



Tree-ring isotopic composition reveals intraspecific variation in water use efficiency of *Pinus pinaster* Ait. provenances grown in common gardens

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Abstract

Key message The physiological responses expressed by variation in carbon and oxygen stable isotopes and iWUE in five provenances of maritime pine grown in four common gardens were primarily determined by genotype differences in phenotypic plasticity and secondarily by genotype.

Abstract Given the impacts of climate change on forest resources and considering the slowness of evolutionary processes in trees, a need arises to understand the interplay between tree species adaptation to climate, genetic variation, and their impact on tree growth and productivity. Broadening knowledge of the capacity of tree populations to respond to climate-related disturbances is a prerequisite for the development of resilience strategies, including assisted migration and climate-smart forestry. This study tests the physiological ability of different maritime pine provenances, comparing Mediterranean (Corsica, Sardinia, and Tuscany) and Atlantic (Portugal) provenances, to adapt to progressively drier conditions that have occurred in the last thirty years. Four provenance trials with randomized blocks of the five maritime pine provenances were used as test sites in Sardinia (Italy). Wood cores were collected from the 40-year-old plants. Cores were split into five-year segments to determine provenance-related variations in carbon and oxygen stable isotopes and provide information on long-term patterns in intrinsic water use efficiency (iWUE). The provenance × site interaction was the most important source of variation, meaning that the genotypes responded differently to the planting sites. Considering the main effects, both genotype and environmental conditions at the planting sites influenced stable isotope composition in tree rings. This suggests that iWUE was determined by phenotypic plasticity that differed among genotypes. In contrast, provenance responses were stable with time, and the provenance × site interaction was stable across time periods. These findings suggest that provenance selection to improve iWUE in maritime pine may need to consider site conditions but point more to soil conditions than to climate. In any case, they limit our ability to recommend maritime pine provenances based on iWUE until the missing site factors can be identified.

Keywords Climate change · Mediterranean pines · Natural disturbances · Provenance trials · Tree rings

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Introduction

Climate change and related disturbance events are expected to have negative effects on forest functions and services by causing a mismatch with the environmental conditions to which individual trees and populations are adapted (Isaac-Renton et al. 2014). The ability of trees to withstand climate-related stressors, such as drought, and maintain their functions determines the overall forest stability. However, the adaptation capacity and phenotypic plasticity may vary among populations across the species distribution ranges and buffer the negative impacts of environmental disturbances on functional traits (Li et al. 2017). Although trees may

physiologically and morphologically respond to changing environmental conditions, the speed and intensity of these changes may exceed the competitive ability and growth capacity of many species under climate change, eventually leading to shifts in species distribution and loss (or migration) of local populations (Aitken et al. 2008). Therefore, unravelling adaptive patterns and plastic responses to environmental conditions and careful selection of seed sources may contribute to maintaining healthy and productive trees and forests in a changing climate (Vázquez-González et al. 2020).

Pinus pinaster Ait. (maritime pine) is a tree species with high economic and ecological importance in south-western Europe and the Mediterranean region, an area characterised by recurrent drought events that are predicted to increase in frequency and intensity in the near future (Spinoni et al. 2017). Maritime pine has been traditionally used for timber and turpentine production, but other main uses of the species are related to recreation and soil protection. In sand dune areas, maritime pine plays an important ecological role in protecting habitats from salty winds and marine aerosols (Mazza et al. 2014). Drier and warmer conditions in the distribution of maritime pine may increase tree mortality due to vascular damage and hydraulic failure and/or through depletion of internal carbon reserves (McDowell et al. 2008), threatening the provision of ecosystem services. Under declining soil water reserves, a pronounced reduction of stomatal conductance is expected for this isohydric species (Picon et al. 1996). Previous studies on maritime pine examined intraspecific differences in water-use behaviours (e.g., Aranda et al. 2010; Corcuera et al. 2010; de Miguel et al. 2012; Sánchez-Gómez et al. 2010). Populations from drier climates often exhibit conservative growth strategies, such as larger biomass allocation to roots (Corcuera et al. 2012) and slower height or needle growth (de la Mata et al. 2014). Furthermore, higher water-use efficiency (Correia et al. 2008) and higher osmotic adjustment (Nguyen-Queyrens and Bouchet-Lannat 2003) were found in populations from dry climates in comparison with those from mild climates. Magnani et al. (2008), found a negative relationship between leaf-specific hydraulic conductance and tree height in maritime pine stands, suggesting a reduction in stomatal conductance and a role of hydraulic constraints in the decline in annual growth.

As maritime pine trees grow taller, $\delta^{13}\text{C}$ should increase due to a combination of greater light intensity increasing assimilation rates and lower water potential at greater evaporative demand, inducing stomatal closure, in turn reducing stomatal conductance (Delzon et al. 2004). Factors that affect CO_2 supply by changing stomatal conductance also influence tree-ring $\delta^{13}\text{C}$ values, while $\delta^{18}\text{O}$ variation in tree rings tracks water sources and leaf evaporative conditions (Roden and Siegwolf 2012). In the dual-isotope model, $\delta^{18}\text{O}$

variation is not affected by photosynthetic rates and, therefore, can be used to infer the effects of stomatal conductance on $\delta^{13}\text{C}$ (Scheidegger et al. 2000). However, additional factors may influence evaporative enrichment at leaf level and modify isotope signature in tree rings, which make these relationships complex to interpret. Regardless of the dual-isotope approach limitations and though $\delta^{18}\text{O}$ cannot be directly related to changes in stomatal conductance (Roden et al. 2022), plotting $\delta^{18}\text{O}$ versus $\delta^{13}\text{C}$ can still be useful to make differences across different genotypes emerge, when trees growing with similar access to source water diverge in $\delta^{13}\text{C}$ and, thus, iWUE. Indeed, iWUE provides information on the ratio of CO_2 assimilation to stomatal conductance, remaining relatively vague about which of the two changes and to what extent (Saurer and Voelker 2022); still, different seed sources may exhibit different degrees of coupling between iWUE and $\Delta^{18}\text{O}$, depending on provenance-specific sensitivity of stomatal behaviour in response to variation in water availability. Brendel et al. (2002) observed that, in maritime pine, the significant phenotypic correlation between $\delta^{13}\text{C}$ and tree ring width was not determined by the genetic component but was attributable to the environment.

In maritime pine, wide differentiation in secondary growth and survival occurs across the natural range of this species (Harfouche et al. 1995). Indeed, populations vary in adaptive functional traits (de la Mata et al. 2012), which are associated with growth responses to drought (Rozas et al. 2011). In addition to the assessment of ‘traditional’ growth traits, such as radial or basal area increment, or tree height, stable isotope ratios in tree ring cellulose or whole wood are increasingly used to understand ecophysiological processes and their response to changing environmental conditions (e.g., Marshall and Monserud 1996; McCarroll and Loader 2004; Saurer et al. 1997; Treydte et al. 2001, 2007). In this context, common garden experiments, or provenance trials, in which conifer trees of the same species originating from different geographical areas are grown in test sites each having uniform environmental conditions (Evans et al. 2018; Tognetti et al. 2000; Zhang and Marshall 1994, 1995), may provide insights on intraspecific variation and interannual patterns in water-use strategies and tree growth (Jansen et al. 2013; Suvanto et al. 2016; Taeger et al. 2013).

