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ORIGINAL PAPER

Substrate Enrichment with Clinoptilolite: Limits and Possibilities for Container-Grown Seedling Propagation

Aleš Kučera[1] · **Jiří Volánek**[1] · **Gabriela Tomášová**[1] · **Ladislav Holík**[1] · **Marie Balková**[1] · **Tomáš Vichta**[1] · **Pavel Samec**[1] · **Jana Rosíková**[1] · **Valerie Vranová**[1]

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Abstract

This study examines the benefits of adding clinoptilolite (zeolite) to forest nursery substrates (growth medium) to optimise cultivation of European beech (*Fagus sylvatica* L.) seedlings. Ten growth medium variants were tested, each consisting of a basic peat substrate (nutrient-enriched / non-enriched) with the addition of different quantities of zeolite in different forms (ammonium nitrate and potassium sulphate-enriched / non-enriched). Zeolite had a limiting effect on plant growth, with greater seedling growth in substrates with a low proportion of zeolite (5%), and lower root biomass and root: shoot ratios in substrates with higher proportions. In the latter case, exchangeably-bound divalent base cations (Ca2 + and Mg2+) were enriched in both the sorption complex and aqueous solution due to cation exchange. At the same time, antagonistic K^+ cations were depleted in leaves, despite high concentrations in the substrate. Higher doses also reduced above-ground height/root collar diameter, with substrate type as main cause, irrespective of zeolite content. These findings confirm a dose-dependent effect of zeolite, with low concentrations (5%) stimulating seedling growth and higher doses (especially 20%) leading to impaired rood development, primarily due to K^+ deficiency caused by Ca^{2+} and Mg^{2+} antagonism. By identifying physiological thresholds for zeolite use in silvicultural substrates, we provide important new information for ensuring the success of European beech seedling cultivation for climate-resilient regeneration projects.

Keywords European beech · Artificial substrates · Forest nursery · Plant nutrition · Seedling biometry · Zeolite

1 Introduction

Reforestation, an integral part of forest management for centuries, is currently a highly topical issue due to widespread forest dieback brought about through climate change (Pretzsch et al. 2014; Schuldt et al. 2020). The emergence of climatically and edaphically extreme localities places increasing demands on the quality of planting stock, both in terms of vitality and plantability (Bernier et al. 1995; Haase 2007; Grossnickle and MacDonald 2018). While the need for resilient, high-quality planting stock has grown, efforts to improve fertilisation rates have not always resulted in improved seedling quality and can suppress root development (Jacobs et al. 2004).

In Central European forest adaptation strategies, European beech is widely perceived as a promising alternative species for monoculture and mixed forests over a wide range of habitats due to its ability to survive the increasing occurrence of dry seasons predicted under climate change (Baumbach et al. 2019; Kramer et al. 2010). Any large-scale planting programme, however, will require maximum survival of the planting stock used. In part because of this, production of forest tree planting stock has undergone numerous changes over recent decades, both in terms of production processes and final product quality requirements, as well as morphological characteristics, physiological conditions, out-planting ability and resistance to stressors (see review by Grossnickle and MacDonald 2018). Several studies have also highlighted the role of substrate nutrient release rate for optimal production technologies (Jurásek et al. 2008), suggesting the need for new studies focusing on monitoring and selection for improved rates of nutrient release as a

✉ Gabriela Tomášová
gabrielatomasova20@gmail.com

[1] Department of Geology and Soil Science, Faculty of Forestry and Wood Technology, Mendel University, Brno, Czech Republic

means of maximising uptake efficiency, stimulating favourable physiological states and minimising excessive leaching into the substrate liquid phase after mobilisation.

At present, the preferred method of seedling establishment is through the use of ‘air cushion containers’, which ensure an optimal root system shape, supports the development of root hairs and minimises the risk of root ball overwetting by irrigation or rain (Amoroso et al. 2010; Nelson 2012; Wichter et al. 2020). At the same time, container-grown forest tree seedlings require optimal conditions for plant growth and development over the short-term (Ayan et al., 2005). In particular, the growth medium used should provide the optimal concentration, ratio and release rate of nutrients, supporting growth of both aboveground plant elements and the underground root system. In the case of container-grown forest trees, the most used substrate is based on upland peat, supplemented with organic and/or mineral components (Milosevic and Milosevic 2009; Jankauskienė et al. 2015; Argüello et al., 2018). In recent years, zeolites (a family of hydrated aluminosilicate minerals) have gained in importance as mineral substrate components as, in their natural form, they display high sorption, water retention and cation exchange capacities (Cattivello, 1994; Ramesh and Reddy 2011). Furthermore, when used in large quantities, they naturally contain exchangeably-bound cations such as Ca^{2+}, Na^{+}, K^{+} and Na^{2+} (Bogdanov et al. 2009). Zeolites have numerous applications in plant cultivation, including acting as sorption mediums for increasing water retention, ensuring the gradual release of nutrients, optimising water: air ratios (Argüello et al., 2018) and stimulating root hair development (Datta et al. 2011; Milosevic and Milosevic 2009; Yılmaz et al., 2014). Several naturally occurring zeolites have already been studied (Polat et al. 2004), with clinoptilolite, for example, being widely applied as a cation exchanger (Butorac et al. 2002; Bogdanov et al. 2009; Kroutil et al. 2021), an odour sorbent, a sorbent of chemicals such as ammonia (NH_4^{+}), making them more available to plants (Perrin et al. 1998; Li et al. 2013; Czekaj and Sobuś 2024), and generally as a component for silvicultural substrates and for restoration and remediation of soils (Bogdanov et al. 2009). Another important use is as a source of nutrients in fertilisers. In addition to absorbing NH_4^{+} readily, clinoptilolite can also be used to adjust the efficiency of nutrient uptake, thereby promoting plant growth (Perrin et al. 1998; Polat et al. 2004; Bogdanov et al. 2009). It is also an especially effective source of N and K (along with Ca, Mg and microbioelements) and thus is often used to support effective transition of seedlings to nutrition (Mumpton 1999). When combined with soluble fertilisers such as ammonium nitrate (NH_4NO_3) or potassium sulphate (K_2SO_4), zeolites utilise exchange mechanisms to fix nutrients, thereby supplying essential macroelements for biomass growth and stress resistance (Binkley 1986). On the other hand, this has been linked with an increased risk of nutrient leaching (e.g. nitrate NO_3^{-}) or substrate salinisation (e.g. sulphates SO_4^{2-}) (Chong 2005). To address this, nutrients can be temporarily immobilised through physicochemical sorption, thereby supplying nutrients while mitigating against related risks.

The sorption properties of clinoptilolite (hereafter referred to as zeolite) were previously described by Kroutil et al. (2021), using N as the strategic nutrient. Here, we build on that work by providing a comprehensive assessment of the role of clinoptilolite-based zeolite amendments in peat substrates used for growing European beech under air-cushion container cultivation, using K (sorbed onto the colloidal phase of the growth medium as K^{+}) as the strategic nutrient. While zeolites have been widely studied in agriculture and horticulture, their targeted use in forest nurseries, particularly in relation to beech seedling propagation, remains largely unexplored. Furthermore, unlike previous studies, which have typically applied zeolites in large-scale agronomic and silvicultural settings, the aim of this study was to assess the effect of the different enriched substrates on container-grown planting stock over one vegetation season, focusing on the impact of different zeolite proportions. We hypothesise that the addition of zeolite will have a stimulating effect on seedling growth and nutritional status, though higher proportions could potentially have a negative effect on some seedling parameters, reflected in biometric characteristics and/or nutritional status.

