Contrasting ecophysiological strategies related to drought: the case of a mixed stand of Scots pine (*Pinus sylvestris*) and a submediterranean oak (*Quercus subpyrenaica*)

Paula Martín-Gómez[1], Mònica Aguilera[1], Jesús Pemán[1], Eustaquio Gil-Pelegrín[2], Juan Pedro Ferrio[1,3]*

[1]Dept. Crop and Forest Sciences-AGROTECNIO, Universitat de Lleida, Lleida, Spain.
[2]Unidad de Recursos Forestales, CITA de Aragón, Zaragoza, Spain.
[3]Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile.

* Corresponding author:
Juan Pedro Ferrio
Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile.
e-mail: pitter.ferrio@gmail.com

ABSTRACT

Submediterranean forests are considered an ecotone between Mediterranean and Eurosiberian ecosystems, and are very sensitive to global change. A decline of Scots pine (*Pinus sylvestris* L.) and a related expansion of oak species (*Quercus* spp.) have been reported in the Spanish Pre-Pyrenees. Although this has been associated with increasing drought stress, the underlying mechanisms are not fully understood, and suitable monitoring protocols are lacking. The aim of this study is to bring insight into the physiological mechanisms anticipating selective decline of the pines, with particular focus on carbon and water relations. For this purpose, we performed a sampling campaign covering two growing seasons in a mixed stand of *P. sylvestris* and *Q. subpyrenaica* E.H del Villar. We sampled seasonally twig xylem and soil for water isotope composition ($\delta^{18}O$ and $\delta^{2}H$), leaves for carbon isotope composition ($\delta^{13}C$) and stems to quantify non-structural carbohydrates (NSC) concentration, and measured water potential and leaf gas exchange. The first summer drought was severe for both species, reaching low pre-dawn water potential (-2.2 MPa), very low stomatal conductance (12 $\pm$1.0 mmol m^{-2} s^{-1}) and near-zero or even negative net photosynthesis, particularly in *P. sylvestris* (-0.6 $\pm$0.34 μmol m^{-2} s^{-1} in oaks, -1.3 $\pm$0.16 μmol m^{-2} s^{-1} in pines). Hence, the tighter stomatal control and more isohydric strategy of *P. sylvestris* resulted in larger limitations on carbon assimilation, and this was also reflected in carbon storage, showing two-fold larger total NSC concentration in oaks than in pines (7.8 $\pm$2.4 % and 4.0 $\pm$1.3 %, respectively). We observed a faster recovery of predawn water potential after summer drought in *Q. subpyrenaica* than in *P. sylvestris* (-0.8 MPa and -1.1 Mpa, respectively). As supported by the isotopic data, this was probably associated with a deeper and more reliable water supply in *Q. subpyrenaica*. In line with these short-term observations, we found a more pronounced negative effect of steadily increasing drought stress on long-term growth in pines compared to oaks. All these observations confer evidence of early warning of *P. sylvestris* decline and indicate the adaptive advantage of *Q. subpyrenaica* in the area.

INTRODUCTION

In Europe, the transition zone between the Mediterranean and Eurosiberian region is generally called submediterranean ecotone (Abadía et al. 1996). The change between both regions is mostly gradual and has intermediate climatic characteristics between those (e.g. Sánchez de Dios et al. 2009). The main community in this region is composed by marcescent oaks (*Q. faginea*, *Q. humilis*, and their hybrid form, *Q. subpyrenaica*) and conifers, either from natural regeneration or reforested (*P. sylvestris*, *P. nigra* subsp. *salzmannii* and *P. halepensis*). Both groups may appear in the form of pure or mixed stands.

In particular, in the medium-high mountains of the Spanish Pyrenees, one typical association is formed by the marcescent oak (*Quercus subpyrenaica* E.H del Villar) and Scots pine (*Pinus sylvestris* L.). These co-ocurring species show different functional traits associated with contrasting water-use strategies. Scots pine, as an evergreen conifer, has a tracheid-bearing xylem with low hydraulic conductivity and isohydric behavior; based on a tight stomatal control, limiting transpiration when soil moisture deficit reaches a threshold (e.g. Irvine et al. 1998, Poyatos et al. 2008, Martínez-Vilalta et al. 2009). Meanwhile, *Q. subpyrenaica* as its parental species (*Q. faginea* x *Q. humilis*) is a marcescent oak with ring-porous xylem with high maximum hydraulic conductivity and a typical anisohydric behavior, i.e. maintaining higher transpiration rates despite soil drought incidence (Nardini and Pitt 2002, Corcuera et al. 2004, Himrane et al. 2004, Tognetti et al. 2007). Additionally, the different physiological behavior between these species is partly associated with their root system distribution (e.g. Filella and Peñuelas 2003, Moreno-Gutiérrez et al. 2012b, West et al. 2012, Barbeta et al. 2015); thus, oaks are able to maintain their continuous physiological activity by relying on stable pools like groundwater or deep soil layers while pines develop an opportunistic strategy of fast use of precipitation water based on a high root density in top soil layers (e.g. Poyatos et al. 2008, Klein et al. 2013, del Castillo et al. 2016, Grossiord et al. 2016). Also, because of the ability of oaks to reach lower water potentials than pines, during dry periods they can have access to more tightly-bound water pools in the soil (Tang and Feng 2001). In this regard, the analysis of the isotopic composition of xylem water has been widely applied to determine the source of water used by plants, under the assumption that there is no fractionation during water uptake and transport along the xylem (Ehleringer and Dawson 1992). Nevertheless, this is not always the case, and a fractionation during root water uptake has been described in some xerophytic and halophytic species (Ellsworth and Williams 2007), and evaporative enrichment in the stem may cause divergences from source values during periods of limited sap flow (Dawson and Ehleringer 1993; Martín-Gómez et al. 2017). In spite of this, the analysis of water isotopes in soil and xylem water still provides useful information on the water uptake dynamics, and the interaction among co-occurring species (Máguas et al. 2011; Moreno-Gutiérrez et al. 2012b; del Castillo et al. 2016).

Climate-change predictions include increasing temperatures and a reduction of spring and summer precipitation for the next decades (Christensen et al. 2007). In the Mediterranean basin, water is largely the main limiting factor for plant and tree development; thus, the predicted changes in water availability, even subtle, may affect the ecosystem functioning modifying the species composition and dynamic of current

forests systems (e.g. Martínez-Vilalta et al. 2002, Poyatos et al. 2008, Allen et al. 2010, Granda et al. 2014). Considering its transitional nature, the submediterranean ecotone is thought to be particularly sensitive to global change (Gosz 1992). In this regard, there is increasing evidence that extreme drought events are causing extensive tree decline in Scots pine populations, particularly in its southern limit of distribution (e.g. Martínez-Vilalta and Piñol 2002, Gómez-Aparicio et al. 2011, Poyatos et al. 2013, Sánchez-Salguero et al. 2015). Conversely, coexisting deciduous or marcescent oaks generally remain unaffected or even expanded towards higher or fresher locations (e.g. Poyatos et al. 2008, Benito Garzón et al. 2008, Galiano et al. 2010). There are also some prediction models that suggest an important reduction in the distribution area of marcescent oaks and a displacement towards higher altitudes in favor of Mediterranean evergreen oaks; e.g the holm oak, *Q. ilex* (Sánchez de Dios et al. 2009, Carnicer et al. 2013a, Carnicer 2014). In this context, it remains unclear how the different physiological and adaptive responses of pines and oaks could determine the composition and structure of submediterranean forests in a climate-change scenario. Besides, whether pine decline in the Mediterranean is mainly driven by hydraulic failure (due to cavitation) or carbon starvation (due to stomatal limitations) is still a matter of debate (e.g. Poyatos et al. 2008; Galiano et al. 2011, 2012; Sangüesa-Barreda et al. 2012; Voltas et al. 2013; Camarero et al. 2015; Salmon et al. 2015). Thus, further research is needed on the species-specific strategies to cope with drought at the submediterranean ecotone, particularly in those areas lacking a clear dominance of one functional group over the other, as a way to identify the most suitable early-warning traits.

We hypothesize that a combination of physiological measures related to carbon and water use across different time-scales may provide early warning indicators of incipient, species-specific tree decline in mixed submediterranean forests. In this study, we analyzed the seasonal evolution of water uptake patterns and physiological response in terms of water relations and carbon dynamics, in a mixed forest of *P. sylvestris* and *Q. subpyrenaica* in the submediterranean area of the Spanish Pyrenees. The study spanned over two growing seasons with contrasting water availability. The main objectives were to: (i) understand the physiological advantages (if any) of marcescent oaks in comparison to Scots pine and (ii) anticipate the underlying mechanisms explaining Scots pine decline in the area.

