Fluxes of $^{15}$N, soil nitrogen in sod-podzolic soil in spring wheat crops under inoculation with Rhyzoagrin

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Abstract. In a microfield experiment using a labeled nitrogen $^{15}$N fertilizer on sod-podzolic light loamy soil for 11 years during the cultivation of spring wheat and the use of a biological product based on a strain belonging to the genus Agrobacterium radiobacter (strain 204), nitrogen flows in the fertilizer – soil – plant system were studied. The utilization rate of spring wheat labeled with $^{15}$N ammonium nitrate averaged 40% of the amount applied. The absolute size of the immobilization in the structure of the labeled nitrogen balance of the mineral fertilizer was 27-30%. Inoculation with Rhyzoagrin does not significantly affect the nitrogen immobilization of the mineral fertilizer. The gaseous losses of labeled nitrogen of the mineral fertilizer were 33% of those applied to the background of the phosphorus and potash fertilizers and decreased with inoculation with Rhyzoagrin to 29%. The use of an integral assessment showed that the agroecosystem functioned in the resistance mode on sod-podzolic light-loamy soil when using mineral nitrogen fertilizer. The dependence of the indicator RI: M, % of the HTC (hydrothermal coefficient) expressed by the equation $y = -24.506x^2 + 43.462x + 15.251$ at $R^2 = 0.7416$ is established.

1. Introduction

The soil is a self-regulating system, which is characterized by the desire for homeostasis and a certain dynamic balance of nitrogen content, therefore, its natural response to fertilization is to activate the processes of the intrasoil transformation cycle of this element, including its mineralization and immobilization [1].

One of the important indicators characterizing the agroecosystem, the management of its development, as well as assessing the anthropogenic impact in the process of agricultural use is “sustainability”. This property of the agroecosystem is determined not only by structural changes occurring in it, but by the ability to manage them. The complexity of material flow management is largely related to soil and climatic conditions (soil type, meteorological conditions). The most acute problem of nitrogen cycle management is in agrophytocenoses on soddy-podzolic soils, which are not only prone to erosion due to low humus content, its fulvate type, significant nitrogen losses both in the process of soil erosion during a leaching type of water regime, and as a result of denitrification - nitrification, a high degree of soil nitrogen mineralization and a low ability to immobilize fertilizer nitrogen [2-4]. This reduces the share of nitrogen participation in the production process of crops grown, affects their productivity and leads to a decrease in product quality. It is possible to estimate nitrogen fluxes in an agroecosystem using a stable nitrogen isotope $^{15}$N [5, 6].
The aim of the work is to determine the parameters of the soil nitrogen cycle and mineral fertilization in the agrophytocenosis of spring wheat during inoculation with Rhyzoagrin.

2. Research methodology

The study was carried out for 11 years (1998-2000 and 2014-2019) in the Smolensk and Moscow regions on sod-podzolic light loamy soil with ammonium nitrate (\(^{15}\)NH\(_4\)\(^{15}\)NO\(_3\), N\(_{an}\)), enriched with labeled nitrogen \(^{15}\)N (54.04 at. %) in a microfield experiment in vessels without a bottom with an area 0.018 m\(^2\) grown 10 spring wheat plants (Triticum aestivum L.) varieties Zlata. The predecessors of spring wheat are potatoes and buckwheat. Ammonium nitrate was introduced in the spring when the vessels were packed at a dose of 180 mg per vessel, which corresponds to 4.5 g/m\(^2\), or 45 kg N/ha.

Agrochemical indicators of the soil of the experimental site: humus content (according to Tyurin) – 1.91-2.04%, movable forms P\(_2\)O\(_5\) and K\(_2\)O (according to Kirsanov) – respectively 125.1-140.8 and 129.0-166.0 mg / kg soil, pH\(_{KCl}\) 5.6-5.7; the experiment was repeated 4 times. In order to better perceive the results of the experiment, the corresponding indicators are given in terms of g / m\(^2\).

Rhyzoagrin – created on the basis of a strain belonging to the genus Agrobakterium radiobakter (strain 204) and are characterized by a number of advantages: they form active associations between plants and microorganisms, are able to fix atmospheric nitrogen and convert it into an easily assimilated form of nitrogen-containing compounds. Their high competitiveness in relation to phytopathogenic fungi increases the resistance of plants to diseases.

