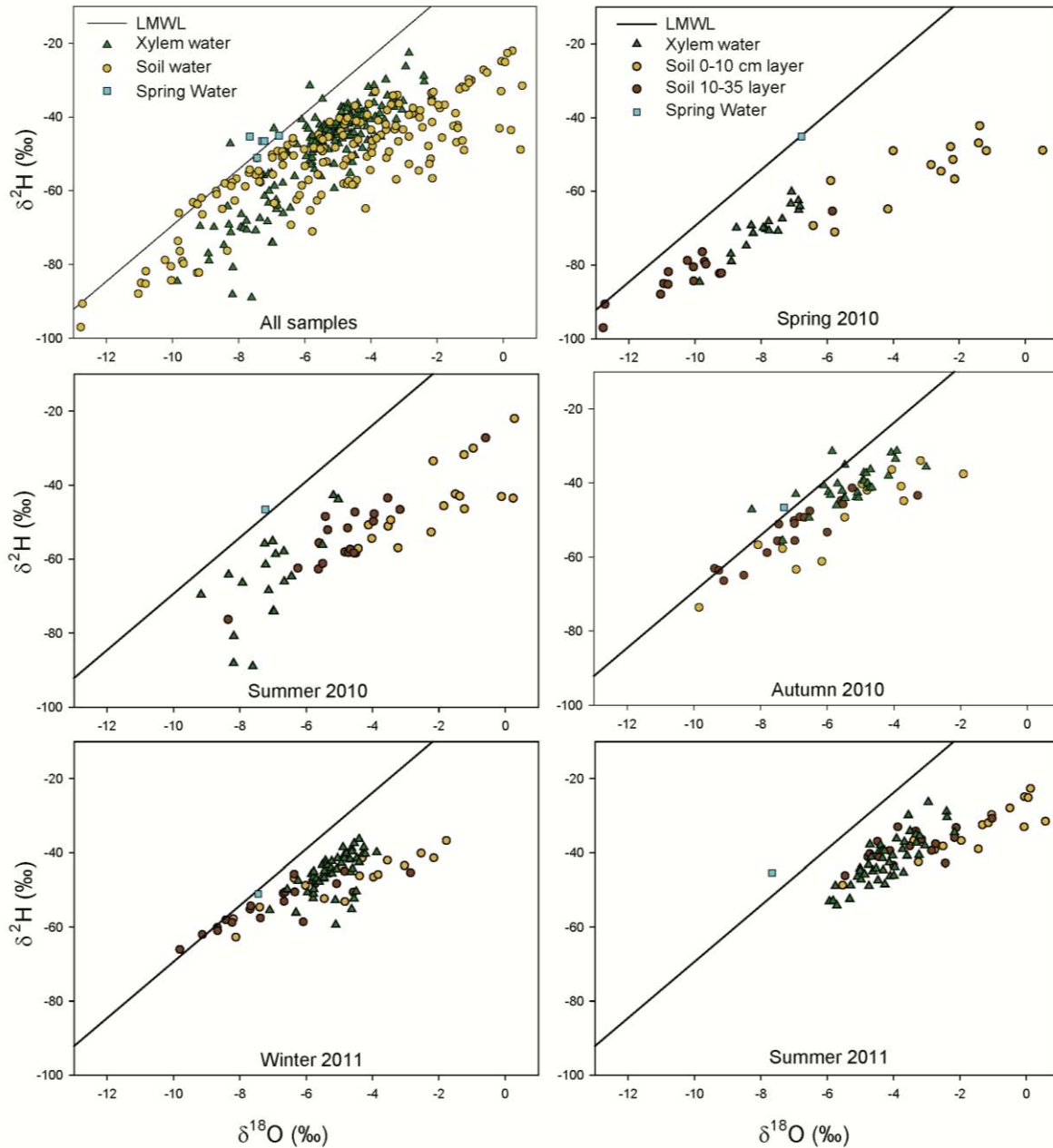**Figure 3.** Water isotopes for all samples of xylem (triangles), soil (circles) and spring (squares) water. All samples are plotted in the upper left panel, with the remaining panels corresponding to single seasons. The line in the panels is the local meteoric water line (LMWL), corresponding to $\delta^2H=6.62+7.60*\delta^{18}O$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

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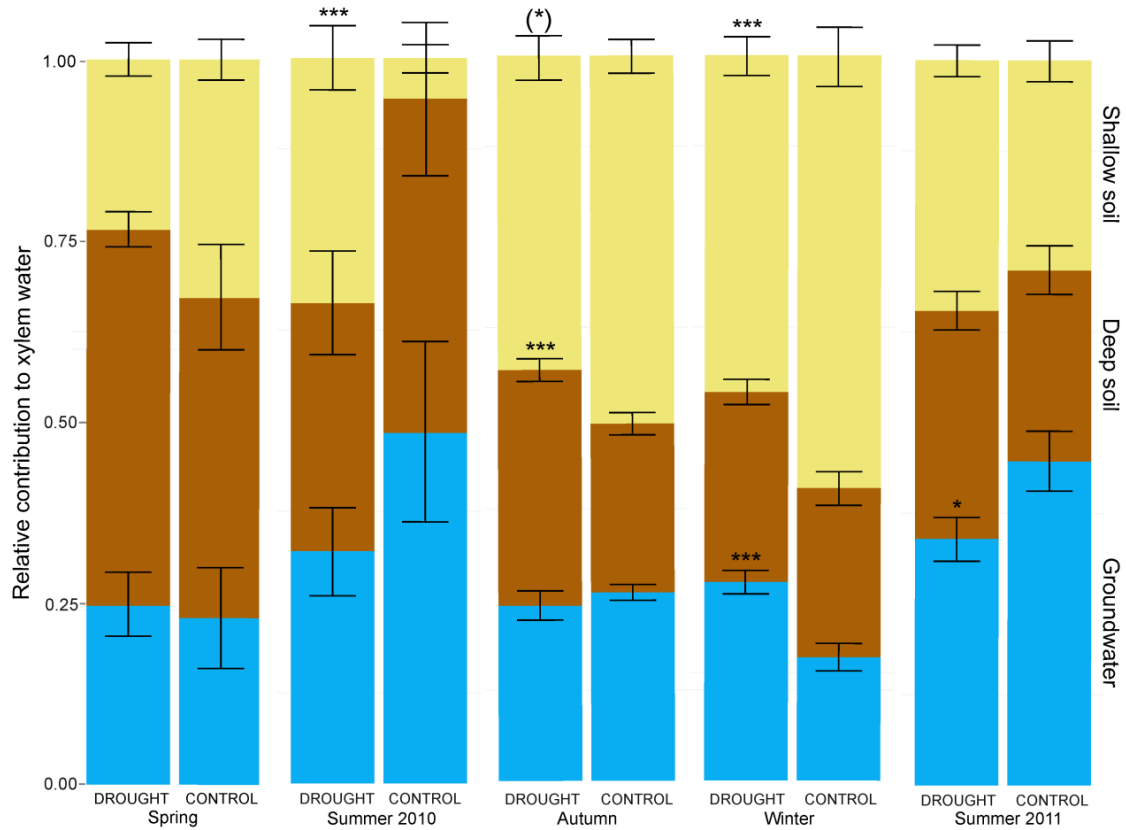
Figure 4. Mean contributions of plant-water sources for each season in the control and drought treatments obtained by siar Bayesian mixing models. The error bars are the standard errors of the means. The asterisks denote significance levels for the comparisons between the control and drought treatments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

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

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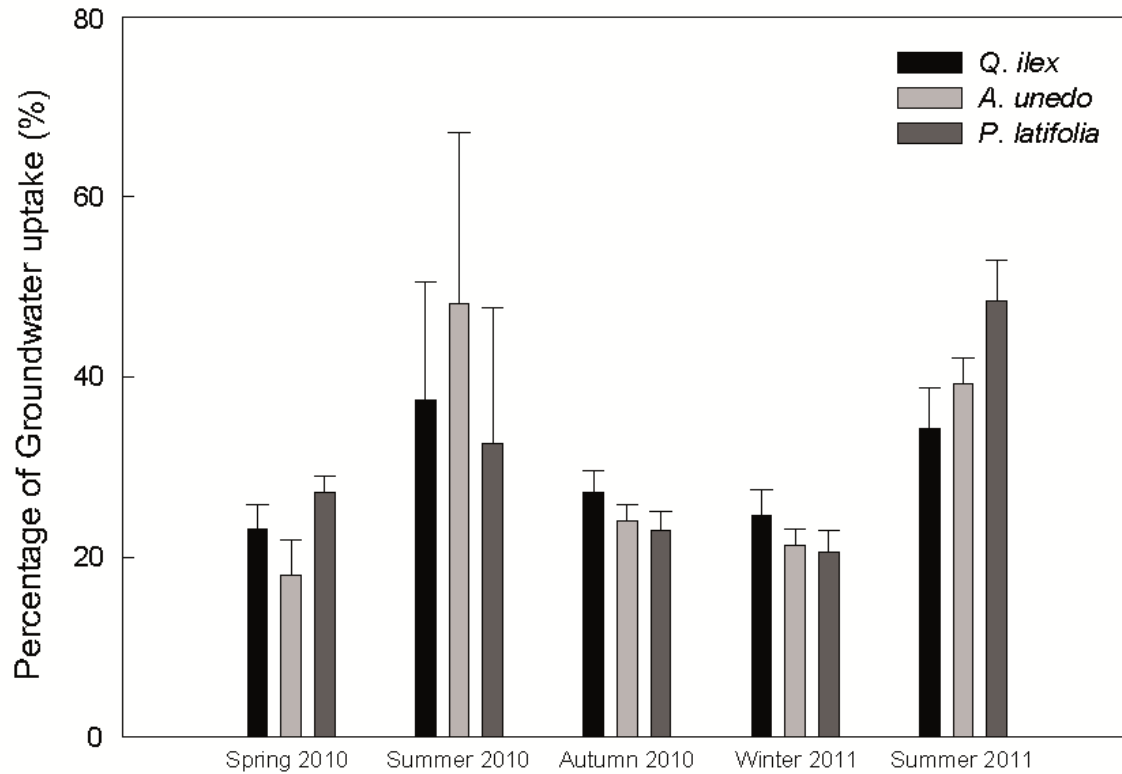
Figure 5. Seasonal percentages of groundwater uptake in the three species for each season. The errors bars are the standard errors of the means.

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).

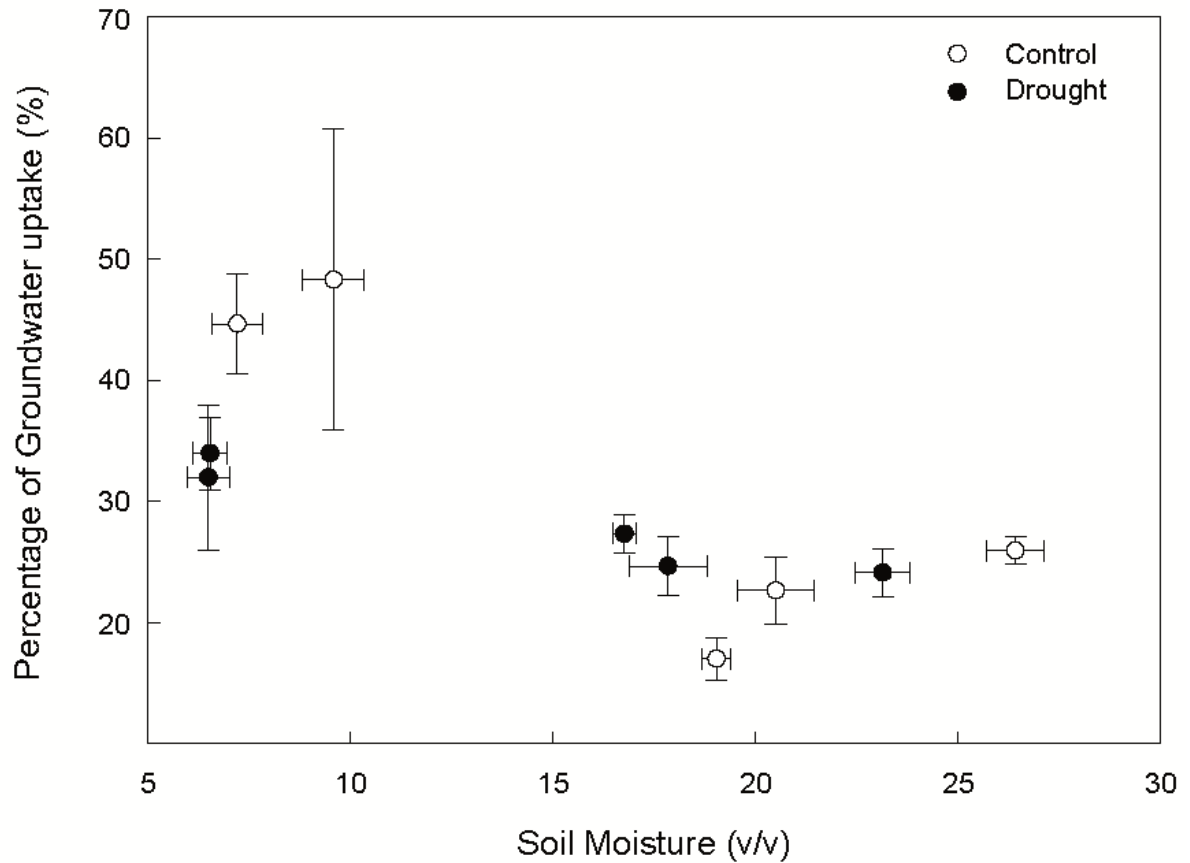**Figure 6.** Relationship between percentage of groundwater uptake and soil moisture in the two treatments. The Y-axis values are the mean seasonal proportions of groundwater uptake for each treatment, and the three species are pooled. The error bars are the standard errors of the means.

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).
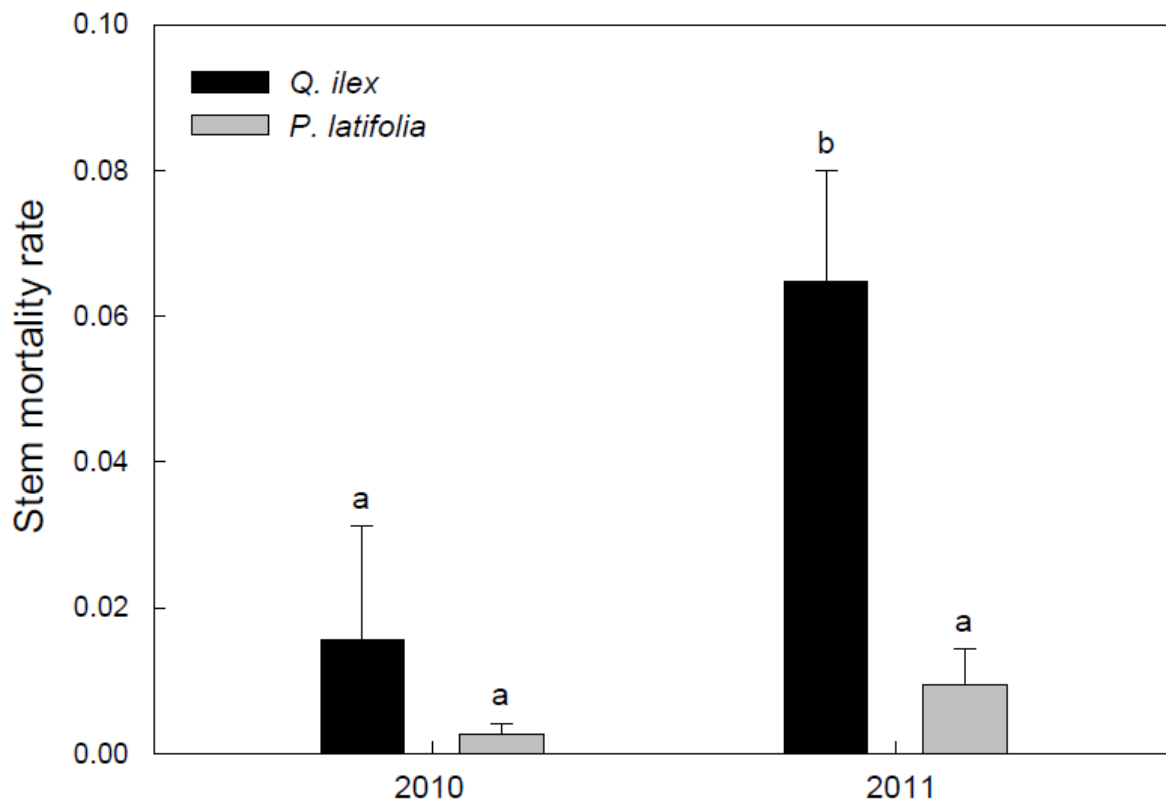
Figure 7. Stem mortality rates for *Quercus ilex* and *Phillyrea latifolia* (2010 and 2011) calculated for the plots where the isotope samples were collected. Different letters indicate significantly different stem mortality rates, which were assessed by ANOVAs (*p<0.05*).
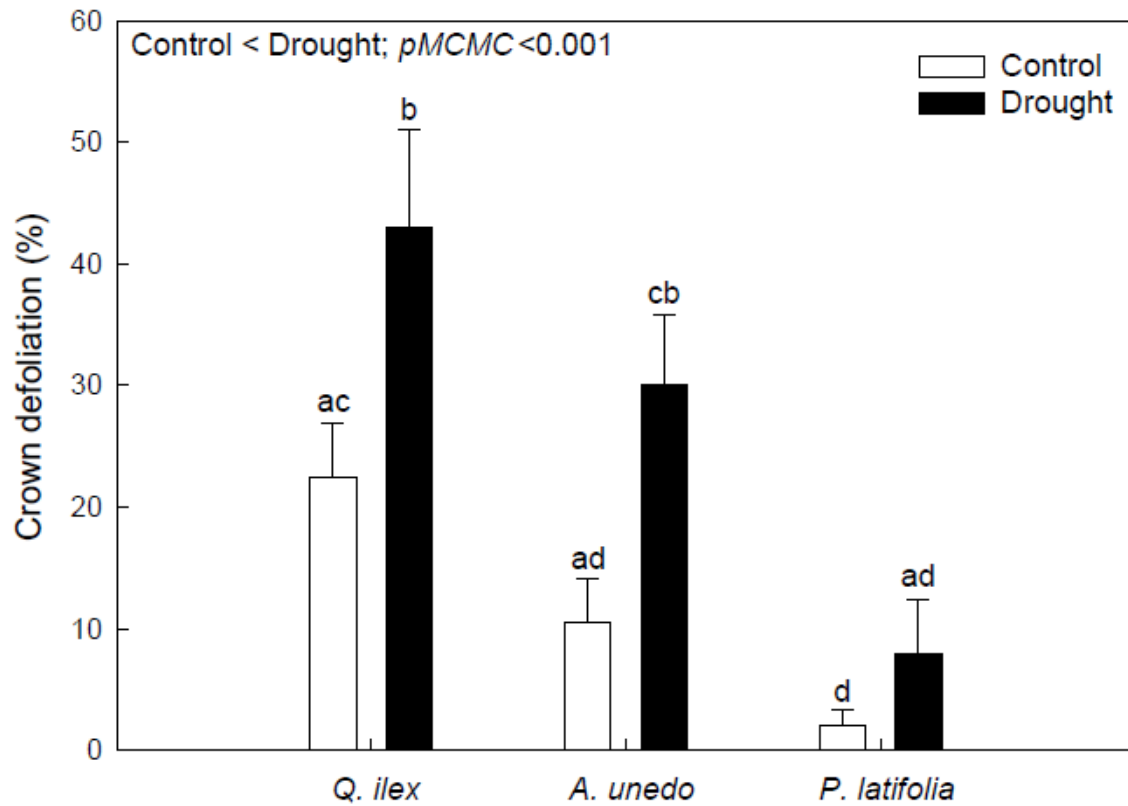
Figure 8. Crown defoliation (%) following the extreme drought in 2011 for each species and treatment for the plots where the isotope samples were collected. The error bars are the standard errors of the means (n=10). Different letters indicate significant differences between group percentages, assessed 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.

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.*, 2013c; 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, 2003a), 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; Sternberg *et al.*, 1996; Moreno & Cubera, 2008; Maeght *et al.*, 2013). 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; Filella & Peñuelas, 2003; 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, 2007a; 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.*, 2013c), 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.

ACKNOWLEDGEMENTS

REFERENCES

Allison GB (1982) The relationship between 18O and deuterium in water in sand columns undergoing evaporation. Journal of Hydrology, 55, 163–169.

Anderegg WRL, Berry JA, Field CB (2012) Linking definitions, mechanisms, and modeling of drought-induced tree death. Trends in Plant Science, 17, 693–700.

Anderegg WRL, Kane JM, Anderegg LDL (2013a) Consequences of widespread tree mortality triggered by drought and temperature stress. Nature Climate Change, 3, 30–36.

Anderegg LDL, Anderegg WRL, Abatzoglou J, Hausladen AM, Berry JA (2013b) Drought characteristics' role in widespread aspen forest mortality across Colorado, USA. Global Change Biology, 19, 1526–37.

Araya YN, Silvertown J, Gowing DJ, Mcconway KJ, Linder HP, Midgley G (2011) A fundamental , eco-hydrological basis for niche segregation in plant communities. New Phytologist, 189, 253–258.

Barbeta A, Ogaya R, Peñuelas J (2012) Comparative study of diurnal and nocturnal sap flow of Quercus ilex and Phillyrea latifolia in a Mediterranean holm oak forest in Prades (Catalonia , NE Spain). Trees, 26, 1651–1659.

Barbeta A, Ogaya R, Peñuelas J (2013) Dampening effects of long-term experimental drought on growth and mortality rates of a Holm oak forest. Global Change Biology, 19, 3133–44.

Breshears DD, Myers OB, Meyer CW et al. (2009) Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. Frontiers in Ecology and the Environment, 7, 185–189.

Brolsma RJ, van Vliet MTH, Bierkens MFP (2010) Climate change impact on a groundwater-influenced hillslope ecosystem. Water Resources Research, 46, 1–15.

Brooks JR, Barnard HR, Coulombe R, McDonnell JJ (2009) Ecohydrologic separation of water between trees and streams in a Mediterranean climate. Nature Geoscience, 3, 100–104.

Canadell J, Zedler PH (1995) Underground structures of woody plants in Mediterranean ecosystems of Asutralia, California and Chile. In: Ecology and Biogeography of Mediterranean Ecosystems in Chile, California and Australia (eds Kalin Arroyo MT, Zedler PH, Fox MD), pp. 177–210. Springer New York.

Canadell J, Jackson RB, Ehleringer JB, Mooney H a., Sala OE, Schulze E-D (1996) Maximum rooting depth of vegetation types at the global scale. Oecologia, 108, 583–595.

Canadell J, Djema A, López B, Lloret F, Sabate S, Siscart D, Gracia CA (1999) Structure and dynamics of the root system. In: Ecology of Mediterranean Evergreen Oak Forests (eds Rodà F,
Retana J, Gracia CA, Bellot J. , pp. 47–59. Springer Berlin / Heidelberg.

Carnicer J, Barbeta A, Sperlich D, Coll M, Peñuelas J (2013a) Contrasting trait syndromes in angiosperms and conifers are associated with different responses of tree growth to temperature on a large scale. Frontiers in plant science, 4, 409.

Carnicer J, Brotons L, Herrando S, Sol D (2013b) Improved empirical tests of area-heterogeneity tradeoffs. Proceedings of the National Academy of Sciences of the United States of America, 110, E2858–60.

Christensen JH, Hewitson B, Busuioc A et al. (2007) Regional Climate Projections. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (eds Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL). Cambridge University Press, Cambridge, UK. New York, USA.

David TS, Henriques MO, Kurz-Besson C et al. (2007a) Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. Tree Physiology, 27, 793–803.

David TS, Henriques MO, Kurz-Besson C et al. (2007b) Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. Tree Physiology, 27, 793–803.

David TS, Pinto CA, Nadezhdina N et al. (2013) Root functioning, tree water use and hydraulic redistribution in Quercus suber trees: A modeling approach based on root sap flow. Forest Ecology and Management, 307, 136–146.

Dawson TE, Pate JS (1996) Seasonal water uptake and movement in root systems of Australian phraeatophytic plants of dimorphic root morphology: a stable isotope investigation. Oecologia, 107, 13–20.

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable Isotopes in Plant Ecology. Annual Review of Ecology and Systematics, 33, 507–559.

Eckhardt K, Ulbrich U (2003) Potential impacts of climate change on groundwater recharge and streamflow in a central European low mountain range. Journal of Hydrology, 284, 244–252.

Eggemeyer KD, Awada T, Harvey FE, Wedin DA, Zhou X, Zanner CW (2009) Seasonal changes in depth of water uptake for encroaching trees Juniperus virginiana and Pinus ponderosa and two dominant C4 grasses in a semiarid grassland. Tree Physiology, 29, 157–69.

Ehleringer JR, Dawson TE (1992) Water uptake by plants: perspectives from stable isotope composition. Plant Cell and Environment, 15, 1073–1082.

Fan Y, Li H, Miguez-Macho G (2013) Global patterns of groundwater table depth. Science, 339, 940–3.

Filella I, Peñuelas J (2003) Partitioning of water and nitrogen in co-occurring Mediterranean woody shrub species of different evolutionary history. Oecologia, 137, 51–61.

Galiano L, Martínez-Vilalta J, Sabaté S, Lloret F (2012) Determinants of drought effects on crown condition and their relationship with depletion of carbon reserves in a Mediterranean holm oak forest. Tree Physiology, 32, 478–489.

Goldsmith GR, Muñoz-Villers LE, Holwerda F, McDonnell JJ, Asbjornsen H, Dawson TE (2012) Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical montane cloud forest. Ecohydrology, 5, 779–790.

Gray AN, Spies TA (1995) Water content measurement in forest soils and decayed wood using time domain reflectometry. Canadian Journal of Forest Research, 25, 376–385.

Hadfield JD (2010) MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software, 33.

Kukowski KR, Schwinning S, Schwartz BF (2013) Hydraulic responses to extreme drought conditions in three co-dominant tree species in shallow soil over bedrock. Oecologia, 171, 819–30.

Kurz-Besson C, Lobo-do-vale R, Rodrigues ML et al. (2014) Cork oak physiological responses to manipulated water availability in a Mediterranean woodland. Agricultural and Forest Meteorology, 184, 230–242.

Leuzinger S, Luo Y, Beier C, Dieleman W, Vicca S, Körner C (2011) Do global change experiments overestimate impacts on terrestrial ecosystems? Trends in Ecology & Evolution, 26, 236–41.

Limousin JM, Rambal S, Ourcival JM, Rocheteau A, Joffre R, Rodríguez-Cortina R (2009) Long-term transpiration change with rainfall decline in a Mediterranean Quercus ilex forest. Global Change Biology, 15, 2163–2175.

Limousin J, Longepierre D, Huc R, Rambal S (2010) Change in hydraulic traits of Mediterranean Quercus ilex subjected to long-term throughfall exclusion, 30, 1026–1036.

Lin GH, Sternberg L da SL (1993) Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In: Stable isotopes and plant carbon-water relations. (eds

Ehleringer JR, Hall AE, Farquhar GD). Department of Biology, University of Miami, Coral Gables, FL, 33124, USA.

Lloret F, Siscart D, Dalmases C (2004) Canopy recovery after drought dieback in holm-oak Mediterranean forests of Catalonia (NE Spain). Global Change Biology, 10, 2092–2099.

Lloret F, Escudero A, Iriondo JM, Martínez-Vilalta J, Valladares F (2012) Extreme climatic events and vegetation: the role of stabilizing processes. Global Change Biology, 18, 797–805.

López B, Sabaté S, Gracia CA (2001) Annual and seasonal changes in fine root biomass of a Quercus ilex L . forest. Plant and Soil, 125–134.

Martínez-Vilalta J, Prat E, Oliveras I, Piñol J (2002) Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. Oecologia, 133, 19–29.

Martínez-Vilalta J, Mangirón M, Ogaya R, Sauret M, Serrano L, Peñuelas J, Piñol J (2003) Sap flow of three co-occurring Mediterranean woody species under varying atmospheric and soil water conditions. Tree Physiology, 23, 747–58.

Martin-Stpaul NK, Limousin JM, Vogt-Schilb H, Rodríguez-Calcerrada J, Rambal S, Longepierre D, Misson L (2013) The temporal response to drought in a Mediterranean evergreen tree: comparing a regional precipitation gradient and a throughfall exclusion experiment. Global Change Biology, 19, 2413–26.

Matesanz S, Valladares F (2013) Ecological and evolutionary responses of Mediterranean plants to global change. Environmental and Experimental Botany, 103, 53-67.

Mcdowell N, Pockman WT, Allen CD et al. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought. New Phytologist. 178: 719–739.

Moritz C, Agudo R (2013) The Future of Species Under Climate Change: Resilience or Decline? Science, 341, 504–508.

Mueller RC, Scudder CM, Porter ME, Talbot Trotter R, Gehring C a., Whitham TG (2005) Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. Journal of Ecology, 93, 1085–1093.

Nadezhdina N, Ferreira MI, Silva R, Pacheco CA (2007) Seasonal variation of water uptake of a Quercus suber tree in Central Portugal. Plant and Soil, 305, 105–119.

Neal C, Neal M, Warrington A, Ávila A, Piñol J, Rodà F (1992) Stable hydrogen and oxygen isotope studies of rainfall and streamwaters for two contrasting holm oak areas of Catalonia , northeastern Spain. Journal of Hydrology, 140, 163–178.

Oerter E, Finstad K, Schaefer J, Goldsmith GR, Dawson T, Amundson R (2014) Oxygen isotope fractionation effects in soil water via interaction with cations (Mg, Ca, K, Na) adsorbed to phyllosilicate clay minerals. Journal of Hydrology, 515, 1–9.

Ogaya R, Barbeta A, Basnou C, Peñuelas (2014) Satellite data as indicators of tree biomass growth and forest dieback in a Mediterranean holm oak forest. Annals of Forest Science, 1-10.

Ogaya R, Peñuelas J (2003) Comparative field study of Quercus ilex and Phillyrea latifolia: photosynthetic response to experimental drought conditions. Environmental and Experimental Botany, 50, 137–148.

Ogaya R, Peñuelas J (2006) Contrasting foliar responses to drought in Quercus ilex and Phillyrea latifolia. Biologia Plantarum, 50, 373–382.

Oshun J, Dietrich WE, Dawson TE, Fung I (2014) Dynamic, structured heterogeneity of water isotopes inside hillslopes. Nature GeoSciences. Submitted.

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PloS one, 5, e9672.

Peñuelas J, Filella I, Llusia J, Siscart D, Piñol J (1998) Comparative field study of spring and summer leaf gas exchange and photobiology of the mediterranean trees Quercus ilex and Phillyrea latifolia. Journal of experimental botany, 49, 229–238.

Peñuelas J, Filella I, Lloret F, Piñol J, Siscart D (2000) Effects of a severe drought on water and nitrogen use by Quercus ilex and Phillyrea latifolia. Biologia Plantarum, 43, 47–53.

Peñuelas J, Terradas J, Lloret F (2011) Solving the conundrum of plant species coexistence: water in space and time matters most. New Phytologist, 189, 5–8.

Peñuelas J, Sardans J, Estiarte M et al. (2013) Evidence of current impact of climate change on life: a walk from genes to the biosphere. Global change biology, 19, 2303–38.

Poyatos R, Aguadé D, Galiano L, Mencuccini M, Martínez-Vilalta J (2013) Drought-induced defoliation and long periods of near-zero gas exchange play a key role in accentuating metabolic decline of Scots pine. New Phytologist, 200, 388–401.

R Core Development Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0, URL: www.r-project.org.

Rosas T, Galiano L, Ogaya R, Peñuelas J, Martínez-Vilalta J (2013) Dynamics of non-structural carbohydrates in three Mediterranean woody species following long-term experimental drought. Frontiers in Plant Science, 4, 1–16.