METHODS

Site description

This study was conducted in an adult mixed stand located in the north-west part of the Spanish Pre-Pyrenees (Boalar Forest - Jaca - Huesca, 30T 693606 4714041, 800m high). The main species in the stand are Scots pine (*Pinus sylvestris* L.) and a Mediterranean marcescent oak (*Quercus subpyrenaica* E.H del Villar) with a 1:1 stand density of 1200 trees/ha aproximately, with 70% canopy cover (local source data, see Fig. S1a). Besides, in the area there is a dense (80% in soil cover) understory composed mainly by evergreen *Buxus sempervirens* L. Mixed stands of Scots pine and oak in the area mainly originated from natural regeneration, and forest age structure reflects their history. In the past, Iberian forests experienced strong land-use pressures (logging, grazing) until the 1950s, when massive migration of rural population led to an extensive abandonment of cultivated land and in consequence to an enhancement of forest encroachment and increase of canopy and understory cover (Garcia-Ruiz et al. 1996, Ameztegui et al. 2016).

The climate in the region is described as transitional Mediterranean or Submediterranean (Sánchez de Dios et al. 2009). The mean annual precipitation and temperature are 806 mm and 11.7°C, respectively. The summer is mild and relatively dry (mean summer precipitation of 143 mm, mean average temperature of 20.1 °C), registering drought periods mainly in July. About 60% of annual precipitation falls during autumn and spring (261 and 220 mm, respectively). Winter precipitation, often in the form of snow, accounts for 23% of annual precipitation (182 mm). According to National Geologic Institute (IGME, Geologic map 1:50.000) the dominant geological substrate in the area is a sedimentary combination of sandstone and grey marlstone. In situ soil analyses resulted in a clayey-loam texture with 32% of clay particles and non-limiting calcium carbonate content (<0.3% in most of the cases), electric conductivity (<0.1 dS/m) and organic matter content (1.9%). Average pH was 7.3 and soil water content at field capacity (0.33 MPa) and wilting point (1.5 MPa) was 21.4% and 13.1% respectively.

Remote sensing and meteorological data

Biweekly spectral variation of the Normalized Difference Vegetation Index (NDVI) for the study period were obtained from the MODIS Land Subsetted Products at the MODIS web service (2014). We selected the pixel containing the study stand (250×250 m^2). Seasonal changes in soil water content (SWC) were derived from data acquired from the ESA´s Soil Moisture and Ocean Salinity (SMOS) mission, available at http://www.smos-bec.icm.csic.es; the data is a high resolution (1 x 1 km) soil moisture delayed product, which contains two layers per day, corresponding to SMOS ascending (6 A.M.) and descending (6 P.M.) passes. Although remote-sensing estimates are restricted to the upper soil surface (0-5 cm, actual penetration depends also on the soil type and water content), previous studies have shown that they can be used as a good proxy of soil water availability (see e.g. Sánchez et al. 2016). We selected the four closest pixels containing the study site. These maps are obtained using a downscaling algorithm, which combines the brightness temperature measurements from ESA-SMOS with Land Surface Temperature and NDVI data from Terra/Aqua MODIS.

Meteorological data was obtained from the nearest meteorological station (Instituto de Formación Agroambiental de Jaca, < 500m).

Field sampling and measuring campaigns

We selected ten pairs of apparently healthy and codominant neighbor pine – oak trees, distributed along a 40 m x 42 m plot. The studied individuals in the stand showed an even age and size structure, with diameter at breast height (dbh, mean ± SD) of 30.0 ± 4.4 cm for pines and 19.4 ± 3.4 cm for oaks, and tree height of 12.2 ± 1.5 m and 12.5 ± 1.0 m, for pine and oak respectively, with average age around 55-60 years for both species. Along 2012 and 2013, we performed five intensive field campaigns: on July 31st, October 9th (2012), March 21st, June 27th and August 9th (2013) (Fig. 1). For each sampling time, we collected xylem samples from sun-exposed twigs of the upper third of the crown at two aspects (north and east, Fig. S1a). In the middle of each pair of trees, soil was sampled at three different depths (0-10 cm, 10-30 cm and 30-50 cm, Fig. S1b). For xylem sampling, bark and phloem were removed and the peeled xylem was immediately placed in air-tight glass tubes (Duran GL-18). Soil samples were also rapidly placed in air-tight glass tubes after collection. All samples were placed on dry ice directly after sampling, and kept frozen until processing. Twig xylem sampling was complemented with pre-dawn and midday measurements of xylem water potential with a pressure chamber (Scholander et al. 1965). In adjacent twigs, gas exchange measurements were determined with an infra-red gas analyzer (Walz GFS-3000, Heinz Walz GmbH, Effeltrich, Germany). The system was equipped with a LED-Array/PAM-Fluorometer 3055-FL and a cuvette for conifers 3010-V80. Chamber conditions were set to mimic ambient conditions in the upper canopy (CO_2 concentration: 380 ppm; photosynthetic photon flux density: 1000-1500 μmol m^{-2} s^{-1}; cuvette temperature and relative humidity: 18-28ºC, 40-70%). All parameters were recalculated to actual leaf area using the equations from Von Caemmerer and Farquhar (1981). Leaf area in oaks was determined by calculating the projected area of a single leaf, normally filling all the chamber surface – 8cm^2; in pines it was calculated by multiplying the total number of needles inside the chamber by the area of an average needle, considered as a thin rectangle (length × width).

Water collection, distillation and isotope analyses

Precipitation was collected at the closest meteorological station (Instituto de Formación Agroambiental de Jaca, < 500m), between August 2012 and October 2013. We used a hand-crafted rain collector with paraffin oil to prevent evaporation; samples were collected biweekly and immediately stored in the fridge until analysis (following IAEA protocol for precipitation collection; http://www-naweb.iaea.org/napc/ih/documents/other/gnip_manual_v2.02_en_hq.pdf). After the sampling campaigns, groundwater samples were collected from a nearby fountain (Atarés, <500 m from the study site) and its isotopic value was in accordance with other fountains along the medium-high mountains in the Spanish Pyrenees (-8.49 ± 0.10‰ for $\delta^{18}O$ and -57.57 ±0.70‰ for δ^2H; Oliván et al. 2011). The extraction of water from the soil and xylem samples was performed by cryogenic vacuum distillation (Dawson and Ehleringer 1993) at the Dept. of Crop and Forest Sciences, Universitat de Lleida (Spain). The extraction system consisted of 10 sample tubes connected with Ultra-Torr™ fittings (Swagelok Company, Solon, Ohio, USA) to 10 U-shaped collection tubes specifically designed for this system. The sample tubes were submerged in

mineral oil at a constant temperature (110-120ºC) to evaporate water and the U-tubes were cooled with liquid nitrogen to condense the water vapour. The system was connected to a vacuum pump (model RV3; Edwards, Bolton, UK) to ensure the flow of water vapour from the sample tubes to the collection tubes, and to prevent contamination with atmospheric water vapour. The entire system maintained constant vacuum pressures of *ca.* 10^{-2} mbar. Distillation time was 1.5 hour for xylem samples and 2 hours for soil samples. A preliminary test with a subset of soil and xylem samples showed that after distillation the samples were dry enough to gain weight (i.e. rehydrate), when placed in the drying oven at 60ºC for 24h, and no significant improvements in distillation performance were found using longer distillation times (for further details see Martín-Gómez et al. 2017).

We analysed the isotope composition of water samples by Cavity Ring-Down Spectroscopy (CRDS) in a Picarro L2120-*i* isotopic water analyser (Picarro Inc., Sunnyvale, CA, USA) at the Serveis Científico-Tècnics of the Universitat de Lleida (Lleida, Spain). The analyser was coupled to a high-precision vaporiser (A0211) through a Micro-Combustion Module^{TM} (MCM), integrated in-line between the vaporiser and the analyser. The MCM removes the contaminants through oxidation, in a way that only pure water arrives to the analyser (Picarro 2012). After calibration with three internal standards, isotope composition was expressed in per mil notation ($\delta^{18}O$ and δ^2H, for oxygen and hydrogen, respectively), relative to VSMOW (Vienna Standard Mean Ocean Water). The estimated precision for the L2120-*i*, based on the repeated analysis of 4 reference water samples was 0.10‰ and 0.40‰, for $\delta^{18}O$ and δ^2H, respectively.

As described in Martín-Gómez et al. (2015), residual organic compounds in the distilled water can interfere with the analysis of plant and soil samples using CRDS. As a quality assessment of the level of contamination among our samples, we compared the results with and without post-processing correction. We found a very strong correlation between corrected and uncorrected values (R^2=0.998 for $\delta^{18}O$; R^2=0.989 for δ^2H, N=620) agreeing with low levels of contamination. For consistency, and taking into consideration that some samples were analysed before the MCM installation, we used software-corrected results for all samples.

