

RESEARCH ARTICLE

Multifactorial diagnosis of drought-induced decline in Mediterranean conifer forests

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Abstract

1. Drought-induced tree mortality is a complex process involving interdependent shifts in tree water, carbon and nutrient relations, alongside adjustments in growth and trait variability. Anticipating dieback under increasing drought severity requires mechanistic insights into the underlying physiological processes.
2. We applied a tree-level, multi-proxy approach to identify factors associated with vigour decline in mortality hotspots of Mediterranean *Pinus sylvestris*, *Pinus pinaster* and *Pinus halepensis* forests located in north-eastern Spain. Tree-ring metrics, foliar carbon and oxygen stable isotopes ($\delta^{13}\text{C}$, $\Delta^{18}\text{O}$) and elemental composition and soil properties were integrated to assess drought impact on growth ($DGLi$), intrinsic water-use efficiency ($iWUE$), $\Delta^{18}\text{O}$ -inferred stomatal conductance ($G_{\Delta^{18}\text{O}}$) and nutrient status of trees.
3. Declining trees had consistently higher $DGLi$ than non-declining trees. In *P. sylvestris* and *P. pinaster*, elevated $DGLi$ was linked to reduced $G_{\Delta^{18}\text{O}}$ and increased $iWUE$, indicating more severe drought stress in declining trees. Declining *P. sylvestris* and *P. halepensis* also exhibited pronounced nutrient imbalances—particularly in P, K, Zn, Fe, S and Ca—and all species showed disrupted Fe:Cu ratios. Differences in vigour across species were also associated with variation in needle size and morphology, tree height and soil pH, with declining *P. sylvestris* and *P. halepensis* displaying contrasting adjustments in leaf mass per area.
4. *Synthesis.* Together, these results highlight that interactions among hydraulic impairment, nutrient imbalance, foliar senescence and unfavourable soil conditions amplify climatic stress in the studied populations of Mediterranean pines. Such

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potential feedbacks may accelerate crown defoliation and ultimately contribute to tree death under increasing drought severity.

KEYWORDS

dendroecology, drought-induced dieback, nutrient imbalances, *Pinus halepensis*, *Pinus pinaster*, *Pinus sylvestris*, plant-climate interactions, stable isotopes, stomatal conductance

1 | INTRODUCTION

Episodes of forest dieback and tree mortality triggered by compound heat and drought events have been documented across all forested biomes (Gazol, Pizarro, et al., 2025; Hammond et al., 2022). Rising atmospheric evaporative demand, alongside increasingly severe drought events, poses a critical threat to forest persistence worldwide (Allen et al., 2015; Grossiord et al., 2020; Vicente-Serrano et al., 2020). There is thus an imperative need to improve forecasts of forest dieback and tree mortality, which requires a mechanistic understanding of the carbon–water balance in trees. Hydraulic failure—a progressive deterioration of the function of xylem conducting tissue—and carbon starvation—an impairment of C sources or fluxes to sustain metabolic activity—have been proposed as the two primary mechanisms explaining tree mortality due to drought stress (McDowell et al., 2008). So far, a mechanistic understanding of the processes that induce drought decline is far from being achieved, due to the interaction of multiple factors, including environmental, biotic and tree intrinsic features (McDowell et al., 2022; Trugman et al., 2021). In this context, adopting a multi-proxy approach that explicitly considers tree water, carbon and nutrient status provides a powerful framework for advancing our understanding of tree physiological responses to escalating drought stress (Camarero et al., 2025; Colangelo, Camarero, Battipaglia, et al., 2017).

Trees generally reduce growth under drought stress, and declining or dead trees often (though not always) show growth reductions or stagnation prior to mortality (Cailleret et al., 2017; Camarero et al., 2018). More vulnerable trees and populations often exhibit a stronger climate-growth coupling, which can serve as an early-warning signal of forest dieback (Camarero et al., 2015). Tree-ring analysis provides a unique annually resolved record of past and present growth dynamics that constitutes a valuable tool to assess tree performance under climatic stress (Fritts, 1976). Alongside growth metrics, morphological traits and tree size have been widely examined across species, revealing trade-offs between acquisitive and conservative resource-use strategies (e.g. Reich, 2014). However, intraspecific patterns remain less clear, with mixed evidence regarding whether traits such as leaf mass per area (LMA) or tree height can modulate drought vulnerability within species, and could also in turn change in response to greater drought vulnerability (Fajardo & Siefert, 2018; Muñoz-Gálvez et al., 2025; Trugman et al., 2021).

While growth and morphological traits provide essential information on tree performance, they do not fully capture the physiological mechanisms underlying decline. The combined measurement of the carbon and oxygen isotopic composition ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) of plant tissues integrates information on stomatal regulation, photosynthetic rates and water-use efficiency, thereby reflecting environmental constraints (Gessler et al., 2018). Carbon isotopic fractionation arises during CO_2 diffusion, influenced by stomatal and mesophyll conductance, and from the enzymatic discrimination against ^{13}C by Rubisco during carboxylation (Cernusak et al., 2013). Oxygen isotopic fractionation, in contrast, is primarily driven by the isotopic composition of source water—reflecting precipitation $\delta^{18}\text{O}$ as well as soil water evaporation—and by evaporative enrichment at the leaf level, which is strongly modulated by stomatal conductance (Barbour, 2007). By combining $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ measurements in leaves (dual-isotope approach), it is possible to infer the water-use strategies of trees and their response to drought stress (Muñoz-Gálvez et al., 2025; Scheidegger et al., 2000).

Water-use patterns are closely linked to nutrient status, as drought-induced stomatal closure reduces the transpiration-driven uptake of nutrients from soil (Salazar-Tortosa et al., 2018; Schlesinger et al., 2016). In addition, shifts in root water uptake towards deeper soil/bedrock layers due to topsoil desiccation can alter tree nutrition because water and nutrient availability are often vertically decoupled along soil/bedrock profiles in water-limited ecosystems (Querejeta et al., 2021; Ryel et al., 2008). Drought also impacts soil nutrient availability and acquisition by plants, owing to lowered ion mobility, impaired microbial activity, reduced nutrient mineralization and functional decline of fine roots and mycorrhizal fungi (Kreuzwieser & Gessler, 2010; León-Sánchez et al., 2018, 2020; Rozas & Sampedro, 2013). Adequate plant nutrition is essential for photosynthesis, hydraulic function and redox metabolism, among other processes, thereby underpinning drought resistance and resilience (da Silva et al., 2011). Consequently, nutritional impairment plays a central role in determining tree performance and survival under drought stress (Gessler et al., 2017). Consistent patterns of nutrient imbalance under drought—such as altered phosphorus (P) and potassium (K) stoichiometry and deficiencies or accumulations of micronutrients, for example iron (Fe), copper (Cu) and manganese (Mn)—have been reported across forest ecosystems (e.g. González de Andrés et al., 2022; He et al., 2024; Hevia et al., 2019). In addition,

soil properties—including pH, texture, depth and nutrient availability—modulate the nutritional response of trees to water deficit and ultimately affect overall performance during drought (Liu et al., 2017; Marschner & Rengel, 2012).

In this study, we applied a multi-proxy integrative approach—incorporating growth, morphological traits and isotopic and elemental composition as proxies of water, carbon and nutrient dynamics—to more comprehensively assess tree vulnerability to drought-induced canopy dieback under ongoing climate change in pine forests in Spain. Mediterranean conifer forests are among the most severely affected by climate change and face the highest risk of nutrient deficiency (Bussotti & Pollastrini, 2020; He et al., 2024), owing to the increasing frequency of extreme compound climatic events and inherently low soil nutrient availability (Gazol & Camarero, 2022; Peñuelas et al., 2020). In recent decades, widespread conifer mortality—particularly in populations located near the warm and dry rear edges of species ranges—has been frequently reported across the Mediterranean Basin (e.g. Camarero et al., 2015; Rubio-Cuadrado et al., 2021). Our research focused on mortality hotspots of *Pinus sylvestris* L., *Pinus pinaster* Ait. and *Pinus halepensis* Mill in north-eastern Spain. Our objectives were (1) to characterize healthy and declining trees of each species in terms of morphological traits, drought impact on growth and foliar isotopic composition; (2) to discern changes in leaf nutrient content during drought-induced dieback and relate them to tree water-use strategies and soil properties; and (3) to determine which structural, physiological, nutritional and soil-related factors are associated with crown defoliation as a proxy of vigour decline. We hypothesised that (1) declining trees would exhibit more severe drought stress levels (i.e. tighter stomatal regulation and higher water use efficiency) compared to healthy trees; (2) dieback would be associated with P, K and micro-nutrient (Fe, Cu, Mn, Zn) imbalance; and (3) the combination of factors explaining tree vigour variation among coexisting individuals will depend on species characteristics (e.g. nutrient- and water-use strategies) and site-specific conditions (e.g. soil properties).

2 | MATERIALS AND METHODS

2.1 | Study species and sites

We studied four mortality hotspots (sensu Senf et al., 2020) of pine species located in northeastern Spain (Figure 1a) along an aridity gradient (Figure 1b and Figure S1a). In particular, we examined two Scots pine (*Pinus sylvestris* L.) forests, one maritime pine (*Pinus pinaster* Ait.) forest and one Aleppo pine (*Pinus halepensis* Mill.) forest (Table S1). They were all natural stands, subjected to light thinning in the past, but no management has been performed since the 1950s.

