University of Agriculture in Kraków
Faculty of Forestry

DOCTORAL THESIS

Odunayo James Rotowa
M. Sc. Forest Resources Management

The impact of seedling production in a container nursery on their growth on crop

Wpływ produkcji sadzonek w szkółce kontenerowej na ich wzrost w uprawie

work carried out under the supervision of
prof. dr. hab. eng. Stanisław Małek
University of Agriculture in Kraków
Faculty of Forestry
Department of Ecology and Silviculture

Kraków, 2025

Dedication

This work is dedicated to Almighty God who knows the end from the very beginning and from whom all blessings flows. He is the source of knowledge that profits. This same God, in His infinite mercy has given me another unmerited grace to complete this work even in the face of all odds, the grace so enormous that it might be confused for brilliance. Also, to all born and unborn lovers of knowledge.

I would like to express my heartfelt gratitude to:

prof. dr. hab. eng. Stanisław Małek,
my supervisor, for his immeasurable style of imparting knowledge through his untiring motivation and attentive mentorship. He has indeed demonstrated how highly committed he is to seeing that this work comes to a reasonable conclusion. He is a remarkable teacher to whom I owe great respect and admiration.

dr. hab. eng. Anna Klamerus-Iwan, prof. URK.

for inspiring my interest in the PhD School of this University and for kindly introducing me to my doctoral supervisor.

All the teaching and non-teaching staff of the Department of Ecology and Silviculture,
for their scientific and technical support, the creation of a conducive working environment, the spirit of collaboration, and their continuous kindness and assistance throughout this journey.

The Rotor's,
for their unwavering love, encouragement, and resilience. Your constant support has been a source of strength and motivation.

Friends,
who makes my stay in Poland comfortable and enjoyable, those who supported in data collection and entry, those who render words of encouragement during moments of fatigue through my PhD journey, all these meant so much to me. I am deeply grateful.

Abstract

This study examines how innovative peat-free substrates and different fertilization regimes affect the early growth of container-grown *Fagus sylvatica* L. and *Quercus robur* L. seedlings. Focus was placed on above-ground biometry, root system traits, and biomass–nutrient allocation. Seedlings were cultivated in conventional peat-based substrate and three innovative peat-free substrates (R20, R21, R22), each combined with either traditional solid fertilizer (as used in Forest District Daleszyce) or novel liquid fertilizer developed by the University of Agriculture in Kraków. The materials were developed under the NCBiR-funded POIR.04.01.04−00−0016/20 project, led by Prof. Stanisław Małek. After nursery cultivation, seedlings were planted in Miechów Forest District, and evaluated a year later. Solid fertilizer consistently led to better growth performance across species. Among the substrates, R22 showed the closest growth results to peat, particularly with solid fertilizer. Although peat-free variants supported good survival, shoot and biomass growth varied with species and treatments. Root morphological analysis indicated strong effects of substrate-fertilizer interactions. In beech, very fine roots traits (≤ 0.50 mm) correlated with shoot growth, suggesting a surface-oriented, phototropic strategy. In oak, total root length was more predictive of shoot development, reflecting a deeper, gravitropic strategy. Nutrient allocation patterns showed species-specific responses. Peat and R22, especially under solid fertilization, promoted higher N, P, and K accumulation. Very fine roots were critical for above-ground growth in beech but less so in oak. The results stress the importance of species-specific nursery protocols. Peat-free mixes like R22 are promising for sustainable forestry, but fertilization optimization remains key. The findings offer practical insights for climate-smart reforestation and environmentally conscious nursery practices.

Keywords: forest establishment, biometric traits, peat-free substrates, seedling survival, nutrient allocation, root diameter classification

Streszczenie

Niniejsze badanie analizuje wpływ innowacyjnych, beztorfowych podłoży szkółkarskich oraz różnych reżimów nawożenia na wczesny wzrost siewek buka zwyczajnego (*Fagus sylvatica* L.) i dębu szypułkowego (*Quercus robur* L.) uprawianych w kontenerach szkółkarskich. Szczególną uwagę poświęcono biometrii części nadziemnych, cechom systemu korzeniowego oraz alokacji składników pokarmowych w biomasie. Siewki uprawiano w konwencjonalnym podłożu torfowym oraz w trzech innowacyjnych podłożach beztorfowych (R20, R21, R22), w połączeniu z tradycyjnym nawozem stałym (stosowanym w Nadleśnictwie Daleszyce) lub nowatorskim nawozem płynnym opracowanym przez Uniwersytet Rolniczy w Krakowie. Materiały te zostały opracowane w ramach projektu POIR.04.01.04−00−0016/20, finansowanego przez NCBiR i kierowanego przez prof. Stanisława Małka. Po zakończeniu uprawy w szkółce, siewki zostały posadzone w Nadleśnictwie Miechów i ocenione po roku. Nawożenie stałe wykazywało konsekwentnie lepsze wyniki wzrostu u obu gatunków. Spośród badanych podłoży, R22 wykazało wyniki najbardziej zbliżone do torfu, zwłaszcza w połączeniu z nawozem stałym. Choć warianty beztorfowe zapewniały dobrą przeżywalność, wzrost pędów i biomasy był zróżnicowany w zależności od gatunku i zastosowanego wariantu uprawy. Analiza wykazała silny wpływ interakcji pomiędzy podłożem a nawożeniem na morfologię systemu korzeniowego. U buka cechy bardzo drobnych korzeni (≤ 0,50 mm) korelowały ze wzrostem pędu, co sugeruje strategię powierzchniową, fototropową. U dębu większe znaczenie dla rozwoju pędu miała całkowita długość korzeni, co odzwierciedło strategię głębokiego zakorzenienia i geotropizmu dodatniego. Wzorce alokacji składników pokarmowych wykazały reakcje swoiste dla danego gatunku. Podłoża torfowe i R22, szczególnie przy nawożeniu nawozem stałym, sprzyjały wyższemu nagromadzeniu azotu (N), fosforu (P) i potasu (K). Bardzo drobne korzenie były kluczowe dla wzrostu nadziemnego u buka, natomiast u dębu miały mniejsze znaczenie. Wyniki podkreślają konieczność dostosowania postępowania hodowlanego do specyfiki gatunku. Podłoża beztorfowe, takie jak R22, są obiecującą alternatywą dla zrównoważonego leśnictwa, jednak optymalizacja nawożenia pozostaje kluczowa. Uzyskane wyniki dostarczają praktycznych wskazówek dla leśnictwa odpornego na zmiany klimatu oraz środowiskowo odpowiedzialnych praktyk szkółkarskich.

Słowa kluczowe: odnawianie lasu, cechy biometryczne, podłoża beztorfowe, przeżywalność siewki, alokacja składników pokarmowych, klasyfikacja korzeni

Content

List of Abbreviations

Abbreviation	Definition
ANOVA	Analysis of Variance
ARD	Average Root Diameter
Ca	Calcium
C	Carbon
DMRT	Duncan’s Multiple Range Test
EU	European Union
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
K	Potassium
LECO CNS	Carbon-Nitrogen-Sulfur Analyzer (LECO Corporation)
Mg	Magnesium
N	Nitrogen
PCA	Principal Component Analysis
P	Phosphorus
RV	Root Volume
RSA	Root Surface Area
R20, R21, R22	Peat-Free Organic Substrates
SC	Peat-Based Control Substrate with Solid Fertilizer
SPC	Seedlings in Perfect Condition
SR20, SR21, SR22	Peat-Free Organic Substrates with Solid Fertilizer
TRL	Total Root Length
TSS	Total Survived Seedlings
UC	Peat-Based Control Substrate with Liquid Fertilizer
UR20, UR21, UR22	Peat-Free Organic Substrates with Liquid Fertilizer
VFL	Very Fine Root Length ($\leq$ 0.5 mm)
VFSA	Very Fine Root Surface Area
1YAP	One Year After Planting

1. Work structure

The doctoral dissertation takes the form of a thematic collection of works published in scientific journals:

Rotowa, O.J. Małek, S. Banach J. Pach, M. (2023). Effect of different innovative substrate mediums on roots characterization of European beech (*Fagus sylvatica* L.) and Pedunculate oak (*Quercus robur* L) seedlings. Sylwan, 167 (9).
(MNiSW=140; IF:0.5)

Rotowa, O.J., Małek, S., Jasik, M., Staszel-Szlachta, K. (2025). Effect of innovative peat-free organic growing media and fertilizer on nutrient allocation in pedunculate oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.) seedlings after nursery production cycle. New Forests 56:171-22
(MNiSW=100; IF:1.9)

Rotowa, O.J. Małek, S. Jasik, M. Staszel-Szlachta, K (2005). Substrate and Fertilization Used in the Nursery Influence Biomass and Nutrient Allocation in *Fagus sylvatica* and *Quercus robur* Seedlings After the First Year of Growth in a Newly Established Forest. Forests, 16, 511.
(MNiSW=100; IF:2.4)

Rotowa, O.J. Małek, S. Kupka D. Pach, M. Banach J. (2025). Innovative peat-free organic substrates and fertilizers influence growth dynamics and root morphology of *Fagus sylvatica* L. and *Quercus robur* L. seedlings one year after planting. Forests, 16, 800.
(MNiSW=100; IF:2.4)

I played a leading role in the research and reporting activities that formed the basis of these publications. My responsibilities included study design, experimental layout, field data collection (biometric measurements, root system analysis), and laboratory preparation for chemical analysis. I conducted most of the statistical analyses and led the drafting of manuscripts, including figures, tables, and literature synthesis. I also contributed to data interpretation, co-authored key sections, and managed the submission and revision process. As both lead and corresponding author, I ensured consistency across the four core manuscripts. Detailed contributions are outlined in the attached *Author Contribution Declarations* document.

The research was carried out under the project titled *"Innovative technologies for the production of substrate and fertilizer from indigenous resources for the cultivation of forestry tree seedlings"* (Project No. POIR.04.01.04-00-0016/20) carried out at the Department of Ecology and Silviculture, University of Agriculture in Krakow. Funded by the National Centre for Research and Development through national resources and the European Regional Development Fund, the project was led by prof. dr. hab. eng. Stanisław Małek and focused on developing sustainable, locally sourced solutions for nursery production of forestry seedlings.

2. Introduction

Global efforts to combat climate change, promote biodiversity, and restore degraded ecosystems have brought reforestation and afforestation to the forefront of environmental policy and research agendas (Pérez-Ramos *et al.,* 2010). Central to the success of these initiatives is the use of high-quality planting stock capable of surviving and thriving in newly established forest environments. Among the most influential factors shaping early seedling performance are nursery substrate composition and fertilization strategies, which directly affect plant morphology, physiology, and resilience post-transplantation (Grossnickle, 2012; Banach *et al.*, 2020). Reforestation and afforestation remain pivotal strategies for mitigating climate change, enhancing biodiversity, and restoring degraded ecosystems. A key determinant of success in such forest restoration efforts is the early performance of seedlings after the out-planting process. This is largely shaped by nursery practices during the seedling production (Pérez-Ramos et al., 2010; Grossnickle, 2012). In particular, the method of seedling cultivation in containerized nursery systems has gained global attention due to its potential to standardize plant quality, improve root morphology, and increase post-planting survival rates (Ivetić & Škorić, 2013; Banach *et al.*, 2020).

Using container-grown seedlings offers numerous advantages, including better root plug integrity, reduced transplant shock, and increased planting flexibility. However, translating these nursery gains into long-term forest performance requires a deeper understanding of how nursery production inputs especially substrate composition and fertilization type impact on root development and above-ground growth over time (Rotowa *et al.*, 2023; Kormanek *et al.*, 2023). Traditional nursery substrates are largely peat-based, valued for their consistency. Simultaneously, fertilization practices in forestry nurseries are evolving. While slow-release solid fertilizers have traditionally supported robust seedling growth, recent studies suggest that novel liquid fertilizers may improve nutrient uptake efficiency, reduce environmental runoff, and better match the dynamic needs of growing plants (Priya *et al.*, 2024; Rotowa *et al.*, 2025b). However, the effectiveness of these liquid formulations especially in combination with peat-free substrates remains poorly understood.

Peat-based substrates have long dominated nursery production due to their exceptional water retention capacity, nutrient buffering ability, and favorable physical structure. However, peat extraction has become increasingly controversial due to its ecological consequences, including habitat loss, carbon release, and soil degradation (Schmilewski, 2013; EPAP, 2021). Between 2012 and 2022, peat extraction in Europe increased by over 300%, from 6 thousand tons to 20 million tons annually, prompting EU Member States to enforce stricter regulations and promote the use of sustainable alternatives (EPAP, 2021; EU, 2018). This growing environmental imperative has accelerated the search for innovative, organic, peat-free substrates that maintain or improve plant performance without compromising ecological sustainability. Several recent studies, including Rotowa *et al.* (2023, 2025a), have introduced innovative substrate blends based on composted woody materials, such as coniferous shavings, wood chips, and bark, enriched with silage and perlite. These substrates, when coupled with tailored liquid fertilization regimes, have demonstrated potential in controlled nursery conditions. Yet, the translation of nursery performance into successful field establishment and early growth remains a critical gap. Notably, while above-ground biometrics such as height and diameter are often prioritized in such studies, root system morphology which plays a pivotal role in water and nutrient uptake, anchorage, and stress tolerance is frequently overlooked (Makita *et al.*, 2011; Zhang & Wang, 2015; Prasad *et al*., 2023).

Root system architecture, particularly the development of very fine roots (≤ 0.5 mm), plays a vital role in water and nutrient uptake, carbon storage, and overall plant health (Makita et al., 2011; Pregitzer et al., 2002). Yet, this critical aspect is often underrepresented in seedling quality assessments, which focus on shoot height and stem diameter alone. Root diameter classification is particularly important for predicting below-ground carbon responses (Zhang and Wang, 2015). Very fine roots (less than 0.5 mm in diameter) serve as better indicators of root function than the traditional category of roots less than 2 mm in diameter (Makita, *et al*, 2011). Very fine roots exhibit species-specific traits and can adapt their nutrient and water uptake potential in response to soil depth through plasticity in root biomass and length, adjusting their morphology and physiology accordingly (Park *et al.*, 2008; Makita, *et al*, 2011; Pregitzer *et al*, 2022). Fine roots (0.5 mm - 2 mm in diameter) are critical for nutrient cycling in terrestrial ecosystems; acting as both sinks and sources of nutrients (Ding *et al*., 2019; Zhou *et al*., 2021) they are dynamic and play a crucial role in carbon cycling and accumulation in forest ecosystems. These fine roots exhibit remarkable plasticity, adjusting their biomass and architecture in response to environmental cues and contributing significantly to nutrient cycling and carbon storage

(Pregitzer *et al.*, 2002; Park *et al.*, 2008) but comprised less than 5% of forest biomass (Makita, *et al*, 2011). In contrast, coarse roots (> 2.0 mm in diameter) differ markedly in morphology, nutrient concentrations, functions, and decomposition mechanisms. They often reflect aboveground biomass and factors such as tree size and age have been suggested as predictors of their size

This PhD research addresses the gap by investigating how nursery-based interventions specifically containerized production using innovative peat-free substrates and contrasting fertilizer types affect the early growth dynamics and root development of *Fagus sylvatica* L. (European beech) and *Quercus robur* L. (pedunculate oak) after their first year of growth in a newly established forest. Drawing on a series of nursery and field trials, the study examined species-specific responses in both shoot and root traits to determine: (i) whether novel peat-free nursery substrates and fertilizers (solid or liquid) can match or exceed the performance of conventional peat-based substrate. (ii) how nursery production influence root morphology and nutrient allocation after one year growth on crop; and (iii) which root characteristics best predict above-ground growth during first year after forest establishment.

3. Justification for the choice of the research topic, the aim of the work and the research hypotheses

The sustainability and success of forest restoration are increasingly dependent on the quality and adaptability of seedlings used during plantation establishment. In this context, the method of seedling production particularly container nursery systems have emerged as a critical factor influencing post-planting performance. However, despite their widespread adoption, the long-term field outcomes of container-grown seedlings are still not fully understood, particularly under varying environmental and silvicultural conditions. This research responds directly to this gap by evaluating how nursery production practices impact seedling performance after field planting, with a specific focus on biomass and nutrient allocation, root system development and shoot growth dynamics.

Globally, climate change and land-use degradation have prompted intensified reforestation efforts, requiring more efficient and ecologically sound seedling production methods. Conventional nursery practices often rely on peat-based substrates and solid fertilizers, which, although effective, present ecological and regulatory challenges. Peat extraction contributes significantly to greenhouse gas emissions and biodiversity loss, leading to increasing restrictions on its use across Europe and other parts of the world (EPAP, 2021; Gruda, 2012). Furthermore, the nutrient delivery efficiency of traditional solid fertilizers is being questioned, especially in light of new, more targeted fertilization strategies such as liquid applications. Thus, evaluating sustainable alternatives like peat-free organic substrates and novel liquid fertilizers is essential to ensure the environmental compatibility of forest nursery systems.

While several studies have examined the growth of seedlings under various substrate and fertilizer combinations in controlled nursery environments (Stewart *et al.*, 2018; Banach *et al.,* 2020; Madrid-Aispuro *et al.*, 2020; Harayama *et al.*, 2021; Popović *et al.*, 2015) relatively few have traced these effects into the field. Yet, the transition from nursery to forest is a critical period in seedling development, often determining long-term survival and growth potential. The current research, therefore, justifies its relevance by bridging this knowledge gap: it evaluates the carry-over effects of nursery inputs on field performance, particularly in terms of survival, biomass allocation, and root-shoot coordination strategies in two ecologically significant species *Fagus sylvatica* and *Quercus robur*.

Moreover, the functional role of root morphology especially the development of very fine roots is increasingly recognized in forest ecology for its contribution to nutrient uptake, water acquisition, and nutrient cycling. Yet, few studies incorporate detailed root architecture assessments into evaluations of seedling quality and field performance. This research addresses that limitation by integrating root trait classification and morphometric analysis to better understand how nursery decisions influence field outcomes. Finally, from a practical and policy perspective, this study supports the urgent need for climate-smart forestry practices. By exploring environmentally friendly alternatives to conventional peat based substrates and fertilizers, and linking nursery performance to forest establishment, the findings have the potential to inform forestry guidelines, nursery production, and reforestation policies both locally and globally.

The primary purpose of this study is to examine the long-term effects of nursery production practices specifically container-based cultivation using different innovative substrates and fertilization methods on the growth performance and development of forest seedlings after planting in the forest. As global forestry moves toward more sustainable practices, there is an urgent need to assess whether eco-friendly nursery inputs, such as peat-free substrates and liquid fertilizers, can match or exceed the effectiveness of traditional materials (peat based substrate and solid fertilizer) in supporting early seedling establishment and resilience in forest environments. This study focuses on two ecologically and commercially important tree species *Fagus sylvatica* and *Quercus robur* to investigate how different nursery substrate compositions (peat-based vs. innovative peat-free blends) and fertilizer types (solid vs. liquid) affect: biometric growth: including seedling height, stem diameter, and survival rates one year after forest planting; root system development: characterized through detailed morphometric parameters such as total root length, root surface area, average diameter, and volume, including root diameter class analysis (very fine, fine, coarse); biomass and nutrient allocation: evaluating how treatments influence carbon and nutrient partitioning and accumulation post-transplant.

The study tested the following hypothesis:

1) influence of various innovative peat-free substrate media on the morphological development and diameter classification traits of the root systems of the studied species at the end of seedling production in nursery (just before planting on crop in forest) and after one year growing on the crop can match or exceed the performance of conventional peat-based substrates;

2) innovative substrate composition and fertilization practices have lasting legacy effects on seedling overall growth performance one year after planting in the field;

3) novel liquid fertilizer can provides more stable and effective nutrient delivery than traditional solid fertilization, enhancing seedling biomass production, nutrient use efficiency, and long-term growth potential across different substrate compositions;

4) innovative peat-free treatments would support root traits comparable to those observed under conventional peat-based methods. Given their differing growth strategies, the studied species were anticipated to exhibit species-specific responses to these treatments. Furthermore, specific root morphological traits were hypothesized to vary in their relationship with early shoot development between the two species.

4. Materials and methods

The research included the following stages (the location of the activities carried out is given in brackets):

1. preparation of the experiment - containers with different substrates (Department of Forest Utilization, Forest Engineering and Technology) under the supervision of dr. hab. eng. Mariusz Kormanek, prof. URK;

2. sowing of containers with beech and oak seeds and growing of seedlings (Suków Papiernia Nursery Farm, Daleszyce Forest District under the supervision of dr. hab. eng. Jacek Banach, prof. URK);

3. establishment of forest experimental plantation, protection and seedling management (Barbarka, Miechów Forest District under the supervision of prof. dr. hab. eng. Stanisław Małek, in collaboration with Miechów Forest District);

4. laboratory analyses of the substrate and plant material:

a) biometric measurements (Department of Forest Utilization, Forest Engineering and Technology under the supervision of dr. hab. eng. Mariusz Kormanek, prof. URK and Department of Ecology and Silviculture, under the supervision of prof. dr. hab. eng. Stanisław Małek;

b) root system scanning and diameter classification (Laboratory of Forest Biotechnology, Department of Ecology and Silviculture, under the supervision of prof. dr. hab. eng. Stanisław Małek in cooperation with, dr. hab. eng. Jacek Banach prof. URK);

c) soil and chemical analyses (Laboratory of Forest Environment Geochemistry and Land Intended for Reclamation, Department of Ecology and Silviculture under the supervision of prof. dr. hab. eng. Stanisław Małek);

5. development of the obtained results (under the supervision of prof. dr. hab. eng. Stanisław Małek in collaboration with the team members of each publication).

4.1. Preparation of the experiment

The methodology, taking into account the division into species and type of research (concerning biometry, root system and chemical analyses), was included in all works constituting the basis of this doctoral dissertation.

The experiment used a peat substrate produced by the Nursery Farm in Nędza (N 50.167964, E 18.3138334) with the following composition: peat 93%, perlite 7%, with the addition of dolomite (3 kg per 1 m^3 of substrate) to obtain pH = 5.5. The peat used to prepare the substrate was characterized by a maximum degree of decomposition of 15%, organic matter content >85%, granulometric composition: 10.1÷20 mm – 2.5%, 4.1÷10 mm – 12.5%, 2.1÷4.0 mm – 12.5%, <2.0 mm – 72.5%, air capacity 15÷25% vol. water capacity 70-80% vol., at 10 cm H_2O, total porosity 85÷95% vol., humidity approx. 65%, pH in H_2O 3.0÷4.5, salinity up to 0.12 m^S·cm (based on the report of the Institute of Horticulture in Skierniewice for the Container Nursery in Nędza). The research used HIKO V300 polypropylene containers, with dimensions of 650x3126x150 mm (length, width, height), with 53 cells, each with a volume of 275 cm^3. The diameter of the entrance hole to the cell is 5.2 cm, while the diameter of the outlet hole from the cell is 2.5 cm with an average flow area of 13.1 cm^2 for both beech and oak. The cell containers narrow towards the bottom and are equipped with internal guide ribs preventing spinalizations of the root system (BCC HIKO). It was planned to obtain substrate with different levels of compaction in the containers (Photo 1).

***Photo 1**. Container filled with substrate and sown (Kormanek and Małek, 2023)*

The peat-free substrates (R20, R21, and R22), derived primarily from coniferous woody material (mainly pine) were composed of varying mixtures of shavings, wood chips, straw, bark, perlite, core wood, and mixed silage, with specific proportions detailed in Table 1. Four substrate types were used in total: the three peat-free variants and a traditional peat-based substrate, which served as the control. Each substrate was tested under two fertilization regimes: standard solid fertilizer used in Daleszyce Forest District (S) and an innovative liquid fertilizer (U) developed by the University of Agriculture in Kraków. This resulted in treatment variants SR20, SR21, SR22 (solid fertilizer), and UR20, UR21, UR22 (liquid fertilizer), with the peat substrate designated as SC and UC, respectively. The specific peat components used in the control substrate are listed in Table 2, while the particle sizes prior to sowing varied across treatments, as indicated in Table 3. Although the initial nutrient content of all substrates was standardized before sowing, differences in nutrient composition emerged by the end of the seedling growing period (Table 4).

Table 1. *Properties of the organic peat-free substrate [%] (Rotowa et al., 2023).*

Substrate	Sawdust	Wood bark	Perlite	Core wood	Mixed silage	Wood chips	Straw
R20	73	10	4	2	1	10	-
R21	20	10	4	2	1	63	-
R22	50	33	4	2	1	-	10

Table 2. *Physicochemical properties of substrates used in seedling growth in the Nursery. (±SD).* *[abcd] – significance differences between means (DMRT) (Rotowa et al., 2023).*

Substrate	Water capacity (%)	Water outflow rate (litre/min)	Bulk density (g/cm3)	Solid density (g/cm3)	Air capacity (%)	Porosity (%)
R20	40.5±2.9[b]	0.595 ±0.150[b]	0.115 ±0.009[a]	0.64±0.08[a]	52.1±3.19[c]	92.6±0.60[d]
R21	33.1±2.5[d]	0.781±0.114[a]	0.098±0.014[c]	1.74±0.07[a]	60.8±3.06[a]	93.6 ±0.87[c]
R22	37.8±5.1[c]	0.594±0.150[b]	0.104±0.020[b]	1.66±0.11[a]	55.8±5.58[b]	93.9±0.98[b]
Control	57.7±5.4[a]	0.417 ±0.145[c]	0.085±0.007[d]	1.69±0.14[a]	37.0±5.72[d]	94.7±0.42[a]
F	387.45	56.32	65.81	1.0717	295.79	76.48
p	0.0000	0.0000	0.0000	0.3870	0.0000	0.0000

Letters with different alphabet indicate statistically significant differences between means (p < 0.05 t-test).

Table 3. *Granulometric composition of the substrate before sowing (Rotowa et al., 2023).*

Substrate	> 10	10 < 5	5 < 2	2 < 1	1 < 0.5	0.5 < 0.25	0.25 < 0,1	> 0.1
					[mm]			
R20	0.05±0.10	3.77±1.57	14.45±5.90	30.53±9.72	24.45±2.24	17.72±7.49	7.71±4.16	1.69±0.98
R21	0.00±0.00	6.40±1.37	25.44±1.91	30.90±1.11	19.11±0.90	12.16±0.31	5.12±0.41	0.96±0.11
R22	0.08±0.13	3.03±0.45	14.15±2.36	33.36±2.36	25.11±1.07	17.02±3.21	7.11±1.72	1.48±0.28
Control	0.00±0.00	11.27±0.37	25.08±1.18	27.77±1.05	16.20±1.05	8.42±0.56	3.81±0.43	1.88±0.21

Table 4. *Nutrient content of substrates before seed sowing and after seedling production (Rotowa et al., 2023).*

Substrate	C	N	P	K	Ca	Mg	Na	pH (H_2O)	µS/cm
				%					
Before sowing									
R 20	48.01	0.297	0.031	0.159	0.452	0.055	0.040	5.64	277
R 21	46.34	0.507	0.068	0.271	0.601	0.072	0.035	5.99	240
R 22	48.90	0.447	0.043	0.404	0.857	0.059	0.042	5.85	416
Control	45.85	0.709	0.015	0.058	1.307	0.585	0.068	5.65	147
After seedling production									
F. sylvatica									
SR20	42.167	0.596	0.093	0.129	0.721	0.068	0.018	5.863	469.2
SR21	39.978	0.996	0.134	0.161	0.985	0.087	0.020	5.979	695.1
SR22	42.167	0.756	0.110	0.156	1.463	0.086	0.023	5.72	648.7
SC	40.987	0.844	0.096	0.162	1.695	0.476	0.075	5.458	831.8
UR20	44.148	0.434	0.030	0.066	0.677	0.055	0.016	6.154	169.7
UR21	42.518	0.5323	0.049	0.072	0.854	0.055	0.015	5.931	214.5
UR22	42.93	0.578	0.043	0.074	1.179	0.066	0.018	5.987	211.6
UC	39.784	0.651	0.019	0.071	1.543	0.525	0.072	5.855	222.9
Q. robur									
SR20	45.455	0.519	0.059	0.991	0.589	0.056	0.014	5.751	382.7
SR21	43.313	0.942	0.121	1.626	0.879	0.088	0.020	5.896	580.3
SR22	45.096	0.872	0.114	1.703	1.139	0.081	0.018	5.567	844.8
SC	41.425	0.805	0.076	1.798	1.424	0.441	0.069	5.525	729.3
UR20	44.703	0.383	0.028	0.563	0.594	0.065	0.015	5.873	185.2
UR21	44.969	0.418	0.032	0.597	0.627	0.042	0.015	5.771	175.2
UR22	45.422	0.493	0.032	0.650	0.966	0.060	0.015	5.954	163.3
UC	41.863	0.654	0.016	0.654	1.392	0.472	0.060	5.796	177.9

SR= state substrate and fertilization, UR= University substrate and fertilization

4.2. Seed sowing and germination

Seed sowing took place on April 19–20th, 2022, and was conducted by workers at the container nursery Sukowie Papiernia in the Daleszyce Forest District. To improve germination, oak seeds were scarified immediately before sowing by removing approximately one-third of the cotyledon portion. In contrast, beech seeds were subjected to stratification without a substrate medium, maintained at a constant temperature of +3°C and relative humidity of 31%. All seeds used across the different substrate treatments, irrespective of species, originated from the same provenance and were accompanied by individual certificates of origin (MR/65848/21/PL for oak and MR/63313/20/PL for beech). After sowing, the containers were placed in a vegetation hall for four weeks before being moved to an outdoor production bed. Manual weeding was carried out during the growing period, and the seedlings were cultivated for five months following standard nursery procedures (Szabla and Pabian, 2009). Due to low total rainfall during this period, only 78 mm of supplementary irrigation was provided using an automated RATHMAKERS Gartenbautechnik sprinkler ramp to compensate for the water deficit (Photo 2).

Photo 2. *Supplementary irrigation system* *Photo by; Odunayo Rotowa*

4.3. Fertilization treatments

Osmocote fertilizer was applied once during substrate preparation prior to sowing, at a total rate of 3 kg·m^{-3} for each substrate type. This consisted of a blend of Osmocote 3–4M (2 kg) and Osmocote 5–6M (1 kg). The nutrient composition of Osmocote 3–4M was: total nitrogen (N) – 16%, including 7.1% nitrate nitrogen ($N–NO_3^-$) and 8.9% ammonium nitrogen ($N–NH_4^+$); phosphorus (P_2O_5) – 9%; potassium (K_2O) – 12%; magnesium oxide (MgO) – 2.0%; and trace elements (B, Fe, Cu, Mn, Zn, Mo). Osmocote 5–6M contained: N – 15%, including 6.6% $N–NO_3^-$ and 8.4% $N–NH_4^+$; P_2O_5 – 9.0%; K_2O – 12%; MgO – 2.0%; and the same set of microelements. The novel liquid fertilization regime was also implemented using two distinct formulations. The first variant contained 4.78% N, 1% P_2O_5, 2.64% K_2O, 2.65% CaO, 1.4% MgO, 0.71% SO_3, and 0.14% Na_2O. It was initially applied at a total volume of 3.14 dm^3 (0.048 dm^3·m^{-2}). The second variant included 0.798% N, 0.166% P_2O_5, 0.440% K_2O, 0.441% CaO, 0.234% MgO, 0.118% SO_3, and 0.023% Na_2O, and was administered at a total volume of 15.09 dm^3 (0.229 $dm^3 \cdot m^{-2}$). Throughout the seedling production period, the first liquid fertilizer was applied eight times at 10-day intervals, while the second was applied fifteen times at 5-day intervals. Importantly, the fertilization protocols were identical for both *Fagus sylvatica* and *Quercus robur* seedlings.

4.4. Laboratory analyses of soil and plant material

Prior to plantation establishment, soil samples were collected from two depths (0–10 cm and 10–20 cm) and analyzed for pH (in water and KCl), hydrolytic and exchangeable acidity, and exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) using ammonium acetate extraction. Total carbon and nitrogen were determined using a LECO CNS analyzer. Macroelement concentrations in plant tissues (N, P, K, Ca, Mg) were measured using inductively coupled plasma emission spectrometry (ICP-OES) following wet acid digestion. After the production cycle in the nursery, all parts of the seedlings (leaves, shoots and roots) were dried at 65°C (48 h). After drying, the dry mass was determined on an analytical balance with an accuracy of ±0.1 mg. The dried plant material of both species from different combinations of treatments was ground in a laboratory grinding machine into powder (Photos 3-5), and then analyzed for the content of N and S elements using a CNS TruMac analyzer (LECO Corporation) and P, K, Ca, Mg using an ICP-OES iCAP 6500DUO emission spectrometer (Thermo Fisher Scientific) after prior microwave mineralization in a mixture of nitric (V) and hydrochloric acids. The concentration of elements was obtained in % (g of element per 100 g of dry sample). The analyses were performed at the Laboratory of Geochemistry of the Forest Environment and Areas Designated for Reclamation, Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Kraków.

Before drying and grinding of root samples, all root samples (three per sub-plot, totalling 144 samples across both species) were processed as follows: root systems were carefully separated from soil and organic debris to preserve root integrity and maintain connections to larger roots (>2 mm diameter). The intact roots were gently rinsed with tap water and then with deionized water to remove residual soil without damaging delicate root tips. Morphological traits and three diameter classes: <0.5 mm (very fine), 0.5–2.0 (fine) and >2.0 mm (coarse) (Makitai *et al*., 2011;

Farahnaki *et al.*, 2020) were analyzed using the WinRhizo™ Pro 2003b image analysis system (Regent Instruments Inc., Quebec City, QC, Canada) (Photo 6). The same procedure was repeated on seedlings after one year of growth in the forest using destructive sampling methods.

Photo 3. *Seedling samples*

Photo 4. *milling machine*

Photo 5. *Powdered form of seedling organs*

Photo 6. *Root samples analysed*

Photos by; Odunayo Rotowa

4.5. Plantation establishment and seedling collection

At the end of the nursery phase, seedlings were transplanted into a 0.7-hectare afforestation site in Barbarka, within the Miechów Forest District in southern Poland (Photo 7) on September 5, 2022. The site, formerly planted with *Populus spp.*, featured uniform soil properties and was divided into subplots for each treatment. The field experiment was arranged in a randomized complete block design, comprising 8 treatments with 3 replications each, resulting in 24 subplots per species. In each subplot, 49 seedlings were planted at an inter- and intra-row spacing of 1 × 1.7 meters, totalling 147 seedlings per treatment and species. Altogether, 2,352 seedlings were established across both species. At the end of the 2023 growing season, 144 seedlings (3 per subplot) were selected for laboratory analysis, based on the average height of each subplot. These seedlings were carefully uprooted to preserve the entire root system. To protect the young plantation from animal disturbance, the area was fenced after establishment. Above-ground data collected included plant height, collar diameter, number of seedlings in perfect condition (SPC), and total survived seedlings (TSS). Below-ground development was assessed through root morphological characteristics: total root length (TRL), root surface area (RSA), average root diameter (ARD), and root volume (RV). Root diameters were further categorized into three classes: very fine (≤0.5 mm), fine (0.5–2.0 mm), and coarse roots (>2.0 mm).

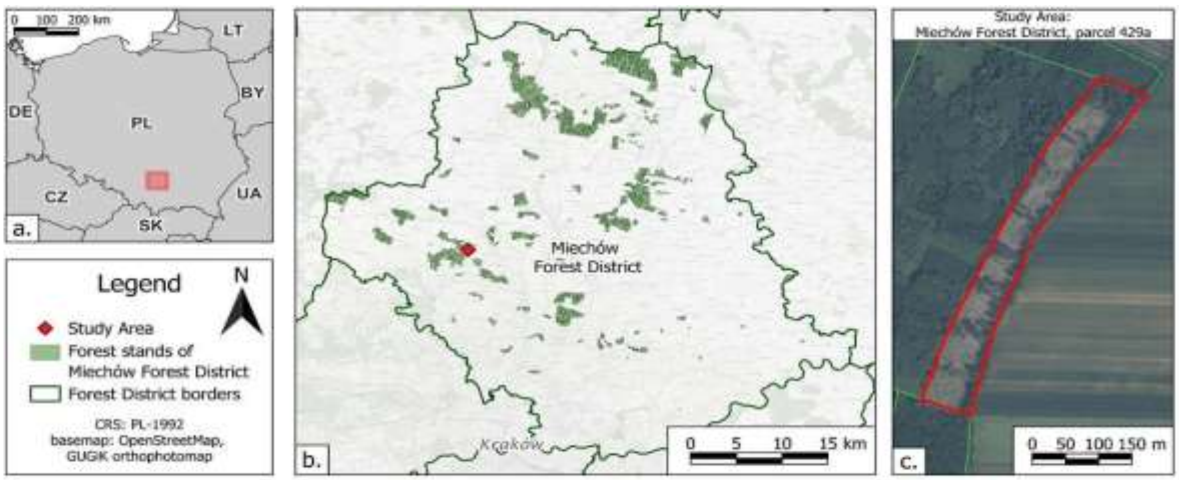

Photo 7. *The study area. a. Geographic location of the study site. b. Topographic map of the forest area. c. Satellite imagery showing the experimental plot layout. (source; QGIS 3.34)*

4.6. Statistical and data analysis

A comprehensive suite of statistical techniques was applied to evaluate the effects of nursery treatments on seedling morphology, nutrient allocation, and field performance. All analyses were conducted separately for *Fagus sylvatica* and *Quercus robur*. Prior to inferential statistics, data were assessed for normality using the Shapiro–Wilk test, and homogeneity of variances was verified. A two-way analysis of variance (ANOVA) was performed to test the main effects and interaction of substrate type and fertilizer regime. Where significant differences were identified, Duncan's Multiple Range Test (DMRT) was applied for pairwise comparisons, with statistical significance accepted at $p < 0.05$. Pearson correlation analysis was conducted to explore the relationships among growth variables and root morphological traits, and results were visualized using correlation matrices and heatmaps. In addition, Principal Component Analysis (PCA) was used to reduce dimensionality and identify the primary root and shoot variables that contributed to overall variation among treatment groups. To further assess predictive relationships, multiple linear regression models were developed using shoot height and root collar diameter as dependent variables, and root morphological traits such as total root length (TRL), average root diameter (ARD), root volume (RV), and root surface area (RSA) as predictors. All statistical analyses and visualizations were performed using Python (version 3.10, Python Software Foundation, Wilmington, DE, USA) and IBM SPSS Statistics (version. 26, IBM Corporation).

5. Results

5.1. Oak – results of root system studies after nursery production

The results of root system studies were published in the article: **Rotowa, O.J.,** Małek, S., Banach, J., Pach, M. (2023). *Effect of different innovative substrate mediums on roots characterization of European beech Fagus sylvatica L. and pedunculate oak Quercus robur L. seedlings*. Sylwan, 167(09).

Among the treatments, the peat-based substrate with solid fertilization (SC) yielded the strongest root collar diameter (RCD), indicating the most vigorous stem base development. This was followed by the SR22 variant, with the R22 peat-free substrate. In contrast, the poorest performance was observed in the UR20 treatment, where the combination of the R20 substrate and liquid fertilizer resulted in the weakest stem thickness. Statistically, there were significant differences among treatments for RCD ($p = 0.00$), indicating that substrate and fertilizer combinations had a measurable impact on stem thickness. The longest root development was recorded in seedlings treated with UR20, showing that the liquid fertilizer in combination with the R20 substrate effectively promoted root elongation. The second-best performance was observed in the SC treatment. On the other hand, UC treatment produced the shortest total root length (TRL), reflecting limited root extension when peat was combined with the university formulated liquid fertilizer.

Seedlings grown under the SC treatment exhibited the largest root surface area (RSA), closely followed by the UR21 treatment. However, the UC treatment had the lowest surface area among all variants. Peat combined with liquid fertilizer (UC treatment) produced a larger average root diameter (ARD). This was followed by the UR21 treatment, while the thinnest roots appeared in the UR20 variant. Seedlings in the SC treatment displayed the largest root volume (RV). The UR21 treatment ranked second. The smallest root volume was recorded under the UR20 treatment, reflecting relatively lower root mass production under this substrate-fertilizer combination. There was no significant effect observed among treatments in root morphological parameters. The observed descriptive variations could be due to natural variability rather than treatment effects (Table 5).

Table 5. *Mean and standard deviation of studied root system parameters of Q. robur (±SD).* [abcd] *– significance differences between means (DMRT) (Rotowa et al., 2023)*

Treatment	RCD (mm)	TRL (cm)	RSA (cm²)	ARD (mm)	RV (cm³)
SR20	1.66 ±0.32[c]	730.57 ±177.88[a]	113.94 ±18.81[a]	0.53 ±0.06[a]	1.51 ±0.33[a]
UR20	0.96 ±0.07[d]	894.97 ±112.22[a]	106.18 ±12.40[a]	0.38 ±0.01[a]	1.00 ±0.12[a]
SR21	1.19 ±0.13[d]	631.41 ±90.58[a]	111.33 ±12.35[a]	0.57 ±0.04[a]	1.60 ±0.22[a]
UR21	1.16 ±0.19[d]	619.11 ±125.43[a]	122.63 ±19.92[a]	0.67 ±0.14[a]	2.16 ±0.57[a]
SR22	2.15 ±0.49[b]	603.21 ±78.10[a]	109.63 ±11.48[a]	0.60 ±0.04[a]	1.61 ±0.19[a]
UR22	0.87 ±0.06[d]	820.04 ±179.87[a]	110.89 ±15.09[a]	0.46 ±0.04[a]	1.24 ±0.16[a]
SC	2.78 ±0.54[a]	843.29 ±134.75[a]	167.23 ±27.81[a]	0.64 ±0.08[a]	2.67 ±0.48[a]
UC	1.15 ±0.19[d]	494.63 ±126.36[a]	83.23 ±17.95[a]	0.99 ±0.49[a]	1.45 ± 0.33[a]
Total	1.49 ±0.69	704.65 ±128.15	115.63 ±16.69	0.61 ±0.16	1.65 ±0.44
P-value.	0.00**	0.38[ns]	0.12 [ns]	0.46[ns]	0.39[ns]

SR – State forest substrate and fertilization, UR – University substrate and fertilization, RCD – root collar diameter, TRL – total root length, RSA – surface area, ARV – avg. diameter, RV – root volume. The same letters in the same column are not significantly different while figures with different letter are significantly different at p=0.05

5.2. Oak – results of biometrics and root system studies one year after planting in the forest

The results of biometric and root system studies after nursery production circle was published in the article: **Rotowa, O.J**. Małek, S. Kupka D. Pach, M. Banach J. (2025). *Innovative peat-free organic substrates and fertilizers influence growth dynamics and root morphology of Fagus sylvatica L. and Quercus robur L. seedlings one year after planting*. Forests 2025, 16, 800.

The beech and oak sites exhibited slightly acidic soils typical of forest environments, with lower pH values observed at a 20 cm depth compared to 10 cm. The oak site maintained consistent N levels across both depths, while C content and P decreased with soil depth at both sites. The C/N ratio remained stable, indicating balanced soil nutrient cycling. Exchangeable cations were more concentrated at the shallower depth, suggesting better nutrient availability near the surface for early seedling growth. Statistical analysis showed no significant differences in soil properties between the sites, allowing for a controlled evaluation of treatment effects on seedlings (Table 6).

