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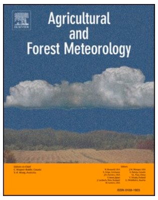

Die-off after an extreme hot drought affects trees with physiological performance constrained by a more stressful abiotic niche

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ABSTRACT

Forest die-off has become more frequent under climate change, making crucial to understand the physiological mechanisms of forest mortality. We analyzed *Quercus ilex* die-off after a record-setting hot drought in two open woodlands without previous signs of decline. To understand physiological performance of trees we compared observations of radial growth dynamics, xylem hydraulic architecture, sapwood nutrient content and δ^{13}C and δ^{18}O in wood cellulose, with model simulations of tree carbon and water fluxes. We also assessed climate-growth responses across a *Q. ilex* network including sites with and without increased mortality. Past extreme droughts triggered multidecadal growth declines consistently in dead trees, which suggests long-term vulnerability of dead *Q. ilex* independent of the mortality process or causal factor. In the two studied woodlands, trends in xylem cellulose δ^{18}O suggest that both dead and surviving trees increasingly relied on deeper water sources as stress increased under climate change. Dead and surviving trees followed different functional strategies reflecting chronic abiotic niche-related differences in stress. Dead trees invested similar or larger amounts of carbon in xylem reservoir tissues and less in xylem conductive tissues compared to surviving trees, yet exhibited an impaired nutrient status. Xylem hydraulic architecture differed in surviving and dead trees. The latter formed more efficient xylems with higher vessel density and larger or similar vessel sizes. The isotopic proxies suggested that dead trees systematically maintained tighter stomatal regulation and were forced to rely on deeper water likely sourced from the fractured granite bedrock. Isotopic proxies and simulations of water and carbon dynamics further suggest that surviving trees benefitted from soils with higher water-holding capacity contributing to buffer water stress. Dead trees expressed a functional paradox. Although their long-term functional strategy successfully coped with higher baseline water stress, they failed to withstand the additional increase in stress during an unprecedented hot drought.

1. Introduction

Forest mortality triggered by compound heat and drought events (‘hot-drought’) has increased worldwide (Choat et al., 2018; Anderegg et al. 2020; McDowell et al. 2020; Hartmann et al. 2022). Heatwaves and droughts can drive conversion of forest communities through reductions in plant density and changes in species composition (Jacobsen and Pratt 2018; Brodribb et al. 2020; Hammond et al. 2022; McDowell et al. 2022). It is necessary to assess the abiotic and biotic stress factors that drive the spatial distribution of mortality and the temporal expressions of different mortality syndromes (Anderegg et al. 2015; McDowell et al. 2018). Soil characteristics determining soil water availability greatly mediate spatial distribution of species mortality under drought (Lloret et al. 2004; Tai et al. 2017; Johnson et al. 2018; Preisler et al. 2019; Bachofen et al. 2024). Increased mortality can be expressed in both higher diffuse annual mortality and/or higher frequency of episodic or sporadic die-off events after major disturbances. Increased mortality can be focused on individuals and species with less resilient strategies (Meddens et al. 2015; Anderegg et al. 2020; McDowell et al. 2020). Understanding the functional strategies that increase tree resilience to

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heat and drought requires linking tree mortality with the underlying physiological mechanisms of the coupled carbon and hydraulic functions in the tree (e.g. Sevanto et al. 2014; Mencuccini et al. 2015; Hammond et al. 2019; McDowell et al. 2018, 2020, 2022).

Drought stress leave negative physiological legacies in trees that can ultimately drive mortality (Gessler et al. 2020; Kannenberg et al. 2020; Zweifel et al. 2020; Gea-Izquierdo et al. 2023). To understand variability in the underlying physiological mechanisms of tree mortality it is essential to consider complete spatio-temporal and organizational plant scales (Sevanto et al. 2014; McDowell et al. 2020, 2022; Hammond et al. 2022). Numerous studies have analyzed tree mortality addressing single or paired functional traits such as growth and xylem ^{13}C dynamics (e.g. McDowell et al. 2010; Levanic et al. 2011; Camarero et al. 2015; Wang et al. 2021). However, multifunctional approaches concomitantly analyzing different complementary traits, functions and plant scales are less common, yet necessary (e.g. Mencuccini et al. 2015; Colangelo et a. 2017; Gessler et al. 2017, 2018; McDowell et al. 2018, 2022). By combining dendrochronological dynamics of growth, xylem anatomical functional traits (i.e. variability in hydraulic architecture) and wood stable isotopes (particularly δ^{13}C and δ^{18}O), we can gain a multifunctional and long-term perspective of the physiological response of trees under environmental stress, and identify functional strategies underlying mortality (Levanic et al. 2011; Gessler et al. 2018; Camarero et al. 2020; Wang et al. 2021).

Trees reduce growth under water stress, with dead trees often exhibiting a growth decline of variable time length preceding mortality interpreted as expressing long-term carbon limitation (e.g. Suárez et al. 2004; Camarero et al. 2015; Cailleret et al. 2017; Gea-Izquierdo et al. 2021a). Radial growth is related to the tree xylem architecture, which greatly regulates the tree functional strategy to manage water stress (Tyree and Ewers 1991; Hacke and Sperry 2001; Tyree and Zimmerman 2002; Liang et al. 2021). There is no clear trade-off between xylem safety and efficiency because xylem vulnerability to water stress is mostly explained by pit and cell-wall physical and chemical characteristics, rather than by any mechanistic link with size-related conduit anatomical features (Delzon et al. 2010; Gleason et al. 2016; Lens et al. 2022; but see Isasa et al. 2023). However, the formation of xylem conductive elements responds to changes in water stress (e.g. Fonti et al. 2010; Gea-Izquierdo et al. 2012; Pellizzari et al. 2016), which may suggest a relationship between conduit sizes and pit characteristics (Pittermann et al. 2006). The relationship between water stress and xylem architecture may be greater in angiosperms with high levels of vessel connectivity, clustering or density (but see Kaack et al. 2021; Pereira et al. 2023). Thus, intra- and inter-specific variability in xylem architecture needs to be considered to better understand the susceptibility of trees to drought-induced mortality (Gessler et al. 2018; Borghetti et al. 2020; Puchi et al. 2021; Isasa et al. 2023).

Assessment of isotopic composition and nutrient content in plant tissues can add novel insights into the mechanisms of tree mortality (He and Dijkstra 2014; Gessler et al. 2017; Querejeta et al. 2021; Dalling et al. 2024). The dual-isotope conceptual model (δ^{13}C– δ^{18}O) reduces uncertainty in the interpretation of plant isotopic composition as a proxy for photosynthesis (A_n) and leaf stomatal conductance (g_s) (Scheidegger et al. 2000; Siegwolf et al. 2023). Before carbon is fixed in plant cell walls, carbon isotopic fractionation occurs during CO_2 diffusion into the substomatal cavities and the sites of carboxylation in chloroplasts as affected by stomatal and mesophyll conductance, and also due to Rubisco discrimination against ^{13}C during photosynthesis (Farquhar et al. 1989; McCarroll and Loader 2004; Cernusak et al. 2013; Gessler et al. 2014). This fractionation scheme allows using ^{13}C natural abundance in plant tissues (δ^{13}C) as a proxy for the ratio between A_n and g_s, i.e. the intrinsic water use efficiency (iWUE. Farquhar et al. 1989; Beer et al. 2009; Medlyn et al. 2017). The isotopic fractionation pathway during oxygen assimilation depends on the $^{18}O/^{16}O$ ratio of the original water source from precipitation, soil water evaporation, xylem water isotopic composition, evaporative demand at the leaf boundary layer, back diffusion from the leaf, stomatal conductance and post-assimilation isotopic exchange between xylem and phloem water (Barbour 2007; Gessler et al. 2014; Treydte et al. 2014; Cernusak et al. 2022). Plant δ^{18}O can be dynamically modified in seasonal ecosystems with high evaporative demands like those under Mediterranean climates (Flexas et al. 2014; Niinemets and Keenan 2014; Mas et al. 2024). Thus, similar ^{18}O abundance (δ^{18}O) in plant tissues can be reached with different combinations of soil water depth (δ^{18}O in water decreasing with soil depth) and leaf evaporative demands (high vapor pressure deficit, VPD, inducing δ^{18}O enrichment) (Farquhar et al. 2007; Grossiord et al. 2017; Brinkmann et al. 2018; Martínez-Sancho et al. 2023; Holloway-Phillips et al. 2023). Wood cellulose δ^{18}O can be used as a tracer of water uptake depth by trees, given that source water from upper soil layers is generally enriched in ^{18}O compared to deeper pools (McCulley et al. 2004; Sarris et al. 2013; Bachofen et al. 2024). However, access to deeper soil/bedrock sources to buffer the impact of water stress can reduce nutrient uptake. Impaired nutrient status can constrain tree physiological performance and increase the tree mortality risk (Gessler et al. 2017; Sardans et al. 2020; Querejeta et al. 2021; González de Andrés et al. 2021; Dalling et al. 2024).

