NITRIFICATION IN THE ROOT ZONE OF WINTER RYE UNDER THE INFLUENCE OF MINERAL NITROGEN AND PRESOWING SEED BACTERIZATION

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The paper studies the influence of mineral nitrogen and presowing seeds bakterization on the activity of autotrophic and heterotrophic nitrification in rhizosphere soil of winter rye plants. Increasing doses of mineral nitrogen enhance nitrification activity. Application of microbial preparation Diazobakteryn has a prolonged influence and improves nitrification process, mainly due to the increase of heterotrophic nitrification activity.

Keywords: *inoculation, autotrophic nitrification, heterotrophic nitrification, Diazobakteryn, winter rye.*

Plants nitrogen nutrition level can limit optimal course of crop production processes demanding application of nitrogen fertilizers in agricultural practices. However, even at application of mineral nitrogen into the soil there are certain conditions when assimilation of active ingredients of fertilizers by plants is low and followed by the intensive soil pollution. Thus, ammonia from the ammonium fertilizers can be utilized by nitrifying microorganism and oxidized during the process of nitrification. Plants assimilate formed nitrates, which is a positive process. At the same time, excess of nitrates is washed out to the water reservoirs, and starts another process of nitrogen cycle – biological denitrification. Therefore, nitrogen fertilizers should be used efficiently not only from the standpoint of economic impact but also with environmental considerations, which determines the urgency of studies of biological transformation of nitrogen compounds in the soil.

Nitrification process appear to be the least studied process among the other important parts of nitrogen cycle in nature. Its course in soils depends on many factors while the role of some of them remains yet unknown. Thus, it is believed that autotrophic nitrification dominates in agrocenosis soils, since the presence of ammonia and lack of organic matter provides optimum living conditions for autotrophic nitrifiers. According to M. Umarov [1] in cultivated agrocenosis soils almost 84 – 99% of ammonium oxidation is caused by autotrophic nitrifiers. At the same time, the main vector of biochemical activity in soil are plants that excrete into the root zone a large number of organic compounds, including those that contain nitrogen. That, theoretically, might create good conditions for heterotrophic nitrification progress in the area of plant roots.

In this context, the aim of our study was to determine the characteristics of autotrophic and heterotrophic nitrification processes in the root zone of winter rye plants at different levels of nitrogen nutrition.

Materials and methods. Nitrification activity of rhizosphere soil of winter rye plants (*Secale cereale* L.) on various mineral backgrounds was studied in field

experiments. It was assumed that artificial activation of heterotrophic microorganisms in the root zone can be an additional possible impact factor on the course of studied processes. Therefore, the pre-sowing seed bacterization with Diazobakteryn (biological agent – *Azospirillum brasilense* 18-2) was applied [2].

Field experiments were conducted during 2010 – 2012 on sod-podzolic sandy-loam soil of Institute of Agricultural Microbiology and Agricultural Production NAAS (pH – 7.2, humus – 1.2%, easily hydrolyzable nitrogen (by Cornfield) – 54.9 mg/kg, P₂O₅ – 330 mg/kg, K₂O – 148 mg/kg).

The experiments scheme:

I. Without bakterization:

1. Without fertilizers;
2. N₃₀K₂₀ (N₁₀ in autumn + N₂₀ in early spring);
3. N₆₀K₄₀ (N₃₀ in autumn + N₃₀ in early spring);
4. N₉₀K₆₀ (N₃₀ in autumn + N₃₀ in early spring + N₃₀ in tubing phase);
5. N₁₂₀K₈₀ (N₃₀ in autumn + N₄₅ in early spring + N₄₅ in tubing phase);

II. Bakterization with Diazobakteryn:

- 6 – 10 (same fertilization options).

Estimation of fertilizers doses was carried out taking into account the content of the nutrition elements in the soil. Due to the high content of mobile phosphates in the soil phosphate fertilizers were not applied. The highest fertilizer's dose in the experiment was justified by carry-over of the NPK elements at expected harvest of 35 kg/ha. Size of field experiments plots was 9 sq.m. Harvesting and yield record was performed by direct method (weighing yield from whole plot). Winter rye cultivar – Synthetic-38.