In soil and plant samples: N\(_{\text{total}}\) – dry combustion method according to Dumas on the Flesh EA model 1112 analyzer, atomic % \(^{15}\)N – on a mass spectrometer "Delta V" (Germany). Nitrogen fluxes in the agroecosystem were calculated using the method [7]. Calculation of the hydrothermal coefficient according to G.T. Selyaninova (HCA) was determined as the ratio of the amount of precipitation (O) in mm for the period with average daily air temperatures above 10 °C to the sum of temperatures (∑\(t\)) during the same time, reduced by a factor of 10, i.e., the HCA = \(O / 0.1 \sum t\) [8].

Statistical analysis of the experimental data was carried out by the dispersion method according to the model of a two-factor field experiment using the EXCEL and STATISTICA programs. The significance of differences was assessed by Fisher's F-test.

The meteorological conditions during the study period were significantly different. Hydrothermal coefficient according to G.T. Selyaninov (HCA) ranged from 0.64 to 1.78.

3. Results and discussion

As a result of the studies carried out, it was found that on soddy-podzolic soil, the nitrogen pool in spring wheat crops is formed due to soil nitrogen. With the introduction of nitrogen fertilizers, the mineralization of soil organic matter increases and therefore the availability of soil nitrogen to plants increases. By the time of harvesting in the variants with nitrogen fertilizers, the assimilation of soil nitrogen increased by almost 1.2 times in comparison with the variant P\(_{30}\)K\(_{45}\), and its share in the removal was 84% (Table 1). Using NH\(_4\)NO\(_3\) nitrogen consumption by plants increases 1.4 times. The maximum amount of nitrogen in fertilizers and soil was consumed by the spring wheat plant when applying ammonium nitrate and inoculating seeds with Rhyzoagrin (1.5 times). The calculated share of additional nitrogen ("extra" -nitrogen) over the years of research under different weather conditions varied from 5 to 22% and averaged 11% of the removal when applied NH\(_4\)NO\(_3\) and 8% with the combined use of nitrogen fertilizer and biological product.

Inoculation of seeds with Rhyzoagrin in spring wheat crops provided fixation of 1.0 g / m\(^2\) nitrogen air. The action of diazotrophs is subject to the influence of soil temperature and moisture; therefore, fluctuations in the fixation of atmospheric nitrogen by microorganisms were observed over the years from 0.4 in a dry year to 2.0 g / m\(^2\) in humidification conditions are close to optimal. The application of nitrogen fertilizers somewhat reduces the use of associative nitrogen, the fixation volume of which was 0.7 g / m\(^2\).
Table 1. Nitrogen consumption of fertilizer and soil in spring wheat.

| Variant                | Total takeaway N, g/m² | N of fertilizer | N of soil | Extra nitrogen | Associative N, g/m² |
|------------------------|------------------------|-----------------|-----------|----------------|--------------------|
|                        | 1          | 2               | 1         | 2               | 1          | 2               |
| 1. P₃₀K₄₅—background   | 7,95      | –               | 7,95     | 100             | –         | –               |
| (B)                    |           |                 |          |                 |           |                 |
| 2. B+ Rhyzoagrin       | 8,94      | –               | 8,94     | 100             | –         | 0,99            |
| 3. B + N₄₅             | 10,95     | 1,80            | 9,15     | 84              | 1,20      | 11              |
| 4. B + N₄₅ + Rhyzoagrin| 11,68     | 1,84            | 9,84     | 84              | 0,90      | 8               |

\(P\), \% 3
LSD₀₅, g/m² 0,47

Note: in column 1 - g/m², 2 – %

Balance calculations (Table 2) showed that during inoculation with Rhyzoagrin there is a positive trend in the use of mineral fertilizer nitrogen by spring wheat. Utilization factor (UF) for spring wheat \(^{15}\)N ammonium nitrate varied within 19-50% under changing weather conditions and averaged 40% of the applied amount. At the same time, in the studies carried out, an increase in the fixation of fertilizer nitrogen in the soil from 27 to 30% was noted, which ultimately leads to a decrease in the loss of mineral nitrogen \(N_{aa}\).

