



Fertilization of saplings: growth and hydraulic response of three temperate tree species

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Abstract

Fertilization plays a crucial role in sapling production and quality, especially in the face of increasing drought frequency driven by climate change. However, its effects on sapling growth and hydraulic traits remain debated, with inconsistent findings across species and conditions. We examined the effects of annual fertilization on growth, gas exchange, and hydraulics of *Pinus sylvestris*, *Fagus sylvatica*, and *Quercus robur* saplings over three years. Fertilization increased sapling growth with species-specific responses. Net photosynthesis (P_n) and stomatal conductance (g_s) increased across species, though with varying timing and magnitude. Hydraulic traits, such as turgor loss point (Ψ_{tlp}), stem embolism resistance (Ψ_{50}), and drought-induced leaf damage (Ψ_{EL50}), were unaffected by fertilization. This suggests limited plasticity in drought resistance traits under well-watered conditions. However, *F. sylvatica* exhibited increased stem-specific conductivity (k_s) in the final year, indicating species-specific adjustments in water transport. Additionally, fertilization led to a shift in biomass allocation, with reduced root: shoot ratios across all species, raising concerns about potential trade-offs with drought resilience. These findings underscore the importance of species-specific nutrient responses in sapling production and forest management. Further research is needed to assess the long-term effects of fertilization under field conditions, particularly in the context of increasing drought.

Keywords embolism resistance · gas exchange · drought tolerance · hydraulic properties

Introduction

Fertilization is a common practice in forestry nurseries aiming at producing saplings of high quality. It can promote the growth of saplings, and improve physiological traits beneficial for their future field performance (Puértolas et al. 2003; Bigler et al. 2006; Villar-Salvador et al. 2008; Oliet et al. 2009; Luis et al. 2010). This is of importance under stressful conditions, such as drought events (Demchik and Sharpe 2000), where the quality of saplings plays a critical role in the success of reforestation programs (Luis et al. 2009).

The increase in drought and heat events observed during the last decades (and predicted to become more frequent in the future; IPCC 2021), has had a severe impact on many European forest ecosystems (Hammond et al. 2022), with hydraulic failure (i.e., dysfunction of the water transport) being a major risk to trees under drought (Brodrribb and Cochard 2009; Kursar et al. 2009; McDowell et al. 2008). Water transport in the xylem is driven by a pressure gradient which enables the continuous flow of water from roots to leaves (Tyree and Zimmermann 2002). As soil dries and/or

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evaporative demand increases, the pressure may exceed the 'air seeding' threshold, triggering embolism events (Choat et al. 2012). During embolism formation, an air bubble is pulled into a xylem conduit, rapidly expands, and blocks it, disrupting water transport (Tyree and Zimmermann 2002). When embolism becomes widespread, it can lead to desiccation of distal tree organs (Brodribb et al. 2021; Cardoso et al. 2020) and ultimately tree mortality (Hammond et al. 2019). Resistance to embolism formation thus has been strongly associated with drought tolerance in trees (Brodribb and Cochard 2009; Choat et al. 2012). Another critical trait linked to drought tolerance is the turgor loss point (Ψ_{tlp} , negative values), which represents the water potential at which leaf cells lose turgor pressure. Below Ψ_{tlp} , leaves lose their structural integrity, stomatal function becomes impaired, and photosynthesis declines (Bartlett et al. 2012). Species with a lower Ψ_{tlp} (more negative) tend to be more drought-tolerant, as they can maintain cellular function and structural integrity at lower water potentials under drought.

Fertilization was reported to increase Ψ_{tlp} (less negative, e.g., Beikircher et al. 2019), stomatal conductance (e.g., Fangmeier et al. 1994), net photosynthesis (e.g., Evans and Clarke 2019), and height growth (e.g., Wright et al. 2011). It can also result in increased vessel diameters (e.g., Hacke et al. 2010), enhance stem hydraulic conductivity (e.g., Medeiros et al. 2016), lower ratios of root to leaf area (e.g., Kozłowski and Pallardy 2002), and reduce embolism resistance (e.g., Plavcová et al. 2013). These shifts in plant hydraulic traits likely boost the growth and facilitate the hydraulic efficiency, but may decrease the hydraulic safety margin (i.e., difference between field water potentials and embolism thresholds) and, in consequence, increase the risk of hydraulic failure under drought conditions. For example, Beikircher et al. (2019) demonstrated that increases in stomatal conductance, net photosynthesis, hydraulic conductivity, Ψ_{tlp} , and shoot growth under fertilization were associated with increased embolism risk. In addition, higher biomass accumulation under fertilization may elevate carbon costs during periods of carbon starvation, as respiration is often less sensitive to drought than photosynthesis (Flexas et al. 2006), if not compensated by increased carbon storage. Importantly, fertilization not only increases total biomass but often alters its allocation among plant organs, as fertilized saplings tend to allocate more biomass to aboveground components, such as stems and leaves, and less to roots (Müller et al. 2000; Faustino et al. 2013, 2015; Guillemot et al. 2021). Increased investment in leaves can enhance leaf area expansion (Hacke et al. 2010; Plavcová et al. 2013) or lead to thicker, more resource-conservative leaves (Poorter et al. 2009). The shift in biomass allocation can enhance resource capture aboveground but, due to the reduction in root: shoot ratio, compromise drought resilience by limiting

water uptake capacity (Mokany et al. 2006). There is experimental evidence that high nitrogen supply increases drought sensitivity of European beech mainly due to reduced root biomass (Dziedek et al. 2016).

In contrast to the responses described above, fertilization may also reduce the risk of embolism formation and increase the safety margin, depending on how nutrient availability alters plant functional traits (e.g., Villagra et al. 2013). Fertilization can lower embolism risk through generally reduced stomatal conductance and/or more restrictive stomatal regulation, thereby limiting minimum xylem water potentials (Goldstein et al. 2013), as well as through adjustments in xylem structure (Beikircher et al. 2019). However, as fertilization often enhances growth, leaf area, and hydraulic efficiency, which can increase transpiration demand and xylem tension, the effect of fertilization on embolism vulnerability depends on the balance between growth-related increases in hydraulic demand and compensatory adjustments in stomatal control, xylem properties, and tissue water relations. Consequently, fertilization effects vary with fertilizer type and among plant species and organs. For example, embolism resistance of hybrid poplar has been found to decrease upon nitrogen, slightly increase with phosphorus, and to be unaffected by potassium application (Harvey and Driessche 1997, 1999; Hacke et al. 2010; Plavcová and Hacke 2012; Plavcová et al. 2013). Furthermore, an increase in embolism resistance of roots but no effect on branches were observed on *Pinus taeda* upon fertilization (Ewers et al. 2000). Fertilization has also been shown to stabilize cell turgor through osmotic adjustment, lowering Ψ_{tlp} (Fang et al. 2018). Also, enhanced cell wall elasticity under fertilization improves drought tolerance by reducing Ψ_{tlp} (Beikircher et al. 2013, 2019).

Overall, fertilization can have significant effects on tree hydraulics, but responses vary widely in magnitude and direction. Most studies on young trees to date have focused on short-term experiments lasting days to months (Oddo et al. 2011; Luis et al. 2010; Faustino et al. 2013; Beikircher et al. 2019). The present study thus aimed to investigate the long-term effects of fertilization on the hydraulics, gas exchange and growth of saplings over three years. We analyzed saplings of *Pinus sylvestris*, *Fagus sylvatica* and *Quercus robur*, which are important European forest species and exhibit differences in drought resistance and nutrient demand (Brunet et al. 2010; Yeste et al. 2025; Svensson et al. 2023). We hypothesized that (i) fertilization would enhance hydraulic efficiency, gas exchange and growth, but reduced drought resistance in fertilized saplings (ii) responses of the parameters studied would be species-specific and (iii) the magnitude of the responses would increase over time with repeated fertilization.

This study should enable new insights into the complex effects of fertilization on saplings during their development. It should also provide information useful to tree nurseries and help to improve fertilization treatment of saplings, thereby optimizing reforestation material.

