The productivity of spring wheat, the streams of nitrogen and agroecosystem stability with the application of fertilizers and biopreparation

Alexey Alferov*  
1Pryanishnikov All-Russia Research Institute for Agrochemistry, Moscow 127550, Russia

Abstract. To form spring wheat grain at the insufficient bringing of nitric fertilizers the additional sources of nitric feed are necessary. They can be filled in due to the inoculation of seeds with microbial preparation of Rhizoagrin (RA) created on the basis of associative bacteria. The inoculation of seeds of RA provided the increase of grain mass by 15 %. Biomass of spring wheat on sod-podzolic soil largely forms due to soil nitrogen, the share of which reaches four-fifths of the total removal of the element when using mineral fertilizers. Inoculation increases the fertilizer nitrogen use efficiency by 4.5% and reduces losses by 7%; there is a trend to increase the immobilization of N fertilizers. Stability of an agroecosystem is characterized by nitrogen flows. The amount of mineralized nitrogen depending on the fertilizer amounts to 17.4-18.0 g/m², while the amount of reimmobilized nitrogen is 4.4-4.9 g/m² and net-mineralization (N-M) is 13.1 g/m². The inoculation of seeds with Rhizoagrin does not significantly affect the processes of mineralization (M) and reimmobilization (RI) in anything of nitrogen in the soil. The use of nitrogen fertilizer brings the agroecosystem into a state of resistance (the maximum threshold limit of exposure (RI : M = 25% and N-M : RI = 3)). On average, over the years of research, inoculation of seeds with Rhizoagrin did not change the indicators of an agroecosystem's stability with the application of the fertilizers.

1 Introduction

All existing agroecosystems, despite the existing diversity, have such common characteristics as development, flows, energies, food chains, management, cycles or cycles of biophilic elements. As a result of anthropogenic impact, soil regimes change, and the agroecosystem it is of self acquires a number of specific characteristics [1]. In existing agroecosystems, the biosphere function of soils is mainly associated with the formation of biogeochemical cycles of nutrients - nitrogen and carbon [2]. Experimental studies evaluating the functioning, conditions not only of individual components, but also of the ecosystem as a whole, were

*Corresponding author alferov72@yandex.ru
carried out using different methods [3-11]. Of particular interest to scientists are instruments developed based on various optical methods [12–20] and nuclear magnetic resonance phenomena [21–28]. Such an integrated approach makes it possible to comply with studies in the global concept for determining the state of the ecosystem [29-31].

The goal of the present work is to determine the effects that fertilizers and biopreparations of rhizospheric diazotrophs have on the plant’s use of nitrogen, including biological, of fertilizers, and the soil nitrogen, and on agroecosystem stability, and productivity of spring wheat.

2 The methods

The effect of rhizospheric diazotroph biopreparations (Rhizoagrin) on nitrogen balance and the role that various sources of plant nitrogen nutrition play in the establishment of a spring wheat crop was studied in a microfield experiment according to the scheme presented in Table 1. The sown spring wheat seeds Triticum aestivum L. of Zlata cultivar were inoculated with biopreparation from rhizospheric bacteria [32, 33]. The experiment was conducted in 2014–2018 in 0.018-m² in area bottomless containers with fourfold replication on sod-podzolic, light, loamy soil from Smolensk oblast featuring the following agrochemical properties: 1.98-2.04% humus content (after Tyurin), 5.1 - 5.2 pH KCl, and 57.6-67.8 and 153.1-161.4 mg/kg of soil, respectively, active P₂O₅ and K₂O content (after Kirsanov). Potato was a predecessor of spring wheat in the field. Fertilizers were introduced while packing the containers with soil. For nitrogen fertilizer, tagged fertilizer was used in the form of 15NH₄15NO₃ salt enriched to 54.04 at % at a dose of 81 mg/container, which corresponds to 45 kg N/ha. Double superphosphate and potassium chloride were applied as the control at doses of Р₆₀К₆₀. On the day of sowing, spring wheat seeds were treated with Rhizoagrin biopreparation (RA) formulated based on the 204 strain assigned to Agrobacterium radiobacter.

To calculate the balance and flow of soil nitrogen in the soil-microorganism-fertilizers-plants-atmosphere system, we utilized the data obtained using the 15N isotope and relying on proportionality in distribution of tagged and mineralized soil nitrogen [1, 3, 32]. The calculations were performed according to the following formulas:

\[
N_c = N_a \cdot \frac{15N_c}{15N_a}; \quad N_d = N_a \cdot \frac{15N_d}{15N_a};
\]
\[
M = N_a + N_b + N_c + N_d; \quad H-M = N_a + N_b + N_d;
\]
\[
RI = M - N-M,
\]

where nitrogen flows are (a) used by plants, (b) residual mineral in the soil, (c) immobilized, (d) losses, (M) mineralized nitrogen, (N-M) net mineralized nitrogen, and (RI) reimmobilized nitrogen.

