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Nutrient and Growth Response of *Fagus sylvatica* L. Saplings to Drought Is Modified by Fertilisation

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Abstract: The increased frequency of climate change-induced droughts poses a survival challenge for forest trees, particularly for the common beech (*Fagus sylvatica* L.). Drought conditions adversely affect water supply and nutrient uptake, yet there is limited understanding of the intricate interplay between nutrient availability and drought stress on the physiology, growth, and biomass accumulation in young trees. We aimed to address this knowledge gap by examining the effects of irrigation and fertilisation and their interaction with various parameters in common beech saplings, including foliar and root N, P, and K concentrations; height and diameter increments; and aboveground and belowground biomass production. Our findings revealed that a higher fertilisation dose increased nutrient availability, also partially mitigating immediate drought impacts on foliar N concentrations. Also, higher fertilisation supported the post-drought recovery of foliar phosphorus levels in saplings. Prolonged drought affected nitrogen and potassium foliar concentrations, illustrating the lasting physiological impact of drought on beech trees. While drought-stressed beech saplings exhibited reduced height increment and biomass production, increased nutrient availability positively impacted root collar diameters. These insights have potential implications for forest management practices, afforestation strategies, and our broader understanding of the ecological consequences of climate change on forests.

Keywords: common beech; foliar and root nutrients; biomass; post-drought recovery; prolonged drought effect

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1. Introduction

The recent increase in extreme drought and heatwave events has had a severe impact on many forest ecosystems worldwide [1], and the severity and frequency of such extreme climate events in the near future will be a significant limiting factor for tree growth and survival at least for boreal and temperate areas [2]. The current and projected adverse effects of climate change on the stability, structure and biodiversity of forest ecosystems in Europe present risks to essential ecosystem services and functions such as water protection, biodiversity, carbon sequestration, and timber production [3–6]. Given that common beech (*Fagus sylvatica* L.) is an economically and ecologically important species in Europe, with a total area of beech-dominated forests covering between 14 and 15 million hectares [7], its survival and ability to withstand changing climate conditions are of great importance. Despite its relatively high plasticity [8–10], common beech prefers a temperate climate with mild winters and humid summers [8,11,12], while a pronounced continental (cold/dry) climate restricts its distribution [13–16].

The latest research shows that recent droughts have seriously impacted beech forests [17–19]. Depleted soil moisture conditions during drought can significantly affect tree physiology, affecting tree growth and mortality rates [20,21].

In common beech, drought was found to trigger various physiological processes, including decreased leaf water potential, turgor loss, reduced transpiration, and photosynthesis [14,22–24]. This may lead to an alteration of anatomical characteristics, affecting factors such as xylem sap flow and other physiological performances, ultimately causing a reduction in the rate of photosynthesis, decreasing stomatal conductance [25], and constraining nutrient uptake and assimilation [26,27].

During drought, the reduction of nutrient availability from the soil can promote impairment of the plant's nutritional status and its general functioning [28]; for instance, lower foliar and root phosphorus and potassium concentrations in beech can lower its biomass production [29].

On the other hand, plants that are able to restore their nutrient uptake and allocation efficiently will more successfully re-establish their physiological functions [30,31] following drought. Besides physiological processes, drought significantly affects morphological traits in beech.

Drought-exposed beech saplings exhibit reduced height, stem diameter [32], fine-root biomass and root growth [33–35]. Reduced root growth and function, as well as altered nutrient allocation patterns, can also affect the uptake of essential elements, such as nitrogen and phosphorus [36]. The negative impact of drought is particularly significant for afforestation projects with young beech trees [37], although in some cases beech saplings have successfully adapted to changed climate conditions [38]. The adaptive strategies of beech saplings are very complex, depending on drought severity [3], duration [39] and soil nutrient availability [27], influencing different functional [40] and morphological adaptive traits [41]. Conditions of low and high nutrient availability have different impacts on tree drought resilience. Trees growing in conditions of low nutrient availability could be more drought resistant due to better-developed root systems, reduced aboveground biomass and smaller vessel diameter, reducing cavitation risk [30,42]. However, poor beech nutritional status may hinder drought endurance [28], potentially causing carbon starvation and impeding tree recovery [30,42]. Conversely, high nutrient availability can increase vulnerability to carbon starvation or hydraulic failure during drought, which is associated with higher biomass production and cavitation risk [30,43–45]. On the other hand, an adequate nutrient supply boosts water use efficiency, nutrient uptake, and distribution during drought, leading to quicker post-drought recovery [30].

One of the ways to increase nutrient availability in the soil, and consequently enhance nutrient uptake by plants even under drought conditions is the application of mineral fertilisers [46,47]. The effects of increased nutrient availability on different physiological and morphological traits such as root functioning, gas exchange, growth, and foliar nitrogen were investigated in different tree species [31,48–50], yet there is no general consensus on the possible role of nutrition in alleviating the negative effects of drought in trees.

Therefore, the objective of this study was to investigate the interaction between nutrient availability and drought on nutrition, growth, and biomass accumulation in beech saplings during drought and after drought release. Additionally, the research aimed to explore the connection between nutrient status and the potential for recovery and assess the prolonged effects of drought.

We hypothesised that a higher dose of fertilisation would:

I. Increase nutrient availability, partially mitigating the negative effects of drought on nutrition by maintaining adequate foliar concentrations of nitrogen, phosphorus, and potassium during drought.
II. Alleviate the prolonged effect of drought on the foliar concentrations, growth, and biomass production of beech saplings by maintaining them at the level of regularly watered saplings.

