Increasing carbon discrimination rates and depth of water uptake favor the growth of Mediterranean evergreen trees in the ecotone with temperate deciduous forests

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Abstract
Tree populations at the low-altitudinal or -latitudinal limits of species’ distributional ranges are predicted to retreat toward higher altitudes and latitudes to track the ongoing changes in climate. Studies have focused on the climatic sensitivity of the retreating species, whereas little is known about the potential replacements. Competition between tree species in forest ecotones will likely be strongly influenced by the ecophysiological responses to heat and drought. We used tree-ring widths and \( \delta^{13}C \) and \( \delta^{18}O \) chronologies to compare the growth rates and long-term ecophysiological responses to climate in the temperate-Mediterranean ecotone formed by the deciduous Fagus sylvatica and the evergreen Quercus ilex at the low altitudinal and southern latitudinal limit of F. sylvatica (NE Iberian Peninsula). F. sylvatica growth rates were similar to those of other southern populations and were surprisingly not higher than those of Q. ilex, which were an order of magnitude higher than those in nearby drier sites. Higher Q. ilex growth rates were associated with high temperatures, which have increased carbon discrimination rates in the last 25 years. In contrast, stomatal regulation in F. sylvatica was proportional to the increase in atmospheric CO\(_2\). Tree-ring \( \delta^{18}O \) for both species were mostly correlated with \( \delta^{18}O \) in the source water. In contrast to many previous studies, relative humidity was not negatively correlated with tree-ring \( \delta^{18}O \) but had a positive effect on Q. ilex tree-ring \( \delta^{18}O \). Furthermore, tree-ring \( \delta^{18}O \) decreased in Q. ilex over time. The sensitivity of Q. ilex to climate likely reflects the uptake of deep water that allowed it to benefit from the effect of CO\(_2\) fertilization, in contrast to the water-limited F. sylvatica. Consequently, Q. ilex is a strong competitor at sites currently dominated by F. sylvatica and could be favored by increasingly warmer conditions.

Keywords
climate change, dendroecology, ecophysiology, ecotones, Mediterranean forests, temperate forests, tree growth, tree water use, tree-ring isotopes

1 | INTRODUCTION

Reports of poleward and upward altitudinal shifts in the distributional ranges of plant species in response to warmer conditions are increasing (Jump, Mátayás, & Peñuelas, 2009; Chen, Hill, Ohlemüller, Roy, & Thomas, 2011). The accumulated evidence suggests that these shifts are a worldwide phenomenon; shifts have been observed in plant species from arctic (Myers-Smith, Forbes, & Wilmking, 2011), temperate (Lenoir, Gégout, Pierrat, Bontemps, & Dhôte, 2009), Mediterranean (Penuelas & Boada, 2003), and (Allen...
logical processes (McCarroll & Loader, 2004). Trees take up CO2 and produce carbohydrates that are fixed in the annual rings. The resulting isotopic footprint in the wood incorporates both the signals of past environmental conditions and tree ecophysiology (McCarroll & Loader, 2004; Gessler et al., 2014). Retrospectively analyzing tree-level physiological processes is therefore possible using annual series of isotopic ratios of tree-ring carbon and water (commonly 13C/12C and 18O/16O).

Carbon isotopic ratios in wood are sensitive to the impacts of gas exchange (Francey & Farquhar, 1982; Scheidegger, Saurer, Bahn, & Siegwolf, 2000). 13C can be postphotosynthetically discriminated (Gessler et al., 2009; Offermann et al., 2011), but 13C/12C ratios can be used as a proxy of the ratio between atmospheric (C3) and intracellular (C4) CO2 concentrations in C3 plants (Saurer, Siegwolf, & Schweingruber, 2004). Temperate tree species have maintained a moderately constant CI/Ca ratio under the current sustained increase in atmospheric CO2 concentration, suggesting a partial CO2-induced stomatal closure (Frank, Poultier, & Saurer, 2015). This regulation has led to a more efficient use of water; less water is transpired per unit of carbon assimilated, but this decreased transpiration has generally not increased tree growth in temperate forests (Peñuelas, Canadell, & Ogaya, 2011), nor in water-limited forests (Andreu-Hayles et al., 2011; Battipaglia et al., 2014; Granda, Rossatto, Camarero, Voltas, & Valladares, 2014; Olano et al., 2014). Relict tree populations at the low-altitudinal and -latitudinal limits of their distributional ranges have experienced drought-related declines in growth in recent years, albeit with higher water-use efficiencies (lower discrimination rates (Δ13C)) (Peñuelas, Hunt, Ogaya, & Jump, 2008; Linares, Delgado-Huertas, Camarero, Merino, & Carreira, 2009; Heres, Voltas, Lopez, & Martinez-Vilalta, 2014). Drought-affected trees have stronger stomatal control but lower growth than nonaffected trees at the stand level, as shown by lower carbon discrimination rates (Voltas et al., 2013). The relationship between carbon discrimination rates (i.e., regulation of gas exchange) and growth rates, however, can be highly species-specific in these water-limited environments (Ferrio, Florit, Vega, Serrano, & Voltas, 2003). Species-specific differences in the strategies of water use and in the associated plant traits strongly modulate the climatic sensitivity of Δ13C (Shrestakova, Aguilera, Ferrio, Gutierrez, & Voltas, 2014).