P. sylvestris is a widely distributed Eurasian pine that reaches its south-western distribution limit in the Iberian Peninsula, where it experiences strong drought stress at its rear-edge populations (Martínez-Vilalta et al., 2012). Stands of *P. sylvestris* are located in the Iberian System in the provinces of Teruel and Guadalajara, and they grow under continental Mediterranean climate conditions. Soil conditions were similar between the two stands sampled (Figure S2). Mortality of *P. sylvestris* peaked in response to the 2005, 2012 and 2022 droughts, with drought-induced growth decline persisting thereafter (Valeriano, Gazol, Colangelo, & Camarero, 2021). *P. pinaster* is a Mediterranean pine species that shows high plasticity in its drought responses throughout its distribution range in the Western Mediterranean Basin (Sánchez-Salguero et al., 2018). The studied *P. pinaster* forest was situated in the Iberian Range of Zaragoza province where climate is continental Mediterranean. Forest dieback started after the severe drought episode recorded in 2017 in this area (Moreno-Fernández et al., 2021). *P. halepensis* is a drought-adapted Mediterranean pine that thrives in semi-arid environments but remains highly vulnerable to extreme drought events (Klein et al., 2011). The *P. halepensis* study site is located in the semi-arid and continental middle Ebro Basin, in Zaragoza province. Drought-induced dieback of *P. halepensis* has been observed

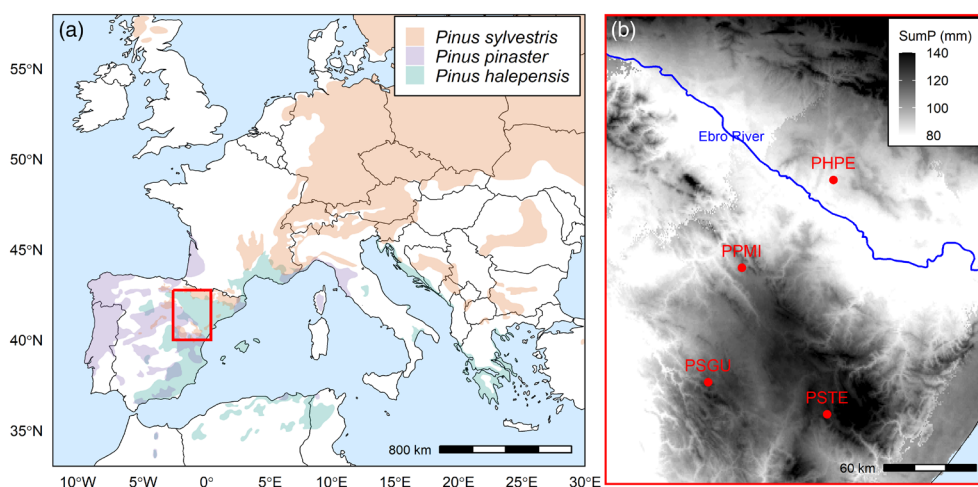


FIGURE 1 (a) Distribution of study species in Europe and north Africa (Caudullo et al., 2017). The red insert indicates the location of study region in north-eastern Spain. (b) Location of the study sites in Iberian System and Ebro Basin. The background map represents spatial variability of historical summer precipitation (SumP, period 1970–2000) from WorldClim dataset (Fick & Hijmans, 2017).

since the early 2010s (Camarero et al., 2015), and mortality peaked in 2012 (Valeriano, Gazol, Colangelo, & Camarero, 2021). A more detailed description of the study sites can be found in [Appendix S1](#) of the Supporting Information.

To assess drought severity, we used the Standardized Precipitation Evapotranspiration Index (SPEI, Vicente-Serrano et al., 2010), calculated from precipitation and potential evapotranspiration data obtained from the ~4 km resolution TerraClimate dataset (Abatzoglou et al., 2018). The SPEI is a multi-scalar drought index, where negative values indicate dry conditions and positive values indicate wet conditions. Monthly SPEI values were computed for the period 1961–2023 at timescales ranging from 1 to 24 months using the *SPEI* package (Beguería & Vicente-Serrano, 2023) in R software version 4.5.2 (R Core Team, 2025). Mean annual temperature (MAT) was highest in the *P. halepensis* stand (14.9°C) and lowest in the *P. sylvestris* forests (9.4°C), whereas the opposite pattern was found regarding total annual precipitation (TAP), which was highest in *P. sylvestris* stands (580 mm) and lowest in the *P. halepensis* site (408 mm). The *P. pinaster* stand showed intermediate climatic conditions ([Table S1](#)). Climate records identify 2022 and 2023 as years of extreme drought in Spain (Serrano-Notivol et al., 2023), affecting the study forests ([Figure S1b](#)) and contributing to the acceleration of dieback and mortality processes.

2.2 | Field sampling and morphological traits

In total, 90 dominant and codominant trees were selected for the present study, comprising 30 individuals per species. Tree vigour was assessed through crown defoliation, quantified as the proportion of leaf area lost relative to a fully foliated reference tree from the same stand. Defoliation is strongly related to climatic stress and provides an early-warning indicator of tree death (Camarero et al., 2025; Dobbertin, 2005). It was estimated in 5% incremental defoliation classes based on the methodology of the European ICP Forest network (Eichhorn et al., 2016). Trees were then categorized as non-declining (ND) if defoliation was below 40%, and as declining (DD) if they exhibited more than 60% crown defoliation. These thresholds are widely used in Mediterranean drought-related decline studies (e.g. Camarero et al., 2015) and provide robust basis for distinguishing DD and ND trees, although this approach may under-represent individuals of intermediate vigour.

For each selected tree, we collected leaves, wood and soil samples, and we measured diameter at breast height (DBH) and tree height using tape and a laser range finder (Nikon Forestry Pro II, Nikon, Japan), respectively. To analyse the chemistry and morphology of needles, a sun-exposed branch was cut using a telescopic pole pruner or a canopy slingshot device (Notch Equipment, USA), which allowed safe cutting and retrieval of branch material from the uppermost third of the canopy. Fully expanded, undamaged needles from the 2022 cohort were collected, placed in sealed plastic bags and transported to the laboratory. The leaf area of 15 needles per tree was scanned and measured with ImageJ software (Schneider

et al., 2012) while fresh. Afterwards, the same needles were oven-dried for 72 h at 70°C prior to obtaining dry weights. Then, leaf mass per area (LMA), which is a measure of biomass investment in leaves per unit light interception and gas exchange area (Poorter et al., 2009), was calculated by dividing leaf dry weight by leaf area. Needle samples from all species and wood cores of *P. sylvestris* were collected during summer of 2023, whereas dendrochronological sampling of *P. pinaster* and *P. halepensis* was conducted in autumn 2020.

2.3 | Soil physical and chemical properties

Soil samples were taken with Edelman soil augers (7 cm diameter and 15 cm depth; Royal Eijkelpamp, The Netherlands) around each tree (less than 50 cm away from the trunk). Prior to the collection of the samples, the litter layer was removed carefully, and three soil samples were collected in the vicinity of each selected tree from the uppermost 15 cm, where most fine roots are found (Janssens et al., 2002). Samples were air-dried and sieved with a 2-mm mesh size. Soil texture was determined with a laser diffraction method in a particle analyser (Coulter Mastersizer 2000, Malvern Panalytical, Spectris, London, UK), and clay content was corrected following Taubner et al. (2009). Soil carbon (organic and total) and total nitrogen (N) were determined with an elemental analyser (Element Analyzer VarioMAX N/CM, Hanau, Germany). Assimilable phosphorus (P), calcium (Ca), potassium (K) and magnesium (Mg) concentrations were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES; Varian 720-ES, Palo Alto, CA, USA). Physical and chemical properties of soils were analysed at IRNASA-CSIC (Spain). A detailed description of the soil properties is provided in [Appendix S2](#) of the Supporting Information. Across sites, soils in *P. pinaster* stands were more acidic and had lower nutrient availability, whereas soils in *P. sylvestris* and *P. halepensis* stands were more alkaline (due to their high CaCO₃ content) and showed higher nutrient availability ([Table S2](#)).

2.4 | Drought impacts on tree growth

We extracted two wood cores from each selected tree at 1.3 m height using 5.15 mm Pressler increment borers (Haglöf, Sweden) and processed them following standard dendrochronological procedures (Fritts, 1976). Cores were scanned at a resolution of 2400 dpi (Epson Expression 10,000XL Scanner; Epson, Suwa, Japan) and processed using CooRecorder and CDendro v.9.8.1 software (Larsson & Larsson, 2022). Samples were visually cross-dated, and ring widths were measured to the nearest 0.001 mm. The quality of cross-dating was checked using the COFECHA software, which calculates moving correlations between individual series of ring-width values and the mean sites series (Holmes, 1983). Dendrochronological statistics were calculated following Wigley et al. (1984) and Briffa and Jones (1990) (see [Appendix S3](#)).

Year-to-year variability in radial growth was evaluated using residual ring-width series (RWI). To calculate RWIs, we first detrended each series with a 25-year spline (50% frequency cut-off) and subsequently removed temporal autocorrelation using autoregressive modelling. Site chronologies were calculated using the Tukey bi-weight robust mean for each species, site and vigour class. Growth–climate relationships were assessed using Pearson correlation coefficients between site RWI and the SPEI drought index using 1–24 month timescales and 25-year moving windows. Correlations were also calculated at the tree level and validated against site-level chronologies (Figure S3) to ensure the reliability of tree-level assessments of drought impacts on subsequent growth analyses.

To address growth trajectories, we transformed ring-width series to basal area increment (BAI), as it is considered a more reliable indicator of tree growth (Klesse & Bigler, 2025). To calculate BAI, we used Equation (1) assuming concentric rings:

$$\text{BAI} = \pi (R_t^2 - R_{t-1}^2), \quad (1)$$

where R is the radius of the tree and t is the year of tree-ring formation. Linear models were fitted to individual BAI as a function of calendar year within 25-year moving windows from 1961 to 2022 (*P. sylvestris*) or 2020 (*P. pinaster* and *P. halepensis*), with the slope representing the growth trend.