Table 6. *Soil properties of sampled plot of F. sylvatica and Q. robur at Barbarka experimental site (±SD). (Rotowa et al., 2023)*

Soil Uptake Level (cm)	**pH (H_2O)**	**N**	**C**	**P_2O_5**	**C/N**	**Exchangeable Cations**			
						Ca	**K**	**Mg**	**Na**
				***Fagus sylvatica* L. site**					
0–10	5.21 ± 0.55	0.36 ± 0.10	2.41 ± 0.21	3.14 ± 0.21	13.78 ± 0.32	5.51 ± 0.52	0.18 ± 0.01	0.57 ± 0.06	0.70 ± 0.08
10–20	5.10 ± 0.57	0.34 ± 0.09	1.94 ± 0.15	2.87 ± 0.14	12.90 ± 0.32	4.81 ± 0.39	0.16 ± 0.01	0.42 ± 0.03	0.63 ± 0.06
Total	5.15 ± 0.56	0.35 ± 0.10	2.16 ± 0.12	2.99 ± 0.12	13.30 ± 0.23	4.22 ± 0.32	0.15 ± 0.01	0.49 ± 0.03	0.55 ± 0.07
p-value	0.187^{ns}	0.170^{ns}	0.061^{ns}	0.286^{ns}	0.065^{ns}	0.064^{ns}	0.062^{ns}	0.062^{ns}	0.062^{ns}
				***Quercus robur* L. site**					
0–10	5.14 ± 0.59	0.45 ± 0.95	2.14 ± 0.22	4.70 ± 0.45	13.31 ± 0.33	5.11 ± 0.53	0.23 ± 0.02	0.55 ± 0.06	0.81 ± 0.26
10–20	5.03 ± 0.61	0.44 ± 0.09	1.83 ± 0.14	4.22 ± 0.40	12.23 ± 0.31	4.65 ± 0.36	0.21 ± 0.02	0.46 ± 0.03	0.82 ± 0.27
Total	5.08 ± 0.59	0.45 ± 0.10	1.55 ± 0.13	4.44 ± 0.30	12.73 ± 0.23	3.32 ± 0.32	0.22 ± 0.01	0.38 ± 0.03	0.82 ± 0.29
p-value	0.208^{ns}	0.945^{ns}	0.061^{ns}	0.427^{ns}	0.069^{ns}	0.061^{ns}	0.363^{ns}	0.061^{ns}	0.989^{ns}

^{ns} – non significance

After one full growing season in the forest, *Q. robur* seedlings displayed impressive biometric increases in certain treatments. Solid-fertilized seedlings C and R22 achieved mean shoot heights exceeding 57 cm, with collar diameters of 8.93 mm and 8.62 mm, respectively. Compared to their nursery values, these seedlings recorded absolute height increases of approximately 26 cm and collar diameter gains of 3–3.5 mm, equivalent to height increment rates of 77–82% and diameter increases of up to 64% .Interestingly, even some liquid treatments (e.g., C and R22) yielded moderate height increases (up to 45 cm), but these came with consistently lower diameter growth (7.68–7.96 mm), indicating a weaker basal thickening response (Table 7)

Table 7. *Biometric and increment rate of Quercus robur seedlings after one year on crop (±SD). ^{a,ef} – significance differences between means (DMRT) (Rotowa et al., 2025c)*

Treatment	Fertilization type	SPC	TSS	RSS (%)	After 1 year in the forest		After nursery production cycle		Absolute Increment	
					Height (cm)	Diameter (mm)	Height (cm)	Diameter (mm)	Height %	Diameter %
SR20	Solid	129	134	91	56.76±9.28^{a}	8.72±1.89^{a}	31.15±2.97^{a}	5.53±1.56^{a}	82	58
SR21		123	130	88	55.74±7.95^{a}	8.61±1.78^{a}	31.73±3.47^{a}	5.25±1.43^{a}	76	64
SR22		132	140	95	56.79±7.74^{a}	8.62±2.03^{a}	30.95±2.99^{a}	5.42±1.39^{a}	77	59
SC		145	145	99	57.07±6.89^{a}	8.93±2.11^{a}	31.09±2.99^{a}	5.66±1.55^{a}	77	59
Total					55.58±8.89	8.72±1.85	31.23±1.34	5.47±1.67		
p-value.					**0.103^{ns}**	**0.473^{ns}**	**0.564^{ns}**	**0.271^{ns}**		
UR20	Liquid	139	142	97	41.72±8.44^{f}	7.22±1.10^{e}	31.51±2.74^{e}	5.45±0.89^{e}	32	32
UR21		134	140	95	41.03±8.26^{f}	7.79±1.22^{e}	30.43±2.55^{e}	5.52±0.92^{e}	35	41
UR22		139	144	98	42.69±9.67^{f}	7.81±1.54^{e}	31.49±2.86^{e}	5.47±.095^{e}	36	43
UC		132	138	94	45.19±9.93^{e}	7.94±1.44^{e}	31.51±3.02^{e}	5.66±0.96^{e}	43	40
Total					42.66±9.08	7.69±1.32	31.23±2.94	5.52±0.94		
p-value.					**0.007****	**0.978^{ns}**	**0.305^{ns}**	**0.231^{ns}**		

SPC– Number of seedlings in perfect condition, TSS–Total survived seedlings, RSS– rate of seedling survival. S – State Forests solid fertilization, U – University novel liquid fertilization, R – novel substrates, C – control substrate (peat−perlite) (N =147)

After one year of growth in the freest, SR treatments consistently outperformed UR treatments in promoting root morphological properties 1YAP. There were statistically significant differences in total root length (TRL) across treatments liquid fertilization, while no significant variation was maintained under solid fertilization 1YAP. Among solid-fertilized treatments, SR21 and SR22 produced the longest roots, closely followed by SR20 and SC, with no significant difference between them. Liquid fertilizer treatments generally resulted in shorter root lengths, with UR22 recording the least. This indicates that solid fertilization supports better root elongation than liquid variants under both peat and peat-free conditions. Root surface area (RSA) showed significant differences across treatments. Among solid fertilized groups, SR21 and SR20 again led with the highest root surface area, reflecting a well-developed lateral root spread. Conversely, the liquid fertilized treatments especially UR22 and UR21recorded the lowest RSA values (Table 8).

Differences in average root diameter (ARD) were statistically significant for both seedlings. SR21, SC, and SR20 showed the thickest average root diameters under solid fertilization. Although UR22 had the highest ARD among liquid treatments, other liquid groups showed reduced root thickness. Surprisingly, root volume (RV) did not differ significantly across liquid fertilizer treatment, but was very highly significant in solid fertilizer treatment, 1YAP. While root length, area, and diameter changed, the total root biomass volume remained statistically similar across both fertilization types. Among all, SR21 stood out as the best-performing treatment across most parameters (Table 8).

Table 8. *Morphological parameters of the root system of Quercus robur seedlings under different substrate fertilizer treatment after one year in the forest. (±SD).* ^{abc,efg} *– significance differences between means (DMRT) (Rotowa et al., 2023)*

Treatment	**fertilization type**	**TRL (cm)**	**RSA (cm^2)**	**ARD (mm)**	**RV (cm^3)**
SR20		$1396.90±81.03^{a}$	$218.23±38.22^{a}$	$0.97±0.12^{a}$	$4.66±0.56^{b}$
SR21		$1445.48±83.89^{a}$	$219.83±30.32^{a}$	$1.03±0.13^{a}$	$6.01±1.07^{a}$
SR22	**solid**	$1443.91±95.26^{a}$	$189.41±18.21^{b}$	$0.79±0.14^{b}$	$3.68±0.48^{c}$
SC		$1386.20±82.95^{a}$	$187.28±20.75^{b}$	$1.03±0.14^{a}$	$4.24±0.76^{bc}$
Total		1418.13±86.59	203.69±31.00	0.95±0.16	4.65±1.13
P-value.		**0.336** ^{ns}	**0.024***	**0.001****	**0.000****
UR20		$1329.14±59.57^{f}$	$144.58±19.36^{f}$	$0.99±0.32^{ef}$	$3.41±0.48^{e}$
UR21		$1345.08±44.24^{f}$	$125.69±25.66^{g}$	$0.81±0.06^{f}$	$2.44±0.49^{e}$
UR22	**liquid**	$1248.43±68.06^{g}$	$109.37±15.36^{g}$	$1.07±0.19^{e}$	$2.99±0.11^{e}$
UC		$1453.88±65.88^{e}$	$164.48±10.42^{e}$	$0.87±0.18^{ef}$	$3.36±1.19^{e}$
Total		1344.13±93.91	136.03±27.42	0.94±0.23	3.05±1.43
P-value.		**0.000****	**0.000****	**0.051***	**0.471** ^{ns}

TRL- Total root length, RSA- Root surface area, ARD- Average root diameter, RV- Root volume. Letters with different letters indicate statistically significant differences between means (p < 0.05). Letters 'a, b' and 'c' denote homogeneous groups under solid fertilization and 'e, f' and 'g' denote homogeneous groups under liquid fertilization.

The comparison between the nursery and one-year post-planting phases reveals meaningful differences in the performance of *Quercus robur* seedlings under various substrate and fertilization treatments. During the nursery phase, seedlings grown in the peat-based substrate combined with solid fertilizer demonstrated significantly superior performance across most root system parameters. These results positioned SC as the best-performing treatment at the initial stage of development. However, after one year in the field, this early advantage did not hold, as seedlings from the peat-free substrate R21 with solid fertilizer (SR21) surpassed SC in key metrics. This shift suggests that while peat-based treatments promote early growth, certain peat-free formulations, particularly R21 with solid fertilizer, offer better adaptation and sustained root development in natural field conditions 1YAP. The observed transition in performance indicates that an initial size advantage conferred during the nursery phase does not necessarily predict long-term success in the field. Furthermore, the performance of seedlings under liquid fertilization treatments (UR20, UR21, UR22, and UC) declined over time. In the nursery phase, some of these seedlings exhibited comparable root traits to those in solid-fertilized variants. However, after one year in the forest, their values were significantly lower. Finally, the adaptability of certain peat-free substrates, particularly R21, when combined with solid fertilizer was ascertained (Table 8).

Regression results show only parameters with significant predictor. Total root length was the only significant predictor of oak height, while no root-class trait predicted diameter, reinforcing the notion that multiple factors govern oak stem thickening post-transplant. The result of root system traits at 1YAP shows that root architecture continued to reflect species-specific patterns. The highest TRL and coarse root volume (>2 mm) were recorded in SC and SR21, affirming the effect of solid fertilization in promoting robust anchorage systems. In contrast, while promoting fine root traits, liquid treatments development, confirming oak's preference for deeper structural rooting (Table 9).

Table 9. *Model estimates for above ground parameters of F. sylvatica and Q.robur one year after planting in the forest (Rotowa et al., 2025c)*

Species/ Dependent Variable	Predictor	Coefficient	Std. Error	P-value	CI Lower	CI Upper	Adjusted R^2
F. sylvatica/ Plant height	VFL	0.061	0.017	0.001	0.027	0.095	0.619
	VFSA	-3.195	1.213	0.011	-5.623	-0.766	0.619
F. sylvatica/ Plant diameter	VFL	0.011	0.002	0.000	0.006	0.016	0.644
	VFSA	-0.351	0.169	0.043	-0.689	-0.011	0.644
Q.robur/ Plant height	TRL	-0.034	0.013	0.009	-0.059	-0.009	0.530

TRL- Total root length (cm), VFL (≤ 0.50) - Very-fine length (cm), VFSA (≤ 0.50)- Very fine surface area (cm^2)

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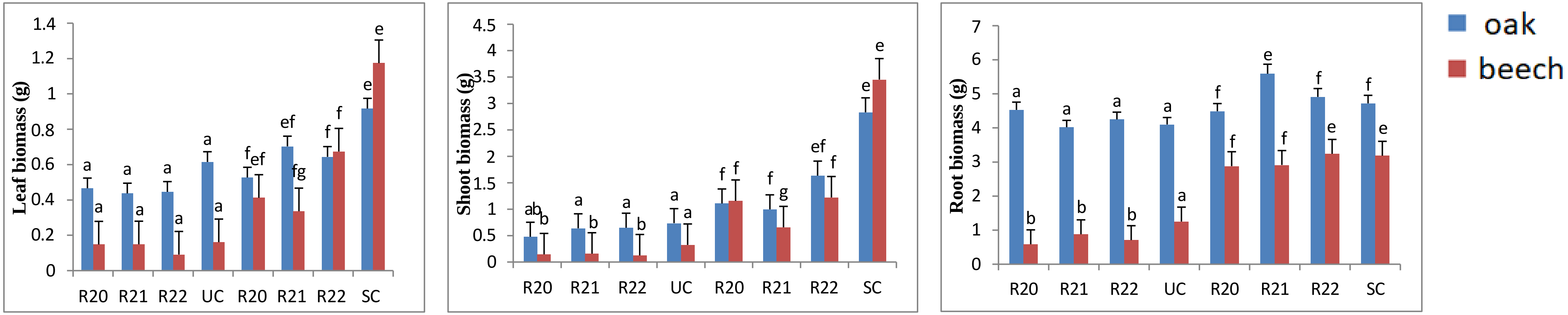
5.3. Oak – biomass and element content studies after nursery production

The results of biomass and element content after the nursery production cycle were published in the article:**Rotowa, O.J.,** Małek, S., Jasik, M., Staszel-Szlachta, K. (2025). Effect of innovative peat-free organic growing media and fertilizer on nutrient allocation in pedunculate oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.) seedlings after nursery production cycle. New Forests 56:171-22.

During the nursery phase, *Quercus robur* seedlings demonstrated a clear response to both substrate type and fertilization method in terms of biomass accumulation and nutrient allocation. Seedlings treated with solid fertilizer produced higher total biomass in all organs (shoot, leaf, and root) compared to those under liquid fertilizer. Notably, the SC (peat-based) and SR22 (peat-free with bark and perlite) treatments yielded the highest dry matter accumulation in oak seedlings (Fig. 1a-c). Principal Component Analysis (PCA) highlighted that fertilization method, rather than substrate, was the dominant factor shaping nutrient profiles in oak root tissues, as observed in clear separation patterns in the PCA plots (Figures 3-5). Analysis of nutrient content in seedling organs revealed substantial variation across treatments. Under liquid fertilization, the R22 substrate promoted the highest macronutrient concentrations of nitrogen (N), phosphorus (P), and potassium (K) in roots and shoots, while under solid fertilizer, the control SC treatment supported the greatest overall accumulation C, N, P, K, Ca across organs (Table 10). Correlational analysis revealed strong positive relationships between biomass and virtually all the studied elements (Table 11).



A. Liquid fertilizer Solid fertilizer B. Liquid fertilizer Solid fertilizer C. Liquid fertilizer Solid fertilizer

Letters 'a' and 'b' denote homogeneous groups under liquid fertilization and 'e,f' and 'g' denote homogeneous groups under solid fertilization.

Fig. 1a-c: *biomass allocation across different substrate in Quercus robur L. and Fagus sylvatica L. species (Rotowa et al., 2025a)*

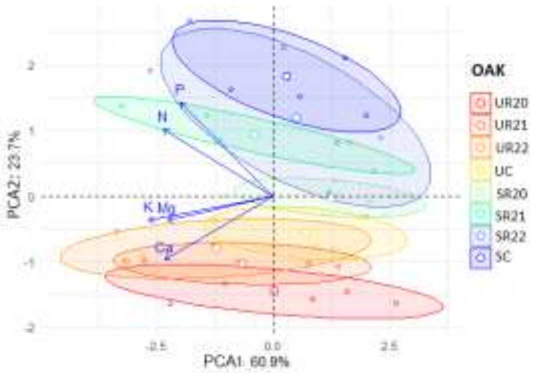


Fig. 2. *Nutrient allocation in oak root grown on different growing medium (Rotowa et al., 2025a)*

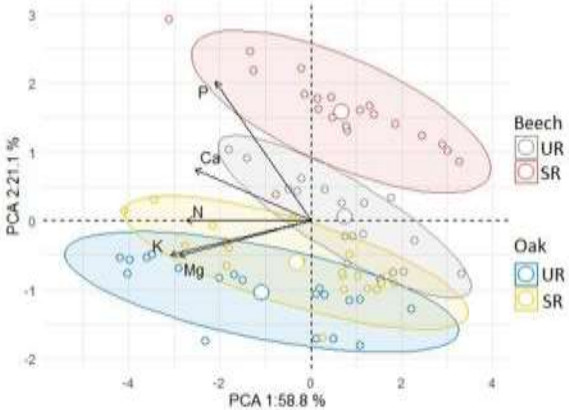


Fig. 3. *Nutrient allocation in oak and beech root grown on different fertilization methods (Rotowa et al., 2025a)*

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Table 10. *Mean values showing the allocation of macro elements content in different parts of Quercus robur seedlings for each treatment. (±SD).* $^{abc, ef}$ *– significance differences between means (DMRT) (Rotowa et al., 2025a).*

Treatment	Part	Fertilizer type	C (g/kg)	N	P	K	S	Ca	Mg
						(mg/kg)			
	Roots								
UR20		**Liquid**	4.09±0.69^{a}	36.8±7.10^{a}	6.00±1.10^{ab}	32.6±5.40^{a}	4.10±0.60^{b}	33.10±6.00^{bc}	10.80±1.90^{a}
UR21			5.48±0.69^{a}	48.3±6.71^{a}	6.00±0.50^{ab}	36.4±4.20^{a}	5.10±0.50^{ab}	37.80±5.20^{ab}	10.90±1.40^{a}
UR22			5.94±0.60^{a}	50.7±5.20^{a}	7.00±0.80^{a}	42.6±4.30^{a}	6.20±0.60^{a}	49.80±4.50^{a}	13.50±1.50^{a}
UC			4.05±0.44^{a}	42.8±5.40^{a}	2.90±0.30^{b}	26.2±2.50^{a}	4.20±0.60^{b}	22.00±2.90^{c}	13.00±1.30^{a}
Total			4.86±0.34	44.27±6.17	5.64±0.86	34.3±3.41	4.90±0.61	35.32±4.25	11.77±1.63
p-value			**0.069** ^{ns}	**0.408**^{ns}	**0.046***	**0.078** ^{ns}	**0.050***	**0.005****	**0.673**^{ns}
SR20		**Solid**	3.16±0.36^{e}	36.0±10.2^{e}	5.10±1.30^{e}	22.1±6.10^{e}	4.20±1.40^{e}	29.80±4.20^{e}	8.40±2.00^{e}
SR21			4.15±0.74^{e}	49.8±14.9^{e}	8.20±1.40^{e}	34.0±5.50^{e}	4.00±0.70^{e}	31.30±4.80^{e}	9.70±1.60^{e}
SR22			3.46±0.23^{e}	44.6±16.2^{e}	7.20±1.80^{e}	27.6±4.20^{e}	4.50±0.50^{e}	25.70±4.10^{e}	9.40±1.70^{e}
SC			2.76±0.38^{e}	51.7±9.20^{e}	7.70±0.70^{e}	25.8±3.60^{e}	4.70±0.80^{e}	20.80±3.00^{e}	11.10±1.20^{e}
Total			3.37±0.24	45.5±15.8	7.06±1.40	27.4±4.47	4.36±0.72	26.87±3.08	9.64±1.50
p-value			**0.238**^{ns}	**0.429**^{ns}	**0.323**^{ns}	**0.263**^{ns}	**0.893**^{ns}	**0.325**^{ns}	**0.612**^{ns}
	Shoots								
UR20		**Liquid**	0.85±0.07^{ab}	10.9±0.80^{a}	1.20±0.12^{a}	4.90±0.60^{a}	1.00±0.10^{a}	17.70±1.90^{b}	3.00±0.20^{b}
UR21			0.77±0.03^{ab}	9.30±0.80^{a}	1.00±0.10^{ab}	4.30±0.20^{ab}	0.90±0.10^{a}	15.20±1.50^{b}	2.10±0.10^{c}
UR22			0.93±0.07^{a}	10.6±0.80^{a}	1.20±0.20^{a}	5.20±0.50^{a}	1.00±0.60^{a}	18.40±0.90^{b}	2.60±0.20^{ab}
UC			0.76±0.02^{b}	10.3±0.40^{a}	0.61±0.03^{d}	3.40±0.20^{b}	0.90±0.10^{a}	10.10±1.00^{a}	3.30±0.30^{a}
Total			0.83±0.04	10.29±0.35	0.99±0.08	4.45±0.35	0.97±0.04	15.34±0.98	2.75±0.20
p-value			**0.118**^{ns}	**0.423**^{ns}	**0.015***	**0.030***	**0.629**^{ns}	**0.003****	**0.002****
SR20		**Solid**	0.94±0.09^{f}	12.4±1.10^{f}	1.40±0.20^{f}	4.90±0.50^{f}	1.30±0.20^{f}	14.50±1.80^{f}	2.50±0.30^{f}
SR21			0.86±0.12^{f}	12.6±1.70^{f}	1.30±0.20^{f}	5.00±0.70^{f}	1.20±0.20^{f}	15.50±1.80^{f}	2.70±0.40^{f}
SR22			0.96±0.04^{f}	17.8±3.40^{f}	1.60±0.20^{f}	5.70±1.05^{f}	1.60±0.20^{f}	13.68±1.30^{f}	2.20±0.20^{f}
SC			1.96±0.26^{e}	34.1±6.30^{e}	3.20±0.40^{e}	11.9±1.30^{e}	3.60±0.60^{e}	22.21±3.70^{e}	7.40±1.10^{e}
Total			1.29±0.07	14.8±1.50	1.40±0.24	5.70±0.94	1.40±0.32	15.90±2.80	3.20±0.83
p-value			**0.003****	**0.002****	**0.000****	**0.000****	**0.000****	**0.007****	**0.000****
	Leave								
UR20		**Liquid**	1.36±0.05^{a}	21.20±3.60^{a}	1.70±0.30^{a}	9.2±1.40^{a}	2.06±0.39^{a}	58.8±4.58^{a}	8.40±0.18^{a}
UR21			0.84±0.10^{b}	11.30±1.30^{b}	0.70±0.10^{b}	7.2±1.020^{ab}	1.16±0.17^{c}	33.8±4.55^{b}	3.6±0.40^{b}
UR22			0.78±0.09^{b}	19.40±2.80^{b}	0.80±0.10^{b}	8.3±1.30^{ab}	0.92±0.13^{c}	33.8±3.73^{b}	4.0±0.54^{b}
UC			0.82±0.13^{b}	15.20±1.60^{b}	0.50±0.20^{b}	6.0±0.95^{b}	1.54±0.87^{b}	28.1±3.10^{b}	8.2±0.90^{a}
Total			0.94±0.09	14.24±2.37	0.95±0.13	7.69±1.98	1.42±0.11	38.62±4.05	6.05±0.57
p-value			**0.000****	**0.003****	**0.000****	**0.050****	**0.000****	**0.000****	**0.000****
SR20		**Solid**	1.49±0.11^{e}	32.80±2.90^{e}	4.90±0.40^{e}	19.1±1.70^{e}	3.80±0.30^{e}	64.9±6.4^{e}	7.5±0.61^{f}
SR21			1.10±0.09^{f}	26.80±2.80^{e}	3.70±0.60^{e}	15.8±2.30^{e}	3.20±0.40^{e}	40.1±5.8^{e}	5.8±0.90^{f}
SR22			1.26±0.03^{e}	38.80±3.70^{e}	4.70±1.00^{e}	19.8±3.80^{e}	3.50±0.70^{e}	35.0±3.7^{e}	5.2±0.20^{f}
SC			1.728±0.33^{e}	74.10±4.90^{e}	6.90±2.30^{e}	26.3±8.50^{e}	6.30±1.80^{e}	49.3±7.5^{e}	13.6±2.20^{e}
Total			2.10±0.16	29.50±2.10	3.60±0.30	16.8±2.20	3.00±0.20	30.0±15	7.00±0.40
p-value			**0.114**^{ns}	**0.107**^{ns}	**0.390**^{ns}	**0.497**^{ns}	**0.150**^{ns}	**0.150**^{ns}	**0.001***

S - State Forest fertilization, U - University fertilization, R - novel substrate, C - controls substrate (peat-perlite).
Letters 'a' and 'b' denote homogeneous groups under liquid fertilization, 'e, f' and 'g' denote homogeneous groups under solid fertilization. p=0.05.

Table 11. *Correlation analysis between chemical elements and biomass of Quercus robur (Rotowa et al., 2025a)*

	C	**N**	**P**	**K**	**S**	**Ca**	**Mg**
N	0.649**						
P	0.727**	0.890**					
K	0.899**	0.857**	0.910**				
S	0.754**	0.959**	0.895**	0.905**			
Ca	0.339**	0.447**	0.436**	0.495**	0.518**		
Mg	0.791**	0.797**	0.735**	0.847**	0.843**	0.590**	
Biomass	0.914**	0.607**	0.700**	0.839**	0.687**	0.288**	0.729**

** *Correlation is significant at the 0.01 level*

5.4. Oak – biomass and element content studies after one year in the forest

The results of biomass and element content after one year in the forest was published in the article: **Rotowa, O.J.** Małek, S. Jasik, M. Staszel-Szlachta, K. (2025) *Substrate and Fertilization Used in the Nursery Influence Biomass and Nutrient Allocation in Fagus sylvatica and Quercus robur Seedlings after the First Year of Growth in a Newly Established Forest*. Forests, 16, 511.

At 1YAP, the impact of nursery treatments continued to manifest in oak biomass allocation and nutrient profiles. Solid-fertilized seedlings in (SC and SR22) again ranked highest in shoot and root biomass. Destructive sampling of 72 oak seedlings (three per subplot) revealed that roots consistently held the highest concentrations of nutrients, followed by shoots and leaves. Macronutrient accumulation, particularly for N, P, and K, were again most pronounced in the C and R22 substrates of solid fertilized treatments. Liquid-fertilized seedlings generally showed lower nutrient content across organs, though UR22 treatments performed comparably to peat-based controls in some cases (Figure 4). Therefore, *Q. robur* seedlings benefited most from peat-based (SC) and peat-free (SR22) substrates under solid fertilization, both in the nursery and one-year post-planting. These treatments produced the highest biomass across all organs and macronutrient concentrations in roots and shoots (Fig. 4).

Nutrient allocation patterns revealed by heatmap analysis showed that roots accumulated the highest nutrient levels across treatments. Solid fertilizers consistently promoted higher nutrient concentrations in all organs compared to liquid fertilizers. Oak seedlings grown in the peat-based SC treatment exhibited the highest nutrient. The R22 novel substrate showed comparable nutrient levels, signifying its potential as an effective alternative, especially for N, P, and K. In contrast, leaves and shoots treated with liquid fertilizers had lower nutrient concentrations, with novel substrates occasionally outperformed peat under liquid treatments (Fig. 5). Correlation analysis highlighted a strong relationship between biomass production and nutrient concentration with weaker correlations observed between Ca and other elements (Fig. 6).

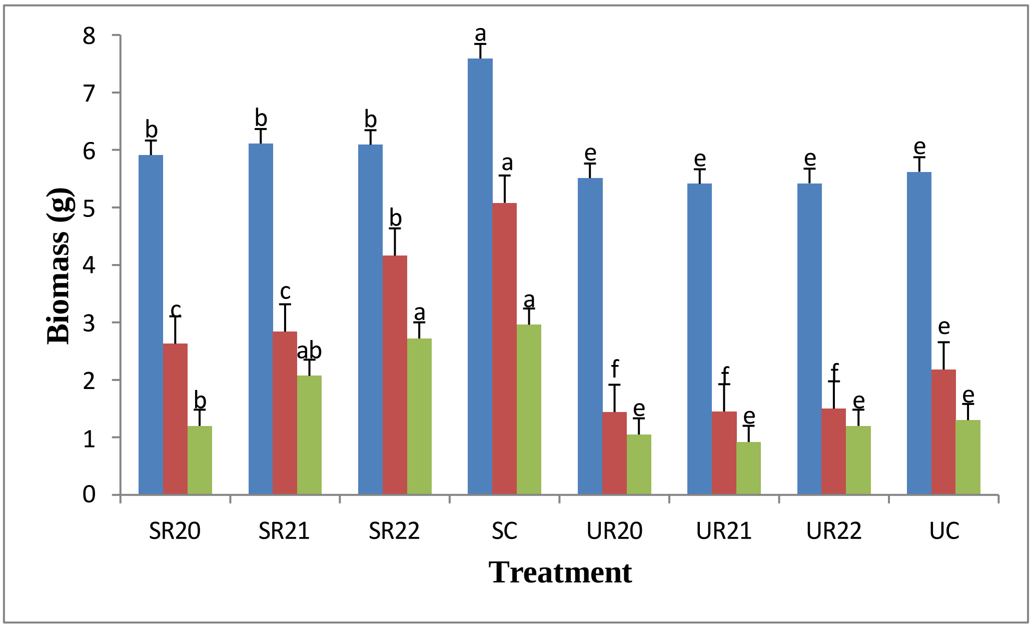
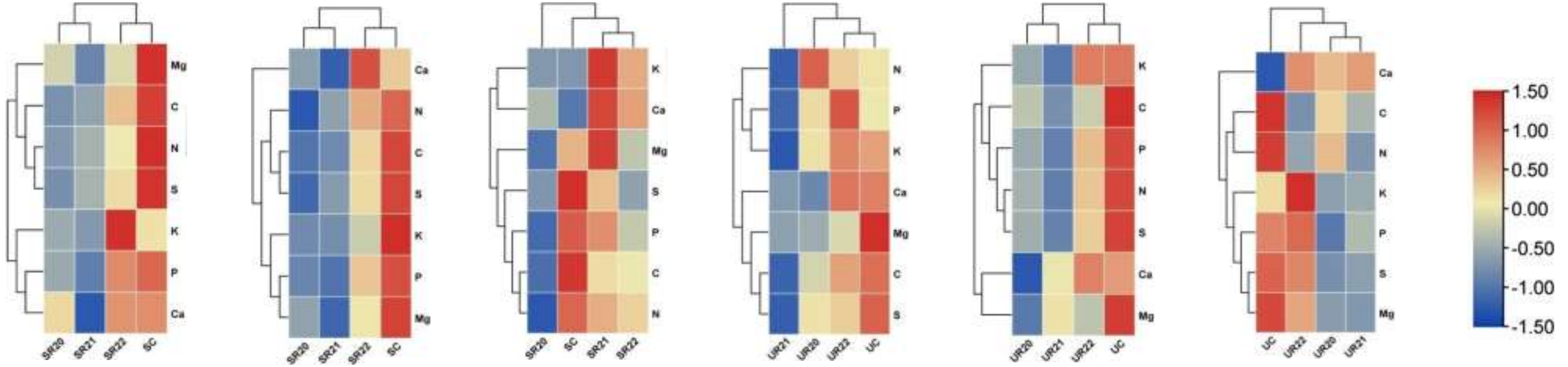


Fig. 4. *Distribution of biomass across different treatments for Quercus robur (Rotowa et al, 2025b) Letters 'a–c' denote homogeneous groups under solid fertilization and 'e' and 'f' denote homogeneous groups under liquid fertilization; S—solid fertilization; U—liquid fertilization; R—novel substrates; C— controls substrate (peat–perlite).*



(**a**) Leave (**b**) Shoot (**c**) Root (**d**) Leave (**e**) Shoot (**f**) Root

Traditional solid fertilizer **Novel liquid fertilizer**

Fig. 5. *Allocation of nutrients in different parts of Quercus robur seedlings for each treatment (mg/kg). (Rotowa et al., 2025b). S—State Forest fertilization; U—University fertilization; R—novel substrates; C—control substrate (peat).*

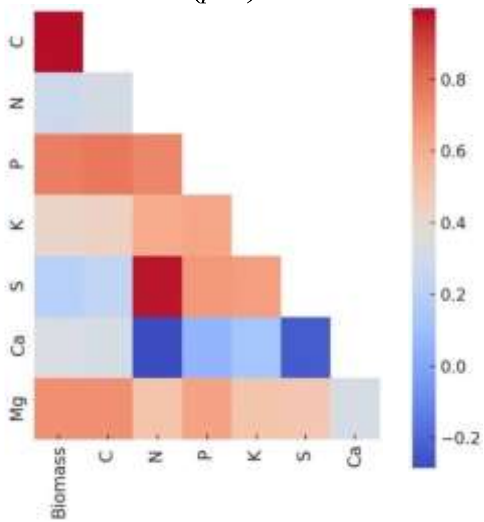


Fig. 6. *Correlation matrix of nutrient and biomass in Quercus robur.*
(Rotowa et al., 2025b)

5.5. Beech – results of root system studies after nursery production

The results of root system studies were published in the article: **Rotowa, O.J**., Małek, S., Banach, J., Pach, M. (2023). *Effect of different innovative substrate mediums on roots characterization of European beech Fagus sylvatica L. and pedunculate oak Quercus robur L. seedlings*. Sylwan, 167 (09).

The root system parameters of *Fagus sylvatica* seedlings at the end of the nursery production phase, focusing on the effects of different substrate and fertilization treatments are presented in table 12. Each parameter shows statistically significant differences among the treatments, as indicated by the p-values ($p < 0.05$). The peat-free substrate under liquid fertilizer (UC) recorded the highest value, indicating the thickest stem base, while SR20 showed the smallest diameter. The differences among treatments were statistically significant with the results suggesting that although UC produced the thickest root collars, SR22 also had a relatively large RCD and may offer a more balanced performance across other parameters. The highest TRL values were observed in UR20 and SR20, both exceeding 1400 cm on average. These two treatments promoted extensive root elongation. On the other hand, UR21 and UR22 recorded the lowest root lengths, indicating that the specific combination of university substrate and fertilization in these cases had limited root elongation with significant differences.

The highest values of RSA were recorded in SR20, SR22, and UR20, all statistically grouped together as the best performers. These treatments supported the development of a more extensive root network, contributing to better water and nutrient absorption capacity. In contrast, UR22 and UC had the lowest RSA, with statistically significant differences across treatments. Looking at ARD, UR21 produced the thickest roots, closely followed by SR22 and UC. The thinnest roots were observed in UR20 and SR20. These differences were significant, and indicated varying root strategies among treatments. Lastly, for RV, SR22 performed best, producing the largest root biomass volume, followed by UR21 and SR20. The lowest RV values were observed in UR22 and SC with significant difference. Overall, the data show that peat-free treatments (especially SR22) with solid fertilizer perform comparably to peat-based controls across several root traits, with consistently significant statistical variation (Table 12).

Table 12. *Mean and standard deviation of studied root system parameters of Fagus sylvatica (±SD).* *[abc, de] – significance differences between means (DMRT) (Rotowa et al., 2023)*

Treatment	RCD (mm)	TRL (cm)	RSA (cm^2)	ARD (mm)	RV (cm^3)
			F. sylvatica		
SR20	4.25 ±0.69 [c]	1412.40 ±199.04 [ab]	140.61 ±22.21 [a]	0.31 ±0.01 [de]	1.12 ±0.16 [bc]
UR20	4.35 ±0.77 [d]	1436.84 ±145.29 [a]	134.99 ±12.58 [a]	0.30 ±0.01 [e]	1.01 ±0.16 [b]
SR21	5.26 ±0.87 [d]	825.19 ±70.63 [ab]	102.22 ±10.78 [ab]	0.39 ±0.02 [ab]	1.02 ±0.16 [bc]
UR21	5.16 ±0.57 [d]	647.47 ±37.07 [b]	95.24 ±8.41 [ab]	0.47 ±0.04 [a]	1.15 ±0.16 [ab]
SR22	5.95 ±0.75 [b]	1067.23 ±204.39 [a]	137.49 ±15.90 [a]	0.43 ±0.04 [ab]	1.47 ±0.16 [a]
UR22	4.00 ±0.58 [d]	656.20 ±98.60 [b]	72.24 ±6.29 [b]	0.36 ±0.02 [bc]	0.64 ±0.16 [c]
SC	4.37 ±0.42 [d]	897.99 ±134.33 [bc]	96.09 ±14.86 [ab]	0.34 ±0.02 [cd]	0.83 ±0.16 [ab]
UC	5.51 ±0.84 [a]	686.06 ±175.92 [a]	82.93 ±18.69 [b]	0.41 ±0.02 [ab]	0.81 ±0.16 [bc]
Total	4.86 ±0.92	953.67 ±66.99	107.73 ±6.13	0.38 ±0.01	1.01 ±0.16
P-value.	0.00**	0.00**	0.00**	0.00**	0.002*

SR – state forest substrate and fertilization, UR – University substrate and fertilization, RCD – Root collar diameter, TRL – Total root length, RSA – root surface area, ARV – average root diameter, RV – root volume. The same letter in the same column are not significantly different while letters with different letter are significantly different at p=0.05

5.6. Beech – results of biometrics and root system studies one year after planting in the forest

The results of biometric and root system studies after nursery production circle were published in the article: **Rotowa, O.J.** Małek, S. Kupka D. Pach, M. Banach J. (2025). *Innovative peat-free organic substrates and fertilizers influence growth dynamics and root morphology of Fagus sylvatica L. and Quercus robur L. seedlings one year after planting*. Forests, 16, 800.

After transplanting into the forest, *Fagus sylvatica* exhibited remarkable post-nursery performance in certain solid-fertilized treatments. The SC and SR22 treatments led to the highest absolute height increases, from 30.88 cm in the nursery to 62.25 cm at 1YAP, representing a growth increment of over 102%. Collar diameter also increased from 5.72 mm to 9.61 mm, a gain of nearly 68%. Liquid-fertilized treatments recorded lower overall height and diameter increments, with R22 substrate recording best performance of 61% and 47%, respectively, after the control treatment. This study confirms that while survival rates were acceptable across treatments, solid-fertilized seedlings consistently outperformed liquid once in both shoot elongation and basal thickening (Table 13). Root system metrics at 1YAP reinforced the trend from the nursery. *Fagus sylvatica* continued to invest heavily in the very fine root fraction. Treatments such as SR22 supported both long TRL and high VFSA, enhancing water and nutrient uptake capacity in the upper soil layers. Regression analysis from confirms that VFL and VFSA were the strongest predictors of both height and collar diameter in beech, with adjusted R^2 values above 0.61 and p-values < 0.01 (Table 9).

Table 13. *Biometric and increment rate of F. sylvatica seedlings after one year on crop. (±SD).* $^{ab, ef}$ *– significance differences between means (DMRT) (Rotowa et al., 2025c)*

Treatment	fertilization type	SPC	TSS	RSS (%)	After 1 year in the forest		After nursery production cycle		Absolute Increment	
					Height (cm)	Diameter (mm)	Height (cm)	Diameter (mm)	Height %	Diameter %
					Fagus sylvatica					
SR20	Solid	112	123	84	58.12±9.89^{b}	9.11±2.23^{a}	31.46±3.56^{a}	5.63±1.48^{a}	85	62
SR21		135	142	97	59.01±10.42^{ab}	8.93±2.01^{a}	30.25±3.43^{a}	5.70±1.62^{a}	95	55
SR22		138	143	97	61.92±11.61 ^{a}	9.22±1.96^{a}	31.64±3.29^{a}	5.58±1.15^{a}	96	58
SC		133	143	97	62.25±12.02^{a}	9.61±2.48^{a}	30.88±3.23^{a}	5.72±1.23^{a}	102	68
Total					60.32±11.18	8.94±2.44	31.06±3.23	5.66±1.26		
P-value.					**0.033***	**0.203^{ns}**	**0.473^{ns}**	**0.341^{ns}**		
UR20	Liquid	138	143	97	44.21±7.77^{f}	7.33±1.98^{e}	30.55±3.23^{e}	5.22±0.95^{e}	45	40
UR21		130	136	93	43.32±7.18^{f}	7.41±2.04^{e}	30.24±2.98^{e}	5.36±1.24^{e}	44	38
UR22		113	135	92	50.07±8.49^{e}	7.68±1.86^{e}	31.18±3.19^{e}	5.21±1.07^{e}	61	47
UC		118	135	92	54.04±8.52^{e}	7.96±1.49^{e}	31.07±3.08^{e}	5.28±0.99^{e}	56	51
Total					49.41±3.19	7.60±1.82	30.76±3.17	5.27±1.07		
P-value.					**0.024***	**0.176^{ns}**	**0.253^{ns}**	**0.316^{ns}**		

SPC– Number Seedlings in perfect condition, TSS–Total survived seedlings, RSS– rate of seedling survival. S – State Forests solid fertilization, U – University novel liquid fertilization, R – novel substrates, C – control substrate (peat−perlite) (N =147)

A comparison of solid and liquid fertilization approaches reveals that solid-fertilized treatments generally outperformed liquid-fertilized ones in most root parameters after one year in the forest. Under solid fertilization, there were no statistically significant differences among treatments for TRL, RSA, or ARD ($p > 0.05$), although RV showed a significant difference ($p = 0.035$). Despite the lack of significance in most parameters, some trends can still be observed. SR20 recorded the longest roots, followed by SR22, SC, and SR21, although all values were statistically similar. In terms of RSA, SC had the highest surface area, indicating the most expansive root network, but differences were not statistically significant. For ARD, SC again had the highest value, followed by SR20 and SR21, while SR22 had the smallest root diameter. As for RV, SC performed best, closely followed by SR20 and SR21, while SR22 had the least volume among the solid-fertilized group (Table 14).

In contrast, liquid fertilization showed statistically significant differences for RSA, ARD, and RV, although TRL differences were not significant. Among the treatments, UR20 consistently outperformed others across all traits: it had the longest roots, highest surface area, thickest average diameter, and greatest root volume. UR21 and UR22 were similar in their performance, showing lower RSA, ARD, and RV. UC was slightly better than UR21 and UR22 in RSA and ARD, but it still produced the lowest RV overall. At the nursery stage, significant differences in all root traits indicated strong treatment effects, with solid-fertilized seedlings particularly SR22 and SR20 showing superior root development (Table 12). After one year in the forest, these differences diminished for some traits, especially under solid fertilization, though RV still shows significant effect 1YAP. Liquid-fertilized seedlings showed more pronounced variability over time, with UR20 remaining competitive (Table 14). Therefore, solid fertilization supported more consistent and long-term root development in beech, affirming its suitability for enhancing seedling establishment in forest environments.

Table 14. *Morphological parameters of root system of Fagus. sylvatica under different substrate fertilizer treatment after one year in the forest. (±SD).* $^{ab,\, ef}$ *– significance differences between means (DMRT) (Rotowa et al., 2025c)*

Treatment	fertilization type	TRL (cm)	RSA (cm^2)	ARD (mm)	RV (cm^3)
			F. sylvatica		
SR20	**solid**	1673.27±252.94^a	171.66±41.43^a	0.85±0.15^a	3.59±0.77^b
SR21		1265.52±151.19^a	168.37±39.55^a	0.82±0.17^a	3.51±1.32^b
SR22		1424.69±265.10^a	182.79±62.43^a	0.72±0.16^a	3.37±1.58^b
SC		1377.56±104.39^a	226.28±48.05^a	0.93±0.19^a	4.10±1.59^a
Total		1335.26±207.75	187.28±52.15^a	0.83±0.18^a	3.89±1.48
p-value.		**0.285^{ns}**	**0.061^{ns}**	**0.087^{ns}**	**0.035***
UR20	**liquid**	1132.69±130.54^e	113.87±19.61^e	0.83±0.10^e	2.39±0.89^e
UR21		1037.03±92.90^e	88.98±14.17^f	0.72±0.08^f	1.80±0.26^f
UR22		1029.58±88.39^e	89.30±6.50^f	0.72±0.09^f	1.61±0.56^f
UC		1056.09±98.93^e	122.22±10.22^e	0.74±0.06^f	1.53±0.42^f
Total		1063.85±107.76	99.09±16.65	0.75±0.09	1.83±0.65
p-value.		**0.157^{ns}**	**0.001****	**0.024***	**0.015****

TRL- Total root length, RSA- Root surface area, ARD- Average root diameter, RV- Root volume
Letters with different alphabet indicate statistically significant differences between means ($p < 0.05$). Letters 'a, b' and 'c' denote homogeneous groups under solid fertilization and 'e, f' and 'g' denote homogeneous groups liquid fertilization.

5.7. Beech – biomass and element content studies after nursery production

The results of biomass and element content in beech after the nursery production cycle were published in the article: **Rotowa, O.J**., Małek, S., Jasik, M., Staszel-Szlachta, K. (2024). *Effect of innovative peat-free organic growing media and fertilizer on nutrient allocation in pedunculate oak (Quercus robur L.) and European beech (Fagus sylvatica L.) seedlings after nursery production cycle*. New Forests, 56:171-22.

This preliminary result revealed that, *Fagus sylvatica* seedlings showed a strong treatment response in biomass and nutrient content, driven largely by the type of fertilization. Solid-fertilized seedlings in the SC and SR22 treatments produced the highest shoot and root biomass, with total dry mass values significantly surpassing those recorded under liquid fertilization (Figure 1a-c). Leaf biomass was more stable across treatments, but even here, the UC (peat-liquid) and R22-liquid treatments maintained competitive values. In terms of nutrient allocation, under liquid fertilizer, the R22 substrate performed best for roots and shoots, while R21 was most effective under solid fertilizer for enhanced root nutrient contents. Under liquid fertilizer, the control substrate achieved the highest nutrient accumulation. Again, SC consistently produced the highest concentrations across all tested macroelements (Table 15).

