1 **Scarce population genetic differentiation but substantial spatiotemporal phenotypic**

2 **variation of water-use efficiency in *Pinus sylvestris* at its western distribution range**

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Abstract

Water and carbon fluxes in forests are largely related to leaf gas exchange physiology varying across spatiotemporal scales and modulated by plant responses to environmental cues. We quantified the relevance of genetic and phenotypic variation of intrinsic water-use efficiency (WUE_i, ratio of net photosynthesis to stomatal conductance of water) in *Pinus sylvestris* L. growing in the Iberian Peninsula as inferred from tree-ring carbon isotopes. Inter-population genetic variation, evaluated in a provenance trial comprising Spanish and German populations, was low and relevant only at continental scale. In contrast, phenotypic variation, evaluated in natural stands (at spatial level) and by tree-ring chronologies (at temporal inter-annual level), was important and ten- and three-fold larger than the population genetic variance, respectively. These results points to preponderance of plastic responses dominating variability in WUE_i for this species. Spatial phenotypic variation in WUE_i correlated negatively with soil depth (r= -0.66; $p<0.01$), while temporal phenotypic variation was mainly driven by summer precipitation. At the spatial level, WUE_i could be scaled-up to ecosystem-level WUE derived from remote sensing data by accounting for soil water holding capacity (r= 0.63; $p<0.01$). This outcome demonstrates a direct influence of the variation of leaf-level WUE_i on ecosystem water and carbon balance differentiation. Our findings highlight the contrasting importance of genetic variation (negligible) and plastic responses in WUE_i (large, with changes of up to 33% among sites) on determining carbon and water budgets at stand and ecosystem scales in a widespread conifer such as *Pinus sylvestris*.

Keywords: genetic variation; phenotypic plasticity; *Pinus sylvestris*; remote sensing; tree rings; intrinsic water-use efficiency

Introduction

The carbon and water cycles on Earth are coupled with the composition and functioning of forest ecosystems (Pan et al. 2011; Ukkola et al. 2016). Forests represent a net sink of 1.1×10^{15} g of carbon per year (Pan et al. 2011) and contribute to *ca.* 56% of terrestrial evapotranspiration (Schlesinger and Jasechko 2014). Ecosystem water-use efficiency (WUE), defined as the ratio between gross primary productivity and evapotranspiration, is a key characteristic determining the carbon and water balance of forests. Spatial heterogeneity, temporal fluctuations and their interactions over a broad spectrum of scales determine the multifaceted variability of WUE (e.g. Saurer et al. 2014). In order to monitor climate-vegetation feedbacks, an in-depth understanding of factors and processes that determine variation of WUE has become an urgent priority in ecology and biogeosciences (Keenan et al. 2013; Knauer et al. 2016).

Attempts to relate ecosystem WUE to leaf-level plant responses are pursued by the estimation of intrinsic water-use efficiency (WUE_i), or the ratio of net photosynthesis to stomatal conductance of water. The diffusion equation $[WUE_i = A/g_s = (C_a - C_i)/1.6]$ links carbon assimilation rate (A) to stomatal conductance (g_s) to the difference in atmospheric (C_a) and inner leaf (C_i) CO_2 concentration, irrespective of atmospheric water demand (Farquhar et al. 1989a). Detailed information on leaf-level physiology can be gained through the analysis of stable isotopes in tree rings (McCarroll and Loader 2004). Particularly, the ratio of the heavy to light carbon isotopes ($^{13}C/^{12}C$) depends on factors affecting CO_2 uptake, being directly related to WUE_i (Farquhar et al. 1989b). Alternatively, eddy covariance fluxes, remote sensing data and dynamic global vegetation models provide estimates of WUE at ecosystem scale (e.g. Frank et al. 2015; Dekker et al. 2016).

The spatiotemporal dynamics of WUE are conditional to the functional characteristics of the vegetation and, particularly, of the dominant tree species as fundamental component of

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74 forests. In this regard, it is expected that WUE_i fluctuations of a prevailing tree species could

75 be a determinant factor of WUE variation, hence strongly influencing water and carbon

76 economy of the whole ecosystem. Quantifying the spatial and temporal dimensions of the

77 variation of a functional characteristic such as WUE_i in forest trees is experimentally

78 challenging. Trees can respond to changes in the environment through phenotypic plasticity,

79 i.e. the array of phenotypes that an individual shows in response to different growing

80 conditions (Nicotra et al. 2010). Phenotypic plasticity allows plants to adjust their functional

81 traits to environmental changes, up to a level in which the extent of plastic responses (or

82 range of potential acclimation) does not suffice to cope with substantial variations in external

83 factors (Bussotti et al. 2015). Concurrently, different selective pressures may also engender

84 variation in functional traits within the genetic pool of a species through localised selection of

85 genotypes that perform better under particular conditions (i.e. genetic adaptation, Alberto et

86 al. 2013). Intra-specific phenotypic variation of a functional trait is thus the result of the co-

87 occurring effects of phenotypic plasticity and genetic adaptation, which have been long

88 proposed as partially independent mechanisms shaping plants' responses to the environment

89 (Bradshaw, 1965).

90   So far, a comprehensive investigation of the magnitude of phenotypic variation and its

91 components (spatiotemporal and genetic) is mostly lacking for WUE_i, even for widely

92 distributed tree species. The analysis of genotype by environment interaction in multi-

93 environment trials (i.e. the assessment of the performance of individuals in common gardens

94 across different environments) is well suited to this task, but their availability in forest trees is

95 limited and their records are often extremely unbalanced, which further complicates

96 partitioning such effects in a straightforward manner.

97   In this study, we focus on Scots pine (*Pinus sylvestris* L.), the most widespread conifer

98 of the Northern Hemisphere. Scots pine is found under very diverse climatic conditions and

99 ecological habitats from Boreal forests at high latitudes to Alpine biomes and meso-

100 Mediterranean areas at mid-latitudes, with some populations at the trailing edge of

101 distribution (e.g., Mediterranean basin) subjected to chronic summer drought (Irvine et al.

102 1998; Sánchez-Salguero et al. 2015). As most pines, Scots pine is considered a drought-

103 avoidant and isohydric species (Irvine et al. 1998), that is, prone to close stomata under water

104 shortage to maintain approximately constant leaf water potentials. During the last decades, the

105 southernmost populations of this species have experienced drought-induced decline in growth

106 and die-back episodes (Hereş et al. 2012; Camarero et al. 2018). Data derived from common

107 garden experiments indicate the existence of inter-population genetic differentiation in traits

108 such as nutrient acquisition (Oleksyn et al. 2003), phenology (Notivol et al. 2007), survival

109 (Benito-Garzón et al. 2011) or carbon allocation (Bachofen et al. 2018). Some evidences also

110 suggest specific adaptation of southern populations to drought (Taeger et al. 2013; Matías et

111 al. 2014). In addition to genetic differentiation, phenotypic plasticity drives variation in traits

112 such as hydraulic conductivity (Irvine et al. 1998), radial growth and carbon isotope

113 composition (Eilmann et al. 2010), and gas exchange physiology (Feichtinger et al. 2017) in

114 this species.

115 Despite the wealth of information on functional traits for *P. sylvestris*, little is known

116 about how phenotypic variation of WUE_i is structured for this species. A few studies based on

117 carbon isotopes have investigated the relevance of genetic variation of WUE_i through

118 common garden tests and clone bank experiments (Palmroth et al. 1999; Brendel et al. 2002),

119 finding low intra-specific genetic variation for this trait. However, these studies did not

120 include populations from the trailing edge of distribution of the species, where trees are

121 subjected to frequent summer droughts (Sánchez-Salguero et al. 2015). A persistent selective

122 pressure on water conservation might have led to an appreciable population genetic

123 differentiation in WUE_i. Conversely, Brendel et al. (2002) concluded that the main source of

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124 phenotypic variation of WUE_i is related to a plastic response of individuals to climate, as

125 expected for a species with high stomatal sensitivity to water shortage (Irvine et al. 1998),

126 which can be found across a vast array of conditions and environments. However, the

127 magnitude and relevance of the spatial and temporal components of phenotypic variation

128 remain unclear. Several studies have characterized temporal variation of WUE_i in *P. sylvestris*

129 (e.g. Andreu-Hayles et al. 2011; Voltas et al. 2013), but mainly focusing on the long-term

130 consequences of rising atmospheric CO_2 and climate on tree performance rather than on

131 interpreting the relevance of temporal variation in relation to spatial and genetic effects.

132 Here, we use carbon isotope discrimination ($\Delta^{13}C$) in tree rings sampled in a

133 provenance trial and in natural stands of *P. sylvestris* aiming at quantifying the importance of

134 inter-population genetic variation of WUE_i in relation to the overall spatiotemporal

135 phenotypic variation of the species near its south-western distribution limit (Pyrenees range,

136 north-eastern Iberian Peninsula). Our main objective is to understand how phenotypic

137 variation in carbon assimilation rate and stomatal conductance is structured in a widespread

138 conifer and to identify the external drivers of such changes. We hypothesize that: 1) the inter-

139 population genetic variation of WUE_i is of little relevance for *P. sylvestris* owing to a reduced

140 selective pressure towards water conservation as compared with other pine species that are

141 more xeric and, therefore, subjected to extreme and recurrent drought events (e.g. *Pinus*

142 *halepensis* [Voltas et al. 2008]); and 2) the spatiotemporal phenotypic variation of WUE_i is of

143 comparatively much more relevance than the extent of population genetic differentiation,

144 mainly owing to the relevance of plastic effects for this functional trait. To better understand

145 how leaf-level responses of a dominant tree species may contribute to the overall water and

146 carbon balance in conifer forests, WUE_i records of phenotypic variation obtained across

147 natural stands are compared to several indicators of ecosystem-level WUE derived from

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148 satellite data. In this way, we evaluate the feasibility of upscaling tree-ring-based high-resolution spatial estimates of WUE_i over the whole forest stand.

149 resolution spatial estimates of WUE_i over the whole forest stand.

150

Material and methods

Provenance trial

153 A provenance trial located in Aragüés del Puerto (NE Iberian Peninsula; 42°44'N, 00°37'W, 1350 m a.s.l.) and comprising sixteen Spanish and six German populations (Fig.1a; Online Resource 1) was sampled to quantify the importance of inter-population genetic differentiation of WUE_i. The mean annual temperature at the site is 7.4°C and the mean annual precipitation is 1086 mm (Fig. 1a), of which *ca*. 22% fall in summer (1960-1990 period) (WorldClim database; Hijmans et al. 2005). The trial has optimal growing conditions for this species in the Iberian Peninsula, and can be considered representative of the average (albeit slightly warmer) climate conditions encountered by *P. sylvestris* across Europe (Fig. 1b). Two-years-old seedlings were planted in 1992 according to a randomized complete block design with four replicates. Each replicate plot consisted of 16 trees of the same population, spaced 2.5 × 2.5 m, resulting in square-shaped experimental units.

164 The Spanish populations are representative of the species distribution in the Iberian Peninsula (south-western edge of its present range). The German populations are distributed across the country (i.e. approximately the centre of the European distribution range for this species) and were chosen to compare their performance against those of the Spanish populations (for further details see Alía et al. [2001]) (Fig. 1b). At age 20 (year 2010), survival rate (%) per replicate plot was recorded and height and diameter at breast height (DBH) were measured using telescopic measuring sticks and tapes respectively. Mean tree-ring width (TRW) of each tree was estimated as the radius divided by age. At age 25 (year 2015), wood cores were sampled at 1.3 m using 5-mm Pressler increment borers. We selected two adjacent blocks having relatively uniform growing conditions and six trees per population

were sampled (i.e. three trees per block). Tree rings were visually cross-dated and rings corresponding to the 2005-2014 period were pooled together and used for carbon isotope analysis.

