Dendroecological and genetic insights for future management of an old-planted forest of the endangered Mediterranean fir *Abies pinsapo*

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Abstract

Pinsapo fir (*Abies pinsapo* Boiss.) is an endangered Mediterranean conifer that has raised strong conservation interest as a paradigmatic example of species characterized by small and fragmented populations. We studied an old reforestation stand composed of *A. pinsapo*, *Pinus nigra* and *Pinus sylvestris* established in the 1910s in central-eastern Spain (about 500 km north of the species native distribution range), with the aim of evaluating the stand’s suitability as an *ex situ* conservation unit for the fir. To this end, we investigated the long-term performance of the stand and assessed genetic diversity of *A. pinsapo*. Tree-ring width (TRW) and carbon isotope discrimination ($\Delta^{13}C$) were used to characterise growth dynamics and intrinsic water-use efficiency ($WUE_i$), respectively. Furthermore, 42 pinsapo firs were genotyped at five microsatellite loci to compare their genetic variation with published data on natural populations. *A. pinsapo* showed ca. two-fold higher radial growth than pines in the last 80 years; however, a growth decrease was observed for all species from the 1990s onwards. Indexed TRW was positively associated with $\Delta^{13}C$ at the species level, denoting inter-annual growth dependence on water availability. Overall, $\Delta^{13}C$ was higher for *A. pinsapo* compared to pines, indicating lower $WUE_i$, but $\Delta^{13}C$ significantly decreased over the last 50 years for all species, likely as the result of tighter stomatal regulation of water loss, resulting in $WUE_i$ increases of about 25%. Recently, however, *A. pinsapo* showed reduced $WUE_i$ increase in concord with growth slowdown, suggesting a threshold response for stomatal regulation. Although genetic diversity of *A. pinsapo* was about half of natural populations, the old-planted stand could be important for the conservation of this endemic species considering its good long-term growth and physiology. The latest decrease in performance of *A. pinsapo*, however, asks for urgent management measures aimed at reducing the competition for water and promoting growth and natural regeneration. This study illustrates the potential of
combining tree-ring-based long-term physiological information with genetic data to ascertain the prospects of artificial stands for conservation purposes.

Keywords

*Abies pinsapo*; carbon isotopes; conservation unit; genetic diversity; microsatellites; tree rings
1. Introduction

Mediterranean forest ecosystems are a biodiversity hotspot, harbouring an important number of endemic tree species. The case of the genus *Abies* is an example of such diversity, being present around the Mediterranean basin with at least eight species, many of them with a very limited distribution (Caudullo and Tinder, 2016). This richness is threatened by the on-going climate change, especially in the case of species characterized by small and fragmented populations (Aussenac, 2002). Among Mediterranean firs, the pinsapo fir (*Abies pinsapo* Boiss.) is endemic to the Baetic range of southern Spain, where it grows between 900 and 1800 m a.s.l. on north-facing slopes. Due to its endemic and endangered status, *A. pinsapo* has been subjected to intense conservation efforts (Arista et al. 2011). Currently, most of the natural distribution of *A. pinsapo* is included in a network of protected areas, and its most emblematic forests have been declared Biosphere Reserves by UNESCO.

The pinsapo fir is considered a drought-sensitive conifer, although it has greater efficiency of water transport than the European silver fir (*Abies alba* Mill.) (Linares et al., 2012; Peguero-Pina et al., 2012). Its high physiological plasticity (Linares et al., 2009a; Sancho-Knapik et al., 2014; Serichol-Escobar et al., 2016) allows the presence of *A. pinsapo* in areas exposed to summer drought. While past conservation efforts have been focused on the *in situ* maintenance of the remaining natural stands, recent episodes of warming-induced die-back and forest fires (Linares et al., 2009b; Arista et al., 2011) raise the necessity of coupling initiatives involving extant populations with *ex situ* conservation efforts. Additional concerns are related to the low genetic diversity of *A. pinsapo*, which is regarded as a potential limitation for future adaptation to new climatic conditions (Liepelt et al., 2010; Dering et al., 2014). *Ex situ* strategies are recommended in case of particularly endangered species or if on-going environmental changes point to
the unsuitability of *in situ* conservation measures (Ledig, 1986; Serra-Valera et al., 2017). In this regard, old-planted stands established for reforestation purposes outside of the species’ distribution range might be of potential interest in conservation programs (Koskela et al., 2013; Schueler et al., 2014; Santini et al., 2018; Mataruga et al., 2020), thereby complementing other *ex situ* strategies such as seed banks or arboreta (Ledig, 1986).

Yet, very few artificial stands, which might be used as *ex situ* conservation units, are present outside the distribution range of *Abies pinsapo*. One remarkable exception is the stand assessed in this study, which was established about 100 years ago in central-eastern Spain. The outstanding performance and regeneration of the pinsapo fir led to the recent declaration of the Orcajo stand as “Arboleda Singular” (singular grove) by the Regional Government of Aragón. However, an in-depth characterisation of pinsapo fir growth and physiology is lacking at the Orcajo stand. For this reason, we used a dendrochronological approach to conduct a retrospective study aiming at characterising the performance of *A. pinsapo* in relation to the two main pine species of the planted stand (*Pinus nigra* subsp. *salzmannii* (Dunal) Franco and *Pinus sylvestris* L.), which show consistently inferior growth. To this end, radial growth dynamics was inferred through ring-width records while indirect information on leaf-level physiology was obtained through analysis of stable isotopes in wood (McCarroll and Loader, 2004).

The ratio of the heavy to light carbon isotopes ($^{13}\text{C}/^{12}\text{C}$) depends on factors affecting CO$_2$ uptake, mainly controlled by photosynthetic rate ($A$) and stomatal conductance ($g_s$), as expressed in the ratio $A/g_s$ (intrinsic water-use efficiency, WUE$_i$; Farquhar et al., 1989). Temporal trends in $\Delta^{13}\text{C}$ (and WUE$_i$) are dependent on plant physiological reactions to both increasing atmospheric CO$_2$ and changes in climate factors, and can be interpreted following Saurer et al. (2004): (i) a positive $\Delta^{13}\text{C}$ trend
indicates a weak or null stomatal response to atmospheric CO₂ stimulation, implying a nearly constant WUEᵢ through time; (ii) a constant Δ¹³C denotes the proportional regulation of photosynthesis and stomatal conductance, indicating a constant ratio of internal to external CO₂ concentration and improved long-term WUEᵢ; and (iii) a negative Δ¹³C trend suggests a large stomatal response that leads to constant internal CO₂ concentration and a strong rise in WUEᵢ. Particularly, if drought becomes more limiting through time, a decreasing Δ¹³C trend would be expected as indicative of increasing stomatal constraints on leaf gas exchange (Gessler et al., 2014). We analyzed Δ¹³C trends at Orcajo to disentangle tree physiological responses to climate change and to rising CO₂ concentration. Indeed, a careful examination of tree-ring-based Δ¹³C trajectories can provide clues on physiological mechanisms that may compromise the future survival of forest stands in the context of climate change (Gessler et al., 2018). In addition to growth and physiological analyses, we also conducted a quantification of genetic diversity for A. pinsapo individuals growing in the stand using five microsatellite loci.