The weather conditions varied throughout the study years. The greater part of the 2014 growing season was characterized by increased temperature and extremely uneven pattern of precipitation alternating between the periods of dry weather and falling of showers with the hydrothermal coefficient (HTC) of 1.33. The growing season of 2015 was dry in terms of the amount of precipitation with the temperatures above the long-term annual average and HTC of 0.64. In 2016, the air temperature was above the climate normal by 1°C with considerable falling of precipitation and HTC = 1.63. The growing season of 2017 was characterized by the lack of precipitation with temperatures above the climatological normal. In 2018, weather
conditions were characterized by an uneven distribution of precipitation - a deficiency in May, August and a significant excess in July (267% to the monthly rate) at an average monthly temperature of the growing season 1.1 °C higher than the climatic norm, HTC = 1.72

3 The results and discussion

The grain mass of spring wheat varied from fertilizers and the use of RA, and also varied due to changing hydrothermal conditions during the growing season (Table 1). Against the background of the application of PK fertilizers, the grain weight was 3.66 g / bottomless containers. Due to the improvement of the nitrogen nutrition of plants with the introduction of the fertilizer of the same name, the grain mass increased to 5.32 g / bottomless containers, or the increase reached 45%. When sowing on the PK-control with inoculated seeds, the increase in grain weight was 15.5%, its variation under changing weather conditions of the growing season was: in hot and arid 6.7%; with excess moisture 16.3%, under optimal conditions 19%. On average, over five years, the increase from inoculation of RA seeds was less than from the introduction of nitrogen fertilizer, which is associated with a low content of nitrogen compounds available for plants in the soil [30]. When sowing inoculated seeds in NPK grain mass increase amounted to the P60K60 (Control) – 2.78, to option NPK – 1.12 to option PK + RA – 2.21 g / bottomless containers (Table 1). That is, the effect of the biological product is significantly more effective when sowing inoculated seeds against the background N45 due to the better supply of plants with nitrogen in the initial phases of development, when the flow of horse secretions of plants into the rhizosphere is minimal [32, 33].

Table 1. Spring wheat productivity and nitrogen content when applying nitrogen fertilizer and inoculating seeds with Rhizoagrin

| Option                          | weight       | Cecon | Content of N |
|---------------------------------|--------------|-------|--------------|
|                                 | g/ bottomless containers |       | %            |
| 1. P60K60 (Control)             | 3.66         | 6.22  | 0.37         | 1.96 | 0.38 |
| 2. Control + N45                | 5.32         | 8.96  | 0.37         | 2.09 | 0.42 |
| 3. Control + Rhizoagrin (RA)    | 4.23         | 6.87  | 0.38         | 2.04 | 0.41 |
| 4. Control + N45+ RA            | 6.44         | 9.39  | 0.41         | 2.12 | 0.42 |
| HCP05 for partial differences   | 0.59         | 0.94  | 0.03         | 0.07 | 0.04 |

Cecon - Coefficient of economic efficiency of the crop

The mass of spring wheat straw, as well as grain, changed against the P60K60 (Control) under various meteorological conditions of the growing season and averaged 6.22 g / bottomless containers. Its maximum amount was obtained under optimal weather conditions, the lack of moisture, as well as its excess, reduced it by 25-30%. The use of N-fertilizer contributed to the increase in the mass of straw by 44%. Inoculation of RA seeds increased straw mass by 10% on the average. In all the years of the experiment, improving the nutritional conditions of plants from nitrogen fertilizer and biological product had a positive effect on the mass of straw, which increased by 51% on the average.

Coefficient of economic efficiency of the crop (Cecon) ranged from 0.37 to 0.42. The use of chemicals and biologization provided only a tendency to increase the proportion of grain
in the general biological crop when sowing inoculated seeds against the background of N-fertilizer.

