1 Introduction

Intensive agriculture can significantly affect the movement of mineral components not only within the soil profile, but also between the soil, atmosphere and hydrosphere. The main processes involved are leaching of the components to the ground water, emission of greenhouse gases to the atmosphere and mineralization of organic matter (Hester et al., 1996; Ju et al., 2006; Cameron et al., 2013; Watanabe et al., 2018). All this results in soil deterioration and reduction in the environmental quality. Therefore, at present, various methods to retain nutrients in soils are being discussed in the scientific literature. One of such methods is application of biochar to agricultural soils. Biochar application can improve physical, chemical and biological properties of soils (Glaser et al., 2002; Juriga and Simansky, 2019), which has an indirect effect on increased yields (Liu et al., 2013). Also soil ammendment with biochar has many environmental benefits, including waste reduction, carbon sequestration, water resource protection (Dias et al., 2010, Igaz et al., 2018). Biochar can interact with microorganisms, soil mineral components, dissolved organic and inorganic compounds, roots, root exudates and gases (Grossman et al., 2010). Beneficial influences of biochar on soil properties resulted in biochars being used for soil fertilisation and reclamation (Beesley et al., 2011, Hale et al., 2013). However, some research show (Hale et al., 2012) that biochar can contain dangerous inorganic and organic contaminants making agricultural utilisation of biochars questionable.

The purpose of this study was to find out whether toxicity of a commercially produced slow pyrolysis biochar was affecting mineral nitrogen leaching from arable sandy loam Albic Luvisol and whether it was affecting water retention capacity of the soil and root growth at the very early stage of root development. Two laboratory experiments were conducted. The leaching experiment included four treatments: S – control soil; SB20 – soil + biochar (20 t ha-1); SF – soil + fertiliser (120 kg N ha-1); SB20F – soil + biochar (20 t ha-1) + fertiliser (120 N kg ha-1) with spring wheat (Triticum aestivum L.) sown. The leachate from the soil with the treatments was analysed for pH and available nitrogen in ammonium (NH4+) and nitrate (NO3-) forms. The water retention capacity of the soil after the leaching experiment was measured at water potentials between -5, and -300 kPa. In the second experiment watercress (Lepidium sativum L.) was used to study the root growth in the aqueous extracts from the studied soil with different amendments. The results of the conducted studies have shown that the tested biochar did not help in preventing NO3- or NH4+ leaching from the arable sandy loam Albic Luvisol. Water-holding capacity of the studied soil was increasing after biochar application only at water potentials of -5 kPa and -10 kPa that corresponded to the soil capillary water. The extract from the biochar had a stimulating effect on the watercress root growth.
2 Material and methods

2.1 Leaching experiment

Soil material from the ploughed layer (0–23 cm) of the arable sandy loam Albic Luvisol of North-Western Russia was collected in the field, dried and sieved through a 2 mm sieve in autumn 2018. The initial properties of the soil are given in Table 1. Pots with a volume of 2,400 cm³ and surface area of 201 cm² were used for the experiment in the laboratory. The experiment included four treatments in four replicates: 1) S – control soil; 2) SB20 – soil + biochar (20 t ha⁻¹); 3) SF – soil + fertiliser (120 kg N ha⁻¹); 4) SB20F – soil + biochar (20 t ha⁻¹) + fertiliser (120 N kg ha⁻¹). The soil (3,135 g) was mixed, according to the experiment setup, with biochar (40.2 g). The bulk density of the soil in all the pots was 1.3 g cm⁻³, which was the average bulk density of the topsoil in the field conditions. The soil water content was adjusted to field capacity (20%) with 627 ml of distilled water. The pots were left for three days for stabilisation and during that time the soil water content was kept at the field capacity level gravimetrically. Spring wheat (Triticum aestivum L.) of Ester variety was sown to all the experimental pots on the day three (ten seeds per pot). The soil in the pots was regularly watered to keep the water content at the field capacity level and the wheat was left to grow for seven weeks. When the wheat plants were strong with well-developed root systems, mineral fertiliser (2.41 g) was added to the soil in liquid form according to the experiment setup. Where no fertiliser was required, distilled water of the same volume was added to the soil. On the days 1, 3, 7, 14, 21, 28 and 41 after the fertilizer application high rates of water (400 cm³) were applied to the soil in the pots. This amount of water corresponded to 20 mm of rain which is high daily precipitation observed in the natural conditions of the area. The leachate was collected and was tested for acidity (pH) using the potentiometric method, for available nitrogen in ammonium form (NH₄⁺) and in nitrate form (NO₃⁻) using photometric method.