Assessment of evaporative processes in soil and xylem water
Potential evaporation processes in xylem and soil water were visually assessed by plotting δ^2H against $\delta^{18}O$ (Fig. 2). In a previous work (Martín-Gómez et al. 2017) we stated that evaporation of soil and xylem water causes a progressive enrichment in the heavy isotopes of the remaining water pools, but this effect is larger in $\delta^{18}O$ than in δ^2H. Consequently, isotopic composition increases along an evaporation line, with a flatter slope than the meteoric water in the $\delta^{18}O$ - δ^2H scatterplot (Craig 1961). Under a particular set of environmental conditions, water samples from the same meteoric origin should move along the same evaporation line, whereas distinct evaporation lines would indicate different origins. As a reference, we used the Global Meteoric Water Line (GMWL), which represents the relationship between the two isotopes in global precipitation (δ^2H= 10 + 8×$\delta^{18}O$; Craig 1961), and a Local Meteoric Water Line (LMWL) built from precipitation records (August 2012 - October 2013).

Leaf $\delta^{13}C$ isotopic analysis

For each field campaign, the leaves belonging to every xylem sample collected were immediately frozen in dry ice and stored at -24ºC until processing. The samples were freeze-dried for 24 hours in order to stop cellular activity and subsequently oven-dried for 48 hours at 50ºC. For carbon isotope analysis, 0.9–1.1 mg of dry matter was weighted into tin capsules and analyzed by mass spectrometry at the Stable Isotope Facility of the University of California, Davis (USA) using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). $^{13}C/^{12}C$ ratios were calibrated against internal standards and expressed relative to the international standard Vienna PeeDee Belemnite (VPDB). The accuracy of the analyses (Standard deviation of working standards) was 0.06‰.

Total non-structural carbohydrate concentrations in twig xylem

Once the water was extracted from the twigs, xylem was also analysed for soluble sugars (SS) and starch concentrations. For this purpose the dry xylem samples were grounded to fine powder and analysed twice. Soluble sugars were extracted from 50 mg samples with 80% (v/v) ethanol. The extraction was done in a shaking water bath at 60 °C. After centrifugation, the concentration of SS was determined colorimetrically at 490 nm using the phenol–sulphuric method of Dubois et al. (1956), as modified by Buysse & Merckx (1993). For starch concentration, the pellet remaining after ethanol extraction was digested with an enzyme mixture containing amyloglucosidase to convert starch into glucose, as described in Palacio et al. 2007. Concentration of starch-derived glucose was then determined colorimetrically using the same method as for SS. Concentration of total nonstructural carbohydrates (NSC, % dry matter) was calculated as the sum of SS and starch concentrations.

Tree-ring width

After the last sampling campaign we extracted cores of the studied trees in order to assess their long-term growth patterns. We extracted 10 trees per specie, but 1 pine and 1 oak were discarded as outliers for the chronology. Tree rings were visually cross-dated using a binocular microscope coupled to a PC using the software WinDendro™ (Regent Instruments. Canada Inc., 2009). The COFECHA™ program (Holmes 1983) was used to evaluate the visual cross-dating. Individual tree-ring width was then converted into Basal Area Increment (BAI, in mm²/year), in order to remove variations in radial growth attributable to size and age effects (e.g. Piovesan et al. 2008, Moreno-Gutiérrez et al. 2012a). BAI was calculated from radial increments assuming a cylindrical shape with the formula: $BAI = \pi \times (r^2_t - r^2_{t-1})$, where r is the radius and t is the year of the tree-ring formation.

Statistical analyses

We tested significant differences in time and between species for all physiological variables. In all cases, generalized linear mixed models were based on Restricted Maximum Likelihood - REML ($\alpha = 0.05$). Differences among sampling times for each species were tested with the Tukey test ($\alpha = 0.05$). The association between isotope composition in xylem and soil water at different depths was assessed by simple *Pearson* correlations. The relationship among physiological variables (water potential, stomatal

conductance, net assimilation) and NSC was described by fitting exponential and linear regressions, respectively. All the statistical analyses were performed with JMP Pro 11 (SAS Inc., Cary, NC, USA) with the exception of the relation between water potential, g_s and NSC that was adjusted with Sigma Plot version 12.5 (Systat Software, Inc., San Jose California USA). For water potential and gas exchange variables, March sampling-time was excluded from analysis as only data for pine was available. Unless otherwise stated; means are shown together with their associated standard error of the mean.

RESULTS

Historical evolution of Basal Area and growth patterns
The long-term evolution of Basal Area Increment (BAI, Fig. 1) indicated differential patterns in growth for both species, although they had similar age (around 55-60 years old). Growth rates in both species were higher and more variable during the first years of establishment. However, pine oscillation was higher and the slope of the decreasing trend with age was higher in comparison to oaks (-12.64 in pines, -1.27 in oaks). Conversely, BAI in oaks remained nearly constant (also slower) throughout the study period, and seemed less affected by the accumulation of non-favorable climatic conditions. Furthermore, after the extreme dry years around mid-nineties, pine decrease in BAI was exacerbated, in contrast with the lack of response in the oaks.

Environmental conditions during the seasonal sampling
The year 2012 was a dry year throughout the Iberian Peninsula (Peguero-Pina et al. 2015). In Jaca, the annual total precipitation in 2012 was only 5% under average (806mm, calculated from 1983 to 2011); however, summer precipitation (June-August) was 43% lower than the average (143mm). Besides, the preceding winter of 2012 growing season was relatively dry, with very scarce snow events and spring rains. Thus, the scarce precipitation in 2012, along with high summer temperatures and plant water consumption, resulted in a very dry and long drought period. In this regard, we observed a clear reduction in SWC and a sharp decline in NDVI starting from June until late September. At this point, autumn rains increased, soil water reserves were filled again and vegetation recovered physiological activity (Fig. 2). In contrast, 2013 was a very wet year (1189 mm annual). In particular, summer precipitation was twice (330 mm) the historical average, and previous winter and spring precipitation events were also abundant. Consequently, SWC in summer was higher than in 2012, and only a short and slight decline in NDVI was found in July-August (Fig. 2).

Seasonal changes in the isotopic composition of precipitation, soil and xylem water
Isotopic composition of precipitation events was very variable in time, ranging from -13.90‰ for $\delta^{18}O$ and -99.26‰ for δ^2H in the end of winter period to +5.17‰ for $\delta^{18}O$ and +13.22‰ for δ^2H in summer, whereas the soil remained fairly stable throughout the sampling campaigns (-9.06 ± 0.10‰ for $\delta^{18}O$ and -64.41±0.69‰ for δ^2H; Fig. S2). Usually, soil water composition did not correspond strictly to the preceding precipitation events and was only in autumn and after the abundant winter precipitation when the soil approached isotopic composition of previous rains (Fig. S2). Groundwater values (-8.41‰ for $\delta^{18}O$ and -56.36‰ for δ^2H) were similar to soil recharge precipitation in autumn (October and November average values: -8.77±0.30‰ for $\delta^{18}O$ and -58.13±2.48‰ for δ^2H; Fig. S2). Despite in most cases the top soil was more enriched than deeper soil layers, the evaporative gradient along the soil vertical profile was not very pronounced (average values for surface soil enrichment: +1.03 ± 0.14‰ for $\delta^{18}O$ and +4.51 ± 0.87‰ for δ^2H; Fig. 3). During the wettest period (spring 2013, Fig. 3c), surface soil values were slightly more depleted than deeper soil layers, due to the more depleted isotope signature of recent rains (01/13 and 03/13 in Fig. 3f). Additionally, xylem water showed in most cases a clear deviation towards more enriched values with respect to soil isotopic values (+2.01±0.21‰ and +2.47±0.29‰ in

$\delta^{18}O$; +7.31±0.96‰ and 8.91±1.43‰ in δ^2H for pines and oaks respectively; Fig. 3), particularly during the driest period for pines (+4.08±0.29‰ in $\delta^{18}O$ and +14.82±1.38‰ in δ^2H; Fig. 3a), and during leafless stage in oaks (+5.91±0.25‰ in $\delta^{18}O$ and +26.34±1.30‰ in δ^2H; Fig. 3c). This can be attributed to isotopic enrichment in the stem xylem, following a typical evaporative line (Fig. 3a-e), and generally overlapping with the one formed by soil water composition with depth. Only in September 2013 (Fig. 3e), xylem and soil isotopic signature were clearly coupled, coinciding with moderate precipitation events occurring several days before the sampling campaign. As a result of the broadly-found enrichment of xylem in respect to soil isotopic composition, we could not make a quantitative assignation of the relative contribution of different soil depths as water sources for the two species to water source, e.g. based on mixing models (Parnell et al. 2010). However, comparing inter-tree variability for a given species and time, we would expect better correlations between xylem and soil water for the soil depth constituting the predominant source of water. As shown in Table 1, the isotope composition of xylem water in pines was best correlated with intermediate depths (0.64 for δ^2H at 30 cm), whereas the oaks were better correlated with the deepest soil layer (0.64 for δ^2H at 50cm). It should be noted that we found better correlations for δ^2H than $\delta^{18}O$ (Table 1) because evaporative enrichment within the stem xylem was the main cause for the observed soil-xylem uncoupling, and δ^2H is less affected by evaporative processes than $\delta^{18}O$ (Martín-Gómez et al. 2017).