This study tests the physiological ability of different maritime pine provenances to acclimate to drought conditions. We focus on five provenances (one each for Portugal, Corsica, and Tuscany, and two Sardinian, i.e., Telti and Limbara) planted in four provenance trials that were started in the early 1980s in Sardinia (Italy). Geographic variation in monoterpene composition, stem diameter growth, and carbon isotope discrimination ($\Delta^{13}\text{C}$) were assessed in the same provenance trials when plants were 16 years old (Tognetti et al. 2000). The Portuguese provenance, which showed the highest potential for growth amongst the five

seed sources, had a distinct terpene pattern and a tendency for lower $\Delta^{13}\text{C}$ in needles (higher intrinsic water-use efficiency; iWUE) than the other provenances. Our objective was to determine whether physiological responses (variation in carbon and oxygen isotopes in tree rings, providing information on temporal patterns in meteorological conditions and physiological attributes, namely water-use efficiency) have followed different trends among the provenances as stands have aged and grown taller. We first hypothesized that differential sensitivity might imprint on stable isotopes in contrasting ways, depending on different selection pressures at the sites of origin, namely determined by variation in water availability, resulting in differences in water-use efficiency among provenances. We also expected a stronger association of the relationship between iWUE ($\Delta^{13}\text{C}$) and $\Delta^{18}\text{O}$ ($\delta^{18}\text{O}$) in Mediterranean provenances in comparison with the Atlantic seed source of this isohydric forest tree species.

Materials and methods

Study area and climate setting

Maritime pine has a fragmented distribution, from which the isolated populations exhibit genotype by environment interactions for growth performance and disturbance susceptibility when brought to a common planting site (Caminero et al. 2018; Di Matteo and Voltas 2016). This study was conducted in four provenance trials in Sardinia (Italy), each

comprising five provenances of maritime pine. Two-year-old seedlings, identified as the Corsica, Limbara (Sardinia), Portugal, Telti (Sardinia), and Tuscany populations, were planted in 1981 at four trial sites (Montarbu, Montes, Uatzo, and Usinavà). Seedlings were transplanted at 2.5-m spacing; at each site, singular provenances were assigned to 25-tree square plots, which were randomized within five blocks, except in Uatzo where the replicates were three (see Tognetti et al. 2000). Characteristics of the trial sites are reported in Table 1. Additional information on soil traits and environmental conditions at the trial sites is reported in Giannini et al. (1992; Table 2) and in Lisella et al. (2022; Table 1). In synthesis, soil texture is generally loam, only in Usinavà the texture is sandy loam, while fertility is higher in Montes and lower in Usinavà, with Uatzo and Montarbu in between the two extremes. Understorey vegetation consists of sparse and low evergreen sclerophyllous shrubs.

Sardinia is an island with a typical Mediterranean climate characterized by mild and wet winter and hot and dry summer (Canu et al. 2015). Daily climate records of precipitation and minimum and maximum temperatures for each site were obtained from the Agenzia Regionale per la Protezione dell'Ambiente della Sardegna (ARPAS), available at the site (www.sardegnaambiente.it/index.php). Temperature and precipitation were averaged every five years. Moreover, the seasonal minimum and maximum temperatures and cumulative precipitation were calculated. Winter was defined as December of the previous year to February of the current year; spring was from March to May of the current year; summer, from June to August of the current year; autumn,

Table 1 Environmental setting of the trial sites and main stand characteristics

Site	Latitude N	Longitude E	Altitude (m a.s.l.)	Tree density (n/ha)	DBH (SD) (cm)	Height (SD) (m)	BAI (SD) ($\text{mm}^2 \text{y}^{-1}$)
Montarbu	39° 54' 25"	9° 23' 13"	900	1239	29.45 (± 3.59)	14.80 (± 1.81)	2160.75 (± 719.31)
Montes	40° 07' 48"	9° 22' 37"	985	748	31.71 (± 4.71)	17.24 (± 2.47)	2596.44 (± 624.58)
Uatzo	39° 58' 49"	9° 08' 34"	770	1109	31.50 (± 2.83)	18.86 (± 1.75)	2235.63 (± 1025.08)
Usinavà	40° 41' 40"	9° 34' 48"	750	696	31.58 (± 5.87)	15.43 (± 2.02)	2259.78 (± 540.99)

DBH diameter at breast height (1.30 m), *Height* total tree height, *BAI* basal area increment (mean for the whole lifespan of trees), *SD* in brackets standard deviation

Table 2 Main climate characteristics, reported as annual mean for the period, of the trial sites in the period 1988–2017

Site	Annual precipitation (mm)	Min temperature ($^{\circ}\text{C}$)	Max temperature ($^{\circ}\text{C}$)	De Martonne Aridity Index
Montarbu	843	7.39	18.6	36.7 (35.0 \leq 55.0 very humid) a
Montes	921	8.04	17	41 (35.0 \leq 55.0 very humid) a
Uatzo	827	9.56	18.2	34.6 (28.0 \leq 35.0 humid) a
Usinavà	671	11.5	23.1	24.5 (24.0 \leq 28 semi-humid) b

Type of climate according to the De Martonne Aridity Index (adapted after Pellicone et al. 2019). Results of multi-comparison tests are reported: different letters indicate a difference in the Aridity Index

from September to November of the current year. Among the trial sites, total annual precipitation (671 mm) was the lowest and the average annual temperature was highest (17.3 °C) in Usinavà (Table 2). At the other extreme, annual precipitation (921 mm) was highest and the average annual temperature (12.5 °C) was lowest at Montes. The De Martonne Aridity Index, which decreases with aridity, showed the highest value in Montes and the lowest value in Usinavà (Table 2). Multiple *t* tests showed differences among sites for the climatic variables (Table 2); in summary, Usinavà was clearly more arid than the other sites. These values were classified from very humid to semi-humid, following De Martonne (1926; Pellicone et al. 2019). Minimum and, particularly, maximum temperatures increased during the period 1988–2017 (Fig. 1). Annual precipitation showed a slight increase with variation in the same period.