The macroelement composition in the roots of beech seedlings appeared consistent across the substrates, with points clustered closely together. This implies that variation in growing medium had minimal impact on the macroelement profiles in the roots (Fig. 7). In contrast, the impact of U and S fertilizers was particularly noticeable, as shown by the diverging points in Fig. 3. This indicated a significant effect of fertilization on nutrient content for beech species, with the points being spatially separated. The variation observed in these graphs suggests that the primary factor influencing growth is the type of fertilizer treatment rather than the growing medium. Significant differences in nutrient contents were recorded, especially for shoots and leaves under both liquid and solid fertilizers, emphasizing the substantial impact of the fertilizer type on nutrient allocation (Table 15). Correlation analysis showed strong positive relationships between total biomass and nutrient content in leaves and roots (Table 16).

Table 15. *Mean values showing the allocation of macro elements content in different parts of Fagus sylvatica seedlings for each treatment (±SD).* ^{abc,ef} *– significance differences between means (DMRT) (Rotowa et al., 2025a)*

Treatment	**Part**	**Fertilizer type**	**C (g/kg)**	**N**	**P**	**K**	**S**	**Ca**	**Mg**
						(mg/kg)			
	Roots								
UR20		**Liquid**	3.31±0.60^{a}	34.40±2.50^{a}	3.50±0.21^{c}	21.80±3.50^{b}	3.30±0.30^{a}	30.80±2.19^{a}	7.20±0.98^{a}
UR21			3.09±0.51^{a}	34.20±2.90^{a}	6.10±0.90^{b}	23.50±4.00^{ab}	3.50±0.50^{a}	35.00±6.30^{a}	8.10±1.60^{a}
UR22			3.49±0.56^{a}	40.60±3.40^{a}	9.30±1.00^{a}	25.50±5.70^{a}	4.20±0.40^{a}	34.10±5.60^{a}	9.30±1.70^{a}
UC			2.66±0.61^{b}	42.10±3.90^{a}	2.10±0.30^{c}	16.30±3.01^{c}	3.10±0.30^{a}	13.30±6.00^{b}	7.40±1.90^{a}
Total			3.12±0.62	36.92±3.31	2.89±0.74	20.56±3.89	3.52±0.19	28.92±9.38	7.95±1.69
p-value			**0.125^{ns}**	**0.470^{ns}**	**0.000****	**0.000****	**0.194^{ns}**	**0.000****	**0.135^{ns}**
SR20		**Solid**	3.25±0.74^{e}	38.80±4.20^{e}	8.20±0.80^{e}	22.10±5.30^{e}	3.80±0.60^{e}	35.80±4.50^{e}	8.40±1.90^{e}
SR21			3.20±0.94^{e}	38.50±5.50^{e}	9.80±1.60^{e}	21.10±7.20^{e}	3.50±0.90^{e}	39.50±7.50^{e}	9.60±3.70^{e}
SR22			2.30±0.86^{ef}	40.80±8.10^{e}	6.60±0.80^{e}	20.30±4.80^{e}	3.10±0.90^{e}	30.10±1.90^{e}	8.40±2.20^{e}
SC			1.99±0.86^{f}	38.00±4.70^{e}	7.00±0.80^{e}	18.40±7.10^{e}	3.40±0.80^{e}	12.00±2.10^{f}	7.30±1.90^{e}
Total			2.68±0.93	38.40±5.60	6.60±0.85	22.10±5.10	3.50±0.80	28.80±3.90	8.20±2.30^{a}
p-value			**0.049***	**0.988^{ns}**	**0.186^{ns}**	**0.797^{ns}**	**0.715^{ns}**	**0.003****	**0.620^{ns}**
	Shoots								
UR20		**Liquid**	0.65±0.05^{a}	11.91±0.79^{a}	1.31±0.04^{b}	4.55±0.61^{a}	0.85±0.01^{a}	11.08±1.08^{a}	1.83±0.05^{b}
UR21			0.70±0.05^{a}	12.28±1.04^{a}	1.88±0.23^{ab}	5.61±0.84^{a}	0.89±0.05^{a}	11.86±0.36^{a}	1.97±0.05^{b}
UR22			0.73±0.10^{a}	12.64±3.46^{a}	2.38±0.34^{a}	7.21±1.74^{a}	0.95±0.12^{a}	12.56±3.56^{a}	2.02±0.55^{b}
UC			0.91±0.15^{a}	13.43±6.47^{a}	0.92±0.13^{c}	5.09±1.26^{a}	1.08±0.19^{a}	9.93±2.14^{a}	2.84±0.96^{a}
Total			0.75±0.04	13.56±4.14	1.63±0.16	5.62±0.98	0.95±0.06	11.35±2.21	2.16±0.25
p-value			**0.242^{ns}**	**0.105^{ns}**	**0.001****	**0.166^{ns}**	**0.518^{ns}**	**0.287^{ns}**	**0.047***
SR20		**Solid**	1.31±0.11^{f}	14.80±11.3^{b}	2.50±0.30^{f}	5.80±0.60^{f}	1.00±0.80^{f}	15.70±2.40^{e}	2.90±0.80^{f}
SR21			0.82±0.07^{f}	16.50±1.20^{b}	2.40±0.10^{f}	6.00±0.50^{f}	0.90±0.10^{f}	16.20±2.00^{e}	2.10±0.77^{f}
SR22			0.88±0.09^{f}	22.50±1.60^{ab}	4.70±1.60^{ef}	9.80±0.80^{e}	2.00±0.60^{e}	22.80±3.00^{e}	3.20±0.93^{ef}
SC			2.79±0.85^{e}	24.40±0.70^{e}	6.50±0.10^{e}	9.30±0.80^{e}	3.70±0.12^{e}	24.70±3.40^{e}	5.10±0.50^{e}
Total			1.10±0.15	17.80±1.10	3.80±0.30	7.80±0.67	1.30±0.17	19.50±2.20	2.85±0.30
p-value			**0.018***	**0.013***	**0.007****	**0.007****	**0.028***	**0.280^{ns}**	**0.006****
	Leaves								
UR20		**Liquid**	0.50±0.01^{b}	7.50±1.80^{b}	0.55±0.05^{b}	4.90±0.15^{a}	0.74±0.08^{b}	24.68±3.06^{a}	3.07±0.42^{b}
UR21			0.49±0.04^{b}	7.60±1.80^{b}	0.79±0.14^{b}	4.20±0.13^{a}	0.76±0.09^{b}	24.86±6.07^{a}	2.93±0.74^{b}
UR22			0.44±0.07^{b}	6.10±1.50^{b}	1.01±0.13^{b}	5.50±0.30^{a}	0.67±0.09^{b}	22.75±7.42^{a}	2.48±0.96^{b}
UC			0.71±0.07^{a}	15.10±2.33^{a}	0.59±0.03^{a}	4.60±0.28^{a}	1.35±0.12^{a}	23.08±4.49^{a}	4.30±1.03^{a}
Total			0.54±0.03	9.21±1.75	0.74±0.06	4.68±0.23	0.87±0.08	23.84±5.15	3.19±1.03
p-value			**0.011***	**0.000****	**0.019****	**0.227^{ns}**	**0.001****	**0.901^{ns}**	**0.019***
SR20		**Solid**	0.51±0.07^{f}	17.40±2.50^{g}	2.20±0.30^{f}	7.80±0.85^{f}	3.30±0.55^{f}	19.60±3.50^{f}	3.00±0.30^{f}
SR21			0.72±0.11^{f}	20.50±9.80^{g}	2.50±0.49^{f}	9.20±1.00^{f}	3.70±0.70^{f}	33.70±3.80^{f}	3.40±0.55^{f}
SR22			1.03±0.18^{ef}	29.50±2.20^{f}	4.50±0.80^{e}	11.00±1.90^{ef}	3.93±0.82^{ef}	42.80±4.00^{e}	4.20±0.84^{ef}
SC			1.20±0.22^{e}	34.20±2.90^{e}	4.10±0.78^{e}	15.50±1.22^{e}	4.50±0.88^{e}	43.00±4.39^{e}	5.10±0.90^{e}
Total			0.70±0.06	19.80±2.80	2.10±0.63	7.80±1.07	3.50±0.72	27.40±3.30	3.80±0.40
p-value			**0.028***	**0.002****	**0.053 ***	**0.019***	**0.006***	**0.030***	**0.002****

S - State Forest solid fertilization, U - University novel liquid fertilization, R - novel Substrate, C - controls substrate (peat-perlite).

Letters 'a' and 'b' denote homogeneous groups under liquid fertilization, 'e, f' and 'g' denote homogeneous groups under solid fertilization. p=0.05.

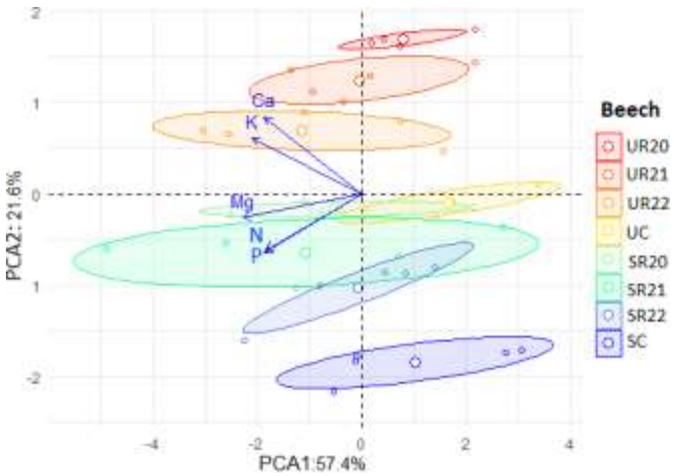


Fig. 7. *Nutrient allocation in beech root grown on different substrate medium (Rotowa et al., 2025a)*

Table 16. *Correlation analysis between chemical elements and biomass Fagus sylvatica (Rotowa et al., 2025a)*

	C	N	P	K	S	Ca	Mg
N	0.770**						
P	0.829**	0.753**					
K	0.925**	0.841**	0.873**				
S	0.862**	0.938**	0.831**	0.933**			
Ca	0.491**	0.608**	0.590**	0.579**	0.624**		
Mg	0.860**	0.913**	0.818**	0.908**	0.956**	0.669**	
Biomass	0.997**	0.783**	0.833**	0.932**	0.872**	0.489**	0.876**

***** Correlation is significant at the 0.01 level***

5.8. Beech – biomass and element content studies after one year in the forest

The results of biomass and element content after one year in the forest were published in the article: **Rotowa, O.J**. Małek, S. Jasik, M. Staszel-Szlachta, K (2025). *Substrate and Fertilization Used in the Nursery Influence Biomass and Nutrient Allocation in Fagus sylvatica and Quercus robur Seedlings after the First Year of Growth in a Newly Established Forest*. Forests, 16, 511.

One year after planting in the field, *F. sylvatica* seedlings retained clear treatment effects. Solid-fertilized treatments (especially SC and SR22) continued to produce the highest total biomass across roots, stems, and leaves (Figure 8). Roots remained the dominant sink for dry matter, but shoot and leaf mass also increased substantially compared to nursery values. The nutrient allocation analysis revealed that roots accumulated the highest concentrations of macronutrients in all treatments (Figure 9). Solid-fertilized SC and SR22 maintained superior concentrations of nitrogen, phosphorus, calcium, and magnesium, particularly in root and stem compartments with dendrograme showing the same homogeneous group. In contrast, liquid fertilized seedlings exhibited lower nutrient accumulation, especially in shoots and leaves. However, UR22 still achieved moderate nutrient values, indicating some efficacy of liquid feeding when paired with optimized substrate composition. A consistent grouping pattern by fertilizer type was revealed, with clear clustering of solid-fertilized treatments showing higher nutrient saturation. Correlation analysis (Figure 10) showed strong associations between biomass accumulation and nutrient concentrations in all elements, showing greater synchrony between nutrient uptake and growth in this species.

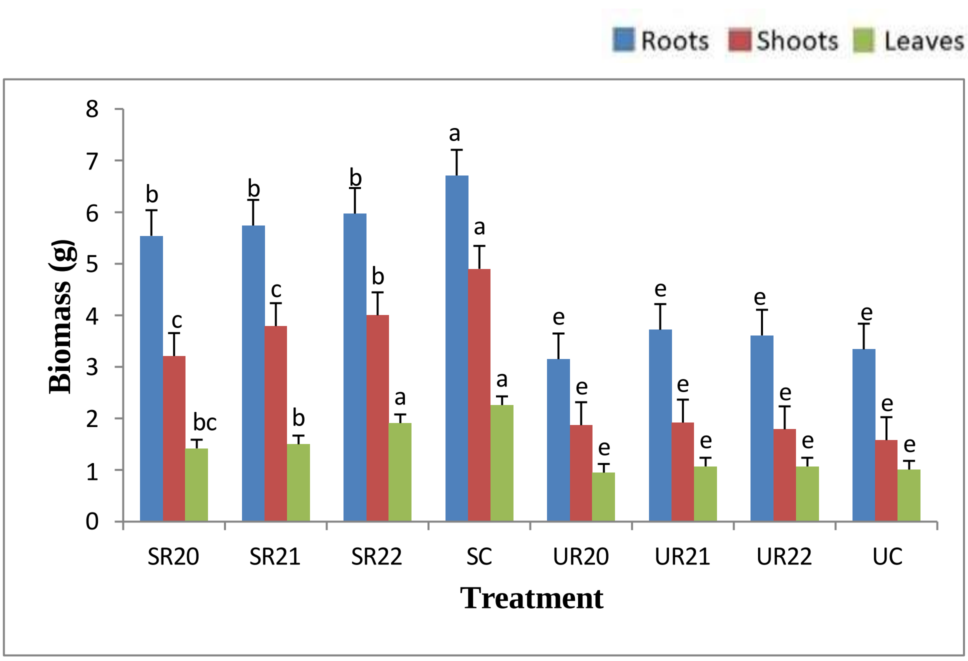
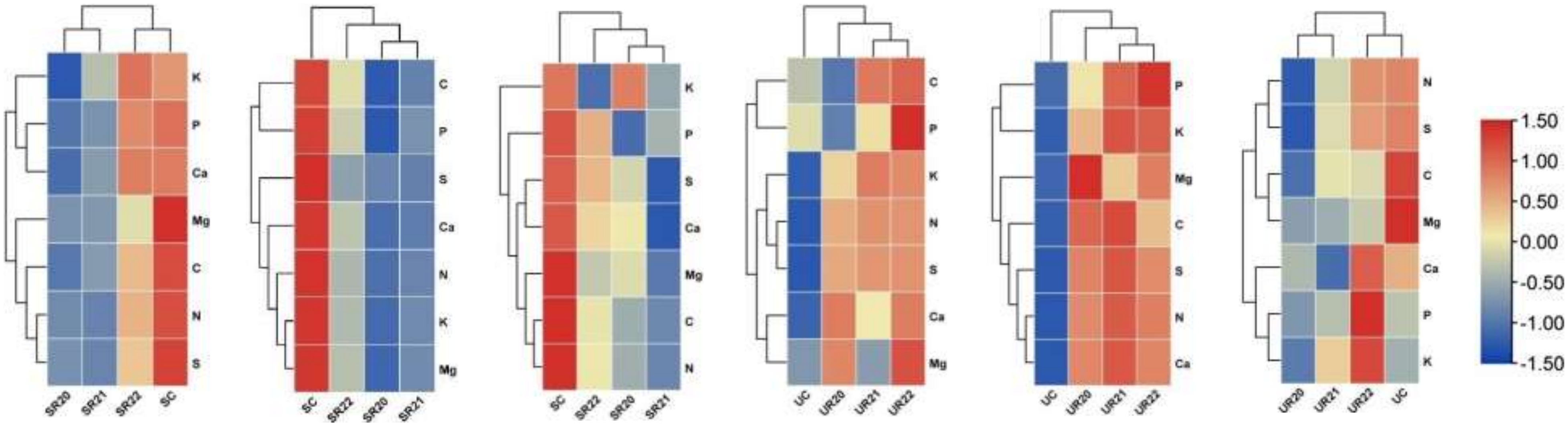


Fig. 8. *Distribution of biomass across different treatments for Fagus sylvatica L. (Rotowa et al., 2025b). Letters 'a, b and c' denote homogeneous groups under solid fertilization and 'e'-denote homogeneous groups under liquid fertilization; S—solid fertilization; U—liquid fertilization; R—novel substrates; C—controls substrate (peat–perlite).*



(**a**) Leave (**b**) Shoot (**c**) Root (**d**) Leave (**e**) Shoot (**f**) Root

Traditional solid fertilizer **Novel liquid fertilizer**

Fig. 9. *Matrix of nutrients in different parts of Fagus sylvatica seedlings for each treatment (mg/kg). S—State Forest fertilization; U—University fertilization; R—novel substrates; C—control substrate (peat). (Rotowa et al., 2025b)*

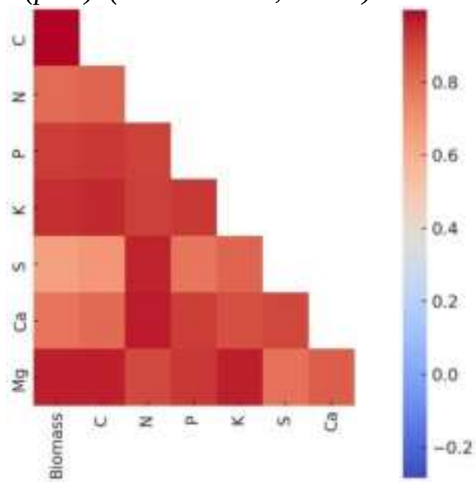


Fig. 10. *Correlation matrix of nutrient and biomass in Fagus sylvatica. (Rotowa et al., 2025b)*

5.9. Species comparison – biometric, root systems, biomass allocation and elements of oak and beech seedlings

A comparative evaluation of *Qeuerus robur* and *Fagus sylvatica* seedlings revealed notable species-specific responses in biometric traits, root system characteristics, biomass accumulation and nutrient allocation to substrate and fertilization treatments. These contrasts were evident both at the nursery stage and one year after planting (1YAP), and they significantly inform nursery and field management recommendations. These differences reflect inherent ecological strategies and root system architecture that influence how each species responds to substrate and fertilization treatments.

Biometric changes and growth strategies: Field data shows that beech seedlings under SC and SR22 treatments achieved the highest absolute height increment up to 102%**,** compared to 77-82% in oak. However, collar diameter gains were more substantial in oak (up to 3.5 mm) than in beech (3.8 mm), with beech displaying slightly greater variability under liquid fertilizer (Tables 7 and 12).

Root morphological strategies: Root architecture diverged severely between the species. *F. sylvatica* developed dense networks of VFR ($\leq$ 0.5 mm) with high surface area, especially under solid fertilization (SC, SR22). These roots supported efficient nutrient searching in upper soil layers, a trait consistent with beech's phototropic growth strategy. On the other hand, *Q. robur* favored deeper rooting via coarse roots and longer TRL, often under the same treatments, indicating a gravitropic response to substrate and field conditions. When analyzed by root class, beech seedlings displayed significantly higher surface area in the VFR class, particularly under SC and SR22 treatments. Oak, in contrast, showed higher values in RV and TRL**,** particularly under SR21 and SC. Despite some enhancement of fine root traits under liquid fertilizer in both species (e.g. UR22), solid fertilizer was more consistently effective across all metrics (Table 17). Multiple linear regression, confirmed that in beech**,** VFRL and surface area were strong predictors of both height and collar diameter ($R^2 > 0.60$; $p < 0.01$). In contrast, oak growth was predicted only by total root length**,** with no significant associations with fine or coarse root traits (Table 9).

Biomass allocation: At the nursery stage, solid fertilization consistently led to greater total biomass across all seedling organs in both species. However, oak seedlings accumulated more biomass in the root system, whereas beech seedlings showed a slightly more balanced shoot-to-root distribution (Fig. 1). After outplanting, these trends continued: oak seedlings under SC and SR22 treatments had the highest root and stem dry weights (Fig. 4), while beech seedlings under the same treatments excelled in leaf and shoot biomass (Fig. 8). The increase in biomass was substantial in both species. Regression and correlation analysis revealed stronger inter-organ biomass relationships in beech (especially between shoot and root dry mass). Oak, in contrast, displayed a more compartmentalized pattern with root biomass acting as a dominant sink, particularly under solid fertilizer regimes.

Nutrient allocation patterns: Species-specific strategies were even more evident in nutrient partitioning. In both nursery and field phases, oak seedlings accumulated more calcium and magnesium in roots, aligning with their structural rooting and long-term support functions (Table 11). In contrast, beech seedlings showed higher nitrogen and phosphorus concentrations, particularly in leaves and shoot, supporting rapid growth and metabolic activity (Table 15). PCA analyses indicated that fertilizer type was the main driver of nutrient distribution patterns (Fig 3). Solid fertilizers (SC and SR22) yielded higher macronutrient concentrations across organs, especially for N and K, in both species. However, beech seedlings had a stronger correlation between nutrient content and biomass production compared to oak (Fig. 10 and 6 respectively).

Table 17. *Root diameter classification of beech and oak seedlings under different treatments (±SD).* $^{abc,\ ef}$ *– significance differences between means (DMRT) (Rotowa et al., 2025c)*

Treatment	fertilization type	Length < 0.5 mm	Length 0.5 - 2.0 mm	Length >2.0mm	Surface area < 0.5 mm	Surface area 0.5-2.0 mm	Surface area > 2.0 mm	Volume < 0.5 mm	Volume 0.5-2.0 mm	Volume > 2.0 mm
					Fagus sylvatica					
SR20	Solid	465.22±94.62^{b}	144.40±30.07^{b}	36.04±6.30^{ab}	23.72±6.32^{a}	42.28±9.08^{b}	58.19±11.61^{b}	0.17±0.02^{a}	1.13±0.27^{ab}	10.59±2.31^{b}
SR21		463.26±99.38^{b}	118.89±41.23^{b}	28.82±8.58^{b}	21.27±8.28^{a}	33.90±11.14^{b}	57.00±16.68^{b}	0.14±0.06^{a}	0.90±0.35^{b}	10.05±3.55^{b}
SR22		472.41±109.16^{ab}	133.92±57.69^{b}	30.40±7.53^{b}	24.48±7.03^{a}	39.49±19.08^{b}	59.43±9.28^{b}	0.15±0.05^{a}	0.97±0.38^{b}	10.89±1.73^{b}
SC		493.66±109.71^{a}	194.83±67.13^{a}	40.70±9.64^{a}	26.32±5.46^{a}	58.74±20.40^{a}	72.39±15.11^{a}	0.18±0.04^{a}	1.52±0.39^{a}	14.40±4.54^{a}
Total		471.14±111.71	148.01±56.76	33.99±9.11	23.19±6.87	43.60±17.71	60.75±14.61	0.16±0.04	1.13±0.35	11.38±3.03
p-value		**0.048***	**0.021***	**0.015***	**0.376**^{ns}	**0.013***	**0.043***	**0.1236**^{ns}	**0.045***	**0.032***
UR20	Liquid	325.34±118.82^{e}	88.38±25.80^{ef}	22.79±7.43^{e}	21.22±6.78^{ef}	23.21±7.00^{ef}	42.16±8.81^{e}	0.14±0.04^{e}	0.56±0.20^{f}	6.97±1.40^{f}
UR21		302.71±94.01^{e}	81.96±30.18^{f}	23.84±7.70^{e}	20.07±5.66^{f}	21.24±8.24^{ef}	40.79±11.58^{e}	0.13±0.04^{e}	0.50±0.21^{f}	6.31±2.02^{f}
UR22		360.41±103.54^{e}	112.89±45.33^{ef}	22.83±3.78^{e}	24.60±7.39^{ef}	29.68±12.54^{f}	39.07±4.52^{e}	0.17±0.06^{e}	0.71±0.33^{e}	7.13±1.08^{f}
UC		382.04±101.77^{e}	123.33±41.95^{e}	24.88±5.05^{e}	26.77±5.66^{e}	34.31±17.49^{e}	47.47±8.63b^{e}	0.18±0.05^{e}	0.72±0.35^{e}	8.32±1.66^{e}
Total		342.62±105.03	101.63±39.12	23.58±6.00	23.16±6.70	27.11±12.66	42.36±8.96	0.16±0.04	0.62±0.27	6.89±1.54
p-value		**0.394**^{ns}	**0.070**^{ns}	**0.876**^{ns}	**0.124**^{ns}	**0.103***	**0.222**^{ns}	**0.285**^{ns}	**0.022***	**0.015***
					Quercus robur					
SR20	Solid	597.88±151.78^{b}	130.18±19.66^{a}	37.64±8.96^{ab}	33.85±2.83^{b}	37.49±4.85^{a}	47.87±5.43^{a}	0.24±0.07^{a}	0.99±0.15^{a}	6.38±0.48^{a}
SR21		664.72±156.56^{a}	145.75±27.53^{a}	34.94±12.46^{bc}	39.58±3.23^{a}	40.86±3.84^{a}	56.42±16.35^{a}	0.26±0.06^{a}	1.09±0.21^{a}	8.25±1.81^{a}
SR22		670.63±193.31^{a}	134.17±48.93^{a}	46.29±5.92^{a}	29.60±6.76^{b}	38.72±3.33^{a}	59.85±13.16^{a}	0.22±0.09^{a}	1.04±0.36^{a}	7.62±1.46^{a}
SC		672.41±112.37^{a}	152.96±17.73^{a}	36.57±9.13^{c}	31.33±6.61^{b}	41.46±4.77^{a}	48.78±15.77^{a}	0.22±0.07^{a}	1.03±0.19^{a}	8.69±1.41^{a}
Total		641.41±157.42	140.76±31.07	36.36±11.48	33.59±6.27	39.63±7.56	53.23±13.81	0.23±0.06	1.04±0.22	7.74±1.29
p-value		**0.012***	**0.395**^{ns}	**0.001***	**0.002***	**0.674**^{ns}	**0.184**^{ns}	**0.191**^{ns}	**0.204**^{ns}	**0.152**^{ns}
UR20	Liquid	443.93±225.91^{e}	55.89±29.77b^{ef}	12.29±2.96^{e}	25.54±11.89^{e}	15.28±7.80^{ef}	12.53±3.73^{f}	0.16±0.07^{e}	0.39±0.21^{f}	0.95±0.51^{e}
UR21		421.33±202.95^{e}	56.70±28.37b^{ef}	13.08±4.37^{e}	17.63±10.41^{e}	13.71±8.49^{ef}	13.25±4.45^{ef}	0.11±0.06^{e}	0.38±0.24^{f}	1.12±0.46^{e}
UR22		474.44±91.74^{e}	71.06±35.94^{e}	13.49±5.74^{e}	24.22±6.32^{e}	21.22±9.63^{e}	11.91±5.49^{f}	0.15±0.07^{e}	0.59±0.24^{e}	1.24±0.43^{e}
UC		437.25±94.64^{e}	54.60±18.55^{f}	15.68±2.94^{e}	21.92±4.97^{e}	19.11±5.25^{f}	17.42±4.45^{e}	0.13±0.06^{e}	0.23±0.15^{f}	1.67±0.58^{e}
Total		444.24±171.88	52.06±30.71	13.54±4.19	22.33±9.01	14.83±8.78	13.78±4.89	0.14±0.06	0.40±0.09	1.25±0.49
p-value		**0.147**^{ns}	**0.042***	**0.349**^{ns}	**0.267**^{ns}	**0.025***	**0.054***	**0.062**^{ns}	**0.042***	**0.097**^{ns}

S – State Forests Solid fertilization, U – University novel liquid fertilization, R – novel substrates, C – control substrate (peat−perlite)
Letters with different alphabet indicate statistically significant differences between means (p < 0.05).
Letters 'a' and 'b' denote homogeneous groups under state fertilization and 'e' and 'f' denote homogeneous groups under novel liquid fertilization

Table 18. *Summary of species differences one year after planting in the forest*

Sn.	Parameter	*Fagus sylvatica*	*Quercus robur*
1.	Root strategy	Phototropic (surface foraging)	Gravitropic (depth-focused)
2.	Key root predictor	Very fine root length and surface area	Total root length only
3.	Root branching	Highly branched, fine roots	Coarser, vertically aligned
4.	Sensitivity to treatment	High (especially substrates)	Moderate (more resilient)
5.	Best-performing treatment	SC and SR22	SC and SR20, SR21, SR22
6.	Biomass-to-nutrient correlations	Stronger	Strong

6. Discussion

The findings provide clear support for earlier stated research hypotheses. First, the results confirmed that peat-free substrates such as R22, when combined with solid fertilization, matched the performance of the conventional peat-based substrate in promoting shoot growth, root development, and nutrient uptake. Second, the influence of nursery treatments continued after transplanting, as evidenced by significant increases in shoot height, collar diameter, and root system expansion one year after planting. Third, species-specific root traits shows that, very fine root length and surface area in beech, and total root length in oak were strongly predictive of field growth, validating the functional importance of root system quality in determining early plantation success. These outcomes confirm that targeted nursery interventions can deliver both immediate and long-term benefits for forest regeneration.

The findings of this study provide strong evidence that nursery production decisions create lasting legacy effects on seedling performance beyond the nursery phase. Seedlings of both *Fagus sylvatica* and *Quercus robur* that were raised in peat-based (SC) and peat-free (SR22) substrates, combined with solid fertilization, consistently outperformed other treatments not only in the nursery but also one year after planting (1YAP). This was evidenced by significant height gains of over 100% in beech and 77–82% in oak, confirming that early nursery advantages persist in the field environment. These legacy effects extended beyond shoot growth to include root system expansion and plasticity. Beech seedlings maintained dense, fine-root networks that enhanced surface nutrient capture, while oak seedlings preserved deep, coarse-root structures that facilitated moisture access and anchorage. Regression models (Table 9) confirmed that very fine root traits in beech and total root length in oak were the strongest predictors of post-planting growth performance, validating the functional importance of root architecture pre-conditioned in the nursery.

An important but often overlooked factor influencing seedling development is the physical constraint imposed by containerized nursery systems. In this study, both *Fagus sylvatica* and *Quercus robur* were raised in V300 Styrofoam containers, which provided a controlled root space but may have also restricted lateral root expansion. This constraint appeared to amplify species-specific root responses. These patterns are consistent with earlier findings that container geometry can significantly influence root morphology, potentially affecting post-planting root exploration capacity (Sung *et al*., 2019; Korbik, *et al*., 2025). While container systems are essential for standardizing seedling production, they may also lead to root deformation or root circling, which can impair root-soil contact and hydraulic function after planting (Salifu *et al*., 2005). However, the consistent post-planting performance of seedlings in this study suggests that well-designed container systems can successfully promote functional root architecture when combined with appropriate substrate and fertilization management. This finding highlights the need for further optimization of container design, including volume, shape, and depth, to better align with the root system strategies of different species. Such improvements could enhance root system quality, minimize post-planting transplant shock, and improve early establishment success.

Seedlings raised in these substrates shows stable nutrient supply biomass expansion across all organs after planting, with SC and SR22 treatments leading in total dry mass production. These findings align with earlier work by Grossnickle (2012) and Ivetić and Škorić (2013), who highlighted the role of nursery quality in sustaining early field establishment success. Importantly, these results emphasize that nursery grading systems should go beyond visual seedling size, such as height or collar diameter alone, and consider root trait quality as a critical predictor of long-term field performance. The ability of seedlings to exploit soil resources effectively after planting is directly linked to the morphological and physiological traits shaped during nursery production. This underscores the practical importance of nursery management decisions in ensuring both short- and long-term plantation successes.

This study also demonstrated that both substrate composition and fertilization regime are critical drivers of seedling quality at the nursery stage and influence biomass development after planting (Velázquez *et al*., 2016; Pascual *et al*., 2018). The consistent success of R22 substrate, when combined with solid fertilization, highlights the feasibility of using sustainable, organic-based growing media without compromising seedling performance. Seedlings grown in SR22 and SC substrates produced the highest total dry mass, both in the nursery and one year after planting.

This performance was evident in shoot, leaf, and root biomass, confirming that a balanced supply of nutrients and physical substrate structure supports both above- and below-ground development (Wang, *et al.*, 2023). In contrast, seedlings raised with liquid fertilization exhibited more variable performance, with generally lower total biomass.

Nutrient allocation patterns varied both by species and treatment, reflecting differential uptake efficiencies and metabolic demands. Solid fertilizers provided higher macronutrient concentrations (N, P, K, Mg) in in the studied organ particularly in SC and SR22 treatments. Beech seedlings demonstrated higher concentrations of N and P, particularly in leaf and shoot, both in the nursery phase (Table 14) and one year after planting (Fig. 9). These nutrients are essential for rapid metabolic activity, photosynthesis, and fine-root maintenance, supporting beech's strategy of maximizing early resource uptake in nutrient-rich surface soils (Makela *et al.*, 2008; Pérez-Ramos *et al.*, 2010). In contrast, oak seedlings consistently accumulated Ca and Mg in roots, reflecting their structural growth priority and stress-buffering capacity (Crowley and Lovett, 2017; Park *et al.*, 2008). These elements contribute to cell wall stability, woody tissue formation, and long-term nutrient storage, enabling oak to build a robust framework for future canopy development and hydraulic stability.

Beyond immediate growth performance, the biomass partitioning patterns observed in this study provide deeper insights into the functional survival strategies of *Fagus sylvatica and Quercus robur*. The consistent investment of beech in leaf and shoot biomass, paired with its dense fine root systems, reflects a resource-acquisitive strategy designed for rapid canopy expansion and short-term dominance in resource-rich environments (Simon *et al.*, 2014). This makes beech highly competitive in nutrient-rich and moist sites, where fast light capture and space occupation are critical for survival. However, this strategy may come at the cost of reduced structural investment, potentially making beech more vulnerable to drought or mechanical stress if not properly supported by substrate and nutrient management (Schume *et al.*, 2004; Packham *et al.*, 2012). In contrast, oak's root- and stem-dominated biomass allocation pattern represents a conservative, stress-tolerant strategy, investing in long-term structural resilience and deep resource acquisition (Donovan *et al.*, 2000; Kasper *et al.*, 2002; Rodríguez-Calcerrada *et al.*, 2008). This makes oak particularly suited to drought-prone or nutrient-poor environments, where mechanical stability and deep water access are more critical than rapid above-ground growth. While oak may establish more slowly than beech in favorable conditions, its structural investment supports long-term survival and stand stability, especially in climate-stressed or marginal sites (Leuschner *et al.*, 2001; Rotowa *et. al* 2025c).

Interestingly, while both species performed best under solid-fertilized SC and SR22 treatments, the nutrient allocation pathways differed markedly. Beech showed stronger biomass-to-nutrient correlations, particularly between N and shoot growth (Fig. 10), suggesting higher nutrient use efficiency. Oak, on the other hand, displayed more stable nutrient concentrations across treatments, but with weaker correlations to above-ground biomass (Fig. 6), reflecting a more conservative nutrient strategy. Therefore, these differences imply that species-specific fertilization protocols may be required to optimize nutrient efficiency and field performance. For beech, strategies that promote rapid nutrient uptake and turnover are critical, while for oak, stable nutrient provisioning that supports structural development may be more beneficial. This highlights the importance of matching nutrient delivery systems to species-specific functional traits when designing nursery production protocols.

These results have practical implications for forest nursery management, particularly in the context of European Union directives aimed at reducing peat consumption to mitigate greenhouse gas emissions from land use change (EU, 2018; EPAP, 2021). The peat-free R22 substrate provided structural stability, aeration, and moisture retention comparable to peat-based controls. Its strong performance supports current European and national policies that encourage the transition toward bio-based, renewable growing media (Hirschler *et al.*, 2022). In addition to substrate selection, nutrient delivery system choice was a significant factor. Solid fertilization, using slow-release granules incorporated into the substrate, provided stable nutrient availability throughout the seedling's early growth stages. This stability supported greater biomass accumulation compared to the irregular nutrient supply provided by liquid fertilization. These findings align with previous reports emphasizing the role of consistent nutrient delivery in improving plant health and growth outcomes (Makela *et al.*, 2008; Pérez-Ramos *et al.*, 2010). Importantly, these results demonstrate that nursery practices combining well-structured, peat-free substrates with solid fertilization can achieve high-quality planting stock while reducing environmental impacts. This dual benefit positions peat-free, solid-fertilized production systems as a sustainable and effective strategy for modern nursery management and large-scale forest restoration.

The studied species displayed distinct biomass allocation strategies that reflect their ecological adaptations and functional growth priorities. These differences were consistently observed across both nursery and field stages, providing valuable insights into how species-specific traits influence growth dynamics. Beech seedlings exhibited a balanced biomass distribution between roots, stems, and leaves, with a noticeable investment in leaf biomass and canopy development. This strategy supports early light capture and photosynthetic efficiency, enabling rapid shoot elongation and competitive canopy closure in shaded or nutrient-rich environments (Eissenstat, *et al.,* 2000; Leuschner, *et al.,* 2001; Gessler, *et al.,* 2005; Brunner, *et al*., 2015).. The strong correlation between shoot biomass and nutrient content, particularly N and P, further emphasizes beech's dynamic nutrient-driven growth strategy. In contrast, oak seedlings followed a root-dominated biomass allocation pattern, with a higher proportion of dry mass consistently directed toward roots especially the coarse portion of the root (Kozlowski, and Pallardy, 2002; Wilson, *et al.,* 2007; Iversen, 2010; Allen, 2015). This conservative growth strategy prioritizes mechanical stability and long-term structural resilience, rather than immediate canopy expansion. Even when oak seedlings received favorable nursery treatments, root mass remained the dominant biomass sink, confirming the species' reliance on below-ground structural development.

These contrasting strategies imply different ecological roles and site suitability for the two species. Beech may perform better on nutrient-rich, moisture-retentive sites where rapid canopy formation is desired to outcompete other vegetation. In contrast, oak is better suited to drought-prone or structurally demanding sites, where deep rooting and mechanical support provide long-term survival advantages (Pregitzer *et al.,* 2002; Park *et al.,* 2008). Understanding these species-specific biomass strategies is essential for nursery and forest managers seeking to match planting stock to site conditions. Tailoring nursery practices to reinforce these natural allocation patterns can improve plantation success and ecosystem resilience in diverse forest landscapes.

In addition to their contrasting biomass allocation patterns, *Fagus sylvatica* and *Quercus robur* exhibited distinct root system strategies that explain their species-specific responses to nursery treatments and field conditions. Beech seedlings demonstrated a phototropic, fine-root-dominated strategy, characterized by the development of very fine roots ($\leq$ 0.5 mm) with extensive surface area. This shallower, highly absorptive root network maximizes nutrient and water uptake in the upper soil layers, supporting the species' rapid nutrient-driven growth (Makita *et al.,* 2011; Zhang and Wang, 2015). Oak seedlings in contrast, displayed a gravitropic rooting strategy, emphasizing the development of coarse roots and deeper root penetration. This architecture enhances mechanical anchorage and water access from deeper soil layers, making oak better suited to structurally demanding (Pregitzer *et al*., 2002; Park *et al.,* 2008).

These findings have important implications for nursery production and forest restoration programs. The clear differences in species response suggest that uniform nursery protocols may not be optimal. Instead, customized treatments matching substrate properties and fertilization types to species-specific traits can maximize seedling quality and early field performance. For beech, peat-free substrates such as R22 paired with solid fertilization can ensure both ecological sustainability and growth efficiency. For oak, focus should be placed on substrates that support deeper root elongation and structural development.

Furthermore, these results highlight the significance of nursery-driven root plasticity in enabling seedlings to exploit diverse soil niches upon planting. Fine-root expansion in beech supported rapid nutrient capture, while deeper rooting in oak facilitated moisture access and nutrient buffering. Both responses demonstrate that morphological adaptations initiated in the nursery persist into the forest phase, influencing growth trajectories beyond the first year. Ultimately, the observed increases at 1YAP validate the critical role of nursery substrate and fertilization choices in ensuring vigorous seedling development. They reinforce the need for forest managers to select production systems that promote root trait expression aligned with species-specific ecological strategies. These findings complement earlier conclusions that the physiological quality of planting stock is a reliable predictor of long-term plantation success (Grossnickle, 2012; Rotowa *et al.*, 2023; Rotowa *et al.*, 2025a).

The contrasting growth strategies of *Fagus sylvatica* and *Quercus robur* identified in this study provide valuable insights for mixed-species plantation design, particularly in the context of climate change adaptation and ecosystem resilience. Combining species with complementary resource acquisition and stress tolerance traits offers a strategic way to diversify ecosystem functions and reduce plantation vulnerability to environmental stressors (Pretzsch *et al.*, 2013a; 2013b; 2015; 2016). For example, beech's rapid canopy expansion can be leveraged to suppress ground competition and accelerate microclimate stabilization, while oak's deep rooting and mechanical stability can provide long-term stand resilience against drought and windthrow. This functional complementarity proposes that mixed planting of beech and oak could optimize both early site capture and long-term ecosystem stability in our world of increasing climatic variability.

7. Summary and conclusions

This PhD study evaluated the effect of novel nursery substrate composition and fertilization systems on the early growth dynamics, root morphology, biomass distribution, and nutrient allocation of *Quercus robur* and *Fagus sylvatica* seedlings, both in controlled nursery conditions and after one year of forest establishment. The key conclusions are outlined below in direct response to the study's hypotheses.

1. This study provides strong statistical evidence that peat-free organic substrates, particularly the SR22 formulation, are effective and environmentally sustainable alternatives to conventional peat-based growing media in forest nursery production. Seedlings of both *F. sylvatica* and *Q. robur* grown in SR22 achieved comparable performance in biometric traits, root system development, and nutrient accumulation compared to those grown in the commercial peat-based substrate (SC). These results were consistent across both the nursery phase and one year after planting, confirming the functional viability of peat-free substrate.

2. The results of this study provide convincing evidence that the influence of nursery production decisions extends well beyond the nursery phase, shaping seedling performance one year after planting. Seedlings of *F. sylvatica* and *Q. robur* raised on peat and R22 substrates, when combined with solid fertilization, achieved the highest increases in shoot height, collar diameter, and total biomass not only in the nursery but also in the field. Beech seedlings achieved height increments exceeding 100%, while oak achieved increases of 82%, confirming that early nursery advantages translate into sustained field growth. These legacy effects were not limited to above-ground traits but also extended to root system architecture.

3. Solid fertilizers consistently outperformed liquid fertilization**,** delivering a more stable and accessible nutrient supplies that aided superior growth in both species. This was evident in higher biomass accumulation, nutrient concentrations, and root development in solid-fertilized seedlings across all treatments. This finding hope that, the current formulation of the liquid fertilizer may require optimization, particularly through the addition of essential nutrients like N, to enhance its effectiveness to better support seedling growth and survival beyond preliminary stage.

4. Peat-free substrates particularly R22 demonstrated root growth and survival rates comparable to those of conventional peat-based controls. The studied species exhibited contrasting responses, reflective of their distinct ecological foraging strategies. These results highlight the predictive value of root morphological traits in understanding early developmental dynamics under novel substrate and fertilizer regimes.

8. Literature

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Declaration of author contribution:

Declaration of author contribution:

Rotowa, O.J. Malek, S. Banach J. Pach, M. (2023). Effect of different innovative substrate mediums on roots characterization of European beech (*Fagus sylvatica* L.) and Pedunculate oak (*Quercus robur* L) seedlings. Sylwan, 167 (9): doi.org/10.26202/sylwan.2023075.

Contribution of individual authors to the above publication:

prof. dr hab. eng Stanisław Małek. - study concept and design, methodology, article proofreading, reviewing, and editing, funding acquisition project administration, approval of the final version of the article

Contribution: 20%

dr hab. eng Jacek Banach, prof. URK. - concept and design, methodology, supervision over the correctness of root system data, formal analysis of data, proofreading of the article.