We hypothesised that (i) A. pinsapo is growing under favourable ecological conditions in the planted stand, as fingerprinted in its physiological performance, in relation to the two main accompanying species (P. nigra and P. sylvestris), and (ii) the genetic diversity of the existing plantation is adequate for this stand to be utilized for the conservation of forest genetic resources of A. pinsapo. Specifically, our objectives were: a) to characterise differences in radial growth among species and their associated changes over the last century; b) to evaluate tree carbon and water relations through WUEᵢ during the last 50 years across species; c) to understand the patterns of climatic dependence of these species with time, both through stable carbon isotopes and radial growth; and d) to assess the genetic diversity of A. pinsapo in the planted stand and to compare it to that present in
natural populations. The final goal was to evaluate the potential of this stand as \textit{ex situ} conservation unit of this emblematic Mediterranean conifer.

2. Material and methods

2.1. Study site and field sampling

The site is a naturalised reforestation stand planted in Orojao municipality (southern Iberian range, Zaragoza province, Spain), ca. 500 km north of the closest natural population of \textit{A. pinsapo} (Figure 1a; Figure A.1). It is located on a gentle slope facing northeast at an altitude of about 1150 m (coordinates 41°05’ N, 1°30’ W). The mean annual temperature is +10.1°C, with January being the coldest (+1.8°C) and July the warmest (+19.8°C) month (period 1920-2017). The mean annual precipitation is 556 mm, and is relatively well-distributed throughout the year, with summer precipitation accounting for 18% of total precipitation (Figure 1a).

The natural vegetation in the area is a sparse forest of \textit{Quercus pyrenaica} Wild. and \textit{Quercus faginea} Lam. The original reforested area is a mixed-species plantation of 3.65 ha, and includes \textit{A. pinsapo} (24% of adult trees), \textit{P. nigra} (22%) and \textit{P. sylvestris} (32%) intermingled, with minor representation of \textit{P. halepensis} and \textit{P. pinaster}. The density of adult individuals is 640 trees ha\(^{-1}\) and tree distance varies between 3.2 and 10 m (mean ± SD = 4.2 ± 2.1 m). No written documents remain about the seed sources employed in the plantation, or the reasons for using pinsapo fir, an unusual species for the area. A recent forest inventory provides evidence of abundant fir regeneration (with different existing cohorts), and no evidence of natural regeneration of \textit{Pinus} ssp.

Field sampling was performed in October 2018. The inspection of the stand did not reveal evidence of natural or anthropic perturbations (fires, logging, diseases and pests, etc.). For the dendrochronological analysis, we selected around 20 representative trees of
each species from the original reforestation in groups of three individuals (one per species) separated by a maximum of 10 m. The sampled groups of trees were at least 25 meters apart, to cover the variability within the stand. Two cores per tree were sampled at breast height (1.30 m) using a 5-mm increment borer. For each selected tree, the cores were extracted from the opposite cross-slope sides of the trunk within a vertical distance of about 5 cm. A total of 63 trees were sampled. Height and diameter at breast height (DBH) were measured with a clinometer and tree calliper, respectively.

2.2. Sample preparation and dendrochronological methods

Wood samples were dried at 35°C for 72 h and the surface of one core per tree (from the same trunk side) was sanded with sandpaper of progressively smaller grain until the tree rings were clearly visible. The remaining core per tree was kept intact for carbon isotope analysis. Tree rings were visually cross-dated and measured using high-resolution images produced on a flat-bed scanner coupled with WinDendro™ software (Regent Instruments, 2012). The COFECHA program (Holmes, 1983) was used to evaluate the quality of cross-dating. All available trees were used for ring-width measurements (Table 1). Each ring-width series was detrended using a cubic smoothing spline with a 50% frequency cut-off of 50 years (high-pass filtering which corresponds to approximately 2/3 of the series length; Cook and Peters [1981]). This procedure eliminates non-climatic fluctuations, reducing growth variability that is not common to most trees as well as the effects of stand dynamics. An autoregressive model was also applied to each detrended series to remove temporal autocorrelation. Finally, a biweight robust mean was used to obtain indexed tree-ring width (TRW_i) chronologies per species consisting of stationary (mean = 1) dimensionless indices. Chronology development and standardisation were performed using the ARSTAN v.44 h2 (Cook and Krusic, 2005).
We evaluated the quality of the chronologies by calculation of the mean inter-series correlation (\(R_{\text{bar}}\)) and the expressed population signal (EPS) statistics. \(R_{\text{bar}}\) informs on the strength of the common signal captured by a chronology (Wigley et al., 1984). EPS indicates the suitability of a chronology for capturing the hypothetical population signal, which is typically checked against a threshold value of 0.85 (Wigley et al., 1984). The chronologies reached the EPS threshold value in 1935 (\(P. \text{sylvestris}\)), 1938 (\(P. \text{nigra}\)) and 1941 (\(A. \text{pinsapo}\)) (Table 1).

The annual basal area increment (BAI) was used to characterise the absolute radial growth trends for the set of cross-dated trees at the species level. BAI was calculated from tree-ring series according to:

\[
\text{BAI} = \pi (R_t^2 - R_{t-1}^2)
\]

where \(R\) is the radius of the tree and \(t\) is the year of tree-ring formation. Finally, a biweight robust mean was computed to provide a mean BAI chronology for each species.

