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Title: The “isohydric trap”: a proposed feedback between water shortage, stomatal regulation and nutrient acquisition drives differential growth and survival of European pines under climatic dryness

Running head: Climate-induced nutrient imbalance in pines

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Keywords: Climatic change, hotter drought, stable isotopes, nutrients, stoichiometry, stomatal behaviour, water use efficiency

Type of paper: Primary Research Articles
Abstract

Climatic dryness imposes limitations on vascular plant growth by reducing stomatal conductance, thereby decreasing CO₂ uptake and transpiration. Given that transpiration-driven water flow is required for nutrient uptake, climatic stress-induced nutrient deficit could be a key mechanism for decreased plant performance under prolonged drought. We propose the existence of an “isohydric trap”, a dryness-induced detrimental feedback leading to nutrient deficit and stoichiometry imbalance in strict isohydric species. We tested this framework in a common garden experiment with 840 individuals of four ecologically-contrasting European pines (*Pinus halepensis*, *P. nigra*, *P. sylvestris*, and *P. uncinata*) at a site with high temperature and low soil water availability. We measured growth, survival, photochemical efficiency, stem water potentials, leaf isotopic composition (δ¹³C, δ¹⁸O), and nutrient concentrations (C, N, P, K, Zn, Cu). After two years, the Mediterranean species *Pinus halepensis* showed lower δ¹⁸O and higher δ¹³C values than the other species, indicating higher time-integrated transpiration and water-use efficiency (WUE), along with lower predawn and midday water potentials, higher photochemical efficiency, higher leaf P and K concentrations, more balanced N:P and N:K ratios, and much greater dry-biomass (up to 63-fold) and survival (100%). Conversely, the more mesic mountain pine species showed higher leaf δ¹⁸O and lower δ¹³C, indicating lower transpiration and WUE, higher water potentials, severe P and K deficiencies and N:P and N:K imbalances, and poorer photochemical efficiency, growth, and survival. These results support our hypothesis that vascular plant species with tight stomatal regulation of transpiration can become trapped in a feedback cycle of nutrient deficit and imbalance that exacerbates the detrimental impacts of climatic dryness on performance. This overlooked feedback mechanism may hamper the ability of isohydric species to respond to ongoing global change, by
aggravating the interactive impacts of stoichiometric imbalance and water stress caused
by anthropogenic N deposition and hotter droughts, respectively.

Introduction

Plant survival and performance is determined largely by soil water availability, since
water is a major limiting resource for primary production in many regions (Adams et
al., 2017; Cramer & Hoffman, 2015; Lawlor & Cornic, 2002). The responses of plant
species and individuals to water shortage span a variety of mechanisms that either tend
to increase water uptake (e.g. enhanced root growth) or reduce water loss (e.g. stomatal
closure). Stomatal regulation is the quickest mechanism to cope with drought, leading to
two contrasting strategies termed anisohydry and isohydry, characterized by relaxed vs.
tight stomatal control of transpiration in response to decreases in plant water potential
(Moran, Lauder, Musser, Stathos, & Shu, 2017; Tardieu & Simonneau, 1998; but see
Martínez-Vilalta & Garcia-Forner, 2017). These strategies are clearly the opposite
extremes of a continuous ecophysiological gradient (Klein, 2014) but, overall, the
degree of stomatal regulation of transpiration is currently considered a key functional
trait that explains not only individual plant response to drought, but also forest
persistence under current and future climatic conditions (McDowell et al., 2011).

Although the role of stomatal control as a key mechanism in the regulation of
plant carbon balance and hydraulic functioning under drought and heat stress has been
the focus of much research attention in recent years (Garcia-Forner, Biel, Savé, &
Martínez-Vilalta, 2016; Martínez-Vilalta & Garcia-Forner, 2017; McDowell et al.,
2013), less attention has been paid to plant nutrient dynamics as a potential key factor
influencing plant survival and growth under conditions of prolonged climatic dryness
(Gessler, Schaub, & McDowell, 2017; Hartmann et al., 2018). Nutrient availability and
plant nutrient status influence forest productivity through photosynthetic, allocation, and stoichiometric effects (Marschner, Kirkby, & Cakmak, 1996; Sardans & Peñuelas, 2012). Drought decreases soil nutrient availability for plants due to reduced ion mobility and microbial activity, which can lead to impairment of the plant’s nutrient status and growth (Kreuzwieser & Gessler, 2010). Plant nutrient acquisition from the soil is tightly linked to water uptake and movement in soils, as plants rapidly deplete nutrients from the rhizosphere, which must be replenished by dissolved nutrients carried in the transpiration-driven mass flow of water to plant roots (Cabrera-Bosquet, Sánchez, & Araus, 2009; Lambers, Chapin, & Pons, 2008; Voltas, Romagosa, Muñoz, & Araus, 1998). Plant nutrient uptake is therefore heavily dependent on the existence of a negative water potential gradient from the soil to the roots that is driven by leaf transpiration (Lambers, Chapin, & Pons, 2008). Furthermore, plants need to maintain particular nutrient stoichiometric relations in their tissues for proper ecophysiological functioning (Güsewell, 2004; Koerselman & Meuleman, 1996; Marschner et al., 1996; Sardans & Peñuelas, 2012). Given that nutrient mobility in the soil matrix may differ by several orders of magnitude among various essential macro- and micronutrients (Lambers et al., 2008), environmental conditions of dryness forcing stomatal closure may severely impair the nutrient balance and stoichiometric ratios of plant tissues. Thus, the interplay and interdependence between plant water relations, nutrient status and stoichiometric relations should be considered a potential important mechanism contributing to plant mortality or reduced performance in models that seek to predict the impact of drier climatic conditions on plant communities.

Here, we propose the existence of an “isohydric trap” that occurs when vascular plant species with a strict isohydric behaviour fall under prolonged climatic dryness conditions, leading to a detrimental feedback loop between water stress, tight stomatal
control, and nutrient uptake and status that can be detrimental to plant physiological function including growth and survival (Figure 1). In this conceptual model, strict isohydric species exhibit early and prolonged stomatal closure under dry conditions (Klein, 2014; Moran et al., 2017), thereby drastically reducing cumulative transpiration, and hence mass flow of water and in-solution nutrients to roots. As a result, nutrient uptake decreases and plants become prone to macro- and micronutrient deficiency and stoichiometric imbalance, which in turn further decreases stomatal conductance and carbon assimilation through reductions in photochemical efficiency and water use efficiency (Figure 1; blue arrow). These feedbacks eventually decrease carbon availability for supporting root and ectomycorrhizal activity and growth, further reducing the capacity for plant water and nutrient uptake (León-Sánchez et al., 2017) and thereby further impairing the plant’s ability to cope with prolonged climatic dryness (Figure 1; green arrow).