Contribution: 10%

dr hab. eng Maciej Pach prof. URK - results visualization, translation, article proofreading, editing, and review

Contribution: 10%

M. Sc. Odunayo James Rotowa - paper concept, literature review, data development, writing the article, proofreading, participation in field studies, conducting laboratory and statistical analyses: 60%

Rotowa, O.J., Małek, S., Jasik, M., & Staszel-Szlachta, K. (2024). Effect of innovative peat-free organic growing media and fertilizer on nutrient allocation in pedunculate oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.) seedlings after nursery production cycle. New Forests (2024) 56:171-22 https://doi.org/10.1007/s11056-024-10079-1

Contribution of individual authors to the above publication:

prof. dr hab. eng Stanisław Małek. - study concept and design, methodology, supervision, article proofreading, reviewing, and editing, funding acquisition project administration, approval of the final version of the article

Contribution: 20%

dr eng Michał Jasik - Investigation, Data collection, Data editing and validation, writing, review.

Contribution: 10%

dr eng Karolina Staszel-Szlachta. – data collection, data curation, data analysis, review.

Contribution: 10%

M. Sc. Odunayo James Rotowa - paper concept, literature review, methodology, manuscript preparation, data collection, entry and statistical analysis, writing the article,

Contribution: 60%

Rotowa, O.J. Malek, S. Jasik, M. Staszel-Szlachta, K. Substrate and Fertilization Used in the Nursery Influence Biomass and Nutrient Allocation in *Fagus sylvatica* and *Quercus robur* Seedlings After the First Year of Growth in a Newly Established Forest. Forests 2025, 16, 511.
doi.org/10.3390/f16030511

Contribution of individual authors to the above publication:

prof. dr hab. eng Stanisław Małek. - study concept and design, methodology, validation, supervision, article proofreading, reviewing, and editing, funding acquisition project administration, assisted in field layout and forest plantation establishment approval of the final version of the article

Contribution: 20%

dr eng Michał Jasik - review and editing, formal analysis, proofreading of the article, validation.

Contribution: 10%

dr eng Karolina Staszel-Szlachta. – data curator, formal analysis proofreading of the article, validation.

Contribution: 10%

M. Sc. Odunayo James Rotowa - paper concept, literature review, data development, field layout, forest plantation establishment, data collection, entry and analysis, writing the article, conducted laboratory and statistical analyses

Contribution: 60%

Rotowa, O.J. Małek, S. Kupka D. Pach, M. Banach J. (2025). Innovative peat-free organic substrates and fertilizers influence growth dynamics and root morphology of *Fagus sylvatica* L. and *Quercus robur* L. seedlings one year after planting. Forests 2025, 16, 800. https://doi.org/10.3390/f16050800

Contribution of individual authors to the above publication:

prof. dr hab. eng Stanisław Małek. - study concept and design, methodology, validation, supervision, article proofreading, reviewing, and editing, funding acquisition project administration, assisted in field layout and forest plantation establishment approval of the final version of the article

Contribution: 15%

....

dr eng Dawid Kupka- methodology, review and editing, assisted in field layout and forest plantation establishment, data collection and recording, formal analysis.

Contribution: 10%

....

dr hab. eng Jacek Banach, prof. URK. – Data curator, formal analysis proofreading of the article.

Contribution: 10%

....

dr hab. eng Maciej Pach- results visualization, article proofreading, editing, and review

Contribution: 10%

....

M. Sc. Odunayo James Rotowa - paper concept, literature review, data development, field layout, forest plantation establishment, data collection, entry and analysis, writing the article, conducted laboratory and statistical analyses

Contribution: 55%

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sYLwaN 167 (9): 535–548, September 2023
https://doi.org/10.26202/sylwan.2023075
Journal homepage: https://sylwan-journal.pl

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Effect of different innovative substrate mediums on roots characterization of European beech *Fagus sylvatica* L. and pedunculate oak *Quercus robur* L. seedlings

Odunayo James Rotowa✉, Stanisław Małek, Jacek Banach, Maciej Pach

Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Krakow, Al. 29 Listopada 46, 31–425 Kraków, Poland

ABSTRACT

The development of a root system is crucial for the effective establishment of forest tree seedlings. There are various seedling production methods in nurseries adopted by professionals and foresters to guarantee quality root systems aimed at successful forest plantations. This study evaluated the effect of different innovative, peat–free organic substrates (R20, R21 and R22) on the root system and nutrient content in the root zone of European beech and pedunculate oak seedlings. This was done to examine if the newly designed substrate and liquid fertilizer formulated by the University of Agriculture in Krakow (UAK) would successfully grow seedlings that meet the existing characteristics of those raised with peat substrate and solid fertilizer. Although the properties and granulometric composition of the substrates were different during the production process of the seedlings, two different Osmocote fertilizers (solid 3–4M and 5–6M) were applied. Fertilization used in the State Forest nurseries based on the set standard was represented with SR20, SR21 and SR22, while the novel fertilizer developed by UAK was represented with UR20, UR21 and UR22. Meanwhile, SC and UC represent the control substrates (peat) in both cases, respectively. The substrates developed by UAK were adapted to the nutritional requirements of the forest tree seedlings and their suitability was monitored using nursery technology with a covered root system in multi–pot containers. The experiment was laid out in a 2×2×4 (2 species, 2 types of fertilizers and four different substrates) experimental design using five seedlings per treatment. The results of the study indicated that the innovative substrate and fertilizer support root system development and aid sufficient macro element content for seedling production in the nursery. Treatment UR20 recorded the highest mean value of total root length in both species. A significant variation was observed from the analysis of nutrients in the root system. Conclusively, substrate mediums developed under this study have proven to possess qualities not worse than the substrate based on peat because the root system is adequately well developed. This guarantees the quantity and reliability of supplies and could replace high peat in the substrate formula.

KEY WORDS

forest seedlings, peat, peat–free organic substrate, root

✉e-mail: odunrotowa@yahoo.com

Received: 4 September 2023; Revised: 18 November 2023; Accepted: 21 November 2023; Available online: 20 December 2023

Introduction

Forest tree seedlings can be grown in conventional (ground) nurseries or unique compartments often called container nurseries. Irrespective of the method adopted, seedling development relies on several factors for survival such as the availability and accessibility to light, water, and mineral supplements as well as the type of substrate medium used and its physical properties to grow the seedlings (Alameda and Villar, 2009; Perez–Ramos *et al*., 2010; Kormanek, 2013, Pająk *et al*., 2022a). Different standards are utilized to evaluate the seedlings' suitability for plantation establishment. This could be based on attributes such as height and dry weight. However, previous studies have also suggested other factors including seedling sturdiness quotient (SQ) and shoot–root index (S/R) which better indicate the capability of seedlings for utilization (Haase, 2007; Grossnickle, 2012; Ivetić and Skorić, 2013; Banach *et al*., 2020, 2021).

Peat serves as the primary component in nursery substrates as it is known for its exceptional physical, chemical and biological properties. Its remarkable water retention capacity and consistent, high–quality attributes make it an ideal medium for plant cultivation. However, peat soils accumulate a substantial amount of carbon over time which could have profound climate implications. While forests typically sequester carbon, peatlands can inadvertently release it into the atmosphere. This poses a significant challenge since peatlands store more soil carbon, equivalent to over one–third of the world's total, surpassing even the combined carbon storage of all global forests. When peat is spread on plantations, it quickly transforms into carbon dioxide contributing to elevated greenhouse gas levels and endangering precious ecosystems. The annual excavation of 20,000 cubic meters of peat according to Gruda (2012) further exacerbates environmental degradation. As a result of global environmental concerns associated with the use of peat as a standard nursery substrate, peatland should rather be preserved and not destroyed. The growing emphasis on environmental sustainability necessitates the need to design an innovative peat–free organic substrate with materials that are sustainable, cheap and ecologically friendly as alternatives to peat.

Historically rooting space is measured as a plant resource, yet research on biomass portion versatility related to root volume (RV) is uncommon. However, root volume can be considered an asset for plant growth (McConnaughay and Bazzaz, 1991). A decrease in rooting volume can alter entire plant development based on nutrient accessibility. Mechanical limitations forced to root growth and development by the volume of a container has been a significant issue of concern for forest plants (Landis, 1990; Ferree *et al*., 1992; Beeson, 1993; NeSmith and Duval, 1998; Aphalo and Rikala, 2003; Dominguez–Lerena *et al*., 2006). Root limitation lessens crop development and expansion in shoot/root biomass proportion (NeSmith *et al*., 1992; Hsu *et al*., 1996; Clemens *et al*., 1999). The impact of root limitation in different species has been studied (Endean and Carlson, 1975; Carlson and Endean, 1976; Lamhamedi *et al*., 1998; South *et al*., 2005; Dominguez–Lerena *et al*., 2006). The growth response of seedlings to compact rooting volume may be based on species (NeSmith and Duval, 1998; Climent *et al*., 2008).

In Poland, coniferous monocultures have been intensively restructured due to the declining health and quality of trees. European beech *Fagus sylvatica* L. is an Atlantic climate species found throughout central and western Europe (Jaworski, 2019). Oak *Quercus robur* L. is a significant tree species in Polish forests and the majority of European temperate vegetation types. Due to their excellent wood quality, beech and oak are becoming more competitive than several conifers as they are the preferred tree genera in adaptation strategies to climate change for both ecological and economic reasons in Europe (Rotowa *et al*., 2023). Hence, it is of paramount importance to intensify efforts to raise the health and the quantity of sustainable forest stands of these highly sought species.

Therefore, a comparison of the root biomass allocation and ontogenetic parameters of beech and oak seedlings of contrasting substrate treatments were used as the basis for this study. The following hypothesis was tested for beech and oak seedlings grown in a container nursery using organic substrate: the features of beech and oak seedlings grown on a peat–free substrate and liquid fertilizer developed by the University of Agriculture in Krakow are similar to those grown on a standard substrate (peat plus solid fertilizer).

Materials and methods

SUBSTRATE COMPOSITION AND PREPARATION. The peat substrate used for this study as the control variant (C) was produced at Nursery Farm in Nędza (50.167964 N, 18.3138334 E). The substrate composition included peat 93% and perlite 7% with the addition of dolomite (3 kg per m^3 of the substrate) to obtain a pH of 5.5. The peat–free substrates (R20, R21, R22) consisted of a blend of various components including scobs, wood chips, straw, bark, perlite, core wood and mixed silage [%]. These components were combined in varying proportions as shown in Table 1. The peat–free substrates and liquid fertilizer used in the study were prepared under the project POIR.04.01.04–00–0016/20 funded by the National Centre for Research and Development (NCBiR) from National Resources and the European Regional Development Fund entitled 'Innovative technologies for the production of substrate and fertilizer produced from indigenous resources for the production of forestry tree seedlings' which was led by the Department of Ecology and Silviculture, Forest Faculty, the Agricultural University of Krakow. Four substrates (R20, R21, R22 and peat) and two fertilization variants were used. The first was a standard fertilizer in the Suków container nursery (SR20, SR21 and SR22 variants) and the second was a novel liquid fertilizer designed by the University of Agriculture in Krakow (UR20, UR21 and UR22). The peat substrate used in both fertilizer scenarios included two control variants (SC and UC). The substrates were mineralized with a microwave mineralizer MARS CEM in a mixture of HCl (35–38%) and HNO_3 (65%) acids at the Laboratory of Forest Environment Geochemistry and Land Intended for Reclamation in the Department of Ecology and Silviculture and Faculty of Forestry, University of Agriculture in Krakow. In each experimental variant seedlings of both species were grown in 75 Marbet V300 polystyrene containers each containing 53 cells with a volume of 275 cm^3 (Fig. 1). The cells were tapered downward and were equipped with vertical guides for the root systems. The components of the peat that were adapted for the preparation of the substrate used for this study are shown in Table 2, although the particle sizes of the substrate before seed sowing were different (Table 3). The nutrient content present in the substrate was the same before seed sowing, however, it became different at the end of seedling production (Table 4).

SEED SOWING AND GERMINATION. After filling the containers with the various substrates, beech and oak seeds were sown manually in the Suków–Papierna Nursery Farm (Daleszyce Forest District). The seeds were sown on April 19–20, 2022 with the preparation and sowing of seeds

Table 1.
Properties of the organic peat free substrate

Substrate	Scobs	Wood chips	Straw	Wood bark	Perlite	Core wood	Mixed silage
				[%]			
R20	73	10	–	10	4	2	1
R21	20	63	–	10	4	2	1
R22	50	–	10	33	4	2	1

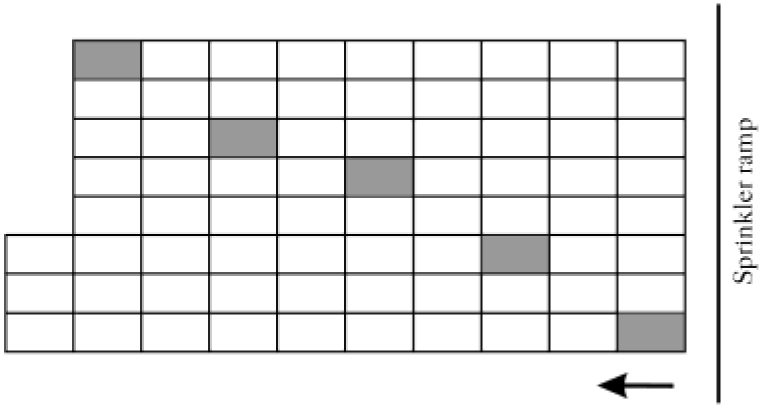

Fig. 1.
Distribution of containers in the production field for one experimental variant; the containers from which seedlings were taken for analysis are marked in grey

Table 2.
Mean and standard deviation values of the organic substrate properties

Substrate	Water capacity [%]	Average [litre/min]	Variation factor [%]	Bulk density [g/cm³]	Solid density [g/cm³]	Air capacity [%]	Porosity [%]
R20	53.02 ±2.42	0.595 ±0.150	25.2	0.127 ±0.009	1.56 ±0.000	38.90 ±2.90	91.85 ±0.60
R21	45.39 ±3.60	0.781 ±0.114	14.6	0.103 ±0.013	1.61 ±0.000	48.14 ±4.20	93.62 ±0.83
R22	50.71 ±2.11	0.594 ±0.150	25.3	0.113 ±0.009	1.62 ±0.000	42.35 ±2.61	93.04 ±0.55
Control	71.44 ±2.83	0.417 ±0.145	34.9	0.091 ±0.006	1.59 ±0.000	22.89 ±3.15	94.25 ±0.39

Table 3.
Mean and standard deviation values of granulometric composition of the substrate before sowing

Substrate	>10 mm	10–5 mm	5–2 mm	2–1 mm	1–0.5 mm	0.5–0.25 mm	0.25–0.1 mm	>0.1 mm
R20	0.05 ±0.10	3.77 ±1.57	14.45 ±5.90	30.53 ±9.72	24.45 ±2.24	17.72 ±7.49	7.71 ±4.16	1.69 ±0.98
R21	0.00 ±0.00	6.40 ±1.37	25.44 ±1.91	30.90 ±1.11	19.11 ±0.90	12.16 ±0.31	5.12 ±0.41	0.96 ±0.11
R22	0.08 ±0.13	3.03 ±0.45	14.15 ±2.36	33.36 ±2.36	25.11 ±1.07	17.02 ±3.21	7.11 ±1.72	1.48 ±0.28
Control	0.00 ±0.00	11.27 ±0.37	25.08 ±1.18	27.77 ±1.05	16.20 ±1.05	8.42 ±0.56	3.81 ±0.43	1.88 ±0.21

carried out by workers at the container nursery. To enhance the germination process, oak seeds were scarified just before sowing which involved the removal of approximately one–third of the seed in the cotyledon part. In contrast, beech seeds underwent a stratification process without the use of a stratification medium with temperature maintained at +3°C and humidity at 31%. The seeds used for all substrate variants, regardless of species, were sourced from the same provenance and came with separate certificates of origin (MR/65848/21/PL for oak and MR/63313/20/PL for beech). After sowing, the containers were placed in a vegetation hall for four weeks and then transported to an external production field. During the growth of the seedlings manual weeding was employed. The seedlings were grown for five months following the procedure used in the container nursery (Szabla and Pabian, 2009). During the seedling growth period the total rainfall was only 78 mm, therefore, to replenish the water deficit irrigation was applied using an automatic RATHMAKERS Gartenbautechnik sprinkler ramp.

Osmocote fertilizer was applied once during substrate preparation before sowing at a total dose of 3 kg m^{-3} of each substrate medium, prepared as a mixture of Osmocote 3–4M (2 kg) and

Table 4.
Nutrient content of substrates before seed sowing and after seedling production

Substrate	C	N	P	K	Ca	Mg	Na
	[%]						
Before sowing							
R 20	48.01	0.297	0.031	0.159	0.452	0.055	0.040
R 21	46.34	0.507	0.068	0.271	0.601	0.072	0.035
R 22	48.9	0.447	0.043	0.404	0.857	0.059	0.042
Control	45.85	0.709	0.015	0.058	1.307	0.585	0.068
After seedling production							
Beech							
UR20	44.148	0.434	0.030	0.066	0.677	0.055	0.016
UR21	42.518	0.5323	0.049	0.072	0.854	0.055	0.015
UR22	42.93	0.578	0.043	0.074	1.179	0.066	0.018
UC	39.784	0.651	0.019	0.071	1.543	0.525	0.072
SR20	42.167	0.596	0.093	0.129	0.721	0.068	0.018
SR21	39.978	0.996	0.134	0.161	0.985	0.087	0.020
SR22	42.167	0.756	0.110	0.156	1.463	0.086	0.023
SC	40.987	0.844	0.096	0.162	1.695	0.476	0.075
Oak							
UR20	44.703	0.383	0.028	0.563	0.594	0.065	0.015
UR21	44.969	0.418	0.032	0.597	0.627	0.042	0.015
UR22	45.422	0.493	0.032	0.650	0.966	0.060	0.015
UC	41.863	0.654	0.016	0.654	1.392	0.472	0.060
SR20	45.455	0.519	0.059	0.991	0.589	0.056	0.014
SR21	43.313	0.942	0.121	1.626	0.879	0.088	0.020
SR22	45.096	0.872	0.114	1.703	1.139	0.081	0.018
SC	41.425	0.805	0.076	1.798	1.424	0.441	0.069

S – State Forests fertilization, U – University fertilization, R – novel substrates, C – control substrate (peat–perlite)

Osmocote 5–6M (1 kg). The composition of the Osmocote fertilizer 3–4M was the following: N – 16% including 7.1% N–NO_3^- and 8.9% N–NH_4^+; P_2O_5 – 9%, K_2O – 12%; MgO – 2.0%, and microelements (B, Fe, Cu, Mn, Zn, Mo); 5–6M: N – 15%; including 6.6% N–NO_3^- and 8.4% N–NH_4^+; P_2O_5 – 9.0%; K_2O – 12%; MgO – 2.0%; and microelements (B, Fe, Cu, Mn, Zn, Mo). The novel liquid fertilizer applied was based on two distinct compositions. The first variant consisted of N at 4.78%, P_2O_5 at 1%, K_2O at 2.64%, CaO at 2.65%, MgO at 1.4%, SO_3 at 0.71%, and Na_2O at 0.14%. This fertilizer was administered initially with a total volume of 3.14 dm^3 (0.048 $dm^3 \cdot 1\ m^{-2}$). The second fertilizer variant contained N at 0.798%, P_2O_5 at 0.166%, K_2O at 0.440%, CaO at 0.441%, MgO at 0.234%, SO_3 at 0.118%, and Na_2O at 0.023%. The second fertilizer was applied with a total volume of 15.09 dm^3 (0.229 $dm^3 \cdot 1\ m^{-2}$). Over the course of seedling production the first fertilizer variant was applied eight times at 10–day intervals, while the second variant was applied fifteen times at 5–day intervals. It is important to note that the fertilization regimes remained consistent for both beech and oak seedlings.

Parameter assessment and analysis of nutrients. At the end of the nursery's production cycle, a thorough examination of seedlings was conducted. Due to limitations stemming from the availability of seedling parts for laboratory testing, a specific selection process was employed. Five seedlings, characterized by standard vigor and biometric parameters, were carefully chosen from each of the eight treatment groups for the purpose of data collection. This resulted in a total assessment of 40 seedlings for the two species used in the experiment (beech and oak). To obtain seedlings for measurements, five containers in each treatment were selected with 1 seedling taken from each container. These selected containers were distributed diagonally across the experimental field. After measuring the height and root collar diameter of the seedlings, one representative seedling was chosen from each of these subsamples for further analysis which included the evaluation of root system parameters and nutrient content.

Biometric data was collected on root collar diameter (RCD), total root length (TRL), root surface area (RSA), average root diameter (ARD), and root volume (RV). All these root parameters were analyzed using WinRHIZO software in the Laboratory of Forest Biotechnology, Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Krakow, Poland.

Subsequently, the roots of the sampled seedlings were dried at 105°C for 48 h, ground into a powder form and analysed for their N, S, and C content using a LECO CNS TruMac analyzer and P, K, Ca, and Mg contents using a Thermo iCAP 6500DUO ICP–OES following mineralization in nitric and hydrochloric acids at a ratio of 3:1. The concentrations of most chemical elements (expressed in percentages) were determined using spectrometer ICP OES with the exception of C and N which were determined using the TruMac LECO apparatus. The analyses were performed at the Laboratory of Forest Environment Geochemistry and Land Intended for Reclamation in the Department of Ecology and Silviculture and Faculty of Forestry, University of Agriculture in Krakow, Poland.

STATISTICAL ANALYSIS. The experiment consisted of two species (beech and oak), two fertilizers (solid and liquid) and four substrates for each variant (R20, R21, R22 and control). This was laid out in a 2×2×4 experimental design using five seedlings per treatment. To show the comparative performance between the treatments, the collected data (after verifying that it met the assumptions of ANOVA) were subjected to mean and variance analysis (ANOVA). At the same time, the Duncan Multiples Range Test (DMRT) was applied to locate where the significant difference occurred in the treatments at $p<0.05$. This was done to confirm the substrate variant that differed significantly from the others according to the selected biometric features and elemental content in the root systems of both species. A correlation test was further carried out to quantify the strength of the linear relationship between the analyzed variables.

Results

ROOT CHARACTERISTICS OF BEECH AND OAK SEEDLINGS GROWN IN DIFFERENT TYPES OF ORGANIC SUBSTRATES. The root collar diameter of beech seedlings showed evident variation among the eight treatments. Treatment SR22 recorded the highest mean value of 5.95 ±0.75 mm followed by 5.51 ±0.84 mm recorded in SC, while the lowest mean value of 4.00 ±0.58 mm was recorded in the UR22 samples. The results of variance analysis showed that significant variations exist among the treatments. The results for total root length revealed a different trend from the results of root collar diameter. Treatment UR20 recorded the highest mean value (1436.84 cm) followed by the SR20 variant, and the lowest value for this parameter was recorded in the UR21 variant which had a significant difference. The analysis of average root diameter and volume showed that the highest values were recorded in variant UR21 of 0.47 mm and 1.15 cm^3, respectively (Table 5).

Treatment SC recorded the highest mean value of 2.78 mm for the root collar diameter of oak seedlings. The results of the parameters assessed revealed that UR variants has competitive growth and in some cases performs better especially on total root length and surface area. Excellent growth trend was also recorded in the SC variant, especially in average root diameter and root volume.

Table 5.
Mean and standard deviation of studied root system parameters of *F. sylvatica* and *Q. robur*

Treatment	RCD [mm]	TRL [cm]	RSA [cm²]	ARD [mm]	RV [cm³]
			F. sylvatica		
SR20	4.25 ±0.69c	1412.40 ±199.04ab	140.61 ±22.21a	0.31 ±0.01de	1.12 ±0.16bc
UR20	4.35 ±0.77d	1436.84 ±145.29a	134.99 ±12.58a	0.30 ±0.01e	1.01 ±0.16b
SR21	5.26 ±0.87d	825.19 ±70.63 ab	102.22 ±10.78ab	0.39 ±0.02ab	1.02 ±0.16bc
UR21	5.16 ±0.57d	647.47 ±37.07b	95.24 ±8.41ab	0.47 ±0.04a	1.15 ±0.16ab
SR22	5.95 ±0.75b	1067.23 ±204.39a	137.49 ±15.90a	0.43 ±0.04ab	1.47 ±0.16a
UR22	4.00 ±0.58d	656.20 ±98.60b	72.24 ±6.29b	0.36 ±0.02bc	0.64 ±0.16c
SC	4.37 ±0.42d	897.99 ±134.33bc	96.09 ±14.86ab	0.34 ±0.02cd	0.83 ±0.16ab
UC	5.51 ±0.84a	686.06 ±175.92a	82.93 ±18.69b	0.41 ±0.02ab	0.81 ±0.16bc
Total	4.86 ±0.92	953.67 ±66.99	107.73 ±6.13	0.38 ±0.01	1.01 ±0.16
P–value.	0.00**	0.00**	0.00**	0.00**	0.02*
			Q. robur		
SR20	1.66 ±0.32c	730.57 ±177.88a	113.94 ±18.81a	0.53 ±0.06a	1.51 ±0.33a
UR20	0.96 ±0.07d	894.97 ±112.22a	106.18 ±12.40a	0.38 ±0.01a	1.00 ±0.12a
SR21	1.19 ±0.13d	631.41 ±90.58a	111.33 ±12.35a	0.57 ±0.04a	1.60 ±0.22a
UR21	1.16 ±0.19d	619.11 ±125.43a	122.63 ±19.92a	0.67 ±0.14a	2.16 ±0.57a
SR22	2.15 ±0.49b	603.21 ±78.10a	109.63 ±11.48a	0.60 ±0.04a	1.61 ±0.19a
UR22	0.87 ±0.06d	820.04 ±179.87a	110.89 ±15.09a	0.46 ±0.04a	1.24 ±0.16a
SC	2.78 ±0.54a	843.29 ±134.75a	167.23 ±27.81a	0.64 ±0.08a	2.67 ±0.48a
UC	1.15 ±0.19d	494.63 ±126.36a	83.23 ±17.95a	0.99 ±0.49a	1.45 ± 0.33a
Total	1.49 ±0.69	704.65 ±128.15	115.63 ±16.69	0.61 ±0.16	1.65 ±0.44
P–value.	0.00**	0.38ns	0.12 ns	0.46ns	0.39ns

S – State Forests fertilization, U – University fertilization, R – novel substrates, C – control substrate (peat–perlite), RCD – Root collar diameter, TRL – Total root length, RSA – Surface area, ARV – Avg. Diameter, RV – Root Vol. The same letter in the same column are not significantly different while figures with different letter are significantly different at p=0.05

According to the results of ANOVA, significant variation was recorded in the entire root collar diameter based on different substrate formulations. Beyond the root collar diameter and total root length, root surface area, average root diameter, and root volume also varied based on different treatments (Table 5).

A significant positive correlation was observed between root collar diameter and average root diameter and root surface area for beech roots. A negative relationship was observed for oak between root length and average root diameter which was substantial, while most of the other parameters showed significantly positive correlations. Additionally, all assessed parameters consistently exhibited a negative correlation with the substrate treatments with the implication that as the seedlings grow older, the rate of nutrient absorption reduces. This observed negative correlation indicates an inverse relationship between the substrate treatments and the root biometrics parameters (Table 6).

CONTENT OF MACRO ELEMENTS. The concentration of elements in the root systems differed between treatments and tree species. The highest macro element concentration in the beech root system was in the C variant with a total mean value of 46.23%. The highest mean value of C content was 47.56% recorded in treatment UR20, and the lowest mean value (44.71) was recorded in treatment UR21. The result was the same for oak roots. The highest mean value of 46.04% was recorded in treatment UR20, and the lowest mean value (44.22%) was recorded in treatments SR22 and UR22, respectively. The root system's lowest macro element concentration was in S with a total average mean value of 0.047% in beech and 0.042% in oak roots. The highest mean

Table 6.
The results of correlation analysis of biometric root parameters

Parameter assessed	Root collar diameter	Root surface area	Average root diameter	Root volume	Root length	Treatment
			Beech			
Root collar diameter	1					
Surface area	0.337*	1				
Average root diameter	0.371*	−0.160	1			
Root volume	0.530**	0.821**	0.419**	1		
Root length	0.092	0.901**	−0.552**	0.498**	1	
Treatment	−0.310	−0.175	0.058	−0.118	−0.154	1
			Oak			
Root collar diameter	1					
Surface area	0.362*	1				
Average root diameter	0.058	−0.223	1			
Root volume	0.407**	0.718**	0.391*	1		
Root length	0.041	0.745**	−0.491**	0.145	1	
Treatment	−0.706**	−0.256	−0.043	−0.285	−0.0004	1

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level

S values (0.067%) were recorded for beech roots in treatment UC while that of oak (0.055%) was recorded in SR22. The N content in roots was highest in variants SC and SR22 of beech and oak roots, respectively. N showed significant variation amongst treatments in beech but not in oak roots (Table 7). A significant negative correlation exists between N and Mg in beech roots, between N and P, N and S, and P and K in oak roots. (Table 8)

Discussion

The analysis of biometric features and content of macro elements of the root systems of the beech and oak seedlings under study suggests that the innovative substrate mediums and fertilizers utilized effectively promote root system development. In essence, the characteristics of beech and oak seedlings cultivated in a peat–free substrate, coupled with the liquid fertilizer developed by the University of Agriculture in Krakow, closely resemble those of seedlings grown on the conventional substrate comprising peat and solid fertilizer.

In the present study, however, a significant positive correlation was discovered to exist among the biometrics features. Analysis of variance showed considerable variation in the entire root collar diameter. This could be attributed to the variation in properties and granulometric composition of the substrate since the lower density of substrates allows better root penetration and nutrient transportation (Arvidsson, 1999; Pająk *et al.*, 2022a). Beyond the root collar diameter and total root length, root surface area, root average diameter, and root volume also varied between treatments resulting in the significant formation of a viable root system. This is consistent with the results of previous studies by Kormanek *et al*. (2015) in the root growth of *Quercus petraea* (Matt.) Liebl. seedlings. Tworkoski *et al*. (1983) reported a reduction in the growth of *Quercus alba* L. with variations in the compartment medium and density. It can further be deduced from the results obtained that these seedlings could be planted in the forest as the root system is adequately well–developed. These results, therefore, corroborate other studies on forest tree species grown in containers (Sands and Bowen, 1978; Corns, 1988; Pająk *et al*., 2022a).

There is no doubt that when tree seedlings are well nourished it increases their performance in the forest. To this end, nourishment has been considered a significant property in seedling

Table 7.
Percentage of macro elements in the roots of beech and oak seedlings for each treatment (±SD)

Treat–ment	C	N	P	K	S	Ca	Mg
	[%]						
			F. sylvatica				
SR20	45.68 ±2.00a	0.622 ±0.011ab	0.145 ±0.057a	0.389 ±0.115a	0.037 ±0.011a	0.494 ±0.115a	0.119 ±0.057a
UR20	47.56 ±2.00a	0.663 ±0.115ab	0.071 ±0.011a	0.323 ±0.115a	0.049 ±0.011a	0.648 ±0.057a	0.158 ±0.088a
SR21	45.75 ±2.00a	0.563 ±0.011abc	0.148 ±0.011a	0.434 ±0.115a	0.031 ±0.011a	0.499 ±0.115a	0.120 ±0.115a
UR21	44.71 ±2.00a	0.340 ±0.057c	0.054 ±0.011a	0.408 ±0.115a	0.032 ±0.011a	0.408 ±0.057a	0.087 ±0.005a
SR22	46.61 ±2.00a	0.695 ±0.115a	0.134 ±0.057a	0.402 ±0.115a	0.057 ±0.011a	0.442 ±0.057a	0.111 ±0.057a
UR22	45.98 ±3.06a	0.388 ±0.057bc	0.060 ±0.011a	0.374 ±0.115a	0.042 ±0.011a	0.367 ±0.011a	0.092 ±0.001a
UC	47.18 ±2.00a	0.682 ±0.115a	0.039 ±0.011a	0.305 ±0.115a	0.067 ±0.011a	0.243 ±0.057a	0.127 ±0.057a
SC	46.38 ±2.00a	0.689 ±0.115a	0.137 ±0.057a	0.390 ±0.115a	0.064 ±0.011a	0.251 ±0.057a	0.154 ±0.115a
Total	46.23 ±2.00	0.580 ±0.037	0.098 ±0.014	0.378 ±0.035	0.047 ±0.004	0.419 ±0.034	0.121 ±0.014
p–value	0.801ns	0.051*	0.201ns	0.993ns	0.237ns	0.22ns	0.948ns
			Q. robur				
SR20	44.860 ±2.00a	0.501 ±0.173a	0.087 ±0.005a	0.464 ±0.115a	0.036 ±0.011a	0.382 ±0.115a	0.108 ±0.057a
UR20	46.043 ±1.50a	0.414 ±0.000a	0.049 ±0.011a	0.418 ±0.115a	0.032 ±0.011a	0.364 ±0.115a	0.105 ±0.057a
SR21	44.790 ±2.00a	0.690 ±0.115a	0.158 ±0.057a	0.682 ±0.115a	0.044 ±0.011a	0.357 ±0.115a	0.119 ±0.057a
UR21	44.530 ±2.00a	0.509 ±0.115a	0.109 ±0.057a	0.492 ±0.115a	0.036 ±0.001a	0.364 ±0.115a	0.121 ±0.011a
SR22	44.220 ±2.00a	0.831 ±0.115a	0.163 ±0.057a	0.576 ±0.115a	0.055 ±0.005a	0.362 ±0.057a	0.107 ±0.057a
UR22	44.220 ±2.00a	0.412 ±0.577a	0.055 ±0.011a	0.585 ±0.115a	0.048 ±0.011a	0.389 ±0.115a	0.100 ±0.057a
SC	44.600 ±3.00a	0.688 ±0.115a	0.113 ±0.057a	0.446 ±0.115a	0.054 ±0.011a	0.232 ±0.011a	0.130 ±0.057a
UC	44.380 ±1.00a	0.436 ±0.115a	0.035 ±0.011a	0.408 ±0.000a	0.036 ±0.011a	0.300 ±0.057a	0.139 ±0.057a
Total	44.705 ±2.13	0.560 ±0.449	0.096 ±0.015	0.509 ±0.036	0.042 ±0.003	0.344 ±0.030	0.116 ±0.016
p–value	1.000ns	0.126ns	0.286ns	0.605ns	0.702ns	0.944ns	0.999ns

S – State Forests fertilization, U – University fertilization, R – novel substrates, C – control substrate (peat–perlite); figures with the same letter in the same column are not significantly different while figures with different letter are significantly different at p=0.05, separately for each species

Table 8.
The results of correlation analysis of macro element content

Elements	C	N	P	K	S	Ca	Mg
			Beech Root				
C	1						
N	0.598	1					
P	−0.241	0.418	1				
K	−0.767*	−0.350	0.652	1			
S	0.683	0.682	−0.144	−0.593	1		
Ca	0.042	−0.001	0.194	0.094	−0.547	1	
Mg	0.419	0.825*	0.431	−0.234	0.607	−0.212	1
			Oak Root				
C	1						
N	0.037	1					
P	0.156	0.880**	1				
K	0.002	0.482	0.768*	1			
S	−0.345	0.746*	0.630	0.467	1		
Ca	−0.064	−0.263	0.081	0.410	−0.334	1	
Mg	0.177	0.069	−0.154	−0.372	−0.066	−0.780*	1

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level quality (Burdett, 1983; Landis, 1985; Puttonen, 1989; Ritchie *et al*., 2010; Hawkins, 2011). Although the results of nutrient content show no significant differences among the substrates and fertilizer treatments (except N in beech), the percentage value of nutrient content amongst the species and treatments falls within the commonly used percentage value that meets the needs of plant growth which is consistent with studies by Baule and Fricker (1973) who reported the demand for Ca and K as high in forest trees. Dzwonko (1990) reported that beech seedlings develop better in a substrate rich in Ca, Mg, and K. Balcar *et al*. (2011) and Pająk *et al*. (2022b) reported that the use of dolomite (which contains both Mg and Ca) to fertilize beech trees has a positive effect on the growth of the seedlings, especially root systems.

Other studies have reported that the use of Mg has enhanced yields by 8.5% in numerous experiments across different nations of the world regardless of the species of tree, soil and substrate conditions, and other factors in China (Wang *et al*., 2020) and Poland (Pająk *et al*., 2022b). Furthermore, Wang *et al*. (2020) reported that using Mg fertilizers is more efficient at improving growth and yields than using N, P, and K. The average mean value of Mg in this study (0.121% and 0.116% for beech and oak root, respectively) falls slightly below 0.165% as reported by Pająk *et al*. (2022b) on the root system of European beech. Banach *et al.* (2013) reported that the structure of the substrate was vital for proper growth in beech seedlings. The type of fertilizer used for seedling production is also very important for this species, as demonstrated by Banach *et al*. (2021). It can, therefore, be said that the substrate medium and fertilizer developed and used to raise these forest seedlings is rich in essential macro elements, especially the control variants of the treatments possibly due to the addition of dolomite additives that were added to increase the pH of the substrates.

The results of various element contents obtained from the root systems of the studied beech and oak seedlings indicates that the substrates mediums used and fertilizers applied (especially the novel design) enhanced the root system in a manner that has the ability to increase Mg uptake by seedlings further as they grow beyond nursery stage. This could be related to the low density of the organic substrates used. This is supported by the results reported of Pająk *et al*. (2022b) that an improved root system resulted in increased Mg uptake in *F. sylvatica* seedlings from lower substrate densities which in turn resulted in a better proportion of dry-weight above- to below-ground parts of the seedlings. Potassium likewise plays a significant role in stacking the phloem and carbohydrate transportation in plants. Its deficiency may likewise result in an expansion in the dry-weight S: R proportion as reported for *Phaseolus vulgaris* L. and *Betula pendula* Roth cuttings (Cakmak, 2013). Treatment with dolomite decreases soil acidification and increases the Mg content in the plant. Additionally, dolomite promotes enzymatic action in the peat substrate as confirmed by different studies carried out in forest nurseries on F. *sylvatica* (Lasota *et al*., 2021; Pająk *et al*., 2022a) and *Q. robur* (Lasota *et al.,* 2021).

Conclusions

The results of the root biometric features indicate that the different substrate treatments caused significant variation in root length, root surface diameter, and average root volume in beech and oak seedlings. The effects of these treatments were visible in differences in root formation and macro element concentrations in the root system. Interestingly, the novel peat-free organic substrate and fertilizer mediums developed by the University of Agriculture in Krakow have shown strong competitiveness with organic peat. The newly designed substrate and liquid fertilizer formulation by the University of Agriculture in Krakow for beech and oak seedlings grown in container nurseries using organic substrate met the existing characteristics of those raised with peat substrate and solid fertilizer. The root system was adequately well developed to tap into the soil for nutrients and water necessary for plant growth which further guarantees plant growth and survival.

Authors contributions

O.J.R. – paper concept, methodology, manuscript preparation, data collection, statistical analysis, literature review, writing; S.M. – funding acquisition, project administration, reviewing, and editing; J.B. – data editing, writing, review; M.P. – literature review, writing, editing, and review.

Conflict of interest

The authors declare no conflict of interest.

Funding source

The author did not receive support from any organization to conduct the research presented in the paper. The seedlings used for the analyses were grown in project POIR.04.01.04–00–0016/20 funded by the National Centre for Research and Development from National Resources and the European Regional Development Fund.

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STrEszCzENIE

Wpływ różnych podłoży na charakterystykę korzeni sadzonek buka zwyczajnego *Fagus sylvatica* L. i dębu szypułkowego *Quercus robur* L.

W celu zagwarantowania rozwoju systemów korzeniowych szkółkarze stosują wiele metod produkcji materiału szkółkarskiego. W pracy oceniono wpływ innowacyjnych beztorfowych podłoży organicznych (R20, R21 i R22) na rozwój systemu korzeniowego i zawartość składników pokarmowych w korzeniach sadzonek buka zwyczajnego i dębu szypułkowego. Zastosowane podłoża różniły się właściwościami fizycznymi, składem granulometrycznym oraz zawartością składników pokarmowych (tab. 1–4). W trakcie hodowli sadzonek zastosowano 2 warianty nawożenia: standardowe nawożenie mieszaniną 2 nawozów Osmocote (3–4M i 5–6M) oraz nawożenie nawozem dolistnym opracowanym przez Uniwersytet Rolniczy w Krakowie (URK). Warianty podłoża ze standardowym nawożeniem (S) zostały oznaczone jako SR20, SR21 i SR22, natomiast z nawożeniem URK (U) jako UR20, UR21 i UR22. W obu przypadkach jako warianty kontrolne (SC, UC) zastosowano substrat torfowo–perlitowy. Sadzonki obydwu gatunków były hodowane w kontenerach styropianowych w szkółce kontenerowej Suków–Papiernia (Nadleśnictwo Daleszyce) w 8 wariantach substratowo–nawożeniowych. Schemat ustawienia kontenerów na polu produkcyjnym dla wariantu oraz miejsce pobierania sadzonek do analiz przedstawiono na rycinie 1. Sadzonki użyte do analiz wyhodowano w projekcie POIR.04.01.04–00–0016/20 finansowanym przez Narodowe Centrum Badań i Rozwoju ze środków krajowych oraz Europejskiego Funduszu Rozwoju Regionalnego „Innowacyjne technologie produkcji substratów i nawozów produkowanych z rodzimych surowców do produkcji sadzonek drzew leśnych". Po zakończeniu produkcji dla każdego z 8 wariantów wybrano po 25 sadzonek (5 z kontenera) o standardowym wigorze i parametrach biometrycznych. Każdej z nich zmierzono średnicę w szyjce korzeniowej (RCD) suwmiarką elektroniczną, natomiast przy użyciu oprogramowania WinRHIZO określono całkowitą długość korzeni (TRL), powierzchnię korzeni (RSA), przeciętną średnicę korzeni (ARD) oraz ich objętość (RV). Korzenie każdej sadzonki wysuszono i zmielono, a następnie oznaczono (w %) zawartość N, S i C przy użyciu analizatora LECO CNS TruMac oraz P, K, Ca i Mg przy użyciu analizatora Thermo iCAP 6500DUO ICP–OES. Analizy chemiczne przeprowadzono w Laboratorium Geochemii Środowiska Leśnego i Terenów Rekultywowanych, natomiast biometryczne

w Laboratorium Biotechnologii Leśnej Katedry Ekologii Lasu i Hodowli Lasu Uniwersytetu Rolniczego w Krakowie.

Stwierdzono, że rodzaj podłoża w połączeniu ze sposobem nawożenia wpłynął na rozwój systemu korzeniowego oraz na zawartość makroelementów w korzeniach analizowanych gatunków. U sadzonek dębu i buka wyhodowanych w wariancie UR20 odnotowano najwyższą średnią wartość całkowitej długości korzeni (tab. 5). Analizowane parametry biometryczne korzeni buka istotnie dodatnio korelowały ze sobą, natomiast z RCD istotnie dodatnio korelował tylko parametr ARD. W przypadku dębu RCD dodatnio korelował z dwoma parametrami: RSA i RV. W przeciwieństwie do buka odnotowano u dębu ujemną korelację między TRL i ARD, natomiast większość pozostałych parametrów wykazywała istotną dodatnią korelację (tab. 6).

Analiza zawartości makroskładników w systemie korzeniowym sadzonek wyhodowanych w poszczególnych wariantach nawożenia i zastosowanego substratu wykazała różny ich poziom, ale w zbliżonych zakresach. Jedynie u buka stwierdzono istotny wpływ wariantu produkcyjnego na zawartość N. Korzenie buka miały wyższą zawartość C niż dąb, z maksimum w wariancie UR20, natomiast zawartość N i K była na ogół wyższa w korzeniach dębu (tab. 7). Nie stwierdzono istotnej pozytywnej interakcji pomiędzy analizowanymi elementami, z wyjątkiem kilku przypadków, zwłaszcza dla N i P w sadzonkach dębu (tab. 8). Na podłożach innowacyjnych (beztorfowych) z zastosowaniem nawożenia dolistnego zaproponowanego przez URK uzyskano zbliżone wartości analizowanych parametrów systemów korzeniowych w wariancie z substratem torfowym. Wskazuje to na możliwość częściowego lub całkowitego zastąpienia torfu wysokiego nowym składnikiem w składzie podłoża szkółkarskiego.