Forests in the Mediterranean Region are among the most negatively affected by global change. Increased mortality of oak species such as *Quercus ilex* L. and *Quercus suber* L. since the 1980s (Brasier 1996; Camilo-Alves et al. 2013; Ruiz-Gómez et al. 2019) together with scarce seedling regeneration threatens these ecosystems in warm and dry locations (Moreno-Fernández et al. 2019; Acácio et al. 2021). Oak decline has been attributed to infection by introduced pathogenic oomycete fungi in combination with other stress factors including drought and land-use (Camilo-Alves et al. 2013; Ruiz-Gómez et al. 2019; Gea-Izquierdo et al. 2021a). Accelerated mortality is also observed at dry locations in other forest species like *Pinus pinaster* Ait. and *Pinus sylvestris* L. However, accelerated pine mortality is solely attributed to increased climatic stress and not to any biotic factor (Gea-Izquierdo et al. 2019, 2021b). The increase in both oak and pine mortality rates is diffuse in time, meaning that there is raised annual incidence of mortality with inter-annual variability linked to the severity of summer climatic conditions and different legacy effects. Additionally, these forests eventually suffer episodic or sporadic mortality events after major environmental disturbances (Brodribb et al. 2020; Hammond et al. 2022). In this study we examined a die-off event in two *Q. ilex* woodlands in Central Spain after a record-setting hot drought in 2019. The two studied forests did not exhibit any decline symptoms or increased background diffuse mortality rates in the years previous to this lethal hot drought. We characterized the functional strategy of surviving and dead trees to analyze whether dead trees were predisposed by a more stressful abiotic niche. We hypothesize that dead trees expressed a differential functional response to long-term stress recorded in their xylem functional traits, showing that oak mortality was concentrated in soils with lower water-holding capacity. Combining xylem anatomical and isotopic proxies with model simulations of water and carbon dynamics, specifically we assessed: (1) whether dead trees show multi-decadal growth declines triggered by past drought under climate change regardless of the stress factor (biotic or abiotic) producing death; (2) whether lower radial growth in dead trees is offset by formation of more efficient xylems; (3) whether dead trees exhibited sustained long-term stomatal constraints on carbon assimilation (assessed through C and O isotopic proxies) suggesting predisposition by abiotic stress legacies previous to the lethal hot-drought; and (4) whether surviving trees were able to buffer the impact of drier climatic conditions by dynamically accessing water from deeper soils.

2. Material and methods

2.1. Study sites and dendroecological sampling

The two studied forests 'DP-Dehesa del Pinar' and 'DS-Dehesa de la Serna' are open woodlands dominated by *Q. ilex* near the city of Ávila, in Central Spain (40.1°N, 4.7°W. Appendix S1) at a similar elevation of 1132±42 m (mean±standard deviation). Tree-tree aboveground competition is nil and belowground competition very reduced or nil in these open woodlands (Gea-Izquierdo and Cañellas 2009). The trees grow on poor sandy soils of granitic origin with abundant rock outcrops indicating a matrix of soils with variable depth over a fractured granite bedrock (Appendix S2). *Q.ilex* is a resprouting evergreen species with high resistance both to drought and other disturbances such as fire (Martín-Sánchez et al. 2022). Contrary to more Southern and warmer forests of the same species with widespread signs of tree decline and high annual diffuse mortality since the 1980s (Brasier 1996; Camilo-Alves et al. 2013), the two studied forests do not show signs of recent oak decline and *Phytophthora cinnamomi* Rands has not been isolated there yet (Moreno-Fernández et al. 2019; Ruiz-Gómez et al. 2019). The studied forests experience a continental Mediterranean climate (i.e. dry-hot summers and cold winters), with mean annual temperature of 10.9 °C, maximum mean temperature in July of 28.1 °C, minimum mean temperature in January of -1.3 °C, and mean annual precipitation of 424.6 mm. The Mediterranean Region is a climate change hotspot that has suffered pronounced warming since the early 1980s (Lionello and Scarascia 2018). In 2018–2019, the study area experienced the most extreme hot drought of the last +100 years as expressed by absolute minimum 12-month standardized precipitation-evapotranspiration index in July (SPEI$_{12July}$) caused by simultaneous very low precipitation and very high temperatures (Appendix S3). In fall 2019 there was widespread *Q. ilex* mortality with complete canopy dry-out. To analyze the long-term functional response of trees in relation with this mortality event we collected stem cross sections from 57 dead trees: 31 from DP and 26 from DS. To be sure that trees were truly dead and did not resprout after the hot drought in 2019, we sampled trees in late fall 2020, one year after the mortality event. Trees presented single stems with heights 5.7 ± 1.6 m in DS and 6.8 ± 1.6 m in DP, tree diameters at 1.30 m of 27.6 ± 8.4 cm and 34.9 ± 14.4 cm; and ages 100±21 years and 134±30 years, respectively (Appendix S4, S5). The complex xylem in *Q. ilex* with thick medullary rays, variable semi ring-porous structure, discontinuous and often unclear ring-boundaries, and abundant wedging and missing rings, makes imperative the use of stem cross sections in dendroecological studies. For this reason and because the species is protected, we collected cross sections from main branches from the upper crown receiving full light from 19 surviving trees and a random subsample of 22 dead trees of the 57 for which we collected stem cross sections. We used main stem cross sections in dead trees to analyze growth trends and the sensitivity to climate of dead trees. Branch cross sections from surviving and dead trees were used to study sapwood nutrient content and dynamics of xylem hydraulic architecture and isotopic composition.

2.2. Growth trends and sensitivity to climate in Q. ilex

The 57 cross sections from main stems of dead trees (Appendix S4, S5) were processed using standard dendroecological techniques to build annual growth series. All cross sections were progressively sanded until annual rings were clearly distinguishable. Then they were visually and statistically crossdated and their annual growth (tree-ring width, TRW) measured in at least 2 radii per sample. To analyze growth trends, we estimated basal area increments (BAI, cm^2 year^{-1}) by progressively subtracting TRW (mm year^{-1}) from measured DBH corrected for bark thickness. To analyze the high-frequency response of growth to climate, individual TRW series were detrended using splines with 50% variance cut-off of 50 years and mean residual chronologies were calculated using ratios. Growth trends from DP and DS dead trees were compared with seven published *Q. ilex* tree-ring chronologies from live and dead trees from a latitudinal gradient in Western Spain. Dead trees from this gradient come from stands with oak decline resulting on high annual diffuse background mortality (Gea-Izquierdo et al. 2021a. Appendix S1, S4, S5).

2.3. Xylem architecture dynamics in dead and surviving trees

Branch cross sections were processed to estimate radial growth in the same manner as explained above for stem cross sections. Branches were older than expected for the diameters sampled: 48±12 years, for a mean diameter of 4.5 ± 1.3 cm (maximum 109 years for an 8.9 cm branch. Appendix S6). After branch TRWs had been measured, we selected a subsample of 20 branches (5 for each health status and site) for anatomical and isotopic analyses. We split each of the 20 selected branches in two slices: one for xylem anatomical analyses and one for isotopic analyses. To analyze dynamics in xylem architecture, we built annual time series for the period 1989–2019 (i.e. 30 years) of different functional traits from image analysis of wood microsections following the protocol in Férriz et al. (2023). First, we cut wood blocks from the border through the pith of each cross section and then split them into 5-cm long pieces to fit into glass slides. Each 5-cm xylem piece was embedded in paraffin and 10-µm thick transversal microsections were cut using a rotary microtome. Histological microsections were fixed in glass slides and stained with safranin and astrablue to highlight lignified cells in red (dead conductive elements and fibers) and non-lignified cells in blue (living cells, mainly parenchyma). Slides were then photographed at 100 × magnification with a digital camera coupled to a microscope. On these images, for each identified annual ring we used ROXAS (von Arx and Carrer 2014) to measure the following xylem anatomical traits related to the hydraulic function: vessel lumen areas (LA), and average of the vessel radial and tangential diameters (d). We calculated annual vessel density within the conductive area between two consecutive medullary parenchyma rays (VD, vessels mm^{-2}. Fig. 1A). Additionally, for each annual ring we calculated the hydraulic diameter ($HD = \sum d^5 / \sum d^4$) and the theoretical xylem hydraulic conductivity (kg m^{-1} s^{-1} MPa^{-1}) according to Hagen-Pouseuille (HP, Tyree and Zimmermann 2002): $K_s = (\pi\rho/128\eta A)\cdot\sum nd^4$, where ρ is the density of water at 20 °C (998.2 kg m^{-3}), η is the viscosity of water at 20 °C (1.002×10^{-9} MPa), A is the image area and d is the tracheid lumen diameter. Finally, we estimated the percentage of area occupied by parenchyma as a proxy to reservoir carbohydrates in trees (Plavcová and Jansen 2015).