2.3. Carbon isotope analysis

The analysis of carbon isotope composition (\(\delta^{13}\)C) in tree rings was performed on core segments spanning the period 1965-2017 (i.e., within sapwood) in order to avoid a possible juvenile imprint on the isotope values (average age of sampled trees at 1.30 m was 72 ± 10 years, 77 ± 8 years and 80 ± 9 years for \(A. \text{pinsapo}, P. \text{nigra}\) and \(P. \text{sylvestris}\), respectively; mean ± SD). Tree rings were identified with the aid of a wet soft brush and excised using a scalpel. For this purpose, the best five cross-dated trees per species were used. Rings were pooled together on an annual basis for isotopic analysis (Leavitt, 2008), since it has been shown that the bias produced by the relative mass contribution of the trees is negligible with regard other sources of uncontrolled variability related to sample preparation and analysis (e.g. \(P. \text{nigra}\) [Scymczak et al., 2012], \(Picea likiangensis\) [Shi et
In addition, we selected five years (one per decade as follows: 1970, 1980, etc.) in which the rings were analysed separately for each tree, to obtain an estimate of inter-individual variability. The set of rings of the same year and species were ground together to a fine powder with a mixer mill (Retsch MM301, Haan, Germany), except those rings that were analysed independently, which were ground alone. An aliquot of about 1 mg was weighed and encapsulated into tin capsules for mass spectrometry analysis. We did not perform cellulose extraction since many studies have found that whole wood material from the sapwood of conifers contains the same climate signal as cellulose with regard of carbon isotopes (e.g. *A. alba* [Weigt et al., 2015], *P. sylvestris* [Mischel et al., 2015], *P. halepensis* [Ferrio and Voltas, 2005] or *Pseudotsuga menziesii* [Taylor et al., 2008]). Samples underwent combustion using a Flash EA-1112 elemental analyser interfaced with a Finnigan MAT Delta C isotope ratio mass spectrometer (Thermo Fisher Scientific Inc., MA, USA). Isotope ratios were expressed as per mil deviations using the δ notation relative to Vienna Pee Dee Belemnite (VPDB). The accuracy of the analyses (SD of standards) was 0.05‰.

To account for changes in δ¹³C of atmospheric CO₂ (δ¹³C_{air}), we calculated carbon isotope discrimination (Δ¹³C) from δ¹³C_{air} and wood δ¹³C (δ¹³C) following Farquhar et al. (1989):

\[
\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C}{1 + \delta^{13}C}
\]  

(2)

δ¹³C_{air} applied to the samples varied between -8.44‰ and -7.09‰ (in the period 1965-2017) (Ferrio et al. 2005).

Trends in Δ¹³C were evaluated at the species level by simple linear regression or, alternatively, by broken-line regression (Robbins et al. 2006). In this last case, a simple two straight-line, one breakpoint model was fitted to the data. This model accounted for the observed decreasing slope in Δ¹³C records over time at values less than an estimated
breakpoint year, and either a plateau at values above the breakpoint or a second line with increasing slope.

Based on the best-fit model, the $\Delta^{13}$C records were detrended and indexed $\Delta^{13}$C chronologies ($\Delta^{13}$Ci) were obtained at the species level consisting of stationary (mean = 1) dimensionless indices. No significant autocorrelation was found for $\Delta^{13}$Ci. The $\Delta^{13}$Ci chronologies were then used to examine the relationships between high-frequency fluctuations in tree-ring width and carbon isotopes and, also, to calculate correlations with climate. The NLIN procedure of SAS/STAT software (ver. 9.4, SAS Inc., Cary, NC, USA) was used for fitting various broken-line regression models.

Using $\Delta^{13}$C data, intrinsic water-use efficiency (WUEi) was estimated according to:

$$WUE_i = \left( C_a \times (b - \Delta^{13}C) \right) \left[ 1.6 \times (b - a) \right]$$

where $C_a$ represents the atmospheric CO$_2$ concentration, $a$ is fractioning during diffusion through stomata (~4.4‰) and $b$ is fractioning during carboxylation by Rubisco and PEP carboxylase (~27‰) (Farquhar et al., 1989). The factor 1.6 denotes the ratio of the diffusivities of water vapour and CO$_2$ in the air. $C_a$ values were taken from the National Oceanic and Atmospheric Administration (NOAA) Earth System Research Laboratory.

2.4. Genetic data and analysis

To estimate the genetic diversity of A. pinsapo at Orcajo, adult trees were genotyped at five microsatellite loci (SF 1, SF b4, SF b5, SF 78 and SF 239) developed by Cremer et al. (2006). For this purpose, needles were sampled from 44 trees separated at least 25 meters apart using telescopic loppers. Samples were frozen in the field and kept at -80℃. For DNA extraction, the needles were disrupted with a TissueLyser (Qiagen, Hilden, Germany) using two 4 mm tungsten beads (Brammer Iberia S.A., Spain). DNA extraction was performed using an E.Z.N.A. plant DNA kit (Omega, Bio-tek, GA, USA). All PCR
reactions were performed with PCR profile A (2.5 mM MgCl$_2$ and 60°C starting annealing temperature) described by Cremer et al. (2006). The PCR products were analysed using an ABI 3730XL sequencer (Applied Biosystems, CA, USA) and the allele size was determined using GeneMarker software (SoftGenetics, State College, USA).

The genotype records were added to a dataset including respectively 28 and 29 individuals from two out of the three existing natural populations of *A. pinsapo* (Sierra de Zafalgar and Serranía de Ronda), which had been previously genotyped by Dering et al. (2014) using the same markers. In the two natural populations, Dering et al. (2014) sampled trees spaced at least 50 meters, to cover as much as possible the genetic diversity present in the stands. The similar sampling strategy and the same set of markers used to genotype the individuals allowed for a direct comparison between the genetic diversity present in the artificial stand and that of natural populations of the species. The frequency of null alleles was estimated for the three stands (i.e., two natural and one planted) using the EM algorithm (Dempster et al., 1977) implemented in the program freeNA (Chapuis and Estoup, 2007). Standard genetic parameters were calculated to describe within-population variation. Specifically, the mean number of alleles per locus ($N_a$), the heterozygosity based on Hardy-Weinberg expectations ($H_e$), the observed heterozygosity ($H_O$) and the fixation index ($F_{IS}$) i.e., $1-H_O/H_E$ (Nei, 1977) were calculated using GenAlex software (Peakall and Smouse, 2006). Since the sampling effort differed between populations, the allelic richness based on rarefaction at 52 gene copies ($A_{R52}$) was calculated using the program FSTAT (Goudet, 1995). Paired $t$-tests were performed to test for differences in genetic parameters ($N_a$, $H_e$, $H_O$, $F_{IS}$ and $A_{R52}$) between the three populations.
2.5. Meteorological data and climate analysis

Mean temperature and precipitation records were obtained on a monthly basis from the nearest meteorological station to the planted stand (Daroca, Zaragoza province, 41°06’N, 1°25’W, 782 m a.s.l., located 7 km away from the study site). The standardised precipitation evapotranspiration drought index (SPEI; Vicente-Serrano et al., 2010) was also calculated at time scales of one (abbreviated as SPEI1), three (SPEI3) and six months (SPEI6) to monitor droughts with respect to different usable water resources. The climate records covered the period of 1920-2017.