The analysis of tree-ring δ18O provides additional information on tree-water relationships (McCarrroll & Loader, 2004; Sternberg, 2009), which is essential for identifying the ecophysiological controls on long-term growth trends and thus on forest productivity. Analyses of the oxygen isotopes (δ18O) in the organic matter of C3 plants have been used to distinguish between the effects of assimilation rates and stomatal conductance, because δ18O is not substantially affected by photosynthesis (Scheidegger et al., 2000). A dual-isotope approach including both tree-ring δ13C and δ18O could therefore be useful for studying stomatal behavior over time, but only under certain conditions (Barnard, Brooks, & Bond, 2012). The variability in tree-ring δ18O encompasses the effects of source-water δ18O, leaf-level enrichment, exchanges between carbohydrate pools and the postphotosynthetic exchange of oxygen in stem
water during phloem transport and the deposition of cellulose (Gessler et al., 2014). The interpretation of tree-ring δ18O under uncontrolled environmental conditions may thus not always be able to reliably reconstruct leaf-level processes (Rodén & Farquhar, 2012; Rodén & Siegwolf, 2012). Besides, the water vapor δ18O also influences leaf water δ18O and consequently the δ18O of organic matter (Dubbert, Kübert, & Werner, 2017). In contrast, the δ18O signal of the source water was more important than the effect of leaf-level water enrichment on tree-ring δ18O of conifer species (Treydte, Boda, & Graf Pannatier, 2014). Tree-ring δ18O may reflect the patterns in the tree water sources (Leonelli, Battipaglia, & Cherubini, 2016; Sargeant & Singer, 2016), indicating contrasting strategies of water uptake between coexisting species (Shrestakova et al., 2014; Churakova Sidorova, Saurer, Bryukhanova, Siegwolf, & Bigler, 2016). Drought can decrease tree-ring δ18O in deep-rooted Mediterranean species by forcing the use of deeper water sources more depleted in 18O (Sarris, Siegwolf, & Körner, 2013). Additionally, drought can trigger a premature growth cessation and reduce the amount of wood formation in summer, which usually has more enriched values (Pflug et al., 2015). Despite our understanding of the processes influencing tree-ring δ18O is improving but not yet complete, studies focused on the long-term effects of increasing drought can extract relevant information from this isotopic archive.

We retrospectively analyzed the effect of climate on growth rates and the ecophysiology of an ecotone formed by one of the southernmost populations of the temperate deciduous European beech (F. sylvatica) and the Mediterranean evergreen broadleaved Holm oak (Quercus ilex L.). F. sylvatica populations at low altitude in the Mediterranean region have declined in growth linked to increasing temperatures and drought (Jump et al., 2006). The forest compositions at these sites indicate a progressive replacement of F. sylvatica by the more drought-resistant, slower growing and generally smaller Q. ilex, which dominates the immediate lower altitudes (Penuelas, Ogaya, Boada, & Jump, 2007). Most studies, however, have focused on the species that is potentially retreating, in this case, F. sylvatica. A shift in vegetation, though, would only be possible if Q. ilex out-competed F. sylvatica in this ecotone by adapting better to the current and future climates. We hypothesized that a decline in F. sylvatica growth would be paralleled by an increase in Q. ilex growth. This tree species, however, is at the coldest limit of its distribution in this ecotone, so we expected that its growth would be limited by the minimum temperatures in early spring and winter. We analyzed the growth–climate relationships and tree-ring δ13C and δ18O to understand the ecophysiological mechanisms driving the observed decline in growth of F. sylvatica and the hypothesized increased growth of Q. ilex. Specifically, we (i) compared the growth rates of F. sylvatica and Q. ilex and their relationship with climate, (ii) analyzed tree-ring δ13C and δ18O chronologies to identify the ecophysiological mechanisms driving the growth patterns of the tree rings and (iii) investigated the climatic determinants of tree-ring isotopes and their links with physiological traits of the tree species.

2 | MATERIAL AND METHODS

2.1 Study site, sampling, and tree-ring chronologies

This study was conducted in one of the southernmost populations of F. sylvatica, in the Montseny Mountains (NE Iberian Peninsula, 41°49′28″N, 2°24′20″E). This population is thus at the drier and warmer edge of the F. sylvatica distributional range. The stand is in the ecotone between broadleaved temperate and evergreen Mediterranean forests. F. sylvatica dominates the higher elevations (750–1690 m a.s.l.) of this mountain range, whereas Q. ilex is the most abundant tree species at the lower elevations (300–1300 m a.s.l.) (De Bolós, 1983). Regional and altitudinal distributional ranges are further detailed in Fig. S1 and Fig S2. The altitude of this ecotone varies with the aspect but is 1000–1100 m a.s.l. in the study area (facing NW). The climate is temperate, without a dry season and with warm summers (Cfb in the Köppen-Geiger climatic classification, Fig. S3). The soils are typically Dystric Cambisols and Dystric Regosols established over granodiorite lithology (Penuelas et al., 2008). Climatic data for 1996–2011 were obtained from a meteorological station 2 km from the sampling area (Viladrau; 950 m a.s.l.; 41°50′22″N, 2°25′10″E). Data from the Viladrau station were correlated with data from the Turó de l’Home meteorological station on the highest peak of these mountains (1707 m a.s.l.), 6 km from Viladrau (41°46′37″N, 2°26′04″E). Monthly means for maximum and minimum temperatures and the monthly precipitation were correlated between the two meteorological stations (R2 = 0.99, 0.99 and 0.65, respectively) to produce a series spanning from 1920 to 2011 (Figure 1a). Monthly temperature and precipitation data were also used to calculate series of D, the balance between precipitation and potential evapotranspiration (Thornthwaite, 1948). The D series were later used to compute the Standardized Precipitation and Evapotranspiration Index (SPEI; see Supporting Information SI for more details), a multiscalar drought index that includes the effect of temperature in the water balance. The δ18O of precipitation for the period 2000–2010 was obtained from a station of the Global Network of Isotopes in Precipitation (GNIP) located 30 km away from the sampling plots.