A fine fraction (<1 mm) of biochar commercially produced from the wood of broad-leaved trees (Table 2) and complex mineral fertiliser: (NH₄)₂SO₄ + (NH₄)₂HPO₄ + K₂SO₄ with concentration of N ~ 10%, were used in the experiment. The laboratory experiment was conducted at the average air temperature of 20 °C and the average air humidity of 30% under artificial light with twelve-hour-long day and twelve-hour-long night.

The water retention capacity of the undisturbed soil samples, collected from the pots with different treatments after the laboratory experiment, was measured using a pressure membrane apparatus (Soil moisture Equipment Corp., USA) at water potentials of -5,-10, -30, -50, -100 and -300 kPa according to the standard procedure.

2.2 Toxicity test

The aqueous extracts from the studied soil with different amendments (H₂O – distilled water/control, S – soil, B – biochar, SB10 – soil + biochar (10 t ha⁻¹), SB20 – soil + biochar (20 t ha⁻¹), SB10F – soil + biochar (10 t ha⁻¹) + fertiliser (120 kg N ha⁻¹), SB20F – soil + biochar (20 t ha⁻¹) + fertiliser (120 N kg ha⁻¹)) used in the laboratory experiment (made in ratio 1 : 5 for the soil with the amendments and 1 : 25 for the biochar) were prepared by shaking the soil/water mixtures for two hours. The extracts were tested for toxicity using watercress (Lepidium sativum L.) seeds. For the test the seeds were put into petri dishes (twenty per dish) with 5 ml of the studied extracts and left in the thermostat with constant temperature of 23 °C for seven days. After that the length of the longest root of every seed was measured and average values were calculated.

Gross content of heavy metals was measured in the soil, biochar and fertiliser using atomic adsorption spectrometer; 3,4-benzo(α)pyrene – with liquid chromatograph Lumachrom (Rus), petroleum products – with liquid analyzer Fluorate-02-3M (Rus) and Hg concentration – with PA-915M, Rus.

| Table 1 | Initial properties of the sandy loam Albic Luvisol |
|---------|-----------------------------------------------|
| FC (%)  | BD (g cm⁻³) | pH₄Cl | C₄₁₈ (%) | N₄₁₈ (%) | N₄₁₈ (mg kg⁻¹) | P₄₁₈ (mg kg⁻¹) | K₄₁₈ (mg kg⁻¹) |
|---------|-------------|-------|----------|---------|----------------|----------------|----------------|
|          |             |       |          |         | 20             | 1.3            | 6.7            | 1.9            | 0.14           | 15.7           | 237            | 131            |
| FC – field capacity; BD – bulk density; pH₄Cl – soil pH; C₄₁₈ – total carbon content; N₄₁₈ – total nitrogen content; N₄₁₈ – mineral nitrogen content; P₄₁₈ – exchangeable phosphorus content; K₄₁₈ – exchangeable potassium content |

| Table 2 | Chemical properties of biochar |
|---------|--------------------------------|
| C₄₁₈ (%) | N₄₁₈ (%) | H (%) | O (%) | C : N | H : C | O : C | pH₄Cl | W (%) | Ash (%) | S (m² g⁻¹) | P (%) |
|---------|---------|-------|-------|-------|-------|-------|-------|-------|-------|---------|--------|-------|
|          |         | 78.6  | 0.3   | 5.2   | 4.2   | 302   | 0.06  | 0.05  | 7.21  | 3.92    | 21.4   | 16.2  | 81    |
| C₄₁₈ – total carbon content; N₄₁₈ – total nitrogen content; H – total hydrogen content; O – total oxygen content; pH – acidity; W – water content; Ash – ash content; S – specific surface area; P – porosity |

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2.3 Statistical analysis
The Shapiro-Wilk test was used to find out whether the obtained results were normally distributed and as the distribution was not normal, the nonparametric statistics was used for the data analyses (Kruskal-Wallis test) and Mann Whitney U-test.