We quantified drought impact on growth using the drought growth impact index (DGLI) based solely on tree rings and following Gazol, Tamudo-Minguez, et al. (2025). This harmonized approach integrates RWI–SPEI correlations and growth trends based on the assumption that stronger growth–climate relationships and more negative growth trends indicate greater drought impact on growth (Gazol, Tamudo-Minguez, et al., 2025). Further rationale and calculation details for this index are provided in Appendix S3 of the Supporting Information. Before calculating the DGLI, variables were rescaled to range between 0 and 1, where 0 corresponds to the most positive growth trend and the most negative SPEI–growth correlation as follows:

$$\text{BAI trend scaled} = 1 - (\text{BAI trend} + 1) / 2, \quad (2)$$

$$\text{RWI – SPEI correlation scaled} = (\text{RWI – SPEI correlation} + 1) / 2, \quad (3)$$

The DGLI was calculated then as the product between BAI trend scaled and the RWI–SPEI correlation scaled. The DGLI index ranges between 0 and 1, with high values representing negative growth trajectories and strong climate–growth coupling, suggesting a strong drought impact on growth. As done for RWI–SPEI correlations and growth trends, the DGLI was calculated at the individual tree level using 25-year moving windows for the period 1961–2022 (*P. sylvestris*) or 1961–2020 (*P. pinaster* and *P. halepensis*). Tree-ring width processing and growth–climate correlations were performed using the R packages *dpIR* (Bunn et al., 2024) and *treeclim* (Zang & Biondi, 2015).

2.5 | Foliar isotopic and elemental composition

Leaf carbon and oxygen isotopic composition ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$), along with foliar nutrient concentrations, were used as time-integrated proxies for carbon/water relations and nutrient status of trees, respectively. The collected needles from all selected trees were oven-dried, ground in a ball mill (Retsch ZM1, Haan, Germany) and encapsulated (tin capsules for $\delta^{13}\text{C}$ and silver capsules for $\delta^{18}\text{O}$). The $\delta^{13}\text{C}$ and C and N concentrations of leaves were determined by continuous flow dual-isotope analysis using a CHNOS elemental analyser interfaced to an IsoPrime100 mass spectrometer. Leaf $\delta^{13}\text{C}$ isotopic composition is expressed in delta notation (‰) relative to the Vienna Pee Dee Belemnite standard (V-PDB). The $\delta^{18}\text{O}$ was determined in continuous flow using an Elementar PYRO Cube interfaced to a Thermo Delta V mass spectrometer. Leaf $\delta^{18}\text{O}$ isotopic composition is expressed in delta notation (‰) relative to VSMOW for $\delta^{18}\text{O}$. Long-term external precision values for leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ determinations are $\pm 0.10\text{‰}$ and $\pm 0.20\text{‰}$, respectively. All stable isotope analyses were conducted at the University of Lisbon (Portugal).

Leaf $\delta^{13}\text{C}$ was employed to calculate intrinsic water-use efficiency (iWUE), which represents the ratio between the photosynthetic rate and stomatal conductance, as follows (Farquhar et al., 1982):

$$\text{iWUE} = Ca \times [1 - (Ci / Ca)] \times 0.625, \quad (4)$$

where Ca and Ci are CO_2 concentrations in the atmosphere and the intercellular space, respectively, and 0.625 in the relation among conductance of H_2O and CO_2 . To determine Ci , we used Equation (3).

$$Ci = Ca [(\delta^{13}\text{C}_{\text{leaf}} - \delta^{13}\text{C}_{\text{atm}} + 1) / (b - a)], \quad (5)$$

where $\delta^{13}\text{C}_{\text{leaf}}$ and $\delta^{13}\text{C}_{\text{atm}}$ are the leaf and atmospheric $\delta^{13}\text{C}$, respectively, a is the diffusion fractionation across the boundary layer and the stomata (+4.4‰), and b is the fractionation by the Rubisco enzyme (+27.0‰). Values of $\delta^{13}\text{C}_{\text{atm}}$ and Ca were retrieved from published databases (Belmecheri & Laverne, 2020). Values of iWUE were calculated using the R package *isocalcR* (Mathias & Hudiburg, 2022), which applies an elevation correction to account for differences in temperature and atmospheric pressures among study sites (Cernusak et al., 2013).

To remove the influence of geographic variation in precipitation $\delta^{18}\text{O}$ values, we calculated leaf oxygen isotopic enrichment above source water ($\Delta^{18}\text{O}$) as the difference between leaf $\delta^{18}\text{O}$ and the isotopic composition of rainwater at each site. We used mean annual precipitation $\delta^{18}\text{O}$ data, calculated as rainfall amount-weighted averages (Bowen & Revenaugh, 2003). Rainfall $\delta^{18}\text{O}$ data were obtained from the Online Isotopes in Precipitation Calculator (OIPC) (IAEA/WMO, 2015) using each site's coordinates and elevation.

We used leaf $\Delta^{18}\text{O}$ to assess time-integrated stomatal conductance ($G_{\Delta^{18}\text{O}}$) following the dual-isotope conceptual framework (Scheidegger et al., 2000), which assumes that variation in $\Delta^{18}\text{O}$ primarily reflects evaporative enrichment of leaf water, based on the

idea that conspecific trees within the same stand use water with similar isotopic composition. Although we examined carbon–oxygen isotope relationships to test coupling between leaf-level processes and $\Delta^{18}\text{O}$ (Roden & Farquhar, 2012), we acknowledge that leaf $\Delta^{18}\text{O}$ may still be influenced by other environmental factors, including temporal shifts in source water $\delta^{18}\text{O}$, or microclimate differences among individuals (Roden & Siegwolf, 2013).

Leaf phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and nickel (Ni) concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP–OES; Thermo Elemental Iris Intrepid II XDL) after a microwave-assisted digestion with $\text{HNO}_3\text{:H}_2\text{O}_2$ (4:1, volume/volume). Needles were not washed prior to digestion. Elemental composition analyses were conducted at the CEBAS-CSIC Ionomics laboratory (Murcia, Spain). Elemental concentrations were converted to nutrient content per unit leaf area, which is associated with higher carboxylation and photosynthetic capacity (Querejeta et al., 2022; Wright et al., 2003). In addition, we calculated five meaningful stoichiometric ratios N:P, N:K, K:Ca and Fe:Cu, which provide insights into important physiological processes, including growth and hydraulic function (He & Dijkstra, 2014; Hevia et al., 2019; Salazar-Tortosa et al., 2018).

2.6 | Data analysis

Statistical analyses were conducted using the R software version 4.5.2 (R Core Team, 2025). To assess differences in leaf isotopic and elemental composition, traits and growth patterns and drought responses between ND and DD trees, we performed linear models considering vigour class as the predictor variable. In the *P. sylvestris* models, site was included as a fixed effect to account for differences between sampling sites. The relationship between iWUE and $\Delta^{18}\text{O}$ at the species level was assessed using linear models. We evaluated associations of isotope signals with growth, needle morphology, and nutrient contents using Spearman correlations with Bonferroni corrections to adjust the *p*-value during multiple comparisons.

We assessed the variation in foliar nutrient status within species using principal component analysis (PCA) (Legendre & Legendre, 2012) considering the covariance–variance matrix of the nutrient contents per unit leaf area for each species. Significant differences between vigour classes were tested with permutational multivariate analysis of variance (PERMANOVA, 1000 permutations) considering a Euclidean distance between observations (Anderson, 2001). To test the relationship of leaf multi-elemental composition with isotopic composition and radial growth, we correlated the nutritional PCA axes with iWUE, $\Delta^{18}\text{O}$ and BAI, and projected into the ordination space. Additionally, we performed correlations between soil sand percentage, pH and available P with axes of nutritional PCA to assess the relationships between leaf multi-elemental composition and soil properties.

The ordination analyses were conducted using the *vegan* package (Oksanen et al., 2024).

To relate crown defoliation, as a proxy of tree vigour, with predictor variables we fitted partial least squares regression (PLS) models for each species separately. This regression method is a multivariate technique that extracts uncorrelated (orthogonal) components from the predictor variables (i.e. structural, physiological, nutritional and soil-related factors) that maximize the explained variance in the response variable. This technique is appropriate to deal with collinearity of predictor variables and low number of observations compared to predictors (Carrascal et al., 2009). PLS models were fitted using the *pls* R package (Liland et al., 2024). The number of PLS components was selected using cross-validation based on minimizing the root mean squared error of prediction (RMSEP), and all optimal models included two components. The goodness-of-fit and predictive performance of models were evaluated by the coefficient of determination (R^2) and cross-validated coefficient of determination (Q^2), respectively (Mevik & Cederkvist, 2004). Predictors were standardized prior to analyses. To assess the stability and uncertainty of model coefficients, we conducted nonparametric bootstrap resampling with 1000 iterations. Predictors whose 95% confidence intervals did not include zero were considered to have statistically significant and robust effects on defoliation. To account for the effect of different sampled stands, the factor site was included as a predictor in *P. sylvestris* models. To obtain a more general understanding of defoliation patterns across species, the relationship between defoliation and the predictors was additionally tested using a random forest model applied to all species combined, as described in Appendix S4.

3 | RESULTS

3.1 | Morphological traits and growth responses to drought

In *P. sylvestris* mortality hotspots, crown defoliation ranged from ~33% in non-declining trees (ND) to ~58% in declining trees (DD). Similarly, in *P. pinaster* and *P. halepensis* hotspots, ND trees showed mean defoliation of ~19% and ~15%, respectively, whereas DD trees reached 79% and 73%. Mean tree age (measured at 1.3 m height) was 138, 90 and 101 years for *P. sylvestris*, *P. pinaster*, and *P. halepensis* stands, respectively, with no significant differences between vigour classes. Non-declining *P. sylvestris* trees had smaller DBH and height compared to DD trees. In contrast, ND individuals of *P. pinaster* and *P. halepensis* were thicker and taller than their DD neighbours (Table 1). Mean individual needle size (leaf area) differed between vigour classes in *P. pinaster* and *P. halepensis*, so that DD trees consistently showed smaller needles. Regarding LMA, DD *P. sylvestris* trees had greater LMA than ND individuals, while the opposite pattern was found in *P. halepensis* (Table 1).

Since 1960, the basal area increment (BAI) exhibited declining trends in both ND and DD trees from mortality hotspots across

TABLE 1 Morphological traits, leaf isotopic composition, growth patterns and drought responses calculated at the individual tree-level.