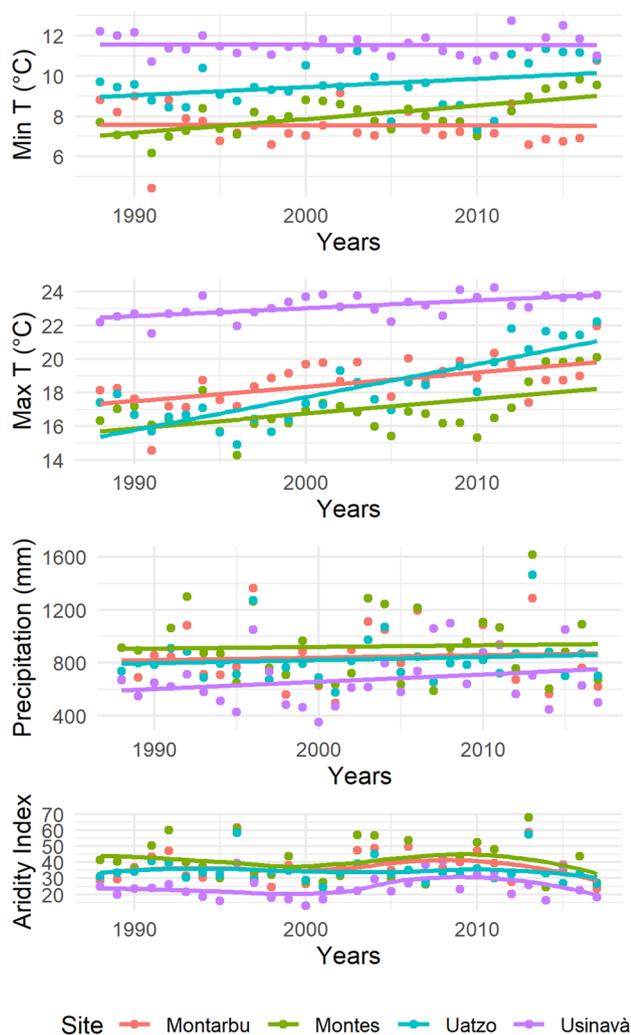


Fig. 1 Temporal variation of minimum temperature (Min T), maximum temperature (Max T), total precipitation, and De Martonne Aridity Index at the study sites from 1988 to 2017

The Aridity Index was the least variable during 1988–2002 and decreased in the last five years (2013–2017) (Fig. 1). Geographic locations of the seed sources are reported in Giannini et al. (Table 1; 1992); seeds were collected from at least 50 plants at least 100 m from each other, in autochthonous stands. The Portuguese seed source has Atlantic influences, Sardinia and Corsica have a Mediterranean climate characterized by hot and dry summers and mild and wet winters, and Tuscany is affected by westerlies. Indeed, total annual precipitation at the sites of origin ranges from 653 mm in both Sardinia sites to 953 mm in Portugal, while annual mean temperature is the lowest in Corsica and the highest in Portugal (data obtained by the annual mean for the period 1960–1990, as reported in Lisella et al. 2022; Table 3). The De Martonne Aridity Index at the locations of the origin of the provenances, see Lisella et al. (2022) was used as a covariate in the statistical analyses (Table 3), along with the Aridity Index of the trial site.

Tree ring and stable isotope analyses

We measured diameter at breast height (DBH) and height for all trees in the trial sites in the spring of 2018 and 2019. Additionally, 15 increment cores were collected from healthy trees for each provenance in each site. Core sampling and processing were performed using standard dendrochronological techniques (Speer 2010). Tree ring width was converted into tree basal area increment (BAI) using the function `bai.out` in the `dplR` package in R (Bunn et al. 2022). This function converts ring-width series (mm) to ring-area series (basal area increments) based on the diameter of the tree and the width of each ring moving towards the pith of the tree. This method was developed according to Biondi (1999) and Biondi and Qeadan (2008).

Five cores for each provenance in each site were selected for isotope analyses. This number of samples is generally considered sufficient for isotope studies (Leavitt 2010). Each core was split into pentads (combining five rings) with a scalpel under a binocular microscope, yielding six groups that cover the last 30 years (1988–2017). Once the tree rings were separated, the material was homogenised with a ball mill (Retsch MM400, Germany), dried at 70 °C in an oven,

Table 3 The De Martonne aridity index classification at the locations of origin of the provenances (cf. Lisella et al. 2022; De Martonne 1926)

Site of origin	De Martonne Aridity index value	Classification
Corsica	35.38	Very Humid (VH)
Portugal	37.35	Very Humid (VH)
Telti & Limbara	28.21	Humid (H)
Tuscany	36.13	Very Humid (VH)

and used for analysis in the elemental analyser (Flash 2000, Thermo-Scientific) and its coupled isotope ratio mass spectrometer (Delta V Advantage, Thermo-Scientific). Results are expressed as per-mil notation (δ , ‰), relative to the international standards V-PDB (Vienna-PeeDee Formation Belemnite), and V-SMOW (Vienna-Standard Mean Ocean Water) for $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, respectively.

From $\delta^{13}\text{C}$ of tree ring samples ($\delta^{13}\text{C}_{\text{plant}}$), the photosynthetic carbon isotope fractionation ($\Delta^{13}\text{C}$) was calculated, according to the Farquhar equation (Farquhar et al. 1982):

$$\Delta^{13}\text{C} = a + (b - a)C_i/C_a = \frac{(\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}})}{(1 + \delta^{13}\text{C}_{\text{plant}})}$$

where C_i and C_a are the intercellular and ambient CO_2 concentration, respectively, a is the fractionation during diffusion through stomata and leaf intercellular space (4.4‰) and b is the carbon isotope discrimination during carboxylation by ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (27‰). The advantage of $\Delta^{13}\text{C}$ is that it removes variation due to $\delta^{13}\text{C}_{\text{air}}$ and provides a clearer picture of plant response. Data about the variation of $\delta^{13}\text{C}$ of tropospheric CO_2 ($\delta^{13}\text{C}_{\text{air}}$) for the whole studied period was obtained from the CU-INSTAAR/NOACMDL network for atmospheric CO_2 (<http://www.esrl.noaa.gov/gmd/>). We derived C_i from the following equation:

$$C_i = C_a \frac{\delta^{13}\text{C}_{\text{plant}} - \delta^{13}\text{C}_{\text{air}} + a}{a - b}$$

We estimated iWUE as (cf. Saurer and Voelker 2022):

$$\text{iWUE} = (C_a - C_i)/1.6 = C_a \frac{(b - \Delta^{13}\text{C})}{1.6(b - a)}$$

The factor 1.6 corresponds to the ratio of molecular diffusion coefficient for water relative to CO_2 .

Similarly, Barbour (2007) and Barbour et al. (2004); suggested that the interpretation of $\delta^{18}\text{O}_{\text{plant}}$ can be simplified by removing spatial and temporal variation in source water $\delta^{18}\text{O}_{\text{precipitation}}$. This enables the identification of variation owing to leaf water enrichment and isotopic exchange. Variation in the isotope composition of source water may be removed from $\delta^{18}\text{O}_{\text{plant}}$ by presenting the composition as an enrichment above source water ($\Delta^{18}\text{O}_{\text{plant}}$). Thus, $\Delta^{18}\text{O}_{\text{plant}}$ was calculated from:

$$\Delta^{18}\text{O} = \frac{(\delta^{18}\text{O}_{\text{precipitation}} - \delta^{18}\text{O}_{\text{plant}})}{(1 - \delta^{18}\text{O}_{\text{plant}})}$$

Data on $\delta^{18}\text{O}_{\text{precipitation}}$ was obtained from the IsoMap site (<https://isomap.rcac.purdue.edu/>). Data were then downscaled differentially for each site and year, averaging across five years, i.e., those associated with the tree rings.