In this study, we seek to demonstrate that the detrimental impact of this “isohydric trap” on plant nutrient status and stoichiometry is a key mechanism behind the response of drought-sensitive plant species to prolonged climatic dryness. To test this theoretical framework, we conducted a two-year common garden experiment where juveniles of four pine species with contrasting ecological niche and ecophysiological behaviour were grown under the same xeric environmental conditions. Plants were monitored for survival and growth, along with photochemical efficiency, water potential, leaf nutrient concentrations, and leaf δ¹⁸O and δ¹³C signatures, which were used as surrogates of time-integrated stomatal conductance and water use efficiency, respectively. We predicted that exposure to prolonged climatic dryness will impose strong constraints on cumulative transpiration and nutrient uptake in pine species with strict isohydric behaviour. Due to the tight coupling between water and nutrient uptake
by roots, reduced transpiration will hamper the mass flow and diffusion of nutrients to roots and the uptake of dissolved nutrients, eventually leading to nutrient deficiency and severe N:P:K stoichiometric imbalance. This will hinder plant carbon balance, transpiration and water use efficiency, ultimately leading to decreased growth and survival. In contrast, drought-tolerant pine species with less strict stomatal control of transpiration will escape this “isohydric trap” by allowing water potential to drop while maintaining greater stomatal aperture and transpiration under prolonged drought stress, which will allow greater nutrient uptake and a more balanced plant nutrient status and stoichiometry. We seek to expand current knowledge on plant responses to increasing frequency of hotter droughts (Allen, Breshears, & McDowell, 2015) by examining the importance of drought-induced nutrient starvation and stoichiometric imbalance, which to-date has been largely overlooked or underestimated in both conceptual and empirical models of plant responses to climate change (Gessler et al., 2017).

Material and methods

Species and plant material

The studied species were Pinus halepensis Mill., P. nigra Arnold, P. sylvestris L., and P. uncinata Ram. These species are native in Europe, altogether cover a wide geographic range across the continent (circa 30% of its forest area; Köble & Seufert, 2001) and segregate clearly along aridity gradients in the order P. halepensis > P. nigra > P. sylvestris > P. uncinata, whereas their resistance to cold stress follows the opposite trend (Fernández-Pérez, Villar-Salvador, Martínez-Vilalta, Toca, & Zavala, 2018; Ruiz de la Torre, 2006; Tapias, Climent, Pardos, & Gil, 2004). Pinus halepensis is widely distributed throughout the Mediterranean basin from sea level to 1200 m a.s.l., P. nigra from 800 to 2000 m a.s.l. (Mediterranean and Alpine distribution), P. sylvestris from...
1000 to 2100 m a.s.l. (Boreal-Alpine/Eurosiberian distribution), and *P. uncinata* is distributed from 1400 to 2200 m a.s.l. in the Alps, Pyrenees, and other high Iberian mountains (Richardson 2000; altitudinal ranges for Southern Europe). In general, pines are considered isohydric species compared to other taxa such as oaks or junipers (Meinzer, Woodruff, Marias, McCulloh, & Sevanto, 2014; Zweifel, Steppe, & Sterck, 2007). However, several evidences support that there is a gradient in stomatal behaviour in response to water stress among the studied species. First, these species show differences in the regulation of plant water potential, which is associated with stomatal control (Klein, 2014; Sperry, Hacke, Oren, & Comstock, 2002; Tardieu & Simonneau, 1998), with *P. halepensis* reaching the lowest water potentials, followed by *P. nigra*, *P. sylvestris* and *P. uncinata* (Choat et al., 2012; Matías, Castro, Villarí-Salvador, Quero, & Jump, 2017; Oliet, Planelles, López Arias, & Artero, 2002). Second, leaf-level measurements suggest the existence of large interspecific differences in stomatal sensitivity to low plant water potentials, with *P. halepensis* showing the lowest water potentials at stomatal closure, followed by *P. nigra* and *P. sylvestris* (Martin-StPaul, Delzon, & Cochard, 2017). Finally, previous measurements at whole-plant level in the study site showed that *P. halepensis* exhibits less tight stomatal control and higher transpiration rates under dry conditions than *P. nigra* and *P. sylvestris* (Salazar-Tortosa et al., 2018). Therefore, we may assume that the studied species can be ordered along an iso-anisohydric gradient from *P. uncinata* (most isohydric), *P. sylvestris*, *P. nigra* to *P. halepensis* (most anisohydric).

Seeds of the four species were collected when ripe from certified provenance regions of the Iberian Peninsula (Appendix S1, Table S1). The seeds were stored under cold, dry conditions until sowing. Seeding was done in winter 2012 using 300-mL plastic containers filled with fertilized peat (White 420 F6 Kekkilä, Finland; pH 4.7).
containing 0.8-1 kg/m³ of a slow-release fertilizer NPK 16-10-20. They were initially
grown in a greenhouse of the Centro Nacional de Recursos Genéticos Forestales “El
Serranillo” (Guadalajara, Spain, 40° 39’ 56.14” N, 3° 10’ 15.20” W) to avoid frost
damage. In mid-May 2012, the seedlings were moved outdoors and cultivated under
optimal forest nursery conditions until 15 February 2013, when they were transferred to
the common garden site. Nutrient content and isotopic composition at the time of
transplanting indicates that seedlings had not been subjected to any water or nutrient
stress during the nursery stage (Appendix S1, Table S1).