Variables	<i>Pinus sylvestris</i>		<i>Pinus pinaster</i>		<i>Pinus halepensis</i>	
	ND trees	DD trees	ND trees	DD trees	ND trees	DD trees
Traits						
DBH (cm)	34.81 ± 1.44a	40.42 ± 1.77b	25.69 ± 0.90a	21.97 ± 0.96b	35.07 ± 3.46a	29.54 ± 2.71a
Tree height (m)	13.13 ± 0.43a	14.29 ± 0.38b	6.32 ± 0.15a	5.83 ± 0.16b	8.70 ± 0.25a	6.90 ± 0.28b
Individual leaf area (mm ²)	49.86 ± 3.02a	49.05 ± 5.44a	316.3 ± 17.9a	274.6 ± 12.8b	127.8 ± 4.5a	93.52 ± 4.47b
LMA (g m ⁻²)	245.4 ± 14.4a	284.9 ± 19.6b	440.6 ± 7.8a	438.8 ± 7.0a	256.1 ± 4.1a	235.7 ± 4.3b
Radial growth ^a						
BAI (cm ²)	4.01 ± 0.47a	3.59 ± 0.40a	1.98 ± 0.16a	1.33 ± 0.13b	3.17 ± 0.52a	1.13 ± 0.19b
BAI trend	-0.12 ± 0.06a	-0.22 ± 0.06a	-0.10 ± 0.06a	-0.42 ± 0.05b	-0.37 ± 0.10a	-0.56 ± 0.11b
RWI-SPEI correlation	0.63 ± 0.02a	0.71 ± 0.01b	0.73 ± 0.02a	0.69 ± 0.03a	0.69 ± 0.01a	0.73 ± 0.02b
DGLi	0.46 ± 0.03a	0.52 ± 0.02b	0.48 ± 0.02a	0.60 ± 0.02b	0.57 ± 0.06a	0.67 ± 0.02b
Leaf isotopic composition						
iWUE (μmol mol ⁻¹)	116.99 ± 2.02a	123.01 ± 2.2b	125.87 ± 1.62a	131.5 ± 1.54b	120.54 ± 2.12a	122.02 ± 1.13a
Δ ¹⁸ O (‰)	39.17 ± 0.33a	39.77 ± 0.41b	36.35 ± 0.63a	38.27 ± 0.33b	35.18 ± 0.36a	34.95 ± 0.40a

Note: Different letters indicate significant differences ($p < 0.05$) according to linear models and highlighted in bold. ND, non-declining trees; DD, declining trees. Values are means ± SE.

Abbreviations: Δ¹⁸O, leaf oxygen isotopic enrichment above source water; BAI, basal area increment; DBH, diameter at breast height; DGLi, drought growth impact index; iWUE inferred from δ¹³C, intrinsic water-use efficiency; LMA, leaf mass per area; RWI, ring-width index; SPEI, Standardized Precipitation Evaporation Index.

^aCorresponds to the last 25-year period.

all study species (Figure 2a). These downward trends were significantly steeper in DD trees compared to ND trees in *P. pinaster* and *P. halepensis*. Mean BAI over the most recent 25-year moving window was greater in ND *P. pinaster* and *P. halepensis* trees than in their DD counterparts (Table 1). All populations responded to the SPEI drought index, but season and time scales of correlations varied between species and populations (Table S1; Figure S4). The growth response of *P. sylvestris* showed the longest time scales (8–10 months) and was influenced by the period from the previous autumn and winter to current early summer (June–July). The strongest correlation of *P. pinaster* growth was found with mid-term SPEI (5 months) from previous winter to current spring, and the response did not differ between ND and DD trees. Growth of *P. halepensis* showed responses to short-term (2 months) current winter SPEI, and they were stronger in DD trees compared to ND trees. Correlations with SPEI were stronger in DD trees than in ND trees in *P. sylvestris* and *P. halepensis*, but not in *P. pinaster* (Table 1). RWI–SPEI correlations varied over time. In *P. sylvestris*, they exhibited a clear upward trend. In *P. pinaster*, the growth–drought relationships followed a U-shaped pattern, reaching its lowest values in the 1990s. For *P. halepensis*, ND trees showed a slight increase, whereas DD trees displayed no clear trend (Figure 2b). The drought growth impact index (DGLi) was higher in *P. halepensis* trees compared with *P. sylvestris* and *P. pinaster* (Table 1). DGLi trajectories differed among species. In *P. sylvestris*, ND and DD trees showed similar patterns, with moderate declines until the mid-1990s followed by sustained increases. In *P. pinaster*, values followed a U-shaped pattern, declining sharply to a minimum in the early 1990s and then rising steadily, with DD trees

consistently exceeding ND trees. In *P. halepensis*, both ND and DD trees increased DGLi throughout the study period, but maintaining a persistent gap of DD over ND trees (Figure 2c).

3.2 | Isotopic composition and nutrient content of leaves

Intrinsic water-use efficiency (iWUE) was lowest in *P. sylvestris* ($119.4 \pm 1.6 \mu\text{mol mol}^{-1}$), followed by *P. halepensis* ($121.1 \pm 1.2 \mu\text{mol mol}^{-1}$) and *P. pinaster*, which showed the highest value ($128.7 \pm 1.2 \mu\text{mol mol}^{-1}$). Comparing within species, ND *P. sylvestris* and *P. pinaster* trees showed lower iWUE than DD conspecifics, which suggests lower water stress in healthy trees (Table 1; Figure 3). Regarding leaf oxygen isotopic enrichment above source water (Δ¹⁸O), we found the highest values in *P. sylvestris* ($39.4 \pm 0.3 \text{‰}$), followed by *P. pinaster* ($37.4 \pm 0.4 \text{‰}$) and *P. halepensis* ($35.1 \pm 0.2 \text{‰}$). In *P. sylvestris*, DD trees exhibited significantly higher Δ¹⁸O values than ND trees, suggesting lower time-integrated stomatal conductance ($G_{\Delta 18\text{O}}$) in declining individuals. Similarly, ND *P. pinaster* trees had lower Δ¹⁸O than DD trees, which also indicates lower $G_{\Delta 18\text{O}}$ in declining individuals. In contrast, *P. halepensis* showed no significant differences in leaf isotopic composition between vigour classes (Table 1). Moreover, we found significant and positive relationships between iWUE and Δ¹⁸O across vigour classes in *P. sylvestris* and *P. pinaster*, but not in *P. halepensis* (Figure 3).

In *P. sylvestris*, BAI showed negative correlations with both iWUE and Δ¹⁸O, suggesting lower stomatal constraints on photosynthesis

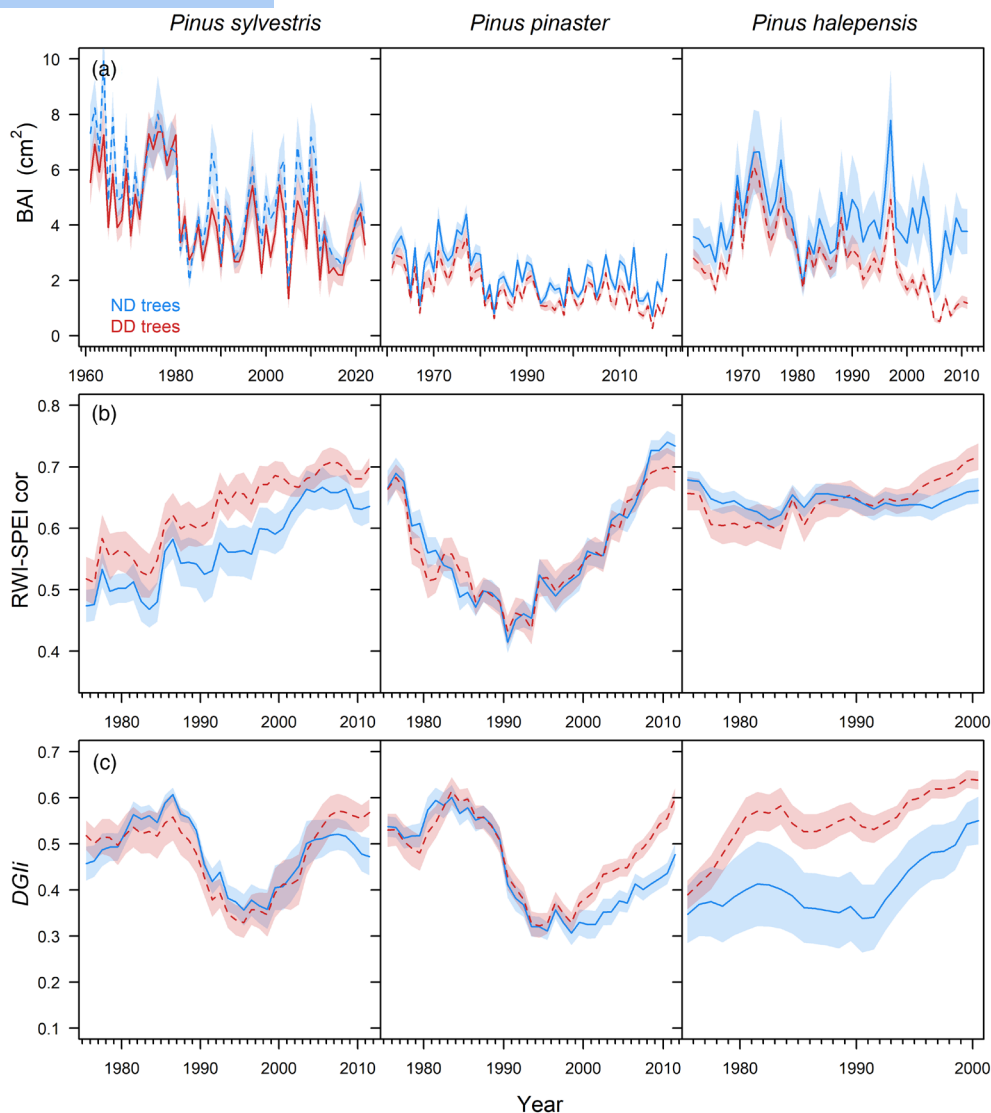


FIGURE 2 Tree-level growth and drought response variability of study species: Basal area increment (BAI, a), correlation of ring-width index and drought index SPEI (RWI-SPEI cor, b), and drought growth impact index (DGII, c). In plots (b) and (c), values represent 25-years moving windows. Different colours indicate non-declining trees (ND; blue solid lines) and declining trees (DD; red dashed lines). Lines are means, and the shaded areas around them are the standard error of the mean.

in trees with greater radial growth. In this species, $\Delta^{18}\text{O}$ was also negatively correlated with needle size and positively correlated with LMA, indicating higher $G_{\Delta^{18}\text{O}}$ in trees with larger and thinner, less sclerophyll needles. Meanwhile, the drought impact on growth (DGII) was negatively associated with iWUE in *P. halepensis* (Figure S5), indicating that radial growth of individuals achieving higher water-use efficiency at leaf level is less sensitive to drought in this xeric-adapted pine species.