Schäfer KVR, Renninger HJ, Clark KL, Medvigy D (2013) Hydrological responses to defoliation and drought of an upland oak / pine forest. Hydrological Processes. DOI: 10.1002/hyp.10104.

Schwinning S (2010) The ecohydrology of roots in rocks. Ecohydrology, 245, 238–245.

Schwinning S, Starr BI, Ehleringer JR (2005) Summer and winter drought in a cold desert ecosystem (Colorado Plateau) part I: effects on soil water and plant water uptake. Journal of Arid Environments, 60, 547–566.

Suarez ML, Ghermandi L, Kitzberger T (2004) Factors predisposing episodic drought-induced tree mortality in Nothofagus – site , climatic sensitivity and. Journal of Ecology, 92, 954–966.

Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours Franc. Journal of experimental botany, 49, 419–432.

Terradas J (1999) Holm Oak and Holm Oak Forests: An Introduction. In: Ecology of Mediterranean Evergreen Oak Forests (eds Rodà F, Retana J, Gracia C, Bellot J), pp. 3–14. Springer Berlin / Heidelberg, Berlin.

Vicente-Serrano SM, Gouveia C, Camarero JJ et al. (2013) Response of vegetation to drought time-scales across global land biomes. Proceedings of the National Academy of Sciences of the United States of America, 110, 52–7.

West AG, Patrickson SJ, Ehleringer JR (2006) Water extraction times for plant and soil materials used in stable isotope analysis. Rapid Communications in Mass Spectometry, 20, 1317–1321.

West AG, Goldsmith GR, Brooks PD, Dawson TE (2010) Discrepancies between isotope ratio infrared spectroscopy and isotope ratio mass spectrometry for the stable isotope analysis of plant and soil waters. Rapid Communications in Mass Spectrometry, 24, 1948–1954.

West AG, Goldsmith GR, Matimati I, Dawson TE (2011) Spectral analysis software improves confidence in plant and soil water stable isotope analyses performed by isotope ratio infrared spectroscopy (IRIS). Rapid Communications in Mass Spectrometry, 25, 2268–74.

West AG, Dawson TE, February EC, Midgley GF, Bond WJ, Aston TL (2012) Diverse functional responses to drought in a Mediterranean-type shrubland in South Africa. New Phytologist, 195, 396–407.

Witty JH, Graham RC, Hubbert KR, Doolittle JA, Wald JA (2003) Contributions of water supply from the weathered bedrock zone to forest soil quality. Geoderma, 114, 389–400.

Wu Z, Dijkstra P, Koch GW, Peñuelas J, Hungate BA. (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Global Change Biology, 17, 927–942.

Zegelin SJ, White I, Jenkins DR (1989) Improved field probes for soil water content and electrical conductivity measurement using time domain reflectometry. Water Resources Research, 25, 2367–2376.

Zeppel M (2013) Convergence of tree water use and hydraulic architecture in water-limited regions: a review and synthesis. Ecohydrology, 6, 889–900.

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	0.77	
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	0.41	
Spring 2010 - Summer 2010	0.12	0.14	0.12	
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	0.80	
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	0.90	
Spring 2010 - Summer 2010	0.07	0.16	0.72	
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	0.95	
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	0.99	
Autumn 2010 - Winter 2011	0.02	0.11	0.97	
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)			
Season	**Factor**	**Df**	**SS**	**Mean Sq**	***F***	***p***	
Spring 2010	**Drought**	1	0.034	0.034	1.97	0.1	
	Species	2	0	0	0.01	0.99	
	Drought x Species	2	0.024	0.012	0.7	0.52	
	Residuals	11	0.192	0.017			
Summer 2010	**Drought**	1	0.319	0.319	81.88	<0.001	***
	Species	2	0.003	0.001	0.32	0.73	
	Drought x Species	2	0.061	0.03	7.8	0.01	
	Residuals	12	0.047	0.004			
Autumn 2010	**Drought**	1	0.041	0.041	3.12	0.09	(*)
	Species	2	0.028	0.014	1.06	0.36	
	Drought x Species	2	0.002	0.001	0.08	0.93	
	Residuals	24	0.312	0.013			
Winter 2011	**Drought**	1	0.279	0.279	10.94	0.002	**
	Species	2	0.126	0.063	2.46	0.1	
	Drought x Species	2	0.079	0.04	1.55	0.22	
	Residuals	45	1.147	0.026			
Summer 2011	**Drought**	1	0.035	0.035	2.48	0.12	
	Species	2	0.022	0.011	0.76	0.47	
	Drought x Species	2	0.055	0.028	1.96	0.15	
	Residuals	39	0.55	0.014			

Table S2. Continued.

Season	Factor	Df	SS	Mean Sq	*F*	*p*	
	Deep soil (10-35 cm)						
	Drought	1	0.021	0.021	0.77	0.4	
Spring 2010	**Species**	2	0.013	0.007	0.24	0.79	
	Drought x Species	2	0.051	0.026	0.93	0.42	
	Residuals	11	0.304	0.028			
	Drought	1	0.057	0.057	0.31	0.59	
Summer 2010	**Species**	2	0.024	0.012	0.06	0.94	
	Drought x Species	2	0.105	0.052	0.28	0.76	
	Residuals	12	2.219	0.185			
	Drought	1	0.063	0.063	15.99	<0.001	***
Autumn 2010	**Species**	2	0.005	0.002	0.58	0.57	
	Drought x Species	2	0.001	0	0.08	0.92	
	Residuals	24	0.094	0.004			
	Drought	1	0.016	0.016	1.6	0.21	
Winter 2011	**Species**	2	0.037	0.019	1.89	0.16	
	Drought x Species	2	0.041	0.021	2.09	0.14	
	Residuals	45	0.447	0.01			
	Drought	1	0.029	0.029	1.39	0.25	
Summer 2011	**Species**						
	Drought x Species	2	0.011	0.005	0.26	0.78	
	Residuals	39	0.827	0.021			

Table S2. Continued.

| Season	Factor	Df	SS	Mean Sq	F	p	
			Groundwater				
	Drought	1	0.002	0.002	0.33	0.58	
Spring 2010	**Species**	2	0.014	0.007	1.51	0.26	
	Drought x Species	2	0.021	0.01	2.17	0.16	
	Residuals	11	0.053	0.005			
	Drought	1	0.107	0.107	0.63	0.44	
Summer 2010	**Species**	2	0.033	0.017	0.1	0.91	
	Drought x Species	2	0.074	0.037	0.22	0.81	
	Residuals	12	2.038	0.17			
	Drought	1	0.002	0.002	0.5	0.49	
Autumn 2010	**Species**	2	0.01	0.005	1.02	0.37	
	Drought x Species	2	0.002	0.001	0.21	0.82	
	Residuals	24	0.117	0.005			
	Drought	1	0.162	0.162	26.48	<0.001	***
Winter 2011	**Species**	2	0.026	0.013	2.14	0.13	
	Drought x Species	2	0.006	0.003	0.51	0.61	
	Residuals	45	0.275	0.006			
	Drought	1	0.128	0.128	4.49	0.04	*
Summer 2011	**Species**	2	0.135	0.067	2.35	0.11	
	Drought x Species	2	0.025	0.013	0.44	0.65	
	Residuals	39	1.117	0.029			
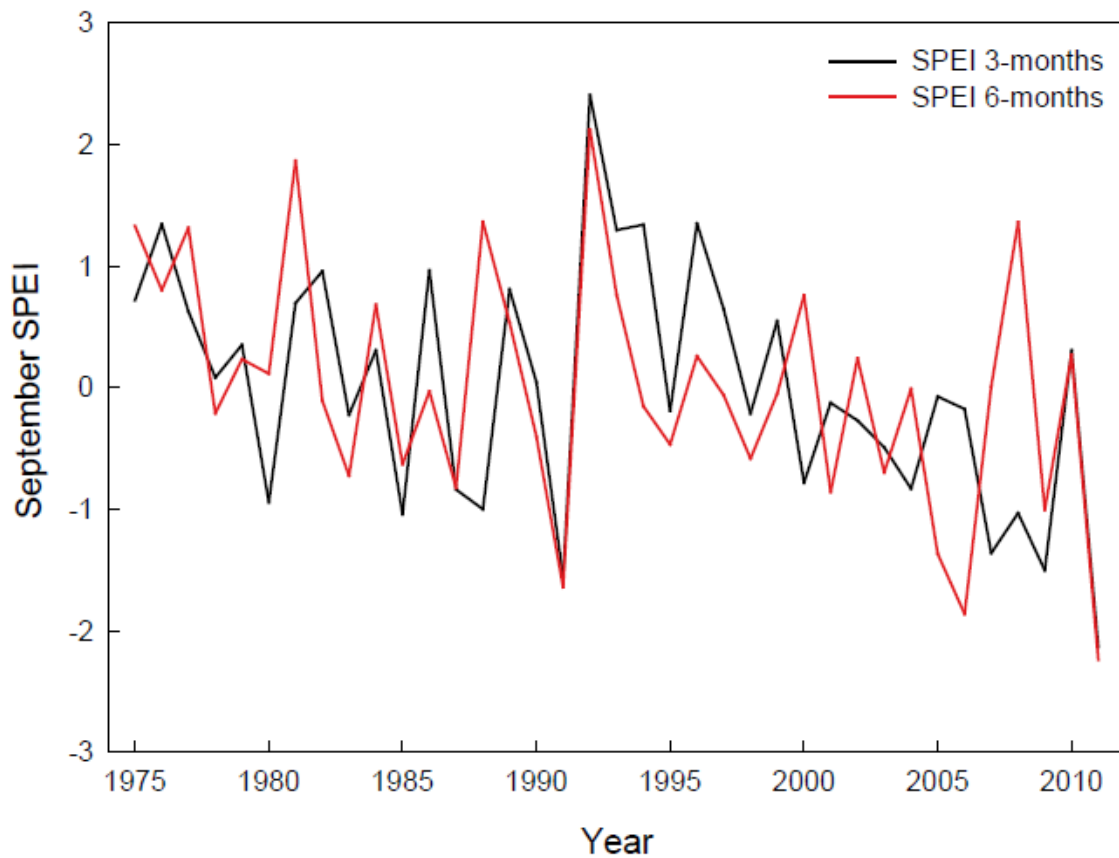
Figure S1. Temporal series (1975-2011) of the Standardized Precipitation and Evapotranspiration Index (SPEI) for September calculated for two timescales: 3 months (black line), which integrates the water balances of July, August and September, and 6 months (red line), which integrates the water balances of April, May, June, July, August and September. Note that both indices reached the period's minimum in 2011.
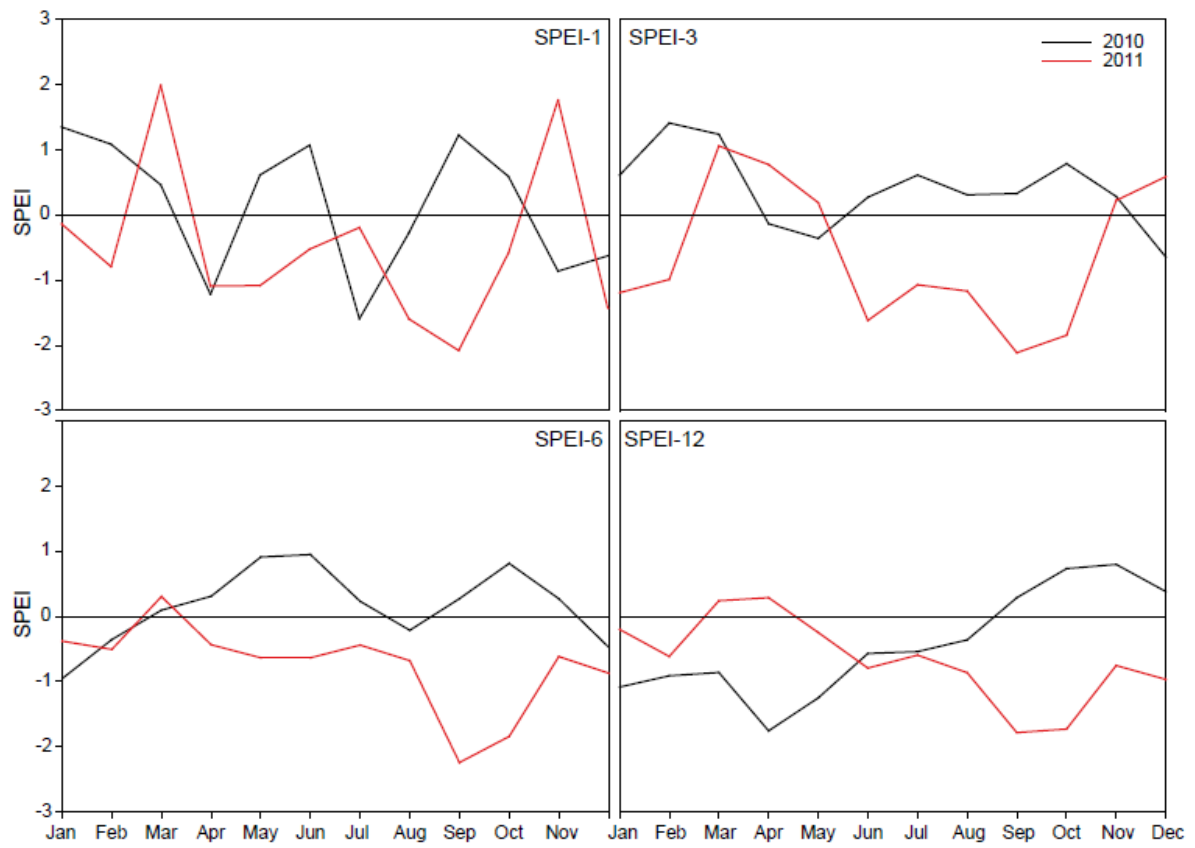
Figure S2. Monthly values of the Standardized Precipitation and Evapotranspiration Index (SPEI) during the study period (2010, black line; 2011, red line). Each panel corresponds to the timescale at which the index was calculated (1 month, 3 months, 6 months and 12 months).

Comparative study of diurnal and nocturnal sap flow of *Quercus ilex* and *Phillyrea latifolia* in a Mediterranean holm oak forest in Prades (Catalonia)

Adrià Barbeta, Romà Ogaya and Josep Peñuelas

ABSTRACT

Droughts are a cyclical disturbance in Mediterranean ecosystems and climate change models forecast an increase of their frequency and severity. Some experimental and observational studies have shown that co-occurring species may cope with drier conditions with different strategies and present different responses. Here, we investigate sap flow response to drought in order to explain the already observed differential growth and mortality of *Quercus ilex* and *Phillyrea latifolia* at Prades Holm oak forest (NE Spain). We measured sap flow of these species and compared their diurnal, nocturnal and seasonal patterns and their relationship with environmental variables. Both species described qualitatively similar daily patterns, either during daylight or night. Sap flow rates were significantly higher in *P. latifolia* except in autumn and spring. *P. latifolia* was more sensitive to soil moisture. Nocturnal sap flow was detected in both species with no significant differences and hourly rates suffered a progressive increase from 3 am to dawn in most sampled nights. Our results indicate a better adaptation of *P. latifolia* to this site as it can take better advantage of wet periods while maintaining higher sap flow rates during dry periods. Along with previous ecophysiological studies at the same location it may be inferred that at its drier distributional limit *Q. ilex* would be in disadvantage respect other species like *P. latifolia*, as the latter would cope better with increasing xeric conditions already occurring and further predicted for Mediterranean ecosystems. Our results also show nocturnal sap flow to be relevant in individual water losses in these two species as they can be up to 35-40% of daily sap flow. Further research on the underlying mechanisms of this nocturnal sap flow is required since it may also enhance early morning CO_2 fixation or nutrient supply to leaves.

KEYWORDS

INTRODUCTION

Water scarcity represents one of the major constraints for plant communities and its importance is likely to increase in the next decades as projected by global circulation models (IPCC 2007). Main consequences of droughts include growth decline, reduced primary production, widespread defoliation, tree mortality and changes of species distribution ranges (Allen *et al.*, 2010, Carnicer *et al.*, 2011, Mueller *et al.*, 2005, Peñuelas & Boada, 2003, Peñuelas *et al.*, 2007). This is especially crucial for Mediterranean ecosystems where rising temperatures and the projected decrease in rainfall will magnify drought risk, as pointed out by many studies (David *et al.*, 2007, Lindner *et al.,* Lloret *et al.*, 2009, Ogaya & Peñuelas, 2007b). Although most of Mediterranean species are relatively well adapted to drought, they do not cope equally with the longer dry periods predicted by models. Specific water use strategies are thus of significance to understand possible changes on stand composition and species' distribution range.

Holm oak (*Quercus ilex*) seems to effectively tolerate drought effects by reducing its leaf area and by stomatal closure control (Limousin *et al.*, 2009, Ogaya & Peñuelas, 2006, Villar-Salvador *et al.*, 1997). Nevertheless, photosynthetic and transpiration rates during drought are not always lower than those from co-occurring species with different water use strategies (Quero *et al.,* 2011). However, it has been reported that nearby its drier distributional limit *Q. ilex* would be in disadvantage respect *Phillyrea latifolia*, a tall shrub more resistant to drought, which could eventually lead to a shift in community composition (Martínez-Vilalta *et al.*, 2003, Martínez-Vilalta *et al.*, 2002a, Ogaya & Peñuelas, 2003, Ogaya & Peñuelas, 2006, Ogaya & Peñuelas, 2007a, Ogaya & Peñuelas, 2007b, Ogaya *et al.*, 2011, Ogaya *et al.*, 2003, Peñuelas *et al.*, 2000, Peñuelas *et al.*, 1998). Sap flow measurements can provide further insights on the water use strategies of these two species and on possible future changes on stand composition for these areas, where differential responses of the leaf water potential, hydraulic conductivity and net photosynthetic rates of these two species to drought have been already demonstrated (Martínez-Vilalta *et al.*, 2003, Ogaya & Peñuelas, 2003).

All around the Mediterranean basin, several studies have already focused on sap flow of these species either together or separately. *Q. ilex* diurnal sap flow has been well studied at several different sites. It usually reaches a similar hourly maxima at any time of the year

except at the end of the dry season (Infante et al. 2003) and decreases with summer drought (Infante *et al.*, 2003, Infante *et al.*, 2001, Limousin *et al.*, 2009, Tognetti *et al.*, 1998, Van der Zande *et al.*, 2009). After a midsummer minimum and after the rainfalls in late summer or autumn, sap flow peaks recover. Summer reduction in transpiration follows the reduction in soil water content, and is driven by stomata closure control (Martínez-Vilalta *et al.,* 2003) in parallel to an increase of hydraulic resistance in the soil-plant-atmosphere continuum (Tognetti *et al.,* 1998). Sometimes this is accompanied by a decline in leaf area (Limousin *et al.*, 2009, Ogaya & Peñuelas, 2006, Villar-Salvador *et al.*, 1997). By contrast, there are not many studies of *P. latifolia*'s sap flow dynamics and results disagree even when conducted on climatically similar locations. While some authors observed that sap flow remained relatively stable over the course of the year (Gucci *et al.,* 1999), others found declines in peaks of sap flow as summer drought progressed (Schiller *et al.,* 2002), similarly than *Q. ilex*. Specific differences in sap flow quantities and seasonal variability are partly explained by differences in hydraulic architecture that provide *P. latifolia* with a vulnerability to xylem embolism lower than in *Q. ilex* (Martínez-Vilalta *et al.,* 2002b). Martínez-Vilalta et al. (2003) monitored sap flow of both species in these mountains and reported a greater sensitivity of *Q. ilex* to soil moisture along with a more important decrease in whole-plant hydraulic conductivity coupled with drought. These specific water use behaviour may partly explain the different physiological and demographical responses that were found in a nearby site submitted to the same experimental drought for 5 years (Ogaya and Peñuelas, 2007b). In the latter study, *Q. ilex* suffered a reduction in mean annual stem diameter increment at drought plots, while no significant differences were detected in *P. latifolia*. *P. latifolia* experienced also the lowest mortality. Furthermore, in another comparative study in the same experimental system, *P. latifolia* was the only species to increase water use efficiency by reducing transpiration losses during midday and the net photosynthetic rate showed no response to water availability above the threshold of 17% of volumetric soil water content (Ogaya & Peñuelas, 2003). In the frame of current and future climate change, it is now warranted to elucidate wheter *Q. ilex* and *P. latifolia* sap flow have similar patterns or not in environmentally more extreme sites for *Q. ilex*. Furthermore, sap flow seasonal patterns are well studied in *Q. ilex* but not extensively enough in *P. latifolia*, a locally abundant species that is in competition with the usually more dominant and widespread *Q. ilex*.

Nocturnal water loss can represent a significant fraction of the total daily water used (maximums between 20-50%) when VPD and soil water content are high (Caird *et al.*, 2007, Daley & Phillips, 2006, Dawson *et al.*, 2007, Phillips *et al.,* Zeppel *et al.*, 2010) and may constitute an important factor for water relations in Mediterranean ecosystems, where water availability represents a major environmental constraint (Peñuelas *et al.,* 1998). Adaptive meanings of this phenomenon include facilitation of early morning carbon fixation, nutrient supply to distal parts of the crown and delivery of dissolved O_2 via the parenchyma to woody tissue sinks, all of them mediated by a partial stomata aperture (Caird *et al.*, 2007, Daley & Phillips, 2006, Scholz *et al.*, 2007, Snyder *et al.*, 2003). Although there are no studies focused on nocturnal sap flow of *P. latifolia* or *Q. ilex*, Fisher *et al.,* (2007) reported relative percentages of nighttime transpiration up to 20% of the whole-day flow in *Quercus douglasii* at the Californian oak-savannah. Furthermore, drought seemed to reduce absolute nighttime water losses in saplings of live oaks (*Quercus virginiana* and *Quercus oleoides*) (Cavender-Bares *et al.,* 2007). In the same study, water losses appeared as a strict function of VPD and thus it was likely an unavoidable and non-adaptive process. Zeppel *et al.,* (2010) found water losses to be a greater contribution to nocturnal sap flow than stem refilling in two evergreen species (*Angophora bakeri* and *Eucalyptus parramattensis*) and mainly through stomata rather than the cuticle as Howard and Donovan (2007) observed in *Helianthus* species. Nocturnal water losses have been documented in a wide range of genera and life forms (Caird *et al.*, 2007, Dawson *et al.*, 2007), hence, the investigation of the magnitude of nocturnal sap flow in *Quercus ilex* and *Phillyrea latifolia* is warranted as it could suppose a substantial part of plant water budget.

The aim of this study was to characterize sap flow of these two co-occurring species by comparing diurnal, nocturnal and seasonal sap flow patterns and relate them to environmental variables, mainly soil water availability and vapor pressure deficit (atmospheric evaporative demand), in order to gain knowledge on the mechanisms that drive to the drought effects on growth and mortality described on these populations by Ogaya and Peñuelas (2003, 2007b). We aimed to conduct such characterization in an environmentally extreme site for *Q. ilex*. Our initial hypothesis was that the more drought tolerant *P. latifolia* would maintain higher relative sap flow quantities during dry periods, but would not increase them significantly during wet periods. On the other hand, we expected that *Q. ilex* would suffer a more drastic reduction in sap flow during summer drought while being more water

consuming in wet periods thanks to their wider xylem conduits (Martínez-Vilalta *et al.,* 2002b). Regarding nocturnal sap flow, we expected to observe higher rates in nights with high VPD and high soil moisture and in nights following days with high sap flow rates, since the diurnal depletion of water stores would require its subsequent refilling at night.

MATERIAL AND METHODS

Study site and species

The present study was carried out in Prades holm oak forest in Southern Catalonia (NE Spain) (41º21'N, 1º2'E), at 950 m asl and on a south-facing slope (25% slope). The climate is Mediterranean, with a mean annual rainfall of 610 mm and a mean temperature of 12.2ºC during the period of experimentation (1999-2009). The soil is a Dystric Cambisol over Paleozoic schist, and its depth ranges from 35 to 90 cm. The forest studied has a very dense multistem crown (20700 stems ha^{-1}) and is dominated by *P. latifolia* (11700 stems ha^{-1}) and *Q. ilex* (7700 stems ha^{-1}), with limited presence of other evergreen species well adapted to dry conditions (*Arbutus unedo* L., *Erica arborea* L., *Juniperus oxycedrus* L., *Cistus albidus* L.). This forest has not suffered any significant disturbance since 60 years ago, and the maximum height of the dominant species is about 3–4m tall (Ogaya *et al.,* 2011).

Sap flow and meteorological measurements

Four sap flow sensors (Sap Flow Meter P4.2, Jiri Kucera-Environmental Measuring Systems, Brno, Czech Republic) were initially installed on two trees of each species studied, *Q. ilex* and *P. latifolia*. These sap flow sensors are based on the tissue heat balance model (THB) with internal heating and sensing. They measure and log the sap flow in kg hr^{-1}. The heat balance of xylem through which the sap flow passes can be described by the general equation:

$$Q = \frac{P}{c_\mathrm{w} \times d \times \mathrm{d}T} - \frac{z}{c_\mathrm{w}}$$

where Q is the sap flow rate (kg s^{-1}), P is the heat input power (W), dT is the temperature difference in the measuring point, c_w is the specific heat of water (J kg^{-1}) and z is the coefficient of heat losses from the measuring point (W degree^{-1}). Q value is obtained per cm of stem circumference, from the triangle-shaped heated space among the four sensors (d). In order to

obtain the whole tree sap flow rate in terms of kg hr^{-1}, the initial values were extrapolated using the following formula:

$$Q_{\text{tree}} = Q \times (A - 6.28 \times B)$$

where A is the stem circumference (with bark) (cm) and B the bark plus phloem thickness [cm]. Q_{tree} was later corrected by the proportion of sapwood area of each individual which is the effective conducting section. Finally, the values obtained (kg.h^{-1}) were normalized dividing them by the individual basal area.

Sap flow sensors were powered by a battery, and when they did not receive enough energy to measure properly, they stopped recording automatically in order to avoid unreal fluctuations caused by low power. Thus, we only monitored sap flow from representative days per each season. An automatic meteorological station installed in the study site monitored temperature, photosynthetic active radiation, air humidity, precipitation and wind speed and direction each half-hour. Soil moisture at 25cm depth was measured continuously with ECH2O probes (Decagon Devices Inc., Pullman, WA, USA) (Fig. 1).

Statistical analyzes

We performed a Repeated Measures ANOVA comparing species sap flow daily quantities. From the regression equation of sap flow hourly quantities as a function of vapor pressure deficit (VPD), we extracted the coefficient β for each day and registered its corresponding soil moisture. After that, we conducted a regression analysis of coefficient β (Sap flow vs VPD) and soil moisture. We assessed species specific differences in the response of β to soil moisture by using a separate slopes model after ensuring that the interaction between species and soil moisture was significant. Moreover, we conducted a multiple regression model for each species including sap flow hourly quantities as a dependent variable and continuous values of VPD and soil moisture as predictor variables. We used sap flow measured in the night (when PAR was zero) as our nocturnal sap flow variable. All statistical tests were conducted with the software Statistica (version 6.0, StatSoft, Tulsa, OK, USA).

RESULTS

Both species followed similar daily sap flow patterns. Diurnal sap flow began before dawn and increased rapidly during the first two hours of light. Then, it presented a sustained rise along midday and usually reached a peak just before nightfall (Fig.2). Also nocturnal sap flow was noticeable and only in very few occasions sap flow was totally stopped. In both *Quercus ilex* and *Phillyrea latifolia*, sap flow rates were low and stable until 3 am, where a progressive rise until dawn occurred. Such increase of sap flow rates was observed at different magnitudes in all seasons but spring (Fig. 3). As during daytime, there were no remarkable differences between species.

The mean of daily sap flow of both species varied among seasons spring > autumn > midsummer > late summer for *P. latifolia* and spring > midsummer > autumn > late summer for *Q. ilex*) although differences were not significant (Fig. 4). Nonetheless, some seasons presented high fluctuations among days. The maximum daily sap flow rates per cm2 of basal area were found in midsummer (*Q. ilex*) and autumn (*P. latifolia*). However, in both species the seasonal mean of daily sap flow was higher in spring, matching up with the rainy seasons in Prades which at the same time had days with practically no flow due to a low vapor pressure deficit, the driving force of sap flow (Fig. 2). Late summer was the least active period for either species and also the maximum daily values were the lowest (Fig. 2, Fig. 4). Sap flow series described different patterns depending on the season. The curve of sap flow was wider in midsummer than in autumn in response to the longer photoperiod (Fig. 2).

Table 1. Characteristics of the monitored trees.

	Diameter at 50 cm height (cm)	Leaves weight (kg)	Aboveground biomass (kg)	Sapwood area (cm²)	Sapwood width (cm)
P. latifolia 1	9.33	0.94	17.16	33.11	1.5
P. latifolia 2	10.60	1.29	23.52	36.06	1.4
Q. ilex 1	11.20	1.95	32.92	30.18	1.2
Q. ilex 2	12.03	2.20	38.72	40.60	1.4

Aboveground and leaves biomass were obtained with the allometric relationships calculated by Ogaya *et al.,* (2003) at the same location.
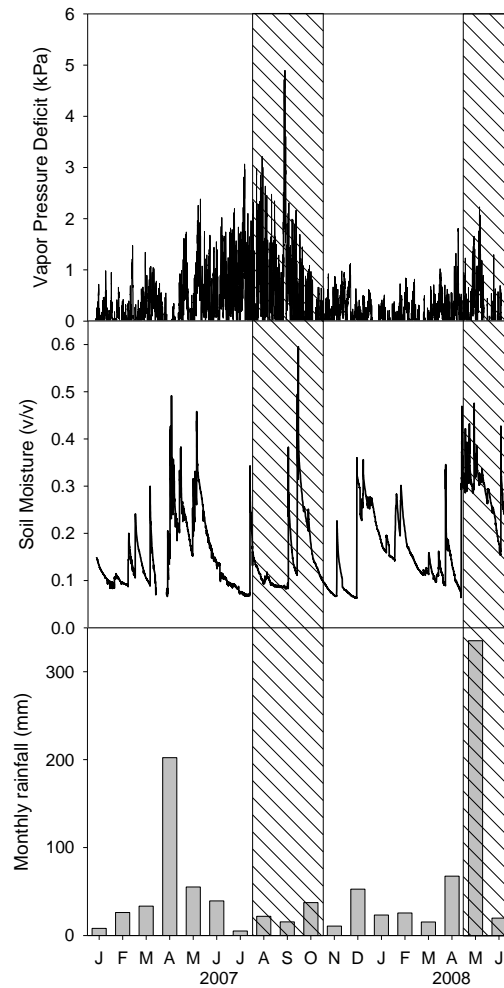
Figure 1. Environmental conditions (Vapor pressure deficit, Soil moisture and Monthly rainfall) from January 2007 to June 2008. Sampling periods are highlighted.