*Study site and experimental design*

The common garden experiment was conducted at the “Huerta de La Paloma” farm (37°
10’ 03.43” N, 3° 36’ 57.80” W; Granada, Southern Spain), a flat (slope ca. 2%),
agricultural terrain at 649 m a.s.l. The climate is Mediterranean with hot, dry summers
and precipitation concentrated in autumn and spring. The mean annual rainfall is
394±38 L m⁻² y⁻¹ and the mean annual temperature is 15.3±0.1ºC, with a mean
maximum of the hottest month of 35.7±0.2ºC and a mean minimum of the coldest
month of -0.1±0.2ºC (period 2006-2015; climatic data from a meteorological station
located 1.5 km away at IFAPA Research Field Station). These climatic conditions can
be regarded as dry and hot for *P. nigra*, *P. sylvestris* and *P. uncinata* when compared to
the prevailing climatic conditions in their native ranges (Christensen, 1987; Enescu, de
Rigo, Caudullo, Mauri, & Houston-Durrant, 2016; Houston-Durrant, de Rigo, &
Caudullo, 2016), whereas they fall within the optimal ecological range of *P. halepensis*
(Mauri, Di Leo, de Rigo, & Caudullo, 2016). The soil is deep with a loamy texture, and
average values of 44.8% sand, 41.8% silt, and 13.3% clay, and a soil water content of
13% at wilting point and 33% at field capacity (-1.5 and -0.033 MPa, respectively;
means for the profile down to 1 m deep; no marked horizons in soil profile; analyses
done in the Laboratorio Agroalimentario de la Junta de Andalucía, Atarfe, Granada,
official laboratory for the Regional Agricultural Service). The soil-nutrient content at 0-
15 cm depth (N, P and K) showed adequate values for plant growth (Appendix S1,
Table S2).

On 15 February 2013, the one-year-old seedlings grown under nursery
conditions were transplanted to the common garden site using a randomized-block
design. Three blocks of 500 m² were located side by side, separated by 2.5 m. In each
block, we planted a total of 70 individuals of each species (70 x 3 blocks x 4 species =
840 seedlings in total). Within each block, seedlings were regularly planted at 1.25-m
distance from each other, and individuals of each pine species where distributed
randomly within the planting scheme. Weeds were removed manually and with a
cultivator as needed to prevent competition. We did not find any competition effect
from neighbours on either survival or growth (P > 0.53 in both cases; Appendix S1,
Table S3). The initial size of each seedling (length of the leader shoot and stem-root
collar diameter) was measured just after planting as a baseline for aboveground growth
estimations (Appendix S1, Table S1). Soil-water content was measured regularly
throughout the summers (June-September) at 10, 20, 30, 40, 60, and 100 cm depth using
a PR-2/6 Soil Moisture Profile Probe (Delta T, Cambridge, UK). Water content in the
soil profile remained above the permanent wilting point throughout the hot, dry
(summer) season (Appendix S1, Table S4).

Seedling survival and growth

Survival was monitored eight times from 13 June 2013 to 9 September 2014. Seedling
growth was measured non-destructively for all the plants in September 2013 and 2014,
considering leader shoot length and stem diameter (increment relative to initial values measured after planting). Stem volume was calculated for each year assuming a conical shape for the stem, with basal diameter given by the average of two perpendicular measurements at the root collar and height given by the maximum height of the leader shoot. Growth patterns for both years were similar and hence only the data from 2014 are reported. In the third year (September 2015), height, stem-root collar diameter, and fresh weight were measured in a random subsample of five pines per species and block (*P. uncinata* not included due to small sample size; 45 pines in total). Survival was not monitored in the third growing season of the experiment, given that a destructive harvesting of seedlings was performed the previous year for leaf isotopic and nutrient analyses (see below), and thus the remaining plants might not represent a random sample for this variable.

**Physiological variables**

The effective photochemical quantum yield of photosystem II (Y(II), termed quantum yield from now on), relative electron transport rate (rETR), photochemical quenching (qP), non-photochemical quenching (qN), maximum photochemical efficiency of photosystem II (F_v/F_m), and leaf-water potential (Ψ) were measured for a subsample of nine randomly selected seedlings per species and block in July of 2014. Quantum yield, qP, and qN were measured by means of a portable junior PAM fluorometer (Heinz Walz GmbH Germany), and rETR was calculated by means of the following equation (Schreiber, 2004):

\[
\text{rETR} = \text{PAR} \times \text{ETR-Factor} \times \frac{P_{PSII}}{P_{PPS}} \times Y(II),
\]
where PAR is the photosynthetically active radiation during the measurements; ETR-Factor is the absorptance of photons by photosynthetic pigments which is considered to be 0.84 as a reasonable match to the average absorptance in the visible range (400-700 nm); \( P_{\text{PSII}}/P_{\text{PPS}} \) is the ratio between the photons absorbed by PS II and photons absorbed by photosynthetic pigments, with a value of 0.5 assuming only linear electron transport, that is, equal transfer rates through PS I and PS II, and comparable photochemical quantum yields of PS I and PS II under strongly light-limiting conditions; and \( Y(\text{II}) \) is the effective photochemical quantum yield of PS II, as described above.

The \( F_v/F_m \) was measured at predawn and midday using a portable fluorometer (FMS2, Hansatech Instruments, UK). Plant water potential (\( \Psi \)) was also measured at predawn and midday (except for \textit{P. uncinata} which was only measured at predawn due to the low number of surviving individuals) with a pressure chamber (SKPM 1400, Skye Instruments, UK). Measurements were made in lateral branches in most cases. Photosynthetic fluorescence parameters were always measured between 12:00 and 16:00 h (solar time), except \( F_v/F_m \) predawn measurements.

\textit{Leaf isotopic composition}

We used leaf \( \delta^{13}\text{C} \) and \( \delta^{18}\text{O} \) as time-integrated proxy measures of intrinsic water-use efficiency (iWUE, which is the ratio between net photosynthetic rate and stomatal conductance; Farquhar et al., 1989) and stomatal conductance, respectively (Barbour, 2007; Farquhar et al., 2007). Given that all the target pine species had very similar needle size and morphology and were exposed to the same environmental conditions in the common garden (including air temperature, vapour-pressure deficit, and soil-moisture content), we assume that interspecific differences in leaf \( \delta^{18}\text{O} \) should primarily reflect differences in time-integrated cumulative transpiration derived from species-
specific patterns of stomatal regulation of leaf-gas exchange (Barbour, 2007; Farquhar et al., 2007). Likewise, we assume that interspecific differences in leaf δ¹³C should primarily reflect differences in iWUE, rather than differences in irradiance or soil-water availability (as these were the same across species; Dawson et al., 2002; Farquhar et al., 1989).

Both δ¹⁸O and δ¹³C were measured on fully expanded leaves harvested in late August 2014 (thus after two growing seasons in the field) from 15 individuals per species and replication block (thus totalling 45 individuals per species); in the case of *P. uncinata* we could only sample 13 individuals due to low survival rate. We harvested pine needles produced during the current year 2014, discarding those from previous year cohorts to minimize potential legacy effects from the nursery. The pine individuals were sampled randomly, although we disregarded those with clear symptoms of decay (close to death) and those previously used to monitor physiological performance in order to avoid any bias due to experimental manipulation (e.g. increased physiological stress after cutting of branches for water-potential measurements in the previous month). After harvesting the needles for isotopic and nutrient analysis, the whole-aboveground biomass of the plant was harvested for dry-biomass production measurement. Samples were oven dried at 60 °C until constant weight and afterwards the two weights (leaves used for isotopic analysis plus the rest of the plant) of each individual were added together for the statistical analyses of aboveground dry-biomass production.