The sampling area was along the limit between Q. ilex and F. sylvatica dominance at altitudes ranging from 1100 to 1150 m. Trees of both species were on the NW face of a hill, but Q. ilex tended to occupy the more exposed sites close to the top of the hill, and F. sylvatica tended to occupy more sheltered areas a few meters lower. We randomly selected 30 dominant or codominant trees of each species along this altitudinal range in January 2014 without focusing on a particular size class to avoid biases due to size (see Brienen, Gloor, and Ziv (2016)). We extracted cores of some F. sylvatica at breast height (1.30 m) with a 4.3-mm increment borer and others with a 12-mm increment borer to ensure obtaining enough material for subsequent isotopic analyses of the wood. The wood anatomy of Q. ilex requires the analysis of entire stem disks, as for other Mediterranean species (Cherubini et al., 2003). Tree rings are usually not sufficiently clear, and their thick parenchymal radii can occupy
large sections of the increment cores and complicate the identification of tree-ring boundaries (Gea-Izquierdo, Martín-Benito, Cherubini, & Cañellas, 2009). We cut basal stem disks of Q. ilex at a site of recent selective logging. Both tree cores and stem disks were air-dried, sanded and polished. The even wood surfaces were then scanned at 1200 dpi, and tree rings were measured using WinDendro (Regent Instruments Inc., Québec, Canada) with the assistance of a stereomicroscope. The samples were cross-dated visually using WinDendro and statistically validated using COFECHA (Holmes, 1983). We used only 28 F. sylvatica and 18 Q. ilex individuals of the 30 initially sampled trees, because we discarded trees with poor correlations with the rest of the population and because a few series for Q. ilex could not be reliably dated.

Tree-ring widths (mm) were transformed to basal area increments (BAI, in mm²) at yearly intervals by subtracting the basal area accumulated during the previous years to the basal area accumulated by year t (BAI_t = πR_t² − πR_{t−1}², where R is the tree radius) and assuming a circular stem (Biondi, 1999). BAI series of dominant trees usually have an initial suppressed phase with low BAI rates followed by a release phase where trees reach mature growth due to canopy dominance. A 9-year running mean was calculated for the BAI chronology of each species to identify the postrelease inflection point (Figure 2), assuming that this point represented the time that the mature phase of growth was reached (Jump et al., 2006). Growth trends were therefore assessed from this point. The inflection point was in 1952 for F. sylvatica and in 1992 for Q. ilex. The shorter chronology (66 years) of Q. ilex tree-ring widths resulted in a BAI trend dominated by the suppressed phase for the first 46 years of the series. We therefore decided to assess climate–growth and isotope–growth relationships using the ring-width index (RWI), not the BAI. Individual series of tree-ring widths were detrended to remove age-related trends and autocorrelations by applying the Friedman supersmooth spline (Friedman, 1984). The resulting RWI series were used to build a chronology using a Tukey’s bi-weight robust mean. Both the BAI

**FIGURE 1** Climatic conditions during the study period. (a) Annual precipitation and mean annual temperature. The line corresponds to the period in which the linear regression of temperature over time was significant (1976–2001). (b) The course of the Standardized Precipitation and Evapotranspiration Index (SPEI), calculated for July and comprising the water balance of the 24 months before July of each year.
FIGURE 2 Mean basal area increment (BAI) for *F. sylvatica* (green) and *Q. ilex* (maroon) during the study period. The error bars are the standard errors of the means, and the lines are the 9-year running means of the BAI for each species.

and the RWI series were calculated with functions from the `dplR` package (Bunn, 2008) in R (R Core Development Team, 2012).

### 2.2 Tree-ring isotopes

We selected five of the sampled trees of each species that had clear tree-ring boundaries and no false or missing rings (which are common in *Q. ilex*). The 12-mm cores (*F. sylvatica*) and stem-disk surfaces (*Q. ilex*) were cut with a high-precision diamond wafering saw to obtain pieces 0.5–2 mm thick and 1 cm wide. The length of the pieces was the maximum for each sample (up to 30 cm); the tree rings for 1987–2011 (the target period) were always within the first 6 cm of wood. These wood pieces were subsequently used for isotopic analysis. Recent studies have shown that whole wood and α-cellulose of beech sapwood retain comparable environmental signals (Weigt, Bräunlich, & Zimmermann, 2015), but we decided to extract cellulose from whole wood, because the potential effect of lignin deposition interfering with the annual climatic signals can be larger in oaks, and because no studies have compared the signatures from whole wood and cellulose for *Q. ilex*. Wood pieces up to 6 cm long (containing the targeted period of tree rings) were enclosed in Teflon punching-sheet holders (Kagawa, Sano, Nakatsuka, Ikeda, & Kubo, 2015; Schollaen, Baschek, Heinrich, & Helle, 2015). The enclosed samples were submerged in 5% sodium hydroxide (2 × 2 h, 60°C) and then 7% sodium chloride (pH 4–4.5 (acetic acid), 36 h, 60°C) using a peristaltic pump with two silicon tubes for continuous circulation and easy renewal of chemical solutions and gentle washing with deionized water. The extracted cellulose samples were freeze-dried and cut under a binocular microscope with a scalpel and with the support of digital images of untreated wood. Tree-ring boundaries were clearly distinguishable, as shown by previous studies (Kagawa et al., 2015). We removed cellulose fibers spanning the entire width of the tree rings (representing both early and late wood), which were subsequently enclosed in tin (carbon analysis) or silver (oxygen analysis) capsules to maximum masses of 180–220 μg for carbon analysis and 130–165 μg for oxygen analysis. Carbon isotopic ratios (13C/12C) were measured by combustion at 1080°C using an elemental analyzer (Model NA 1500; Carlo Erba, Milan, Italy) coupled online to an isotopic ratio mass spectrometer (IRMS) (Isoprime Ltd, Cheadle Hulme, UK). Oxygen isotopic ratios (18O/16O) were determined using a high temperature TC/EA pyrolytic oven at 1340°C coupled online to an IRMS (Delta V Advantage; Thermo Fisher Scientific, Bremen, Germany). The isotopic ratios are given with the delta (δ) notation, relative to the Vienna Pee Dee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW) standards for δ13C and δ18O, respectively (Craig, 1957). The laboratory analyses were conducted at the GFZ German Center for Geosciences in Potsdam, Germany.