By identifying optimal zeolite concentrations, our findings will help inform nursery practice and substrate formulation strategies in Central European forestry, thereby improving seedling quality and reducing nutrient leaching and supporting successful reforestation efforts in areas vulnerable to climate change.

2 Materials and methods

2.1 Experimental Set-Up

This study took place at the Dykovy školky forest nursery at the Training Forest Enterprise Masaryk Forest, Křtiny (Czech Republic, 49°31’83”N, 16°73’13”E; 530 m a.s.l.) over the 2019 vegetation season. The commercial clinoptilolite (Zeocem a.s., Slovakia) used had a particle size of <200 µm, a porosity of 24–32%, a maximum water sorption level of 68% vol. and a cation exchange capacity for Ca2+, K+, Mg2+and Na+of 0.64–0.98, 0.22–0.45, 0.06–0.19 and 0.01–0.19 mol kg−1, respectively.

Clinoptilolite enrichment was carried out using 20 M NH_4NO_3 and 0.6M K_2SO_4. After stirring the suspension for two days, unabsorbed salts were removed by washing repeatedly with distilled water until leachate electrical conductivity had decreased to 3.5 mS cm − 1. Individual experimental variants were then prepared by mixing both non-enriched pure zeolite (ZP) and enriched zeolite (ZE) at four concentrations (0, 5, 10 and 20%) with a standard growing medium. This base substrate consisted of white peat 55%, perlite 0–6 mm 15%, lignofibre 10%, black peat 20%, granular clay (glauconite, 2–4 mm) 25 kg m^{-3}, microelements 150 g m^{-3}, wetting agents 300 ml m^{-3} and cattle horn shavings 2.0 kg m^{-3}. The clay dose of 25 kg m^{-3} refers to the addition of fine mineral clay in dry weight, calculated per cubic metre of the substrate mixture. Two base substrate variants were used: a pure substrate (SP) and enriched substrate (SE), the latter containing 3.0 kg m^{-3} of mineral fertiliser with an NPK (MgO) composition of 15-9-10 (+2) (Table 1). The term “substrate” in this study refers to an artificially prepared growing medium including the listed components and does not include mineral soil.

Following nursery practice, for each variant, stratified beech seeds were sown in ten 28-cell multipots (4 × 7 rows, providing a total of 280 seeds/treatment), each cell having a volume of 0.37 l. The multipots were then placed under a plastic sheet for five weeks, after which they were repositioned outside. All seedlings were treated according to the standard practices of the nursery, with irrigation, fungicide application and shading provided as required.

2.2 Collection and Calculation of Field Biometric Characteristics

Seedling height was measured monthly from July to October, while root collar diameter and health status were measured monthly from August to October. Seedling health status was assessed based on a subjective scale of 1–5, where 1 = optimal health status and 5 = dead. In each case, the same seedlings were measured each month. Peripheral cells were excluded from the measurements, and the arithmetic means of biometric and health status measurements calculated for the remaining five seedling pairs.

Table 1 List of experimental variants (SP = pure substrate, SE = enriched substrate, ZP = pure zeolite, ZE = enriched zeolite)

Code	Treatment name	Substrate type	Zeolite type	Zeolite volume percentage
1	SP_ZE_0	SP	ZE	0
2	SP_ZP_5	SP	ZP	5
3	SP_ZP_10	SP	ZP	10
4	SP_ZE_10	SP	ZE	10
5	SP_ZE_20	SP	ZE	20
6	SE_ZE_0	SE	ZE	0
7	SE_ZP_5	SE	ZP	5
8	SE_ZP_10	SE	ZP	10
9	SE_ZE_10	SE	ZE	10
10	SE_ZE_20	SE	ZE	20

Destructive measurements were performed in the laboratory on 28 randomly selected seedlings from each treatment at the end of the experiment (October). For each seedling, the following parameters were determined: length of the aboveground part; dry weight of the aboveground part; diameter of the root collar; length and width of the leaf blade, based on three randomly selected leaves per seedling; dry weight of 25 randomly selected leaves; and root weight for roots < 1 mm and roots > 1 mm. To determine plant nutritional status, leaf biomass samples were obtained from the upper third of the seedling crown for 50 seedlings of each variant.

2.3 Laboratory Analysis

Pedochemical characteristics for zeolite were determined before and after enrichment (ZP, ZE), and for the substrate at the start and end of the experiment. Active pH(H_2O) and potentially exchangeable pH(KCl) reactions were determined at a substrate: leaching agent ratio of 1:2.5 w/v for mineral samples and 1:5 w/v for organic samples.

Available nutrients (P, Mg, Ca, K) in the substrate were determined from the Göhler leachate for organic substrates (0.52 M acetic acid (CH_3COOH) and 0.05 M sodium acetate ($C_2H_3NaO_2$)), at a substrate: leaching agent ratio of 1:10 w/v (Soukup 1987), while acceptable nutrients (P, Mg, Ca, K) in the substrate were determined from the aqueous leachate using norm standard number CSN EN 13,652 (BSI 2001).

Nitrogen as ammonium (N-NH_4^+) was determined after previous distillation with sodium hydroxide (NaOH) steam, N distillation being preceded by mineralisation of the aqueous extract and conversion to the NH_4^+ form with sulphuric acid (H_2SO_4) distillate collected in an Erlenmeyer flask with boric acid (H_3BO_3) indicator solution and determined by titration with standard acid (Bremner and Keeney 1965).

Content of P, Mg, Ca, K and Na in the ZP and ZE variants was determined from the Mehlich II leachate (Mehlich 1978). Determination of leachate elements was performed differentially, with P content [mg kg^{-1}] determined spectrophotometrically (Houba 2008) and Ca, Mg, K and Na [mg kg^{-1}] through flame adsorption spectrophotometry. Hydrogen concentration (H^+) was determined by double pH measurement [mmol kg^{-1}] (Adams, Evans 1962), while aluminium (Al^{3+}) concentration [mmol kg^{-1}] was determined according to Sokolov (1939). Cation exchange capacity (*CEC*) [$mmol_+$ kg^{-1}] was calculated using the summation method, based on the equation $CEC = Ca^{2+} + Mg^{2+} + K^+ + Na^+ + H^+ Al^{3+}$, while base saturation [%] was determined as the percentage of base cations (Ca, Mg, K, Na) from total *CEC*. The Mg: K ratio was determined based on content of these elements [mg kg^{-1}] in the leachate. Content of S [g kg^{-1}] was determined according to European Community (EC) Directive 2003/2003, methodology 8.2 (extraction of sulphur in various forms), while determination of S as sulphate ($S\text{-}SO_4^{2-}$) [mg kg^{-1}] was performed after extraction with water at a substrate: leaching agent ratio of 1:5 (w/v). Organic carbon (C_{org}) [%] was determined spectrophotometrically after endothermic oxidation in a chromosulphuric mixture, and total N (N_t) [%] according to the Kjeldahl method (Kirk et al. 1950), after which the C: N ratio was calculated.

