

Sequence of plant responses to droughts of different time scales: lessons from holm oak (*Quercus ilex*) forests

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Abstract

The functional traits of plants in regions of the world with a Mediterranean climate have been shaped to tolerate periods of water deficit. These species are adapted to summer droughts but may not be able to cope with future increases in drought intensity, duration, and/or frequency. Here we review the mechanisms and traits of drought resistance and recovery of the well-studied holm oak (*Quercus ilex*), which we propose as a model species for Mediterranean-type ecosystems. Our aim was to understand the differences and links between the responses of *Q. ilex* to summer droughts, extreme droughts, and long-term drought experiments. A main goal was to provide an integral picture of drought responses across organisational and temporal scales for identifying the most relevant processes that are likely to contribute to determining the future of Mediterranean vegetation. Evidence from long-term drought experiments showed that acclimation processes from the molecular (e.g. epigenetic changes) to the ecosystem level (e.g. reductions in stand density) mitigate the effects of drought. Changes in leaf morphology and hydraulics, leaf-to-shoot allometry and root functioning are among the key mechanisms for overcoming increasing drought. The duration of drought determines its severity in terms of canopy loss and stem mortality. Although *Q. ilex* can vigorously resprout after such episodes, its resilience may be subsequently reduced. In the future, higher frequency of return of extreme droughts will challenge thus the capacity of these forests to recover. The insights provided by this review of the complex interplay of processes that determine the response of trees to droughts of different duration, intensity, and frequency will also help us to understand the likely responses of other resprouting angiosperms in seasonally dry ecosystems that share similar functional traits with *Q. ilex*.

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

Introduction

The increasing length and intensity of droughts is producing dieback in all forested regions of the world (Allen *et al.* 2010, 2015). 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 during the evolutionary histories of the trees. Longer, more intense droughts are forcing trees to display their phenotypic plasticity to its maximum (Matesanz and 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 drought effects on plants) factors would thus improve our ability to predict potential changes in the structure and function of vegetation.

In this review, we discuss the effect of drought on a tree species, from the tissue level to stand or ecosystem or level, at range of time scales. Our chosen model tree is the holm oak (*Quercus ilex* L.), an extensively studied Mediterranean forest tree. *Q. ilex* possesses some adaptations to drought which the species shares with many other species of Mediterranean-type ecosystems. It is a keystone species in vast areas of the western Mediterranean Basin, and its dominance is expected to increase to the detriment of other forest species that appear to be more sensitive to aridity, and in response to land abandonment, followed by secondary regrowth by *Q. ilex* (Carnicer *et al.* 2014). The numerous studies on the responses of *Q. ilex* to drought have allowed us to construct a case study to understand the drought-induced physiological and ecological processes that all vary in their response times. We explore changes in forest structure and function, including the different feedbacks that result from extreme drought episodes and a sustained increase in aridity.

The World Meteorological Organisation (1992) provides two different definitions of drought. The first one defines drought as a prolonged absence or marked deficiency of precipitation. In the regions with a Mediterranean climate, the period with the lowest

precipitation coincides with the warmest temperatures every year. This is referred to summer drought in this review, and implies weather conditions similar to the climatic average for a given site. However, drought is also defined as a period of abnormally dry weather, sufficiently long to cause a serious hydrological imbalance. We call this second definition an extreme drought in this study. We also review the response of holm oak forests to drought periods in which the aridity is created or exacerbated by abnormally warm weather (Allen *et al.* 2015).

We first discuss the resistance mechanisms that operate during the summer droughts that typically occur in Mediterranean regions and which last three to four months. Second, we review the effects of droughts that exceed the average climatic variability of a given location in terms of duration, seasonality, intensity, or coincide with heat waves. Third, we report the effects of persistent droughts, such as those produced by experimental rain exclusions. We demonstrate how this information is useful for a better understanding of the response of *Q. ilex* to environmental change, but also to show how the temporal scale is crucial for predicting the responses of plants to the increases in drought projected by global circulation models (IPCC 2013). The mechanisms and traits that *Q. ilex* possesses to resist and recover from droughts (Table 1) are shared by many plant species from Mediterranean-type ecosystems and other drought-prone ecosystems, especially by resprouting angiosperms (Zeppel *et al.* 2015). Therefore, the insights provided by this review are, to some extent, representative of the ecology of Mediterranean vegetation under current climate and can serve for indicating expected climate change responses.

1.1 *Quercus ilex* as a paradigmatic Mediterranean species.

The insights from published studies provided by the present synthesis on the effect of drought at different timescales may be relevant not only for *Q. ilex* conservation and management but also for woody Mediterranean flora, especially resprouting angiosperms. *Q. ilex* shares many morphological, physiological, and phenological traits related to the response to water stress (Table 1) with other species from regions with a Mediterranean climate, such as in California, Chile, Australia, or South Africa. The maximum rooting depth of holm oak is similar to the global average of species in sclerophyllous forests and shrublands (Canadell *et al.* 1996) whereby high investments in below-ground biomass are common in these seasonally dry ecosystems. Similarly, sclerophyllous leaves are also a typical feature of Mediterranean vegetation, indicating the convergent evolution of plant form by several selective forces, including drought (Mooney and Dunn 1970). Mediterranean summer drought stops growth in *Q. ilex*, resulting in bimodal secondary growth patterns (Lempereur *et al.* 2015). A transient

quiescence of growth has also been reported for Mediterranean conifers (Camarero *et al.* 2010; Vieira *et al.* 2014) and forbs (Olano *et al.* 2013). Large below- and above-ground bud banks are also characteristically associated with Mediterranean vegetation (Canadell and López-Soria 1998; Klimešová and Klimeš 2007) and its capacity to recover from fires but are also relevant after extreme droughts (Zeppel *et al.* 2015). Furthermore, resprouting species share a suite of ecophysiological and anatomical traits (Hernández *et al.* 2011, Vilagrosa *et al.* 2014), so their response to drought should be similar, albeit with differences in sensitivity.

Mediterranean summer droughts and the adaptive mechanisms and traits of *Q. ilex*

Plants in Mediterranean ecosystems have to cope with a double limitation or stressful period: summer drought and winter cold (Terradas 1999). The high summer temperatures increase evapotranspiration, which, together with the low precipitation, produce periods with a negative water balance (the difference between precipitation and water losses through evapotranspiration and runoff). The summer drought, within the distributional range of forests dominated by *Q. ilex*, typically lasts three to four months (Figure 1). The duration and intensity of the summer droughts, however, are highly variable between years (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 that colonisation in areas with an oceanic climate is recent (Delzon *et al.* 2013)(Figure 2). Pollen records indicate that *Q. ilex* persisted during the last glacial period in climatic refugia in the wettest part of its current distribution and gained early dominance in the Holocene in more semi-arid areas of current Morocco and southern Spain (Reille and Pons 1992). Human activity may have contributed to the expansion of *Q. ilex* in the wettest areas of its distribution during the second half of the Holocene (Reille and Pons 1992). Furthermore, this tree species will likely expand its distribution in the future as the climate becomes warmer and the availability of water decreases.