Study of nitrification process and evaluation of autotrophic and heterotrophic nitrifying microorganisms contribution in the nitrates formation the composting method of D. Zvyagintsev was used [3, 4]. Mean sample of rhizosphere soil was prepared and sieved through a 2 mm diameter holes. 10 g of sample was placed into the 40 ml vials and watered to 60-70% of the total field water capacity. Samples were incubated for 21 days at 26 – 28 °C. Humidity was maintained by addition of water after control weighing of vials. Activity of autotrophic nitrification was inhibited by nitrification inhibitor 4-amino-1,2,4-triazole (100 mg / g of soil). It is known that aminotriazol (ATG) in the concentration exceeding its specific activity by almost 2 orders does not reduce the growth rate, biomass accumulation and production of nitrate in heterotrophic microorganisms. Nitrate contents was determined after 21 days using ion-selective electrodes. Experiments replication – 3-fold.

Planning and conducting of field experiments, statistical analysis of experimental data were performed According to Dospekhov [5].

Results and discussion. According to the data obtained the nitrification intensity increases at application of mineral nitrogen fertilizers. Thus, in variants with N₁₂₀ fertilizer dose the nitrification activity has increased on 38% in the tubing phase, 54% – in the flowering and on 93% in the milk ripeness phases comparing to control (without fertilizer).

Application of aminotryazol for inhibition of autotrophic nitrifiers have shown a significant reduction of the nitrification process intensity. Thus, the inhibitory approach allows to estimate the differential involvement of auto-and heterotrophic nitrifiers in the oxidation link of nitrogen cycle.

The study of process activity at first sampling period (after 36 days of early spring application of mineral nitrogen) has revealed that the activity of autotrophic nitrification in rhizosphere soil of winter rye plants grown at low mineral background had significantly exceeded the corresponding values of heterotrophic nitrification (Table 1).

TABLE 1. Nitrification activity in rhizosphere soil of winter rye plants, 2012, tubing phase (36 days after the early spring application of mineral nitrogen) mg N-NO₃ / g soil

Variants	Soil (total activity of processes)	Soil + inhibitor of autotrophic nitrification
<i>Without inoculation</i>		
Control without fertilizers	40,87	6,60
N ₃₀ K ₂₀	43,47	7,13
N ₆₀ K ₄₀	49,20	7,60
N ₉₀ K ₆₀	49,40	19,80
N ₁₂₀ K ₈₀	56,93	22,87
<i>Inoculation with Diazobakteryn</i>		
Control without fertilizers	50,67	12,00
N ₃₀ K ₂₀	51,87	14,00
N ₆₀ K ₄₀	50,67	14,33
N ₉₀ K ₆₀	58,13	25,73
N ₁₂₀ K ₈₀	59,47	26,80
HIP ₀₅ experiment	11,15	4,17
fertilizer	4,99	1,87
inoculation and interaction	6,44	2,41

However, increasing doses of mineral fertilizers in rhizosphere soil of rye plants enhance growth of both active autotrophs and heterotrophic nitrifiers, and last

one with a greater extent. Thus, the nitrate portion came from the heterotrophic nitrifiers was 16% of the total process activity in control (without fertilizers). At the same time, this value was 40% in the variant with the highest dose of fertilizer. We explain this with the possible increase of volume of root exudates and contents of nitrogenous organic compounds under such conditions. Experiments by Margel A. et al. [6] confirms these findings. They have indicated 9.5 times gain in root exudation of photoassimilates under the application of mineral nitrogen as comparing to the volume of exudates in control plants (without nitrogen fertilizers). At this, the variation of nitrogen compounds content was observed in the root secretions depending on the amount of fertilizers applied [7].

Application of microbial preparation Diazobakteryn in the technologies of rye growing increases nitrification values. Comparison of autotrophic and heterotrophic nitrification indicators suggests that noted increase was caused by activation of heterotrophic microorganisms. Thus, contribution share of heterotrophic nitrifiers to the total process scale ranged from 24% in controls to 45% in the variant with high fertilizer doses (as it was already noted, the corresponding values in the variants without bacterization were 16 and 40%). Since the amount of mineral nitrogen (as well as other factors) in the soil were the same as in variants without inoculation the observed effect can be explained by the increasing volume of root exudates of inoculated rye plants, which can be an additional source of organic matter containing nitrogen compounds in addition to carbon. This assumption is satisfactory, taking into the account numerous literature data on the influence of pre-inoculation on the activity of the process of photosynthesis of inoculated plants.

