Environmental and Experimental Botany 247 (2026) 106381

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Research Paper

Biomass, water and carbon economy shifts in oak saplings across temperate and Mediterranean bioclimatic zones

Elisa Spennati [a], Sara Gargiulo [b,*], Valentino Casolo [b], Patrizia Trifilò [c], Andrea Nardini [d], Marcello Vitale [a]

[a] Dipartimento di Biologia ambientale, Università di Roma La Sapienza, Piazzale Aldo Moro 5, Roma 00185, Italy
[b] Dipartimento di Scienze Agrarie, Ambientali e Animali, Università di Udine, Via delle Scienze 206, Udine 33100, Italy
[c] Dipartimento di Scienze Botaniche, Università di Messina, Salita Sperone 31, Messina S. Agata 98166, Italy
[d] Dipartimento di Scienze della Vita, Università di Trieste, Via L. Giorgieri 10, Trieste 34127, Italy

ARTICLE INFO

Keywords:
Hydraulics
Non-structural carbohydrates
Drought
Latitudinal gradient
Intraspecific variability
Juvenile trees

ABSTRACT

Tree growth and survival depend on how effectively trees adjust functional traits to environmental variation. Saplings are especially vulnerable to water scarcity; yet their morphological and physiological responses across contrasting climates are far from being completely understood. We investigated functional responses in four-year-old *Quercus pubescens* Willd. and *Quercus ilex* L. saplings of different localities transplanted in the corresponding sites along a latitudinal gradient in Italy, spanning from Temperate to Mediterranean climates. We integrated measurements of morphology and water relations during the summer season, as well as seasonal non-structural carbohydrate partitioning across all plant organs. Mediterranean sites underwent clear trait shifts: saplings prioritised biomass allocation belowground, reflecting an avoidance strategy under drier conditions. Functional traits also diverged between the Mediterranean sites, with saplings at the southern latitude showing a lower turgor loss point and stomatal conductance under similar drought stress levels. Results indicated organ-specific seasonal differences in non-structural carbohydrates; in particular, twigs and stems appeared to be the organs primarily involved in drought responses. Lower winter non-structural carbohydrate allocation in drier sites, particularly in *Q. ilex*, suggests reduced accumulation relative to synthesis, potentially affecting sapling drought resilience. Taken together, these findings and the higher mortality at Mediterranean sites indicate their potential increased survival risk. Evaluating carbon metabolism and water relations of locally sourced saplings under natural conditions at the whole-tree level is therefore pivotal as an initial step to inform restoration plans in increasingly water-limited environments.

1. Introduction

Understanding systematic patterns of functional and morphological diversity among trees is essential for predicting their ability to persist in their current distribution ranges under ongoing climate change (Nicotra et al., 2010; Violle et al., 2012). This diversity arises from two non-mutually exclusive mechanisms: phenotypic plasticity and genetic differentiation (Bradshaw, 1965; Nicotra et al., 2010). Plant phenotypic responses to environmental changes are commonly quantified through functional traits, as they represent characters closely tied to plant performance and fitness (Benito Garzón et al., 2019; Moran et al., 2016; Violle et al., 2012). Intraspecific functional trait variability accounts for a considerable proportion of global functional diversity, with some traits contributing 30–40% of the variance across plant communities (Kattge et al., 2011; Siefert et al., 2015). Notably, trait variability differs between saplings and adult trees, with the former typically showing faster phenotypic responses. This pattern is mainly due to their underdeveloped root systems, which increase their susceptibility to environmental fluctuations (Matías et al., 2014; Ramírez-Valiente et al., 2021). The establishment of new saplings is a key mechanism contributing to forest resilience; for this reason, it is pivotal to understand how tree saplings respond to contrasting environmental forcing and which traits can be used to evaluate plant fitness. These insights are crucial for guiding conservation strategies, anticipating future forest structure and composition, and forecasting recruitment dynamics (Hoffmann and Sgrò, 2011; Lloret et al., 2004).

* Corresponding author.
E-mail address: sara.gargiulo@uniud.it (S. Gargiulo).

https://doi.org/10.1016/j.envexpbot.2026.106381
Received 15 January 2026; Received in revised form 14 May 2026; Accepted 28 May 2026
Available online 30 May 2026

Water availability and temperature are key environmental factors that shape tree establishment and drive intraspecific variability in functional traits (Leites and Benito Garzón, 2023; Moles et al., 2014; Silva et al., 2019). Under increasing water deficit and elevated temperatures, embolism resistance (xylem safety), generally expressed as water potential causing 50% loss of hydraulic conductivity, sets critical thresholds for plant survival. Previous empirical studies reported intraspecific variability of this trait (Blackman et al., 2014; Larter et al., 2017; Rosas et al., 2019). However, this response generally explains greater variation in drought tolerance between than within species (Larter et al., 2017; Lobo et al., 2018; López et al., 2013). Other traits linked to drought tolerance, such as leaf water potential at the turgor loss point, were found to significantly shape intraspecific plant variability (Bartlett et al., 2012b; Rosas et al., 2019). Morphological, organ-level, and integrative traits can also be relevant for water-regulation responses along climatic gradients. For instance, sapwood-to-leaf area ratio (Huber value), a trait linked to carbon and water economy, tends to increase with lower water availability (Martínez-Vilalta et al., 2009; Mencuccini and Bonosi, 2001).

While evidence on intraspecific trait variability is accumulating (Aranda et al., 2018, 2015; Salazar Zarzosa et al., 2021; Wang et al., 2021), it remains far from being fully understood (Brodribb et al., 2020; Rosas et al., 2019). Functional responses related to water relations may provide valuable insights into plant drought tolerance and fitness at the intraspecific level (Brodribb, 2017; Skelton et al., 2015). Complexity arises because drought tolerance is often inversely related to growth rate, suggesting a trade-off or a decoupling between these two processes (Brodribb et al., 2020; Gazol et al., 2017; Hajek et al., 2016; Leites and Benito Garzón, 2023). Accordingly, previous evidence shows that as the climate becomes drier, trees generally shift their functional strategy from acquisitive to conservative along the overarching 'fast-slow' economic spectrum (Reich, 2014). The interplay between plant drought tolerance and growth is likely associated with non-structural carbohydrate (NSC) reserves, as they are essential in both processes (Nawaz et al., 2025).

NSCs are partitioned into starch and sugars, each playing distinct roles. Sugars are the substrates used for growth, respiration, plant signalling, defence, phloem transport, and osmoregulation (Martínez-Vilalta et al., 2016). Starch functions as a transitory and long-term carbohydrate storage that can be hydrolysed into sugars when internal carbon demand outpaces assimilatory supply. This is likely to occur under drought conditions, where decreases in stomatal conductance constrain photosynthetic activity and carbon demand increases (Hartmann and Trumbore, 2016; MacNeill et al., 2017). Therefore, NSC seasonal differences reflect organ-specific shifts between carbon assimilation or storage and its utilisation to support survival under given environmental conditions (Kozlowski, 1992; Martínez-Vilalta et al., 2016). Evidence shows that NSC pools are crucial for tree recovery from drought, as trees with higher NSC concentrations exhibit higher survival rates (O'Brien et al., 2014). Thus, it is essential to assess summer-to-winter changes in non-structural carbohydrates to investigate the legacy effects of drought on NSC allocation in tree species, and the implications for their recovery over time. However, our understanding of how individual organs contribute to seasonal differences in NSC allocation in conspecific tree saplings across different climates remains limited and inconsistent (Furze et al., 2019; Wang and Wang, 2025). This is likely to depend on the modulation of several factors on NSC allocation and storage, such as abiotic conditions, plant size, drought tolerance (Wang and Wang, 2025), the organ being considered (Furze et al., 2019), and stomatal behaviour (Kannenberg et al., 2022). Specifically, the tighter control of leaf water potential through stomatal regulation at one end of the isohydry-anisohydry spectrum is expected to impose more substantial limitations on carbon assimilation into NSCs (Kannenberg et al., 2022; McDowell, 2011). Conversely, lower stomatal regulation at decreasing water potentials at the other end of the spectrum is hypothesised to increase their exposure to hydraulic damage under high evaporative demand (Kannenberg et al., 2022; McDowell, 2011). The integration of water relations and carbon metabolism processes can enhance the understanding of the phenotypic traits that shape tree sapling establishment and survival under contrasting temperature and precipitation regimes.

To provide new evidence on these functional aspects, we investigated intraspecific variation in traits associated with carbon metabolism, water relations, and morphology in tree saplings of different origins distributed along a latitudinal gradient with distinct bioclimates. We focused on four-year-old saplings of *Quercus ilex* L. and *Quercus pubescens* Willd. that, despite belonging to the same genus, exhibit distinct drought tolerance (Kiorapostolou et al., 2019; Moreno et al., 2024), which is reflected in their distributions (de Rigo and Caudullo, 2016; Pasta et al., 2016). We hypothesised that saplings in Mediterranean sites would display: (1) coordinated functional responses towards a more negative turgor loss point, greater biomass allocation to roots, greater Huber value, and conservative leaf function; (2) greater summer-to-winter changes of sugars in all organs in response to seasonal drought stress in both species.

2. Materials and methods

2.1. Study sites and experimental design

The study was carried out at three sites in Italy: "A. Savadei" Farm, University of Udine in the north (46° 2′ 9.41" N, 13° 13' 33.03" E), Palo Laziale Woodland in the centre (41°56′24″ N, 12°06′03″ E), and a privately owned garden in Messina in the south (38°16'12.6"N, 15°37'27.3" E). Based on the thermotype and ombrotype typologies of the bioclimatic classification system defined by Rivas-Martínez et al. (2002), Udine falls within the humid meso-temperate bioclimate, Palo Laziale in the dry upper meso-Mediterranean, while Messina falls in the subhumid lower thermo-Mediterranean. We conducted the analyses during the summer of 2024, focusing on four-year-old plants of *Q. ilex* and *Q. pubescens*. Three-year-old saplings of these species were provided by public forestry nurseries in Friuli Venezia Giulia (Vivai Pascul, Regional Forestry Service, Tarcento, Italy), Latium (ARSIAL, Regional Agency for Agricultural Development and Innovation), and Sicily (Vivaio Ziriò, Regional Forestry Service, Saponara, Messina, Italy). In January 2023, only locally sourced saplings were planted in the corresponding locality, with seventy saplings per species planted at each site, randomly spaced and maintained under standardised growing conditions (planting system, light exposure) and without regular irrigation (Fig. S1). Supplemental watering was applied only as an emergency measure to prevent sapling mortality during prolonged drought conditions in the first year after transplanting. In 2024, no water supply was provided. At each site, saplings were planted in mixed species groups at least 1 m apart to limit interspecific competition for water and resources. We focused on conspecific tree saplings of the same age but distinct origin to address their functional trait variability and drought vulnerability under local, natural conditions to help inform forest restoration plans.

Meteorological conditions were monitored at each site with a weather station that recorded daily maximum, minimum, and mean temperature (°C), precipitation (mm), and mean relative humidity (%) (Fig. S2). For the two Mediterranean sites, precipitation was greater in Messina than in Palo Laziale, especially during summer (Fig. S2; Table S1), contributing to higher seasonal water availability at the former site, consistent with the corresponding bioclimatic classification. Therefore, despite the sites being situated along a latitudinal gradient, water availability does not strictly follow latitude. Vapour pressure deficit (kPa) was derived from daily mean air temperature and relative humidity (Andersson-Sköld et al., 2008) for each month and site (Table S2). Growing degree days were calculated over the past 30 years (GDD, McMaster and Wilhelm, 1997) and revealed higher GDD at the Mediterranean sites, especially in Messina (Fig. S3).

We measured the leaf water potential and percentage loss of

hydraulic conductivity to evaluate plant drought stress across sites; the turgor loss point and stomatal conductance to assess drought resistance strategies; leaf mass per area, Huber value, root mass fraction, and plant biomass to evaluate morphological shifts linked to water regulation and resource use; non-structural carbohydrates to evaluate their variation in partitioning and storage with respect to the intensity of drought stress at each site. The traits were assessed on five saplings per species and site at the time of highest and most comparable drought stress across sites (August in Udine and Palo Laziale and July in Messina). Sampling time was determined using site-specific meteorological data, particularly mean daily temperatures above 25 °C and precipitation equal to or below 5 mm during the preceding 10 days. Sampling was further guided by leaf water potential measurements to track drought stress progression and, where site meteorological conditions allowed, approach the common mean water potential associated with 50% loss of hydraulic conductivity for each species (Robert et al., 2017; Table S3). Non-structural carbohydrates were measured in five saplings per species and site in summer and at the end of the growing season (December in all sites) to assess seasonal changes in NSC levels from peak summer drought to winter dormancy (Fig. S3). Plant biomass was evaluated on the same plants in winter.