3 Results and discussion
3.1 Leaching experiment
Leachate pH from the soil with the studied treatments was slightly acidic to almost neutral and was, in average, 6.32, 6.33, 6.14 and 6.08 for S, SB20, SB20F and SF treatments, respectively. During the entire experiment the lowest pH values were measured in the leachate from the soil with SF treatment, while the highest – from the untreated soil or the soil with SB20 treatment with no significant difference between the latter two. The differences in leachate pH values between fertilised and unfertilised treatments were significant for three weeks after the beginning of the experiment. After that the acidity of the leachate was about the same from the soil with all the studied treatments (Figure 1). It is a well-known fact that mineral fertiliser application to soils can result in soil and soil solution acidification. In our earlier short-term experiment with more acidic soil (Abramova, Buchkina, 2022) we have shown (the data are not provided), that leachate pH values were significantly increasing after high rate of biochar application (20 t ha⁻¹) to the fertilised soil. Based on the results of this experiment we can see that for the less acidic soil the increase was not always statistically significant and in 41 day after the beginning of the measurements, by the end of the experiment, the leachates from all the studied treatments had the same pH values. The results of our both studies are in line with the results received by Zhang et al. (2019) who have shown that the most pronounced effect of biochar application on soil pH increase was found for most acidic soils (in their experiment it was yellow-brown soil and fluvo-aquic soil). For soils with originally higher pH values the effect of biochar application on soil pH increase was often insignificant.

The experimental soil was not rich with mineral nitrogen at the beginning of the experiment and by the time of the leaching experiment most of the mineral nitrogen was consumed by the developing plants. That was, presumably, the main reason why leaching of both NO₃⁻ and NH₄⁺ from the unfertilised soil with or without biochar was very low compared to the two fertilised treatments (Figure 2).

The experimental soil was amended with mineral fertiliser containing N in ammonium form and that must have been the main reason of the significant increase in the concentration of NH₄⁺ in the leachate from the fertilized treatments compared to the unfertilized. Concentration of NH₄⁺ in the leachate was quite high for only a very short period of time: on the seventh day of measurements the concentrations of this ion in the leachate was very low and did not differ from the concentration of it in the

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Figure 1 Acidity (pH values) of the leachate during the experiment
S – soil (control), SB20 – soil + biochar (20 t ha⁻¹), SB20F – soil + biochar (20 t ha⁻¹) + fertiliser (120 kg N ha⁻¹), SF – soil + fertiliser (120 kg N ha⁻¹)
leachates of unfertilised soil with or without biochar. Biochar, applied into the fertilised soil, did not stop NH$_4^+$ from leaching in long-term, which is similar to our earlier findings (Abramova, Buchkina, 2022).

Concentration of NO$_3^-$ in the leachate of two fertilised treatments was slowly increasing for three weeks after the beginning of measurements and then decreasing by the end of the experiment. The increase was probably related to the nitrification process and conversion of NH$_4^+$, applied to the soil with the mineral fertiliser, to NO$_3^-$ while the decrease – with N-consumption by the plants. The fertilised soil with biochar (SB20F) was always containing smaller amounts of NO$_3^-$ but the difference with the SF treatment was almost always insignificant due to high variability between the replicates.

It was shown earlier that NO$_3^-$ leaching under the effect of biochar was significantly dependent on soil texture (Ghorbani et al., 2019) and affected by soil hydraulic conductivity (Li et al., 2018): the more permeable was the soil the higher concentrations of nitrate were found in the leachate. The soil in our experiment was of sandy loam texture and this probably was the main reason why biochar did not have any significant effect on NO$_3^-$ removal from the soil with the leachate.

### 3.2 Water retention capacity

The results of the study have shown that the water retention capacity of the soil, collected from the experimental pots when the laboratory experiment was over, in the range of moisture potentials from -5 to -300 kPa (plant-available water), changed from 20.4 to 13.3%,
while for the biochar treated soil (SB20) it changed from 23 to 14% (Figure 3). It was found that the significantly ($p < 0.05$) higher content of water was retained by the soil with SB20 and SB20F treatments at a moisture potential of -5 kPa and -10 kPa. As the moisture potential decreased to -300 kPa, the differences between the biochar treated and untreated soil were becoming insignificant. The results are similar with the data of the authors working with soils of the same area (Rizhia et al., 2015, Mukhina et al., 2019) – biochar, due to its porous structure and high surface area, increases the water-holding capacity of the soil in the range of plant-available water. Fertilisation of the soil (SF treatment) resulted in a slight decrease of the water-holding capacity of the studied soil but the differences were not statistically significant compared to the unfertilised soil.