2.4. Dual δ^{13}C− δ^{18}O dynamics in the xylem of dead and surviving trees

We used the dual δ^{13}C-δ^{18}O conceptual model to indirectly assess differences between dead and live trees in A_n, g_s and iWUE (Scheidegger et al. 2000; Siegwolf et al. 2023). Acknowledging that δ^{13}C and δ^{18}O in earlywood and latewood can differ due to modifications in the fractionation pathways within the year (Werner et al. 2012; Gessler et al. 2014), we built time series of xylem isotopes pooling samples every 3 years rather than building annual time series for the following reasons: (1) we were interested in analyzing differences in long-term functional acclimation between dead and live trees more than analyzing inter-annual variability in isotopic composition; (2) branch growth was very low, often making indistinguishable the boundary between earlywood and latewood (if existing); (3) missing rings were often identified towards the end of the cross sections. We separated annual rings with a scalpel under magnification, then pooled them every 3-years for a period of 30 years (1989–2019). Then, we extracted cellulose from wood samples following Andreu-Hayles et al. (2019). Cellulose samples (200 ±20 µg) were encapsulated in silver capsules. δ^{18}O and δ^{13}C were

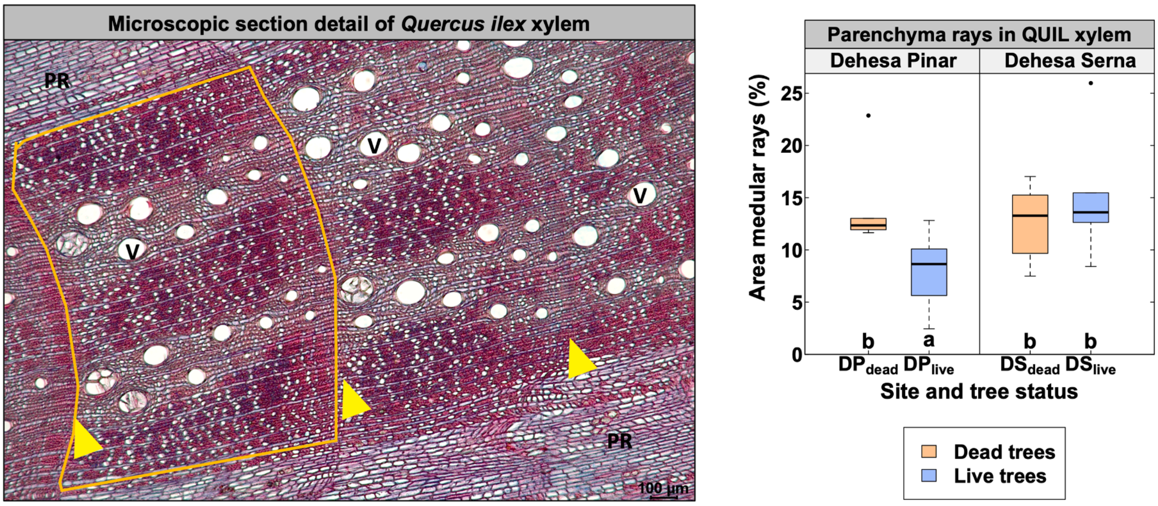

Fig. 1. The picture shows an example of a xylem microsection of one *Q. ilex* individual illustrating how we measured annual potential conductivity for a given year (within the orange-lined polygon) excluding parenchyma rays. In the graph, we show the percentage of area of medullary rays measured as an indirect measure of NSC sapwood storage in the 20 branches used for anatomical and isotopic analyses. PR=parenchyma ray in xylem. *V*=vessel. Some vessels are filled with tyloses expressing their loss of functionality. Yellow triangles mark annual ring growth borders.

measured simultaneously in the Stable Isotope Laboratory at the Department of Geology, University of Maryland as described in Evans et al. (2016). Overall precision for these analyses, based on 2-point corrected replicate working standards was 0.09‰ for δ^{13}C with respect to the VPDB standard, and 0.28‰ for δ^{18}O with respect to the VSMOW standard (Evans et al. 2016). Analyzer linearity was better than 0.05- 0.06 ‰/nA over the collector amplitude range 2.6–14.5 nA, across all batch runs.

The oxygen stable isotopic composition is reported and further analyzed as δ^{18}O. The carbon stable isotopic composition was used to estimate C isotope discrimination (Δ^{13}C) and iWUE. Carbon isotope discrimination between plant material ($\delta^{13}C_{plant}$) and air ($\delta^{13}C_{atm}$) was estimated as $\Delta^{13}C = (\delta^{13}C_{atm} - \delta^{13}C_{plant})/(1+ \delta^{13}C_{plant}/1000)$ to correct for increasing atmospheric CO_2 (C_a) from fossil fuel burning, which is depleted in ^{13}C with respect to preindustrial levels (i.e., *Suess* effect. Cernusak et al. 2013; Keeling et al. 2017). In C_3 plants, Δ^{13}C can be approximated as $\Delta^{13}C = a + (b - a)\, C_i/C_a$, where *a* is the fractionation associated with diffusion of CO_2 through the stomata (≈ 4.4‰), *b* is the fractionation by Rubisco during carboxylation (≈ 27‰), and C_i is the intercellular CO_2 concentration (μmol mol^{-1}, Farquhar et al. 1989). These estimates neglect several fractionation processes from the atmosphere to carboxylation centers, including those of mesophyll conductance and photorespiration (Farquhar et al. 1989; Werner et al. 2012; Lavergne et al. 2019). We obtained $\delta^{13}C_{atm}$ time series data from McCarroll & Loader (2004) and Mauna Loa (Lan et al. 2024). Finally, iWUE was calculated as:

$$iWUE = \frac{A}{gs} \cong \frac{g_{CO_2}(C_a - C_i)}{g_s} \cong \frac{1}{1.6}\,(C_a - C_i).$$

2.5. Sapwood nutrient content in dead and surviving trees

In order to compare nutrient content in dead and surviving trees, over 3 mg of dry sapwood was cut from each of the 41 branches sampled, then milled and homogenized. An aliquot of wood powder was used to estimate nutrient concentrations of 31 elements including Phosphorus (P), Potassium (K), Aluminum (Al), Boron (B), Calcium (Ca), Cobalt (Co), Copper (Cu), Iron (Fe) Magnesium (Mg), Manganese (Mn), Sodium (Na), Silicon (Si) and Strontium (Sr). These concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP−OES; Thermo Elemental Iris Intrepid II XDL) after a microwave--assisted digestion with HNO2:H2O2 (4:1, v/v). Nitrogen (N) and Carbon (C) concentrations in sapwood were measured with a C/N elemental analyzer (Flash EA 1112 Series, Leco Truspec). Differences in individual nutrients between dead and live trees were analyzed using non-parametric Kruskal-Wallis tests. Analysis of the tree nutrient fingerprint was performed using principal components analysis (PCA). The capacity of discrimination of the first principal component between dead and surviving trees was tested with a logistic model (Legendre and Legendre 1998).

2.6. Analysis of xylem trait variability and growth sensitivity to climate

We analyzed differences between dead and surviving live trees in xylem functional adjustment in response to increased water stress and warming in the last decades. To assess growth trends previous to tree death and identify tipping points in years triggering growth decline we used breakpoint analyses (Muggeo, 2008) on the dead stem chronologies from DP and DS. To assess climate sensitivity of radial growth we calculated tree-by-tree individual correlations with covariates related to water and temperature stress in different seasons. We used both daily and monthly climatic data from E-OBS (Haylock et al. 2008) for the period 1920–2022. With monthly data we calculated SPEI for 6- and 12-month periods (Beguería et al. 2014). To analyze differences in xylem traits (anatomical and isotopic) we used linear mixed models with random intercepts for 'tree' and 'year'. We compared dead and surviving trees for HD, VD, K_s, δ^{13}C proxies and δ^{18}O during the period 1989–2019.

2.7. Model simulations of carbon and water dynamics in Q. ilex woodlands

As a complement to the previous analyses with observational data and to further assess physiological performance in trees, we simulated stand carbon and water dynamics using the process-based model MAIDEN as described in detail in Gea-Izquierdo et al. (2015). The model calculates water and carbon dynamics at the tree level using as input daily meteorological data, species-specific parametrization and functional traits, and soil characteristics (including soil depth and structure). We run the model for the period 1950–2019 to produce simulations of daily photosynthesis (A_n), stomatal conductance (g_s), total tree transpiration (E), and gross (GPP) and net primary productivity (NPP). From model outputs we calculated daily time series of Δ^{13}C and different measures of water use efficiency: leaf $iWUE = A_n/g_s$; and tree

WUE=GPP/E (Beer et al. 2009; Medlyn et al. 2017; Lavergne et al. 2019). Modeled daily outputs were integrated and reported annually. We ran simulations for two contrasting soil depths to assess the hypothesis of tree mortality being concentrated on shallower soils with lower water holding capacity. Models were run using the parameterization specifically developed for *Q. ilex* (Gea-Izquierdo et al. 2021a), and the same daily meteorological data (i.e. same VPD) for the two simulations runs. Therefore, input for model runs only differed in soil depth: 1.5 m for ‘deep soil’, and 0.7 m for ‘shallow soil’. Additionally, we simulated $\delta^{18}O$ dynamics across its fractionation pathway from precipitation, to soil water, xylem water, leaf water and xylem cellulose using MAIDENiso (https://dendro-eco.uqat.ca/maiden/), as described in detail in Hermoso de Mendoza et al. (2022). We discuss model simulations with the observed xylem traits to assess functional strategies in dead and surviving trees.

