



Latitudinal variation in the functional response of *Quercus suber* seedlings to extreme drought



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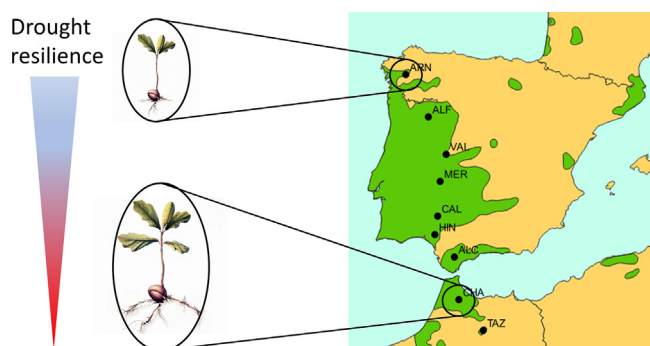
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HIGHLIGHTS

- Local adaptations might reduce the impact of increased drought on plant populations.
- We test drought effects on *Q. suber* across its complete distribution range.
- We investigate the role of local adaptations through functional trait-based approach.
- Southern plants were bigger which implied higher water loss and reduced survival.
- This study identifies drought-resistant *Q. suber* populations and morphological traits.

GRAPHICAL ABSTRACT



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ABSTRACT

Many plant species are being threatened by increasingly drought conditions due to current climate change at planetary scale. This global trend is leading to the scientific community to investigate the potential role of local adaptations through intraspecific differences in functional traits that may boost conservation strategies by modulating the plant responses to reduced water availability. We assessed under controlled conditions the effect of four different drought intensities on the survival time and morphological traits of *Quercus suber* seedlings collected from nine populations covering the complete latitudinal distribution of the species. Functional morphological traits related to biomass allocation and leaf and root display were analyzed. We then related these traits with the survival time after a terminal desiccation, used as a drought-resistance proxy and expressed as survival time without watering. Abundant watering availability allowed seedlings to survive for a longer period compared to drier conditions. Further, all morphological traits differed across watering levels, showing a very plastic response. Acorns from southern latitudes produced very large seedlings compared to those gathered from northern latitudes. However, the larger biomass implied higher evaporative water loss, inducing lower survival of southern populations under extreme drought conditions. We further found a clear trend toward maximizing those traits related with belowground growth (i.e., root surface area, root average diameter and root volume) in southern populations aimed to increase water uptake, overcoming the most limiting factor for plant growth in that area. Our results support that increased root development allow cork oak to maintain its functioning after being subjected to damage caused by reduced water availability, whereas high aerial biomass allocation is a handicap for survival under drought stress conditions. This study identifies drought-resistant populations and morphological traits related to drought resistance, which can be applied to improve restoration actions under a warmer climate.

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1. Introduction

Climate is regarded as a major driver of changes in ecosystems and biodiversity (IPBES, 2019) and is increasingly becoming an overwhelming environmental stressor (Willeit et al., 2019). The Mediterranean basin, hotspot of biodiversity (Myers et al., 2000) but also of vulnerability (Ochoa-Hueso et al., 2017), is one of the European regions expected to be most impacted by climate change. The latest IPCC report highlighted that the Mediterranean region is currently getting warmer and drier (Masson-Delmotte et al., 2021). Droughts are forecasted to occur more often, being of greater duration and intensity, aggravated by the situation that they might not be followed by wet winters in some areas as the Iberian Peninsula (Böhnisch et al., 2021). As summer drought is one of the most important bottlenecks for plant establishment in the Mediterranean region (e.g. Pérez-Ramos et al., 2012), the overall decrease in water availability resulting from increased evapotranspiration and reduced rainfalls might pose a threat to the future of plant communities in these areas. Namely, drought stress has negative effects on both plant morphology (limiting the stem length, leaf size and vegetative growth) and physiology (reducing the leaf chlorophyll and increasing the secondary metabolites and antioxidants) (Pérez-Girón et al., 2022).

The projected abrupt increase of extreme climatic events is expected to have many negative implications for Mediterranean tree species, which have developed different mechanisms to cope with environmental changes (Limousin et al., 2012; Pérez-Ramos et al., 2013; Soheili et al., 2023). Increased phenotypic plasticity and intra-specific genetic variation for adaptive traits (i.e., local adaptations) seem to be the most relevant strategies to this end (Matesanz and Valladares, 2014; Pérez-Ramos et al., 2019). Phenotypic plasticity represents the ability of a genotype to express a range of different phenotypes adapted to certain environmental conditions (Bongers et al., 2017; Bradshaw, 1965), enabling to respond to potential environmental changes, which is essential for the acclimation of plant species (Bussotti et al., 2015). Local adaptations are the results of natural selection acting over a number of genotypes better fitted to local environmental conditions, which entail competitive advantage regarding other genotypes (Peguero-Pina et al., 2014; Savolainen et al., 2007). However, the way in which tree species can take advantage of these two mechanisms to cope with the ongoing changes in climate and their relative importance is still

unclear, especially in Mediterranean areas where number of studies are scarce compared to more mesic ecosystems.

A very common procedure to mimic the environmental conditions imposed by climate change is the functional analysis of natural plant populations coming from areas with contrasting environmental conditions. This method allows to assess the phenotypic responses of various populations by identifying which tree populations grow better and are more resistant to adverse climatic conditions through the analyses of key morphological characteristics commonly used as indicators of tree responses to environmental constraints (Pérez-Harguindeguy et al., 2013). In tree species whose natural distribution encompass a wide range of environmental conditions and geographic gradients, it can be expected that long-term natural selection and genetic drift have led to a high level of genetic variation among populations concerning adaptive traits (Magri et al., 2006). Thus, these populations could have developed morphological adaptations to local conditions giving rise to ecotypic differentiation in several key functional traits (Kawecki and Ebert, 2004; Savolainen et al., 2007).