Natural stands

The spatial extent of phenotypic variation in WUE_i, which comprises genetic variation and phenotypic plasticity, was evaluated in natural stands of *P. sylvestris*. The sampling sites consisted of 30 unmanaged, monospecific and even-aged stands located in the central and eastern Pyrenees mountains (north-eastern Iberian Peninsula) (Fig. 1a; Online Resource 2). The stands cover a wide range of local climatic, physiographic and edaphic conditions (Fig. 1b; Online Resource 2). They are supposed to share adaptive characteristics as they belong to two neighbouring Spanish provenance regions (or adaptive units in which phenotypically or genetically similar stands are found; European Council Directive 1999/105/EC). Each stand was characterized for the following features: elevation, slope, hillslope position (footslope, backslope or shoulder) and aspect, carbonate content in soil, stand density, stand basal area and soil depth (maximum dig depth before finding a compact rock layer). The Harmonized World Soil Database (FAO, 2009) classifies the soils of the stands as humic or calcaric cambisols with a resolution of 1 km^2. Soil texture is relatively uniform among sites (range = 37-42% sand, 23-44% silt and 19-36% clay).

Wood cores were sampled at 1.3 m using 5-mm Pressler increment borers in 2008. A variable number of representative trees (two to ten) were taken per stand (tree age = 68±18 years; mean ± SD). Tree rings were visually cross-dated and tree age was calculated. The rings corresponding to the period of 2000-2007 were identified and used to estimate mean tree-ring width (TRW). These rings were then pooled together and used for carbon isotope analysis. Height and DBH of sampled trees were also measured.

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Tree-ring chronologies

At temporal level, the magnitude of phenotypic variation was evaluated in two representative sites (Arcalís, 42°22'N, 01°11'E, 1150 m a.s.l.; Seira, 42°31'N, 0°23'E, 1538 m a.s.l.) out of the 30 sites comprising the sampled region (Fig. 1a; Online Resource 2). Tree-ring chronologies were built in these sites for carbon isotopes analysis. Arcalís is drier and warmer compared with Seira (Fig. 1b) and is located in an area where recent die-off episodes have been observed (Hereş et al. 2012). Wood cores were sampled from 40 and 20 representative, adult and healthy trees in Arcalís and Seira respectively. Tree rings were cross-dated and TRW was measured using WinDENDRO™ (Regent Instruments Inc., Ville de Québec, Canada) with a resolution of 0.01 mm. The accuracy of cross-dating was checked with the COFECHA program (Holmes 1983). The quality of the resulting chronologies was evaluated by the expressed population signal (EPS) statistic, which resulted adequate (>0.85) for the study period (1975-2009). Individual rings from a subset of ten cores in Arcalís and five cores in Seira were separated with a scalpel under a binocular microscope to carry out carbon isotope analysis with annual resolution. Approximately the last 30-35 rings (corresponding to 1975-2008 for Arcalís and 1980-2009 for Seira) were used for isotopic analysis, hence avoiding juvenile imprints on the carbon isotope signature (tree age = 68±19 and 66±11 years in Arcalís and Seira respectively; mean ± SD).

Carbon isotope analysis and leaf-level intrinsic water-use efficiency

Wood samples (i.e. pooled rings from the provenance trial and sampled sites, annual rings from tree-ring chronologies) were milled to a fine powder with a mixer mill (Retsch MM301, Haan, Germany) and purified to α-cellulose (Ferrio and Voltas 2005). An aliquot of 0.9-1.1 mg of α-cellulose was weighted and encapsulated into tin capsules that underwent combustion using a Flash EA-1112 elemental analyser interfaced with a Finnigan MAT Delta

224 C isotope ratio mass spectrometer (Thermo Fisher Scientific Inc., MA, USA). Carbon isotope

225 composition ($\delta^{13}C$) was calculated as:

226 $\delta^{13}C\ (\text{‰}) = (R_{sample} / R_{standard} - 1) \times 1000$ (1)

227 where R_{sample} and $R_{standard}$ are the isotope ratios ($^{13}C/^{12}C$) of the sample and of the Vienna Pee

228 Dee Belemnite (VPDB) standard respectively.

229   The $\delta^{13}C$ values were then used to estimate carbon isotope discrimination ($\Delta^{13}C$)

230 following Farquhar et al. (1989b):

231 $\Delta^{13}C\ (\text{‰}) = (\delta^{13}C_a - \delta^{13}C_s)/(1 + \delta^{13}C_s/1000)$ (2)

232 where $\delta^{13}C_a$ and $\delta^{13}C_s$ are the carbon isotope composition of atmospheric CO_2 and sample

233 respectively. $\delta^{13}C_a$ was inferred by interpolating a range of data from Antarctic ice-core

234 records, as described by Ferrio et al. (2005). According to these records, the $\delta^{13}C_a$ value

235 applied to α-cellulose $\delta^{13}C$ was -8.20‰ (provenance trial samples; 2005-2014 period), -

236 8.09‰ (sampled sites; 2000-2007 period) and ranged between -7.30‰ and -8.23‰ (annually-

237 resolved tree rings; 1975-2009 period).

238       $\Delta^{13}C$ is proportional to the ratio of intercellular (C_i) to atmospheric (C_a) CO_2

239 concentrations in C3 plants as follows:

240 $\Delta^{13}C\ (\text{‰}) \approx a + (b - a) \times (C_i/C_a)$ (3)

241 where a is the fractionation during diffusion through stomata (~4.4‰) and b is the

242 fractionation due to carboxylation by Rubisco (~27‰) (Farquhar et al. 1989b). The previous

243 equation is valid for the primary photosynthetic assimilates, but it does not consider further

244 fractionation occurring downstream from photosynthesis to α-cellulose formation (Gessler et

245 al. 2014). Indeed, leaves are usually less enriched in ^{13}C than wood α-cellulose. To estimate

246 $\Delta^{13}C$ at the leaf level, we added a value of +2.1‰ to our α-cellulose $\Delta^{13}C$ values, as average

247 difference between leaf $\Delta^{13}C$ and α-cellulose $\Delta^{13}C$ reported by Frank et al. (2015). We

248 assumed near-constancy of differences between chloroplastic and intercellular CO_2

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249 concentration at the intra-specific level and a linear relationship between g_s and internal

250 conductance in response to varying water availability. Anyhow, the high internal conductance

251 of *Pinus sylvestris* suggests low mesophyll limitations of photosynthesis (Vernomann-

252 Jürgenson et al. 2017).

253   WUE_i was obtained from leaf-level $\Delta^{13}C$ according to Farquhar et al. (1989b):

254 $$WUE_i = (C_a \times (b - \Delta^{13}C)) / (1.6 \times (b - a)) \tag{4}$$

255   C_a was obtained from National Oceanic and Atmospheric Administration (NOAA)

256 Earth System Research Laboratory data (http://www.esrl.noaa.gov/). We considered the

257 average C_a corresponding to the time period of the analysed tree rings (ten years for the

258 provenance trial, eight years for the sampled sites and annually-resolved values for

259 characterising temporal variation). Further analyses were performed using leaf-level WUE_i

260 estimates.

261

Climate data

263   Long-term (1960-1990) averages of precipitation, maximum and minimum

264 temperatures were obtained on a monthly basis from the WorldClim database with a

265 resolution of 1 km^2 (Hijmans et al. 2005). They were used to infer mean annual temperature

266 (T_{an}), total annual precipitation (P_{an}) and summer (June to August) precipitation (P_s) of each

267 geographic origin of populations evaluated at the provenance trial (Online Resource 1) and of

268 each natural stand (Online Resource 2). Annual potential evapotranspiration (PET_{an}) was

269 derived from monthly temperatures and precipitation according to the Hargreaves method

270 (Hargreaves and Samani 1982). Climate at the Spanish provenances is in general less humid

271 than that of German provenances. Particularly, Spanish populations originated from climates

272 having a significantly lower P_s (143±49 mm *vs*. 242±89 mm respectively; mean ± SD), lower

273 P_s to P_{an} ratio (0.19±0.16 *vs*. 0.32±0.21) and higher PET_{an} (880±69 mm *vs*. 762±62 mm) than

274 their German counterparts ($p<0.05$; two-tailed t-tests). In comparison, the climate conditions

275 in the provenance trial are intermediate between those of German and Spanish populations

276 (P_s=238 mm, P_s to P_{an} ratio=0.22, PET_{an}=747 mm).

277 Precipitation, temperature and the Standardised Precipitation-Evapotranspiration Index

278 (SPEI) corresponding to the site chronologies were obtained on a monthly basis from the

279 CRU TS3.22 dataset (Harris et al. 2014). CRU TS3.22, despite its lower spatial resolution

280 compared with WorldClim, provides yearly records of monthly climate data suitable to

281 investigate relationships between climate and annually-resolved WUE_i estimates. The climate

282 records, available on a 0.5º latitude/longitude grid for global land areas, covered the period in

283 which annual estimates of WUE_i were available (1975-2008 for Arcalís, 1980-2009 for Seira).

284

Statistical analyses

286 The magnitudes of population genetic differentiation and phenotypic variation (spatial

287 and temporal) in WUE_i were quantified independently using the three different sources of

288 data: provenance trial for population differentiation, natural stands for spatial variation, and

289 tree-ring chronologies for temporal variation. To this end, we used linear mixed-effects or

290 random-effects models through restricted maximum likelihood (REML) (see below for a

291 detailed description). The significance of variance components of random effects was

292 evaluated through likelihood ratio tests. These models were fitted using SAS/STAT (ver. 9.4,

293 SAS Inc., Cary, NC, USA). The suitability of analysis of variance (ANOVA) was evaluated

294 through normal quantile plots of the residuals (Online Resource 3).

295

Provenance trial

297 The provenance trial was subjected to mixed-effects analysis of variance (ANOVA)

298 with a fixed block effect, a random population effect, a random block × population interaction

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299 (intra-block error) and a random variation of trees within a plot (intra-plot error or residual).

300 The random population term provided an estimate for the variance component associated to

301 inter-population genetic variation in WUE_i. This term was further partitioned into a fixed term

302 accounting for differences between large regions (or countries of origin, Germany *vs*. Spain)

303 and a random effect quantifying the variation among populations nested to country of origin.

304 This was done to determine the extent by which population genetic differentiation for the trait

305 could be attributed to differences between contrasting ecoregions. An equivalent procedure

306 was also applied to tree height, mean TRW and survival (expressed in per cent values on a

307 plot basis). In this last case, an angular transformation was used to achieve normality.

308

Natural stands

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310 A full random-effects ANOVA was fitted to WUE_i and TRW records testing for

311 significant variation among stands. The stand (site) effect was declared as random and the

312 associated variance component was used as an estimate of the spatial dimension of

313 phenotypic variation for these traits. The random variation of trees within a site was used as

314 residual or error term. We used simple correlations to test for the effects of physiography,

315 climate and soil characteristics on site WUE_i and TRW. The associations between WUE_i,

316 TRW and height, and the effect of tree age on WUE_i and TRW were also tested at tree level

317 by simple correlations.