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 (Table 1). Summer drought depletes the water in the topsoil, but moisture can still be found in deeper soil layers. 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 exploit fissures in the bedrock to access 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 (Ferrio *et al.* 2003, Comas *et al.* 2015). This ability to tap deep water tables and thus have permanent access to water has also been confirmed at the ecosystem level by data for synchronous eddy covariance and soil water content (Reichstein *et al.* 2002). The possession of this morphological character has led to the categorisation of *Q. ilex* as a drought-avoiding species (David *et al.* 2004). Tapping deep water table is maximised where soils lay on permeable or fractured bedrock in a 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 ratio of total root biomass relative to above-ground biomass is associated with water availability. Holm oaks in xeric sites have a higher percentage of below-ground biomass than those in mesic sites; roots account for more than half the total tree biomass in some cases (Canadell *et al.* 1999). Fine roots have been shown to 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 of resources for the growth of below-ground structures to the detriment of aerial organs. As a result, *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 above-ground to below-ground biomass.

The morphology of aerial organs has been evolutionarily shaped by summer drought to promote a conservative use of water. Xylem conduits in *Q. ilex* are narrower than in *Quercus* deciduous tree species (Villar-Salvador *et al.* 1997), resulting in lower conductivity, although this may be an adaptation to winter cold (Cavender-Bares *et al.* 2005). In contrast, *Q. ilex* have wider xylem conduits compared to sympatric Mediterranean evergreen species from other genus (Martínez-Vilalta *et al.* 2002), and thus transpire at higher rates than those. Sap flow during summer drought, however, must be substantially reduced (Martínez-Vilalta *et al.* 2003; Barbeta *et al.* 2012) to avoid xylem cavitation, because the xylem anatomy of *Q. ilex* is not more resistant to embolism than that of deciduous *Quercus* species in temperate forests (Choat *et al.* 2012). Once water reaches the canopy, sclerophyllous leaves with high specific mass and thickness promote the conservation of cellular water (Bartlett *et al.* 2012). Together, the morphologies of roots, xylems, and leaves partly determine the water-use strategy of *Q. ilex* and thus its performance under drought.

When morphological adaptations to dry environments are not sufficient to avoid completely any physiological water stress, plants activate several physiological mechanisms,

most relevantly stomatal control (Martínez-Vilalta *et al.* 2014). *Q. ilex* may be the most isohydric species of the genus, and more isohydric than sympatric sclerophyllous shrubs, according to a recent review (Martínez-Vilalta *et al.* 2014). Accordingly, it will reduce stomatal conductance to avoid conductivity losses at less negative water potentials than other congeneric or co-occurring species. Indeed, *Q. ilex* uses water very conservatively during summer drought, with almost completely closed stomata at midday (Ogaya, Llusà, *et al.* 2014), and the trees consequently have a lower risk of xylem embolism. Nevertheless, embolism in *Q. ilex* xylem is not rare and can occur either during summer drought (Aguadé *et al.* 2015) or winter frost (Cavender-Bares *et al.* 2005). The soluble fraction of non-structural carbohydrates has been hypothesised to be used for maintaining the integrity of the vascular system by osmotic regulation (Sala *et al.* 2012). While it has not been observed in *Q. ilex*, the unloading of sugars from the phloem to embolised xylem vessels has been proposed as a possible mechanism of embolism repair (Nardini *et al.* 2011). Xylem refilling in Mediterranean plants such as *Olea europaea* and *Laurus nobilis* has recently been shown to be ion-mediated (Trifilò *et al.* 2014). Interestingly, the proportion of soluble sugars increases during summer drought in the branches (Rosas *et al.* 2013) and leaves (Rivas-Ubach *et al.* 2014) of *Q. ilex*, suggesting a role for these solutes in drought resistance. Rivas-Ubach *et al.* (2014) have also found an increase in foliar potassium concentration in summer, which may also be related to osmotic regulation (Babita *et al.* 2010).

Common with a number of other species, in *Q. ilex* 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 (Mitrakos 1980, Lempereur *et al.* 2015). The phenology of primary, secondary, and reproductive growth are thus linked to summer drought and winter cold. A bimodal pattern of stem growth that peaks in spring and autumn is common in *Q. ilex* (Montserrat-Martí *et al.* 2009, Gutiérrez *et al.* 2011). The onset of the summer cessation of growth has been 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 summers are sufficiently wet (Albuixech *et al.* 2012). Primary growth, including foliar flushing (Ogaya and 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). *Q. ilex* thus takes advantage of periods with favourable conditions for growth (Albuixech *et al.* 2012). In contrast to deciduous Mediterranean oaks, it has a peak of growth in autumn, either of the stems (Corcuera *et al.* 2004, Gutiérrez *et al.* 2011, Albuixech *et al.* 2012) or the leaves (Ogaya and Peñuelas 2006). In

addition, several cohorts of buds up to 3-4 years old are maintained in the branches, which may burst in years with favourable conditions (Alla *et al.* 2012). Overall, *Q. ilex* has large phenological plasticity of growth-related traits to cope with the marked seasonality in water supply of Mediterranean ecosystems.

The various morphological, physiological adaptations to drought and phenological patterns in response to water shortage manifest themselves at a range of time scales (Table 1). Above-ground to below-ground biomass ratios reflect the compound effects of environmental conditions experienced during the lifetime of individuals. For example, contrasting micro-topographical conditions can produce morphological differentiation between genetically closely related populations in terms of allocation to roots and above-ground organs (Canadell *et al.* 1999). The environmental conditions experienced during an individual's lifetime thus contribute to defining its structure and functioning. However, foliar and xylem morphology are determined mainly by the environmental conditions of the relevant periods of development - spring for leaves (Ogaya and Peñuelas 2006) and summer for xylem (Abrantes *et al.* 2013). Physiological mechanisms can be activated at even shorter times; the degradation of starch to sugars occurs on a daily basis (Patakas *et al.* 2002), and stomatal response, mediated by ... is almost instantaneous (Mcadam and Brodribb 2012). Most of these mechanisms are transient responses and can be reversed when the limitation disappears (*sensu* Wolkovich *et al.* (2014), Table 1). For example, at the leaf-level, stomata can open fully again once soil moisture availability increases, or leaves initiated in wet years may grow less sclerophyllic than those in dry years. However, other features, such as modified xylem anatomy (e.g. the vessel area and density) 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). In those traits that form over longer periods, the plasticity to respond to sudden climatic extremes is thus limited.