The subsample of pine needles used for isotopic analyses was finely ground to a fine powder with a ball mill. For leaf δ¹³C, we used 4 mg weighed in tin capsules. Samples were analysed using an Isoprime isotope ratio mass spectrometer (IRMS; Isoprime Ltd, Cheadle Hulme, Stockport, UK) coupled to a CN elemental analyser (EA;
Eurovector, Pavia, Italy) with continuous flow, at the Department of Biology, University of Copenhagen. For foliar δ\(^{18}\)O analyses, 0.7-0.8 mg were weighed in silver capsules and analysed at the Stable Isotope Facility of the University of California at Davis (USA). A Heckatech HT Oxygen Analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK) was used following the method described in Kornexl, Gehre, Höfling, & Werner (1999). Leaf samples were decomposed in a glassy carbon reactor at 1400°C to CO and H\(_2\)O, and oxygen was analysed as CO. We expressed the isotopic composition of the samples in delta notation as:

\[ \delta^{xx} = \left( \frac{R_{\text{samp}}}{R_{\text{stand}}} - 1 \right) \times 1000 \]

where \(^{xx}\)E is the heavy isotope which is compared to the lighter one; R refers to the molar ratio of the heavy to the light isotope (i.e. \(^{13}\)C:\(^{12}\)C or \(^{18}\)O:\(^{16}\)O); “samp” refers to the sample; and “stand” refers to an international standard (V-PDB for C and V-SMOW for O). International and internal standards were used for validating the quality and precision of isotopic analyses.

**Nutrient analysis**

The concentrations of several essential macro- and micronutrients (C, N, P, K, Zn, and Cu) were measured on the same finely ground leaf samples used for isotopic analyses. Foliar C and N concentrations were measured with a CN elemental analyser as described above. Leaf K, P, Cu and Zn concentrations were measured by inductively coupled plasma emission spectrometry using a Perkin-Elmer 5500 ICP.
Data analysis

Analyses were performed using R, version 3.3.2 (R Core Team, 2016). We explored different approaches to control for spurious statistical effects induced by spatial heterogeneity. Note that the number of blocks is too low to consider this variable a random factor in a standard mixed-model approach (random factors need to have at least five levels; Crawley, 2002). Therefore, we controlled for spatial heterogeneity including the number of columns and rows as continuous variables in linear models (Appendix S2). Differences across species for all the variables measured, in any case, followed similar patterns whatever the model used.

Differences in seedling survival were estimated with a Cox regression model using the survival R package (Therneau, 2015; Therneau & Grambsch, 2000). The rest of response variables (growth, physiological variables, leaf isotopic composition and nutrient concentrations) were analysed with linear models. Significant differences between species were tested using Tukey’s test. The stem-volume increment was used as a proxy of overall plant growth because it exhibited a close correlation with both plant height (P ≤ 0.001; ρ ≥ 0.8 in all species) and stem diameter (P ≤ 0.001; ρ ≥ 0.6 in all species).

Results

Demography

Across species, a total of 627 (74.7%) seedlings survived after two growing seasons. Survival rate differed among species (P < 2.2e-16), with an overall value of 100% for P. halepensis, 92.9% for P. nigra, 80% for P. sylvestris, and 25.8% for P. uncinata (different superscript letters indicate significant differences among species, P < 0.05 after Tukey’s multiple comparison).
Growth (stem-volume increment) after two growing seasons also differed greatly among species (P < 2.2e-16), with a much higher value for *P. halepensis* (223.14±11.47 cm³), followed by *P. nigra* (9.41±0.48 cm³), *P. sylvestris* (5.06±0.29 cm³), and *P. uncinata* (2.91±0.43 cm³). Interspecific differences in growth increased even further after the third growing season (September 2015), with mean stem volume of 3000.56±330.25, 45.26±5.90, and 16.50±2.20 cm³, and mean fresh weight of 11723±700, 322±4, and 110±14 g for *P. halepensis*, *P. nigra*, and *P. sylvestris*, respectively. *Pinus uncinata* was not sampled in 2015 due to the small number of surviving individuals, but their size was visually the lowest of all the species. See Appendix S1, Table S5 for species mean values of stem-root collar and leader shoot height in both years.

**Physiological variables**

Overall, there were large differences in physiological parameters between *P. halepensis* and the rest of the species, with *P. uncinata* in particular showing the poorest performance under the common garden conditions (Table 1). *Pinus halepensis* showed significantly lower predawn and mid-day stem water potentials than the other species during the summer dry season (Table 1), indicating a more anisohydric behaviour. *Pinus halepensis* also showed the highest values for most photochemical parameters including predawn and midday *Fv/Fm*, quantum yield, rETR, and photochemical quenching, as well as the lowest values of non-photochemical quenching (although without statistically significant differences from *P. nigra* and/or *P. sylvestris* for the latter two variables). On the contrary, *P. uncinata* showed the lowest values for most of these photochemical parameters, with significant differences from the rest of species for quantum yield and rETR but without significant differences from *P. nigra* and/or *P.*
sylvestris for the remaining fluorescence variables (Table 1).

Leaf isotopic composition and nutrient concentrations

Mean leaf $\delta^{13}$C values differed significantly among pine species (Table 2), with $P. halepensis$ showing the highest value (indicative of higher time-integrated water use efficiency), followed by $P. sylvestris$, $P. nigra$ and $P. uncinata$. There were also large differences in mean leaf $\delta^{18}$O values among species, with $P. halepensis$ showing the lowest mean value by far (indicative of higher stomatal conductance and cumulative transpiration), followed by $P. nigra$, $P. sylvestris$, and $P. uncinata$ (Table 2).

Leaf N concentration differed only slightly among pine species, with values ranging from 12.8 mg g$^{-1}$ for $P. nigra$ to 18.3 mg g$^{-1}$ for $P. sylvestris$ (Table 2). In contrast, leaf P, K, Cu and Zn concentrations differed sharply among species, with $P. halepensis$ showing about 2-fold (P), 1.7-fold (K), 1.5-fold (Zn) and 1.8-fold (Cu) higher mean concentrations than the other species (Table 2). As a result, mean foliar N:P and N:K ratios were over 2-fold lower in $P. halepensis$ than in the other pine species (Table 2). Leaf C:N ratios were less variable across species and ranged from 26.5 in $P. sylvestris$ to 35.6 in $P. nigra$.