Nitrogen accumulation was calculated through determining its total concentration in grain and straw, while the sources participating in the crop establishment were identified using nitrogen isotope analysis 15 [33]. Due to the improvement of nitrogen nutrition with the use of nitrogen fertilizers and biological products, the nitrogen content in the grain increases by 2.04-2.12%. The spring wheat crop (grain mass + straw mass) was developing due to the soil nitrogen alone in the PK control setting. With the application of N-fertilizers, the total removal of nitrogen, including the soil nitrogen and N fertilizers, by crop grew by 57% (Table 2). Inoculation of seeds with Rhizoagrin (RA) promoted a 17% increase in the total nitrogen accumulation compared with the PK-control setting and a 4.5% increase in its use efficiency by plants from N fertilizers toward the crop establishment. As a result of soil microorganism activity, application of N fertilizers gives rise to the processes of organic matter mineralization, leading to the formation of "extra" nitrogen [33], which is similarly used by the plants toward the crop establishment. The use of the 15N isotope made it possible to isolate the fraction of “extra” nitrogen, which is 13% with the N fertilizer application and 12% with N-fertilizer + RA of the total removal.

**Table 2.** Fertilizer and soil nitrogen consumption in spring wheat

| Test option                  | Total removal, g/m² | Including N of fertilizers | Additional N | FNUE       |
|------------------------------|---------------------|-----------------------------|--------------|------------|
|                              | g/m² | %    | g/m² | %    | g/m² | %    | %    |          |
| 1. P6K6 (Control)            | 5,3   | –          | 5,3  | 100 | –          |           |           |          |
| 2. Control + N₄₅             | 8,3   | 1,9        | 6,4  | 77  | 1,1        | 13        | 42,2      |          |
| 3. Control + RA              | 6,4   | –          | 6,4  | 100 | 1,1        | 17        | –          |          |
| 4. Control + N₄₅+ RA         | 9,4   | 2,1        | 7,3  | 78  | 2,0        | 21        | 46,7      |          |

FNUE is fertilizer nitrogen use efficiency.

The share of additional nitrogen in the wheat crop establishment reached 17% due to the seed inoculation with Rhizoagrin and 21% when sowing the seeds inoculated with the biopreparation in the presence of N fertilizer.

Calculation of the sizes of associative nitrogen fixation in crops of a cultivated plant allows us to evaluate the agrotechnical technique.

The amount of nitrogen fixed by *Agrobaktcnum radiohakter* (strain 204) (Rhizoagrin) in the sod podzolic soil was calculated applying the modified difference method with the use of 15N in fertilizers. In the planted spring wheat, seed inoculation with the biopreparation ensured fixation of 1.1 g/m² of atmospheric nitrogen on average. The application of nitrogen fertilizers did not have a significant effect on a decrease in the amount of the associated nitrogen (0.9 g/m²) use.

Calculation of nitrogen balance of applied fertilizers (Table 3) over the years of research, on average, showed that about 42% of ammonium nitrate nitrogen was used by spring wheat plants and was immobilized in a layer of 0-20 cm 29% NH₄NO₃.

Nitrogen losses are an important item of its balance. Nitrogen losses from soil through conversion to the gaseous forms and leaching to the underlying layers are the primary cause of a decrease in the efficiency of nitrogen use by plants and effectiveness of fertilizers in all the national climate zones [32, 33]. Unaccounted fertilizer nitrogen losses amounted to 31 %
due to leaching to the underlying soil layers and gaseous losses (molecular nitrogen, its oxides, and ammonia) in our experiment.

Table 3. Fertilizer nitrogen balance in spring wheat cultivation

| Option          | N used by plants | Fixed in the 20 cm soil layer | Losses from the 20 cm soil layer |
|-----------------|------------------|-------------------------------|---------------------------------|
| P<sub>60</sub>K<sub>60</sub> + N<sub>45</sub> | 1.9/42.2         | 1.2/26.7                      | 1.4/31.1                        |
| P<sub>60</sub>K<sub>60</sub> + N<sub>45</sub> + RA | 2.1/46.7         | 1.3/28.9                      | 1.1/24.4                        |

Inoculation of seeds with Rzizoagrin increased the use of nitrogen from NH<sub>4</sub>NO<sub>3</sub>, by 4.5%, did not significantly change its fixation in the 20-cm soil layer, and decreased the nitrogen losses primarily associated with gaseous forms from 31 to 24% [30, 33].

A systematic analysis of soil nitrogen transformation revealed that the functioning mode of the agroecosystem with the use of fertilizers and Rzizoagrin depended on the balance of the flows of net-mineralized (N–M) and (re) immobilized (RI) nitrogen in a pool of mineralized soil nitrogen (M). As a result of studies using isotope 15N revealed the flow of nitrogen fertilizer and soil (fig. 1, Table 4). During the growing season, the amount of mineralized soil nitrogen was 17.4 g / m<sup>2</sup>, while the content of reimmobilized nitrogen is 4.3 g / m<sup>2</sup>. Inoculation of seeds did not significantly affect the processes of mineralization and reimmobilization; a positive tendency of their growth was noted (+ 3.5% mineralization and + 14.0% immobilization).