The isotopic values of atmospheric CO2 (δ13Cair) have declined in recent decades due to land-use changes and the combustion of fossil fuels, becoming less enriched in the heavier isotope 13C. We calculated the carbon discrimination ratios (Δ13C) in tree-ring cellulose following Farquhar, O’Leary, and Berry (1982) to account for these changes:

\[ \Delta^{13}C = \left( \delta^{13}C_{air} - \delta^{13}C_{cel} \right) / \left( 1000 - \delta^{13}C_{cel} \right) \times 1000 \]

where δ13Cair is the isotopic composition of atmospheric CO2, and δ13Ccel is the isotopic composition of tree-ring cellulose. The δ13Cair and the CO2 concentrations in ppm were obtained from the Global Monitoring Division of the Earth System Research Laboratory (NOAA, USA, [http://www.esrl.noaa.gov/gmd/index.html](http://www.esrl.noaa.gov/gmd/index.html)). The discrimination rate associated with the fixation of CO2 (Δ13C) is correlated with \( C_s \) and \( C_h \) by (Farquhar et al., 1982):

\[ \Delta^{13}C = a + (b - a) \times (C_i/C_s) \]

where \( a \) is the discrimination against 13CO2 during CO2 diffusion through stomata \( (a = 4.4\% \text{, O’Leary, } 1981) \), and \( b \) is the discrimination associated with carboxylation \( (b = 27\% \text{, Farquhar et al., } 1982) \). We then obtained \( C_i \) at annual resolutions for each species. The species-specific trends in \( C_i \) for 1987–2011 were used to assess potential changes in the relationship between \( C_i \) and \( C_s \) as proposed by Saurer et al. (2004). Plant gas exchange can be regulated to maintain a constant \( C_s \) (strong stomatal regulation, scenario 1), to maintain a constant \( C_a \) (weak stomatal regulation, scenario 2) or to maintain a constant \( C_s - C_a \) in which increases in \( C_s \) match increases in \( C_a \) (weak stomatal regulation, scenario 3). In contrast to \( \delta^{13}C_{cel} \), the isotopic composition of tree-ring cellulose oxygen, \( \delta^{18}O_{cel} \), obtained from the analyzers was directly used in the statistical analyses.

### 2.3 Statistical analyses

We tested the temporal changes in mean air temperatures, precipitation, and the drought index by fitting a regression model with segmented relationships and estimated break-points (segmented package in R) for 1920–2011. The trend in BAI was assessed for *F. sylvatica* using a linear regression but was not assessed for *Q. ilex* due
to the shortness of the mature-growth phase. Species-specific differences in BAI for 1987–2011 were statistically tested with general linear models in the nlme package in R, accounting for temporal autocorrelation. *Q. ilex* reached the mature phase of growth in 1992, so we added a factor to differentiate between the suppressed and mature phases. All basic statistics for the tree-ring chronologies were calculated using the dplR package. The relationships between monthly climatic variables (mean maximum and minimum temperatures and total precipitation) and the chronologies of the ring-width indices were calculated using bootstrapped correlation functions in the treeclim package in R (Zang & Biondi, 2015). The temporal changes in tree-ring isotopes ($\Delta^{13}$C and $\delta^{18}$O) and in $C_3$/Ca and $C_4$–$C_3$ were assessed by fitting linear regressions. To identify the environmental determinants of the tree-ring isotopes, we built two structural equation models (path analysis; sem package in R) for each species using tree-ring isotopic data and tree-ring widths (standardized) as endogenous variables and monthly climatic variables (mean maximum and minimum temperatures and total precipitation) as exogenous variables. Models were selected based on the Akaike Information Criterion (AIC), where models with an AIC decrease of two or more points were selected, and the most parsimonious of those with similar AICs (difference <2) was chosen. The beta coefficients are standardized estimates used to compare the strength of the relationship among dependent and independent variables. In addition, seasonal values of rainfall $\delta^{18}$O were correlated with tree-ring $\delta^{18}$O using a linear regression for each species. Correlations between relative humidity and tree-ring isotopes were also calculated using bootstrapped correlation functions in the treeclim package in R, but for the period for which data for relative humidity were available (1996–2011). We calculated the SPEI drought index using temperature and precipitation data from the Vladrarlu meteorological station (Vicente-Serrano, Beguería, & López-Moreno, 2010). This index was calculated at monthly resolutions and with different temporal scales. We correlated the isotopic composition in tree-ring cellulose with this index at scales ranging from 1 to 24 months with the same bootstrapped correlation functions (treeclim) used for the RWI.

3 | RESULTS

The mean annual temperature in the study area for 1920–2011 was 10.6°C, and the mean annual precipitation was 1027 mm (Fig. S3). The mean annual temperature increased at an average rate of 0.013°C y⁻¹ ($p < .001$) for the same period. The segmented regression model indicated that this significant increase was mainly driven by the period 1976–2001 (Figure 1a), when it increased at a rate of 0.09°C y⁻¹. In contrast, we did not detect any trend for the precipitation data. The long-term water balance, indicated by the SPEI (calculated at a scale of 24 months and in July), however, decreased significantly (drier conditions) after 1973 ($p < .05$) (Figure 1b).

The sampled trees had mean diameters of 34.7 ± 13.6 and 33.7 ± 11.2 cm for *F. sylvatica* and *Q. ilex*, respectively (Fig. S4). The chronological statistics are detailed in Table 1. Both populations were relatively young, with the chronologies spanning 1925–2011 and 1945–2011 for *F. sylvatica* and *Q. ilex*, respectively. The *F. sylvatica* population had a stronger common signal, as shown by the higher average correlation between series ($r_{bar}$) and the expressed population signal. The mean tree-ring width was slightly larger in *Q. ilex*. The BAI of *Q. ilex* had only 15 years of mature growth (sensu Jump et al., 2006). In contrast, the *F. sylvatica* population reached the mature phase of growth in 1952. BAI increased significantly in *F. sylvatica* by 0.13 cm² y⁻¹ for 1952–2011 ($p < .001$). Twenty-two of the 28 individual trees, though, had not reached the mature phase of growth by 1952. *Q. ilex* did not reach the mature phase of growth until 1992. We compared the BAI for 1987–2011 (coinciding with the isotopic data) but acknowledging the change in growth phase of *Q. ilex*. Mean BAI during 1987–1992 averaged 6.91 cm² larger for *F. sylvatica* than *Q. ilex* ($p < .001$). The BAI of the two species, however, did not differ significantly when *Q. ilex* reached the mature growth phase (1992–2011).