318

Tree-ring chronologies

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320 WUE_i and TRW time series of the site chronologies showed long-term trends (Online

321 Resource 3). For the purpose of studying high-frequency variation in WUE_i, estimates were

322 re-calculated considering an average C_a value for a 30-year period instead of using annually-

323 resolved C_a records. In this way, the direct effect of CO_2 fertilization enhancing WUE over

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324 time was excluded from the temporal trend. For the same purpose, TRW was investigated by

325 high-pass filtering using cubic smoothing splines with 50% frequency cut-off at 15 years

326 (ARSTAN program; Cook and Krusic 2005). The resulting WUE_i time series (C_a-constant

327 WUE_i) and the residuals of TRW splines (TRW_{res}) were considered free of increased

328 atmospheric CO_2 concentration and ontogenic effects, thus retaining primarily inter-annual

329 variation. Thus, only the fraction of temporal variation associated to climate effects was

330 presumably quantified by mixed-effects ANOVAs. These models consisted of a fixed site

331 chronology effect and random year, random year by site interaction and random tree-within-

332 site effects. The random year by tree-within-site interaction was used as a residual term. The

333 variance component for the year effect was taken as an estimate of the temporal dimension of

334 phenotypic variation for these traits. Indexed TRW records (TRW_i) were obtained by

335 applying an autoregressive model to each TRW_{res} series to remove the autocorrelation related

336 to the growth of the previous year and later calculating the ratio of observed to predicted

337 values of the cubic splines. Bootstrapped correlations between annual C_a-constant WUE_i or

338 TRW_i and monthly climate factors (precipitation, temperature and SPEI) from the October of

339 the previous year to September of the ring year were calculated using the software

340 DendroClim (Biondi and Waikul 2004). Statistical significance of correlations was estimated

341 by drawing 1000 bootstrapped samples with replacement from the initial data set.

342

Ecosystem water-use efficiency

344   We correlated the estimates of leaf-level WUE_i of the natural stands with four

345 ecosystem-level indicators of water-use efficiency. Ecosystem water-use efficiency (EWUE)

346 is a common indicator calculated as the ratio between ecosystem gross primary productivity

347 (GPP) and its associated evapotranspiration (ET) (Huang et al. 2015). Soil water-use

348 efficiency (SWUE) is a recently proposed indicator calculated as the ratio between GPP and

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soil water content (SWC) (He et al. 2017). We took advantage of available high-resolution (1 km^2) remote sensing data to calculate the two indices for each sampled site. Mean GPP and ET data for the period of 2000-2014 were derived from Moderate Resolution Imaging Spectroradiometer (MODIS) on board NASA's Terra satellite, available at https://daacmodis.ornl.gov/data.html. Mean SWC for the period of 2010-2016 was derived from the ESA's Soil Moisture and Ocean Salinity (SMOS) mission data, downscaled at 1 km^2 resolution as described in Merlin et al. (2013). SWC data are available at http://cp34-bec.cmima.csic.es/data/data-access/. We also calculated an "inherent" WUE (IWUE) multiplying EWUE by the average vapour pressure deficit (VPD) estimated at each site. IWUE accounts for atmospheric water demand and it approximates WUE_i better compared with EWUE (Beer et al. 2009). Average VPD was calculated from altitude and monthly temperature and precipitation following Ferrio and Voltas (2005). As SWC is given in relative units as m^3 of water per m^3 of soil, we estimated the total amount of water available along the soil profile by multiplying SWC by soil depth at site level. We then recalculated SWUE as the ratio between GPP and the corrected (total) SWC (SWUE'). Finally, we correlated leaf-level WUE_i with EWUE, SWUE, IWUE or SWUE' across sites.

RESULTS

Inter-population genetic variation inferred from the provenance trial

We found low inter-population genetic variation for WUE_i and radial growth in *P. sylvestris* as indicated by small and poorly estimated variance components of population effects, which were non-significant (Table 1). However, when the population effect was partitioned into a fixed effect accounting for differences between countries of origin and a remaining random population effect, significant differences in WUE_i emerged between Spanish and German populations ($p<0.05$). In particular, German populations showed a 3.2%

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374 higher WUE$_i$ than Spanish populations (Fig. 2). On the other hand, there were no significant

375 differences in TRW between countries (Fig. 2), but Spanish populations showed a

376 significantly lower tree height than German ones (9.12±0.27 m *vs*. 10.84±0.28 m respectively;

377 mean ± SE) and experienced lower mortality (11.2±0.05% *vs*. 19.2±0.12 % respectively).

378

Spatial phenotypic variation inferred from natural stands

380 The spatial phenotypic variation for WUE$_i$ was highly significant and *ca*. ten-fold

381 higher than the magnitude of inter-population genetic variation estimated at the provenance

382 trial (Table 1). WUE$_i$ varied across sites between 85.26 μmol CO_2 mol^{-1} H_2O (Vallfogona)

383 and 113.01 μmol CO_2 mol^{-1} H_2O (Estamariu II), which translated into a relative maximum

384 WUE$_i$ difference of 33% among stands. Mean TRW varied between 0.51 mm (Valls de Valira

385 II) and 2.51 mm (Gombreny), which translated into a relative maximum TRW difference of

386 *ca*. 400% among stands. The spatial component of phenotypic variation for TRW was highly

387 significant and about five-fold higher than that of inter-population genetic variation (Table 1).

388 WUE$_i$ did not correlate with any physiographic, climatic or edaphic characteristic

389 across stands (Online Resource 4). The only exception was soil depth, which was negatively

390 associated with WUE$_i$ (Fig. 3a). WUE$_i$ did not correlate with TRW across stands (Fig. 3b). On

391 the other hand, TRW was negatively correlated with the ratio of summer to total precipitation,

392 with annual potential evapotranspiration and with stand density (Online Resource 4). At tree

393 level, we did not find significant relationships between WUE$_i$ and age or height, but TRW

394 was negatively associated with tree age (Online Resource 4).

395

Temporal phenotypic variation inferred from tree-ring chronologies

397 WUE$_i$ increased significantly over the last 30 years, with similar rates in Arcalís and

398 Seira (Online Resource 5). Particularly, WUE$_i$ was 17% (Arcalís) and 11% (Seira) higher in

the period of 2004-2008 than at the beginning of the 1980s (1980-1984 period). Due to the positive effect of increased atmospheric CO_2 concentration on WUE_i over time, the temporal (year) variance calculated through annually-resolved C_a values was much higher than the inter-population genetic variance (over ten-fold higher) and slightly higher than the spatial phenotypic variance (Online Resource 6). Instead, the high-frequency signal of time-varying WUE_i was estimated using an average C_a value over the study period (1975-2008 in Arcalís and 1980-2009 in Seira). After recalculation, the increasing trend disappeared in Seira (Online Resource 5). The resulting C_a-constant mean WUE_i was 77.79 µmol CO_2 mol^{-1} H_2O in Seira and 85.00 µmol CO_2 mol^{-1} H_2O in Arcalís, and the temporal phenotypic variance became four-fold lower (9.57 *vs*. 40.77 (µmol CO_2 mol^{-1} H_2O)^2) and only marginally significant (p=0.11). Also, it was about four-fold lower than the magnitude of spatial variation, but still three-fold higher than the magnitude of inter-population genetic variation (Table 1). The variance component of year by site (spatiotemporal) interaction was not significant and had about the same magnitude of the variance component of population (genetic) effects (Table 1).

TRW showed a significant increase over time in Seira, but a significant decrease in Arcalís (Online Resource 5). The common temporal variance estimated across sites on non-detrended (absolute) TRW records was negligible, but the magnitude of year by site interaction was very relevant (higher than those of inter-population genetic variation and of spatial phenotypic variation) (Online Resource 6). After high-pass filtering, the common temporal variance for TRW_{res} became sizeable (0.04 ± 0.02 mm^2) and similar to the variance associated with year by site interaction (Table 1). In this regard, the temporal component of phenotypic variation for TRW_{res} was also similar to that of inter-population genetic variation (Table 1).

18

The relationships between C_a-constant WUE_i or TRW_i and monthly climatic variables are summarized in Fig. 4. In Seira, C_a-constant WUE_i correlated positively with March precipitation and negatively with summer (June and August) precipitation and SPEI drought index. At this site, TRW_i correlated negatively with October temperature and positively with November temperature and August precipitation and SPEI. In Arcalís, C_a-constant WUE_i correlated negatively with spring to early-summer precipitation and SPEI (April and June) and positively with July temperature. It also showed a strong negative correlation with winter (previous December and current January) precipitation, SPEI and temperature. TRW_i correlated positively with January SPEI and April temperature, and with precipitation and SPEI in May-June.

Ecosystem-level WUE

The average ecosystem WUE (EWUE) estimated for the period of 2000-2014 varied between 2.17 g C mm^{-1} H$_2$0 (Seira) and 3.66 g C mm^{-1} H$_2$0 (Sant Llorenç) across sites. Alternatively, the average soil WUE (SWUE) varied between 12.63 g C kg^{-1} H$_2$O (Espot) and 19.68 g C kg^{-1} H$_2$O (St. Joan Abadesses) across sites. Neither EWUE nor SWUE correlated with leaf-level WUE_i (Fig. 5a,b). IWUE (i.e. EWUE corrected by atmospheric water demand) varied between 11.02 g C hPa mm^{-1} H$_2$O (Seira) and 23.00 g C hPa mm^{-1} H$_2$O (Sant Llorenç). IWUE did not correlate with leaf-level WUE_i (Fig. 5c). The modified SWUE values taking into account soil depth at stand level, that is, accounting for the total amount of water potentially available along the soil profile, varied between 19.44 g C kg^{-1} H$_2$O (Espot) and 76.48 g C kg^{-1} H$_2$O (St. Joan Abadesses). In this case, a significant positive correlation was found between SWUE' and WUE_i (Fig. 5d).

19

DISCUSSION

449 **The extent of genetic and phenotypic variation of WUE_i in *Pinus sylvestris***

450   Phenotypic diversity in functional traits, including intra-specific genetic variation and

451 phenotypic plasticity, is called upon to play a central role in assisting forest populations to

452 withstand future environmental conditions (Nicotra et al. 2010; Alberto et al. 2013; Bussotti

453 et al. 2015). Our results demonstrate very limited genetic divergence in WUE_i among *P.*

454 *sylvestris* populations of the western part of the species distribution, in line with previous

455 studies reporting lack of genetic differences in $\Delta^{13}C$ among populations of this species

456 (Palmroth et al. 1999; Brendel et al. 2002). *P. sylvestris* is not subjected to pervasive droughts

457 and, thus, selective pressure towards a strong stomatal regulation of water losses may be

458 irrelevant to shape physiological adaptations among populations for this species (Matías et al.

459 2017). In comparison, other European pines thriving in Mediterranean regions and exposed to

460 substantial water shortage, such as *Pinus pinaster* or *P. halepensis*, are known to bear a large

461 intra-specific genetic variation in WUE_i (Voltas et al. 2008; Aranda et al. 2010). Specifically,

462 Voltas et al. (2008) reported differences of *ca*. 26 μmol CO_2 mol^{-1} H_2O among populations of

463 the drought-avoidant *P. halepensis*, which is almost three-fold higher than the range we

464 estimated for *P. sylvestris* (*ca*. 10 μmol CO_2 mol^{-1} H_2O). However, our results are not

465 conclusive of lack of intra-specific genetic variation in WUE_i in *P. sylvestris*. Indeed, they do

466 not allow for an assessment of intra-population genetic variation in relation to that present

467 among populations. Such a comparison would require testing the performance of different

468 progenies within each population in a provenance/progeny trial, which is currently

469 unavailable at the south-western distribution range of *P. sylvestris*.

470   On the other hand, we could identify a significant, albeit low, genetic differentiation in

471 WUE_i after grouping populations according to their country of origin, which might suggest a

472 role of selection on functional traits that contribute to WUE_i (i.e. assimilation rate and stomata

473 control), but only at large (i.e. continental) scales. Particularly, German populations showed

474 increased WUE_i coupled with a greater height growth compared with Spanish populations.

475 This outcome agrees with an evidence for genetic differentiation in WUE reported in *P.*

476 *sylvestris* for a subset of European populations, although at the seedling stage (Bachofen et al.