Materials and methods

Plant material, experimental design, and study site

Measurements were carried out on three ecologically and economically important European forest species: *Pinus sylvestris* L. (evergreen, coniferous), *Fagus sylvatica* L. (deciduous, diffuse-porous) and *Quercus robur* L. (deciduous, ring-porous). In autumn 2020, a total of 216 plants (72 per species), approximately 30 to 50 cm in height, were purchased from Tiroler Landesforstgärten. At the time of purchase, *P. sylvestris* saplings were three years old, whereas *F. sylvatica* and *Q. robur* saplings were two years old, and saplings of similar size were selected within each species, respectively. All saplings had relatively small root systems, which were planted in 2 L pots filled with a standard soil mixture designed for alpine plants (leaf mold: ground soil: coconut fiber: sand: horticultural lava: rock meal; 5: 2: 2: 2: 1: 0.15). The substrate provided sufficient baseline nutrient availability for sapling establishment prior to treatment. Pots were arranged in a randomized design at the Innsbruck Botanical Garden (affiliated to the Department of Botany, University of Innsbruck) and shifted once a month to avoid edge effects. All pots were watered regularly to avoid dehydration.

In spring 2021, saplings of each species were evenly divided into three groups (24 saplings per group) and fertilized with 0 g (control, C), 6 g (low fertilization treatment, LF) or 19 g (high fertilization treatment, HF) standard NPK fertilizer per pot. *P. sylvestris* received COMPO conifer fertilizer (COMPO GmbH, Germany) containing 16% N and 13.5% K₂O, while *F. sylvatica* and *Q. robur* were fertilized with COMPO standard fertilizer containing 7% N, 3% P₂O₅ and 3% K₂O (in 2021 and 2023), and GPI fertilizer (gpi green partners international GmbH&Co. KG, Germany) containing 9% N, 4% P₂O₅ and 7% K₂O (in 2022; depending on availability of fertilizer). All fertilizers used in this study were slow-release formulations with a release duration of 3 to 6 months, ensuring gradual nutrient availability over time and minimizing nutrient leaching. In summer 2021, 8 saplings per fertilization treatment and per species were randomly selected and subjected to a series of measurements including total nitrogen content of soil, hydraulics (pressure-volume curves, embolism resistance of stem, stem-specific conductivity, drought-induced leaf damage),

gas exchange (net photosynthesis, stomatal conductance, stomatal density, stomatal pore length), and growth (sapling height and stem diameter, biomass, specific leaf area, root: shoot). In spring 2022, the remaining saplings were transplanted to 4 L pots to avoid limitations in root growth (with the same soil used in autumn 2020) and fertilized again with the same protocol as in 2021. In summer 2022, 8 saplings per fertilization treatment and per species were collected and subjected to the same series of measurements as in 2021. In 2023, the remaining saplings (8 saplings per fertilization treatment and per species) were again fertilized in spring and used for final measurements in summer.

Soil nitrogen content

Nitrogen, which is the primary growth limiting nutrient, was determined. When saplings were removed from the pots for biomass measurements, the corresponding soil was air-dried, thoroughly mixed, and sieved with a 2-mm sieve ($n=3$ per fertilization treatment and per species). Then, ca. 10 g per pot was oven-dried at 80 °C for 48 h and grounded with a TissueLyser II (QIAGEN, Germany). The total nitrogen content was determined by an elemental analyzer (Flash 2000, Thermo Fisher Scientific, US; detail please see <https://documents.thermofisher.com/TFS-Assets/CMD/posters/PN-42227-OEA-Nitrogen-Carbon-Soils-Plants-Pittcon2015-PN42227-EN.pdf>).

Hydraulic measurements

Pressure-volume curves

Five small terminal twigs (*P. sylvestris*) or five leaves (*F. sylvatica*, *Q. robur*) per fertilization treatment and per species were randomly selected to measure pressure-volume curves (pv curves), which describe the relationship between inversely transformed leaf water potential ($1/\Psi_{\text{leaf}}$) and relative water deficit (RWD) of leaf tissues. While several parameters can be derived from pv curves, we primarily focused on turgor loss point (Ψ_{tlp}) as it represents a critical physiological threshold for leaf-level drought tolerance and turgor maintenance across diverse species. Saplings were well watered the evening before the measurement to ensure full saturation. Twigs or leaves were cut on the next morning (ca. 8:00 am) and immediately transferred to the lab to measure the turgid weight (TW) with an analytical balance (BP61S, Sartorius AG, Germany). Then the twigs or leaves were dehydrated on the bench and, at intervals, the fresh weight (FW) was measured after evaluating the respective leaf water potential (Ψ_{leaf}) with a pressure chamber (1505D, PMS Instrument Company, US). Once Ψ_{leaf} reached -4 to -5 MPa, twigs or leaves were oven dried at 80 °C for 48 h

to obtain the dry weight (DW), and the RWD was calculated as:

$$RWD = 100 - 100 * \frac{FW - DW}{TW - DW}$$

The R package “pvldcurve” (Raesch 2020) was applied to construct the pv curves and determine the turgor loss point (Ψ_{tlp}) for each single twig and leaf, respectively.

Embolism resistance and stem-specific conductivity

Four to six stems per fertilization treatment and per species were harvested at about 8:30 to measure the embolism resistance. The parameter Ψ_{50} , which indicates the xylem water potential (Ψ_x) at which 50% of the xylem has been embolised, was calculated from either vulnerability curves (VCs, *F. sylvatica*, *P. sylvestris*) or extraction curves (ECs, *Q. robur*). VCs describe the relationship between Ψ_x and percent loss of conductivity (LC), indicating reduced water flow through stems as embolisms form. ECs, on the other hand, represent embolism formation by quantifying the percent loss of volume (LV) as water is released from stems at decreasing Ψ_x . ECs are suitable for species with long vessels, to avoid “open vessel” artifact that can affect LC measurements (Cochard et al. 2010, 2013; Martin-StPaul et al. 2014; Pivovarov et al. 2016). In both curves, Ψ_{50} represents the critical Ψ_x where 50% of either LC (in VCs) or LV (in ECs) occurs.

VCs were measured by using the Cavitron method (Cochard 2002; Cochard et al. 2005; Beikircher et al. 2010). Briefly, under water a 27-cm-long segment was cut from a stem, and side branches and leaves were removed. Both the distal and basal end (ca. 5 cm) were debarked, then the segment was placed in a custom-built rotor of a modified centrifuge (Sorvall RC-5, Thermo Fischer Scientific, US) with both ends submerged in reservoirs filled with distilled, filtered and degassed water (containing 0.005% v/v, Micro-pur Forte MF 1000 F, Katadyn Products, Switzerland). Reservoirs had a spill hole at 10 mm and 16 mm distance from the base, respectively. Under centrifugal force, a water potential gradient was generated across the stem segment by the difference in water levels between the two reservoirs. This gradient enabled the measurement of stem-specific hydraulic conductivity (k_s). The calculation of k_s , accounting for stem length and cross-sectional area, was done by using a standard regression method (Wang et al. 2014). The stem segment was first spinned at an RPM set to a target Ψ_x of -0.25 MPa (10–15 min for equilibration), followed by the initial k_s measurement. Subsequently, the stem segment was subjected to a series of k_s measurements after step-wise increasing RPM, at intervals of about -0.5 MPa, until

LC > 95% was reached. The R package “fitplc” (Duursma and Choat 2017) was employed to fit VCs using the Weibull function and determine the Ψ_{50} .