2. Materials and Methods

2.1. Experimental Design and Treatments

The greenhouse experiment was set up in the nursery of the Croatian Forest Research Institute, Jastrebarsko, Croatia (45°40′03″ N, 15°38′26″ E). A total of 1120 one-year-old potted common beech saplings were transplanted into 6 litre square pots in a substrate of 1:3 sand: peat and placed in an open-sided greenhouse equipped with roof-blocking precipitation, an automated drip irrigation system (Irritrol Total Control® with six stations, Irritrol Systems, Riverside, CA, USA), and polyethylene shade nets with a light permeability of 50% (Figure S1).

The experiment was set up in April 2020 as a Latin square with four different treatments: WH—regular watering, high dose of fertiliser, DH—induced drought, high dose of fertiliser, WL—regular watering, low dose of fertiliser, and DL—induced drought, low dose of fertiliser. Each treatment had 8 replicates, with 35 randomly selected pots/saplings per replicate (Figure S2). The timeline of measurements and sampling is shown in Figure 1.

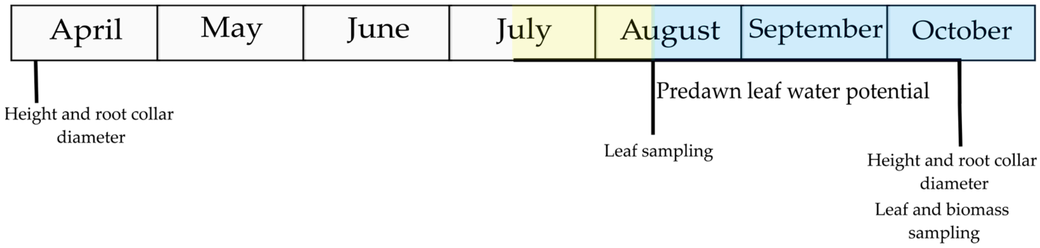

Figure 1. Timeline of measurements and sampling. The yellow square depicts the induced drought period, the blue line depicts the start of rewatering and the blue square depicts the post-drought period.

Controlled-release fertiliser (Osmocote Exact Standard 5–6 M, 8.6% NH_4-N and 6.4% NO_3-N, 9% P, 12% K, 2% MgO, 0.47% Fe, 0.07% Mn, 0.06% Cu, 0.03% Zn, 0.02% B, and 0.02% Mo) was mixed into the substrate in two different doses: 2.0 g/L for low-dose fertilisation and 4.0 g/L treatment for high-dose fertilisation, i.e., low and optimal fertiliser dose for growing potted beech saplings [51]. For the first eight weeks of the experiment, all saplings were well-watered to absorb fertiliser and recover from transplant shock.

The saplings in regularly watered treatments (WH, WL) received 1 L of water once a week throughout the experiment (April–October). The amount of 1 litre of water per week/pot was calculated as one sixth of the mean difference between the weight of six pots filled with substrate and watered to field capacity and the weight of the same pots one week later, which was performed at the beginning of the experiment.

Saplings in the drought treatments (DH, DL) were not watered for 32 days during the induced drought period (14 July–14 August), i.e., until the first visible symptoms of drought stress (wilting leaves) were found on most plants treated by drought.

2.2. Response Parameters

Sapling water status was determined by predawn leaf water potential (Ψ_{pd}) measurements. They were performed weekly from the beginning of the induced drought (Figure 1) on four randomly selected plants from each replicate of each treatment (128 saplings in total), which were excluded from foliar and biomass sampling. Ψ_{pd} was measured on one mature leaf per sapling, utilising a Scholander pressure chamber (Pressure Chamber

Instruments Model 600, PMS Instrument Company, Albany, OR, USA). Samples for the foliar analysis of nitrogen (N), phosphorus (P), and potassium (K) were collected during the induced drought period just before rewatering and again in the post-drought period, 9 weeks after rewatering. A medium-sized leaf was sampled from the upper part of each sapling in each replicate. A composite sample was made of all leaves sampled in 1 replicate—altogether 32 samples, 4 samples per replicate. Samples were dried at 105 °C to a constant mass and then milled (Fritsch Pulverisette 14 Mill, Fritsch GmbH Manufacturers of Laboratory Instruments, Idar-Oberstein, Germany) [52]. The concentration of total N was determined on an elemental analyser (Leco CNS 2000, LECO Corporation, St. Joseph, MI, USA), P was determined using a UV/VIS spectrophotometer (UVS-2700 LaboMed Inc., Los Angeles, CA, USA) [52], and K on the atomic absorption spectrometer (Perkin-Elmer Aanalyst 700, PerkinElmer Inc., Waltham, MA, USA) [52], following digestion with a combination of HNO_3 and H_2O_2 in a microwave oven (Milestone Ethos One, Milestone Srl, Sorisole, Italy). Foliar nutrient concentrations were compared with available reference values for beech [53]. The temporal change of N, P and K foliar concentrations between drought and post-drought periods within each treatment was calculated using the following formula:

$$\text{Temporal change} = \text{foliar concentration (post drought period)} - \text{foliar concentration (drought period)} \quad (1)$$

The height (measured from the root collar to the top) and diameter of the root collar of all saplings were measured before leaves emerged (h_{start}, d_{start}) and at the end of the experiment (h_{end}, d_{end}) using a measuring rod and a digital calliper, respectively. Because of initial differences in height and root collar diameter, relative height increment (i_h) and relative root collar diameter increment (i_d) were calculated using the following formula:

$$i_h = (h_{end} - h_{start}/h_{start}) \times 100,\ i_d = (d_{end} - d_{start}/d_{start}) \times 100 \quad (2)$$

At the end of the experiment (October 2020) and before the onset of autumnal yellowing, three average-sized saplings were sampled from each replicate (96 saplings in total) to determine aboveground (leaves and stems) and belowground (coarse roots > 2 mm diameter, fine roots < 2 mm diameter) biomass. The roots were washed from substrate residues and separated into coarse roots and fine roots. Leaves, stems, and coarse and fine roots were dried to constant mass and weighed, resulting in the following measurements: leaf biomass (LB), stem biomass (SB), coarse-root biomass (CRB), fine-root biomass (FRB), aboveground biomass (AGB), belowground biomass (BGB), and total biomass (TB). A composite sample was made of fine roots from each replicate and then ground in a Fritsch Pulverisette 14 Mill [52]. The concentration of total N, P and K were determined as described earlier for foliar concentrations.