2.2. Hydraulic measurements

2.2.1. Leaf water potential and gas exchanges

We measured leaf water potential at predawn (ψ_{PD}) and midday (ψ_{MD}) on the same set of plants, using five leafy twigs per species and site, collected from different individuals. We performed the measurements with a Scholander-type pressure chamber (PMS Instrument Co.). We measured leaf gas exchange at midday on the same five plants, immediately before sampling the twigs to determine ψ_{MD}. Specifically, we recorded stomatal conductance to water vapour per unit leaf area (g_S, mmol m^{-2} s^{-1}) on three leaves per twig, using one twig per plant (five plants in total per species and site). For these measurements, we selected healthy, mature leaves from the current year. Measurements were carried out using a leaf gas exchange analyser in Palo Laziale and Messina and a leaf porometer in Udine (Ciras−2, PP Systems; Licor−600, LI-COR Biosciences, USA). For the leaf gas exchange analyser, the following conditions were applied: photosynthetically active radiation of 1000 μmol m^{-2} s^{-1}, a reference CO_2 concentration of 425 ppm, a relative humidity of 60%, and a leaf chamber temperature at ambient values. As leaf porometers and infra-red gas analysers can yield slightly different absolute values of stomatal conductance, we applied the correction proposed by Rizzo and Bailey (2026) to the values measured at the Udine site with the leaf porometer and used the updated values for comparison.

2.2.2. Leaf water potential at turgor loss point

Within the same two-week period as leaf water potential and gas exchange measurements, we measured the osmotic potential at full turgor (π_0, MPa) and we estimated the leaf water potential at turgor loss point (ψ_{TLP}, MPa) on five leafy twigs per species and site. We collected the twigs in the early morning and maintained their cut basal ends immersed in water until they reached full turgor. After the samples reached full turgor, we wrapped four leaves per twig in clingfilm, immersed them in liquid nitrogen for 2 min, and then ground them. The samples were kept at −20 °C until measurement. From the same plants, we collected four additional leaves per individual to determine leaf dry matter content (LDMC, mg g^{-1}), calculated as the ratio of leaf dry mass after drying at 60 °C for 3 days to leaf saturated mass. We measured π_0 using a dew-point hygrometer (WP4, Decagon Devices Inc.), and estimated Ψ_{TLP} from π_0 as described by Petruzzellis et al. (2019), which improved the equations proposed by Bartlett et al. (2012a).

2.2.3. Stem xylem embolism

We harvested five plants per species and site at predawn, kept them with their cut basal end immersed in water, and promptly transported them to the laboratory for measurement. We assessed the percentage loss of hydraulic conductivity (PLC, %) gravimetrically on the main stem, as outlined by Trifilò et al. (2014), using a similar set-up to that described by Lo and Salleo (1991). We cut the stems to obtain segments of 40 cm, which corresponded to at least 1.5 times the maximum length of xylem vessels, as determined from preliminary measurements. To prevent spurious air from entering the xylem conduits, we trimmed 1 cm cuts from the basal cut end of the stems while submerged in water, before connecting them to the apparatus. We perfused the samples with a mineral water solution containing 10 mM KCl (Nardini et al., 2017), supplied from a reservoir located 80 cm above the samples, resulting in a water head pressure of 0.008 MPa. We measured initial xylem hydraulic conductivity (K_i) gravimetrically by collecting the flow at the stem distal end in 1.5 mL Eppendorf Tubes containing a piece of sponge. We assessed K_i each minute for at least eight minutes until the flow rate stabilised. We determined the maximum hydraulic conductivity (K_{max}) analogously after flushing the stem segments at 0.2 MPa for 10 min to remove embolisms. We calculated PLC as: $(1 - (K_i/K_{max})) \times 100$.

2.3. Morphological measurements and mortality assessment

We measured plant dry biomass in summer (July-August) and winter (December) 2024 on five individuals per species and site. Samples were oven-dried at 70 °C for 72 h, and dry weight (DW, g) was determined separately for the aerial part and the root system. For the individuals harvested at the end of the vegetative period (winter), we calculated total biomass as the sum of these components. Plant biomass from summer samples was used to estimate root mass fraction (RMF), defined as the ratio of root dry mass to total plant dry biomass. These samples corresponded to the same individuals used for PLC determination, as well as for leaf mass per area (LMA, g m^{-2}) and Huber value (Hv, cm^2 m^{-2}). For LMA, we randomly collected three healthy and mature leaves per plant, scanned the same day, and quantified their leaf area (LA, m^2) using ImageJ (Schindelin et al., 2012). We oven-dried the leaves to obtain dry mass (DW, g), and computed LMA as DW/LA. We estimated total leaf area per plant as the mean leaf area multiplied by the total number of leaves. We calculated the xylem cross-sectional area at the stem base as $\pi(d/2)^2$, where d is xylem diameter, and stem Hv was derived as the ratio of xylem area to total leaf area. At each site, we calculated species-specific mortality as the proportion of individuals that died between spring and autumn out of 35 plants per species. We classified saplings as dead when both the crown and phloem were brown and dry, with no buds present (Cregg, 1994).

2.4. Non-structural carbohydrates

We performed NSC measurements in summer and winter 2024 on five plants per species and site. We quantified summer NSCs on the same plants used for PLC and biomass measurements. Concentrations of sugars and starch were determined separately for each plant organ. For each plant, we sampled three randomly selected, healthy leaves; twigs of uniform age (3 years old); the stem at a standardised height (10 cm above the root collar); and tertiary roots to capture NSC changes following transplantation. After collecting the samples at predawn, we microwaved them on the same day at 700 W for 3 min to stop enzymatic activity, oven-dried them at 70 °C for 48 h and ground them in a ball mill (MM400; Retsch GmbH, Haan, Germany). The protocol of NSC analyses followed the standardised procedure proposed by Landhäusser et al. (2018) and Quentin et al. (2015), modified for small amounts as proposed in Casolo et al. (2023). In brief, to separate the sugars from the starch, we suspended 15 ± 1 mg of each dried ground sample in 80% ethanol, incubated them at 80 °C for 30 min, then centrifuged them at 14,000 rpm for 3 min. We transferred the resulting supernatant into labelled 1.5 mL Eppendorf tubes and repeated sugar extraction twice using this procedure. We analysed sugars following the Anthrone assay

(Yemm and Willis, 1954). We dried the extracted supernatant, resuspended it in 50 mM Tris-HCl (pH 7.5) and centrifuged it at 14,000 rpm for 3 min before spectrophotometric quantification at 620 nm (Victor3, PerkinElmer, Boston, MA, USA).

To allow starch gelatinisation, we suspended pellets in 10 mM Tris-HCl (pH 6.7) and incubated at 100 °C for one hour. Starch hydrolysis was performed at 70 °C by adding 100 U/sample of α-amylase dissolved in 10 mM Tris-HCl (pH 6.7) and 25 U/sample of γ-amylase dissolved in 25 mM Na Acetate (pH 4.5). We analysed starch through the enzymatic method proposed by Bergmeyer and Bernt (1974). We quantified glucose obtained by starch hydrolysis as NADPH formation by the action of 0.2 U per sample of hexokinase and 0.5 U per sample of glucose−6-phosphate dehydrogenase in a buffer solution (50 mM NAD^+, 0.4 M NaATP, and 2 M $MgCl_2$). We performed the enzymatic reaction at 32 °C and read NADPH at 340 nm. We converted the absorbance values to glucose and starch concentrations (mg mg^{-1} DW) using calibration curves with known amounts of glucose and commercial potato amylose, respectively. The amylose followed the same hydrolysis protocol as the starch samples.

2.5. Statistical analysis

To address our first research question, we tested for within-species variation in mean trait values across sites using one-way analysis of variance (ANOVA). Before analyses, we assessed normality and homogeneity of variances for each physiological variable, species, and site, using quantile–quantile plots and Levene's test, respectively. Significant ANOVAs were followed by the Tukey post hoc test.

To evaluate the variability in leaf water potentials and percentage loss of hydraulic conductivity along the precipitation-to-evapotranspiration index (P: PET) imposed by our experimental design, we fitted separate linear mixed-effects models for each variable, with the physiological parameter as the response variable and the aridity index as a fixed effect. To account for the non-independence of observations, we included random intercepts for species and site. Specifically, we calculated the P: PET for the sampled summer month at each site using the Hargreaves–Samani method (Hargreaves and Samani, 1982), as implemented in the SPEI R package (Beguería and Vicente-Serrano, 2025) to estimate the potential evapotranspiration.

To address our second research question, we performed a two-way ANOVA to examine seasonal variation in NSCs across sites. We conducted separate analyses for each species, plant organ and NSC fraction (sugars, starch, and their sum). Before investigating the effect of meteorological factors on the variation in non-structural carbohydrates, we summarised the data using the principal component analysis (PCA) with the psych R package (Revelle, 2025). This analysis included daily data from the NSC sampling months for the following scaled meteorological variables: minimum, maximum and mean temperature; vapour pressure deficit; relative humidity; and precipitation. We then extracted the eigenvalues of the first principal component (PC1), and we fit linear mixed-effects models, treating each NSC fraction (sugars and starch) as the response variable and PC1 as the fixed effect. Site and season were included as random effects to account for non-independence among observations. To specifically evaluate NSC seasonal variation with P: PET, we calculated this index for the sampled summer and winter months at each site. Similarly to PC1, we then fitted separate linear mixed-effects models for each species. To examine hypothesised relationships between stem PLC and stem sugars, we fitted linear mixed-effects models for each species with site included as a random effect, using the *lme4* package (Bates et al., 2015). All traits met the model assumptions, as indicated by diagnostic plots of the models' residuals. We performed all statistical analyses in RStudio (R Core Team, 2023, version 4.3.2).

3. Results

3.1. Morphological and water relations trait differences along the latitudinal gradient

Overall, we found significant shifts in measured traits across sites in both species. Leaf water potential measurements indicated greater drought stress at Mediterranean sites for both species compared with the Temperate site. In *Q. pubescens*, Ψ_{PD} values were similar between the Palo Laziale and Messina sites, and significantly more negative than those of conspecifics in Udine ($P = 0.001$). By midday, leaf water potential of plants in Udine declined to values comparable to conspecifics in Messina but not to Palo Laziale, which exhibited the lowest values ($P = 0.02$; Fig. 1). In *Q. ilex*, Ψ_{PD} differed significantly across all sites, with plants in Udine showing the least negative ($P < 0.001$) and those in Messina the most negative ($P < 0.001$; $P = 0.04$) values. At midday, leaf water potential values of conspecifics at Palo Laziale and Messina were similar and both significantly lower than those at the Udine site ($P = 0.001$; Fig. 1).

Water relations traits varied between the sites. Both species exhibited significant shifts of Ψ_{TLP} toward more negative values in Messina (Fig. 1). In *Q. pubescens*, plants in Udine had the highest g_S compared to conspecifics at Palo Laziale ($P = 0.01$; Fig. 1) and Messina ($P < 0.001$; Fig. 1), while the latter exhibited the lowest values. In *Q. ilex*, g_S was also significantly higher in Udine than in both Palo Laziale ($P = 0.01$) and Messina ($P = 0.001$; Fig. 1).

Plants also displayed pronounced morphological variation across sites. Root biomass was greater in plants grown at Mediterranean sites than conspecifics at the temperate location, as reflected by higher RMF in *Q. pubescens* ($P < 0.01$; $P = 0.001$; Fig. 1), while in *Q. ilex*, this difference was significant only for the Palo Laziale site ($P = 0.03$; Fig. 1). In contrast, biomass at the end of the vegetative period was comparable across sites in both species (Fig. S4). In *Q. pubescens*, LMA values were similar between the sites, while in *Q. ilex*, the values at Palo Laziale were significantly lower than those at Udine ($P = 0.01$) (Fig. 1). In *Q. pubescens*, the Hv showed no significant variation across sites (Fig. 1), whereas in *Q. ilex*, plants in Messina exhibited a higher sapwood-to-leaf area ratio than conspecifics in Palo Laziale ($P = 0.03$; Fig. 1).