We also observed significant differences in leaf elemental composition between vigour classes in the target species (Table 2). These differences persisted after including LMA as a covariate (Table S3), suggesting that differences in nutrient content are not dependent on leaf area. For *P. sylvestris*, N, Ca, S, and Fe content per unit leaf area, as well as N:P and Fe:Cu ratios, were higher in DD trees compared to ND trees. Conversely, leaf Zn content was higher in needles of ND trees than in DD trees. In *P. pinaster*, only the Fe:Cu ratio differed

between classes, with higher values in DD trees. For *P. halepensis*, the N:K and Fe:Cu ratios were higher in DD trees relative to ND trees, whereas K, Cu and Zn contents were higher in the leaves of ND trees than in DD trees.

Patterns observed for individual nutrients were further supported by ordination analyses, which showed significant differences in leaf multi-elemental composition between vigour classes in *P. sylvestris* and *P. halepensis*, but not in *P. pinaster* (Figure 4). The first two PCA axes (PC1 and PC2) accounted for 73%, 59%, and 55% of the total variability in leaf nutrient content for *P. sylvestris*, *P. pinaster* and *P. halepensis*, respectively. In *P. sylvestris*, PC1 (accounting for over 60% of variability) was positively associated with most nutrients and negatively to Zn content, clearly separating vigour classes: ND trees clustered at lower values and DD trees at higher values along PC1 (Figure 4a). Both PC1 and PC2 were negatively correlated with $\Delta^{18}\text{O}$ ($r=0.293$, $p=0.018$), primarily reflecting its negative

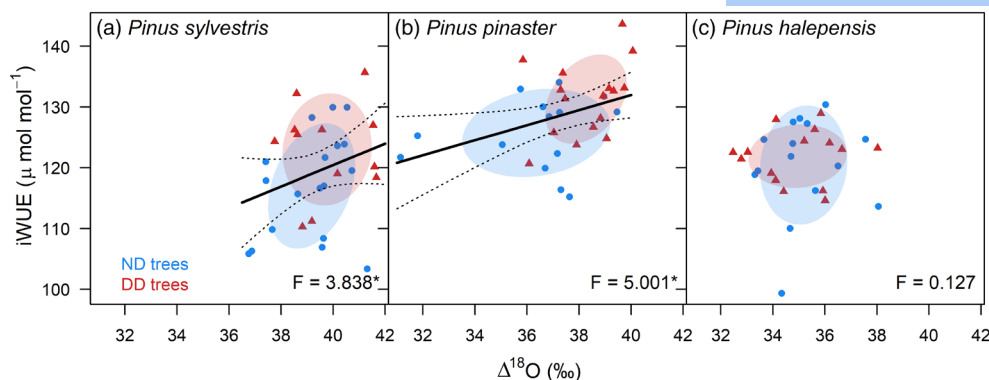


FIGURE 3 Relationships between intrinsic water-use efficiency (iWUE) and leaf oxygen isotopic enrichment above source water ($\Delta^{18}\text{O}$) of (a) *Pinus sylvestris*, (b) *Pinus pinaster*, and (c) *Pinus halepensis*. Different colours indicate non-declining trees (ND; blue dots) and declining trees (DD; red triangles). Shaded areas represent the centroid of the values for each vigour class. Solid lines represent significant relationships between variables and dotted lines are 95% confidence intervals. The F-statistic is shown, asterisk indicate significant regression. A positive relationship indicates that inter-tree differences in iWUE are primarily driven by differences in time-integrated stomatal regulation among individuals. Within species, higher iWUE and $\Delta^{18}\text{O}$ indicate more severe drought stress.

TABLE 2 Nutrient contents per leaf area.

Variable	<i>Pinus sylvestris</i>		<i>Pinus pinaster</i>		<i>Pinus halepensis</i>	
	ND trees	DD trees	ND trees	DD trees	ND trees	DD trees
N (g m^{-2})	2.87 ± 0.21a	3.71 ± 0.26b	3.47 ± 0.11a	3.37 ± 0.11a	2.73 ± 0.07a	2.62 ± 0.10a
P (g m^{-2})	0.18 ± 0.02a	0.22 ± 0.02a	0.21 ± 0.01a	0.24 ± 0.01a	0.13 ± 0.01a	0.13 ± 0.01a
K (g m^{-2})	1.24 ± 0.10a	1.43 ± 0.15a	1.35 ± 0.11a	1.41 ± 0.08a	0.74 ± 0.03a	0.63 ± 0.06b
Ca (g m^{-2})	1.07 ± 0.11a	2.29 ± 0.45b	1.37 ± 0.15a	1.32 ± 0.10a	1.58 ± 0.12a	1.59 ± 0.12a
Mg (g m^{-2})	0.25 ± 0.02a	0.33 ± 0.07a	1.11 ± 0.08a	1.05 ± 0.06a	0.39 ± 0.03a	0.36 ± 0.03a
S (g m^{-2})	0.20 ± 0.01a	0.38 ± 0.03b	0.35 ± 0.02a	0.37 ± 0.02a	0.26 ± 0.02a	0.24 ± 0.01a
Fe (mg m^{-2})	26.48 ± 2.15a	45.96 ± 4.92b	17.56 ± 1.53a	17.87 ± 0.62a	32.51 ± 1.24a	34.45 ± 1.72a
Mn (mg m^{-2})	18.68 ± 2.16a	28.44 ± 7.03a	121.9 ± 11.9a	118.6 ± 11.6a	9.72 ± 0.56a	9.75 ± 0.77a
Cu (mg m^{-2})	0.78 ± 0.15a	0.95 ± 0.07a	0.97 ± 0.19a	0.75 ± 0.03a	0.57 ± 0.04a	0.49 ± 0.02b
Zn (mg m^{-2})	12.69 ± 0.97a	9.19 ± 0.75b	10.43 ± 0.45a	11.19 ± 0.66a	5.27 ± 0.62a	3.53 ± 0.29b
Ni (mg m^{-2})	3.94 ± 0.47a	3.10 ± 0.55a	3.54 ± 1.59a	4.68 ± 2.02a	1.03 ± 0.28a	1.29 ± 0.37a
N:P	16.14 ± 0.69a	17.28 ± 0.91b	16.50 ± 0.59a	15.67 ± 0.64a	21.39 ± 0.97a	21.27 ± 1.12a
N:K	2.46 ± 0.20a	2.81 ± 0.20a	2.78 ± 0.23a	2.5 ± 0.16a	3.82 ± 0.19a	4.59 ± 0.44b
K:Ca	1.30 ± 0.14a	0.92 ± 0.22a	1.19 ± 0.20a	1.17 ± 0.13a	0.50 ± 0.04a	0.42 ± 0.05a
Fe:Cu	35.22 ± 3.08a	49.47 ± 5.28b	20.90 ± 1.34a	24.37 ± 1.23b	58.18 ± 3.02a	71.32 ± 3.12b

Note: Different letters indicate significant differences ($p < 0.05$) according to linear models and highlighted in bold. ND, non-declining trees; DD, declining trees. Values are means ± SE.

association with Ca and S contents rather than Zn (Figure S5). In *P. pinaster*, PC1 was negatively related to S, Mg, Ca, Cu, Mn, and Fe, while PC2 was positively associated with P, K, Zn, N, and Ni contents (Figure 4b). Notably, PC2 also showed a positive correlation with iWUE ($r = 0.234$, $p = 0.034$), consistent with positive relationships between iWUE and P and K contents observed in this species (Figure S5). In *P. halepensis*, PC1 was negatively associated with most nutrients, whereas PC2 showed positive and negative associations with Fe and K, respectively, among others. Vigour classes separated along both axes, with DD trees showing an overall reduction in leaf nutrient contents but elevated Fe content compared to ND trees (Figure 4c). Both PC1 and PC2 were positively correlated with $\Delta^{18}\text{O}$

($r = 0.215$, $p = 0.042$), which is in accordance with the negative correlations found between $\Delta^{18}\text{O}$ and K, Zn, and Cu, indicating greater foliar contents of these essential nutrients in individuals exhibiting higher $G_{\Delta^{18}\text{O}}$ (Figure S5).

Correlations with soil properties revealed significant associations of pH and available P in *P. pinaster* and *P. halepensis*. In *P. pinaster*, higher soil pH was linked to increased contents of P, N, Zn and Ni ($r = 0.218$, $p = 0.044$), while higher available P was associated with lower contents of Cu, Mg, Fe and other elements ($r = 0.221$, $p = 0.039$). In *P. halepensis*, available P showed a strong positive correlation with most foliar nutrient contents ($r = 0.563$, $p = 0.001$), whereas pH had the opposite effect ($r = 0.276$, $p = 0.015$) (Figure S6).