Response of *Q. ilex* to extreme drought

Summer droughts in Mediterranean ecosystems occur every year but with high inter-annual variability in intensity and duration. However, as climatic change is increasing temperatures and their variability (IPCC 2013), a positive *trend effect* (Jentsch *et al.* 2007) is likely to increase the intensity of summer droughts. Extreme droughts, in addition to the cyclic summer droughts, are also expected to become more frequent in the Mediterranean Basin (IPCC 2013; Wolkovich *et al.* 2014). Droughts occurring in or extending into the typically wet seasons in the

Mediterranean Basin (spring and autumn) have recently increased and these extreme drought events may have larger impacts than more intense summer droughts, because spring and autumn are the optimum periods for growth in holm oaks (Camarero *et al.* 2015).

Extreme droughts are episodes that (i) represent statistically rare events regarding their intensity, duration, and/or timing and that (ii) cause negative effects that involve at least partial crown damage or an increase in tree mortality rates above the baseline levels. The available literature on drought-induced dieback of holm oaks has reported a great diversity of drought characteristics. Several extreme droughts have been recorded recently in the Iberian Peninsula. For example, the 1993-1995 drought in the north-eastern Iberian Peninsula lasted for 10 months, during which time precipitation was only half of the historical average (Peñuelas *et al.* 2000, Lloret *et al.* 2004). In 2005 drought in the southern Pyrenees was very intense and aseasonal, peaking in the spring, while another in the same region in 2012 was characterised by less intense but lasted longer (Camarero *et al.* 2015). Other, more local droughts - such as the drought in southern Catalonia in 2011 - had severe effects on the vegetation but were restricted to smaller geographic areas (Poyatos *et al.* 2013, Ogaya, Barbeta, *et al.* 2014). All these events had in common the rarity of the historical climatic series, either in drought duration, intensity, and/or timing. *Q. ilex* populations will suffer important alterations in their 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 of Q. ilex.

Leaf shedding occurs in *Q. ilex* simultaneously with bud burst (Ogaya and Peñuelas 2006), whereby some of the old leaves are abscised and substituted by new leaves. Leaf shedding can also be a short-term response to drought, whereby plants adjust their transpiratory area to decreased water availability. Leaf abscission may be able to act as a safety valve to avoid extensive embolism in the xylem of stems, where carbon investment is more expensive (Bucci *et al.* 2012, Pivovarov *et al.* 2014). Some level of leaf shedding during a 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 relations. High foliage loss, however, reduces photosynthetic capacity and may compromise the survival of trees in the long term (Galiano *et al.* 2012). Extensive canopy loss is thus the first indicator of drought-induced forest dieback.

The crown defoliation of *Q. ilex* stands in the last 15 years has been correlated with a progressive increase in water stress (Carnicer *et al.* 2011) and with extreme droughts (Peñuelas *et al.* 2000, Lloret *et al.* 2004, Galiano *et al.* 2012, Ogaya, Barbeta, *et al.* 2014,

Barbeta *et al.* 2015). These events were noticeable by the browning of leaves and the desiccation of entire branches, and, in some cases, of all above-ground organs of an individual. Temperature has increased in most parts of the world over the last 100 years, but no such consistent trend has been observed for precipitation (IPCC 2013). As a result, the vulnerability of forests to drought is increasing because hotter temperatures enhance drought severity (Allen *et al.* 2015). Therefore, the inclusion of temperature in the calculation of a drought index is required for comparing the conditions of current and past droughts (Vicente-Serrano *et al.* 2010).

The timing of crown defoliation or stem mortality triggered by drought has been variable (Table 2); some events (1994 and 2011) were produced by long-lasting water deficits (Lloret *et al.* 2004; Barbeta *et al.* 2015), whereas others caused a much more negative water balance but for shorter periods (2005) (Barbeta *et al.* 2013). Stem mortality after the event reported by Barbeta *et al.* (2013) was 5.4% (short-term acute water shortage) , but the prolonged drought in 1994 caused 51.3% of stem mortality in stands over the same bedrock, suggesting that *Q. ilex* has more tolerance to short-term and acute droughts. 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 as the lack of resprouting the year after the drought event, because the isolation and identification of genets was not possible. In contrast, Galiano *et al.* (2012) categorised trees (multi-stemmed individuals) into classes of crown damage and assessed mortality at the genet level in another site. Harmonising the metrics of the effects of drought on forests to provide comparable datasets would be desirable in future studies and useful for assessing large-scale patterns.

Drought characteristics alone (represented in Table 2 by the multi-scalar drought index) do not appear to fully explain the variability in crown defoliation or stem mortality. The effects of a drought event depend on 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 a multi-stemmed individual but increases individual stem mortality and defoliation (Galiano *et al.* 2012). Taller trees (Lloret *et al.* 2004) with larger basal areas (Galiano *et al.* 2012; Barbeta *et al.* 2013) are the least affected during these events. The form of tree growth (multi-stems, in particular) is affected by management practices and thus

has an impact on drought survival. Topography is another critical factor that affects the levels of mortality and defoliation in *Q. ilex*. The fluctuations of climate are offset, to some extent, by the deep root system of *Q. ilex* (Canadell *et al.* 1999), but rooting depth can be spatially heterogeneous. Soil is rockier, shallower, and has a lower capacity to store water on steep slopes than on flat ground or in valley bottoms, so the survival of the trees varies across the landscape (Galiano *et al.* 2012). Similarly, access to groundwater is critical for surviving summer drought (Barbeta *et al.* 2015), and the mortality rates during an extreme drought are substantially higher on bedrock types that do not allow the penetration of roots in fissures (Lloret *et al.* 2004).

Trends of crown defoliation and their relationship with drought characteristics

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 carried out a correlative analysis, using 25 years of data on crown defoliation in the Iberian Peninsula (ICP (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests), (Carnicer *et al.* 2011, de la Cruz *et al.* 2014)) and the global Standardised Precipitation Evapotranspiration Index (SPEI) database (Vicente-Serrano *et al.* 2010). Using an updated version of the data analysed by Carnicer *et al.* (2011) but only for *Q. ilex*, we correlated plot-level data on the annual relative change of crown defoliation with gridded (0.25°) SPEI values at all different months and temporal scales (periods ranging from 1 to 24 months). The correlation heat map presented in Figure 3 indicates that the SPEI and the annual relative change in defoliation level were best correlated for SPEI scales longer than seven months and during summer months (June, July, and August). More particularly, June SPEI-23 (which is calculated using the water balance, P-PET, of the 23 months prior to June) 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.