Moreover, for even-aged trees grown in the same common garden, differences in source water should be minor. Stable isotope analyses were done on whole wood samples to retain the strongest climatic signals (Loader et al. 2003).

Statistical analysis

All the analyses were done in an R statistical environment (R Core Team, 2021). Normality of the variables— $\Delta^{13}\text{C}$, $\Delta^{18}\text{O}$, and iWUE—was tested using the Shapiro–Wilk test and the Levene test for homoscedasticity was performed. ANOVA test for unbalanced design (Uatzo had a different number of samples), was used to test the effect of provenances, sites, and periods (six groups of five years) on $\Delta^{13}\text{C}$, $\Delta^{18}\text{O}$, and iWUE, using ‘car’ package (Fox and Weisberg 2019). Moreover, to understand if the climate of the trial sites or at the locations of origin of the provenances characterised the response on $\Delta^{13}\text{C}$, $\Delta^{18}\text{O}$, and iWUE, the De Martonne Aridity Index was included in the ANOVA test. Tukey’s HSD test was performed for multiple comparisons among provenances, sites, and periods for $\Delta^{13}\text{C}$, $\Delta^{18}\text{O}$, and iWUE.

Isotope signals were correlated with weather data, (i.e., cumulative monthly precipitation, maximum and minimum mean temperatures) from the trial sites, using both yearly average values and seasonal values. Correlations of isotope signals with long-term averaged climate data (from 1961 to 1990) at the sites of origin of the seed sources were also determined. The statistical significance of correlations was tested using Pearson’s correlation method, through ‘rcorr’ function from the ‘Hmisc’ R package, to obtain p value (Harrell 2021).

Results

Patterns of isotopic signals within provenances and at the trial sites

Results of ANOVA showed a significant effect of provenances, sites, and periods on $\Delta^{13}\text{C}$ and iWUE, while provenances had no effect on $\Delta^{18}\text{O}$ (Table 4; Fig. 2). More relevant were the interactions. The provenance \times site (genotype \times environment) interaction was significant ($p < 0.001$) and the most important source of variation of the variables for all isotope traits (Table 4), which indicated that provenances responded differently in $\Delta^{13}\text{C}$, $\Delta^{18}\text{O}$, and iWUE among the planting sites. The interaction provenance \times period did not significantly impact the stable isotope values and iWUE (Table 4) or the interaction among the three components. Likewise, the interaction site \times period was not significant, which means that the provenance ranking did not change with time periods. Thus, provenances responded individually

Table 4 Results of ANOVA for $\Delta^{13}\text{C}$ (carbon isotope discrimination), $\Delta^{18}\text{O}$ (oxygen isotope discrimination), and iWUE (intrinsic water-use efficiency)

	Sum of square	Mean of square	Df	F value	Pr(> F)
$\Delta^{13}\text{C}$					
Provenance	4.36	1.089	4	2.682	0.0313 *
Site	13.34	4.446	3	10.949	6.37e-07***
Time period	20.40	4.080	5	10.048	4.53e-13***
Provenance:Site	37.31	3.109	12	7.656	8.30e-13 ***
Provenance:Time period	3.93	0.197	20	0.484	0.9768
Site: Time period	10.68	0.712	15	1.754	0.2813
Provenance:Site:Time period	6.55	0.109	60	0.269	0.9999
Trial site Aridity Index	5.02	1.26	4	3.092	0.0159 *
Residuals	163.65	0.406	403		
iWUE					
Provenance	441	110.3	4	2.646	0.0332 *
Site	1339	446.4	3	10.711	8.8e-07***
Time period	4950	989.9	5	23.749	<2e-16 ***
Provenance:Site	3811	317.6	12	7.620	9.8e-13***
Provenance:Time period	387	19.3	20	0.465	0.9818
Site: Time period	1035	69	15	1.655	0.3548
Provenance:Site:Time period	668	11.1	60	0.267	0.9999
Trial site Aridity Index	505	126.2	4	3.027	0.0177*
Residuals	16,798	41.7	403		
$\Delta^{18}\text{O}$					
Provenance	2.02	0.51	4	1.080	0.368
Site	208.01	69.34	3	148.256	<2e-16 ***
Time Period	235.34	47.07	5	100.642	<2e-16 ***
Provenance:Site	27.06	2.25	12	4.821	7.18e-08***
Provenance:Time period	6.02	0.30	20	0.644	0.884
Site:Time period	152.17	10.14	15	21.692	<2e-16***
Provenance:Site:Time period	11.72	0.20	60	0.418	0.9999
Trial site Aridity Index	56.63	14.16	4	30.171	<2e-16***
Residuals	188.48	0.47	403		

One, two, and three asterisks correspond to significant differences at $p < 0.05$, < 0.01 , and < 0.001 , respectively. Aridity Index refers to the trial sites; the Aridity Index at the locations of origin of the provenances was automatically excluded due to multicollinearity

to the environmental variation among sites but responded uniformly to the environmental variation over time periods.