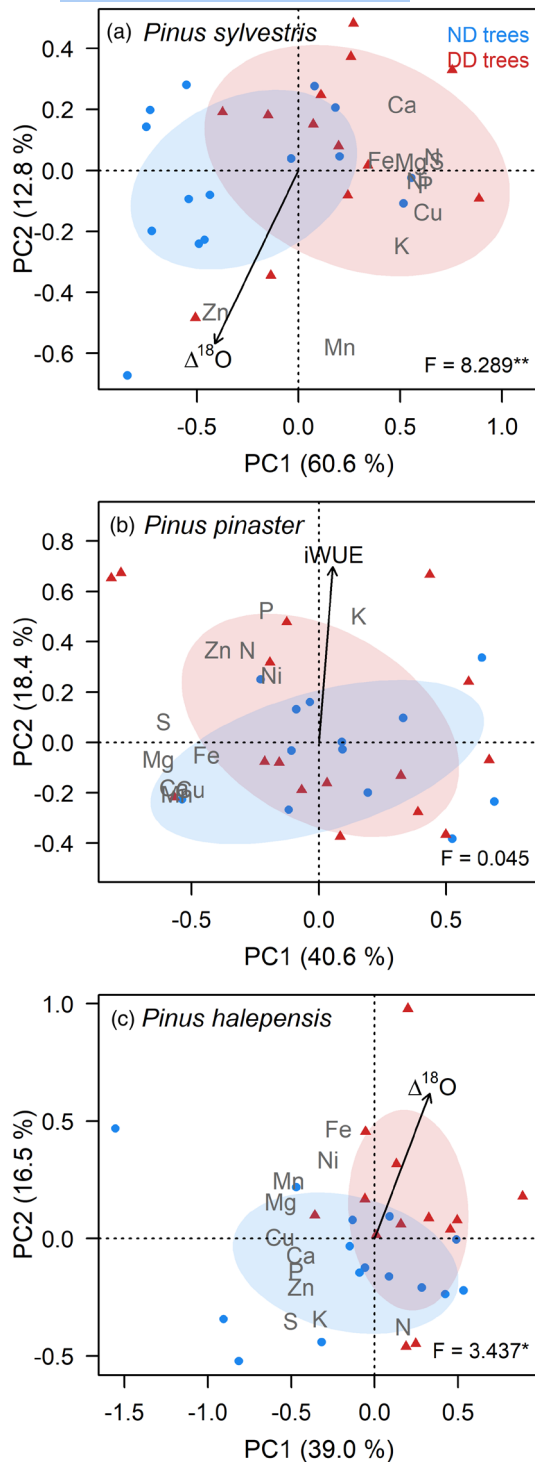


FIGURE 4 Principal component analysis (PCA) biplot of nutrient content per unit leaf area of non-declining (ND; blue dots) and declining trees (DD; red triangles) of (a) *Pinus sylvestris*, (b) *Pinus pinaster*, and (c) *Pinus halepensis*. Grey labels indicate loadings of nutrient contents. The significant correlations between intrinsic water-use efficiency (iwUE), leaf oxygen isotopic enrichment above source water ($\Delta^{18}\text{O}$), and growth over the last 25 years (BAI) and PC axes were projected in the ordination diagram and represented with black arrows. Shaded areas represent the centroid of the values for each vigour class and the F -statistic of the PERMANOVA test comparing vigour classes are shown. Asterisk indicates significant differences.

3.3 | Covariation of variables and impacts on crown defoliation

At the species level, the multivariate PLS analyses explained a substantial proportion of variance in the calibration data ($R^2=0.51\text{--}0.70$), but cross-validated performance was lower ($Q^2=0.25\text{--}0.34$), indicating limited predictive ability. Prediction errors were not negligible (RMSEP=14%–18%, Table S4). The sole fully consistent and significant pattern among the three pine species was a robust negative association between BAI and defoliation (Figure 5). In *P. sylvestris*, LMA and iwUE showed positive associations with defoliation. Higher Fe content per unit leaf area, as well as higher N:P, N:K and Fe:Cu ratios, were also associated with increased defoliation in *P. sylvestris* (Figure 5a). In *P. pinaster* and *P. halepensis*, tree height and defoliation were negatively related. Isotopic signals (iwUE and $\Delta^{18}\text{O}$) and leaf S content showed positive associations with defoliation in *P. pinaster*. Regarding soil properties, higher available P was associated with higher crown defoliation in *P. pinaster* (Figure 5b). Needle size and LMA were negatively related to defoliation in *P. halepensis*. In this species, leaf Ca content and Fe:Cu ratio showed positive associations with defoliation, while Cu and Zn were negatively related to defoliation. Regarding soil properties, pH was positively related to defoliation and available P was negatively associated with defoliation (Figure 5c). Analysis across species widely supported species-level associations (Figures S7 and S8).

4 | DISCUSSION

In this study, we assessed the tree-level intrinsic factors (radial growth, leaf morphological traits, isotopic signals and elemental composition) and environmental conditions (soil properties) associated with drought-induced dieback of three pine species. Declining *Pinus sylvestris* and *Pinus pinaster* trees exhibited lower time-integrated stomatal conductance inferred from leaf $\Delta^{18}\text{O}$, coupled with higher LMA, indicating more severe drought stress in declining than healthy individuals, and thereby supporting our first hypothesis. Our second hypothesis was partially supported since differences in nutrient contents between vigour classes were evident in the mortality hotspots of *P. sylvestris* and *P. halepensis*, which showed evidence of N:P and N:K imbalances and shifts in Zn contents. Weaker nutritional responses in *P. pinaster* suggest that canopy dieback in this population was mainly driven by carbon-water balance processes. Declining trees of all species showed nutrient profiles indicative of an advanced foliar senescence stage and enhanced oxidative stress metabolism, mainly related to Fe accumulation and imbalances. Our multi-proxy approach detected patterns of factors associated with tree vigour decline in Mediterranean pine populations. These included temporal radial growth dynamics, needle size and morphology, tree height, stomatal regulation stringency, nutrient imbalances and soil pH (under calcareous conditions), although with distinct population-specific patterns, thus supporting our third hypothesis.

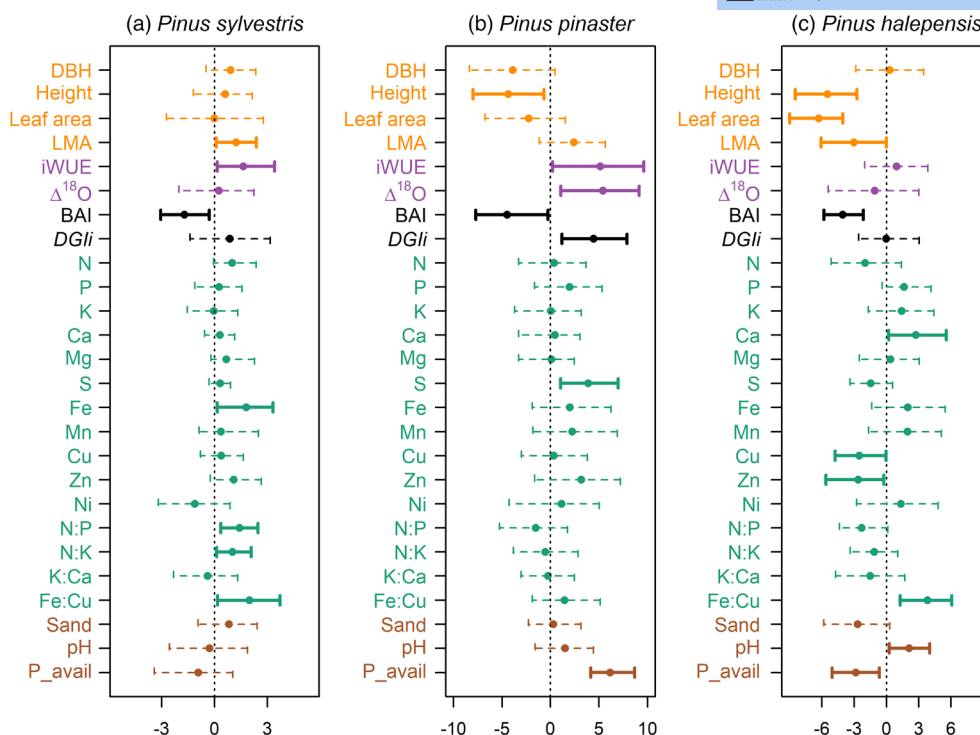


FIGURE 5 Bootstrapped coefficients for the Partial Least Squares regression (PLS) models for defoliation of (a) *Pinus sylvestris*, (b) *Pinus pinaster*, and (c) *Pinus halepensis*. Dots and bars represent the mean and the 95% confidence intervals with continuous thick lines corresponding to significant variables ($p < 0.05$). Different categories of predictor variables are represented with colours: Traits (DBH, height, leaf area and LMA; orange), isotopic composition (iWUE and $\Delta^{18}\text{O}$; purple), radial growth patterns (BAI and DGLi; black), leaf nutrient concentrations and ratios (green) and soil properties (sand, pH and P available; brown).

4.1 | Growth and isotopic signals are linked to crown condition

Long-term declines in radial growth and low growth rates are well-established indicators of drought-induced dieback and mortality (Cailleret et al., 2017; Camarero et al., 2015, 2018). In *P. pinaster* and *P. halepensis*, divergent radial growth trajectories between non-declining and declining trees emerged two to three decades ago, most likely triggered by major drought events—specifically the 1994–1995 drought for *P. pinaster* and the 1986 drought for *P. halepensis* (Camarero et al., 2015; Valeriano, Gazol, Colangelo, & Camarero, 2021). In contrast, the lack of clear divergence in *P. sylvestris* may reflect mortality driven by an extreme climate event that surpasses tolerance thresholds in the studied populations, resulting in rapid hydraulic failure and death (Arend et al., 2021; Rubio-Cuadrado et al., 2025). The more negative growth trajectories observed in declining trees of mortality hotspots align with their stronger drought–growth correlations (*P. sylvestris* and *P. halepensis*) and with the higher drought growth impact index values detected across all studied forests. Dieback processes in conifers inhabiting Mediterranean environments are often linked to heightened drought sensitivity, reflected in stronger climate–growth relationships and reduced resistance and resilience to drought (Lloret et al., 2022; Voltas et al., 2013). Although lower responsiveness to occasional periods of greater water availability in declining trees has also been reported (e.g. Valeriano,

Gazol, Colangelo, & Camarero, 2021; Valeriano, Gazol, Colangelo, González de Andrés, et al., 2021).