3. Results

3.1. Growth trends and sensitivity to climate of dead Q. ilex trees

Tree growth from dead trees in the two study sites (DS and DP) was slower than growth across the network of *Q. ilex* open woodlands (Fig. 2A, B) located along a latitudinal gradient including warmer, less continental, and more humid sites (Appendix S3, S4). Dead trees from DP and DS expressed multidecadal declines in radial growth prior to death (Fig. 2C, D), equal to those expressed by dead *Q. ilex* but in contrast to surviving *Q. ilex* along the latitudinal gradient analyzed from the published network (Fig. 2A, B). Negative growth trends in DP and DS became particularly evident after an extreme drought in 1992 (Fig. 2D, Appendix S3, S7). As for the other sites analyzed across the *Q. ilex* network, radial growth of dead trees in DS and DP was highly sensitive to water stress at seasonal and, particularly, hydrological year time scales (Fig. 3). Tree performance was limited by the double winter-summer stress periods in Mediterranean climates, as shown by the positive influence of warm winter and spring temperatures and the negative effect of hot summers on growth (Fig. 3).

3.2. Xylem functional traits in surviving and dead trees

Medullary parenchyma occupied a larger proportion of the xylem of dead than live trees in DP, and similar in DS (Fig. 1B). BAI growth was similar in dead and live oak branches but TRW was higher in DP in the

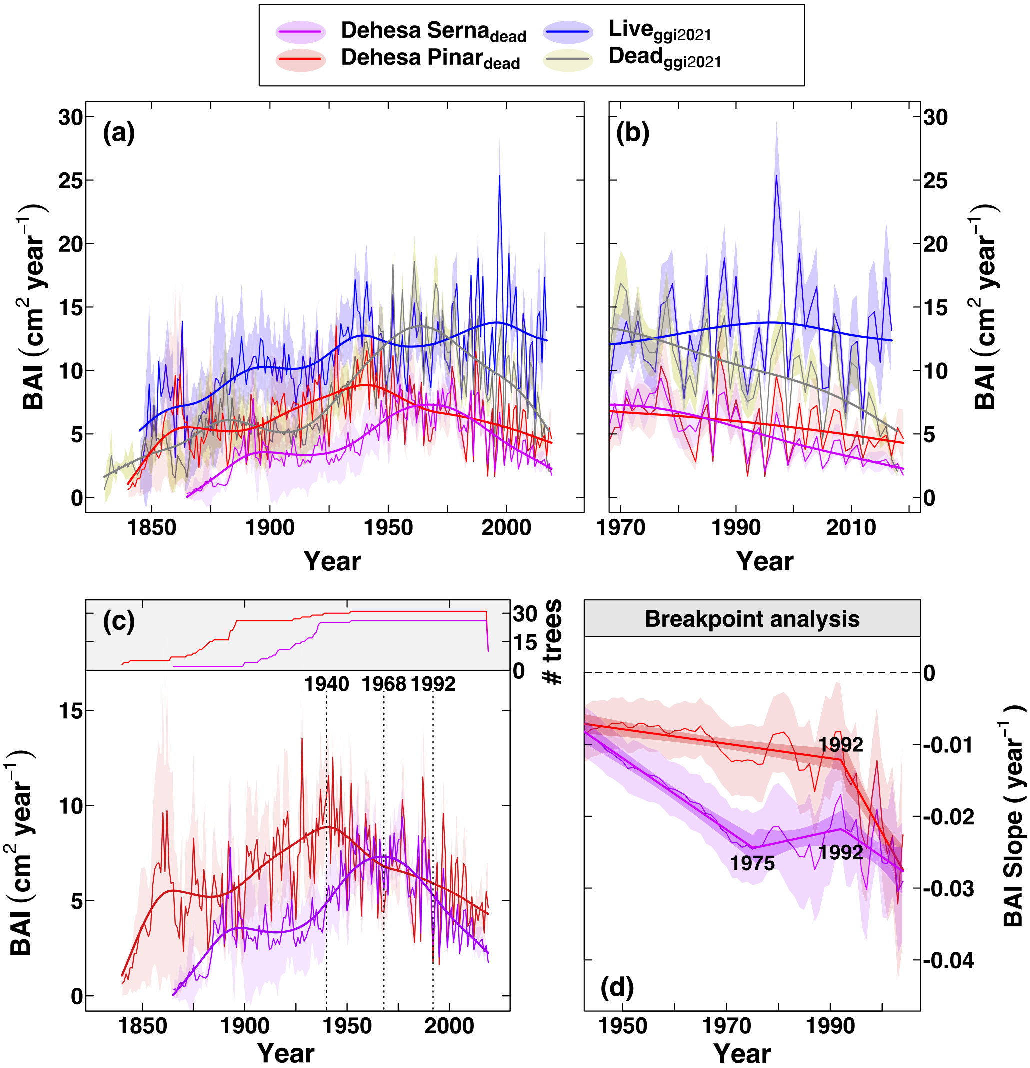

Fig. 2. Growth trends (BAI, basal area increment) of live and dead *Q. ilex*: (a) Mean BAI for four different live and dead chronologies; (b) Detail of mean growth trends from (a); (c) BAI from the two sites DS and DP; (d) Slopes breakpoint analyses for DS and DP. In (c) years with maximum growth estimated with the smoother and 1992, as the tipping point marking the onset of growth decline according to (d) are highlighted. (a) to (c) are smoothed with 50-year splines to highlight growth trends. Live$_{ggi2021}$= mean of 5 sites with live oak trees from Western Spain. Dead$_{ggi2021}$=mean of two dead sites with isolation of *Phytophthora cinnamomi* (Gea-Izquierdo et al. 2021a) (Appendix S1, S2).

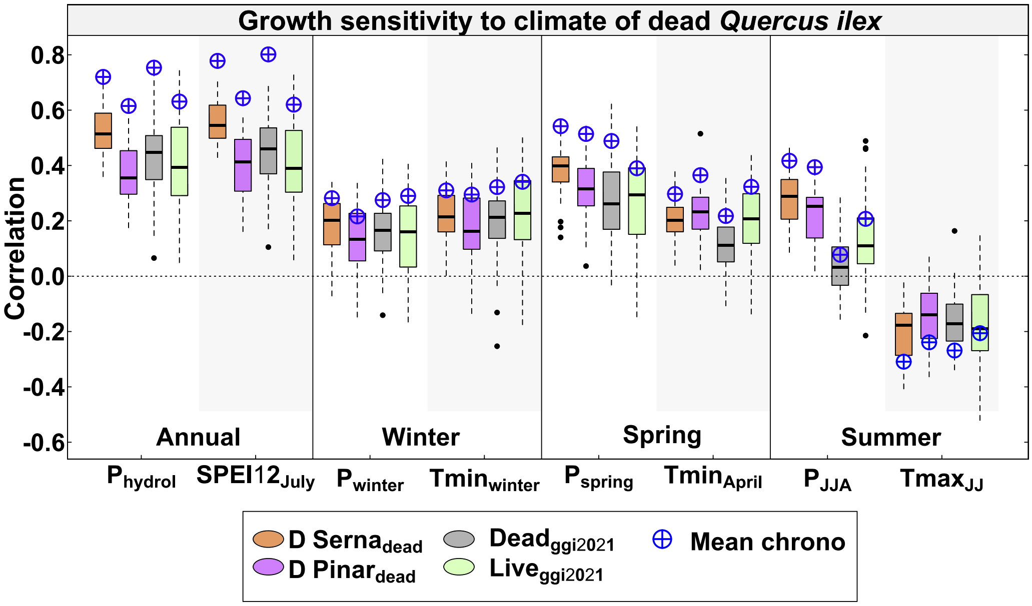

Fig. 3. Annual and seasonal sensitivity to climate of oak trees from Dehesa del Pinar (D Pinar), Dehesa de la Serna (D Serna), $\text{Dead}_{\text{ggi2021}}$ and $\text{Live}_{\text{ggi2021}}$(as from Fig. 2). Boxplots represent tree individual correlations with climatic indices and periods of maximum response. Blue symbols correspond to calculated correlations between mean chronologies and climatic indices. P_{hydrol}=precipitation of hydrological year (from October to September); $\text{SPEI12}_{\text{July}}$=July SPEI for 12-month period; *P*=precipitation; Tmin=minimum temperature; Tmax=maximum temperature; winter=January-March; Spring=April-June; JJA=June, July, August; JJ=June-July. $\text{Dead}_{\text{ggi2021}}$ correspond to dead oaks from two different locations in the Extremadura Region from Gea-Izquierdo et al. (2021a) where *Phytophthora cinnamomi* was isolated from dead tree roots.

same live trees presenting less medullary parenchyma (Appendix S8). Dead trees formed xylems with higher vessel density and higher potential conductivity (K_s) than those in surviving trees (Fig. 4). Although mean vessel size was similar in dead and live oaks (Fig. 4B), the vessel diameter distribution of dead trees from DP included a higher abundance of vessels in the largest diameter classes (Fig. 4G). There was little interannual variability in vessel diameter compared to that in vessel density (VD) and K_s. Live oaks exhibited a positive time trend in vessel size (HD, LA) and K_s but not in VD. In contrast, VD of dead oaks increased in the last 30 years, but not vessel size and K_s (Fig. 4; Appendix S9).