The dry-biomass of pines harvested for isotopic analysis followed the same trend described above for shoot volume and fresh weight after three years: 660.9±32.2 for $P. halepensis$, 32.0±2.0 for $P. nigra$, 19.2±1.1 for $P. sylvestris$ and 10.4±1.6 g for $P. uncinata$.

Relationships among plant response variables across and within species

Across pine species, leaf $\delta^{18}$O was strongly negatively correlated with aboveground biomass and leaf P, K, Cu, and Zn concentrations (Figure 2), while it was positively
associated with leaf N and N:P ratio (Figure 3), overall indicating increased nutrient status, stoichiometric balance and growth with increasing time-integrated stomatal conductance and transpiration. Conversely, leaf δ\textsuperscript{13}C exhibited strong positive correlations with biomass production and leaf P, K, Cu, and Zn concentrations across species, indicating increasing water use efficiency with increasing leaf nutrient status and growth. Leaf δ\textsuperscript{13}C was negatively associated with N:P ratios, but was unrelated to leaf N concentration across species. Interestingly, leaf δ\textsuperscript{13}C and δ\textsuperscript{18}O were negatively associated with each other across all four pine species (ρ = -0.48; P = 1.70E-09), which suggests that interspecific variation in δ\textsuperscript{13}C was primarily driven by variation in photosynthesis (rather than in stomatal conductance).

Similar correlation patterns were found also at the within-species level for P. nigra and P. sylvestris: aboveground biomass and stem volume increment were positively associated with leaf P concentration and negatively associated with leaf δ\textsuperscript{18}O, N, and N:P in at least one of the two species (Appendix S1, Figure S1). Similarly, leaf δ\textsuperscript{13}C was positively correlated with leaf P and Cu concentrations and negatively associated with N:P ratio. Leaf δ\textsuperscript{18}O was negatively associated with leaf P, Cu, and Zn concentrations and positively correlated with N:P ratio in one or the two species (Appendix S1, Figure S2).

Plant water potentials and photochemical fluorescence variables were also significantly correlated with leaf nutrients and isotopes across species. Mean midday water potential was negatively associated with mean leaf K concentration (ρ = -0.991; P = 0.0088). Mean leaf Zn concentration was positively associated with mean quantum yield (ρ = 0.968; P = 0.032) and predawn F\textsubscript{v}/F\textsubscript{m} (ρ = 0.994; P = 0.0063), while it was negatively associated with qN (ρ = -0.984; P = 0.016), which overall suggests increased photochemical performance with increased leaf Zn status across...
species. In addition, mean leaf $\delta^{13}$C was positively correlated with mean quantum yield and predawn $F_v/F_m$, and was negatively associated with $qN$ (Figure 5), which suggests increased water use efficiency with increased photochemical efficiency across species. Finally, mean leaf $\delta^{18}$O was negatively associated with mean quantum yield, predawn $F_v/F_m$ and rETR (and positively with mean $qN$), thus revealing an improved photochemical efficiency with increasing stomatal conductance and cumulative transpiration across species (Figure 6).

Discussion

We found that the thermophilous, drought-tolerant pine species $P. halepensis$ exhibited 100% survival and far greater growth than the other more drought-sensitive mountain pine species evaluated in the common garden experiment. Unsurprisingly, pine species originating from wetter and cooler habitats such as $P. uncinata$ and, to a lesser extent, $P. sylvestris$, showed the poorest growth and survival under the xeric common garden conditions. These results are expected according to the contrasting ecological requirements of each species and the relatively warm and dry climatic conditions at the study site. However, our study provides insights into the physiological mechanisms underlying the contrasting responses among pine species, and supports the contention that the tight stomatal regulation typical of drought-sensitive mountain pine species makes them fall into an “isohydric trap”, in which high stomatal sensitivity to soil water shortage and high atmospheric evaporative demand trigger stomatal closure and lead to a detrimental feedback loop that eventually causes severe nutrient starvation and stoichiometric imbalance under prolonged dry conditions (Figure 1).

The large interspecific differences in leaf isotopic ratios and stem water potential indicate that pine species differ in stomatal control under prolonged climatic dryness.
According to the dual isotope conceptual model (Grams, Kozovits, Häberle, Matyssek, & Dawson, 2007; Scheidegger, Saurer, Bahn, & Siegwolf, 2000), the combination of high $\delta^{18}$O and low $\delta^{13}$C values in the drought-sensitive mountain pine species (compared to *P. halepensis*) indicates low time-integrated stomatal conductance and water use efficiency, as well as low photosynthesis rates (Querejeta, Allen, Caravaca, & Roldán, 2006), which is consistent with the poor growth of these species. This interpretation of isotope data is in strong agreement with gas exchange measurements conducted at whole plant level with transient-state closed chambers in the same common garden experiment, which showed higher stomatal conductance and transpiration, photosynthetic rates and water use efficiency in *P. halepensis* than in the other pine species during the dry season (Salazar-Tortosa et al., 2018). Furthermore, the potential influence of the use of different water sources among species can be discarded as the lower predawn water potential of *P. halepensis* compared to other species would be incompatible with the alternative explanation that it was using a more $\delta^{18}$O depleted source water stored in deeper, wetter soil layers (Nardini et al., 2016; Voltas, Lucabaugh, Chambel, & Ferrio, 2015; West et al., 2012).

The combination of high predawn and midday stem water potentials at the peak of the dry season with low stomatal conductance and cumulative transpiration (inferred from high leaf $\delta^{18}$O values) indicates a typical isohydric behaviour (Martínez-Vilalta & Garcia-Forner, 2017) in *P. nigra*, *P. sylvestris* and *P. uncinata*. Under low water availability and high temperature and evaporative demand conditions, plants with isohydric behaviour close their stomata early to maintain relatively high water potentials and prevent dehydration (Klein, 2014; McDowell et al., 2008; Tardieu & Simonneau, 1998). Ultimately, this reduction of cumulative transpiration negatively affects the plant’s nutrient status given the importance of active transpiration flux along the soil-
plant-air continuum for effective soil nutrient uptake in drying soil (Cramer et al., 2009; Lambers et al., 2008; Rouphael et al., 2012; Sardans, Peñuelas, Prieto, & Estiarte, 2008; Sardans, Peñuelas, Coll, Vayreda, & Rivas-Ubach, 2012). The strong negative correlations between leaf $\delta^{18}$O (proxy of stomatal conductance) and foliar P, K, Cu, Zn concentrations and positive correlation of leaf $\delta^{18}$O with N:P and N:K ratios across species (as well as within species for P and N:P; Figure 3, Appendix S1 Figure S2) indicate that soil nutrient uptake was severely constrained by low cumulative transpiration in the drought-sensitive mountain pine species under prolonged dryness, relative to *P. halepensis*.