These growth responses are likely linked to the greater drought stress experienced by declining trees, as suggested by the carbon isotopic composition in *P. sylvestris* and *P. pinaster*. In both species, declining trees showed higher iWUE than healthy individuals, and in *P. sylvestris* growth was negatively correlated with iWUE. These findings are consistent with the general pattern of $\delta^{13}\text{C}$ enrichment and iWUE increase under water stress (Gessler et al., 2018) and find support in previous research on pine species (e.g. Corcuera et al., 2012; Pellizzari et al., 2016; Timofeeva et al., 2017; Voltas et al., 2013). The positive association between iWUE and $\Delta^{18}\text{O}$ across vigour classes in both *P. sylvestris* and *P. pinaster* points to more stringent stomatal regulation of water loss as the primary mechanism underlying the iWUE increase in declining trees (Roden & Farquhar, 2012). Accordingly, the dual-isotope framework suggests that the higher $\Delta^{18}\text{O}$ values in declining trees indicate lower $G_{\Delta^{18}\text{O}}$ and thus tighter stomatal constraints on carbon assimilation, compared to healthy neighbours within the same forest stand (Scheidegger et al., 2000; Siegwolf et al., 2023). These physiological differences were likely amplified in our dataset because the sampled needles corresponded to 2022, a year characterized by an extreme compound drought and heatwave event that swept across Europe during summer (Serrano-Notivol et al., 2023). Similar evidence for reduced stomatal conductance in declining individuals has been reported in other declining pine populations

(Bose et al., 2024; Timofeeva et al., 2017). However, whether this altered carbon–water balance primarily results from reduced carbon assimilation—leading to depletion of non-structural carbohydrate (NSC) reserves (Arend et al., 2021; Poyatos et al., 2013)—or other processes such as drought-induced phloem impairment (Sala et al., 2010) remains unresolved.

In the *P. halepensis* population, foliar isotopic signals showed no relationship with crown defoliation or tree decline, suggesting that all individuals in this semi-arid stand may experience severe, chronic water stress under recurrent drought. Nevertheless, the frequent and strong positive $\delta^{13}\text{C}$ – $\delta^{18}\text{O}$ correlations previously documented in this species under semi-arid conditions (e.g. Moreno-Gutiérrez et al., 2011; Voltas et al., 2008) indicate a predominant stomatal control on leaf $\delta^{18}\text{O}$ composition, while drought-related mortality of this species has been primarily associated with hydraulic dysfunction (Morcillo et al., 2022). Instead, we found that coupled adjustments in needle morphology and nutrient profiles may modulate the impacts of drought stress on tree vigour in this *P. halepensis* mortality hotspot (Juan-Ovejero et al., 2022; Querejeta et al., 2008).

4.2 | Tree nutrition is driven by stomatal regulation impacts on tree water flux and soil properties

Nutritional impairment during drought-induced dieback has been documented in multiple forest tree species (e.g. Gea-Izquierdo et al., 2025; González de Andrés et al., 2022; González de Andrés, Suárez, et al., 2021; Hevia et al., 2019; Juan-Ovejero et al., 2022, 2024; Salazar-Tortosa et al., 2018). In this study, the *P. sylvestris* and *P. halepensis* populations exhibited differences in elemental composition between declining and healthy trees, consistent with the tight coupling of nutrient status and drought-stress responses in these species (Juan-Ovejero et al., 2022; Querejeta et al., 2008). In contrast, the comparatively weak evidence of drought-induced nutritional impairment in declining *P. pinaster* suggests that mortality in this stand may be exclusively driven by hydraulic or carbon-related limitations, with little or no contribution from drought-induced nutrient deficits or imbalances (Adams et al., 2017; McDowell et al., 2011). This pattern may also partly reflect distinctive soil conditions at this site relative to the other stands.

In our dataset, declining trees consistently showed altered Fe:Cu ratios across the three studied species. In *P. sylvestris*, this pattern was accompanied by needle accumulation of Fe, Ca and S; in *P. pinaster*, higher S content was associated with increased crown defoliation; whereas in *P. halepensis*, the pattern mainly reflected reduced Cu content in needles of defoliated trees. These findings point to alterations in the Fe:Cu ratio as a potential novel indicator of nutritional stress under dieback conditions, and suggest that shifts in micronutrient stoichiometry may play a previously overlooked role in the nutritional responses of drought-stressed conifers. Although Fe, Ca and S are essential for leaf physiology (Broadley et al., 2012), their excessive accumulation can disrupt

electron transport and photoprotection and impair antioxidant metabolism (Foyer & Noctor, 2009; Kaufholdt et al., 2025; Li et al., 2022), thereby accelerating leaf senescence and abscission. In line with this, the progressive accumulation of excess Ca, S and Fe in older leaves may reflect heightened stress and an advanced stage of foliar senescence, particularly in dry Mediterranean ecosystems (Prieto & Querejeta, 2020).

The previous patterns may be linked to multiple, interconnected processes. Reduced transpiration-driven mass flow and diffusion of nutrients to roots in trees with lower stomatal conductance—as suggested by isotopic evidence—combined with severe crown defoliation, can constrain nutrient uptake (Salazar-Tortosa et al., 2018; Schlesinger et al., 2016). Concurrently, drought-induced reductions in phloem transport and cambial activity may limit internal nutrient remobilization, reduce dilution effects, and promote the sequestration of low-mobility elements into structural and defence-related compounds (Gessler et al., 2017; Sala et al., 2010; Skirycz & Inzé, 2010). In addition, soil properties such as pH strongly regulate nutrient availability (Marschner & Rengel, 2012), and thus influence plant elemental composition (Wright et al., 2003). Because our study focused on one or two mortality hotspots per species mostly located on alkaline soils, it remains difficult to disentangle the relative contributions of hydraulic constraints versus site-specific edaphic factors to the observed nutritional shifts. Nevertheless, the consistent patterns across both intra- and interspecific analyses suggest that these stoichiometric shifts may represent downstream effects of oxidative imbalance in senescing foliage following hydraulic failure. Future research should encompass a broader range of environmental and soil conditions within species to better resolve these interactions. Finally, although foliar elemental composition can be influenced by atmospheric deposition of dust-derived nutrients, which is particularly high in Mediterranean regions (Lokshin et al., 2026), this process is likely to affect all trees within a stand similarly and therefore cannot readily explain the observed differences between vigour classes.

Consistently, N:P and N:K imbalances were related to defoliation patterns in the *P. sylvestris* and *P. halepensis* mortality hotspots, while lower K content was detected in declining *P. halepensis* trees. Impaired P and K nutrition has been frequently reported during tree dieback (e.g. González de Andrés, Suárez, et al., 2021; León-Sánchez et al., 2020; Sardans & Peñuelas, 2007). Phosphorus deficiency constrains hydraulic and photosynthetic functions, whereas K deficiency increases xylem cavitation risk and impairs stomatal regulation and water-use efficiency (Juan-Ovejero et al., 2022; Sardans & Peñuelas, 2015; Trifilò et al., 2014). Notably, our results also revealed lower Zn contents in declining *P. halepensis* and *P. sylvestris* trees, in agreement with previous studies (González de Andrés, Suárez, et al., 2021; He et al., 2024). Zinc is crucial for drought tolerance through its role in maintaining membrane stability and supporting plant water relations under water deficit (Umair Hassan et al., 2020), thus contributing to protection against drought-induced oxidative damage (Gill & Tuteja, 2010).

4.3 | Multifactorial pathways to diagnose drought-induced decline of tree vigour

Radial growth emerged as the most consistent trait related to crown defoliation across mortality hotspots of all study species, supporting the tight association between low growth rates and vulnerability to drought-induced canopy dieback (Cailleret et al., 2017; Camarero et al., 2025). In *P. sylvestris* and *P. pinaster*, defoliation was positively associated with *i*WUE and $\Delta^{18}\text{O}$, and the growth of *P. sylvestris* trees negatively correlated with both variables. These patterns are consistent with drought-induced stomatal closure, which limits carbon assimilation and depletes NSC reserves (Hartmann et al., 2018; Sala et al., 2010). Resulting carbon shortages may constrain foliage production and maintenance, promoting leaf senescence and shedding as a drought-avoidance strategy to reduce transpirational demand (Choat et al., 2018; Nadal-Sala et al., 2021). Recurrent or severe stomatal closure during severe drought episodes can therefore trigger extensive defoliation, which not only signals decline but also reinforces it by further restricting photosynthesis and whole-tree carbon balance (McDowell et al., 2022; Poyatos et al., 2013). Therefore, our results point to an association between low $\Delta^{18}\text{O}$ -inferred time-integrated stomatal conductance, reduced growth and a stronger drought impact on growth with increased susceptibility to dieback (Martin-Benito et al., 2025; Petrucco et al., 2017). These patterns could have been particularly evident during the extreme summer of 2022, when compounded heat and drought likely intensified stomatal closure, accelerated needle loss, and aggravated carbon imbalance. Such recent extreme weather conditions exemplify how ongoing climatic variability can irreversibly tip drought-stressed trees beyond recovery thresholds.

Foliar nutrient profiles observed in declining trees, particularly in *P. sylvestris* and *P. halepensis*, have been associated with increased stress and diminished photosynthetic capacity and hydraulic performance (Gessler et al., 2017; Hunziker et al., 2024), patterns that have been linked to carbon reserves depletion and increased mortality risk (Poyatos et al., 2013). We observed, however, contrasting patterns between mortality hotspots of the two species. In *P. sylvestris*, the positive association of LMA and *i*WUE with defoliation points to more severe canopy dieback in individuals exhibiting more sclerophyllous needles and tighter stomatal constraint on photosynthesis (Martínez-Vilalta et al., 2009), as inferred from higher $\Delta^{18}\text{O}$ values in declining trees and the positive LMA- $\Delta^{18}\text{O}$ correlation. Nutrient imbalances in N:P:K stoichiometry may further impair hydraulic performance (Juan-Ovejero et al., 2022, 2024; Salazar-Tortosa et al., 2018) of declining *P. sylvestris* trees, which also accumulated senescence-related nutrients (Fe, Ca, S) in their needles (Prieto & Querejeta, 2020). In contrast, *P. halepensis* displayed the opposite pattern: higher LMA was associated with reduced defoliation, and higher *i*WUE correlated with weaker drought impact on growth. Healthy *P. halepensis* trees also showed higher foliar K, Zn and Cu contents, which are essential nutrients for osmoregulation, drought resistance and antioxidant protection (Sardans & Peñuelas, 2015; Umair Hassan et al., 2020). Notably, positive associations between

*i*WUE and P and K in *P. pinaster* point to improved carboxylation and photosynthetic capacity in nutrient-rich individuals (Querejeta et al., 2022). Although these results may point to species-specific adaptive strategies consistent with their biogeographical distributions (boreo-alpine vs. Mediterranean), our dataset does not allow us to draw broader conclusions at the species level.