3.3. *Xylem isotopic composition and sapwood nutrient content in surviving and dead trees*

Δ^{13}C was lower and iWUE higher in dead oaks (Fig. 5). There was no time trend in Δ^{13}C whereas iWUE increased in both dead and surviving trees. A decreasing trend in δ^{18}O in both surviving and dead trees was evident in recent years. Overall δ^{18}O was higher in live than dead oaks, although this difference was less constant along time than that in traits calculated from δ^{13}C (Fig. 5). δ^{18}O in surviving trees remained above that in dead trees except for years when the lowest Δ^{13}C values were observed (i.e. the most severe drought stress conditions). For those years, δ^{18}O values in xylem tissues of dead and surviving trees converged (Fig. 6). The negative relationship between δ^{18}O and Δ^{13}C (decreasing δ^{18}O with increasing Δ^{13}C) was steeper in dead than surviving trees (Fig. 6B). This suggests tighter stomatal regulation of water flux and carbon assimilation with increasing water stress (i.e. increasing VPD and decreasing soil moisture) in dead than surviving trees. This tighter stomatal response in dead trees was more evident for low δ^{18}O levels as expressed by its relationship with iWUE (Fig. 6A). iWUE increased steeply in dead trees with increasing drought stress and decreasing stomatal conductance up to a certain threshold ($\delta^{18}O = 27.7$ ‰), above which there were no further gains in iWUE (Fig. 6A). This suggests photosynthesis impairment under drought in dead trees and, together with the combined interpretation of Fig. 5A and E, that the stomatal signal in δ^{18}O is weaker than that of the water source.

When sapwood elemental concentrations were analyzed individually, Fe was the only nutrient that significantly differed between dead (lower Fe) and live trees ($\chi^2 = 5.17$; $p = 0.023$). Yet, a multivariate analysis including six of the most important elements and the N:P ratio explained almost 60% of the variance in the first two principal components (PC). PC1 enabled discrimination between dead and surviving trees ($\chi^2 = 5.48$; $p = 0.019$). Surviving trees were negatively correlated with the sapwood N:P ratio and positively correlated with all the other sapwood nutrients included in the PCA (Fig. 7).

3.4. *Simulation of carbon and water dynamics in trees growing on deep vs. shallow soils*

Model simulations suggest that leaf g_s would be lower whereas A_n would be similar in *Q. ilex* growing on shallow relative to trees growing on deep soils (Fig. 8A, 8C). This would result in higher iWUE, consequently lower Δ^{13}C, in trees growing on shallow than deep soils (Fig. 8E, 8G). Leaf area index in the model stabilized at higher values for trees growing on deep than shallow soils (1.1 m^2 m^{-2} vs. 0.6 m^2 m^{-2}, respectively). Therefore, simulations for trees growing on deep soils yielded higher NPP and transpiration (E) than for trees on shallow soils (Fig. 8B, 8D). Despite differences in iWUE, simulated whole-tree WUE (i.e. GPP/E) was similar on average for trees growing on the two contrasting soil depths considered (Fig. 8F). Simulated tree ring cellulose δ^{18}O was higher for *Q. ilex* growing on deep than on shallow soils (Fig. 8H).

4. Discussion

Dead and surviving trees after an extreme hot-drought exhibited different strategies in xylem functional adjustment to chronic water stress prior to the event (Fig. 9). Differences in xylem architecture and isotopic composition between surviving and dead trees were consistent through time, showing that dead trees experienced greater chronic life constraints prior to the mortality-causing hot drought in 2019. Die-off could have been produced by hydraulic failure in susceptible trees

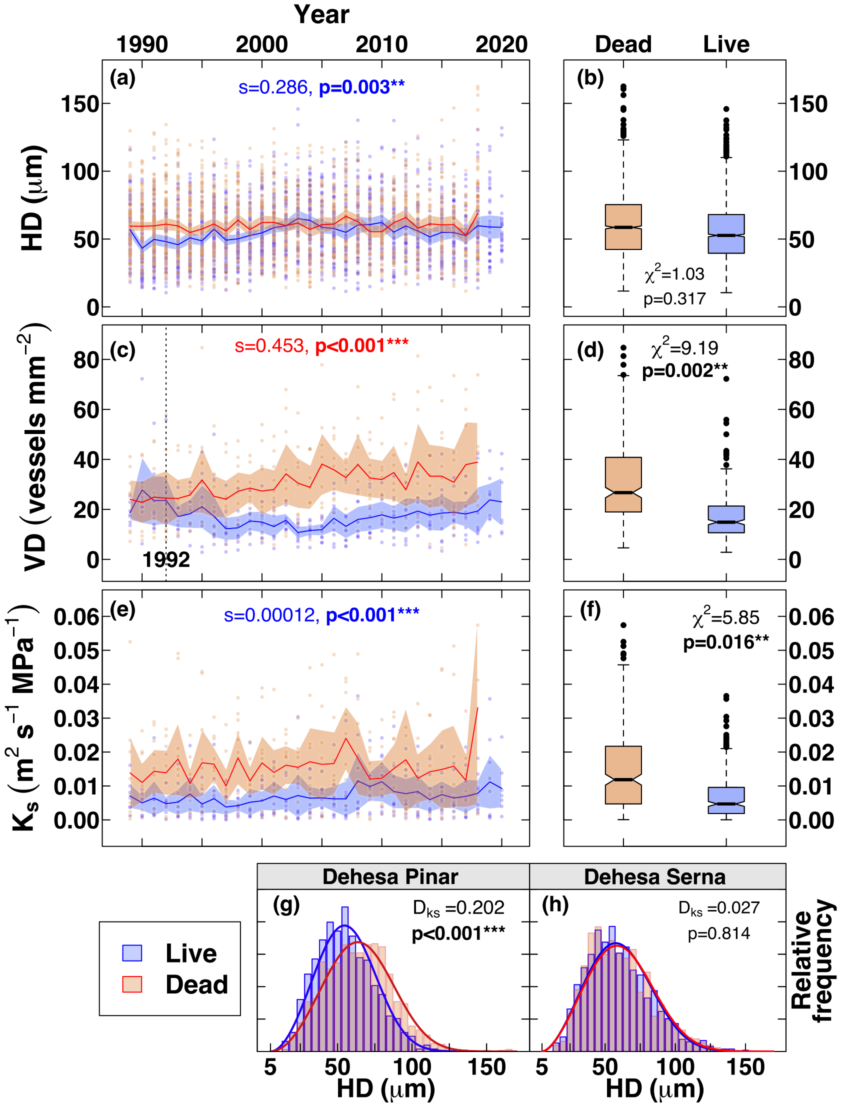

Fig. 4. Differences in functional xylem traits between dead and live oaks: (a)-(b) Hydraulic diameter (HD); (c)-(d) Vessel density (VD); (e)-(f) HP conductivity (Ks); (g)-(h) Hydraulic diameter probability density functions (pdfs) with Weibull pdfs (lines) and Kolmogorov-Smirnov tests (D_{ks}) comparing dead vs. live pdfs for DP and DS. s=slope for linear regressions (only shown when significant). To compare mean values by health status, we show χ^2 tests and p-values. Significant differences at 0.05 are represented with ** and those at 0.01 with ***. 1992 is a tipping point for growth decline (Fig. 2D).

with constrained physiological performance limiting carbon assimilation and leaving negative ecological legacies for decades prior to mortality. Tree mortality preferentially affected trees suffering more severe baseline water stress levels because they were likely growing on more stressful locations where trees could not buffer the sharp increase in water stress forced by the hot drought. Because surviving and dead trees were exposed to the same climate and similar low levels of inter-tree competition, we argue that chronic abiotic-niche differences were responsible for the observed coordination of xylem functional traits mediating individual tree vulnerability to the lethal hot drought.

4.1. *Constrained carbon dynamics but more efficient hydraulic systems in dead trees*

Differences in iWUE and its relationship with δ^{18}O suggest that leaf gas exchange of dead trees was chronically more constrained by tighter stomatal regulation (Ferrio et al. 2003; Barbour 2007; Gessler et al. 2014; Siegwolf et al. 2023). The carbon isotopic proxies suggest that dead trees reduced g_s comparatively more than A_n during periods of high evaporative demand and low soil moisture availability, whereas the gas exchange of surviving trees remained comparatively more homeostatic. Absolute carbon depletion is seldom identified in dying trees probably because trees may die after crossing C thresholds above zero or after depletion of specific key carbon compounds, or because drought limits the ability of trees to mobilize stored carbohydrates (Hartmann et al. 2013; Voltas et al. 2013; Choat et al. 2018; Gessler et al. 2018; Arend et al. 2021; 2022). Nevertheless, chronic low carbon assimilation jeopardizes plant performance in the long-term (Flexas et al. 2014; Niinemets and Keenan 2014). Trees increase investment in carbon storage under climatic stress (Plavcová and Jansen al. 2015; Arend et al. 2022). Dead trees may have invested a greater carbon cost in xylem storage tissues (i.e. parenchyma rays) (but see Kotowska et al. 2020) than in xylem conductive tissues (i.e. intervessel area).