Daily sap flow rates were 11.44±3.65 kg.d^{-1} for *Quercus ilex* and 14.29±6.12 kg.d^{-1} for *Phillyrea latifolia* in the sampled days. *P. latifolia* had significantly higher daily sap flow rates per cm^2 of basal area than *Q. ilex* throughout the sampling season (0.23±0.08 vs 0.15±0.04 kg.cm^{-2}.d^{-1}, Repeated Measures ANOVA (RMA), Wilks' Lambda (*WL*)=0.82, p<0.01). Although means of *P. latifolia* were higher for each season alone, differences were statistically significant in midsummer (0.27±0.05 vs 0.20±0.02 kg.cm^{-2}.d^{-1}, RMA, *WL*=0.60, p<0.05), late summer (0.15±0.02 vs 0.11±0.02 kg.cm^{-2}.d^{-1}, RMA, *WL*=0.54, p<0.001), and autumn (0.29±0.11 vs 0.16±0.02 kg.cm^{-2}.d^{-1}, RMA, *WL*=0.64, p=0.056). Likewise, spring values were 33% lower for *Q. ilex* than for *P. latifolia* but a large variance prevented to obtain significant

differences (0.31 ± 0.10 vs 0.21 ± 0.04 kg.cm^{-2}.d^{-1}, RMA, *WL* =0.77, p=0.53). We used allometric relationships calculated by (Ogaya *et al.*, 2003) at the same site to estimate total leaf area and from that to obtain sap flow relative to leaf area. Overall differences increased because *P. latifolia* has a lower leaf area (*P. latifolia*=1.97 ± 0.26 kg.d^{-1}.m^{-2} and *Q. ilex*=1.14 ± 0.16 kg.d^{-1}.m^{-2}).
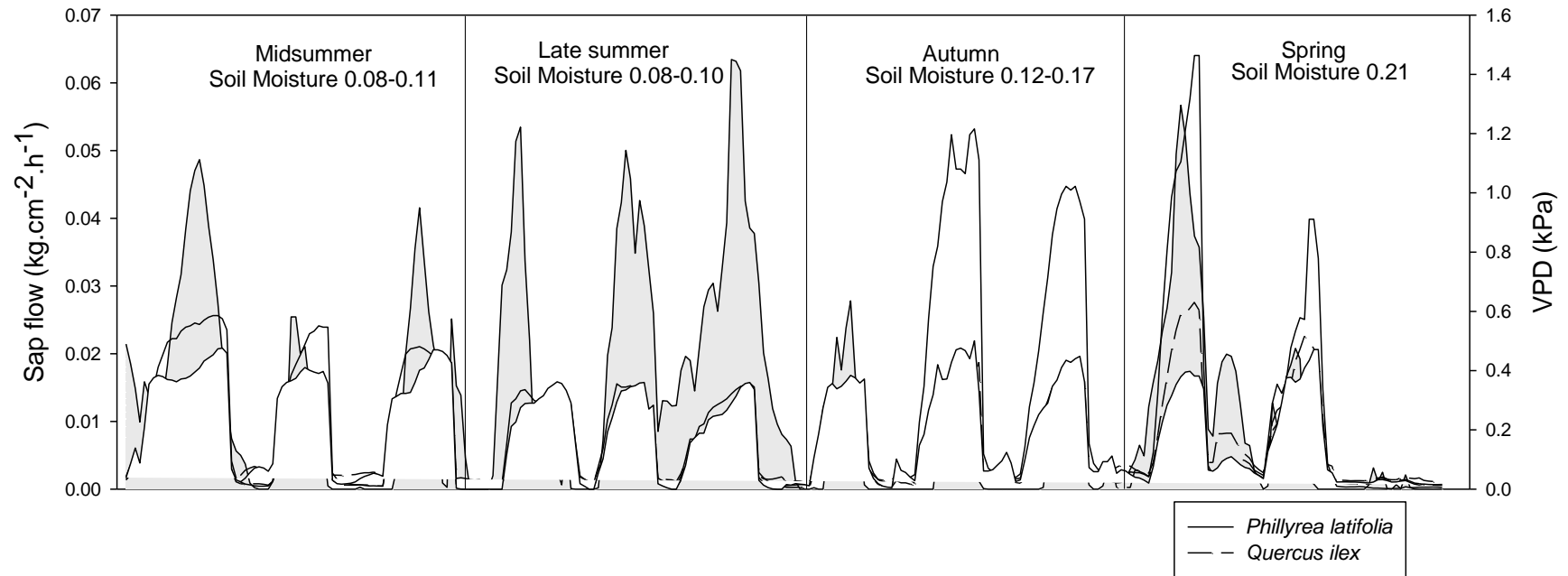
Figure 2. Daily cycles of sap flow of two individuals of each species (solid line *P. latifolia* and *Q. ilex* dashed line). Three representative days per season are shown. Filled area corresponds to VPD (right axis) and soil moisture ranges for each three days period are depicted under season's name.
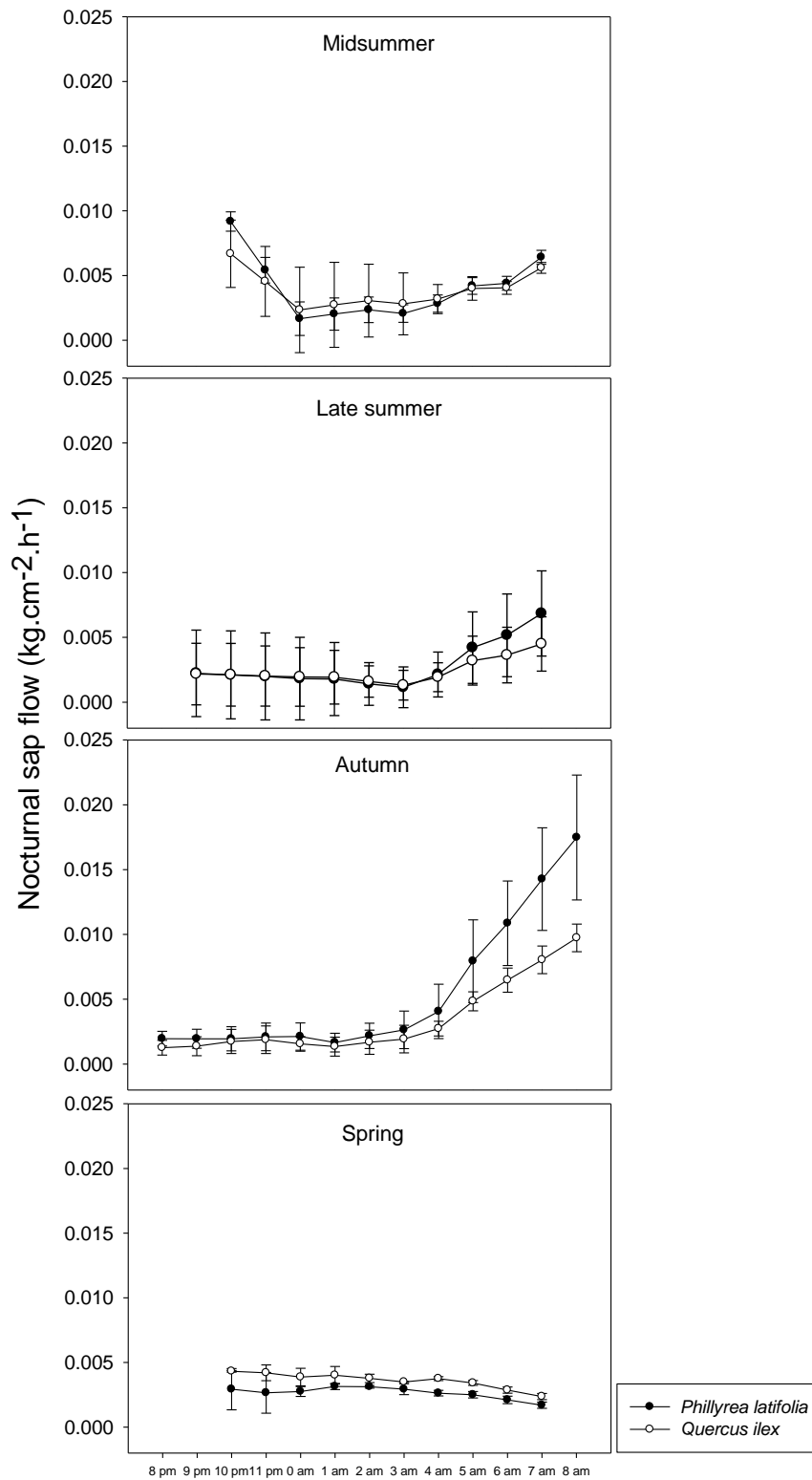
Figure 3. Hourly sap flow rates at nighttime for the four sampling periods. Error bars are standard errors of the mean (n=2).

Night-time sap flow was detected in both species. Unlike the overall sap flow rates commented above, there were no differences between the two species in the whole sampling season nor when analyzing each season separately. The amount of nighttime sap flow was considerable since some days it represented up to 38% (*P. latifolia*) and 39% (*Q. ilex*) of whole-day flow. The average mean for the sampled days were 18.2±8.9% (*P. latifolia*) and 22.6±7.5% (*Q. ilex*). No significant differences were found among seasons although autumn values were typically higher than the others (Fig. 5). VPD alone could not explain the detection of sap flow at night globally nor seasonally, as even when VPD fell to 0 sap flow was appreciable, and in addition, hourly values of VPD and sap flow were not correlated.
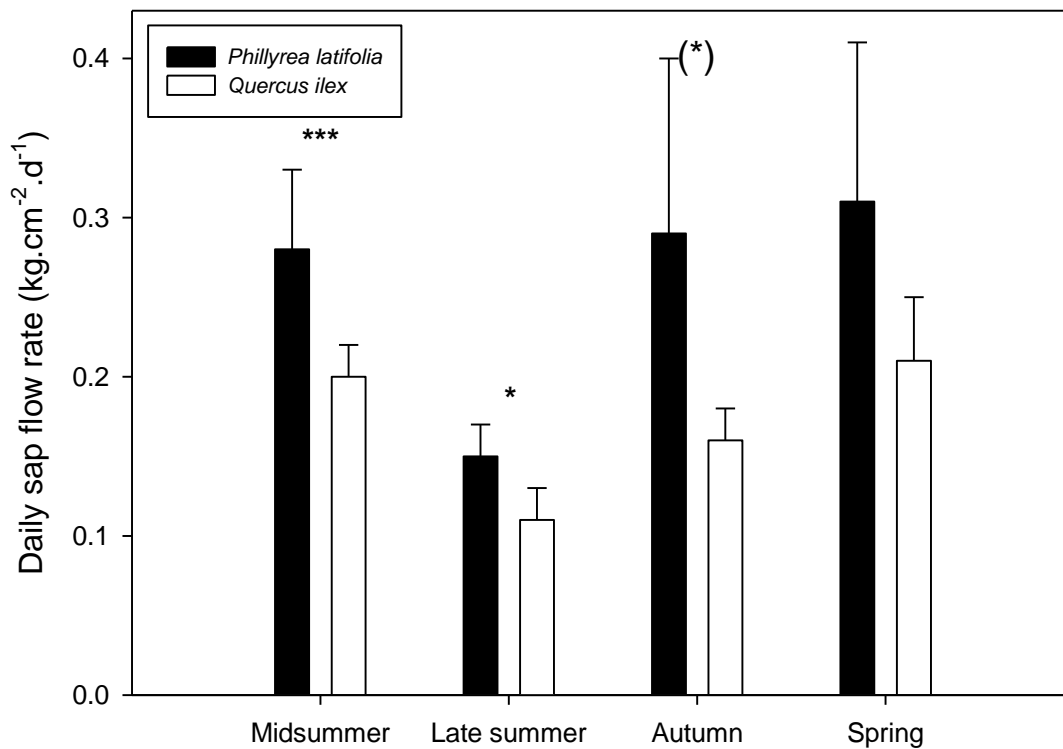

Figure 4. Seasonal means of daily sap flow rates. The error bars are standard error of the mean (n=2). Significant differences are marked with *$p<0.05$, **$p<0.01$, ***$p<0.001$ and (*) $p=0.056$.

Daily sap flow of both species seemed to be mainly determined by the atmospheric evaporative demand (VPD) and by soil moisture. However, the relationship of daily sap flow with these two environmental variables differed between *Q. ilex* and *P. latifolia* (Fig.6). Since
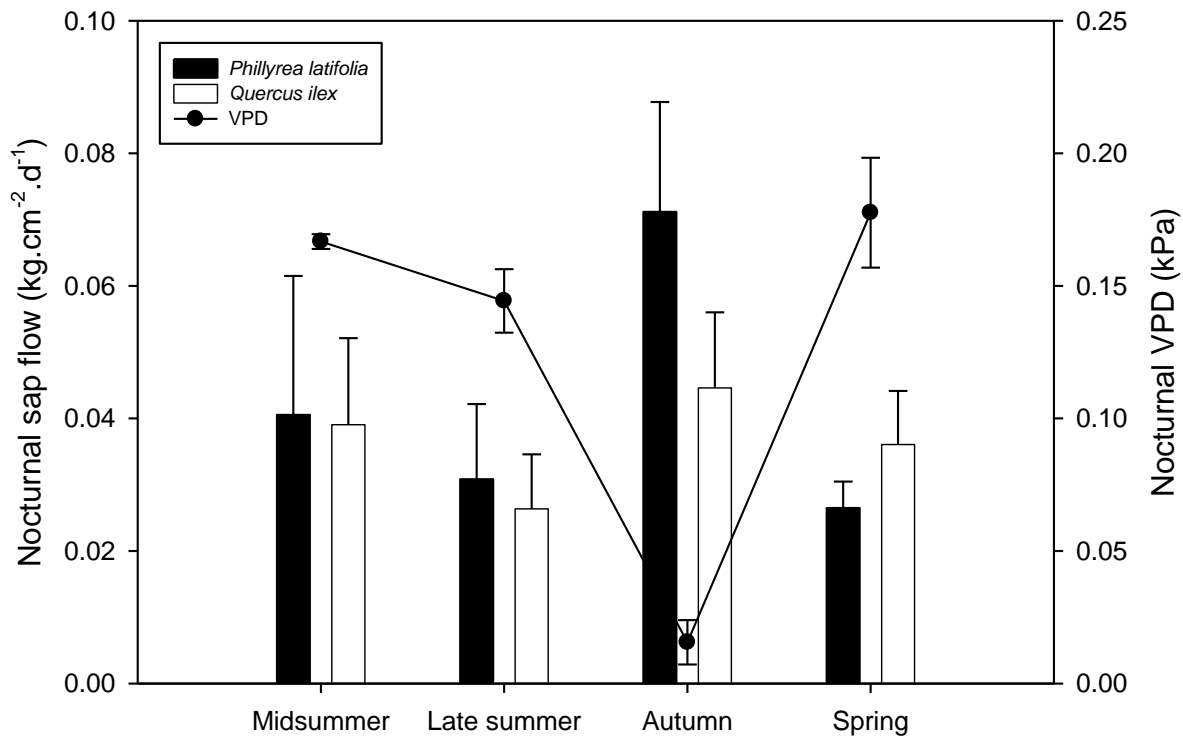
Figure 5. Mean VPD and mean nocturnal sap flow rates per season and species. Error bars are standard errors of the mean (n=2).

VPD is the driving force of sap flow, we evaluated changes in the relationship between sap flow and VPD at different levels of soil moisture (Fig. 7). Linear regression for *P. latifolia* fitted better than for *Q. ilex* (R^2=0.62 vs R^2=0.35, $p<0.01$ and $p<0.05$ respectively) and furthermore, coefficient β was higher for *P. latifolia* (0.16 vs 0.05, Separate slope design with F=12.56 and $p<0.001$). In an exploratory analysis, we also performed a multiple regression model to assess soil moisture and VPD weight on sap flow hourly quantities. Results showed soil moisture as predominant on *P. latifolia* (Soil Moisture β=0.34, VPD β=0.23, $p<0.001$). On the other hand, in *Q. ilex* there was a similar influence of VPD and soil moisture on sap flow performance (Soil Moisture β=0.32, VPD β=0.32, $p<0.001$). Nevertheless, when soil moisture was below 10%, sap flow peaks of both species did not follow VPD patterns.
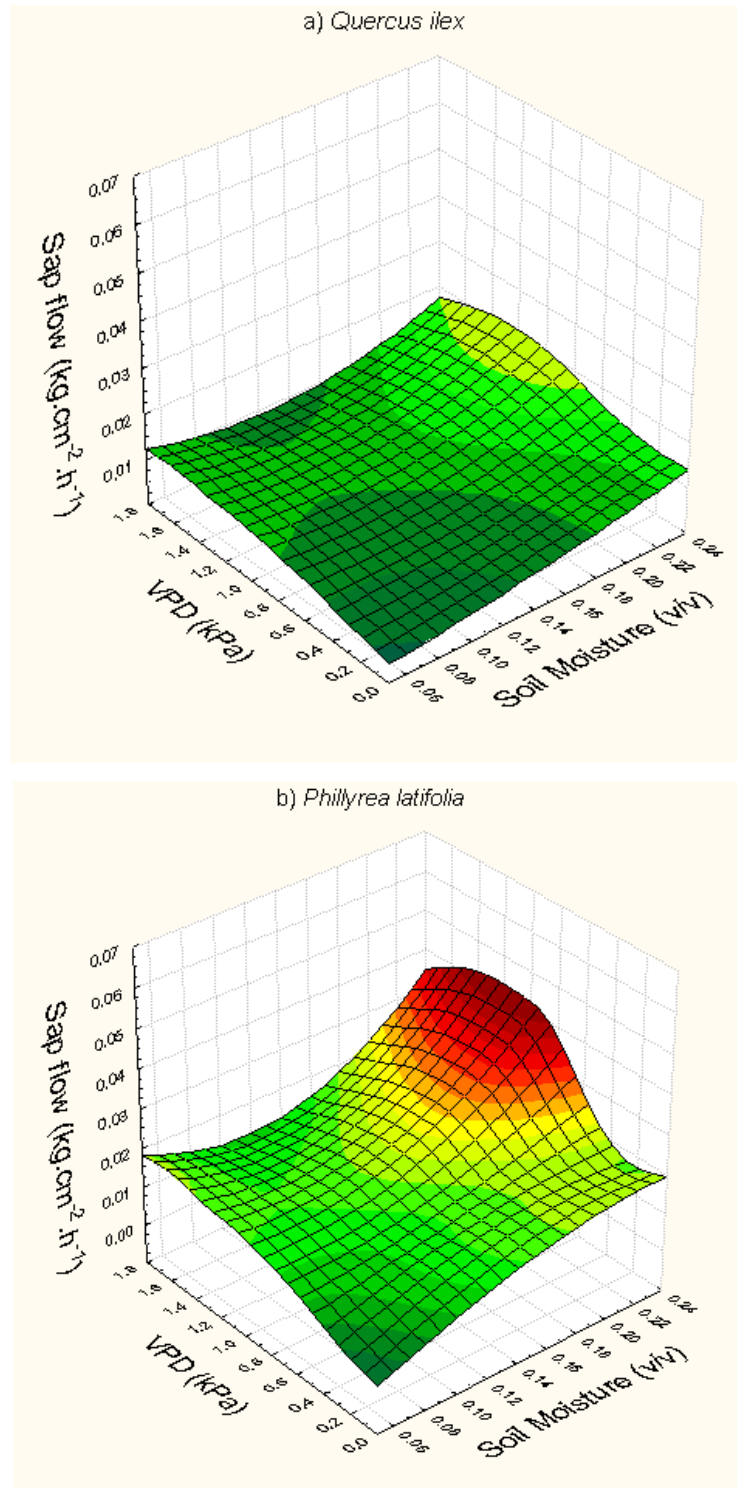
Figure 6. Sap flow response to instantaneous values of VPD and soil moisture for a) *Q. ilex* and b) *P. latifolia*. Z axis units are hourly integrated estimations.
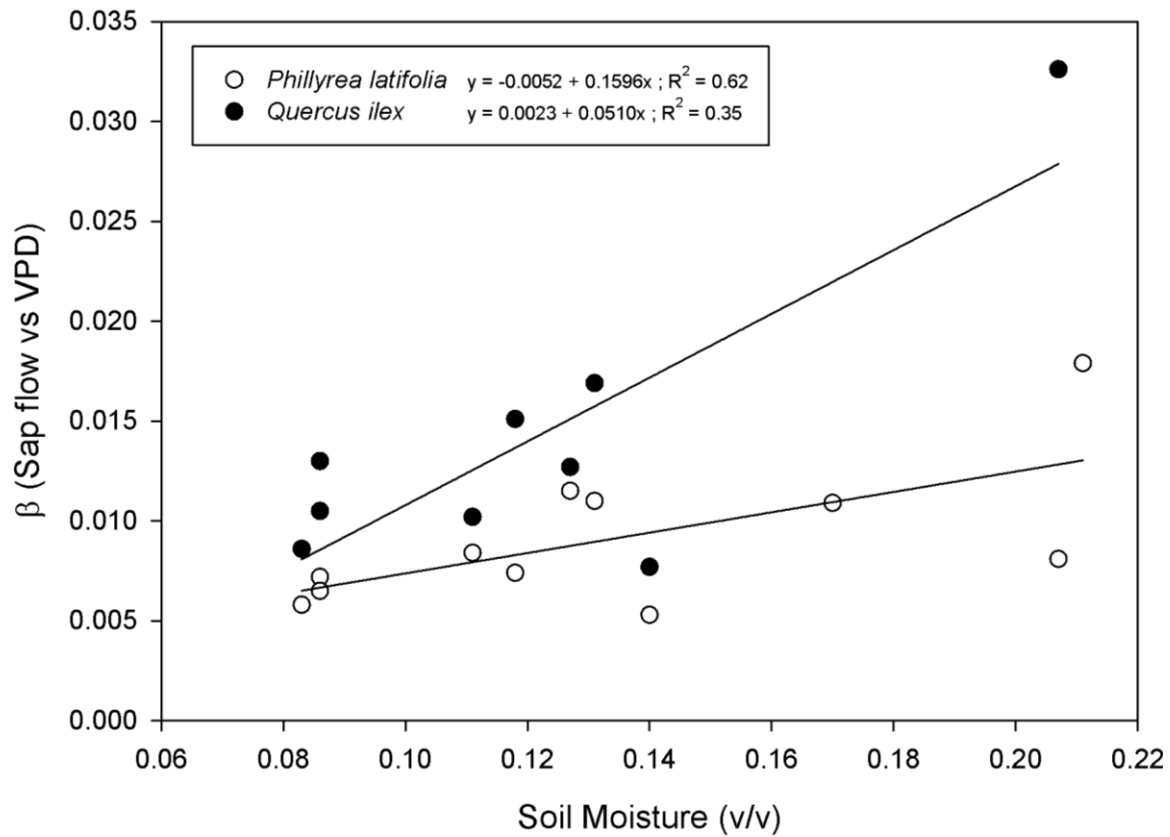
Figure 7. Daily β coefficients of the sap flow versus VPD regressions at increasing soil moistures. Only β from significant (p<0.05) sap flow vs VPD relationships were used. Soil moisture is the mean for each day.

DISCUSSION

Overall sap flow rates of *P. latifolia* were unexpectedly higher than those of *Q. ilex* all along the sampling seasons although only clearly significantly in mid and late summer, the driest periods. Martínez-Vilalta et al. (2003) observed a similar difference between both species at the end of summer at a nearby site. In our study, *P. latifolia* integrated quantities and peaks of sap flow were clearly higher during wet periods (spring and autumn) than in mid and late summer (Fig. 3). Hence, it may be deduced that at least at this site *P. latifolia* is more water spender regardless of the environmental conditions. However, daily sap flow reduction in the peak of dry respect wet periods were 64.3% in *P. latifolia* and 47.6% in *Q. ilex* whilst Martínez-Vilalta et al. (2003) found 57.8% and 87.1% respectively. Also Tognetti et al. (1998) found higher sap flow reduction due to summer drought for *Q. ilex* than in our study (approximately

75%), which is likely due to the lower and stable quantities that we obtained during favorable conditions. Moreover, during our sampling season (Fig. 1), the lowest soil moisture did not coincide with the highest VPD due to a couple of rainfall episodes in early and mid August, so the strongest water stress took place in late summer and was thus probably less severe in comparison to the cited studies. Our results on seasonality of sap flow for *Q. ilex* agree with Infante et al. (2001, 2003) at an oak-savannah, where they described similar patterns with minimum quantities at the end of summer instead of midsummer. Furthermore, *Q. ilex* showed the highest sap flow daily mean in spring, coinciding with the maximum radial increment observed in a nearby forest (Gutierrez *et al.,* 2011).

This study also demonstrates a species-dependent response to environmental conditions. Sap flow needs evaporative demand (VPD) to take place but at varying soil moisture we detected higher slopes of the sap flow versus VPD relation in *P. latifolia* than in *Q. ilex* and (Fig. 7). Such difference indicates a greater sensitivity of *P. latifolia* to increases in soil moisture. This greater sensitivity of *P. latifolia* disagrees with our expectations based on previous studies of hydraulic properties of both species. Martínez-Vilata et al. (2002) described wider xylem conduits and higher hydraulic conductivity of *Q. ilex* respect to other Mediterranean woody species, among them, *P. latifolia*. Consequently, we expected that under favorable environmental conditions *Q. ilex* would be able to conduct higher amounts of sap flow as a response to the soil moisture increases after autumn and spring rainfalls, but instead, at this site of Prades forest, *P. latifolia* presented a higher plasticity in its sap flow rates, which is also in disagreement with findings of Gucci et al. (1999) who found similar sap flow peaks throughout the seasons except for summer in a meteorologically similar location. This higher fluctuation of *P. latifolia* sap flow rates may be explained by their shallower root system in comparison to *Q. ilex*, as the latter may have access to deeper soil layers with more constant moisture. Moreover, this morphological difference seemed to allow *Q. ilex* to have similar average daily sap flow amounts in midsummer and spring despite a large difference in soil moisture (Fig.4). As drought progressed, deeper soil layers also dry out and *Q. ilex* would not be able to maintain the same hydraulic conductivity, resulting in lower sap flow rates in late summer (Fig.4).

The nocturnal sap flow in both species represented a similar percentage of the whole-day flow than reported in the study of (Fisher *et al.,* 2007) at the Mediterranean oak-savannah

of deciduous *Q. douglasii* (they found 18% and we calculated a 22.6±7.5% for *Q. ilex* and 18.2±8.9% for *P. latifolia*). Nevertheless, our data lacks winter measures while Fisher *et al.,* (2007) monitored almost a whole-year. With our data, it is difficult to discern whether nocturnal sap flow responds to water losses or to refilling of water stores or both, since they can occur simultaneously. This is usually tested by coupling nocturnal sap flow with VPD (Cavender-Bares *et al.*, 2007, Fisher *et al.*, 2007). In our study, the hourly rates of nocturnal sap flow shown in Fig. 3 did not follow VPD fluctuations. Therefore, our data does not clarify the mechanism that drives both species to either transpire or refill water stores during the night. It is possible that premature stomata aperture would enhance early morning carbon assimilation or facilitate nutrient supply to distal parts of the crown (Daley & Phillips, 2006, Dawson *et al.*, 2007, Scholz *et al.*, 2007). These potential ecological meanings of nocturnal sap flow were further supported by the rise of sap flow since 3 am observed in all sampling seasons but spring (Fig.3), which has been also detected for stomatal conductance in many species and seems to respond to endogenous circadian rhythms (Caird *et al.*, 2007, Dodd *et al.*, 2005, Donovan *et al.*, 2003, Howard & Donovan, 2007). On the other hand, plants could suffer simply unavoidable water leaks through the leaf cuticle or stomata under high VPD as observed by Cavender-Bares *et al.,* (2007) in live oaks under drought and by Zeppel *et al.,* (2010) in two evergreen *Eucalyptus* species. Our results may reveal a pre-dawn activation of sap flow and warrant further research to elucidate the drivers of nocturnal sap flow and the ecological significance for these species, if any. Moreover, if plant transpiration is different to zero, the assumption that plant water potential reaches equilibrium with the soil at night will have to be reconsidered and thus will limit our ability to assess plant water status from predawn water potential measurements (Bucci *et al.*, 2004, Dawson *et al.*, 2007).

In summary, *P. latifolia* seemed to cope better with drought conditions and transpired generally greater amounts of water. This might partly explain and support the more severe effects of an experimental drought (on average 15% decreased soil moisture) on *Q. ilex* than on *P. latifolia* observed at the same site (Ogaya & Peñuelas, 2007b). In that experiment, *Q. ilex* presented lower mean annual stem diameter increments and higher mortality rates than *P. latifolia* (Ogaya & Peñuelas, 2007b). These results provide additional evidence that *P. latifolia* may out-compete *Q. ilex* at the drier edge of its distributional range due to the increase in the intensity and frequency of summer droughts, as stated in several previous studies (Martínez-Vilalta *et al.*, 2003, Martínez-Vilalta *et al.*, 2002a, Ogaya & Peñuelas, 2003, Ogaya & Peñuelas,

2006, Ogaya & Peñuelas, 2007a, Ogaya & Peñuelas, 2007b, Ogaya *et al.*, 2011, Ogaya *et al.*, 2003, Peñuelas *et al.*, 2000, Peñuelas *et al.*, 1998). The results also showed a significant role of nocturnal sap flow in both species with a frequent pattern of increase from 3 am to dawn that warrants further and in depth research.

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REFERENCES

Allen CD, Macalady AK, Chenchouni H et al. (2010) A global overview of drought and heat induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management, 259, 660-684.

Bucci SJ, Scholz FG, Goldstein G, Meinzer Fc, Hinojosa Ja, Hoffmann Wa, Franco Ac (2004) Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. Tree Physiology, 24, 1119-1127.

Caird MA, Richards JH, Donovan LA (2007) Nighttime stomatal conductance and transpiration in C-3 and C-4 plants. Plant Physiology, 143, 4-10.

Carnicer J, Coll M, Ninyerola M, Pons X, Sanchez G, Peñuelas J (2011) Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proceedings of the National Academy of Sciences of the United States of America, 108, 1474-1478.

Cavender-Bares J, Sack L, Savage J (2007) Atmospheric and soil drought reduce nocturnal conductance in live oaks. Tree Physiology, 27, 611-620.

Daley MJ, Phillips NG (2006) Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. Tree Physiology, 26, 411-419.

David TS, Henriques MO, Kurz-Besson C et al. (2007) Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. Tree Physiology, 27, 793-803.

Dawson TE, Burgess SS, Tu KP et al. (2007) Nighttime transpiration in woody plants from contrasting ecosystems. Tree Physiology, 27, 561-575.

Dodd AN, Salathia N, Hall A et al. (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science, 309, 630-633.

Donovan LA, Richards JH, Linton MJ (2003) Magnitude and mechanisms of disequilibrium between predawn plant and soil water potentials. Ecology, 84, 463-470.

Fisher JB, Baldocchi DD, Misson L, Dawson TE, Goldstein AH (2007) What the towers don't see at night: nocturnal sap flow in trees and shrubs at two AmeriFlux sites in California. Tree Physiology, 27, 597-610.

Gucci R, Massai R, Casano S, Mazzoleni S (1999) Seasonal changes in the water relations of Mediterranean co-occurring woody species. Plant Biosystems, 133, 117-128.

Gutierrez E, Campelo F, Julio Camarero J, Ribas M, Muntan E, Nabais C, Freitas H (2011) Climate controls act at different scales on the seasonal pattern of Quercus ilex L. stem radial increments in NE Spain. Trees-Structure and Function, 25, 637-646.

Howard AR, Donovan LA (2007) Helianthus nighttime conductance and transpiration respond to soil water but not nutrient availability. Plant Physiology, 143, 145-155.

Infante JM, Domingo F, Ales RF, Joffre R, Rambal S (2003) Quercus ilex transpiration as affected by a prolonged drought period. Biologia Plantarum, 46, 49-55.

Infante JM, Mauchamp A, Fernandez-Ales R, Joffre R, Rambal S (2001) Within-tree variation in transpiration in isolated evergreen oak trees: evidence in support of the pipe model theory. Tree Physiology, 21, 409-414.

Limousin JM, Rambal S, Ourcival JM, Rocheteau A, Joffre R, Rodriguez-Cortina R (2009) Long-term transpiration change with rainfall decline in a Mediterranean Quercus ilex forest. Global Change Biology, 15, 2163-2175.

Lindner M, Maroschek M, Netherer S et al. (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. Forest Ecology and Management, 259, 698-709.

Lloret F, Peñuelas J, Prieto P, Llorens L, Estiarte M (2009) Plant community changes induced by experimental climate change: Seedling and adult species composition. Perspectives in Plant Ecology Evolution and Systematics, 11, 53-63.