The sensitivity of *Q. ilex* to drought indicated by crown defoliation was similar to the sensitivity of secondary growth, indicated by tree-rings (Pasho *et al.* 2011, Camarero, Sangüesa-Barreda, *et al.* 2015). The correlation between crown defoliation and the SPEI became stronger by June and when accounting for the water balance of the previous winter and spring (Figure 3). In those tree-ring studies (Gea-Izquierdo *et al.* 2009, Pasho *et al.* 2011, Camarero, Sangüesa-Barreda, *et al.* 2015), growth rates were correlated highest with the water balance in summer. In contrast, crown defoliation was better correlated with longer periods of the water balance (i.e. timescales of the SPEI longer than seven months), spanning

nearly 2 years. This is in agreement with previously reported drivers of defoliation in Iberian tree species at the large-scale, which found that the duration of drought is a statistically significant factor (de la Cruz *et al.* 2014). The good correlations of defoliation with the SPEI over long periods are likely to be a result of tapping of groundwater by *Q. ilex* (Reichstein *et al.* 2002, David *et al.* 2004, Barbeta *et al.* 2015). Certainly, the level of groundwater reserves responds to the accumulated water balance of longer periods than the level of surface soil moisture (Vicente-Serrano *et al.* 2010). Surface soil moisture is subject to strong short-term fluctuations responding to single events of rainfall and to the atmospheric evaporative demand. In contrast, deeper soil layers containing groundwater are recharged during wet and cold periods and are less affected by evaporation. Growth is mainly dependent on the climate of the growing climate that determines the surface soil moisture whereas, groundwater reserves that have accumulated over periods of more than one year are crucial to survive those droughts that deplete surface soil moisture completely.

Winter rainfall may also be related to the replenishment of tree carbon reserves. Sap flow is higher in winter than in summer, but trees do not allocate carbon to above-ground growth in winter (Sánchez-Costa *et al.* 2015). Thus carbon assimilated during winter is likely to be directed to other functions, such as defence or cold hardening. Such winter carbon could be also stored in the form of starch that may then be remobilised and used during summer drought events 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 1990s peaked during the droughts of 1994-1995 (Figure 4a). The defoliation levels have since stabilised at around 20%. The SPEI values indicated that droughts of similar intensity occurred after this peak (e.g. 2001 and 2005) but did not trigger larger increases in defoliation than those in the mid-1990s, indicated by the relative change in crown defoliation (Figure 4b). These data suggest that increasing aridity has led to a diminished canopy cover, but also that once a certain level of defoliation was reached, droughts of equal intensity did not further decrease leaf area. A reduction in leaf area to sapwood area and/or in the ratio of above- and below-ground biomass prompted by droughts could be maintained in the long term. Forests have been hypothesised to acclimate through stabilising processes at the individual (Barbeta *et al.* 2015) and community levels (Lloret *et al.* 2012, Barbeta *et al.* 2013). Other studies, however, have suggested 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 below) and long-term studies on the recovery of affected stands following extreme drought events can help to elucidate whether it is the case of stabilising processes or rapid

ecosystem change. In addition to the likely acclimation of forest leaf area as a result of reduced water availability, a potential sampling-effect on the quantification of defoliation should not be ruled out. Defoliation within the ICP forests programme (Carnicer *et al.* 2011, de la Cruz *et al.* 2014) is estimated in relation to a reference tree supposed to be completely healthy. Over the years, these reference trees may have also suffered some defoliation while still being the relatively healthiest at the plot-level. This would have led to the underestimation of defoliation in the most recent years.

Resprouting after drought-induced biomass loss and the legacy of droughts

Q. ilex has the capacity to resprout from pre-existing buds after a disturbance that causes the loss of biomass (Espelta *et al.* 1999). Resprouts can emerge either from above-ground (canopy) or below-ground (lignotuber) buds, allowing a rapid recovery of the photosynthetic area after a disturbance. Fire, overgrazing, firewood extraction, and coppicing have been the most common disturbances triggering resprouting in *Q. ilex* stands historically, and extreme droughts will likely increasingly frequent in the coming years, and *Q. ilex* will likely resprout in response. Extreme droughts cause low tree mortality compared to other disturbances such as wildfires (Espelta *et al.* 1999). However, extreme droughts can impact roots by causing hydraulic dysfunction (Anderegg *et al.* 2012, Meinzer and McCulloh 2013) and deplete below-ground reserves of non-structural carbohydrates (NSC) (Galiano *et al.* 2012) and weaken the resprouting capacity of trees. Still, *Q. ilex* has shown full canopy recovery within a year after an extreme drought (Ogaya, Barbeta, *et al.* 2014) that induced extensive branch desiccation (Liu *et al.* 2015). The presence of long-lived apical buds confers *Q. ilex* the capacity to resprout from stems and twigs (Table 1, Alla *et al.* (2012)). The resilience of trees to drought relies on these above-ground meristemic tissues that are not usually damaged by drought.

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. Forty percent 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 of their 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, which has been associated with the levels of NSC in lignotubers, the below-ground swollen structures that accumulate starch and form buds (Canadell *et al.* 1999). Galiano *et al.* (2012) were the first to

demonstrate that the resilience of crown condition 7 years after an extreme drought was associated with the amount of NSC 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, confirming this organ as an important NSC reservoir for overcoming the effects of drought. The period of return of extreme drought appears to be a key factor for determining the resilience of *Q. ilex* to these droughts. If lignotuber NSC levels are not fully recovered by the following drought, the survival of the tree will be compromised. *Q. ilex* needs an estimated 20 years to fully replenish the NSC levels of its lignotubers (López *et al.* 2009), so the current frequency of extreme droughts (see time-lapse between droughts of similar intensity in Figure 2) is already threatening the future resilience of *Q. ilex* stands. These extreme droughts will have tangible effect on the levels of NSC reserves only if they result in extensive or total loss of the photosynthetic area, impairing the use of newly assimilated carbon. It is known that the levels of NSC reserves are not constrained by the source activity (*i.e.* the photosynthetic activity) and that carbon acquisition can be maintained during periods of drought or low temperatures (Körner 2003). Therefore, droughts that drastically reduce or stop growth but do not produce leaf shedding will have little effect on the NSC reserves and consequently, the capacity of a tree to resprout in response to drought will remain intact.