The relationships between the TRW$_i$ or $\Delta^{13}$C$_i$ chronologies and monthly climatic variables were assessed through bootstrapped correlations using the R package ‘treeclim’ (Zang and Biondi, 2015). To ensure that results were driven by local climate rather than by long-term warming, the monthly climatic series exhibiting a linear trend over time were detrended by linear regression and the residuals were kept for correlation analysis. The relationships between chronologies and climatic variables were investigated from June of the previous year to November of the current year over the period of 1965-2017. Temporal stability of these associations was assessed through moving correlations for 30-year segments lagged by one year.

3. Results

3.1. Climate trends

The site experienced a consistent warming since the 1980s, which rendered an increase of 1.1°C in mean temperature for the last 30 years (Figure 1b). This tendency was compounded by a parallel decrease in precipitation for the same period. The temperature increase was statistically significant for most of the months since 1920 (excluding February, April and December), but a concomitant decrease in precipitation was
detectable only in December (Figure A.2). These patterns translated into consistently lower than average values for SPEI1 since the 1990s (Figure 1b), pointing to increased intensity and duration of drought events, especially in the summer (Figure A.2).

3.2. BAI, Δ\(^{13}\)C and WUE\(_i\) trends

For the common period with \(\varepsilon\)PS>0.85 (1941-2017), the highest BAI was observed for \(A. \text{pinsapo}\) (7.97 ± 0.59 cm\(^2\) year\(^{-1}\); mean ± SE), followed by \(P. \text{nigra}\) (5.05 ± 0.58 cm\(^2\) year\(^{-1}\)) and \(P. \text{ sylvestris}\) (3.92 ± 0.58 cm\(^2\) year\(^{-1}\)). A general trend towards increasing radial growth was evident from 1960 until the 1980s (Figure 2), which was followed by a significant \((p< 0.05)\) growth decrease for all the species during the period of 1990-2017 (BAI reduction= -0.09 cm\(^2\) year\(^{-1}\) \([A. \text{pinsapo}]\) to -0.04 cm\(^2\) year\(^{-1}\) \([P. \text{ sylvestris}]\)). Despite the recent negative trends, \(A. \text{pinsapo}\) still showed the largest BAI during the 21\(^{st}\) century (7.09 ± 0.52 cm\(^2\) year\(^{-1}\) compared with 4.73 ± 0.51 and 2.40 ± 0.50 cm\(^2\) year\(^{-1}\) for \(P. \text{nigra}\) and \(P. \text{ sylvestris}\) respectively). \(A. \text{pinsapo}\) showed consistently higher absolute basal area than \(P. \text{nigra}\) (from the 1940s) and \(P. \text{ sylvestris}\) (from the 1950s) (Figure A.3).

The highest mean \(\Delta^{13}\)C was observed for \(A. \text{pinsapo}\) (18.06 ± 0.10‰), followed by \(P. \text{ sylvestris}\) (17.19 ± 0.10‰) and \(P. \text{nigra}\) (16.90 ± 0.10‰). The outcome of the regression analysis indicated that broken-line regression models fitted the \(\Delta^{13}\)C records better than simple linear regressions, and indicated the presence of breakpoint years (denoting a change in slope) during the early 1990s for all species (Table A.1). \(\Delta^{13}\)C decreased significantly from 1965 to the early 1990s (Figure 3a), with slopes ranging from -0.068 %\(\_\)year\(^{-1}\) \([A. \text{pinsapo}]\) to -0.046 %\(\_\)year\(^{-1}\) \([P. \text{nigra}]\). \(\Delta^{13}\)C increased in pinsapo fir (i.e. increasing slope) or remained approximately stable in pines thereafter (Table A.1). The inter-individual variability of the isotopic signal was low (standard deviation, SD =
0.58‰, 0.51 ‰ and 0.40‰ for *P. nigra*, *P. sylvestris* and *A. pinsapo* respectively) compared to the magnitude of $\Delta^{13}C$ changes through time (Figure 3a).

$WUE_i$ increased by about 25% between the first (1965-1969) and the last (2013-2017) five years of the study period (Figure 3b), but there was an apparent change in $WUE_i$ trends from ca. 1990 onwards, coinciding with the breakpoint years for $\Delta^{13}C$. Since then, trees showed null (*A. pinsapo*) or limited $WUE_i$ increase (*P. nigra* and *P. sylvestris*). Changes in $WUE_i$ over time were unrelated to BAI over the period of 1965-2017. This indicated that the trees did not respond to an increase in $WUE_i$ by enhancing their radial growth, suggesting lack of CO$_2$ fertilization effects. On the contrary, $WUE_i$ became negatively related to BAI from the $\Delta^{13}C$ breakpoint year onwards in the case of the pine species (Figure 4).

### 3.3. Tree-ring width and $\Delta^{13}C$ chronologies

The TRW$_i$ chronologies showed good agreement among species, with significant correlation coefficients ranging from 0.77 (*A. pinsapo* vs. *P. nigra*) to 0.86 (*P. nigra* vs. *P. sylvestris*). Similarly, the $\Delta^{13}C_i$ chronologies showed significant correlations, varying between 0.58 (*P. nigra* vs. *P. sylvestris*) and 0.63 (*A. pinsapo* vs. *P. sylvestris*). At the species level, the correlation between TRW$_i$ and $\Delta^{13}C_i$ was positive and significant, with the highest and lowest values found for *P. nigra* and *P. sylvestris*, respectively (Figure 5).