Cork oak (*Quercus suber* L.) is an evergreen sclerophyllous tree species native to western Mediterranean region, mainly distributed along southwest Europe and northwest Africa (Fig. 1). It is well adapted to non-severe droughts and high temperatures, being regarded as a transitional species between the high drought tolerant evergreen oaks (such as *Quercus ilex* or *Q. coccifera*, David et al., 2007) and the mesic deciduous oaks (such as *Q. faginea* or *Q. pyrenaica*, Mediavilla and Escudero, 2004). Cork oak forests play an important ecological role in key ecosystem functions such as net primary production, carbon sequestration, soil formation, hydrological flow regulation and reservoir for biodiversity (Bugalho et al., 2011; Torralba et al., 2016; Reis et al., 2021), while they are also of critical economic importance due to their large contribution to the global cork market (Pereira, 2007). Even though cork oaks can adapt to environmental conditions and their genetic variability allow them to cope with climatic variation (Leite et al., 2019), the ongoing climate change scenarios of higher water deficit in combination with other natural and human-induced factors such as diseases caused by exotic pathogens are alarmingly threatening their natural populations (Aguilera et al., 2020; González et al., 2020; Serrano et al., 2021). As a result, a severe reduction in cork oak forests has been occurring over the last decades (Costa et al., 2009; Pinto-Correia et al., 2011) and they have recently been included as a natural habitat

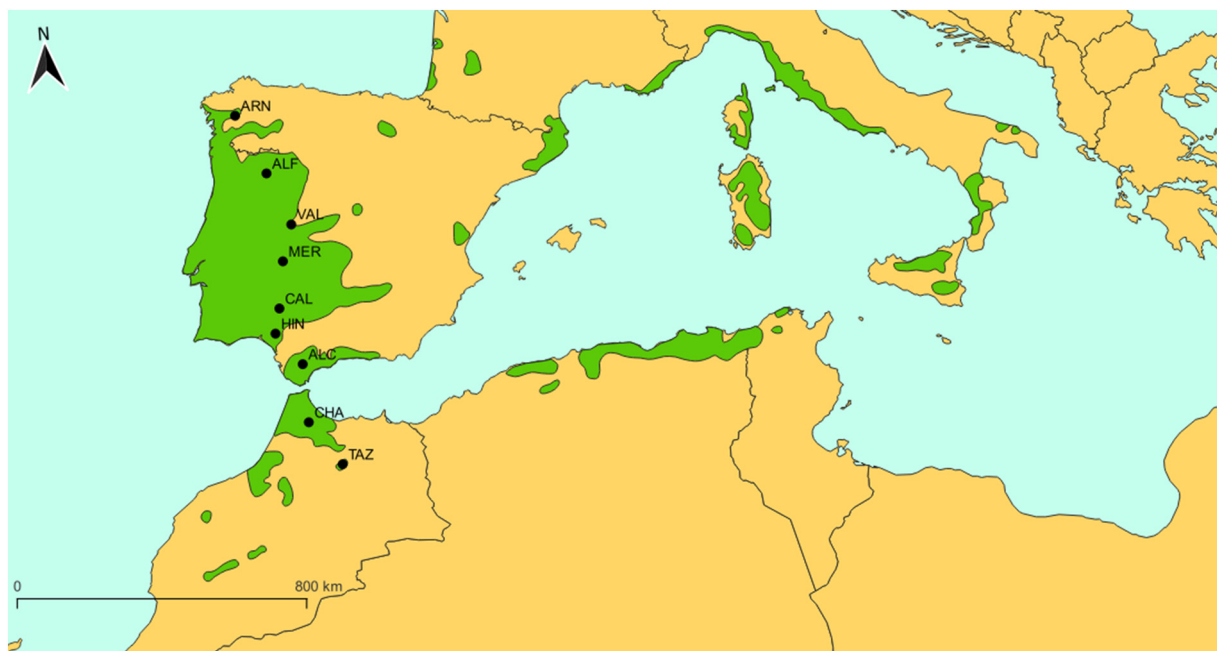


Fig. 1. Distribution map of *Quercus suber*. The map shows the native range of *Quercus suber* (green area) and the location of the source populations are indicated by black dots (see population names and characteristics in Table A.1). Distribution map obtained from Caudullo et al. (2017).

type of community-wide interest within the EU Habitats Directive, which stress both their value and fragility.

Adaptations developed by cork oaks to deal with Mediterranean summer drought are related to increased water acquisition capacity such as higher biomass investment in the root system through thinner and deeper roots that allow to explore higher soil volumes (David et al., 2007; Piayda et al., 2014), or higher efficiency in the use of water as a better stomatal control (Besson et al., 2014; David et al., 2007). Variations in these functional traits imply a clear advantage for tree survival (Gimeno et al., 2009; Matías et al., 2014) and could be locally selected at the most arid areas within the distribution range of a tree species. Thus, the identification of adaptive morphological traits along natural environmental gradients could be of paramount importance for exploration of the plastic responses of cork oaks aimed to increase the resistance and resilience of their populations under future scenarios of increased aridity.

The main goals of this study were to (i) analyze the functional responses of natural populations located along the cork oaks' latitudinal distribution to extreme droughts and to (ii) explore the morphological traits involved in drought resistance and their prevalence along the species' distribution. To do so, we carried out a greenhouse common-garden experiment using acorns coming from nine populations of cork oak spanning the complete latitudinal distribution of the species, and their resulting seedlings were further subjected to different levels of water availability. Survival time after a terminal drought was used as an indicator of drought resistance and was related to the different morphological traits to determine their role in the response to drought stress. This approach is useful to detect the most vulnerable populations to water stress and provides new tools to be applied in plan management strategies aimed to mitigate the negative consequences of climate change on tree populations. We hypothesized that (i) high water availability during the growing season will result in increased survival and traits maximizing aboveground growth but reducing root development; (ii) populations located in drier areas will invest more resources in water uptake (e.g. through higher root biomass); and (iii) morphological traits related to water uptake such as higher development of the root system are expected to be significantly related to drought resistance. Finally, we predicted that (iv) populations located close to the southern species' distribution limit will have developed local adaptations to aridity and will harbor drought-resistant genotypes, which imply some kind of competitive advantage with respect to other genotypes, making them less vulnerable to the expected changes in climate compared to populations located in the central or northern parts of the distribution.