The highest PLC values in *Q. pubescens* occurred in the Palo Laziale site ($P = 0.03$; $P = 0.02$; Fig. 1), while in *Q. ilex*, the highest values occurred in both Mediterranean sites ($P < 0.001$; Fig. 1). When data were pooled across sites, PLC and stem sugars correlation only approached statistical significance in *Q. pubescens* (Fig. S5). The mortality assessment revealed the highest mortality in Mediterranean sites, especially for *Q. pubescens* (Table 1). We detected significant relationships between the aridity index, the absolute value of Ψ_{MD} and PLC, with both parameters increasing with decreasing P: PET (increasing aridity) (Fig. 2).

3.2. Spatial and temporal variability of non-structural carbohydrates

Overall, the site had a significant effect on total NSC concentrations, with higher levels in twigs and stems at Mediterranean sites during summer (Fig. S6). This pattern was driven by the consistently higher sugar concentrations in these two organs of Mediterranean plants of both species, whereas starch concentrations remained broadly comparable across sites (Figs. 3–4). In leaves, no differences were found in either sugar or starch concentrations across sites during summer for the two oak species (Figs. 3–4). In fine roots, sugar concentrations were lower at Palo Laziale than at Udine during summer in both *Q. pubescens* ($P < 0.01$; Fig. 3) and *Q. ilex* ($P = 0.01$; Fig. 4), while starch concentrations did not differ between sites (Figs. 3–4).

During winter, total NSCs in *Q. pubescens* were largely comparable across sites, except for higher concentrations in twigs at Messina ($P = 0.01$; $P < 0.001$) and lower concentrations in stems at Palo Laziale ($P = 0.01$; $P = 0.04$) (Fig. S6). In *Q. ilex*, total NSCs were higher in Udine

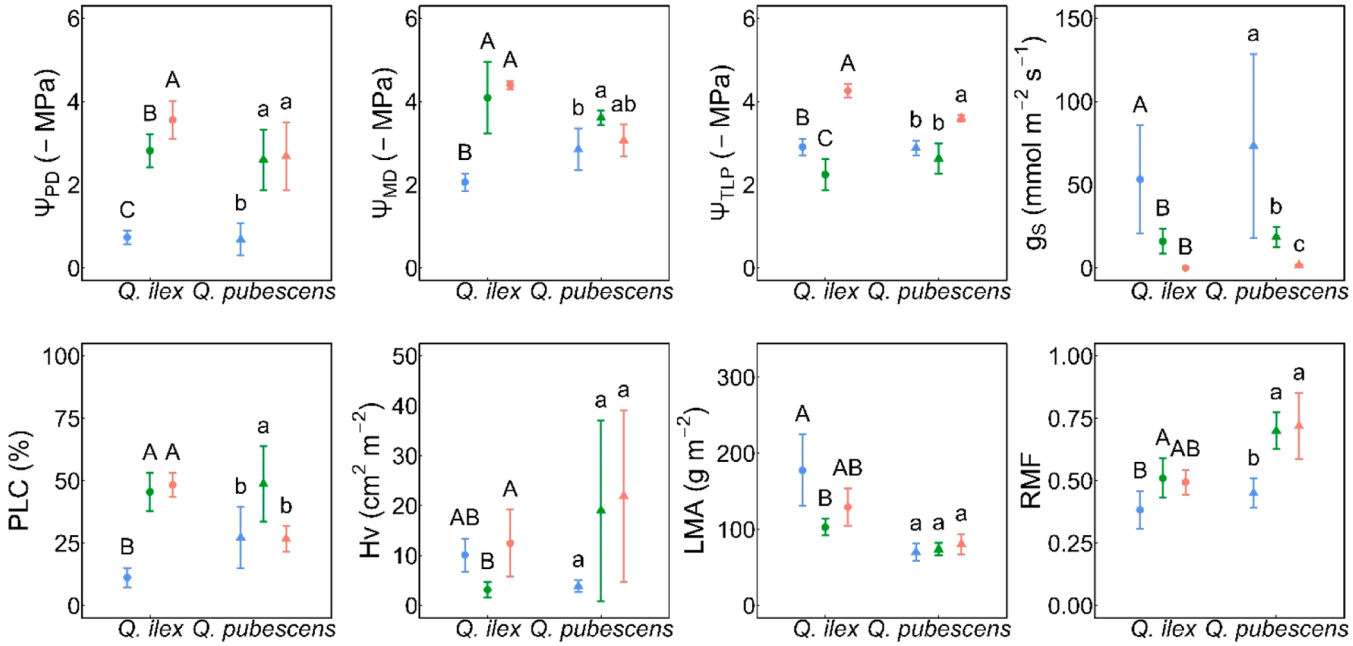

Fig. 1. Functional trait variability in Q. ilex (circle) and Q. pubescens (triangle) in summer 2024 across sites: Udine, Palo Laziale, and Messina. Triangles and circles indicate mean trait values ± standard deviation. Colours denote sites: Udine (blue), Palo Laziale (green), Messina (red). Letters above bars indicate differences between the sites within-species based on ANOVA and Tukey's post hoc tests. Letters in uppercase refer to intraspecific differences in Q. ilex, whereas letters in lowercase refer to intraspecific differences in Q. pubescens. ψ_{PD}: predawn leaf water potential, ψ_{MD}: midday leaf water potential, ψ_{TLP}: turgor loss point, g_S: stomatal conductance, PLC: percentage loss of hydraulic conductivity, Hv: Huber value, LMA: leaf mass per area, RMF: root mass fraction.

Table 1
Percentage of mortality from spring to autumn 2024 for *Q. pubescens* and *Q. ilex* saplings at each site (n = 35).

Site	Mortality (%) *Q. pubescens*	Mortality (%) *Q. ilex*
Udine	0	0
Palo Laziale	23	6
Messina	16	7

than in the Mediterranean sites in leaves and roots (all $P < 0.001$), and higher than in Messina, specifically in twigs ($P = 0.01$) and stems ($P = 0.02$) (Fig. S6). For *Q. pubescens*, winter sugar concentrations were similar across sites in all organs, whereas starch concentrations were higher in twigs at Messina (all $P < 0.001$) and lower in stems ($P = 0.01$; $P < 0.001$) and roots ($P = 0.02$; $P < 0.01$) at Palo Laziale (Fig. 3). In *Q. ilex*, sugar concentrations were consistently higher in Udine across all organs (Fig. 4), whereas starch concentrations were comparable, except higher concentrations in twigs at Palo Laziale (all $P < 0.001$) and in roots at Udine compared to the other sites ($P = 0.02$; $P < 0.01$) (Fig. 4).

Overall, sugar and starch concentrations in twigs and stems varied significantly with P: PET in both species. Specifically, sugar concentrations decreased with increasing P: PET (i.e., decreasing aridity), whereas starch concentrations increased (Fig. 5). In leaves, sugars showed a slight increase with P: PET in *Q. ilex*, while no significant relationships with P: PET were detected for roots in any species (Fig. 5). The meteorological variables were mostly summarised by the first principal axis of PCA, which explained 64.3% of variability and was mostly driven by mean and maximum temperature and VPD, while the second principal axis of PCA explained 21.3% of variability and was mainly driven by

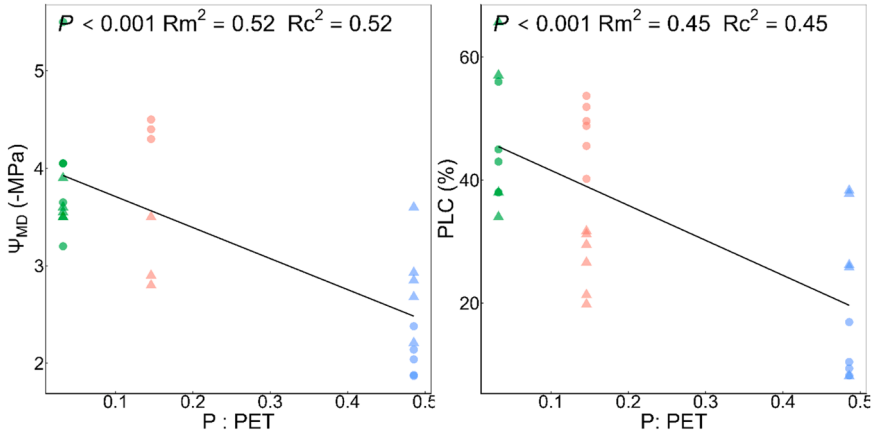

Fig. 2. Trait–environment relationships. Marginal (Rm^2) and conditional (Rc^2) R^2 values and P-values (P) are reported in the top-left corner of each panel. Black solid lines indicate significant trait–environment relationships. Trait values are represented with different symbols to distinguish the species: *Q. ilex* (circles) and *Q. pubescens* (triangles), and colours to denote sites: Udine (blue), Palo Laziale (green), Messina (red). P: PET, precipitation-to-potential evapotranspiration index. Ψ_{MD}: midday leaf water potential, PLC: percentage loss of hydraulic conductivity.

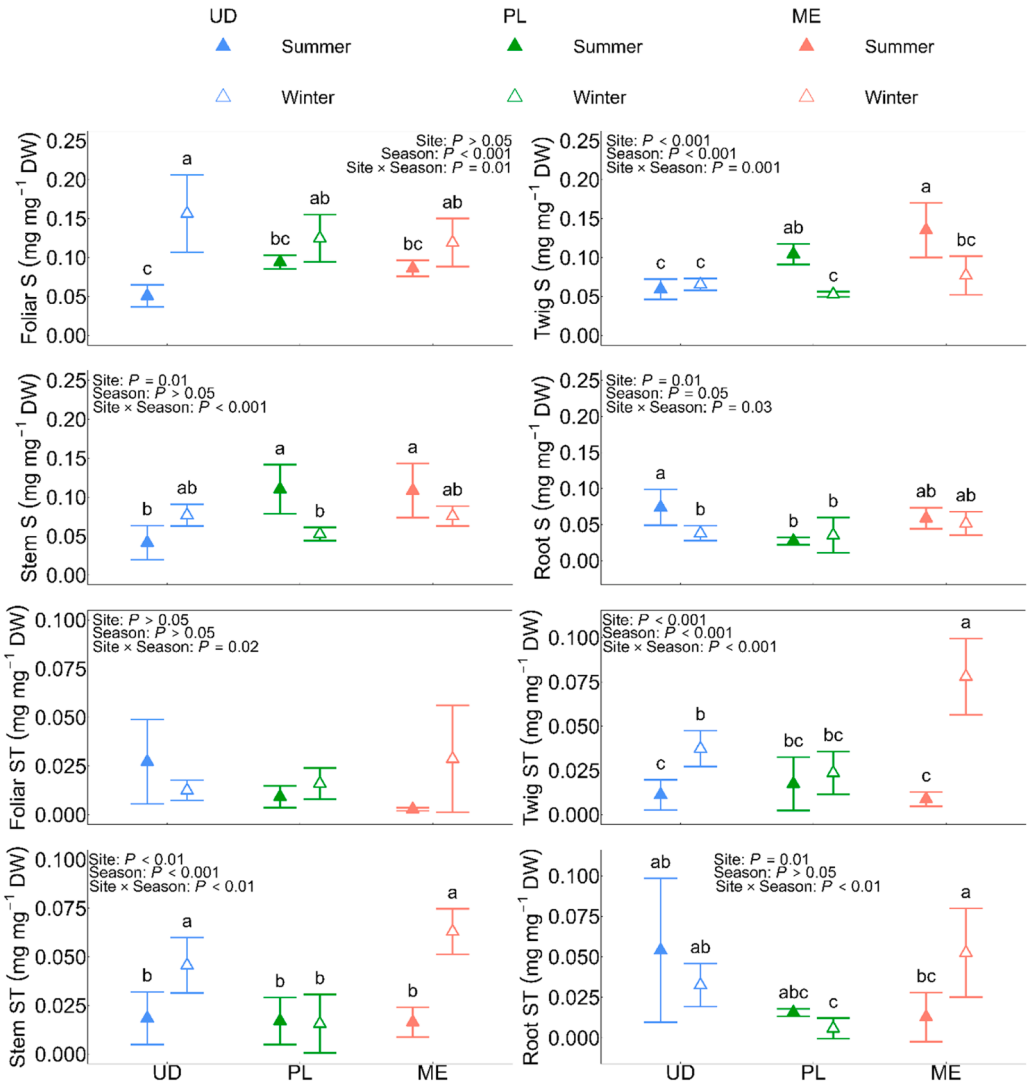

Fig. 3. Seasonal and site variation in non-structural carbohydrates in different organs of *Q. pubescens* saplings, shown by organ and non-structural carbohydrate fraction (S, sugars; ST, starch). Triangles indicate mean values ± standard deviation. ANOVA results are shown inside each panel. Different letters denote significant differences according to Tukey's post hoc test. Filled triangles correspond to summer data, empty triangles to winter data, and colours indicate sites: Udine (UD) (blue), Palo Laziale (PL) (green), Messina (ME) (red).

rainfall and relative humidity (Fig. S7). Foliar sugars significantly declined in both species with increasing temperatures and VPD (higher values of PC1), while significantly increased in twigs of *Q. pubescens*, stem of *Q. ilex* and fine roots of *Q. pubescens*. Regarding starch, it remained nearly stable, except in twigs, where it decreased significantly with increasing temperatures and VPD in both species and increased in fine roots of *Q. pubescens* (Fig. 6).