The correlation between the RWI series and the monthly climatic variables for 1960–2011 identified species-specific differences in the sensitivity of growth to climate. The correlation coefficient for *F. sylvatica* growth was highest with the precipitation for June and July (Figure 3a). Growth was also correlated positively with the maximum and minimum temperatures for February but negatively with June and July maximum temperatures, July minimum temperatures and September precipitation. In contrast, *Q. ilex* growth was sensitive only to precipitation. Growth was correlated positively with June precipitation and more weakly but nonetheless significantly negatively with September and October precipitation of the year previous to the tree-ring growth (Figure 3b).

We analyzed the temporal trends and species-specific differences in tree-ring carbon discrimination rates ($\Delta^{13}$C) and oxygen ratios $\delta^{18}$O for 1987–2011. $\Delta^{13}$C averaged significantly higher in *Q. ilex* than *F. sylvatica* ($p < .01$) (Figure 4a). In turn, the coefficient of variance (CV) (0.057 vs. 0.036) was higher for the *F. sylvatica* $\Delta^{13}$C series, indicating greater inter-annual variability than *Q. ilex*. *Q. ilex* had a significant and increasing temporal trend of $\Delta^{13}$C with higher values at the end of the period. *F. sylvatica* $\Delta^{13}$C did not have a

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<th>Descriptive statistics for the tree-ring chronologies</th>
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<td><em>Fagus sylvatica</em></td>
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<td><em>Quercus ilex</em></td>
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significant temporal trend, unlike Q. ilex. F. sylvatica had a higher average tree-ring δ¹⁸O (more enriched in the heavy isotope) (p < .01) (Figure 4b). The F. sylvatica δ¹⁸O series had a higher coefficient of variance (CV) (0.030 vs. 0.011), indicating greater inter-annual variability than Q. ilex. The Q. ilex series of tree-ring δ¹⁸O tended to decrease over time (p = .069). Again, no significant temporal trend was detected for F. sylvatica tree-ring δ¹⁸O.

Ci was derived from tree-ring Δ¹³C and Ca, as described above. We evaluated the response of Ci to the increase in Ca during 1987–2011 (from 349.2 to 391.6 ppm) under the theoretical framework proposed by Saurer et al. (2004). Ci progressively and significantly increased in both species from 1987 to 2011 (Figure 5a). F. sylvatica did not show any significant trend in the Ci/Ca ratio during the same period (Figure 5b), reflecting a proportional regulation of carbon assimilation and stomatal conductance in response to increasing Ca. The proportional regulation of Ci relative to Ca due to the increase in Ca led to a higher water-use efficiency in F. sylvatica. However, the Ci/Ca ratio increased significantly and steadily for Q. ilex from 1987 to 2011, indicating that the response of stomatal conductance to the increase in Ci was weaker for Q. ilex than F. sylvatica. The temporal trends of Ci and Ca did not differ for either species (Figure 5c).

The structural equation models for 1987–2011 indicated that the climatic determinants of tree-ring growth, Δ¹³C and δ¹⁸O differed between the species (Figure 6). These models assessed the direct and indirect effects within a multivariate design, in contrast to the bivariate relationships between the monthly climatic variables and growth (Figure 3). The accumulated precipitation for June and July had a large effect on F. sylvatica, which significantly determined the three response (i.e., endogenous) variables. June and July precipitation particularly had a positive effect on tree-ring growth (R² = .50), a positive effect on tree-ring Δ¹³C (R² = .41) and a negative effect on tree-ring δ¹⁸O (R² = .55) (Figure 6a). No indirect effects were identified. The model was more complex for Q. ilex. Similar to F. sylvatica, Q. ilex tree-ring growth was positively correlated with the
significant (F. sylvatica and Q. ilex respectively). Ca and Ci for 1987 for February, mediated by the tree-ring was also indirectly positively affected by the minimum temperature for April and the tree-ring growth, though, were determined by the temperatures experienced prior to or at the very beginning of the growing season (April). Relative humidity had contrasting effects on the tree-ring isotopes of F. sylvatica and Q. ilex for a shorter period, 1996–2011. \( \Delta^{13}C \) for F. sylvatica was correlated positively with the relative humidity from May to August and negatively with the relative humidity for October from the year previous to the ring growth, whereas \( \Delta^{13}C \) for Q. ilex was not significantly correlated with relative humidity (Figure 7). The opposite pattern was observed for \( \delta^{18}O \); \( \delta^{18}O \) was significantly positively correlated with relative humidity during the growing season (April to September) for Q. ilex but not F. sylvatica (Figure 7). F. sylvatica tree-ring \( \delta^{18}O \) showed a strong correlation with the \( \delta^{18}O \) of the precipitation from February to April \( (R^2 = .78, p < .001) \), whereas Q. ilex tree-ring \( \delta^{18}O \) had a marginally significant correlation with the \( \delta^{18}O \) of the precipitation from January to May \( (R^2 = .29, p = .09) \), Figure 8).