Predawn and midday leaf water potentials

Overall, throughout the study period, oaks reached lower water potential than pines at midday (ψ_{md}), but recovered to higher water potential at predawn (ψ_{pd}), indicating a preferential access to wet soil layers, together with more active transpiration during the day (Fig. 4). During the dry summer in 2012, both species reached very low water potentials for midday (ψ_{md}) and predawn (ψ_{pd}), in accordance with generally dry soil conditions (ψ_{md} = -2.39±0.12 MPa; ψ_{pd} = -2.14±0.11 MPa). In October 2012, despite the milder temperatures and some recent rains, the drought developed during summer was still reflected in the physiological status, particularly for the pines (Fig. 4). On the one hand, the higher ψ_{pd} in the oaks suggest than the pines had more limited access to the wetter soil (Fig. 4a); on the other hand, the oaks showed much lower values of ψ_{md}, presumably associated with higher transpiration rates (Fig. 4b). Contrarily, oaks were reacting faster to the more favorable autumn conditions, showing a clear recovery in predawn water potential (ψ_{pd} = -0.80±0.07 MPa) and more negative midday water potential, indicative of higher transpiration rates (ψ_{md} = -2.27±0.07 MPa). Although midday water potentials in summer 2013 were as low as in summer 2012, predawn values were significantly higher, indicating higher soil water availability and transpiration rates for both species.

Gas exchange and water use efficiency

Pines showed consistently higher $\delta^{13}C$ values than oaks, associated with a greater intrinsic water use efficiency (WUE_i) and tighter stomatal regulation (Fig. 5a,c). In the dry summer of 2012, oak $\delta^{13}C$ values were high and close to those of pines, indicating similar levels of stress in both species. In 2013, the new oak leaves maintained low $\delta^{13}C$ values from early spring to summer. Conversely, the pines showed a sharp decline of $\delta^{13}C$ in early spring, rising in June-August to similar levels to those found in 2012.

Although the pine needles sampled in 2013 were not current-year needles, their higher $\delta^{13}C$ cannot be only attributed to a memory-effect of previous-year conditions, considering the highly dynamic changes observed.

In summer 2012, both species showed minimal g_s (Fig. 5c), and near-zero or even negative A_n (Fig. 5b) indicating that respiration was in the same order of magnitude as photosynthesis. In October, after the first rain events, both species recovered physiological activity, showing a moderate increase in g_s, and clearly positive A_n. Pine photosynthesis during the following early spring (before budburst in oaks) indicated only moderate physiological activity. Despite the wet conditions of previous winter-spring 2013, neither oak nor pine showed high physiological activity in late spring (June), probably due to relatively low temperatures, and it was not until August that both species reached a maximum in g_s and A_n. At this point, oaks showed significantly higher g_s and A_n than pines. For the leaves showing positive A_n, we also calculated instantaneous WUE_i (A_n/g_s, Fig. S3). Overall, whereas in the oaks temporal fluctuations in A_n/g_s generally agreed with changes in $\delta^{13}C$ (Fig. S3a), the pines showed more limited variations in A_n/g_s, and lower values of instantaneous WUE_i than what would be expected, according to their $\delta^{13}C$ (Fig. S3b).

Non-structural carbohydrates concentration in the xylem As compared to pines, oaks showed significantly higher NSC, particularly during drought and recovery in the case of SS (July and October 2012, Fig. 6a), and throughout the study period in the case of starch (Fig. 6b). . Seasonal evolution of SS concentration was rather stable with time in pines, whereas in the oaks SS concentration showed a maximum during the dry season and declined progressively until June and August 2013 (Fig. 6a). In pines, starch concentration increased progressively from July 2012 to a peak in June 2013, followed by a new minimum in August 2013 (Fig 6b). . Conversely, the oaks showed a clear peak in starch concentration in October 2012, and minimum values during July 2012, although the latter did not differ significantly from the values in March, June and August 2013 (Fig. 6b).

Drought limitation of photosynthesis

We found a significant exponential relation between ψ_{pd} and gas exchange parameters (Fig. 7a − A_n, Fig. 7b − g_s) for pines, showing higher g_s and A_n for higher ψ_{pd}, i.e. associated with higher soil water content. Contrarily, this relationship was not found for oaks, for which gas exchange parameters were largely independent from water potential. In this regard, we also found a significant correspondence between ψ_{pd} and Total NSC for pines, and not for oaks, which could be linked to stomatal limitation and thus reduced assimilation in pines. Otherwise, we did not find significant relation between ψ_{pd} and starch or soluble sugars separately. Additionally, when comparing these variables with ψ_{md}, we only found a significant relationship with A_n in pines ($r^2=0.42$, $p = 0.002$).

DISCUSSION

As summarized in Fig. 8, we found species-specific seasonal patterns on physiological variables and isotope composition, reflecting the contrasting climatic conditions during the two measured growing seasons, and the distinct physiological strategies and ability to cope with drought. 2012 was a very dry year with limited autumn precipitation and scarce snow events, whereas 2013 was a very wet year with important rain and snow events in autumn and winter. The marcescent oak (*Q. subpyrenaica*), in comparison to Scots pine (*P. sylvestris*), was able to reach lower midday water potential throughout the studied period, thus being able to maintain transpiration and physiological activity under drier soil conditions (Picon et al. 1996, Poyatos et al. 2008, Klein et al. 2013, Forner et al. 2014). Despite the lower values in midday water potential, the oaks showed higher water potential overnight, indicating that they could also have access to deeper (and wetter) soil layers than the pines (e.g. Poyatos et al. 2008, Klein et al. 2013, del Castillo et al. 2016, Grossiord et al. 2016). Indeed, transpiration was only restricted in oaks during the extremely dry conditions in summer 2012 (Fig. 8a), but rapidly recovered after the first rains in early autumn (Fig. 8b). In this sense, marcescence is a well-known phenomenon of extending physiological activity during early and even late autumn when climatic conditions still allow sufficient gas exchange (e.g. Abadía et al. 1996; Peguero-Pina et al. 2015). Although a partial recovery was also noticeable for Scots pine in early autumn, full recovery was only evidenced in early spring 2013 (Fig. 8c). During the wet summer in 2013, near-optimal conditions allowed for a complete recovery of stomatal conductance, leading to higher photosynthetic rates in both species, and a tight coupling between soil and xylem isotope values (Fig. 8d).

The ability of oaks to reach low water potentials at midday and recover water status overnight is partly associated with their root system distribution and water uptake patterns (e.g. Filella and Peñuelas 2003, Moreno-Gutiérrez et al. 2012b, West et al. 2012, Barbeta et al. 2015). Generally, oaks are able to maintain their conservative strategy and continuous physiological activity by relying on stable water pools like groundwater, deep soil layers or more tightly-bound water in the soil, while the pines develop an opportunistic strategy of fast use of precipitation water based on a high root density on top soil layers (e.g. Poyatos et al. 2008, Klein et al. 2013, del Castillo et al. 2016, Grossiord et al. 2016). In this sense, despite the offset between soil and xylem isotopic composition in our data, the analysis of correlation for all the studied period showed maximum δ^2H correlations for soil water at 50 cm depth for oaks and at 30 cm for pines, reinforcing the general knowledge of oak reliance on deeper soil water pools in comparison to pines (e.g. David et al. 2007, Barbeta et al. 2015). In support of the more opportunistic strategy of Scots pine, a study in the Eastern Pyrenees (Poyatos et al. 2008) reported larger sap flow restrictions during summer drought in a Scots pine stand than in a plot with pubescent oak (*Quercus humilis*), but a faster increase in pine transpiration after the first rain events. Besides the differences in water uptake patterns, pines are generally regarded as more isohydric species, with quicker and tighter stomatal control (e.g. Irvine et al. 1998, Poyatos et al. 2008, Martínez-Vilalta et al. 2009) . However, the more dynamic stomatal control of pines does not always imply a faster recovery of physiological activity after drought, since it could be linked to greater xylem vulnerability (Martínez-Vilalta et al. 2004). Reported stem vulnerability curves for *Q. subpyrenaica* show 50% loss of conductivity in twigs at rather low water potentials (-5.0 MPa, Peguero-Pina et al. 2015), which contrasts with the much higher