477 2018). These results may indicate improved carbon uptake through photosynthesis as

478 mechanism enhancing WUE_i at the genetic level (and hence, primary growth) for this species

479 (Fardusi et al. 2016).

480 We estimated, for a small Scots pine area (~5,000 km^2), a phenotypic variation of

481 WUE_i over ten-fold larger than the extent of variation related to inter-population genetic

482 differentiation for two major European regions occupying together over 800,000 km^2.

483 Phenotypic variation in natural stands is the result of the interacting effects of genetic

484 variation and phenotypic plasticity, but our approach could not directly disentangle the

485 relative importance of these components. Despite this caveat, results from the provenance trial

486 suggests lack of relevant population differentiation in WUE_i continent-wide for *P. sylvestris*,

487 making unlikely the presence of a significant inter-population differentiation at the much

488 smaller scale of the sampled natural stands. In this regard, two populations evaluated in the

489 provenance trial, Morrano and Pobla de Lillet (Online Resource 1), which originated from the

490 area where the 30 natural stands were sampled, did not differ in terms of WUE_i (89.09±2.46

491 μmol mol^{-1} *vs*. 91.14±2.62 μmol mol^{-1}), TRW (3.32±0.26 mm *vs*. 3.47±0.26 mm) and height

492 (8.67±0.74 m *vs*. 8.41±0.74 m). This observation supports a lack of genetic differentiation

493 among stands for the study traits. Although it can be argued that our results do not quantify

494 within-population genetic variation in WUE_i, the sampling strategy adopted in the natural

495 stands, consisting of a number of representative trees sampled per site, buffered possible

496 genetic differences among trees. Therefore, the reported phenotypic variation among natural

497 stands is most probably indicative of plastic responses to divergent growing conditions. The

498 large phenotypic plasticity for *P. sylvestris* is somewhat expected since pines are known to be

499 extremely plastic organisms (e.g. Tapias et al. 2004), and suggests that Scots pine

500 performance is primarily modulated by plastic responses in functional characteristics such as

501 WUE$_i$ (Feichtinger et al. 2017), rather than by genetic adaptation. Other drought-related traits

502 such as leaf-to-sapwood area ratio (Martínez-Vilalta et al. 2009) or wood anatomy (Eilmann

503 et al. 2009; Martín et al. 2010) also show high plasticity in *P. sylvestris*.

504 Notably, climate characteristics did not correlate with WUE$_i$ across stands, despite the

505 sampled sites differed considerably in terms of total precipitation, evapotranspiration and

506 summer drought intensity. As a comparison, Ferrio and Voltas (2005) found that WUE$_i$

507 negatively correlated with annual precipitation across an aridity gradient of the xeric *P.*

508 *halepensis* in eastern Iberian Peninsula. Coupling our data with those from Ferrio and Voltas

509 (2005), a decay pattern of plastic WUE$_i$ responses to annual precipitation is evident across

510 species (Fig. 6). The environmental conditions in which these two isohydric pines overlap in

511 the Iberian Peninsula (~800 mm of annual precipitation) roughly agree with the threshold at

512 which WUE$_i$ (and stomatal sensitivity) becomes unresponsive to precipitation (Fig. 6) or

513 water deficit (Fig. 6 insert), as observed for *P. halepensis* (del Castillo et al. 2015). Within

514 such a continuous distribution of pines, precipitation would mainly drive phenotypic variation

515 of WUE$_i$ in *P. halepensis*, while *P. sylvestris* would be found in conditions where

516 precipitation is no longer a fundamental determinant of spatial variation in gas exchange.

517 Instead, the driving force of phenotypic variation in WUE$_i$ for *P. sylvestris* was the

518 amount of water available to roots, as indicated by the negative relationship between soil

519 depth and WUE$_i$. Because of methodological difficulties, the study of rooting patterns for

520 elucidating adaptations to edaphic conditions is a much less explored field of research

521 compared with the analysis of above-ground tree characteristics. However, the distribution of

522 water along the soil profile is known to be extremely relevant in modulating plastic responses

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of forest trees to drought (Lebourgeois et al. 2010; Song et al. 2015; Voltas et al. 2015). Song et al. (2015) found a lower increase of WUE_i under drought stress in old plantations of *P. sylvestris* compared to young stands, and explained the difference as a higher rooting depth of older trees and, therefore, an easier access to deep water sources. In our case, the depth of rooting zone, which can be regarded as proxy of water holding capacity, seems a critical characteristic for *P. sylvestris* growing at its south-western distribution edge (Mellert et al. 2017). However, other soil factors such as nutrient availability and its interactions with climate may also determine changes in WUE_i across sites, hence warranting further examination (Silva et al. 2015).

WUE$_i$ increased over the last 30 years by 11% and 17% at the mesic (Seira) and xeric site (Arcalís) respectively. These rates are consistent with previous results reporting similar WUE_i increments in European forests (Saurer et al. 2014) and in other *P. sylvestris* stands from the Iberian Peninsula (Andreu-Hayles et al. 2011; Voltas et al. 2013). After reappraising WUE_i for constant C_a, we observed increases over time only at Arcalís, indicating that warming-induced drought effects may effectively contribute to long-term WUE_i changes in drought-prone areas for this species. In fact, most sampling sites of the central and eastern Pyrenees showed higher mean WUE_i than Arcalís over the same period (93 μmol CO_2 mol^{-1} H_2O) (*cf*. Fig. 3a), which suggests that water availability is the most limiting factor for regional tree performance. It can therefore be hypothesised that a long-term temporal variation of WUE_i related to increasing drought effects (Shestakova et al. 2017) may be common to most *P. sylvestris* stands of the region.

The lack of interaction between spatial and temporal plastic responses in terms of WUE_i anticipates the existence of common temporal patterns across sampling sites. Although this point would need further assessment, the fact that Arcalís and Seira shared the dependence of WUE_i on summer precipitation indicates a predominant role of summer water

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548 availability influencing inter-annual variation of WUE_i. However, WUE_i was also negatively

549 correlated with winter precipitation at the xeric site (Arcalís), which can be interpreted as the

550 effect of groundwater recharge on tree performance during the growing season (Shestakova et

551 al. 2014). Indeed, water stress could be delayed or supressed during the growing season in

552 years with important snow coverage, resulting in low WUE_i. Thus, our results suggest that

553 soil water plays an important role in modulating inter-annual variation of WUE_i, at least at the

554 xeric site, which is supportive of a strong dependence of spatial variation of WUE_i on soil

555 depth for *P. sylvestris*.

556

Inter-population genetic differentiation and phenotypic variation of radial growth

558 The extent of phenotypic variation and the magnitude of inter-population genetic

559 differentiation in WUE_i were compared with those of radial growth as indicator of overall

560 performance in forest trees. Tree growth is indeed influenced by many biotic, abiotic and

561 stand (local) factors, but climate plays a major role in driving growth variation in *P. sylvestris*

562 (Matías and Jump 2012; Sánchez-Salguero et al. 2015). Broadly speaking, the phenotypic

563 variation in radial growth is predominantly driven by drought at the southern edge of the

564 species distribution (Camarero et al. 1998; Matías and Jump 2012) and by low temperatures

565 in boreal latitudes (Grace and Norton 1990; Persson and Beuker 1997; Matías and Jump

566 2012). However, there is less clear-cut information quantifying the contribution of plastic and

567 genetic responses to this variation. In our study, we estimated the amount of inter-population

568 genetic variation in radial growth to be about one-fifth and one-half the spatial and temporal

569 phenotypic variation respectively. As for WUE_i, this finding suggests preponderance of

570 plastic variation (especially at spatial level) over population differentiation, but with smaller

571 differences in the case of radial growth.

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Upscaling WUE$_i$ to the ecosystem level

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574   To understand the relevance of leaf-level WUE$_i$ in determining ecosystem-level WUE,

575   we attempted at scaling up our long-term spatial WUE$_i$ estimates based on tree rings (2000-

576   2007 period) using remote sensing data. This could be undertaken owing to comparatively

577   homogeneous stand conditions across sites. Indeed, the sampled forests were mature, even-

578   aged and unmanaged, with large basal areas and crown covers exceeding 65%, hence

579   suggesting comparable leaf area indices (Whitehead 1978).

580   We used high resolution (~1 km^2) estimates of GPP and ET derived from MODIS

581   satellite data, which are considered reliable at large (i.e. regional or continental) scales. The

582   use of MODIS GPP and ET at local (i.e. site) scale is instead more problematic. However,

583   reliable site-level estimates of MODIS GPP and ET have been reported in evergreen-needle

584   forests similar to our stands (Turner et al. 2006a; Turner et al. 2006b; Kim et al. 2012;

585   Cristiano et al. 2015). Anyhow, absolute values of productivity and evapotranspiration have

586   relatively little importance in our study, which is addressed to assess variation in carbon-water

587   balance among stands. In this regard, a bias in GPP or ET estimations, if present, would likely

588   be consistent across sites due to homogenous vegetation type and stand structure.

589   Although estimates of ecosystem WUE (EWUE) varied considerably among stands,

590   they were similar to values reported for evergreen-needle forests derived from either flux

591   tower measurements (Tang et al. 2014) or remote sensing data (He et al. 2017). It should be

592   noted, however, that EWUE is not able to directly catch the influence of available soil water

593   on ecosystem performance, which was instead the primary driver of variation in WUE$_i$ along

594   the study area. He et al. (2017) proposed a new indicator (SWUE, or ratio of gross

595   productivity to soil water content) to potentially improve the information on how ecosystems

596   use available water. In the present study, SWUE was calculated from remotely sensed data of

597   soil water content (SWC) previously validated for the north-eastern Iberian Peninsula (Merlin

598　et al. 2013). The derived SWUE values were much higher than those reported by He et al.

599　(2017). The authors found an average SWUE of 3.4 g C kg^{-1} H$_2$O for evergreen-needle

600　forests, while our values varied between 12.63 and 19.68 g C kg^{-1} H$_2$O. Such discordance

601　may be related to the very large difference in data resolution (1 km^2 in this work compared

602　with ~ 600 km^2 in He et al. [2017]), which may have influenced site-level SWUE estimation.

603　This issue, however, would require specific attention that is beyond the scope of our study.

604　　　None of EWUE, SWUE or IWUE (i.e. inherent WUE) (Beer et al. 2009) were related

605　to variation in WUE$_i$ among stands. On the other hand, when SWUE was corrected to account

606　for soil depth, the resulting SWUE' significantly correlated with WUE$_i$ ($p<0.01$), indicating a

607　tight relation between leaf-level physiology and gross productivity per unit of soil water

608　available. This outcome suggests that the range of variation in leaf-level WUE$_i$, as inferred

609　from tree rings, can be upscaled to ecosystem level if accounting for soil water holding

610　capacity *via* soil depth. Indeed, many studies have demonstrated the influence of soil water

611　availability on canopy conductance, an effect that is remarkably similar across tree species

612　(e.g. Granier et al. 2000). Our finding suggests a direct influence of plastic variation of WUE$_i$

613　of a major forest species in determining ecosystem water and carbon balances. On the other

614　hand, local factors controlling WUE$_i$ (such as rooting depth) must be taken into account when

615　scaling up from leaf to ecosystem WUE.

616

CONCLUSIONS

618　　　Accurate predictions of water-use efficiency changes in forests are crucial to

619　understand how climate change will affect terrestrial carbon and water balances. While the

620　two components −genetic and plastic− of phenotypic variation of WUE$_i$ can potentially

621　influence ecosystem WUE, our results point to a minor role of genetic differentiation in the

622　variation of WUE$_i$ in *P. sylvestris*. Instead, a maximum 33%−difference in WUE$_i$ among

26

stands indicates a strong spatial divergence in the water and carbon economy of pinewoods in a small area of *ca*. 5000 km^2. We also showed that long-term spatial differentiation in ecosystem WUE can be directly coupled to carbon uptake and stomatal responses for closed forest canopies in an isohydric species such as *P. sylvestris*. The key role of soil water holding capacity modulating WUE at both leaf and ecosystem levels, at least in *P. sylvestris*, emphasizes the necessity to incorporate belowground information when forecasting forest responses to climate (Ostle et al. 2009; van der Putten et al. 2013).