We calculated the correlations between the tree-ring isotopes and the SPEI drought index at different temporal scales to gain more insight into the coupling of tree-ring isotopes with climate beyond monthly averages. The correlations between the SPEI values spanning from 1 to 24 months and from January to October with the carbon discrimination rates for F. sylvatica and Q. ilex are shown in Fig. 5a and 5b, respectively. \( \Delta^{13}C \) for F. sylvatica was positively correlated most highly with July SPEI-2, in agreement with the structural equation model (Figure 6). \( \Delta^{13}C \) was positively correlated with the water balance (wetter conditions) during May to August of the year of ring growth. Similarly, the long-term water balance encompassing the previous winter or even the entire previous year was positively correlated with \( \Delta^{13}C \) (Figure 5a). \( \Delta^{13}C \) for Q. ilex was less well correlated with the water balance, with a positive correlation only with a short-term (1 month) water balance in June (Fig. 5b). Tree-ring \( \delta^{18}O \) was generally more strongly correlated than \( \Delta^{13}C \) with the water balance for both species. All correlations were negative, implying that positive water balances depleted tree-ring \( \delta^{18}O \). The correlations for F. sylvatica were strongest during late spring and the summer months (June, July, and August), for both short- and long-term water balances (Fig. 5). Correlations were significant for all months at longer SPEI timescales (more than a year). Tree-ring \( \delta^{18}O \) was less dependent on the water balance for Q. ilex than F. sylvatica, as shown by the lower number of significant correlations. The correlation was strongest with the short-term water balance for February (1 month). The mid-term water balance (4 to 10 months) for May and June was also significantly correlated with \( \delta^{18}O \) (Fig. 5).
4 | DISCUSSION

4.1 | Growth patterns in a climate-sensitive ecotone

This dendroecological study conducted in the ecotone between Mediterranean evergreen and temperate broadleaved forests indicated that the similar growth rates of the drought-resistant evergreen Q. ilex and the temperate deciduous broadleaved F. sylvatica were associated with the recent warming trend. The growth rates of mature F. sylvatica (17.7 ± 0.7 cm² y⁻¹) were similar to those of other populations along the drier edge of the distributional range and were clearly lower than those of core populations (Cavin & Jump, 2016). In contrast, the Q. ilex average growth rates were an order of magnitude higher than the average growth rates in other Q. ilex stands at sites with more pronounced summer droughts. The average growth rate of mature Q. ilex was 20.8 ± 0.04 cm² y⁻¹, whereas other studies have reported rates <2 cm² y⁻¹ (Barbeta, Ogaya, & Peñuelas, 2013; Granda, Camarero, Gimeno, Martínez-Fernández, & Valladares, 2013; Camarero, Sangüesa-Barreda, & Vergarechea, 2015; Lempereur et al., 2015). The single-stemmed structure of this stand, the larger tree sizes and the high rainfall may have contributed to this large difference. The tree-ring widths of Q. ilex were more similar, albeit still wider, than those of low-density oak-savannas (dehesas) containing old and large trees (Gea-Izquierdo et al., 2009; Gea-Izquierdo, Cherubini, & Cañellas, 2011). The ecotone studied represents the upper altitudinal limit of Q. ilex in the region, so the exceptionally high growth rates suggest that the climate at this range limit has recently become optimum for this species. Q. ilex could thus potentially expand upslope, but this expansion would be subject to internal ontogenic and demographic processes (Rabasa, Granda, & Benavides, 2013) and to the direct competition for light and water with F. sylvatica during early life stages. We thus did not detect a decline in the growth of F. sylvatica, such as in nearby sites where this species is also limited by the dominance of Q. ilex (Jump et al., 2006), perhaps because the trees in our study were at higher altitudes, and many of the sampled trees were younger. In any case, our sampling design does not allow us to make strong inferences from long-term growth trends (Nehrbass-Ahles et al., 2014).

Our hypothesis that the recent growth enhancement of Q. ilex, leading to mature growth rates similar to those of F. sylvatica, was mediated by higher temperatures was supported by the sensitivity of growth to climate. High temperatures in early summer decreased the growth of F. sylvatica, whereas Q. ilex growth was insensitive to temperature (Figure 2). The growth of both species increased in years with a wet June, but only F. sylvatica responded positively to July precipitation, which alleviated the summer drought. This sensitivity of F. sylvatica growth to summer drought is not limited to southern populations, because it is found throughout the species’ distribution (Hacket-Pain, Cavin, Friend, & Jump, 2016). Southern populations could accordingly be even better adapted to summer drought, demonstrated by the absence of growth sensitivity to summer drought of beech stands at higher altitudes of the nearby mountains (Jump, Hunt, & Peñuelas, 2007). Increasing summer drought in the ecotone in our study, though, may favor the drought-resistant Q. ilex. Q. ilex growth at drier sites has been negatively correlated with high temperatures (Corcuera, Camarero, & Gil-Pelegrín, 2004; Gea-Izquierdo et al., 2009; Granda et al., 2013), but warm winters have enhanced growth at the coldest sites (Gea-Izquierdo et al., 2011; Camarero et al., 2015). The stand in our study was at the upper altitudinal limit of Q. ilex (see SI), but we detected no effect of winter temperatures, contrary to our expectations. Thermal inversions in
winter in this area may have mitigated the negative effects of frosts (Dot & Jiménez, 2002). The variability of inter-annual growth for *Q. ilex* was a function of June precipitation, but climatic factors generally did not constrain the growth of this evergreen species in the face of the current warming, in contrast to *F. sylvatica*.

4.2 | Species-specific differences in long-term gas exchange

The species-specific differences in the sensitivity of growth to climate are consistent with the temporal trends in the physiological parameters identified by the tree-ring isotopes. *Q. ilex* discrimination rates ($\Delta^{13}C$) increased throughout the study period, suggesting either a disproportional increase in stomatal conductance or rather unlikely a decrease in photosynthesis. $\Delta^{13}C$ was lower for *F. sylvatica* than *Q. ilex* (Figure 4), indicating a stronger regulation of $C_i$ by the deciduous species (Saurer et al., 2004). To do so, *F. sylvatica* proportionally regulated $C_i$ in response to increasing $C_a$ (Figure 5b) probably by reducing stomatal conductance and maintaining or slightly decreasing assimilation rates, as reported for other sites (Duquesnay, Bréda, Stievenard, & Dupouey, 1998; Peñuelas et al., 2008). Stomatal control, though, was weaker for *Q. ilex*, indicated by the increasing $C_i/C_a$ ratio over time, which in turn may have boosted *Q. ilex* photosynthetic activity. Lower $\delta^{18}O$ and/or higher water-use efficiency have previously been reported in drought-affected trees of *F. sylvatica* (Peñuelas et al., 2008) and of other species at the driest limits of their distributions (Voltas et al., 2013; Hereş et al., 2014). Most temperate European trees, however, tend to maintain a constant $C_i/C_a$ ratio and thus have increased their water-use efficiencies over recent decades (Frank et al., 2015). Thus, the temporal changes in the regulation of gas exchange alone are therefore not evidence of an increased vulnerability of *F. sylvatica* to climate. On the other hand, the improvement in the regulation of gas exchange in *Q. ilex* indicates the successful physiological acclimation of this species to the ongoing warming and increasing $C_a$.