Leaf macrobioelement content (N [%], P, K, Ca, Mg, S [g kg^{-1}]) was determined as dry biomass (Čižmárová 2014) after mineralisation of the sample in H_2SO_4 and hydrogen peroxide (H_2O_2). The ratios of K: Mg, K:Ca, N:S, N:Ca and N: Mg were then calculated from individual macrobioelement contents.

2.4 Data Treatment

All data were processed using R v. 4.0.3 and R studio software for statistical computing v. 1.3.1093 (R Core Team 2021), with a statistical significance level of a = 0.05.

Following application of the Shapiro-Wilks test of normality, differences between categorical variables were determined using either parametric ('aov' package) or non-parametric ('kruskal.test' package) analysis of variance. Box plots (median, 0.25 and 0.75 quantiles) were constructed using the R 'plot' function, with the results of multiple comparisons displayed using the 'plotmeans' function from the 'gplots' package v. 3.1.1 (Möller 2020).

The influence of individual experimental variants on biometric quantities was evaluated separately for individual categorical variable components, with the influence of substrate type (SP/SE) and zeolite impact (ZP/ZE) being assessed using the Student's t-test ('ttest' package), and the effect of zeolite quantity in the substrate (0, 5, 10 or 20%) evaluated using ANOVA without interaction.

Following data standardisation, a principal component analysis (PCA) was performed using the 'vegan' package v. 2.5–7.5 (Oksanen et al. 2020), with the PCA factorial plane displaying continuous variables reduced by strongly correlated and categorical variables, i.e. treatments. Relationships between variables were also examined using two-tailed correlation analysis with a Pearson's correlation coefficient critical value of 0.576 for $n = 10$. The evolution of biometric characteristics (shoot height and collar diameter) over time was visualised using the packages 'ggplot2' v. 3.3.2 (Hadley et al. 2020) and 'ggrepel' v. 0.9.1 (Slowikowski et al. 2020).

A two-step generalised linear model (GLM) was used to evaluate the effect of individual treatment components on leaf nutrient content (N, P, K, Mg, Ca, S). As a first step, the following general formula was used:

$$\mathrm{Nutr}_{\mathrm{leaf}} \sim \mathrm{ST} + \mathrm{ZTA}$$

where $\mathrm{Nutr}_{\mathrm{leaf}}$ is leaf nutrient content, ST is substrate type and ZTA is zeolite type and amount (see Table 1). After calculating model significance, pH(H_2O) and pH(KCl) and content of P, Mg, Ca and K in the Göhler and water leachates at the start and end of the experiment were included as continuous variables to explain nutrient concentration. As ST and ZTA were significant for P content, the following general formula was used:

$$\mathrm{Nutr\_P} \sim \mathrm{ST} + \mathrm{ZTA} + \mathrm{CV}$$

where Nutr_P is leaf P content and CV is the continuous variable (Mg or Ca content in Göhler leachate at the start of the experiment).

For each sampling month, the influence of individual treatment components (substrate type, zeolite type, zeolite amount added to growth medium) on seedling shoot height and diameter was assessed using GLMs including the data transformation family 'Gamma', link = 'identity', under the general formula:

$$\mathrm{Incr.} \sim \mathrm{ZT} + \mathrm{ZA} + \mathrm{ST} + \mathrm{M}$$

where Inc. is increment (either shoot height or collar diameter), ZT is zeolite type, ZA is zeolite amount added to the growth medium, ST is substrate type and M is month of biometric measurement. As in the first step, as the model evaluated SP as non-significant for shoot height, the formula was simplified to:

$$\mathrm{Incr.} \sim \mathrm{ZT} + \mathrm{ZA} + \mathrm{M}$$

3 Results

3.1 Zeolite Characteristics after Enrichment

After mixing zeolite with the NH_4NO_3 and K_2SO_4 enrichment solutions (ZE) supplying NH_4^+ and K^+, concentrations of all base cations in the leachate were enhanced, including those not supplied directly during enrichment (Table 2). Concentration of K^+ cations, for example, increased twofold in the Mehlich II leachate and fourfold in the water leachate. There was also a slight decrease in pH during the enrichment process, especially when measuring active pH(H_2O).

This increase in base cations was the result of significant exchange during the enrichment process due to the high levels of K^+ released from the K_2SO_4. As a result, considerable amounts of divalent bases (especially Ca) were transformed into their water-soluble form, resulting in approx. 50

Table 2 Physico-chemical and chemical properties of pure zeolite and zeolite after enrichment. ZP = pure zeolite, ZE = enriched zeolite.

Leachate	Unit	Parameter	Zeolite type	
			ZP	ZE
Water		pH	7.7	6.2
KCl		pH	6.3	5.8
	%	C_{org}	1.4	1.4
		N_t	< 0.02	1.1
		C/N	> 70	1.0
Mehlich II	mg kg^{-1}	P	10.0	10.0
		Mg	205.0	404.0
		Ca	8495.0	12240.0
		K	7595.0	15960.0
		Na	1377.0	360.0
	mmol kg^{-1}	H^+	11.0	11.0
		Mg	16.9	33.3
		Ca	423.9	610.8
		K	194.7	409.2
		Al	< 1	< 1
		CEC	646.5	1064.3
		Bc	635.5	1053.3
	%	BS	98.3	99.0
Water	mg kg^{-1}	P	7.0	4.0
		Mg	22.0	401.0
		Ca	116.0	5791.0
		K	69.0	3018.0
		N-NH_4^+	81.2	1126.0
		N tot	107.0	1221.0
		S-SO_4^{2-}	13.0	6460.0
	g kg^{-1}	S	2.9	9.0

times higher Ca concentrations and approx. 18 times higher Mg concentrations (Table 2). The same process increased the proportion of K^+ adsorbed on the sorption complex from 30% to almost 40%, though at the expense of Ca^+, the proportion of which decreased by more than 8% after enrichment. The proportion of Mg^{2+} cations in the leachate increased least, though they were still present in the resulting substrate at very high levels (Supplementary file 1). Despite its relatively unfavourable position in the lyotropic series, a similar process is also likely in the case of NH_4^+ ions released from the NH_4NO_3 solution.

3.2 Growth Medium Characteristics

All treatment variants exhibited declines in pH(H_2O) and pH(KCl), with the greatest decline in substrates with highest proportions of ZE (Supplementary file 1). While all ZP and ZE variants displayed lower C_{org} levels at the end of the experiment (apart from sample SE_ZE_10), C: N ratios increased across the board, apparently due to high N uptake by the plants. Despite this, soil depletion is unlikely to become an issue due to (i) the high overall N content at the start of the experiment, and (ii) the relatively short period (i.e. one season) that seedlings are grown in the substrate. All SE variants displayed higher concentrations of available P in the Göhler leachate, though levels decreased with increasing proportion of zeolite (Supplementary file 1). This same trend was observed at both the start and end of the experiment.