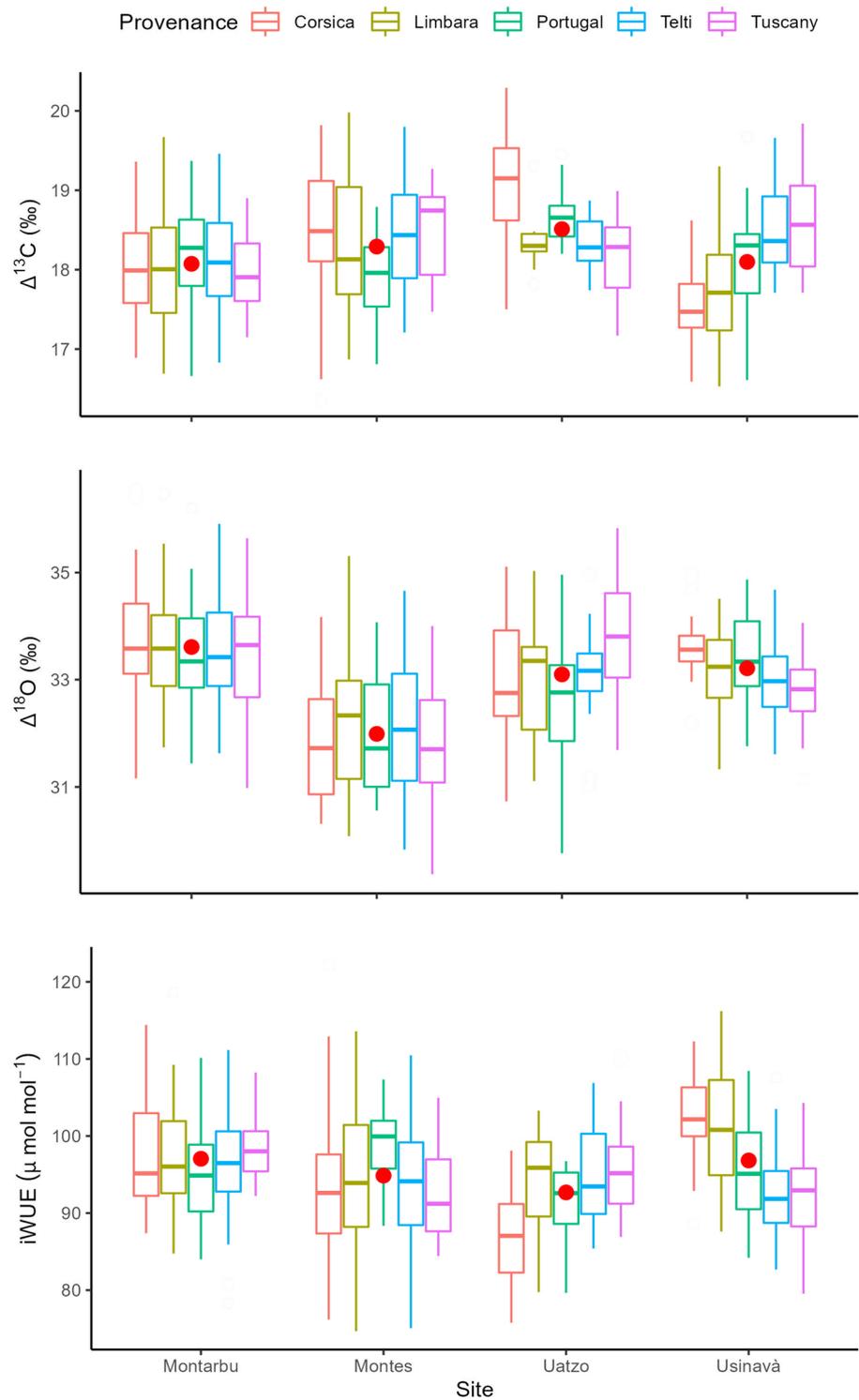
Among the tested provenances, Telti showed the highest $\Delta^{13}\text{C}$ (18.3 ‰) and Limbara the lowest (18.1‰) values, with significant differences between these two provenances ($p = 0.023$; SI_Table 2). While considering the whole period and all provenances, $\Delta^{13}\text{C}$ ranged from 18.1‰ in Usinavà and Montarbu to 18.5‰ in Uatzo (Fig. 2). $\Delta^{13}\text{C}$ in Montarbu differed significantly from Montes and Uatzo, the latter also differing from Usinavà (SI_Table 3).

Considering the provenance \times site interaction, Corsica, in Uatzo and Usinavà, differed from the other provenances in the other sites in terms of $\Delta^{13}\text{C}$ (Fig. 2; SI_Table 4). In the relatively more arid site, Usinavà, the provenance \times site interaction highlighted differences between Corsica and Portugal, between Telti and Tuscany, and between Limbara and Telti or Tuscany (Fig. 2; SI_Table 4).

Differently from $\Delta^{13}\text{C}$, ANOVA for $\Delta^{18}\text{O}$ did not show differences among provenances ($p > 0.05$; Table 4), although Portugal displayed the lowest $\Delta^{18}\text{O}$ value (32.90‰), while Corsica had the highest (33.06‰). Comparing the planting sites, the lowest $\Delta^{18}\text{O}$ occurred in Montes (31.99‰) (Fig. 2) but did not differ from other sites. Montarbu differed significantly from Uatzo and Usinavà in terms of $\Delta^{18}\text{O}$ (SI_Table 3). Yet, for $\Delta^{18}\text{O}$, Tukey's test showed significant provenance \times site interaction in several comparisons (SI_Table 5), indicating differences in phenotypic plasticity among provenances. This was especially true in Usinavà and Uatzo, where Tuscany differed from Corsica or Portugal, respectively.

Mean iWUE values were ranked in inverse order from $\Delta^{13}\text{C}$, being the lowest in Telti (94.4 $\mu\text{mol mol}^{-1}$) and the highest in Limbara (97.0 $\mu\text{mol mol}^{-1}$). iWUE ranged between 89.0 $\mu\text{mol mol}^{-1}$ in the period 1988–1992 and

Fig. 2 Mean values of isotopic traits at each site are indicated by red dots; boxplots refer to the different provenances of maritime pine: carbon isotope discrimination ($\Delta^{13}\text{C}$), intrinsic water-use efficiency (iWUE), and oxygen isotope discrimination ($\Delta^{18}\text{O}$). The boxplots represent the median and standard deviation (bars) of stable isotope traits



102.9 $\mu\text{mol mol}^{-1}$ in the last period. iWUE was lowest in Uatzo (92.7 $\mu\text{mol mol}^{-1}$) and the highest in Montarbu (97.1 $\mu\text{mol mol}^{-1}$). Usinavà and Montarbu did not differ from each other, in terms of iWUE ($p > 0.05$), as well as Usinavà and Montes or Montes and Uatzo. The provenance \times site interaction was significant and, as for $\Delta^{13}\text{C}$ in Usinavà,

Tukey's test highlighted differences between Corsica and Portugal, between Telti and Tuscany, and between Limbara and Telti or Tuscany (SI_Table 6).

Across provenances and periods, $\Delta^{13}\text{C}$ ranged between a minimum of 17.1‰ for Portugal, for the first period in Montes, to 19.5‰ for Corsica, for the last period in the

Uatzo site (Fig. 3). $\Delta^{13}\text{C}$ increased progressively during most of the study period. The exception was in the last period, 2013–2017, when all the provenances showed a decreasing trend in $\Delta^{13}\text{C}$ at all sites, except for Uatzo. $\Delta^{18}\text{O}$ decreased until the period 1998–2002, after which values increased in all provenances, except for Usinavà (Fig. 3). In Uatzo, $\Delta^{13}\text{C}$ increased more than in all other sites; while, in Usinavà, $\Delta^{18}\text{O}$ reached higher values than in Montes. Comparing the subperiods, significant differences were found in isotopic traits and the last period differed from the others.

Climate variability, growth, and correlations with isotopic signals

The Aridity Index of the trial sites affected the isotope traits, while the Aridity Index at the locations of origin of

the provenances was excluded automatically due to multicollinearity (Table 4). Isotopic signals of the five provenances showed significant relationships with interannual variation of weather at the trial sites (Table 5), while no significant correlations with climate variables at the sites of origin of the seed sources were found (SI_Table 1). Annual and seasonal cumulative precipitation (except for summer) showed a positive association with $\Delta^{13}\text{C}$, but there were no correlations with minimum mean temperatures. Conversely, $\Delta^{18}\text{O}$ showed positive relationships with minimum and maximum mean temperatures, as expected, and negative correlations with both winter and autumn cumulative precipitation. Moreover, iWUE was positively correlated with temperature and negatively with precipitation. The Aridity Index, which decreases with aridity, was negatively linked to $\Delta^{18}\text{O}$ and iWUE, but positively correlated with $\Delta^{13}\text{C}$ (Table 5).