The hydraulic deterioration produced by drought-induced xylem cavitation accumulates and persists over several years in aspen trees (Anderegg *et al.* 2013). This so-called cavitation fatigue has been shown to increase the vulnerability of the xylem to embolism of previously drought-affected stems in several species (e.g. *Populus angustifolia*, *P. tremuloides* or *Helianthus annuus*), but in others (e.g. *Acer negundo* and *Alnus incana*) stems do not suffer any change in vulnerability (Hacke *et al.* 2001). Other woody plants (*Ligustrum vulgare* and *Viburnum lantana*) are even able to acclimate to drought by increasing their resistance to embolism (Beikircher and Mayr 2009). This does not seem to be the case of *Quercus ilex*, as was shown by a long-term drought experiment where its vulnerability of xylem to cavitation has slightly but not significantly increased (Limousin, Longepierre, *et al.* 2010). During extremely dry years, such as 1994 in the Iberian Peninsula, the vessels produced by *Q. ilex* present a smaller diameter than during average years (Corcuera *et al.* 2004). Smaller diameters imply a reduction in the hydraulic conductance and this persists for several years (Table 1, (Martínez-Vilalta *et al.* 2003)). It is highly likely thus that *Q. ilex* trees will carry over a drought legacy not only in their transpiring area and the levels of NSC but also in their hydraulic function. This legacy will negatively predispose trees to maintain an adequate

hydraulic functioning by reducing the potential conductance and increasing the vulnerability to embolism of xylem in the stems.

In addition to the drought legacies related to carbon and water use, drought may also predispose trees to suffer attacks from insects and fungal pathogens (Desprez-Loustau *et al.* 2006, Oliva *et al.* 2014). Importantly, biotic agents are considered a contributing factor in drought-induced forest dieback (Worrall *et al.* 2010). The combined effect of fungal infections and drought-induced defoliation has been associated with lower growth, sapwood depth and reduced conductivity in *Pinus sylvestris* individuals (Aguadé *et al.* 2015). Consequently, infected trees will have a lower capacity to recover from drought, exacerbating the hydraulic dysfunction and the NSC depletion. A common fungal pathogen that causes defoliation and mortality in *Q. ilex* is *Phytophthora cinnamomi* (Sánchez *et al.* 2002). There is yet no information available on the potential initiating or contributing role of this fungal pathogen in drought-induced forest dieback of *Q. ilex* or during the recovery after these events. Nonetheless, an experimental study conducted with *Q. ilex* seedlings demonstrated that plants subjected to drought were more likely to die after being inoculated with this pathogen (Corcobado *et al.* 2014). Interestingly, the most damaging treatment included a prior water-logging treatment, followed first by the inoculation and then by drought treatment. Hence, the sequence of the meteorological extremes matters, as it may interact with the activity of biotic agents and amplify the damage of droughts. For this reason, it is required to conduct experimental and field studies to understand the role of pathogens previously, during and after drought-induced forest dieback of *Q. ilex*.

The role of forest structure in episodes of forest dieback

The spatial variation in growth and mortality rates of some Mediterranean tree species is largely related to forest structure, more so than to climate (Vilà-Cabrera *et al.* 2011). Indeed, the demographic response by forests to climate disturbances (e.g. extreme droughts) is determined by structural characteristics, such as tree density and stand basal area (Lloret *et al.* 2012, Carnicer *et al.* 2014). The abandonment of forest management practices over the last decades has resulted higher stand densities, which, in turn, increased competition for resources (Vayreda *et al.* 2012). The widely distributed *Quercus ilex* forests are a good example of this process. Coll *et al.* (2013) showed competition constraints on growth illustrated by lower growth rates of *Q. ilex* in stands with high basal area, which is supported by less noticeable effects of drought on tree water use in low density stands compared to high density stands (Moreno and Cubera 2008). In the Iberian Peninsula, many forests are recovering from

overexploitation during the 1950s and 1960s, so they are relatively young forests that are increasing in biomass (Vayreda *et al.* 2012). Along with the accumulation of biomass, there is a progressive but slow self-thinning (López *et al.* 2009). Extreme droughts may partly accelerate self-thinning by killing stems of multi-stemmed trees and by suppressing the smaller individuals (Galiano *et al.* 2012; Barbeta *et al.* 2013). Consequently, the stands with high stem density and basal area (and thus with a potential larger degree competition for water) are a priori more prone to suffering large impacts during extreme drought episodes. These impacts may involve extensive biomass loss and the death of individuals and thus, the surviving trees will have less stems per individual, and the stand basal area will be concentrated in a reduced number of individuals. It is likely therefore that the forest structure resulting from a drought impact will present more resistance to drought episodes in the long-term. The convergence towards structures adapted to the new, lower availability of water and more recurrent drought episodes can be expected. Research addressed to predict which the most suitable forest structure is for each sub-type of climate and topography should be a primary goal in order to adapt management practices to the ongoing climate change.

Sequence of responses 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 (north-eastern Iberian Peninsula) and the other in 2003 in the Puébadon State Forest (southern France). Both experimental designs excluded throughfall over ca. 30% of the plot surface (Limousin, Misson, *et al.* 2010), and runoff at the Prades site was intercepted by ditches along the top edges of the plots (Ogaya and Peñuelas 2003). These experiments have produced a large body of information on the responses of *Q. ilex* forests to imposed moderate but continuous drought, from leaf-level physiology to ecosystem-level demography and fluxes of energy and matter. In fact, the drought imposed by this throughfall exclusion matches the predictions of increasingly drier conditions and the decrease in soil moisture for the western Mediterranean Basin for the next decades (IPCC, 2013). 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 coupled climate-vegetation models. Seedlings and adults from the same species may also have different water-use strategies (Mediavilla and Escudero 2004), so the results of experiments on seedlings may not be comparable with those on adults. Furthermore, the strength of the effect on ecosystems of the same level of stress induced by an pulse type 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 from these two experimental systems (Table 3; Figure 5).

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). An analogous reduction of ca. 28% throughfall significantly increased water stress in the drought plots of Puébachon, as it induced a consistent decrease in predawn leaf water potentials of droughted plants (Limousin *et al.* 2009, 2012, Limousin, Misson, *et al.* 2010). Predawn water potential is often considered a surrogate of soil water potential (Ritchie and Hinckley 1975, Barbeta *et al.* 2012). Midday leaf water potentials, though, did not generally differ between treatment and control (Limousin *et al.* 2009, Ogaya, Barbeta, *et al.* 2014, Barbeta *et al.* 2015). As a short-term response to an increase in xylem tension produced by lower soil-water potentials, 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), shortly after the treatment had commenced (Limousin *et al.* 2009), showing a rapid acclimation of plant functioning to the new drier conditions. The consistent decrease in total transpiration was not accompanied by significant modifications of foliar gas exchange, neither the short term (after 1-4 years of drought treatment (Limousin, Misson, *et al.* 2010, Ogaya, Llusià, *et al.* 2014), nor the long term (after 14 years (Ogaya, Llusià, *et al.* 2014, Sperlich *et al.* 2015), indicating 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.* 2015). These authors have provided evidence that mesophyllic conductance, in addition to stomatal conductance, can acclimate to drought conditions by facilitating the diffusion of CO₂ 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.