We considered different effects of irrigation and fertilisation treatments based on values from final leaf/biomass sampling: a prolonged effect of drought was considered when drought treatments had lower values in comparison with values recorded in regularly watered treatments, always comparing the same level of fertilisation.

Recovery was considered only for foliar nutrient concentrations if there were no differences between drought and regularly watered treatments. Here, two separate sampling campaigns allowed us to crosscheck the presumed recovery in the post-drought period against the effects at the peak of drought.

2.3. Statistical Analysis

All statistical analyses were performed with R-studio, version 4.1.1 [54]. Data normality and residual homogeneity were checked for all data before analysis.

To determine differences in foliar concentrations between treatments (WH, DH, WL, DL) during each period, we used the pairwise comparisons *t*-test with Holm–Bonferroni correction. The temporal change of N, P and K foliar concentrations was analyzed with a one-way analysis of variance to determine significant differences between periods within each treatment.

Differences among individual treatments (WH, DH, WL, DL) for biomass and fine roots N, P and K concentrations were assessed by using analysis of variance (ANOVA) followed by a Tukey post hoc test. In addition, results were tested for an influence of the main factors "irrigation" and "fertilisation," as well as their interactions using two-way ANOVA.

In the case of non-normally distributed data (height and root collar diameter increment), the Kruskal–Wallis test was followed by the Dunn multiple comparisons test.

3. Results

3.1. Water Status of Saplings

Drought-treated saplings (DH and DL treatments) maintained predawn leaf water potentials (Ψ_{pd}) at the same level as regularly watered saplings (WH and WL treatments) during the first three days of the drought period (Figure 2). A week later, the Ψ_{pd} of the DH and DL saplings had already become significantly lower than in WH and WL. The values of Ψ_{pd} in drought-treated saplings continued to decrease afterwards, reaching mean values of −2.1 MPa (±0.2 SE) in DH and −1.8 MPa (±0.2 SE) in DL in mid-August (day 32 of induced drought).

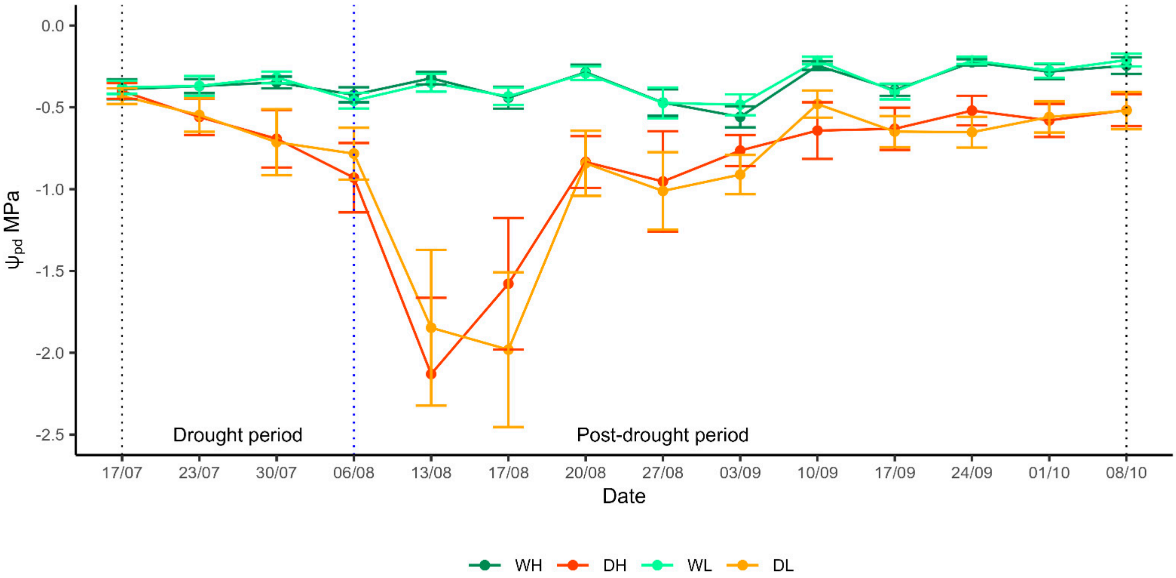

Figure 2. Seasonal pattern of predawn leaf water potential (Ψ_{pd}), in the drought and post-drought period for each treatment; regular watering and high dose of fertiliser (WH), regular watering and low dose of fertiliser (WL), induced drought and high dose of fertiliser (DH) and induced drought and low dose of fertiliser (DL). The first vertical dotted line (black) indicates the beginning of the drought, the second vertical dotted line (blue) indicates the time point of rewatering and the beginning of the post-drought period, and the third vertical dotted line (black) indicates the end of the experiment. Dots indicate mean values and vertical bars indicate ± confidence intervals.

After rewatering, the Ψ_{pd} of drought-treated saplings began to gradually increase. At the end of the experiment, 78% of saplings in DH and 94% of saplings in DL treatment had recovered their Ψ_{pd} to values to close to, but never fully matching, the Ψ_{pd} of WH and WL saplings. Saplings in the WH and WL treatments maintained their Ψ_{pd} between −0.5 MPa and −0.2 MPa (mean value −0.3 MPa ± 0.01 SE) throughout the experiment.