xylem vulnerability reported for Mediterranean populations of *P. sylvestris* (50% loss of conductivity in twigs at -2.7 MPa, in Aguadé et al. 2015; 50% xylem embolism at -1.5 MPa, according to Salmon et al. 2015). Moreover, roots are generally more vulnerable to cavitation than stems, and this difference tends to be larger in conifers than in angiosperms (Johnson et al. 2016). In the case of *P. sylvestris*, for example, Aguadé et al. 2015 reported 50% loss of conductivity in root xylem at -1.7 MPa. Therefore, after long and severe drought periods, the greater vulnerability to xylem embolism (particularly in the roots, see e.g. Aguadé et al. 2015) may lead to more limited recovery of transpiration flow in pines, as compared to oaks (e.g. Poyatos et al. 2008, Salmon et al. 2015). Furthermore, in mixed stands, as in the present study, the risk of xylem embolism in Scots pine might be enhanced due to the larger ability of oak species to exhaust soil water (Willaarts 2012, Klein et al. 2013). In agreement with our findings, del Castillo et al. (2016) found a faster recovery after a long summer drought in the evergreen oak *Quercus ilex*, with respect to the Mediterranean pine *Pinus halepensis*. Similar to our study, the larger water uptake capacity of the evergreen oaks also resulted in a tighter association between soil and xylem water isotope composition during drought and subsequent recovery. This can be explained by the enhanced effect of evaporative enrichment of stem xylem water under conditions of limited sap flow, as has been recently shown for the same studied species (Martín-Gómez et al. 2017). In consequence, pines in the summer 2012, and leafless oaks showed maximum divergence between soil and xylem values. Oaks recovered physiological activity during the first rains after summer, and hence xylem water approached soil isotopic signature, whereas only from spring 2013, when the soil was completely hydrated, xylem water in pines was clearly in the range of soil values.

The limited ability of pines to access deep water reservoirs was also evidenced by the response of gas exchange parameters to ψ_{pd}. Over the entire growth period, we found a significant relationship between soil water status (as reflected in ψ_{pd}) and g_s and A_n in pines, showing that photosynthesis was largely constrained by drought due to stomatal closure, in accordance with previous studies (Gallego et al. 1994, Irvine et al. 1998, Perks et al. 2002, Poyatos et al. 2008, Klein et al. 2013, Salmon et al. 2015, Nardini et al. 2016). Hence, when pines reached levels of ψ_{pd} below -1.5 MPa, stomatal conductance was lower than 30 mmol m^{-2} s^{-1}, and consequently carbon assimilation was heavily affected. Similarly, Salmon et al. (2015) showed a decrease in g_s from about 120 mmol m^{-2} s^{-1} at ψ_{pd} =-1.3 MPa to values below 20 mmol m^{-2} s^{-1} at ψ_{pd} =-1.8 MPa, whereas Poyatos et al. (2008) and Perks et al. (2002) showed that midday g_s was already reduced to 10-20% of its maximum value with ψ_{pd} below -0.8 MPa. Similarly, oaks also showed a tendency towards lower ψ_{pd} associated with lower g_s and A_n, but this was only significant for A_n, and after excluding the data from June, when new leaves were still not fully active. This suggests a lesser restriction of water availability for this species; and hence, continued physiological activity despite the harsh climatic conditions. Poyatos et al. (2008) also found a rather scattered response of g_s against ψ_{pd} in pubescent oaks, which maintained g_s above 30% of their maximum over the entire range of ψ_{pd} recorded (from -0.2 to -0.8 MPa). On the other hand, Gallego et al. (1994) did not find substantial changes in g_s and ψ_{pd} of *Q. pyrenaica* as soil water decreased, concluding that this was a consequence of the ability of this species to access deep water reservoirs. Indeed, in the two sites included in their study, soil water potential at 20 cm declined up to -4.0 MPa during summer drought, whereas ψ_{pd} was generally

above -1.0 MPa, coinciding with that found in the soil at 40-60 cm depth. Notably, this range of soil depth agrees with our observation of a tight association between isotope composition in oak xylem water and soil water at 50 cm.

The generally higher $\delta^{13}C$ values in the pines than in the oaks would agree with their tighter stomatal control. However, whereas the oak, as expected, showed the highest $\delta^{13}C$ during the dry summer in 2012, the pine $\delta^{13}C$ reached nearly the same values in 2012 and 2013. This might be partly explained by a larger memory effect in the evergreen pines, as compared with the deciduous oaks. Nevertheless, this apparent uncoupling between WUE_i and current environmental conditions in the pines was also evidenced in gas-exchange derived instantaneous $WUEi$ (A_n/g_s), which showed much more limited response in the pines than in the oaks. On top of that, we found a better agreement between gas-exchange and $\delta^{13}C$ in oaks than in pines, and comparatively higher instantaneous WUE_i in the oaks, contrary to what would be expected from $\delta^{13}C$ values. We cannot rule out some uncertainty due to the different time-integration of $\delta^{13}C$ and instantaneous data, as well as the need to estimate effective leaf area in conifers. However, the observed divergences would agree with the larger mesophyll limitation in conifers, as compared to deciduous oaks (see e.g. Flexas et al. 2012). Hence, mesophyll conductance for CO_2 might deserve further attention in the study of carbon limitations associated with tree decline, particularly in conifers (see e.g. Salmon et al. 2015).

As expected, we found lower concentrations of both SS and starch for pines in comparison to oaks throughout the studied period. This is in accordance with general knowledge that stem in conifers has very low content of NSC relative to angiosperm species (Martínez-Vilalta et al. 2016) and it is associated with the lower amount of parenchyma in conifer sapwood (Morris et al. 2016, Plavcová et al. 2016). Our results for *P. sylvestris* (average NSC content: $3.99 \pm 1.34\%$) are in the range of those presented for defoliated individuals in Poyatos et al. (2013), but higher than other studies under water-limited conditions, such as Voltas et al. (2013) (<1%) and Sangüesa-Barreda et al. (2012) (1% of starch, 1.5% SS) in the submediterranean ecotone, or Gruber et al. (2012) (1-2%) in a xeric Alpine valley. Conversely, compared with the literature, NSC values ($7.76 \pm 2.39\%$) in oaks were relatively high; see e.g. Nardini et al. (2016) for *Q. humilis* (1.5% of starch, 3.5% SS), or Klein et al. 2016 for *Q. petraea* (6.1% of starch, *ca.* 1% SS). Overall, even considering the additional uncertainty associated with different methods of carbohydrate quantification (Quentin et al. 2015), the values obtained suggest a moderate NSC depletion in the studied pines, and very scarce carbon limitations in the oaks. This is further confirmed when we consider drought responses and seasonal trends in NSC. In pines, as expected from the strong drought-induced limitation of carbon uptake (A_n), we found a positive association between ψ_{pd} (as an integrated proxy for tree water status) and total NSC, suggesting that drought periods actually imposed limitations to carbon storage in this species. Conversely, in the oaks SS concentration increased during the summer drought, but at the expense of starch concentration, most likely as a mechanism to supply increasing carbon demand in the stem for xylem repair (see e.g. Salleo et al. 2009). These opposite trends in SS and starch in response to drought would be partly responsible for the less consistent association between total NSC and water status (as indicated by ψ_{pd}) in oaks.

Nardini et al. (2016) found decreasing water potential and NSC associated with hydraulic failure and drought-induced decline. In our study, however, this trend was only significant after excluding the data from the wettest months (June and August 2013), when 90% of the samples showed ψ_{pd}>-0.5 MPa. During this period, NSC values were lower than in October 2012, and in the range of the dry season values (July 2012). Our results agree with the phenological trends described by Klein et al. (2016) in *Q. petraea*, with minimum starch concentration (1.3%) during leaf expansion in spring, and maximum values during autumn storage (11.9% starch in September), but superimposed by a pattern of starch-sugar interconversion in response to drought (Salleo et al. 2009). Overall, despite the existence of certain level of photosynthetic limitation imposed by water availability, the seasonal dynamic of total NSC in oaks appears to be dominated by phenology patterns, rather than by physiological status. This suggest that the site conditions (e.g. deep soils, co-dominance) are favourable enough for oaks in order to keep sufficient storage pools, minimizing drought effects and allowing a rapid recovery after a severe drought. In this regard, the close link between carbon pools and xylem repair indicate that large storage pools may contribute to the drought-tolerance strategy of Mediterranean oaks (Tyree et al. 1999, Salleo et al. 2009, Secchi and Zwieniecki 2011, Johnson et al. 2012, Brodersen and McElrone 2013).