### 3.4. Relationships with climate

TRW$_i$ correlated negatively with the current May-June temperature for all species and with the current August temperature for *P. nigra* only (Figure 6a). The three species reacted positively to winter-spring precipitation (January to June) and negatively to drought stress (SPEI1) in the same months, with the exception of *A. pinsapo*, which was
sensitive to drought from April to June only. The species were also sensitive to longer
drought episodes occurring from February to August (SPEI3), and from January (P. nigra), February (P. sylvestris) and April (A. pinsapo) to September (SPEI6)

There was a shorter temporal sensitivity to climate for Δ13C, as compared with TRW, (Figure 6b). Δ13C correlated negatively with late winter (March) temperature for all species and with spring (May) temperature for both pines. Positive correlations were detected between Δ13C and late winter to early spring precipitation (February to April), but A. pinsapo was also sensitive to January precipitation and both pines to May precipitation. The relationships involving SPEI1 resembled those of precipitation. The accumulated drought over three months (SPEI3) was related to Δ13C over January to June (A. pinsapo), February to July (P. nigra, which showed the highest overall sensitivity) and February-June (P. sylvestris). The three conifers were also sensitive to longer drought episodes (SPEI6) occurring from March to August.

The associations between TRW, and climate were unstable over the last five decades, and progressively longer temporal relationships were observed with variables related to water availability. In particular, the temporal sensitivity of TRW, to precipitation increased across species from three (April to June) to five months (February to June). Likewise, the temporal sensitivity to SPEI3 increased from five (April to August) to seven months (February to August). This behaviour is exemplified for A. pinsapo in Figure 7, but similar responses were also observed for both pines (Figures A.4 and A.5). For Δ13C, a variable dependence on water availability was observed with time only in A. pinsapo (Figure 7). In particular, the sensitivity to the three-month accumulated drought (SPEI3) was reduced from six to four months, shifting from March through August to February through May at the end of the study period. This considerable adjustment was not observed for any of the pine species (Figures A.4 and A.5).
3.5. Genetic diversity of A. pinsapo

The 44 sampled individuals were added to a published dataset comprising two natural populations of southern Spain. All loci were polymorphic and the frequency of null alleles was low in the three populations (0.00-0.16 across loci and populations; Table A.2). The two natural populations showed comparable levels of genetic variation (Table 2; Fig. A.6), and paired t-tests indicated non-significant differences in $Na$ ($p = 0.75$), $Ar_{52}$ ($p = 0.94$), $He$ ($p = 0.18$), $Ho$ ($p = 0.68$) and $F_{IS}$ ($p = 0.98$). On the other hand, the genetic parameters indicated reduced diversity of the artificial stand compared to the natural populations (Table 2; Fig. A.6). In particular, the $Na$ of the artificial stand took about half the value of the natural populations, and the value of $Ar_{52}$ was similarly reduced in the artificial stand. Paired t-tests indicated that $Na$ and $Ar_{52}$ were significantly lower at Orcajo compared to the natural populations ($p < 0.05$). Similarly, both expected and observed heterozygosity were lower in the artificial stand compared to the natural populations ($p < 0.05$). However, the Orcajo stand showed a fixation index comparable to that of the natural populations. Indeed, paired t-tests showed a non-significant difference in $F_{IS}$ between Orcajo and both natural populations ($p = 0.88$ and 0.16 respectively), indicating that reduction in heterozygosity due to inbreeding was not higher in the plantation.

4. Discussion

4.1. Growth dynamics indicates better performance of A. pinsapo

The growth dynamics of the Orcajo stand could be traced back to the late 1920s through dendrochronological analysis, thereby pointing to the decade of 1910 as the most likely period of establishment, as previously suggested (Pérez-Soba Díez del Corral, 2010). A. pinsapo showed a growth rate comparable or slightly higher than that observed for natural populations of the species (Linares et al., 2009c; Linares et al., 2010). This
finding is consistent with the scarce available information reporting good performance of this endangered fir outside of its distribution range under ecologically related conditions (i.e., south-eastern France; Morel, 1983). Interestingly, precipitation is considerably less abundant at Orcajo than across the natural distribution of A. pinsapo (Pérez Latorre and Cabezudo, 2012). The high growth of this species at Orcajo is likely related to the homogenous distribution of precipitation occurring throughout the year in the planted stand which, together with the abundance of horizontal precipitation, may mitigate drought stress in summer. In this regard, a growth decrease was observed since 1990. This reduction is consistent with that reported in recent decades at the lower altitudinal limit of the species’ distribution range (Linares et al., 2009b; Linares et al., 2011). Although this suggests a negative effect of increasing summer drought, the higher growth rate of A. pinsapo compared to both pine species has been maintained starting from the 1940s until present. Taking into account that pine growth is comparable to that observed in nearby stands (Shestakova et al., 2017), we speculate that pinsapo fir can show excellent growth outside of its native range, even in mountain areas subjected to chronic summer drought. However, it must be stressed that our inferences are based solely on the analysis of a single stand, and that the good growth potential of A. pinsapo in areas outside its original distribution needs further confirmation under additional ecological conditions.

4.2. Trends in $\Delta^{13}C$ and WUE$_i$ suggest increased vulnerability to warming

The low WUE$_i$ of A. pinsapo is likely related to the high stomatal conductance of shade-tolerant species such as firs (Serichol-Escobar et al., 2016), especially if compared to heliophilous pines (Maier et al., 2004). Irrespective of tree species, however, the combined effects of increased atmospheric CO$_2$ concentration and an active physiological response to water shortage produced large increases in WUE$_i$ (ca. 25%) in the last five
decades. These increases mainly occurred between 1965 and 1990. During this period, a consistent Δ13C decrease through time indicates drought-induced stomatal closure as main contributing factor to rising WUEi across species (Gessler et al., 2014). Although similar responses have been occasionally observed in the western Mediterranean, e.g. P. sylvestris (Hereş et al., 2014) or P. halepensis (Choury et al., 2017), such a strong increasing rate in WUEi is infrequent and has rarely been reported in literature for European forests (Saurer et al., 2004). Instead, proportional regulation of photosynthesis and stomatal conductance (i.e. constant ratio of internal to external CO2 concentration) is preponderant in temperate and mountain forests, denoting a preferential set point for gas exchange (Saurer et al., 2014). The distinct performance of the densely planted Orcajo stand suggests that, as trees grew older, exacerbated competition for water was forcing these trees to adopt an extreme water saving strategy.