4. Discussion

4.1. Trait shifts in plant morphology and water relations across sites

Under drier conditions, we expected to observe morphological shifts towards higher leaf mass area, Huber value and root mass fraction, reflecting increased biomass investment in leaves, sapwood and roots. However, we found that, at the Mediterranean sites, oak saplings preferentially allocated their limited carbon reserves to belowground biomass, while not showing substantial morphological shifts aboveground (Fig. 1). This finding highlights the importance of the belowground compartment in juvenile trees with underdeveloped root systems for increasing water uptake and enabling them to avoid severe drought stress (Volaire, 2018).

Similar leaf mass area in *Q. pubescens* saplings across sites (Fig. 1) contrasts with our hypothesis and previous evidence on adult trees, which indicated greater values of this trait under more xeric conditions (Alonso-Forn et al., 2020; Rosas et al., 2019). Our result may imply that the evolution of a conservative resource strategy under dry conditions might not consistently occur in trees at their early life stage (Solé-Medina and Ramírez-Valiente, 2023). On the other hand, the higher LMA of *Q. ilex* saplings in Udine (Fig. 1) can be explained by the positive association between this trait and leaf longevity, with optimal leaf longevity typically declining with increasing growing-season mean temperature in evergreen plants (Dong et al., 2020; Kikuzawa et al., 2013). Alternatively, this finding may reflect slightly higher summer VPD in Udine than in the other localities, despite higher rainfall (Tables S1, S2), given the positive relationship between growing-season VPD and LMA (Dong et al., 2020).

While the Huber value was expected to be higher at drier sites as a mechanism to increase supply capacity per unit of water demand (Anderegg et al., 2021; Robert et al., 2017), our results did not support this pattern. The high within-site variability of Hv in *Q. pubescens* saplings grown in Mediterranean sites (Fig. 1) might subtend high

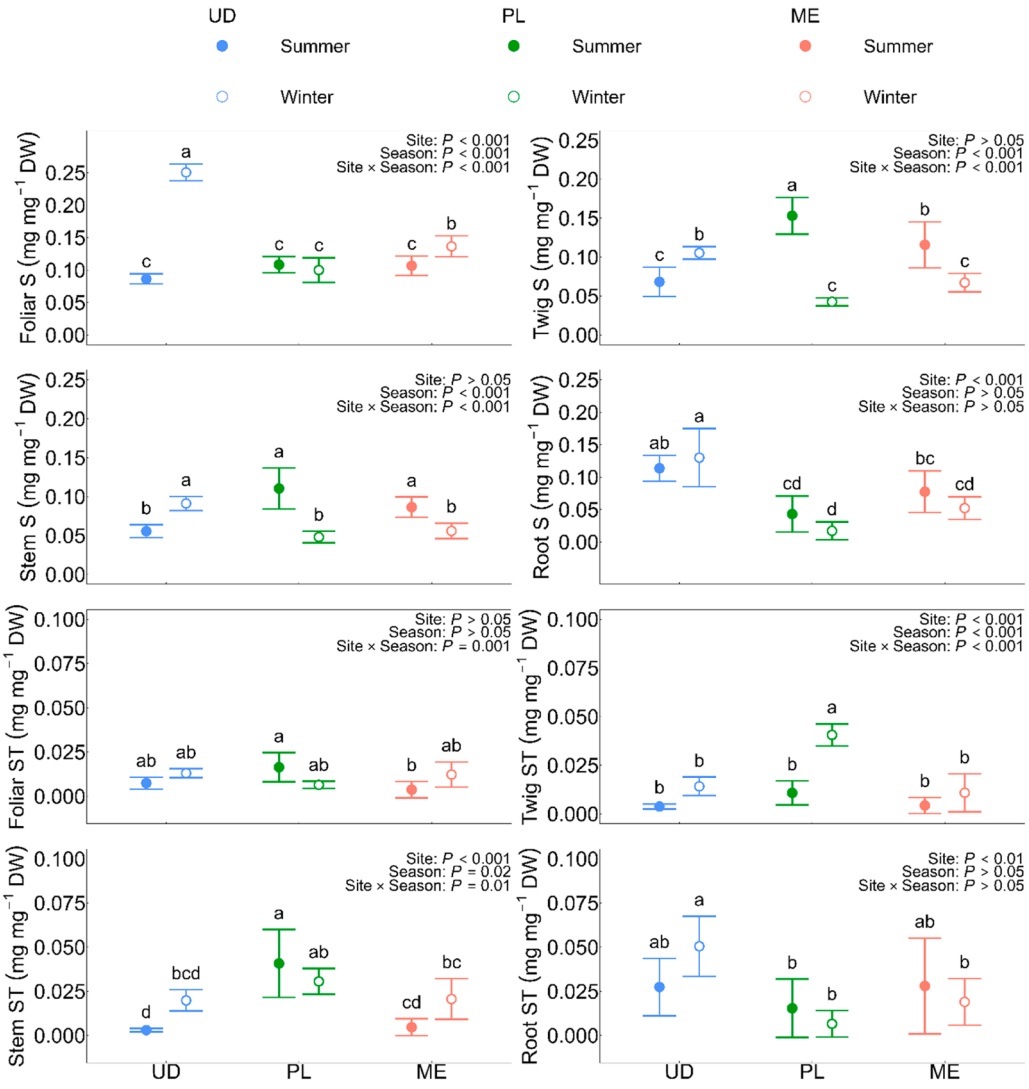

Fig. 4. Seasonal and site variation in non-structural carbohydrates in different organs of *Q. ilex* saplings, shown by organ and non-structural carbohydrate fraction (S, sugars; ST, starch). Circles indicate mean values ± standard deviation. ANOVA results are reported inside each panel. Different letters denote significant differences according to Tukey's post hoc test. Filled circles correspond to summer data, empty circles to winter data, and colours indicate sites: Udine (UD) (blue), Palo Laziale (PL) (green), Messina (ME) (red).

phenotypic or genotypic variation in this species under drier conditions. Accordingly, previous studies found high intra-population variability in this hydraulic efficiency-related trait (Fuchs et al., 2021; Rosas et al., 2019). In *Q. ilex* saplings, which showed lower within-site variability, the absence of higher Hv in Mediterranean sites (particularly in Palo Laziale) may suggest that other characteristics, such as RMF, exert a greater influence at this ontogenetic stage.

A general trade-off is expected between traits associated with drought-avoidance strategies, such as high RMF, and drought-tolerance strategies, such as more negative Ψ_{TLP} (Kannenberg et al., 2022). However, saplings, especially of *Q. pubescens*, at the Messina site exhibited both greater root mass fraction and more negative turgor loss point values (Fig. 1). Although drought stress levels were similar at the two Mediterranean sites, lower turgor loss point only at the southern location suggests site-specific responses, contrary to our initial expectation. Based on previous studies, we speculate that this difference might be shaped by site-specific long-term differences in drought-stress regimes or by phylogeographic history (Di Pietro et al., 2021; Peguero-Pina et al., 2014; Ramírez-Valiente and Cavender-Bares, 2017).

Higher leaf Ψ_{TLP} values (i.e., less negative) can make leaves more susceptible to wilting during drought, but they can also extend hydraulic safety margins by prompting earlier stomatal closure (Bartlett et al., 2016; Martin-StPaul et al., 2017). However, *Q. pubescens* saplings in Palo Laziale showed higher stomatal conductance than conspecifics in Messina at comparable leaf water potentials beyond their turgor loss point (Fig. 1). This, alongside the drier summer conditions at Palo Laziale, may explain the higher percentage losses of hydraulic conductance and mortality observed in *Q. pubescens* at this site. Conversely, saplings of *Q. ilex* both in Messina and Palo Laziale surpassed their turgor loss point, possibly because this species typically remains physiologically active despite relatively low leaf gas exchanges during summer drought (Ugolini et al., 2012). This result and the typically high embolism resistance of *Q. ilex* (Kiorapostolou et al., 2019) can explain why similar embolism levels were observed in this species at Mediterranean sites, with lower mortality than in *Q. pubescens*. Given the observed higher mortality at Mediterranean sites, we can hypothesise that under projected future warmer and more water-limited conditions, the observed functional shifts may not allow plants to keep pace with environmental variability.

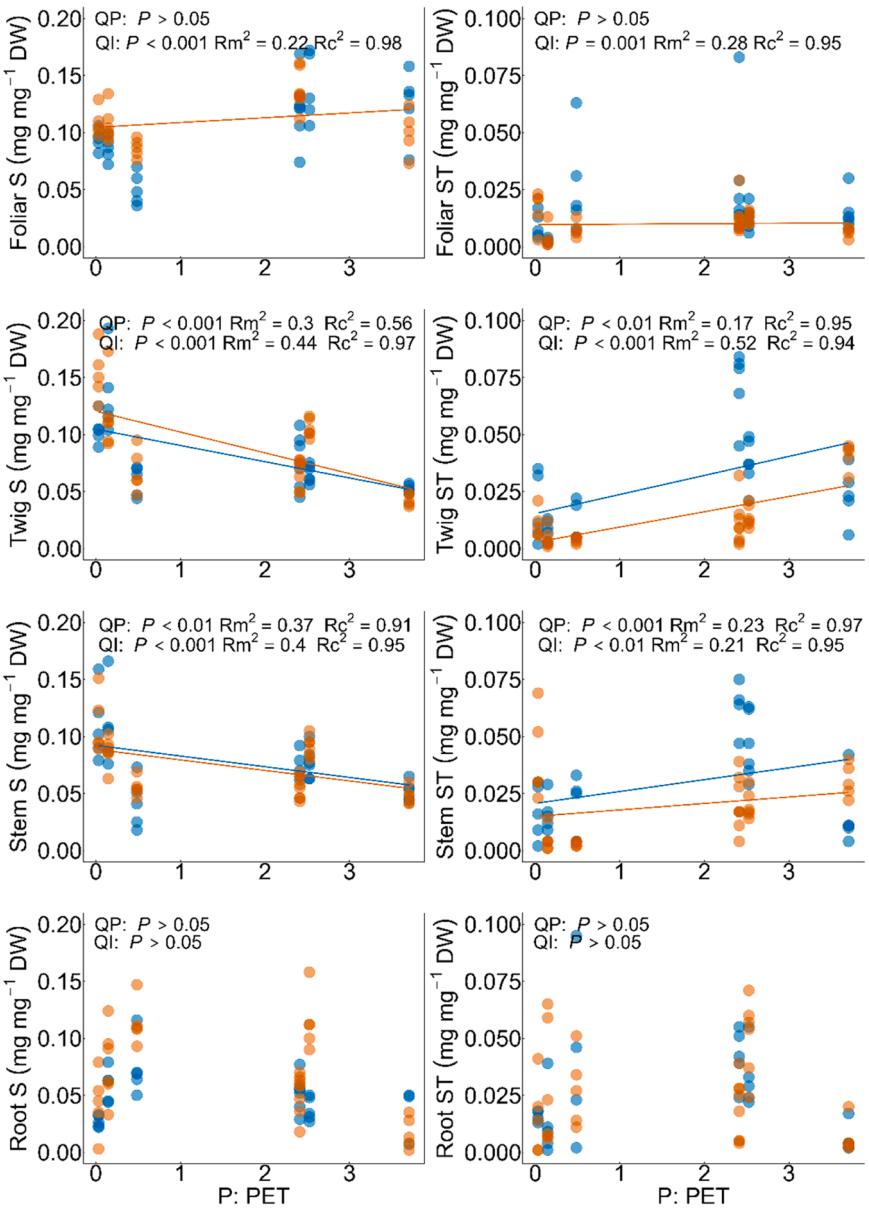

Fig. 5. Linear regressions between non-structural carbohydrates divided by fraction: sugars (S), and starch (ST) and the precipitation-to-potential evapotranspiration index (P: PET). Marginal (Rm^2) and conditional (Rc^2) R^2 values and p-values for each species (QP: *Q. pubescens*, QI: *Q. ilex*) are reported in the top-left corner of each panel for significant relationships, whereas only p-values are shown for non-significant ones. Only significant regressions are shown. Colour of regression lines and dots denotes the species: blue (*Q. pubescens*) and orange (*Q. ilex*).