Stomatal conductance and transpiration will determine the rate of water extraction from soil, so that species with higher transpiration rates will deplete rhizosphere soil water faster and will hence experience greater reductions in predawn water potentials during dry periods (Martínez-Vilalta & Garcia-Forner, 2017), as found in *P. halepensis*. This ability to allow water potentials to drop while sustaining high stomatal aperture and conductance (i.e. a more anisohydric strategy) may have enabled *P. halepensis* to continue extracting water and dissolved nutrients from soil during prolonged dry periods. A recent study has also reported strong negative correlations between leaf $\delta^{18}$O and foliar nutrient concentrations across contrasting plant species exposed to the same environmental conditions, suggesting a heavy dependence of plant nutrient uptake and status on stomatal conductance and cumulative transpiration (Prieto, Querejeta, Segrestin, Volaire, & Roumet, 2017). Nitrogen, by contrast, did not seem to be involved in the observed pattern, as this nutrient was negatively associated with biomass and positively associated with $\delta^{18}$O (Figure 2, 3, Appendix S1 Figure S1), which suggests that the interspecific differences in leaf N concentration were mainly a consequence of differential growth dilution effects (Sabaté & Gracia, 1994) and not of
stomatal behaviour. In addition, the species with lower growth (*P. sylvestris* and *P. uncinata*) could have constitutively high leaf N as supported by higher N concentration for *P. uncinata* at nursery, and by previous studies for *P. sylvestris* (Sardans et al., 2011).

The unusually high leaf N:P and N:K ratios found in the more drought sensitive, mountain pine species are indicative of strong P and K limitation of growth (Güsewell, 2004; Lawniczak, Güsewell, & Verhoeven, 2009; See Table 2 for a comparison with reference values of each species). The severe nutrient deficit and imbalance observed in these drought-sensitive pine species appeared to be a direct consequence of climatic drought stress, as seedling nutrient status was optimal at the beginning of the experiment across species (Appendix S1, Table S1), and given that the soil at the common garden site was not deficient in any nutrient (Appendix S1, Table S2). Indeed, soil nutrient availability in this fertile agricultural field was sufficient to support a balanced leaf stoichiometry with optimal N:P ratios and vigorous biomass growth in *P. halepensis*. Despite much larger growth-dilution effects, leaf P, K, Zn and Cu concentrations were higher and N:P and N:K ratios were much lower in *P. halepensis* than in the other species, indicating that severe nutrient starvation and stoichiometric imbalance in the other species was largely the result of climatic stress, rather than the result of low soil nutrient availability “per se”. Deficiency of P, K, Zn, and Cu and severe N:P:K stoichiometric imbalance can impair photosynthesis, transpiration (Figure 1; blue arrow), water-use efficiency and growth, as reported in this study (see also Güsewell, 2004; Sardans & Peñuelas, 2015; Talbott & Zeiger, 1996). This idea is supported by the strong correlations of leaf nutrient concentrations and stoichiometric ratios with both leaf δ¹⁸O and δ¹³C ratios and with aboveground biomass across and within species (Figures 2, 3, 4, Appendix S1, Figures S1, S2). We also found a negative
association between leaf K concentration and water potential across species, which may
be explained by the major role that K plays in drought tolerance (Rivas-Ubach, Sardans,

The rather extreme N:P:K stoichiometric imbalance observed in the drought-
sensitive mountain pine species may be explained by differences in mobility and
availability in the soil solution among nutrients, given that PO$_4^{3-}$ and K$^+$ have diffusion
coefficients that are lower by orders of magnitude than that of NO$_3^-$ (Lambers et al.,
2008; Marschner & Rengel, 2012). Our study suggests that uptake of nutrients with
limited mobility and diffusion rates in soil (e.g. phosphate, potassium, zinc, copper)
may be particularly vulnerable to severe decreases in transpiration and mass flow to
roots, compared to highly mobile nutrients like nitrate (Rengel & Marschner, 2005).

Besides, atmospheric deposition is several orders of magnitude higher for N than for
other nutrients (Peñuelas, Sardans, Rivas-Ubach, & Janssens, 2012), which may also
lead to higher foliar N uptake. Therefore, in a global scenario of climate warming
combined with increasing rates of anthropogenic N deposition (Güsewell, 2004; Jonard
et al., 2015) we should expect plant P status (along with K and micronutrients like Cu or
Zn) to be particularly vulnerable to decreases in transpiration fluxes during prolonged
periods of climatic dryness, whereas plant N status may be less responsive. In addition,
the reduced carbon assimilation exhibited by drought-sensitive mountain pine species at
the common garden site (Salazar-Tortosa et al., 2018) could lead to low carbon
availability to support the growth and activity of fine roots and ectomycorrhizal (EMF)
fungi (Gessler et al., 2017; Matías et al., 2017; Moran et al., 2017). This could hamper
even more the assimilation of low mobility nutrients, whose absorption has high energy
and carbon costs such as the production of extramatrical EMF mycelium, the secretion
of phosphatases and organic acids by roots and mycorrhizae for solubilisation and
mineralization of inorganic and organic P, or rhizosphere priming effects (Achat, Augusto, Gallet-Budynek, & Loustau, 2016; Kreuzwieser & Gessler, 2010). Moreover, limited carbohydrate availability and transfer to roots could also constrain the supply of energy and carbon skeletons for nutrient assimilation (Kreuzwieser & Gessler, 2010). Soil moisture content remained above the permanent wilting point throughout the summer dry period in both years of the experiment (Appendix S1, Table S4), which suggests that high temperature and evaporative demand may have also been key drivers of the contrasting responses observed among pine species (McDowell et al., 2015; McDowell & Allen, 2015; Salazar-Tortosa et al., 2018; Williams et al., 2013). In fact, mean summer temperature at the common garden site was considerably higher than that experienced by the mountain pine species in their original habitat (P. nigra, P. sylvestris, and P. uncinata). Heat stress can limit stomatal conductance, as plants close their stomata to prevent excessive transpiration and water loss when atmospheric demand for water increases with rising temperatures (Novick et al., 2016; Urban, Ingwers, McGuire, & Teskey, 2017; Zhang, Wollenweber, Jiang, Liu, & Zhao, 2008). Stomatal closure in response to heat and drought stress reduces evaporative leaf cooling (Cook, Dixon, & Leopold, 1964), which may favour heat-induced damage of the photosynthetic machinery that decreases photochemical efficiency (Sharkey, 2005). Lower predawn Fv/Fm values as well as larger predawn Fv/Fm vs. midday Fv/Fm differences in mountain pine species (compared to P. halepensis) support the idea that heat stress in combination with high light levels led to both chronic photoinhibition as well as reversible reduced photochemical efficiency during the hottest time of the day in summer (Table 1). Moreover, the detrimental impacts of the combination of high temperatures and drought stress on leaf photochemical efficiency and carboxylation capacity were likely compounded by the effects of macro- and micronutrient deficiency
and stoichiometric imbalance in mountain pine species (Cakmak, 2005; Casimiro, Barroso, & Pais, 1990; Eller, Jensen, & Reisdorff, 2016), which may explain the association of low photochemical efficiency (as indicated by low rETR, quantum yield and predawn \( F_v/F_m \) and high \( qN \)) with low cumulative transpiration (high \( \delta^{18}O \)) and water use efficiency (low \( \delta^{13}C \)) across species (Figures 5, 6). Overall, the results of this study suggest that intensified drought stress from higher air temperatures—the “hotter drought” effect (Allen et al., 2015)—might lead to severe nutrient deficit, stoichiometric imbalance, and photosynthetic impairment mediated by reduced cumulative transpiration in drought-sensitive species.