3.2. Nutritional Status of Saplings

3.2.1. Foliar Concentrations

Foliar nitrogen (N) concentrations were within or above the normal range for beech [53] both in the drought and the post-drought period (Figure 3A), but concentrations were significantly lower in drought-treated saplings compared to regularly watered plants. In the drought period, a significant effect of fertilisation was observed in both drought-treated and regularly watered saplings (Table S1), resulting in higher foliar N concentrations in saplings treated with a high fertiliser dose. In the post-drought period, foliar N concentrations were lower in drought-treated saplings than in regularly watered saplings indicating the prolonged effect of drought in both fertilisation treatments. The influence of fertilisation was not as pronounced as during the drought period, with no significant differences established between fertilisation treatments. Foliar N concentrations in drought treatments decreased significantly in the post-drought period but remained within the normal range (Table 1).

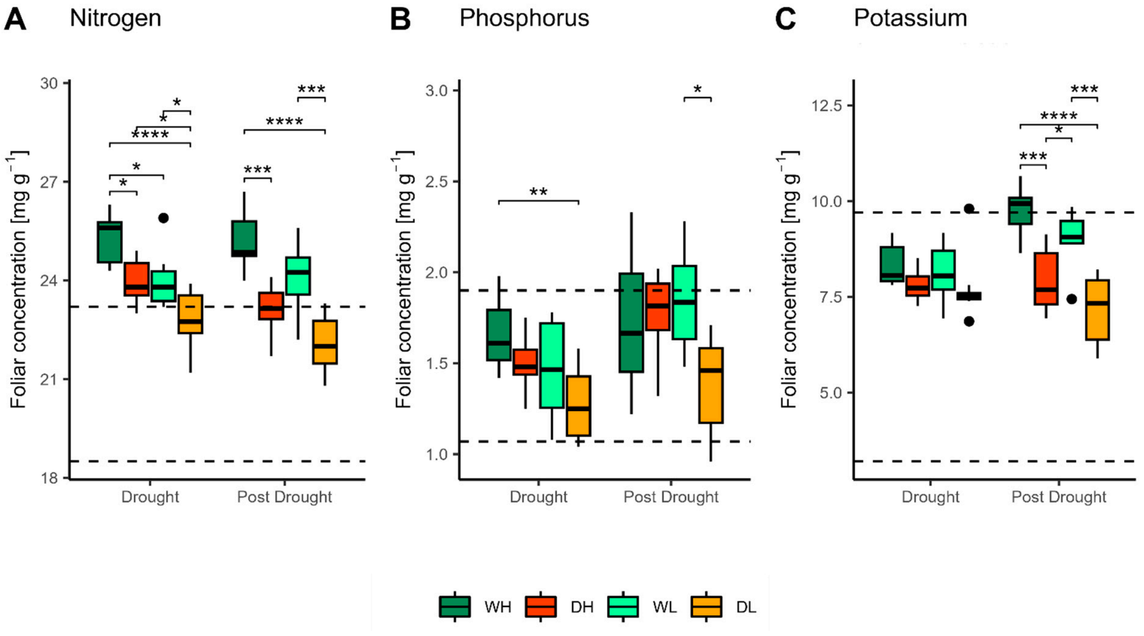

Figure 3. Nitrogen (**A**), phosphorus (**B**) and potassium (**C**) foliar concentrations in the drought and post-drought period, for each treatment; regular watering and high dose of fertiliser (WH), regular watering and low dose of fertiliser (WL), induced drought and high dose of fertiliser (DH) and induced drought and low dose of fertiliser (DL). Levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$. Dotted lines represent an optimal range of foliar N, P and K concentrations for common beech according to [53].

Table 1. Temporal change of foliar concentrations of N, P, K, between the drought and post-drought period calculated by formula: foliar concentration (post drought)—foliar concentration (drought) within each treatment: regular watering and high dose of fertiliser (WH), regular watering and low dose of fertiliser (WL), induced drought and high dose of fertiliser (DH) and induced drought and low dose of fertiliser (DL).

	N	**P**	**K**
WH	−0.64 *	0.20 ns	1.36 **
DH	−1.04 ***	0.14 ns	−0.10 ns
WL	0.59 *	0.25 *	0.98 *
DL	−0.54 ns	0.24 *	−0.34 ns

Data represent mean values of temporal change. Statistical significance calculated with one-way analysis of variance is indicated as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant.

Both in the drought and the post-drought period, foliar phosphorus (P) concentrations (Figure 3B) were in the normal range, according to [53]. The most significant difference between treatments was observed during the drought period, with P concentrations in the WH treatment being significantly higher than in the DL treatment, indicating a significant influence of both drought and fertilisation (Figure 3C, Table S1). In the post-drought period, the only significant differences in foliar P values were established between DL and WL treatments. Foliar P concentrations in the DH saplings did not differ from those of the WH and WL saplings in either period. P concentrations generally increased in the post-drought period, but this was significant only in low-fertilised saplings, indicating partial recovery (Table 1).

Foliar potassium (K) concentrations of DH, WL, and DL were mostly within the normal range [53] (Figure 3C). No significant effect of drought or fertilisation was found on foliar K concentrations in the drought period; significant differences were established only in the post-drought period, with WH concentrations suggesting a luxury supply of K (Table S1). Foliar K concentrations were significantly reduced in saplings previously exposed to drought compared to regularly watered saplings, indicating the prolonged effect of drought on foliar K and the importance of water for K nutrition in beech (Table S1). After drought release, increased foliar K concentrations were observed for regularly watered treatments, but not in the induced drought treatments (Table 1).