ECs were measured using the same modified centrifuge and stem segment preparation method. The stem segment was placed in the rotor, with both ends submerged in reservoirs. The volume of solution added to the reservoirs (same type used in VCs measurements but without holes) varied slightly due to differences in apical and basal stem diameters, but both reservoirs were filled to a consistent height of 1 cm. The rotational speed was first set to 800 rpm (corresponding to -0.08 MPa) for 10–20 min for equilibration. Once the menisci in both reservoirs stopped moving and merged into a single visible meniscus, its position was recorded using an ocular micrometer scale. The RPM was then increased in steps of 0.5 MPa from -0.1 to -6.1 MPa. As the meniscus moved radially toward the axis of rotation due to water being extracted from the stem, its new position was recorded after each step. The menisci generally stabilized within 10–30 min, with a small decline in extracted water volume observed, which indicated residual evaporation. At this point, the meniscus position was recorded and rpm increased. Some EC measurements were also done with a ChinaTron centrifuge (H2100R, Cence, China) equipped with a digital camera (Scout Sc640gm, Basler AG, Germany), which enabled image acquisition to analyze menisci positions with a C-mount lens (HF16 HA-1B, Fujinon Corp., Japan), a standardized lens mount commonly used to couple industrial cameras with optical lenses. The respective software recorded RPM and provided the pixel position of the meniscus vs. time and saved all relevant data to a text file on a hard disk. Preliminary tests confirmed that both centrifuges yielded consistent results, as they utilize identical rotors and water extraction principles, ensuring that any bias arising from the use of different equipment was avoided. In the R package “fitplc” (Duursma and Choat 2017), the Weibull function was used to fit the ECs (LV versus Ψ_x), and Ψ_{50} was subsequently determined (Peng et al. 2019; Du et al. 2019). The k_s measurement was not possible in ECs measurements.

Drought-induced leaf damage

Drought-induced leaf damage was analyzed on leaves or needles using a modified electrolyte leakage method based on Morin et al. (2007) and Ganthaler and Mayr (2015). Saplings were well watered the evening before the measurement. Leaves or needles were harvested in the morning and dried on the bench to different Ψ_{leaf} between 0 and -7 MPa. Once the desired Ψ_{leaf} was reached, 15 pieces of segments (5 mm in length) were cut from the needle pair of each bundle using with a razor blade or 10 circular segments (5 mm

in diameter) per leaf were cut using a cork borer, and put into a 2 ml Eppendorf safe-lock tube filled with 1.5 ml distilled water. Tubes were placed in a shaker with thermoregulation (Thermomixer C, Eppendorf, Germany) and shaken for 1 h at 25 °C. The electric conductivity of the solution (C1) was measured with a conductivity meter (LAQUAtwin EC-22, Horiba, Japan). Then the tubes were again shaken for 30 min at 100 °C followed by another 30 min-shaking at 25 °C before the electric conductivity of the solution (C2) was analyzed. The relative electrolyte leakage (REL) was then calculated as:

$$REL = \frac{C1}{C2}$$

Percentage cellular lysis of each sample (up to 40 samples per fertilization treatment and per species) was calculated as:

$$\text{cellular lysis (\%)} = \frac{REL - REL_c}{REL_m - REL_c} \times 100$$

where REL_c referred to samples between 0 and −0.5 MPa (i.e., control samples at (nearly) full saturation), and REL_m referred to maximum values, calculated from samples dried on the bench for 2 (*F. sylvatica*, *Q. robur*) or 7 (*P. sylvestris*) days. Cellular lysis data was plotted against the corresponding Ψ_x to generate electrolyte leakage curves, and Ψ_{EL50} value (the Ψ_{leaf} at which 50% cellular lysis occurred) was calculated using the R package “fitplc” (Duursma and Choat 2017), as applied to xylem vulnerability curves. Ψ_{EL50} value was used as a proxy for the short-term drought intensity causing substantial leaf damage.

Gas exchange

Net photosynthesis and stomatal conductance

Three to five saplings per fertilization treatment and per species were randomly selected to measure maximum net photosynthesis (P_n) and stomatal conductance (g_s). Measurements were conducted on sunny days at 12:00–15:00, and saplings were well watered the evening before. Two needles (*P. sylvestris*) or a fully expanded leaf (*F. sylvatica* and *Q. robur*) were attached to a gas exchange system Li-6400 (LI-COR Environmental GmbH, US, 2021 and 2022) or Li-6800 (LI-COR Environmental GmbH, US, 2023). Preliminary tests between the two systems confirmed consistent results, and both instruments utilize the same infrared gas analyzer (IRGA) and calculation models, ensuring that any bias arising from the use of different equipment was avoided. The measuring parameters were set to ambient

temperature, humidity and CO₂ concentration, and a flow of 500 $\mu\text{mol s}^{-1}$. Preliminary tests were carried out to determine the light saturation point for each species. These tests were conducted on several saplings of different fertilization treatments within each species, and showed that fertilization did not alter the shape of light response curves. Based on these tests, photosynthetically active radiation (PAR) was set to 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *F. sylvatica* and *P. sylvestris*, and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *Q. robur*. Data was logged when P_n and g_s were stable (after ca. 3 min).

Stomatal density and pore length

Stomatal density and pore length were analyzed on *F. sylvatica* and *Q. robur*. Five leaves were randomly selected from each fertilization treatment and per species. Transparent nail varnish (Essie Cosmetics, Ltd.) was applied to the abaxial side of leaves, and once dried, it was peeled off using adhesive tape, placed on a microscope slide, and photographed under 40 X magnification with a light microscope (Olympus BX 41, System Microscope, Austria) interfaced with a digital microscope camera (ProgRes CT3, Jenoptik, Germany). An automated, high-throughput tool (StoManager1 v.1.0.0, Wang et al. 2024) was used to analyze the stomatal density (SD, number/mm²) and the pore length (l, μm). These measurements were not conducted on *P. sylvestris*, as the narrow and recessed stomatal pores of conifer needles hinder accurate analysis using this method.

Growth

Sapling height and stem diameter, biomass, root: shoot

The sapling height was determined with a measuring tape, and the stem diameter (1 cm above soil) was measured with a caliper. At the end of each measurement campaign, saplings were separated into roots, stems and leaves. The material was oven dried at 80 °C for 48 h and then weighed with a balance (0.01 g accuracy, ED4202S, Sartorius AG, Germany) to obtain biomass of different sapling parts and calculate the ratio of root: shoot.

Specific leaf area

Two bundles of needles or two leaves were harvested from the distal part of five sapling per fertilization treatment and per species. The needles or leaves were photographed with a scale, then the leaf area was measured with Image J (Fuji, 2.15.1). After oven-drying at 80 °C for 48 h, the specific leaf area (SLA) was calculated as dry weight divided by leaf area.

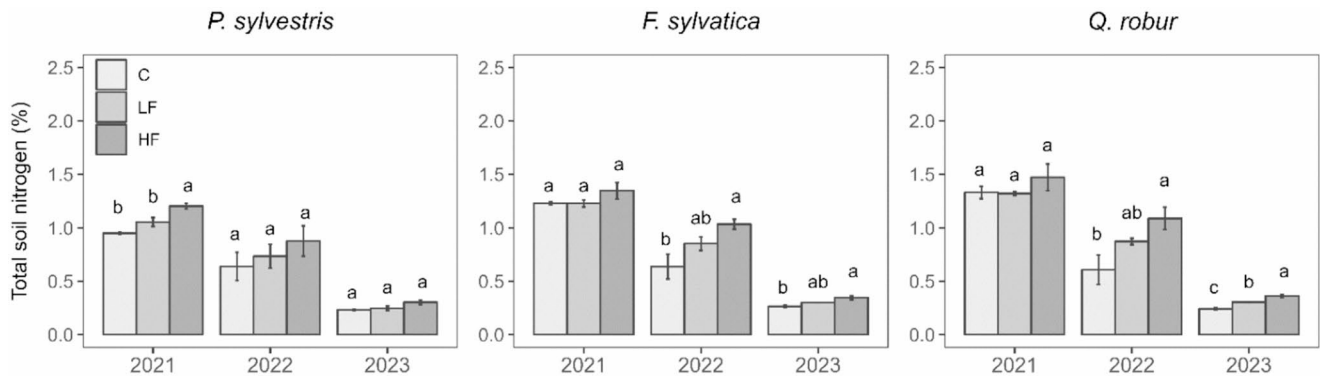


Fig. 1 Percentage of total nitrogen content in the soil of control (C), low (LF) and high (HF) fertilization treatments. Mean $\pm$ SE. Different letters indicate significant differences among fertilization treatments within the same year ($p < 0.05$, $n = 3$ per treatment and per species)