The decrease in transpiration reported by Limousin *et al.* (2009) may seem inconsistent with the results of other drought treatments (Martin-StPaul *et al.* 2013, Ogaya,

Llusà, *et al.* 2014), but the droughted plants at both the Prades and Puébachon experimental sites had reduced leaf areas after 3 years (Ogaya and Peñuelas 2006) and 7 years (Limousin *et al.* 2012) of treatment. This reduction was not accompanied by a reduction in sapwood area, and thus the ratio of leaf to sapwood area was decreased at the shoot level (Limousin, Longepierre, *et al.* 2010, Limousin *et al.* 2012), 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 observed in Prades (Liu *et al.* 2015). Indeed, leaf-specific conductivity has increased in the long term in droughted plants as a consequence of 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, individual leaf area and hydraulic conductivity and stem anatomy and hydraulic properties have changed little. Root and branch xylem vulnerability and stem specific conductivity did not differ significantly in the trees at Puébachon after 6 years of the drought treatment (Limousin, Longepierre, *et al.* 2010). 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 & Peñuelas 2006), and leaf-to-shoot allometry (Limousin, Longepierre, *et al.* 2010) to drier conditions 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 2 years (Ogaya *et al.* 2003) and 5 years (Ogaya and Peñuelas 2007a) 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 and Peñuelas 2007b). In contrast, stem growth was never affected in Puébachon after 9 years of drought treatment. Indeed, a recent analysis of a longer data series (11 years) in Prades found that the differences in stem growth tended to dampen over time (Barbeta *et al.* 2013), which has been linked to a potential increase 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 has led to lower competition for water. Furthermore, the physiological and morphological mechanisms of acclimation described above may have provided to droughted plants a greater resistance to naturally

590 occurring extreme droughts, such as those in 2005-2006 and 2011 in Prades (Ogaya, Barbeta,
591 *et al.* 2014). Additionally, the self-thinning related to extreme drought and the drought
592 treatment kills those stems that had previously lower growth rates (Barbeta *et al.* 2013).
593 Consequently, stems with higher growth rates would have been selected by self-thinning in the
594 drought plots, influencing the difference between treatments.

595 The responses to drought after a decade or more after the onset of the experiments
596 differed slightly from those early in the experiments. *Q. ilex* has shown it to be able to display
597 some mechanisms of acclimation to drought, which would be useful for its persistence under a
598 future drier climate. Overall forest productivity tended to decrease (Liu *et al.* 2015) with the
599 forests reducing their biomass in accordance with the artificially imposed ca. 30% reduction in
600 throughfall. Less dense forests with sparser canopies would thrive under the projected 15%
601 decrease in soil moisture in the Mediterranean Basin (IPCC 2013), but as we described above,
602 the effects of extreme droughts are more severe than those of drought experiments that
603 substantially reduce the water availability permanently. Because both an increase in the
604 frequency of extreme drought events and generally lower water availability are likely to occur
605 in the future, continuing investigating the interaction between extreme drought and the
606 acclimation mechanisms of *Q. ilex* identified by the long-term experiments is necessary. One
607 could expect that a forest adapted to lower water availability would be more resistant to an
608 extreme drought. Trees suffering the effects of a long-term drought experiment, however,
609 stored similar NSC than the control trees (Rosas *et al.* 2013) so their resilience would be
610 equivalent after droughts that cause total canopy loss. In contrast, groundwater contributed
611 less to transpiration in the trees in the control plot during the drought of 2011 (after 12 years
612 of drought treatment), which indicated a relatively stronger depletion of groundwater reserves
613 (Barbeta *et al.* 2015). These reserves respond to the water balance over longer periods, so the
614 chronic water stress imposed by the experiment would prevent them from being ready and
615 available to minimise the negative effects of drought.

616 **Concluding remarks**

617 The objective of this review was to shed light on the key factors that determine potential
618 changes in the distribution, structure, and function of *Q. ilex* forests in response to increasing
619 droughts, either in the form of extreme events or as a sustained decrease in water availability.
620 This is a critical issue not only for *Q. ilex* forests but also for other ecosystems dominated by
621 resprouting angiosperms in all the Mediterranean regions of the world that share many of the
622 life-history traits and physiological mechanisms presented in Table 1. Analysing the responses

to droughts along different timescales, some of the key determinants of drought responses 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 *Q. ilex*. 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.* 2013). This seasonal compensation is not only relevant for species sharing similar phenological patterns and life-history traits with *Quercus ilex* such as Mediterranean woody angiosperms, but also for winter- and drought-deciduous species in temperate and subtropical environments.

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 could be weakened. This weakened resprouting capacity has been observed in the field following episodes of extreme drought. However, the permanent reduction of water availability imposed by field drought experiments does not seem to reduce lignotuber reserves and thus the resprouting capacity (Rosas *et al.* 2013). Still, this does not directly imply that the resistance to extreme drought events associated with the current climatic change remains unchanged after this permanent reductions of water availability. 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 has estimated that growth could be reduced for 4 years after a single event (Anderegg *et al.* 2015). In addition, the broad-scale analysis of the drought characteristics that triggered *Q. ilex* forest dieback presented here shows that the duration of drought is the key parameter in determining the severity of drought effects on *Quercus ilex* forests. The multi-year dynamics of non-structural carbohydrates and/or the accumulated hydraulic dysfunction may be behind the fact that persistent droughts cause more crown defoliation and mortality on *Q. ilex* forests than droughts of short duration regardless of their intensity.

Individuals and plant communities have an extraordinary capacity to accommodate to environmental changes. This is especially true for *Quercus ilex* and the forests that it dominates. Such long-lived woody species have thrived in the same geographical area at least since the Middle Würm (Reille and Pons 1992), facing contrasting climatic conditions. A characteristic that may have allowed such plants to survive these climatic periods is their extensive root system, but when the access to below-ground water pools is impaired by long-lasting droughts forest dieback may occur (Barbeta *et al.* 2015). To sum up, 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 *Q. ilex* forests and perhaps other Mediterranean vegetation types. We recommend that future research should keep on addressing the combined effect of consecutive extreme droughts and drier average conditions on the structure and function of plant communities, but with a special emphasis on the resilience after crown damage and on the access to the vital long-lasting deep water pools.