At the end of the experiment, the ratio of adsorbed monovalent and divalent base cations showed opposing trends, with the proportion of K^+ in the aqueous and Göhler leachates increasing in samples with higher zeolite content and the proportion of Ca^{2+} and Mg^{2+} decreasing (Supplementary file 1). K^+ levels were high in all treatment variants, even in the SP variant with no added zeolite, due to both cation exchange taking place between the substrate liquid phase and the colloidal phase throughout the growing season and the antagonism of base cations. At this point, cation exchange favoured the dominant K^+ cations, which were released from soluble bonds in the salts and displaced divalent bases. On the other hand, in the Göhler leachate, both Ca^{2+} and Mg^{2+} were found at highest levels in both SP and SE ZE_20 variants at the start of the experiment but were lowest in these variants by the end of the experiment, while levels in the water leachate were similar to the other variants. In the SP_ZE_0 variant, however, Mg concentration on the sorption complex was seen to increase, despite levels decreasing significantly in the other variants (there was also a slight decrease in the SE_ZE_0 variant, such that both were at the same level by the end of the experiment). Though less obvious, the same trend was observable for Ca, with initial concentrations clearly corresponding to the quantity of zeolite added (dependence potentially related to the high Ca content in zeolite), and final concentrations being inversely proportional to the quantity of zeolite.

3.3 Nutritional Characteristics of Seedlings

Nutritional status for all treatment variants, based on total concentration of macrobioelements in leaves, lay within referenced optimal ranges (Table 3; Forber 1990). Individually, K^+ was recorded at lowest concentrations in all variants, with lowest values in the SE_ZP_5 and SP_ZP_5 variants, and highest in ZE variants. Indeed, K^+ concentrations were so low that there was a risk of deficiency in all experimental treatments. Ca content, on the other hand, was lowest in SP_ZE_10, followed closely by SP_ZE_20 and SE_ZP_10, while Mg content was lowest in SP_ZE_20 and highest in SE_ZE_0.

K deficiency was also reflected in its low ratios with Mg and Ca, despite targeted supply of K from the mineral fertiliser. This disproportion was especially noticeable for K: Ca, particularly in variants containing ZP. Note that the low ratio with Ca was due in part to the high concentrations of Ca in zeolite. The S: N ratio was also low owing to high uptake

Table 3 Leaf macrobioelement content (g kg^{-1}) for different variants (treatment name: SP = pure substrate, SE = enriched substrate, ZP = pure zeolite, ZE = enriched zeolite; number in the variant formula = zeolite volume percentage).

Element	SP_ZE_0 (C)	SP_ZP_5	SP_ZP_10	SP_ZE_10	SP_ZE_20	SE_ZE_0	SE_ZP_5	SE_ZP_10	SE_ZE_10	SE_ZE_20
N	1.88	2.35	2.72	2.39	2.66	2.50	2.40	2.36	2.56	2.49
P	1.36	1.62	1.69	1.67	1.70	1.73	1.76	1.87	1.99	2.07
K	7.42	6.25	7.12	8.81	8.77	7.23	6.57	7.38	9.09	8.11
Ca	12.70	13.40	13.40	11.10	11.50	13.50	15.50	13.40	11.80	12.10
Mg	3.22	2.78	2.83	2.45	2.40	3.27	3.05	2.66	2.83	2.44
S	1.46	1.26	1.37	1.59	1.41	1.53	1.38	1.02	1.14	1.25
K/Mg	2.30	2.25	2.52	3.60	3.65	2.21	2.15	2.77	3.21	3.32
K/Ca	0.58	0.47	0.53	0.79	0.76	0.54	0.42	0.55	0.77	0.67
N/S	12.88	18.65	19.85	15.03	18.87	16.34	17.39	23.14	22.46	19.92
N/Ca	1.48	1.75	2.03	2.15	2.31	1.85	1.55	1.76	2.17	2.06
N/Mg	5.84	8.45	9.61	9.76	11.08	7.65	7.87	8.87	9.05	10.20
Ca/Mg	3.94	4.82	4.73	4.53	4.79	4.13	5.08	5.04	4.17	4.96

of N, concentrations of which were predominantly supra-optimal. Lowest N concentrations were recorded in the SP_ZE_0 treatment (classed as slightly deficient), while highest concentrations were observed in SP_ZP_10 and SP_ZE_20.

The PCA (Fig. 1a) revealed a clear relationship between high proportions of ZE in the substrate and high rates of K nutrition, as well as antagonism of K uptake against divalent bases (represented by Ca). There was also a significant positive correlation between N and P with zeolite-rich substrates (less so for K and P) and, conversely, a negative correlation with S, i.e. S concentrations were highest in zeolite-poor substrates, e.g. SP_ZE_0 (Fig. 1b).

The GLM revealed a significant dependence between P content in leaves and substrate type, zeolite type and percentage zeolite addition, with P content increasing with increasing richness of the growing medium (Tables 4 and 5). Overall, lowest P concentrations were recorded in SP samples, and highest in the SE_ZE_20 variant. In contrast, nutritional status showed the opposing trend, with P content decreasing with increasing zeolite content (Tables 3 and 4). Likewise, P content in leaves was negatively correlated with both Mg and Ca content, though values were only close to significant in Göhler leachate, i.e. water-soluble Mg and Ca forms had a significantly greater negative impact on P uptake (Table 4). No other significant dependencies were observed for macrobioelements.

3.4 Biometric Characteristics

3.4.1 Field Biometric Measurements

Differences between treatments for health status, root collar diameter and, especially, seedling height increased visibly over the vegetation season (Fig. 2). Average seedling height at the end of the season (October) ranged from 32 to 45 cm (av. 37.5 cm), with lowest values in SE_ZE_20 (31.75 cm) and highest in SE_ZP_5 (44.45 cm), while average root collar diameter was 4.67 mm, with lowest values again recorded in SE_ZE_20 (4.16 mm) and highest in SP_ZP_5 (5.15 mm). While collar diameter also increased close to the average value in SE_ZE_0 (4.56 mm), growth increment rates in SE_ZE_0, SP_ZP_10 and, partially, SP_ZP_5, declined markedly between September and October. Health status was notably better in SE_ZE_20 throughout the measuring period. Aside from ZP_ZE_20, SP_ZE_0 and, especially, SP_ZE_10, health status showed a progressive decrease, with lowest values again in SE_ZE_20.

Overall, GLMs indicated that decreasing zeolite levels resulted in higher shoot increments, with highest incremental growth at the lowest zeolite addition level (5%; Table 5). Even after removal of non-significant parameters (i.e. substrate type) from GLM 1, significance of the remaining parameters changed relatively little, even with equal coefficients of determination. Thus, quantity and type of zeolite had a more significant effect on seedling height than substrate type. Both substrate type and, less so, zeolite type, had a significant positive effect on root collar increment; however, as with seedling height, increasing zeolite proportion had an increasingly negative limiting effect on root collar growth (Table 5). Following removal of non-significant parameters from GLM 3 (i.e. zeolite type), the lowest proportion of zeolite (5%) had a positive impact on growth, while higher zeolite concentrations (10 and 20%) continued to have a negative influence.