In summary, we found that the degree of stomatal regulation of transpiration is tightly linked to plant nutrient status and stoichiometry under dry conditions, and that reduced nutrient uptake is a key mechanism to consider when assessing poor plant growth and survival under prolonged climatic dryness. We propose the existence of a detrimental feedback loop leading to severe P and K starvation and N:P:K imbalance in strictly isohydric vascular plants undergoing prolonged drought stress, such as some of our study pine species. These drought-sensitive species close their stomata at relatively high soil and plant water potentials, and thus are not able to maintain adequate transpiration and nutrient uptake during prolonged drought periods, which leads to severe nutrient imbalance that might exacerbate and accelerate the onset of carbon starvation, hydraulic failure, phloem dysfunction, and their multiple interplays (McDowell et al., 2011; Sala, Piper, & Hoch, 2010; Sevanto, McDowell, Dickman, Pangle, & Pockman, 2014; Zwieniecki & Holbrook, 2009). In contrast, relatively drought-tolerant plant species with a more anisohydric behaviour such as \( P. \) halepensis can maintain open stomata and transpiration at comparatively lower soil and plant water potentials, thereby escaping this detrimental feedback and avoiding nutrient deficit and
imbalance. This study highlights the intimate interplay and interdependence between stomatal regulation, transpiration, carbon assimilation and nutrient status in the response of vascular plants to long periods of climatic dryness (Gessler et al., 2017). We advocate consideration of this proposed conceptual framework (Fig. 1) in order to better understand and predict the impacts of ongoing global change on the performance and survival of pines and other plant species with tight stomatal regulation and strict isohydric behaviour, with special attention to the role of macronutrients with low mobility in soil, such as P and K.

**Acknowledgements**

This work was supported by the projects ECOLPIN (AGL2011-24296), CGL2013-48753-R, the network REMEDINAL 3 (S2013/MAE-2719) of the CAM, by FPU (FPU13/03410) and Juan de la Cierva (FPDI-2013-15867) fellowships from the Spanish Ministry of Education, Culture and Sport to DS and LM, respectively, and by a grant from IES Abroad. We are also grateful to the Centro Nacional de Recursos Genéticos Forestales ‘El Serranillo’ (MAGRAMA) for cultivating the plants during the nursery stage. Craig D. Allen and five anonymous reviewers provided insightful comments that improved the manuscript.
Bibliography


*Tables*

Table 1 Mean values ± SE of physiological variables measured in the four target pine species during mid-summer (dry season) of the second year after planting. F and P values of the “species” factor are shown for each variable. Significant p values are indicated in bold, while differences between species (P < 0.05 after Tukey’s multiple comparison) are shown with different superscripts.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sample size</th>
<th>P. halepensis</th>
<th>P. nigra</th>
<th>P. sylvestris</th>
<th>P. uncinata</th>
<th>F</th>
<th>Pr(&gt;F)</th>
</tr>
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<tbody>
<tr>
<td>Predawn Water Potential (MPa)</td>
<td>71</td>
<td>-1.26±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.86±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.87±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>43.9</td>
<td>2.63E-11</td>
</tr>
<tr>
<td>Midday Water Potential (MPa)</td>
<td>131</td>
<td>-2.37±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-1.88±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.84±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.92±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.6</td>
<td>6.46E-14</td>
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<tr>
<td>Predawn fluorescence (F&lt;sub&gt;v&lt;/sub&gt;/F&lt;sub&gt;m&lt;/sub&gt;)</td>
<td>132</td>
<td>0.8709±0.0024&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.839±0.005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.836±0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.825±0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.2</td>
<td>5.17E-09</td>
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<td>Midday fluorescence (F&lt;sub&gt;v&lt;/sub&gt;/F&lt;sub&gt;m&lt;/sub&gt;)</td>
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<td>0.721±0.017&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.657±0.022&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.3</td>
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<td>Yield</td>
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<td>0.599±0.017&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.598±0.017&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.545±0.024&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.1</td>
<td>7.35E-11</td>
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<td>rETR (µM e/m²s)</td>
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<td>455.0±10.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>396.1±13.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>384.9±13.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>324.4±15.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.7</td>
<td>3.85E-09</td>
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<td>Photochemical quenching</td>
<td>125</td>
<td>0.972±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.968±0.004&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.958±0.005&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.953±0.005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6</td>
<td>1.66E-02</td>
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<td>Non-Photochemical quenching</td>
<td>126</td>
<td>0.044±0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.067±0.010&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.071±0.009&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.083±0.013&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2</td>
<td>2.53E-02</td>
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Table 2 Mean values ± SE of leaf isotopic composition, nutrient concentrations and stoichiometric ratios measured in the four target pine species in mid-summer (dry season) of the second year after planting. F and P values of the “species” factor are shown for each variable. Significant p-values are indicated in bold, while differences between species (P < 0.05 after Tukey’s multiple comparison) are shown with different superscripts. As a reference, macronutrient concentration and stoichiometric ratios observed in natural populations of the studied species in Northeast of the Iberian Peninsula are shown in brackets (data obtained from Sardans, Rivas-Ubach, & Peñuelas, 2011).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sample size</th>
<th>P. halepensis</th>
<th>P. nigra</th>
<th>P. sylvestris</th>
<th>P. uncinata</th>
<th>F</th>
<th>Pr(&gt;F)</th>
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<tr>
<td>Foliar δ¹³C (‰)</td>
<td>186</td>
<td>-25.50±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-27.68±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-27.50±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-28.38±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.8</td>
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<tr>
<td>Foliar δ¹⁸O (‰)</td>
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<td>26.72±0.11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>28.59±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.55±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.12±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.3</td>
<td>&lt; 2.2E-16</td>
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<td>Nitrogen (mg g⁻¹)</td>
<td>181</td>
<td>14.3±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.8±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.3±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.5±0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>26.8</td>
<td>1.06E-13</td>
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<td>Phosphorus (mg g⁻¹)</td>
<td>185</td>
<td>0.85±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>122</td>
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<td>58.4</td>
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<td>12.3±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.3±1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.1</td>
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<tr>
<td>Copper (ppm)</td>
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<td>C:N ratio</td>
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<td>99</td>
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<td>N:K ratio</td>
<td>181</td>
<td>3.13±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>7.84±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.20±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.1</td>
<td>&lt; 2.2E-16</td>
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Figure captions