Fig. 1. Indicators of the integrated assessment of the functioning of the soil-plant system during the cultivation of spring wheat.

Table 4. Integral estimate indicators of the soil-plant system functioning during the spring wheat growing

| Option          | P<sub>60</sub>K<sub>60</sub> + N<sub>45</sub> |
|-----------------|--------------------------------------------|
|                 | no inoculation | inoculation |
| RI: M, %        | 24.7          | 27.2        |
| N-M : RI        | 3.0           | 2.7         |
Based on the integral estimate, the agroecosystem on sod-podzolic light loamy soil functions in a regime of resistance (maximum threshold limit of exposure) with the application of ammonium saltpeter (RI: M = 25%, N-M : RI = 3), while merely exhibiting a trend of increase in its stability with the use of Rhizoagrin.

The stability of agroecosystems using fertilizers is influenced by meteorological conditions during the cultivation of spring wheat. The study showed that the stability of the agroecosystem, or its ability to maintain structure and functions (indicator RI: М, %), in changing weather conditions when applying nitrogen fertilizer, it was higher at optimal air temperature and humidity (HTC =1.0-1.2). Arid (HTC <0.9), as well as humid (HTC> 1.6) weather conditions similarly reduce the agroecosystem stability, which is supported by the regression equation, i.e., strong relationship in terms of closeness and curvilinear in shape:

\[ Y = -27.348x^2 + 47.768x + 15.352 \]

where \( Y \) is the ratio of reimmobilized nitrogen to the amount of mineralized, %; \( x \) is HTC over the growing season; \( r_{yx} \) is a correlation coefficient; \( n = 9 \).

When inoculating seeds with Rhizoagrin and application N45 the dependence does not change much:

\[ Y = -28.731x^2 + 52.874x + 11.612 \]

Thus, the formation of biomass of spring wheat on sod-podzolic soil is carried out mainly due to soil nitrogen, whose share reaches 4/5 of the total removal of the element with the use of mineral fertilizers, the participation of “extra” nitrogen in the production process is limited (13% of the total removal). Under these conditions, seed inoculation with Rhizoagrin biopreparation ensures the additional use of nitrogen by spring wheat in the amount of 9-11 kg/ha.

The regime of agroecosystem functioning with the application of nitrogen fertilizer and Rhizoagrin depends on a balance between the flows of net mineralized and (re)immobilized nitrogen in the soil. The agroecosystem functions in a regime of resistance (maximum threshold limit) on the sod-podzolic light loamy soil. With the introduction of nitrogen fertilizer, the agroecosystem functioning considerably changes with the meteorological conditions over the growing season. Its stability is reduced in dry, as well as humid, weather conditions, which is confirmed by the regression equation, i.e., a strong relationship in terms of closeness and curvilinear in shape (\( r_{yx} = 0.82, t_\eta > t_\alpha \)).

4 Conclusions

1. On sod-podzolic soils with an average humus content, the leading role in increasing the yield of spring wheat grain belongs to mineral fertilizers, the increase from the use of N45 is not less than 45%. The increase from Rhizoagrin on average is 15.5%.

2. Nitrogen of mineral fertilizer in the soil is included in cyclic mineralization-immobilization transformations. High immobilization of nitrogen fertilizers in sod-podzolic soil is a key process that determines the stability of the agroecosystem. Absolute sizes of immobilization in structure of balance of labeled nitrogen of mineral fertilizer make 26-29% from the brought quantity. Inoculation of wheat seeds with Rhizoagrin does not affect the immobilization of nitrogen fertilizers.

3. The mode of functioning of the agroecosystem in the application of nitrogen fertilizer and Rhizoagrin depends on the balance of flows of net mineralized and (re)immobilized...
nitrogen in the soil. On sod-podzolic loamy soil with the use of ammonium nitrate agroecosystem operates in the mode of resistance (maximum permissible level). In years with increased moisture agroecosystem goes into a zone of repression. Significant differences in the indicator of functioning of agroecosystems between variants with inoculation of spring wheat seeds with and without Rhizoagrin were not established.

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**Compliance with ethical standards**

The author declares that he has no conflict of interest. This article does not contain any studies involving animals or human participants performed by the author.

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