### 3.3 Toxicity test

The results of the toxicity test (Figure 4) have shown that the extract of pure biochar (B) was significantly stimulating watercress root growth: the roots in this treatment were in average 2.2 cm longer than the roots under the control (H$_2$O) treatment and 1.2–4.0 cm longer than in any other treatment of the experiment. The extracts from the soil mixed with both rates of the biochar (SB10 and SB20) had less pronounced stimulating effect on the root growth and the effect was statistically insignificant compared to the control and the pure soil extract. Under the treatment SB20F the root growth reduction was found: the average length of the roots in this treatment was 4 cm shorter than in the case of B treatment. The difference was statistically significant compared to the B, SB10 and SB20 treatments.

Gross content of heavy metals, 3,4-benzopyrene and petroleum products in the soil, fertiliser and biochar, used in the experiment, are given in Table 3. Concentration of these chemical substances in the soil did not exceed the threshold limit values accepted in Russia. In the fertilizer the content of petroleum products exceeded the threshold limit values while in the biochar Cd, Zn and petroleum products contents were higher than the threshold limit values.

Rogovska et al. (2012) evaluated the use of standard germination tests as an indicator of biochar quality while Oleszczuk et al. (2013) was studying toxicity of different biochars using different living organisms including watercress. The authors (Rogovska et al., 2012) suggested that some biochars contained water-soluble organic compounds (PAHs) that can inhibit seed germination. It was found that aqueous biochar extracts were quite effective at identifying biochars that contain phytotoxic compounds. It was also found that biochars produced at relatively low temperatures ($<500$ °C) had the lowest total concentrations of PAHs and were more suitable for agronomic use while biochar produced by high-temperature gasification and pyrolysis contained compounds that suppressed seedling growth. Solaiman et al. (2012) in their paper highlighted that biochars can be produced from a wide range of organic materials with varying nutrient, heavy metals and PAHs concentrations. They recommended, before making irreversible applications of biochar to soil, to conduct preliminary
ecotoxicological assessment of biochars to identify potential toxicity of biochars on seed germination and early plant growth. In our experiment the biochar was commercially produced by slow pyrolysis from wooden material at relatively low temperature. According to the results of the germination test this particular biochar was not in any way restricting the growth of watercress roots and was suitable to be used in agriculture for soil amelioration purposes.

4 Conclusions
The results of the conducted experiments have shown that the studied biochar did not help in preventing NO₃⁻ or NH₄⁺ leaching from sandy loam Albic Luvisol and did not significantly change the leachate acidity during the six-week period following the mineral fertiliser application.

Water-holding capacity of the sandy loam Albic Luvisol of North-Western Russia studied in the experiment was increasing after biochar application only at water potentials of −5 kPa and −10 kPa what corresponds to the soil capillary water.

The results have also shown that the extracts from the commercially produced biochar studied in the experiment had a stimulating effect on the watercress root growth despite of the fact that concentration of Cd, Zn and petroleum products in the biochar was higher than threshold limit values for sandy soils. Content of 3,4-benzopyrene in the studied biochar was very low and not limiting the use of this material in agriculture.

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Table 3  Gross content of heavy metals, 3,4-benzopyrene and petroleum products in the soil, fertiliser and biochar, used in the experiment

| Parameter                                      | Cd     | Ni     | Cu   | Pb   | Zn   | As    | Mn   | Hg   | 3,4-benzo[α]pyrene | Petroleum products |
|-----------------------------------------------|--------|--------|------|------|------|-------|------|------|-------------------|------------------|
| Soil                                          | 0.16   | 1.2    | 4.0  | 3.1  | 24.9 | 0.72  | 227.9| 0.026 | 0.008             | 39               |
| Fertiliser                                    | 0.54   | 2.1    | 1.8  | 0.8  | 6.1  | <0.05 | 188.7| 0.146 | <0.005            | 1,014            |
| Biochar                                       | 1.40   | 0.5    | 4.2  | <0.1 | 142.8| <0.05 | 731.2| <0.005| <0.005            | 3,799            |
| Threshold limit value for sandy loam soils    | 0.5    | 20     | 33   | 32   | 55   | 2     | 1,500| 2.5   | 0.02              | 500              |
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