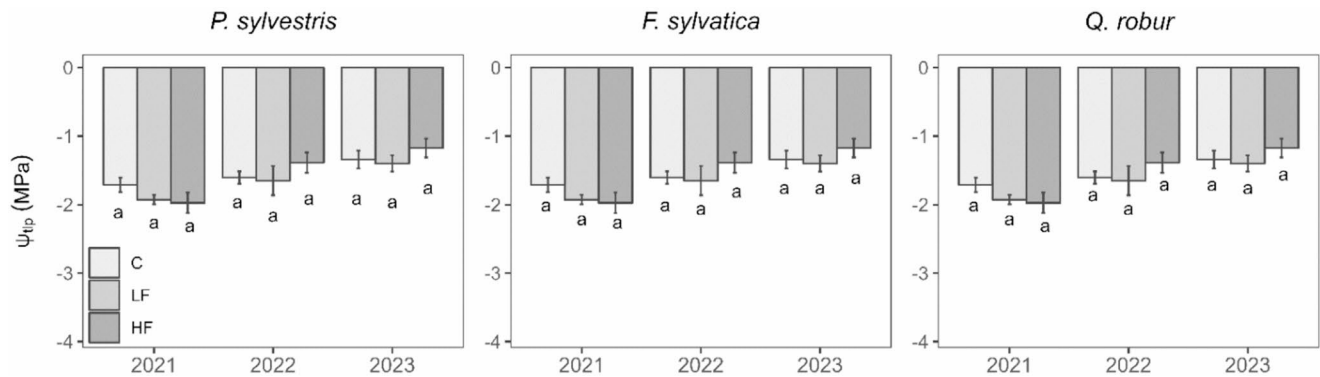


Fig. 2 Turgor loss point (Ψ_{tlp}) in twigs (*P. sylvestris*) and leaves (*F. sylvatica*, *Q. robur*) over three years of control (C), low (LF) and high (HF) fertilization saplings. Mean $\pm$ SE. Different letters indicate significant

ifferences among fertilization treatments within the same year ($p < 0.05$, $n = 5$ per treatment and per species)

Statistical analysis

All analyses were conducted in R version 4.2.1 (R Development Core Team, 2022). Differences between fertilization treatments of each species and year were tested with one-way ANOVA followed by pairwise Tukey HSD test. Model assumptions were checked by testing the normality of ANOVA residuals (Shapiro-Wilk test) and homoscedasticity of variances (Levene's test). In addition, to test whether species differed in their response to fertilization (i.e., species $\times$ fertilization), fertilization effects were expressed as log-response ratios (lnRR) relative to the control mean within each species and year. This standardization was performed to allow for a direct comparison of fertilization sensitivity among species. The lnRR values were compared among species using ANOVA ($p < 0.05$). All tests were performed at a probability level of 5%, and data is presented as mean $\pm$ SE.

Results

Soil nitrogen content

The total nitrogen content (%) in the soil showed a consistent trend across all three species over the study period, ranging from approximately 0.3 to 1.5% (Fig. 1). Higher fertilization levels corresponded to increased nitrogen contents, with the highest values observed in the HF, followed by the LF and C. Within each fertilization level, nitrogen content decreased over time. However, the magnitude of the fertilization response did not differ among species in any study year ($p > 0.19$, Table S1).

Hydraulic measurements

Pressure-volume curves

Across the three years, Ψ_{tlp} values showed no significant changes due to fertilization in any of the three species studied (Fig. 2). In the final year (2023), Ψ_{tlp} in all species showed least negative values in the HF, though these

differences remained statistically insignificant. *P. sylvestris* and *F. sylvatica* ranged between -2.0 to -1.5 MPa and -1.5 to -1.1 MPa, respectively, showing minimal variation across fertilization treatments each year. *Q. robur*, in contrast, showed more variable Ψ_{tlp} values between -2.0 to -1.0 MPa but with no significant differences across treatments. InRR analysis likewise revealed no inter-species differences in fertilization response in any year ($p > 0.228$, Table S1).

Embolism resistance and stem-specific conductivity

The embolism resistance, as indicated by Ψ_{50} values, showed species-specific responses across years and

fertilization treatments (Fig. 3). The Ψ_{50} of *P. sylvestris* exhibited an overall decrease over time but was around -3 MPa across all fertilization treatments in 2021 and 2022. In 2023, the Ψ_{50} of the C reached -3.56 ± 0.03 MPa, which was more negative than that of the LF and HF (around -3.37 MPa). In *F. sylvatica*, Ψ_{50} showed an overall decrease across the years as well, with most negative value was found in C (-3.37 ± 0.11 MPa) compared to LF and HF (-3.18 ± 0.10 MPa and -3.17 ± 0.05 MPa) in 2023. But no significant differences between fertilization treatments were found in any given year. In *Q. robur*, Ψ_{50} was also approx. -3 MPa across fertilization treatments without major changes over years. Among species, differences in fertilization response were minor in 2021 ($p = 0.426$, Table S1),

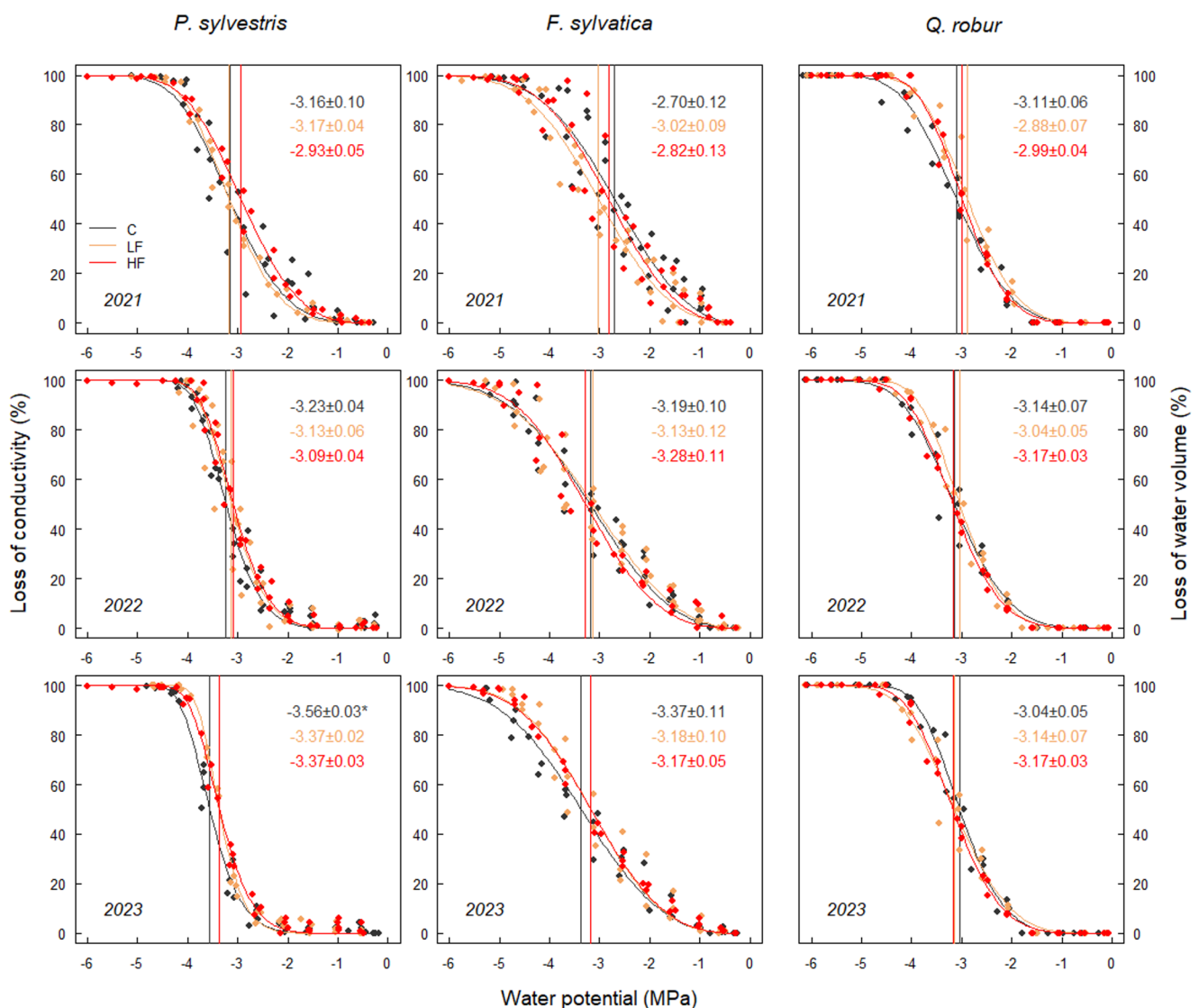


Fig. 3 Vulnerability curves of *P. sylvestris* and *F. sylvatica*, and extraction curves of *Q. robur* over three years of control (C, grey), low (LF, yellow) and high (HF, red) fertilization treatments ($n=4-6$ per treatment and per species). Embolism resistance, indicated by Ψ_{50} (the

xylem pressure at which 50% loss of conductivity or water volume occurs), is indicated by vertical lines, and Ψ_{50} values (mean $\pm$ SE) are also given in the panels. Asterisks (*) indicate significant differences from the other two Ψ_{50} values of the plot ($p < 0.05$)

and small ($|\ln RR| < 0.06$) but significant in 2022 ($p=0.016$, Table S1) and 2023 ($p=0.004$, Table S1).