Figure 1 Proposed “isohydric trap” conceptual model of reduced plant physiological performance and growth under hotter drought, in which vascular plants with a strong stomatal regulation of transpiration undergo early and prolonged closure of the stomata under heat and drought stress. This strict isohydric behaviour triggers a series of physiological changes that culminate in severe nutrient imbalance and a reduction of the capacity to cope with further drought stress, thereby becoming a feedback to in earlier stomatal closure under a subsequent event of water stress (the “isohydric trap”). Sub-loops are shown with arrows of different colours: i) Transpiration-nutrient loop in blue; ii) Transpiration-root growth loop in green.

Figure 2 Spearman’s rank coefficients (ρ) for the correlations of pine sapling growth after two years with leaf δ¹⁸O, δ¹³C and nutrient concentration across species. Significant associations are indicated with asterisks (* for P < 0.05 and ** for P < 0.01).

Figure 3 Correlations between leaf nutrient concentrations and foliar δ¹⁸O (proxy of stomatal conductance) across species. Spearman’s rank correlation coefficients along with p and S value are shown for each correlation. Abbreviations: H = P. halepensis, N = P. nigra, S = P. sylvestris, U = P. uncinata.

Figure 4 Correlations between foliar δ¹³C (proxy of water-use efficiency) and leaf nutrient concentrations across pine species. Spearman’s rank correlation
coefficients together with p and S value are shown for each correlation. Species abbreviations as in Figure 3.

Figure 5 Correlation between mean values per species of leaf $\delta^{13}$C (proxy of water-use efficiency) with photosynthetic fluorescence parameters across pine species. Pearson’s correlation coefficients along with p and t value are shown for each correlation. Error bars for both axes represent ±SE. Species abbreviations as in Figure 3. Variables abbreviations: Yield = Quantum yield, qN = Non-photochemical quenching.

Figure 6 Correlation between mean values per species of leaf $\delta^{18}$O (proxy of stomatal conductance) with photosynthetic fluorescence parameters across pine species. Pearson’s correlation coefficients together with p and t value are shown for each correlation. Error bars for both axes represent ±SE. Species abbreviations as in Figure 3. Variables abbreviations: Yield = Quantum yield, rETR = relative electron transport rate, qN = Non-photochemical quenching.
\[ \rho(\log(\text{biomass})) \]

\[ \begin{array}{cccccccc}
\delta^{18}O & \delta^{13}C & N & P & K & Zn & Cu & N:P & N:K \\
-0.9 & -0.5 & -0.1 & 0.3 & 0.5 & 0.7 & 0.9 \\
\end{array} \]

\[ \rho(\Delta \text{Stem volume}) \]

\[ \begin{array}{cccccccc}
\delta^{18}O & \delta^{13}C & N & P & K & Zn & Cu & N:P & N:K \\
-0.9 & -0.5 & -0.1 & 0.3 & 0.5 & 0.7 & 0.9 \\
\end{array} \]
Foliar δ¹³C (‰) vs.
- Water-use efficiency +

**Nitrogen (mg g⁻¹)**

- **ρ = 0.0367**
- **p.value = 0.666**
- **S = 450052**

**Phosphorus (mg g⁻¹)**

- **ρ = 0.641**
- **p.value = 3.66e−18**
- **S = 182261**

**Potassium (mg g⁻¹)**

- **ρ = 0.501**
- **p.value = 1.19e−10**
- **S = 258823**

**Zinc (ppm)**

- **ρ = 0.525**
- **p.value = 1.18e−11**
- **S = 241228**

**Copper (ppm)**

- **ρ = 0.683**
- **p.value = 3.99e−21**
- **S = 157667**

**N/P ratio**

- **ρ = −0.623**
- **p.value < 2.2e−16**
- **S = 742178**
Yield

\[
\text{Foliar } \delta^{13} \text{C (‰)} = 0.989, \quad p.value = 0.0108, \quad t = 9.56
\]

Predawn \( F_v/F_m \)

\[
\text{Foliar } \delta^{13} \text{C (‰)} = 0.991, \quad p.value = 0.0087, \quad t = 10.7
\]

\[
\text{Foliar } \delta^{13} \text{C (‰)} = -0.981, \quad p.value = 0.0186, \quad t = -7.23
\]

- Water-use efficiency +
Foliar δ^{18}O (‰)

* Yield
  - $r = -0.966$, $p.value = 0.0343$, $t = -5.26$

* Predawn Fv/Fm
  - $r = -0.981$, $p.value = 0.0194$, $t = -7.08$

* qN
  - $r = 0.987$, $p.value = 0.013$, $t = 8.69$

* rETR
  - $r = -0.953$, $p.value = 0.0471$, $t = -4.44$

Stomatal conductance

+ ↔ -