Fig. 3 Carbon isotope discrimination ($\Delta^{13}\text{C}$), oxygen isotope discrimination ($\Delta^{18}\text{O}$), and intrinsic water-use efficiency (iWUE) of different provenances in each site during the investigated period, considering the five-year tree ring segments: coloured lines represent different provenances

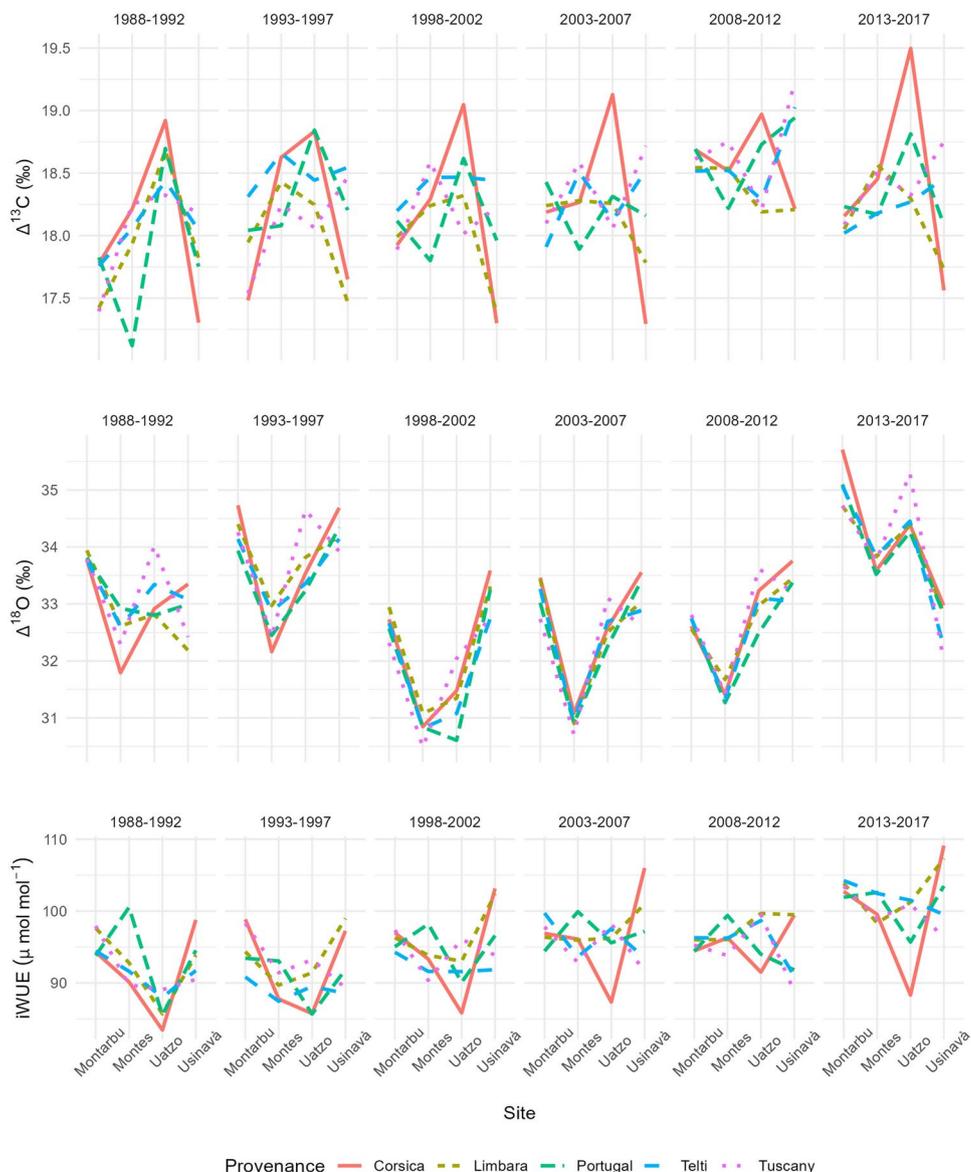


Table 5 Pearson's correlation coefficients among $\Delta^{13}\text{C}$ (carbon isotope discrimination), $\Delta^{18}\text{O}$ (oxygen isotope discrimination), and iWUE (intrinsic water-use efficiency), and climate variables (total precipitation and mean temperature) at the trial sites

Variables	$\Delta^{13}\text{C}$	$\Delta^{18}\text{O}$	iWUE
Annual precipitation	0.165	-0.071	-0.034
Winter precipitation	0.090	-0.150	0.023
Spring precipitation	0.184	0.067	-0.112
Summer precipitation	-0.081	0.078	-0.104
Autumn precipitation	0.214	-0.117	-0.094
Annual min temperature	0.002	0.140	0.069
Winter min temperature	-0.058	0.309	0.086
Spring min temperature	-0.001	0.141	0.075
Summer min temperature	0.059	-0.064	0.012
Autumn min temperature	-0.022	0.168	0.094
Annual max temperature	-0.053	0.303	0.221
Winter max temperature	-0.090	0.364	0.168
Spring max temperature	-0.052	0.257	0.202
Summer max temperature	-0.006	0.241	0.278
Autumn max temperature	-0.061	0.339	0.221
Aridity Index	0.121	-0.161	-0.088

Significant correlations ($p < 0.05$) are indicated in bold

Table 6 Pearson's correlation coefficients among $\Delta^{13}\text{C}$ (carbon isotope discrimination), $\Delta^{18}\text{O}$ (oxygen isotope discrimination), and iWUE (intrinsic water-use efficiency), and main characteristics of maritime pine trees

Variables	$\Delta^{13}\text{C}$	$\Delta^{18}\text{O}$	iWUE
$\Delta^{18}\text{O}$			
Provenance	-0.1985		
Corsica	-0.2303		
Limbara	-0.2205		
Portugal	0.0299		
Telti	-0.2153		
Tuscany	-0.3019		
iWUE	-0.83465	0.22901	
DBH	-0.17737	0.04172	0.17099
Height	0.11683	-0.14968	-0.11002
BAI	-0.11231	-0.02405	-0.25578

The relationships between $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ were also reported for each provenance. Significant correlations ($p < 0.05$) are indicated in bold
DBH diameter at breast height, *Height* tree height, *BAI* basal area increment

Unexpectedly, $\Delta^{13}\text{C}$ showed a positive association with tree height, while negative correlations were shown with DBH and BAI (Table 6); these correlations, though significant because of the large sample size, were relatively weak. Conversely, a positive association was found between DBH and iWUE, the latter negatively linked to

height and BAI. Finally, $\Delta^{18}\text{O}$ showed a negative correlation with tree height (Table 6).