In addition to the temporal trends, the inter-annual variability in $\Delta^{13}C$ identified contrasting sensitivities to drought and temperature between the species. The correlation between the water balance at
the peak of the growing season and $\Delta^{13}C$ was much stronger in $F. \text{sylvatica}$ than $Q. \text{ilex}$ (Fig. S5). The water balance for periods up to two years was correlated with $F. \text{sylvatica} \Delta^{13}C$, which could be due either to the use of soil water stored for long periods or to residual effects of drought (Anderegg, Schwalm, & Biondi, 2015; Gazol et al., 2016). Similar to previous studies, $\Delta^{13}C$ for $F. \text{sylvatica}$ was increased by high precipitation (Saurer, Borella, Schweingruber, & Siegwolf, 1997; Saurer et al., 2008) and relative humidity (Hemming, Switsur, Waterhouse, Heaton, & Carter, 1998) during the growing season, whereas the correlation of $\Delta^{13}C$ with climate was clearly weaker for $Q. \text{ilex}$. Furthermore, both the growth and $\Delta^{13}C$ of $Q. \text{ilex}$ were positively affected by high minimum temperatures in spring. Future increases in temperature leading to more negative water balances may further restrict $F. \text{sylvatica}$ gas exchange, and the more drought-resistant $Q. \text{ilex}$ could benefit from the fertilization effect of CO$_2$, because water conditions at the site are well above the minimum requirements of $Q. \text{ilex}$ (around 400 mm y$^{-1}$; Terradas, 1999).

4.3 | Ecophysiological interpretation of tree-ring $\delta^{18}O$: beyond stomata control

The uncontrolled conditions of this study prevented the use of tree-ring $\delta^{18}O$ to distinguish between the relative roles of assimilation rate and stomatal conductance in driving the patterns in $\Delta^{13}C$ (Roden & Siegwolf, 2012). Our results, though, add to the growing evidence supporting a predominant effect of the variability in source-water $\delta^{18}O$ on tree-ring isotopic signals (Sarris et al., 2013; Treydte et al., 2014; Leonelli et al., 2016). Indeed, $F. \text{sylvatica}$ tree-ring $\delta^{18}O$ was strongly correlated with the $\delta^{18}O$ of early spring precipitation (Figure 8). Similarly, more than half of the variance in $Q. \text{ilex}$ tree-ring $\delta^{18}O$ was explained by the temperature in February, long before the onset of tree-ring growth (Lemperreur et al., 2015). The tree-ring $\delta^{18}O$ signal was thus decoupled from leaf-level processes during the growing season. $Q. \text{ilex}$ is a deep-rooted species adapted to use groundwater or deep soil water during the summer (Barbeta et al., 2015). Deep water pools are usually replenished in winter, so these waters carry the isotopic signal of winter precipitation (Brooks, Barnard, Coulombe, & McDonnell, 2010). The correlation of tree-ring $\delta^{18}O$ with the temperature (Figure 6b) and water balance (Fig. S5b) for February may indicate that $Q. \text{ilex}$ extensively used deep water pools. Deep water pools are usually more depleted in $\delta^{18}O$ than topsoil water (Dawson, 1996) and so was the tree-ring $\delta^{18}O$ of $Q. \text{ilex}$ (Figure 4). The tree-ring $\delta^{18}O$ data thus suggest that $Q. \text{ilex}$ was more likely to use deeper water than $F. \text{sylvatica}$. Topsoil $\delta^{18}O$ is usually more depleted in the lighter, more volatile isotopes because it is more exposed to evaporation rates (Sprenger, Leistert, Gimbel, & Weiler, 2016). Moreover, topsoil water strongly fluctuates depending on the climate for the same reason. The inter-annual variability of tree-ring $\delta^{18}O$ was low for $Q. \text{ilex}$, contrasting with the more variable and enriched $\delta^{18}O$ values for $F. \text{sylvatica}$ (Figure 4b). It is thus plausible to hypothesize that $F. \text{sylvatica}$ has a relatively shallower root system compared to $Q. \text{ilex}$, at least in this site.

The strong influence of precipitation (Figure 6), long-term water balance during the growing season (Fig. S6a) on tree-ring $\delta^{18}O$ for $F. \text{sylvatica}$ could be partly caused by the canopy conditions during the formation of the rings (Gessler et al., 2014). If so, $\delta^{18}O$ should be negatively correlated with relative humidity during the growing season. Moister air would reduce the ratio of water vapor in the atmosphere and the intracellular space, minimizing foliar evaporative enrichment (Offermann et al., 2011); relative humidity has been reported to decrease the $\delta^{18}O$ of foliar organic matter in $F. \text{sylvatica}$ (Keitel, Matzarikis, Rennenberg, & Gessler, 2006). The lack of correlation between tree-ring $\delta^{18}O$ and relative humidity in our study (Figure 7) thus suggests that the foliar enrichment signal was dampened by postphotosynthetic processes, such as a mixing of old and new carbohydrate pools (Helle & Schleser, 2004) and oxygen exchange with nonenriched xylem water during phloemic transport and the synthesis of cellulose (Gessler et al., 2014). Our findings consequently suggest that tree-ring $\delta^{18}O$ is not a good proxy of foliar conditions and thus cannot be used to reconstruct past stomatal behavior, at least in $F. \text{sylvatica}$. The tree-ring $\delta^{18}O$ of this species, though, could serve as a proxy of the hydroclimate, as shown by the consistent correlations with the drought