Although the importance of inter-population genetic variation of WUE$_i$ seems marginal for understanding *P. sylvestris* responses to environmental changes, this observation could not hold true for other widespread isohydric pines (e.g. *P. halepensis* or *Pinus pinaster*) in which strong genetic variation for this trait is acknowledged (Voltas et al. 2008; Aranda et al. 2010). Altogether, rigorous assessments of global change impacts on forests should consider potential additive effects of genetic variation and phenotypic plasticity, as well as their interactions, in modulating phenotypic variation in functional traits such as WUE$_i$ (Benito-Garzón et al. 2011; Klein et al. 2013).

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare no conflict of interest.

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FIGURES CAPTIONS

957

958 **Fig. 1** (a) Location of the provenance trial (Aragüés del Puerto, Spanish Pyrenees) (black

959 square) and geographic origin of the German (green squares) and Spanish (red squares)

960 populations evaluated in the trial. The climograph of the trial site is also included (left insert).

961 The right insert shows the study area in which the natural stands (blue circles) were sampled.

962 Sites chosen to build temporal chronologies (Arcalís and Seira) are indicated with empty blue

963 circles in the right insert. (b) Mean annual precipitation and temperature for the distribution

964 range of *P. sylvestris* in Europe (grey circles) calculated in 10' resolution grids from the

965 WorldClim database (period 1960-1990). The species range is derived from the EUFORGEN

966 distribution map (http://www.euforgen.org/species/pinus-sylvestris). Temperature and

967 precipitation of the trial site (black square), of German and Spanish sites of origin (red and

968 green squares), of natural stands (blue circles) and of two chronologies sites (open blue

969 circles) are shown

970

971 **Fig. 2** Mean intrinsic water-use efficiency (WUE$_i$) and tree-ring width (TRW) of Spanish and

972 German populations of the provenance trial. Error bars indicate standard errors. The asterisk

973 indicates a statistically significant difference at $p < 0.05$

974

975 **Fig. 3** Relationships between intrinsic water-use efficency (WUE$_i$) and soil depth (a) and

976 between WUE$_i$ and tree-ring width (TRW) (b) in the 30 natural stands composing the study

977 area. Each dot indicates one particular stand. Open dots indicate the two sites where temporal

978 chronologies were available. Error bars indicate standard errors

979

41

980 **Fig. 4** Bootstrapped correlations between intrinsic water-use efficiency (WUE$_i$, top panels) or

981 indexed tree-ring width (TRW$_i$, bottom panels) and precipitation, temperature and SPEI

982 drought index in Arcalís (1975-2008 period) and Seira (1980-2009 period). Correlations were

983 calculated on a monthly basis from October of the previous year to September of the current

984 year. Filled bars represent significant correlations

985

986 **Fig. 5** Relationships between WUE$_i$ and ecosystem water-use efficiency (EWUE) (a), soil

987 water-use efficiency (SWUE) (b), inherent water-use efficiency (IWUE) (c) and soil water-

988 use efficiency corrected for soil depth (SWUE') (d) in the 30 natural stands composing the

989 study area. Each dot indicates one particular stand. Open dots indicate the two sites where

990 temporal chronologies were available

991

992 **Fig. 6** Regression plot (negative exponential function) of WUE$_i$ on mean annual precipitation

993 combining records of *Pinus sylvestris* (present study; closed circles) and *Pinus halepensis*

994 stands (original data published in Ferrio and Voltas (2005); open circles) sampled across

995 north-eastern Iberian Peninsula. WUE$_i$ was estimated from carbon isotope discrimination

996 ($\Delta^{13}C$) of tree rings corresponding to different time periods (2000-2007 for *P. sylvestris*;

997 1975-1999 for *P. halepensis*). WUE$_i$ values were estimated using the mean atmospheric CO_2

998 concentration (C$_a$) for the period of 1975-2007 in order to allow a direct comparison of both

999 datasets. We assumed constant $\Delta^{13}C$ values over time (i.e. C$_i$/C$_a$ is maintained constant), as

1000 previously observed in the Iberian Peninsula for Scots pine (Voltas et al. 2013; Hereş et al.

1001 2014). The insert shows the regression plot of WUE$_i$ on the ratio of annual precipitation to

1002 annual potential evapotranspiration. Both regressions are significant at $p < 0.01$ (**)

TABLES

Table 1. Linear mixed-effects models for intrinsic water-use efficiency (WUE$_i$) and tree-ring width (TRW). Data were obtained from the provenance trial of Aragüés del Puerto, a set of 30 natural stands located in the central and eastern Pyrenees mountains and two tree-ring chronologies from the same area. For chronologies, variance components were estimated based on C_a-constant WUE$_i$ values or high-pass filtered TRW residuals (TRW$_{res}$). Only the random effects of the models are shown.

	Source of variation	Variance component	% of total variance	Chi-Square value[a]	Likelihood ratio test (*p*-value)[a]
WUE$_i$					
Provenance trial					
	Population	3.38±3.73[b]	11.20	0.87	*p*=0.35
	Population×Block	4.14±4.50	13.73	1.14	*p*=0.28
	Residual	22.68±3.87	75.07		
Natural stands					
	Site	34.47±12.37[c]	50.77	38.63	*p*<0.01
	Residual	33.42±5.41	49.23		
Chronologies					
	Tree [Site]	20.53±8.40	40.65	52.45	*p*<0.01
	Year	9.57±5.27[d]	18.94	2.45	*p*=0.11
	Year×Site	3.69±3.26[e]	7.30	3.52	*p*=0.06
	Residual	16.72±2.34	33.11		
TRW					
Provenance trial					
	Population	0.03±0.02[b]	3.97	3.70	*p*=0.05
	Population×Block	0.07±0.02	10.31	32.81	*p*<0.01
	Residual	0.58±0.03	85.72		
Natural stands					
	Site	0.15±0.05[c]	54.27	31.67	*p*<0.01
	Residual	0.13±0.02	45.73		
Chronologies					
	Tree [Site]	0.00±0.00	0.00	0.00	*p*=1.00
	Year	0.04±0.02[d]	10.11	7.64	*p*<0.01
	Year×Site	0.03±0.01[e]	7.30	30.66	*p*<0.01
	Residual	0.32±0.01	82.59		

[a] Test for the null hypothesis of variance component being equal to 0; [b] estimate of inter-population genetic variation; [c] estimate of spatial phenotypic variation; [d] estimate of temporal phenotypic variation; [e] estimate of spatiotemporal phenotypic variation

Electronic Supplementary Material

Online Resource 1 Characteristics of the 22 Scots pine populations evaluated in the provenance trial.

Online Resource 2 Characteristics of the 30 Scots pine natural stands.

Online Resource 3 Normal quantile plots of the ANOVAs residuals

Online Resource 4 Pearson correlations between WUE_i or TRW and climatic, edaphic and physiographic characteristics of the 30 Scots pine natural stands.

Online Resource 5 WUE_i and TRW chronologies.

Online Resource 6 Variance components estimated for absolute TRW and for WUE_i obtained from annually-resolved C_a values