New Forests (2025) 56:17
https://doi.org/10.1007/s11056-024-10079-1

RESEARCH

Effect of innovative peat-free organic growing media and fertilizer on nutrient allocation in pedunculate oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.) seedlings after nursery production cycle

Odunayo James Rotowa[1,2] · Stanisław Małek[1] · Michał Jasik[1] · Karolina Staszel-Szlachta[1]

Received: 30 April 2024 / Accepted: 18 November 2024

Abstract
This study evaluates the effects of novel peat-free organic growing media and a novel liquid fertilizer on the biometric features and macronutrient allocation of *Quercus robur* and *Fagus sylvatica* seedlings with the view to compare biomass and nutrient allocation of plant organs in seedlings cultivated on peat growing medium against those grown on novel peat-free growing medium and fertilizer. The experimental setup involved four growing medium variants, including peat as the control (R20, R21, R22 and C). The novel growing medium and fertilizer were designed and formulated by the University of Agriculture in Kraków, Poland (UAK). Fertilization used in the state forest nurseries was represented as SR20, SR21, and SR22, while the novel fertilizer of UAK was represented as UR20, UR21, and UR22; meanwhile, SC and UC represented the control growing medium (peat) in both cases, respectively. The experiment was laid in a 2 × 2 × 4 experimental design using five seedlings per treatment. Seedlings were assessed for roots, shoots, and leaves biomass after the nursery production cycle. The allocation patterns highlighted the variability of nutrient allocation within the plants, with more nutrients allocated to the root system. Interestingly, treatment UR22 yielded the highest mean root values, root biomass, and virtually all macroelement allocation. The SC solid fertilizer treatment and the UR22 liquid fertilizer treatment consistently showed superior performance across both species and different plant organs. These findings suggest that these treatments are particularly effective in enhancing the nutrient content of oak and beech seedlings, making them suitable choices for optimizing the growth and health of these species.

Keywords Peat-free growing media · Liquid fertilizer · Forest tree seedlings · Sustainable cultivation

✉ Odunayo James Rotowa
odun.james.rotowa@student.urk.edu.pl

[1] Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Kraków, Al. 29 Listopada 46, Kraków 31-425, Poland

[2] Department of Forestry and Wildlife Management, Faculty of Agriculture, Nasarawa State University, Keffi, Nigeria

Published online: 05 December 2024

Introduction

The decline of natural resources, including forest, is one of the greatest environmental problems faced in Europe and on other continents. The nutrient cycle plays a vital role in the nourishment and proper development of trees (Cambi et al. 2015). The proper development and high vitality of forest seedlings can be accomplished with an adequate amount and extent of nutrients adapted to the species' requirements (Muschler, 20l6). Mineral fertilizers are the main source of nutrients for seedlings growing in forest nurseries, affecting their physiological and morphological properties (Wani et al. 2016). Appropriate mineral fertilization has been reported to enable better nourishment of seedlings, and the aggregated stores of nutrients promote better development, ability to adjust to ecological circumstances, and imperviousness to stress in difficult periods after planting (Loewe-Muñoz, 2024). Moreover, alternative growing medium used in nurseries are prepared based on ingredients such as high peat, bark, sawdust, perlite, vermiculite, or sand, which are poor in mineral compounds and therefore require fertilization (Bosiacki 2009).

In Poland, containers are commonly used for the production of tree seedlings in the nursey (Kormanek and Małek 2023). Over the years, coniferous monocultures have been intensively restructured due to trees' declining health and quality. *Fagus sylvatica* L. is a temperate species found throughout central and Western Europe (Jaworski 2019). *Quercus spp*., including *Quercus robur* L. are a major tree genus in Polish forests. Oak and beech forests play a significant role in the functioning of the biosphere. They produce and accumulate biomass, produce oxygen, regulate the composition of the atmosphere of the planet, and more (Romanov et al. 2022). Meanwhile, the decline and reduction of forests dominated by these species have been observed in Poland and across Europe. Because of their excellent wood quality, beech and oak are becoming more commercially desirable than several conifers because they are the preferred tree genus in any adaptation strategies to climate change for both ecological and economic reasons (Rotowa et al. 2023a). Under the circumstances of global change, it has been established that shifts in tree species and their symbiotic associations impact biogeochemical processes (Crowley et al. 2016; Crowley and Lovett 2017; Yu, 2023). Hence, it is of paramount importance to intensify efforts to raise the health and sustainability of forest stands of these highly sought-after species, especially in this era of transition in Polish forests from pine to other species.

The distribution of dry matter among plant organs is one of the key variables influencing the survival, competitive ability, and productivity of individual plants. It is influenced by several factors, including the availability of minerals in the rhizosphere and the flow of nutrients to the roots (Millner and Kemp 2012; Cortina et al. 2013; Chu et al. 2020; Pająk et al. 2022a). There is no doubt that; the distribution of macronutrients among plant organs plays a critical role in regulating growth rates and is an essential ecological process related to the history of plant life (Tripathi et al. 2014). Earlier studies have predominantly focused on biomass allocation (Vicca et al. 2012; Poorter et al. 2012a, b; Wei et al. 2013; Zhu et al. 2016; Pająk et al. 2022a) or nutrient investment in leaves only (Reed et al. 2012; Richardson et al. 2004; Hu et al. 2014). Aoyagi and Kitayama (2016) took a novel approach by examining how nitrogen and phosphorus investment in plant organs changes in Bornean rain forests to depletion of each element. Notably, Rotowa et al. (2023b) reported the effects of peat-free organic growing medium on the root systems of *F. sylvatica* and *Q. robur*. However, the specific allocation of nutrients between plant organs when grown on novel growing medium has rarely been demonstrated.

Peat is widely recognized as a foundational component within nursery growing medium due to its exceptional good physical, chemical, and biological properties. Its outstanding water-retention capabilities and consistently high quality make it a favored medium for nurturing plants (Gruda 2012). However, the release of carbon from peat soils over time raises concerns about peat's environmental impact. Peat excavation in Europe saw an extraordinary increase from 20,000 cubic meters in 2012 (equivalent to approximately 6,000 tonnes) to 20 million tonnes by 2022 (Gruda 2012; Hirschler and Osterburg 2022). This represents an increase of around 333% over the decade, highlighting a significant expansion in peat excavation activities, which further amplifies environmental degradation (van Beek, 2023). As a result, EU Member States has been on the outlook to reduce peat consumption (FCS, 2012; EPAP 2021; NMCE 2021) and because of upcoming restrictions on the availability of this material (EU, 2018) (GME 2003, Schmilewski, 2015) there is an urgent need to find a material/materials to replace it either partially or completely. To achieve this, it is important to be able to sample the liquid penetrating through the growing medium (both with and without plants growing) during periods of irrigation, fertilization or chemical application. Our technology solution makes this possible.

Therefore, this study aimed to analyze the effect of innovative peat-free organic growing medium and liquid fertilizer developed by the University of Agriculture in Kraków, Poland on the allocation of macroelements in different parts (leaves, shoots, and roots) of *Q. robur* and *F. sylvatica* seedlings toward the end of nursery production, just before planting to the forest site. This research is a preliminary investigation into the effects of innovative peat-free organic growing media and fertilizers on nutrient allocation in *Quercus robur* and *Fagus sylvatica* seedlings. Due to the small sample size, the results should be interpreted as suggestive, laying the groundwork for more extensive studies in the future. The hypotheses put forward assumed that the novel growing medium and fertilizer would effectively support the allocation of nutrients in different parts of individual seedlings, and that the uptake of macronutrients in both species raised on a novel growing medium and liquid fertilizer would enhance the biomass of these seedlings compared to those grown on a standard growing medium (peat plus solid fertilizer).

Materials and methods

Composition and formulation of growing medium

The properties of the organic peat-free growing medium, granulometric composition of the growing medium before sowing, and nutrient content of growing media before seed sowing and after seedling production are shown in Tables 1, 2, 3 and 4. The growing medium, based on peat rich in sphagnum used for this study as the control variant (C) was produced at Nursery Farm in Nędza (50.167964 N, 18.3138334 E) Poland. The following granulometric composition, declared by the producer as percentage content of the fraction in a unit of volume: 2.5% of the 10.1–20 mm fraction, 12.5% of the 4.1–10 mm fraction, 12.5% of the 2.1-4. 0 mm, 72.5% of < 2.0 mm; maximum degree of decomposition 15%; organic matter content > 5%; and elemental content (g/g of 100% dry weight of the growing medium at the

Table 1 Properties of the organic peat free growing medium

Growing medium	Saw dust (%)	Wood chips (%)	Straw (%)	Wood bark (%)	Perlite (%)	Core wood (%)	Mixed silage (%)
R20	73	10	-	10	4	2	1
R21	20	63	-	10	4	2	1
R22	50	-	10	33	4	2	1

Woody materials were sourced from coniferous (mainly *Pinus sylvestris* L)

Table 2 Mean and standard deviation values of properties of the organic growing medium

Growing medium	Water capacity (%)	Water Outflow Rate (litre/min)	Coefficient of Variation (%)	Bulk density (g/cm3)	Solid density (g/cm3)	Air capacity (%)	Porosity (%)
R20	53.02 ± 2.42	0.595 ± 0.150	25.2	0.127 ± 0.009	1.56 ± 0.000	38.90 ± 2.90	91.85 ± 0.60
R21	45.39 ± 3.60	0.781 ± 0.114	14.6	0.103 ± 0.013	1.61 ± 0.000	48.14 ± 4.20	93.62 ± 0.83
R22	50.71 ± 2.11	0.594 ± 0.150	25.3	0.113 ± 0.009	1.62 ± 0.000	42.35 ± 2.61	93.04 ± 0.55
Control	71.44 ± 2.83	0.417 ± 0.145	34.9	0.091 ± 0.006	1.59 ± 0.000	22.89 ± 3.15	94.25 ± 0.39

beginning of the experiment) of 37.99 ± 0.69 (C), 0.74 ± 0.01 (N), 0.02 ± 0.01 (P). On the other hand, the peat-free growing medium (R20, R21, R22) were formulated using a mixture of various components in varying percentage proportions (Table 1). In total, four growing media (R20, R21, R22, and peat) were utilized, each subjected to two fertilization (S and U) variants. The first set received standard solid fertilization (SR20, SR21, and SR22 variants), while the second set was treated with a novel liquid fertilizer also developed by the University of Agriculture in Kraków (UR20, UR21, and UR22). The peat growing medium served as the control in both fertilization scenarios, designated as SC and UC variants.

The properties of the growing medium were calculated according to (Jasik et al. 2023) where the volume of these medium was determined by measuring the top surface of the container using a ruler along with the dimensions of the upper surface of the cell opening and the height of the growing medium, the unoccupied volume (Ve, in cm^3) within the container was calculated. By subtracting Ve from the total volume of the cell (V, in cm^3) next, a container was placed beneath the cell to collect both the growing medium and the water that dripped out. The water outflow rate (W (N·s − 1) was determined as the ratio of the water weight increase (w) to the time (t) taken for this increase (Bilderback 2022; Kormanek and Małek 2023). The time interval for the differential quotient was set at 60 s. The growing medium was removed from the top using a plastic spoon, and any remaining material that couldn't be spooned out was pushed into the container below. The collected samples from the cell and the container were weighed to determine the wet growing medium weight (mw, in g). After drying the growing medium, its dry mass (ms, in g) was measured. These measurements calculated the dry bulk density (BD, in g/cm^3) and the wet bulk density (WBD, in g/cm^3). $BD = ms/Vs$ and $WBD = mw/Vs$. In each experimental variant, seedlings of each species underwent cultivation in 75 Marbet V300 polystyrene containers. Each container comprised 53 cells with a volume of 275 cm3.

Table 3 Mean (%) and standard deviation values of granulometric composition of the growing media before sowing

Growing medium	> 10 mm	10 < 5 mm	5 < 2 mm	2 < 1 mm	1 < 0.5 mm	0,5 < 0.25 mm	0,25 < 0.1 mm	> 0.1 mm
R20	0.05 ± 0.10	3.77 ± 1.57	14.45 ± 5.90	30.53 ± 9.72	24.45 ± 2.24	17.72 ± 7.49	7.71 ± 4.16	1.69 ± 0.98
R21	0.00 ± 0.00	6.40 ± 1.37	25.44 ± 1.91	30.90 ± 1.11	19.11 ± 0.90	12.16 ± 0.31	5.12 ± 0.41	0.96 ± 0.11
R22	0.08 ± 0.13	3.03 ± 0.45	14.15 ± 2.36	33.36 ± 2.36	25.11 ± 1.07	17.02 ± 3.21	7.11 ± 1.72	1.48 ± 0.28
Control	0.00 ± 0.00	11.27 ± 0.37	25.08 ± 1.18	27.77 ± 1.05	16.20 ± 1.05	8.42 ± 0.56	3.81 ± 0.43	1.88 ± 0.21

Seed sowing and germination

The containers were mechanically filled with various growing media, beech and oak seeds were sown manually in the nursery in Suków-Papierna (Daleszyce Forest District). The seeds were sown on April 19 and 20, with the preparation and sowing being carried out by the workers of the container nursery. To improve the germination process, oak seeds were scarified just before sowing, which involved the removal of about one-third of the seed in the cotyledon part. In contrast, beech seeds were stratified without using a stratification medium at a temperature of + 3 °C and a humidity of 31%. Regardless of species, the seeds used for all growing medium variants came from the same origin and were accompanied by separate certificates of origin (MR/65848/21/PL for oak and MR/63313/20/PL for beech). After sowing, the containers were placed in the green house for 4 weeks and then transported to an external production field. During the growth of the seedlings, manual weeding was conducted. The seedlings were grown for 5months according to the procedure used in the container nursery (Szabla and Pabian 2009). During the seedling growth period, the total rainfall was only 78 mm, therefore irrigation was applied using an automatic RATHMAKERS Gartenbautechnik sprinkler ramp to replenish the water deficit.

Osmocote fertilizer was applied once during preparation of growing medium before sowing at a total dose of 3 kg m-3 of each growing medium, prepared as a mixture of Osmocote 3-4 M (2 kg) and Osmocote 5-6 M (1 kg). The composition of the Osmocote 3-4 M fertilizer was as follows *N* –16% including 7.1% N-NO3- and 8.9% N-NH_4+; P_2O_5 –9%, K_2O –12%; MgO –2.0% and microelements (B, Fe, Cu, Mn, Zn, Mo); 5-6 M: *N* –15% including 6. 6% N-NO_3- and 8.4% N-NH_4+; P_2O_5 –9.0%; K_2O –12%; MgO –2.0%; and microelements (B, Fe, Cu, Mn, Zn, Mo). The new liquid fertilizer used was based on two different compositions. The first one consisted of *N* – 4.78%, P_2O_5 – 1%, K_2O – 2.64%, CaO – 2.65%, MgO – 1.4%, SO^3 – 0.71% and Na_2O – 0.14%. This fertilizer was initially applied with a total volume of 3.14 dm3 (0.048 dm3 -1 m-2). The second fertilizer contained N at 0.798%, P_2O_5 at 0.166%, K_2O at 0.440%, CaO at 0.441%, MgO at 0.234%, SO_3 at 0.118% and Na_2O at 0.023%. The second fertilizer was applied with a total volume of 15.09 dm3 (0.229 dm3 -1 m-2). In the course of seedling production, the first fertilizer variant was applied eight times at 10-day intervals, while the second variant was applied 15 times at 5-day intervals. It is important to note that the fertilization regimes remained consistent for both beech and oak seedlings.

Parameter assessment and nutrient analysis

After the nursery's production cycle, a thorough examination of multiple seedlings was conducted. However, due to limitations stemming from the availability of seedling parts for laboratory testing, a specific selection process was employed by Rotowa et al. (2023b). Five seedlings, characterized by standard vigor and biometric parameters, were carefully chosen from each of the eight treatment groups for data collection. This resulted in a total assessment of 80 seedlings for both species in the experiment. The selected containers were distributed diagonally across the experimental field. Data were collected on the biomass of the different parts of the plant organs. These parts (leaves, shoots, and roots) of the sampled seedlings were dried at 65 °C for 48 h. After drying, the samples were ground and mineralized. From each part of the organ, 0.5 g was placed into a flask for mineralization with an

Table 4 Nutrient content (%) of growing media before seed sowing and after seedling production

Growing medium	C	*N*	*P*	K	Ca	Mg	Na
Before sowing							
R 20	48.01	0.297	0.031	0.159	0.452	0.055	0.040
R 21	46.34	0.507	0.068	0.271	0.601	0.072	0.035
R 22	48.90	0.447	0.043	0.404	0.857	0.059	0.042
Control	45.85	0.709	0.015	0.058	1.307	0.585	0.068
After seedling production							
Fagus sylvatica							
UR20	44.148	0.434	0.030	0.066	0.677	0.055	0.016
UR21	42.518	0.532	0.049	0.072	0.854	0.055	0.015
UR22	42.93	0.578	0.043	0.074	1.179	0.066	0.018
UC	39.784	0.651	0.019	0.071	1.543	0.525	0.072
SR20	42.167	0.596	0.093	0.129	0.721	0.068	0.018
SR21	39.978	0.996	0.134	0.161	0.985	0.087	0.020
SR22	42.167	0.756	0.110	0.156	1.463	0.086	0.023
SC	40.987	0.844	0.096	0.162	1.695	0.476	0.075
Quercus robur							
UR20	44.703	0.383	0.028	0.563	0.594	0.065	0.015
UR21	44.969	0.418	0.032	0.597	0.627	0.042	0.015
UR22	45.422	0.493	0.032	0.650	0.966	0.060	0.015
UC	41.863	0.654	0.016	0.654	1.392	0.472	0.060
SR20	45.455	0.519	0.059	0.991	0.589	0.056	0.014
SR21	43.313	0.942	0.121	1.626	0.879	0.088	0.020
SR22	45.096	0.872	0.114	1.703	1.139	0.081	0.018
SC	41.425	0.805	0.076	1.798	1.424	0.441	0.069

S - State Forest fertilization (solid), U - University novel fertilization (liquid), R - novel growing media, C - controls growing medium (peat-perlite)

added mixture of acids. The mineralized samples were later filtered into a 50 ml flask and the concentration of elements was determined using the ICP-OES apparatus. The samples were analyzed for their nitrogen, sulfur, and carbon contents using a LECO CNS TruMac analyzer and phosphorus, potassium, calcium, and magnesium contents using a Thermo iCAP 6500 DUO ICP-OES spectrometer following mineralization in nitric and hydrochloric acids at a ratio of 3:1. The analyses were performed at the Laboratory of Forest Environment, Geochemistry, and Land Intended for Reclamation in the Department of Ecology and Silviculture and Faculty of Forestry at the University of Agriculture in Kraków, Poland. Before chemical analyses, the root parameters were analyzed using WinRHIZO software in the Laboratory of Forest Biotechnology of the same department.

Statistical analysis

The experiment was laid in a 2 × 2 × 4 experimental design, consisting of two species (beech and oak), two fertilizer types (solid and liquid), and four growing medium in each treatment (R20, R21, R22, and control) using five seedlings per treatment. Data analysis was conducted using a multifaceted approach. In order to show the comparative performance between the treatments, the collected data (after verifying that it met the assumptions of ANOVA) were subjected to mean and analysis of variance (ANOVA). Duncan Multiples

Range Test (DMRT) was applied to locate where the significant difference occurs among the treatments at $p < 0.05$. PCA was employed to reduce the dimensionality of the dataset, identifying key variables that collectively explained the variance in the data. Correlation test was further carried out to quantify the strength of the linear relationship between the analysed variables.

Results

Comparative analysis of biomass in *Q. robur* and *F. sylvatica*

The preliminary results indicate that growing medium may influence biomass production in both *Q. robur* and *F. sylvatica* seedlings, with variations observed across different organs. However, due to the small sample size ($n = 80$), these findings is interpreted as trends rather than conclusive evidence. Solid fertilizer appears to enhance biomass production more consistently than liquid fertilizer in both species and across all seedling organs. The studied species both show enhanced growth under solid fertilizer, but the magnitude of this enhancement varies between the organs (root, shoot, leaf) and types of growing medium. Liquid fertilizer results in consistent leaf and root biomass across growing medium with moderate shoot biomass. While solid fertilizer results in a higher biomass in all organs in oak, While in beech, liquid fertilizer results in lower biomass across seedling organs and more consistent in leaf Solid fertilizer significantly increases root, shoot and leaf biomass.

The observed consistency in leaf biomass among oak seedlings raised with liquid fertilizers suggests a potential stabilizing effect of the treatments (Fig. 1a) Shoot dry mass increases slightly from UR20 to UC, with UR20 being significantly lower (Fig. 1b). Root biomass also shows consistent allocation showing no significant variation (Fig. 1c). In solid fertilizer however, highest biomass was recorded in SC showing slight variations in root, significant increases in shoot and leaves. From the result of beech seedlings raised on liquid fertilizer, leaf biomass is fairly consistent across growing medium. Shoot dry mass is highest in UC and Root dry mass shows a notable increase in UC compared to other growing media. From solid fertilizer treatment, leaf and shoot biomass follows the same trend, with SC having the significantly higher values compared to others, root dry mass is higher in SR22 and SC (Fig. 1). The distinct responses of oak and beech to the treatments stress the importance of fertilization in forestry seedlings and the restoration of forest ecological systems and management.

The result further shows that there exist positive correlations between the below-ground and above-ground organs of the seedlings The relationship between root and shoot biomass indicates balanced growth, which is critical for healthy plant development, Similarly, the relationship between root and leaf biomass indicates efficient nutrient uptake, which is essential for healthy leaf development. In the shoot-leaf biomass correlation among treatments reflects resource allocation strategies influenced by the properties of growing medium and fertilization methods (Fig. 2). Although the result showed large variation, significant differences observed on the SC growing medium but not on others suggested that certain growth characteristics were more sensitive on SC than on others. This means that the novel growing media also affected the biomass of the seedlings toward the end of seedling production just before planting in the forest.

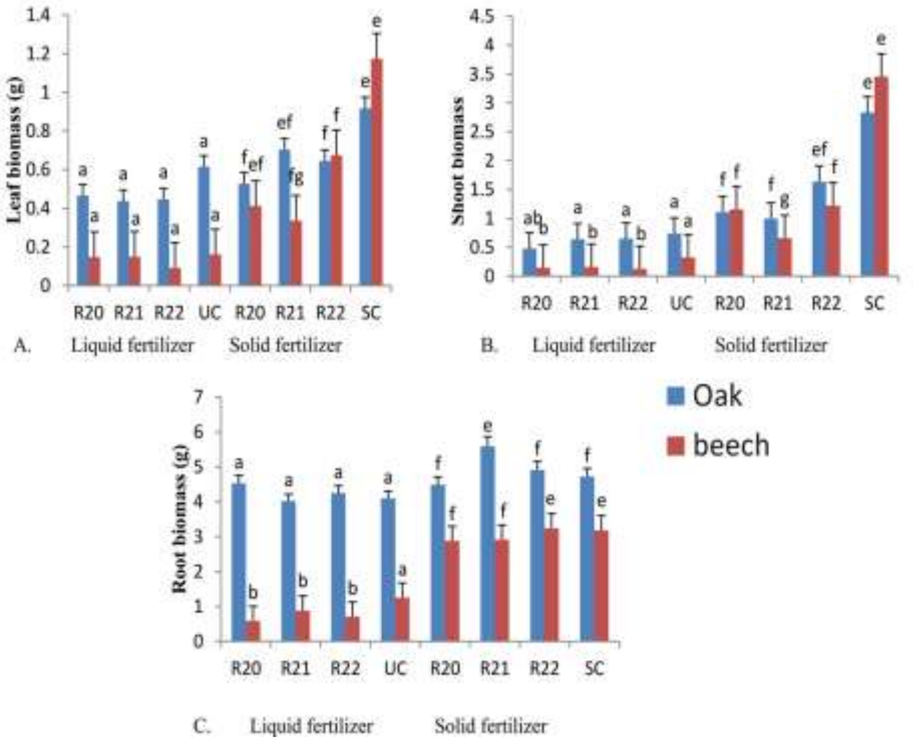

Fig. 1 Alphabets 'a' and 'b' denote homogeneous groups under liquid fertilization and 'e, f' and 'g' denote homogeneous groups under solid fertilization. **A-C** biomass allocation across different growing medium in oak and beech species

Analysis of the allocation of nutrients in *Q. robur* and *F. sylvatica* seedlings as affected by treatments

The results in Tables 5 and 6 show the allocation of macroelement contents in different parts (root, shoot, leaf) of *Q. robur* and *F. sylvatica* seedlings, respectively. In the roots of oak seedlings, the UR22 treatment under liquid fertilizer demonstrated the highest accumulation of crucial macro-elements, including NPK. This suggests that UR22 was the most effective liquid fertilizer treatment for promoting root nutrient content in oak seedlings. Among solid fertilizers, the SC treatment outperformed others, particularly in nitrogen and calcium content, indicating its superior efficacy for root nutrient uptake in this species. For the shoots of oak seedlings, UR22 again emerged as the best-performing liquid fertilizer treatment, yielding the highest levels of C, P, K and Ca. This indicates that UR22 not only supports root development but also enhances nutrient accumulation in the above-ground biomass. Among solid fertilizers, the SC treatment was particularly effective, producing the highest concentrations of C, N, P, K, and Ca in the shoots, making it the most robust solid fertilizer treatment for shoot growth. In the leaves, the UR20 liquid fertilizer treatment led to the highest accumulation of all tested macro-elements. This suggests that UR20 is especially beneficial for leaf nutrient content in oak seedlings. Similarly, the SC solid fertilizer treat-

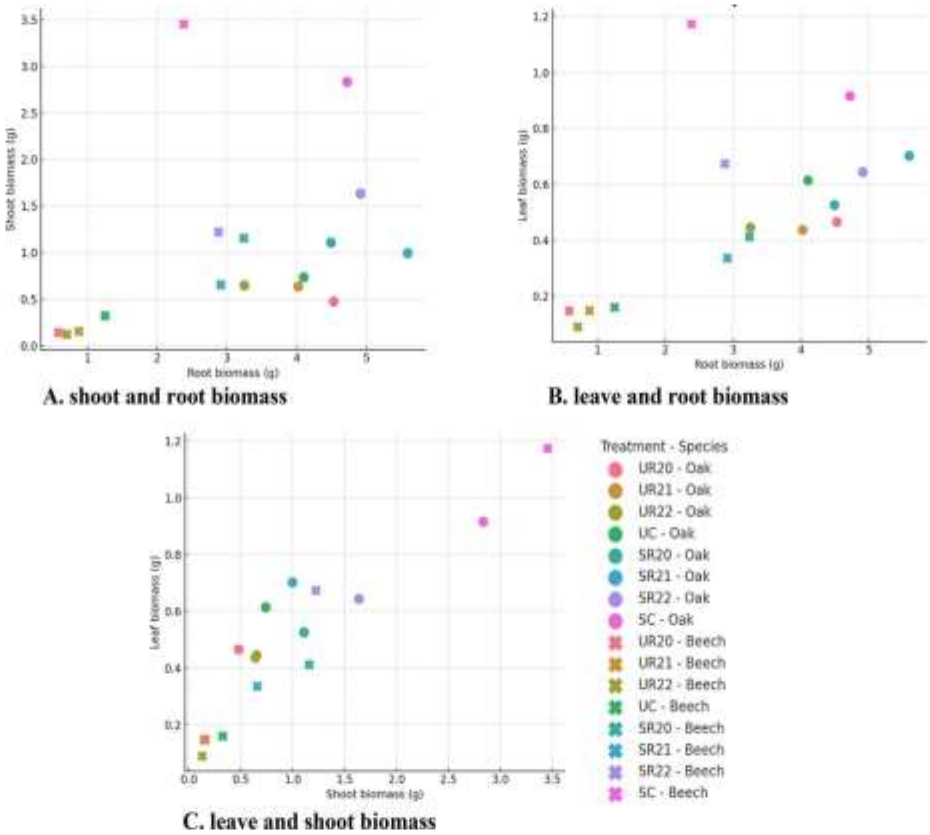

Fig. 2 **A-C** correlation among assessed biomass of seedling organs. S - State Forest solid fertilization, U - University novel liquid fertilization, R - novel growing media, C - controls growing medium (peat-perlite)

ment outperformed others, achieving the highest levels of all macro-elements in the leaves, which underscores its overall superiority among the solid fertilizer options. The result of analysis of variance revealed significant differences in macro-element content among the treatments. For oak seedlings, significant differences were observed in the nutrient content of shoots and leaves under liquid fertilizers, particularly for elements such as P, K, Ca and Mg. Under solid fertilizers, significant differences were also noted in the nutrient content of both shoots and leaves, indicating that the type of growing medium and fertilizer significantly influenced nutrient allocation in these organs (Table 5).

In the roots of beech seedlings, the UR22 treatment under liquid fertilizer was most effective, resulting in the highest levels of crucial nutrient including NPK. This indicates that UR22 is the most suitable liquid fertilizer for enhancing root nutrient content in beech seedlings. Among solid fertilizers, SR21 was the most effective, particularly in increasing N, P, Ca and Mg. For the shoots of beech seedlings, the UR22 liquid fertilizer treatment once again proved superior, showing the highest accumulation of phosphorus, potassium, and calcium. This highlights its consistent performance across different organs of the plant. In contrast, the SC solid fertilizer treatment led to the highest concentrations of C, N, P, K and Mg in the shoots, confirming its strong overall performance for shoot nutrient accumu-

Table 5 Mean and SD analysis showing the allocation of macro elements content in different parts of *Q. Robur* seedlings for each treatment

Treatment	Part	Fertilizer type	C (g/kg)	*N*	*P*	K	S	Ca	Mg
			(mg/kg)						
	Roots								
UR20		**Liquid**	4.09 ± 0.69[a]	36.8 ± 7.10[a]	6.00 ± 1.10[ab]	32.6 ± 5.40[a]	4.10 ± 0.60[b]	33.10 ± 6.00[bc]	10.80 ± 1.90[a]
UR21			5.48 ± 0.69[a]	48.3 ± 6.71[a]	6.00 ± 0.50[ab]	36.4 ± 4.20[a]	5.10 ± 0.50[ab]	37.80 ± 5.20[ab]	10.90 ± 1.40[a]
UR22			5.94 ± 0.60[a]	50.7 ± 5.20[a]	7.00 ± 0.80[a]	42.6 ± 4.30[a]	6.20 ± 0.60[a]	49.80 ± 4.50[a]	13.50 ± 1.50[a]
UC			4.05 ± 0.44[a]	42.8 ± 5.40[a]	2.90 ± 0.30[b]	26.2 ± 2.50[a]	4.20 ± 0.60[b]	22.00 ± 2.90[c]	13.00 ± 1.30[a]
Total			4.86 ± 0.34	44.27 ± 6.17	5.64 ± 0.86	34.3 ± 3.41	4.90 ± 0.61	35.32 ± 4.25	11.77 ± 1.63
p-value			**0.069[ns]**	**0.408[ns]**	**0.046***	**0.078[ns]**	**0.050***	**0.005****	**0.673[ns]**
SR20		**Solid**	3.16 ± 0.36[e]	36.0 ± 10.2[e]	5.10 ± 1.30[e]	22.1 ± 6.10[e]	4.20 ± 1.40[e]	29.80 ± 4.20[e]	8.40 ± 2.00[e]
SR21			4.15 ± 0.74[e]	49.8 ± 14.9[e]	8.20 ± 1.40[e]	34.0 ± 5.50[e]	4.00 ± 0.70[e]	31.30 ± 4.80[e]	9.70 ± 1.60[e]
SR22			3.46 ± 0.23[e]	44.6 ± 16.2[e]	7.20 ± 1.80[e]	27.6 ± 4.20[e]	4.50 ± 0.50[e]	25.70 ± 4.10[e]	9.40 ± 1.70[e]
SC			2.76 ± 0.38[e]	51.7 ± 9.20[e]	7.70 ± 0.70[e]	25.8 ± 3.60[e]	4.70 ± 0.80[e]	20.80 ± 3.00[e]	11.10 ± 1.20[e]
Total			3.37 ± 0.24	45.5 ± 15.8	7.06 ± 1.40	27.4 ± 4.47	4.36 ± 0.72	26.87 ± 3.08	9.64 ± 1.50
p-value			**0.238[ns]**	**0.429[ns]**	**0.323[ns]**	**0.263[ns]**	**0.893[ns]**	**0.325[ns]**	**0.612[ns]**
	Shoots								
UR20		**Liquid**	0.85 ± 0.07[ab]	10.9 ± 0.80[a]	1.20 ± 0.12[a]	4.90 ± 0.60[a]	1.00 ± 0.10[a]	17.70 ± 1.90[b]	3.00 ± 0.20[b]
UR21			0.77 ± 0.03[ab]	9.30 ± 0.80[a]	1.00 ± 0.10[ab]	4.30 ± 0.20[ab]	0.90 ± 0.10[a]	15.20 ± 1.50[b]	2.10 ± 0.10[c]
UR22			0.93 ± 0.07[a]	10.6 ± 0.80[a]	1.20 ± 0.20[a]	5.20 ± 0.50[a]	1.00 ± 0.60[a]	18.40 ± 0.90[b]	2.60 ± 0.20[ab]
UC			0.76 ± 0.02[b]	10.3 ± 0.40[a]	0.61 ± 0.03[d]	3.40 ± 0.20[b]	0.90 ± 0.10[a]	10.10 ± 1.00[a]	3.30 ± 0.30[a]
Total			0.83 ± 0.04	10.29 ± 0.35	0.99 ± 0.08	4.45 ± 0.35	0.97 ± 0.04	15.34 ± 0.98	2.75 ± 0.20
p-value			**0.118[ns]**	**0.423[ns]**	**0.015***	**0.030***	**0.629[ns]**	**0.003****	**0.002****
SR20		**Solid**	0.94 ± 0.09[f]	12.4 ± 1.10[f]	1.40 ± 0.20[f]	4.90 ± 0.50[f]	1.30 ± 0.20[f]	14.50 ± 1.80[f]	2.50 ± 0.30[f]
SR21			0.86 ± 0.12[f]	12.6 ± 1.70[f]	1.30 ± 0.20[f]	5.00 ± 0.70[f]	1.20 ± 0.20[f]	15.50 ± 1.80[f]	2.70 ± 0.40[f]
SR22			0.96 ± 0.04[f]	17.8 ± 3.40[f]	1.60 ± 0.20[f]	5.70 ± 1.05[f]	1.60 ± 0.20[f]	13.68 ± 1.30[f]	2.20 ± 0.20[f]
SC			1.96 ± 0.26[e]	34.1 ± 6.30[e]	3.20 ± 0.40[e]	11.9 ± 1.30[e]	3.60 ± 0.60[e]	22.21 ± 3.70[e]	7.40 ± 1.10[e]
Total			1.29 ± 0.07	14.8 ± 1.50	1.40 ± 0.24	5.70 ± 0.94	1.40 ± 0.32	15.90 ± 2.80	3.20 ± 0.83
p-value			**0.003****	**0.002****	**0.000****	**0.000****	**0.000****	**0.007****	**0.000****
	leave								

Table 5 (continued)

Treatment	Part	Fertilizer type	C (g/kg)	*N*	*P*	K	S	Ca	Mg
			(mg/kg)						
UR20		**Liquid**	1.36 ± 0.05^{a}	21.20 ± 3.60^{a}	1.70 ± 0.30^{a}	9.2 ± 1.40^{a}	2.06 ± 0.39^{a}	58.8 ± 4.58^{a}	8.40 ± 0.18^{a}
UR21			0.84 ± 0.10^{b}	11.30 ± 1.30^{b}	0.70 ± 0.10^{b}	7.2 ± 1.020^{ab}	1.16 ± 0.17^{c}	33.8 ± 4.55^{b}	3.6 ± 0.40^{b}
UR22			0.78 ± 0.09^{b}	19.40 ± 2.80^{b}	0.80 ± 0.10^{b}	8.3 ± 1.30^{ab}	0.92 ± 0.13^{c}	33.8 ± 3.73^{b}	4.0 ± 0.54^{b}
UC			0.82 ± 0.13^{b}	15.20 ± 1.60^{b}	0.50 ± 0.20^{b}	6.0 ± 0.95^{b}	1.54 ± 0.87^{b}	28.1 ± 3.10^{b}	8.2 ± 0.90^{a}
Total			0.94 ± 0.09	14.24 ± 2.37	0.95 ± 0.13	7.69 ± 1.98	1.42 ± 0.11	38.62 ± 4.05	6.05 ± 0.57
p-value			**0.000****	**0.003****	**0.000****	**0.050****	**0.000****	**0.000****	**0.000****
SR20		**Solid**	1.49 ± 0.11^{e}	32.80 ± 2.90^{e}	4.90 ± 0.40^{e}	19.1 ± 1.70^{e}	3.80 ± 0.30^{e}	64.9 ± 6.4^{e}	7.5 ± 0.61^{f}
SR21			1.10 ± 0.09^{f}	26.80 ± 2.80^{e}	3.70 ± 0.60^{e}	15.8 ± 2.30^{e}	3.20 ± 0.40^{e}	40.1 ± 5.8^{e}	5.8 ± 0.90^{f}
SR22			1.26 ± 0.03^{e}	38.80 ± 3.70^{e}	4.70 ± 1.00^{e}	19.8 ± 3.80^{e}	3.50 ± 0.70^{e}	35.0 ± 3.7^{e}	5.2 ± 0.20^{f}
SC			1.728 ± 0.33^{e}	74.10 ± 4.90^{e}	6.90 ± 2.30^{e}	26.3 ± 8.50^{e}	6.30 ± 1.80^{e}	49.3 ± 7.5^{e}	13.6 ± 2.20^{e}
Total			2.10 ± 0.16	29.50 ± 2.10	3.60 ± 0.30	16.8 ± 2.20	3.00 ± 0.20	30.0 ± 15	7.00 ± 0.40
p-value			**0.114**^{ns}	**0.107**^{ns}	**0.390**^{ns}	**0.497**^{ns}	**0.150**^{ns}	**0.150**^{ns}	**0.001***

S - State Forest fertilization, U - University fertilization, R - novel growing media, C - controls growing medium (peat-perlite)

alphabets ‘a’ and ‘b’ denote homogeneous groups under liquid fertilization, ‘e, f’ and ‘g’ denote homogeneous groups under solid fertilization. $p = 0.05$

Table 6 Mean and SD analysis showing the allocation of macro elements content in different parts of *F. Sylvatica* seedlings for each treatment

Treatment	Part	Fertilizer type	C (g/kg) (mg/kg)	*N*	*P*	K	S	Ca	Mg
	Roots								
UR20		**Liquid**	3.31 ± 0.60^{a}	34.40 ± 2.50^{a}	3.50 ± 0.21^{c}	21.80 ± 3.50^{b}	3.30 ± 0.30^{a}	30.80 ± 2.19^{a}	7.20 ± 0.98^{a}
UR21			3.09 ± 0.51^{a}	34.20 ± 2.90^{a}	6.10 ± 0.90^{b}	23.50 ± 4.00^{ab}	3.50 ± 0.50^{a}	35.00 ± 6.30^{a}	8.10 ± 1.60^{a}
UR22			3.49 ± 0.56^{a}	40.60 ± 3.40^{a}	9.30 ± 1.00^{a}	25.50 ± 5.70^{a}	4.20 ± 0.40^{a}	34.10 ± 5.60^{a}	9.30 ± 1.70^{a}
UC			2.66 ± 0.61^{b}	42.10 ± 3.90^{a}	2.10 ± 0.30^{c}	16.30 ± 3.01^{c}	3.10 ± 0.30^{a}	13.30 ± 6.00^{b}	7.40 ± 1.90^{a}
Total			3.12 ± 0.62	36.92 ± 3.31	2.89 ± 0.74	20.56 ± 3.89	3.52 ± 0.19	28.92 ± 9.38	7.95 ± 1.69
p-value			**0.125**^{ns}	**0.470**^{ns}	**0.000****	**0.000****	**0.194**^{ns}	**0.000****	**0.135**^{ns}
SR20		**Solid**	3.25 ± 0.74^{e}	38.80 ± 4.20^{e}	8.20 ± 0.80^{e}	22.10 ± 5.30^{e}	3.80 ± 0.60^{e}	35.80 ± 4.50^{e}	8.40 ± 1.90^{e}
SR21			3.20 ± 0.94^{e}	38.50 ± 5.50^{e}	9.80 ± 1.60^{e}	21.10 ± 7.20^{e}	3.50 ± 0.90^{e}	39.50 ± 7.50^{e}	9.60 ± 3.70^{e}
SR22			2.30 ± 0.86^{ef}	40.80 ± 8.10^{e}	6.60 ± 0.80^{e}	20.30 ± 4.80^{e}	3.10 ± 0.90^{e}	30.10 ± 1.90^{e}	8.40 ± 2.20^{e}
SC			1.99 ± 0.86^{f}	38.00 ± 4.70^{e}	7.00 ± 0.80^{e}	18.40 ± 7.10^{e}	3.40 ± 0.80^{e}	12.00 ± 2.10^{f}	7.30 ± 1.90^{e}
Total			2.68 ± 0.93	38.40 ± 5.60	6.60 ± 0.85	22.10 ± 5.10	3.50 ± 0.80	28.80 ± 3.90	8.20 ± 2.30^{a}
p-value			**0.049***	**0.988**^{ns}	**0.186**^{ns}	**0.797**^{ns}	**0.715**^{ns}	**0.003****	**0.620**^{ns}
	Shoots								
UR20		**Liquid**	0.65 ± 0.05^{a}	11.91 ± 0.79^{a}	1.31 ± 0.04^{b}	4.55 ± 0.61^{a}	0.85 ± 0.01^{a}	11.08 ± 1.08^{a}	1.83 ± 0.05^{b}
UR21			0.70 ± 0.05^{a}	12.28 ± 1.04^{a}	1.88 ± 0.23^{ab}	5.61 ± 0.84^{a}	0.89 ± 0.05^{a}	11.86 ± 0.36^{a}	1.97 ± 0.05^{b}
UR22			0.73 ± 0.10^{a}	12.64 ± 3.46^{a}	2.38 ± 0.34^{a}	7.21 ± 1.74^{a}	0.95 ± 0.12^{a}	12.56 ± 3.56^{a}	2.02 ± 0.55^{b}
UC			0.91 ± 0.15^{a}	13.43 ± 6.47^{a}	0.92 ± 0.13^{c}	5.09 ± 1.26^{a}	1.08 ± 0.19^{a}	9.93 ± 2.14^{a}	2.84 ± 0.96^{a}
Total			0.75 ± 0.04	13.56 ± 4.14	1.63 ± 0.16	5.62 ± 0.98	0.95 ± 0.06	11.35 ± 2.21	2.16 ± 0.25
p-value			**0.242**^{ns}	**0.105**^{ns}	**0.001****	**0.166**^{ns}	**0.518**^{ns}	**0.287**^{ns}	**0.047***
SR20		**Solid**	1.31 ± 0.11^{f}	14.80 ± 11.3^{b}	2.50 ± 0.30^{f}	5.80 ± 0.60^{f}	1.00 ± 0.80^{f}	15.70 ± 2.40^{e}	2.90 ± 0.80^{f}
SR21			0.82 ± 0.07^{f}	16.50 ± 1.20^{b}	2.40 ± 0.10^{f}	6.00 ± 0.50^{f}	0.90 ± 0.10^{f}	16.20 ± 2.00^{e}	2.10 ± 0.77^{f}
SR22			0.88 ± 0.09^{f}	22.50 ± 1.60^{ab}	4.70 ± 1.60^{ef}	9.80 ± 0.80^{e}	2.00 ± 0.60^{e}	22.80 ± 3.00^{e}	3.20 ± 0.93^{ef}
SC			2.79 ± 0.85^{e}	24.40 ± 0.70^{e}	6.50 ± 0.10^{e}	9.30 ± 0.80^{e}	3.70 ± 0.12^{e}	24.70 ± 3.40^{e}	5.10 ± 0.50^{e}
Total			1.10 ± 0.15	17.80 ± 1.10	3.80 ± 0.30	7.80 ± 0.67	1.30 ± 0.17	19.50 ± 2.20	2.85 ± 0.30
p-value			**0.018***	**0.013***	**0.007****	**0.007****	**0.028***	**0.280**^{ns}	**0.006****
	leave								

Table 6 (continued)

Treatment	Part	Fertilizer type	C (g/kg)	*N*	*P*	K	S	Ca	Mg
			(mg/kg)						
UR20		**Liquid**	0.50 ± 0.01^{b}	7.50 ± 1.80^{b}	0.55 ± 0.05^{b}	4.90 ± 0.15^{a}	0.74 ± 0.08^{b}	24.68 ± 3.06^{a}	3.07 ± 0.42^{b}
UR21			0.49 ± 0.04^{b}	7.60 ± 1.80^{b}	0.79 ± 0.14^{b}	4.20 ± 0.13^{a}	0.76 ± 0.09^{b}	24.86 ± 6.07^{a}	2.93 ± 0.74^{b}
UR22			0.44 ± 0.07^{b}	6.10 ± 1.50^{b}	1.01 ± 0.13^{b}	5.50 ± 0.30^{a}	0.67 ± 0.09^{b}	22.75 ± 7.42^{a}	2.48 ± 0.96^{b}
UC			0.71 ± 0.07^{a}	15.10 ± 2.33^{a}	0.59 ± 0.03^{a}	4.60 ± 0.28^{a}	1.35 ± 0.12^{a}	23.08 ± 4.49^{a}	4.30 ± 1.03^{a}
Total			0.54 ± 0.03	9.21 ± 1.75	0.74 ± 0.06	4.68 ± 0.23	0.87 ± 0.08	23.84 ± 5.15	3.19 ± 1.03
p-value			**0.011***	**0.000****	**0.019****	**0.227**^{ns}	**0.001****	**0.901**^{ns}	**0.019***
SR20		**Solid**	0.51 ± 0.07^{f}	17.40 ± 2.50^{g}	2.20 ± 0.30^{f}	7.80 ± 0.85^{f}	3.30 ± 0.55^{f}	19.60 ± 3.50^{f}	3.00 ± 0.30^{f}
SR21			0.72 ± 0.11^{f}	20.50 ± 9.80^{g}	2.50 ± 0.49^{f}	9.20 ± 1.00^{f}	3.70 ± 0.70^{f}	33.70 ± 3.80^{f}	3.40 ± 0.55^{f}
SR22			1.03 ± 0.18^{ef}	29.50 ± 2.20^{f}	4.50 ± 0.80^{e}	11.00 ± 1.90^{ef}	3.93 ± 0.82^{ef}	42.80 ± 4.00^{e}	4.20 ± 0.84^{ef}
SC			1.20 ± 0.22^{e}	34.20 ± 2.90^{e}	4.10 ± 0.78^{e}	15.50 ± 1.22^{e}	4.50 ± 0.88^{e}	43.00 ± 4.39^{e}	5.10 ± 0.90^{e}
Total			0.70 ± 0.06	19.80 ± 2.80	2.10 ± 0.63	7.80 ± 1.07	3.50 ± 0.72	27.40 ± 3.30	3.80 ± 0.40
p-value			**0.028***	**0.002****	**0.053 ***	**0.019***	**0.006***	**0.030***	**0.002****

S - State Forest solid fertilization, U - University novel liquid fertilization, R - novel growing media, C - controls growing medium (peat-perlite). alphabets ‘a’ and ‘b’ denote homogeneous groups under liquid fertilization, ‘e, f’ and ‘g’ denote homogeneous groups under solid fertilization. $p = 0.05$

lation in beech seedlings. In the leaves, the UC liquid fertilizer treatment performed best. For solid fertilizers, the SC treatment demonstrated the highest levels of all tested macro-elements. ANOVA result showed significant differences in the nutrient content of roots and leaves, especially under solid fertilizers (Table 6).