During next research period – flowering phase (62 days after the early spring application of mineral nitrogen and 25 days after fertilization of plants in their corresponding variants), the above described features of nitrates formation depending on mineral background due to the nitrification were observed. However, there were also some differences. The share of heterotrophic nitrifiers into the formation of nitrate pool during this period has reduced. Although it is possible to follow the bacterization effect – values difference was more than doubled, it should be concluded that autotrophic nitrification dominates even under these conditions. At this, its share have reached 83 – 86% (Table 2).

Reduced activity of heterotrophic nitrification during the flowering phase in the variants with Diazobakteryn is associated with certain interaction patterns of host plants and inoculant. Thus, it is known that introduced bacterial strains actively colonize the root areas of inoculated plants in the initial phases of plants organogenesis, predominating among microorganisms. Over the time, their relative number in microbial community is decreasing due to the tendency of young ecosystem to stabilize. Thus, the number of introduced bacteria is gradually reduced to its normal environmental level [8]. Naturally in this case is the reduction of bacterization impact both on the production process of agricultural crops and on soil biochemical processes in the root zone of plants over the time.

TABLE 2. Nitrification activity in rhizosphere soil of winter rye plants, 2012, flowering phase (62 days after the early spring application of mineral nitrogen and 25 days after plants fertilization) mg N-NO₃ / g soil

Variants	Soil (total activity of processes)	Soil + inhibitor of autotrophic nitrification
<i>Without inoculation</i>		
Control, without fertilizers	36,00	3,68
N ₃₀ K ₂₀	40,00	3,69
N ₆₀ K ₄₀	43,13	3,40
N ₉₀ K ₆₀	46,00	3,72
N ₁₂₀ K ₈₀	55,53	4,65
<i>Inoculation with Diazobakteryn</i>		
Control, without fertilizers	50,33	8,60
N ₃₀ K ₂₀	55,60	8,87
N ₆₀ K ₄₀	64,33	9,20
N ₉₀ K ₆₀	64,80	9,40
N ₁₂₀ K ₈₀	67,33	10,00
HIP ₀₅ experiment	9,88	1,37
fertilizer	4,42	0,61
inoculation and interaction	5,70	0,79

At the end of the vegetative season, nitrification activity slows down, which may be due to the exhaustion of ammonium (substrate for autotrophs) and organic nitrogen compounds (substrate for heterotrophic nitrifiers) in rhizosphere soil of winter rye plants (Table 3). Interesting, that in variants with bacterization activity of nitrification (both autotrophic and heterotrophic) is lower than the corresponding values in variants without inoculation. Obviously, this is because: firstly, initiated plants actively absorb nutrients [9, 10], which contributes to their rapid uptake from soil, and secondly, inoculated rye plants, as indicated by long-term observations, promptly passes organogenesis phases and mature earlier. Accordingly, the biochemical activity in the root zone of these plants will also be reduced.

TABLE 3. Nitrification activity in rhizosphere soil of winter rye plants, 2012, milk ripeness phase (91 days after the early spring application of mineral nitrogen and 54 days after plants fertilization) mg N NO₃ / g soil

Variants	Soil (total activity of processes)	Soil + inhibitor of autotrophic nitrification
<i>Without inoculation</i>		
Control, without fertilizers	13,00	2,63
N ₃₀ K ₂₀	12,93	2,67
N ₆₀ K ₄₀	13,53	3,52
N ₉₀ K ₆₀	16,73	4,73
N ₁₂₀ K ₈₀	25,13	7,53
<i>Inoculation with Diazobacteryn</i>		
Control, without fertilizers	11,47	3,80
N ₃₀ K ₂₀	13,07	2,74
N ₆₀ K ₄₀	13,20	2,89
N ₉₀ K ₆₀	13,80	4,19
N ₁₂₀ K ₈₀	13,33	4,32
HIP ₀₅ experiment	3,01	1,33
fertilizer	1,35	0,60
inoculation and interaction	1,74	0,77

Observed peculiarities of nitrification processes were similar for all years of the research.

The activity of nitrification in the root zone of plants of winter rye rises together with increasing doses of mineral nitrogen. Autotrophic nitrification substantially prevailing the activity of heterotrophic process. Moreover, its relative contribution into the formation of nitrate pool in rhizosphere soil of plants rises over the vegetative season. Application of Diazobakteryn promotes intensification of heterotrophic nitrifiers, especially during the initial stages of the vegetative season.