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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

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	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 muchbrowning
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
Camarero et al 2015	2011	Formicó del Bajo	-1.28	-0.64	-1.25	-1.50	-1.37	-0.56	-0.89	Calcareous	

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	Mesophylllic conductance	Unchanged	13	Sperlich et al. unpublished results.
Leaf	Carbon relations	Night respiration	Unchanged	13	Sperlich et al. unpublished results.
Leaf	Carbon relations	Ratio mesophylllic/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 mesophylllic 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)
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)
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)

Figure captions

Figure 1. Monthly average difference between the monthly precipitation and the monthly potential evapotranspiration (the climatic water balance) for two forests dominated by *Quercus ilex*. Data was obtained from meteorological stations located at Torners Valley in the Prades Mountains (930 m a.s.l.; 1998-2014) and at Viladrau in the Montseny Mountains (950 m a.s.l. 1995-2014). Potential Evapotranspiration is calculated with the *SPEI* package in R, based on monthly temperature and incoming solar radiation (Thornthwaite 1948). Error bars are the standard errors of the means.

Figure 2. Map of the current distributional range of holm oak (*Quercus ilex*) in the Mediterranean Basin.

Figure 3. Correlation heatmap between the annual relative change in crown defoliation and the SPEI values for Iberian *Quercus ilex* forests (1987-2013, 233 plots). The SPEI duration on the x-axis represents the number of months used to calculate the water balance (e.g. an SPEI duration of 3 in June is calculated using the water balance of the previous April and May and the June itself). The y-axis is the month in which the calculation of SPEI departs from. The panel on the right top indicates the correspondence of the colours in the heatmap with the Person's r coefficient of the correlation between SPEI and defoliation.

Figure 4. Time courses of (a) accumulated crown defoliation and (b) annual change in crown defoliation relative to defoliation from the 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).

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. The rectangles that surpass 12 years indicate effects that lasted till the present day of the experiment. The position on the x-axis represents the approximate time of appearance. Rectangles with dashed contour lines indicate potential effects that require further study.