The comparative analysis of the average allocation of various elements in photosynthetic (leaves) and non-photosynthetic (shoots and roots) organs of oak and beech seedlings established that the allocation of nutrients varied significantly within and between both species. These results suggest that oak seedlings have a higher overall demand for or efficiency in absorbing nutrient, and that they allocate more resources to growth and development compared to beech seedlings, especially in roots, which play a key role in the adaptive process of crops after planting. The response to different treatments also highlights the species-specific adaptations and needs for nutrients. Oak generally showed a higher allocation of nutrient in its roots compared to beech, suggesting that the oak roots possessed a higher nutrient uptake and storage capacity than beech. Overall, the SC solid fertilizer treatment and the UR22 liquid fertilizer treatment consistently showed superior performance across both species and different plant organs.

Correlation analysis of nutrient components

The correlation analysis of nutrient components revealed a linear relationship among the assessed variables. The correlation coefficient between carbon and biomass shows a perfect positive relationship, meaning that biomass was highly related to the carbon and other element content of the samples, indicating that higher allocation of these elements was associated with higher biomass. Significant positive relationships were shown to also exist between nitrogen and other elements. This could imply that different treatments resulted in different patterns of nutrient allocation in seedlings of oak and beech. This trend suggests a strong positive relationship between the treatment applied and the allocation metric may continue beyond the nursery growth (Table 7).

Result of principal component analysis of *Q. robur*, the *F. sylvatica*

The results of the PCA showed the distribution of macroelement allocation in the root system of oak and beech seedlings under different fertilizer treatments. The influence of growing medium was less significant, as illustrated in Figs. 3 and 4. Here, the macroelement composition in the roots of both oak and beech seedlings appeared consistent across different growing medium, with points clustered closely together. This implies that variation in growing medium had minimal impact on the macroelement profiles in the roots (Figs. 3 and 4). In contrast, the impact of UR and SR fertilizers was particularly noticeable, as shown by the diverging points in Fig. 5. This indicated a significant effect of fertilization on nutrient content for both species, with the points being spatially separated. The variation observed in these graphs suggests that the primary factor influencing growth is the type of fertilizer treatment rather than the growing medium treatment.

Table 7 Correlation analysis between chemical elements and biomass

	C	*N*	*P*	K	S	Ca	Mg
Oak							
N	0.649**						
P	0.727**	0.890**					
K	0.899**	0.857**	0.910**				
S	0.754**	0.959**	0.895**	0.905**			
Ca	0.339**	0.447**	0.436**	0.495**	0.518**		
Mg	0.791**	0.797**	0.735**	0.847**	0.843**	0.590**	
Biomass	0.914**	0.607**	0.700**	0.839**	0.687**	0.288**	0.729**
Beech							
N	0.770**						
P	0.829**	0.753**					
K	0.925**	0.841**	0.873**				
S	0.862**	0.938**	0.831**	0.933**			
Ca	0.491**	0.608**	0.590**	0.579**	0.624**		
Mg	0.860**	0.913**	0.818**	0.908**	0.956**	0.669**	
Biomass	0.997**	0.783**	0.833**	0.932**	0.872**	0.489**	0.876**

** Correlation is significant at the 0.01 level

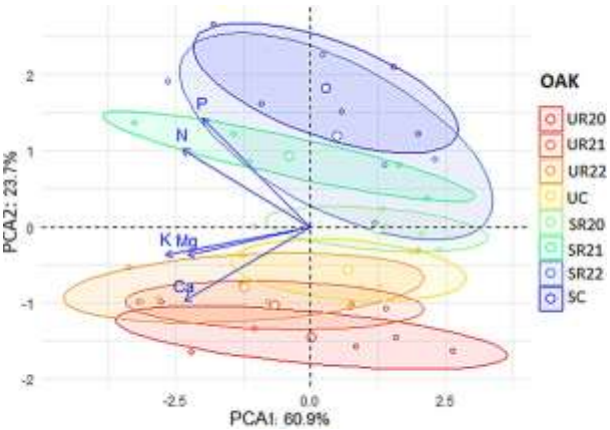

Fig. 3 Nutrient allocation in oak root grown on different growing medium

Discussion

The results of this study provide information on how different growing medium treatments affect the growth (biomass) and nutrient allocation (macroelements) in and within oak and beech seedlings after production in the nursery (just before establishing them as a crop). This pilot study provides some preliminary evidence that innovative peat-free growing media and liquid fertilizers can result in an improved nutrient status of *Q. robur* and *F. sylvatica* seedlings, with the UR22 treatment being particularly promising in this case. Although the findings provide some evidence of potential benefits for nursery management in relation to reduced peat use. In essence, the aboveground characteristics of the seedlings cultivated on the peat-free growing medium, coupled with the liquid fertilizer developed by the University of Agriculture in Kraków, were in every respect close to those of the seedlings grown

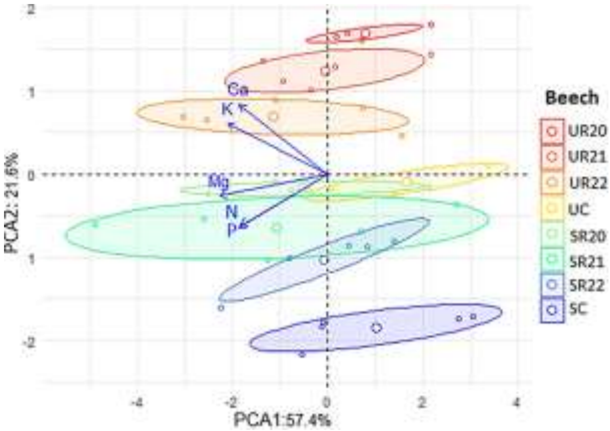

Fig. 4 Nutrient allocation in beech root grown on different growing medium

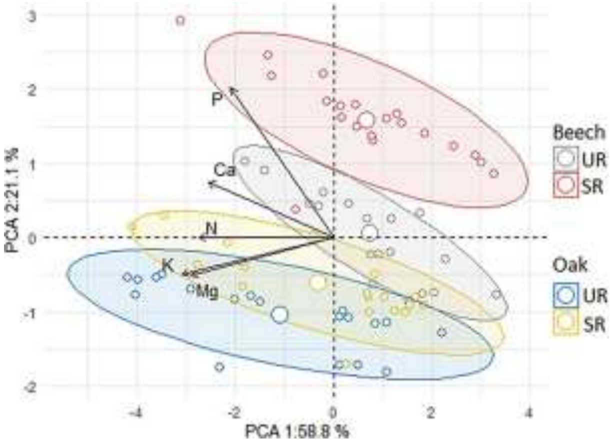

Fig. 5 Nutrient allocation in oak and beech root grown on different fertilization methods

on the state forest growing medium comprising peat and solid fertilizer and these supports the earlier stated hypothesis. The novelty of this research is the examination of sustainable peat substitutes for use in forestry nurseries.

The distribution of plant biomass has been identified as a crucial factor that aids allocation of nutrients in plant organs (Rumpf et al. 2011; Meiwes et al. 2012; Freschet et al. 2015; Husmann et al. 2018; Klimešová et al. 2018; Yue et al. 2021). The importance of nitrogen and phosphorus partitioning between plant organs as a critical factor in regulating growth rate has been highlighted in previous studies (Laliberté et al. 2012; Minden and Kleyer 2014; Tang et al. 2018; Malhotra et al. 2018; Zhang et al. 2018; Yang et al. 2021) The results of this study show contrasting trends in nutrient allocation between nitrogen and phosphorus as both were influenced from negative side of distribution to positive as a result of the effect of fertilization. A similar divergence in nutrient availability was observed by Aoyagi and Kitayama (2016) in their investigation of nutrient allocation between plant organs in Bornean rainforests. In contrast to the findings of Zhao et al. (2020) that nutrient allocation among organs of tree species is intimately related to it demand, the application of this novel fertilizer, as depicted in Fig. 5, appears to be markedly different. The storage of carbon and nutrients in long-lived organs, such as shoots and roots, is considered essential to compensate for biomass losses due to fallen leaves and branches (Aoyagi and Kitayama 2016), or to natural enemies (Fricke et al. 2014; Comita and stump 2020) It is important to note that although the role of shoot phosphorus is less well established in oak, the shoot could potentially act as a storage organ, as previously suggested by Sardans and Peñuelas (2015).

Although the allocation of C, N and P in the studied species recorded higher values than those reported by Aoyagi and Kitayama (2016) However, assessment of nutrient allocation in the studied organs are similar with the values reported by Wei et al. 2013; Pająk et al. 2022a, 2022b; Marušić et al. 2023. The lower concentrations of elements especially, N,P, K and Mg observed in leaves agrees with the study of Hidaka and Kitayama (2011) indicating a withdrawal of nutrient into stable organs, while the calcium content was high because it is immobile in leaves (Peuke and Rennenberg 2011; Loewe-Muñoz et al. 2024). As observed in this study, the seedlings performed well on R22 growing medium, this could be as a result of the addition of straw in the formulation which has been reported to be rich in nutrient content (Xie et al. 2012; Viera et al. 2017; Liu et al. 2018; Guan et al. 2020) Beech seedlings have been reported to grow better in media rich in calcium, magnesium, and potassium (Pająk et al. 2022b). Moreover, Balcar et al. (2011) used dolomite (containing calcium and magnesium) to fertilize beech trees in plantations, and reported a positive effect on their survival and growth.

Variety in nutrient allocation was not only found to exist between the tree species, but also within different organs of each species. This concurs with various investigations on European beech and pedunculate oak (Poorter et al. 2012a, b; André 2010, Pretzsch 2014, Husmann et al. 2018). Unlike beech, oak displayed essentially higher nutritional accumulation in the roots, shoots, and leaves. This study also re-established that nutrient allocation is generally higher in below ground organ than those that are above ground and this applied to the two species (Haase & Jacobs 2013, Liu et al. 2016). Prominently, nutrient response efficiencies varied significantly among the studied species, with treatment on R22 of novel growing medium and UAK fertilizer formulation accumulating more nutrients in the root. This corroborates previous studies (Freschet et al. 2015; Husmann et al. 2018; Klimešová et al. 2018; Rumpf et al. 2011; Meiwes et al. 2012; Pająk et al. 2022b; Rotowa et al. 2023b) that focused solely on the nutrient content of aboveground and belowground biomass.

The different growing media exhibited significant effects on the biomass characteristics of seedlings, especially in oak. This agrees with other studies that have highlighted the influence of peat growing medium on nutrient contents. For example, Pająk et al. (2022a) reported higher macronutrient allocation in root of European beech seedlings grown on differently compacted peat growing medium in a container nurseryRotowa et al. (2023b) reported the influence of various growing medium and fertilizers on the root systems of oak and beech. In addition, Banach et al. (2013) experiment conducted on *F. sylvatica* and *Abies alba* seedlings in a sawdust–peat growing medium, finding that well-aerated growing medium are essential for good growth in beech seedlings. Additionally, Freschet et al. (2015) reported that nutrient allocation additionally relied on the quality of the medium on which they were grown. Rotowa et al. (2023b) in a study carried out on the root systems of the same seedlings, reported treatment UR20 to be best in root morphology and characterization. In this study, however, the treatment UR22 showed the best performance in nutrient allocation in the roots of both species' seedlings. This therefore implies that peat-free growing medium with the recommended dosage of fertilizer (Pająk et al. 2022a; Jasik et al. 2023;

Rotowa et al. 2023b) could be a viable alternative to traditional peat-based medium, offering a sustainable approach to seedling cultivation in forest nurseries.

Conclusions

The confirmation of the earlier-stated hypotheses highlights the efficacy of the peat-free organic growing medium for supporting the growth of *Q. robur* and *F. sylvatica* seedlings in the nursery. The first hypothesis, predicting an improvement in nutrient allocation across different parts (leaves, shoots, and roots) of the seedlings with the novel growing medium and fertilizer, was confirmed. According to the PCA results, the utilization of the novel growing medium and liquid fertilizer indeed resulted in an efficient distribution of nutrients in a way that was not worse than the traditional practice within individual crops. Moreover, the study validated that the uptake of macroelements in both species under the improved root system was comparable and in some cases better than those grown on a standard growing medium (peat plus solid fertilizer). This affirmation emphasizes the potential of the novel growing medium and fertilizer.

Based on the trends observed in this pilot study, the use of peat-free organic growing media and fertilizers developed by the University of Agriculture in Kraków shows potential to support nutrient allocation and growth in pedunculate oak and European beech seedlings at the end of the growing season in the nursery. Significant variations were evident across the different novel growing media treatments especially UR22. Moreover, the use of liquid fertilizer also formulated by the University of Agriculture in Kraków, for the needs of the seedlings, further supports nutrient allocation in various organs. Our concept of growing media and novel liquid fertilizers has potential to provide a new approach of growing tree seedlings for establishing forest plantations in Europe. Furthermore, it may offer a sustainable alternative to peat growing media in the long run.

Author contributions OJR - Paper concept, Methodology, manuscript preparation, Data collection, statistical analysis, Literature review, writing. SM - Funding acquisition, Project administration, Supervision, reviewing, and editing. MJ – Investigation, Data collection, Data editing and validation, writing, review. KS - Data collection, Data curation, data analysis, review.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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forests

Article

Substrate and Fertilization Used in the Nursery Influence Biomass and Nutrient Allocation in *Fagus sylvatica* and *Quercus robur* Seedlings After the First Year of Growth in a Newly Established Forest

Odunayo James Rotowa [1,2,*], Stanisław Małek [1], Michał Jasik [1] and Karolina Staszel-Szlachta [1]

[1] Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Kraków, Al. 29 Listopada 46, 31-425 Kraków, Poland; stanislaw.malek@urk.edu.pl (S.M.); michal.jasik@urk.edu.pl (M.J.); karolina.staszel@urk.edu.pl (K.S.-S.)

[2] Department of Forestry and Wildlife Management, Faculty of Agriculture, Nasarawa State University, Keffi, Nigeria

* Correspondence: odun.james.rotowa@student.urk.edu.pl

Abstract: This study evaluates the efficacy of innovative peat-free organic substrates and liquid fertilizers as alternatives to traditional peat substrates in the cultivation of *Fagus sylvatica* L. and *Quercus robur* L. seedlings in a newly established forest in Southern Poland. The experiment was conducted in a 2 × 2 × 4 experimental layout using a randomized complete block design, comprising eight treatments that combined four substrate types (three novel organic substrates and one peat-based control) with two types of fertilizers (solid and liquid). After one year of growth, biomass and nutrient allocation in the roots, shoots, and leaves of the seedlings were analyzed. The results showed that while solid fertilization enhances biomass accumulation, liquid fertilization supports more uniform growth across different substrates, particularly in oak seedlings. Also, peat substrates recorded the highest nutrient allocation. However, one novel substrate (R22) performed comparably, indicating its potential as a viable peat alternative. Significant interspecies differences were observed, with beech seedlings allocating more biomass to aboveground organs, while oak seedlings favored belowground nutrient allocation. These findings suggest that while peat substrates and solid fertilizers currently provide better outcomes, the innovative R22 substrate shows promise for sustainable forestry practices. Further refinement of the liquid fertilizer was recommended to enhance effectiveness.

Keywords: nutrient allocation; beech and oak seedlings; organic substrate; forestry practice; tree seedling growth

Academic Editor: Wenjie Wang

Received: 7 February 2025
Revised: 9 March 2025
Accepted: 12 March 2025
Published: 14 March 2025

Citation: Rotowa, O.J.; Małek, S.; Jasik, M.; Staszel-Szlachta, K. Substrate and Fertilization Used in the Nursery Influence Biomass and Nutrient Allocation in *Fagus sylvatica* and *Quercus robur* Seedlings After the First Year of Growth in a Newly Established Forest. *Forests* **2025**, *16*, 511. https://doi.org/10.3390/f16030511

1. Introduction

Environmental factors exert a profound influence on physiological traits, thereby demonstrating key aspects of developmental plasticity in plants [1–3]. The success of forest plantations is contingent upon the traits of their seedlings. Biomass from forests has the potential to provide a continuous, largely carbon-neutral supply of materials for the forestry industry [4]. The cultivation practices employed in nurseries can have a significant impact on the functional characteristics and field performance of seedlings. These practices can influence post-transplant rooting and early growth, as evidenced by the studies conducted by Villar-Salvador et al. [5] and Grossnickle and MacDonald [6]. Conversely, some research indicates that seedlings with limited nutrient availability may exhibit greater resilience to transplant shock and summer drought [7,8]. Fertilization can enhance plant survival through a variety of mechanisms. For example, root growth potential and hydraulic conductance are enhanced by phosphorus (P) and nitrogen (N) availability [9,10] which increases the capacity of fertilized seedlings to absorb soil water [11]. N- and P-deficient plants frequently exhibit alterations in their biomass accumulation and allocation patterns [2,12].

In Poland, the extensive restructuring of coniferous monocultures has been necessitated by the declining health and quality of the trees. European beech (*Fagus sylvatica* L.), a temperate species that is widely distributed across central and Western Europe [13], along with oaks (*Quercus* spp., including *Quercus robur* L.), are the dominant species in Polish forests and constitute a significant portion of European temperate vegetation. Beech and oak forests are of great importance to the biosphere, contributing to biomass production, oxygen generation, atmospheric regulation, and more [14]. Due to their superior wood quality, beech and oak have become more commercially desirable than several conifer species and are favored in climate change adaptation strategies for both ecological and economic reasons in Europe [15]. Global changes have demonstrated that shifts in tree species and their symbiotic associations have an impact on biogeochemical processes [16–18]. Consequently, it is imperative to intensify efforts to enhance the health and sustainability of forest stands dominated by these highly valued species, particularly as Polish forests transition from pine to species such as beech and oak.

Peat is widely recognized as a foundational component in nursery substrates due to its exceptional physical, chemical, and biological properties. Its remarkable water-retention capabilities and consistent quality make it a preferred medium for the nurturing of plants [19,20]. Nevertheless, the release of carbon (C) from peat soils over time has the potential to give rise to environmental concerns. In contrast to forests, which act as C sinks, peatlands have the potential to release stored C into the atmosphere, which can have a significant impact on climate change. Given that peatlands store an estimated one-third of the world's soil C, which exceeds the combined capacity of global forests, the rapid conversion of peat to CO_2 in plantations has the potential to elevate greenhouse gas levels, thereby threatening our treasured ecological systems [21]. Gruda [22] notes that Europe excavates approximately 20,000 cubic meters of peat annually, which has the effect of exacerbating environmental degradation [23]. In light of the urgent global environmental challenges associated with peat use in nursery substrates, it is of the utmost importance to prioritize the preservation of peatlands over their destruction. These, therefore, underscore the urgent need for sustainable alternatives.

Over the years, there has been an increased adoption of compost utilization in place of or as a mixture with peat [24,25]. The process of production is time-consuming and labor-intensive. If not carried out correctly, compost can harbor pathogens, weed seeds, and plant diseases [26]. As a result of the contemporary emphasis on sustainability and environmental awareness, there is a pressing need to develop a novel peat-free organic substrate using sustainable, cost-effective, and eco-friendly materials as viable alternatives to peat. The novel substrate used in this study was designed to overcome these challenges, with properties, including water capacity, bulk density, and solid density, meeting those of standard peat substrates [27].

In previous studies, the novel substrates and liquid fertilizer used in this research demonstrated promising physicochemical properties, such as water retention capacity, bulk density, and nutrient content, comparable to those of traditional peat substrates [27]. Specifically, the R22 substrate exhibited enhanced structural stability and nutrient availability after the nursery production cycle [28]. Building on these findings, this study extends the evaluation to assess the performance of seedlings after one year of growth in a forest environment. This approach allows us to determine whether the promising outcomes observed in the nursery translate into sustainable growth and nutrient allocation under field conditions as recommended by Rotowa et al. [28]. Integrating these advancements, our study aims to explore the practical potential of these innovations for sustainable nursery and forestry practices.

The objective of this study is to evaluate the impact of novel peat-free organic substrate and liquid fertilizer, developed by the University of Agriculture in Kraków, Poland, on the nutrient allocation and biomass production in *Fagus sylvatica* and *Quercus robur* seedlings after one year of growth in a forest environment. This study hypothesizes that novel peat-free organic substrates and liquid fertilizers will again result in comparable or superior seedling growth, biomass allocation, and nutrient distribution compared to traditional peat substrates and solid fertilizers. This hypothesis is grounded in the demonstrated physicochemical benefits of the innovative substrates and fertilizers after the nursery production cycle. By evaluating these plant materials under both nursery and field conditions, the study seeks to bridge the gap between experimental advancements and practical forestry applications.

2. Materials and Methods

2.1. Study Site

The study site was situated in Barbarka, within the Miechów Forest District (Figure 1). The research area is situated at an altitude of approximately 370 m above sea level in the Olkuska Upland, southern Poland (50°15′54.2′′ N 19°53′36.5′′ E). It is located within a forest complex managed by the National Forest Holding. The area in question encompasses several gaps created by the clear-cutting of a *Populus* spp. plantation. The Miechów Forest District is distinguished by its diverse upland landscape. The Olkuska Upland is a compact karst plateau composed of limestone and marl. The climate is continental, exhibiting a notable temperature range of 21 °C and a considerable amount of precipitation during the growing season. The mean annual air temperature in the Forest District is 8.2 °C, with July being the warmest month (19.6 °C) and January the coldest (−3.0 °C).

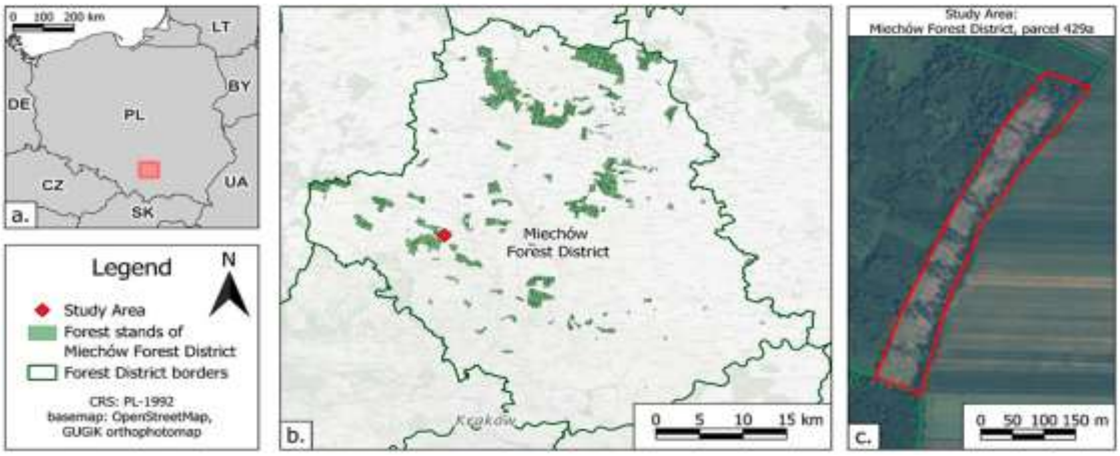


Figure 1. Study area of the experiment in Barbarka, Miechów Forest District, Poland. (**a**) Geographic location of the study site. (**b**) Detailed topographic map of the forest area. (**c**) Satellite imagery showing the experimental plot layout.

2.2. Substrate Composition and Seedling Production

At the nursery stage of the experiment, the control variant (C) peat substrate was produced at the Nursery Farm in Nędza (50.167964 N, 18.3138334 E). The substrate was composed of 93% peat and 7% perlite, with the addition of dolomite (3 kg per m^3 of substrate) to achieve a pH of 5.5. The novel peat-free substrates (R20, R21, and R22) were manufactured from diverse components from coniferous woody materials (mainly *Pinus sylvestris* L.), with specific proportions designed to optimize water retention and nutrient availability (Table 1). Physicochemical properties, such as water capacity, bulk density, and porosity, were measured following standardized protocols to ensure comparability (Table 2). The peat-free substrates and liquid fertilizer used in this study were developed under the project POIR.04.01.04-00-0016/20, which was funded by the National Centre for Research and Development (NCBiR) from national resources and the European Regional Development Fund. This project was spearheaded by the Department of Ecology and Silviculture, University of Agricultural in Kraków. The comprehensive procedure for preparing the novel substrate and fertilizer was previously described by Rotowa et al. [27].

Table 1. Properties of the organic peat-free substrate [%].

Substrate	Sawdust	Wood Bark	Perlite	Core Wood	Mixed Silage	Wood Chips	Straw
R20	73	10	4	2	1	10	-
R21	20	10	4	2	1	63	-
R22	50	33	4	2	1	-	10

Table 2. Mean and standard deviation of physicochemical properties of substrates used in seedling growth in the nursery.

Substrate	Water Capacity (%)	Water Outflow Rate (L/min)	Bulk Density (g/cm^3)	Solid Density (g/cm^3)	Air Capacity (%)	Porosity (%)
R20	40.5 ± 2.9 ^{b}	0.595 ± 0.150 ^{b}	0.115 ± 0.009 ^{a}	0.64 ± 0.08 ^{a}	52.1 ± 3.19 ^{c}	92.6 ± 0.60 ^{d}
R21	33.1 ± 2.5 ^{d}	0.781 ± 0.114 ^{a}	0.098 ± 0.014 ^{c}	1.74 ± 0.07 ^{a}	60.8 ± 3.06 ^{a}	93.6 ± 0.87 ^{c}
R22	37.8 ± 5.1 ^{c}	0.594 ± 0.150 ^{b}	0.104 ± 0.020 ^{b}	1.66 ± 0.11 ^{a}	55.8 ± 5.58 ^{b}	93.9 ± 0.98 ^{b}
Control	57.7 ± 5.4 ^{a}	0.417 ± 0.145 ^{c}	0.085 ± 0.007 ^{d}	1.69 ± 0.14 ^{a}	37.0 ± 5.72 ^{d}	94.7 ± 0.42 ^{a}
F	387.45	56.32	65.81	1.0717	295.79	76.48
p	0.0000	0.0000	0.0000	0.3870	0.0000	0.0000

Letters with different alphabet indicate statistically significant differences between means (p < 0.05).

In each experimental variant, seedlings of both species were cultivated in 75 Marbet V300 polystyrene containers, each containing 53 cells with a volume of 275 cm^3. Subsequently, the various substrates were filled into containers, and beech and oak seeds were manually sown at the Suków-Papierna Nursery Farm (Daleszyce Forest District) on 19–20 April 2022. To enhance germination, seeds of both species were scarified before sowing. After sowing, the containers were transferred into a vegetation hall for four weeks, after which they were relocated to an external production field. Manual weeding was conducted during the seedling growth phase. The seedlings were cultivated for five months following the procedures employed in the container nursery, as outlined by Szabla and Pabian [29].

Osmocote fertilizer was incorporated into the substrate during its preparation prior to sowing, with a total application rate of 3 kg m^3 for each medium. The mixture comprised Osmocote 3–4 M (2 kg) and Osmocote 5–6 M (1 kg). The Osmocote 3–4 M formulation contained 16% N (7.1% N-NO_3^- and 8.9% N-NH_4^+), 9% P_2O_5, 12% K_2O, 2.0% MgO, and microelements (B, Fe, Cu, Mn, Zn, and Mo). The Osmocote 5–6 M formulation included 15% N (6.6% N-NO_3^- and 8.4% N-NH_4^+), 9.0% P_2O_5, 12% K_2O, 2.0% MgO, and the same microelements. A novel liquid fertilizer regime was introduced, utilizing two distinct compositions. These application rates were determined based on the nutrient demand of the seedlings at different growth stages and the need to ensure adequate nutrient availability without causing leaching losses. The first liquid fertilizer contained 4.78% N, 1% P_2O_5, 2.64% K_2O, 2.65% CaO, 1.4% MgO, 0.71% SO_3, and 0.14% Na_2O. It was initially applied at a total volume of 3.14 dm^3 (0.048 $dm^3\ m^{-2}$) to provide an immediate supply of essential macronutrients required for early root and shoot development. The second liquid fertilizer contained 0.798% N, 0.166% P_2O_5, 0.440% K_2O, 0.441% CaO, 0.234% MgO, 0.118% SO_3, and 0.023% Na_2O, applied at a total volume of 15.09 dm^3 (0.229 $dm^3\ m^{-2}$). This staggered approach was designed to sustain nutrient availability throughout the critical growth phases, promoting steady biomass accumulation and nutrient allocation. During seedling production, the first liquid fertilizer was applied eight times at 10-day intervals, while the second was applied 15 times at 5-day intervals. These fertilization schedules were consistently maintained for both European beech and pedunculate oak seedlings to optimize nutrient use efficiency while minimizing environmental impact. This approach aligns with previous research findings on staged nutrient applications to enhance seedling performance in forest nurseries [27,29].

2.3. Experimental Layout

After the nursery production cycle, the seedlings were transported and planted into the forest on 5 September 2022. The experimental design employed a 2 × 2 × 4 factorial layout within a randomized complete block design (RCBD), comprising four substrate types (three novel and one peat-based control) and two fertilizer types (solid and liquid), resulting in eight treatment combinations. Each treatment was replicated three times within blocks to minimize spatial variability. The blocks were stratified based on topographic and soil characteristics to ensure uniform environmental conditions. Subplots, each containing 49 seedlings were established with consistent spacing (1 × 1.7 m) to standardize growing conditions across treatments. A total of 24 subplots were established for each species. Four substrates (R20, R21, R22, and peat) and two fertilizer treatments were employed to cultivate the seedlings. The first fertilizer treatment was a solid fertilizer utilized in the Suków container nursery (SR20, SR21, and SR22 variants), while the second was a novel liquid fertilizer developed by the University of Agriculture in Kraków, Poland (UR20, UR21, and UR22 variants). In both fertilization cases, the peat substrate served as the control variant (SC and UC).

2.4. Soil Sample Collection and Plantation Establishment

The research plot, comprising 0.7 ha, was established on a *Populus* spp. harvest site with similar parent material and soil type to that of an old-growth forest. Based on the primary active root zones of young seedlings; soil samples were collected from five different locations within each subplot at two layers. The top mineral layer at depths of 0–10 cm and the second layer at 10–20 cm were collected using polyvinyl chloride (PVC) tubes. The layer of the top 0–10 cm is not only conducive to initial root growth and early seedling establishment, but rich organic matter nutrients also make it essential. This layer is also the primary domain of microbial activity and nutrient cycles, and the 10–20 cm layer was chosen to test beyond the immediate root zone the availability of nutrients, as well as the potential effects of leaching from fertilization. The inclusion of these two depths provides a comprehensive analysis of nutrient dynamics within the soil, ensuring that the study captures both immediate and longer-term soil fertility impacts on seedling growth [30]. A total of 480 soil samples were collected. Each sample was then air-dried, sieved through a 2 mm mesh, ground, and prepared for the analysis of its soil properties following standard soil preparation protocols [31]. To ensure consistency in analysis, the soil samples were air-dried, sieved, and ground. Air-drying stabilized the samples and prevented microbial activity that could alter nutrient content. Sieving through a 2 mm mesh removed debris and homogenized the samples, ensuring uniformity across all the analyses. Grinding the samples into a fine powder increased surface area, enhancing the accuracy of nutrient extractions and spectrometric measurements. This process maintained comparability between soil samples, ensuring that the results accurately reflected the nutrient composition of the study site.

The physicochemical properties of the samples were determined following established methods described by Ostrowska et al. [31] and Staszel et al. [30]. The pH values of the soil were measured potentiometrically in both water and 1 M KCl. Hydrolytic acidity was evaluated using the Kappen method, while exchangeable acidity and content were estimated using the Sokołow method. The total N and C contents were determined using a LECO CNS True Mac Analyzer (Leco, St. Joseph, MI, USA). To quantify alkaline cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}), 1 M ammonium acetate was employed (iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, UK), utilizing inductively coupled plasma optical emission spectrometry (ICP-OES). The analyses were conducted at the Laboratory of Forest Environment Geochemistry and Land Intended for Reclamation, Department of Ecology and Silviculture, and the Faculty of Forestry at the University of Agriculture in Krakow, Poland.

The field experiment was conducted using a randomized complete block design. In each subplot, 49 seedlings were planted following a standardized spacing of 1 × 1.7 m both between and within rows, ensuring consistent plant density across all the treatments. Before transplantation, the seedlings were selected based on specific morphological criteria to ensure uniformity and quality. The selection criteria included a minimum height of 25 cm, a root system length of at least 15 cm, a well-developed collar diameter, and healthy stem and leaf formation. These parameters were used to enhance transplant success and minimize variability in initial seedling vigor. The planting depth was standardized to ensure that the root collar was level with the soil surface, a technique aimed at optimizing root establishment and reducing transplant shock. For both species combined, a total of 2352 seedlings were established (147 seedlings per treatment). At the end of the 2023 growing season, three seedlings were selected from each of the 49 seedlings in every subplot (nine seedlings per treatment). The seedlings were assessed for root, shoot, and leaf biomass and nutrient allocation. These seedlings were carefully uprooted to preserve an intact root segment. The mean height of the seedlings in each subplot was recorded and this mean value formed the choice of the selected seedling. The seedlings were selected from each of the eight treatment groups for root, shoot, and leaf biomass and the subsequent laboratory analysis for nutrient allocation, leading to the assessment of 144 seedlings for both species in the laboratory experiment. To protect the new forest from animal interference, the area was fenced after the plantation was established.

2.5. Parameter Assessment and Nutrient Analysis

Following the conclusion of the 2023 growing season, the biomass of different plant organs, including leaves, shoots, and roots, was quantified. To validate the hypothesis, biomass allocation (roots, shoots, and leaves) and nutrient concentrations (C, N, P, K, S, Ca, and Mg) were measured after one year of growth in a newly established forest. These parameters were chosen based on their relevance to seedling survival and productivity under field conditions. The samples were dried at 65 °C for 48 h to achieve a constant weight, then ground and homogenized. For each organ, 0.5 g of the ground sample was placed into a flask and subjected to acid digestion using a 3:1 mixture of nitric and hydrochloric acids. After digestion, the samples were filtered into a 50 mL flask, and the concentration of elements was determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Quality control measures were implemented throughout the nutrient analysis process to ensure accuracy and reliability. Calibration standards were prepared and run alongside the samples to verify the accuracy of the ICP-OES readings. Additionally, blank samples and duplicates were included in the analysis to monitor and correct for any potential contamination or analytical drift. The samples were analyzed for N, sulfur, and C contents using a LECO CNS TruMac analyzer (Leco, St. Joseph, MI, USA). The phosphorus, potassium, calcium, and magnesium (P, K, Ca, and Mg) contents were analyzed using a Thermo iCAP 6500 DUO ICP-OES spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) following mineralization in a 3:1 mixture of nitric and hydrochloric acids. These analyses were conducted at the Laboratory of Forest Environment, Geochemistry, and Land Intended for Reclamation in the Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Kraków, Poland.

2.6. Statistical Analysis

Data analysis was conducted using a multifaceted approach. A descriptive analysis was conducted to compare the performance across treatments. The Shapiro–Wilk [32] and Levene [33] tests were employed to verify compliance with normality, homogeneity of variance, and linearity assumptions. To reveal significant differences between treatments for each measurement, post hoc analyses were performed with DMRT. The significance of these relationships and the differences between species combinations were revealed using ANOVA. Before performing each model, the homogeneity of variances and the normality of residuals were assessed, and data were log-transformed where necessary. A clustered heatmap was used to identify the hierarchy clustering using colors to represent values. The row (nutrient) and the column (substrate and fertilizer treatment) of the data matrix were ordered according to the output of clustering. The values were standardized based on mean and standard deviation using color to show the relative expression of individual nutrient allocation between individual treatments. A dendrogram was used to separate the homogeneous relation within the row and column. This dendrogram represents the hierarchical clustering of treatments based on nutrient allocation patterns in the studied species. Clusters were generated using Ward's method, with Euclidean distance as the similarity metric. Branches indicate treatments with similar nutrient allocation profiles, and the length of the branches reflects the degree of similarity. Heapmap Pearson correlation coefficients (r) were further calculated to examine linear relationships between biomass and nutrient variables. The relationships were deemed significant at the 0.05 level [34]. Correlation coefficients were interpreted as follows: $r \leq 0.35$ indicated low or weak correlations (deep blue color) r from 0.36 to 0.67 indicated modest or moderate correlations (light blue to light red color) r from 0.68 to 1.0 indicated strong or high correlations (red color) Regression analysis focused on nitrogen (N) and phosphorus (P) due to their primary role in plant growth, nutrient allocation, and physiological processes. Data visualizations were performed using Python (version 3.10, Python Software Foundation, Wilmington, DE, USA).

3. Results

3.1. Soil Properties

Both the beech and oak sites exhibit slightly acidic soils, which are characteristic of forest environments. The pH values are marginally lower at a depth of 20 cm compared to 10 cm. The oak site demonstrates a consistent N level across both depths. The C content was observed to be higher at the 10 cm depth for both sites, with a subsequent decrease at 20 cm. Furthermore, P levels also decrease with depth at both sites. The carbon/nitrogen (C/N) ratio remains stable across depths, indicating balanced C and N cycling within the soil. Exchangeable cations, including Ca, K, Mg, and Na, were more concentrated at the 10 cm soil depth than at 20 cm across both sites. This suggests that nutrient availability in the upper soil layer may play a crucial role in seedling nutrient uptake, particularly during the early stages of growth. A statistical analysis of soil properties revealed no significant differences between the two sites despite minimal numerical variations across the soil depths (Table 3). This suggests that soil composition was relatively uniform across the study area, allowing for a controlled comparison of the different treatments' effects on seedling growth.

Table 3. Soil properties of sampled plot of *F. sylvatica* and *Q. robur* at Barbarka experimental forest of Poland.

Soil Uptake Level (cm)	pH (H_2O)	N	C	P_2O_5	C/N	Exchangeable Cations			
						Ca	K	Mg	Na
				Fagus sylvatica L. site					
0–10	5.21 ± 0.55	0.36 ± 0.10	2.41 ± 0.21	3.14 ± 0.21	13.78 ± 0.32	5.51 ± 0.52	0.18 ± 0.01	0.57 ± 0.06	0.70 ± 0.08
10–20	5.10 ± 0.57	0.34 ± 0.09	1.94 ± 0.15	2.87 ± 0.14	12.90 ± 0.32	4.81 ± 0.39	0.16 ± 0.01	0.42 ± 0.03	0.63 ± 0.06
Total	5.15 ± 0.56	0.35 ± 0.10	2.16 ± 0.12	2.99 ± 0.12	13.30 ± 0.23	4.22 ± 0.32	0.15 ± 0.01	0.49 ± 0.03	0.55 ± 0.07
p-value	0.187 ns	0.170 ns	0.061 ns	0.286 ns	0.065 ns	0.064 ns	0.062 ns	0.062 ns	0.062 ns
				Quercus robur L. site					
0–10	5.14 ± 0.59	0.45 ± 0.95	2.14 ± 0.22	4.70 ± 0.45	13.31 ± 0.33	5.11 ± 0.53	0.23 ± 0.02	0.55 ± 0.06	0.81 ± 0.26
10–20	5.03 ± 0.61	0.44 ± 0.09	1.83 ± 0.14	4.22 ± 0.40	12.23 ± 0.31	4.65 ± 0.36	0.21 ± 0.02	0.46 ± 0.03	0.82 ± 0.27
Total	5.08 ± 0.59	0.45 ± 0.10	1.55 ± 0.13	4.44 ± 0.30	12.73 ± 0.23	3.32 ± 0.32	0.22 ± 0.01	0.38 ± 0.03	0.82 ± 0.29
p-value	0.208 ns	0.945 ns	0.061 ns	0.427 ns	0.069 ns	0.061 ns	0.363 ns	0.061 ns	0.989 ns

Mean ± SD; C and N (%); P_2O_5 (mg/100g) Ca, K, Mg, Na (cmol (+) kg^{-1}) ns = not significance.