2. Materials and methods

2.1. Study species

Focal species in this study was cork oak (*Quercus suber* L.), a sclerophyllous evergreen tree species with a wide distribution in the western area of the Mediterranean region. The densest cork oak forests are located at the south-western part of the Iberian Peninsula and at the north of Morocco, with more disperse populations toward the northern part of the distribution (Fig. 1). The wide distribution of this species was exploited to assess local adaptations to drought by selecting nine populations along its latitudinal gradient distribution and separated approximately 1° in latitude (Fig. 1, Table A.1). In late 2020 and following the natural ripening period of the species across its distribution range, five trees separated by a minimum of 50 m were selected per population to cover the spatial variability of the sampling area, and 50 mature acorns per tree were collected. Viability of acorns was tested by the flotation method following Gribko (1995), and viable acorns were stored in wet vermiculite at 5 °C until sowing.

2.2. Experimental design

This experiment was conducted from January to September 2021, at the greenhouse facilities of the University of Seville (CITIUS II), located at the city of Seville (Spain; 37°21'31"N 5°59'13"W). These facilities allowed to

maintain stable temperature throughout the experiment (25 °C at day and 15 °C at night) and mimic the light conditions of forest understory (PAR 516.7 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 50.13$) with natural daily duration in Seville. The experiment followed the natural phenology of the species, accounting for the first spring and summer of life of cork oak seedlings, which is the most critical stage for the establishment of Mediterranean woody species (Mendoza et al., 2009; Pérez-Ramos et al., 2012). In January 2021, a total of 360 experimental pots (15 cm in diameter and 20 cm height) were filled with a mixture of peat and sand in a ratio of 3 to 1 per volume, to allow a non-limiting nutrient availability and adequate drainage. As seed size strongly vary geographically in this species (Matías et al., 2019), all acorns were individually weighed before sowing. One individual acorn was sown per pot at 2 cm depth, 8 pots per mother tree, making a total of 40 pots per latitudinal population. To promote mycorrhization, all pots were watered with 300 ml of a soil microbial inoculum obtained from the maceration of 10 kg of soil collected under the canopy of *Q. suber* on 100 l of water for 48 h (Matías and Jump, 2014). This soil was gathered from a population central to the latitudinal gradient of distribution (Doñana National Park, SW Spain), which was previously analyzed to assure the absence of the soil-borne pathogen *Phytophthora cinnamomi* according to the protocol developed by Sánchez et al. (2002). Soils in all pots were irrigated at 100 % of water holding capacity (WHC) until 80 % of the seedlings emerged, which occurred after 13 weeks (April 2021).

Following this initial stage in which water availability was not a limiting factor, the initial 40 seedlings per provenance were subjected to four different precipitation scenarios, each of them with 10 replicates: 1) 100 % of WHC, simulating a rainy spring season where soils remain wet most of the time, corresponding with a weekly irrigation until field capacity; 2) 50 % of WHC, simulating average water availability conditions in a cork oak forest during the growing season (Pérez-Ramos and Marañón, 2012); 3) 25 % of WHC, simulating a reduction of ca. 30 % over the previous scenario and according with the projections of the A1B scenario for the Mediterranean region at the end of the current century (Giorgi and Lionello, 2008); 4) 10 % of WHC, simulating an exceptionally dry spring, an extreme event that is expected to increase in frequency during the coming decades (Mildrexler et al., 2018). Seedlings were subjected to watering treatments by adding a constant amount of water to all pots within the same watering level from 12 April to 1 July 2021, simulating the natural growing season.

After this watering treatment application, seedlings were subjected to a progressive drought until death (terminal drought henceforth) to determine seedling resistance to extreme drought across provenances and water availabilities. To do so, watering was stopped from this point onwards, simulating the arrival of summer. Soil volumetric water content was weekly measured in all pots using a TDR probe (ML3 ThetaProbe, Delta-T, Cambridge) at the top 10 cm. The position of every pot was rotated monthly to avoid the effect of possible small differences in environmental conditions. Seedling survival was weekly monitored until all seedlings were dead, being progressively harvested as they died. Terminal soil water content (TSWC), defined as soil moisture measured at the time of the plant death, was recorded for every seedling.

2.3. Measurements of morphological traits in response to drought

At the end of the watering period, five fully developed leaves were collected and scanned from five random seedlings per provenance and watering treatment combination for the determination of leaf area using the software Image-Pro Plus v 4.5 (Media Cybernetics, Silver Spring, MD, USA). Then, all leaf samples were oven-dried at 60 °C for 72 h until constant weight. This procedure allowed for computation of specific leaf area (SLA).

Once harvested at the end of the experiment, dead seedlings were separated into stem, leaf and root fractions. We measured maximum length of the shoot and root with a rule, and weighted separately the dry biomass of each fraction (shoot, leaves and roots) using a precision balance (AX-80, Cobos Precision S.L, Barcelona). All roots were scanned using a Root Scanner STD4800 (Reagent Instruments Inc., Canada) and their

morphology was analyzed using the software WinRhizo (Reagent Instruments Inc., Canada). Based on these primary data, a set of morphological traits was computed following Pérez-Harguindeguy et al. (2013). This dataset included: total biomass (g), root:shoot ratio (R:S ratio; root mass/aboveground mass, $g\ g^{-1}$), root mass ratio (RMR; root mass/total plant mass, $g\ g^{-1}$), leaf mass ratio (LMR; leaf mass/total plant mass, $g\ g^{-1}$), stem mass ratio (SMR; stem mass/total plant mass, $g\ g^{-1}$), specific root area (SRA; total root area/total root mass, $m^2\ kg^{-1}$), specific leaf area (SLA; total leaf area/total leaf mass, $m^2\ kg^{-1}$), root surface area (cm^2), root average diameter (mm) and root volume (cm^3). The functional roles of these traits are related to biomass allocation (total biomass, R:S ratio, RMR, LMR and SMR), root and leaf display (SRA and SLA) and uptake of soil resources (root surface area, root average diameter and root volume), respectively (Poorter, 1999).