Dead trees formed xylems with higher vessel density and similar or larger vessel size distributions, which implies higher potential hydraulic conductivities per xylem conductive unit area, i.e. more efficient xylems (Hacke and Sperry 2001; Hacke et al. 2006; Tyree and Zimmermann 2002; Sperry et al. 2008). We observed higher variability in vessel density than in vessel size, which suggests that, at the xylem level, trees mostly modified their vessel density to adjust to environmental stress

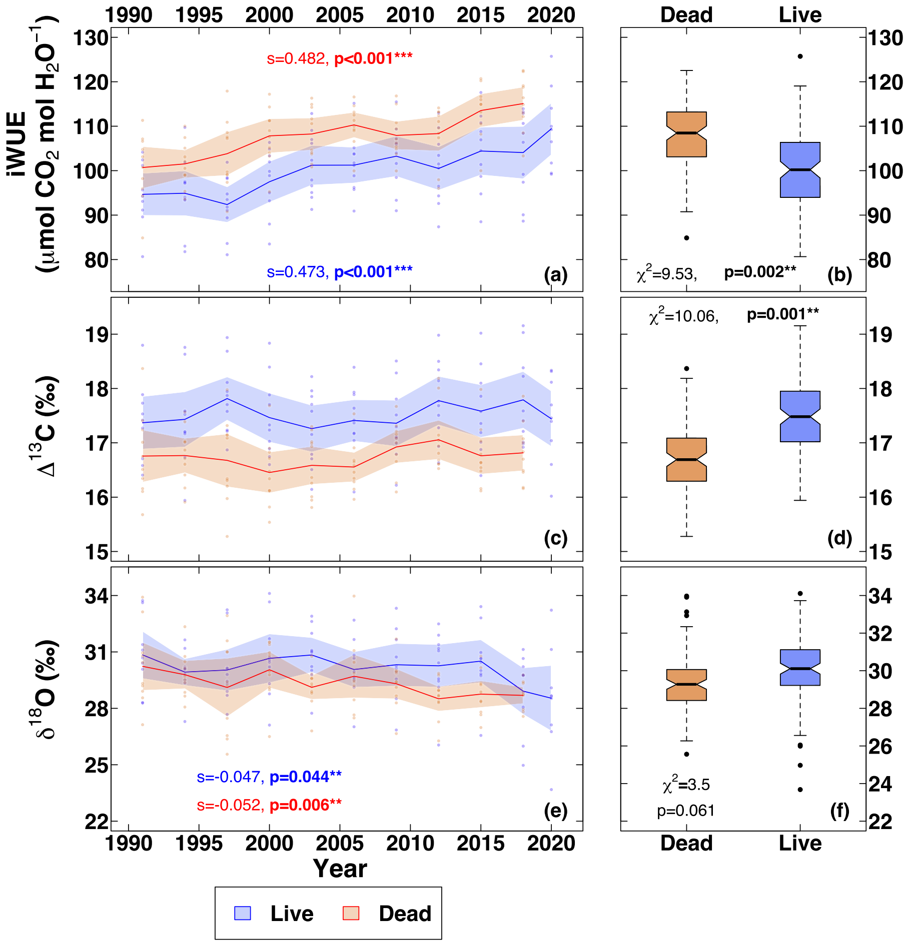

Fig. 5. Mean profiles and boxplot of dead and surviving *Q. ilex* trees for: (a)-(b) iWUE; (c)-(d) Δ^{13}C; and (e)-(f) δ^{18}O. On the right column p-values and tests for differences between dead and live trees are shown, with significant differences at 0.05 represented with ** and at 0.001 with ***. *s*=slope for linear regressions (only shown when significantly different from 0).

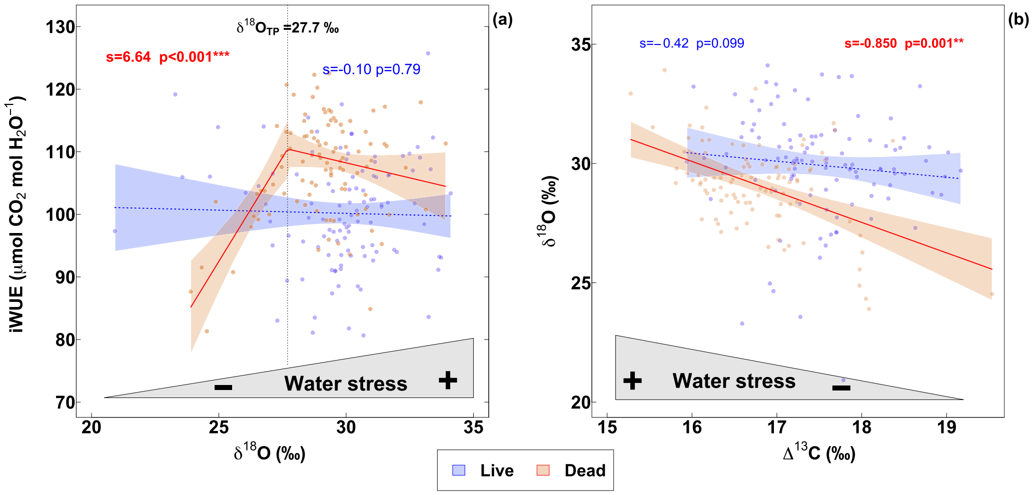

Fig. 6. Dual isotope analysis: (a) relationship between δ^{18}O and intrinsic water use efficiency (iWUE); (b) relationship between δ^{18}O and Δ^{13}C (inversely related to iWUE). Significant piecewise (a) and linear (b) relationships are depicted with solid lines. The conceptual gray triangles refer to hypothetical trends in water stress (VPD + soil water stress) along the x-axis. *s*=slope for linear regressions (in bold when significantly different from 0). The vertical dashed line in panel (a) represents a tipping point in the relationship between iWUE and δ^{18}O for dead trees.

(Corcuera et al. 2004; Levanic et al. 2011; Gea-Izquierdo et al. 2013; Castagneri et al. 2017). Higher vessel density increasing conduit proximity (i.e. xylem redundancy) could have increased xylem vulnerability in dead trees by allowing embolism to spread through increased interconnectivity of conductive elements (Tyree and Ewers 1991; Choat et al. 2008; Borghetti et al. 2020, but see Sperry et al. 2008 for the opposite

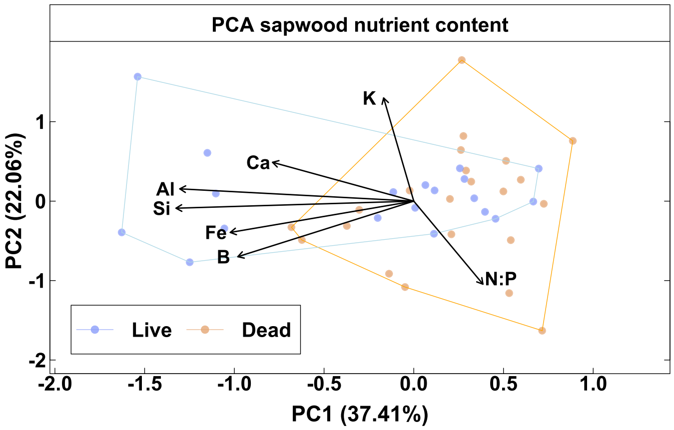

Fig. 7. PCA estimated for the N:P ratio and the 6 nutrients (Al, B, Ca, Fe, K, Si) explaining more variability. The percentage of variance explained by PC1 and PC2 is shown between parentheses The result of a logistic model analyzing the relationship between health status (i.e. dead-live) and PC1 was significant ($p = 0.019$).

effect). This would imply accepting the ‘rare pit hypothesis’ at the intraspecific level, which however has been much contested in interspecific and modelling studies (Kaack et al. 2021; Lens et al. 2022; Pereira et al. 2023). A vessel size-vulnerability link does not hold globally because hydraulic safety depends more on pits and the membrane ultrastructure than on vessel size features (Choat et al. 2008; Gleason et al. 2016; Lens et al. 2022, but see Isasa et al. 2023). This means that the xylem of surviving trees was not necessarily more resistant to drought-induced hydraulic collapse. Surviving trees may have experienced less intense water stress levels during the hot drought because they grew in soil pockets with greater water availability.

4.2. *Functional mechanisms and physiological legacies driving die-off under drought*

The science community struggles to understand the underlying physiological mechanisms of forest mortality under global change to mitigate the related threats to forest conservation (Anderegg et al. 2020; Brodribb et al. 2020; Hammond et al. 2022; Hartmann et al. 2022). Interrelated limitations in carbon assimilation and the hydraulic function are necessarily involved in tree mortality (Sevanto et al. 2014; Meddens et al. 2015; McDowell et al. 2020, 2022). However, isolating the effect of these mechanism is complicated because, in response to different combinations of abiotic and biotic stress factors they can be expressed differently between and within species: (1) at different organizational scales (e.g. cellular, leaf, xylem or tree individual level); (2) in different physiological processes, (e.g. photosynthesis, carbon allocation, hydraulic function); and (3) at different mortality time scales (diffuse vs. episodic or sporadic, slow vs. sudden). Negative legacies from past chronic stress can further constrain plant’s physiological performance under posterior sudden stress ultimately affecting mortality (Gessler et al. 2020; Zweifel et al. 2020; Kannenberg et al. 2020; Arend et al. 2022; Gea-Izquierdo et al. 2023). A better identification of these legacies in key functional traits is necessary to understand forest dynamics under increasing climatic stress.