**FIGURE 8** Linear relationship between annually resolved tree-ring $\delta^{18}O$ and rainfall $\delta^{18}O$ for $F. \text{sylvatica}$ (a) and $Q. \text{ilex}$ (b), for the period 2000–2010 (only period with rainfall $\delta^{18}O$ data). Each species had best correlations with rainfall $\delta^{18}O$ of different periods; February to April for $F. \text{sylvatica}$, and January to May for $Q. \text{ilex}$, in both cases with the year of ring growth.
index and the June and July rainfall (Figure 6a; Fig. S6) and with the δ18O of early spring rainfall (Figure 8). The correlations between climate and tree-ring δ18O are most likely mediated by varying rates of soil evaporation that modify source-water δ18O caused by drying-wetting cycles in soil pore water (Sprenger et al., 2016).

The positive correlation between tree-ring δ18O and relative humidity for Q. ilex throughout the growing season (April to September) was unexpected, because relative humidity is usually negatively correlated with tree-ring δ18O (Tsuji, Nakatsuka, & Takagi, 2006; Li, Nakatsuka, Kawamura, Liu, & Song, 2011; Roden & Farquhar, 2012). This negative correlation found in the literature has several causes. First, foliar evaporative enrichment is minimized in moist air (Roden & Farquhar, 2012), so the sugars synthesized in leaves under such conditions contain less δ18O. Second, a high air relative humidity results in precipitation water being depleted in δ18O (Dansgaard, 1964), and this depletion is transferred to the soil water, the tree source-water. Third, the soil water evaporative enrichment is dampened during periods with high relative humidity, resulting in more depleted topsoil water compared to periods with dry air. Still, the effects of relative humidity on soil water δ18O are mostly restricted to the topsoil, which is subjected to evaporation (Sprenger et al., 2016). Q. ilex uses deep soil water in periods with high evaporative demand, but shifts to topsoil water when evaporative demand is low (Barbeta et al., 2015). It is thus possible that when relative humidity is high and the atmospheric evaporative demand is low, this plant would mainly rely on topsoil, more enriched water, resulting in the positive correlation between tree-ring δ18O and relative humidity that we found. The use of deep soil water depleted in δ18O during dry periods is also probably behind the negative correlation between δ18O and Δ13C in Q. ilex (Figure 6). Lower Δ13C caused by reduced stomatal conductance in dry years would coincide in time with deeper tree water uptake.

Tree-ring δ18O for Q. ilex tended to decrease over time (Figure 4). Warming would cause an opposite pattern (enriched source-water δ18O over time), so declining trends in tree-ring δ18O has been attributed to the use of deep water that is depleted in δ18O in Mediterranean pines (Maseyk, Hemming, Angert, Leavitt, & Yakir, 2011; Sarris et al., 2013). The effect of relative humidity on tree-ring δ18O (Figure 7) is consistent with this hypothesis, since the fluctuations in tree-ring δ18O would follow shifts in the depth of water uptake. In contrast, the tree-ring δ18O of F. sylvatica was more variable inter-annually (Figure 4b) and presented higher temporal correlations with the water balance (Fig. S6). It cannot be ruled out that increasingly drier conditions could have produced a decrease in tree-ring δ18O mediated by a premature cessation of growth in summer, when the assimilates used for secondary growth are more enriched in δ18O (Pflug et al., 2015). However, we should then expect a stronger temporal decrease in tree-ring δ18O of the more drought sensitive F. sylvatica. In addition, the period of basal increment is quite similar in both species (Sánchez-Costa, Poyatos, & Sabaté, 2015; Martínez del Castillo et al., 2016). Q. ilex can have a second growth peak in autumn (Gutiérrez et al., 2011), so warmer temperatures could also have extended the period of autumn growth, producing more depleted tree-ring δ18O ratios (assuming that autumn rains are more depleted in the heavy isotope than summer rains). Climate change-induced shifts in growth phenology should be taken into account in tree-ring isotope studies dealing with climate-sensitive tree populations.

### 4.4 Concluding remarks

Tree-ring isotopes identified the physiological determinants of the surprisingly similar growth rates of F. sylvatica and Q. ilex at the warmest and coldest limit of their respective distributional ranges. The contrasting stomatal responses to increasing C3 were associated with species-specific strategies of water use, which allowed Q. ilex to achieve much higher growth rates compared to nearby drier sites. The deep root system of Q. ilex, together with other physiological and morphological traits for coping with drought (Barbeta & Peñuelas, 2016), enabled this species to take advantage of the effect of CO2 fertilization; Q. ilex may have increased the relative contribution of groundwater over the studied period. In contrast, F. sylvatica growth and rates of gas exchange were largely constrained by summer drought, probably because its maximum rooting depth rarely exceeds 1.5 m (Lebourgeois, Bréda, Ulrich, & Granier, 2005), shallower than the 3.7 m of Q. ilex (Canadell et al., 1996). This difference is consistent with the global patterns of rooting depth of Mediterranean and temperate forests (Canadell et al., 1996). In the case that in this site, climatic factors are more influential than biotic interactions or management practices, the future evolution of this ecotone in the face of current climate change is likely to favor Q. ilex. The climatic sensitivity of reproductive and establishment processes, however, can strongly differ from the growth and physiological sensitivity of the dominant trees (Lloret, Escudero, Iriondo, Martínez-Vilalta, & Valladares, 2012; Zhu, Woodall, & Clark, 2012; Rabasa et al., 2013). A comparative assessment of the early life stages of trees, such as seed germination and seedling establishment, would thus be required to infer whether evergreen Mediterranean forests would replace temperate deciduous forests.

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