Figure 1

Figure 2

Figure 3

Figure 4

Click here to access/download;Figure;Fig. 4.tif ±

Figure 5

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Figure 6
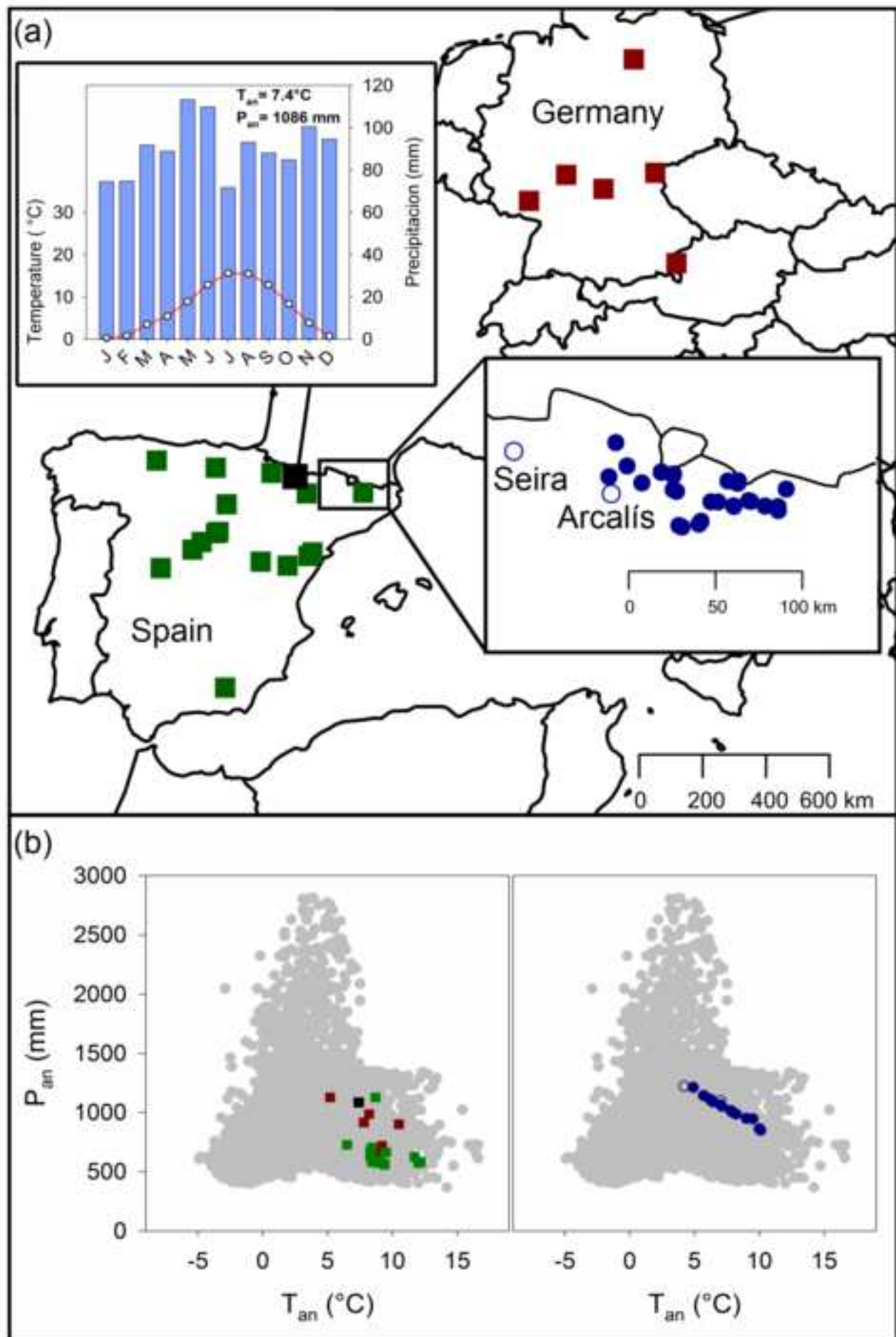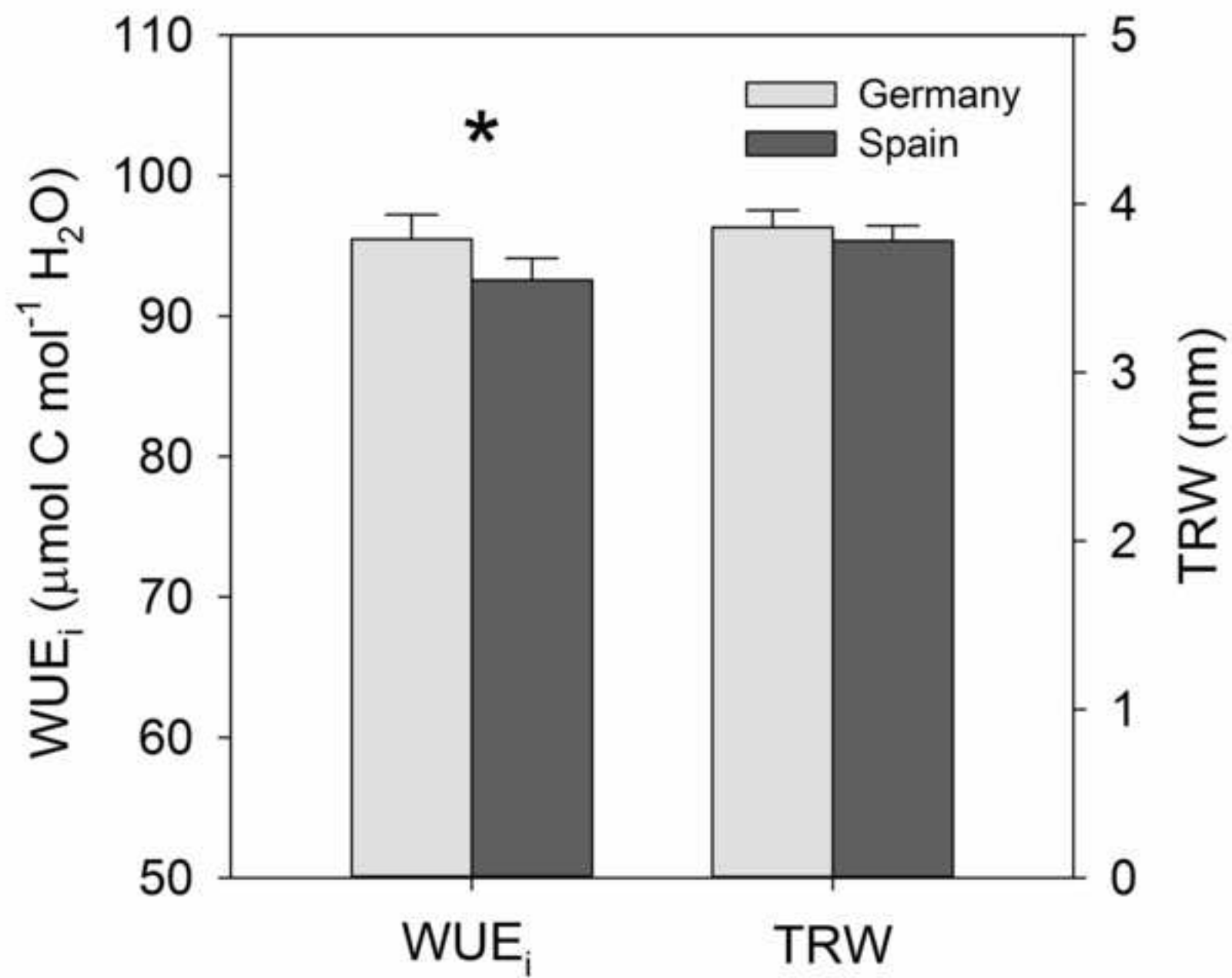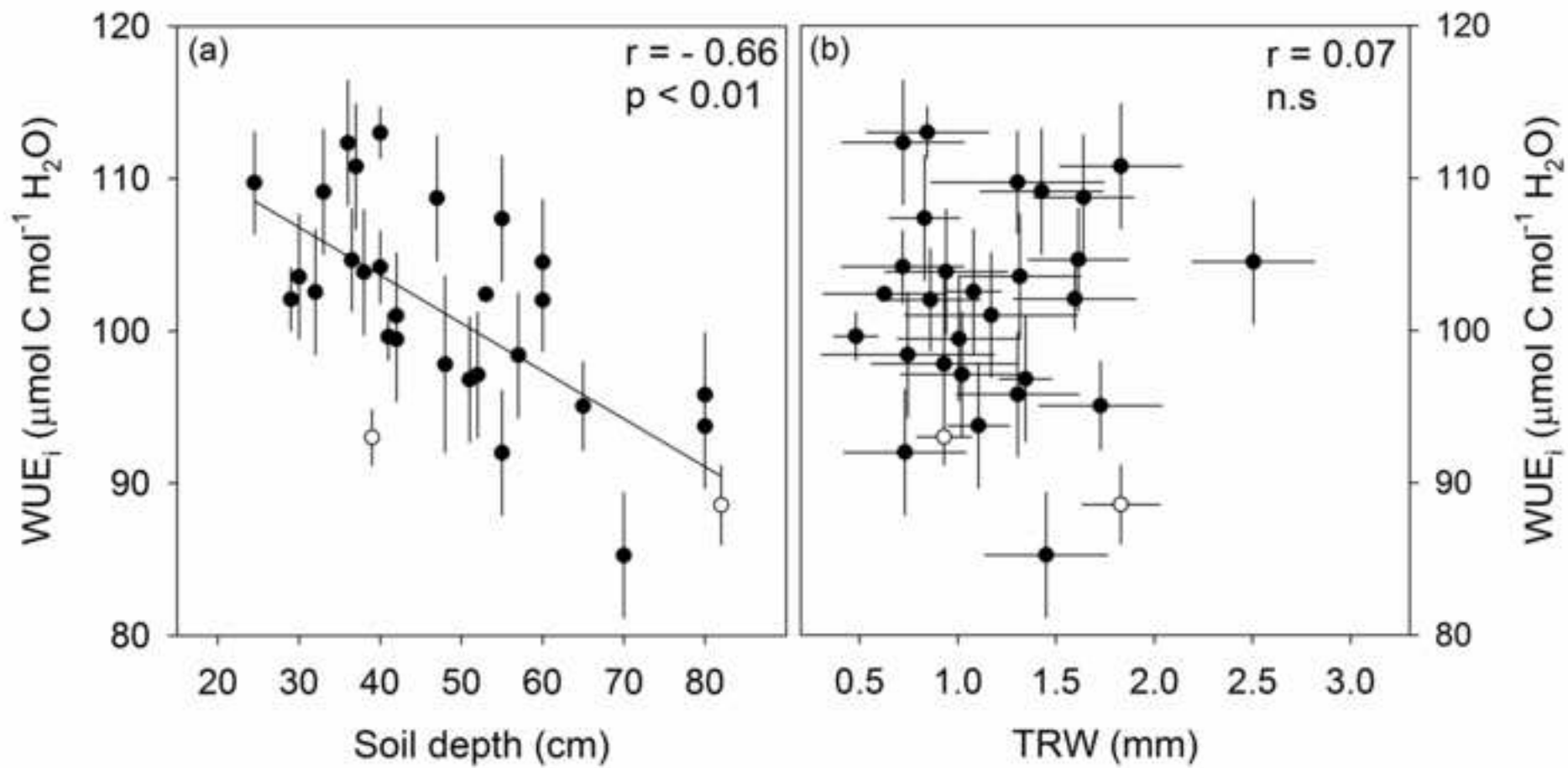
Title Scarce population genetic differentiation but substantial spatiotemporal
phenotypic variation of water-use efficiency in *Pinus sylvestris* at its western distribution

Authors: F. Santini, J.P. Ferrio, A-M. Hereş, E. Notivol, M. Piqué, L. Serrano, T.A. Shestakova, E. Sin, P. Vericat, J. Voltas

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Main characteristics of the 22 populations of *Pinus sylvestris* evaluated in the provenance trial of Aragüés del Puerto (Spanish Pyrenees). Data refer to the site of origin of each population.

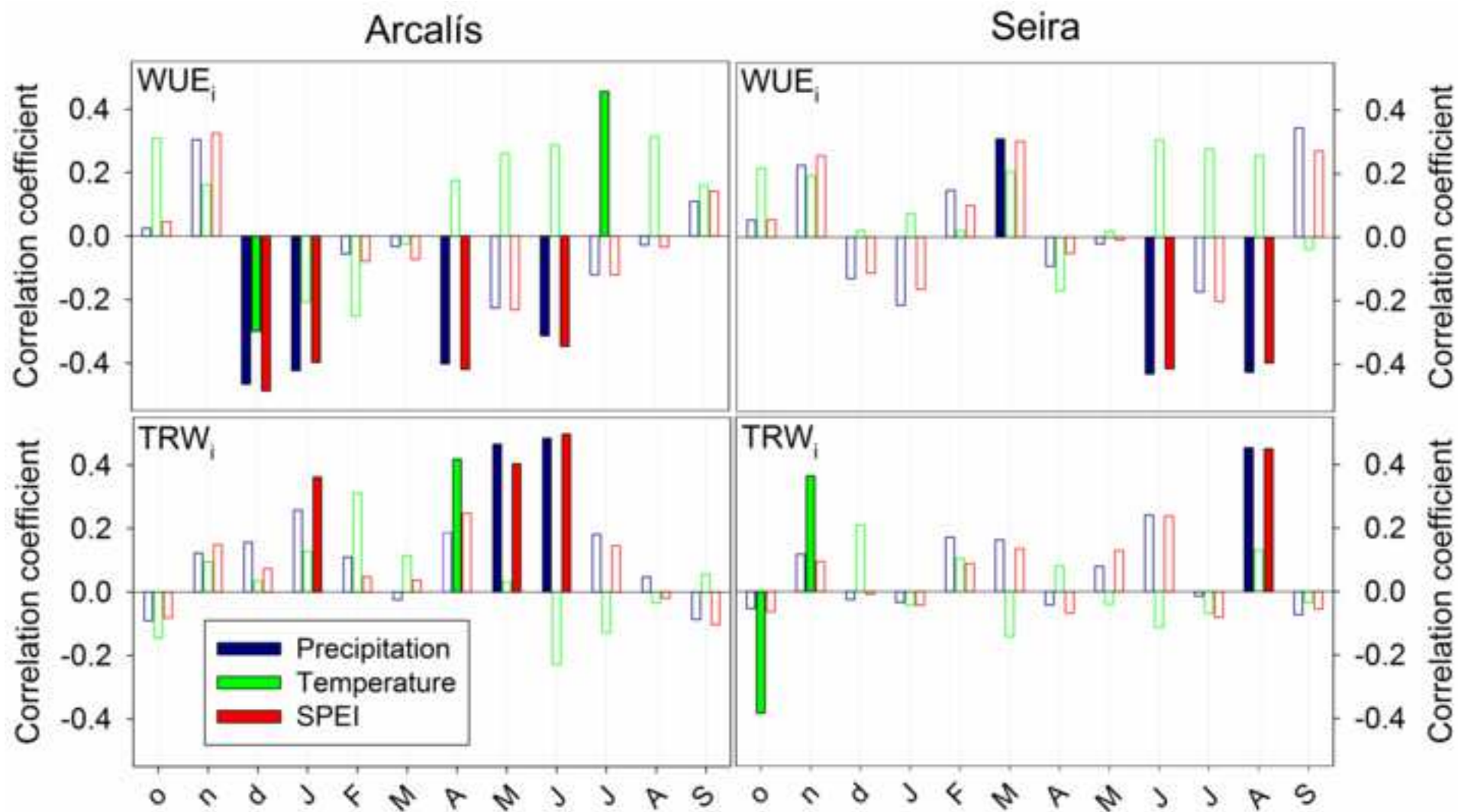| Country	Provenance	Latitude	Longitude	Elevation (m a.s.l.)	T_{an} (°C)	P_{an} (mm)	P_s (mm)	PET_{an} (mm)
Germany	Gartow	53° 02' 03" N	11° 25' 03" E	50	8.7	582	186	698
Germany	Laufen	47° 57' 08" N	12° 54' 00" E	430	8.7	1113	423	839
Germany	Otterberg	49° 30' 03" N	07° 45' 02" E	300	8.5	681	205	764
Germany	Selb	50° 12' 03" N	12° 10' 03" E	570	6.5	702	235	683
Germany	Wiesentheid	49° 48' 03" N	10° 21' 03" E	220	8.8	611	203	793
Germany	Wolfgang	50° 09' 03" N	09° 03' 02" E	177	9.5	662	204	795
Spain	Baza	37° 22' 31" N	02° 51' 34" W	2050	8.1	783	96	1000
Spain	Borau	42° 41' 56" N	00° 34' 44" W	1550	5.9	1093	238	737
Spain	Campisábalos	41° 14' 54" N	03° 05' 34" W	1400	8.5	591	113	930
Spain	Castell de Cabres	40° 38' 57" N	00° 03' 19" E	1150	10.4	649	132	876
Spain	Covaleda	41° 56' 41" N	02° 48' 40" W	1550	7.6	748	147	889
Spain	Galve de Sorbe	41° 13' 14" N	03° 10' 45" W	1400	8.9	592	105	938
Spain	Gúdar	40° 24' 41" N	00° 41' 04" W	1700	7.3	710	163	815
Spain	La Sènia	40° 44' 50" N	00° 11' 29" E	1100	9.9	638	142	858
Spain	Morrano	42° 12' 42" N	00° 05' 54" W	700	8.9	593	178	851
Spain	Navafría	41° 00' 20" N	03° 47' 56" W	1600	10.6	679	78	976
Spain	Navarredonda	40° 21' 18" N	05° 06' 50" W	1550	8.6	603	84	950
Spain	Orihuela	40° 30' 47" N	01° 37' 46" W	1750	6.9	691	150	874
Spain	Pobla de Lillet	42° 13' 46" N	01° 57' 30" E	1100	9.7	895	245	788
Spain	Puebla de Lillo	43° 03' 35" N	05° 15' 12" W	1550	8	1005	161	850
Spain	San Zadornil	42° 51' 11" N	03° 11' 43" W	1000	9.8	935	162	837
Spain	Valsaín	40° 48' 43" N	04° 00' 33" W	1550	8.3	589	100	911