However, the speed of WUEi increase dwindled (P. nigra, P. sylvestris) or even ceased (A. pinsapo) since the early 1990s, and this response was coupled with a slowdown in radial growth. In dry environments, a response to increased atmospheric CO2 concentration changing from active to passive (as in the case of A. pinsapo) is often interpreted as the progressive attainment of a physiological threshold in the tree’s capacity to increase WUEi through stomatal closure in response to water scarcity (Linares and Camarero, 2012; Choury et al., 2017). This passive response has been recognised as an early die-back signal in A. alba (Linares and Camarero, 2012) and P. sylvestris (Hereş et al., 2014) in the Iberian Peninsula. Altogether, our findings anticipate the future vulnerability of A. pinsapo if temperatures continue to increase, a concern that has been already raised for natural populations of the species (Arista et al., 2011; Linares et al., 2011) and that calls the suitability of the stand for conservation purposes into question.
4.3 Plasticity of climate responses of A. pinsapo compared to the pine species

As observed in other studies (e.g. Shestakova et al., 2019), the positive relationship between TRW$_i$ and $\Delta^{13}$C$_i$ corroborates that drought stress is a key common factor influencing leaf physiology and growth in the stand. Negative relations of TRW$_i$ with June temperature and positive associations with winter-spring precipitation for the three species indicated a general negative impact of water deficit on xylogenesis. Unlike in pines, however, the influence of short- (SPEI1) and long-term winter drought (SPEI6) on TRW$_i$ was less important in A. pinsapo, which suggests a less plastic cambial phenology for the pinsapo fir (Pérez Latorre and Cabezudo, 2012). The response of $\Delta^{13}$C$_i$ to climate indicates low reliance on summer precipitation in the case of A. pinsapo, as reported for natural populations of this species (Linares et al., 2011), being $\Delta^{13}$C$_i$ more dependent on winter precipitation. Moreover, the lack of climate responses observed during July-September for TRW$_i$ and $\Delta^{13}$C$_i$ across species suggests summer cambium quiescence, as already observed in natural populations of A. pinsapo (Linares et al., 2009a), P. nigra and P. sylvestris (Camarero et al., 2010) in the Iberian Peninsula.

The recent rise in temperatures and associated drought effects modified tree responses to climate. An increase in competition for water and resources due to the increase in total basal area over time and the presence of abundant regeneration of A. pinsapo likely modulated these responses (Linares et al., 2010). Specifically, during the 21st century there was increased growth dependence on water availability in the early growing season, including the late winter months during which A. pinsapo shows photosynthetic activity (Sánchez-Salguero et al., 2015). Changes in the climate dependence of $\Delta^{13}$C$_i$ were also observed in recent decades, although these were evident only for A. pinsapo (SPEI3). This result suggests higher plasticity of A. pinsapo for gas exchange during favourable periods of water availability, which currently appear to be restricted to late winter-spring
(Sancho-Knapik et al., 2014). This plasticity was also reflected in a progressive loss of \( \Delta^{13}C \) signal during summer, as indicated by moving correlations between \( \Delta^{13}C \) and climatic conditions. Such temporal tuning of leaf gas exchange might be a direct consequence of \( A. \) pinsapo recently reaching a threshold in its ability to increase WUE.

4.4 Limited genetic diversity of \( A. \) pinsapo in the stand

The good performance demonstrated by \( A. \) pinsapo in terms of growth, physiological plasticity and natural regeneration may justify the interest of the Orcajo plantation as \textit{ex situ} conservation unit (Rotach, 2005). However, the genetic analysis revealed a genetic diversity quantifiable as about half the diversity observed for two natural populations of the species (Dering et al., 2014). Less genetic diversity in old-planted forests is expected due to the restricted number of trees typically used in early reforestation programs as seed source, which likely did not cover the entire genetic diversity of the population of origin (Lefèvre, 2004). For this reason, if additional efforts are undertaken aimed at increasing the current underrepresentation of the species’ genetic diversity, this might warrant the preservation of this stand for conservation purposes. Since \( A. \) pinsapo shows low inter-population genetic differentiation (Dering et al., 2014; Cobo-Simón et al., 2020), a more representative sample of the existing genetic diversity could be collected at origin with relative ease and planted in the stand.

However, prior to the introduction of new individuals, a more accurate monitoring of the stand’s genetic dynamics and of the adaptively relevant genetic diversity should be carried out using a set of more informative markers. A higher number of markers would allow determining the precise origin of the planted trees, which is important for planning management strategies of old-planted stands for conservation purposes (Santini et al. 2018; Mataruga et al., 2020). Moreover, the inclusion of the offspring in such analysis
would enable to determine the approximate number of adult trees contributing to natural regeneration and to monitor potential losses of genetic diversity between adult individuals and their offspring (Kavaliauskas et al, 2018).

4.5 Implications for future performance and management recommendations

Despite the relatively low genetic diversity of *A. pinsapo* at Orcajo, our study has revealed good performance of the species over most of the 20th century, indicating the potential of the planted stand as *ex situ* conservation unit. However, it also suggests poor prospects for the resilience and future development of the stand, where the increase in competition among individuals, coupled with the ongoing rise in temperatures, is affecting growth and physiology of pinsapo fir. Unfortunately, this phenomenon has also been observed in natural populations of the species (Linares et al., 2009c; Linares et al., 2010). Management actions are urgently needed given the stand’s protected status. In this regard, low intensity thinning (e.g. 30%-50% removal of basal area) may stimulate radial growth of adult *A. pinsapo* individuals (Lechuga et al., 2018). This effect is related to reduced competition and increased water availability, which positively affect photosynthesis and tree vigour. A possible intervention could involve the complete removal of *P. sylvestris* individuals, the species showing the poorest growth performance at Orcajo, resulting in a basal area reduction of about 25-30%, without affecting the density and distribution of the pinsapo fir to avoid declines in genetic diversity and undesirable effects on seed production (Arista and Talavera, 1996). The conversion to a mixed forest of *A. pinsapo* and *P. nigra* could favour the resilience of adult individuals and the natural regeneration of *A. pinsapo*. Combined with the introduction of new individuals (i.e. enrichment planting), this strategy can promote natural stand dynamics and, simultaneously, preserve or increase its genetic diversity (Kavaliauskas et al, 2018).
5. Conclusions

We retrospectively characterised radial growth and gas exchange physiology of *A. pinsapo* in a rare example of old reforestation involving a threatened Mediterranean fir outside of its natural range. The good long-term performance of the species is in favour of the use of the Orcajo stand as *ex situ* conservation unit of *A. pinsapo*, but the recent increasing negative effects of temperature rise and associated drought stress require urgent silvicultural interventions to secure stand’s resilience. In addition, measures aimed to increase the limited genetic diversity of *A. pinsapo* at Orcajo are needed (Koskela et al., 2013; Fady et al., 2016). This study suggests the feasibility to carry out *ex situ* conservation efforts in *A. pinsapo* well away of their endangered natural habitats. Although our study included only one stand and any generalization to other plantations of endangered forest species must be taken carefully, similar conservation approaches could possibly be applied to other threatened, taxonomically-close Mediterranean firs such as *A. maroccana*, *A. tazaotana* (Morocco) or *A. numidica* (Algeria), as is currently done in *A. nebrodensis* (Ducci, 2014).