Overall, our study on the seasonal patterns of physiological variables suggest that, despite the lack of visible symptoms of tree decline, the pines in our study site are largely limited by drought conditions, and cannot recover easily from severe drought episodes. In line with this, in both species radial growth patterns were affected by contrasting climatic years, but *Pinus sylvestris* showed higher variations and far more pronounced decreasing trend with time. In this sense, it is well known that carbon storage has priority over growth because ultimately survival depends more on carbon demands for metabolism than for growth (McDowell 2011, Sala et al. 2012, Palacio et al. 2013). Therefore, since we observed an effect of drought on NSC patterns, we would expect an even larger effect on growth. In this regard, *Pinus sylvestris*, which showed a long-lasting accumulated effect of drought limitation on NSC patterns, was also the most affected in terms of growth, particularly after the extreme dry years around mid-nineties. In fact, Galiano et al. (2012) probed in *P. sylvestris* that extreme drought events can induce long-term physiological disorders that would cause a slower recovery of the survival trees, and point to carbohydrate reserves as a key factor determining tree survival.

CONCLUSIONS

Our results provide evidence of a number of physiological traits that confer marcescent oaks a significant advantage for survival and adaptation to increasing drought trends, as compared to the widely distributed conifer Scots pine. This is not only reflected in the short-term, but has already clear consequences on long-term trends in secondary growth. Despite the repeated drought events reported in the area, *Q. subpyrenaica* seems to stay within its 'confort zone' of physiological performance, being able to survive and grow steadily; anyhow, *P. sylvestris* seems to be largely drought-limited in the driest conditions. The isohydric strategy of *P. sylvestris* confers a very sensitive behavior, where hydraulic and carbon limitations are difficult to separate; soil dryness affects stomatal closure, which in turn influences carbon assimilation and in consequence carbon storage. Also, these lower NSC concentrations in conifer sapwood may result in lower capacity to repair embolism in xylem conduits. Along with this, even though the studied pine trees did not exhibit clear symptoms of decline, the physiological and isotopic data reported here constitute an early warning evidence, confirming our initial hypothesis that these variables could be used to anticipate potential tree decline. Our results encourage the implementation of long-term studies to monitor the evolution of mixed stands in the area, in order to validate whether forest decline of *P. sylvestris* is consistently associated with the climatic and physiological conditions reported here.

Author contributions

P.M.G., M.A., J.P., E.G.P. and J.P.F. designed the research and collected the samples. P.M.G and J.P.F analysed the data and wrote the manuscript.

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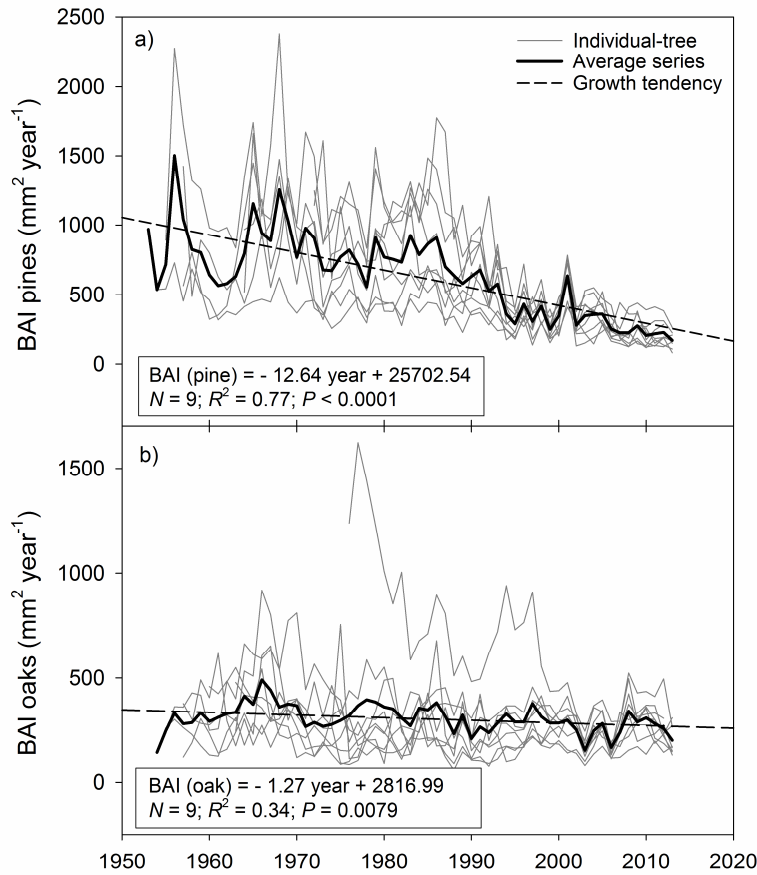
FIGURES