Table 2. Nitrogen balance of fertilizers when growing spring wheat inoculated with Rhyzoagrin.

| Variant                  | Used by plants N | Anchored in 20 cm soil layer | Loss from 20 cm soil layer |
|--------------------------|------------------|------------------------------|---------------------------|
| Background + N₄₅         | 1,80             | 1,20                         | 1,50                      |
|                          | 40               | 27                           | 33                        |
| Background + N₄₅ + Rhyzoagrin | 1,84              | 1,35                         | 1,31                      |
|                          | 41               | 30                           | 29                        |

\(P\), \% 3
LSD₀₅, g/m² 0,09

Note: numerator – g/m², denominator – % from fertilized

Nitrogen fluxes during the cultivation of spring wheat were characterized by the following parameters (Table 3). The share of net mineralized nitrogen was 72%, and that of reimmobilized soil nitrogen was 28%. Inoculation of spring wheat with Rhyzoagrin increases the amount of mineralized soil nitrogen by 15%, net mineralized nitrogen by 12%, and reimmobilized nitrogen by 21%. It should be noted that, at the ecosystem level, changes in the subsurface nitrogen cycle are manifested in the formation of flows of net mineralized and reimmobilized nitrogen and their ratio \((N−M : RI)\), whose indicators are used to assess the mode of functioning of the agroecosystem and anthropogenic load. Studies have shown that inoculation with a strain-based drug \(Agrobacterium radiobakter\) promotes an increase in the mineralization-immobilization process of N in the soil, but does not significantly affect the ratio \(N−M : RI\).

Table 3. Nitrogen fluxes of labeled fertilizers \(^{15}\)N, soils and indicators of integral assessment of the functioning of the soil-plant system

| Indicator                      | B + N₄₅ | B + N₄₅ + Rhyzoagrin |
|--------------------------------|---------|----------------------|
| Mineralized nitrogen, g/m² (M) | 26,2    | 30,3                 |
| Net mineralized nitrogen, t/m² (N – M) | 18,9 | 21,2                 |
| Reimmobilized nitrogen, g/m² (RI) | 7,4    | 9,0                  |
| RI : M, %                       | 28      | 30                   |
| N – M : RI                      | 2,6     | 2,3                  |
The functioning of spring wheat agrophytocenosis with nitrogen fertilization largely depends on weather conditions. The stability of the agroecosystem in wheat crops or its ability to maintain structure and function (RI : M,%) was higher under optimal temperature and humidity conditions, both dry and humid weather conditions reduce the stability of the agroecosystem, which is confirmed by the regression equation - strong in tightness and curvilinear in shape (Figure 1a). Presumably, the reaction of the agroecosystem to weather conditions is due to the coherent interaction of all components (metabolic processes, direct and feedback links). First of all, weather conditions affect the intensity of (re) immobilization of nitrogen and its recirculation in the subsurface cycle (“return” at the exit), which is an important condition for maintaining dynamic equilibrium in the agroecosystem. A systematic analysis of the transformation of soil nitrogen (regression equation) revealed that the mode of functioning of the agroecosystem depends on the balance of flows of net mineralized and (re) immobilized nitrogen. With significant precipitation, the balance of the flows of net mineralized and (re) immobilized nitrogen changes towards a decrease in the intensity of nitrogen immobilization. Already at HCA≥1,3 the agroecosystem goes into a functioning mode designated as "resistance" (the level of impact is the maximum permissible), and when HCA≥1,65 is experiencing adaptive exhaustion. With excessive moisture (HCA≥1,8…2,0) the applied nitrogen fertilization puts the agroecosystem into a repression mode, which leads to a decrease in the degree of nitrogen participation of the mineral fertilizer in the production process of spring wheat.
When spring wheat seeds are inoculated with Rhyzoagrin against the background of nitrogen fertilization, the observed state of functioning of the agroecosystem hardly differs from the option of using only nitrogen fertilizer (Figure 1b).

4. Conclusion
As a result of the study carried out on sod-podzolic light loamy soil, the parameters of the nitrogen cycle of fertilizer and soil were determined during inoculation of spring wheat with a preparation based on the strain *Agrobacterium radiobakter*. The utilization factor (UF) of $^{15}$N labeled ammonium nitrate by spring wheat averaged 40% of the applied amount. The absolute size of immobilization in the structure of the balance of the labeled nitrogen of the mineral fertilizer was 27-30%. Inoculation with Rhyzoagrin does not significantly affect the immobilization of nitrogen in mineral fertilizers.

The gaseous losses of the labeled nitrogen of the mineral fertilizer amounted to 33% of the applied on the background of the RA and decreased upon inoculation with Rhyzoagrin to 29%.

The use of an integral assessment showed that on sod-podzolic light loamy soil, the agroecosystem functioned in a resistance mode when using mineral nitrogen fertilizer. No significant differences in the indicator of the functioning of agroecosystems (the ratio of $R_I : M$ and $N - M : R_I$) between the variants with inoculation of spring wheat with Rhyzoagrin and without it have not been established.

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