3.4.2 Laboratory-Based Biometric Measurements

Highest assimilating biomass production (i.e. leaf length and width and quantity of dry matter at the end of the experiment) was mainly observed in ZE and ZP variants with 5% zeolite, though levels in SE variants were also high (Figs. 3a, b, c; see also Table 6). Variants with lower zeolite content also tended to have higher values for other biometric parameters. For example, both root hair biomass (Fig. 3d) and, especially, total root biomass (Fig. 3e), were highest in

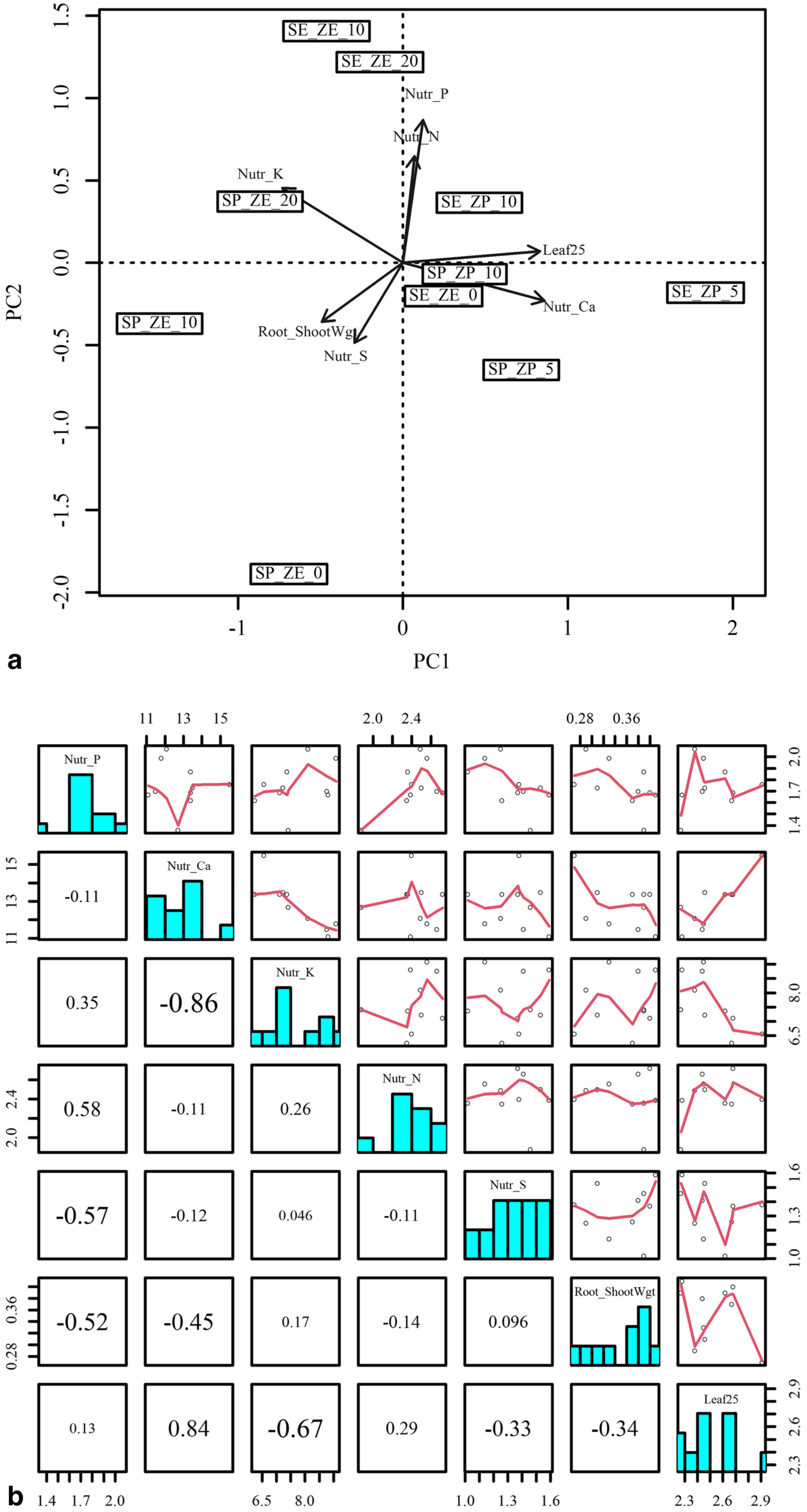

Fig. 1 Biometric characteristics and relationships between macrobioelements in leaves of each variant. (**a**) PCA analysis (1st and 2nd main component), showing continuous and categorical (individual treatment) variables; (**b**) correlation matrices, with scatter plots and smooth curves above the diagonal with correlation coefficients (critical value = 0.576; see Methods) below the diagonal. Nutr_N, Nutr_P, Nutr_Ca, Nutr_K and Nutr_S = nutrient content in leaves; Root_ShootWgt = root:shoot ratio; Leaf25 = weight of 25 randomly chosen leaves; SP = pure substrate, SE = enriched substrate, ZP = pure zeolite, ZE = enriched zeolite; number in the variant formula = zeolite volume percentage.

Table 4 Generalised linear model outputs explaining phosphorus nutrition depending on substrate properties (substrate type, zeolite type and percentage proportion; ZP = pure zeolite, ZE = enriched zeolite, SP = pure substrate) and bivalent base cation content (Ca^{2+}, Mg^{2+}) in Göhler or water leachates of growth medium at the start of the experiment. Asterisks indicate levels of significance, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS – non-significant parameter; Est. (estimate) indicates either lower or higher value of parameter in relation to Int. (intercept). Number in the variant formula (Parameter column) = zeolite volume percentage

	Leachate: Göhler				Leachate: water			
	Mg		Ca		Mg		Ca	
Parameter	*p*-value	Est.	*p*-value	Trend	*p*-value	Trend	*p*-value	Trend
Intercep	**	(+)	**	(+)	***	(+)	***	(+)
SP	*	(-)	**	(-)	**	(-)	**	(-)
ZE10	**	(+)	*	(+)	**	(+)	**	(+)
ZE20	**	(+)	*	(+)	**	(+)	**	(+)
ZP10	NS	(+)	*	(+)	**	(+)	**	(+)
ZP5	*	(+)	*	(+)	**	(+)	**	(+)
Mg	NS	(-)			*	(-)		
Ca			0.079	(-)			*	(-)
R^2	0.9836		0.9797		0.9926		0.9920	

Table 5 Linear regression analysis for categorical variables (substrate type, zeolite type, measurement month) and continuous variables (seedling height, root collar diameter). ZT = zeolite type, ZA = zeolite quantity, ZTZA = combination of ZT and ZA in one categorical variable, M = month, ZP = pure zeolite, ZE = enriched zeolite, ST = substrate type, SP = pure substrate, SE = enriched substrate). The model intercepts include differentiated variables according to specific models (see Table footnote). Asterisks indicate levels of significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS – non-significant; Est. (estimate) indicates either lower or higher value of parameter in relation to Int. (intercept). Number in the variant formula = zeolite volume percentage.

Parameter	Shoot height				Collar diameter			
	GLM 1		GLM 2		GLM 3		GLM 4	
	y~ZTZA+ST+M		y~ZT+ZA+M		y~ZTZA+ST+M		y~ZA+ST+M	
	p-value	Est.	*p*-value	Est.	*p*-value	Est.	*p*-value	Est.
Intercept*	***	(-)	***	(-)	***	(-)	***	(-)
ZP			***	(-)				
Z5			***	(+)			*	(+)
Z10			*	(-)			**	(-)
Z20			***	(-)			***	(-)
SP	NS	(+)			**	(+)	**	(+)
ZP5	*	(+)			*	(+)		
ZP10	***	(-)			**	(-)		
ZE10	*	(-)			*	(-)		
ZE20	***	(-)			***	(-)		
M	***	(+)	***	(+)	***	(+)	***	(+)
R^2	0.737		0.737		0.478		0.478	

*** Model intercept**

GLM 1 and GLM 3: ZE, Z0, SE

GLM 2: ZE, Z0

GLM 4: Z0, SE

SP_ZP_5 and lowest in the richest substrate, i.e. SE_ZE_20. Likewise, the root: shoot ratio also showed a declining trend with ZE enrichment, with highest average ratios observed in 5% variants and lowest in SE_ZE_20 (Fig. 3f).