Figure 1. Historical evolution of Basal Area Index (BAI in mm/year) for the same individuals included in the seasonal sampling: (a) pines (*n*=9) and (b) oaks (*n*=9). Grey thin lines, individual-tree values; bold thick line average values for the 9 trees; linear regression in a thick dashed line represents the decreasing trend for BAI (named growth tendency). The regression equation, number of chronologies (*N*), regression coefficient (R^2) and significance (*P*) is represented in each panel.
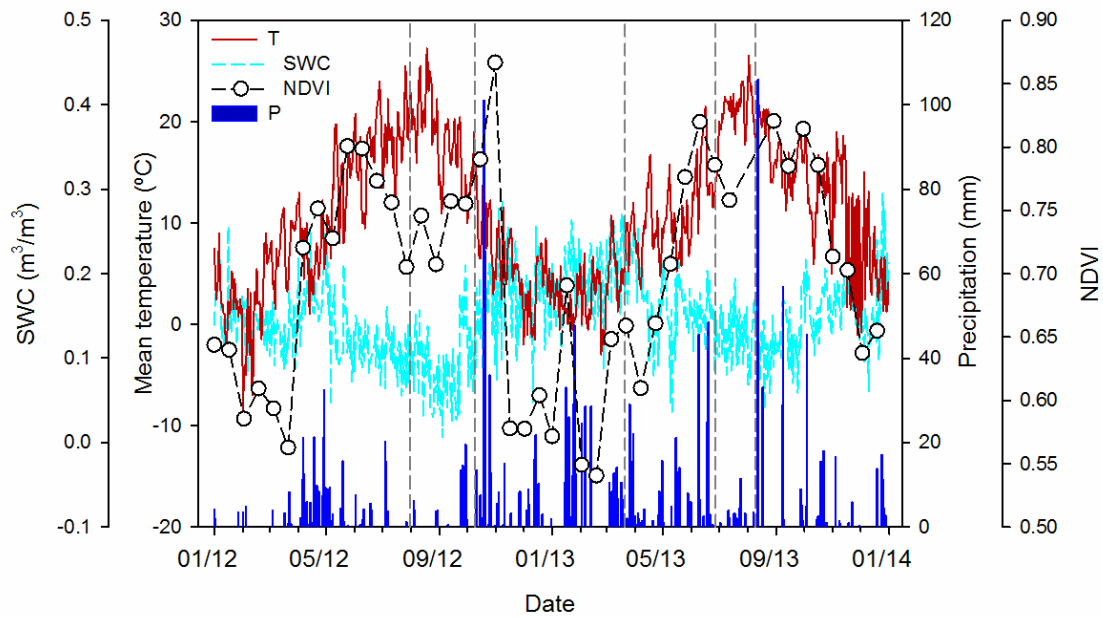
Figure 2. Physiological and environmental variables of the study site during the 2012-2013 seasonal sampling: soil water content (SWC) derived from ESA-SMOS remote sensing data for the four closest pixels (50×50 m^2) to the study site, daily mean temperature (T) and precipitation (P), and vegetation index (NDVI) from MODIS data of the pixel containing the study site (250×250 m^2). Dotted vertical lines indicate the five sampling campaigns.
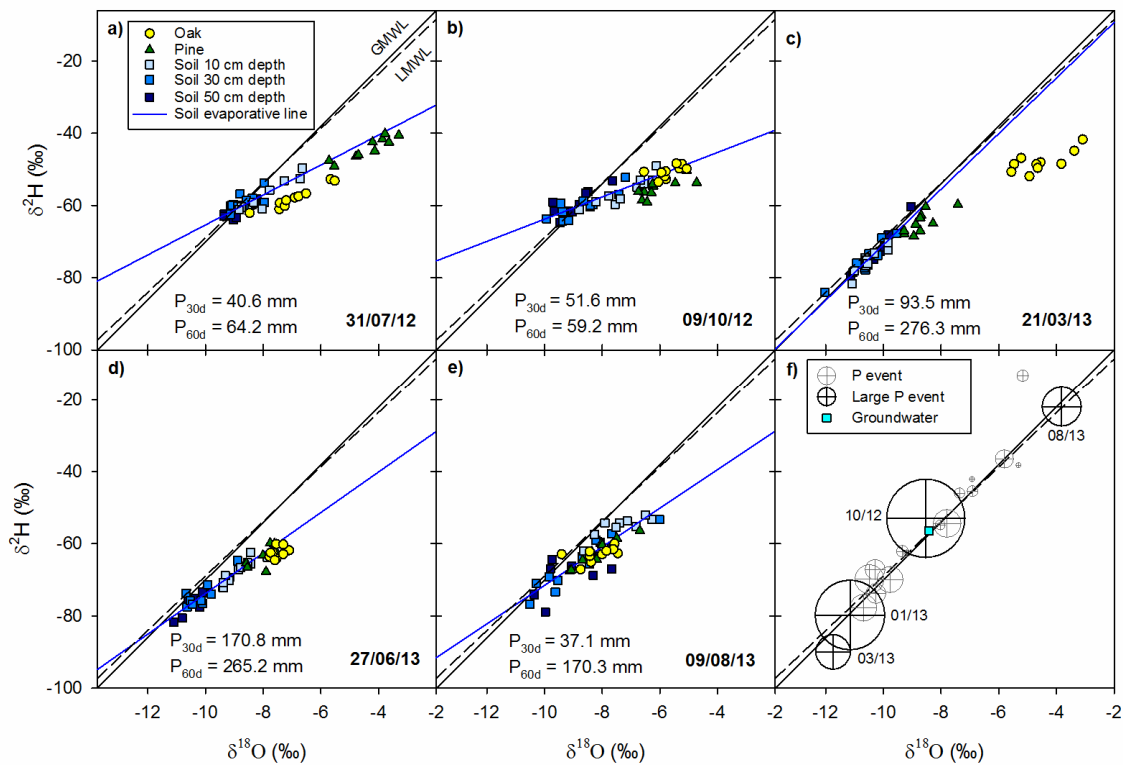
Figure 3. Bi-plots representing the seasonal evolution of the isotope composition of oxygen ($\delta^{18}O$) and hydrogen ($\delta^{2}H$) of precipitation, groundwater, and water extracted from twig xylem of pines and oaks and from the 10, 30 and 50 cm deep soil layers during the five sampling campaigns. Crossed circles in f panel, $\delta^{18}O$ and $\delta^{2}H$ of precipitation during the study period collected in the closest climatic station to the forest; size proportional to precipitation quantity; the largest precipitation events are highlighted with thicker and black lines and date (month/year) is indicated. The precipitation of the previous 30 and 60 days to the sampling date is displayed. Black diamond in f; δ-values for groundwater collected in a fountain within the forest. In a-e panels, black triangles, pine; white circles oak; light to dark grey, 10, 30 and 50 cm depth soil, respectively. Solid and dashed lines indicate, respectively, the global (GMWL, solid) and local (LMWL, dashed) meteoric water lines. Dotted line represents soil water evaporative line.
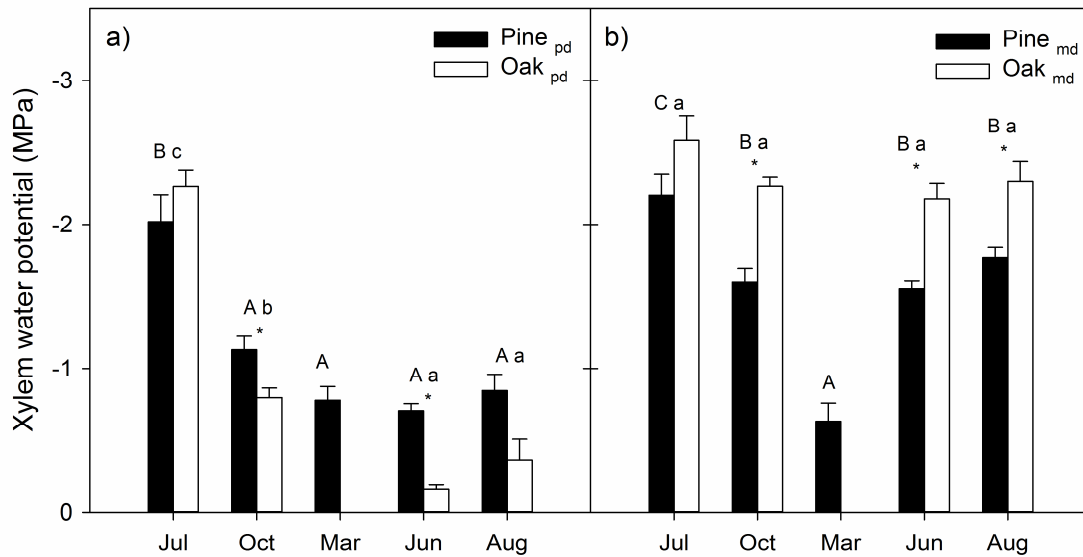
Figure 4. Seasonal evolution of pre-dawn (a) and midday (b) xylem water potential (MPa) of the two species, pine (black) and oak (white). Error bars represent standard errors. Differences were calculated based on Restricted Maximum Likelihood - REML (α = 0.05). Asterisks denote significant differences between species for each time. Letters show significant differences among sampling times for each species (Tukey test, $P < 0.05$); capital letters, pine; lower case, oak.

Figure 5. Seasonal evolution of leaf-level carbon and water balance for the two studied species, pine (black) and oak (white): (a) carbon isotope composition ($\delta^{13}C$) of bulk leaves, (b) photosynthetic rate (A_n), and (c) stomatal conductance (g_s). Error bars represent standard errors. Differences were calculated based on Restricted Maximum Likelihood - REML ($\alpha = 0.05$). Asterisks denote significant differences between species for each time. Letters show significant differences among sampling times for each species (Tukey test; $P < 0.05$): capital letters, pine; lower case, oak.

Figure 6. Seasonal evolution of soluble sugars (a) and starch (b) concentration in xylem for the two studied species, pine (black) and oak (white). Error bars represent standard errors. Differences were calculated based on Restricted Maximum Likelihood - REML ($\alpha = 0.05$). Asterisks denote significant differences between species for each time. Letters show significant differences among sampling times for each species (Tukey test; $p < 0.05$): capital letters, pine; lower case, oak.

