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ORIGINAL PAPER

Effect of nitrogen deposition on root systems and exudates of seedlings of beech *Fagus sylvatica* L. in a temperate climate

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ABSTRACT

The purpose of this experiment was to determine the effect of deposition of different doses of nitrogen on the root systems of common beech *Fagus sylvatica* L. seedlings and their exudates. We tried to explain how different doses of nitrogen can affect the assimilation of nutrients necessary for seedling growth, as well as the morphology of fine roots. The experiment was conducted on a nursery at the Forest Experimental Station of the Agricultural University of Kraków. Three different nitrogen doses were used in the experiment: 0.75 kg·ha^{-1}, 2.25 kg·ha^{-1}, 4.5 kg·ha^{-1} and a control variant without nitrogen. The experiment was conducted from May to September 2021. Seedlings with their root systems and their secretions were taken twice, at the beginning and end of the experiment. The content of micro- and macro-nutrients was determined in the above- and below-ground parts of the seedlings. In addition, the basic chemical properties and enzymatic activity of the substrate in which the seedlings grew were determined. In the study, we showed that a higher nitrogen dose influenced a higher amount of carbon released with exudates from fine roots, which was related to the overall root morphology. Higher specific root length (SRL) and specific root area (SRA) parameters showed a positive correlation with root exudates. In addition, a higher nitrogen dose had a positive effect on the nutritional status of the seedlings.

KEY WORDS

beech, enzyme activity, nitrogen deposition, seedlings, soil properties

Introduction

Mineral nutrients in the soil are needed to ensure adequate growth and metabolic processes occurring in the plant (Soares *et al*., 2019). Nitrogen is one of the macronutrients necessary for plant life. Nitrogen is the building material of proteins and is part of vitamins, nucleotides, nucleic acids, alkaloids and chlorophyll. This element stimulates the growth of aboveground parts, and additionally regulates the consumption of potassium, phosphorus and other nutrients. Many studies have focused on the effect of fertilization on seedling yield. Previous research indicates that intensive fertilization can lead to excessive vegetative growth (Shen *et al*., 2010) which can result in greater water and energy consumption, which is reflected in greater leaching of N beyond the reach of root systems (Rudnick *et al*., 2017; Wang *et al*., 2017).

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Root systems play an extremely important role for plants, primarily enabling them to anchor in the soil. During the growth stage, root development is very plastic and is mainly dependent on nutrient distribution and water availability (Hodge *et al.*, 2009). Among others, the morphology of the roots and the amount of exudates secreted into the soil change in response to the amount of nutrients (Richardson *et al.*, 2009; Staszel *et al.*, 2022c). Above all, fine roots show high physiological activity in response to environmental changes such as drought or excessive acidity of soils (Guo *et al.*, 2004; Hirano *et al.*, 2007). Research by Trachsel *et al.* (2013) confirm that the production of fine and long roots, as well as their distribution in the soil, contributes to transporting more nutrients due to drought or low nitrogen deposition. Root development is additionally influenced by the form of nitrogen, which, with the participation of microorganisms, is converted into an oxidized and reduced readily available nitrate form (Philippot *et al.*, 2007).

Depending on the availability of certain soil nutrients, the activity of microorganisms can vary significantly (Razaq *et al.*, 2017). In particular, excessive nitrogen fertilization can affect the respiration processes, competitiveness, diversity and biomass of microorganisms as well as change their life strategies (Zhou *et al.*, 2015). It is known from research that soil microbiota is mainly shaped by soil properties such as nutrient availability, pH, salinity and soil moisture, as a result of which they can quickly respond to a change in environmental conditions (Li *et al.*, 2014; Zhao *et al.*, 2014). The abundance and activity of microorganisms, expressed by enzymatic activity, can be used to assess the quality and fertility of soils, the correctness of nutrient cycling and the changes that occur in the soil environment (Piaszczyk *et al.*, 2019; Błońska *et al.*, 2021).

The common beech *Fagus sylvatica* L. is one of the most widespread and socio-economically valuable species for European forest ecosystems (Kolář *et al.*, 2017). The high reproductive and productive potential of beech, as well as its relatively broad ecological value, is the reason for beech planting in Central European areas affected by the decline of spruce in recent years (Ammer *et al.*, 2008). The purpose of this study was to determine the effects of deposition of different nitrogen doses on the root systems of beech *F. sylvatica* seedlings and their exudates. We tried to explain how different doses of nitrogen could affect the assimilation of nutrients necessary for seedling growth, as well as the morphology of fine roots. We assumed that the nitrogen doses applied would affect the morphology of the roots and their exudates. We suppose that as a result of the applied nitrogen doses, the degree of nutrition of beech seedlings will change.