3.2.2. Fine-Root Nutrient Concentrations

N concentrations in fine-roots were affected only by fertilisation (Table S2), while P and K fine-root concentrations were affected by both irrigation and fertilisation. Saplings in high-fertilisation treatments had significantly higher root N concentrations than low-fertilisation treatments (Table 2). P concentrations were progressively diminishing in the order WH > DH > WL > DL. For K, only DL treatment values were different (lower) from the rest (Table 2). We did not observe any prolonged effect of drought on fine-root nutrient concentrations.

Table 2. Mean values ± SD for fine-root concentrations of nitrogen (N), phosphorus (P) and potassium (K) in each treatment; regular watering and high dose of fertiliser (WH), regular watering and low dose of fertiliser (WL), induced drought and high dose of fertiliser (DH) and induced drought and low dose of fertiliser (DL).

	WH	**DH**	**WL**	**DL**
N (mg/g)	16.71 ± 0.53 a	17.66 ± 0.53 a	14.73 ± 0.42 b	14.80 ± 0.42 b
P (mg/g)	2.09 ± 0.11 a	1.93 ± 0.09 ab	1.75 ± 0.08 bc	1.45 ± 0.06 c
K (mg/g)	7.91 ± 0.22 a	7.74 ± 0.23 a	7.41 ± 0.25 a	6.48 ± 0.18 b

Different small letters indicate significant differences between treatments at $p < 0.05$, according to the Tukey post hoc test.

3.3. Growth Responses

The relative height increment (i_h) of WH saplings was significantly higher than the i_h of saplings in all other treatments, while no difference in i_h was noted between DH and DL saplings (Figure 4). Regularly watered saplings had higher i_h values than drought-treated saplings. The prolonged effect of drought was found for saplings in both fertilisation treatments. At the end of the experiment, the difference in total height was quite apparent between the WH and other treatments, although this was not significant between WH and WL. Absolute height and root collar diameter values are shown in Table 3. Contrary to i_h, we found that drought-exposed saplings treated with a high fertiliser dose (DH) had higher relative root collar diameter increment (i_d) values than drought-exposed saplings treated with a low fertiliser dose (DL). There was no significant difference between the i_d of DH and WL saplings, indicating a partial compensatory effect of fertilisation vs. adequate water supply. As expected, and similar to height increment, saplings in the WH treatment had the highest i_d. A prolonged drought effect was established for both growth parameters and in both fertilisation levels.

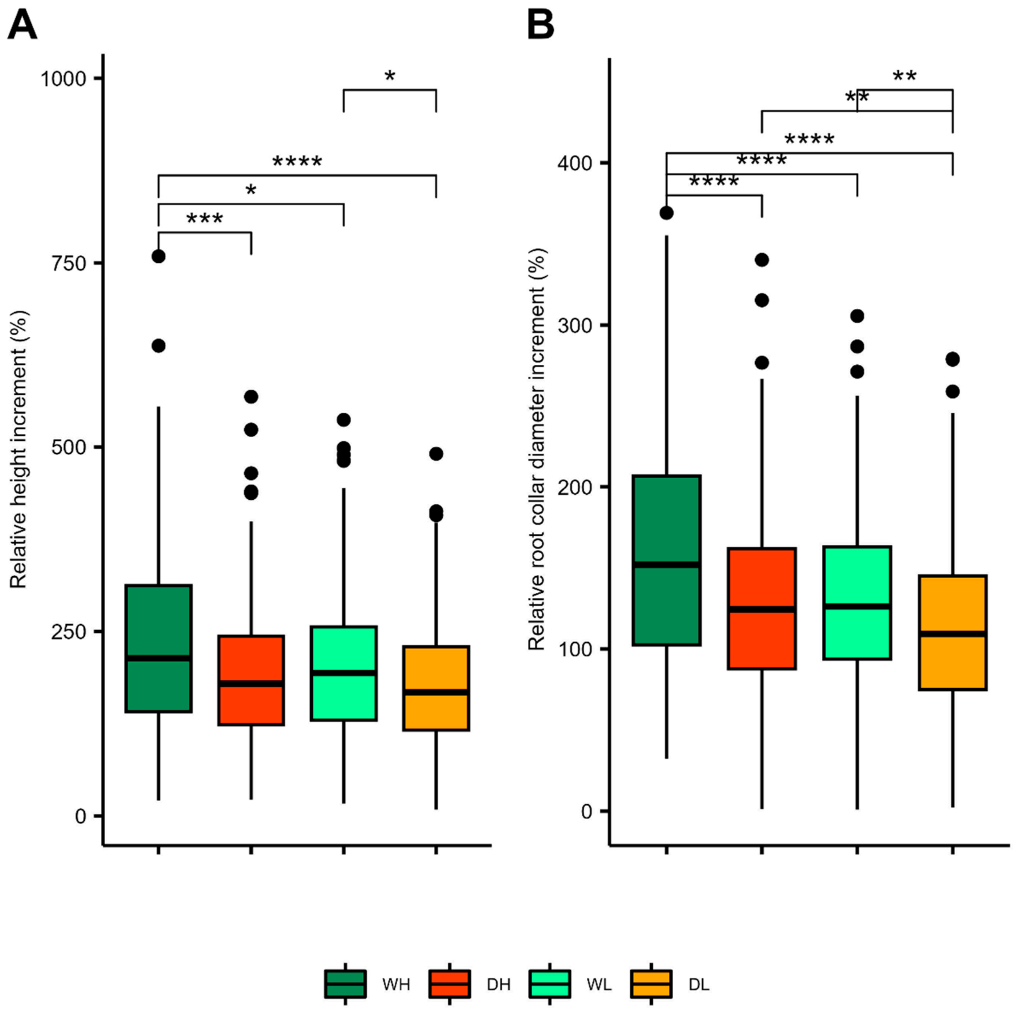

Figure 4. (**A**) Relative height increment (i_h) and (**B**) relative root collar diameter increment (i_d) in each treatment; regular watering and high dose of fertiliser (WH), regular watering and low dose of fertiliser (WL), induced drought and high dose of fertiliser (DH) and induced drought and low dose of fertiliser (DL). Levels of significance in differences among treatments are indicated as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$.