4.2. Seasonal non-structural carbohydrate partitioning in saplings across sites

Demand for soluble NSCs is expected to increase during dry periods in xeric environments (Signori-Müller et al., 2021) to buffer drought stress, by increasing cell turgor via osmoregulation (Ali et al., 2023; Tomasella et al., 2025) and potentially to recover xylem hydraulic functioning (Trifilò et al., 2017). In this study, we tested whether this general pattern applies to both above- and belowground plant compartments, anticipating greater seasonal differences in soluble NSCs in all plant organs at the drier locations in response to summer drought. However, results indicated a summer increase and winter decline in soluble NSCs at the Mediterranean sites only in the twigs and stems of *Q. pubescens* and *Q. ilex* saplings (Figs. 3–4), implying organ-specific physiological responses. This finding can be explained by the functional characteristics of twigs and stems. Specifically, young woody stems are generally photosynthetically active (Natale et al., 2023; Pfanz et al., 2002), and parenchyma rays in the bark are a critical source of NSCs in responding to abiotic stresses (Gargiulo et al., 2024; Hartmann et al., 2018) and, in particular, drought (Trifilò et al., 2019). Accordingly, the increase in sugar concentrations in twigs and stems with water deficit (i.e., decreasing P: PET) and, to a lesser extent, temperature and VPD (PC1) supports the view that seasonal NSC differences were linked to physiological responses to drier conditions (Figs. 5–6). Despite greater total NSCs in twigs and stems during summer under drier conditions, plant biomass at the end of the growing season was not significantly lower at the Mediterranean sites (Figs. S4, S6). Therefore, we found no clear evidence of a growth–storage trade-off under carbon limitation (Dietze et al., 2014; Fajardo and Piper, 2021).

The winter decline in twig and stem sugars at Mediterranean locations (Figs. 3–4), anticipated in our first hypothesis, reflected distinct NSC allocation patterns across sites and species. Reduced twig and stem sugar and total NSC concentrations in *Q. ilex* saplings grown at Mediterranean sites, particularly in Messina, compared to Udine (Figs. 4, S6),

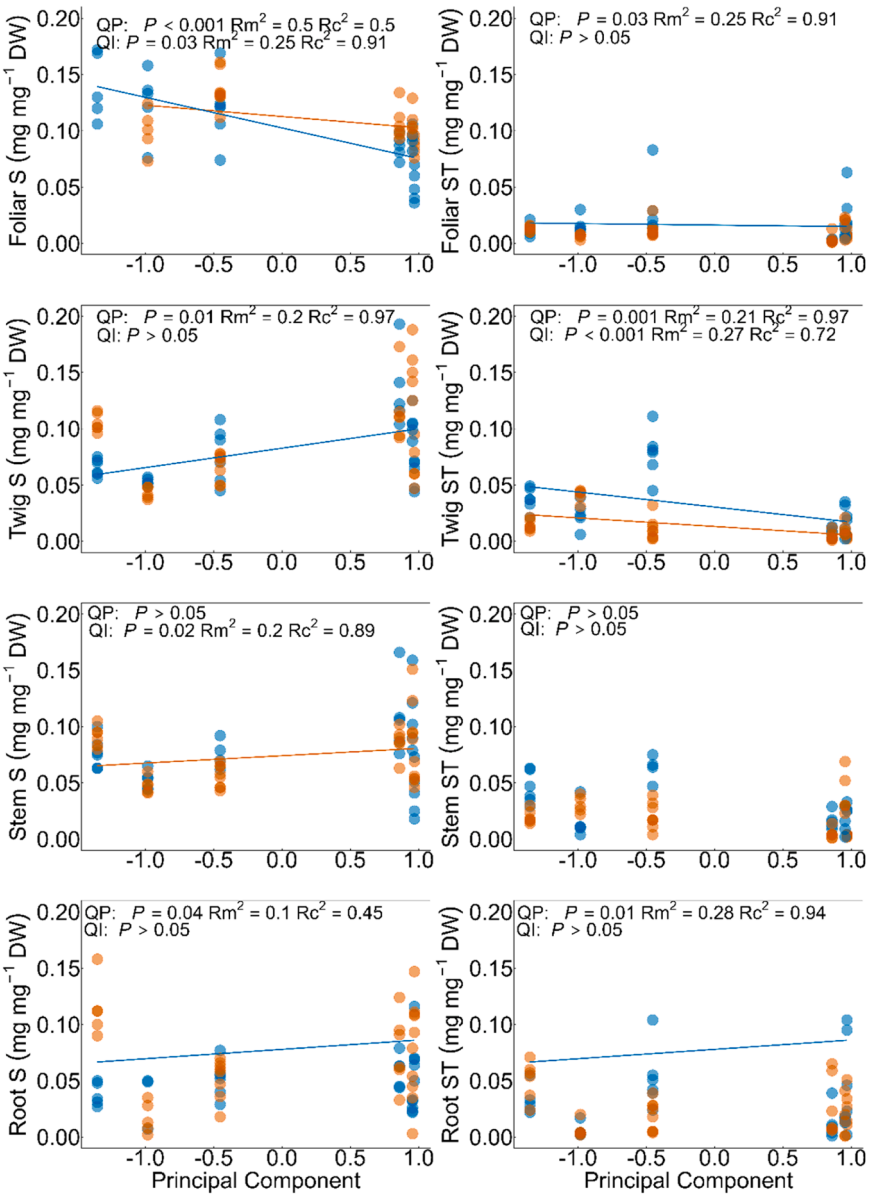

Fig. 6. Linear regressions between non-structural carbohydrates divided by fraction: sugars (S), and starch (ST) and climate dryness (first axis of principal components analysis (PCA) in the Supporting Information, Fig. S7). Marginal (Rm^2) and conditional (Rc^2) R^2 values and p-values for each species (QP: *Q. pubescens*, QI: *Q. ilex*) are reported in the top-left corner of each panel for significant relationships, whereas only p-values are shown for non-significant ones. Only significant regressions are shown. Colour of regression lines and dots denotes the species: blue (*Q. pubescens*) and orange (*Q. ilex*).

indicate higher sugar consumption than synthesis, under these harsher drought conditions. Analogously, we found the same pattern in the stems of *Q. pubescens* saplings at Palo Laziale, which aligns with their greater PLC and likely associated post-drought sink activities (Figs. 1, 3, S6) (Tomasella et al., 2017; Trugman et al., 2018). Conversely, twig and stem total NSCs did not vary seasonally in *Q. pubescens* saplings in Messina, but sugar levels declined, and starch increased from summer to winter, suggesting a seasonal conversion between soluble and insoluble NSC fractions (Figs. 3, S6) (Kannenberg and Phillips, 2020).

In leaves, we expected similar NSC patterns to those observed in twigs and stems, reflecting physiological responses to buffer summer drought stress. Conversely, foliar sugar concentrations generally increased from summer to winter in both species, especially in Udine, where *Q. pubescens* leaves had already begun to senesce (Figs. 3–4). This finding suggests that NSC accumulation exceeded utilisation in saplings grown in Udine, likely due to milder summer drought conditions (Figs. 1, S2) and potentially limited phloem sugar translocation at the time of sampling (Ray and Savage, 2021). Accordingly, the remobilisation of sugars from source to sink organs via the phloem can continue at the onset of and during leaf senescence (Parida et al., 2018). However, dead leaves in the litter can still contain significant quantities of non-remobilised NSCs (Bufacchi et al., 2020; Wu et al., 2023). Furthermore, the observed winter increase in foliar sugars in saplings of the evergreen species at the Udine site is consistent with a cryoprotective function (Martínez-Vilalta et al., 2016). Accordingly, the significant correlation between foliar sugar seasonal differences and temperature, in addition to aridity, supports this interpretation (Figs. 5–6).

Regarding fine roots, the seasonal NSC patterns across sites differed from those detected in the remaining organs, contrary to our initial expectation. Overall, the soluble and insoluble NSC fractions remained stable across seasons and were unaffected by P: PET (Figs. 3–4, 5). While this result disagrees with previous evidence indicating substantial seasonal NSC differences in fine roots (Hartmann et al., 2018), it corroborates other studies which found smaller drought responses in roots than

in stems (He et al., 2020). On the other hand, the significant positive correlation between fine root NSCs, temperature and VPD (PC1) in *Q. pubescens* may indicate carbon allocation to support increased respiratory and metabolic demand under these environmental conditions (Fig. 6) (Blessing et al., 2015). Accordingly, sink activities in fine roots require maintaining a certain pool of free carbon, in contrast to aerial organs (Zhang et al., 2024). However, the decline in winter total NSC pools in fine roots of *Q. ilex* saplings at the drier sites suggests that more NSCs were consumed for sink activities (e.g., respiration or post-drought root growth; Hikino et al., 2022) than were translocated via the phloem and stored in this organ (Figs. 4, S6).

Overall, reduced NSC storage in all organs of *Q. ilex* saplings in winter at the Mediterranean sites may exert legacy effects on tree saplings' capacity to cope with recurrent drought spells over multiple years (Figs. 4, S6) (Sapes et al., 2019; Tomasella et al., 2017). Conversely, *Q. pubescens* saplings in Mediterranean areas appeared to be less affected in terms of winter NSC storage, suggesting that the species' physiological characteristics can play a role in regulating carbon metabolism (Figs. 3, S6). Previous evidence indicated that NSC changes can be shaped by the distinct NSC storage requirements between species with contrasting leaf habits and/or xylem anatomy (Barbaroux and Bréda, 2002; Hoch et al., 2003). Given the role of NSCs in drought tolerance, recovery, and leaf sprouting (Tomasella et al., 2020) and the observed substantial variation within and between species in response to drought stress, it is crucial to understand the mechanisms subtending this variability.

5. Conclusions

Understanding systematic patterns of whole-tree physiological responses to drought of locally sourced saplings under local environmental conditions is a key prerequisite for evaluating the effectiveness of assisted migration. In this study, we showed that assessed oak saplings increased their biomass allocation belowground at drier sites without exhibiting substantial aboveground morphological changes. Although there were detectable functional trait differences across sites, including a lower turgor loss point at the southern location, these shifts were overall insufficient to offset the heightened hydraulic risk and mortality observed at Mediterranean sites. The results also uncovered seasonal, organ-specific differences in NSCs across locations, indicating that twigs and stems were subject to different physiological regulation than leaves and fine roots. We further identified a consistent reduction in winter sugars and total NSCs in above- and belowground organs of *Q. ilex* saplings grown under drier conditions. We propose that this depletion of NSCs could potentially serve as an early warning signal of drought vulnerability and should therefore be considered in revegetation planning.

Common-garden experiments would provide evolutionary insights into the causes of phenotypic intraspecific variability. Moreover, radiation, soil texture and its associated hydraulic properties should be explicitly considered in further experimentation to help explain functional differences among localities (Simpson et al., 2016). Despite these uncertainties, our study provided detailed information on trait variation at the whole-plant level and hydraulic risks across distinct bioclimates in two closely related oak species with contrasting drought tolerance, which may help future forest restoration outcomes.

Authors' contribution

ES, SG, VC, PT, AN, and MV designed the work; ES, SG, and PT acquired the data; SG performed NSC measurements; ES performed the data analysis; ES, SG, and VC interpreted the results and conceptualised the discussion with the contribution of all authors; ES wrote the first version of the draft. All the authors contributed to the revised version and agreed with the final version.

CRediT authorship contribution statement

Sara Gargiulo: Writing – review & editing, Investigation, Data curation, Conceptualization. **Valentino Casolo:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Data curation. **Elisa Spennati:** Writing – original draft, Investigation, Data curation. **Marcello Vitale:** Writing – review & editing, Supervision, Funding acquisition. **Patrizia Trifilò:** Writing – review & editing, Visualization, Validation, Investigation, Conceptualization. **Andrea Nardini:** Writing – review & editing, Methodology, Conceptualization.

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.

Acknowledgement

We gratefully acknowledge the EU project LIFE PRIMED (LIFE17 NAT/GR/000511). We sincerely thank Dr Paolo de Angelis (University of Tuscia) for providing one of the Scholander Chambers used in this study. We are also grateful to master's student Alessandra Conti (University of Rome La Sapienza) for valuable assistance with data collection at Palo Laziale and to Mr Francesco La Spada for providing access to his private property for the experimental site in Messina.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2026.106381.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Ali, O., Cheddadi, I., Landrein, B., Long, Y., 2023. Revisiting the relationship between turgor pressure and plant cell growth. New. Phytol. 238, 62–69. https://doi.org/10.1111/nph.18683.