Materials and methods

STUDY AREA AND SOIL SAMPLING. The research was carried out at the Forest Experimental Station Krynica of the Agricultural University of Kraków on the nursery in Kopciowa. The vegetation period was 164 days. The research was conducted on beech *Fagus sylvatica* seedlings fertilized with three different doses of nitrogen 0.75 $kg{\cdot}ha^{-1}$, 2.25 $kg{\cdot}ha^{-1}$, 4.5 $kg{\cdot}ha^{-1}$ and a control variant. Each variant contained 5 replicates (20 plots). The experiment was conducted from May to September 2021. In May, beech *F. sylvatica* seeds were sown into the substrates in plastic boxes. A standard mixture of fir-spruce sawdust and high peat at a ratio of 1:1 was used as a substrate. We used the same substrate in all experimental variants. In July, the first series of exudates secreted by the root systems over 24 h was taken, in which dissolved organic carbon was determined. The exudates were collected using a culture-based cuvette system (Philips *et al.*, 2008). A second series was taken at the end of the experiment in September. Root exudates were collected from a single branched thin section of root of similar length and branching from 5 seedlings in each variants. The extracted solutions were analysed using a Shimadzu TOC-Total Organic Carbon analyzer (Shimadzu, Japan). In parallel with the exudates, root systems were sampled, 5 from each variant for the determination of basic root parameters. In addition, substrates were sampled to determine enzymatic activity, enzymes involved in the cycling of C, N and P. At the end of the experiment in September, the morphology of seedling roots was analyzed in all variants and C, N, macro and micronutrient content were determined. The detailed analysis included 30 seedlings per plot (about 600 seedlings in total). In addition, the properties of the substrates were analyzed.

Root analysis. Extracted root systems collected were scanned at 400 dpi resolution and then analyzed using a WinRhizo™ Pro 2003b image analysis system (Regent Instruments Inc., Ville de Québec, QC, Canada) to determine diameter, length, and projected area. Air-dried roots were further desiccated at 65°C for 24 hours to constant weight and then weighed. Root tissue density (RTD [$kg \cdot m^{-3}$]), specific root area (SRA [$m^2 \cdot kg^{-1}$]) and specific root length (SRL [$m \cdot g^{-1}$]) were then calculated as described by Ostonen *et al.* (1999).

Chemical analysis. After drying to an air-dried state, all substrate samples were sieved through a 2-mm mesh. Physicochemical properties were determined in these prepared samples (Ostrowska *et al.*, 1991). pH was determined by the potentiometric method in water and 1M KCl. Hydrolytic acidity (Y) was determined by the Kappen method. Total nitrogen and carbon content was determined using a LECO CNS True Mac Analyser (Leco, St. Joseph, MI, USA). The cation concentrations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) were extracted with ammonium acetate and determined through inductively-coupled plasma analysis (ICP-OES Thermo iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, UK). Soil samples for the determination of enzymatic activity were stored at 4°C. The activity of four extracellular enzyme [β-glucosidase (BG), β-D-cellobiosidase (CB), N-acetyl-β-D-glucosaminidase (NAG), phosphatise (PH)] were determined using fluorogenically labelled substrates (Pritsch *et al.*, 2004; Turner, 2010; Sannaullah *et al.*, 2016). Fuorescence was measured on a multi-detection plate reader (Biotek Synergy) with excitation at 355 nm and emission at 460 nm wavelengths.

In the leaves and roots samples, the concentration of macro and microelements was determined by an ICP (ICP-OES Thermo iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, U.K.). Dried samples of leaves and roots were mineralized in a mixture of HNO_3 and $HClO_4$ (3:1). Carbon (C) and nitrogen (N) in the leaves and roots samples were measured with an elemental analyzer (LECO CNS TrueMac Analyzer (Leco, St. Joseph, MI, USA)).

Statistical analysis. Spearman correlation coefficients between individual root parameters and root exudates were calculated. Principal component analysis (PCA) was used to evaluate the relationship between root parameters and nitrogen doses. In addition, a cluster analysis was performed. Regression lines were used to test the relationship between root exudates and the SRL parameter. A *post-hoc* test was used to evaluate differences between the mean values of the traits. Results were considered statistically significant at $\alpha<0.05$. All statistical analyses were carried out using R statistical software (R Core Team, 2022), R Studio (RStudio Team, 2022) and Statistica 13 software (Tibco Software Inc., 2017).