Martínez-Vilalta J, Mangiron M, Ogaya R, Sauret M, Serrano L, Peñuelas J, Pinol J (2003) Sap flow of three co-occurring Mediterranean woody species under varying atmospheric and soil water conditions. Tree Physiology, 23, 747-758.

Martínez-Vilalta J, Pinol J, Beven K (2002a) A hydraulic model to predict drought-induced mortality in woody plants: an application to climate change in the Mediterranean. Ecological Modelling, 155, 127-147.

Martínez-Vilalta J, Prat E, Oliveras I, Pinol J (2002b) Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. Oecologia, 133, 19-29.

Mueller RC, Scudder CM, Porter ME, Trotter RT, Gehring CA, Whitham TG (2005) Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. Journal of Ecology, 93, 1085-1093.

Ogaya R, Peñuelas J (2003) Comparative field study of Quercus ilex and Phillyrea latifolia: photosynthetic response to experimental drought conditions. Environmental and Experimental Botany, 50, 137-148.

Ogaya R, Peñuelas J (2006) Contrasting foliar responses to drought in Quercus ilex and Phillyrea latifolia. Biologia Plantarum, 50, 373-382.

Ogaya R, Peñuelas J (2007a) Species-specific drought effects on flower and fruit production in a Mediterranean holm oak forest. Forestry, 80, 351-357.

Ogaya R, Peñuelas J (2007b) Tree growth, mortality, and above-ground biomass accumulation in a holm oak forest under a five-year experimental field drought. Plant Ecology, 189, 291-299.

Ogaya R, Peñuelas J, Asensio D, Llusia J (2011) Chlorophyll fluorescence responses to temperature and water availability in two co-dominant Mediterranean shrub and tree species in a long-term field experiment simulating climate change. Environmental and Experimental Botany, 71, 123-127.

Ogaya R, Peñuelas J, Martínez-Vilalta J, Mangiron M (2003) Effect of drought on diameter increment of Quercus ilex, Phillyrea latifolia, and Arbutus unedo in a holm oak forest of NE Spain. Forest Ecology and Management, 180, 175-184.

Peñuelas J, Boada M (2003) A global change-induced biome shift in the Montseny mountains (NE Spain). Global Change Biology, 9, 131-140.

Peñuelas J, Filella I, Lloret F, Pinol J, Siscart D (2000) Effects of a severe drought on water and nitrogen use by Quercus ilex and Phillyrea latifolia. Biologia Plantarum, 43, 47-53.

Peñuelas J, Filella I, Llusia J, Siscart D, Pinol J (1998) Comparative field study of spring and summer leaf gas exchange and photobiology of the Mediterranean trees Quercus ilex and Phillyrea latifolia. Journal of Experimental Botany, 49, 229-238.

Peñuelas J, Ogaya R, Boada M, Jump As (2007) Migration, invasion and decline: changes in recruitment and forest structure in a warming-linked shift of European beech forest in Catalonia (NE Spain). Ecography, 30, 829-837.

Phillips NG, Lewis JD, Logan BA, Tissue DT. 2010. Inter- and intra-specific variation in nocturnal water transport in Eucalyptus. Tree Physiology, 30, 586-596.

Quero JL, Sterck FJ, Martínez-Vilalta J, Villar R (2011) Water-use strategies of six co-existing Mediterranean woody species during a summer drought. Oecologia, 166, 45-57.

Schiller G, Ungar EDd, Cohen Y (2002) Estimating the water use of a sclerophyllous species under an East-Mediterranean climate I. Response of transpiration of Phillyrea latifolia L. to site factors. Forest Ecology and Management, 170, 117-126.

Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F (2007) Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. Tree Physiology, 27, 551-559.

Snyder KA, Richards JH, Donovan LA (2003) Night-time conductance in C-3 and C-4 species: do plants lose water at night? Journal of Experimental Botany, 54, 861-865.

Tognetti R, Longobucco A, Miglietta F, Raschi A (1998) Transpiration and stomatal behaviour of Quercus ilex plants during the summer in a Mediterranean carbon dioxide spring. Plant Cell and Environment, 21, 613-622.

Van Der Zande D, Mereu S, Nadezhdina N, Cermak J, Muys B, Coppin P, Manes F (2009) 3D upscaling of transpiration from leaf to tree using ground-based LiDAR: Application on a Mediterranean Holm oak (Quercus ilex L.) tree. Agricultural and Forest Meteorology, 149, 1573-1583.

Villar-Salvador P, Castro-Diez P, Perez-Rontome C, Montserrat-Marti G (1997) Stem xylem features in three Quercus (Fagaceae) species along a climatic gradient in NE Spain. Trees-Structure and Function, 12, 90-96.

Zeppel M, Tissue D, Taylor D, Macinnis-Ng C, Eamus D (2010) Rates of nocturnal transpiration in two evergreen temperate woodland species with differing water-use strategies. Tree Physiology, 30, 988-1000.

Chapter V

Sequence of drought responses in holm oak forests: lessons from experiments to extreme droughts.

Adrià Barbeta and Josep Peñuelas

ABSTRACT

Longer, severer droughts are threatening the persistence of the current structure and function of Mediterranean forests. Plants in this region have already adapted to summer droughts, but they may not be able to cope with current and future increases in drought intensity, duration, or/and frequency. Here we review the mechanisms of drought resistance (and recovery) and the traits of the well-studied and widespread tree *Quercus ilex* from the tissue to the stand level using the temporal dimension as a conceptual framework. We have synthesised the available literature on these mechanisms and traits operating at different timescales in relation to (i) summer droughts, (ii) extreme droughts, and (iii) long-term drought experiments. The developmental time of some morphological traits is too long to respond to abrupt increases in drought. The phenological plasticity of *Q. ilex*, however, may allow it to adapt to changing drought regimes. The reports of forest dieback in *Q. ilex* suggest that the forcing droughts were extremely long rather than intense. *Q. ilex*, though, can vigorously resprout after such events. Long-term experiments can identify the processes of acclimation at the individual and stand level, but the capacity of *Q. ilex* to recover may be endangered if extreme droughts become more frequent.

KEYWORDS

Quercus ilex, drought stress, defoliation, acclimation, water availability, climate change, temporal scales.

INTRODUCTION

The increasing length and intensity of droughts is producing forest declines around the globe. The vulnerability of forests to droughts of a certain length and intensity depends largely on the climate of the region to which the structure and function of the forests are adapted (Vicente-Serrano *et al.*, 2013). Tree species have evolved to cope with climatic variability (including the variability of drought duration), so the mechanisms to resist drought have been adjusted to the climatic variability with in the evolutionary histories of the trees. Longer, more intense droughts are forcing trees to display their phenotypic plasticity to its maximum (Matesanz & Valladares, 2013). Severe modifications of tree function or forest structure may often occur, however, when drought reaches a certain threshold of length and/or intensity. Furthermore, the capacity of recovery after such a disturbance may also be influenced by prior disturbances and by the length of the period until a potential subsequent disturbance (Anderegg *et al.*, 2015). The mechanisms of drought resistance, the responses to severe and extreme droughts, and the subsequent recovery include processes from the molecular to the stand level, which operate at different timescales. A focus on the temporal dimension of both the abiotic (duration and intensity of droughts) and biotic (drought-resistance mechanisms and effects on plants) factors would thus improve our ability to predict potential changes in the structure and function of vegetation.

The diversity of the relationships between drought timescales and response mechanisms is as extensive as species diversity and may be more so due to high intraspecific variability (Anderegg, 2015). This diversity challenges the assessment of global patterns. In this review, we discuss the effect of different timescales of drought on plant life, from the tissue level to the ecosystemic or stand level of a tree species. Our chosen model tree is the holm oak (*Quercus ilex*), an extensively studied Mediterranean forest tree considered by some authors as a circum-Mediterranean species (Barbero *et al.*, 1992) that possesses some adaptations to drought. It is a keystone species of vast areas of the western Mediterranean Basin, and its dominance is expected to increase to the detriment of other forest species that seem to respond more negatively to the present increase in aridity, and in response to land abandonment and advancing succesional stages (Carnicer *et al.*, 2014). The numerous available studies on the responses of *Q. ilex* to drought allowed us to use it as a case study to understand how the drought timescale is tightly linked to the species-specific mechanisms of drought resistance. We also explore the modification of forest structure and function and the introduction of feedbacks of

either sign from the interaction between the sequence of discrete droughts and the anthropogenic, sustained increase in aridity.

We review the mechanisms and effects of drought resistance in *Q.* ilex, categorising them into three main sections corresponding to the timescale of the forcing drought. First, we discuss the resistance mechanisms operating during the seasonal droughts that typically occur in Mediterranean regions and which last only a few months and coincide with the season with the highest temperatures. Second, we review the effects of droughts exceeding the climatic variability of a given location in terms of duration, seasonality, intensity, or coincidence with heatwaves. Third, we report the effects of persistent droughts such as those produced by experimental systems. We believe that an overview of this information will be useful not only to better understand the response of *Q. ilex* to environmental change, but also to show how the temporal dimension is crucial for predicting the responses of plants to the increases in drought projected by global circulation models (IPCC, 2013).

MEDITERRANEAN SUMMER DROUGHTS AND THE ADAPTIVE MECHANISMS AND TRAITS OF *QUERCUS ILEX*

Plants in Mediterranean ecosystems have to cope with a double stress: summer drought and winter cold (Terradas, 1999). The summer drought coincides with the periods with the highest temperatures and the lowest precipitation. The high temperatures increase evapotranspiration, which together with the low precipitation produce periods with a negative water balance (the difference between precipitation and potential evapotranspiration). The summer drought, within the distributional range of forests dominated by *Q. ilex*, typically lasts 3-4 months (Fig. 1). The duration and intensity of the summer droughts, however, are highly inter-annually variable (Piñol *et al.*, 1991), and some *Q. ilex* forests thrive in circum-Mediterranean areas where summer drought is rare, such as the coastal areas of the Bay of Biscay (Niinemets, 2015), and evidence suggests recent colonisation in areas with an oceanic climate (Delzon *et al.*, 2013)(Fig. 2). *Q. ilex* possesses important ecotypic variation (Gratani *et al.*, 2003; Peguero-Pina *et al.*, 2014) but is generally considered as a species that has several morphological and physiological adaptations for surviving summer droughts. In this section, we review these morphological traits and physiological mechanisms, which are summarised in Table 1.
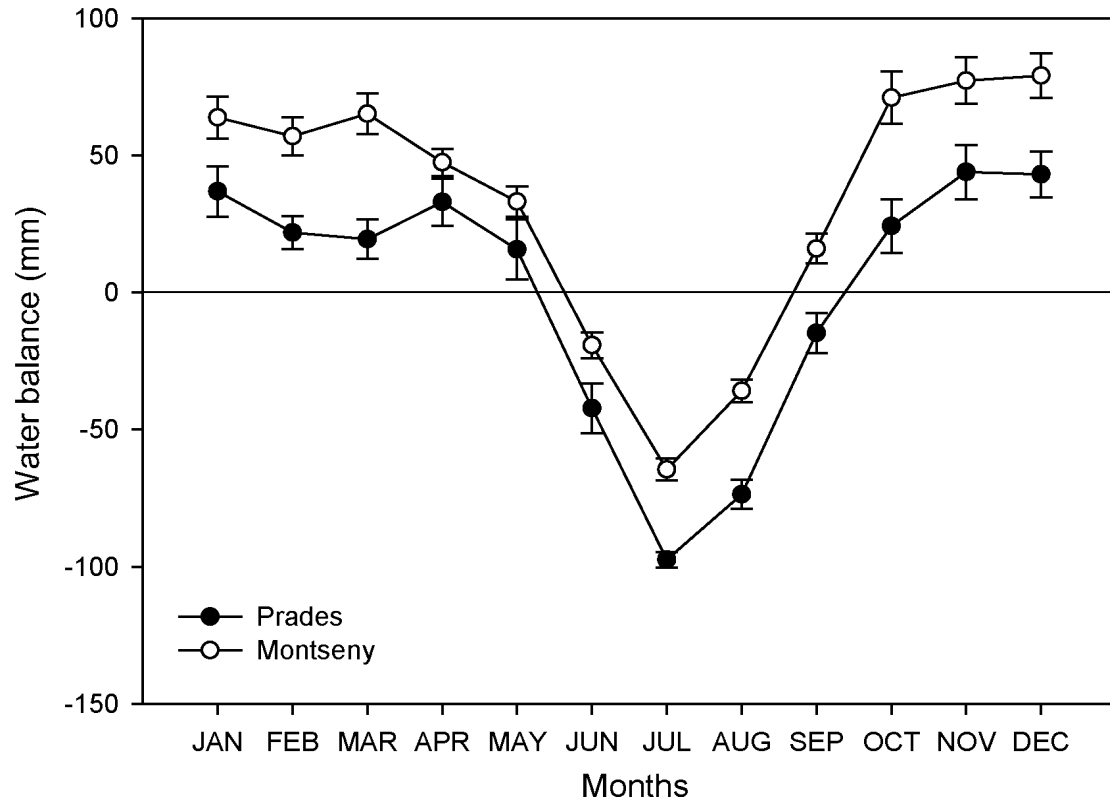
Figure 1. Monthly average water balance (calculated as the difference between monthly precipitation and monthly potential evapotranspiration) for two forests dominated by *Quercus ilex* at their driest (Prades) and wettest (Montseny) distributional limits. Error bars are the standard errors of the means.

Summer drought depletes the water in the topsoil, but moisture can still be found in deeper water pools. Because water is taken up by roots, the development of a deep root system in *Q. ilex* has been regarded as a morphological adaptation to summer drought (Canadell *et al.*, 1999). Woody species in sclerophyllic forests and shrublands (Canadell *et al.*, 1996) also have deeper roots than plants in ecosystems without seasonal drought. The effect of drought at sites with shallow soils is determined by the capacity of roots to penetrate the bedrock in search of moisture pockets (Lloret *et al.*, 2004; Barbeta *et al.*, 2015). Deep roots are thus a crucial morphological feature of holm oaks, conferring a competitive advantage over sympatric species such as Mediterranean pines(Comas *et al.*, 2015). This ability to tap deep water pools and thus to have permanent access to water has

Figure 2. Map of the current distributional range of holm oak (*Quercus ilex*) in the Mediterranean Basin, from Delzon et al (2013). Crosses represent geographically isolated populations.

led in some cases to the categorisation of *Q. ilex* as a drought-avoiding species (David *et al.*, 2004). The trees, however, would only avoid water shortages under certain atypical conditions: when soils lay on permeable or fractured bedrock in relatively flat terrain and with sufficient groundwater recharge during winter months (David *et al.*, 2004). In addition to the depth of the root system, the total root biomass relative to the aboveground biomass is associated with water availability. Holm oaks in xeric sites have a higher percentage of belowground biomass than those in mesic sites, accounting for more than the half of the total tree biomass in some cases (Canadell *et al.*, 1999). The ultimate water-absorbing organs, fine roots, also have a higher relative area in the drier sites of *Q. ilex* (Martin-Stpaul *et al.*, 2013). The variability in the length of summer drought is thus offset by the allocation in this species of resources for the growth of belowground structures to the detriment of aerial organs, especially transpiring organs such as leaves.

Q. ilex trees withstand the typical summer droughts by ensuring access to water through their extensive root systems and also by maintaining a sufficiently low ratio of aboveground to belowground biomass. At the same time, though, the morphology of aerial organs has been evolutionarily shaped by summer drought to promote a conservative use of water. Xylem conduits are narrower than in other *Quercus* tree species (Villar-Salvador

et al., 1997), resulting in lower conductivity, although this maybe an adaptation to winter cold (Cavender-Bares *et al.*, 2005). *Q. ilex* can nevertheless transpire at higher rates than other Mediterranean evergreen plants (Martínez-Vilalta *et al.*, 2002) due to its relatively wider xylem conduits. Sap flow during summer drought, however, must be substantially reduced (Martínez-Vilalta *et al.*, 2003; Barbeta *et al.*, 2012) to avoid xylem cavitation, because its xylem anatomy is not more resistant to embolism than deciduous *Quercus* species intemperate forests (Choat *et al.*, 2012). Once water reaches the canopy, sclerophyllic leaves with high specific mass and thickness promote the conservation of cellular water (Bartlett *et al.*, 2012).

The morphologies of roots, xylems, and leaves partly determine the water-use strategy of a plant and thus its performance under drought. Perhaps more importantly, plants activate several physiological mechanisms under water stress, most relevantly stomatal control (Martínez-Vilalta *et al.*, 2014). *Q. ilex* may be the most isohydric species of the genus, more isohydric than sympatric sclerophyllic shrubs according to a recent review (Martínez-Vilalta *et al.*, 2014), indicating that it will reduce stomatal conductance in response to conductivity losses before the other species. This tree uses water very conservatively during summer drought, with almost completely closed stomata at midday (Ogaya *et al.*, 2014a), and the plants consequently have a lower risk of xylem embolism. Nevertheless, native embolism in *Q. ilex* xylem is not rare, either during summer drought (Aguadé *et al.*, 2015) or winter frost (Cavender-Bares *et al.*, 2005). The soluble fraction of plant non-structural carbohydrates has thus been hypothesised to be used for maintaining the integrity of the vascular system by osmotic regulation (Sala *et al.*, 2012). The unloading of sugars from the phloem to embolised xylem vessels has been proposed as a mechanism of embolism refilling (Nardini *et al.*, 2011). Interestingly, the proportion of soluble sugars increases during summer drought in holm oak branches (Rosas *et al.*, 2013) and leaves (Rivas-Ubach *et al.*, 2014), suggesting a role for these solutes in drought resistance. The latter study also found an increase in foliar K concentration in summer, which may also be related to osmotic regulation (Babita *et al.*, 2010).

Thresholds of water availability (van der Werf *et al.*, 2007) and temperature (Deslauriers *et al.*, 2008) constrain cambial activity and thus govern the phenology of growth and the carbon supply, also in *Q. ilex* (Lempereur *et al.*, 2015). The phenology of primary, secondary, and reproductive growth are thus necessarily influenced by the aforementioned double stress in Mediterranean ecosystems: summer drought and winter cold. A bimodal pattern of stem growth peaking in spring and autumn is common,

particularly in summer drought (Montserrat-Martí *et al.*, 2009; Gutiérrez *et al.*, 2011). The onset of this summer cessation of growth has been directly correlated with spring rainfall (Lempereur *et al.*, 2015). This quiescence might be an adaptation to summer drought, but growth would be expected to be plastic enough to continue when sufficient soil moisture became available. Furthermore, primary growth, including foliar flushing (Ogaya & Peñuelas, 2006) and shoot growth (Camarero *et al.*, 2015), occurs mostly before the summer drought, although foliar maturation and shoot growth are completed in summer (Montserrat-Martí *et al.*, 2009). Holm oaks must thus take advantage of periods with favourable conditions for growth, and in contrast to deciduous Mediterranean oaks have a relevant peak of growth in autumn, either in the stems (Corcuera *et al.*, 2004; Gutiérrez *et al.*, 2011) or leaves (Ogaya & Peñuelas, 2006). In addition, several cohorts of buds up to 3-4 years old are maintained in the canopy, which may burst in years with favourable conditions (Alla *et al.*, 2012).

These adaptive morphological, physiological, and phenological mechanisms of holm oaks to withstand the months of summer drought are developed and/or activated at different timescales (Table 1). The development of tap roots and a low ratio of leaf to root area accumulate throughout the years of growth, producing morphological differentiation between genetically closely related populations growing in contrasting micro-topographical conditions (Canadell *et al.*, 1999). The environmental conditions along an individual's life consequently define its future structure. In the case of foliar and xylem morphology, however, genetics interact with the environmental conditions at shorter timescales to determine an individual's structure (spring for leaves (Ogaya & Peñuelas, 2006) and summer for xylems (Abrantes *et al.*, 2013)). The physiological mechanisms can be activated in even shorter times; the degradation of starch to sugars occurs on a daily basis (Patakas *et al.*, 2002), and stomatal sensitivity is almost instantaneous (Mcadam & Brodribb, 2012). Most of these mechanisms are transient responses and can be reversed when the stress disappears (*sensu* Wolkovich et al. (2014), Table 1). Stomata can open fully again once soil moisture increases, or leaves grown in wet years may become less sclerophyllic. Other features such as modified xylem anatomy (e.g. the vessel area) are persistent responses. Xylem anatomy fluctuates in response to the environmental conditions during the growing season (Campelo *et al.*, 2010), but it influences future water transport because water is conducted by the xylem formed over several years (Martínez-Vilalta *et al.*, 2003). Unprecedented intensities of summer drought enhanced by global warming may thus negatively affect those individuals or stands that have grown under

milder conditions, because morphological characteristics such as xylem anatomy, rooting depth, or the ratio of leaf to root area accumulate over long periods of time.

Table 1. Adaptive morphological, physiological and phenological mechanisms and/or traits of *Quercus ilex* for withstanding summer droughts.

	Organ	Adaptive mechanism or trait	Developmental time	Duration
Morphology	Roots	Tap roots reaching water table or water pools in rock fractures	Individual life	Persistent
		Relatively high belowground biomass	Individual life	Persistent
		Low proportion of fine roots	Seasonal	Transient
	Leaves	Sclerophylly	Seasonal	Transient
Physiology	Leaves	Stomatal closure to reduce xylem tension	Instantaneous	Transient
	Systemic	Osmotic adjustment to maintain cellular turgor	Hours	Transient
Phenology	Stems	Summer growth cessation (bimodal growth pattern)	Seasonal	Transient
	Leaves and shoots	Most growth completed before summer	Seasonal	Persistent
	Buds	Coexistence of several bud cohorts in the canopy	Years	Transient

RESPONSE OF *QUERCUS ILEX* TO EXTREME DROUGHTS

Summer droughts in Mediterranean ecosystems are cyclical, with a period of return of one year but with high inter-annual variability. They thus represent a recurrent abiotic stress for the vegetation. Current climatic change, however, is increasing temperatures (IPCC, 2013), which supposes a positive-trend effect (Jentsch *et al.*, 2007) on the intensity of summer droughts. Extreme droughts, though, are also expected to become more frequent in the Mediterranean Basin (IPCC 2013). In fact, non-cyclical droughts (Wolkovich *et al.*, 2014) will have shorter periods of return, so paradoxically these extreme droughts would be more common in the future. Droughts occurring in or extending into the typically wet seasons in the Mediterranean Basin (spring and autumn) have also recently increased, which may have even larger impacts than more intense summer droughts, because spring and autumn are the optimum periods for growth in holm oaks (Camarero *et al.*, 2015).

Because extreme climatic events can be defined differently depending on the characteristics of the event (rarity, intensity, or duration) or its effects on a system (Smith, 2011), delimiting what an extreme climatic event actually means is mandatory. Here we review the effects of drought on, and the resistance mechanisms of, *Q. ilex* to droughts that (i) represent statistically rare events due to their intensity, duration, and/or timing and that (ii) caused negative effects involving at least partial crown damage or an increase in mortality rates above the baseline levels. The available literature on drought-induced declines of holm oaks has reported a great diversity of drought characteristics. For example, the 1993-1994 drought in the northeastern Iberian Peninsula lasted for 10 months when precipitation was only half of the historical average (Peñuelas *et al.*, 2000; Lloret *et al.*, 2004). In contrast, the 2005 drought in the southern Pyrenees was very intense and aseasonal because it peaked in spring, while another drought at the same site in 2012 was characterised by more persistent dry and warm conditions (Camarero *et al.*, 2015). Other droughts have been restricted to a smaller geographic area but have still had severe effects on the vegetation, such as the drought of 2011 in southern Catalonia (Poyatos *et al.*, 2013; Ogaya *et al.*, 2014b). All these events have in common the rarity of the historical climatic series, although this rarity may be caused by differences in drought duration, intensity, and/or timing. Oak populations will suffer important alterations in their plant use of water and carbon, phenology, primary and secondary growth rates, or mortality rates, depending on the characteristics of the drought.

Drought-induced forest dieback in holm oak stands

Leaf shedding is a phenological event that occurs in *Q. ilex* simultaneously with bud burst (Ogaya & Peñuelas, 2006), in which some of the old leaves are abscissed and substituted by new leaves. Leaf shedding can also be a short-term response to drought by which the plant adjusts its transpiratory area to decreased water availability. Leaf abscission may be able to act as a safety valve to avoid damage in organs such as stems where carbon investment is more expensive (Bucci *et al.*, 2012; Pivovaroff *et al.*, 2014). Some level of leaf shedding during summer drought is thus not necessarily indicative of strong water stress and could be added to Table 1 as a mechanism for regulating the plant-water relationship during summer droughts. High percentages of canopy loss, however, reduce photosynthetic capacity and may compromise the survival of the plant individual in the long term (Galiano *et al.*, 2012). Canopy loss is thus the first indicator of forest dieback.

The extensive crown defoliation of holm oak stands in the last fifteen years has been correlated with a progressive increase in water stress (Carnicer *et al.*, 2011) and with individual extreme droughts (Peñuelas *et al.*, 2000; Lloret *et al.*, 2004; Galiano *et al.*, 2012; Ogaya *et al.*, 2014b; Barbeta *et al.*, 2015). These events were in fact noticeable by the browning of leaves and the desiccation of entire branches and in some cases even of all aboveground organs of an individual. Table 2 shows the values of the drought index (Vicente-Serrano *et al.*, 2010) at different months and timescales for published reports of episodes of forest decline that included stem mortality and/or crown defoliation. A closer look at the water balance for different lengths of time identifies some diversity in the timing of crown defoliation or stem mortality triggered by drought. Comparing drought characteristics is thus possible (Table 2); some events are produced by long-lasting water deficits (Lloret *et al.*, 2004; Barbeta *et al.*, 2015), whereas others cause a much more negative water balance and of greater rarity but for shorter periods (Barbeta *et al.*, 2013). Stem mortality after the event in the latter study was 5.4%, but the 1994 drought caused 23% crown defoliation over the same bedrock (increasing to 76% on less permeable bedrock), suggesting that *Q. ilex* has more tolerance to short and acute droughts, even if their intensity increases in the future. Confirming this premise will require increasing observational efforts and understanding other factors inherent to forest structure and function that interact with drought to determine the magnitude of its effects. Furthermore, the lack of standardisation between the units to measure the effects of drought on vegetation (crown defoliation at plot or individual level, or mortality rates at stem or individual level) prevents a straightforward comparison among events. For example,

Barbeta et al. (2013) assessed mortality at the stem level if no resprouting occurred the year after the event, because the isolation and identification of genetic individuals was not possible in the studied site. In contrast, Galiano et al. (2012) categorised whole trees (several stems) into classes of crown damage and assessed mortality at the individual level in another site.

The number of published reports of drought-induced declines of holm oaks is relatively low, but drought characteristics alone (represented in Table 2 by the multi-scalar drought index) cannot fully explain the variability in crown defoliation or stem mortality. The effects of a drought also depend on the pre-drought forest structure (Carnicer *et al.*, 2011). Trees in denser stands are more likely to die or be defoliated (Carnicer *et al.*, 2011; Galiano *et al.*, 2012). Having more stems increases the survival of the individual but increases stem mortality and defoliation. Taller trees (Lloret *et al.*, 2004) with larger basal areas (Galiano *et al.*, 2012; Barbeta *et al.*, 2013), however, are the least affected during these events. The history of the forest must be considered, because all these characteristics are affected by past management practices. Topography is another critical factor that determines the levels of mortality and defoliation in *Q. ilex*. The fluctuations of climate are offset to some extent by the deep root system of holm oaks (Canadell *et al.*, 1999), but rooting depth can be spatially heterogeneous. Soil in steeper locations is rockier, shallower, and has a lower capacity to store water, so the survival of the trees is also lower (Galiano *et al.*, 2012). Similarly, access to pools of groundwater is critical for surviving summer drought and determines the effects of extreme droughts (Barbeta *et al.*, 2015). The mortality rates during an extreme drought are consequently substantially higher on bedrock types that do not allow the penetration of roots (Lloret *et al.*, 2004).

Table 2. Characteristics of reported drought-induced declines in *Quercus ilex* forests involving crown defoliation and/or stem mortality. CD, crown defoliation.

			SPEI								
Study	**Drought year**	**Site**	**May-3**	**May-6**	**Aug-3**	**Aug-6**	**Oct-9**	**Dec-12**	**Dec-24**	**Bedrock type**	**Reported effects**
Lloret et al 2004	1985	Sant Llorenç de Munt	-0.13	0.14	-0.85	-0.44	-1.06	-0.22	-0.39	Schists, Breccia	Not available
Lloret et al 2004	1994	Sant Llorenç de Munt	0.67	0.53	-1.51	0.10	0.87	0.90	-0.62	Schists (S), Breccia (B).	75.9% of plants without green foliage (CD) in B and 23.2% in S
Galiano et al 2012	2000	Garrotxa	-0.84	-0.59	-0.68	-0.77	-0.96	0.12	-1.14	Conglomerate	73.8% of trees with some level of CD in patches with much browning
Barbeta et al 2013	2005	Prades	-1.90	-1.44	-2.00	-1.87	-1.71	0.43	0.23	Schists	Average of 5.4% stem mortality (SM)
Barbeta et al 2015, Ogaya et al 2015	2011	Prades	-0.43	-0.34	-0.47	-0.76	-1.10	0.90	-0.62	Schists	Averages of 3.9% SM and 22.5% CD

Trends of crown defoliation and their relationship with drought characteristics in the Iberian Peninsula

To identify the more critical drought characteristics (in terms of duration and timing) for crown defoliation (i.e. for *Q. ilex* resistance to drought), we performed a correlative analysis using 25 years of data on crown defoliation in the Iberian Peninsula (ICP forests program, 2006) and the global Standardized Precipitation Evapotranspiration Index (SPEI) database (Vicente-Serrano *et al.*, 2010). Carnicer et al. (2011) reported consistent trends of increasing defoliation associated with warmer temperatures and exacerbated water deficits in southern Europe. Using an updated version of the data but only for *Q. ilex*, we correlated plot-level data on the annual relative change of crown defoliation with gridded SPEI values (0.25°) at all different months and available scales (1 to 24 months). The correlation heatmap presented in Fig. 3 indicated that the SPEI and the annual relative change in defoliation level were best correlated for SPEI scales longer than seven months and for summer months (June, July, and August). More particularly, June SPEI-23 was best (negatively) correlated, followed by July SPEI-21 and July SPEI-15. These data were collected from 25 years (1987-2013) and were based on 233 plots distributed throughout the range of *Q. ilex* in the Iberian Peninsula.
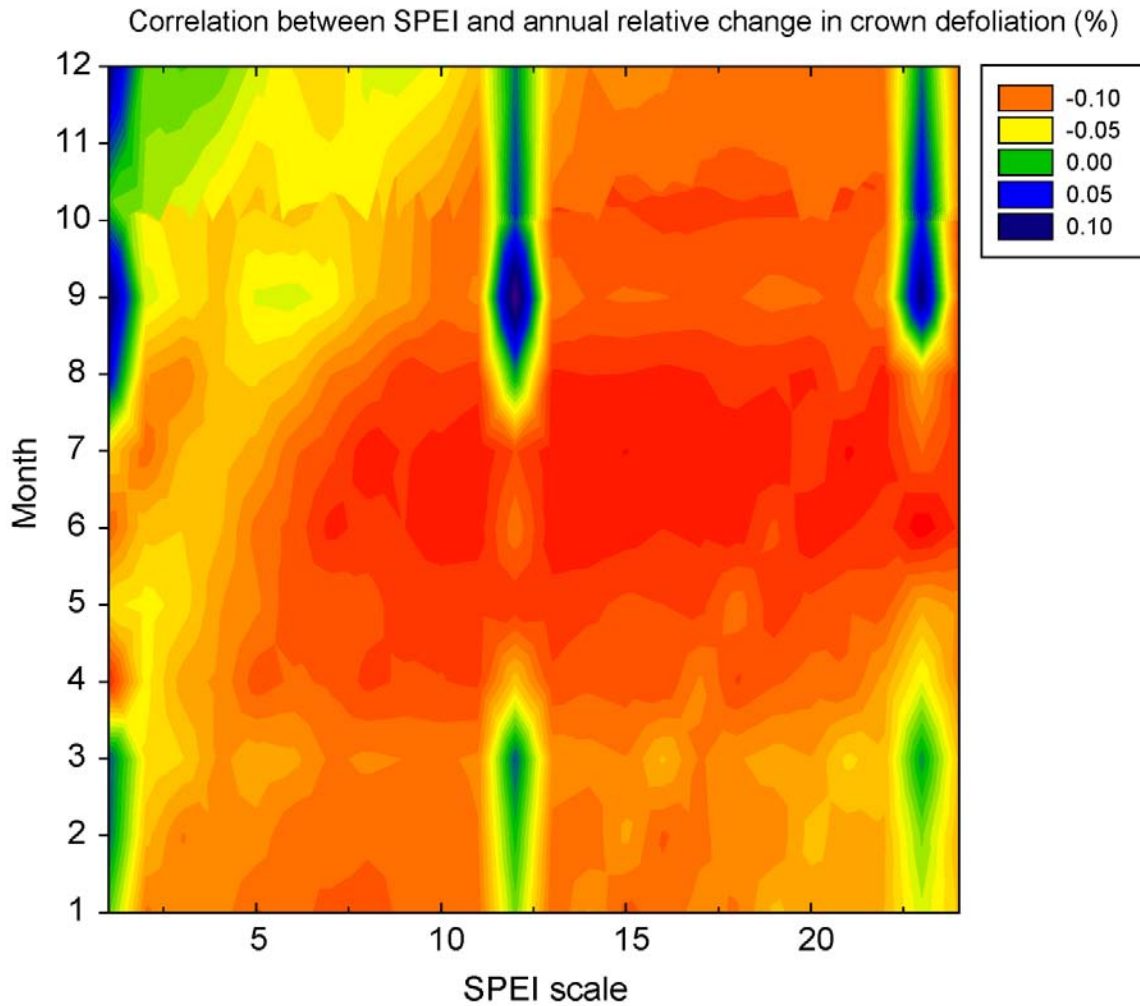
Figure 3. Correlation heatmap between the annual relative change in crown defoliation and the SPEI for Iberian *Quercus ilex* forests (1987-2013, 233 plots). The SPEI scale on the x-axis represents the timescale of the water balance (e.g. an SPEI scale of 3 in June is calculated using the water balance of the previous April and May and of June).