T_{an} (mean annual temperature); P_{an} (total annual precipitation); P_s (June to August precipitation); PET_{an} (annual potential evapotranspiration)

Title Scarce population genetic differentiation but substantial spatiotemporal
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Main characteristics of the 30 *Pinus sylvestris* natural stands composing the study area (central/eastern Pyrenees, NE Spain).

Site	Latitude	Longitude	Elevation (m a.s.l.)	T_{an} (°C)	P_{an} (mm)	P_s (mm)	PET_{an} (mm)	Basal area (m^2 ha^{-1})	Density (tree ha^{-1})	Crown cover (%)	Height (m)	DBH (cm)	Age (year)	Rocky outcrops (%)	Rock fragments in soil (%)
Alàs i Cercs (I)	42° 19' 26" N	01° 30' 32" E	1130	9.2	914	256	804	46.2	2500	75	12.5	15.3	50	0	15
Alàs i Cercs (II)	42° 18' 54" N	01° 31' 55" E	1391	7.6	1021	270	741	56.4	1850	80	11.8	19.7	58	0	20
Alp (I)	42° 22' 08" N	01° 53' 24" E	1356	8.9	949	256	773	42.7	1500	80	15.2	19	48	0	40
Alp (II)	42° 21' 25" N	01° 57' 32" E	1468	7	1065	267	705	29.7	725	72	16.6	22.8	64	30	20
Arcalís	42° 22' 24" N	01° 11' 00" E	1150	7.1	1058	276	810	10	1070	50	10.9	26.5	80	20	20
Castellar de n'Hug	42° 16' 01" N	02° 02' 06" E	1314	8.7	968	252	755	37.5	1100	70	12.3	20.8	76	0	5
Codó	42° 08' 17" N	01° 32' 56" E	1300	7.9	991	258	747	44.2	1760	80	20.2	37.5	62	0	35
Espot	42° 34' 05" N	01° 06' 36" E	1324	7.6	1059	282	764	51.7	1219	75	17.7	23.4	54	5	20
Estamariu (I)	42° 24' 18" N	01° 30' 43" E	1609	6.3	1106	283	697	45.7	950	32	15.6	24.7	96	0	60
Estamariu (II)	42° 23' 53" N	01° 30' 36" E	1558	6.8	1075	279	716	46.8	2100	80	12.1	16.8	51	0	50
Fontanals (I)	42° 21' 43" N	01° 56' 42" E	1702	6.2	1117	274	678	43.1	950	65	14.7	24	56	10	20
Fontanals (II)	42° 22' 12" N	01° 57' 43" E	1265	6.3	1111	272	681	43.1	650	70	17.7	29.1	56	5	40
Gisclareny (I)	42° 15' 40" N	01° 46' 19" E	1405	8.7	950	256	767	50.3	1500	80	15.8	20.7	50	5	30
Gisclareny (II)	42° 15' 36" N	01° 49' 16" E	1082	9.8	882	247	803	36.3	1600	70	14	17	67	0	40
Gombreny	42° 15' 47" N	02° 02' 53" E	1242	8.9	957	250	761	41.2	1250	80	15.5	20.5	48	0	20
Guils	42° 21' 32" N	01° 17' 28" E	1463	7.2	1048	276	740	58	1050	70	18.7	26.5	76	5	65
Guixers (I)	42° 09' 36" N	01° 42' 11" E	1523	7.1	1047	264	712	22.8	550	70	16	23	67	0	50
Guixers (II)	42° 08' 42" N	01° 41' 17" E	1112	9.6	884	244	801	45	1100	75	16.6	22.8	70	0	5
Pobla de Lillet (I)	42° 14' 24" N	01° 55' 44" E	818	11.2	804	235	843	33.6	800	65	19.8	23.1	84	5	10
Pobla de Lillet (II)	42° 14' 13" N	01° 55' 55" E	803	11.1	810	236	840	52.1	750	65	24.4	29.7	74	5	35
Queralbs	42° 14' 20" N	02° 98' 53" E	1552	10.6	869	240	810	70.4	1550	75	15.2	24.1	51	5	35
Rialb	42° 26' 49" N	01° 11' 17" E	1671	6.5	1100	285	717	51.9	1750	80	12.7	19.4	58	15	15
Sant Llorenç	42° 07' 48" N	01° 34' 12" E	1075	9.7	873	242	810	25.2	700	75	15.2	21.4	69	35	35
Seira	42° 31' 24" N	0° 23' 01" N	1538	6.1	1085	267	810	76.2	1025	75	17.7	37.38	66	20	10
Soriguera	42° 21' 00" N	01° 05' 20" E	954	9.7	901	252	854	35.8	750	70	15.1	24.7	61	0	40
St Joan Abadesses (I)	42° 12' 58" N	02° 14' 24" E	921	11.4	849	235	830	30.3	500	70	17.7	27.8	94	20	0
St Joan Abadesses (II)	42° 14' 10" N	02° 14' 20" E	999	10.8	877	239	813	22.7	1162	65	10.3	15.9	72	0	10
Vallfogona	42° 19' 37" N	02° 17' 46" E	1407	8.6	995	247	744	35.5	700	75	18.2	25.4	49	0	0
Valls de Valira (I)	42° 24' 50" N	01° 25' 34" E	1357	7.5	1035	275	744	33.5	700	70	15	24.7	101	0	35
Valls de Valira (II)	42° 24' 58" N	01° 25' 34" E	1451	7.6	1026	274	751	36	2500	70	14.3	13.5	125	0	45