2.4. Data and statistical analysis

We applied linear models including seed mass, provenance, watering treatments and the interaction of the latter two as fixed factors to analyze differences in seedling survival and morphological traits. The Akaike Information Criterion corrected for small sample sizes (AICc) was applied to select the best model, i.e. lower AICc (Burnham and Anderson, 2004). $\Delta AICc$ shows the difference in AICc between the best model and the null model. The proportion of variability in the outcome explained by the predictors (fixed effects) is provided by R^2 . Transformations to assure normality and homoscedasticity of data were used when necessary. When significant interactions between provenance and water treatments were found, the post-hoc Tukey pairwise comparisons for each provenance were performed. When these interactions were not significant, the post-hoc Tukey pairwise comparisons were performed for each variable with the whole dataset. Survival time of seedlings during the extreme drought imposition was also used as an indicator of drought resistance and was related by simple regression to the different morphological traits of the same seedling to look for significant associations. All analyses were performed in R statistical software version 4.2.1 (R Development Core Team, 2022). All values are given as mean \pm SE.

3. Results

3.1. Morphological variations in response to water availability

We found strong effects of both provenance and watering treatments on all the morphological traits measured, except for root diameter that was unaffected by provenance (Tables 1, A.2, A.3 and A.4). Seed mass was found

Table 1

Results from the best linear models for seedling survival and morphological traits. The Akaike Information Criterion corrected for small sample size (AICc) was applied. For each variable, the initial model accounted for provenance (P), water treatment (WT), their interaction ($P \times WT$) and seed mass (SM) as a covariable. For variable "survival", terminal soil water content (i.e., soil moisture measured at the time of the plant death, TSWC) was also included as a covariable. The best model for each variable is the one with the lowest AICc. R:S ratio stands for root to shoot ratio; RMR stands for root mass ratio; LMR mean leaf stands for ratio; SMR stands for stem mass ratio; SRA stands for specific root area; SLA stands for specific leaf area.

Variable	Best model	N	$\Delta AICc$	R^2
Survival	SM + TSWC + P + WT	369	306.68	0.58
Total biomass	SM + P + WT	391	366.49	0.62
R:S ratio	SM + P + WT	392	87.04	0.23
RMR	P + WT	392	123.27	0.29
LMR	P + WT	392	155.58	0.35
SMR	SM + P + WT	392	79.74	0.21
SRA	P + WT	390	79.55	0.21
SLA	P + WT	138	41.8	0.32
Root surface area	SM + P + WT	390	325.94	0.58
Root diameter	SM + WT	390	199.44	0.41
Root volume	SM + P + WT	390	284.09	0.53

to be an important factor for most of these traits when responding to the terminal drought, especially for those traits related to biomass allocation and root development (Tables 1, A.2 and A.4). Seedlings coming from southern populations displayed higher biomass, LMR, SMR, SRA and root development (root surface area, diameter and volume), whereas those coming from northern populations presented higher R:S ratio and RMR (Figs. 2, 3 and 4, and A.1). High water availability consistently yielded increased total biomass, RMR and root development (root surface area, diameter and volume) for all provenances (Figs. 2 and 4), whereas low water availability resulted in higher LMR, SLA and SRA (Figs. 2 and 3). Interestingly, no differences between treatments 50 and 100 % WHC were detected for any of these variables. R:S ratio and SMR were the highest and the lowest, respectively, when subjected to intermediate watering levels (Fig. 2). We found no interactions between provenance and watering treatment for any of the morphological traits analyzed (Table 1). Average values for all watering treatments revealed that northern populations presented higher R:S ratio and RMR, whereas the southern ones tended to show higher values of LMR and root surface area (Fig. A.1).

3.2. Factors influencing seedling survival and its relationship with morphological traits

Seedling survival strongly depended on several factors, including seed mass, soil moisture at the time of the plant death, seed provenance and water availability during the growing period (Tables 1, A.5, Fig. 5). During the terminal drought, seedlings coming from northern and central populations survived longer than those coming from southern populations (Table A.5, Fig. 5). Higher water availability during the growing period increased survival time of seedlings for all provenances. Rising soil water content from 10 to 100 % WHC gradually improved the plants' ability to endure future dry conditions (Table A.5, Fig. 5). We detected no interactions between provenance and watering treatment for seedling survival (Table 1).

We found a number of significant correlations between the seedling's morphological traits and the number of surviving days since the onset of the terminal drought, used as proxy of their drought resistance. The main traits that increased seedling resistance to drought were those related to root development (i.e. root surface area, root diameter, root volume and RMR) and total biomass, whereas LMR was the most important trait negatively related to seedling survival (Table 2).

4. Discussion

Cork oak exhibits a large phenotypic plasticity in response to water availability and provenance, and this morphological variation provides the seedling with a differential ability to cope with drought. On the one hand, cork oak seedlings showed a very plastic response to differential water availability during the growing season, as evidenced by the inclusion of the factor "watering treatment" in the best linear models for seedling survival and all the explored morphological traits. Plastic features ranged from those at the plant level, such as total biomass, R:S ratio, RMR, LMR or SMR, to those at the organ level, such as SLA or SRA. According to our first hypothesis, high water availability resulted in increased seedling survival for all provenances, as these conditions allow to maintain photosynthetic activity and carbon gain over a longer period as compared to seedlings subjected to water restrictions. However, and against our expectations, we did not find maximized aboveground growth or reduced radicular development in seedlings exposed to high moisture levels. Instead, we found that cork oak seedlings devoted a higher proportion of biomass to roots (i.e., higher RMR) and heavily invested in developing their radicular system (i.e., higher root surface area, root diameter and root volume) when subjected to high water availability. It is generally accepted that higher root development is a frequent plant response to water and nutrient limitation (Matías et al., 2016; Padilla et al., 2009), as it enables a deeper exploration of the underground and improves resource uptake. Although similar results were recently reported in the range edges of this species (Matías et al., 2019), further field studies subjecting seedlings to more prolonged drought