3.2. Biomass Allocation in Studied Organs of F. sylvatica and Q. robur Seedlings

A biomass allocation analysis revealed significant interactions between substrate type and fertilization method. *F. sylvatica* demonstrated a stronger dependence on the peat-based control substrate, whereas *Q. robur* exhibited greater adaptability to novel substrates. Solid fertilization was generally more effective in enhancing both species' shoot and root biomass accumulation. In contrast, the seedlings treated with liquid fertilization showed greater adaptability to alternative growing media after one year of growth in a new forest (Figures 2 and 3). For the beech seedlings, those grown in the peat-based control (SC) exhibited superior shoot and root biomass accumulation compared to those grown in novel substrates under solid fertilization. However, biomass allocation in the seedlings grown in novel substrates remained consistent across all the organs under liquid fertilization (Figure 2). The response of oak seedlings was somewhat different. While the peat-based control still supported better overall growth and showed variation in root, shoot, and leaf allocation under solid fertilization. Biomass allocation remained relatively stable in more stable organs (root and shoot), with no significant differences among the seedlings raised with liquid fertilization (Figure 3). In both species, a positive correlation was observed between root biomass and both shoot and leaf biomass, suggesting that root development is closely linked to aboveground growth. Similarly, shoot and leaf biomasses were positively correlated (Figure 4a–c). Notably, the regression lines for beech in root–shoot and shoot–leaf relationships were steeper than those for oak, indicating a stronger dependence of shoot and leaf biomass on root biomass in beech compared to oak.

3.3. Allocation of Nutrients in F. sylvatica and Q. robur Seedlings One Year After Planting in the Forest

The heatmap analysis (Figures 5 and 6) illustrates the impact of different treatments on nutrient allocation in the roots, shoots, and leaves of both *F. sylvatica* and *Q. robur* after one year of growth in a new forest. A consistent trend emerged across all the treatments, with roots generally exhibiting the highest nutrient allocation compared to other organs. Notably, the seedlings treated with traditional solid fertilizers demonstrated significantly higher nutrient concentrations across all organs, whereas those treated with novel liquid fertilizers had consistently lower nutrient levels. These findings emphasize the superior efficacy of solid fertilizers in nutrient uptake.

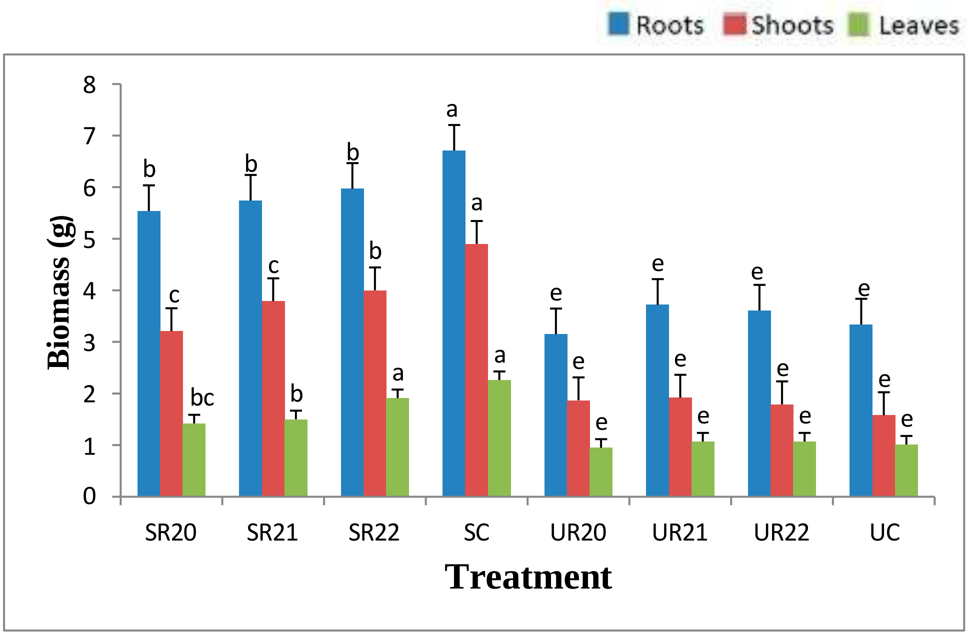


Figure 2. Distribution of biomass across different treatments for *Fagus sylvatica* L. Alphabets 'a–c' denote homogeneous groups under solid fertilization and 'e' denote homogeneous groups under liquid fertilization; S—solid fertilization; U—liquid fertilization; R—novel substrates; C—controls substrate (peat–perlite).

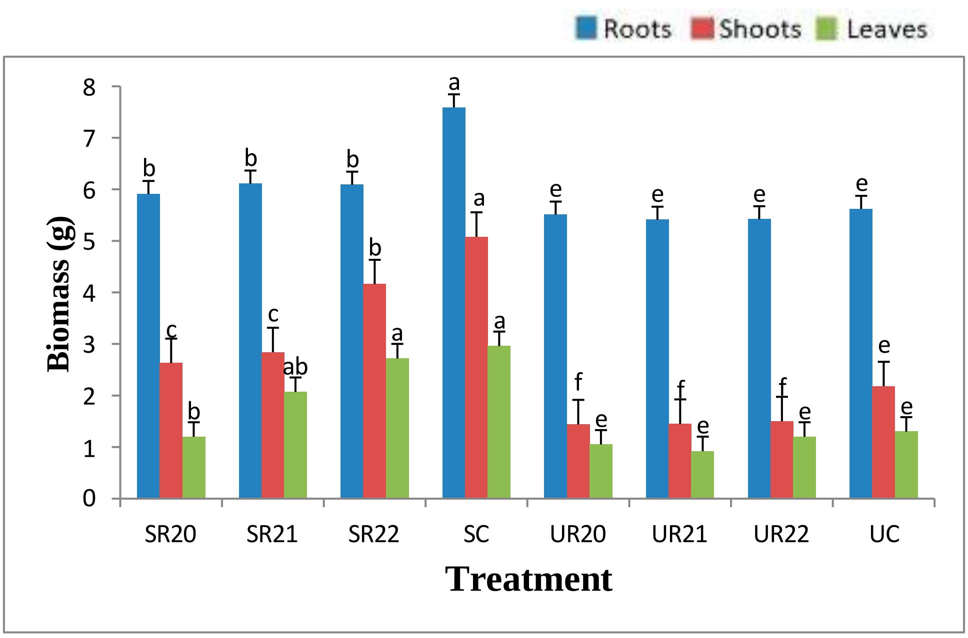
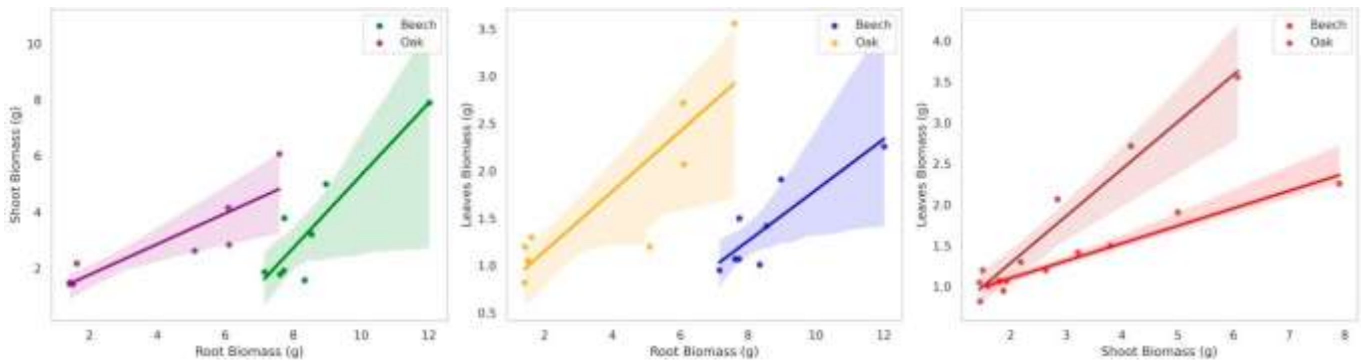


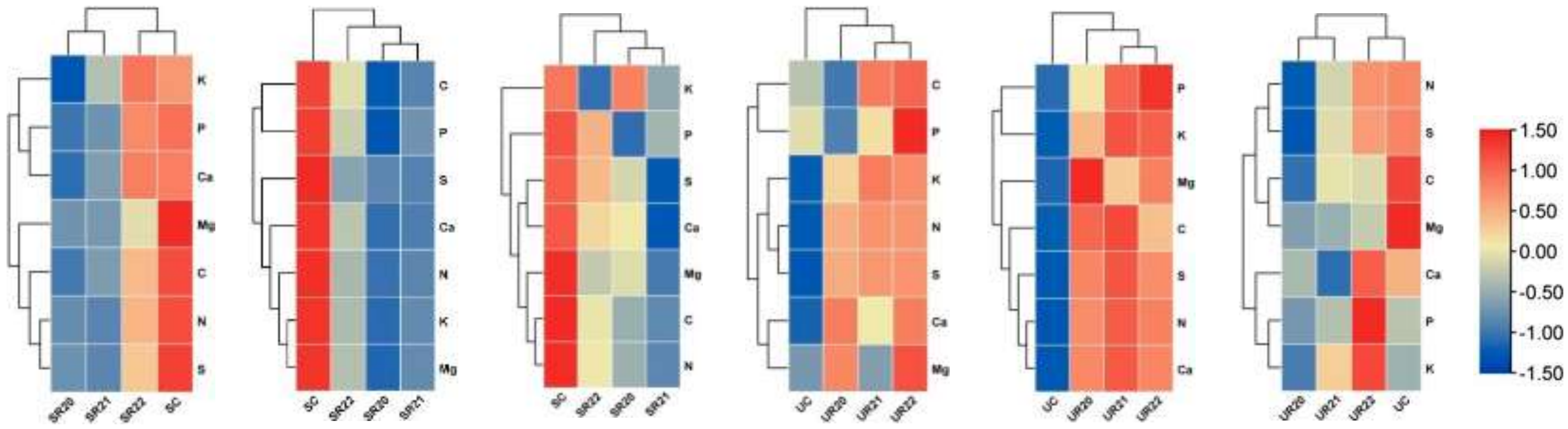
Figure 3. Distribution of biomass across different treatments for *Quercus robur* L. Alphabets 'a–c' denote homogeneous groups under solid fertilization and 'e' and 'f' denote homogeneous groups under liquid fertilization; S—solid fertilization; U—liquid fertilization; R—novel substrates; C—controls substrate (peat–perlite).



(**a**) shoot and root biomass (**b**) leave and root biomass (**c**) leave and shoot biomass

Figure 4. (**a**–**c**) correlation among assessed biomass of seedling organs.

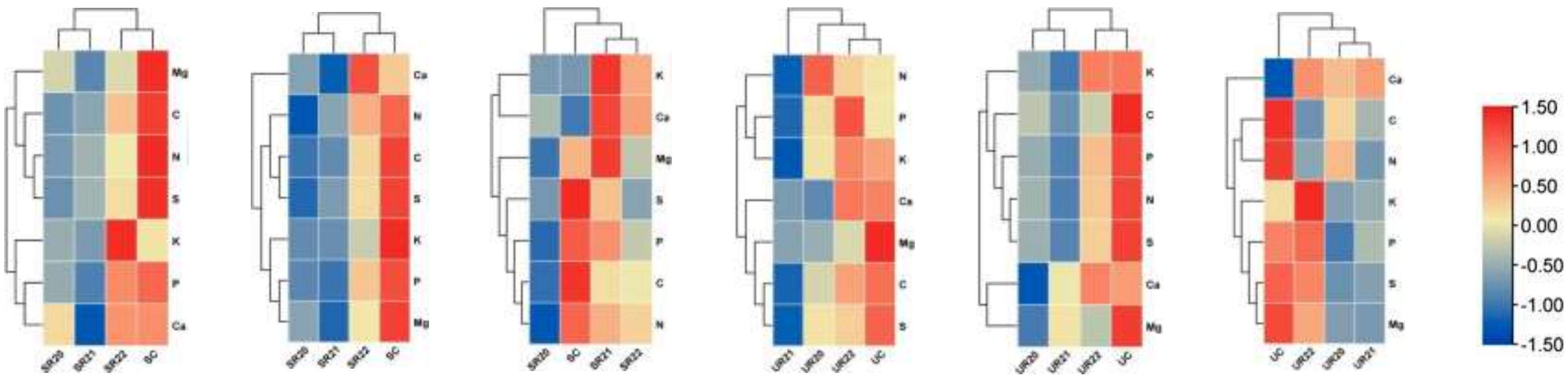
Beech seedlings treated with solid fertilizers, particularly in the SC treatment, exhibited the highest concentrations of all the elements. Interestingly, the novel substrate SR22 showed nutrient levels comparable to those of the peat control (SC), indicating its potential as an effective alternative (Figure 5a). In contrast, the leaves treated with liquid fertilizers showed an opposite trend. It is noteworthy that the three novel substrates perform better than the peat substrate (Figure 5d). The nutrient content in the shoots followed a similar pattern. The shoots in the control treatment (SC) exhibited superior performance, demonstrating the most favorable outcomes among all the treatments. The SR22 treatments under solid fertilization exhibited neutral performance, with close clusters within the treatment groups and among the nutrient levels with the peat (SC) substrate (Figure 5b). The situation with shoots treated with liquid fertilizers was extremely contrary. Peat shoots treated with liquid fertilizers showed reduced nutrient content, with UR21 having a higher concentration of virtually all the elements compared to the other liquid treatments except Mg and P (Figure 5e). Again, the roots of beech seedlings showed the highest nutrient content in the SC treatment, with significant levels of C, N, and Mg (Figure 5c). The roots treated with liquid fertilizers exhibited a more competitive performance. The UR22 treatment performs better in the concentration of crucial nutrients like N, P, and K (Figure 5f). For oak leaves and shoots, the SC and SR22 treatments are tightly grouped in the dendrogram, this suggests that the two treatments have similar effects on nutrient concentration in these organs. In particular, Figure 6a,b indicated that the treatments have a consistent impact on the uptake of N, P, and K. The roots of the oak seedlings showed the highest nutrient content in all the treatments except in SR20 (Figure 6c). The situation was closely similar in the seedlings raised with liquid fertilizer (Figure 6f) The dendrogram groupings for nutrient content in the oak roots demonstrate clear differences among the fertilization methods with respective substrates (Figure 6c). The result of correlation analysis indicates a strong interdependence between biomass and nutrient concentration, including N, P, and K. (Figure 7a,b). Beech exhibits a robust positive correlation between biomass production and the availability of all the nutrients (Figure 7b). In contrast, oak exhibits weaker and more complex correlations between biomass and these nutrients (Figure 7b).



(**a**) Leave (**b**) Shoot (**c**) Root (**d**) Leave (**e**) Shoot (**f**) Root

Traditional solid fertilizer **Novel liquid fertilizer**

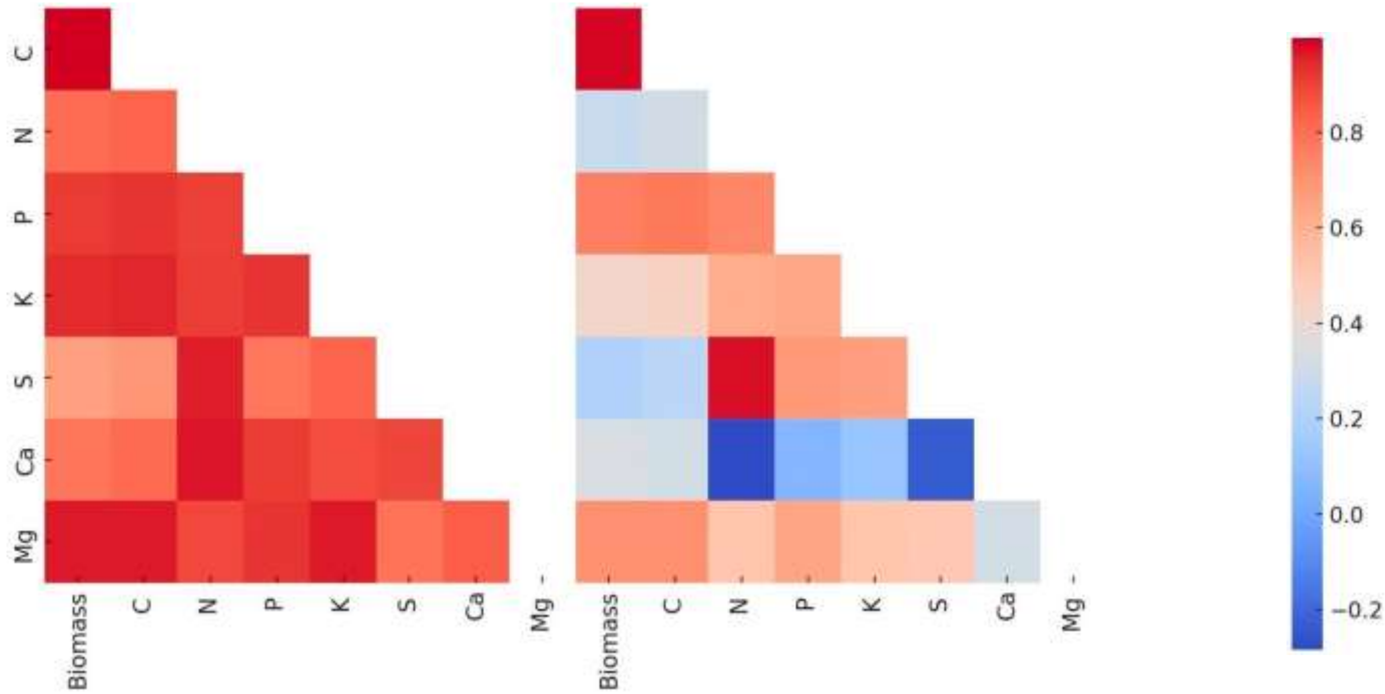
Figure 5. Matrix of nutrients in different parts of *F. sylvatica* seedlings for each treatment (mg/kg). S—State Forest fertilization; U—University fertilization; R—novel substrates; C—control substrate (peat).



(**a**) Leave (**b**) Shoot (**c**) Root (**d**) Leave (**e**) Shoot (**f**) Root

Traditional solid fertilizer **Novel liquid fertilizer**

Figure 6. Allocation of nutrients in different parts of *Q. robur* seedlings for each treatment (mg/kg). S—State Forest fertilization; U—University fertilization; R—novel substrates; C—control substrate (peat).



(**a**) *Fagus sylvatica* (**b**) *Quercus robur*

Figure 7. Correlation matrix analysis of nutrient and biomass in *F. sylvatica* and *Q. robur*.

3.4. Principal Component Analysis (PCA) Results for F. sylvatica and Q. robur

The PCA results revealed the distribution patterns of nutrient allocation in the root system of *F. sylvatica* and *Q. robur* under different fertilization treatments. In both species, the substrate medium had a minimal effect on nutrient composition as, nutrient profiles remained relatively consistent across different substrates, with data points clustering closely together (Figures 8 and 9). This, therefore, implies that variations in the growing medium did not significantly influence nutrient composition in the root system. In contrast, the effect of fertilization treatments (UR and SR) was more pronounced, as illustrated in Figure 10, where data points were more widely dispersed. This separation indicates that fertilization had a significant impact on nutrient content in both the oak and beech seedlings, causing noticeable variation in nutrient allocation at the end of the production cycle. This indicated a significant effect of fertilization on nutrient content for both species, with the points being spatially separated. The variation observed in these graphs suggests that the primary factor influencing growth is the type of fertilizer treatment rather than the growing medium treatment.

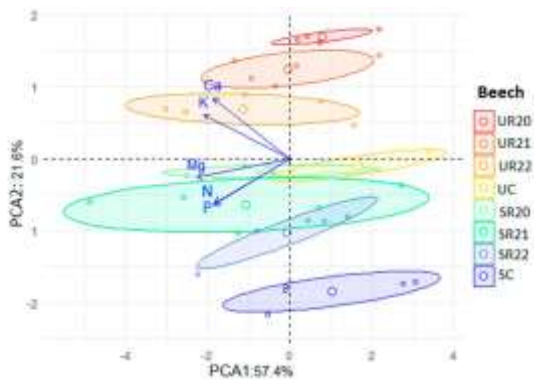


Figure 8. Nutrient allocation in beech root grown on different substrates.

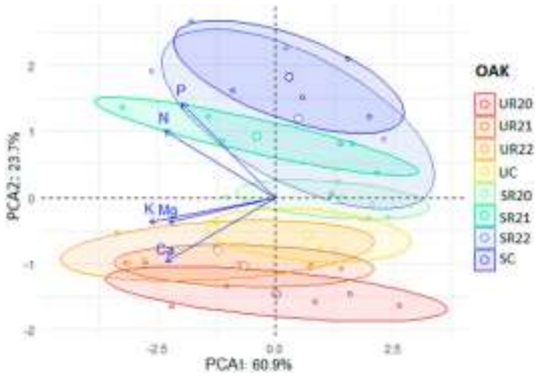


Figure 9. Nutrient allocation in oak root grown on different substrates.

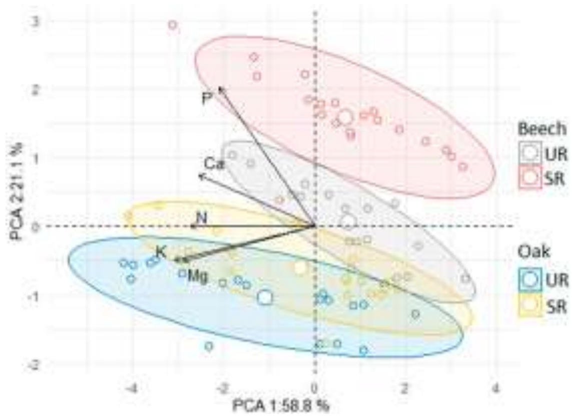


Figure 10. Nutrient allocation in oak and beech root grown on different fertilization methods.

4. Effects of Treatments on N and P Allocation in Beech and Oak Seedlings

Among the analyzed nutrients, N and P showed significant responses to different treatments, influencing nutrient allocation in seedling organs. These two nutrients were, therefore, selected for detailed regression analysis to quantify treatment effects more precisely. The regression analysis revealed significant effects of the treatments and plant parts on the N and P concentrations in the *F. sylvatica* and *Q. robur* seedlings. For the beech seedlings, the N concentration was significantly reduced across all the treatments compared to the peat substrate. Decreases were observed in the liquid fertilizer treatments (UR22, UR20, and UR21), while the solid fertilizer treatment (SR20, SR21, and SR22) resulted in smaller reductions. Similarly, the phosphorus concentration declined under all the treatments, with the most notable reductions in the liquid fertilizer. Across plant organs, the N and P concentrations were significantly lower in the leaves and shoots, indicating preferential nutrient allocation to the root organ. In the oak seedlings, the nitrogen concentration also showed significant reductions, particularly in the liquid fertilizer, with UC (-37.10 mg g^{-1}) also showing substantial declines. Unlike beech, oak leaves exhibited a positive N allocation (7.25 mg g^{-1}), suggesting preferential retention in the foliage, while shoots showed a reduction. For phosphorus, the largest decreases were again observed in UR21, UR20, and UC. Similarly to beech, the oak seedlings exhibited lower P concentrations in comparison to the reference part (Table 4).

Table 4. Modeled estimate of the effects of treatments and plant organs on the N and P concentrations in the beech and oak seedlings.

Species	Nutrient	Treatment/ Organ	*Fagus sylvatica* Estimate (mg g^{-1})	Std. Error	Lower CI	Upper CI	*Quercus robur* Estimate (mg g^{-1})	Std. Error	Lower CI	Upper CI
Beech	N	SR20	−22.03	3.29	−29.09	−14.98	−24.37	4.33	−33.66	−15.08
		SR21	−21.47	3.29	−28.52	−14.41	−20.13	4.33	−29.42	−10.84
		SR22	−12.70	3.29	−19.76	−5.65	−14.53	4.33	−23.82	−5.24
		UC	−38.17	3.29	−45.22	−31.11	−37.10	4.33	−46.39	−27.81
		UR20	−41.80	3.29	−48.86	−34.75	−39.70	4.33	−48.99	−30.41
		UR21	−41.20	3.29	−48.26	−34.15	−42.57	4.33	−51.86	−33.28
		UR22	−42.87	3.29	−49.92	−35.81	−39.43	4.33	−48.72	−30.14
		Leaf	−27.26	2.01	−31.58	−22.94	7.25	2.65	1.56	12.94
		Stem	−21.33	2.01	−25.65	−17.01	−10.36	2.65	−16.05	−4.67
Beech	P	SR20	−2.90	0.76	−4.54	−1.26	−3.03	1.46	−6.17	0.10
		SR21	−2.40	0.76	−4.04	−0.76	−3.00	1.46	−6.14	0.14
		SR22	−1.33	0.76	−2.97	0.30	−0.97	1.46	−4.10	2.17
		UC	−6.00	0.76	−7.64	−4.36	−6.67	1.46	−9.80	−3.53
		UR20	−6.57	0.76	−8.20	−4.93	−7.07	1.46	−10.20	−3.93
		UR21	−6.47	0.76	−8.10	−4.83	−7.30	1.46	−10.44	−4.16
		UR22	−6.43	0.76	−8.07	−4.80	−6.70	1.46	−9.84	−3.56
		Leaf	−5.40	0.47	−6.40	−4.40	−1.96	0.90	−3.88	−0.04
		Stem	−3.46	0.47	−4.47	−2.46	−3.31	0.90	−5.23	−1.39

5. Discussion

The results of this study provide insights into how substrate treatments affect the growth (biomass and macronutrient allocation) within and between beech and oak seedlings after one year of growth in a new forest. The results showed significant variation in the nutrient content across treatments, especially for N, P, and K, which are the most crucial nutrients required for the continuous survival of tree seedlings beyond nursery success. In essence, the novel substrate and fertilizer did not exhibit higher biomass and allocation of more nutrients in the studied organs compared to those raised on traditional peat substrate.

The analysis of soil properties revealed favorable conditions for nutrient availability. Although there were slight numerical variations, these differences were not statistically significant (p-value > 0.05). The C content is observed to be higher at the 10 cm depth for both sites, with a subsequent decrease at 20 cm. This is primarily attributed to the greater proportion of organic matter present in the humus-accumulative A horizon. However, as the pedological profile was not opened and the thickness of the A horizon was not determined, the C/N ratio remains stable across depths, indicating balanced C/N cycling within the soil, which is essential for maintaining soil health and organic matter stability [35,36]. This suggests that the variations in soil properties at different depths and sample areas may not significantly impact the growth and survival of the oak and beech seedlings. The overall similarity in soil properties across both species and depths implies that soil factors were not the primary cause of the observed differences in seedling performance. The interaction between environmental factors and nutrient availability plays a pivotal role in shaping seedling performance in the field [37,38]. Soil properties were relatively uniform across the study site, with higher nutrient concentrations in the upper 0–10 cm layer, highlighting the importance of nutrient-rich surface soils, particularly during early root development stages.

The distribution of plant biomass is a crucial factor in nutrient allocation within plant organs [39–44]. Previous studies have highlighted the significance of N and P partitioning between plant organs as a pivotal factor in regulating growth rates [45–53]. A previous study on the seedlings examined in this investigation, conducted in a nursery setting, indicated that the physical and chemical properties of the novel substrates were not significantly different from those of the peat substrate [15]. However, contrasting trends in nutrient allocation, particularly between N and P, were influenced from negative to positive by fertilization [28]. Consequently, the pronounced variation in seedling response observed after one year in the forest can be attributed to the fertilization methods employed during nursery cultivation.

Both species exhibited comparable responses to varying nutrient availability, yet the magnitude of these responses differed significantly. A shift in biomass allocation from shoots to roots is a well-documented response to nutrient limitation [2,54]. This may enhance the plant's ability to access soil resources [54] and improve water supply to aboveground parts [8,55]. An increased allocation of biomass belowground and a decreased specific leaf area have been linked to a greater ability to withstand stress, dearth, or cold winter [56,57]. These adaptations can reduce water loss and enhance the seedling's capacity to access soil moisture [58]. Furthermore, the observed differences in biomass and nutrient allocation between *F. sylvatica* and *Q. robur* suggest species-specific strategies in responding to environmental stressors [59,60]. The pronounced allocation of nutrients to belowground organs in oak seedlings may enhance resilience to nutrient limitations and water scarcity, whereas beech seedlings' preference for aboveground growth reflects an adaptive response to favorable soil conditions.

Both fertilization methods demonstrated a positive impact on both species. This is consistent with the findings of Trubat et al. [8] who reported that fertilization significantly influences nutrient status, aboveground and belowground biomass accumulation, and biomass allocation patterns. However, seedlings raised with the solid fertilizer treatment exhibited higher nutrient concentrations than those raised with liquid fertilizer, which contradicts the initial hypothesis. The substantial variation observed in response to fertilization effects may be attributed to deficiencies of N and P in the novel fertilizer, which resulted in decreases in leaf area, likely lowering transpiration rates and reducing water demands. Trubat et al. [8] additionally observed that nutrient deprivation can enhance the field performance of woody seedlings. They found that the root–shoot ratio was higher in N- and P-deficient seedlings than in those receiving complete nutrient solutions or slow-release fertilizers. Additionally, solid fertilizers may have provided a more even distribution of nutrients within the substrate, while the timing of application with liquid fertilizers may have been less optimal in relation to the seedlings' growth beyond nursery phases.

Nutrient allocation exhibited considerable variation not only between the two-tree species but also within different organs of each species. This finding is consistent with the findings of various studies on European beech and pedunculate oak [28,42,61–63]. In contrast to beech, oak exhibited significantly higher nutrient allocation in roots, shoots, and leaves. This study reaffirmed that nutrient allocation is generally higher in the belowground organs than in the aboveground ones for both species. Notably, the response efficiencies of the studied species to nutrient treatments varied significantly. The R22 treatment of the novel substrate and UAK fertilizer formulation led to greater nutrient accumulation in the roots. This finding is consistent with previous research on the nutrient content of aboveground and belowground biomass [28,39–43,64].

This study revealed a significant interaction between substrate types, fertilization methods, and nutrient allocation in plant organs. It is of paramount importance to fertilize seedlings in order to ensure their vitality and subsequent success following transplantation. Plant roots serve as the primary storage organs for plant nutrients, particularly during periods of dormancy or reduced metabolic activity, as was the case for the studied seedlings. After the growing season in the nursery, emphasis was placed on the transport of nutrients to the roots for storage. This mobility and reallocation of nutrients is likely to have resulted in a greater allocation to underground growth, as observed in this study. Furthermore, the allocation of nutrients to stable plant organs occurred more rapidly in oak than in beech, which is why more nutrients were directed to the underground root growth of oak compared to beech. The significant enrichment of major elements due to higher N fertilizer indicates that plants efficiently accessed and transported substantial amounts of the applied fertilizer to all the organs, especially the root. These results validate the effectiveness of the fertilization treatment during production at the nursery and explain the enhanced growth and productivity driven by these macronutrients, which are crucial for plant growth and development [50,51,65,66]

Numerous studies across various ecological zones have demonstrated that the application of fertilizers over an extended period, spanning several weeks or multiple years, has a beneficial impact on a diverse range of tree species: *Larix kaempferi* [66,67], *Moringa oleifera* [68], *Khaya senegalensis* [57], *Eucalyptus torelliana* [69], *Fagus sylvatica* [28,60,70–72], and *Quercus robus* [28,71,73]. Furthermore, the effect of fertilization on container-grown seedlings within the context of this recent modern forestry practice has also been exploited, [72,74–77]. However, the continuous survival of these seedlings in the forest has remained underexplored [28]. This study highlights the significant enhancement of total plant biomass through fertilization, underscoring the practical importance of this fertilization regime in forestry practice. It not only produces superior container-grown seedlings but also promotes their growth and survival in the forest, exposed to environmental factors.

F. sylvatica allocates a greater proportion of its resources to aboveground growth when the conditions of its root system are favorable in response to changes in the supply of nutrients. In contrast, *Q. robur* exhibits a more balanced growth strategy, allocating more resources below ground, resulting in a consistent but less steep increase in aboveground biomass. These disparate responses to fertilization methods may be attributed to interspecific differences in nutrient transport and partitioning [78], morphological and anatomical structures [79], and nutrient resorption efficiency [80,81]. Despite the consistent nutrient supply and the absence of variation in forest soil properties, observations from *Quercus ilex* indicate that seedling demands may be supported by nutrient reserves until the end of the first spring after germination [82]. The differing strengths and patterns of correlations observed in biomass and nutrient concentration between beech and oak are likely reflective of their specific adaptations to the new forest environments.

The results indicate that N and P allocation were significantly influenced by fertilizer treatments, consistent with their key roles in plant metabolism. The strong treatment effects observed for N and P suggest that these nutrients are the primary drivers of seedling response under peat substrate. N and P are well known to be critical for plant growth, particularly in nutrient-limited environments [83–85]. Their availability affects biomass accumulation, root growth, and overall survival in seedlings. In this study, the N and P concentrations declined significantly across different treatments, with reductions observed in liquid fertilizer treatments. Unlike previous studies reporting higher N levels as beneficial for seedling growth and survival [86], this study showed a reduction in N allocation across all the treatments, particularly in the seedlings exposed to liquid fertilizer treatments. This pattern suggests a possible limitation in N uptake, potentially affecting long-term seedling establishment. Similarly, while P is widely recognized as a limiting nutrient in forest ecosystems [87,88], our study revealed consistent P reductions under experimental treatments, with liquid fertilizer again exhibiting declines.

The temperate forest environment presents significant challenges for seedling survival due to summer drought, winter frost, and sometimes soil infertility, which can impede successful establishment. Nutrient loading for seedlings has proven to be an effective strategy to alleviate post-planting stresses. Recent studies into the relationship between seedling nutrient levels and out-planting performance has introduced the concept of “nutrient loading” with N. This concept involves the “supercharging” of seedlings with N to improve their survival and growth on forest sites. Nutrient loading involves fertilizing seedlings until their N content reaches levels that meet or exceed their needs. This process has been successful with black spruce (*Picea mariana*) on sites with heavy plant competition, as reported by Timmer [89], Thomas et al. [90], Villar-Salvador et al. [91], and Lin et al. [92]. The adoption of this concept can aid the performance of the studied species if replicated.

6. Conclusions

This study highlights the significant impact of substrate types and fertilization methods on the biomass and nutrient allocation of *Fagus sylvatica* L. and *Quercus robur* L. seedlings after one year of growth in a newly established forest. While traditional peat substrates combined with solid fertilizers yielded the highest overall biomass and nutrient uptake, the novel R22 substrate emerged as a promising alternative, performing comparably in several key metrics. This finding is particularly relevant in the context of sustainable forestry practices, where reducing peat use is increasingly prioritized. The observed species-specific responses emphasize the importance of tailoring forestry practices to the biological characteristics of the target species. The beech seedlings allocated more resources to aboveground growth, reflecting a strategy that could be advantageous in environments with favorable soil conditions. In contrast, the oak seedlings exhibited a more balanced growth strategy, with significant nutrient allocation to belowground organs, which may enhance their resilience in less fertile soils.

Despite the promising performance of the R22 substrate, the study revealed that seedlings treated with solid fertilizers consistently outperformed those treated with novel liquid fertilizers. This suggests that the current formulation of the liquid fertilizer may require optimization, particularly through the addition of essential nutrients like N, to enhance its effectiveness in supporting seedling growth and survival. The findings from this study contribute valuable insights into the development of sustainable forestry practices, particularly in the context of peatland conservation. The R22 substrate, with further refinement, could serve as a viable replacement for peat in the cultivation of forest seedlings. Future research should focus on optimizing liquid fertilizer formulations and exploring the long-term impacts of these treatments on seedling survival and forest establishment.

Although this study primarily focuses on the first year of seedling growth, the promising results observed in biomass accumulation, nutrient allocation, and root development suggest that certain substrates and fertilizer types may have lasting effects on seedling survival and resilience. Given the critical role that early-stage growth plays in determining the long-term success of forest seedlings, these findings provide valuable insights into the potential for sustained growth and establishment over time. For instance, solid fertilizers, which exhibited better performance in terms of biomass and nutrient accumulation, may contribute to stronger root systems and greater overall seedling vigor. This could potentially translate to higher survival rates and improved resilience to environmental stressors, such as drought or nutrient deficiency.

Similarly, while R22 demonstrated comparable performance to peat after one year in the new forest, it is essential to consider its long-term implications. The potential for R22 to sustain seedling growth over multiple years may depend on factors such as its degradation rate and nutrient release. Over time, the accumulation of organic matter and microbial activity in the R22 substrate could further enhance its suitability as a peat alternative; however, it is possible that certain physical or chemical properties may change, affecting the long-term growth of seedlings. Therefore, continuous monitoring of R22's performance across multiple growth stages will be critical to understanding its long-term viability in forest nursery settings. Additionally, the interplay between substrate and fertilizer types in the long term could have important implications for the health and resilience of seedlings. As seedlings mature, their nutrient requirements and stress tolerance may evolve. This highlights the need for future studies that explore the effects of these variables over extended periods, including the impact of environmental conditions and changes in soil quality over time.

Author Contributions: Conceptualization, O.J.R. and S.M.; Methodology, M.J. and K.S.-S.; Validation, K.S.-S.; Formal analysis, O.J.R. and M.J.; Investigation, M.J.; Data curation, O.J.R. and K.S.-S.; Writing—original draft, O.J.R.; Writing—review & editing, S.M., M.J. and K.S.-S.; Supervision, S.M.; Project administration, S.M.; Funding acquisition, S.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. However, The peat-free substrates and liquid fertilizer used in this study were developed under the project POIR.04.01.04-00-0016/20, which was funded by the National Centre for Research and Development (NCBiR) from national resources and the European Regional Development Fund.

Data Availability Statement: Data is available upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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forests

Article

Innovative Peat-Free Organic Substrates and Fertilizers Influence Growth Dynamics and Root Morphology of *Fagus sylvatica* L. and *Quercus robur* L. Seedlings One Year After Planting

Odunayo James Rotowa [1,2,*], Stanisław Małek [1], Dawid Kupka [1], Maciej Pach [1] and Jacek Banach [1]

[1] Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Kraków, Al. 29 Listopada 46, 31-425 Kraków, Poland; stanislaw.malek@urk.edu.pl (S.M.); dawid.kupka@urk.edu.pl (D.K.); maciej.pach@urk.edu.pl (M.P.); jacek.banach@urk.edu.pl (J.B.)

[2] Departments of Forestry and Wildlife Management, Faculty of Agriculture, Nasarawa State University, Keffi P.M.B 1022, Nigeria

* Correspondence: odun.james.rotowa@student.urk.edu.pl

Abstract: This study evaluated the effects of six innovative peat-free substrate formulations, combined with either a conventional solid fertilizer or a novel liquid fertilizer developed by the research team, on the early growth and root morphology of *Fagus sylvatica* L. and *Quercus robur* L. seedlings. Treatments were analyzed through two-way ANOVA and species-specific linear regression models. Following one year of field growth, survival rates remained high across all treatments. While R22 (a peat-free substrate with liquid fertilizer) exhibited the highest mean values for seedling height and diameter, only height showed statistically significant variation among treatments ($p < 0.05$), with no significant differences observed for diameter increment. It was further, revealed that seedlings treated with peat-free substrates and liquid fertilizers exhibited adequate survival, with several combinations especially R22 showing comparable performance to traditional peat-based media with solid fertilizer. Root morphological traits, particularly fine root length (≤0.50 mm) were strong predictors of above-ground growth in *F. sylvatica*, but less so in *Q. robur*, which relied more on total root length. The results highlight species-specific root–shoot coordination strategies, with beech exhibiting above-ground growth pattern and oak a gravitropic one. The findings concluded that R22 substrates confirmed exceptional performance with enhanced root growth comparable to peat after one year of forest planting, indicating strong potential for future development without the environmental concerns associated with peat use.

Keywords: tree seedling growth; forestry management; reforestation techniques; root system architecture; sustainable forestry

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Academic Editor: Mulualem Tigabu

Received: 15 April 2025
Revised: 2 May 2025
Accepted: 9 May 2025
Published: 10 May 2025

Citation: Rotowa, O.J.; Małek, S.; Kupka, D.; Pach, M.; Banach, J. Innovative Peat-Free Organic Substrates and Fertilizers Influence Growth Dynamics and Root Morphology of *Fagus sylvatica* L. and *Quercus robur* L. Seedlings One Year After Planting. *Forests* **2025**, *16*, 800. https://doi.org/10.3390/f16050800

1. Introduction

Reforestation and afforestation initiatives are critical to combating climate change, enhancing biodiversity and stabilizing ecosystems [1,2]. Seedling survival after planting constitutes an essential component of the success of forest restoration programs [3–6]. This survival is strongly influenced by nursery cultural practices and silvicultural techniques, which play an important role in the performance of a seedling immediately after transplanting. [3]. Root system morphology is particularly important because its architecture directly affects water and nutrient uptake, thereby influencing tree seedling's overall health and resilience [7]. The importance of root characteristics, in predicting seedling survival and adaptability to varying environments cannot be overemphasized [8].

Classifying root diameter is essential for understanding below-ground carbon dynamics [9]. Very fine roots, defined as those less than 0.5 mm in diameter, are more accurate indicators of root function than the broader traditional category of roots under 2 mm [10]. These very fine roots display species-specific traits and exhibit remarkable plasticity, adjusting their biomass and length across soil depths to optimize nutrient and water uptake [10–12]. Fine roots, ranging from 0.5 mm to 2 mm in diameter, are vital to nutrient cycling in terrestrial ecosystems [13,14]. Though they make up less than 5% of forest biomass, they are highly dynamic, functioning as both nutrient sources and sinks, and play a key role in carbon cycling and accumulation [10]. In contrast, coarse roots those greater than 2.0 mm in diameter differ significantly in morphology, nutrient content, and decomposition processes. Their size often correlates with aboveground biomass, and factors like tree size and age are commonly used to predict their development [9,15].

Known for its exceptional physical, chemical and biological properties, peat has long been a stable component in nursery substrates. Its superior water-holding capacity and consistent quality make it ideal for plant growth. However, the slow release of carbon from peat soils raises environmental concerns. Europe experienced a dramatic rise in peat excavation, with volumes soaring from 6000 tonnes in 2012 to 20 million tonnes by 2022 [16–18] This represents a staggering 333% increase over a decade, underscoring the expansion of peat extraction and its contribution to environmental degradation [19,20]. Consequently, EU Member States have been actively seeking to reduce peat consumption [21–23]. With impending restrictions on the availability of peat [24–27], the need to find alternative materials to replace peat demand an urgent attention, either partially or entirely. In response to this pressing issue, our team has developed and proposed a substrate designed to provide a sustainable solution [7,28].

Despite increasing efforts to promote peat-free substrates in forest nurseries, existing studies have notable limitations. Many studies evaluating peat-free substrates and fertilization strategies primarily assess above-ground parameters such as height, diameter, and biomass accumulation [28–30]. However, root system development, remains understudied. While solid fertilizers are widely used in forestry nurseries, recent advancements in liquid fertilizers suggest they may offer improved nutrient uptake efficiency [31–35]. These studies further examined fertilization effects in nurseries but rarely in the context of peat-free organic substrates. This study therefore, provides a direct comparison of solid and liquid fertilizers in combination with innovative organic substrates, assessing their effects on above-ground growth and below-ground root morphology one year after planting in a forest environment.

Therefore, this study aimed to evaluate the performance of innovative peat-free substrates in combination with two contrasting fertilization approaches: a conventional solid fertilizer and a novel liquid fertilizer formulation developed by our research team. The liquid fertilizer was designed specifically to complement the nutrient dynamics of organic peat-free substrates and optimize seedling uptake efficiency. The tested research hypotheses assumed that: (i) these innovative treatments would produce root and shoot traits comparable to conventional methods. (ii) *Fagus sylvatica* and *Quercus robur* would respond differently to the treatments due to their distinct growth strategies. (iii) specific root morphological traits may vary in their association with early shoot growth between the studied species.

2. Materials and Methods

2.1. Study Site

The study site was located in Barbarka, Miechow Forest District. The research area is situated at an altitude of approximately 370 m above sea level, in the Olkuska Upland, southern Poland (50°15′54.2′′ N 19°53′36.5′′ E). The experiment was situated in a forest complex managed by the National Forest Holding. The area was established in several gaps resulting from the clear-cutting of a *Populus* spp. plantation. The area of the Miechów Forest District is characterized by a diverse, upland landscape. The Olkuska Upland is a compact karst plateau made of limestone and marl. The climate is continental, characterized by significant temperature amplitude (21 °C) and a significant share of rainfall during the growing season. The average annual air temperature for the Forest District is 8.2 °C. The warmest month is July (19.6 °C), while the coldest is January (−3.0 °C).