The correlation between defoliation and the SPEI became stronger by June and when accounting for the water balance of the previous winter and spring (Fig. 3). The sensitivity of *Q. ilex* to drought indicated by crown defoliation was thus similar to the sensitivity of secondary growth indicated by tree-rings (Pasho *et al.*, 2011; Camarero *et al.*, 2015). In those studies, the more critical period for growth in terms of water balance was also in the summer months. These studies also found that a positive water balance in the previous winter and spring was translated into high growth rates. Nevertheless, crown defoliation was better correlated with even longer timescales of the SPEI, spanning nearly two years. *Q. ilex* can rely on groundwater to cope with summer drought (Barbeta et al. 2105; David et al. 2004), so the good correlations of defoliation with the SPEI at long timescales are likely a result of the use of this groundwater. Indeed, the level of the groundwater

responds to the accumulated water balance of longer periods than the level of surface soil moisture, i.e. longer than seasonal water balances (Vicente-Serrano *et al.*, 2010). In addition to the recharge of groundwater pools, the relevance of the rainfall during winter months could also be related to the replenishment of carbon reserves. Sap flow in evergreen forests is higher in winter than in summer, but there is no growth (Sánchez-Costa *et al.*, 2015). The carbon assimilated during these periods could thus be directed to other functions such as defence or cold hardening but also to storage in the form of starch that may then be used during summer drought to maintain cell turgor and the integrity of the vascular system (Table 1).

The sustained increase in crown defoliation in *Q. ilex* stands since the early 1990 speaked during the droughts of 1994-95 (Fig. 4a). The defoliation levels have since stabilised near 20%. The SPEI values indicated that droughts of similar intensity occurred after this peak (e.g. 2001 and 2005) but did not trigger further increases in defoliation as in the mid-1990s, indicated by the relative change in crown defoliation (Fig. 4b). These data suggest that increasing aridity has led to a diminished canopy cover, but also that once a certain level of defoliation is reached; droughts of equal intensity may not further decrease leaf area. A reduction in leaf per sapwood area and/or in the ratio of above- and belowground biomass initially produced by droughts could remain as a stable morphological shift. Forests have been hypothesised to acclimate through stabilising processes at the individual (Barbeta *et al.*, 2015) and community (Lloret *et al.*, 2012; Barbeta *et al.*, 2013) levels. Other studies, however, suggest that consecutive droughts can weaken trees and compromise their survival in the long-term (Anderegg *et al.*, 2015). The results of long-term drought experiments (see next section) and studies monitoring the recovery of affected stands years after an extreme event can help to elucidate when each of these processes occurs.
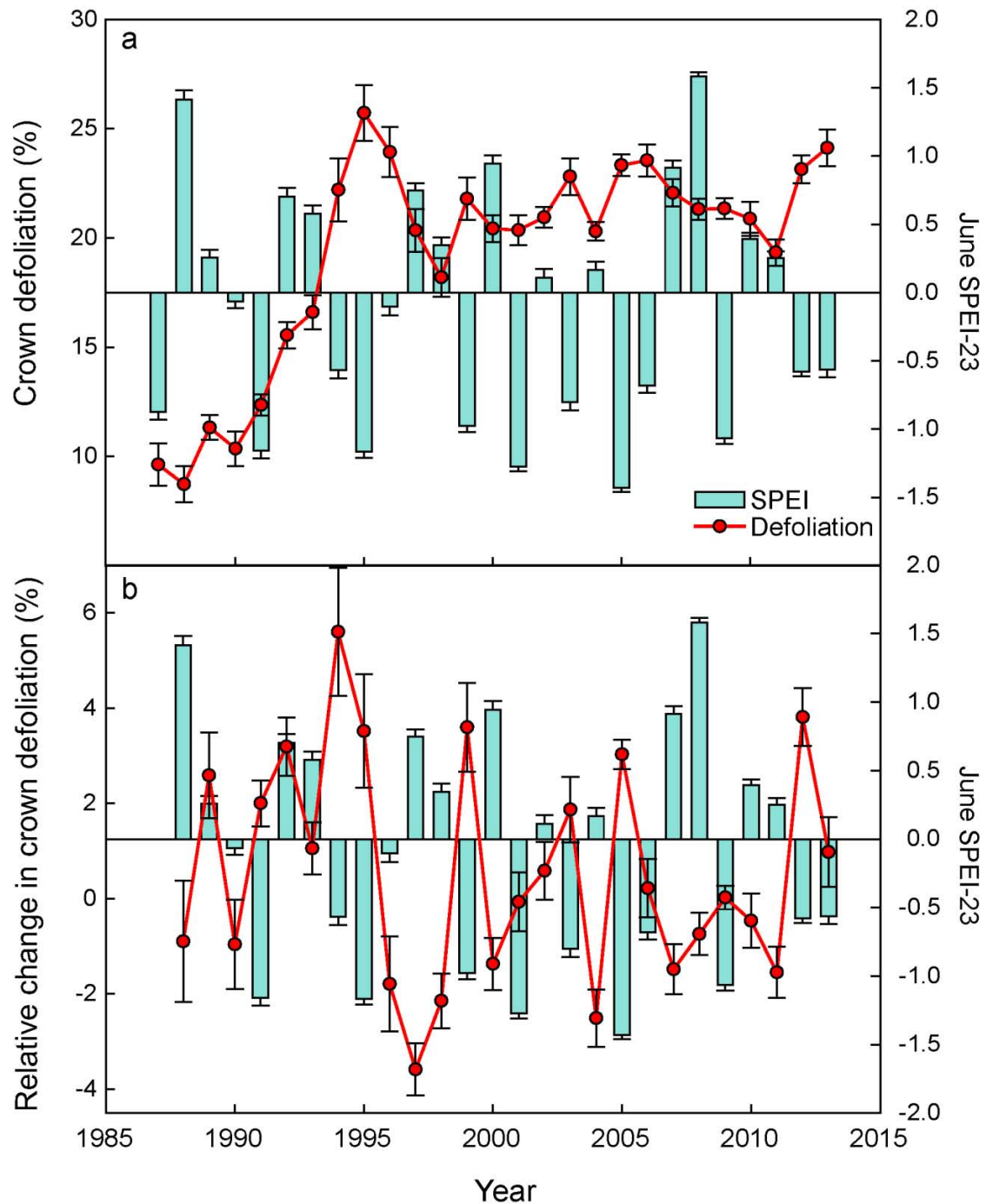
Figure 4. Annual courses of (a) average percentage of crown defoliation and (b) change of crown defoliation relative to previous years (red lines). Blue bars are the June SPEIs calculated at a timescale of 23 months, which was the best correlated index. Error bars are the standard errors of the means (N=233).

Resprouting after drought-induced biomass loss

Holm oaks have the capacity to resprout from pre-existing buds after a disturbance causing the loss of biomass (Espelta *et al.*, 1999). Resprouts can emerge either from aboveground (canopy) or belowground (lignotuber) buds, allowing a rapid recovery of the photosynthetic area after a disturbance. Fire, overgrazing, firewood extraction, and charcoal production have been the most common disturbances triggering resprouting in holm oak stands, but extreme droughts will likely increasingly recur in the coming years, and the oaks will likely resprout in response. Extreme drought causes lower mortality rates than other disturbances (Espelta *et al.*, 1999) but in turn can impact roots by causing hydraulic dysfunction (Anderegg *et al.*, 2012; Meinzer & McCulloh, 2013) and deplete belowground reserves of non-structural carbohydrates (NSCs) (Galiano *et al.*, 2012). The level of the normalised difference vegetation index (NDVI), indicative of canopy cover of a forest dominated by *Q. ilex*, however, fully recovered within a year after a drought (Ogaya *et al.*, 2014b) that induced extensive branch desiccation (Liu *et al.*, 2015). The presence of long-lived apical buds (Table 1, Alla et al. (2012)) thus increases the resilience of trees to drought in cases where aboveground meristemic tissues are not damaged.

Despite the higher resistance to drought-induced mortality of resprouting plants relative to non-resprouting plants (Zeppel *et al.*, 2015), the resilience of the former can be weakened if the frequency of extreme droughts increases. Lloret et al. (2004) reported that plants that resprouted weakly after the 1985 drought were more likely to die in the drought of 1994. Similarly, 40% of the plants that completely lost their green foliage in 1994 had not recovered one year after the end of the drought. In contrast, only 11.2% of the trees that maintained some percentage of green leaves after the 1994 drought were completely defoliated the year after (Lloret *et al.*, 2004). Past droughts thus have a legacy, a carry-over effect, that has been associated with the levels of NSCs in lignotubers, belowground swollen structures that accumulate buds and starch (Canadell *et al.*, 1999). Galiano et al. (2012) were the first to demonstrate that the resilience of crown condition seven years after an extreme drought was associated with the amount of NSCs in the lignotubers. NSC reserves were depleted 60% more in highly damaged than in healthy trees. Rosas et al. (2013) observed significantly lower lignotuber NSC levels in the most defoliated trees after the drought of 2011 (Table 3), confirming this organ as a relevant NSC reservoir for overcoming the effects of drought.

The period of return of extreme droughts appears to be a key factor determining the resilience of *Q. ilex* to these droughts. If lignotuber NSC levels are not fully recovered by the next drought, the survival of a given tree will be compromised. *Q. ilex* needs an estimated 20 years to fully replenish the NSC levels of the lignotubers (López *et al.*, 2009), so the current frequency of extreme droughts (i.e. the timelapse between droughts of similar intensity in Fig. 2) is already threatening the future resilience of *Q. ilex* stands.

SEQUENCE OF RESPONSE OF *Q. ILEX* TO LONG-TERM FIELD EXPERIMENTAL DROUGHT

Two long-term drought experiments have been established in natural forests of *Q. ilex*; the first in 1998 in Prades (Catalonia, Iberian Peninsula) and the other in 2003 in the Puébachon State Forest (southern France). Both experimental designs excluded throughfall over roughly 30% of the plot surface (Limousin *et al.*, 2010b), and runoff at the Prades site was intercepted by ditches along the top edges of the plots (Ogaya & Peñuelas, 2003b). These experiments have produced a profuse body of information on the responses of holm oak forests to imposed moderate but continuous drought, from leaf-level physiology to ecosystem-level demography and fluxes. Severe drought treatments have often been applied to seedlings and/or saplings, providing a useful mechanistic understanding of the sequence of responses to drought. The still relatively short duration of these experiments, however, compromises the implementation of their results in long-term climate-vegetation models. Furthermore, the strength of the effect on ecosystems of the same level of stress induced by an experimental treatment (e.g. warming, drought, or nutrient addition) decreases over time (Leuzinger *et al.*, 2011). In contrast, the results of the long-term drought experiments in Prades and Puébachon would identify more stable effects and responses that can be expected in natural environments. In this section, we review the findings of these two experimental systems, which are summarised in Table 3 and Fig. 5.

Table 3. Summary of the effects identified by studies of long-term experimental drought in *Quercus ilex* forests.

Organ	Aspect	Variable	Drought treatment effect	Exp.drought duration (years)	Reference
Leaf	Carbon relations	iWUE	Unchanged	4	Limousin, Misson, *et al.,* (2010)
Leaf	Carbon relations	J_{max}	Unchanged	4	Limousin, Misson, *et al.,* (2010)
Leaf	Carbon relations	Maximum assimilation rate	Unchanged	4	Limousin, Misson, *et al.,* (2010)
Leaf	Carbon relations	V_{cmax}	Unchanged	4	Limousin, Misson, *et al.,* (2010)
Leaf	Carbon relations	Maximum assimilation rate	Unchanged	9	Martin-StPaul *et al.,* (2013)
Stem	Carbon relations	Stem CO_2 efflux	Unchanged	9	Rodriguez-Calcerrada *et al.,* (2014)
Leaf	Carbon relations	Stomatal conductance	Unchanged	9	Martin-StPaul *et al.,* (2013)
Leaf	Carbon relations	Maximum PSII quantum yield	Unchanged	10	Ogaya *et al.,* 2011
Leaf	Carbon relations	Day respiration	Increased	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	J_{max}	Unchanged	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Maximum assimilation rate	Unchanged	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Mesophyllic conductance	Unchanged	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Night respiration	Unchanged	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Ratio mesophyllic/stomatal conductance	Decreased	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Ratio day/night respiration	Increased	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Stomatal conductance	Unchanged	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Summer maximum assimilation rate	Increased	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Summer carbon-use efficiency	Increased	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Summer mesophyllic conductance	Increased	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Summer stomatal conductance	Increased	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	Triose-phosphate use	Unchanged	13	Sperlich *et al.,* unpublished results.
Leaf	Carbon relations	V_{cmax}	Unchanged	13	Sperlich *et al.,* unpublished results.
Branch	Carbon relations	Branch NSC	Unchanged	14	Rosas *et al.,* (2013)
Leaf	Carbon relations	Leaf NSC	Unchanged	14	Rosas *et al.,* (2013)
Roots	Carbon relations	Lignotuber NSC	Unchanged	14	Rosas *et al.,* (2013)
Leaf	Carbon relations	Maximum assimilation rate	Unchanged	14	Ogaya *et al.,* (2014)
Leaf	Carbon relations	Stomatal conductance	Unchanged	14	Ogaya *et al.,* (2014)
Leaf	epigenetics	Fully methylatedDNAloci	Decreased	12	Rico *et al.,* (2014)
Leaf	Morphology and allometry	Leaf area	Unchanged	3	Ogaya & Peñuelas (2006)
Leaf	Morphology and allometry	Leaf mass per unit area	Decreased	3	Ogaya & Peñuelas (2006)
Leaf	Morphology and allometry	Leaf thickness	Unchanged	3	Ogaya & Peñuelas (2006)
Several	Morphology and allometry	Ratio of leaf to sapwood area (shoot)	Decreased	6	Limousin, Longepierre, *et al.,* (2010)
Stem	Morphology and allometry	Lumen area	Increased	6	Limousin, Longepierre *et al.,* (2010)
Stem	Morphology and allometry	Vessel diameter	Unchanged	6	Limousin, Longepierre *et al.,* (2010)

Table 3. Continued.

Stem	Morphology and allometry	Vessel frequency	Unchanged	6	Limousin, Longepierre *et al.,* (2010)
Stem	Morphology and allometry	Wood density	Decreased	6	Limousin, Longepierre *et al.,* (2010)
Branch	Morphology and allometry	Branch litterfall	Unchanged	7	Limousin *et al.,* (2012)
Leaf	Morphology and allometry	Leaf area index	Unchanged	7	Limousin *et al.,* (2012)
Leaf	Morphology and allometry	Leaf litterfall	Decreased	7	Limousin *et al.,* (2012)
Several	Morphology and allometry	Ratio of leaf to sapwood area (shoot)	Decreased	7	Limousin *et al.,* (2012)
Branch	Morphology and allometry	Shoot length	Unchanged	7	Limousin *et al.,* (2012)
Several	Morphology and allometry	Ratio of leaf to sapwood area (shoot)	Decreased	9	Martin-StPaul *et al.,* (2013)
Leaf	Morphology and allometry	Leaf litterfall	Increased	15	Liu *et al.,* (2015)
Leaf	Water relations	Canopy conductance	Decreased	4	Limousin *et al.,* (2009)
Several	Water relations	Daily transpiration (tree level)	Decreased	4	Limousin *et al.,* (2009)
Leaf	Water relations	Midday leaf water potential	Unchanged	4	Limousin *et al.,* (2009)
Several	Water relations	Tree use of precipitation	Increased	4	Limousin *et al.,* (2009)
Several	Water relations	Whole-tree conductivity	Unchanged	4	Limousin *et al.,* (2009)
Stem	Water relations	Pressure causing 50% loss of conductivity	Unchanged	6	(Limousin, Misson, *et al.,* 2010)
Leaf	Water relations	Predawn water potential	Decreased	6	Limousin *et al.,* (2009)
Leaf	Water relations	Predawn water potential	Decreased	6	Limousin, Longepierre, *et al.,* (2010)
Stem	Water relations	Slope of the vulnerability curve	Unchanged	6	Limousin, Longepierre, *et al.,* (2010)
Stem	Water relations	Stem specific conductivity	Unchanged	6	Limousin, Longepierre, *et al.,* (2010)
Leaf	Water relations	Predawn water potential	Unchanged	7	Limousin *et al.,* (2012)
Leaf	Water relations	Leaf specific conductivity	Increased	9	Martin-StPaul *et al.,* (2013)
Stem	Water relations	Stem specific conductivity	Unchanged	9	Martin-StPaul *et al.,* (2013)
Roots	Water relations	Depth of soil water uptake	Decreased	12	Barbeta *et al.,* (2015)
Roots	Water relations	Midday leaf water potential	Unchanged	12	Barbeta *et al.,* (2015)
Roots	Water relations	Relative groundwater use	Decreased	12	Barbeta *et al.,* (2015)
Leaf	Water relations	Midday leaf water potential	Unchanged	14	Ogaya *et al.,* (2014)
Leaf	Physiology	Nitrogen per unit area	Unchanged	9	Martin-StPaul *et al.,* (2013)
Leaf	Physiology	Nitrogen per unit mass	Unchanged	9	Martin-StPaul *et al.,* (2013)
Seed	Phenology	Flowering	Unchanged	2	Ogaya and Peñuelas (2004)
Seed	Phenology	Fruiting	Unchanged	2	Ogaya and Peñuelas (2004)
Leaf	Phenology	Leaf flushing	Unchanged	2	Ogaya and Peñuelas (2004)
Leaf	Phenology	Leaf unfolding	Unchanged	2	Ogaya and Peñuelas (2004)
Leaf	Phenology	Budburst	Delayed	7	Limousin *et al.,* (2012)

Table 3. Continued.

Leaf	Phenology	Leaf lifespan	Increased	7	Limousin *et al.,* (2012)
Stem	Production	Stem growth	Decreased	2	Ogaya and Peñuelas (2003)
Stem	Production	Stem growth	Decreased	5	Ogaya and Peñuelas (2007)
Stem	Production	Stem mortality	Unchanged	5	Ogaya and Peñuelas (2007)
Seed	Production	Fruit litterfall	Decreased	7	Ogaya *et al.,* (2007)
Stem	Production	Stem growth	Unchanged	9	Martin-StPaul *et al.,* (2013)
Stem	Production	Stem growth	Unchanged	13	Barbeta *et al.,* (2013)
Stem	Production	Stem mortality	Increased	13	Barbeta *et al.,* (2013)
Several	Production	Increase in aboveground biomass	Unchanged	15	Liu *et al.,* (2015)
Seed	Production	Fruit litterfall	Decreased	15	Liu *et al.,* (2015)

The removal of ca. 30% of the throughfall reduced soil moisture by an average of 13% in the drought-treatment plots in Prades (Liu *et al.*, 2015). This reduction significantly increases water stress, which consistently reduced predawn leaf water potentials (Limousin *et al.*, 2009, 2010b, 2012), often a surrogate of soil water potential (Barbeta *et al.*, 2012). Midday leaf water potentials, though, did not generally differ between treatments (Limousin *et al.*, 2009; Ogaya *et al.*, 2014a; Barbeta *et al.*, 2015). As a short-term response to this increase in xylem tension produced by lower soil-water potentials, however, plants down-regulated their canopy conductance and daily transpiration rates (Limousin *et al.*, 2009). Water stress caused by lower amounts of soil moisture thus clearly entailed a decrease in the soil-plant-atmosphere water flow (transpiration), which could occur shortly after the treatment commenced (Limousin et al. 2009). This consistent decrease in total transpiration was not accompanied by significant modifications of foliar gas exchange, neither after 1-4 years of drought treatment (Limousin *et al.*, 2010b; Ogaya *et al.*, 2014a) nor after 14 years (Ogaya, Llusià, et al. 2014, Sperlich et al. unpublished results). The plants thus did not lower their rates of carbon assimilation and gas exchange per unit of leaf area in response to the drought treatment, that is, they exhibited physiological homeostasis (Martin-StPaul et al. 2013). It should be noted, however, that measurements of leaf-level gas exchange are instantaneous. The overall decrease in transpiration may not be detectable by gas-exchange analysers that measure at a scale of seconds. Daily (or a longer timescale) integrals of gas-exchange measurements might be able to identify differences between treatments, as do measurements of sap flow (Limousin et al. 2009). The concept of leaf-level physiological homeostasis has been challenged in a recent study by the measurement of mesophyllic conductance and daytime respiration (Sperlich et al., unpublished results). Sperlich and colleagues have provided evidence that mesophyllic conductance, in addition to stomatal conductance, can acclimate to drought conditions by facilitating the diffusion of CO_2 to

chloroplasts and that, in the long term, the droughted plants can increase the plasticity of their photosynthetic traits to take advantage of environmentally favourable periods.
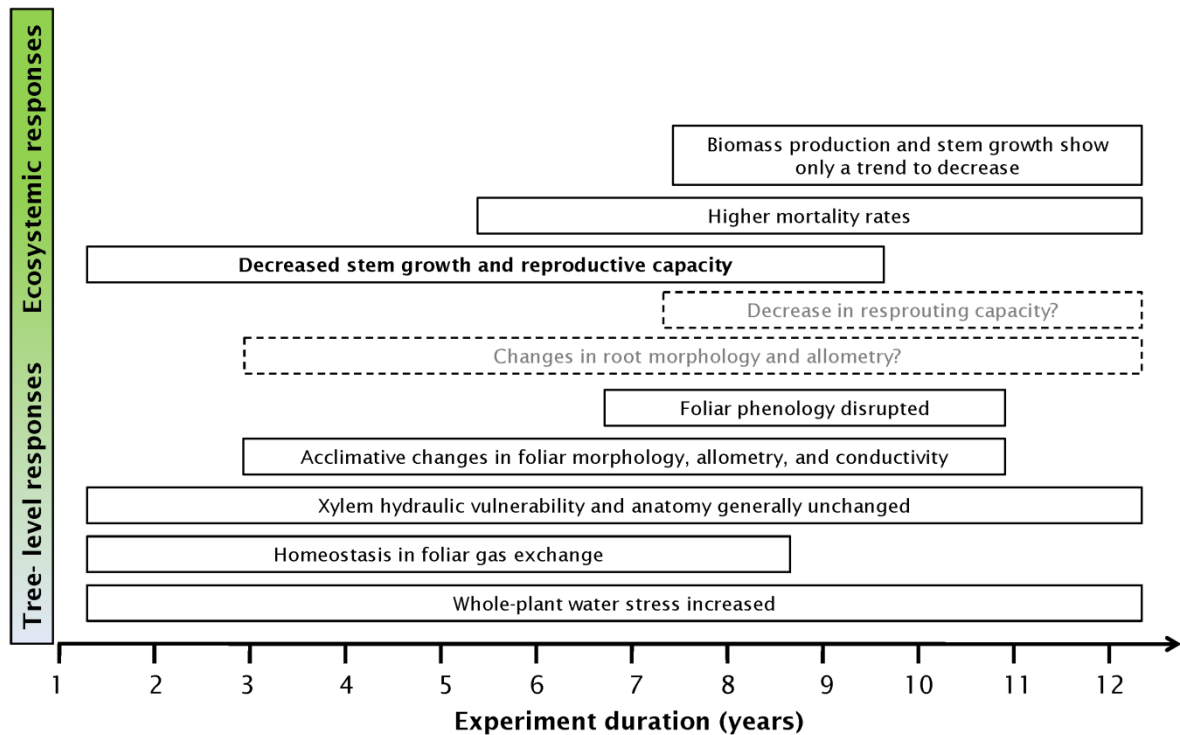

Figure 5. Conceptual scheme of the most relevant effects of the long-term drought experiments in *Quercus ilex* forests at the tree and ecosystemic levels. The span of the rectangles defines the duration of the effects, and the position on the x-axis represents the approximate time of appearance. Rectangles with dashed contour lines indicate potential effects that require further study.

The decrease in transpiration reported by Limousin et al. (2009) may seem inconsistent with the non-significantly lower stomatal conductance in plants in other drought treatments (Martin-StPaul et al. 2013, Ogaya, Llusià, et al. 2014), but the droughted plants at both the Prades and Puébachon experimental sites had reduced leaf areas after three (Ogaya and Peñuelas 2006) and seven (Limousin et al. 2012) years of treatment. This reduction was not accompanied by a reduction in sapwood area, so the ratio of leaf to sapwood area decreased at the shoot level (Limousin et al. 2012, Limousin et al. 2010), indicating that tree-level transpiration was reduced by decreasing the transpiring (leaf) area, which is also in agreement with the long-term increase in leaf litterfall in the drought treatment (Liu et al 2015). Indeed, leaf-specific conductivity is increased in the long term in droughted plants concomitant with a decrease in the ratio of leaf to sapwood area (Martin-StPaul et al. 2013). In parallel with the changes in leaf to shoot allometry, leaf area and hydraulic conductivity and stem anatomy and hydraulic properties changed little.

Root and branch xylem vulnerability and stem specific conductivity did not differ significantly in the trees at Puébachon after six years of the drought treatment (Limousin *et al.*, 2010a). Vessel diameter, frequency, and distribution also did not vary in the same study. Even though wood anatomy is not necessarily associated with xylem vulnerability, the larger picture indicates that the effects of mid- to long-term experimental drought are restricted to foliar anatomy, foliar hydraulic properties and shoot-to-leaves allometry.

The acclimation of tree-level transpiration (Limousin et al. 2009), leaf area (Ogaya and Peñuelas 2006), and leaf-to-shoot allometry (Limousin, Longepierre, et al. 2010) to drier conditions obviously entails a reduction in total photosynthetic area. Despite the lack of significant differences in rates of carbon assimilation per unit leaf area, the amount of carbon assimilated at the individual or stem level should be reduced. The amount of carbon allocated to growth decreased in Prades after two (Ogaya *et al.*, 2003) and five (Ogaya and Peñuelas 2007) years of treatment, indicated by significantly lower rates of stem growth in the drought treatments. Likewise, the allocation of carbon to reproduction was also reduced, indicated by a decrease in fruit litterfall (Ogaya & Peñuelas, 2007b). In contrast, stem growth was never affected in Puébachon after nine years of drought treatment. Indeed, a recent analysis of a longer data series (11 years) at Prades found that the differences in stem growth tended to dampen over time (Barbeta et al. 2013), which has been linked to the release of resources as a consequence of higher mortality rates in the drought plots (Barbeta et al. 2013, Lloret et al. 2012) and to epigenetic acclimation (Rico *et al.*, 2013). That is, the relatively larger decrease in stem density produced by a higher mortality in the drought plots led to lower competition for water resources. Furthermore, the physiological and morphological mechanisms of acclimation reported in the previous paragraphs may have provided to droughted plants a greater resistance to naturally occurring extreme droughts such as those in 2005-2006 and 2011 in Prades (Ogaya *et al.*, 2014b).

The responses to drought after a decade or more after the onset of the experiments differed slightly from those early in the experiments. *Q. ilex* is also able to display some mechanisms of acclimation to drought, which would be useful for its persistence under a future drier climate. Overall forest productivity tended to decrease (Liu et al 2015), with the forests reducing their biomass in accordance with the artificially imposed 30% reduction in throughfall. Less dense forests with sparser canopies would thrive under the projected 15% decrease in soil moisture in the Mediterranean Basin (IPCC, 2013), but as we described in section 3, the effects of extreme droughts are more severe than those of

moderate and continuous drought experiments. Because both an increase in extreme drought and generally lower water availability are likely to occur in the future, investigating the interaction between extreme drought and the acclimation mechanisms of *Q. ilex* identified by the long-term experiments is necessary. One could expect that a forest adapted to lower water availability would be more resistant to an extreme drought. More damaged trees after a drought event, however, stored less NSC than the control trees (Rosas et al. 2013) so would be less resilient after another drought that causes total canopy loss. Similarly, groundwater contributed less to transpiration in the trees in the control plot during the drought of 2011 (after 12 years of drought treatment), which indicated a relatively stronger depletion of groundwater reserves (Barbeta et al. 2015). These reserves respond to the water balance over longer periods, so the chronic water stress imposed by the experiment would prevent them from being ready and available to minimise the negative effects of drought.

CONCLUDING REMARKS

The central objective of this review was to shed light on the key factors that will determine potential changes in the distribution, structure, and function of holm oak forests in response to increasing droughts, either in the form of extreme events or as a sustained decrease in water availability. Using the temporal dimension as the conceptual framework, some of these factors have been described. Gaps in our knowledge that warrant further research, however, still exist. From the dichotomy shown in Table 1 between developmental time and duration of drought-adaptive traits or mechanisms, we can conclude that not all of these traits or mechanisms could be similarly relevant in the future. In particular, the capacity to modify the phenology of growth and carbon assimilation (Lempereur et al. 2015) is a promising compensatory strategy for this species. In this sense, the limits of plasticity in primary and secondary growth in relation to future drier and warmer conditions may be determinants for the persistence of some populations in their current structure and function. We need to determine if an increase in winter photosynthetic activity and an earlier spring budburst could offset the deleterious effects of a longer summer cessation of growth (Carnicer *et al.*, 2013a).

The results of field drought experiments have indicated that trees are able to adjust their tree- and stand-level leaf area to a lower availability of water. This adjustment increases their resistance to drought, but we still do not know if they are also more resilient. The depletion of NSC reserves in resprouting organs such as lignotubers

indicates that the capacity to resprout would be weakened, but we still have no direct evidence of this. We should thus not confound the apparent increase in drought resistance in long-term experiments with a potential higher resistance to extreme droughts associated with the current climatic change. Indeed, a drought in 2011 had a more negative impact on the drought plots (Barbeta et al 2015), and a recent global-scale study evaluating the legacy of extreme droughts on trees estimated that growth could be reduced for four years after the event (Anderegg *et al.*, 2015). We recommend that future research should address the combined effect of consecutive extreme droughts and drier average conditions on the structure and function of forests, with a special emphasis on their resilience after crown damage.