Overall, $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ were somewhat correlated (Table 6), showing generally negative relationships ($r = -0.20$, $p < 0.001$). Considering the provenances separately, negative correlations between $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ were detected only for the Mediterranean provenances; the Atlantic one clearly differentiated from these (Table 6; SI_Fig. 1).

Discussion

In previous work, the combinations of tree ring widths and stable carbon isotopes have been used to investigate adaptive genetic variation to drought in maritime pine provenances, mainly belonging to the core of the species distribution area, i.e., France and Spain (Bogino and Bravo 2014; Brendel et al. 2002; Corcuera et al. 2012; Correia et al. 2008; Marguerit et al. 2014). However, given the climatic changes relative to the slowness of some evolutionary processes, there is a need to better assess the interplay between tree adaptation to climate disturbance and genetic variation, and their impacts on the growth and performance of maritime pine, thus broadening the analysis of provenance trials to marginal populations and locations. The present study provides eco-physiological insights based on a set of four common gardens in the peripheral distribution of maritime pine aimed at comparing provenances that differ in growth characteristics, stem traits, drought adaptation, and frost resistance (Gianini et al. 1992), taking advantage of previous studies in Sardinia Island (Tognetti et al. 2000). These provenances correspond to several geographic locations in Western Europe and the Mediterranean region: Atlantic (Portugal), Mesogeensis (Tuscany), and Corteensis (Corsica and northern Sardinia); in particular, the provenances from northern Sardinia grow in semi-arid conditions. Montes and Usinavà represent the two local climate extremes for humid and arid conditions for maritime pine, respectively. It must be pointed out that these common garden sites have subhumid climates (mean annual precipitation ranging from about 600 to 1000 mm) and the same provenances may exhibit different behaviour in dry (450–600 mm) or semiarid conditions (<450 mm), which might not be suitable for maritime pine. Plant functional traits other than tree-ring stable isotopes, e.g., specific leaf area, stem wood density, or specific root length (e.g., Liu et al. 2021), may provide useful insights to explain the response of different provenances to varying environmental conditions. Since this information was not available, we focused on tree-ring stable isotopes for a retrospective analysis of the ecophysiological behaviour of maritime pine in relation to climate and provenance (Marshall et al. 2022).

Stable isotope signals in tree rings

Tognetti et al. (2000) previously showed different profiles in $\Delta^{13}\text{C}$ signals and $i\text{WUE}$ among the considered maritime pine provenances at age 16. In contrast, only minor differences in $\Delta^{18}\text{O}$ were observed in these 40-year-old trees. Provenance-related differences should be interpreted considering the significant interactions between provenance and site, as well as considering the effects of environmental conditions in the different time periods (site \times time period). Corsica was involved in nearly all the most distinctive interaction means, for both $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$. For $\Delta^{13}\text{C}$, Corsica showed the highest value in Uatzo and the lowest at Usinavà (Fig. 2). For $\Delta^{18}\text{O}$, Corsica showed the highest value in Usinavà and the lowest at Montes and Uatzo. These site-related differences in this provenance contributed to significant interactions shown in SI_Table 4 and SI_Table 5. Corsica was, therefore, far more responsive to environmental conditions than the other seed sources. The same experimental trials revealed weak variation in $\Delta^{13}\text{C}$ among these maritime pine provenances at age 16 (Tognetti et al. 2000). Results obtained in other studies for Scots pine in provenance trials in Spain demonstrated very limited genetic divergence in isotope traits among populations from Spain and Germany (Santini et al. 2018). Likewise, studies of ponderosa pine in the western USA detected no population differences across the vast range of this species (Zhang et al. 1997). Other studies on maritime pine, comparing open-pollinated families from four populations covering a latitudinal cline (France, Spain, and Morocco), and Aleppo pine populations found intraspecific differences in isotopic traits (e.g., Aranda et al. 2010; Voltas et al. 2008). In the present study, instead, isotope signals were more closely related to climatic variation among planting sites, affecting phenotypic adjustment, than to the climate at the sites of origin of the seed sources (see Bogino and Bravo 2014). Environmental changes with time are likely to be dominated by atmospheric conditions, this pattern in the interactions suggests that atmospheric conditions had only additive effects on the provenances, but something else about the sites gave rise to an interaction. For example, the provenances may have responded differently to some set of soil conditions.

A relatively weak correlation between $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ for the Atlantic provenance suggests a higher influence of photosynthetic rate on C_i and $\delta^{13}\text{C}$ (Scheidegger et al. 2000), and lower contribution of the regulation of stomatal conductance to $i\text{WUE}$, in comparison with the *Mesogeensis* and *Corteensis* races (Mediterranean provenances). The slope of such a relationship may vary with seed sources differing in sensitivity to the moisture conditions of the planting sites. Therefore, stomata may remain relatively open during summer, so that stomatal conductance in trees from Portugal is not suppressed and photosynthetic capacity contributes to

control C_i and $\delta^{13}\text{C}$ (Scheidegger et al. 2000), which is risky in environmental conditions subject to recurrent drought events and in a warming scenario. A limited operational range of stomata in the Atlantic provenance would contrast with improved water balance for the Mediterranean provenances over time period at the trial sites. Since climate aridification proceeds rapidly, Mediterranean provenances may presumably move north-westward. Whereas Atlantic provenances lack some of the drought adaptations found in Mediterranean provenances, which may reduce their ability to deal with future climate. A negative relationship between $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ in tree rings was observed in other Mediterranean pines (Voltas et al. 2008), indicating a decrease in stomatal conductance in response to water stress (increase in stomatal control of photosynthesis) or greater utilization of water from deeper soil layers (Sarris et al. 2013). It must be pointed out that these inferences about conductance are based on the dual-isotope conceptual model, which should be considered with caution (Roden and Sigwold 2012), especially in the presence of possible variation in the isotopic composition of source water. We accounted for source-water variation by removing spatial and temporal variation in source water $\delta^{18}\text{O}_{\text{precipitation}}$ from $\delta^{18}\text{O}_{\text{plant}}$ and calculating $\Delta^{18}\text{O}$, considering differences in precipitation. Results evidenced differences among sites, with Usinavà showing higher $\Delta^{18}\text{O}$ in comparison with the other sites, but this site also has lower precipitation and different permeability and texture of soils (Giannini et al. 1992). However, the possibility of different rooting depths, which would also influence $\Delta^{18}\text{O}$, cannot be discounted.