k_s remained stable across fertilization levels and over three years in *P. sylvestris*, averaging approx. $6.0 \text{ E-}04 \text{ kg MPa}^{-1} \text{ m}^{-1}$ (Fig. S1). In *F. sylvatica*, significant differences were observed in 2023, when the LF ($5.99 \pm 0.62 \text{ E-}04 \text{ kg MPa}^{-1} \text{ m}^{-1}$) and HF ($6.52 \pm 0.14 \text{ E-}04 \text{ kg MPa}^{-1} \text{ m}^{-1}$) exhibited higher k_s compared to the C ($3.02 \pm 0.77 \text{ E-}04 \text{ kg MPa}^{-1} \text{ m}^{-1}$). But no significant differences were detected between the LF and HF. Consistently, inter-species differences in fertilization response were significant in 2023 ($p=0.001$, Table S1), driven primarily by the strong increase in k_s in *F. sylvatica*.

Drought-induced leaf damage

The drought-induced leaf damage, as indicated by Ψ_{EL50} values, also showed species-specific responses across years and fertilization treatments (Fig. 4). In *P. sylvestris*, Ψ_{EL50} values showed an overall decrease across all fertilization treatments over the three years, from approx. -3.5 MPa in 2021 to -4.2 MPa in 2023. On average, Ψ_{EL50} was about 0.4 MPa more negative in the C compared to the LF and HF over the study period; however, these differences were not statistically significant. In *F. sylvatica*, Ψ_{EL50} values were highly variable in 2021 but stabilized at around -4 MPa in 2022 and 2023, with no consistent differences among fertilization treatments. In *Q. robur*, Ψ_{EL50} values across

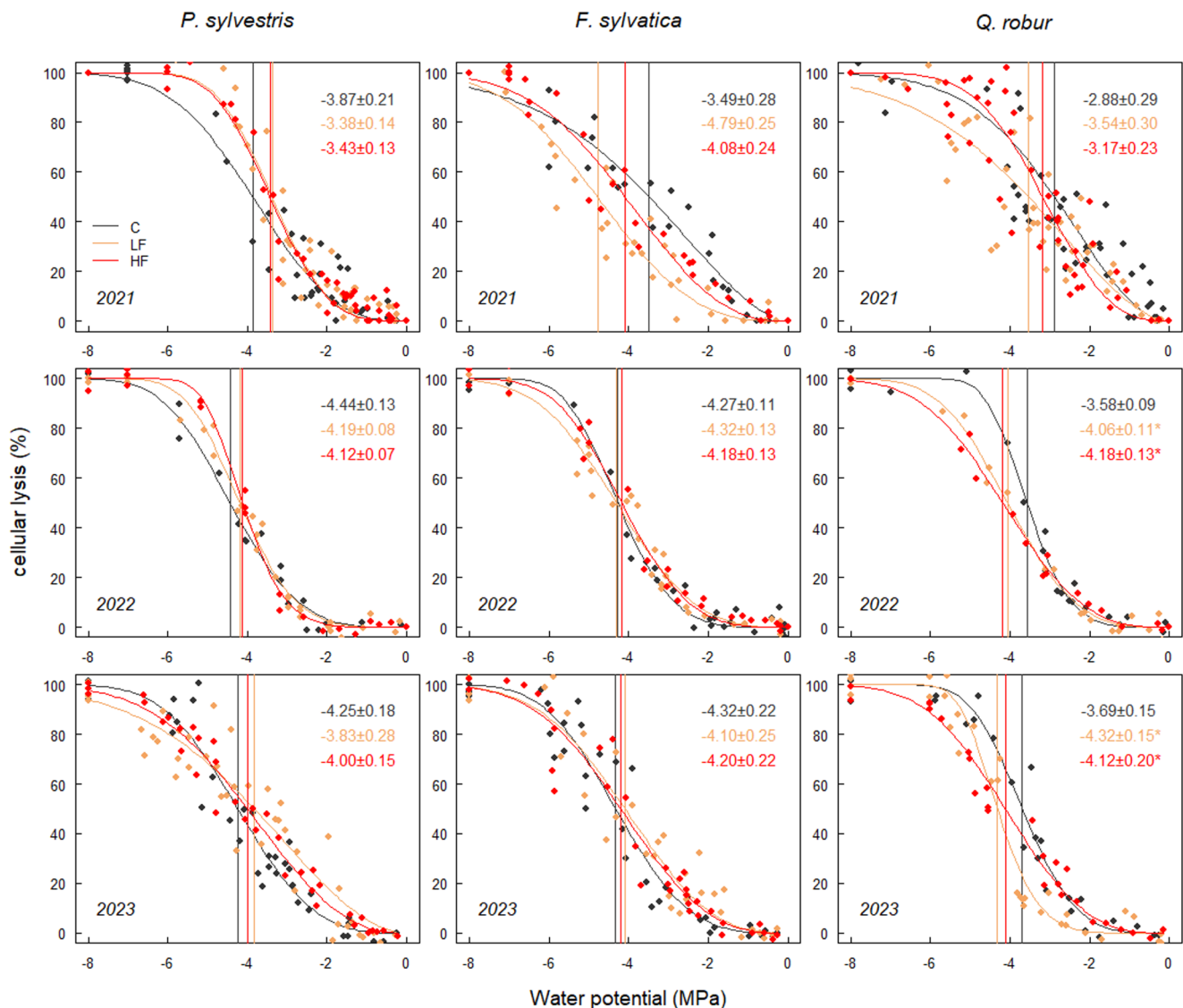


Fig. 4 Percentage cellular lysis versus water potential of leaves of *P. sylvestris*, *F. sylvatica* and *Q. robur* over three years of control (C, grey), low (LF, yellow) and high (HF, red) fertilization treatments (up to 40 samples per fertilization group and per species). Vertical lines

indicate the water potential at 50% cellular lysis (Ψ_{EL50}), and values (mean $\pm$ SE) are also given in the panels. Asterisks (*) indicate significant differences from the other two Ψ_{EL50} values of the plot ($p < 0.05$)

all fertilization treatments also decreased over time, from approx. -3 MPa in 2021 to -4 MPa in 2023. Across all years in *Q. robur*, values in the C were consistently less negative than those in LF and HF, with this trend being more pronounced in 2022 and 2023. For example, in 2022, Ψ_{EL50} was -3.58 ± 0.09 MPa in C compared to -4.18 ± 0.13 MPa in HF, and in 2023, -3.69 ± 0.15 MPa in C compared to -4.12 ± 0.20 MPa in HF. lnRR analysis revealed no inter-species differences in fertilization response in any year ($p > 0.059$, Table S1).

Gas exchange

Net photosynthesis and stomatal conductance

In all fertilization treatments across three species, both P_n and g_s increased over the years, with a more pronounced rise in fertilized treatments, but no significant differences between LF and HF (Fig. 5). In 2023, P_n reached 19.1 ± 2.64 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and g_s was 0.27 ± 0.02 $\text{mol m}^{-2} \text{s}^{-1}$ in the HF of *P. sylvestris*, as opposed to C values of $P_n = 11.6 \pm 1.30$ $\mu\text{mol m}^{-2} \text{s}^{-1}$ and $g_s = 0.16 \pm 0.01$ $\text{mol m}^{-2} \text{s}^{-1}$. In *F. sylvatica*, in 2023, P_n reached 15.7 ± 0.96 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and g_s was 0.32 ± 0.01 $\text{mol m}^{-2} \text{s}^{-1}$ in the HF, compared to the C values of $P_n = 10.2 \pm 0.10$ $\mu\text{mol m}^{-2} \text{s}^{-1}$ and $g_s = 0.22 \pm 0.01$ $\text{mol m}^{-2} \text{s}^{-1}$.