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REFERENCES

Abrantes J, Campelo F, García-González I, Nabais C (2013) Environmental control of vessel traits in Quercus ilex under Mediterranean climate: Relating xylem anatomy to function. Trees - Structure and Function, 27, 655–662.

Aguadé D, Poyatos R, Rosas T, Martínez-vilalta J (2015) Comparative Drought Responses of Quercus ilex L. and Pinus sylvestris L. in a Montane Forest Undergoing a Vegetation Shift. Forests, 6, 2505–2529.

Alla AQ, Camarero JJ, Maestro-Martínez M, Montserrat-Martí G (2012) Acorn production is linked to secondary growth but not to declining carbohydrate concentrations in current-year shoots of two oak species. Trees - Structure and Function, 26, 841–850.

Anderegg WRL (2015) Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. New Phytologist, 205, 1008–1014.

Anderegg WRL, Berry JA, Field CB (2012) Linking definitions, mechanisms, and modeling of drought-induced tree death. Trends in plant science, 17, 693–700.

Anderegg WRL, Schwalm C, Biondi F et al. (2015) Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models W. Science, 349.

Babita M, Maheswari M, Rao LM, Shanker AK, Rao DG (2010) Osmotic adjustment, drought tolerance and yield in castor (Ricinus communis L.) hybrids. Environmental and Experimental Botany, 69, 243–249.

Barbero M, Loisel R, Quézel P (1992) Biogeography, ecology and history of Mediterranean Quercus ilex ecosystems. Vegetatio.

Barbeta A, Ogaya R, Peñuelas J (2012) Comparative study of diurnal and nocturnal sap flow of Quercus ilex and Phillyrea latifolia in a Mediterranean holm oak forest in Prades (Catalonia , NE Spain). Trees, 26, 1651–1659.

Barbeta A, Ogaya R, Peñuelas J (2013) Dampening effects of long-term experimental drought on growth and mortality rates of a Holm oak forest. Global change biology, 19, 3133–44.

Barbeta A, Mejía-Chang M, Ogaya R, Voltas J, Dawson TE, Peñuelas J (2015) The combined effects of a long-term experimental drought and an extreme drought on the use of plant-water sources in a Mediterranean forest. Global Change Biology, 21, 1213–1225.

Bartlett MK, Scoffoni C, Sack L (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: A global meta-analysis. Ecology Letters, 15, 393–405.

Bucci SJ, Scholz FG, Campanello PI et al. (2012) Hydraulic differences along the water transport system of South American Nothofagus species: Do leaves protect the stem functionality? Tree Physiology, 32, 880–893.

Camarero J, Franquesa M, Sangüesa-Barreda G (2015) Timing of Drought Triggers Distinct Growth Responses in Holm Oak: Implications to Predict Warming-Induced Forest Defoliation and Growth Decline. Forests, 6, 1576–1597.

Campelo F, Nabais C, Gutiérrez E, Freitas H, García-González I (2010) Vessel features of Quercus ilex L. growing under Mediterranean climate have a better climatic signal than tree-ring width. Trees - Structure and Function, 24, 463–470.

Canadell J, Jackson RB, Ehleringer JB, Mooney HA, Sala OE, Schulze ED (1996) Maximum rooting depth of vegetation types at the global scale. Oecologia, 108, 583–595.

Canadell J, Djema A, López B, Lloret F, Sabate S, Siscart D, Gracia CA (1999) Structure and dynamics of the root system. In: Ecology of Mediterranean Evergreen Oak Forests (eds Rodà F, Retana J, Gracia CA, Bellot J), pp. 47–59. Springer Berlin / Heidelberg.

Carnicer J, Coll M, Ninyerola M, Pons X, Sánchez G, Peñuelas J (2011) Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proceedings of the National Academy of Sciences of the United States of America, 108, 1474–1478.

Carnicer J, Barbeta A, Sperlich D, Coll M, Peñuelas J (2013) Contrasting trait syndromes in angiosperms and conifers are associated with different responses of tree growth to temperature on a large scale. Frontiers in plant science, 4, 409.

Carnicer J, Coll M, Pons X, Ninyerola M, Vayreda J, Peñuelas J (2014) Large-scale recruitment limitation in Mediterranean pines: The role of Quercus ilex and forest successional advance as key regional drivers. Global Ecology and Biogeography, 23, 371–384.

Cavender-Bares J, Cortes P, Rambal S, Joffre R, Miles B, Rocheteau a (2005) Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: a comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. New Phytologist, 168, 597–612.

Choat B, Jansen S, Brodribb TJ et al. (2012) Global convergence in the vulnerability of forests to drought. Nature, 491, 752–755.

Comas C, Castillo J, Voltas J, Ferrio JP (2015) Point processes statistics of stable isotopes: analysing water uptake patterns in a mixed stand of Aleppo pine and Holm oak. Forest Systems, 24, 1–13.

Corcuera L, Camarero JJ, Gil-Pelegrín E (2004) Effects of a severe drought on Quercus ilex radial growth and xylem anatomy. Trees - Structure and Function, 18, 83–92.

David TS, Ferreira MI, Cohen S, Pereira JS, David JS (2004) Constraints on transpiration from an evergreen oak tree in southern Portugal. Agricultural and Forest Meteorology, 122, 193–205.

Delzon S, Urli M, Samalens JC et al. (2013) Field evidence of colonisation by holm oak, at the Northern Margin of its distribution range, during the anthropocene period. PLoS ONE, 8, 1–8.

Deslauriers A, Rossi S, Anfodillo T, Saracino A (2008) Cambial phenology, wood formation and temperature thresholds in two contrasting years at high altitude in southern Italy. Tree physiology, 28, 863–871.

Espelta J, Sabaté S, Retana J (1999) Resprouting dynamics. In: Ecology of Mediterranean Evergreen Oak Forests, Vol. 454, pp. 61–73. Springer, Berlin.

Galiano L, Martínez-Vilalta J, Sabaté S, Lloret F (2012) Determinants of drought effects on crown condition and their relationship with depletion of carbon reserves in a Mediterranean holm oak forest. Tree Physiology, 32, 478–489.

Gratani L, Meneghini M, Pesoli P, Crescente MF (2003) Structural and functional plasticity of Quercus ilex seedlings of different provenances in Italy. Trees - Structure and Function, 17, 515–521.

Gutiérrez E, Campelo F, Camarero J, Ribas M, Muntán E, Nabais C, Freitas H (2011) Climate controls act at different scales on the seasonal pattern of Quercus ilex L. stem radial increments in NE Spain. Trees Structure and Function, 25, 637–646.

IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (eds Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A,

Xia Y, V. B, Midgley P.). Cambridge University Press, Cambridge, UK. New York, NY, USA.

Jentsch A, Kreyling J, Beierkuhnlein C (2007) A new generation of climate change experiments: events, not trends. Frontiers in Ecology and the Environment, 5, 315–324.

Lempereur M, Martin-stpaul NK, Damesin C, Joffre R, Ourcival J, Rocheteau A, Rambal S (2015) Growth duration is a better predictor of stem increment than carbon supply in a Mediterranean oak forest: implications for assessing forest productivity under climate change. 579–590.

Leuzinger S, Luo Y, Beier C, Dieleman W, Vicca S, Körner C (2011) Do global change experiments overestimate impacts on terrestrial ecosystems? Trends in ecology & evolution, 26, 236–41.

Limousin J-M, Rambal S, Ourcival J-M, Rocheteau A, Joffre R, Rodríguez-Cortina R (2009) Long-term transpiration change with rainfall decline in a Mediterranean Quercus ilex forest. Global Change Biology, 15, 2163–2175.

Limousin J, Misson L, Lavoir A, Martin NK (2010a) Do photosynthetic limitations of evergreen Quercus ilex leaves change with long-term increased drought severity? 863–875.

Limousin J, Longepierre D, Huc R, Rambal S (2010b) Change in hydraulic traits of Mediterranean Quercus ilex subjected to long-term throughfall exclusion. 1026–1036.

Limousin J-M, Rambal S, Ourcival J-M et al. (2012) Morphological and phenological shoot plasticity in a Mediterranean evergreen oak facing long-term increased drought. Oecologia, 169, 565–77.

Liu D, Ogaya R, Barbeta A, Yang X, Peñuelas J (2015) Contrasting impacts of continuous moderate drought and episodic severe droughts on the aboveground-biomass increment and litterfall of three coexisting Mediterranean woody species. Global Change Biology, DOI: 10.1111/gcb.13029.

Lloret F, Siscart D, Dalmases C (2004) Canopy recovery after drought dieback in holm-oak Mediterranean forests of Catalonia (NE Spain). Global Change Biology, 10, 2092–2099.

Lloret F, Escudero A, Iriondo JM, Martínez-Vilalta J, Valladares F (2012) Extreme climatic events and vegetation: the role of stabilizing processes. Global Change Biology, 18, 797–805.

López BC, Gracia C a., Sabaté S, Keenan T (2009) Assessing the resilience of Mediterranean holm oaks to disturbances using selective thinning. Acta Oecologica, 35, 849–854.

Martínez-Vilalta J, Prat E, Oliveras I, Piñol J (2002) Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. Oecologia, 133, 19–29.

Martínez-Vilalta J, Mangirón M, Ogaya R, Sauret M, Serrano L, Peñuelas J, Piñol J (2003) Sap flow of three co-occurring Mediterranean woody species under varying atmospheric and soil water conditions. Tree physiology, 23, 747–58.

Martínez-Vilalta J, Poyatos R, Aguadé D, Retana J, Mencuccini M (2014) A new look at water transport regulation in plants. New Phytologist, 204, 105–115.

Martin-Stpaul NK, Limousin J-M, Vogt-Schilb H, Rodríguez-Calcerrada J, Rambal S, Longepierre D, Misson L (2013) The temporal response to drought in a Mediterranean evergreen tree: comparing a regional precipitation gradient and a throughfall exclusion experiment. Global Change Biology, 19, 2413–26.

Matesanz S, Valladares F (2013) Ecological and evolutionary responses of Mediterranean plants to global change. Environmental and Experimental Botany.

Mcadam S a M, Brodribb TJ (2012) Stomatal innovation and the rise of seed plants. Ecology Letters, 15, 1–8.

Meinzer FC, McCulloh K a (2013) Xylem recovery from drought-induced embolism: where is the hydraulic point of no return? Tree physiology, 33, 331–4.

Montserrat-Martí G, Camarero JJ, Palacio S, Pérez-Rontomé C, Milla R, Albuixech J, Maestro M (2009) Summer-drought constrains the phenology and growth of two coexisting Mediterranean oaks with contrasting leaf habit: Implications for their persistence and reproduction. Trees - Structure and Function, 23, 787–799.

Nardini A, Lo Gullo M a, Salleo S (2011) Refilling embolized xylem conduits: is it a matter of phloem unloading? Plant science: an international journal of experimental plant biology, 180, 604–11.

Niinemets Ü (2015) Is there a species spectrum within the world-wide leaf economics spectrum? Major variations in leaf functional traits in the Mediterranean sclerophyll Quercus ilex. New Phytologist, 205, 79–96.

Ogaya R, Peñuelas J (2003) Comparative seasonal gas exchange and chlorophyll fluorescence of two dominant woody species in a Holm Oak Forest. 08193, 132–141.

Ogaya R, Peñuelas J (2006) Contrasting foliar responses to drought in Quercus ilex and Phillyrea latifolia. Biologia Plantarum, 50, 373–382.

Ogaya R, Peñuelas J (2007) Species-specific drought effects on fl ower and fruit production in a Mediterranean holm oak forest. 80.

Ogaya R, Peñuelas J, Martínez-Vilalta J, Mangirón M (2003) Effect of drought on diameter increment of Quercus ilex , Phillyrea latifolia , and Arbutus unedo in a holm oak forest of NE Spain. Forest Ecology and Management, 180, 175–184.

Ogaya R, Llusià J, Barbeta A, Asensio D, Liu D, Alessio GA, Peñuelas J (2014a) Foliar CO2 in a holm oak forest subjected to 15 years of climate change simulation. Plant Science, 226, 101–107.

Ogaya R, Barbeta A, Başnou C, Peñuelas J (2014b) Satellite data as indicators of tree biomass growth and forest dieback in a Mediterranean holm oak forest. Annals of Forest Science, 135–144.

Pasho E, Camarero JJ, de Luis M, Vicente-Serrano SM (2011) Impacts of drought at different time scales on forest growth across a wide climatic gradient in north-eastern Spain. Agricultural and Forest Meteorology, 151, 1800–1811.

Patakas a., Nikolaou N, Zioziou E, Radoglou K, Noitsakis B (2002) The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. Plant Science, 163, 361–367.

Peguero-Pina JJ, Sancho-Knapik D, Barrón E, Camarero JJ, Vilagrosa A, Gil-Pelegrín E (2014) Morphological and physiological divergences within Quercus ilex support the existence of different ecotypes depending on climatic dryness. Annals of Botany, 114, 301–313.

Peñuelas J, Filella I, Lloret F, Piñol J, Siscart D (2000) Effects of a severe drought on water and nitrogen use by Quercus ilex and Phillyrea latifolia. Biologia Plantarum, 43, 47–53.

Piñol J, Lledó MJ, Escarré a. (1991) Hydrological balance of two Mediterranean forested catchments (Prades, northeast Spain). Hydrological Sciences Journal, 36, 95–107.

Pivovaroff AL, Sack L, Santiago LS (2014) Coordination of stem and leaf hydraulic conductance in southern California shrubs: A test of the hydraulic segmentation hypothesis. New Phytologist, 203, 842–850.

Poyatos R, Aguadé D, Galiano L, Mencuccini M, Martínez-Vilalta J (2013) Drought-induced defoliation and long periods of near-zero gas exchange play a key role in accentuating metabolic decline of Scots pine. New Phytologist, 200, 388–401.

Rico L, Ogaya R, Barbeta A, Peñuelas J (2013) Changes in DNA methylation fingerprint of Quercus ilex trees in response to experimental field drought simulating projected climate change. Plant biology.

Rivas-Ubach A, Gargallo-Garriga A, Sardans J et al. (2014) Drought enhances folivory by shifting foliar metabolomes in Quercus ilex trees. New Phytologist, 202, 874–885.

Rosas T, Galiano L, Ogaya R, Peñuelas J, Martínez-Vilalta J (2013) Dynamics of non-structural carbohydrates in three Mediterranean woody species following long-term experimental drought. Frontiers in Plant Science, 4, 1–16.

Sala A, Woodruff DR, Meinzer FC (2012) Carbon dynamics in trees: feast or famine? Tree Physiology, 32, 1–12.

Sánchez-Costa E, Poyatos R, Sabaté S (2015) Contrasting growth and water use strategies in four co-occurring Mediterranean tree species revealed by concurrent measurements of sap flow and stem diameter variations. Agricultural and Forest Meteorology, 207, 24–37.

Smith MD (2011) An ecological perspective on extreme climatic events: A synthetic definition and framework to guide future research. Journal of Ecology, 99, 656–663.

Terradas J (1999) Holm Oak and Holm Oak Forests: An Introduction. In: Ecology of Mediterranean Evergreen Oak Forests (eds Rodà F, Retana J, Gracia C, Bellot J), pp. 3–14. Springer Berlin / Heidelberg, Berlin.

Vicente-Serrano SM, Beguería S, López-Moreno JI (2010) A multiscalar drought index sensitive to global warming: The standardized precipitation evapotranspiration index. Journal of Climate, 23, 1696–1718.

Vicente-Serrano SM, Gouveia C, Camarero JJ et al. (2013) Response of vegetation to drought time-scales across global land biomes. Proceedings of the National Academy of Sciences of the United States of America, 110, 52–7.

Villar-Salvador P, Castro-Díez P, Pérez-Rontomé C, Montserrat-Martí G (1997) Stem xylem features in three Quercus (Fagaceae) species along a climatic gradient in NE Spain. Trees, 12, 90–96.

Van der Werf GW, Sass-Klaassen UGW, Mohren GMJ (2007) The impact of the 2003 summer drought on the intra-annual growth pattern of beech (Fagus sylvatica L.) and oak (Quercus robur L.) on a dry site in the Netherlands. Dendrochronologia, 25, 103–112.

Wolkovich EM, Cook BI, McLauchlan KK, Davies TJ (2014) Temporal ecology in the Anthropocene. Ecology letters, 17, 1365–1379.

Zeppel MJB, Harrison SP, Adams HD et al. (2015) Drought and resprouting plants. New Phytologist, 206, 583–589.

Relative contribution of groundwater to plant transpiration estimated with stable isotopes

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ABSTRACT

Water stored underground in the saturated zone is an important source of water for plants in water-limited ecosystems. The presence of deep-rooted plants worldwide, however, suggests that the use of groundwater is not restricted to arid and seasonally dry ecosystems. Few modeling and experimental studies, though, include the use of groundwater as a factor. We compiled the available data (98 species) on the relative contribution of groundwater to plant water estimated using stable isotopes and isotope mixing models, which provided information about relative groundwater use, and analyzed their variation across landscape positions, climates, seasons, and plant types. On average, groundwater contributed more than half of the plant water during dry seasons and more than a third during wet seasons. Landscape position, local climate, and their interaction explained 41% of the variance in groundwater uptake. Plants in riparian ecosystems used the highest proportions of groundwater (76% in dry seasons and 44% in wet seasons), and plants in arid ecosystems had the highest use in dry seasons (63%). Groundwater use, however, averaged at least a third of xylem water regardless of landscape position and climate. Our results highlight that the large imbalance between the biomass of deep roots and their contribution to total plant water should be included in models of ecosystemic water budgets. The consistently high percentage of groundwater uptake may have an adaptive significance for perennial plants, which are sessile organisms that must cope with climatic extremes throughout their long lives.

KEYWORDS

Groundwater, water uptake, deep roots, landscape position, stable isotopes, mixing models, drought.

INTRODUCTION

Aquifers influence many terrestrial ecosystems (Fan *et al.*, 2013), and many other ecosystems depend partly on water-saturated fractures in bedrock (Schwinning, 2010; Thomas, 2014). These water pools stored underground in the saturated zone (i.e. groundwater (GW) (Eamus *et al.*, 2015)) are accessed by the deep roots of plants and ultimately transpired to the atmosphere through foliar stomata, sometimes increasing atmospheric humidity and modifying regional climate (Lee *et al.*, 2007). The interactions between the performance of the vegetation and the level of the water table at the watershed scale can modify the contribution of GW to streamflow (Bearup *et al.*, 2014). The fluctuations of water-table levels or the quantity of water trapped within fractured bedrock have different spatiotemporal dynamics than soil moisture but may have a comparable importance for ecosystemic water budgets and tree growth (Sarris *et al.*, 2007). Indeed, the contribution of GW to plant transpiration may be critical in ecosystems with transient or chronic water deficits (Canadell *et al.*, 1996). Few studies of plant-water relations, however, have directly measured the uptake of GW by plants. This neglect has been attributed to technological and economic limitations and to the widespread assumption that deep roots are a secondary component of plants (Maeght *et al.*, 2013).

Water is generally taken up by roots (with remarkable exceptions (Goldsmith, 2013)), so root structure and function should play a central role in research of plant-water relations. A few efforts in the last two decades have compiled and synthesized global-scale data on the maximum rooting depth (Canadell *et al.*, 1996) and the root distribution along soil profiles and across climates and plant types (Schenk & Jackson, 2002) and have modeled the probability of deep rooting globally (Schenk & Jackson, 2005). Arid and seasonally dry ecosystems contain the deepest root systems (Canadell *et al.*, 1996) and some species grow roots to depths of more than 4m, even in temperate and tropical ecosystems (Schenk & Jackson, 2005). Plants around the world (perhaps with the exception of boreal and polar regions) thus grow deep roots. All likely use deep water reserves to some extent, a capacity that will confer a competitive advantage in regions where an increase in drought frequency, intensity, and/or duration is projected (IPCC, 2013). Deep rooting also facilitates the coexistence of species by segregating their hydraulic niches, even in ecosystems where water is not limiting (Peñuelas *et al.*, 2011; Silvertown *et al.*, 2015). The existing literature reporting the use of GW pools by roots is extensive, but we still lack a quantitative assessment of the relative contribution of those pools at a broad geographical scale.

Stable isotopes of water ($\delta^{18}O$ and $\delta^{2}H$) have been used since the 1980s as tracers for the movement of underground water. These isotopes initially appeared able to successfully identify the source of transpired water by a simultaneous comparison of the isotopic compositions of both xylem and source water (Dawson & Ehleringer, 1991). Sources from which plants take up water (soil water at different depths, fog, dew, or GW) usually have different isotopic compositions because of fractionation (Dawson & Pate, 1996) and the rainout effect (Brooks *et al.*, 2009). The uptake of water by roots generally involves little fractionation (but see (Ellsworth & Williams, 2007) for an exception), so the source of xylem water can often be identified from among pools with contrasting isotopic signatures (Dawson & Ehleringer, 1991). This information is only qualitative, and plants commonly use water from two or more sources simultaneously, so that the isotopic signature of the xylem water is mixed. For such cases, end-member mixing models using one or both water isotopes (Thorburn *et al.*, 1993) have been applied to determine the proportional use of each water source. These models, however, could only discern among *n* water sources and required a number of isotopic compositions of *n*+1 (Dawson *et al.*, 2002). Mixing models improved substantially in the early 2000s, culminating in the development of *IsoSource* by Phillips and Gregg (2003) (Phillips & Gregg, 2003), a simple program that has subsequently been widely used. Recently, new isotope mixing models have been developed (SIAR (Parnell *et al.*, 2010) and MixSIR (Moore & Semmens, 2008)) within a Bayesian framework, which provide statistical uncertainties associated with the estimates of source contribution.

A recent multi-site analysis on the isotopic compositions of precipitation, soil and plant xylem, groundwater and streamwater have provided evidence for the ubiquity of the ecohydrologic separation of groundwater and plant xylem water (Evaristo *et al.*, 2015). Plants seem to take up water preferentially from an isotopically distinct water pool than the water that is delivered to aquifers and streams (Brooks *et al.*, 2009). This widespread ecohydrological separation, though, have significant exceptions (20% of the cases studied by Evaristo and colleagues (Evaristo *et al.*, 2015)). Also, it remains unclear whether the separation is (i) physical; plants take up water from tightly bound water in soil micropores while mobile water in macropores contribute to groundwater and streamwater recharge or (ii) temporal; groundwater recharge occurs at a different time than plant water uptake (Bowen, 2015). A better understanding of the ecosystems where the connection between groundwater pools and plant transpiration exists would help elucidate some of the questions arisen from these recent findings.

The availability of GW by plants enhances foliar and canopy-level gas exchange, alleviates xylem tension, and increases overall plant (growth rates and canopy health) and ecosystemic (leaf area index, mortality rates, and plant density)(Eamus *et al.*, 2015 (4) and references there in) performance. Moreover, past climatic changes that decreased GW levels induced the aridification of large areas (Yang *et al.*, 2015). In the face of current climate change, a more comprehensive understanding of the relationship between GW and vegetation is therefore crucial. We have thus quantified the relative contributions of GW pools to plant transpiration by collecting data from studies (Table 1) that applied stable-isotope techniques and isotope mixing models to calculate water-source contributions. We targeted studies evaluating the contributions of GW and sampled the broadest geographical scale possible. By means of a quantitative synthesis, we (i) compared GW use between dry and wet seasons and among contrasting landscape positions, climates, and plant anatomical types, and (ii) assessed the relative contributions of these factors to the variation in GW use.

MATERIAL AND METHODS

We searched the literature in Google Scholar and the Web of Science for the keywords "water uptake", "isotopes", and "mixing model" (last updated 15 June 2015). We assessed all returned studies and selected those that fit the following criteria: (i) used stable isotopes to study plant water sources, (ii) estimated the relative contribution of GW (as defined in (Eamus *et al.*, 2015)) to xylem water using mixing models, and (iii) provided estimates of proportional GW uptake at the species level. We excluded studies that did not include or explicitly quantify GW as a potential source, so we only included studies that directly tested the possibility of roots reaching the GW. Forty-two studies satisfied the selection criteria (Table 1), providing data from 50 sites. Ninety-eight species were represented, including herbaceous plants (*N*=8), shrubs (*N*=26), and trees (*N*=65)(one species had both shrub and tree forms). Data were extracted directly from the tables when possible; we used PlotDigitizer 2.6.6 to extract data from figures. The contribution of GW to xylem water of each species from each field campaign of each study was recorded as a single data point (*N*=623). Each data point was then complemented with categorical information for campaign date, rainfall season (dry or wet), and astronomic season (winter, spring, summer, or autumn). We also defined four categories of landscape position: (i) riparian, plants close to a stream, pond, or oasis; (ii) dunes, plants on sandy dunes either in coastal areas or deserts; (iii) plains, plants in predominantly flat and basal areas but not close to a stream; and (iv) slopes, plants on steep terrain such as hill slopes,

hilltops, or mountain plateaus. The climate at each site was characterized following the Köppen-Geiger climatic classification (Peel *et al.*, 2007). From the main climatic groups defined by this classification, 16 study sites were in arid, four in tropical, 22 in temperate, and eight in cold climates. The dataset also included continuous climatic variables from WorldClim 1.4 (Hijmans *et al.*, 2005). We download 19 bioclimatic variables using the raster package in R (R Core Development Team, 2012). These variables were derived from the monthly temperatures and rainfall. Finally, we recorded the depth to the GW source, the type of saturated zone (rocky or non-rocky), the isotopic approach (single or dual-isotope approach) and the availability of water within the unsaturated zone (categorically).

Table 1. Biomes, species, and sites studied.

Climate	Site	Species	Reference
Arid	Gurbantonggut Desert, Xinjiang, China	*Haloxylon ammodendron, Haloxylon persicum*	Dai *et al.*, 2014
Arid	Mt. Barker, Western Australia, Australia	*Banksia grandis, Eucalyptus camaldulensis, Eucalyptus globulus*	Dawson & Pate, 1996
Arid	Amargosa Valley, Nevada, USA	*Grindelia fraxino-pratensis, Nitrophila mohavensis*	Hasselquist & Allen, 2009
Arid	Oak Creek Canyon, Arizona, USA	*Acer negundo*	Kolb *et al.*, 1997
Arid	Shenmu, North Shaanxi, China	*Artemisia desertorum, Salix psammophila, Caragana korshinskii, Pinus tabuliformis, Populus simonii*	Li *et al.*, 2012
Arid	Ejin Banner, Inner Mongolia, China	*Populus euphratica*	Liu *et al.*, 2015
Arid	Gobabeb, Erongo, Namibia	*Acacia erioloba, Faidherbia albida, Tamarix usneoides*	Schachtschneider & February, 2010
Arid	Ejina, Inner Mongolia, China	*Populus euphratica*	Si *et al.*, 2014
Arid	San Pedro River, Arizona, USA	*Populus fremontii, Prosopis velutina, Salix goodingii*	Snyder & Williams, 2000
Arid	San Pedro River, Arizona, USA	*Prosopis velutina*	Snyder & Williams, 2003
Arid	Chowilla Island, South Australia, Australia	*Eucalyptus camaldulensis, Eucalyptus largiflorens*	Thorburn *et al.*, 1993
Arid	Fukang, Xinjiang, China	*Tamarix ramosissima, Nitraria tangutorum, Reaumuria songarica*	Wu *et al.*, 2013
Cold	Rocky Mountain NP, Colorado, USA	*Salix monticola, Carex* spp.	Alstad *et al.*, 1999
Cold	Pfyn forest, Wallis canton, Switzerland	*Populus nigra, Salix alba, Prunus avium, Alnus glutinosa, Pinus sylvestris*	Bertrand *et al.*, 2014
Cold	Gonghe desert, Tibet, Qinghai, China	*Caragana intermedia*	Jia *et al.*, 2012
Cold	Yellowstone, Wyoming, USA	*Salix geyeriana*	Johnston *et al.*, 2011
Cold	Kerquin, Inner Mongolia, China	*Pinus sylvestris*	Song *et al.*, 2014
Cold	Naiman, Inner Mongolia, China	*Pinus sylvestris*	Yafen *et al.*, 2012
Temperate	Prades, Catalonia, Spain	*Arbutus unedo, Phillyrea latifolia, Quercusilex*	Barbeta *et al.*, 2015
Temperate	Mt. Mitchell, North Carolina, USA	*Abies fraseri, Picea rubens*	Berry *et al.*, 2014
Temperate	Pioneer Valley, Queensland, Australia	*Corymbia clarksoniana, Lophostemon suaveolens, Eucalyptus platyphylla, Melaleuca viridiflora*	Cook and O'Grady 2006
Temperate	Kingaroy, Boonah and Bell, Queensland, Australia	*Casuarina glauca, Eucalyptus camaldulensis*	Cramer *et al.* 1999,
Temperate	Guling, Guangxi, China	*Cyclobalanopsis glauca*	Deng*et al.,* 2014
Temperate	Yalgorup, South Australia, Australia	*Eucalyptus gomphocephala*	Drake *et al.,*2011

Table 1. Continued.

Temperate	Kyabram, Victoria, Australia	*Eucalyptus camaldulensis, Eucalyptus grandis*	Feikema *et al.,* 2010
Temperate	Gantang, Guangxi, China	*Cyclobalanopsis glauca*	Gu *et al.,* 2014
Temperate	Taklimakan, Xinjiang, China	*Populus euphratica*	Hao *et al.,* 2013
Temperate	Western Australia, Australia	*Chamaecytisus proliferus*	Lefroy *et al.,* 2001
Temperate	Menglun, Xishuangbanna, China	*Pometia tomentosa, Gironniera subaequalis*	Liu *et al.,* 2010
Temperate	Xishuangbanna, Menglun, China	*Celtis wightii, Cleistanthus sumatranus, Lasio coccacomberi*	Liu *et al.,* 2014
Temperate	Carriço, Pombal, Portugal	*Acacia longifolia, Corema album, Myrica faya, Pinus pinaster, Salix repens*	Máguas *et al.,* 2011
Temperate	Huanjiang, Guangxi, China	*Alchornea trewioides, Radermachera sinica, Stercolia euosma*	Nie *et al.,* 2010
Temperate	Huanjiang, Guangxi, China	*Alchornea trewioides, Radermachera sinica*	Nie *et al.,* 2012
Temperate	Western Australia, Australia	*Banksia prionotes*	Pate and Dawson 1999
Temperate	Eyre Peninsula, South Australia, Australia	*Allocasuarina verticillata, Eucalyptus diversifolia*	Swaffer *et al.,* 2014
Temperate	Moreton Bay, Queensland, Australia	*Avicennia marina, Casuarina glauca, Melaleuca quinquinervia*	Wei *et al.,* 2013
Temperate	Wolong, Sichuan, China	*Abies faxoniana, Bashania fangiana, Betula utilis*	Xu *et al.,* 2013
Temperate	Swan, Western Australia, Australia	*Hibbertia hypericoides, Banksia attenuata, Banksia ilicifolia*	Zencich *et al.* 2002
Tropical	Merida, Mexico	*Gymnopodium floribundum, Piscidia piscipula*	Estrada-Merino *et al.,* 2013
Tropical	San Salvador Island, Bahamas	*Ipomoea pes-caprae, Scaevola plumieri, Sesuvium portulacastrum,Caesalpinia bondoc, Casasia clusifolia, Coccoloba uvifera, Iva imbricata, Lantana involcrata, Strumphia* sp.	Greaver and Sternberg 2010
Tropical	Yucatán, Mexico	*Brosimum alicastrum, Cordia dodecandra, Enterolobium cyclocarpum, Ficus cotinifolia, Spondias purpurea, Talisia olivaeformis*	Querejeta *et al.,* 2007
Tropical	Everglades, Florida, USA	*Chromolaena frustrata, Conocarpus erectus, Piscidia piscipula, Capparis flexuosa, Eugenia foetida, Batis maritima*	Saha *et al.,* 2015

Statistical analyses

We fitted a general linear model (GLM) with the relative contribution of GW uptake as the dependent variable and with bioclimatic continuous variables, landscape position, season, plant type, the depth to GW, the groundwater type and the number of isotope used as independent predictors. Interactions among the predictors were included in an initially saturated model. We selected the best model (the simplest model that maximized the overall fitting) for our variables using the function *stepAIC* of the R MASS package. The function *calc.relimp* of the R relaimpo package calculated the relative contribution of each independent variable to the variance of of GW uptake. We had identified landscape position, climate, and plant type as significant factors, so we then comparatively tested the levels of these factors to better understand the variability of the relative GW uptake. We treated the relative GW uptake of dry and wet seasons separately, because water-isotope mixing models provide only relative values, and the likely different transpired amounts could lead to a global overestimation of GW uptake when averaging among seasons. All data points from sites with a fully humid climate were assigned to the wet season, and all data points from desert sampling sites lacking any rainy season were assigned to the dry season. Differences among the groups of landscape position and plant type where interpreted from the output of the GLM and the differences among climate types were assessed with ANOVA and TukeyHSD post-hoc tests. Isotopic methodological controls on synthesized data are shown in Fig. S1 and in the Supporting Information. All analyses were performed with R.