The characteristics of a growth decline before tree death provide clues about the factors causing mortality and the physiological processes involved. Multidecadal growth declines preceding tree death are common in many species, and suggest the existence of long-term carbon

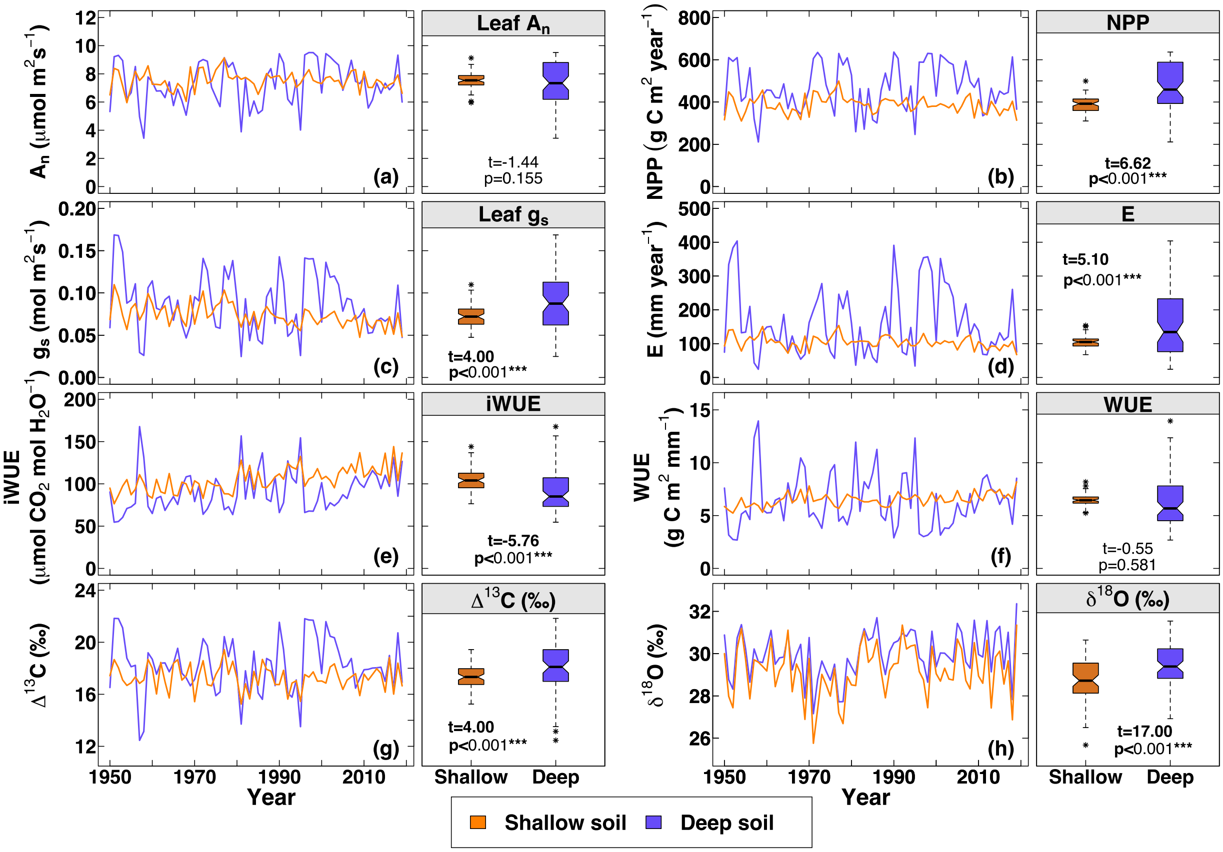

Fig. 8. Model simulations for 0.7 m soil depth (Shallow) and 1.5 m soil depth (Deep) for the period 1950–2019. (a) Mean annual leaf photosynthesis rate (A_n); (b) Annual net primary productivity (NPP); (c) Mean annual leaf stomatal conductance to water vapor (g_s); (d) annual stand transpiration (E); (e) mean annual intrinsic water use efficiency (iWUE=A_n/g_s); (f) stand water use efficiency (WUE=GPP/E); (g) Discrimination against ^{13}C (Δ^{13}C); and (h) simulated δ^{18}O in tree ring cellulose. Significant differences at 0.001 between ’Shallow’ and ’Deep’ simulations for specific traits are represented in bold and with ***.

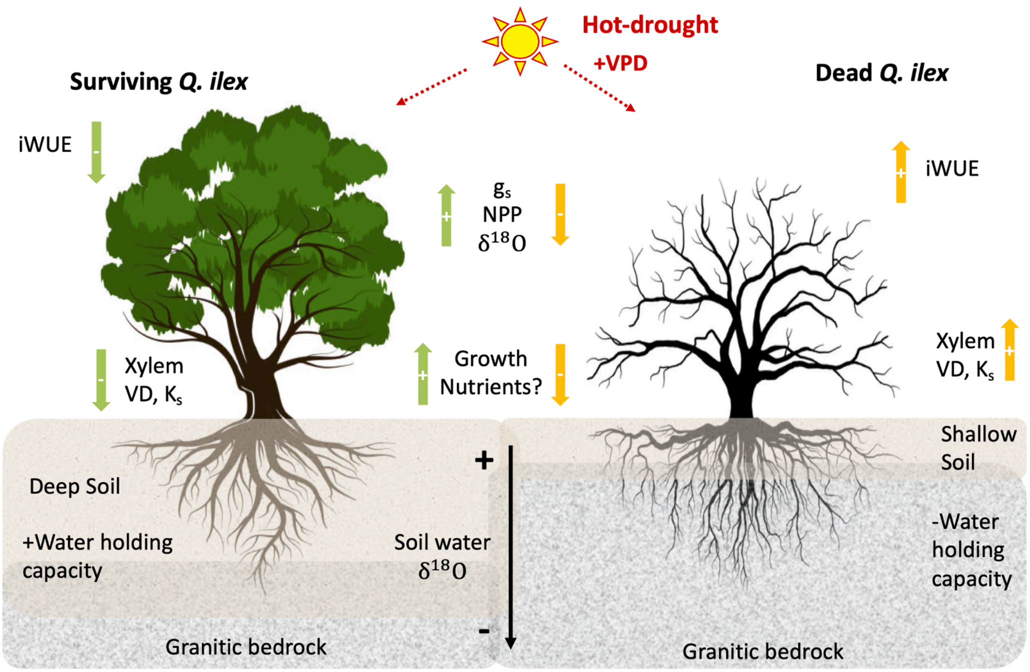

Fig. 9. Proposed conceptual framework of the physiological strategy followed by surviving and dead *Q. ilex* trees in response to their abiotic niche. Colored arrows represent relative trends of functional traits comparing dead and live trees. VD=vessel density in the xylem hydraulic tissues. K_s=potential hydraulic conductivity per unit area in the xylem hydraulic tissues. NPP=net primary productivity. g_s=stomatal conductance to water vapor.

limitation under increased climatic stress (Bigler et al. 2007; Camarero et al. 2015; Cailleret et al. 2017, 2019; Férriz et al. 2021). Similar to other Mediterranean species (Sarris et al. 2013; Castagneri et al. 2017; Gea-Izquierdo et al. 2019), and regardless of the tree health status, *Q. ilex* growth is highly sensitive to combined water and temperature-induced stress at long- and short time scales integrating both the cold winter and hot-dry summer (Gea-Izquierdo et al. 2021a). All dead trees from our network of *Q. ilex* sites exhibited similar multidecadal growth declines initiated right after past droughts within the exceptional warming period starting after the early 1980s (Lionello and Scarasccia 2018). Conversely, live *Q. ilex* trees did not exhibit such growth declines. This suggests that dead *Q. ilex* may have been predisposed by negative past stress independently of the mortality pattern and the causal stress factor. This includes diffuse annual mortality likely produced by pathogenic *Phytophthora* sp. under increased abiotic stress; and episodic or sporadic mortality after major disturbances like the unprecedented hot drought in this study. Both episodic and diffuse mortality patterns coexist in drier and warmer locations within the studied ecosystem, where there is increased baseline stress produced by years of intensified human land-use under climate change (Camilo-Alves et al. 2013; Moreno-Fernández et al. 2019; Acácio et al. 2021). These two mortality patterns can reflect both limitations in the carbon economy and hydraulic function of trees (Sevanto et al. 2014; Meddens et al. 2015; McDowell et al. 2020; 2022).

Different combinations of xylem adjustment and isotopic composition can be found in healthy and dying trees (e.g. Borghetti et al. 2020; Cherubini et al. 2021; Puchi et al. 2021; Wang et al. 2021; Alderotti et al. 2023), which is partly explained by different physiological pathways in trees succumbing to lethal stress (Camarero et al. 2016, 2020; Pellizzari et al. 2016; Limousin et al. 2022). The conceptual mortality model proposed by Gessler et al. (2018) suggests that dead *Q. ilex* trees were trapped within the hydraulic failure-carbon starvation maze (Choat et al. 2018; Hammond et al. 2019; McDowell et al. 2020, 2022). Dead *Q. ilex* trees shared characteristics from mortality related to carbon limitation (i.e. low growth, high iWUE), and mortality associated with higher xylem conductivity but likely lower safety. Our results strongly suggest that dead trees were predisposed by chronic low carbon assimilation, and that they could have ultimately suffered hydraulic failure under extreme water stress from the 2019 hot drought.