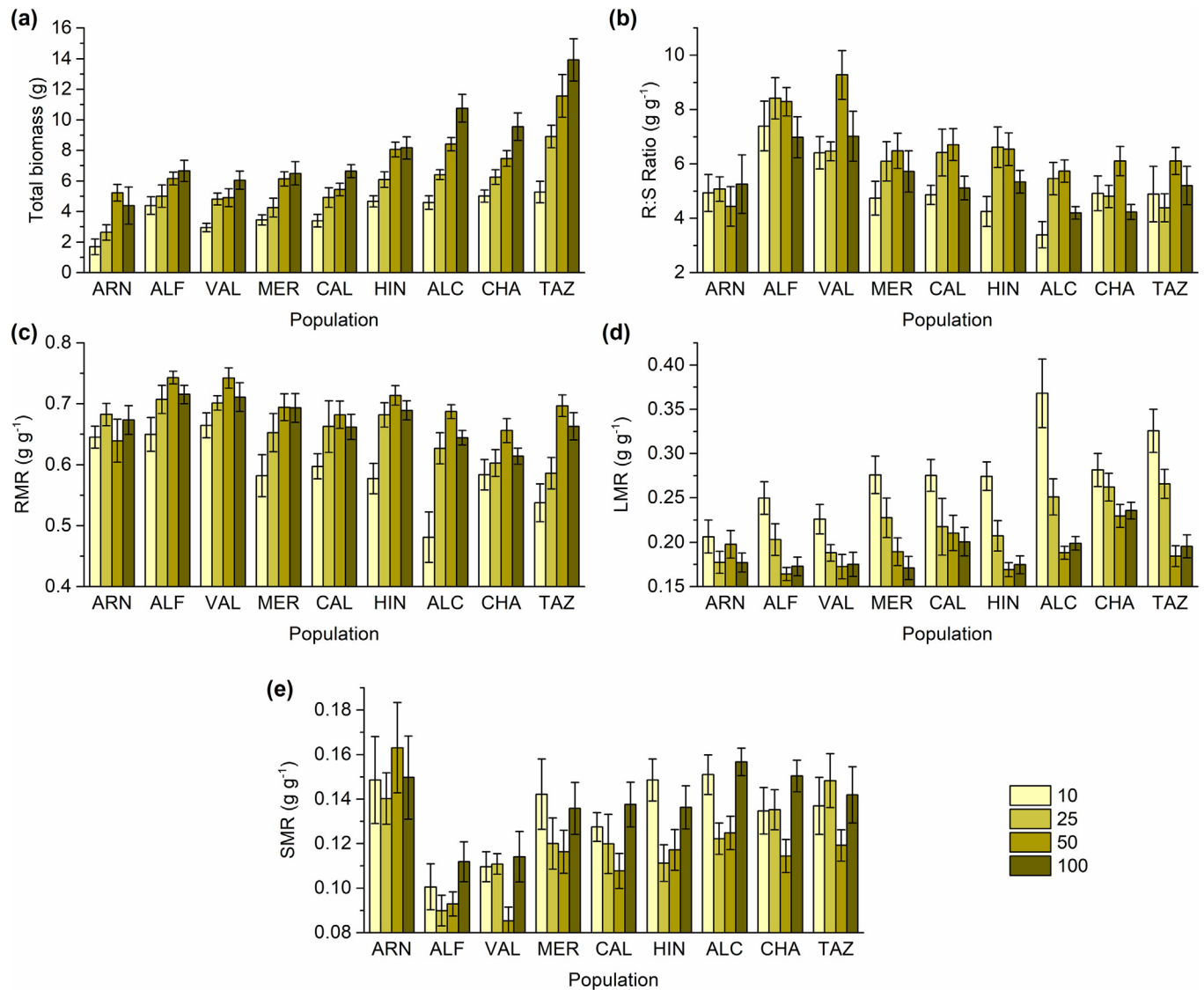


Fig. 2. Differences among provenances and watering treatments for morphological traits. The following traits are shown: total biomass (a), root to shoot ratio (b), root mass ratio (c), leaf mass ratio (d) and stem mass ratio (e). 100, 50, 25 and 10 means saturated soil at 100 %, 50 %, 25 % and 10 % of water holding capacity (WHC), respectively. The x-axis represents the populations where the acorns were collected, sorted out from north to south, being Arn and Taz the northern and southern most populations, respectively. See provenance codes in Fig. 1 and Table A.1. Predictor effects were calculated using equations for best models reported in Table 1 and parameters values in Table A.3. Differences among watering treatments for all provenances after Tukey post hoc test are denoted by different letters: A, B, C, C (a), A, BC, B, AC (b), A, B, C, BC (c), A, B, C, C (d) and AB, AC, C, B (e). Error bars represent \pm SE (standard error).

stress are needed to disentangle the mechanisms behind the trends reported in this experiment.

Also contrary to our expectations, cork oak seedlings displayed higher SLA values under the low water availability treatment for all provenances. SLA is a key trait expressing the trade-off between light interception and water loss, with lower SLA values providing fitness benefits for plants growing in dry environments against their congeneric neighbors (Pérez-Ramos et al., 2019). More sclerophyllous leaves (i.e., lower SLA values) enable a more efficient water use, maintaining photosynthetic activity, carbon gain and growth over a longer period (Aranda et al., 2005; Poorter, 1999; Poorter and Markesteijn, 2008). Previous studies found a higher drought tolerance and lower SLA in *Q. ilex* and *Q. suber* seedlings from drier provenances (Gratani et al., 2003; Pesoli et al., 2003; Ramírez-Valiente et al., 2011). Although this is an expectable strategy for drought-tolerant species and genotypes within a species (Gratani et al., 2003; Leiva and Fernández-Alés, 1998), such relationship has not been always observed (e.g., Aranda et al., 2010; Roupsard et al., 1998). For instance, Matías et al. (2019) attributed the highest SLA values under the driest simulated

scenario for cork oak seedlings to the higher nitrogen concentration reported for that treatment, since SLA is usually correlated with leaf nitrogen (Wright et al., 2004). In this same line, a decreasing pattern of SLA with rainfall has been reported in cork oak by Gouveia and Freitas (2009) and related to a tight correlation between SLA and leaf thickness. However, Andivia et al. (2018) reported contrasting holm oak provenances to show different field performance but similar resilience to drought events despite SLA was consistently unaffected by water shortage (measured 4 different years) in eight years-old Mediterranean plantations.