Alonso-Forn, D., Sancho-Knapik, D., Ferrio, J.P., Peguero-Pina, J.J., Bueno, A., Onoda, Y., Cavender-Bares, J., Niinemets, Ü., Jansen, S., Riederer, M., Cornelissen, J. H.C., Chai, Y., Gil-Pelegrín, E., 2020. Revisiting the Functional Basis of Sclerophylly Within the Leaf Economics Spectrum of Oaks: Different Roads to Rome. Curr. For. Rep. 6, 260–281. https://doi.org/10.1007/s40725-020-00122-7.

Anderegg, L.D.L., Loy, X., Markham, I.P., Elmer, C.M., Hovenden, M.J., HilleRisLambers, J., Mayfield, M.M., 2021. Aridity drives coordinated trait shifts but not decreased trait variance across the geographic range of eight Australian trees. New. Phytol. 229, 1375–1387. https://doi.org/10.1111/nph.16795.

Andersson-Sköld, Y., Simpson, D., Ødegaard, V., 2008. Humidity parameters from temperature: test of a simple methodology for European conditions. Int. J. Climatol. 28, 961–972. https://doi.org/10.1002/joc.1586.

Aranda, I., Cano, F.J., Gascó, A., Cochard, H., Nardini, A., Mancha, J.A., López, R., Sánchez-Gómez, D., 2015. Variation in photosynthetic performance and hydraulic architecture across European beech (*Fagus sylvatica* L.) populations supports the case for local adaptation to water stress. Tree Physiol. 35, 34–46. https://doi.org/10.1093/treephys/tpu101.

Aranda, I., Sánchez-Gómez, D., Cadahía, E., de Simón, B.F., 2018. Ecophysiological and metabolic response patterns to drought under controlled condition in open-pollinated maternal families from a *Fagus sylvatica* L. population. Environ. Exp. Bot. 150 209–221. https://doi.org/10.1016/j.envexpbot.2018.03.014.

Barbaroux, C., Bréda, N., 2002. Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. Tree Physiol. 22, 1201–1210. https://doi.org/10.1093/treephys/22.17.1201.

Bartlett, M.K., Klein, T., Jansen, S., Choat, B., Sack, L., 2016. The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. Proc. Nat. Acad. Sci. 113, 13098–13103. https://doi.org/10.1073/pnas.1604088113.

Bartlett, Scoffoni, C., Sack, L., 2012b. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: A global meta-analysis. Ecol. Lett. 15, 393–405. https://doi.org/10.1111/j.1461-0248.2012.01751.x.

Bartlett, Scoffoni, C., Ardy, R., Zhang, Y., Sun, S., Cao, K., Sack, L., 2012a. Rapid determination of comparative drought tolerance traits: Using an osmometer to predict turgor loss point. Methods Ecol. Evol. 3, 880–888. https://doi.org/10.1111/j.2041-210X.2012.00230.x.

Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67. https://doi.org/10.18637/jss.v067.i01.

Beguería, S., Vicente-Serrano, S.M., 2025. A multiscalar drought index sensitive to global warming: The standardized precipitation evapotranspiration index. J. Clim. 23, 1696–1718. https://doi.org/10.1175/2009JCLI2909.1.

Benito Garzón, M., Robson, T.M., Hampe, A., 2019. ΔTraitSDMs: species distribution models that account for local adaptation and phenotypic plasticity. New. Phytol. 222, 1757–1765. https://doi.org/10.1111/nph.15716.

Bergmeyer, H.U., Bernt, E., 1974. UV-Assay with Pyruvate and NADH. In: Bergmeyer, H. U. (Ed.), Methods of Enzymatic Analysis (Second Edition). Academic Press, pp. 574–579. https://doi.org/10.1016/B978-0-12-091302-2.50010-4.

Blackman, C.J., Gleason, S.M., Chang, Y., Cook, A.M., Laws, C., Westoby, M., 2014. Leaf hydraulic vulnerability to drought is linked to site water availability across a broad range of species and climates. Ann. Bot. 114, 435–440. https://doi.org/10.1093/aob/mcu131.

Blessing, C.H., Werner, R.A., Siegwolf, R., Buchmann, N., 2015. Allocation dynamics of recently fixed carbon in beech saplings in response to increased temperatures and drought. Tree Physiol. 35 (6), 585–598. https://doi.org/10.1093/treephys/tpv024.

Bradshaw, A.D., 1965. Evolutionary Significance of Phenotypic Plasticity in Plants. In: Caspari, E.W., Thoday, J.M. (Eds.), Advances in Genetics. Academic Press, pp. 115–155. https://doi.org/10.1016/S0065-2660(08)60048-6.

Brodribb, T.J., 2017. Progressing from 'functional' to mechanistic traits. New. Phytol. 215, 9–11. https://doi.org/10.1111/nph.14620.

Brodribb, T.J., Powers, J., Cochard, H., Choat, B., 2020. Hanging by a thread? Forests and drought. Science 368, 261–266. https://doi.org/10.1126/science.aat7631.

Bufacchi, P., Bizzo, W.A., Buckeridge, M.S., Franco-Jacome, D.L., Grandis, A., Cambler, A.B., Krieger Filho, G.C., 2020. Thermal degradation of leaves from the Amazon rainforest litter considering non-structural, structural carbohydrates and lignin composition. Bioresour. Technol. Rep. 11, 100490. https://doi.org/10.1016/j.biteb.2020.100490.

Casolo, V., Zancani, M., Pellegrini, E., Filippi, A., Gargiulo, S., Konnerup, D., Morandini, P., Pedersen, O., 2023. Restricted O_2 consumption in pea roots induced by hexanoic acid is linked to depletion of Krebs cycle substrates. Physiol. Plant. 175, e14024. https://doi.org/10.1111/ppl.14024.

Core, T.R., 2023. R: A language and environment for statistical computing (Version 4.3.2). R Foundation for Statistical Computing.

Cregg, B.M., 1994. Carbon allocation, gas exchange, and needle morphology of Pinus ponderosa genotypes known to differ in growth and survival under imposed drought. Tree Physiol. 14, 883–898. https://doi.org/10.1093/treephys/14.7-8-9.883.

Di Pietro, R., Conte, A.L., Di Marzio, P., Fortini, P., Farris, E., Gianguzzi, L., Müller, M., Rosati, L., Spampinato, G., Gailing, O., 2021. Does the genetic diversity among pubescent white oaks in southern Italy, Sicily and Sardinia islands support the current taxonomic classification? Eur. J. For. Res. 140, 355–371. https://doi.org/10.1007/s10342-020-01334-z.

Dietze, M.C., Sala, A., Carbone, M.S., Czimczik, C.I., Mantooth, J.A., Richardson, A.D., Vargas, R., 2014. Non-structural carbon in woody plants. Annu. Rev. Plant. Biol. 65, 667–687. https://doi.org/10.1146/annurev-arplant-050213-040054.

Dong, N., Prentice, I.C., Wright, I.J., Evans, B.J., Togashi, H.F., Caddy-Retalic, S., McInerney, F.A., Sparrow, B., Leitch, E., Lowe, A.J., 2020. Components of leaf-trait variation along environmental gradients. New. Phytol. 228, 82–94. https://doi.org/10.1111/nph.16558.

Fajardo, A., Piper, F.I., 2021. How to cope with drought and not die trying: Drought acclimation across tree species with contrasting niche breadth. Funct. Ecol. 35, 1903–1913. https://doi.org/10.1111/1365-2435.13861.

Fuchs, S., Leuschner, C., Mathias Link, R., Schuldt, B., 2021. Hydraulic variability of three temperate broadleaf tree species along a water availability gradient in central Europe. New. Phytol. 231, 1387–1400. https://doi.org/10.1111/nph.17448.

Furze, M.E., Huggett, B.A., Aubrecht, D.M., Stolz, C.D., Carbone, M.S., Richardson, A.D., 2019. Whole-tree non-structural carbohydrate storage and seasonal dynamics in five temperate species. New. Phytol. 221, 1466–1477. https://doi.org/10.1111/nph.15462.

Gargiulo, S., Boscutti, F., Carrer, M., Prendin, A.L., Unterholzner, L., Dibona, R., Casolo, V., 2024. Snowpack permanence shapes the growth and dynamic of non-structural carbohydrates in *Juniperus communis* in alpine tundra. Sci. Total. Environ. 948, 174891. https://doi.org/10.1016/j.scitotenv.2024.174891.

Gazol, A., Camarero, J.J., Anderegg, W.R.L., Vicente-Serrano, S.M., 2017. Impacts of droughts on the growth resilience of Northern Hemisphere forests. Glob. Ecol. Biogeogr. 26, 166–176. https://doi.org/10.1111/geb.12526.

Hajek, P., Kurjak, D., Von Wühlisch, G., Delzon, S., Schuldt, B., 2016. Intraspecific variation in wood anatomical, hydraulic, and foliar traits in ten European beech provenances differing in growth yield. Front. Plant. Sci. 7. https://doi.org/10.3389/fpls.2016.00791.

Hargreaves, H.G., Samani, A.Z., 1982. Estimating Potential Evapotranspiration. J. Irrig. Drain. Div. 108, 225–230. https://doi.org/10.1061/JRCEA4.0001390.

Hartmann, H., Adams, H.D., Hammond, W.M., Hoch, G., Landhäusser, S.M., Wiley, E., Zaehle, S., 2018. Identifying differences in carbohydrate dynamics of seedlings and mature trees to improve carbon allocation in models for trees and forests. Environ. Exp. Bot. 152, 7–18. https://doi.org/10.1016/j.envexpbot.2018.03.011.

Hartmann, H., Trumbore, S., 2016. Understanding the roles of non-structural carbohydrates in forest trees - from what we can measure to what we want to know. New. Phytol. 211, 386–403. https://doi.org/10.1111/nph.13955.

He, W., Liu, H., Qi, Y., Liu, F., Zhu, X., 2020. Patterns in non-structural carbohydrate contents at the tree organ level in response to drought duration. Glob. Change Biol. 26, 3627–3638. https://doi.org/10.1111/gcb.15078.

Hikino, K., Danzberger, J., Riedel, V.P., Hesse, B.D., Hafner, B.D., Gebhardt, T., Rehschuh, R., Ruehr, N.K., Brunn, M., Bauerle, T.L., Landhäusser, S.M., Lehmann, M. M., Rötzer, T., Pretzsch, H., Buegger, F., Weikl, F., Pritsch, K., Grams, T.E.E., 2022. Dynamics of initial carbon allocation after drought release in mature Norway spruce—Increased belowground allocation of current photoassimilates covers only half of the carbon used for fine-root growth. Glob. Change Biol. 28, 6889–6905. https://doi.org/10.1111/gcb.16388.

Hoch, G., Richter, A., Körner, C., 2003. Non-structural carbon compounds in temperate forest trees. Plant. Cell. Environ. 26, 1067–1081. https://doi.org/10.1046/j.0016-8025.2003.01032.x.

Hoffmann, A.A., Sgrò, C.M., 2011. Climate change and evolutionary adaptation. Nat 470, 479–485. https://doi.org/10.1038/nature09670.

Kannenberg, S.A., Guo, J.S., Novick, K.A., Anderegg, W.R.L., Feng, X., Kennedy, D., Konings, A.G., Martínez-Vilalta, J., Matheny, A.M., 2022. Opportunities, challenges and pitfalls in characterizing plant water-use strategies. Funct. Ecol. 36, 24–37. https://doi.org/10.1111/1365-2435.13945.

Kannenberg, S.A., Phillips, R.P., 2020. Non-structural carbohydrate pools not linked to hydraulic strategies or carbon supply in tree saplings during severe drought and subsequent recovery. Tree Physiol. 40 (2), 259–271. https://doi.org/10.1093/treephys/tpz132.