Warming temperature at the trial sites in Sardinia coincided with a general decrease in $\Delta^{13}\text{C}$ and an increase in $i\text{WUE}$ over time period, especially during the last period, 2013–2017. This warming also coincided with increases in $\Delta^{18}\text{O}$ and $i\text{WUE}$. The steady decrease in $\Delta^{13}\text{C}$ over the last five years would suggest increasingly harsher conditions for tree growth (e.g., Del Castillo et al. 2015). An association between $\Delta^{13}\text{C}$ and precipitation was also found in Aleppo pine (Del Castillo et al. 2015; Ferrio et al. 2003), showing a steeper decrease with increasing aridity.

Maritime pine has a drought-avoiding strategy, i.e., high sensitivity of stomatal conductance to decreases in water potential (Picon et al. 1996). Such a strategy tends to reduce photosynthetic rates, and ultimately growth rates, under drought conditions, which may translate into a negative relationship between tree height and $\delta^{13}\text{C}$ (Corcuera et al. 2010). Correia et al. (2008) found negative correlations between needle $\delta^{13}\text{C}$ and tree height in Atlantic populations, suggesting stronger control of stomatal conductance than photosynthetic assimilation on $\delta^{13}\text{C}$ and high growth with reduced $i\text{WUE}$, whereas low $\delta^{13}\text{C}$ (and $i\text{WUE}$) values were associated with the lowest growth potential in a Mediterranean population, indicating an adaptation to more

xeric environments and less dependency on stomatal control of water loss. Increased height of maritime pine trees may result in higher hydraulic resistance (Magnani et al. 2008), making the xylem more vulnerable to embolism formation, though trees grow taller where there is more water and higher trees may also have deeper roots, which highlights the complexity of these relationships, particularly if the effect of a warming climate at the intraspecific level is considered.

Maritime pine adaptation perspectives

In drought-avoiding, water-saving species Mediterranean species under harsh conditions, iWUE has been considered an adaptive trait linked to the climate of origin (Medrano et al. 2009), however, there is considerable physiological plasticity for this trait (Voltas et al. 2008). Responses of iWUE to increasing atmospheric CO₂ concentrations may outweigh genotypic differences in drought tolerance, limiting the range of tolerance to local aridity and the expression of climate at the population source in this species (Sánchez-Gómez et al. 2017).

Mediterranean tree species have been increasing their iWUE since the 1970s. This increase can be attributed to a greater overall assimilation capacity of species, or to better stomatal control of water losses, where each of these may result from the increased atmospheric CO₂ concentration (Altieri et al. 2015). A negative correlation between $\delta^{13}\text{C}$ and productivity has been reported by Voltas et al. (2008) and Zhang et al. (1997), suggesting that higher water use leads to faster cumulative photosynthesis and growth. A negative correlation between iWUE and productivity, as described here, might indicate that stomatal closure increases iWUE, but at the expense of photosynthesis and growth. This may occur, for example, in the presence of abundant water, where efficient use of the water supply confers little benefit. Trees with low iWUE may also allocate more carbon to the root system and/or show early stomatal closure to escape drought. Indeed, in maritime pine, populations from low precipitation environments have displayed low iWUE (Nguyen-Queyrens et al. 1998). In the case of other pine species, populations with less negative $\delta^{13}\text{C}$ and higher iWUE were found to show either slower growth (Cregg and Zhang 2001) or higher productivity (Guy and Holowachuk 2001), probably depending on whether photosynthetic capacity or stomatal conductance controls iWUE.

Stable isotope differences among these peripheral maritime pine provenances were unrelated to the climate of origin. Considering that the provenance \times site interaction was an important source of variation in this study, the population main effect should be interpreted with caution. However, these results may indicate that both precipitation and temperature (and their seasonality) were of relatively minor importance as selection pressures for iWUE in maritime

pine at the source locations. This conclusion agrees with observations by Sánchez-Gómez et al. (2017), who reported a small variation in iWUE across maritime pine genotypes. Similarly, Warren et al. (2005) did not find an association between variation in stable isotope ratios (both $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$) among eucalyptus populations and precipitation at the origin of the seed sources and questioned the assumption that drought-adapted genotypes might have high iWUE at the intraspecific level. However, this interpretation contrasts with observations made in other studies on maritime pine (Aranda et al. 2010; de Miguel et al. 2012; Marguerit et al. 2014) and other Mediterranean pines (e.g., Voltas et al. 2008). Populations of Aleppo pine from dry areas showed conservative water use, whereas populations from more humid sites displayed lower water-use efficiency (Voltas et al. 2008). Even more different was Douglas-fir, which showed the highest iWUE in provenances from the wettest part of the distribution (Zhang and Marshall 1995). Nevertheless, populations with conservative water use and high iWUE may emerge more clearly when considering drought tolerance characteristics in leaf functional traits (e.g., specific leaf area and leaf nitrogen content), as in ponderosa pine (Zhang et al. 1997), or tree volume, and if a wider range of maritime pine provenances from numerous sites across the natural distribution of the species is compared (Alía et al. 1997; Corcuera et al. 2010). Likewise, if we could explain why the site \times provenance interaction was significant, but the period \times provenance interaction was not, this would help to explain what environmental variables gave rise to the interaction. For the moment, these unexplained interactions leave us unable to recommend seed sources for a new site based on their isotopic characters or iWUE.

Conclusions

Provenance differences in stable isotopes were not associated with the climate of the origin of the seed sources and, therefore, we may reject our initial hypothesis. Maritime pine displays a low level of provenance variation for stable isotopes and iWUE. However, according to our complementary expectation, a weak correlation between $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ for the Atlantic provenance suggests a higher influence of carbon assimilation on $\delta^{13}\text{C}$ and a lower contribution of stomatal regulation to iWUE in comparison with the Mediterranean provenances (cf. Scheidegger et al. 2000). As a result, drought-adapted provenances from the middle of the Mediterranean area would exhibit a conservative water use strategy, in comparison with less conservative provenances from the Atlantic distribution range. The increasing temperature at the trial sites coincided with a general decrease in $\Delta^{13}\text{C}$ and an increase in iWUE and $\Delta^{18}\text{O}$.

These provenance choices, however, are obscured by provenance-specific responses to environmental conditions at the trial sites, which are much stronger than the effect of the climate of origin of these seed sources. This suggests that iWUE should be seen more as a plastic response to some site variable and less as a genetic adaptation to climate at the source. The lack of interaction with period suggests that the site variable causing the interaction is not an atmospheric parameter because atmospheric parameters have changed over the course of this study, but site variables presumably have not. Therefore, the selection of maritime pine populations with higher iWUE for, e.g., assisted migration, based on carbon isotopic discrimination, needs to consider the effect of interactive effects of some site-specific environmental conditions across genotypes. Identifying that site condition will likely be necessary before site-specific genotype recommendations can be made.

Author contribution statement RT and JDM conceptualized the study. SA, GS, MM, and RT made field measurements and sampled trees. SA made the analysis. SA and RT wrote the manuscript. JDM contributed to the structure of the manuscript. All authors contributed to the discussion of results.

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Data availability The data that support the findings of this study are available upon request from the authors.

Declarations

Conflict of interest The authors declare they have no conflict of interest.

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