Results

Root analyses. Between the first and second series of sampling, there were no statistically significant differences between exudation rate and nitrogen dose. The amount of carbon exuded from the roots differed in the second series of sampling. This series showed statistically significant differences between the rate of 2.25 $kg \cdot ha^{-1}$ and 4.5 $kg \cdot ha^{-1}$ (Fig. 1). Root length differed significantly in the second series. The longest root systems were recorded at the 0.75 $kg \cdot ha^{-1}$ rate and the shortest at the 4.5 $kg \cdot ha^{-1}$ rate. Root length varied between 823.03 and 602.38 cm.

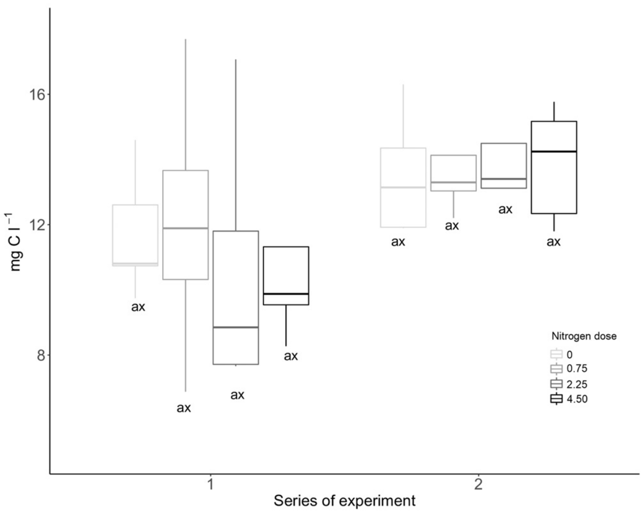

Fig. 1.

The change of total root-exuded carbon (mg C l^{-1}) over time depending on the nitrogen fertilization variant

different lowercase letters (a, b, c) indicate significant differences in parameters between the different nitrogen fertilization; different lowercase letters (x, y) indicate significant differences in parameters between the series; *post-hoc* $p<0.05$

The diameter and weight of roots in the first series of sampling shows no statistically significant differences between the applied doses, while in the second series of sampling the weight of roots differs according to the applied nitrogen dose especially between the dose of 4.5 kg·ha^{-1} and 2.25 kg·ha^{-1}. In the second series of the experiment, SRA differences were recorded between the dose of 2.25 kg·ha^{-1} and 4.5 kg·ha^{-1}. For SRL in the second series of the experiment, differences were recorded at the dose of 0.75 kg·ha^{-1} and 2.25 kg·ha^{-1}. RTD at a dose of 4.5 is significantly different from the other doses (Table 1). Statistical analyses confirmed a significant relationship between the amount of exudates and root parameters. A strong positive correlation was noted between the amount of exudates and SRL and SRA (Figs. 2, 3). A negative statistically significant correlation was found for length, diameter, dry weight, RTD and exudation rate. A strong positive correlation was noted between SRA and SRL (Fig. 2). Cluster analysis carried out taking into account exudation rate, root tissue density (RTD) and specific root length (SRL) distinguished three groups associated with different nitrogen dose (Fig. 4). Factors 1 and 2 distinguished in the PCA analysis together explained 57.8% of the variance in the traits studied (Fig. 5). Factor 1 explained 41.3 % of the variance, factor 2 explained 16.5 % of the variance of the studied parameters. Factor 1 was related to root characteristics and exudation rate, while factor 2 was mainly related to the variant of the experiment.

Chemical analyses. As for the basic physicochemical properties of the substrates used in the experiment, no significant differences were shown as an effect of the applied fertilization (Table 2). The pH values in H_2O as well as in KCl showed no significant differences between the variants included in the experiment. Hydrolytic acidity ranged from 2.30 cmol(+)·kg^{-1} to 2.88 cmol(+)·kg^{-1} for the 0.75 kg·ha^{-1} and 2.25 kg·ha^{-1} application rate, respectively. Exchangeable acidity ranged from 2.18 cmol(+)·kg^{-1} to 2.74 cmol(+)·kg^{-1}, for the 0.75 kg·ha^{-1} application rate and the control variant. For acidity, no statistically significant differences were shown. The amount of accumulated nitrogen and carbon showed little variation between doses. The highest C/N ratio was recorded for the 0.75 kg·ha^{-1} dose and the lowest for the 2.25 kg·ha^{-1} dose.

Table 1.