$\text{m}^{-2} \text{s}^{-1}$. In *Q. robur*, P_n in the HF increased to 14.8 ± 1.11 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and g_s to 0.24 ± 0.05 $\text{mol m}^{-2} \text{s}^{-1}$, while the C treatment had values of $P_n = 9.2 \pm 0.69$ $\mu\text{mol m}^{-2} \text{s}^{-1}$ and $g_s = 0.16 \pm 0.01$ $\text{mol m}^{-2} \text{s}^{-1}$ in 2023. Among species, differences in fertilization response were detected for P_n in 2021 ($p = 0.028$) and 2022 ($p < 0.001$), whereas for g_s such differences were detected only in 2022 ($p = 0.002$, Table S1).

Stomatal density and pore length

F. sylvatica generally exhibited smaller stomatal density and pore length than *Q. robur*, with stomatal density in both species affected by fertilization (Fig. S2). In *F. sylvatica*, stomatal density under fertilization decreased from an average of 300 per mm^2 to around 200 per mm^2 in both 2022 and 2023. Stomatal pore length showed neither relevant change over years nor difference between treatments and was approximately 11 μm . In *Q. robur*, stomatal density tended to increase in response to fertilization, with the most notable effect seen in 2023, where density rose from 359 ± 16.5 per mm^2 in C to 449 ± 9.3 per mm^2 in HF. Stomatal pore length showed a slight upward trend over years, but no consistent differences between fertilization treatments. Accordingly, inter-species differences in fertilization response for stomatal density were significant in 2022 ($p = 0.028$) and 2023

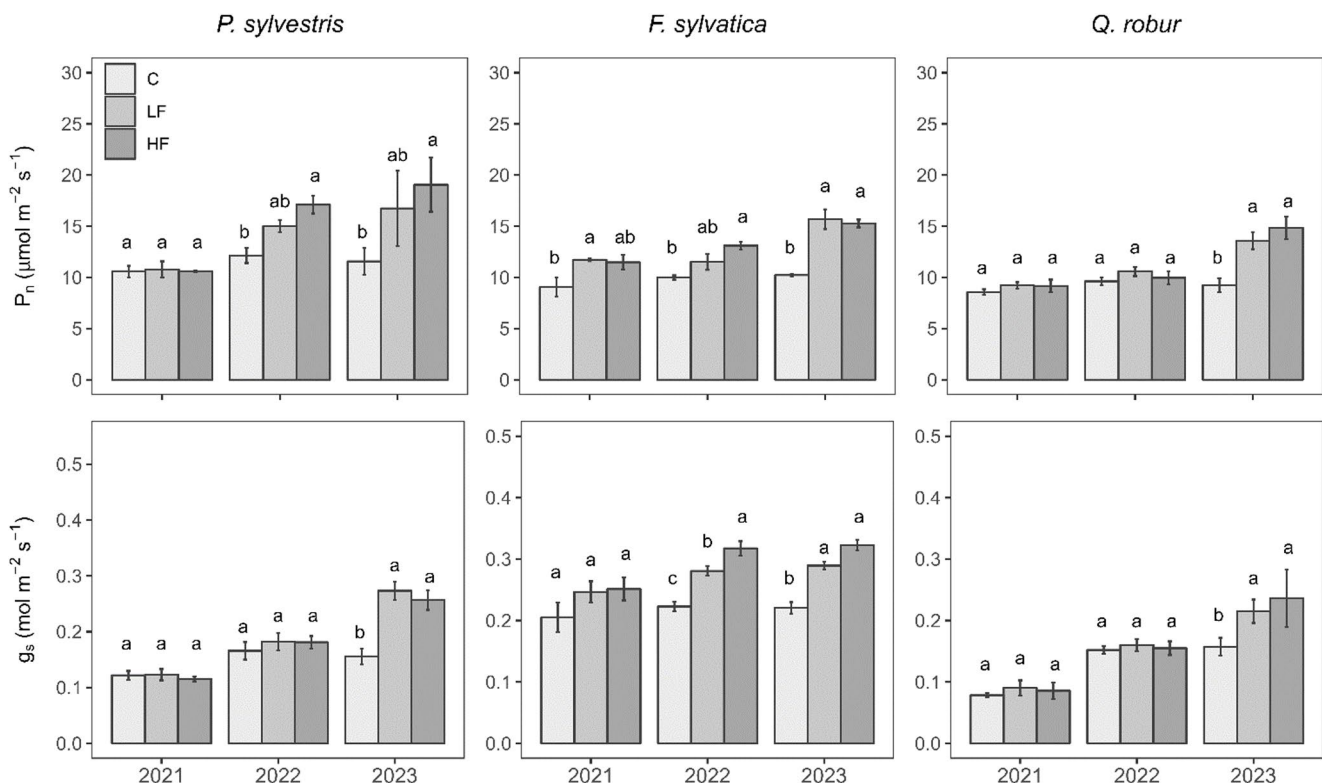


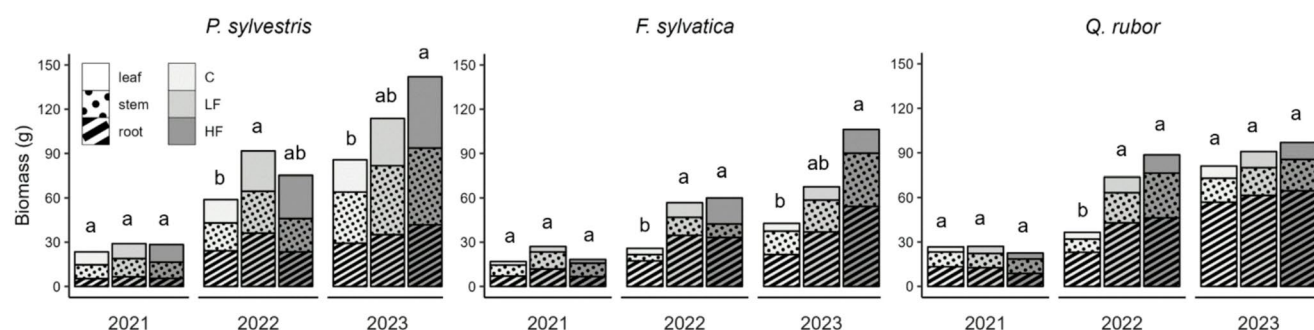
Fig. 5 Net photosynthesis (P_n) and stomatal conductance (g_s) measured over three years of control (C), low (LF) and high (HF) fertilization treatments. Mean $\pm$ SE. Different letters indicate significant differences

among fertilization treatments within the same year ($p < 0.05$, $n = 3-5$ per treatment and per species)

Table 1 Sapling height and diameter, specific leaf area (SLA) and root: shoot ratio under control (C), low (LF) and high (HF) fertilization treatments