RESULTS

The general linear model containing all landscape, climatic, and plant-type predictor variables explained 41.4% of the variance in relative GW uptake (R^2=0.41, F=6.83, P<0.001)(Fig. 1, Table S1). The interaction between landscape position and climate and the depth to GW accounted for 21.0% of the total variance. Landscape position and topography were also significant variables and accounted for 12.3% of the variance. Climatic variables accounted for 7.1% of the total variance, with significant effects of the precipitation (wet or dry) season, the annual precipitation, the precipitation of the wettest quarter and the precipitation of the warmest quarter. The interaction between climate and landscape position showed contrasting effects of climate, depending on the landscape position (Table 1). Finally, plant type explained less than 2% of the variance in relative GW uptake, but trees had a higher contribution of GW than shrubs and herbaceous species.
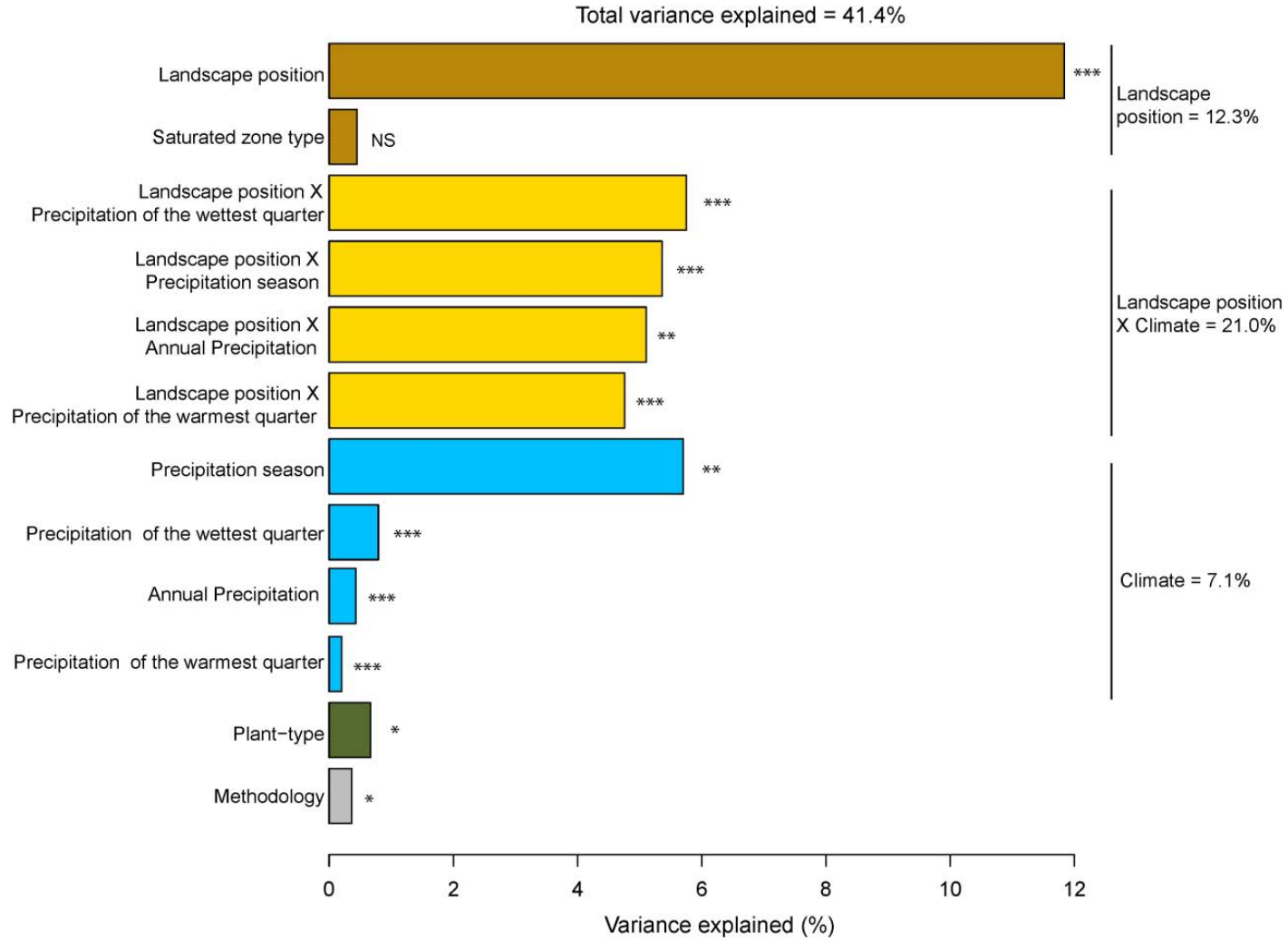
Figure 1. Variance in the relative GW uptake explained by each factor, grouped into three categories: landscape position (brown bars), climate (blue bars), and plant type (green bars). The proportional explained variances were calculated from the general linear model (Table S1) using the *relaimpo* package in R.

The landscape position in which a plant is located partly explained their use of GW (Fig. 1). Among the four defined groups, plants from riparian ecosystems contained a significantly higher proportion of GW in their xylems (Table S1, Fig. 2). This proportion was especially higher during dry seasons, when the average GW use reached 76%. Plants from dunes, plains, and slopes used significantly different proportions of GW depending on the climate (Table 2, Table S1), but all had an overall average of ca. 40%. Plants on riparian ecosystems, plains and slopes, however, increased their GW use in dry seasons, whereas plants in dune systems did not. The use of GW correlated with site climatic characteristics but was not significantly different between Köppen-Geiger climatic classes (Fig. 3). However, plants in arid climates had the highest proportions of transpired GW, and plants in cold and temperate climates had the lowest (Fig. 2). The seasonality of the relative GW uptake was significant for plants in temperate and arid climates, implying that the plants extracted relatively more water from GW sources during dry seasons.
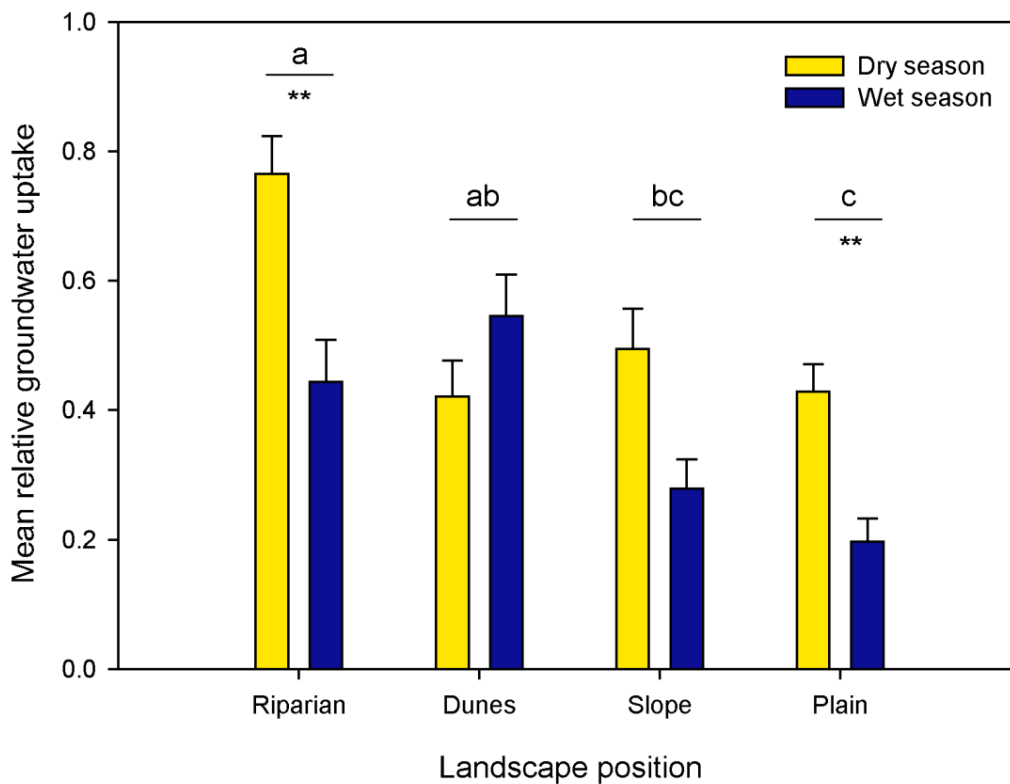

Figure 2. Relative GW uptake and landscape positions in dry and wet seasons. Significant differences between landscape positions are marked with different letters (ANOVA with Tukey HSD post-hoc tests). Significant differences between dry and wet seasons are marked with one ($P<0.05$), two ($P<0.01$), or three ($P<0.001$) asterisks.
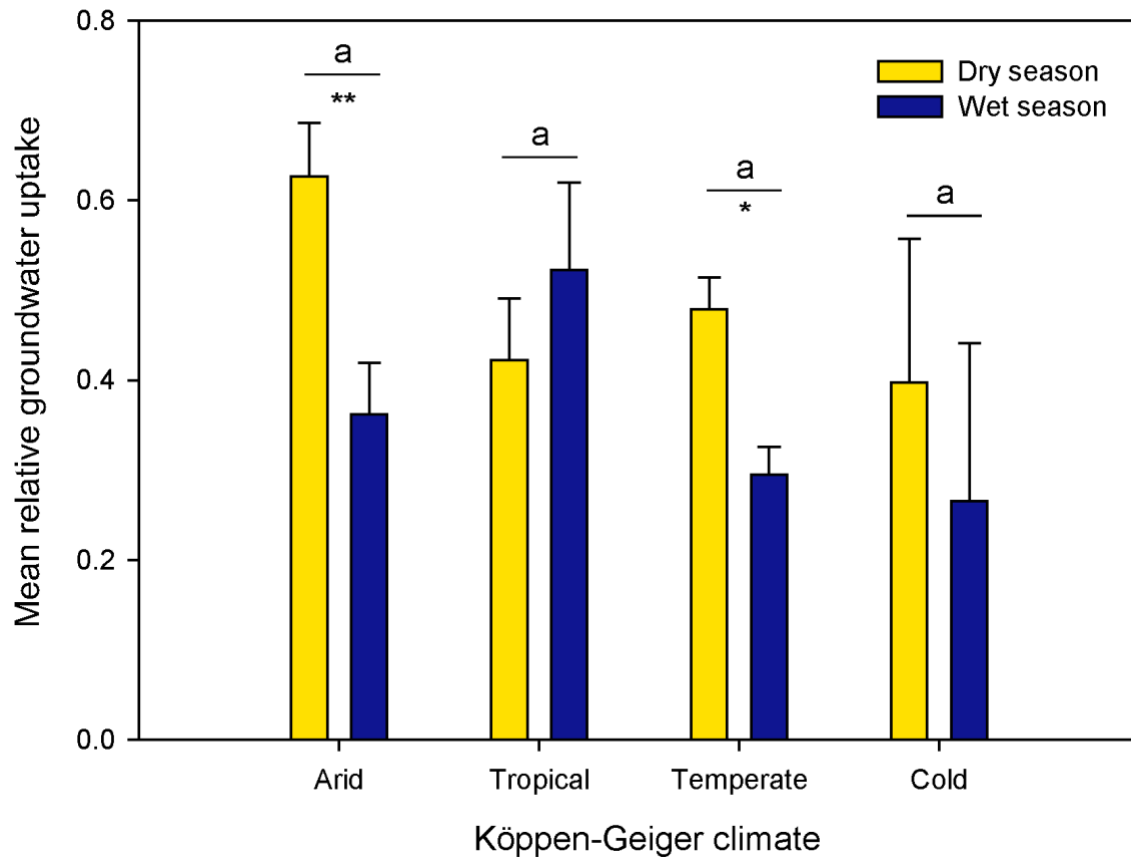
Figure 3. Relative GW uptake and Köppen-Geiger climatic classes in dry and wet seasons. Significant differences between climatic classes are marked with different letters (ANOVA with Tukey HSD post-hoc tests). Significant differences between dry and wet seasons are marked with one ($P<0.05$), two ($P<0.01$), or three ($P<0.001$) asterisks.

Plant anatomical type was a significant factor in the global model (Table S1), but the separate ANOVA and Tukey HSD post-hoc test did not show further differences (Fig. 4). The relative use of GW in herbaceous plants, the least well represented group, did not differ significantly from the uses of shrubs. Trees were the only group to have a marked seasonality. There was a significant effect of the methodology used by each study (Fig. 1) since the relative GW uses estimated with dual-isotope approach were 1.4% higher (Table S2, Fig. S1) than the estimations made with single-isotope approaches. In contrast, the type of matrix that configures the unsaturated zone (rocky vs. non-rocky) did not affect the relative GW use (Fig. S2).

Table 2. Interactive effects on relative GW use between site climatic characteristics and the different landscape positions.

	Riparian	Dunes	Slope	Plain
Mean Annual Precipitation	NS	**+**	**-**	NS
Precipitation of the warmest quarter	**---**	NS	**++**	NS
Precipitation of the wettest quarter	**+++**	NS	NS	**+**

The effects shown were obtained with the GLM depicted in Fig. 1 and detailed in Table S1. Positive effects increasing GW use are marked with a plus sign, and negative effects decreasing GW use with a minus sign. NS means non-significant effects. The number of signs for each pair of variables indicates the level of significane (one sign $P<0.05$, two signs $P<0.01$, three signs $P<0.001$).

The cases in which water within the unsaturated zone was not available for plants revealed a higher dependency on GW (Fig. S3). A GLM run with a subset of data, including only with the cases where the unsaturated zone is extremely dry (water not accessible by plants) explained 70% of the variance in GW use (R^2=69.9%, F=8.44, P<0.001, Table S3). The interaction between landscape position and climate explained nearly 40% of the variance, since riparian ecosystems showed a larger sensitivity to climate of their GW use. Mean annual temperature increased GW use in riparian ecosystems but decreased it in plains and dunes. The effect of precipitation on GW use had the same sign in all landscape positions but was strongest in riparian ecosystems (Table S3).
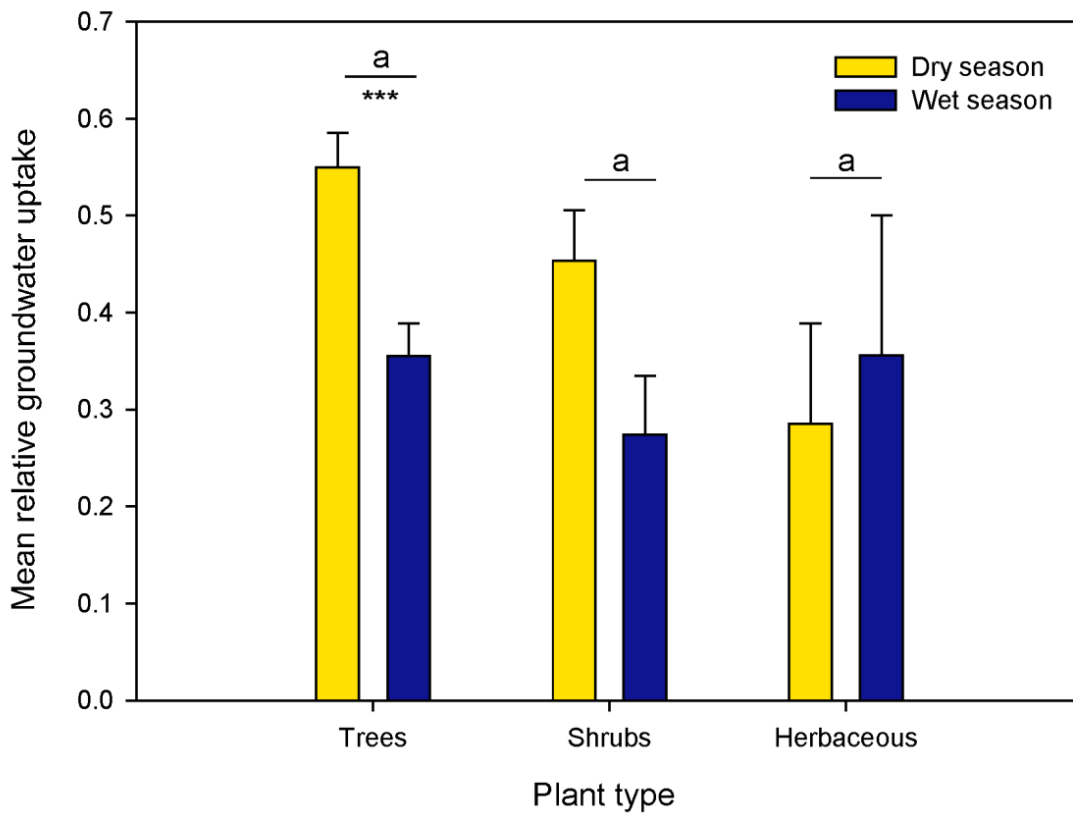
Figure 4. Relative GW uptake and plant types in dry and wet seasons. Significant differences between plant types are marked with different letters (ANOVA with Tukey HSD post-hoc tests). Significant differences between dry and wet seasons are marked with one ($P<0.05$), two ($P<0.01$), or three ($P<0.001$) asterisks.

DISCUSSION

The overall relative contribution of GW to plant water was 51% in dry seasons and 35% in wet seasons. GW uptake was significant in plants from all climatic zones represented in this study and was not restricted to certain landscape positions expected to be more GW-dependent, such as riparian ecosystems. The locations of the studies may have had a bias toward GW-dependent ecosystems, but this analysis included both true phreatophytes (e.g. *Salix goodingii*) and other species with broader distributions along topographical gradients of water availability (e.g. *Pinus sylvestris*). Our results are consistent with the common occurrence of deep roots in woody and herbaceous species in all biomes of the world (Canadell *et al.*, 1996). It has been estimated that 95% of the rooting distribution along the soil profile ranged between 0.3 m in tundra to 1.7 m in Mediterranean woody ecosystems (Schenk & Jackson, 2002). In the studies summarized here, GW was extracted by roots from a mean depth of 3.5±0.3m. A very small percentage of total root

biomass may thus be responsible for more than a third of water uptake, at least in the species represented in this study. GW pools and deep root biomass, therefore, greatly contribute to total plant transpiration (Canadell *et al.*, 1996) and should be included in models of ecosystemic water budgets, which are usually limited to fluctuations in surface-soil moisture and to the biomass of the most superficial roots.

Our results confirm at a broad scale the close relationship between landscape position (that in turn can influence the depth to the saturated zone, the bedrock type, and/or the soil characteristics) and plant GW uptake (Cook & O'Grady, 2006; Engelbrecht *et al.*, 2007); landscape position explained 12.3% of the variance in relative GW uptake (Fig. 1), and their interaction with climate explained 21.0%. This relationship has been previously illustrated at smaller scales by studies investigating the water sources of the same or different species at sites with contrasting landscape and/or topographic characteristics (Zencich *et al.*, 2002; Cook & O'Grady, 2006). Some tree species from desert areas (Snyder & Williams, 2000) but also other climates (Johnston *et al.*, 2011; Hao *et al.*, 2013) are obligate phreatophytes, i.e. they can only grow where GW is permanently available (e.g. riparian areas). Plants in riparian areas where GW levels are shallower thus have larger contributions of GW to plant water, especially in dry seasons (76±6%, Fig. 2). The average relative GW uptake in all other landscape positions was nevertheless 45% in dry seasons, i.e. GW was the most important water source even at sites where roots must penetrate deep into the bedrock to reach the saturated zone (Miller *et al.*, 2010)(Table S1, Fig. S2). GW is certainly a water source that alleviates water stress during dry seasons when surface soil moisture is depleted, because its proportion decreases in wet seasons (Fig. 2).

Climate was an important factor explaining the variance in the relative GW uptake, as expected given that climatic features, especially water balance, are the main factors driving the global distribution of deep roots (Canadell *et al.*, 1996). Water uptake increased significantly in dry seasons (Fig. 3), confirming the prevalence of marked seasonality of water uptake in many species, as reported nineteen years ago for *Eucalyptus* and *Banksia* trees (Dawson & Pate, 1996). The proportion of GW uptake to total plant water was even higher at sites with a more extreme dry season (effect of precipitation of the driest quarter, Fig. 1). Similarly, higher precipitation in the wettest quarter also decreased plants' need for GW supply. The use of GW, though, was positively correlated with annual precipitation at riparian and sloped sites (Table S1). Generally drier climates may induce plants with access to GW to use a higher proportion of it, but our results

suggest that plants in arid and tropical climates use similar proportions of GW (Fig. 3). It has been that rooting could be deeper in tropical plants to support a higher aboveground biomass (Schenk & Jackson, 2002). The seasonality of precipitation, rather than the annual average, is thus a more important factor determining the relative GW use (Fig. 1).

Regardless of the absolute amount of GW extracted, we report that plants use relatively more GW when water is not available within the unsaturated zone (56% vs. 37%, Fig. S3). Plants may reduce their transpiration stream during seasonal (or chronic) droughts involving high evaporative demand and thus depleting surface moisture, and this little transpiration is supported by GW (Pinto *et al.*, 2013). This "relief" use of GW seems to be more affected by site temperature (and other climatic traits) than in the other cases. Therefore, caution must be taken when comparing relative GW uptake between wet and dry seasons, since the absolute values will depend on the transpiration rates. Additionally, the seasonal behavior of GW use suggests that the strong compartmentalization between water pools is at least caused by a temporal disassociation of groundwater recharge and plant transpiration. Reduced transpiration is concomitant to high relative GW uses. In contrast, GW is recharged mainly during winter in temperate and arid (Jasechko *et al.*, 2014).

Our estimates of GW use in nearly 100 species question the dichotomy between plants that tap GW and shallow-rooted plants lacking access to GW. Recent evidence suggests that co-existing plants use different water sources in space and time (ecohydrological niche segregation, (Peñuelas *et al.*, 2011; Silvertown *et al.*, 2015)), but this segregation does not rule out the capacity (and perhaps the necessity) of many plants to use water from the saturated zone at some stage of their lives. Perennial plants, especially trees, are long-lived and sessile organisms unable to migrate (Rabasa *et al.*, 2013) and consequently encounter very diverse environmental conditions during their lives. The capacity to modify root function and even distribution (Barbeta *et al.*, 2015) is thus likely an adaption to the variation in water availability during an individual's life. This premise is supported by the resistance of some plants to experimental drought (Hanson *et al.*, 2001; Barbeta *et al.*, 2013).

We found compelling differences in GW use related to landscape position (Fig 2). Increasing reports of drought-induced mortality have consistently correlated mortality rates and levels of crown defoliation to landscape position and topography (Lloret *et al.*, 2004; Suarez *et al.*, 2004;

Brouwers *et al.*, 2013), in which populations with better GW access had higher survival. At least part of the water stress inducing forest dieback may thus be driven or exacerbated by a depletion of GW reserves. GW pools respond to water balance on long time scales (Vicente-Serrano *et al.*, 2010), so we could expect that long-lasting droughts, rather than short and intense droughts, will more negatively affect species with higher relative GW use. Furthermore, apart from modifications of the precipitation regime, the annual GW extraction is higher than the total renewable GW resources in countries such as Pakistan, Iran, and Saudi Arabia (Eamus *et al.*, 2015), which will further threaten vegetation but not only in riparian areas, given the high proportional GW use in other landscape positions reported here. Although recent global analysis have shown that the connectivity between plant-accessed water pools and water infiltrating and feeding aquifers is lower than expected (Evaristo *et al.*, 2015), our results emphasize that still, GW-dependent ecosystems can be found in many regions and thus the link between groundwater and plant water use should not be underestimated.

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REFERENCES

Barbeta A, Ogaya R, Peñuelas J (2013) Dampening effects of long-term experimental drought on growth and mortality rates of a Holm oak forest. Global change biology, 19, 3133–44.

Barbeta A, Mejía-Chang M, Ogaya R, Voltas J, Dawson TE, Peñuelas J (2015) The combined effects of a long-term experimental drought and an extreme drought on the use of plant-water sources in a Mediterranean forest. Global Change Biology, 21, 1213–1225.

Bearup LA, Maxwell RM, Clow DW, Mccray JE (2014) Hydrological e ects of forest transpiration loss in bark beetle-impacted watersheds. Nature Climate Change, 4, 481–486.

Bowen G (2015) The diversified economics of soil water. Nature, 525, 43–44.

Brooks JR, Barnard HR, Coulombe R, McDonnell JJ (2009) Ecohydrologic separation of water between trees and streams in a Mediterranean climate. Nature Geoscience, 3, 100–104.

Brouwers N, Matusick G, Ruthrof K, Lyons T, Hardy G (2013) Landscape-scale assessment of tree crown dieback following extreme drought and heat in a Mediterranean eucalypt forest ecosystem. Landscape Ecology, 28, 69–80.

Canadell J, Jackson RB, Ehleringer JB, Mooney HA, Sala OE, Schulze ED (1996) Maximum rooting depth of vegetation types at the global scale. Oecologia, 108, 583–595.

Cook PG, O'Grady AP (2006) Determining soil and ground water use of vegetation from heat pulse, water potential and stable isotope data. Oecologia, 148, 97–107.

Dawson TE, Ehleringer JR (1991) Streamside trees that do not use stream water. Nature, 350, 335–337.

Dawson TE, Pate JS (1996) Seasonal water uptake and movement in root systems of Australian phraeatophytic plants of dimorphic root morphology: a stable isotope investigation. Oecologia, 107, 13–20.

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable Isotopes in Plant Ecology. Annual Review of Ecology and Systematics, 33, 507–559.

Eamus D, Zolfaghar S, Villalobos-Vega R, Cleverly J, Huete a. (2015) Groundwater-dependent ecosystems: recent insights, new techniques and an ecosystem-scale threshold response. Hydrology and Earth System Sciences Discussions, 12, 4677–4754.

Ellsworth PZ, Williams DG (2007) Hydrogen isotope fractionation during water uptake by woody xerophytes. Plant and Soil, 291, 93–107.

Engelbrecht BMJ, Comita LS, Condit R, Kursar TA, Tyree MT, Turner BL, Hubbell SP (2007) Drought sensitivity shapes species distribution patterns in tropical forests. Nature, 447, 80–82.

Evaristo J, Jasechko S, McDonnell JJ (2015) Global separation of plant transpiration from groundwater and streamflow. Nature, 525, 91–94.

Fan Y, Li H, Miguez-Macho G (2013) Global patterns of groundwater table depth. Science (New York, N.Y.), 339, 940–3.

Goldsmith GR (2013) Changing directions: the atmosphere – plant – soil continuum. New Phytologist.

Hanson PJ, Todd DE, Amthor JS (2001) A six-year study of sapling and large-tree growth and mortality responses to natural and induced variability in precipitation and throughfall. Tree physiology, 21, 345–358.

Hao XM, Li Y, Deng HJ (2013) Assessment of hydraulic redistribution on desert riparian forests in an extremely arid area. Environmental monitoring and assessment, 185, 10027–38.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology, 25, 1965–1978.

IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (eds Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, V. B, Midgley P.). Cambridge University Press, Cambridge, UK. New York, NY, USA.

Jasechko S, Birks SJ, Gleeson T et al. (2014) The pronounced seasonality of global groundwater recharge. Water Resources Research, 50, 8845–8867.

Johnston DB, Cooper DJ, Hobbs NT (2011) Relationships between groundwater use, water table, and recovery of willow on Yellowstone's northern range. Ecosphere, 2, art20.

Lee J, Oliveira RS, Dawson TE, Fung I (2007) Root functioning modifies seasonal climate. Proceedings of the National Academy of Sciences, 104.

Lloret F, Siscart D, Dalmases C (2004) Canopy recovery after drought dieback in holm-oak Mediterranean forests of Catalonia (NE Spain). Global Change Biology, 10, 2092–2099.

Maeght JL, Rewald B, Pierret A (2013) How to study deep roots-and why it matters. Frontiers in plant science, 4, 299.

Miller GR, Chen X, Rubin Y, Ma S, Baldocchi DD (2010) Groundwater uptake by woody vegetation in a semiarid oak savanna. Water Resources Research, 46, n/a–n/a.

Moore JW, Semmens BX (2008) Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters, 11, 470–480.

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PloS one, 5.

Peel M, Finlayson B, McMahon T (2007) World map of the Köppen-Geiger climate classification updated. Hydrology and Earth System Sciences, 11, 1633–1644.

Peñuelas J, Terradas J, Lloret F (2011) Solving the conundrum of plant species coexistence: water in space and time matters most. New Phytologist, 5–8.

Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: Coping with too many sources. Oecologia, 136, 261–269.

Pinto CA., Nadezhdina N, David JS et al. (2013) Transpiration in Quercus suber trees under shallow water table conditions: The role of soil and groundwater. Hydrological Processes, 6079, 6067–6079.

R Core Development Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rabasa SG, Granda E, Benavides R et al. (2013) Disparity in elevational shifts of European trees in response to recent climate warming. Global change biology.

Sarris D, Christodoulakis D, Körner C (2007) Recent decline in precipitation and tree growth in the eastern Mediterranean. Global Change Biology, 13, 1187–1200.

Schenk HJ, Jackson RB (2002) The global biogeography of roots. Ecological Monographs, 72, 311–328.

Schenk HJ, Jackson RB (2005) Mapping the global distribution of deep roots in relation to climate and soil characteristics. Geoderma, 126, 129–140.

Schwinning S (2010) Ecohydrology Bearings - Invited Commentary The ecohydrology of roots in rocks. Ecohydrology, 245, 238–245.

Silvertown J, Araya Y, Gowing D (2015) Hydrological niches in terrestrial plant communities: A review. Journal of Ecology, 103, 93–108.

Snyder KA, Williams DG (2000) Water sources used by riparian trees varies among stream types on the San Pedro River , Arizona. Agricultural and Forest Meteorology, 105, 227–240.

Suarez ML, Ghermandi L, Kitzberger T (2004) Factors predisposing episodic drought-induced tree mortality in Nothofagus – site , climatic sensitivity and. Journal of Ecology, 92, 954–966.

Thomas FM (2014) Ecology of Phreatophytes. In: Progress in Botany, Vol. 75 (eds Lüttge U, Beyschlag W, Cushman J), pp. 335–375. Springer Berlin Heidelberg.

Thorburn PJ, Hatton TJ, Walker GR (1993) Combining measurements of transpiration and stable isotopes of water to determine groundwater discharge from forests. Journal of Hydrology, 150, 563–587.

Vicente-Serrano SM, Beguería S, López-Moreno JI (2010) A multiscalar drought index sensitive to global warming: The standardized precipitation evapotranspiration index. Journal of Climate, 23, 1696–1718.