Kattge, J., Díaz, S., Lavorel, S., Prentice, I.C., Leadley, P., Bönisch, G., Garnier, E., Westoby, M., Reich, P.B., Wright, I.J., Cornelissen, J.H.C., Violle, C., Harrison, S.P., Van Bodegom, P.M., Reichstein, M., Enquist, B.J., Soudzilovskaia, N.A., Ackerly, D. D., Anand, M., Atkin, O., Bahn, M., Baker, T.R., Baldocchi, D., Bekker, R., Blanco, C. C., Blonder, B., Bond, W.J., Bradstock, R., Bunker, D.E., Casanoves, F., Cavender-Bares, J., Chambers, J.Q., Chapin, F.S., Chave, J., Coomes, D., Cornwell, W.K., Craine, J.M., Dobrin, B.H., Duarte, L., Durka, W., Elser, J., Esser, G., Estiarte, M., Fagan, W.F., Fang, J., Fernández-Méndez, F., Fidelis, A., Finegan, B., Flores, O., Ford, H., Frank, D., Freschet, G.T., Fyllas, N.M., Gallagher, R.V., Green, W.A., Gutierrez, A.G., Hickler, T., Higgins, S.I., Hodgson, J.G., Jalili, A., Jansen, S., Joly, C. A., Kerkhoff, A.J., Kirkup, D., Kitajima, K., Kleyer, M., Klotz, S., Knops, J.M.H., Kramer, K., Kühn, I., Kurokawa, H., Laughlin, D., Lee, T.D., Leishman, M., Lens, F., Lenz, T., Lewis, S.L., Lloyd, J., Llusià, J., Louault, F., Ma, S., Mahecha, M.D., Manning, P., Massad, T., Medlyn, B.E., Messier, J., Moles, A.T., Müller, S.C., Nadrowski, K., Naeem, S., Niinemets, Ü., Nöllert, S., Nüske, A., Ogaya, R., Oleksyn, J., Onipchenko, V.G., Onoda, Y., Ordoñez, J., Overbeck, G., Ozinga, W.A., Patiño, S., Paula, S., Pausas, J.G., Peñuelas, J., Phillips, O.L., Pillar, V., Poorter, H., Poorter, L., Poschlod, P., Prinzing, A., Proulx, R., Rammig, A., Reinsch, S., Reu, B., Sack, L., Salgado-Negret, B., Sardans, J., Shiodera, S., Shipley, B., Siefert, A., Sosinski, E., Soussana, J.F., Swaine, E., Swenson, N., Thompson, K., Thornton, P., Waldram, M., Weiher, E., White, M., White, S., Wright, S.J., Yguel, B., Zaehle, S., Zanne, A.E., Wirth, C., 2011. TRY - a global database of plant traits. Glob. Change Biol. 17, 2905–2935. https://doi.org/10.1111/j.1365-2486.2011.02451.x.

Kikuzawa, K., Onoda, Y., Wright, I.J., Reich, P.B., 2013. Mechanism of leaf longevity patterns. Glob. Ecol. Biogeogr. 22, 982–993. https://doi.org/10.1111/geb.12042.

Kiorapostolou, N., Da Sois, L., Petruzzellis, F., Savi, T., Trifilò, P., Nardini, A., Petit, G., 2019. Vulnerability to xylem embolism correlates to wood parenchyma fraction in angiosperms but not in gymnosperms. Tree Physiol. 39, 1675–1684. https://doi.org/10.1093/treephys/tpz068.

Kozlowski, T.T., 1992. Carbohydrate sources and sinks in woody plants. Bot. Rev. 58, 107–222. https://doi.org/10.1007/BF02858600.

Landhäusser, S.M., Chow, P.S., Turin Dickman, L., Furze, M.E., Kuhlman, I., Schmid, S., Wiesenbauer, J., Wild, B., Gleixner, G., Hartmann, H., Hoch, G., McDowell, N.G., Richardson, A.D., Richter, A., Adams, H.D., 2018. Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. Tree Physiol. 38, 1764–1778. https://doi.org/10.1093/treephys/tpy118.

Larter, M., Pfautsch, S., Domec, J.-C., Trueba, S., Nagalingum, N., Delzon, S., 2017. Aridity drove the evolution of extreme embolism resistance and the radiation of conifer genus *Callitris*. New. Phytol. 215, 97–112. https://doi.org/10.1111/nph.14545.

Leites, L., Benito Garzón, M., 2023. Forest tree species adaptation to climate across biomes: Building on the legacy of ecological genetics to anticipate responses to climate change. Glob. Change Biol. 29, 4711–4730. https://doi.org/10.1111/gcb.16711.

Lloret, F., Peñuelas, J., Estiarte, M., 2004. Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. Glob. Change Biol. 10, 248–258. https://doi.org/10.1111/j.1365-2486.2004.00725.x.

Lo, Gullo, M., Salleo, S., 1991. Three Different Methods for Measuring Xylem Cavitation and Embolism: A Comparison. Ann. Bot. 67, 417–424. https://doi.org/10.1093/oxfordjournals.aob.a088158.

Lobo, A., Torres-Ruiz, J.M., Burlett, R., Lemaire, C., Parise, C., Francioni, C., Truffaut, L., Tomášková, I., Hansen, J.K., Kjær, E.D., Kremer, A., Delzon, S., 2018. Assessing inter- and intraspecific variability of xylem vulnerability to embolism in oaks. For. Ecol. Manag. 424, 53–61. https://doi.org/10.1016/j.foreco.2018.04.031.

López, R., López de Heredia, U., Collada, C., Cano, F.J., Emerson, B.C., Cochard, H., Gil, L., 2013. Vulnerability to cavitation, hydraulic efficiency, growth and survival in an insular pine (*Pinus canariensis*). Ann. Bot. 111, 1167–1179. https://doi.org/10.1093/aob/mct084.

MacNeill, G.J., Mehrpouyan, S., Minow, M.A.A., Patterson, J.A., Tetlow, I.J., Emes, M.J., 2017. Starch as a source, starch as a sink: The bifunctional role of starch in carbon allocation. J. Exp. Bot. 68, 4433–4453. https://doi.org/10.1093/jxb/erx291.

Martínez-Vilalta, J., Cochard, H., Mencuccini, M., Sterck, F., Herrero, A., Korhonen, J.F. J., Llorens, P., Nikinmaa, E., Nolè, A., Poyatos, R., Ripullone, F., Sass-Klaassen, U.,