Table 3. Height (h) and root collar diameter (d) of saplings measured at the start (h_{start}, d_{start}) and at the end (h_{end}, d_{end}) of the experiment in each treatment; regular watering and high dose of fertiliser (WH), regular watering and low dose of fertiliser (WL), induced drought and high dose of fertiliser (DH) and induced drought and low dose of fertiliser (DL).

	WH	**DH**	**WL**	**DL**
h_{start} (cm)	16.17 ± 3.28 a	16.71 ± 4.06 ab	17.26 ± 3.89 b	16.80 ± 3.34 ab
d_{start} (mm)	3.46 ± 0.82 a	3.56 ± 0.91 ab	3.69 ± 0.86 bc	3.68 ± 0.80 c
h_{end} (cm)	52.22 ± 16.86 a	46.89 ± 13.71 b	50.84 ± 16.17 a	45.27 ± 12.20 b
d_{end} (mm)	8.62 ± 1.89 a	7.80 ± 1.36 bc	8.21 ± 1.77 b	7.56 ± 1.31 c

Data represent the mean values ± standard deviations and different letters indicate significant differences between means according to the Tukey post hoc test.

3.4. Biomass Responses

Total biomass (TB) production was strongly affected by irrigation (Table 4), but the prolonged effect of drought was found only for the high-fertilisation treatment (Table 5). The production of aboveground biomass (AGB) was affected by both irrigation and fertilisation, as well as their interaction (Table 4), resulting in the highest aboveground biomass production in WH saplings (Table 5). The dry biomass of both stem (SB) and leaves (LB) in WH was also significantly higher than in the other treatments (Table 5). No significant effects of irrigation or fertilisation were found on the belowground biomass (BGB) (Table 4). However, the belowground biomass of WH saplings was the highest and significantly different from DH, which had the lowest belowground biomass. We found similar relations for coarse-root biomass (CRB) (Table 5). A high dose of fertilisation affected irrigated beech saplings by stimulating aboveground biomass production, while the belowground biomass was not affected by higher fertilisation dose regardless of the irrigation regime (Table 5). Fine-root biomass production was similar in all treatments (Table 5), with no significant effects of either irrigation or fertilisation (Table 4). Belowground to aboveground biomass ratio differed only between WH and DL, while DH and WL treatments had similar ratios of around 1:1 (Table 5).

Table 4. Main effects of irrigation (regular watering vs. induced drought), fertilisation (high dose vs. low dose of fertiliser) and their interaction (irrigation × fertilisation) on dry biomass parameters and allometric relationships, at the end of the experiment, calculated with two-way ANOVA. F values for the factors and their interactions are shown.

Parameters		Irrigation	Fertilisation	Irrigation × Fertilisation
Dry biomass	TB	7.45 **	0.75 ns	5.25 *
	AGB	12.44 ***	3.02 ***	6.66 *
	LB	6.27 *	2.11 ns	11.23 **
	SB	14.14 ***	3.10 ns	4.42 *
	BGB	3.23 ns	0.06 ns	3.69 ns
	CRB	4.78 *	0.01 ns	3.82 ns
	FRB	0.04 ns	0.26 ns	0.90 ns
Allometric relationships	BGB/AGB	9.35 **	9.35 **	0.25 ns
	CRB/SB	5.55 *	6.02 *	0.46 ns
	FRB/LB	5.48 *	5.84 *	8.30 **

Total dry biomass (TB), aboveground dry biomass (AGB), leaf dry biomass (LB), stem dry biomass (SB), belowground dry biomass (BGB), coarse-root dry biomass (CRB), fine-root dry biomass (FRB). Statistical significance is indicated as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant.

Table 5. Mean values ± SD for parameters of dry biomass (g) and its allometric relationship in each treatment; regular watering and high dose of fertiliser (WH), regular watering and low dose of fertiliser (WL), induced drought and high dose of fertiliser (DH) and induced drought and low dose of fertiliser (DL).

Parameters		WH	DH	WL	DL
Dry biomass	TB	27.30 ± 1.68 a	19.7 ± 1.07 b	22.5 ± 1.72 ab	21.8 ± 1.5 ab
	AGB	14.80 ± 0.89 a	9.9 ± 0.51 b	11.3 ± 0.87 b	10.5 ± 0.88 b
	LB	4.5 ± 0.22 a	3.0 ± 0.21 b	3.3 ± 0.28 b	3.5 ± 0.28 b
	SB	10.03 ± 0.70 a	6.8 ± 0.35 b	8 ± 0.61 b	7 ± 0.63 b
	BGB	12.50 ± 0.90 a	9.8 ± 0.65 b	11.2 ± 0.9 ab	11.3 ± 0.71 ab
	CRB	8.6 ± 0.69 a	6.2 ± 0.37 b	7.4 ± 0.67 ab	7.3 ± 0.46 ab
	FRB	3.90 ± 0.29 a	3.6 ± 0.31 a	3.8 ± 0.3 a	4 ± 0.31 a
Allometric relationship	BGB/AGB	0.86 ± 0.22 a	1.02 ± 0.26 ab	1.02 ± 0.17 ab	1.11 ± 0.24 b
	CRB/SB	0.84 ± 0.25 a	0.94 ± 0.25 ab	0.94 ± 0.24 ab	1.11 ± 0.32 b
	FRB/LB	0.88 ± 0.28 a	1.21 ± 0.34 b	1.21 ± 0.34 b	1.18 ± 0.25 c

Total dry biomass (TB), aboveground dry biomass (AGB), leaves dry biomass (LB), stem dry biomass (SB), belowground dry biomass (BGB), coarse-root dry biomass (CRB), fine-root dry biomass (FRB). Different small letters indicate significant differences between treatments at $p < 0.05$, according to the Tukey post hoc test.