Despite the homogeneous effect of the watering treatment in our experiment, it is worth noticing that differences between saturated soil at 100 % and 50 % of water holding capacity were only detected for 2 out of 11 explored variables, which supports the idea that cork oaks are highly adapted to dry conditions. According to our results, a drastic water shortage (25 % of soil water holding capacity) will be needed to produce significant changes in the morphological traits of this drought-tolerant species.

The geographical pattern described for seedling survival supports that cork oak populations differ in their tolerance to drought stress, which

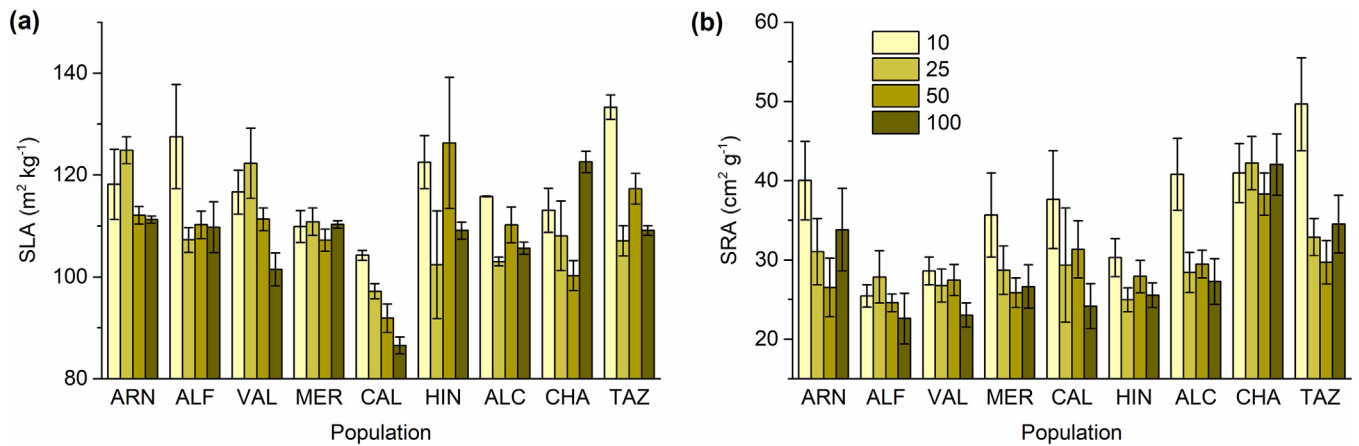


Fig. 3. Differences among provenances and watering treatments for morphological traits. The following traits are shown: specific leaf area (a) and specific root area (b). 100, 50, 25 and 10 means saturated soil at 100 %, 50 %, 25 % and 10 % of water holding capacity (WHC), respectively. The x-axis represents the populations where the acorns were collected, sorted out from north to south, being Arn and Taz the northern and southern most populations, respectively. See provenance codes in Fig. 1 and Table A.1. Predictors effects were calculated using equations for best models reported in Table 1 and parameters values in Table A.4. Differences among watering treatments for all provenances after Tukey post hoc test are denoted by different letters: A, B, B, B (a) and A, B, B, B (b). Error bars represent \pm SE (standard error).

could be due to differential morphological traits resulting from an adaptation to local environmental conditions. Namely, in our second hypothesis we expected that acorns originated in drier areas would invest more resources in traits favoring water uptake compared to those coming from more humid environment. In agreement with our expectations, seedlings with southern provenance consistently presented larger root systems

(i.e., root surface area, root average diameter and root volume) as compared to those coming from central and northern populations. Thus, at the southern edge of cork oaks, where the most arid conditions are being imposed by climate change, a more developed radicular system enables an improved exploration of deeper soil layers for water and nutrient uptake (Nicotra, 2002; Paula and Ojeda, 2009), which has been identified as a key