Yang X, Scuderi LA., Wang X et al. (2015) Groundwater sapping as the cause of irreversible desertification of Hunshandake Sandy Lands, Inner Mongolia, northern China. Proceedings of the National Academy of Sciences, 112, 702–706.

Zencich S, Froend R, Turner J, Gailitis V (2002) Influence of groundwater depth on the seasonal sources of water accessed by Banksia tree species on a shallow, sandy coastal aquifer. Oecologia, 131, 8–19.

SUPPLEMENTARY MATERIALS

Dual vs. single isotope approach

Hydrogen and oxygen isotopic ratios of soil water shift differently as they evaporate (Brooks *et al.,* 2009; Bowen, 2015). Groundwater and soil water could be confused if only one isotope is used, especially during wet and cold seasons, when they may have similar isotopic signals at least in one of their isotopes. Thus, it is recommended to use a dual-isotope approach in determining the source of plant xylem water to minimize overestimations of GW due to similarities of one of the isotopes with soil water. However, 50% of the reviewed cases in this study used only a single isotope to estimate the contribution of GW. For this reason, we included the methodology (single vs. dual-isotope as a fixed factor in the GLM (Table S1), which surprisingly resulted in significantly higher estimations with dual-isotope approaches. Since the signal of precipitation and the effects of evaporation on soil water vary strongly as a function of season, we made an additional analysis split into seasons in order to rule out possible biases on the estimation of GW. Again, we found no general association between astronomic and precipitation seasons and the methodology used affecting the estimations of GW use (Table S3). The exception was dry summers, which appeared to have higher estimations of GW use with the dual-isotope approach. In dry summers, high evaporative demand depletes the upper soil layers in the light isotope. Thus, it is the season when the difference between the isotopic composition of GW (that usually keeps heavier isotopic signals) and that of soil water is higher and as a consequence confusions are less likely. Furthermore, this analysis show that in any case, less accurate single isotope approaches tend to underestimate the relative GW use (Fig. S1).

Table S1. Output of the general linear models correlating the GW contribution to plant water and landscape position, climate, and plant type. Model selection used the Akaike information criterion and avoided multicollinearity among the independent variables. P, precipitation. Significant effects are marked with one (*P*<0.05), two (*P*<0.01), or three (*P*<0.001) asterisks, and marginally significant effects with a point.

	Variance explained (%)	β coefficient	Estimate	Std. Error	*t*-value	
(Intercept)		0.00	0.3099	0.1216	2.55	*
Landscape: Dunes		-0.59	-0.4658	0.1890	-2.46	*
Landscape: Riparian		0.19	0.1484	0.1260	1.18	
Landscape: Slope	11.84	0.53	0.4634	0.2166	2.14	*
Mean Annual P	0.43	-0.46	-0.0004	0.0002	-1.51	
Anatomy: Shrub		0.12	0.0886	0.0801	1.11	
Anatomy: Tree	0.67	0.23	0.1591	0.0791	2.01	*
P season: Wet	5.70	-0.33	-0.2160	0.0564	-3.83	***
P of the wettest quarter	0.79	0.59	0.0010	0.0005	2.08	*
P of the warmest quarter	0.20	-0.21	-0.0003	0.0003	-1.10	
Saturated zone: Non-rocky	0.45	0.04	0.0394	0.0803	0.49	
Methodology: Single-isotope	0.36	-0.18	-0.1156	0.0582	-1.99	*
Landscape: Dunes X Mean Annual P		1.26	0.0012	0.0004	2.70	**
Landscape: Riparian X Mean Annual P		0.30	0.0005	0.0004	1.27	
Landscape: Slope X Mean Annual P	5.11	-0.74	-0.0005	0.0004	-1.33	
Landscape: Dunes X P of the wettest quarter		-0.66	-0.0014	0.0012	-1.14	
Landscape: Riparian X P of the wettest quarter		4.05	0.0165	0.0037	4.46	***
Landscape: Slope X P of the wettest quarter	5.75	-0.54	-0.0008	0.0007	-1.23	
Landscape: Dunes X P of the warmest quarter		0.17	0.0006	0.0005	1.40	
Landscape: Riparian X P of the warmest quarter		-4.18	-0.0185	0.0044	-4.23	***
Landscape: Slope X P of the warmest quarter	4.76	0.94	0.0015	0.0005	2.97	**
Landscape: Dunes X P season: Wet		0.33	0.3508	0.0917	3.83	***
Landscape: Riparian X P season: Wet		0.00	-0.0027	0.0950	-0.03	
Landscape: Slope X P season: Wet	5.70	0.03	0.0406	0.1014	0.40	

Table S2. Output of the general linear models correlating the GW contribution to plant water and landscape position, climate, and plant type for the cases in which water in the unsaturated zone is not available. Model selection used the Akaike information criterion and avoided multicollinearity among the independent variables. P, precipitation. Significant effects are marked with one (*P*<0.05), two (*P*<0.01), or three (*P*<0.001) asterisks, and marginally significant effects with a point.

	Variance explained (%)	Estimate	Std. Error	*t*-value	*P*-value
(Intercept)		-20.16	3.61	-5.58	<0.001
Mean Annual Temperature	1.60	3.09	0.55	5.67	<0.001
Landscape position: Dunes		20.80	3.66	5.69	<0.001
Landscape position: Plain	13.00	21.19	3.62	5.85	<0.001
Mean Annual P	2.73	0.64	0.11	5.81	<0.001
P of the wettest quarter	2.23	-1.19	0.21	-5.76	<0.001
Plant type: herbaceous		-0.23	0.36	-0.63	0.53
Plant type: Shrubs	2.36	-0.24	0.35	-0.70	0.49
Precipitation of the driest quarter	2.64	-0.07	0.02	-3.37	<0.01
Mean Annual Temperature X Landscape position: Dunes		-3.30	0.56	-5.91	<0.001
Mean Annual Temperature X Landscape position: Plain	9.67	-3.07	0.54	-5.67	<0.001
Mean Annual Precipitation X Landscape position: Dunes		-0.62	0.11	-5.64	<0.001
Mean Annual Precipitation X Landscape position: Plain	15.19	-0.63	0.11	-5.72	<0.001
P of the wettest quarter X Landscape position: Dunes		1.15	0.20	5.65	<0.001
P of the wettest quarter X Landscape position: Plain	14.81	1.16	0.20	5.68	<0.001
Plant type: herbaceous X P of the driest quarter		-0.01	0.02	-0.83	0.41
Plant type: Shrubs X P of the driest quarter	5.73	-0.01	0.02	-0.52	0.61

Table S3. Statistical differences (ANOVA with Tukey HSD post-hoc tests) among the relative GW use as estimated with dual and single isotopes across astronomic and precipitation seasons.

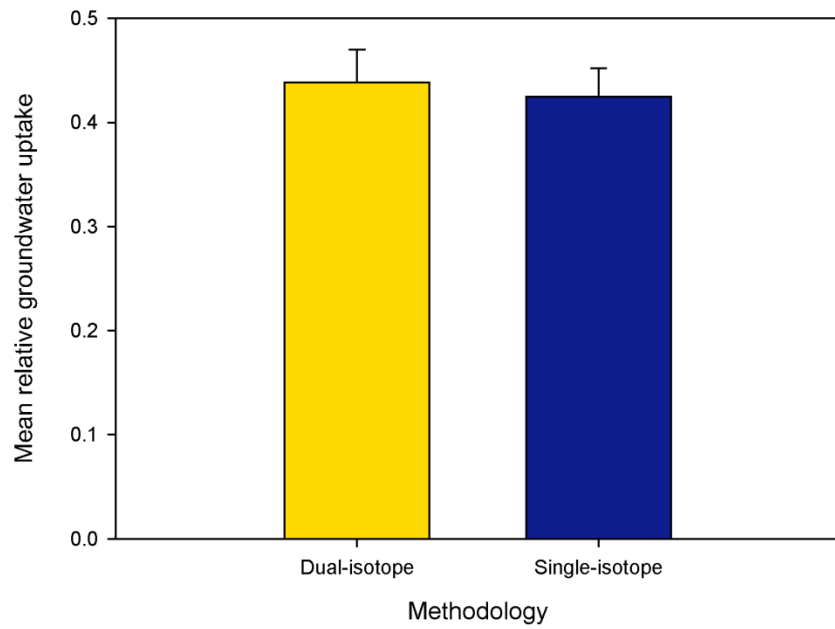|	Dry season		Tukey post-hoc
	Dual isotope	**Single isotope**	*P*-value
Autumn	0.46±0.11	0.50±0.09	1
Spring	0.49±0.06	0.39±0.05	0.99
Summer	0.70±0.06	0.44±0.05	0.04
Winter	0.34±0.11	0.33±0.14	1
	Wet season		
	Dual isotope	**Single isotope**	
Autumn	0.31±0.07	0.26±0.08	1
Spring	0.19±0.04	0.36±0.05	0.77
Summer	0.36±0.06	0.47±0.07	0.99
Winter	0.27±0.08	0.24±0.06	1

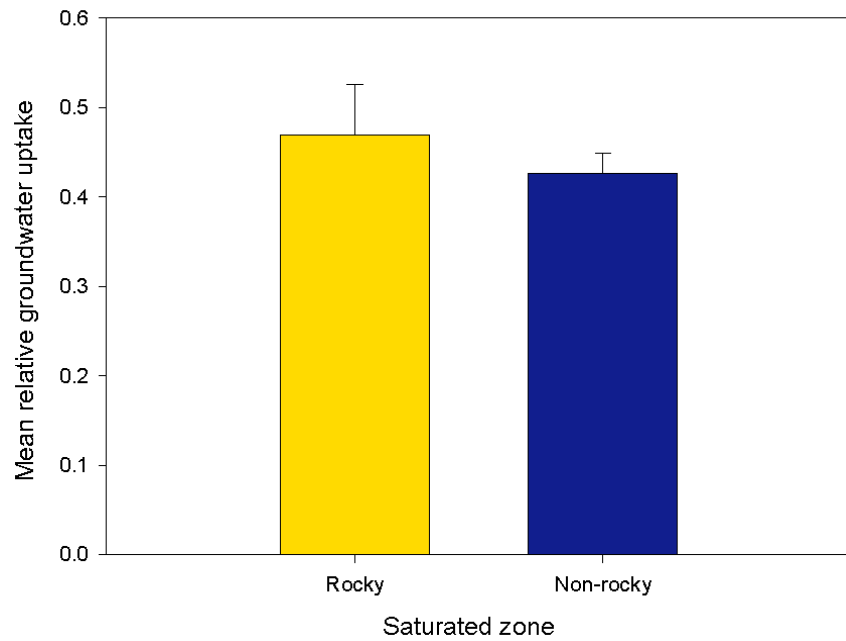**Figure S1.** . Mean relative groundwater uptake depending on the isotopic methodology used.

Figure S2. Mean relative groundwater uptake depending on the characteristics of the saturated zone.
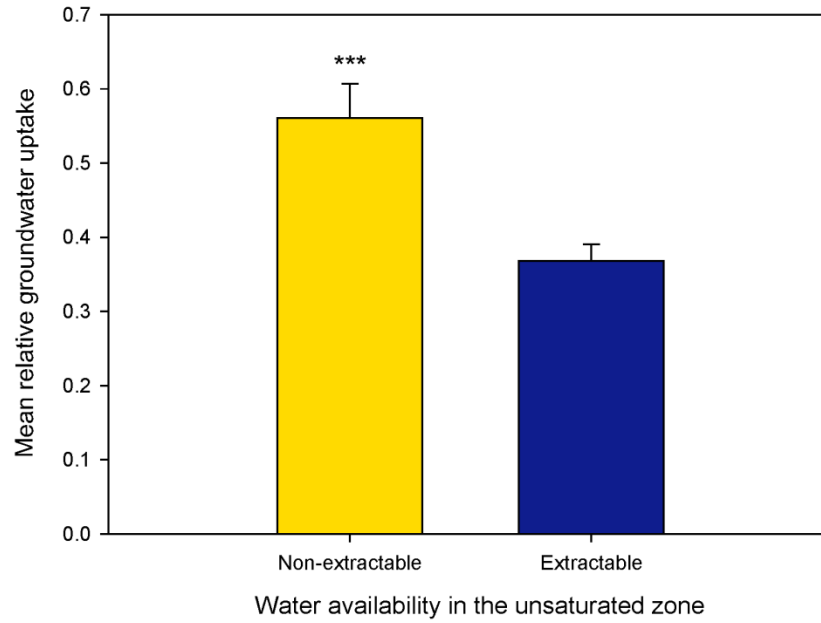
Figure S3. Mean relative groundwater uptake depending on the availability of water within the unsaturated zone.
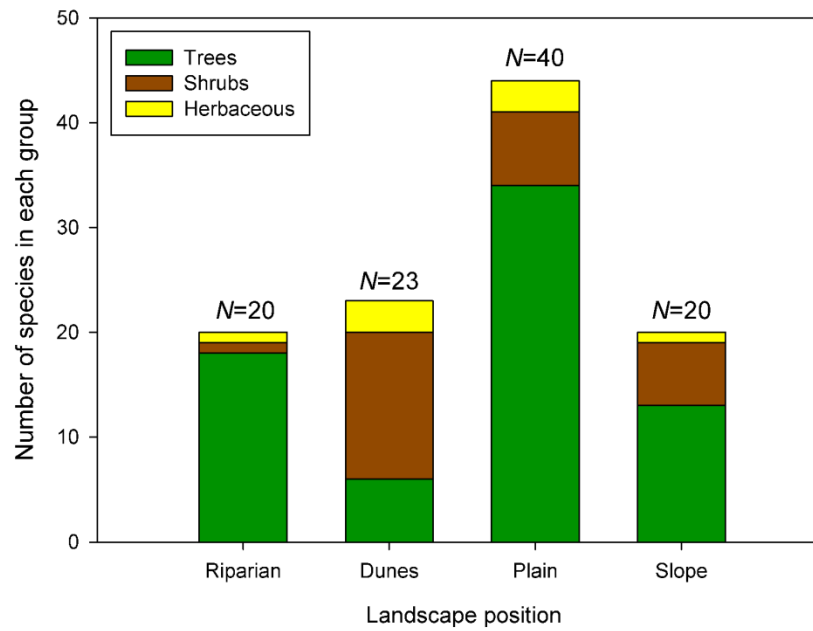

Figure S4. Distribution of the number of species in the data set across landscape positions.
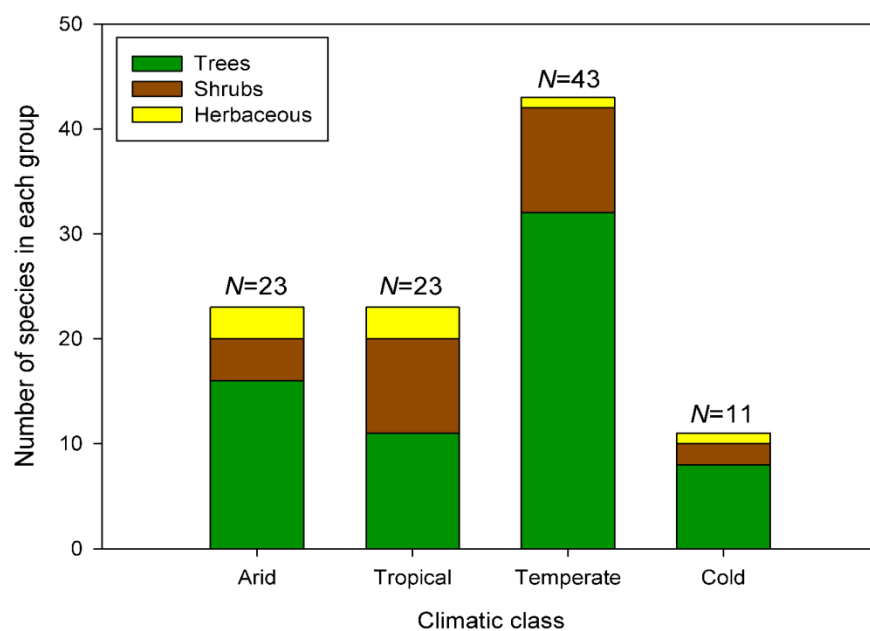
Figure S5. Distribution of the number of species in the data set across climatic classes.
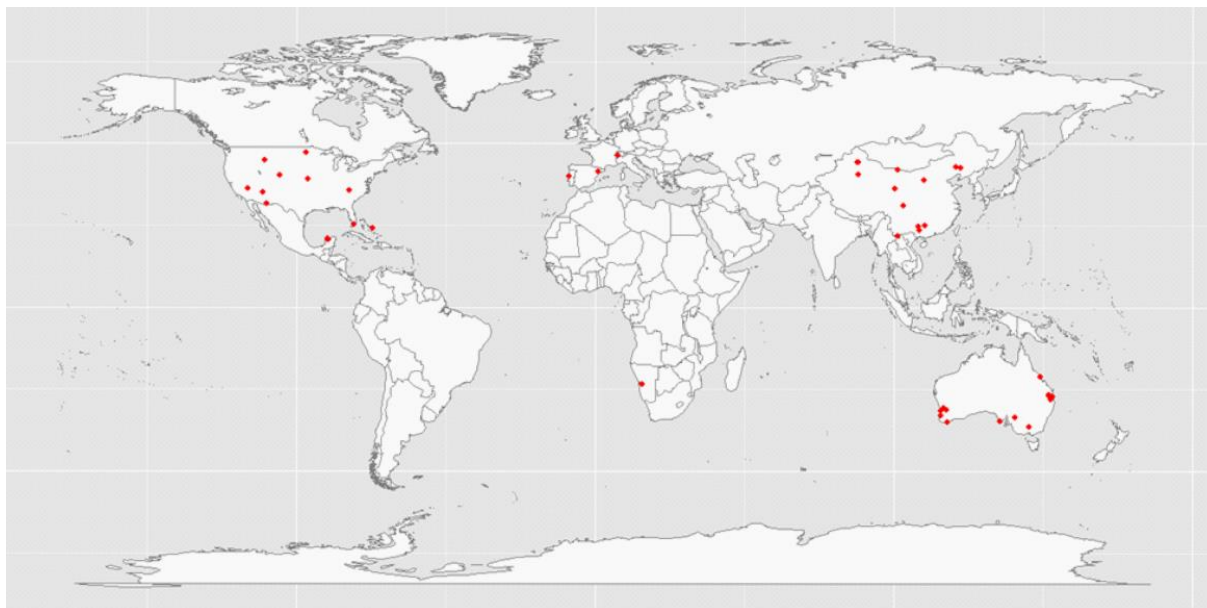

Figure S6. Locations of the study sites.

Chapter VII

General conclusions

FINAL REMARKS

-The results of this thesis confirm that the currently dominant species of holm oak forests (*Q. ilex*) is more sensitive to drought than other co-existing species (*P. latifolia*), since growth and mortality rates were affected the by the long-term experimental drought in *Q. ilex* but remained unaffected in *P. latifolia* (Chapter II). However, this difference was not explained by plant water sources as they both accessed the same belowground water pools in space and time (Chapter III). Therefore, other species-specific physiological and morphological traits besides the patterns in root water uptake are responsible for this asymmetric drought sensitivity, highlighted by species-specific differences in water transport capacity under drought conditions (Chapter IV).

-The course of growth and mortality rates along 13 years of experimental drought provide evidence of stabilizing processes operating at the stand level (Chapter II). Following the application of the drought treatment and the occurrence of several extreme drought events, the subsequent stem mortality reduced the stand-level competition for water resources. Additionally, results from experimental drought systems established in holm oak forests showed that acclimation changes related to morphology, physiology and epigenetics contribute to dampen the negative effects of drought also at the individual-level (Chapters III and V). It is still unclear whether stabilizing processes and acclimation changes would be enough to offset the deleterious effects of the increase in the frequency of extreme drought events.

-A broad-scale analysis of the drought characteristics that triggered holm oak forest declines showed that the duration of drought is the key parameter determining the severity of drought effects (Chapter V). The multi-year dynamics of non-structural carbohydrates and/or the cavitation fatigue may be behind the fact that persistent droughts cause more crown defoliation and mortality on holm oak forests than droughts of short duration regardless of their intensity. In this thesis, an association between the access to groundwater pools and the effects of an extreme drought has been revealed (Chapter III). Indeed, groundwater pools are a significant water source for many plant species (Chapter VI).

- The bottom line of this PhD dissertation is that plant individuals and communities have an extraordinary capacity to accommodate to environmental changes. This is especially true for the tree *Quercus ilex* and the forests that it dominates. Such woody species are long-lived and obviously also sessile and have thrived in the same geographical area along geological ages with contrasting

climatic conditions. A characteristic that may have allowed plants to survive these climatic periods is their extensive root system. However, it is still not clear whether the pace of the current climatic change can be matched by acclimation processes and mechanisms showed here. More particularly, shorter periods of return of extreme droughts may be decisive in determining the fate of holm oak forests, therefore further research in this regard is warranted.

MAIN SPECIFIC CONCLUSIONS OF THE THESIS

Forests increase their resistance to experimental drought over time

- The stem growth of the three studied species iss correlated with the water balance of spring. In contrast, annual stem mortality rates are a function of the water balance of spring and the previous winter in *Q. ilex*, and of current spring and summer in *P. latifolia*.

- The response to drought changes over time. Decreased intra- and interspecific competition after extreme events with high mortality, together with probable morphological and physiological acclimation to drought during the study period dampens the initially stronger response to drought of *Q. ilex* and *A. unedo*.

- After 13 years of drought treatment, the drought-sensitive *Q. ilex* and *A. unedo* suffer higher mortality in the drought plots while *P. latifolia* remains unaffected. The growth of alive stems in 2012 is lower in the drought plots in *A. unedo*, while *Q. ilex* and *P. latifolia* do not show differences.

- The lack of differences in the stem growth of alive stems of *Q. ilex* and *P. latifolia* contrasts with the difference found when accounting with now-dead trees. This is explained by the near-zero growth of *Q. ilex* stems that ended up dying during the experiment period.

- Species-specific different sensitivities of Mediterranean species to more frequent and intense droughts may lead to partial species substitution and changes in forest structure and thus in carbon uptake.

Changes in the root water uptake in response to experimental and extreme drought

- The dominant species of holm oak forests, *Quercus ilex*, *Arbutus unedo* and *Phillyrea latifolia*, all have dimorphic root systems enabling them to access different water sources in space and time, but compete for the same ecohydrologic niche.

- In wet and relatively cold seasons (winter and autumn), all the studied species uptake water preferentially from the top centimeters of soil. In spring, they increase the contribution of water from deeper soil layers. In summer, when soil moisture dropped below the 10%, water stored in bedrock increases its contribution to transpiration.

- Plants subjected to the long-term experimental drought shift water uptake toward deeper soil layers during the wet season and reduce groundwater uptake in summer, indicating plasticity in the functional distribution of fine roots.

- Defoliation and increased stem mortality coincided in time with a reduced access to groundwater in the most affected plants.

Q. ilex is more greatly affected in the driest locations, compared to P. latifolia

- The current dominant tree species *Q. ilex* is being out-competed by the tall shrub *P. latifolia* in the driest limit of its distribution due to a lower water transport capacity under drought conditions.
- The sap flow of *P. latifolia* shows a higher sensitivity to increases in soil moisture and in the atmospheric water demand compared to that of *Q. ilex*.
- Nocturnal sap flow has a significant role in both species with a frequent pattern of increase from 3 am to dawn and was independent of the nighttime atmospheric water demand, which warrants further research in order to understand its potential implications.

Sequence of responses to drought of holm oak forests

- The developmental time of some adaptive morphological traits is too long to respond to abrupt increases in drought.
- The phenological plasticity of *Q. ilex,* especially of growth, may allow it to adapt to changing drought regimes.
- Reported forest dieback episodes in *Q. ilex* suggest that the provoking droughts were extremely long rather than intense.
- *Q. ilex* can vigorously resprout after extreme drought events either from the canopy or the stump. However, it remains unclear if an increase in the recurrence of these events may weaken the capacity to resprout on the long-term.
- Long-term experiments have identified processes of acclimation at the individual and stand level, but the capacity of *Q. ilex* to recover may still be endangered if extreme droughts become more frequent albeit accompanied by acclimation mechanisms and stabilizing processes.

Relative contribution to plant transpiration by groundwater

- Groundwater is a broadly used water source of plants, as it accounts for nearly half of the transpired water in dry seasons and a third during wet seasons.

- The landscape position is a determining factor for groundwater uptake; plants from riparian ecosystems have much higher contributions than plants in dunes, plains or sloped terrain.

- In arid climates, the relative contribution of groundwater to transpiration is the highest.

- The consistently high percentage of groundwater uptake has an adaptive significance for perennial plants, which are sessile organisms that must cope with climatic extremes throughout their long lives.

Publications during the thesis period

PUBLISHED

LIU, D., OGAYA, R., **BARBETA, A.,** YANG, X., PEÑUELAS, J., (2015). Contrasting impacts of continuous moderate drought and episodic severe droughts on the aboveground-biomass increment and litterfall of three coexisting Mediterranean woody species. **Global Change Biology.** DOI: 10.1111/gcb.13029

MARTÍN-GOMÉZ, P., **BARBETA, A.,** VOLTAS, J., PEÑUELAS, J., DENNIS, K., PALACIO, S., DAWSON, T.E., FERRIO, J.P. (2015). Isotope-ratio infrared spectroscopy: a reliable tool for the investigation of plant-water sources? **New Phytologist**. 207: 914–927.

BARBETA A., MEJÍA-CHANG M., OGAYA R., VOLTAS J., DAWSON T.D., PEÑUELAS J. (2015). The combined effects of a long-term experimental drought and an extreme drought on the use of plant-water sources in a Mediterranean forest. **Global Change Biology** 21: 1213-1225.

OGAYA, R., **BARBETA A.,** BASNOU, C., PEÑUELAS, J. (2015) Satellite data as indicators of tree biomass growth and forest dieback in a Mediterranean holm oak forest. **Annals of Forest Science** 72 (1), 135-144.

OGAYA, R., LLUSIÀ, J., **BARBETA, A.,** ASENSIO, D., LIU, D., ALESSIO G.A., PEÑUELAS, J. (2014). Foliar CO_2 in a holm oak forest subjected to 15 years of climate change simulation. **Plant Science** 226, 101-107.

RICO, L., OGAYA, R., **BARBETA, A.,** PEÑUELAS, J. (2014) Changes in DNA methylation fingerprint of Quercus ilex trees in response to experimental field drought simulating projected climate change. **Plant Biology** 16 (2), 419-427.

CARNICER, J., **BARBETA, A.,** SPERLICH, D., COLL, M., PEÑUELAS, J.(2013).Contrasting trait syndromes in angiosperms and conifers are associated with different responses of tree growth to temperature on a large scale. **Frontiers in Plant Science.** 4:409.

BARBETA, A., OGAYA, R., PEÑUELAS, J. (2013) Dampening effects of long-term experimental drought on growth and mortality rates of a Holm oak forest. **Global Change Biology** 19 (10), 3133-3144.

PEÑUELAS, J., SARDANS, J., ESTIARTE, M., OGAYA, R., CARNICER, J., COLL, M., **BARBETA, A**., RIVAS-UBACH, A., LLUSIÀ, J., GARBULSKY, M., FILELLA, I., JUMP, A.S (2013) Evidence of current impact of climate change on life: a walk from genes to the biosphere. **Global Change Biology** 19 (8), 2303-2338.

BARBETA, A., OGAYA, R., PEÑUELAS, J. (2012). Comparative study of diurnal and nocturnal sap flow of Quercus ilex and Phillyrea latifolia in a Mediterranean holm oak forest in Prades (Catalonia, NE Spain). **Trees** 26 (5), 1651-1659.

BARBETA A., PEÑUELAS J., OGAYA, R., JUMP, A.S. (2011). Reduced tree health and seedling production in fragmented *Fagus sylvatica* forest patches in the Montseny Mountains (NE Spain). **Forest, Ecology and Management**. 261:2029-2037.

UNDER REVIEW

BARBETA A., PEÑUELAS J. Relative contribution of groundwater to plant transpiration estimated with stable isotopes. Under review in Proceedings of the National Academy of Sciences of the USA.

BARBETA A., PEÑUELAS J. Sequence of drought responses in holm oak forests: lessons from experiments to extreme droughts. Under review in Plant Ecology and Diversity.

RIVAS-UBACH, A., **BARBETA, A.,** SARDANS, J., GUENTHER, A., OGAYA, R., OROVEC, M, URBAN, O., PEÑUELAS, J. Topsoil depth substantially influences the responses to drought of the tree metabolomes of Mediterranean forests. Under review in Functional Ecology.

SPERLICH, D., **BARBETA, A.,** OGAYA, R., SABATÉ, S., PEÑUELAS, J. Balance between carbon gain and loss under long-term drought: impacts on foliar respiration and photosynthesis in *Quercus ilex*. Under review in Journal of Experimental Botany.

Agraïments

Em sento molt afortunat d'haver pogut gaudir del procés d'aprenentatge que suposa una tesi doctoral. I això és gràcies a les persones que m'han acompanyat o que m'he anat trobat durant aquests quatre anys. Per una banda, destacaria el fet d'haver creat lligams molt forts amb algunes persones, i per l'altra, l'oportunitat de conèixer personatges més que interessants dels quals n'he après tan com he pogut, conscientment o no.

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Durant l'estada a Berkeley em van ajudar molt en Paul, en Todd i en Greg, però també l'Allison i la Stefania, i en un pla més social l'Anthony i la Wendy, el Pierre i la Victoria i el Roberto. Curiosament, de qui vaig aprendre més a Potsdam va ser d'una noia de Granollers! L'Eli, experta dendroecòloga i en comptar vasos de roure. En Heiko i el Gerd també em van donar un bon cop de mà. Gràcies a la Claudia, la Isa i el Marcus per acollir-me i sobretot al Ramon per ser el company de tantes aventures.

Però no només he fet recerca i estades durant aquests anys (ni molt menys). He tingut la sort de treballar al CREAF, tot un privilegi pel bon ambient i companyerisme que hi ha. Començant pel despatx més desastrós, el -116, que tot i que no passaria cap inspecció de riscos laborals ni de sanitat, és un indret en constant ebullició d'on surten tota mena d'idees estrambòtiques i folklòriques de caire rural-sureny, eco-neorural o científico-surrealista, amb les aportacions també de la Janet. Guardo molt bon record de la primera etapa al CREAF, l'època de les festes Gripaues, amb la Maria, la Marta, la Montse, la Rebeca, la Laia, la Txell, la Carla, l'Helena, la Virginia, la Lucía, la Núria, l'Oriol, el Josep, el Jofre, el Lasse, l'Evan, el Guillem, el Dominik, el Gerard, el Pau, l'Anna i l'Albert Rivas. Més recentment, amb l'empenta del procés, també hi he passat estones molt divertides amb la colla dels Miquelets del CREAF, tan la facció carlina; David, Xavi Domene, Ferran, Vicenç, Albert Vilà, com la facció més roja; Joan, Guille, Olga, i els no adscrits; Tere. Capítol a part mereix l'Ander, amb qui portem vides paral·leles. Hem compartit molts moments i converses i crec que al final m'has influït una mica la manera de veure el món.

Més enllà del CREAF, sempre he tingut un gran recolzament per part dels meus amics. Menció especial per en Pol i en Matas, companys de pis i de "latas", i a en Bartu, per passar el xerrac per les alzines del coll de Bordoriol, però també per la resta de la colla de la Garriga (AKA Amics de Sant Antoni). Gràcies Baños, ja que el surf és un esport individual però si no tens ningú per compartir onades i fer la cerveseta després tot comentant la jugada, és infinitament més avorrit. Gràcies a l'Arç, per ajudar-me amb el camp i instruir-me com a boletaire, i també a la resta de la colla berguedana, ens veiem per Patum! Finalment, moltes gràcies Anna, potser l'única persona de fora del CREAF que s'ha interessat per la tesi en si, i ara resulta que potser en faràs una, sort!

Moltes gràcies a tothom!