Zweifel, R., 2009. Hydraulic adjustment of Scots pine across Europe. New. Phytol. 184, 353–364. https://doi.org/10.1111/j.1469-8137.2009.02954.x.
Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., Piper, F.I., Lloret, F., 2016. Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. Ecol. Monogr. 86, 495–516. https://doi.org/10.1002/ecm.1231.
Martin-StPaul, N., Delzon, S., Cochard, H., 2017. Plant resistance to drought depends on timely stomatal closure. Ecol. Lett. 20, 1437–1447. https://doi.org/10.1111/ele.12851.
Matías, L., González-Díaz, P., Jump, A.S., 2014. Larger investment in roots in southern range-edge populations of Scots pine is associated with increased growth and seedling resistance to extreme drought in response to simulated climate change. Environ. Exp. Bot. 105, 32–38. https://doi.org/10.1016/j.envexpbot.2014.04.003.
McDowell, N.G., 2011. Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. Plant. Physiol. 155, 1051–1059. https://doi.org/10.1104/pp.110.170704.
McMaster, G.S., Wilhelm, W.W., 1997. Growing degree-days: one equation, two interpretations. Agric. For. Meteorol. 87 (4), 291–300. https://doi.org/10.1016/S0168-1923(97)00027-0.
Mencuccini, M., Bonosi, L., 2001. Leaf/sapwood area ratios in Scots pine show acclimation across Europe. Can. J. For. Res. 31, 442–456. https://doi.org/10.1139/x00-173.
Moles, A.T., Perkins, S.E., Laffan, S.W., Flores-Moreno, H., Awasthy, M., Tindall, M.L., Sack, L., Pitman, A., Kattge, J., Aarssen, L.W., Anand, M., Bahn, M., Blonder, B., Cavender-Bares, J., Cornelissen, J.H.C., Cornwell, W.K., Díaz, S., Dickie, J.B., Freschet, G.T., Griffiths, J.G., Gutierrez, A.G., Hemmings, F.A., Hickler, T., Hitchcock, T.D., Keighery, M., Kleyer, M., Kurokawa, H., Leishman, M.R., Liu, K., Niinemets, Ü., Onipchenko, V., Onoda, Y., Penuelas, J., Pillar, V.D., Reich, P.B., Shiodera, S., Siefert, A., Sosinski, E.E., Soudzilovskaia, N.A., Swaine, E.K., Swenson, N.G., van Bodegom, P.M., Warman, L., Weiher, E., Wright, I.J., Zhang, H., Zobel, M., Bonser, S.P., 2014. Which is a better predictor of plant traits: Temperature or precipitation? J. Veg. Sci. 25, 1167–1180. https://doi.org/10.1111/jvs.12190.
Moran, E.V., Hartig, F., Bell, D.M., 2016. Intraspecific trait variation across scales: Implications for understanding global change responses. Glob. Change Biol. 22, 137–150. https://doi.org/10.1111/gcb.13000.
Moreno, M., Limousin, J.-M., Simioni, G., Badel, E., Rodríguez-Calcerrada, J., Cochard, H., Torres-Ruiz, J.M., Dupuy, J.-L., Ruffault, J., Ormeno, E., Delzon, S., Fernandez, C., Ourcival, J.-M., Martin-StPaul, N., 2024. Hydraulic plasticity and water use regulation act to maintain the hydraulic safety margins of Mediterranean trees in rainfall exclusion experiments. Plant. Cell. Environ. 47, 4741–4753. https://doi.org/10.1111/pce.15066.
Nardini, A., Savi, T., Losso, A., Petit, G., Pacilè, S., Tromba, G., Mayr, S., Trifilò, P., Lo Gullo, M.A., Salleo, S., 2017. X-ray microtomography observations of xylem embolism in stems of *Laurus nobilis* are consistent with hydraulic measurements of percentage loss of conductance. New. Phytol. 213, 1068–1075. https://doi.org/10.1111/nph.14245.
Natale, S., Tomasella, M., Gargiulo, S., Petruzzellis, F., Tromba, G., Boccato, E., Casolo, V., Nardini, A., 2023. Stem photosynthesis contributes to non-structural carbohydrate pool and modulates xylem vulnerability to embolism in *Fraxinus ornus* L. Environ. Exp. Bot. 210. https://doi.org/10.1016/j.envexpbot.2023.105315.
Nawaz, A.F., Gargiulo, S., Pichierri, A., Casolo, V., 2025. Exploring the Role of Non-Structural Carbohydrates (NSCs) Under Abiotic Stresses on Woody Plants: A Comprehensive Review. Plants 14, 328. https://doi.org/10.3390/plants14030328.
Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., Poot, P., Purugganan, M.D., Richards, C.L., Valladares, F., van Kleunen, M., 2010. Plant phenotypic plasticity in a changing climate. Trends Plant. Sci. 15, 684–692. https://doi.org/10.1016/j.tplants.2010.09.008.
O'Brien, M.J., Leuzinger, S., Philipson, C.D., Tay, J., Hector, A., 2014. Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. Nat. Clim. Change 4, 710–714. https://doi.org/10.1038/nclimate2281.
Parida, A.K., Kumari, A., Panda, A., Rangani, J., Agarwal, P.K., 2018. Photosynthetic pigments, betalains, proteins, sugars, and minerals during *Salicornia brachiata* senescence. Biol. Plant. 62, 343–352. https://doi.org/10.1007/s10535-017-0764-1.
Pasta, S., de Rigo, D., Caudullo, G., 2016. *Quercus pubescens* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A. (Eds.), European Atlas of Forest Tree Species. Publ. Off. EU, Luxembourg, p. e019e5c (+).
Peguero-Pina, J.J., Sancho-Knapik, D., Barrón, E., Camarero, J.J., Vilagrosa, A., Gil-Pelegrín, E., 2014. Morphological and physiological divergences within *Quercus ilex* support the existence of different ecotypes depending on climatic dryness. Ann. Bot. 114 (2), 301–313. https://doi.org/10.1093/aob/mcu108.
Petruzzellis, F., Savi, T., Bacaro, G., Nardini, A., 2019. A simplified framework for fast and reliable measurement of leaf turgor loss point. Plant. Physiol. Biochem. 139, 395–399. https://doi.org/10.1016/j.plaphy.2019.03.043.
Pfanz, H., Aschan, G., Langenfeld-Heyser, R., Wittmann, C., Loose, M., 2002. Ecology and ecophysiology of tree stems: corticular and wood photosynthesis. Naturwissenschaften 89, 147–162. https://doi.org/10.1007/s00114-002-0309-z.
Quentin, A.G., Pinkard, E.A., Ryan, M.G., Tissue, D.T., Baggett, L.S., Adams, H.D., Maillard, P., Marchand, J., Landhäusser, S.M., Lacointe, A., Gibon, Y., Anderegg, W. R.L., Asao, S., Atkin, O.K., Bonhomme, M., Claye, C., Chow, P.S., Clément-Vidal, A., Davies, N.W., Dickman, L.T., Dumbur, R., Ellsworth, D.S., Falk, K., Galiano, L., Grünzweig, J.M., Hartmann, H., Hoch, G., Hood, S., Jones, J.E., Koike, T., Kuhlmann, I., Lloret, F., Maestro, M., Mansfield, S.D., Martínez-Vilalta, J., Maucourt, M., McDowell, N.G., Moing, A., Muller, B., Nebauer, S.G., Niinemets, Ü., Palacio, S., Piper, F., Raveh, E., Richter, A., Rolland, G., Rosas, T., Joanis, B.S., Sala, A., Smith, R.A., Sterck, F., Stinziano, J.R., Tobias, M., Unda, F., Watanabe, M., Way, D.A., Weerasinghe, L.K., Wild, B., Wiley, E., Woodruff, D.R., 2015. Non-structural carbohydrates in woody plants compared among laboratories. Tree Physiol. 35, 1146–1165. https://doi.org/10.1093/treephys/tpv073.
Ramírez-Valiente, J.A., Cavender-Bares, J., 2017. Evolutionary trade-offs between drought resistance mechanisms across a precipitation gradient in a seasonally dry tropical oak (*Quercus oleoides*). Tree Physiol. 37 (7), 889–901. https://doi.org/10.1093/treephys/tpx040.
Ramírez-Valiente, J.A., Solé-Medina, A., Pyhäjärvi, T., Savolainen, O., Cervantes, S., Kesälahti, R., Kujala, S.T., Kumpula, T., Heer, K., Opgenoorth, L., Siebertz, J., Danusevicius, D., Notivol, E., Benavides, R., Robledo-Arnuncio, J.J., 2021. Selection patterns on early-life phenotypic traits in *Pinus sylvestris* are associated with precipitation and temperature along a climatic gradient in Europe. New. Phytol. 229, 3009–3025. https://doi.org/10.1111/nph.17029.
Ray, D.M., Savage, J.A., 2021. Seasonal changes in temperate woody plant phloem anatomy and physiology: implications for long-distance transport. AoB Plants 13, plab028. https://doi.org/10.1093/aobpla/plab028.
Reich, P.B., 2014. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. J. Ecol. 102, 275–301. https://doi.org/10.1111/1365-2745.12211.
Revelle, W., 2025. psych: Procedures for Psychological, Psychometric, and Personality Research_. Northwestern University, Evanston, Illinois. R package version 2.5.6, ⟨https://CRAN.R-project.org/package=psych⟩.
de Rigo, D., Caudullo, G., 2016. Quercus ilex Eur. Distrib. Habitat. Usage Threats.
Rivas-Martínez, S., Rivas-Saenz, S., Penas, Á., 2002. Worldwide bioclimatic classification system. Kerkwerve, The Netherlands. Backhuys Pub.
Rizzo, K.T., Bailey, B.N., 2026. A Psychrometric Temperature Correction for the Positive Bias Observed in Stomatal Conductance Measured by the Open Flow-Through LI-600 Porometer. Plant. Cell. Environ. 49, 1412–1423. https://doi.org/10.1111/pce.70304.
Robert, E.M.R., Mencuccini, M., Martínez-Vilalta, J., 2017. The Anatomy and Functioning of the Xylem in Oaks. In: Oak's physiological ecology, exploring the functional diversity of genus Quercus L, 7. Springer International Publishing AG, pp. 261–302. https://doi.org/10.1007/978-3-319-69099-5_8.
Rosas, T., Mencuccini, M., Barba, J., Cochard, H., Saura-Mas, S., Martínez-Vilalta, J., 2019. Adjustments and coordination of hydraulic, leaf and stem traits along a water availability gradient. New. Phytol. 223, 632–646. https://doi.org/10.1111/nph.15684.
Salazar Zarzosa, P., Diaz Herraiz, A., Olmo, M., Ruiz-Benito, P., Barrón, V., Bastias, C.C., de la Riva, E.G., Villar, R., 2021. Linking functional traits with tree growth and forest productivity in *Quercus ilex* forests along a climatic gradient. Sci. Total. Environ. 786. https://doi.org/10.1016/j.scitotenv.2021.147468.
Sapes, G., Roskilly, B., Dobrowski, S., Maneta, M., Anderegg, W.R.L., Martinez-Vilalta, J., Sala, A., 2019. Plant water content integrates hydraulics and carbon depletion to predict drought-induced seedling mortality. Tree Physiol. 39, 1300–1312. https://doi.org/10.1093/treephys/tpz062.
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019.
Siefert, A., Violle, C., Chalmandrier, L., Albert, C.H., Taudiere, A., Fajardo, A., Aarssen, L. W., Baraloto, C., Carlucci, M.B., Cianciaruso, M.V., de L. Dantas, V., de Bello, F., Duarte, L.D.S., Fonseca, C.R., Freschet, G.T., Gaucherand, S., Gross, N., Hikosaka, K., Jackson, B., Jung, V., Kamiyama, C., Katabuchi, M., Kembel, S.W., Kichenin, E., Kraft, N.J.B., Lagerström, A., Bagousse-Pinguet, Y.L., Li, Y., Mason, N., Messier, J., Nakashizuka, T., Overton, J.M.C., Peltzer, D.A., Pérez-Ramos, I.M., Pillar, V.D., Prentice, H.C., Richardson, S., Sasaki, T., Schamp, B.S., Schöb, C., Shipley, B., Sundqvist, M., Sykes, M.T., Vandewalle, M., Wardle, D.A., 2015. A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. Ecol. Lett. 18, 1406–1419. https://doi.org/10.1111/ele.12508.
Signori-Müller, C., Oliveira, R.S., Barros, F., de, V., Tavares, J.V., Gilpin, M., Diniz, F.C., Zevallos, M.J.M., Yupayccana, C.A.S., Acosta, M., Bacca, J., Chino, R.S.C., Cuellar, G. M.A., Cumapa, E.R.M., Martinez, F., Mullisaca, F.M.P., Nina, A., Sanchez, J.M.B., da Silva, L.F., Tello, L., Tintaya, J.S., Ugarteche, M.T.M., Baker, T.R., Bittencourt, P.R. L., Borma, L.S., Brum, M., Castro, W., Coronado, E.N.H., Cosio, E.G., Feldpausch, T. R., Fonseca, L., d'Agosto, M., Gloor, E., Llampazo, G.F., Malhi, Y., Mendoza, A.M., Moscoso, V.C., Araujo-Murakami, A., Phillips, O.L., Salinas, N., Silveira, M., Talbot, J., Vasquez, R., Mencuccini, M., Galbraith, D., 2021. Non-structural carbohydrates mediate seasonal water stress across Amazon forests. Nat. Commun. 12. https://doi.org/10.1038/s41467-021-22378-8.
Silva, J.C., Potts, B., Harrison, P.A., Bailey, T., 2019. Temperature and rainfall are separate agents of selection shaping population differentiation in a forest tree. Forests 10. https://doi.org/10.3390/F10121145.
Simpson, A.H., Richardson, S.J., Laughlin, D.C., 2016. Soil–climate interactions explain variation in foliar, stem, root and reproductive traits across temperate forests. Glob. Ecol. Biogeogr. 25, 964–978. https://doi.org/10.1111/geb.12457.
Skelton, R.P., West, A.G., Dawson, T.E., 2015. Predicting plant vulnerability to drought in biodiverse regions using functional traits. Proc. Natl. Acad. Sci. U. S. A. 112, 5744–5749. https://doi.org/10.1073/pnas.1503376112.
Solé-Medina, A., Ramírez-Valiente, J.A., 2023. Common garden experiments reveal acquisitive strategies for responding to drought in seedlings of forest tree species: A commentary on "Clinal variations in seedling traits and responses to water availability correspond to seed-source environmental gradients in a foundational dryland tree species. I–II Ann. Bot. 132. https://doi.org/10.1093/aob/mcad115.
Tomasella, M., Häberle, K.-H., Nardini, A., Hesse, B., Machlet, A., Matyssek, R., 2017. Post-drought hydraulic recovery is accompanied by non-structural carbohydrate depletion in the stem wood of Norway spruce saplings. Sci. Rep. 7, 14308. https://doi.org/10.1038/s41598-017-14645-w.

Tomasella, M., Jansen, S., Giacomin, C., Boccato, E., Nardini, A., 2025. Turgor loss in vessel-associated parenchyma cells increases xylem vulnerability to embolism. J. Exp. Bot. 77 (4), 1279–1292. https://doi.org/10.1093/jxb/eraf470.

Tomasella, M., Petrussa, E., Petruzzellis, F., Nardini, A., Casolo, V., 2020. The possible role of non-structural carbohydrates in the regulation of tree hydraulics. Int. J. Mol. Sci. 21 (1), 144. https://doi.org/10.3390/ijms21010144.

Trifilò, P., Casolo, V., Raimondo, F., Petrussa, E., Boscutti, F., Lo Gullo, M.A., Nardini, A., 2017. Effects of prolonged drought on stem non-structural carbohydrates content and post-drought hydraulic recovery in *Laurus nobilis* L.: The possible link between carbon starvation and hydraulic failure. Plant. Physiol. Biochem. 120, 232–241. https://doi.org/10.1016/j.plaphy.2017.10.003.

Trifilò, P., Kiorapostolou, N., Petruzzellis, F., Vitti, S., Petit, G., Lo Gullo, M.A., Nardini, A., Casolo, V., 2019. Hydraulic recovery from xylem embolism in excised branches of twelve woody species: Relationships with parenchyma cells and non-structural carbohydrates. Plant. Physiol. Biochem. 139, 513–520. https://doi.org/10.1016/j.plaphy.2019.04.013.

Trifilò, P., Raimondo, F., Lo Gullo, M.A., Barbera, P.M., Salleo, S., Nardini, A., 2014. Relax and refill: Xylem rehydration prior to hydraulic measurements favours embolism repair in stems and generates artificially low PLC values. Plant. Cell. Environ. 37, 2491–2499. https://doi.org/10.1111/pce.12313.

Trugman, A.T., Detto, M., Bartlett, M.K., Medvigy, D., Anderegg, W.R.L., Schwalm, C., Schaffer, B., Pacala, S.W., 2018. Tree carbon allocation explains forest drought-kill and recovery patterns. Ecol. Lett. 21, 1552–1560. https://doi.org/10.1111/ele.13136.

Ugolini, F., Bussotti, F., Lanini, G.M., Raschi, A., Tani, C., Tognetti, R., 2012. Leaf gas exchanges and photosystem efficiency of the holm oak in urban green areas of Florence, Italy. Urban. For. Urban. Green. 11, 313–319. https://doi.org/10.1016/j.ufug.2012.02.006.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V., Messier, J., 2012. The return of the variance: Intraspecific variability in community ecology. Trends Ecol. Evol. 27, 244–252. https://doi.org/10.1016/j.tree.2011.11.014.

Volaire, F., 2018. A unified framework of plant adaptive strategies to drought: Crossing scales and disciplines. Glob. Change Biol. 24, 2929–2938. https://doi.org/10.1111/gcb.14062.

Wang, F., Israel, D., Ramírez-Valiente, J.-A., Sánchez-Gómez, D., Aranda, I., Aphalo, P.J., Robson, T.M., 2021. Seedlings from marginal and core populations of European beech (*Fagus sylvatica* L.) respond differently to imposed drought and shade. Trees 35, 53–67. https://doi.org/10.1007/s00468-020-02011-9.

Wang, Z., Wang, C., 2025. Dynamics of non-structural carbohydrates during drought and subsequent recovery: a global meta-analysis. Agric. For. Meteorol. 363, 110429. https://doi.org/10.1016/j.agrformet.2025.110429.

Wu, X., Cao, Y., Jiang, Y., Chen, M., Zhang, H., Wu, P., Ma, X., 2023. Dynamics of non-structural carbohydrates release in Chinese fir topsoil and canopy litter at different altitudes. Plants 12 (4), 729. https://doi.org/10.3390/plants12040729.

Yemm, E.W., Willis, A.J., 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508–514. https://doi.org/10.1042/bj0570508.

Zhang, P., Ding, J., Wang, Q., McDowell, N.G., Kong, D., Tong, Y., Yin, H., 2024. Contrasting coordination of non-structural carbohydrates with leaf and root economic strategies of alpine coniferous forests. New. Phytol. 243, 580–590. https://doi.org/10.1111/nph.19678.