Mean morphological characteristics of roots and exudation rate [mg C g-1 day-1] in different nitrogen fertilization variant

Series of experiment	Nitrogen dose	Exudation rate	Length [cm]	Diameter [mm]	Weight [mg]	SRA [m^2 kg^{-1}]	SRL [m kg^{-1}]	RTD [kg m^{-3}]
1	0	0.41±0.08ax	353.21±60.49ax	0.21±0.03ax	206.16±36.66ax	11.20±1.19ax	173.48±28.57ax	175.04±27.53ax
	0.75	0.40±0.11ax	402.44±122.54ax	0.22±0.02ax	217.72±50.58ax	12.97±2.94ax	189.22±55.40ax	145.17±31.14ax
	2.25	0.42±0.09ax	331.57±87.36ax	0.22±0.03ax	182.72±47.86ax	12.62±2.24ax	188.52±51.13ax	149.90±28.67ax
	4.50	0.48±0.15ax	389.33±155.66ax	0.21±0.02ax	198.46±80.83ax	13.90±2.96ax	201.71±42.07ax	140.48±17.19ax
2	0	0.21±0.06aby	621.73±389.15ax	0.24±0.02ax	475.24±104.60abx	9.31±3.76abx	127.87±57.26ax	201.94±61.00ax
	0.75	0.13±0.05by	823.03±201.80ay	0.25±0.02ax	798.12±212.92ay	8.19±2.16aby	107.20±34.65ay	207.48±42.53ay
	2.25	0.16±0.06aby	620.64±212.06ay	0.27±0.03ay	723.72±381.85aby	7.69±1.66by	94.00±32.61ay	198.27±18.25ay
	4.50	0.24±0.10ay	602.38±97.27ay	0.27±0.04ay	443.38±156.85by	12.15±3.34ax	144.91±35.08ax	134.44±49.10bx

Mean ±SD; exudation rate [mg C g^{-1} day^{-1}], specific root area (SRA), specific root length (SRL), root tissue density (RTD); different lowercase letters (a, b, c) indicate significant differences in parameters between different nitrogen doses; letters (x, y, z) indicate significant differences in parameters between series; *post-hoc p*<0.05)

Table 2.

Basic properties and enzymatic activity [nmol MUB.g-1 dry soil .h-1] of soils in which beech seedlings grew after the completed experiment

Nitrogen dose	pH H_2O	pH KCl	Hh	Hex	N	C	C/N	Al	CB	BG	NAG	PH
0	4.87±0.08	4.01±0.05	2.92±0.36	2.74±0.35	0.79±0.05	46.31±1.23	59.1±5.7	0.17±0.02	19.94±30.68ab	295.04±155.02	339.49±102.07a	3317.38±494.98ab
0.75	4.91±0.18	4.07±0.19	2.30±0.43	2.18±0.41	0.76±0.05	46.93±1.16	62.0±6.0	0.12±0.03	34.54±41.94a	204.95±96.39	254.78±45.45b	2975.89±1028.25b
2.25	4.92±0.06	4.03±0.10	2.88±0.46	2.73±0.41	0.80±0.03	46.82±0.73	58.3±1.6	0.15±0.06	24.36±14.00ab	287.05±93.30	372.72±83.82a	3859.47±619.73a
4.50	4.89±0.12	4.05±0.12	2.69±0.56	2.55±0.54	0.79±0.02	46.59±1.19	59.0±2.5	0.14±0.03	2.24±5.01b	187.56±145.03	232.48±87.40b	2099.90±807.20c

Mean ±SD; Hh – hydrolytic acidity [cmol(+)·kg^{-1}], Hex – exchangeable acidity [cmol(+)·kg^{-1}]; C, N, C/N [%]; CB – cellobiosidase, BG – ß-glucosidase, NAG – N-acetyl-ß-D-glucosaminidase, PH – phosphatase; small letters in the upper index of the mean values (a, b, c) mean significant differences in properties between nitrogen doses

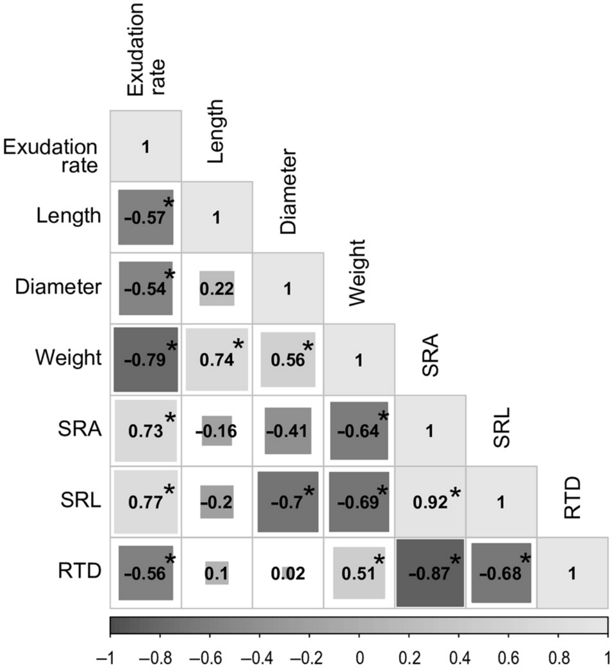

Fig. 2.

Correlation between different root parameters, exudation rate and enzyme activity

RTD – root tissue density, SRA – specific root area, SRL – specific root length, CB – cellobiosidase, BG – β-glucosidase, NAG – N-acetyl-β-D-glucosaminidase, PH – phosphatase; *p<0.05

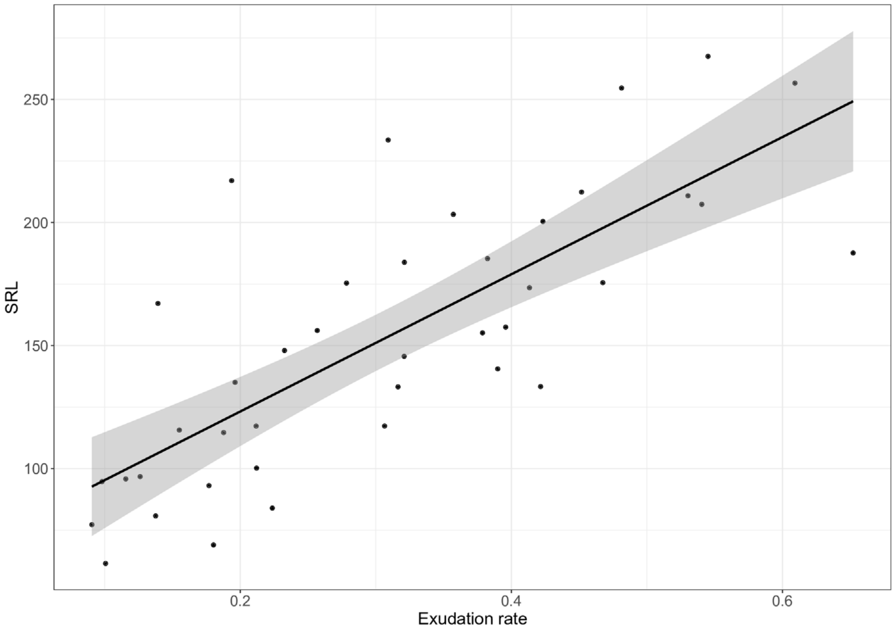

Fig. 3.

The relationship between root exudation rate (mg C g^{-1}·day^{-1}) and specific root length (SRL, m^2·kg^{-1})

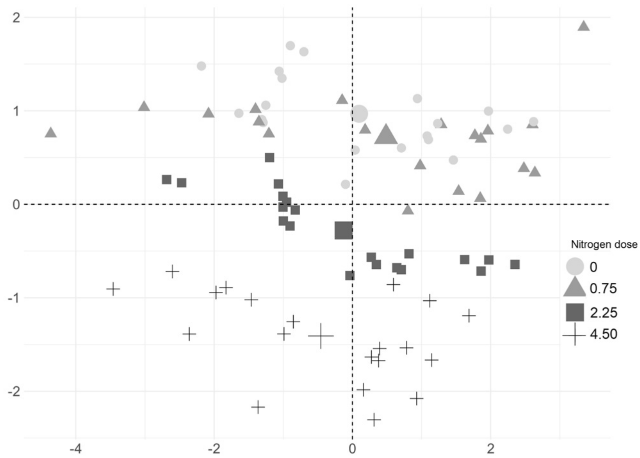

Fig. 4.
Cluster analysis carried out taking into account exudation rate, root tissue density (RTD) and specific root length (SRL)

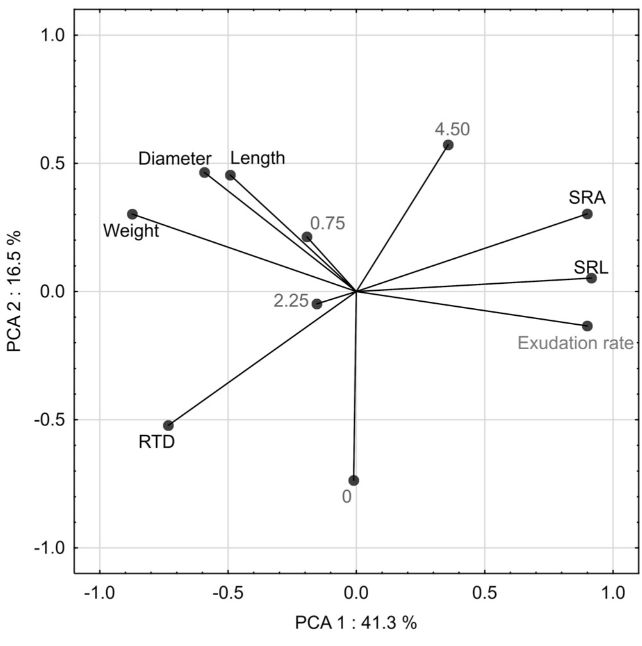

Fig. 5.
Projection of variables on the plane of the first and second PCA factors (root tissue density (RTD), specific root area (SRA), specific root length (SRL), exudation rate, nitrogen doses (0, 0.75, 2.25, 4.5)

In the case of the activity of the enzymes tested, differences were noted between the variants of the experiment. The lowest activity of the four analyzed enzymes was found at the application rate of 4.5 kg·ha^{-1}. In the case of CB, significantly higher activity was recorded in the variant with the application rate of 0.75 kg·ha^{-1}, and the lowest activity was recorded at the application rate of 4.5 kg·ha^{-1} (Table 2). In the case of BG, there was no effect of the applied fertilization variants.

Table 3.

Content of basic elements in leaves, roots and substrate depending on the variant of nitrogen fertilization

	Nitrogen dose	N	C	C/N	Ca	K	Mg	Na	P
Leaves	0	0.82±0.1b	46.60±0.3	57.2±5.2a	8682.5±647.3a	1804.3±361.8b	2600.7±197.0a	453.5±37.6	267.0±58.2b
	0.75	0.86±0.2b	46.97±0.7	58.1±15.7a	8148.0±1100.1ab	1886.1±958.2b	2509.9±239.8a	482.1±102.3	271.9±67.3b
	2.25	1.31±0.2a	46.34±0.7	36.1±5.5b	7034.1±122.5ab	3243.4±359.9a	2238.8±88.2b	479.2±63.9	387.2±63.9a
	4.5	1.21±0.2a	46.37±1.3	39.0±4.9 b	6413.8±766.5b	3405.7±837.1a	2159.5±159.5b	416.0±51.4	414.0±42.9a
Roots	0	0.59±0.2ab	44.98±1.6	80.4±18.0ab	1534.5±229.5b	2958.4±322.4	1237.3±205.9b	357.8±118.5	365.6±31.8
	0.75	0.58±0.1b	46.12±0.6	82.4±17.8a	1969.7±133.5a	3064.8±382.5	1488.3±201.7ab	468.8±49.7	407.2±45.0
	2.25	0.73±0.6ab	45.80±1.2	63.8±10.8ab	1926.6±383.1a	3298.7±125.8	1531.6±177.9a	467.3±47.2	366.0±47.8
	4.5	0.82±0.6a	45.45±0.9	57.5±12.5b	1967.4±277.2a	3193.9±287.7	1423.5±204.4ab	441.2±116.2	359.0±38.9

Mean ±SD; C, N [%]; Ca, K, Mg, Na, P [mg/kg]; small letters in the upper index of the mean values (a, b, c) mean significant differences in properties between nitrogen doses

In the case of NAG, significantly the highest activity was recorded in the variant with a dose of 2.25 kg·ha^{-1}. PH activity varied significantly between the variants tested. Significantly the highest PH activity was recorded in the experimental variant at 2.25 kg·ha^{-1}. The lowest PH activity was recorded in the variant with a dose of 4.5 kg·ha^{-1}.

The elements content of the leaves and roots of the seedlings differed significantly depending on the nitrogen dose applied (Table 3). In the case of leaves, the lowest nitrogen concentration was recorded in the control variant, and the highest in the 2.25 kg·ha^{-1} variant. Significantly the lowest nitrogen content in roots was recorded in the 0.75 kg·ha^{-1} variant, and the highest in the 4.5 kg·ha^{-1} variant. The amount of carbon accumulated in leaves and roots shows little variation. Statistically significant differences in leaves and roots were recorded for the C/N ratio. For leaves and roots, significantly the highest C/N values were recorded in the control variant and in the variant with a dose of 0.75 kg·ha^{-1}. Ca content in leaves ranged from 6413.8 mg/kg for the dose of 4.5 kg·ha^{-1}, to 8682.5 mg/kg for the control variant. For leaves, the highest Ca contents were noted in the control variant and the variant with the lowest nitrogen rate. In the case of roots, the lowest Ca content was recorded in the control variant. The nitrogen doses used in the experiment resulted in changes in the Mg content of the leaves and roots of the tested seedlings. Leaves and roots in variants with higher nitrogen doses, *i.e.*, 2.25 kg·ha^{-1} and 4.5 kg·ha^{-1}, were characterized by significantly higher Mg content. The highest K and P contents in leaves were recorded in the 4.5 kg·ha^{-1} variant, and the lowest in the control variant (Table 3). No significant differences were noted for K and P contents in roots. Na content in leaves and roots was not statistically significantly different between the variants of the experiment (Table 3).

Discussion

The study confirms the effect of deposition of different doses of nitrogen on the root systems of beech *Fagus sylvatica* seedlings and their exudates. The amount of carbon secretion in our experiment ranged from 0.13 to 0.48 mg C g^{-1}·day^{-1} (Table 1).

Similar values of root exudates for the genus *Fagus* were shown in the study by Brzostek *et al.* (2013), the values ranged from 0.35-1.10 mg C g^{-1}·day^{-1}. Higher availability of N in the soil may affect the release of more C by root exudates, which consequently leads to stimulation of microbial activity and acceleration of N transformation (Wang *et al.*, 2021). In our study, there were no significant changes in the physical and chemical properties of the substrate in which beech seedlings grew. As a result of the applied nitrogen doses, changes in the enzymatic activity were observed. It is known that nitrogen directly or indirectly modifies the composition of soil microorganisms, affecting enzymatic activity (Klironomos *et al.*, 2011; Wang *et al.*, 2019). As a result of long-term nitrogen fertilization, there is an accumulation of C in the soil, which leads to a decrease in the rate of decomposition of organic matter and thus to a decrease in the activity of enzymes involved in the decomposition of lignin (Morrison *et al.*, 2018). From a physiological point of view, in the era of climate change, the acceleration of soil N cycling driven by root exudates may be a mechanism regulating forest productivity (Phillips *et al.*, 2012; Wang *et al.*, 2021). Authors of other studies repeatedly point to root exudates as having a significant impact on nutrient cycling and microbial activity (Brzostek *et al.*, 2013; Sun *et al.*, 2021). The differences in the amount of secreted C with exudates between the first and second series of the experiment may be the result of seasonal variation in the rate of C secretion. At the end of the growing season, the rate of physiological processes taking place inside the plant changes due to the slow transition into dormancy caused by lower temperatures (Heide, 1993; Liu *et al.*, 2015). In our study, we showed a strong positive correlation between the amount of root exudates and root parameters such as SRA and SRL. Previous studies also demonstrated that the amount of root exudates is strongly correlated with root morphological characteristics (Yin *et al.*, 2013; Sun *et al.*, 2017; Staszel *et al.*, 2022a, b). Root systems exhibiting higher SRL parameters tend to have more fine roots, which directly translates into root exudates (Ma *et al.*, 2018). Since N is one of the most important components necessary for plants, it directly affects their growth characteristics by increasing shoot and root biomass (Harper, 1974; Zhao *et al.*, 2008). In our study, we found higher SRA and SRL with lower RTD as an effect of increasing the nitrogen rate. Similar relationships were shown by Costa *et al.* (2002) and Makita *et al.* (2012), the higher the nitrogen availability the higher the root area.

The applied fertilization with different doses of nitrogen led to an increase in its content in both leaves and roots of beech seedlings. Significantly higher contents of this element were associated with the highest fertilization doses. According to Zhu *et al.* (2016), N deposition can modify nutrient availability in soils, which consequently is reflected in plant health. In our study, we confirmed the importance of N fertilization on the content of basic components such as Ca, K, Mg and P. In the leaves of the studied seedlings, there was an increase in the content of K and P as a result of fertilization with higher doses of nitrogen. Higher concentrations of N, P and K and, at the same time, lower concentrations of Ca in the leaves of seedlings, were observed in the study of Lambers and Oliveira (2019). For Ca and Mg content in roots, higher values were observed in the fertilized variants compared to the control. Leaves and roots differ in function, but are most commonly used to monitor the effects of nutrient availability and long-term environmental changes on tree nutrition (Adams and Hutchinson, 1992; Duquesnay *et al.*, 2000). According to Vesala *et al.* (2021) nitrogen deposition can affect nutrient cycling. Nutrient allocation in roots can depend on the morphological characteristics of the roots and on their extent (Zhao *et al.*, 2020). In our study, we noted differences in the characteristics of root systems depending on the nitrogen dose which, consequently, may affect the differences in nutrient content in the roots of beech seedlings. In the leaves and roots of seedlings treated with the highest nitrogen doses, there was a reduction in the value of the C/N ratio. The C/N ratio in leaves can be linked to important ecological processes and the ability to adapt to environmental stresses (Woods *et al.*, 2003). C/N ratios in leaves depend on differences in plant physiology (Reich and Oleksyn, 2004). According to Sheng *et al.* (2021) human-induced nitrogen addition can reduce the C/N ratio of leaves by increasing soil nitrogen availability. Zhao *et al.* (2022) highlight the differential responses of fine root traits to N deposition. A global increase in nitrogen (N) deposition affects the underground allocation of plant photosynthesis and the formation of rhizosphere-associated roots and symbionts, as well as the availability of nutrients in the soil, thereby affecting nutrient acquisition by trees (Ma *et al.*, 2021).

Conclusions

- The study showed that the applied doses of nitrogen fertilization influenced the characteristics of the root systems and, consequently, the amount of carbon secreted by the root systems with their exudates.
- In research a strong positive correlation was noted between the amount of C from exudates and SRL (specific root length) and SRA (specific root area).
- Our study confirmed the importance of nitrogen fertilization in shaping the nutrition of beech seedlings. There was a significant increase in N content in the leaves and roots of the tested seedlings as a result of higher doses.
- The results indicate that beech can be used as a plastic species that adapts well to conditions of increased nitrogen deposition.

Supplementary materials

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

Authors' contributions

K.S., E.B., J.L. – conceived and designed the investigation; analysed and visualised the data; E.B., J.L., M.K. – concepts research methodology; K.S., E.B., J.L., M.K. – preparation of manuscript.

Conflicts of interest

The authors declare the absence of potential conflicts of interest.

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STRESZCZENIE

Wpływ depozycji azotu na systemy korzeniowe i wydzieliny siewek buka *Fagus sylvatica* L. w klimacie umiarkowanym

Wielkość systemu korzeniowego wpływa na pobieranie wody i ilości składników pokarmowych, od czego zależy stan odżywienia sadzonek. Systemy korzeniowe, zwłaszcza korzenie drobne, odgrywają ważną rolę w kształtowaniu właściwości gleb. Celem badań było określenie wpływu różnych dawek azotu na systemy korzeniowe siewek buka zwyczajnego *Fagus sylvatica* L. i ich wydzieliny. Próbowano wyjaśnić, w jaki sposób różne dawki azotu mogą wpływać na przyswajanie składników pokarmowych niezbędnych do wzrostu siewek, a także na morfologię drobnych korzeni. Doświadczenie przeprowadzono w szkółce w Kopciowej na terenie Leśnego Zakładu Doświadczalnego Uniwersytetu Rolniczego w Krakowie. W badaniach uwzględniono 4 warianty doświadczenia: 3 różne dawki azotu (0,75 $kg·ha^{-1}$, 2,25 $kg·ha^{-1}$, 4,5 $kg·ha^{-1}$) oraz wariant kontrolny bez azotu. Doświadczenie prowadzono od maja do września 2022 r. Siewki wraz z systemami korzeniowymi oraz ich wydzielinami zostały pobrane dwukrotnie: na początku i na końcu doświadczenia. W wydzielinach korzeniowych oznaczono zawartość węgla organicznego. Systemy korzeniowe poddano szczegółowej analizie z wykorzystaniem programu WinRhizo. Na podstawie uzyskanych parametrów korzeni określono gęstość systemów korzeniowych RTD [$kg·m^{-3}$], właściwą powierzchnię korzeni SRA [$m^2·kg^{-1}$] i właściwą długość korzeni SRL [$m·g^{-1}$]. W nadziemnej i podziemnej części siewek oznaczono zawartość mikro- i makroskładników. Ponadto określono podstawowe właściwości chemiczne oraz aktywność enzymatyczną substratu, w którym wzrastały siewki (tab. 2). W badaniach wykazano, że wyższa dawka azotu wpływała na większą ilość węgla uwalnianego z wysiękami z korzeni drobnych, co było związane z ogólną morfologią korzeni (ryc. 1). Stwierdzono silną dodatnią korelację między ilością węgla z wysięków a SRL i SRA (ryc. 2). Dodatkowo analizy statystyczne potwierdziły istotną zależność pomiędzy ilością wysięków a parametrami korzeni (ryc. 3). Badania wykazały, że zastosowanie różnych dawek azotu miało wpływ na charakterystykę systemów korzeniowych, a co za tym idzie na ilość węgla wydzielanego przez systemy korzeniowe wraz z ich wysiękami (ryc. 4-5). Ponadto badania potwierdziły znaczenie nawożenia azotem w kształtowaniu odżywienia sadzonek buka (tab. 1). W wyniku stosowania wyższych dawek nastąpił znaczny wzrost zawartości N w liściach i korzeniach badanych sadzonek (tab. 3). Uzyskane wyniki wskazują, że buk może być wykorzystany jako gatunek plastyczny, który dobrze adaptuje się do warunków zwiększonej depozycji azotu.