Parametric ANOVA with subsequent multiple comparisons indicated notable differences between treatments for 25-leaf weight, though significant differences were only detected between SP_ZE_10 and SE_ZP_10 (Fig. 3a). Similarly small differences were observed for 25-leaf weight between SP_ZE_0 and SP_ZE_10 against SE_ZP_5 (Fig. 3b). Both absolute biomass and 25-leaf weight variation differed based on substrate, with highest average biomass values observed in SE_ZP_5 and lowest in SP variants, the latter especially showing increased variability, i.e. narrower confidence intervals (Fig. 3b).

Shoot height, roots > 1 mm and the root: shoot ratio were all significantly affected by substrate type (Table 6), with above ground biomass higher ($p<0.001$) in SE variants

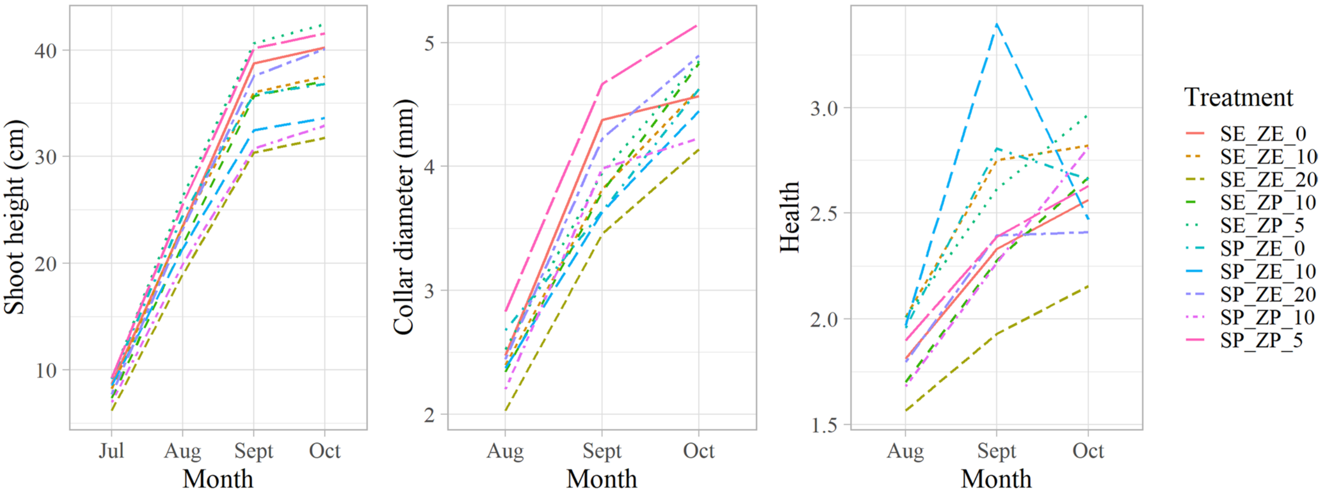

Fig. 2 Monthly measurements of seedling height (**a**), root collar diameter (**b**) and health status (**c**) for different treatment variants. SP = pure substrate, SE = enriched substrate, ZP = pure zeolite, ZE = enriched zeolite; number in the variant formula = zeolite volume percentage.

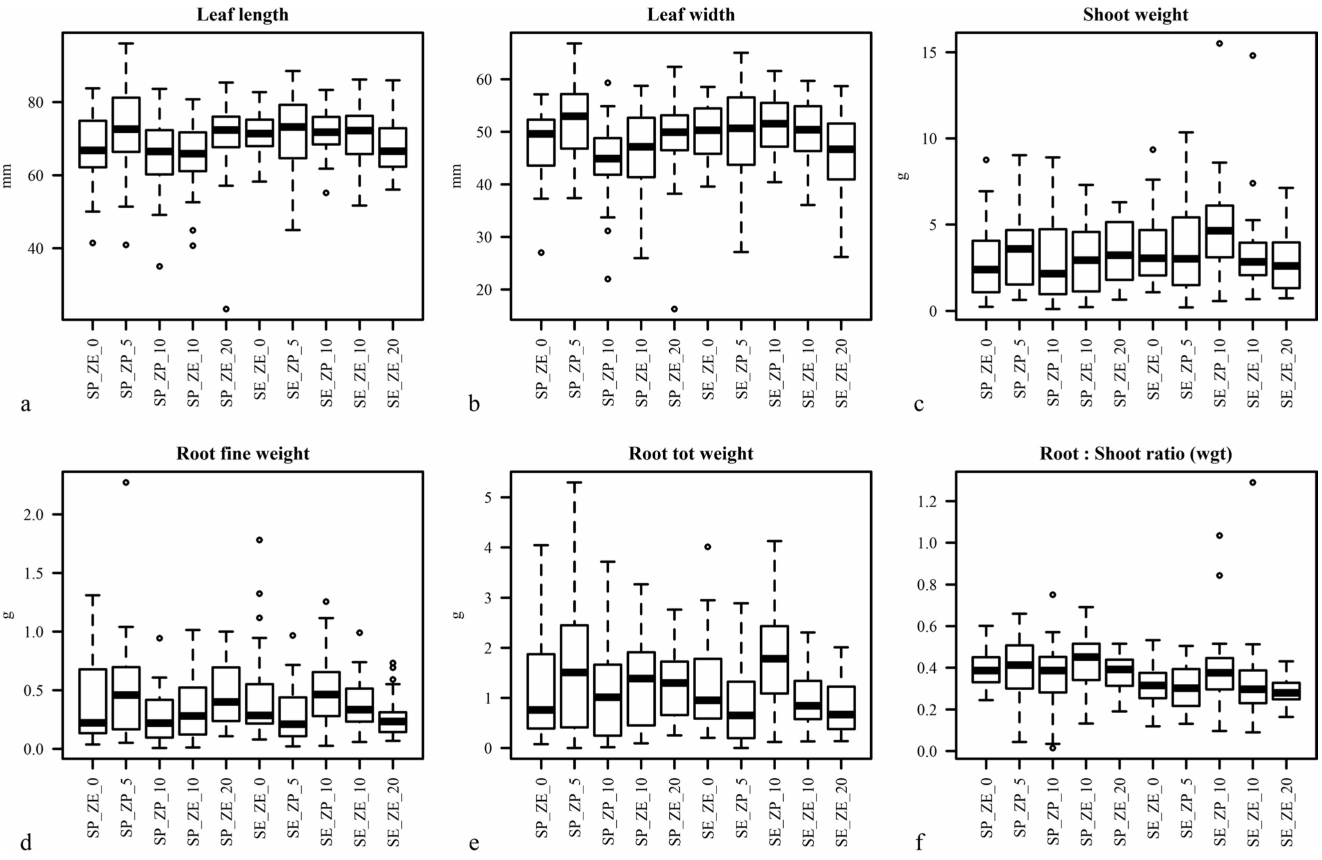

Fig. 3 Box plots (medians with 0.25 and 0.75 quantiles) for (**a**) leaf blade length, (**b**) leaf blade width, (**c**) dry weight of aboveground part, (**d**) dry weight of fine roots, (**e**) dry weight of total root biomass and (**f**) root:shoot weight ratio. SP = pure substrate, SE = enriched substrate, ZP = pure zeolite, ZE = enriched zeolite; number in the variant formula = zeolite volume percentage.

and belowground biomass higher ($p < 0.05$) in SP variants (Table 6). The root: shoot ratio was also significantly higher ($p < 0.05$) in SP variants.