2.2. Substrate Composition and Preparation

The peat rich in sphagnum used as the control variant (C) for growing the seedlings in this study was obtained from the nursery farm in Ne˛dza (50.167964 N, 18.3138334 E). Its composition consisted of 93% peat and 7% perlite, with the addition of dolomite (3 kg per 1 m^3 of substrate) to achieve a pH of 5.5. The elemental content (g/g of 100% dry weight of the growing medium at the beginning of the experiment) of 37.99 ± 0.69 (C), 0.74 ± 0.01 (N), 0.02 ± 0.01 (P) The peat-free substrates (R20, R21, and R22) were sourced from coniferous woody (mainly pine) they composed of a mixture of different components, including shavings, wood chips, straw, bark, perlite, core wood and mixed silage, with varying proportions as shown on Table 1. In total, four substrates (R20, R21, R22, and peat) were utilized, each subjected to two fertilization (S and U) variants. The first set received standard solid fertilization (SR20, SR21, and SR22 variants), while the second set was treated with a novel liquid fertilizer also developed by the University of Agriculture in Kraków (UR20, UR21, and UR22). The peat substrate served as the control in both fertilization scenarios, designated as SC and UC variants (Table 2).

Table 1. Composition of the organic peat free substrate.

Substrate	Saw Dust	Wood Chips	Straw	Wood Bark	Perlite	Core Wood	Mixed Silage
					(%)		
R20	73	10	-	10	4	2	1
R21	20	63	-	10	4	2	1
R22	50	-	10	33	4	2	1

Table 2. Physicochemical properties of substrates used in seedling growth in the Nursery.

Substrate	Water Capacity (%)	Water Outflow Rate (L/min)	Bulk Density (g/cm^3)	Solid Density (g/cm^3)	Air Capacity (%)	Porosity (%)
R20	40.5 ± 2.9 ^{b}	0.595 ± 0.150 ^{b}	0.115 ± 0.009 ^{a}	0.64 ± 0.08 ^{a}	52.1 ± 3.19 ^{c}	92.6 ± 0.60 ^{d}
R21	33.1 ± 2.5 ^{d}	0.781 ± 0.114 ^{a}	0.098 ± 0.014 ^{c}	1.74 ± 0.07 ^{a}	60.8 ± 3.06 ^{a}	93.6 ±0.87 ^{c}
R22	37.8 ± 5.1 ^{c}	0.594 ± 0.150 ^{b}	0.104 ± 0.020 ^{b}	1.66 ± 0.11 ^{a}	55.8 ± 5.58 ^{b}	93.9 ± 0.98 ^{b}
Control	57.7 ± 5.4 ^{a}	0.417 ± 0.145 ^{c}	0.085 ± 0.007 ^{d}	1.69 ± 0.14 ^{a}	37.0 ± 5.72 ^{d}	94.7 ± 0.42 ^{a}
F	387.45	56.32	65.81	1.0717	295.79	76.48
p	0.0000	0.0000	0.0000	0.3870	0.0000	0.0000

Letters with different alphabet indicate statistically significant differences between means ($p < 0.05$).

Prior to filling, the substrate was pre-moistened using a line mixer with spray nozzles, and moisture levels were controlled organoleptically by the line staff to ensure the substrate reached the standard moisture level for container filling. The substrate’s moisture content was 75.9 ± 2.1%. The vibration intensity of the vibrating table was kept constant during the filling process, at 12.0 G maximum acceleration, as measured by the Voltcraft DL-131G device with ±0.5 G accuracy. Throughout the experiment, the line’s efficiency remained stable at 400 containers per hour, which is the standard rate at this nursery. All operating parameters line configuration, containers, and substrate types were consistent with those used in a previous experiment [35].

2.3. Seed Sowing and Germination

Using mechanical methods, the containers were filled with substrates and seeds immediately planted on 19 April 2022, at the Nursery Farm in Suków Papiernia (50.79613, 20.71011), Daleszyce Forest District. The experiment utilized V300 Styrofoam containers, which are commonly used in Poland for cultivating deciduous species such as beech and oak. To improve the germination process, oak seeds were scarified before sowing. After sowing, the containers were placed in a greenhouse for 4 weeks before being transferred to an external production field. During the seedling growth period in the nursery, manual weeding was carried out. The seedlings were cultivated for 5 months, following the standard procedure used in container nurseries [36]. Due to a total rainfall of only 78 mm during this period, irrigation was necessary to address the water deficit, and an automatic RATHMAKERS Gartenbautechnik sprinkler ramp was used for this purpose.

Osmocote fertilizer was incorporated into the substrate before sowing, with a total application rate of 3 kg per cubic meter of substrate. This was a mixture of 2 kg of Osmocote 3-4M and 1 kg of Osmocote 5-6M. The Osmocote 3-4M fertilizer had the following composition: 16% nitrogen (N), with 7.1% as N-NO_3- and 8.9% as N-NH_4+; 9% P_2O_5; 12% K_2O; 2.0% MgO; and included micronutrients (B, Fe, Cu, Mn, Zn, Mo). The Osmocote 5-6M fertilizer contained 15% nitrogen, with 6.6% as N-NO_{3-} and 8.4% as N-NH_4+; 9.0% P_2O_5; 12% K_2O; 2.0% MgO; and similar micronutrients. A new liquid fertilizer regimen was also employed, consisting of two different formulations. The first fertilizer contained 4.78% N, 1% P_2O_5, 2.64% K_2O, 2.65% CaO, 1.4% MgO, 0.71% SO_3, and 0.14% Na_2O. It was initially applied with a total volume of 3.14 dm^3 (0.048 dm^3 per square meter). The second fertilizer composition included 0.798% N, 0.166% P_2O_5, 0.440% K_2O, 0.441% CaO, 0.234% MgO, 0.118% SO_3, and 0.023% Na_2O. This was applied with a total volume of 15.09 dm^3 (0.229 dm^3 per square meter). During the period of seedling production, the first fertilizer variant was applied eight times at 10-day intervals, while the second variant was applied 15 times at 5-day intervals. This fertilization schedule was uniformly applied to both beech and oak seedlings throughout the nursery phase.

2.4. Plantation Establishment and Seedling Collection

After nursery production, the seedling was transported and planted into the forest on 5 September 2022. The field experiment was laid in a randomized complete block design with 8 treatments replicated 3 times. A total of 24 subplots were established for each species. In each subplot, 49 seedlings were planted with 1 × 1.7 m inter and intra-spacing making a total of 147 seedlings per treatment and species. For both species, therefore, were a total of 2352 seedlings were established. The plantation was established at the onset of the autumn season 2022. At the end of the growing season in 2023, 144 seedlings were selected (3 from each subplot) the seedlings were selected according to the average height of each subplot. The seedlings were carefully uprooted to obtain an intact root segment. The mean height of each subplot characterized the selected seedlings. They were carefully chosen from each of the eight treatment groups for onward laboratory analysis. This resulted in a total assessment of 144 seedlings for both species in the laboratory experiment. To reduce the impact of animals on the new forest, the area was fenced after the plantation was established. Above-ground data was collected on plant height, collar diameter, number of seedlings in perfect condition (SPC) total survived seedlings (TSS). The root morphological characteristics examined in this study include total root length (TRL), root surface area (RSA), average root diameter (ARD), and root volume (RV) to assess how different treatments impacted below-ground development. Root morphological diameters were further classified into very fine (≤0.5 mm), fine (0.5–2.0 mm) and coarse root (>2.0 mm).

2.5. Root Sample Preparation and Analyses

In the laboratory, all roots within each block were processed as follows: Root systems were carefully separated from soil and organic matter to keep the root segments intact and maintain attachment to the larger roots (>2 mm in diameter). The intact root segments were then gently rinsed with tap water followed by deionized water to remove residual soil while preserving delicate root tips. Morphological traits of roots in each diameter class were analyzed using WinRhizo™ Pro 2003b image analysis system (Regent Instruments Inc., Ville de Quebec, QC, Canada), an image analysis system specifically designed for root measurements. This analysis was conducted in the Laboratory of Biotechnology, Department of Ecology and Silviculture, University of Agriculture in Kraków.

2.6. Soil Sample Collection and Analysis

A 0.7-hectare research plot was established on a harvested *Populus* spp. site characterized by uniform parent material and soil type. Soil samples were collected from five different points within each subplot at two depth intervals: 0–10 cm and 10–20 cm, representing the top mineral horizons. Samples were placed in polyvinyl chloride (PVC) bags for transport and analysis. In total, 480 soil samples were collected. Each sample was air-dried, passed through a 2 mm sieve, and ground prior to physicochemical analysis. Soil pH was measured using the potentiometric method in both water and 1M KCl. Hydrolytic acidity was determined using the Kappen method, while exchangeable acidity and base content were assessed using the Sokołow method [37]. Total nitrogen and carbon contents were analyzed with a LECO CNS TruMac Analyzer (LECO Corporation, St. Joseph, MI, USA). The concentrations of alkaline cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) were determined using 1M ammonium acetate extraction and quantified via inductively coupled plasma optical emission spectrometry (ICP-OES) with an iCAP 6500 DUO instrument (Thermo Fisher Scientific, Cambridge, UK). All analyses were conducted at the Laboratory of Forest Environment Geochemistry and Land Intended for Reclamation, Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Kraków, Poland.

2.7. Statistical Analyses

To analyze the effect of seedling growth on plantation survival, an overall effect of growth was assessed by fitting all sample units (2352 seedlings) in the population. Using destructive sampling method, nine root samples were selected per treatment, resulting in 72 data points analyzed per species for morphological evaluation. From WinRhizo™ Pro 2003b image analysis system (Regent Instruments Inc., Ville de Quebec, QC, Canada) the data were further separated into very fine, fine and coarse roots (≤0.5 mm, 0.5–2.0 mm, >2.0 mm respectively). To meet normality and homogeneity assumptions for further analyses, the Shapiro-Wilk test for compliance of variable distributions with normal distribution was used. The analyzed variables showed compliance with a normal distribution, and therefore, parametric tests were used. To evaluate treatment effects, on root morphological indices and diameter classification of very fine, fine, and coarse roots, two-way Analysis of Variance (ANOVA) was conducted separately for each species, using substrate type (peat-based vs. peat-free) and fertilizer type (solid vs. liquid) as fixed factors, and block as a random effect in a randomized complete block design. Duncan multiple range test (DMRT) was applied post hoc for pairwise comparisons and significance was set at $p < 0.05$. Multiple linear regression models were developed to explore the predictive relationships between root morphological traits and above-ground performance indicators. All statistical analyses were performed at a significance level of 95% confidence interval.

3. Results

3.1. Soil Properties

At both sites of beech and oak, the soils were slightly acidic, which is typical of forest environments. The pH values at 20 cm are slightly lower compared to those measured at 10 cm. Oak retains a uniform N concentration between both depths. Altogether, C contents were higher both at 10 cm in both sites and decreased at 20 cm as well P levels. Consistent trends in the C/N ratio across depths indicate that C and N cycling within the soil is balanced. Both sites also have more exchangeable cations at 10 cm than at 20 cm indicating that the upper soil layer plays a crucial role in seedling nutrient uptake, particularly in early growth stages. No significant differences were observed between the two sites based on statistical analysis of soil properties, although there were some small numerical differences among depths (Table 3). This implies a similar soil composition across the study area that can serve as a controlled background for comparison of treatment effects on seedling growth.

3.2. Growth Dynamics of F. sylvatica and Q. robur Seedlings One-Year After Forest Plantation

The result of above-ground growth parameters confirm that seedling establishment was successful across all treatments, with survival rates consistently exceeding 70%, the minimum benchmark for acceptable forest regeneration in Poland. The analysis of biometric increments following field transplanting revealed differences in species responses and treatment effectiveness. Notably, seedlings treated with liquid fertilizers exhibited reduced growth in both species, despite showing acceptable survival rates under both fertilization methods. After one year in the forest, treatment had a significant effect on height ($p < 0.05$), with R22 achieved height increment comparable to the peat-based control across both species. SC and SR22 (solid), UC and UR22 (liquid) were statistically grouped as the top performers for both height and diameter in both species (Table 4).

3.3. Root Morphological Response of F. sylvatica and Q. robur Seedlings One Year After Forest Plantation Establishment

The result of root morphological parameters shows that the effect of solid fertilization was more pronounced on root morphological indices than the liquid once after one year of growth in the forest. The result of total root length revealed the R20 substrates recorded the higher mean length in beech regardless of fertilization method. The response of oak was different, showing different response to different fertilizer. Peat substrate recorded the highest mean under liquid fertilization while with significant variation while SR21 performed best with no Signiant variation (Table 5). For *F. sylvatica*, RV was the only parameter to show statistically significant differences across treatments under solid fertilization. Although TRL, RSA, and ARD did not yield statistically significant differences ($p > 0.05$), numerical trends favored the SC treatment. Among the liquid fertilizer treatments, the UC and UR20 demonstrated marginally improved root architecture relative to other liquid combinations, although these differences were less pronounced. The treatment effects were more distinct among solid fertilizer treatments of *Q. robur*. The SC and SR21 treatments consistently produced superior results across these morphological parameters. TRL under solid fertilization did not differ significantly ($p = 0.336$), while the liquid fertilization result shows that TRL varied significantly ($p = 0.000$), with the control variant (UC) and UR20 recording the highest values (Table 5).

Table 3. Mean and SD of soil properties of sampled plot of oak and beech at Barbarka experimental site.

Soil Uptake Level (cm)	pH (H_20)	N (%)	C (%)	P_2O_5 (mg/100g)	C/N	Exchangeable Cations ((cmol[+]/kg)			
						Ca	K	Mg	Na
				Fagus sylvatica site					
0–10	5.21 ± 0.55	0.36 ± 0.10	2.41 ± 0.21	3.14 ± 0.21	13.78 ± 0.32	5.51 ± 0.52	0.18 ± 0.01	0.57 ± 0.06	0.70 ± 0.08
10–20	5.10 ± 0.57	0.34 ± 0.09	1.94 ± 0.15	2.87 ± 0.14	12.90 ± 0.32	4.81 ± 0.39	0.16 ± 0.01	0.42 ± 0.03	0.63 ± 0.06
Total	5.15 ± 0.56	0.35 ± 0.10	2.16 ± 0.12	2.99 ± 0.12	13.30 ± 0.23	4.22 ± 0.32	0.15 ± 0.01	0.49 ± 0.03	0.55 ± 0.07
p-value	0.187 ns	0.170 ns	0.061 ns	0.286 ns	0.065 ns	0.064 ns	0.062 ns	0.062 ns	0.062 ns
				Quercus robur site					
0–10	5.14 ± 0.59	0.45 ± 0.95	2.14 ± 0.22	4.70 ± 0.45	13.31 ± 0.33	5.11 ± 0.53	0.23 ± 0.02	0.55 ± 0.06	0.81 ± 0.26
10–20	5.03 ± 0.61	0.44 ± 0.09	1.83 ± 0.14	4.22 ± 0.40	12.23 ± 0.31	4.65 ± 0.36	0.21 ± 0.02	0.46 ± 0.03	0.82 ± 0.27
Total	5.08 ± 0.59	0.45 ± 0.10	1.55 ± 0.13	4.44 ± 0.30	12.73 ± 0.23	3.32 ± 0.32	0.22 ± 0.01	0.38 ± 0.03	0.82 ± 0.29
p-value	0.208 ns	0.945 ns	0.061 ns	0.427 ns	0.069 ns	0.061 ns	0.363 ns	0.061 ns	0.989 ns

ns = not significance.

Table 4. Biometric and increment rate of *F. sylvatica* and *Q. robur* seedlings after one year on crop.

Treatment	Fertilization Type	SPC	TSS	RSS (%)	After 1 Year in the Forest		After Nursery Production Cycle		Absolute Increment (%)	
					Height (cm)	Collar Diameter (mm)	Height (cm)	Collar Diameter (mm)	Height	Collar Diameter
					Fagus sylvatica					
SR20	Solid	112	123	84	58.12 ± 9.89 b	9.11 ± 2.23 a	31.46 ± 3.56 a	5.63 ± 1.48 a	85	62
SR21		135	142	97	59.01 ± 10.42 ab	8.93 ± 2.01 a	30.25 ± 3.43 a	5.70 ± 1.62 a	95	55
SR22		138	143	97	61.92 ± 11.61 a	9.22 ± 1.96 a	31.64 ± 3.29 a	5.58 ± 1.15 a	96	58
SC		133	143	97	62.25 ± 12.02 a	9.61 ± 2.48 a	30.88 ± 3.23 a	5.72 ± 1.23 a	102	68
Total					60.32 ± 11.18	8.94 ± 2.44	31.06 ± 3.23	5.66 ± 1.26		
p-value.					0.033 *	0.203 ns	0.473 ns	0.341 ns		
UR20	Liquid	138	143	97	44.21 ± 7.77 f	7.33 ± 1.98 e	30.55 ± 3.23 e	5.22 ± 0.95 e	45	40
UR21		130	136	93	43.32 ± 7.18 f	7.41 ± 2.04 e	30.24 ± 2.98 e	5.36 ± 1.24 e	44	38
UR22		113	135	92	50.07 ± 8.49 e	7.68 ± 1.86 e	31.18 ± 3.19 e	5.21 ± 1.07 e	61	47
UC		118	135	92	54.04 ± 8.52 e	7.96 ± 1.49 e	31.07 ± 3.08 e	5.28 ± 0.99 e	56	51
Total					49.41 ± 3.19	7.60 ± 1.82	30.76 ± 3.17	5.27 ± 1.07		
p-value.					0.024 *	0.176 ns	0.253 ns	0.316 ns		
					Quercus robur					
SR20	Solid	129	134	91	56.76 ± 9.28 a	8.72 ± 1.89 a	31.15 ± 2.97 a	5.53 ± 1.56 a	82	58
SR21		123	130	88	55.74 ± 7.95 a	8.61 ± 1.78 a	31.73 ± 3.47 a	5.25 ± 1.43 a	76	64
SR22		132	140	95	56.79 ± 7.74 a	8.62 ± 2.03 a	30.95 ± 2.99 a	5.42 ± 1.39 a	77	59
SC		145	145	99	57.07 ± 6.89 a	8.93 ± 2.11 a	31.09 ± 2.99 a	5.66 ± 1.55 a	77	59
Total					55.58 ± 8.89	8.72 ± 1.85	31.23 ± 1.34	5.47 ± 1.67		
p-value.					0.103 ns	0.473 ns	0.564 ns	0.271 ns		

Table 4. *Cont.*

Treatment	Fertilization Type	After 1 Year in the Forest					After Nursery Production Cycle		Absolute Increment (%)	
		SPC	TSS	RSS (%)	Height (cm)	Collar Diameter (mm)	Height (cm)	Collar Diameter (mm)	Height	Collar Diameter
UR20	Liquid	139	142	97	41.72 ± 8.44 ^{f}	7.22 ± 1.10 ^{e}	31.51 ± 2.74 ^{e}	5.45 ± 0.89 ^{e}	32	32
UR21		134	140	95	41.03 ± 8.26 ^{f}	7.79 ± 1.22 ^{e}	30.43 ± 2.55 ^{e}	5.52 ± 0.92 ^{e}	35	41
UR22		139	144	98	42.69 ± 9.67 ^{f}	7.81 ± 1.54 ^{e}	31.49 ± 2.86 ^{e}	5.47 ± 0.95 ^{e}	36	43
UC		132	138	94	45.19 ± 9.93 ^{e}	7.94 ± 1.44 ^{e}	31.51 ± 3.02 ^{e}	5.66 ± 0.96 ^{e}	43	40
Total					42.66 ± 9.08	7.69 ± 1.32	31.23 ± 2.94	5.52 ± 0.94		
p-value.					0.007 **	0.978 ^{ns}	0.305 ^{ns}	0.231 ^{ns}		

SPC—Number Seedlings in perfect condition, TSS–Total survived seedlings, RSS—rate of seedling survival. S—State Forests solid fertilization, U—University novel liquid fertilization, R—novel substrates, C—control substrate (peat−perlite) (N = 147). Letters with different alphabet indicate statistically significant differences between means ($p < 0.05$). Alphabets 'a' and 'b' denote homogeneous groups under solid fertilization and 'e' and 'f' denote homogeneous groups under liquid fertilization. ns = not significance, * = $p < 0.05$, ** = $p < 0.01$.

Table 5. Morphological parameters of root system of *F. sylvatica* and *Q. robur* seedlings under different substrate fertilizer treatment after one year in the forest.

Treatment	Fertilization Type	TRL (cm)	RSA (cm²)	ARD (mm)	RV (cm³)
			F. sylvatica		
SR20	Solid	1673.27 ± 252.94 [a]	171.66 ± 41.43 [a]	0.85 ± 0.15 [a]	3.59 ± 0.77 [b]
SR21		1265.52 ± 151.19 [a]	168.37 ± 39.55 [a]	0.82 ± 0.17 [a]	3.51 ± 1.32 [b]
SR22		1424.69 ± 265.10 [a]	182.79 ± 62.43 [a]	0.72 ± 0.16 [a]	3.37 ± 1.58 [b]
SC		1377.56 ± 104.39 [a]	226.28 ± 48.05 [a]	0.93 ± 0.19 [a]	4.10 ± 1.59 [a]
Total		1335.26 ± 207.75	187.28 ± 52.15 [a]	0.83 ± 0.18 [a]	3.89 ± 1.48
p-value.		0.285 [ns]	0.061 [ns]	0.087 [ns]	0.035 *
UR20	liquid	1132.69 ± 130.54 [e]	113.87 ± 19.61 [e]	0.83 ± 0.10 [e]	2.39 ± 0.89 [e]
UR21		1037.03 ± 92.90 [e]	88.98 ± 14.17 [f]	0.72 ± 0.08 [f]	1.80 ± 0.26 [f]
UR22		1029.58 ± 88.39 [e]	89.30 ± 6.50 [f]	0.72 ± 0.09 [f]	1.61 ± 0.56 [f]
UC		1056.09 ± 98.93 [e]	122.22 ± 10.22 [e]	0.74 ± 0.06 [f]	1.53 ± 0.42 [f]
Total		1063.85 ± 107.76	99.09 ± 16.65	0.75 ± 0.09	1.83 ± 0.65
p-value.		0.157 [ns]	0.001 **	0.024 *	0.015 **
			Q. robur		
SR20	Solid	1396.90 ± 81.03 [a]	218.23 ± 38.22 [a]	0.97 ± 0.12 [a]	4.66 ± 0.56 [b]
SR21		1445.48 ± 83.89 [a]	219.83 ± 30.32 [a]	1.03 ± 0.13 [a]	6.01 ± 1.07 [a]
SR22		1443.91 ± 95.26 [a]	189.41 ± 18.21 [b]	0.79 ± 0.14 [b]	3.68 ± 0.48 [c]
SC		1386.20 ± 82.95 [a]	187.28 ± 20.75 [b]	1.03 ± 0.14 [a]	4.24 ± 0.76 [bc]
Total		1418.13 ± 86.59	203.69 ± 31.00	0.95 ± 0.16	4.65 ± 1.13
p-value.		0.336 [ns]	0.024 *	0.001 **	0.000 **
UR20	liquid	1329.14 ± 59.57 [f]	144.58 ± 19.36 [f]	0.99 ± 0.32 [ef]	3.41 ± 0.48 [e]
UR21		1345.08 ± 44.24 [f]	125.69 ± 25.66 [g]	0.81 ± 0.06 [f]	2.44 ± 0.49 [e]
UR22		1248.43 ± 68.06 [g]	109.37 ± 15.36 [g]	1.07 ± 0.19 [e]	2.99 ± 0.11 [e]
UC		1453.88 ± 65.88 [e]	164.48 ± 10.42 [e]	0.87 ± 0.18 [ef]	3.36 ± 1.19 [e]
Total		1344.13 ± 93.91	136.03 ± 27.42	0.94 ± 0.23	3.05 ± 1.43
p-value.		0.000 **	0.000 **	0.051 *	0.471 [ns]

TRL—Total root length, RSA—Root surface area, ARD—Average root diameter, RV—Root volume. Letters with different alphabet indicate statistically significant differences between means ($p < 0.05$). Alphabets 'a, b' and 'c' denote homogeneous groups under solid fertilization and 'e, f' and 'g' denote homogeneous groups under liquid fertilization. ns = not significance, * = $p < 0.05$, ** = $p < 0.01$.

3.4. Root Diameter Classification of F. sylvatica and Q. robur Seedlings Under Different Treatments After One Year in the Forest

Root morphological responses across diameter classes showed distinct patterns between *F. sylvatica* and *Q. robur*, and were strongly influenced by fertilizer type and substrate combination (Table 6). In both species, seedlings treated with solid fertilizers, particularly under SC and SR22 treatments, exhibited significantly higher values across most diameter classes, especially in total length and surface area within the very fine root fraction (≤0.50 mm). Surface area and volume in this fine class were not significantly enhanced in these solid fertilizer treatments, but significantly enhanced in these liquid fertilizer treatments. Although, the differences were more pronounced in *Q. robur* of the same class, however, treatments R20 and R21 also demonstrated higher performance in few parameters, after peat-base substrate. Similar to solid once, treatment, UR22 again recorded higher output after UC in most of the accessed root parameters. Across both species, coarse volume (in the >2.00 mm) diameter class was less variable and showed fewer significant differences among treatments (Table 6).

Table 6. Root diameter classification of beech and oak seedlings under different treatments.

Treatment	Fertilization Type	Length < 0.5 mm	Length 0.5–2.0 mm	Length > 2.0 mm	Surface Area < 0.5 mm	Surface Area 0.5–2.0 mm	Surface Area > 2.0 mm	Volume < 0.5 mm	Volume 0.5–2.0 mm	Volume > 2.0 mm
					Fagus sylvatica					
SR20		465.22 ± 94.62 ^{b}	144.40 ± 30.07 ^{b}	36.04 ± 6.30 ^{ab}	23.72 ± 6.32 ^{a}	42.28 ± 9.08 ^{b}	58.19 ± 11.61 ^{b}	0.17 ± 0.02 ^{a}	1.13 ± 0.27 ^{ab}	10.59 ± 2.31 ^{b}
SR21		463.26 ± 99.38 ^{b}	118.89 ± 41.23 ^{b}	28.82 ± 8.58 ^{b}	21.27 ± 8.28 ^{a}	33.90 ± 11.14 ^{b}	57.00 ± 16.68 ^{b}	0.14 ± 0.06 ^{a}	0.90 ± 0.35 ^{b}	10.05 ± 3.55 ^{b}
SR22	Solid	472.41 ± 109.16 ^{ab}	133.92 ± 57.69 ^{b}	30.40 ± 7.53 ^{b}	24.48 ± 7.03 ^{a}	39.49 ± 19.08 ^{b}	59.43 ± 9.28 ^{b}	0.15 ± 0.05 ^{a}	0.97 ± 0.38 ^{b}	10.89 ± 1.73 ^{b}
SC		493.66 ± 109.71 ^{a}	194.83 ± 67.13 ^{a}	40.70 ± 9.64 ^{a}	26.32 ± 5.46 ^{a}	58.74 ± 20.40 ^{a}	72.39 ± 15.11 ^{a}	0.18 ± 0.04 ^{a}	1.52 ± 0.39 ^{a}	14.40 ± 4.54 ^{a}
Total		471.14 ± 111.71	148.01 ± 56.76	33.99 ± 9.11	23.19 ± 6.87	43.60 ± 17.71	60.75 ± 14.61	0.16 ± 0.04	1.13 ± 0.35	11.38 ± 3.03
p-value		0.048 *	0.021 *	0.015 *	0.376 ^{ns}	0.013 *	0.043 *	0.1236 ^{ns}	0.045 *	0.032 *
UR20		325.34 ± 118.82 ^{e}	88.38 ± 25.80 ^{ef}	22.79 ± 7.43 ^{e}	21.22 ± 6.78 ^{ef}	23.21 ± 7.00 ^{ef}	42.16 ± 8.81 ^{e}	0.14 ± 0.04 ^{e}	0.56 ± 0.20 ^{f}	6.97 ± 1.40 ^{f}
UR21		302.71 ± 94.01 ^{e}	81.96 ± 30.18 ^{f}	23.84 ± 7.70 ^{e}	20.07 ± 5.66 ^{f}	21.24 ± 8.24 ^{ef}	40.79 ± 11.58 ^{e}	0.13 ± 0.04 ^{e}	0.50 ± 0.21 ^{f}	6.31 ± 2.02 ^{f}
UR22	Liquid	360.41 ± 103.54 ^{e}	112.89 ± 45.33 ^{ef}	22.83 ± 3.78 ^{e}	24.60 ± 7.39 ^{ef}	29.68 ± 12.54 ^{f}	39.07 ± 4.52 ^{e}	0.17 ± 0.06 ^{e}	0.71 ± 0.33 ^{e}	7.13 ± 1.08 ^{f}
UC		382.04 ± 101.77 ^{e}	123.33 ± 41.95 ^{e}	24.88 ± 5.05 ^{e}	26.77 ± 5.66 ^{e}	34.31 ± 17.49 ^{e}	47.47 ± 8.63b ^{e}	0.18 ± 0.05 ^{e}	0.72 ± 0.35 ^{e}	8.32 ± 1.66 ^{e}
Total		342.62 ± 105.03	101.63 ± 39.12	23.58 ± 6.00	23.16 ± 6.70	27.11 ± 12.66	42.36 ± 8.96	0.16 ± 0.04	0.62 ± 0.27	6.89 ± 1.54
p-value		0.394 ^{ns}	0.070 ^{ns}	0.876 ^{ns}	0.124 ^{ns}	0.103 *	0.222 ^{ns}	0.285 ^{ns}	0.022 *	0.015 *
					Quercus robur					
SR20		597.88 ± 151.78 ^{b}	130.18 ± 19.66 ^{a}	37.64 ± 8.96 ^{ab}	33.85 ± 2.83 ^{b}	37.49 ± 4.85 ^{a}	47.87 ± 5.43 ^{a}	0.24 ± 0.07 ^{a}	0.99 ± 0.15 ^{a}	6.38 ± 0.48 ^{a}
SR21		664.72 ± 156.56 ^{a}	145.75 ± 27.53 ^{a}	34.94 ± 12.46 ^{bc}	39.58 ± 3.23 ^{a}	40.86 ± 3.84 ^{a}	56.42 ± 16.35 ^{a}	0.26 ± 0.06 ^{a}	1.09 ± 0.21 ^{a}	8.25 ± 1.81 ^{a}
SR22	Solid	670.63 ± 193.31 ^{a}	134.17 ± 48.93 ^{a}	46.29 ± 5.92 ^{a}	29.60 ± 6.76 ^{b}	38.72 ± 3.33 ^{a}	59.85 ± 13.16 ^{a}	0.22 ± 0.09 ^{a}	1.04 ± 0.36 ^{a}	7.62 ± 1.46 ^{a}
SC		672.41 ± 112.37 ^{a}	152.96 ± 17.73 ^{a}	36.57 ± 9.13 ^{c}	31.33 ± 6.61 ^{b}	41.46 ± 4.77 ^{a}	48.78 ± 15.77 ^{a}	0.22 ± 0.07 ^{a}	1.03 ± 0.19 ^{a}	8.69 ± 1.41 ^{a}
Total		641.41 ± 157.42	140.76 ± 31.07	36.36 ± 11.48	33.59 ± 6.27	39.63 ± 7.56	53.23 ± 13.81	0.23 ± 0.06	1.04 ± 0.22	7.74 ± 1.29
p-value		0.012 *	0.395 ^{ns}	0.001 *	0.002 *	0.674 ^{ns}	0.184 ^{ns}	0.191 ^{ns}	0.204 ^{ns}	0.152 ^{ns}
UR20		443.93 ± 225.91 ^{e}	55.89 ± 29.77b ^{ef}	12.29 ± 2.96 ^{e}	25.54 ± 11.89 ^{e}	15.28 ± 7.80 ^{ef}	12.53 ± 3.73 ^{f}	0.16 ± 0.07 ^{e}	0.39 ± 0.21 ^{f}	0.95 ± 0.51 ^{e}
UR21		421.33 ± 202.95 ^{e}	56.70 ± 28.37b ^{ef}	13.08 ± 4.37 ^{e}	17.63 ± 10.41 ^{e}	13.71 ± 8.49 ^{ef}	13.25 ± 4.45 ^{ef}	0.11 ± 0.06 ^{e}	0.38 ± 0.24 ^{f}	1.12 ± 0.46 ^{e}
UR22	Liquid	474.44 ± 91.74 ^{e}	71.06 ± 35.94 ^{e}	13.49 ± 5.74 ^{e}	24.22 ± 6.32 ^{e}	21.22 ± 9.63 ^{e}	11.91 ± 5.49 ^{f}	0.15 ± 0.07 ^{e}	0.59 ± 0.24 ^{e}	1.24 ± 0.43 ^{e}
UC		437.25 ± 94.64 ^{e}	54.60 ± 18.55 ^{f}	15.68 ± 2.94 ^{e}	21.92 ± 4.97 ^{e}	19.11 ± 5.25 ^{f}	17.42 ± 4.45 ^{e}	0.13 ± 0.06 ^{e}	0.23 ± 0.15 ^{f}	1.67 ± 0.58 ^{e}
Total		444.24 ± 171.88	52.06 ± 30.71	13.54 ± 4.19	22.33 ± 9.01	14.83 ± 8.78	13.78 ± 4.89	0.14 ± 0.06	0.40 ± 0.09	1.25 ± 0.49
p-value		0.147 ^{ns}	0.042 *	0.349 ^{ns}	0.267 ^{ns}	0.025 *	0.054 *	0.062 ^{ns}	0.042 *	0.097 ^{ns}

S—State Forests Solid fertilization, U—University novel liquid fertilization, R—novel substrates, C—control substrate (peat−perlite). Letters with different alphabet indicate statistically significant differences between means ($p < 0.05$). Alphabets 'a' and 'b' denote homogeneous groups under state fertilization and 'e' and 'f' denote homogeneous groups under novel liquid fertilization. ns = not significance, * = $p < 0.05$.

The multiple linear regression models showed a strong association between below-ground root architecture and above-ground growth performance. Among the assessed variables, the abundance of very fine roots (≤0.50) classified by diameter emerged as the most influential factor driving both height and stem diameter development in beech. In *oak, however,* TRL was the only significant root trait influencing height, while none of the finer root classifications nor did other morphological parameters have a meaningful effect on diameter. This result brings a new perspective to forest nursery evaluations by demonstrating that root traits should not be universally applied as predictors across species. The differentiated role of root classifications especially the functional distinction of very fine, fine and coarse roots offers new practical implications. For beech, promoting fine root proliferation during nursery production may yield tangible benefits in early field performance. For oak, a broader view of establishment factors may be needed, extending beyond root architecture alone (Table 7).

Table 7. Model estimates for above ground parameters of *F. sylvatica* and *Q.robur* one year after planting in the forest.

Species/Dependent Variable	Predictor	Coefficient	Std. Error	*p*-Value	CI Lower	CI Upper	Adjusted R^2
F. sylvatica/Plant height	VFL	0.061	0.017	0.001	0.027	0.095	0.619
	VFSA	−3.195	1.213	0.011	−5.623	−0.766	0.619
F. sylvatica/collar diameter	VFL	0.011	0.002	0.000	0.006	0.016	0.644
	VFSA	−0.351	0.169	0.043	−0.689	−0.011	0.644
Q. robur/Plant height	TRL	−0.034	0.013	0.009	−0.059	−0.009	0.530

TRL—Total Root Length (cm), VFL (≤0.50)—Very-fine length (cm), VFSA (VFSA.≤0.50)—Very fine surface area (cm^2).

4. Discussion

The findings of this study provide viability of innovative peat-free substrates and a novel liquid fertilizer in supporting early seedling development, in *Fagus sylvatica* and *Quercus robur*. Inline with the proposed hypotheses, several peat-free treatments most notably R22 demonstrated shoot growth and survival levels comparable to conventional peat-based controls. Species-specific differences reflected contrasting ecological foraging strategies in both species. *F. sylvatica* exhibited stronger correlations between shoot growth and very fine root development, while *Q. robur's* growth aligned more closely with total root length (TRL). Therefore, root morphological traits predict early developmental dynamics under novel substrate and fertilizer regimes.

The analysis of soil properties showed slight numerical differences; however, these variations were not statistically significant (p-value < 0.05). The differences observed between soil depths and sampling locations did not significantly influence the growth or survival of seedlings [37–40]. The overall consistency in soil characteristics across both sites and depths suggests that soil conditions were not a major factor contributing to variations in seedling performance. Showing high survival rates across sites, beech and oak seedlings responded healthy to the applied treatments after one year of establishment. This agrees with previous research highlighting that the survival of seedlings is enhanced when individuals of the same species and age are planted together [41]. Moreover, successful root development remains a key factor in enabling seedlings to access soil moisture [41–43].

The high survival rates may also be due to the selection of superior seedlings from the nursery, a practice known to enhance field performance [44]. The consistently high survival rate observed across all treatments identified the potential of these innovative materials for successful seedling establishment. The ability of peat-free substrates to support similar survival outcomes to peat established their viability as sustainable alternatives in reforestation practices. This positive relationship between seedling size and survival has been observed in tropical species like *Gmelina arborea* and *Khaya senegalensis* [45,46] and in Mediterranean areas [44,47]. The study found a similar relationship in temperate species, likely due to the balance between root and foliar surface area and the ability to develop deep root systems before leaving the nursery [7,42,48]. However, some studies report no significant or even negative relationships between seedling size and survival [49,50].

The differences observed in seedling performance across treatments are in line with previous study [51] that report generally positive effects of peat-based substrates and solid fertilizers on early seeding development, although in this study, not all such trends reached statistical significance. This contradicts the preliminary report of Rotowa [7] on the same seedling after nursery production circle. Earlier investigation has shown that peat-based media provide favorable water retention, aeration, and structural consistency, which support both root elongation and nutrient uptake during the early growth phase [17]. The compositional analysis of the substrates used in this study confirms that peat substrates had more balanced organic matter and stable texture compared to the peat-free alternatives, which may have contributed to the improved morphological outcomes observed in the control treatments. Similarly, the physicochemical analysis revealed that the peat-based substrates maintained more favorable pH and EC ranges, which are known to influence nutrient availability and uptake efficiency, particularly in forest nursery system.

The limited performance of the novel liquid fertilizers, especially in *Q. robur*, aligns with previous findings that tree species differ in their tolerance to substrate variability and fertilization regimes [51–53]. While some studies report moderate success using composted or wood-based substrates, their performance often depends on precise control of nutrient formulation and substrate stabilization [54,55] These factors were optimized in the current experimental conditions. Furthermore, few studies have explored the role of root diameter classification as a predictor of shoot growth [9–12]. The strong positive association between very fine root length (≤0.50 mm) and shoot development in *F. sylvatica* provides new insight into the functional importance of absorptive root fractions in early seedling establishment. This suggests that simply measuring total root size may overlook key structural parameters that drive above-ground biomass accumulation. While *Q. robur* responded to total root length alone as a significant predictor. The effectiveness of these treatments in promoting increased growth reinforces the earlier recommendation by Rotowa et al., [7] to proceed with forest plantation using these seedlings once adequate root system formation was achieved after nursery production cycle. Though, the translation of those early gains into field performance was less consistent to earlier nursery performance. However, this evenness in results is consistent with the findings of previous studies by Kormanek et al., [56] on root growth of *Quercus petraea* seedlings, as well as studies on forest tree species grown in containers [57–59].

While the distinction between solid and liquid fertilizers was crucial to the experimental design, the observed differences in seedling growth are likely due not only to fertilizer form but also to variations in nutrient composition, availability, and release profiles. The solid fertilizer (Osmocote) used in this study is a controlled-release formulation that provides a balanced supply of essential micronutrients [60]. Its slow nutrient discharge over time ensured consistent availability during this crucial developmental stage, supporting stable root and shoot growth [61–64] In contrast, the liquid fertilizer although applied at regular intervals during nursery production period had lower overall nitrogen content and was more susceptible to leaching, especially in the more porous, peat-free substrates like UR22. Furthermore, previous work by Rotowa et al. [28] reported that this peat-based control substrate exhibited higher baseline nutrient concentrations prior to seed sowing compared to the innovative peat-free mixtures. This initial nutrient advantage, in combination with the solid fertilizer's slow-release properties, likely created more favorable conditions for early seedling establishment in a new forest.

In this study, *Fagus sylvatica* and *Quercus robur* exhibited contrasting responses in below and above-ground coordination, particularly in how specific root traits influenced early shoot development. *F. sylvatica* showed a strong correlation between very fine root length (≤0.50 mm) and above-ground growth metrics, whereas *Q. robur* responded more strongly to TRL as a growth predictor. Although, these findings are consistent with prior studies that emphasize the functional importance of specific root traits in early seedling growth [8,10,11]. However, these variances reflect ecological and eco-physiological strategies of the two species. Beech has been reported to be a shade-tolerant, mesic-adapted species with a typically shallow, fibrous root system that facilitates efficient nutrient acquisition in surface soils [65–68]. Its early growth is often characterized by shoot elongation and crown development, strategies consistent with competitive light acquisition in closed-canopy environments. The observed association between shoot growth and very fine root traits may thus reflect this species' reliance on dense absorptive roots for rapid resource uptake in the upper soil horizon. In contrast, oak has been reported to be more drought-tolerant, and characteristically develops a deep, vertically structured root system to access subsoil moisture, which is crucial for its establishment in open or water-limited sites [69–72]. The weaker correlation between very fine roots and shoot growth, and stronger association with total root length, likely reflects this more conservative growth strategy, which emphasizes rooting depth over fine root proliferation.

For effective reforestation, nursery practices should be tailored to meet species-specific ecological demands, particularly under evolving climate and soil conditions. The species-specific responses and root-shoot dynamics observed in this study offer valuable guidance for nursery management and reforestation planning. *Fagus sylvatica*, promoted fine root development through well aerated and nutrient-balanced substrates. In contrast, *Quercus robur* benefits from practices that support deep, vertically structured root systems, such as using containers and substrates that mimic natural soil conditions.

5. Conclusions

This study provides clear evidence that substrate and fertilizer choices significantly influence the early field performance of *Fagus sylvatica* and *Quercus robur* seedlings, with marked species-specific responses. Solid fertilizers consistently outperformed liquid formulations in promoting root development and shoot growth. The adoption of three root diameter classes identified that, very fine root traits (≤0.50 mm) offered novel predictive insights, especially for *F. sylvatica*, where strong root shoot harmonization was observed. Conversely, *Q. robur* showed a more gravitropic growth strategy, with total root length being the most informative predictor. The root systems of seedlings in the innovative substrates (R20, R21 and R22) were as well healthy, notable root growth was observed in R22, with values closely matching those of the peat-based control; however, these differences were not statistically in most cases after one year of planting into the forest, indicating a guaranteed prospect for future growth without environmental drawbacks like peat. While the study successfully captured early morphological responses under field conditions, limitations such as the absence of root: shoot biomass data and the inability to isolate nutrient form from their composition suggest directions for further research. Future studies should incorporate dry weight measurements, nutrient-matched fertilizer comparisons, and longer-term monitoring to strengthen conclusions on substrate and fertilizer efficacy. Overall, the adoption of these innovative substrates will not only reduce the environmental footprint but also aid biodiversity conservation, providing significant benefits in terms of seedling health and environmental sustainability during the early growth stages of forest seedlings in a new forest.

Author Contributions: Conceptualization, O.J.R. and S.M.; Methodology, O.J.R., S.M., J.B. and D.K.; Validation, S.M., M.P. and J.B.; Formal analysis, O.J.R., S.M., J.B., M.P. and D.K.; Investigation, O.J.R., S.M. and D.K.; Data curation, O.J.R. and J.B.; Writing—original draft, O.J.R.; Writing—review & editing, S.M., M.P., D.K. and J.B.; Supervision, S.M.; Project administration, S.M. and J.B.; Funding acquisition, S.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. However, the peat-free substrates and liquid fertilizer used in this study were developed under the project POIR.04.01.04-00-0016/20, which was funded by the National Centre for Research and Development (NCBiR) from national resources and the European Regional Development Fund.

Data Availability Statement: Data is available upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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