4.3. *Surviving trees thrived in less stressful abiotic niches*

Forest mortality is spatially patchy in part because different trees are able to explore and exploit multiple soil-niches with variable water availability driven by microtopographical heterogeneity (Lloret et al. 2004; Tai et al. 2017; Preisler et al. 2019; Camarero et al. 2020; Bachofen et al. 2024). This may contribute to species conservation because individuals of a given species can find local refugia under climate change and because a patchy distribution of mortality can increase biodiversity and better mimic sustainable ecosystem dynamics (Jacobsen and Pratt 2018). Observed $\delta^{18}O$ values were rather high as expected for a dry ecosystem at mid latitude with high evaporative demand (Barbour 2007; Limousin et al. 2010; Flexas et al. 2014; Martin-Sanchez et al. 2022). Modeled water and carbon dynamics (i.e. simulations for A_n, g_s, iWUE, $\Delta^{13}C$ and $\delta^{18}O$) from shallow (deep) soils were mostly consistent with the functional strategies inferred from the observed anatomical and isotopic traits in dead (surviving) trees. Both model simulations of physiological performance and interpretation of observed isotopic proxies suggest chronic differences in soil water availability between dead and surviving trees. Observed $\delta^{13}C$ suggested greater stomatal constraints on leaf gas exchange in dead trees than in surviving trees in response to more severe chronic stress. Based on these differences in ^{13}C proxies we could have expected higher $\delta^{18}O$ values reflecting lower stomatal conductance in dead trees. Nevertheless, wood of surviving trees was enriched in ^{18}O compared to that in dead trees. This apparent contradiction between $\delta^{13}C$ and $\delta^{18}O$ data can be reconciled considering that wood $\delta^{18}O$ is influenced by both leaf-level evaporative effects (i.e. VPD and transpiration) and by the isotopic composition of the water used by the tree, i.e. wood $\delta^{18}O$ contains a stomatal signal superimposed on a water source signal. The dual analysis of $\delta^{18}O$ and $\delta^{13}C$ suggests that variability in the $\delta^{18}O$ signal in live trees was more influenced by the soil water source than by the evaporative demands. Because the carbon isotopes clearly indicated stronger stomatal constraints on photosynthesis in response to more severe chronic water stress in dead trees (Siegwolf et al. 2023), the most plausible explanation for the lower δ^{18}O in dead trees is that they relied on a greater use of deeper water sources depleted in δ^{18}O (Sarris et al. 2013; Barbeta et al. 2015; González de Andrés et al. 2022, but see Ripullone et al. 2020). Compared to surviving trees, dead trees may have been chronically forced to rely more on deeper water sources from the weathered granitic bedrock (Rose et al. 2003; Carrière et al. 2020; Nardini et al. 2021; Bachofen et al. 2024). Yet, converging δ^{18}O values towards the lower Δ^{13}C range suggest that surviving trees were more capable than dead trees of dynamically exploring deeper soil/bedrock horizons under high water stress. Surviving trees with access to higher soil water sources may had been able to dynamically buffer periods of high evaporative demand (increasing plant δ^{18}O) expressed by decreasing plant Δ^{13}C (reflecting tighter stomatal control with increasing VPD) by increasing water uptake from deeper soil/bedrock (decreasing δ^{18}O). This agrees with our hypotheses and suggests a greater seasonal and/or inter-annual flexibility in surviving trees to explore different water sources at varying depths (Barbeta and Peñuelas, 2017; Grossiord et al. 2017; Brinkmann et al. 2018; Carrière et al. 2020; Illuminati et al. 2022; Bachofen et al. 2024).

Positive trends in iWUE are often observed in forests worldwide in response to rising atmospheric [CO_2] and climate change (Mathias and Thomas 2021). Recent negative trends in δ^{18}O observed in both dead and surviving *Q. ilex* trees under increasing VPD with climate change (that would be expected to increase δ^{18}O) strongly suggest that trees increasingly rely on deeper soil and/or bedrock water sources (Sarris et al. 2013; González de Andrés et al. 2021; Limousin et al. 2022; Bachofen et al. 2024). Although we cannot rule out that dead trees might have grown in poorer microsites, water uptake depth can also influence cumulative plant nutrient uptake (McCulley et al. 2004; Sardans et al. 2020; Querejeta et al., 2021; Illuminati et al. 2022). Heavy tree reliance on nutrient-poor water sources stored in deeper soil/bedrock layers compared to nutrient-rich water stored in upper soil horizons can negatively affect the nutritional status of trees, impairing their physiological performance by reducing assimilation, and ultimately contributing to tree mortality (Gessler et al. 2017; Sardans et al. 2020; Dalling et al. 2024). In particular, plants often exhibit sharp declines in Fe contents under drought stress (León-Sánchez et al. 2020; Querejeta et al., 2021). Dead oak trees had lower Fe content in sapwood than surviving trees, and overall the nutrient status of dead trees was poorer (González de Andrés 2021, 2022). This included higher N:P ratios in dead trees, which is an indication of long-term water shortage (He and Dijkstra 2014). The nutrient impairment observed in dead trees further suggests that they suffered more severe water stress chronically (Coskun et al. 2019; He et al. 2024). Although it may seem contradictory, our results suggest that dead oaks were chronically forced to seek deeper, less accessible water likely from the weathered granitic bedrock. In contrast, live trees likely profited from higher soil water availability, allowing them to dynamically adjust their uptake from different water sources and only rely on deeper sources under high water stress conditions. The hypothesis that dead and surviving trees extract water from different soil depths and how this affects their nutritional status merits further studies specifically measuring soil and xylem water O and H isotopic signal (Werner et al. 2012; Barbeta et al. 2015; Ding et al. 2021; Cernusak et al., 2022; Holloway-Phillips et al. 2023; Mas et al. 2024). Further assessment of this hypothesis will serve to better characterize the functional acclimation of trees to hot and dry climatic conditions, and will also help to elucidate whether the fine-scale spatial distribution of tree mortality can be expected to be concentrated in microtopographic locations and soil-niches with the lowest water storage capacity.

5. Conclusions

All dead oak trees from the two study sites and a regional network expressed long-term vulnerability to increased climatic stress as suggested by multidecadal growth declines prior to mortality independently of the mortality pattern (diffuse, episodic or sporadic), the causal factor (abiotic or biotic) and the plausible physiological mechanism. The onset of radial growth decline began after past drought events framed within the exceptionally warm period starting in the 1980s. Additionally, trees deceased after the extreme hot drought in 2019 expressed negative physiological legacies related to carbon limitation driven by chronic water stress for many years before the mortality event. Dead and surviving trees exhibited different strategies in xylem functional acclimation prior to the mortality event. The hydraulic systems of dead trees were more efficient with higher vessel density and similar or larger vessel distributions. The analysis of C and O isotopic proxies in xylem cellulose suggested that carbon assimilation of dead trees was consistently more constrained by tighter stomatal control than that of surviving trees. Model simulations of tree water and carbon dynamics for shallow (deep) soils were consistent with observations and functional strategies inferred from observations of the different anatomical and isotopic xylem functional traits for dead (surviving) trees. Negative trends in δ^{18}O in both dead and surviving trees warn about greater reliance of trees on deeper water sources with increasing water stress under ongoing climate change. Lower cellulose δ^{18}O in dead trees suggest that they were systematically forced to scout for deeper water sources stored in the weathered or fractured granite bedrock. In contrast, surviving trees likely thriving on soils with greater water availability were able to uptake water more dynamically from deep horizons only under high water stress. Heavier reliance on deeper water sources could have synergistically contributed to nutritional impairment of dead oak trees in the long-term. Observed differences in long-term xylem functional acclimation support the hypothesis of soil-related abiotic niche differentiation between dead and surviving trees. Dead oak trees likely growing in a chronically stressful abiotic niche (i.e. shallow soil pockets underlain by fractured granitic bedrock) expressed a functional paradox: the long-term xylem functional acclimation developed to cope successfully with more severe baseline water stress failed to withstand an additional increase in soil and atmospheric water stress forced by an extreme hot drought. This suggests that dead trees growing on soils with lower water availability could not buffer the further increase in water stress during the extreme hot drought. Therefore, the hydraulic systems of dead trees were not necessarily more vulnerable to drought than those of surviving trees, they could have just suffered higher stress levels.

CRediT authorship contribution statement

Guillermo Gea-Izquierdo: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Macarena Férriz:** Writing – review & editing, Methodology, Data curation. **Maria Conde:** Writing – review & editing, Methodology, Data curation. **Michael N. Evans:** Writing – review & editing, Methodology, Formal analysis. **Jose I. Querejeta:** Writing – review & editing, Methodology, Formal analysis. **Dario Martin-Benito:** Writing – review & editing, Visualization, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Guillermo Gea-Izquierdo reports financial support was provided by Spanish Ministry of Science and Technology. Guillermo Gea-Izquierdo reports a relationship with ICIFOR-INIA, CSIC that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2025.110430.

Data availability

Data will be made available on request.

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