Recent studies have stressed the potential of coupling genetic and dendrological data at the individual level to investigate adaptation and population health in the context of climate change (Heer et al., 2018, Avanzi et al., 2020). In this regard, our results highlight the interest of combining dendroecological and genetic approaches for understanding the status and management options of forest tree species for conservation purposes.

6. Acknowledgements

We are grateful to Maria Josep Pau for technical assistance.
7. Funding

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Scots pine in continental Mediterranean climates: Drought, low temperatures or both?

Agricultural and Forest Meteorology 206, 151–162.


### Table 1. Structural and dendrochronological characteristics of the sampled stand at Orcajo.

<table>
<thead>
<tr>
<th>Tree species</th>
<th>Basal area (m² ha⁻¹)</th>
<th>DBH (cm)</th>
<th>Height (m)</th>
<th>No. trees</th>
<th>Age at coring height (years)</th>
<th>TRW Mean (mm)</th>
<th>EPS &gt;0.85 since</th>
<th>Rbar</th>
<th>Δ¹³C Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Abies pinsapo</em></td>
<td>12.2</td>
<td>36.3 ± 1.2</td>
<td>14.3 ± 0.4</td>
<td>23</td>
<td>72 ± 0.5</td>
<td>1.92 ± 0.02</td>
<td>1941</td>
<td>0.71</td>
<td>18.06 ± 0.10</td>
</tr>
<tr>
<td><em>Pinus nigra</em></td>
<td>7.6</td>
<td>29.7 ± 1.2</td>
<td>12.3 ± 0.4</td>
<td>21</td>
<td>77 ± 0.4</td>
<td>1.35 ± 0.02</td>
<td>1938</td>
<td>0.60</td>
<td>16.90 ± 0.10</td>
</tr>
<tr>
<td><em>Pinus sylvestris</em></td>
<td>10.8</td>
<td>26.8 ± 1.2</td>
<td>10.5 ± 0.4</td>
<td>19</td>
<td>80 ± 0.4</td>
<td>1.25 ± 0.01</td>
<td>1935</td>
<td>0.61</td>
<td>17.19 ± 0.10</td>
</tr>
</tbody>
</table>

DBH, diameter at breast height (1.30 m); EPS, expressed population signal; TRW, tree-ring width; Δ¹³C, carbon isotope discrimination; Rbar, mean inter-series correlation. The variability of mean values is expressed as standard error (±SE).
Table 2. Genetic parameters (mean±SE across loci) of *A. pinsapo* estimated for planted stand at Orcajo and for two natural populations (Sierra de Zafalgar and Serranía de Ronda) of the species characterized by Dering et al. (2014).

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>Na</th>
<th>Ars2</th>
<th>H₀</th>
<th>Hₑ</th>
<th>Fₛₛ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orcajo (planted stand)</td>
<td>44</td>
<td>4.20±1.56</td>
<td>3.64±1.16</td>
<td>0.31±0.11</td>
<td>0.34±0.13</td>
<td>0.05±0.06</td>
</tr>
<tr>
<td>Sierra de Zafalgar</td>
<td>29</td>
<td>8.00±1.92</td>
<td>7.81±1.63</td>
<td>0.58±0.10</td>
<td>0.64±0.08</td>
<td>0.12±0.09</td>
</tr>
<tr>
<td>Serranía de Ronda</td>
<td>28</td>
<td>7.80±1.93</td>
<td>7.58±1.68</td>
<td>0.55±0.09</td>
<td>0.63±0.08</td>
<td>0.13±0.07</td>
</tr>
</tbody>
</table>

N, sampling size; Na, mean number of alleles per locus; Ars2, allelic richness; H₀, observed heterozygosity; Hₑ, expected heterozygosity under Hardy-Weinberg equilibrium; Fₛₛ fixation index, calculated as 1-H₀/Hₑ.
Figure 1
Figure 2
Figure 3

(a) 

\[ \Delta^{13}C (\text{‰}) \]

\[ R^2_{adj} = 0.57 \]
\[ R^2_{adj} = 0.53 \]
\[ R^2_{adj} = 0.36 \]

(b) 

\[ \text{WUE}_i (\mu\text{mol m}^{-2}) \]

\[ \% \text{ change (WUE)} \]

Figure 5
Figure 6

(a) TRW$_i$
- Temperature
- Precipitation
- SPEI1
- SPEI3
- SPEI6

(b) $\Delta^{13}C_i$
- Temperature
- Precipitation
- SPEI1
- SPEI3
- SPEI6

Month
Figure 7
Fig. 1. (a) Geographical location of the planted stand “Pinsapar de Orcajo” and current natural distribution of the species (source: Distribution of *Abies pinsapo*; EUFORGEN, 2011), which comprises ca. 8,000 ha (in about 60% of this territory the species has a token presence of <5% of total trees). A climograph (inset) is also shown from the nearest meteorological station (period 1920-2017), and the meteorological records were corrected for stand altitude according to Gandullo (1994). (b) Annual trends in climate data for the study area (period 1920-2017): mean temperature (top), precipitation (middle) and one-month Standardized Precipitation Evapotranspiration Index (SPEI1) (bottom). Climate data were derived from the nearest meteorological station at Daroca (Zaragoza province). Annual trends of temperature and precipitation (black lines) were smoothed by LOESS fitting (span = 0.5). The horizontal line for temperature and precipitation indicates the average value of the historical series.

Fig. 2. Evolution of mean basal area increments (BAI) for (a) *A. pinsapo*, (b) *P. nigra* and (c) *P. sylvestris*. The sampling size for each chronology is indicated by a dark grey line (secondary Y-axis). The dashed vertical line indicates the starting year (1990) of consistent above-average mean annual temperatures according to Figure 1b.