Substrate type had a significant impact ($p = 0.006$) on shoot height and coarse root (>1 mm) biomass, with higher shoot height values and lower coarse root biomass

Table 6 Differences in biometric characteristics with respect to substrate type (SE, SP), zeolite type (ZE, ZP; analysed by t-test) and proportion of substrate zeolite (0, 5, 10, 20%; analysed by ANOVA without interactions). Significant values ($p<0.05$) in bold. Parameter abbreviations: ShootH_cm = length of the aboveground part; Collar_mm = diameter of the root collar; LeafL_mm and LeafWdt_mm = length and width of the leaf blade, respectively; Shoot_g = dry weight of the aboveground part; RootFine_g and RootCoarse_g = root weight for roots < 1 mm and roots > 1 mm, respectively; Leaf25_g = dry weight of 25 randomly selected leaves; Root_ShootW = root: shoot ratio

Parameter	Substrate type			Zeolite type			Zeolite amount
	SE	SP	*p*-value	ZE	ZP	*p*-value	*p*-value
ShootH_cm	41.70	37.50	**0.0060**	39.11	40.41	0.4133	0.282
Collar_mm	5.03	4.83	0.2171	4.88	5.01	0.4488	0.958
LeafL_mm	70.60	68.64	0.0742	69.25	70.19	0.4257	0.152
LeafWdt_mm	49.42	48.29	0.1907	48.48	49.42	0.305	0.059
Shoot_g	3.66	3.15	0.0686	3.23	3.65	0.1663	0.719
RootFine_g	0.38	0.40	0.6702	0.38	0.39	0.8594	0.606
RootCoarse_g	0.79	0.98	**0.0496**	0.77	1.00	**0.0087**	0.108
Leaf25_g	2.56	2.47	0.2966	2.38	2.72	**0.0002**	**0.008**
Root_Shoot_W	0.33	0.40	**6.923e-05**	0.35	0.37	0.3049	0.109

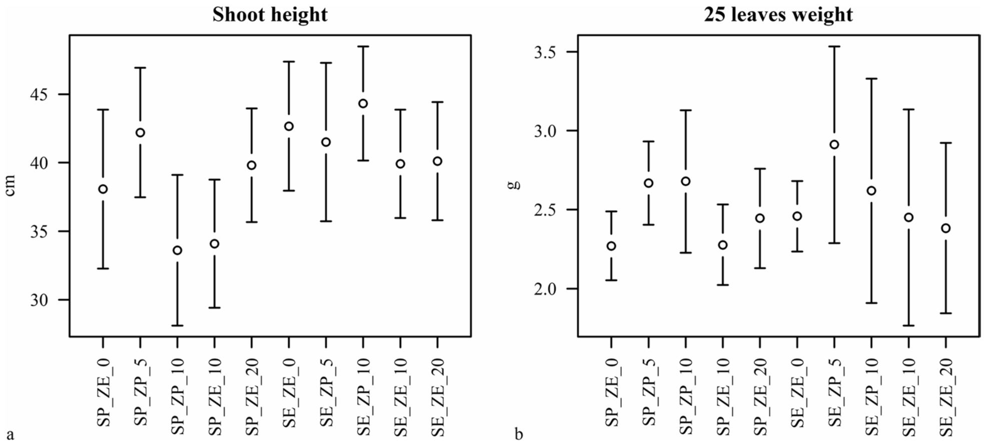

Fig. 4 Multiple comparisons for (**a**) shoot height and (**b**) weight of 25 randomly selected leaves for individual treatment variants. SP = pure substrate, SE = enriched substrate, ZP = pure zeolite, ZE = enriched zeolite; number in the variant formula = zeolite volume percentage.

in SE variants. Furthermore, while mostly non-significant, all other biometric parameters were higher in ZP variants (Table 6). Further analysis confirmed zeolite amount as the significant factor ($p<0.01$) impacting increase in 25-leaf biomass (Table 6, see also Fig. 4).

4 Discussion

In Central and Western Europe, the European beech is one of the main tree species being considered in forestry adaptation strategies for ameliorating the long-term impacts of climate change on commercial tree production (Baumbach et al. 2019). However, successful reforestation using this species will require changes to established practice (Lebourgeois et al. 2013; Pretzsch et al. 2013; Magh et al. 2018), including the provision of suitable forms of nutrient supply during artificial propagation (Jurásek et al. 2008). In this study, we assessed the benefits of adding different concentrations of clinoptilolite, one of the most widely used zeolite minerals, to forest nursery substrates.

Nutrient release and accessibility are important issues in forest seedling production, not only for assessing the chemical and physico-chemical characteristics of growth media but also when evaluating the content and accessibility of macrobioelements in leaf biomass. In the present study, this relationship between substrate richness and nutrition status was especially marked as regards K content. While K concentrations were mainly supraoptimal in the substrate liquid phase and sorption complex, for example, transfer to biomass was limited through antagonism with divalent bases of Mg and Ca. Indeed, PCA not only revealed a clear relationship between high zeolite proportions in substrate and high rates of K nutrition, but also antagonism of K uptake against divalent bases (represented by Ca). Likewise, there was a significant positive correlation between N and P with zeolite-rich substrates (less so for K and P) and, conversely, high S concentrations in zeolite-poor substrates. Enrichment with zeolite has previously been shown to increase the availability of N, K, P, Ca and Mg in growth media, despite zeolite itself being primarily a carrier of Na (Polat et al. 2004; Bogdanov et al. 2009). This was confirmed in this study, with the divalent bases Ca^{2+} and Mg^{2+} becoming more accessible, even in aqueous leachates, despite the zeolite being enriched with K^+ and NH_4^+ cations only. In its natural mineral form, imbalance within the zeolite tetrahedral structure between Al^{3+} and bridging O^{2-} is balanced by divalent bases, which are then displaced from both colloidal and crystalline forms in the presence of excess K^+ in its colloidal form (Johnson et al. 2003).

Regarding biomass production in relation to nutrient supply, our study did not show a clear response of leaf biomass to enriched zeolite application. Instead, the results suggest a more complex interaction. Higher leaf biomass was observed either with pure zeolite or in treatments without any zeolite, indicating a combined influence of both zeolite type and dosage. In contrast, coarse root production appeared to be more strongly influenced by the zeolite type alone.

Similar findings were reported by Maghsoodi et al. (2024), who also did not observe a significant effect of zeolite on shoot biomass production. However, they did report beneficial effects of zeolite use on chlorophyll index and leaf number.