4. Discussion

4.1. Water Status of Saplings during Drought and in the Post-Drought Period

The level of drought stress during the experiment was documented by measuring Ψ_{pd}. While regularly watered saplings did not experience drought stress, drought-treated beech saplings in both fertilisation treatments experienced severe stress, as indicated by Ψ_{pd} values comparable to those associated with a severe water deficit in beech saplings [23] and the onset of native embolism in mature beech trees [18].

Although juvenile beech saplings typically recover Ψ_{pd} levels within one [55] to several days after drought events [23,56], our study uncovered an incomplete recovery, indicating potential non-reversible losses in hydraulic conductance. The persistence of drought effects may be attributed to embolisms formed during water stress, which may not dissolve after rewatering [57–59]. Drought-induced alterations in anatomical characteristics, including xylem vessel size, structure, sap flow, and whole-plant hydraulic conductivity, significantly impact a plant's ability to cope with water scarcity [45,60,61]. These anatomical changes, coupled with physiological responses like reduced photosynthesis and stomatal conductance, collectively contribute to the overall vulnerability of plants to the destructive effects of drought [25].

However, we did not observe more serious damage linked to drought-induced xylem embolism to the plant tissues, such as defoliation [62] or enhanced mortality [18,63]. The Ψ_{pd} value of -0.5 MPa at the end of our experiment indicated a partial recovery of the water status of saplings. Based on these findings, we anticipated finding differences between regularly watered and drought-treated saplings for other investigated parameters in terms of either prolonged drought effects or recovery, underscoring the importance of considering both physiological and anatomical responses to understand a plant's resilience to drought stress.

4.2. Nutritional Status of Saplings in the Drought Period

We recorded a positive effect of fertilisation on nitrogen, phosphorus, and potassium foliar concentrations, with both fertiliser doses enabling optimal foliar nutritional status of common beech saplings during drought. Higher dose of fertiliser significantly increased N and P concentrations, indicating increased nutrient availability. In agreement with our first hypothesis, higher nutrient availability showed a significant impact on reducing the immediate effect of drought on foliar concentrations, but only for nitrogen. Nevertheless, a high dose of fertiliser did not help keep N concentrations in drought-treated saplings at the same level as in regularly watered saplings, which is in agreement with Ouyang et al. [48], who state that drought-stressed saplings cannot benefit from the additional availability of inorganic N. While the drought effect was significant for both N and P, foliar N concentrations were affected comparatively more. This is in line with the results of Netzer et al. [64], who stated that foliar N reflects current drought stress. Multiple studies confirmed an impaired soil-borne uptake of nitrogen during drought, affecting foliar nutrient concentrations [26,65]. During periods of drought, the ability to absorb nutrients containing nitrogen from the soil is generally diminished as a result of the closure of stomata, reduced transpiration, and the mobility of nutrients [66]. However, saplings in our DH treatment did have significantly higher N concentrations in leaves in comparison with low-fertilised, drought-treated saplings, demonstrating the positive effect of fertilisation on foliar N even in drought conditions; this is in accordance with the finding that effective nitrogen nutrition shows the ability to alleviate water stress in crops by sustaining metabolic processes even at low tissue water potential [67]. Also, there is a possibility that higher nitrogen availability prior to drought led to higher foliar N concentrations during drought, as the plants were able to draw on their N reserves to sustain their growth and function. This possibility can be inferred from high N foliar values that were recorded at the peak of the drought, even in DL saplings. Drought also causes a reduction in P absorption and transport in plants, as phosphate ions move through soils primarily through diffusion, and if the water content in the soil decreases, P mobility also decreases [68]. We expected to see some reduction in foliar P with drought; however, at this point, significant differences were only found between the high-fertilisation regularly watered treatment and the low-fertilisation drought treatment. Contrary to our results, a German study that simulated summer drought decreased foliar P concentrations in common beech saplings, while N concentrations were unaffected [69]. Overall, we found that relatively small differences in foliar concentrations between fertilisation treatments could have been the result of the fact that both levels of fertilisation lead to satisfactory sapling nutrition, which may have also influenced the sapling drought response. The effects could have been stronger if the foliar nutrient status of the low-fertilisation treatment had been closer to deficiency.

4.3. Nutritional Status of Saplings in the Post-Drought Period

Nine weeks after restoring regular watering, foliar P concentrations in high-fertilised saplings matched those in regularly watered saplings, supporting the hypothesis that a high fertiliser dose promotes recovery. Previous findings indicate that the application of P can improve drought tolerance, though it is not clear if P has a direct physiological effect on a plant's drought resistance or an indirect nutritional effect on root growth and thus enhanced soil water uptake [70]. A study by da Silva et al. [66] demonstrated that the addition of phosphorus can mitigate the negative impact of drought stress on yield. In our results, we may suspect this to be the reason for the fact that the root collar diameter increment percentage was higher in the drought-treated/high-fertilised saplings than in the drought-treated/low-fertilised saplings.

Saplings in the low-fertilisation treatment have not been able to sufficiently recover their P levels, indicating a prolonged drought effect. The slow diffusion of phosphorus in soils, compounded by reduced mobility during drought [69,71,72], can lead to increased energy demand for phosphorus uptake in plants [69,73]. Prolonged drought stress may consequently hinder energy metabolism and impede vegetative growth, highlighting the critical role of phosphorus dynamics in plant response to drought [69].