T_{an} (mean annual temperature); P_{an} (total annual precipitation); P_s (June to August precipitation); PET_{an} (annual potential evapotranspiration)
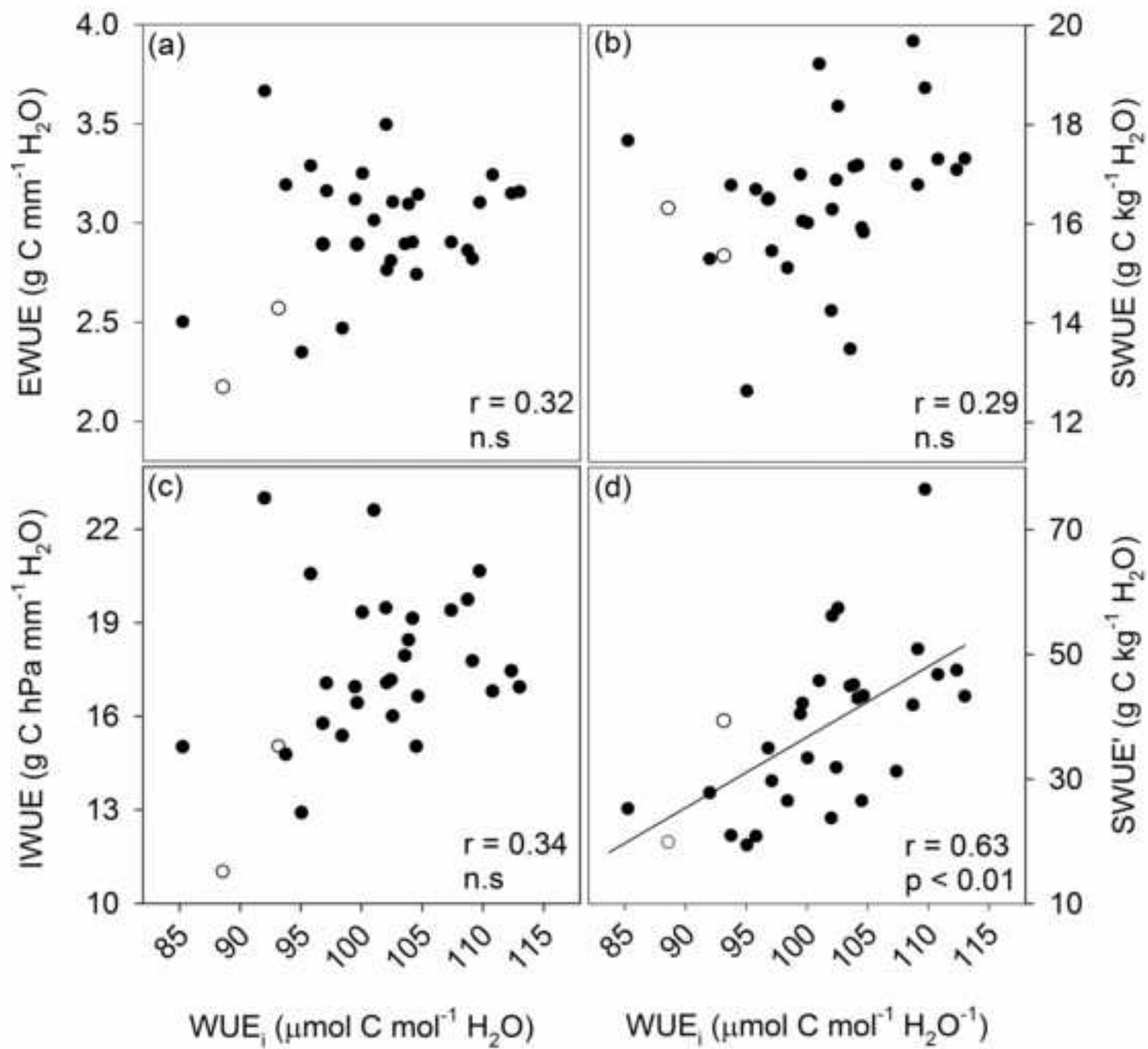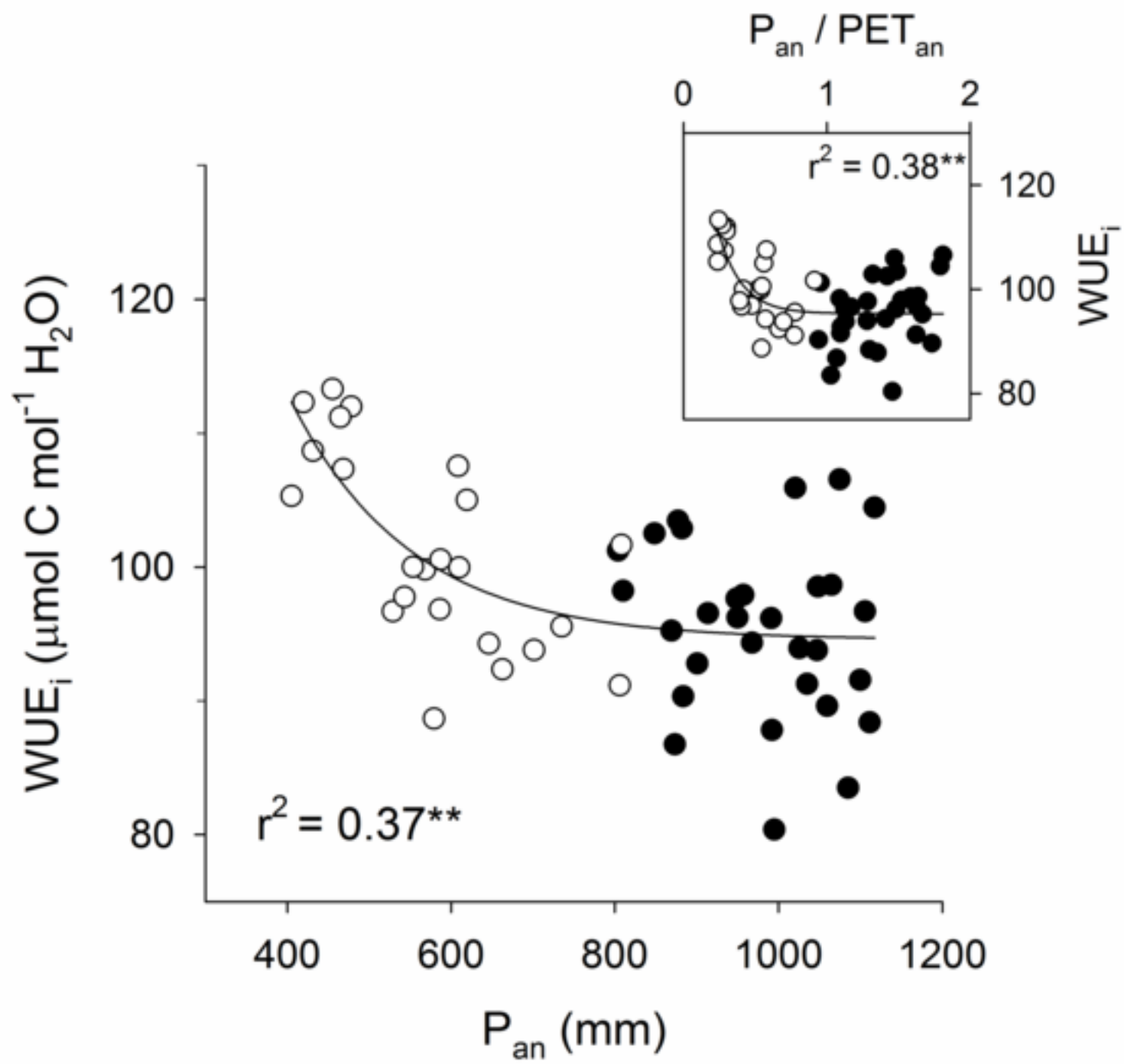
European Journal of Forest Research

Title: Scarce population genetic differentiation but substantial spatiotemporal phenotypic variation of water-use efficiency in *Pinus sylvestris* at its western distribution range

Authors: F. Santini, J.P. Ferrio, A-M. Hereş, E. Notivol, M. Piqué, L. Serrano, T.A. Shestakova, E. Sin, P. Vericat, J. Voltas*

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Figure S1. Normal quantile plots of the residuals of the ANOVAs fitted on WUE_i (a) and TRW (b) in the provenance trial, WUE_i (c) and TRW (d) in the natural sampled sites and WUE_i (e) and TRW (f) in the temporal chronologies

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Pearson correlations between either intrinsic water-use efficiency (WUE_i) or tree-ring width (TRW) and climatic, edaphic and physiographic site characteristics for 30 natural stands of *P. sylvestris*.
Significant correlations ($p < 0.05$) are highlighted in bold.

Variable	r (WUE_i)	p-value	r (TRW)	p-value
Elevation	-0.08	0.66	-0.29	0.12
T_{an}	0.22	0.23	0.19	0.33
T_{max}	0.19	0.31	0.21	0.26
T_{min}	0.25	0.18	0.16	0.41
P_{an}	-0.19	0.3	-0.25	0.18
P_s	-0.1	0.61	-0.05	0.79
P_s/P_{an}	0.28	0.13	**-0.55**	**<0.01**
PET_{an}	0.06	0.77	**-0.4**	**0.03**
Soil depth	**-0.66**	**<0.01**	0.05	0.79
Basal area	0.2	0.31	-0.02	0.93
Density	0.28	0.15	**-0.43**	**0.02**
Slope	-0.09	0.64	-0.23	0.24
Position	-0.03	0.87	-0.13	0.51
Aspect	-0.1	0.61	-0.09	0.67
Soil carbonates	0.22	0.27	-0.1	0.62
Height[a]	-0.18	0.33	0.15	0.42
Age[a]	-0.15	0.12	**-0.39**	**< 0.01**
TRW[a]	-0.04	0.66		

T_{an} (mean annual temperature); T_{max} (average maximum annual temperature); T_{min} (average minimum annual temperature);
P_{an} (total annual precipitation); P_s (June to August precipitation); PET_{an} (annual potential evapotranspiration)
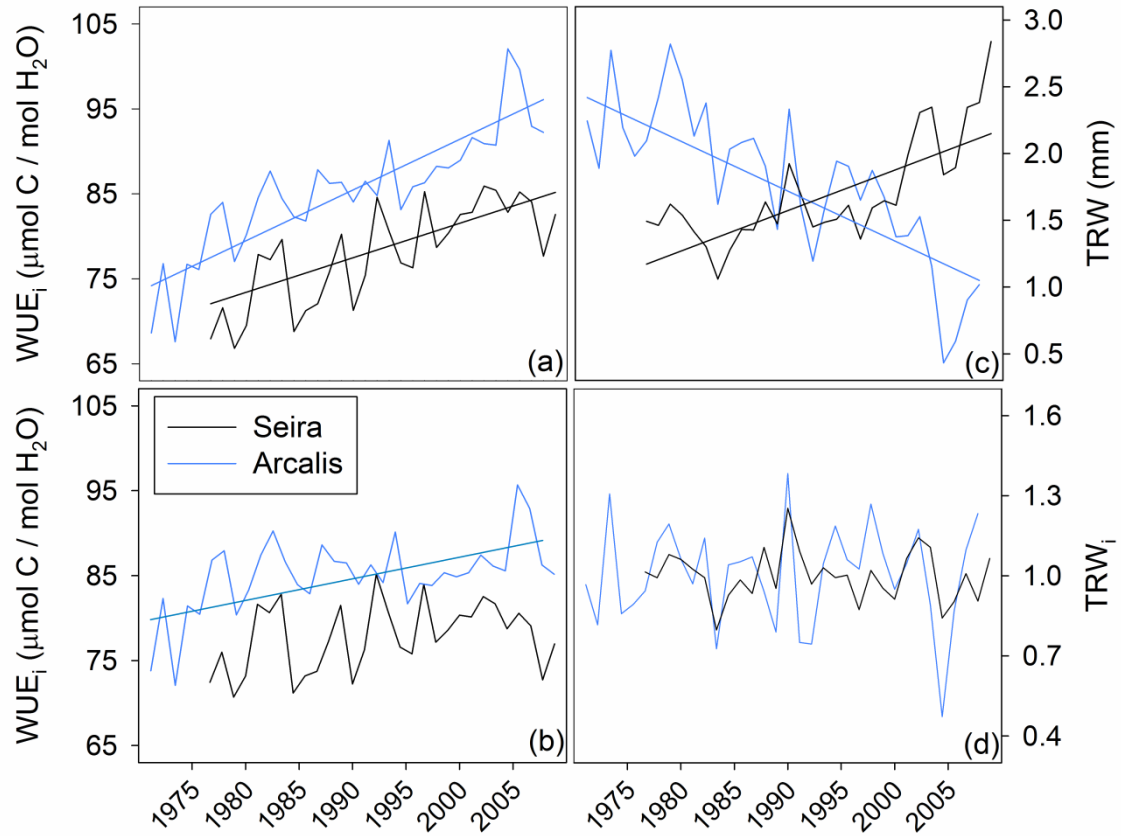
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Figure S2. Intrinsic water-use efficiency (WUE$_i$) and tree-ring width (TRW) chronologies in Seira and Arcalís. Left panels show WUE$_i$ time series estimated using either annually-resolved C$_a$ values (a) or the average C$_a$ over the study period (b). Right panels show absolute TRW records (i.e. not high-pass filtered) (c) and TRW indices (TRW$_i$) after high-pass filtering (d). Significant linear trends are depicted with lines ($p <$ 0.05).

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Linear mixed-effects models for intrinsic water-use efficiency (WUEi) and tree-ring width (TRW) for the provenance trial of Aragüés del Puerto, a set of 30 natural stands located in the central and eastern Pyrenees mountains and two temporal chronologies from the same area. For chronologies, variance components were estimated based on annually-resolved Ca WUEi values or absolute TRW records (not high-pass filtered) Only the random effects of the models are shown.

	Source of variation	Variance component	% of total variance	Chi-Square value[a]	Likelihood ratio test (p-value)[a]
WUE$_i$					
Provenance trial					
	Population	3.38±3.73[b]	11.2	0.87	p=0.35
	Population×Block	4.14±4.50	13.73	1.14	p=0.28
	Residual	22.68±3.87	75.07		
Natural stands					
	Site	34.47±12.37[c]	50.77	38.63	p<0.01
	Residual	33.42±5.41	49.23		
Chronologies[b]					
	Tree [Site]	20.89±8.58d	25.56	102.56	p<0.01
	Year	40.77±12.57e	49.88	19.96	p<0.01
	Year×Site	3.00±2.47	3.67	3.25	p=0.07
	Residual	17.07±2.51	20.89		
TRW					
Provenance trial					
	Population	0.03±0.02[b]	3.97	3.7	p=0.05
	Population×Block	0.07±0.02	10.31	32.81	p<0.01
	Residual	0.58±0.03	85.72		
Natural stands					
	Site	0.15±0.05[c]	54.27	31.67	p<0.01
	Residual	0.13±0.02	45.73		
Chronologies					
	Tree [Site]	0.55±0.10[d]	40.64	1128.67	p<0.01
	Year	0.00±0.00[e]	0	0	p=1.00
	Year×Site	0.25±0.05	18.45	346.96	p<0.01
	Residual	0.55±0.02	40.91		

[a] Test for the null hypothesis of variance component being equal to 0; [b] estimate of inter-population genetic variation;

[c] estimate of spatial phenotypic variation; [d] estimate of temporal phenotypic variation; [e] estimate of spatiotemporal phenotypic variation