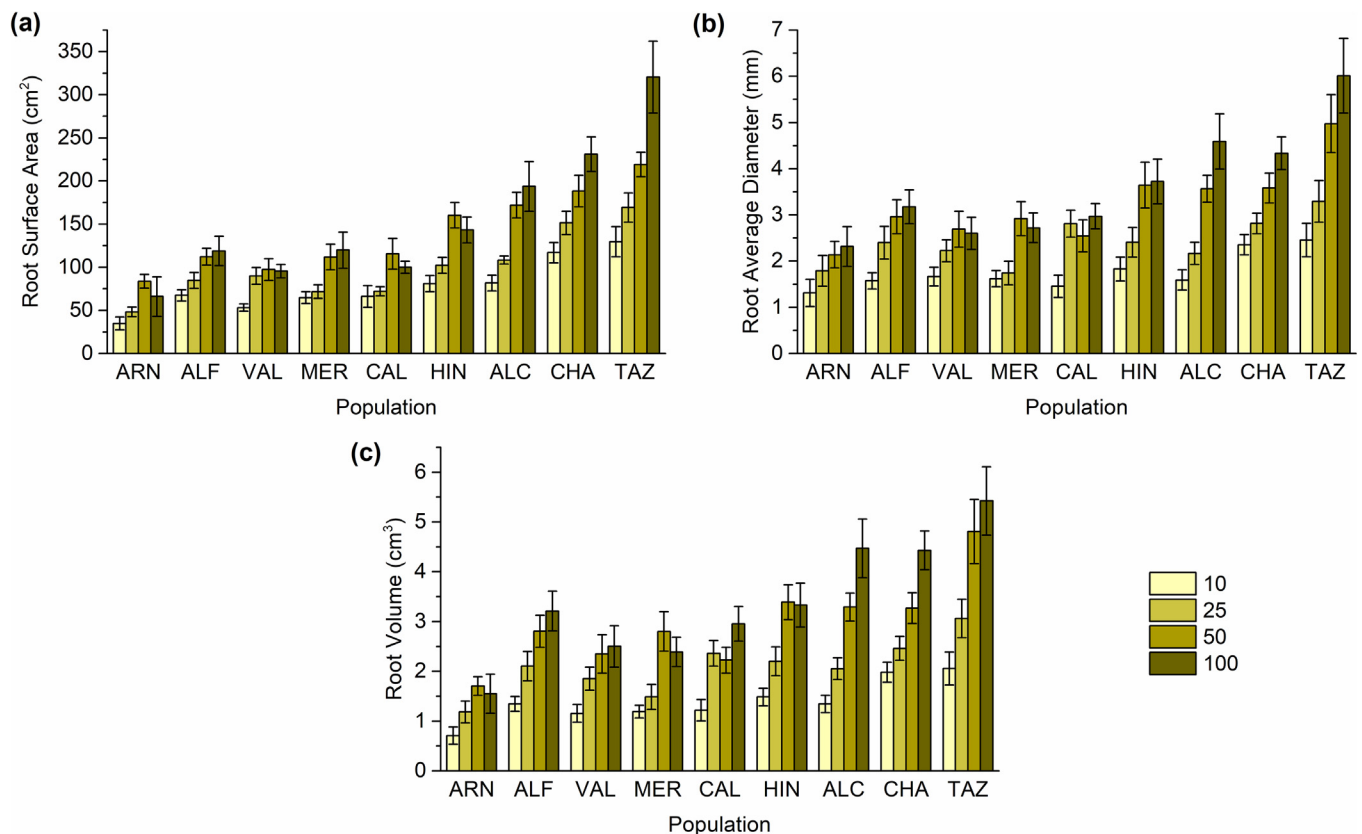


Fig. 4. Differences among provenances and watering treatments for morphological traits. The following traits are shown: root surface area (a), root average diameter (b) and root volume (c). 100, 50, 25 and 10 means saturated soil at 100 %, 50 %, 25 % and 10 % of water holding capacity (WHC), respectively. The x-axis represents the populations where the acorns were collected, sorted out from north to south, being Arn and Taz the northern and southern most populations, respectively. See provenance codes in Fig. 1 and Table A.1. Predictors effects were calculated using equations for best models reported in Table 1 and parameters values in Table A.5. Differences among watering treatments for all provenances after Tukey post hoc test are denoted by different letters: A, B, C, C (a), A, B, C, C (b) and A, B, C, C (c). Error bars represent \pm SE (standard error).

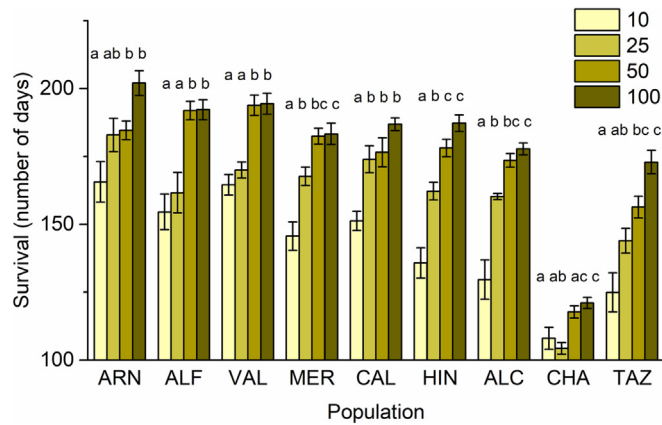


Fig. 5. Number of days that the seedlings survived without watering for each one of the watering treatments. 100, 50, 25 and 10 means saturated soil at 100 %, 50 %, 25 % and 10 % of water holding capacity (WHC), respectively. The x-axis represents the populations where the acorns were collected, sorted out from north to south, being Arn and Taz the northern and southern most populations, respectively. See provenance codes in Fig. 1 and Table A.1. Predictors effects were calculated using equations for best models reported in Table 1 and parameters values in Table A.2. Differences among watering treatments for all provenances after Tukey post hoc test are denoted by different letters: A, B, C, D. Error bars represent ± SE (standard error).

trait related to drought resistance (Markesteijn and Poorter, 2009; Matías et al., 2019, 2014; Pérez-Ramos et al., 2013). A seemingly contradictory result was found for traits describing the distribution between above and belowground biomass allocation of cork oak seedlings: R:S ratio and RMR were minimum in plants with southern provenance and gradually increased with latitude. We expected to find that bigger acorns would lead to higher RMR and R:S ratios in agreement with previous studies (Buckley, 1982; Lloret et al., 1999), because this ratios could improve seedling performance by developing and maintaining more deep and extensive root systems that allow them to access water from deeper soil layers (Dell, 1989). However, we attribute these results to the fact that these indexes are greatly reduced by the large aerial biomass in the southern sites, which was able to decrease these values despite that the radicular system of these plants were more developed than those coming from central and northern populations. More field studies in which radicular growth is unconstrained could contribute to disentangle the factors leading to these apparently inconsistent results. This study shows that cork oak populations exhibit significant levels of phenotypic differentiation in their morphological traits. These results are in line with those found for this species (Gandour et al., 2007) and for other evergreen Mediterranean oaks (Balaguer et al., 2001; Gratani et al., 2003)

Table 2

Correlation coefficient and significance between seedling survival time without watering and the morphological traits assessed in this study. Terminal SWC stands for soil moisture measured at the time of the plant death; R:S ratio stands for root to shoot ratio; RMR stands for root mass ratio; LMR stands for leaf mass ratio; SMR stands for stem mass ratio; SRA stands for specific root area; SLA stands for specific leaf area.

	Rho	p-Value
Terminal SWC	-0.109	0.0304
Seed mass	-0.018	0.7169
Total biomass	0.516	<0.0001
R:S ratio	0.101	0.0446
RMR	0.318	<0.0001
LMR	-0.447	<0.0001
SMR	0.022	0.6609
SRA	-0.195	0.0001
SLA	-0.249	0.0032
Root surface area	0.416	<0.0001
Root diameter	0.500	<0.0001
Root volume	0.548	<0.0001

and point out to differences among populations being related to the climate of the seedling sources (Gandour et al., 2007).