Parameters	Fertilization	<i>P. sylvestris</i>			<i>F. sylvatica</i>			<i>Q. robur</i>		
		2021	2022	2023	2021	2022	2023	2021	2022	2023
Height cm (<i>n</i> =8)	C	50.4±3.3a	76.2±1.4a	93.8±2.2a	36.8±1.8a	60.5±3.8a	68.2±2.9a	52.0±5.2a	66.9±4.0b	78.1±3.0b
	LF	56.3±1.5a	74.8±1.9a	97.5±2.2a	42.6±3.1a	57.0±1.8a	64.5±0.5a	60.9±7.3a	85.6±1.5a	105.0±1.0a
	HF	52.4±3.3a	68.7±3.0a	97.0±2.6a	41.2±2.7a	61.9±3.9a	69.5±1.4a	60.8±6.9a	90.0±3.7a	105.0±3.9a
Diameter cm (<i>n</i> =8)	C	0.90±0.03a	1.19±0.04a	1.48±0.05a	0.92±0.03a	1.13±0.04a	1.21±0.03a	0.77±0.05a	0.87±0.03a	0.98±0.05b
	LF	0.89±0.04a	1.24±0.04a	1.59±0.03a	0.96±0.03a	1.05±0.04a	1.18±0.04a	0.87±0.03a	0.93±0.03a	1.14±0.06a
	HF	0.88±0.03a	1.24±0.03a	1.55±0.04a	0.99±0.08a	1.13±0.03a	1.24±0.02a	0.85±0.05a	0.96±0.04a	1.13±0.07a
SLA cm ² /g (<i>n</i> =10)	C	42±2.6b	45±5.9b	44±3.1b	188±5.8a	183±7.7b	190±10.4b	140±2.8a	156±11.0a	145±9.4b
	LF	55±3.4ab	61±2.5a	64±2.9a	183±6.1a	215±3.2a	281±9.1a	153±11.3a	189±15.3a	207±9.3a
	HF	59±4.9a	65±3.7a	64±4.4a	183±2.4a	210±6.2a	284±6.4a	132±9.7a	160±4.2a	173±10.7ab
root: shoot g/g (<i>n</i> =8)	C	0.29±0.02a	0.68±0.08a	0.53±0.07a	0.74±0.05ab	2.02±0.19a	1.19±0.07a	1.06±0.11a	1.60±0.19a	2.38±0.25a
	LF	0.29±0.02a	0.66±0.04a	0.46±0.03ab	0.83±0.06a	1.58±0.21b	1.03±0.06a	0.91±0.08ab	1.53±0.21ab	2.10±0.05ab
	HF	0.25±0.03a	0.46±0.07b	0.40±0.02b	0.57±0.04b	1.21±0.08c	1.07±0.05a	0.66±0.07b	1.13±0.12b	1.98±0.05b

Mean±SE. Different letters indicate significant differences among fertilization treatments within the same year ($p<0.05$)

**Fig. 6** Biomass distribution among leaves, stems, and roots in *P. sylvestris*, *F. sylvatica*, and *Q. robur* over three years of control (C), low (LF) and high (HF) fertilization treatments ($n=8$ per treatment and

per species). Different letters indicate significant differences in total biomass among fertilization treatments within the same year ($p<0.05$, $n=3-5$ per treatment and per species)

($p=0.023$), whereas no species effect was detected for stomatal pore length in any year ($p>0.13$, Table S1).

Growth

Sapling dimensions and biomass data reflect the growth of the saplings during the experiment (Table 1; Fig. 6). From 2021 to 2023, *P. sylvestris* exhibited an overall increase of ca. 40 cm in height and ca. 0.5 cm in diameter, while *F. sylvatica* showed an overall ca. 30 cm and ca. 0.3 cm height and diameter increment. Neither in *P. sylvestris* nor in *F. sylvatica* any effect of fertilization was observed. In *Q. robur*, fertilized saplings showed higher increase in height (ca. 40 cm) and diameter (ca. 0.3 cm) increment compared to C (ca. 20 cm in height and ca. 0.2 cm in diameter increment), but no differences between fertilization treatments. Among species, fertilization responses differed for height in 2022 ($p<0.001$) and 2023 ($p<0.001$), whereas no species effect for diameter in any year ($p>0.17$, Table S1).

Fertilized saplings exhibited higher SLA in all three species, with this effect being more pronounced in 2023. In *P. sylvestris*, SLA was 64 ± 4.4 cm² g⁻¹ in HF compared to 44 ± 3.1 cm² g⁻¹ in C. In *F. sylvatica*, SLA reached 284 ± 6.4 cm² g⁻¹ in HF versus 190 ± 10.4 cm² g⁻¹ in C. In *Q. robur*, SLA was 207 ± 9.3 cm² g⁻¹ in LF compared to 145 ± 9.4 cm² g⁻¹ in C. Inter-species differences in fertilization response were significant in any year ($p<0.038$, Table S1).

All three species exhibited an increase in total biomass over the years, with a greater increment observed in fertilized treatments (Fig. 6). In *P. sylvestris*, the largest increase occurred in 2023, with total biomass reaching 141.98 ± 14.22 g in HF compared to 85.71 ± 7.78 g in C. Similarly, in *F. sylvatica*, the highest biomass was observed in 2023, with 106.31 ± 20.82 g in HF versus 42.79 ± 12.08 g in C. In *Q. robur*, total biomass also peaked in 2023 (ca. 90 g), though no differences were found between treatments.

No inter-species differences in fertilization response were detected in any year ($p > 0.064$, Table S1).

Fertilized saplings generally allocated more biomass to aboveground organs than to roots across all three species. In *P. sylvestris*, the root: shoot ratio was lower in the HF treatment compared to the C in both 2022 and 2023, with values of 0.46 ± 0.07 in HF versus 0.68 ± 0.08 in C in 2022, and 0.40 ± 0.02 in HF versus 0.53 ± 0.07 in C in 2023. In *F. sylvatica*, significant differences in root: shoot ratio between treatments were observed in 2021 and 2022. In 2021, the ratio was 0.57 ± 0.04 in HF and 0.74 ± 0.05 in C, while in 2022, it was 1.21 ± 0.08 in HF and 2.02 ± 0.19 in C. In *Q. robur*, treatment effects on root: shoot ratio were significant in each year, with the most pronounced difference observed in 2023: 1.98 ± 0.05 in HF compared to 2.38 ± 0.25 in C. However, inter-species differences in fertilization response were not detected in any year ($p > 0.122$, Table S1).

Discussion

Fertilization of *P. sylvestris*, *F. sylvatica*, and *Q. robur* saplings influenced their hydraulics, gas exchange and growth, with these effects intensifying over study years. However, the observed responses were species-specific and varied across studied parameters. This species specificity was supported by lnRR analyses, which revealed inter-species differences for several growth and gas exchange parameters, and minor or inconsistent differences for hydraulic parameters.

The experiment was based on annual fertilization with standard fertilizers as used in tree nurseries. Nitrogen analyses revealed successful fertilization, though the observed decrease in soil nitrogen over the experimental period (Fig. 1) indicated a year-to-year increase of nutrient use due to the growth of saplings. Despite this overall decline, the gradient among fertilization treatments clearly remained, with HF consistently showing the highest nitrogen contents. In a tree nursery, these saplings would have been used as afforestation material in the following season and thus their performance with respect to hydraulics, gas exchange and growth is relevant from a practical viewpoint.

Hydraulics

Nutrient availability or imbalances can impact Ψ_{tlp} by altering osmotic potential and cell wall properties. Potassium, for instance, plays a key role in osmoregulation, and its deficiency can impair turgor maintenance, potentially leading to changes in Ψ_{tlp} (Wang et al. 2013). However, in our study, Ψ_{tlp} showed only weak or no response across fertilization levels (Fig. 2). The limited response may reflect the fact

that, though Ψ_{tlp} exhibits phenotypic plasticity in response to environmental drivers such as drought, light, or temperature (Bartlett et al. 2012; Zhu et al. 2018), its adjustment under non-limiting water conditions is modest. Since the studied species were grown under well-watered conditions, fertilization alone may not have provided a sufficiently strong stimulus to induce marked shifts in Ψ_{tlp} .