Application of zeolite usually affects both plant growth and phenology by promoting biomass development, though this is not necessarily demonstrated through morphological characteristics such as shoot height or root collar diameter (Cattivello, 1994; Ayan et al., 2005; Milosevic and Milosevic 2009; Yılmaz et al., 2014; Ramesh and Reddy 2011). In our study, while biometric characteristics were differentiated in the different variants, variation was not in direct proportion to either the form or quantity of zeolite used in the growth medium. On the contrary, seedlings in variants with high zeolite levels tended to have lowest shoot height and root collar diameter, while highest values were usually recorded in variants with just 5% zeolite. This limiting effect of higher zeolite concentrations was also observed by Ayan et al. (2005), who found growth media with 20% zeolite to be limiting to Eastern spruce (*Picea orientalis* L. (Link.) seedlings, with a negative effect on all morphological characteristics.

The amount of nutrient available to a plant not only affects its physiological processes but also biomass production. This was clearly reflected in our results, with variants with lower zeolite content tending to have higher values for biometric parameters such as root hair biomass and total root biomass. Likewise, the root: shoot ratio showed a declining trend with ZE enrichment, with highest average ratios observed in 5% variants. Overall, our results indicate that high levels of zeolite in growth substrate (indicated by the 20% concentration in this study) negatively affect root system development via both chemical and physical factors, with high zeolite doses significantly increasing the levels of exchangeable Ca^{2+} and Mg^{2+} available, which can antagonise K^+ uptake. This 'blockade' of K uptake via antagonism with divalent bases means that any positive correlation between K nutritional status and the root: shoot ratio can only be non-significant. As shown by Xu et al. (2022), the positive impact of K on root growth (Liu et al. 2024; Sustr et al. 2019a, b) is conditional upon an adequate and consistent K supply, which in turn supports efficient assimilation of other nutrients and thus overall seedling physiology.

As K is essential for root elongation, osmotic regulation and stress response, its limited availability (along with N and Fe deficiency) is likely to reduce both the formation and function of root hairs (Høgh-Jensen and Pedersen 2003). Similar results were also obtained by Jacobs et al. (2004), who studied the effects of fertilisation on development of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) root systems. In such cases, high nutrient availability, together with optimal substrate irrigation, may mean that there is a reduced requirement to increase the absorption area of roots, resulting in a less developed root system and a reduced root: shoot ratio.

High zeolite enrichment levels may also alter the physical structure of the substrate, with higher concentrations leading to decreased porosity and/or negative changes in water retention, thereby reducing aeration and limiting root penetration. Such physical constraints, combined with nutrient antagonism, likely contributed to the reduced root: shoot ratio and overall root biomass observed in this study. These findings align with previous studies demonstrating that high nutrient availability in compact or poorly aerated substrates can suppress root system development by reducing the plant's incentive to expand absorptive structures (Jacobs et al. 2004; Ayan et al., 2005).

In assessing nutritional input of P, it is important to assess both its absolute concentration and its availability in relation to the substrate's chemical make-up (Binkley 1986; Mengel & Kirkby 2001). According to Penn and Camberato (2019), P availability increases significantly at $pH > 6$ due to reduced concentrations of free Fe and Al. In our study, however, we recorded an increase in P availability at a lower pH (approx. 5.5), presumably due to the very high Ca concentrations, as also reported by Penn and Camberato (2019). At the same time, P availability increased noticeably in variants with a high proportion of zeolite, possibly as it interacts with Al at around pH 6 (Price 2006). This high mobility of P due to prevailing substrate chemistry was confirmed by its high concentration in aqueous leachates, both at the start and end of the experiment. In this respect, the experimental growth medium was not behaving like a natural soil environment; rather, it showed certain features typical of low-buffering artificial systems, allowing rapid nutrient release into the aqueous phase. In such an environment, P was not immobilised significantly as its availability is primarily governed by pH-dependent precipitation and solubility. Thus, the behaviour of P reflects a system with limited P fixation capacity and high chemical reactivity, e.g. hydroponic or semi-hydroponic conditions, where a more acidic pH enhances P uptake efficiency due to a dominance of plant-available $H_2PO_4^-$ rather than HPO_4^{2-} (Barrow 2017). In our own experiment, the greatest decrease in pH over the growing season occurred in those variants having highest P concentrations in leaves (Supplementary file 1), this being due to the reaction of P with free Ca^{2+} ions forming calcium phosphate ($Ca_3(PO_4)_2$), and ultimately hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), which then releases significant amounts of H^+ into the substrate solution (see Penn and Camberato 2019). In this case, the correlation with N can only be perceived as marginally related as N itself is bound to the same 10 and 20% zeolite variantsin terms of direct N supply from NO_3 and NH_4 forms.

Interestingly, a marked increase in the C: N ratio (a common indicator of substrate nutritional status) was recorded at the end of the experiment, potentially indicating soil depletion linked with leaching of N-mobile forms. However, this is unlikely to be an issue for sapling growth due to (i) the high overall N content at the start of the experiment, and (ii) the relatively short period that the seedlings are grown in the substrate (i.e. one season).

The temporal impact of zeolite application is emphasised by the study of Szatanik-Kloc et al. (2021), who questioned the effectiveness of zeolite (clinoptilolite) for improving soil conditions after observing no substantial long-term improvement following zeolite addition. In contrast to our own study, however, Szatanik-Kloc et al. (2021) employed relatively low zeolite doses (~0.04%) in mineral soil, whereas we utilised higher application rates in enriched, non-mineral substrates. In comparison, Ozbahce et al. (2015) achieved a similar improvement in yield, quality, and nutrient absorption as our own study after applying a zeolite dose of 90 t/ha, which corresponds well with a dosage of 7% zeolite in the top 10 cm of soil (assuming a bulk density of 1.3 g cm^{-3}).

5 Conclusion

In this study, we examined the effect of enriching growth media for container-grown European beech planting stock with clinoptilolite, a natural zeolite. The results revealed a dose-dependent effect, with lower concentrations (5%) supporting seedling growth and higher concentrations resulting in reduced aboveground growth, root collar diameter, root biomass development and root: shoot ratio, most likely due to imbalances caused by excessive nutrient competition or ionic interactions within the substrate. Furthermore, there was clear differentiation in both biometric and nutritional parameters, with type and amount of clinoptilolite affecting shoot height and type of substrate having a more pronounced effect on root collar thickness. Thus, the results support our original hypothesis.

Although potassium is generally known to promote root development, our results showed a decline in root biomass and root: shoot ratio with increasing clinoptilolite concentration, even when potassium was applied at high rates. This suggests that the potassium added was not fully utilised by the seedlings. The most likely explanation for this would be some form of antagonistic interaction with calcium and magnesium divalent base cations, both of which increased substantially in the sorption complex and aqueous solution with rising clinoptilolite concentration, indicating release triggered by excess potassium in the form of potassium sulfate.

While our findings confirm that addition of 5% non-enriched clinoptilolite enhances growth in container-grown beech seedlings, we nevertheless recommend follow-up studies focussed on a detailed assessment of clinoptilolite's ability to gradually and selectively release nutrients at low percentages, with particular emphasis on plant physiology, vitality and plantability in forest environments.

Beyond its relevance to nursery operations, this research highlights the nuanced trade-offs between substrate enrichment and seedling morphology, particularly root development. Our findings underscore the importance of carefully balancing clinoptilolite/zeolite concentration to avoid suppressing essential belowground growth while maximising nutrient availability. Given the essential role of European beech in Central European forest adaptation strategies, the findings presented here should serve as a preliminary reference for substrate optimisation aimed at improving seedling quality and planting success under future climate scenarios.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42729-025-02794-1.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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