Fig. 3. (a) Carbon isotope discrimination ($\Delta^{13}$C) time series for the period of 1965-2017. The relevant linear trends are highlighted according to the best broken-line regression model (see also Adjusted $R^2$ values in Table A.1), and the breakpoint year is indicated with a vertical line for each species. (b) Temporal absolute trends in intrinsic water-use efficiency (WUE$_i$) and percentage of change with regard to 1965-1970 (secondary y-axis;
the foreground red colour refers to *A. pinsapo*). In both panels, the dashed vertical lines indicate the starting year (1990) of consistent above-average mean annual temperatures according to Figure 2a. *A. pinsapo* (red), *P. nigra* (blue) and *P. sylvestris* (green).

**Fig. 4.** Relationships between BAI and intrinsic water-use efficiency (WUE$_i$) for (a) *A. pinsapo*, (b) *P. nigra* and (c) *P. sylvestris*. Significant correlations between BAI and WUE$_i$ for the period following the breakpoint year (or year of change in slope of $\Delta^{13}$C; Table A.1) are indicated in the case of *P. nigra* and *P. sylvestris*. Years corresponding to the period following the breakpoint year (1992 for *A. pinsapo*, 1995 for *P. nigra* and 1993 for *P. sylvestris*) are identified with coloured dots, while the remaining years are identified with white dots. The horizontal lines indicate the mean BAI for the study period and each species.

**Fig. 5.** *Upper panels.* Regional chronologies for tree-ring width (TRW$_i$; black line) and carbon isotope discrimination ($\Delta^{13}$C$_i$) indices for (a) *A. pinsapo*, (b) *P. nigra* and (c) *P. sylvestris*. *Lower panels.* Relationships between TRW$_i$ and $\Delta^{13}$C$_i$ records for the period 1965-2017 for (d) *A. pinsapo*, (e) *P. nigra* and (f) *P. sylvestris*.

**Fig. 6.** (a) TRW$_i$ and (b) $\Delta^{13}$C$_i$ responses to climate at the species level: *A. pinsapo* (red bars), *P. nigra* (blue bars) and *P. sylvestris* (green bars). Tree ring trait relationships with climate are based on bootstrapped correlations for the period 1965-2017. Significant correlations are shown with filled bars. Lowercase and uppercase letters in the $x$-axes correspond to months of the years before and during tree-ring formation, respectively.
Fig. 7. Moving correlations (30-year periods lagged by one year) between (a) TRW$_i$ or (b) $\Delta^{13}$C$_i$ of *A. pinsapo* and monthly mean temperature (top), precipitation (middle) and three-month drought index SPEI3 (bottom) (period 1965-2017). Asterisks denote significant correlations ($n = 30$ years; $p < 0.05$). Lowercase and uppercase letters on the y-axes correspond to months of the year before and during tree-ring formation, respectively.
Dendroecological and genetic insights for future management of an old-planted forest of the endangered Mediterranean fir *Abies pinsapo*

Filippo Santini, Tatiana A. Shestakova, Svetlana Dashevskaia, Eduardo Notivol, Jordi Voltas

Appendix
Table A.1. Simple linear and broken-line regression models for the explanation of Δ^{13}C values as a function of time (year). Two different broken-line regression models with two segments were applied accounting for either an increasing slope $b_2$ (two-slope model) or a plateau with zero slope $b_2$ above the breakpoint year (single-slope model). An asymptote (A) for the first segment, which defines the lowest predicted Δ^{13}C value, is also included. The $R^2$ is the fraction of variation in the dependent variable explained by the model, and the adjusted $R^2$ adjusts for the number of parameters fitted in the model.

<table>
<thead>
<tr>
<th>Species</th>
<th>Linear regression</th>
<th>Two straight-line, one breakpoint regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$b$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Abies pinsapo</td>
<td>-0.018</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinus nigra</td>
<td>-0.027</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinus sylvestris</td>
<td>-0.025</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table A.2.** Frequencies of null alleles in each microsatellite locus for three populations of *A. pinsapo*.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Orcajo (planted stand)</th>
<th>Sierra de Zafalgar</th>
<th>Serranía de Ronda</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF_1</td>
<td>0.00</td>
<td>0.16</td>
<td>0.06</td>
</tr>
<tr>
<td>SF_b4</td>
<td>0.07</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>SF_b5</td>
<td>0.01</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>SF_78</td>
<td>0.04</td>
<td>0.00</td>
<td>0.13</td>
</tr>
<tr>
<td>SF_239</td>
<td>0.00</td>
<td>0.04</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Fig. A.1. a) General view of the planted stand; b) Mixed conifer forest with *A. pinsapo* occupying the taller canopy stratum; c) *A. pinsapo* trees with an average height of ca. 14 m; d) Detail of abundant *A. pinsapo* regeneration in the understory.
Fig. A.2 Monthly long-term linear changes in mean temperature (top), precipitation (middle) and one-month Standardized Precipitation Evapotranspiration Index (SPEI1) (bottom) for the period 1920-2017. Bars represent slopes of linear regressions of monthly climate factors as a function of time. Filled bars indicate significant linear trends over time ($p < 0.05$). Climate data were derived from the nearest meteorological station at Daroca (Zaragoza province).
Fig. A.3. Evolution of the accumulated mean tree basal area for each species present in the planted stand estimated from the sampled trees. The vertical line indicates the starting year (1990) of consistent above-average mean annual temperatures according to Figure 1b.
Fig. A.4. Moving correlations (30-year periods lagged by one year) between (a) TRW$_i$ or (b) $\Delta^{13}C_i$ of *Pinus nigra* and monthly mean temperature (top), precipitation (middle) and three-month drought index SPEI3 (bottom) (period 1965-2017). Asterisks denote significant correlations ($n = 30$ years; $p < 0.05$). Lowercase and uppercase letters on the y-axes correspond to months of the year before and during tree ring formation, respectively.
Fig. A.5. Moving correlations (30-year periods lagged by one year) between (a) TRW$_i$ or (b) $\Delta^{13}C_i$ of *Pinus sylvestris* and monthly mean temperature (top), precipitation (middle) and three-month drought index SPEI3 (bottom) (period 1965-2017). Asterisks denote significant correlations ($n = 30$ years; $p < 0.05$). Lowercase and uppercase letters on the y-axes correspond to months of the year before and during tree ring formation, respectively.
Fig. A.6. Mean values and standard error of number of alleles per locus ($Na$), allelic richness ($Ar_{S2}$), expected ($H_e$) and observed heterozygosity ($H_o$), and fixation index ($F_{IS}$) across loci in Orcajo plantation and in two natural populations of *A. pinsapo* (Sierra de Zafalgar y Serranía de Ronda). Different letters indicate significant differences according to paired $t$-tests.
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: