Annual dynamics of ammonium and nitrate nitrogen content by using capsulated carbamide in cassettes of one-year-old seedlings of Scots Pine (*Pinus sylvestris*)

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Abstract. Over the past decades of forestry development in Russia, the main trends have been identified, according to which *P. sylvestris* planting material can be effectively grown in a container method with a closed root system, considering the forest-seed area. The purpose of the study was to study the annual dynamics of ammonium and nitrate nitrogen in a soil with *P. sylvestris* with a closed root system using encapsulated urea with hydroquinone and mulch. The objectives of the study were based on the study of the effect of mineral fertilizers on the annual dynamics of ammonium and nitrate nitrogen and their effect on the growth of seedlings. The object of the study was the dynamics of the content of these nitrogen compounds in the soil. The study was carried out by methods of agrochemical, biometric and statistical analysis. Thus, the use of extended-form carbamide makes it possible to cover the need of plants for nitrogen nutrition throughout the growing season, having a significant impact on their growth in stem height. The use of encapsulated carbamide with hydroquinone, 0.5 cm pine mulch, potassium monophosphate and phosphorous flour allows the production of the best *P. sylvestris* seedlings with a maximum height of 188.82±15.41 mm per year due to constant saturation of the soil with available nitrogen, on which a change in the growth rate of seedlings is almost directly dependent (R = 0.99821-0.99988; *Multiple* R = 0.99643-0.99977; *Adjusted* R = 0.98928-0.99930; p < 0.01525-0.05978). At the same time, ammonium nitrogen has the greatest effect on it (p < 0.01633-0.05436).

1 Introduction

The cultivation of high-quality planting material for coniferous crops, including Scots Pine (*Pinus sylvestris* L., 1753), has been an urgent task of forestry for many years, the efficiency and speed of which depends not only on environmental conditions [27], but also on the

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applied complex of fertilizers of various shapes to achieve standard parameters by seedlings [12, 23, 24] for a shorter period of cultivation in forest nurseries, greenhouses, open ground with the creation of the necessary conditions for the seed material of a specific forest-seed region (FSR) of Russia [17, 19, 22].

Recently, planting material with a closed root system (CRS) has been gaining the most popularity [18], which is also reflected in the regulatory legal acts of the country and its regions, in particular in the Rules of reforestation [23], which determines the share of seedlings of CRS up to 30% by 2025 in the total volume of reforestation and afforestation.

Emerging trends in scientific research on the topic of reforestation, in which Russian and foreign authors study the selection of an effective complex of mineral fertilizers, biostimulators, their norms and application dates, demonstrate the results of using a huge variety of different preparations, top dressing [5, 7, 15, 18, 20, 26, 27, 30]. At the same time, many researchers especially highlight long-form fertilizers containing important macronutrients (NPK), a special role among which is played by nitrogenous compounds [2, 27], potentially available for assimilation by coniferous plants. This fact determines the relevance of this study, which analyzes the little-studied issue in Russia of the movement of ammonium and nitrate nitrogen in the soil of containers intended for forest nurseries for growing planting material with CRS, as a rule, up to 1-2 years of age of *P. sylvestris* seedlings.

By itself, the pine of this species is equivalent in relation to the scale of nitrogen richness of the soil mixture and its acidity, as a result of which this crop prefers acidic peat, which is the best substrate for its cultivation [29], which – all together – predetermined the course of our experiment.

2 Materials and Methods

The purpose of the study was to analyze the annual dynamics of ammonium and nitrate nitrogen in the soil of containerized annual seedlings of *P. sylvestris* and its effect on the growth of seedlings height depending on mineral fertilizers with encapsulated urea.

3 Research objectives

1. To study the effect of variations in the applied fertilizer complex using extended-form carbamide on the annual dynamics of changes in the content of ammonium and nitrate nitrogen in the soil;

2. To study the effect of the dynamics of ammonium and nitrate nitrogen in the soil on the change in the height of the stem of containerized seedlings of *P. sylvestris* up to the age of one year.

The object of the study is the dynamics of changes in the content of ammonium and nitrate nitrogen in the soil of cassettes.

The subject of the study is mineral fertilizers and biometric parameters of the growth of the stem height of *P. sylvestris* seedlings.

As part of the study of the issues raised, the following **research methods** were used:

1. Agrochemical analysis of soil in cassettes for ammonium (NH_4^+) and nitrate (NO_3^-) nitrogen by the Bremner method [1] before fertilization and after application in annual dynamics, and exchange acidity according to GOST 11623-89 [11];

2. Biometric accounting with a ruler based on standardized indicators for forest nurseries [13, 21];

3. Mathematical and statistical analysis of experimental options in comparison with control using Microsoft Excel and StatSoft Statistica software products 12.

The experiment was laid in an unheated greenhouse of the Siberian Forest Experimental Station, a branch of the FBI Russian Research Institute for Silviculture and Mechanization of Forestry with an accounting area of 0.89 m^2 for 6 Plantek 81F cassettes (Lessnab LLC) with a total volume of 6,885 cm^3 per 1 cassette, while observing the methods of growing *P. sylvestris* planting material with CRS for forest nurseries [13, 21]. Seedlings were grown from seeds of the XI forest seedling area [22] at t = +20-25°C, sown in May (I rotation) with pre-sowing and post-sowing application of mineral fertilizers (Table 1). Encapsulated carbamide with the urease inhibitor hydroquinone was obtained using a patented technology at the I. D. Komissarov Department of General Chemistry of the FSBEI HE SAU of the Northern Trans-Urals [16, 25].

The single volume of irrigation was 20 liters of water with a temperature of +18-20°C per 0.89 m^2. Watering was carried out twice a day – in the morning before sunrise and in the evening after sunset.

Table 1. Two-time experiment options.

Experiment option	Fertilizer, application form*	Dose of fertilizer	Frequency of fertilization
control C	"Aquarin 5" *foliar top dressing by spraying (in the evening)*	25 g / 10 l (single consumption 4-5 l for 6 cassettes)	1 – 10 days after sowing 2-4 – interval of 15-20 days
variant V1	Encapsulated carbamide ($CO(NH_2)_2$) *at sowing for seeds*	0.1159 kg/m^3	1 – when sowing
V2	Encapsulated carbamide ($CO(NH_2)_2$) with urease inhibitor hydroquinone (*1,4-dihydroxybenzene*) *at sowing for seeds*	0.1162 kg/m^3	1 – when sowing
V3	Encapsulated carbamide ($CO(NH_2)_2$) with urease inhibitor hydroquinone (*1,4-dihydroxybenzene*) and pine mulching 0.5 cm *at sowing for seeds*	0.1162 kg/m^3	1 – when sowing
* in variants *V1-V3*, potassium monophosphate (KH_2PO_4) and phosphorous flour ($Ca_3(PO_4)_2$), ground into dust, in doses of 0.037 and 3.765 kg/m^3, respectively, were also added.			

The conducted agrochemical analysis showed (Table 2) that the content of basic nutrients in the soil is not able to cover the need of seedlings for mineral nutrition, and therefore a complex of fertilizers was introduced in doses according to the variants of the experiment in Table 1.

Table 2. The results of agrochemical analysis of absolutely dry top peat of high acidity before fertilization.

Fertilizing elements	Content (N), mg/kg	The exchange acidity of the soil (pH_{KCl})
Ammonium nitrogen (NH_4^+)	65.3	3.5
Nitrate nitrogen (NO_3^-)	53.9	
Phosphorus oxide (P_2O_5)	70.4	
Potassium oxide (K_2O)	85.2	

The application of fertilizers according to the formula $N_{15}P_{60}K_{60}$, t/ha led to variability in the dynamics of the nitrogen content available to plants in the soil, which affected the dynamics of the development of seedlings in stem height.

4 Results and Discussion

Obtained during laboratory agrochemical analysis, the results of the annual dynamics of NH_4^+ and NO_3^- in the soil of containerized *P. sylvestris* seedlings up to the age of one year with the removal of 2 cells from 1 cassette at each sampling from 05.30.2022 to 05.26.2023, variants *V1-V3* were analyzed in comparison with control *C* for the variability of their content over time during the year (Fig. 1). In total, the selection was carried out in eight-fold repetition throughout the year.

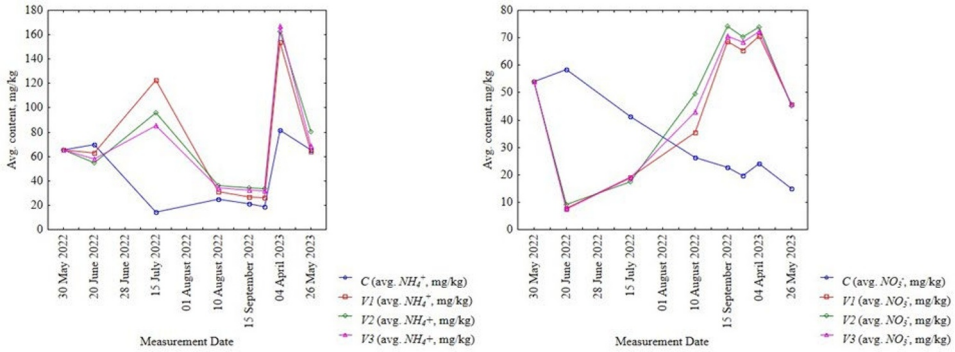

Fig. 1. Graphs comparing the dynamics of NH_4^+ and NO_3^- according to experimental variants (*V1-V3*) and control (*C*).

During sowing, the content of NH_4^+ and NO_3^- in all variants was the same, since the soil was used from one filler package. In the future, an increase occurs in control *C*, as a result of the use of top dressing with "Aquarin 5" fertilizer, which also reflected an increase in the content of NO_3^-. During this period, samples that were grown using encapsulated carbamide have a decrease in NH_4^+ reserves, since the fertilizer is just beginning to act and is used by seedlings.

In June-July 2022, an increase in the NH_4^+ content is recorded, which is associated with the release of nitrogen from the fertilizer and its entry into the soil substrate.

The following sampling showed that the NH_4^+ content in control *C* increased, which is associated with fertilization. The nitrogen content in the remaining variants decreased, since foci of local acidification are created in the acidic soil substrate and N-NH_4^+ can partially decompose to nitrogen oxides and nitric acid, which is confirmed by the research of Turchin F. V. [28]. This phenomenon explains the increase in the content of NO_3^- in the soil during this period of time.

The appearance of high levels of NH_4^+ content from October 2022 to April 2023. In all variants *V1-V3* and control *C*, it can be explained by the decomposition process of the soil substrate consisting of topsoil peat. A fairly warm March and a large amount of precipitation from meltwater accelerate the process of humification, which is accompanied by an increase in the content of soil organic matter and nitrogen, which is subsequently spent on new plant growth.

The lowest NH_4^+ content is observed in control *C*, not exceeding 81.39 mg/kg of absolutely dry soil, whereas in experimental variants, namely *V3*, when using $CO(NH_2)_2$ prolonged action with *1,4-dihydroxybenzene* and pine mulching 0.5 cm – 166.80 mg/kg. Moreover, in all variants there are at least 2 sharp jumps simultaneously for all variants in July 2022 and April 2023 with an increase in the NH_4^+ content to 85.08-122.80 (*V3-V1*) and 154.07-166.80 (*V1-V3*) mg/kg, respectively, and 2 simultaneous sharp declines for all variants in October 2022 and May 2023, when the content decreased to 26.38-32.09 (*V1-V2*) and 64.26-80.11 (*V1-V2*) mg/kg, respectively. The maximum increase in NH_4^+ in the *V1*

variant with the use of encapsulated carbamide is due to the absence of a urease inhibitor in the composition, which inhibits nitrification processes in the soil. At the same time, this variant has more pronounced differences, whereas variants *V1-V2* have relatively similar dynamics among themselves. At the same time, control *C* has one sharp decline in July 2022 to 14.15 mg/kg and one jump in April 2023 to 81.39 mg/kg.

Nitrate nitrogen is very mobile and easily susceptible to leaching from the soil, which we observe in the dynamics of the studied samples during daily watering. Various factors can influence the content of NO_3^- in the soil, including weather conditions, soil type, type and amount of fertilizers used, tillage methods, etc. Thus, at high soil moisture, NO_3^- can be denitrified and washed out of the soil, which leads to a decrease in its content, considering that its content in the soil can vary at different points of the same soil area, which is associated with the heterogeneity of the distribution of organic and mineral substances in the soil [5, 31].

At the same time, the further growth of the graph lines is associated with the release of fertilizer nitrogen and partially the transformation of NH_4^+ into NO_3^-. The maximum content of the latter is observed in variant *V2* with the use of prolonged-acting $CO(NH_2)_2$ and *1,4–dihydroxybenzene* in September 2022 and is 74.11 mg/kg of absolutely dry soil, and the minimum is in control *C* and is 15.00 mg/kg on the last day of sampling on 05.26.2023, whereas in June 2022 control *C* recorded a maximum value of 58.39 mg/kg.

A relatively small loss of NO_3^- in the autumn period is associated with the continued growth of annual seedlings of *P. sylvestris* with a low growth rate in height, as well as a possible antagonistic effect of NH_4^+ on the assimilation of NO_3^- [3, 6].

The increase in NO_3^- in April 2023 is similarly associated with the decomposition of organic matter in the soil substrate. At this stage, the maximum content according to the experimental variants of 74.00 mg/kg was observed similarly in variant *V2*, and the minimum in control *C*, which is reflected in the minimum growth of plants when observing the phenological and ontogenetic development of seedlings to a juvenile state [4, 7, 9, 14].

Analyzing the dynamics of the variability of NH_4^+ content (Fig. 2) in the soil, variants *V1-V3* have approximately the same dynamics in contrast to control *C*, although in comparison of control *C* ($r = -0.1204; p = 0.7764; n = 8$) and variants *V2* ($r = 0.1781; p = 0.6730; n = 8$) and *V3* ($r = 0.1424; p = 0.7366; n = 8$) with variant *V1* ($r = -0.0156; p = 0.9707; n = 8$), there is a polynomial growth trend in the former and a trend in Distance Weighted Least Squares (DWLS) for the latter. The dynamics of NO_3^- (Fig. 2) has a different character of variability over time, which is described by a linear trend for control *C* ($r = -0.9727; p = 0.00005; r^2 = 0.9462; n = 8$), DWLS for variant *V1* ($r = 0.5557; p = 0.1527; n = 8$), and an exponential trend for *V2* ($r = 0.5776; p = 0.1338; n = 8$) and *V3* ($r = 0.5760; p = 0.1351; n = 8$).

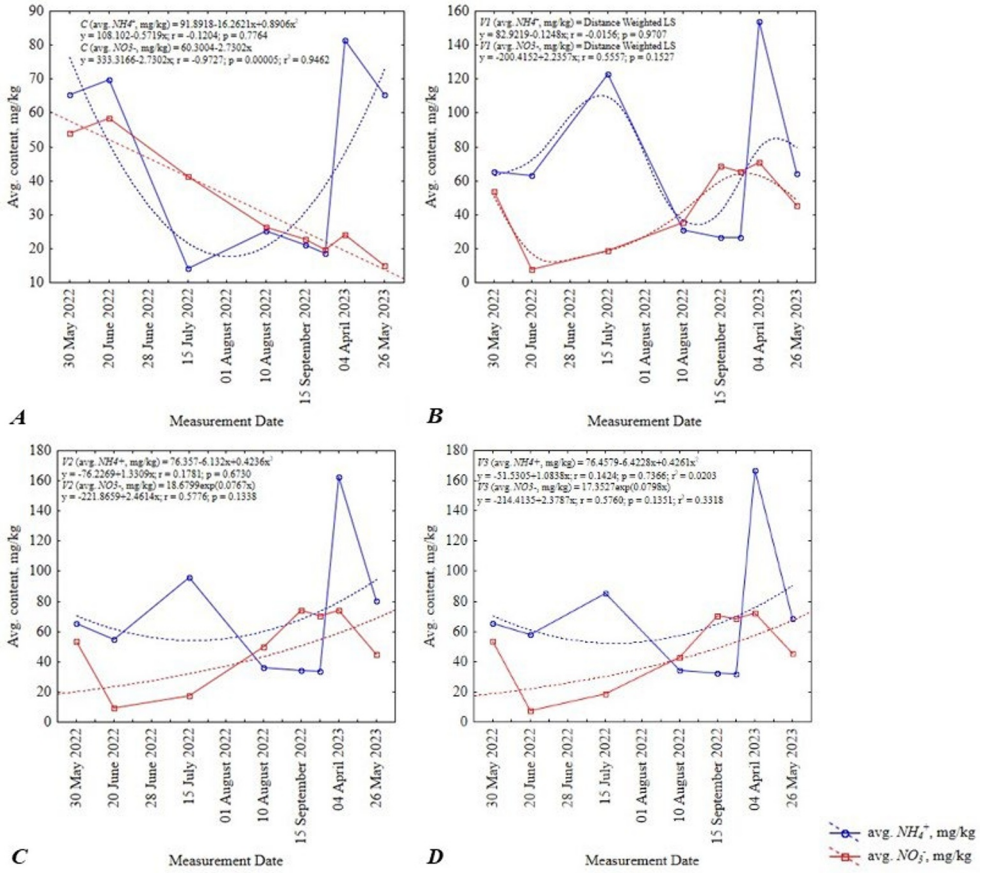

Fig. 2. Annual dynamics of NH_4^+ and NO_3^- content according to the experiment options: *A* – control *C, B* – variant *V1*, *C* – variant *V2*, *D* – variant *V3*.

The content of NH_4^+ and NO_3^- in the soil of *P. sylvestris* seedlings with CRS has a significant effect on the phenological parameters of the planting material [7], in particular on the height of the stem (h_s).

Our regression analysis reliably shows (Table 3) that there is a large relationship between the content of NH_4^+ and NO_3^- in the cassette cells and the increase in h_s of *P. sylvestris* seedlings at $p < 0.05$ for control *C* and variants *V1-V2*, whereas for variant *V3* – $p > 0.05$ with respect to the dependent variable h_s and the content of the NH_4^+ predictor in the soil substrate.

Table 3. Regression analysis results

Statistical parameters	*b**	*Std. Err.* (of *b**)	*b*	*Std. Err.* (of *b*)	*t* (1)	*p*
C (avg. h_s, mm) $R = 0.99995, Multiple\ R = 0.99990, Adjusted\ R = 0.99969$ $F(2,1) = 4866.50, p < 0.01014, Std.Error = 0.10416$						
Intercept			85.2177	0.3258	261.5694	0.00243
C (NH_4^+, mg/kg)	0.7388	0.0101	0.1386	0.0019	72.8501	0.00874
C (NO_3^-, mg/kg)	-0.6494	0.0101	-0.9643	0.0151	-64.0326	0.00994
V1 (avg. h_s, mm) $R = 0.99967, Multiple\ R = 0.99934, Adjusted\ R = 0.99803$ $F(2,1) = 761.61, p < 0.02561, Std.Error = 1.1844$						

Intercept			242.9701	3.7621	64.5828	0.00986
V1 (NH_4^+, mg/kg)	0.6566	0.0261	0.2914	0.0116	25.1620	0.02529
V1 (NO_3^-, mg/kg)	-0.8897	0.0261	-2.0622	0.0605	-34.0955	0.01867
V2 (avg. h_s, mm) $R = 0.99988, Multiple\ R = 0.99977, Adjusted\ R = 0.99930$ $F(2,1) = 2150.60, p < 0.01525, Std.Error = 0.78966$						
Intercept			234.1874	2.2385	104.6162	0.00608
V2 (NH_4^+, mg/kg)	0.5946	0.0152	0.2935	0.0075	38.9816	0.01633
V2 (NO_3^-, mg/kg)	-0.8233	0.0152	-1.7559	0.0325	-53.9728	0.01179
V3 (avg. h_s, mm) $R = 0.99821, Multiple\ R = 0.99643, Adjusted\ R = 0.98928$ $F(2,1) = 139.40, p < 0.05978, Std.Error = 2.3823$						
Intercept			241.5522	7.1871	33.6090	0.01894
V3 (NH_4^+, mg/kg)	0.7089	0.0607	0.2567	0.0220	11.6835	0.05436
V3 (NO_3^-, mg/kg)	-0.8346	0.0607	-1.5367	0.1117	-13.7536	0.04621

Considering the linear trend of variants *V1-V3*, there is a lower trend slope in contrast to control *C*, which indicates a higher growth rate of h_s seedlings depending on the content of NH_4^+ and NO_3^- in the soil (Fig. 3). Thus, with a decrease in their volume, the h_s curve takes on a flattened logarithmic appearance, and vice versa, with an increase it takes on a more elongated upward appearance, whereas the entire curve, in fact, consists of several curves bending logarithmically in proportion to the nitrogen content in the cells and their assimilation by *P. sylvestris*.

To a greater extent, the growth of seedlings in stem height is influenced by NH_4^+, which is explained by regression analysis and is visually marked on the graph considering the confidence interval (CI).

This relationship is especially evident with sharp changes in the heights of the graph lines, which curve down and up along the Y-coordinates. For example, from June 20 to July 15, 2022, at the time of a sharp decrease in the content of NH_4^+ and NO_3^- in the control variant *C* (Fig. 3 *A*) due to the use of nitrogen by plants, a slowdown in the growth of h_s seedlings is observed, continuing in proportion to a further decrease in their content from August 10 to October 16, 2022, due to a lack of nitrogen. In this case, a decrease in the nitrogen content in the soil, tending to zero, is accompanied by a gradual slowdown in the rate of growth of seedlings in height from July 10, 2022, almost reaching the extreme position of the convex line of the logarithmic function by August 14, 2022 – during this period, the rate decreased from 7.37 to 2.45 mm in 19 days and from 2.45 to 0.72 mm in 16 days. Nevertheless, when the NH_4^+ content increases after foliar fertilization with the complex fertilizer "Aquarin 5", the processes of accelerating the rate of development of seedlings according to h_s are noted, which is visible in the periods from May 30 to June 14, when active ontogenetic development processes occur at an increase rate from 4.50 to 17.14 mm in 52 days, from July 15 to August 10, 2022, with an increase rate of 7.37 mm in 11 days, and from October 16, 2022 to April 04, 2023, first, with a slowdown in the growth rate from 4.45 to 2.34 mm in 57 days, and then an increase from 2.34 to 4.08 mm in 17 days. The latter is most likely due to the transition from the autumn-winter period of development and overwintering of seedlings, with a change in the rate of hydrolysis of urea [8] and the reutilization ability of pine to use nitrogen [29], to the spring period of development with an increase in the growth rate of h_s and consumption of NH_4^+ and NO_3^-. Ultimately, seedlings in control *C* reach a maximum average height of 79.80±4.34 mm on May 26, 2023, with a coefficient of variation (CV) of 27%.

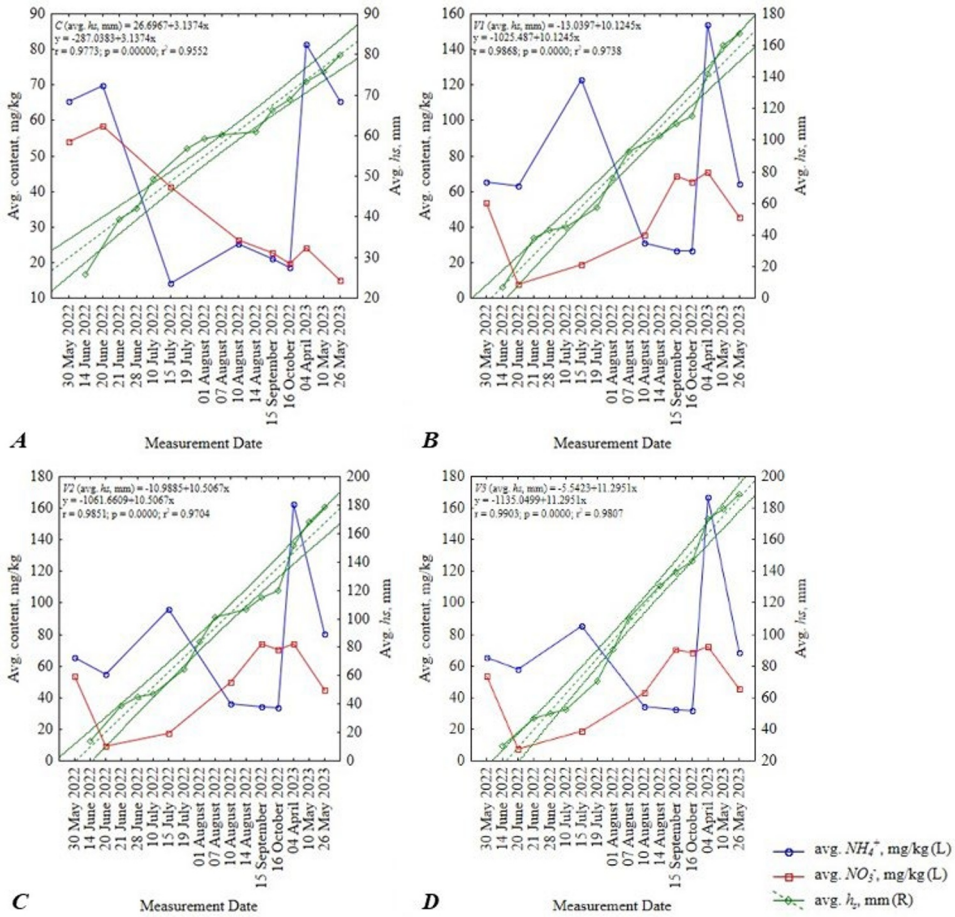

Fig. 3. The relationship between the dynamics of NH_4^+ and NO_3^- content and the stem height (h_s) of *P. sylvestris* seedlings: *A* – control *C*; *B* – variant *V1*; *C* – variant *V2*; *D* – variant *V3*.

It is also noted that the use of the fertilizer "Aquarin 5" [18] by spraying plants practically does not lead to an increase in the content of NO_3^- in the soil substrate.

Unlike control *C*, experimental variants *V1-V3* (Fig. 3 *B-D*) demonstrate a gradual increase in the content of NH_4^+ and NO_3^-, which leads to a more linear trend form, when the growth rate of h_s takes a wave-like shape, reaching a maximum growth rate of 17.38-30.71 mm in 8 days (*V3-V1*) by June 21, 2022 with a further decrease in 21 days to 1.64-2.54 mm (*V1-V3*) by July 10, 2022 and an increase in 24 days to 18.62-19.11 mm (*V1-V2*) by August 01, 2022 and in 31 days to 20.34 mm (*V3*) by August 07, 2022. Moreover, the *V3* variant is represented by a more stable growth rate of h_s. The maximum average height of the stem of seedlings in the studied variants reaches the size of 167.76±13.86 mm at CV = 56% (*V1*), 178.73±14.41 mm at CV = 55% (*V2*), 188.82±15.41 mm at CV = 51% (*V3*), respectively, which, with a relative deviation, is 52.4% more control *C* (*V1*), 55.4% (*V2*) and 57.7% (*V3*).

At the same time, it is known that nitrogen fertilizers have a positive effect on the increase in the length of needles [32], having a complex effect on the growth and development of seedlings, while comparing the planting material of CRS with an open root system (ORS), Russian authors from the Altai Territory note [33] that the former have a greater survival potential and preservation with a certain granulometric composition and specific physico-

chemical properties of soils, as well as when planting seedlings with tillage with the formation of a loosened gap [34].

Similar studies using a slowly soluble form of carbamide were conducted by authors from Kazan [20] and foreign authors from Kazakhstan [15, 26], but demonstrating a lower rate of growth in height of seedlings.

4 Conclusion

As a result of the conducted research, the annual dynamics of ammonium (NH_4^+) and nitrate (NO_3^-) nitrogen in the soil substrate of cells of containerized *P. sylvestris* seedlings with CRS was analyzed using agrochemical, biometric and mathematical-statistical methods, considering the peculiarities of the influence of a complex of mineral fertilizers that affect the increase in stem height, according to experimental variants in calculated dosage for the needs of coniferous crops consisting of potassium monophosphate KH_2PO_4, phosphorous flour $Ca_3(PO_4)_2$ and encapsulated carbamide $CO(NH_2)_2$ (*V1*) in variations with the urease inhibitor hydroquinone *1,4-dihydroxybenzene* (*V2*) and pine mulching with a layer of 0.5 cm from the surface of the soil (*V3*). As a result of the above, the following **conclusions** can be drawn:

1. The introduction of a complex of mineral fertilizers had a significant effect on the content of ammonium and nitrate nitrogen throughout the year of cultivation of *P. sylvestris* seedlings with CRS with prolonged release from encapsulated carbamide, the granules of which are coated with insoluble calcium silicate, the above-mentioned nitrogen compounds available to plants. The use of prolonged fertilizers, in particular carbamide, allows to cover the need for *P. sylvestris* in nitrogen throughout the growing season in forest nurseries of one of the main forest-forming breeds of Western Siberia, the Tyumen Region and the northern regions of Russia, in particular, the Khanty-Mansiysk Autonomous District – Yugra [10], which are part of the XI forest-seed region of the country.

2. The use of prolonged-acting carbamide has a positive effect on the increase in stem height of containerized *P. sylvestris* seedlings with CRS in general and the growth rate in almost direct dependence on the content of NH_4^+ and NO_3^- (R (*V3-V2*) = 0.99821-0.99988; *Multiple R* (*V3-V2*) = 0.99643-0.99977 *Adjusted R* (*V3-V2*) = 0.98928-0.99930; p (*V3-V2*) < 0.01525-0.05978), with a greater influence of the first (p (*V3-V2*) < 0.01633-0.05436). The use of slowly soluble forms of fertilizers with nitrogen content makes it possible to grow seedlings – 57.7% higher than control *C* – with a maximum stem height of up to 188.82±15.41 mm when using a complex of mineral nutrition fertilizers of the *V3* variant, in addition to potassium monophosphate and phosphorous flour, with the addition of encapsulated carbamide with hydroquinone and pine mulch with a layer of 0.5 cm.

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