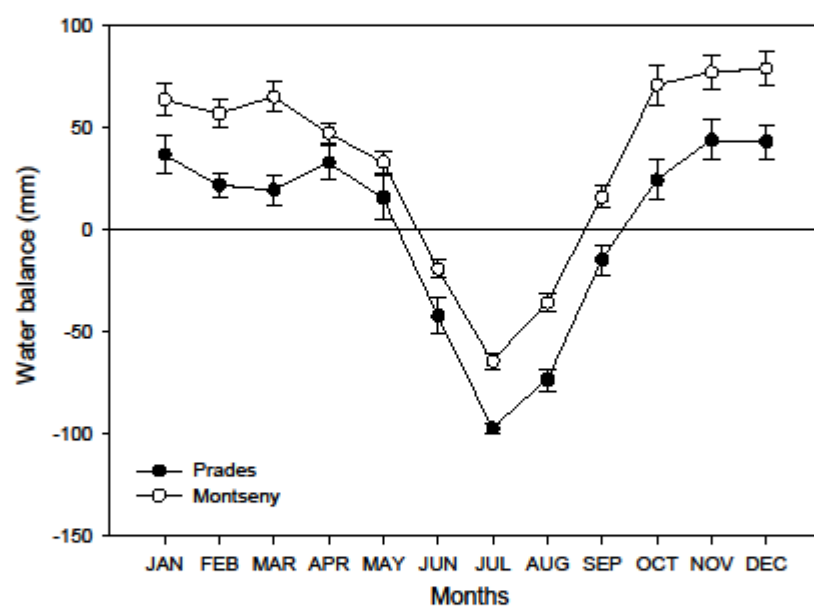


Figure 1

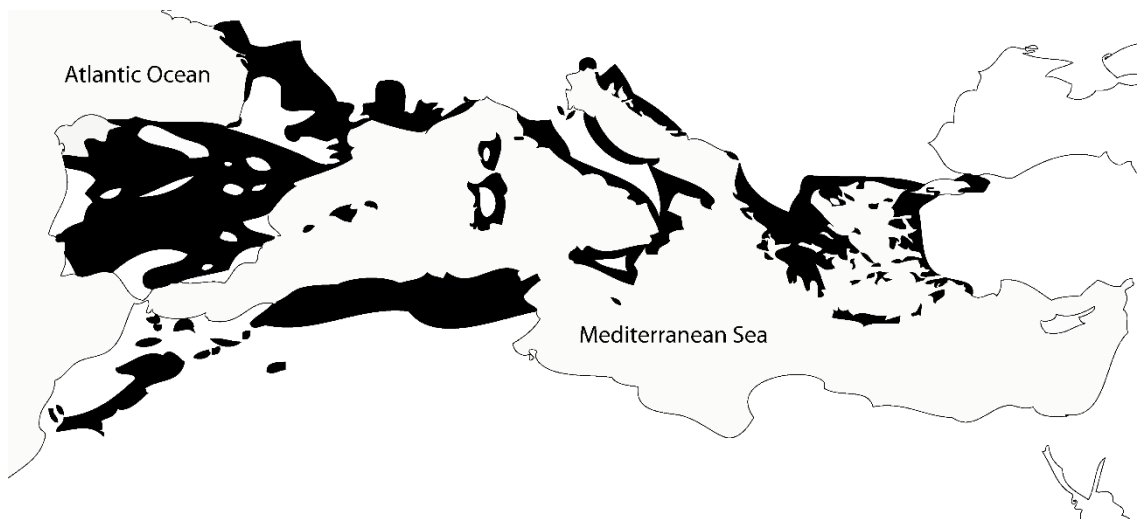


Figure 2

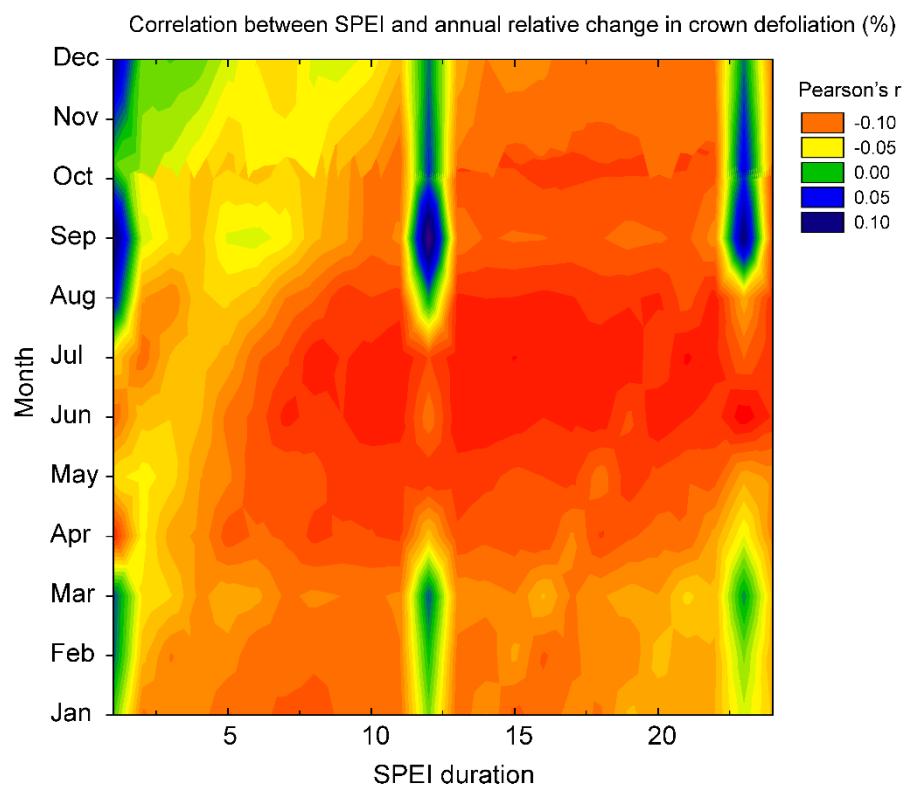


Figure 3

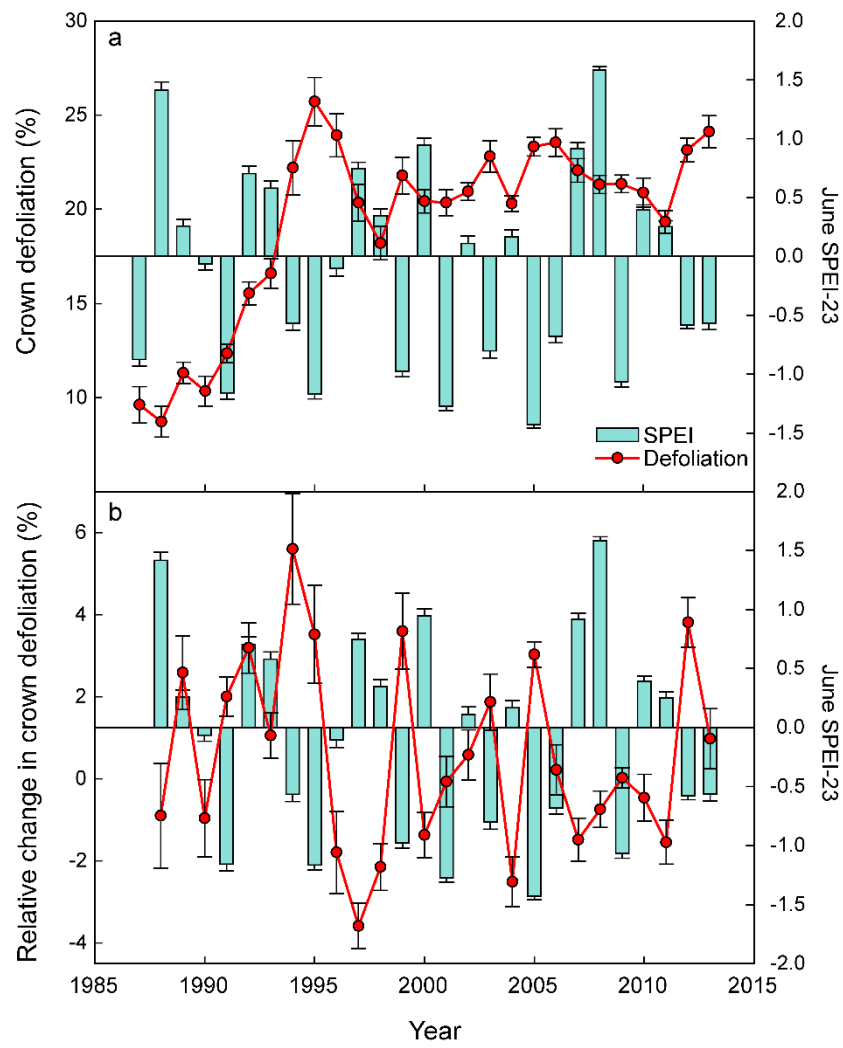


Figure 4

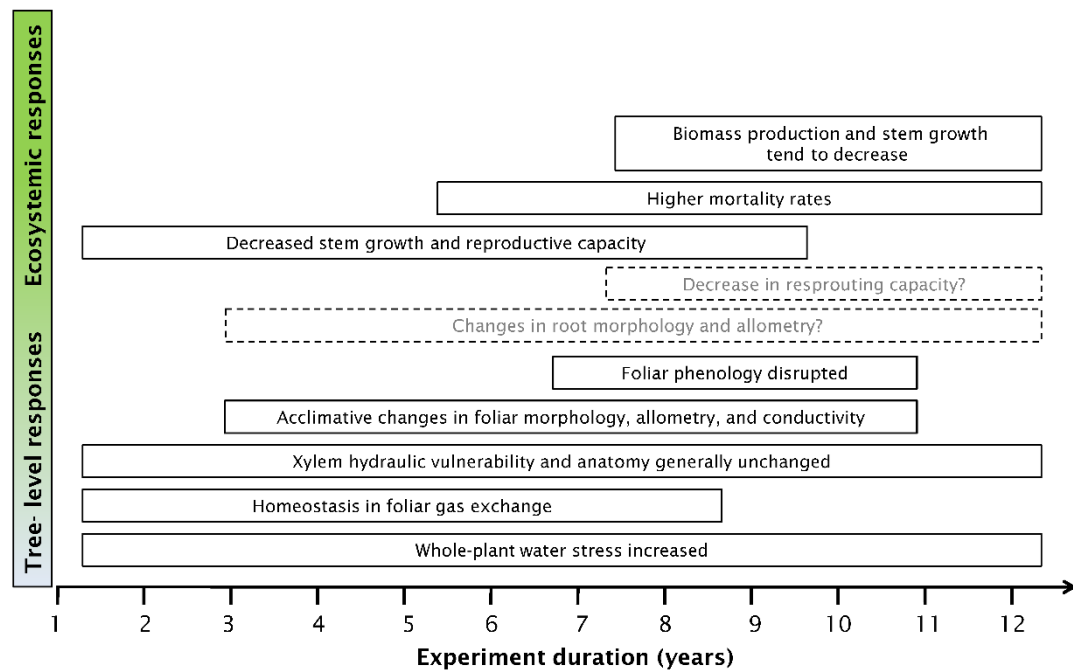


Figure 5