Figure 7. Relationship between predawn water potential and (a) photosynthesis - A_n. (b) stomatal conductance - g_s and (c) total carbohydrates content - Total NSC, for all the sampling times and both species. White circles and dashed line, oaks; black triangles and solid line, pines. The regression coefficient (R^2) and significance (P) is represented in each panel. Dotted circles denote sampling times excluded from the regression in oaks: June 2013 in a) and b); June and August 2013 in c).
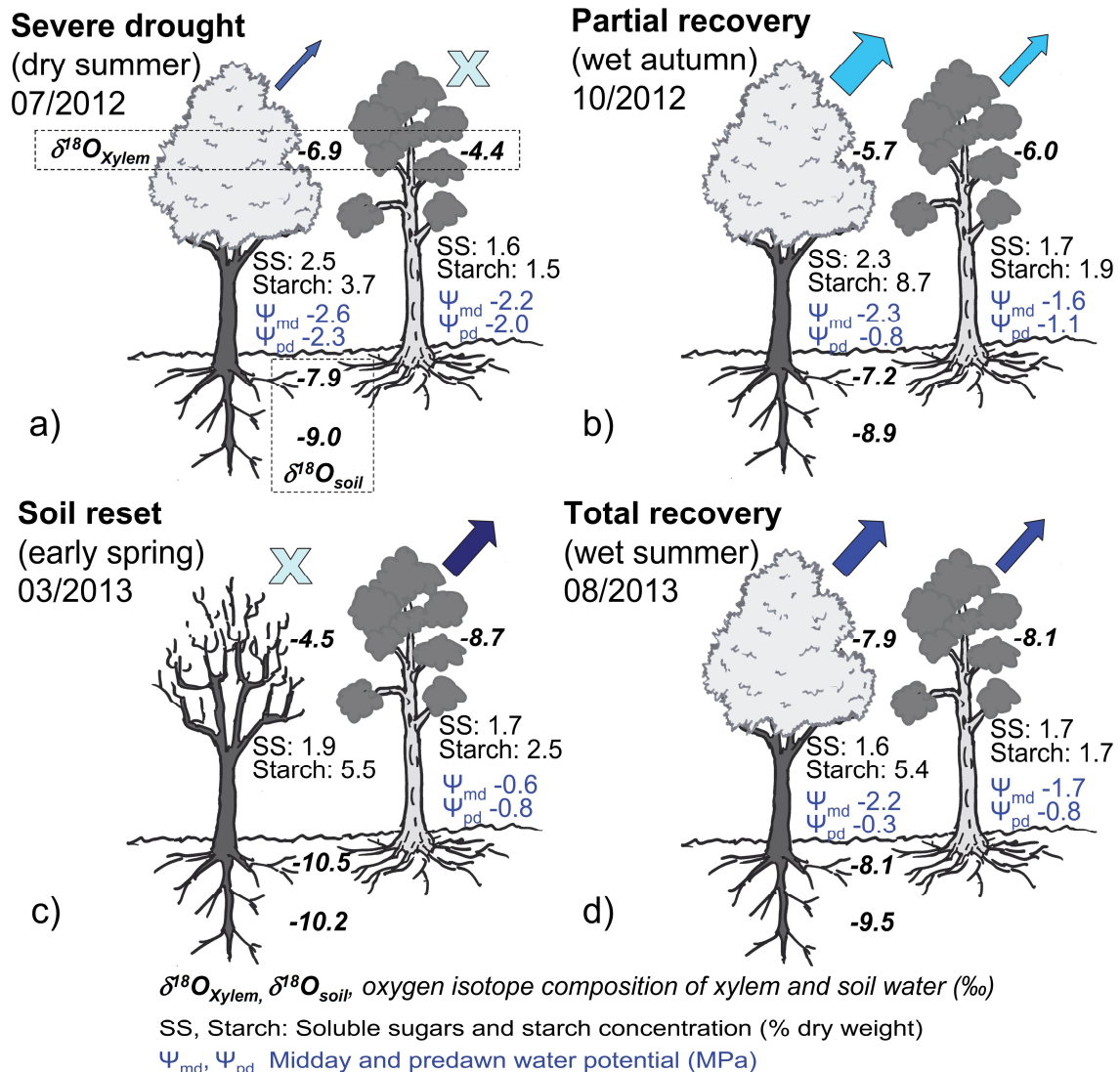
Figure 8. Graphical summary of seasonal changes in soil and xylem water isotope composition ($\delta^{18}O$ in ‰), midday and predawn water potential (Ψ_{md}, Ψ_{pd} in MPa) and carbohydrate concentration in xylem (Ss: soluble sugars; St: starch in %) for oaks and pines during the studied period. The size of the arrows on top of the trees denotes the estimated range of transpiration rates for each species; a cross denotes stomatal closure. Light to dark blue in arrows indicate enriched to depleted isotopic values in xylem water. In the dry summer of 2012 (a) the soil profile shows the strongest evaporative gradient, with more enriched values in the upper layers. In both species the transpiration is minimal and there is not recovery of soil water potential overnight. In early autumn after the first precipitation events (b) there is a recovery of transpiration rates reflected in a lower water potential at predawn and in less enriched xylem water, although still uncoupled. Oak recovery is much more evident than pine. After the soil water recharge phase in winter (c), soil isotopic signature changes to very negative values due to the snow and there is no evaporative gradient. Oaks remained leafless with highly enriched xylem. Pines showed very low water potentials in relation to higher transpiration rates and xylem moving in the direction of new soil isotope values. Finally, in the second summer, were climatic conditions were very wet (d), transpiration is fully recovered and xylem and soil couple.

TREE PHYSIOLOGY, ONLINE SUPPLEMENTARY MATERIAL

Contrasting ecophysiological strategies related to drought: the case of a mixed stand of Scots pine (*Pinus sylvestris*) and a submediterranean oak (*Quercus subpyrenaica*)

by

Paula Martín-Gómez, Mònica Aguilera, Jesús Pemán,
Eustaquio Gil-Pelegrín, and Juan Pedro Ferrio

Figure S1. Field site and sampling campaign. a) soil coring for the determination of the isotope composition of oxygen (δ^{18}O) and hydrogen (δ^{2}H) in soil water. Notice the abundance of (mainly pine) roots in the upper soil layer. b) overview of the upper canopy. Sun-exposed branches were collected from the upper crown for physiological measurements, carbohydrate analysis and isotope determinations.
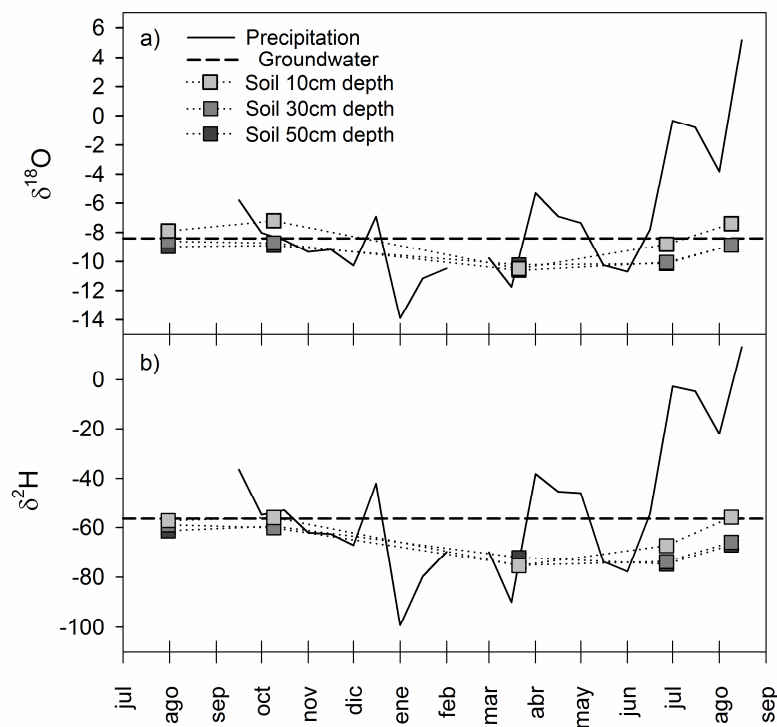
Figure S2. Seasonal evolution of the isotope composition of oxygen (a - $\delta^{18}O$) and hydrogen (b - $\delta^{2}H$) in precipitation, groundwater, and soil water extracted at 10, 30 and 50 cm depth (in this order, light to dark grey squares). Solid and dashed lines indicate, respectively, precipitation and groundwater isotopic values.

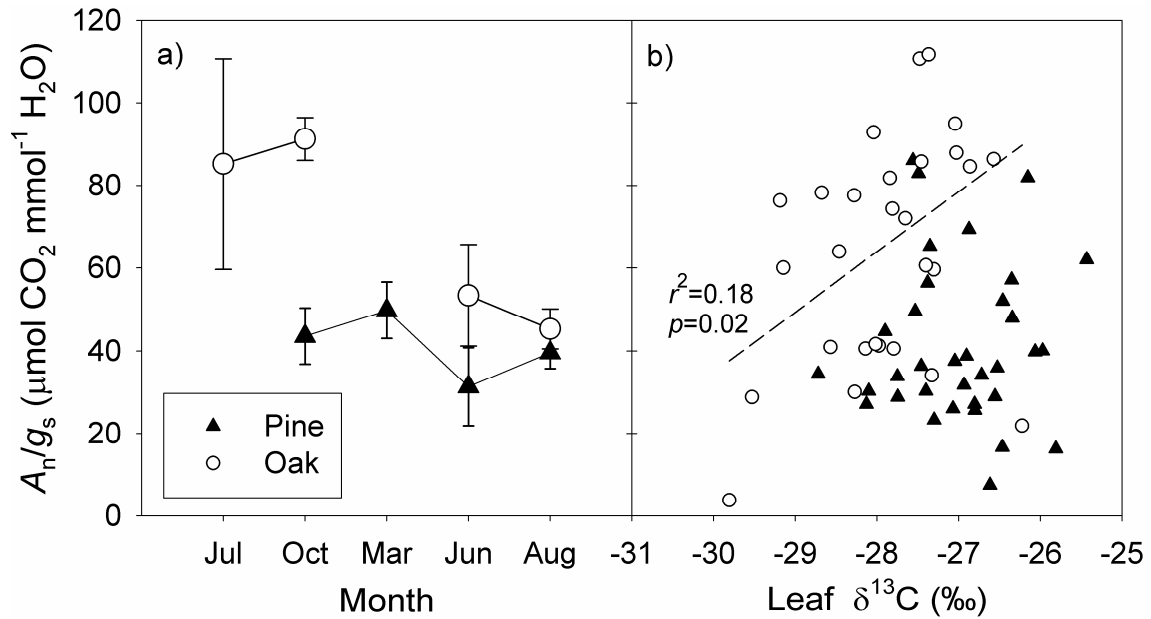**Figure S3.** Instantaneous intrinsic water use efficiency (A_n/g_s) and carbon isotope composition in bulk leaves (leaf $\delta^{13}C$); a) Seasonal evolution of A_n/g_s ; b) relationship between A_n/g_s and leaf $\delta^{13}C$. The trend line denotes the significant linear correlation between leaf $\delta^{13}C$ and A_n/g_s, in oaks. White circles, oak; black triangles, pine.