Contrary to our hypothesis, fertilization also had no substantial effect on embolism resistance (Fig. 3). Previous research has reported both increased embolism resistance following fertilization (e.g., Faustino et al. 2013; Goldstein et al. 2013) and reduced resistance (e.g., Zhang et al. 2018; Beikircher et al. 2019). However, even in studies where fertilization led to statistically significant changes in Ψ_{50} , such as in Scots pine (Martínez-Vilalta et al. 2009) and Mongolian Scots pine (Fang et al. 2018), the absolute differences between fertilized and control treatments were relatively small. Therefore, the effect of fertilization on embolism resistance may be species-specific and mediated by changes in xylem anatomy. Fertilization can promote wider conduits and higher hydraulic efficiency, likely due to adjustments in pit membrane structure, vessel grouping or wood density, traits known to determine vulnerability to air-seeding (Tyree and Zimmermann 2002; Hacke et al. 2001; Choat et al. 2012). To what extent fertilization modifies these anatomical features probably depends on the developmental stage and the balance between growth stimulation and structural reinforcements. Our study focused on saplings, which may exhibit less plasticity in xylem anatomical and physiological traits relative to mature trees, as few tree rings have to ensure the entire water transport in young plants. In larger trees, older rings may lose conductivity after drought or frost, and water transport relies mainly on younger rings. Furthermore, mature trees possess a larger sapwood that may allow for greater anatomical flexibility, whereas age-related differences in the proportion of active xylem and in secondary wall maturation could influence the capacity for anatomical adjustment to fertilization in saplings. To evaluate whether fertilization affects the coordination of drought resistance across tissues, we also examined drought-induced leaf damage, which showed high variability across species and years (Fig. 4), but no consistent fertilization effects in *P. sylvestris* and *Fagus sylvatica* over studied years. In 2022 and 2023, fertilized *Q. robur* saplings exhibited a Ψ_{EL50} approximately 0.5 MPa more negative than controls, while no differences were observed in 2021. Given the observed limited plasticity in both stem embolism resistance and drought-induced leaf damage, it remains to be determined whether prolonged fertilization and/or studies on larger trees would reveal stronger effects in these traits.

Consistent with previous findings that fertilization can significantly increase k_s by modifying xylem anatomical

characteristics (Hacke et al. 2001; Bucci et al. 2006; Villagra et al. 2013; Borghetti et al. 2017; Camarero et al. 2017) or through ion-mediated upregulation of xylem hydraulics (Oddo et al. 2011), we observed up to a 74.3% higher k_s in HF compared to C saplings in *F. sylvatica* in 2023 (Fig. S1). However, in previous years, no clear fertilization effect was observed and also *P. sylvestris* showed no significant differences in k_s between fertilized and C saplings and thus positive effects of fertilization on k_s are not universal (also see Clearwater and Meinzer 2001; Villagra et al. 2013; Faustino et al. 2015; Medeiros et al. 2016; Wang et al. 2016). A study on *P. sylvestris* var. *mongolica* suggested that the lack of k_s response to fertilization may be due to its effect on hydraulic architecture primarily through altering the allometric relationship between water-demanding and water-delivering organs. This adjustment plays a crucial role in maintaining stable or even increased hydraulic capacity per unit leaf area under drier conditions (Bhaskar et al. 2007; Martínez-Vilalta et al. 2009).

Gas exchange

The fertilization enhanced P_n and g_s across all species (Fig. 5), which was in line with previous findings in temperate forest species (Liu and Dickmann 2006; Samuelson et al. 2008). Nitrogen was reported to enhance photosynthetic capacity by increasing investment in photosynthetic enzymes and chlorophyll which can support higher photosynthetic rates (Evans 1989). In *P. sylvestris*, the fertilization-induced increase in g_s was observed in 2023, while P_n of fertilized saplings was already higher in 2022. Such delayed g_s responses have been reported in conifers under fertilization, reflecting physiological trade-offs between maximizing carbon gain and minimizing water loss (Luis et al. 2010). By contrast, *F. sylvatica* exhibited a concurrent increase in both P_n and g_s starting in the first year, indicative of a faster response at the level of stomata that may enhance carbon assimilation under favorable conditions. In *Q. robur*, fertilization effects on P_n and g_s were only visible in 2023. In this species, higher g_s in LF and HF saplings corresponded with higher stomatal density and pore length (Fig. S2). In contrast, stomatal pore length was only slightly higher in *F. sylvatica* and the density was even lower, indicating that increased g_s was also due to other factors (stomatal regulation, leaf architecture etc.) in the latter species.

Overall, differences between the LF and HF treatments in P_n and g_s were small suggesting a threshold beyond which additional nutrients do not further enhance gas exchange, as noted in similar studies (Reich et al. 2006). This plateau effect may reflect either nutrient saturation or physiological limitations in photosynthetic capacity and stomatal regulation. Accordingly, the lack of significant differences in

stomatal pore length between fertilization treatments indicates pore dimensions to be less plastic than stomata density and maybe genetically constrained (Pantin et al. 2012). However, the slight increase in pore length across years within fertilization treatments could indicate a subtle adjustment to support higher gas exchange rates over time.

Growth

Growth showed pronounced fertilization responses in the study species, with increases in sapling height and diameter, SLA and total biomass (Table 1; Fig. 6). This in line with findings from other studies on sapling responses to fertilization (e.g., Wright et al. 2011; Bergh and Hedwall, 2013; Guillemot et al. 2021), although species-specific responses were evident in this study. *P. sylvestris* and *F. sylvatica* exhibited minimal differences in sapling height and diameter between fertilized and unfertilized treatments. Löf (2000) found that beech saplings often experience a prolonged establishment phase and slower initial growth compared to oak, which may limit their structural expansion to increased nutrient availability. Similarly, the response in *P. sylvestris* likely reflects a strategy where nutrient addition is prioritized for foliage development and lateral branching, which was supported by the increase in total biomass, rather than immediate structural growth. In contrast, fertilized *Q. robur* exhibited a pronounced response, particularly in the third year. This agrees with previous studies on oak saplings (Löf 2000; Maillard et al. 2001; Guillemot et al. 2021) that report significant increases in stem growth and biomass following nutrient addition. This suggests that *Q. robur* may have a greater ability to utilize nutrients for radial and height growth, potentially due to enhanced root development (Fig. 6) and higher hydraulic efficiency compared to the other studied species (Tyree and Zimmermann 2002; Choat et al. 2012).

The shift in biomass allocation toward aboveground components is a common response to increased nutrient availability, as plants reduce investment in root growth when nutrient acquisition becomes less limiting (Faustino et al. 2013, 2015; Koç et al. 2022). However, the declining root:shoot ratios observed in all species, particularly in fertilized treatments (Table 1), raise concerns about potential decreasing water uptake capacity and thus compromising drought tolerance (Hartmann et al. 2018; McCarthy et al. 2006). There is experimental evidence that high nitrogen supplies increased drought sensitivity of European beech mainly due to reduced root biomass (Dziedek et al. 2016).

Further explaining the biomass gains, fertilized *P. sylvestris* and *F. sylvatica* showed an increase in SLA (Table 1), a strategy used to optimize light capture when nutrients are abundant (Bergh et al. 2005; Meier and Leuschner 2008;

Poorter et al. 2009). In *P. sylvestris*, the early SLA response (2021, Table 1) suggests a rapid response to fertilization (Fig. 5). In *F. sylvatica*, the substantial SLA increase observed in 2022 and 2023 may reflect a slower nutrient uptake or redistribution process. Conversely, the relatively minor SLA response in *Q. robur* aligns with findings by Catoni et al. (2015), and may reflect a greater allocation of resources to woody structure biomass rather than leaf area expansion (Fig. 6), explaining why its height and diameter increased more significantly than the other species.

Conclusion

In this study, we found that (i) fertilization increased and changed the biomass allocation in all species. This indicates that fertilization was sufficient and positively affected plant growth as expected (and aimed at in nurseries). (ii) Accordingly, also P_n and g_s were increased, maybe because the photosynthetic capacity was enhanced (less source limitation) or because there was more carbon demand (less sink limitation) when other main nutrients were better available. (iii) No consistent changes in hydraulics were observed. Changes in Ψ_{tlp} , Ψ_{50} and Ψ_{EL50} overall were minor and fertilization thus did not lead to changes (neither improved nor decreased as indicated in some studies) in drought resistance of saplings. Increased growth of xylem tissues in stems was obviously sufficient to balance water supply for increased SLA and gas exchange rates so that adjustments to lower water potentials were not required. These responses were species-specific, indicating varying resource-use strategies among the studied species.

Fertilization therefore produced larger saplings with presumably greater reserve pools without detectable reductions in hydraulic drought resistance. However, whether these advantages translate into improved establishment and performance after transplanting should be confirmed under field conditions. These findings underscore the importance of considering species- and plant age-specific nutrient responses in forest management. Future studies should further explore the long-term impacts of fertilization, particularly under field conditions, where environmental stressors such as drought and competition may amplify the observed effects.

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

Declarations

Conflict of interest There are no conflict of interest for this paper.

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