The prolonged effect of drought was also noted in N and K foliar concentrations, regardless of the fertilisation dose, pointing to the long-term effects of drought on beech physiology, in addition to the immediate drought-related effects that plants can mostly remedy based on their reserves. The foliar K concentrations in the post-drought phase displayed significant dependence on water availability, mirroring the findings of a study by da Silva et al. [66]. Interestingly, this effect was present only as the prolonged drought effect, while it was not recorded in the drought period. Some studies have shown that higher levels of K fertilisation may allow crop plants [74,75] to better tolerate water stress, but this has not been confirmed for beech so far.

4.4. Fine-Root Nutrient Concentrations

Similar to our results, Leberecht et al. [76] found no effect of drought on root N in beech saplings. We found that a prolonged drought effect was only relevant for K fine-root concentrations in the low-fertilisation treatment, also showing that higher concentrations of K can help alleviate the effects of drought. This corresponds to the findings of Peuke and Rennenberg [29], who reported decreases in root K of drought-stressed beech saplings and no effect of drought on nitrogen root concentrations. On the other hand, the lack of a prolonged effect of drought for other nutrients, as well as K in HF treatment, may indicate a rapid recovery of beech roots after the drought. During seasonal drought, beech roots largely reflect a "fast" strategy, meaning that beech has an adaptable fine-root system that uses a fast mobilisation of internal storages for new fine-root growth in order to ensure sustained resource uptake [77].

4.5. Effects of Drought and Fertilisation on Sapling Growth and Biomass Partitioning

The growth response of beech saplings to drought was found to be twofold: while drought caused a decreased height increment where fertilisation could not even out the loss to height growth caused by the lack of water, increased nutrient availability had a positive effect on the root collar diameter increment of drought-exposed saplings.

The stimulating effect of increased fertiliser doses on the height and root collar diameter of beech saplings has been documented in previous studies [51,78]. As root collar diameter was shown to predict the survival of young saplings after transplanting [79], we can assume that this effect of fertilisation on root collar diameter can help mitigate the negative effects of drought on the success of afforestation with beech saplings. In contrast, we may expect problems with sapling growth and survival due to drought being more frequent in nutrient-poor soils. In our case, although increased nutrient availability had a positive effect on the root collar diameter increment of drought-exposed saplings, it did not promote diameter growth to the point of fully reducing the effects of drought; the prolonged drought effect was recorded for both height and diameter increments and in both fertiliser treatments. These findings are contrary to our hypothesis that higher fertilisation will alleviate the prolonged effect of drought on growth and biomass production in beech saplings.

Comparisons between saplings with different watering and fertilisation treatments (WH and DL) revealed a lower belowground to aboveground biomass ratio for WH saplings. Trees were shown to invest more assimilates underground after drought [80], increasing root depth and root-to-shoot ratios [81]. Plants in drought conditions generally invest less in the stem and leaves to reduce water loss [82,83]. On the other hand, tree species with a higher investment in leaves and stems tend to maintain stomatal opening and resist stem embolism during a drought event [84], increasing their mortality risk. Our findings align with the concept that plants adjust their biomass allocation in response to changes in nutrient availability and drought conditions [31,85–87].

Although drought typically reduces growth and shifts biomass partitioning to fine-root biomass [88], our study noted an increase in belowground biomass and coarse-root biomass in response to drought, possibly due to the relatively short duration of the drought event and sufficient nutrient availability. Some studies indicate that moderate drought tends to increase fine-root biomass, while long-lasting or extreme drought may have the opposite effect [22,33,81].

The absence of differences in fine-root biomass among treatments may also signify a rapid growth response of fine roots after drought, supporting the concept of ephemeral roots [33]. These thin, ephemeral roots shed during drought and are capable of regrowth upon soil rewetting, providing beech trees with a competitive advantage by enabling rapid proliferation when resources become available [77].

5. Conclusions

Our study emphasises the importance of maintaining optimal nutrient availability for the nutritional status and growth of beech saplings during drought, also shedding light on possible adaptive strategies. Although the benefits of high nutrient availability to combat drought stress seem to be limited, fertilisation played a crucial role in the recovery of phosphorus levels after drought, which is important in the context of a general decline of foliar phosphorus concentrations in Europe. The prolonged drought effect on nitrogen and potassium foliar concentrations also emphasises the enduring physiological impact of drought on beech trees.

Our findings contribute various insights into beech nutrient responses and growth patterns, expanding our understanding of the impact of climate change on beech trees. Regarding the effects of drought on beech saplings such as reduced height increment and biomass production, we propose the adequate fertilisation of saplings to mitigate the physiological consequences of drought, ensuring a larger root collar diameter as a quality measure and a prerequisite for successful afforestation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14122445/s1, Table S1. Main effects of irrigation (regular watering vs. induced drought), fertilisation (high dose vs. low dose of fertiliser) and its interaction (irrigation × fertilisation) on nitrogen (N), phosphorus (P) and potassium (K) foliar concentration in the drought and post-drought period, as calculated with two-way ANOVA. F values for the factors and their interactions are shown; Table S2. Main effects of irrigation (regular watering vs. induced drought), fertilisation (high dose vs. low dose of fertiliser) and its interaction (irrigation × fertilisation) on nitrogen (N), phosphorus (P) and potassium (K) root concentration, at the end of the experiment., as calculated with two-way ANOVA. F values for the factors and their interactions are shown; Figure S1. Pot experiment in the greenhouse; Figure S2. Experimental design layout of the greenhouse pot experiment using a latin square arrangement.

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