A key consideration when interpreting our results is that radial growth was assessed over long-term, multi-year timescales, whereas leaf isotopic and elemental composition were measured in a single year, capturing only a snapshot of tree ecophysiology and nutrient status. However, this approach offers important advantages, as leaf-based measurements are less affected by several sources of uncertainty inherent to tree-ring analyses, including the potential attenuation of stomatal signals during transfer from leaves to wood (Gessler et al., 2014; Treydte et al., 2014), and nutrient translocation within sapwood and among metabolically active tissues (Dalling et al., 2024; Durand et al., 2020), among others. Thus, while tree-ring measurements provide greater temporal depth, leaf measurements yield a more immediate and mechanistically interpretable signal.

Tree height was negatively associated with defoliation in *P. pinaster* and *P. halepensis*, suggesting that taller, dominant trees in these populations might be less vulnerable to drought-induced canopy dieback. Alternatively, this pattern could reflect greater recent height growth in non-defoliated individuals since the onset of the decline process. In any case, this result is consistent with previous findings in Mediterranean tree species (Colangelo, Camarero, Borghetti, et al., 2017; González de Andrés, Rosas, et al., 2021; Laforest-Lapointe et al., 2014) and in other biomes (Ding et al., 2021). Although tall trees may face higher hydraulic resistance due to longer water transport pathways and wider conduits (McDowell & Allen, 2015; Olson et al., 2018), height covaries with traits that can mitigate hydraulic stress. Taller trees often display adjustments in conduit architecture and wood density, maintain larger NSC pools, or develop deeper roots that access subsoil or bedrock moisture (Bachofen et al., 2024; Muñoz-Gálvez et al., 2025; Olson et al., 2021; Ripullone et al., 2020; Trugman et al., 2021), complicating general predictions of height-related mortality risk.

Finally, drought-induced canopy dieback can be further modulated by microsite characteristics. In the *P. halepensis* stand, poorer crown condition was associated with higher microsite soil pH. In calcareous soils, high CaCO_3 content combined with low moisture may constrain fine-root development and reduce the efficiency of ectomycorrhizal associations that facilitate nutrient uptake (Leberecht et al., 2016; León-Sánchez et al., 2018). This finding contrasts with studies reporting positive effects of increasing soil pH on tree growth and drought resilience in forests growing on acidic substrates (González de Andrés et al., 2022; Rozas & Sampedro, 2013; Viet et al., 2013). A similar pattern was found at the *P. pinaster* stand, growing on acidic soil, where higher microsite soil pH was linked to increased foliar concentrations of N, P or Zn. Under these conditions, the positive association observed between crown defoliation and soil P availability is best explained by topsoil enrichment through nutrient-rich litter inputs from declining *P. pinaster*

individuals, whereas healthier trees likely deplete soil nutrient pools more efficiently. Nonetheless, these patterns should be interpreted cautiously, as soil sampling was restricted to the upper 15 cm, while drought-stressed Mediterranean pines may increasingly rely on deeper soil layers for water and nutrient uptake.

5 | CONCLUSIONS

Our findings highlight that drought-induced dieback in Mediterranean pine forests arises from interdependent hydraulic, carbon and nutritional constraints. Across the studied pine populations, declining trees consistently exhibited impaired radial growth and stronger drought impact on growth. Isotopic evidence of increased δWUE and reduced $G_{\Delta 18O}$ in declining *P. sylvestris* and *P. pinaster* trees revealed chronic drought stress and associated carbon limitations. When evaluated alongside nutrient profiles, these isotopic signatures are consistent with a sequence of associated stress responses, in which sustained stomatal closure and soil-driven constraints co-occurs with nutrient imbalances (Fe, Ca, S, Zn and N:P:K stoichiometry), which in turn promote accelerated leaf senescence, hydraulic impairment, and ultimately crown defoliation. In the *P. halepensis* stand, nutritional imbalances and shifts in leaf morphology and physiology (e.g. LMA and δWUE) appear to dominate drought-stress responses. Declining trees of all species showed altered Fe:Cu ratios, suggesting a novel indicator of drought-related nutritional stress. The comparatively weaker nutritional shifts observed in *P. pinaster* indicate that dieback in this population is primarily driven by carbon-water dynamics and/or site conditions.

Overall, our results reveal a potential cascading sequence in which drought-driven canopy loss and mortality emerge from feedbacks among stomatal regulation, carbon depletion, nutritional impairment, oxidative stress and edaphic limitations. They also highlight that only a multi-proxy approach can capture how these mechanisms co-occur and interact. This integrative perspective provides mechanistic indicators that can help detect ecophysiological patterns underlying tree mortality and inform adaptive forest management under intensifying drought. Future research should refine these diagnostic frameworks by incorporating a broader range of environmental and soil conditions, clarifying the roles of key micronutrients and linking nutrient imbalance with water-use traits such as transpiration dynamics and rooting-depth patterns.

AUTHOR CONTRIBUTIONS

Ester González de Andrés, Antonio Gazol, José Ignacio Querejeta and J. Julio Camarero conceived the ideas and designed methodology; Ester González de Andrés, Antonio Gazol, Cristina Valeriano, Michele Colangelo, Christopher Fernández-Blas, Marina Rodes-Blanco and J. Julio Camarero collected the data; Ester González de Andrés analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data used in this study is publicly available at <http://doi.org/10.6084/m9.figshare.33719650> (González de Andrés et al., 2026). No custom code was used in this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Description of study sites.

Table S1. Geographical and climatic characteristics and dendrochronological statistics of sampled non-declining (ND) and declining (DD) trees for each study site and species. Values are means $\pm$ SE. MAT, mean annual temperature; TAP, total annual precipitation; tree-ring width, TRW; first-order autocorrelation, AR1; R_{bar} , mean correlation between tree-ring width series; EPS, Expressed Population Signal; Standardized Precipitation Evapotranspiration Index, SPEI; corr, correlation.

Figure S1. Climatic diagrams of study sites for the 1958–2023 period based on the Terraclimate dataset (Abatzoglou et al., 2018) (a), and August Standardized Precipitation Evapotranspiration Index calculated at a 2-month-long time scale (SPEI2.Aug) (b). The grey background indicates the 2022 and 2023 extreme drought period in the study region. See sites' codes in [Table S1](#).

Appendix S2. Soil properties across and within sites.

Table S2. Soil physical and chemical properties analysed at the tree-level. Different letters indicate significant differences ($p < 0.05$) according to linear models and highlighted in bold. ND, non-declining trees; DD, declining trees. Values are means $\pm$ SE.

Figure S2. Biplots of PCAs conducted with soil physical and chemical properties sampled at individual tree level. Shaded areas represent the centroid of the values non-declining (ND, blue) and declining (DD, red) trees. Significance levels of F values: * $p < 0.05$; *** $p < 0.001$. Some soil properties labels have been excluded to enhance readability.

Appendix S3. Dendrochronological methods.

Figure S3. Comparison between RWI-SPEI correlations calculated at 25-years moving windows at site-level (solid lines) and tree-level (dashed lines). Pearson correlation coefficients and associated p -values between site-level and tree-level series are shown in each graph. See sites' codes in [Table S1](#).

Figure S4. Relationship between growth indices and drought severity measured by the SPEI across species and vigour classes. For each month (previous September to current September, y-axis; lower case letters correspond to the year before ring formation) and scale (1 to 24 months, x-axis). The values show the average correlation where orange colours indicate positive correlations and purple colours indicate negative correlations. Sites' codes are as in [Table S1](#).

Appendix S4. Covariation among variables.

Figure S5. Spearman correlation coefficients of leaf isotope composition ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) with radial growth (BAI), drought impact on growth (DGI), morphological traits and leaf nutrient

contents per unit leaf area, including PCA scores of nutritional ordination. Asterisks indicate significant ($p < 0.05$) correlations after Bonferroni correction for multiple comparisons. Variables' abbreviation are as in Table S2.

Table S3. Linear regression analyses comparing in leaf nutrient content per unit leaf area between tree vigour classes within each study species. Models were fitted both with and without leaf mass per area (LMA) as a covariate, and without considering it. For *P. sylvestris*, site was included as a fixed effect to account for variability between sampling locations. For each variable, the *F*-statistic and associated *p*-value ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$) are shown.

Figure S6. Principal component analysis (PCA) biplot of nutrient content per unit leaf area of non-declining (ND; blue dots) and declining trees (DD; red triangles). Grey labels indicate loadings of nutrient contents. The significant correlations between soil properties and PC axes were projected in the ordination diagram and represented with black arrows: sand percentage (sand), soil pH (pH), and available P (P_{avail}). Shaded areas represent the centroid of the values for each vigour class. Variables' abbreviation are as in Table S2.

Table S4. Loadings of explanatory variables on the first two latent components (Comp1 and Comp2) of the Partial Least Squares (PLS) regressions fitted for crown defoliation. The amount of explained variance by each component and its correlation with defoliation

are also shown (significance levels: $+p < 0.1$; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$). Bold loadings indicate stable variables based on bootstrap resampling. See variables' abbreviations in Table S3.

Figure S7. Variable importance (Z-scores) according to the random forest models fitted for defoliation considering all study species. Blue box-plots correspond to minimal, average and maximum Z-scores of a random predictor. Green, yellow and red box-plots represent Z-scores of important, marginal and discardable predictors, respectively. The proportion of variance explained (R^2) by the model is shown.

Figure S8. Variation in tree vigour (defoliation, %) as a function of the standardized predictors' values according to the random forest model. Values are obtained independently for each predictor, keeping the rest of the predictors constant at the mean. Only variables considered important are shown. See abbreviations in Table S3.

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