Our results show that several morphological traits could allow to predict cork oak responses to drought stress. Functional traits related to plant biomass allocation pattern were found to be the most correlated to drought resistance, expressed as the survival time without watering. As foreseen in our third hypothesis, traits related to maximized root biomass (root surface area, root diameter and root volume) were positively related to drought resistance, whereas increased aerial biomass allocation (LMR) lead to lower survival. This differential morphological development can also result in differences in physiological traits (Matías et al., 2019), although the detailed changes must be explored in detail. Greater aboveground development increases the photosynthetic area and the carbon gain when water is available and light is the limiting factor (Dudley, 1996; Green and Juniper, 2004; Mediavilla and Escudero, 2010). However, this increased light interception capacity has an important side effect in a water-limited context: it increments the evaporative demands of the plant (Poorter and Markesteijn, 2008). Therefore, high values in LMR are largely penalized in plants living in low-water-availability environments expected to face even greater water limitations, such as Mediterranean cork oaks. On the other hand, seedlings maximizing the development of the root system allowing for exploration of more available water in the deep soil layers (Engelbrecht et al., 2005), will be more resilient to drought effects. All in all, our results support that seedlings investing a higher proportion of biomass to the radicular system will increase their survival under drought stress by simultaneously increasing water acquisition and reducing the evaporative surface. These results provide a mechanistic explanation regarding the morphological traits involved in cork oak resistance to drought, as well as describe the geographical differences within the species' distribution in terms of biomass allocation patterns.

Our results showed the important role of local adaptations through the cork oak's distribution range, as the factor "provenance" was included in the best model for almost all variables. Although this finding does concur with our fourth hypothesis, we also foresaw in such hypothesis that southern populations would be more resilient to extreme drought than central and northern populations, which is not supported by our data. Indeed, our results revealed a significant influence of the provenance for a fitness surrogate trait (i.e., number of days that the seedlings survived without watering) when exploring the cork oak's natural distribution range. However, this geographical pattern was opposed to the one expected: seedlings survival decreased from north to south. This finding contrasts not only with our expectation but also with most previous studies, which found that drought-stressed cork oak seedlings from the more xeric provenance tend to have a higher tolerance to drought (Gandour et al., 2007; Gratani et al., 2003; Ramírez-Valiente et al., 2014, 2009; Sampaio et al., 2019). Ramírez-Valiente et al. (2009) attributed this trend to increased acorn size in southern populations, which was regarded as an adaptive trait enhancing stress resistance during plant establishment in Mediterranean ecosystems. As supported by the inclusion of "seed mass" in the best linear model for seedlings survival, acorn size was one of the most important traits influencing the early stages of the life cycle of cork oaks in our study. The relevance of seed size in determining seedling growth and survival has been supported by large empirical evidence (Baraloto et al., 2005; Gómez, 2004), but it has also been found to be temporal (Lopez et al., 2003; Pérez-Ramos et al., 2010; Roach and Wulff, 1987; Schaal, 1984) or even lack of effect (e.g. year 2001 in Ramírez-Valiente et al., 2009). In this same line, we found that cork oak seedlings originating from the southern distribution range, characterized by more xeric environment, exhibited lower survival rates than those from more humid regions located at the northern and central parts of the distribution area. Bigger acorns coming from southern provenance produced very large seedlings with a biomass allocation pattern that tended to maximize aboveground growth (i.e. LMR and SMR). This disproportionately large above-ground biomass has a great water demand that can become an insurmountable handicap under drier scenarios, leading to reduced seedling survival against summer drought. These findings are in line with Vanhove et al. (2021), who predicted that

the southern populations within cork oak's distribution range will be the most sensitive areas to climate change using Gradient Forest analysis, and that trees are expected to find a favourable region in the hinterland of the Iberian Peninsula. In the same line, our results corroborate the forecasts obtained from ecological niche modelling which foresaw an upwards contraction of the cork oak potential distribution range (Vessella et al., 2017). These findings are specially concerning considering that these southern areas are expected to experience the largest climatic disruptions (Masson-Delmotte et al., 2021). These insights support that in order to improve the management and conservation of the forest resources in this urgent scenario, models projecting climate change-driven tree redistribution for the Mediterranean basin (Benito Garzón et al., 2008; Duque-Lazo et al., 2018; Vessella et al., 2017) should account for within-species variation.

5. Conclusions

Our functional trait-based approach allowed us to confirm that not all cork oak populations are equally vulnerable to drought stress. Southern populations are expected to produce very large seedlings with a biomass allocation pattern maximizing aboveground growth (i.e. LMR and SMR), a feature that could become an insuperable handicap under future scenarios of context of climate alterations. Despite the aforementioned biomass allocation pattern, our results indicate a clear selective pressure for fixing in the southern populations those morphological traits aimed to maximize the uptake of soil water (i.e., root surface area, root average diameter and root volume), the most limiting resource in that area. We further reported a very plastic response for all the explored variables resulting in differences in survival and morphological traits across watering levels. Our analyses of morphological traits showed that cork oak resilience to drought stress was benefited by increased radicular development, whereas it was penalized by high aerial biomass allocation. Despite the limitations that our short-term study may have due to the fact that it only accounted for one-year seedlings development, it allowed us to assess their survival during the most critical stage for the establishment of Mediterranean woody species. Thus, selecting the most adapted populations with (re)forestation purposes will be of paramount importance to maximize the species productivity and contribute to its long-term maintenance under ongoing climate changes.

CRedit authorship contribution statement

Lourdes Morillas: Data curation, Formal analysis, Software, Visualization, Writing – original draft. **María José Leiva:** Investigation, Writing – review & editing. **Ignacio M. Pérez-Ramos:** Investigation, Writing – review & editing. **Jesús Cambrollé:** Investigation, Writing – review & editing. **Luis Matías:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing, Supervision.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.164122>.

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