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EXPERIMENTAL ARTICLES
Agroecology

Development of Microorganisms Associated with the Nitrogen Cycle Affected by Reduced Tillage and Direct Seeding in Climatic Conditions of the Southeast of the Central Black Earth Region

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Abstract—A long-term study on the development of groups of microorganisms associated with the nitrogen cycle has been conducted in order to study the changes of soil processes affected by tillage reduction. A general trend to decreasing the activity of these microorganisms with an increasing effect has been revealed in the soil and climatic conditions of the southeast of the Central Asian Republic with the grain crop rotation. The most significant depression of microbiological processes typical for the development of nitrifying bacteria and azotobacter colonies has been associated with the direct seeding.

Keywords: reduced tillage, soil surface treatment, direct seeding, nitrifying microorganisms, *Azotobacter*, ordinary chernozem

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INTRODUCTION

Soil tillage is one of the most costly operations in the technology of growing crops. In modern agricultural technologies, tillage accounts for up to 40% of energy costs and approximately 25% of labor costs [1–4]. Currently, in modern agriculture, economical energy-saving technologies for tillage are widely used. The demand for reduced tillage results from the rising prices for energy resources and the need to reduce the cost of agricultural production. Therefore, in recent years, in science and industry, there has been an increasing interest in reduced tillage, up to the transition to the No-Till agricultural system, direct seeding. At the same time, the inconsistency of existing scientific information regarding the effectiveness of reduced tillage and especially direct seeding increases the severity of the problem and is an urgent issue of modern agriculture [5–10].

Researchers noted that, in almost all soil-climatic zones, reduced tillage and, especially, direct seeding leads to a deficiency of mineral nitrogen and increases the differentiation of the mobile phosphorus content in the arable layer and the pesticide load, which negatively affects the soil biogenic properties and the ecological situation in agricultural landscapes [11–16].

According to many researchers, the soil biological activity is one of the sensitive indicators characterizing changes in soil fertility and its ecological state since microorganisms, along with their active participation in the formation of the soil fertility, are extremely sensitive to the environmental changes [17–22].

Since the soil microbial community actively responds to changes in the soil environments, the study and evaluation of soil microbiological activity allow us to establish the direction of soil processes at the initial stages of agricultural practices. Thus, the monitoring of the changes in soil microbial community is relevant and practically significant.

The aim of this work is to study the dynamics and development trends of microorganisms associated with the nitrogen cycle (nitrifying bacteria and *Azotobacter*) under the conditions of reduced tillage and direct seeding.

MATERIALS AND METHODS

The study was carried out at the Dokuchaev Voronezh Federal Agrarian Scientific Center in a stationary experiment conducted in order to find the most rational methods and systems of tillage in crop rotation, ensuring the preservation of soil fertility and high productivity of crops.

The experimental plots were settled on ordinary chernozem, medium-humus, heavy loamy, with favorable physical and agrochemical characteristics of the 0–30-cm layer: humus content (according to Tyurin's method with Simakov's modification, GOST 2613-91) was 6.48%, total nitrogen (according to Ginzburg's method) was 0.36%, total phosphorus

(according to the method proposed by Ginzburg and Shcheglova) was 0.35%, total potassium (according to Ozhigov's method) was 1.85%, hydrolysable nitrogen (according to the method proposed by Tyurin and Kononova) was 61.2 mg/kg of soil, the sum of absorbed bases (GOST 27821-88) was 66.4 mg/kg of soil, pH_{KCl} 6.99, and hydrolytic acidity was 0.57 meq/100 g soil.

Monitoring the changes in the soil microbiological activity was carried out for 7 years from 2014 to 2020 in the following variants of tillage with crop rotation: traditional tillage, i.e., plowing to a depth of 20–22 cm (control); surface tillage, i.e., plowing to a depth of 6–8 cm; and No-Till, the direct seeding. Soil tillage methods were studied in the crop rotation: peas–winter wheat–corn for grain–barley–annual grasses–winter wheat–sunflower–barley.

The study of changes in soil microbiocenosis was carried out as part of the implementation of the State Task. For analysis, representative composite soil samples were collected from each experimental variant from a layer of 0–20 cm. The number of groups of microorganisms was counted by the classical method of plating on various elective nutrient media according to the Tepper method [23]. Freshly taken samples stored for no more than 24 h at a temperature of 5°C were used for isolation of the microorganisms. The number of nitrifying bacteria was determined on leached agar with ammonium-magnesium salt of phosphoric acid; the number of Azotobacter was determined on soil plates.

Experimental data was processed by the dispersion method of mathematical analysis using the Microsoft Office Excel 2016 software.

The meteorological conditions during the years of the study differed from each other in temperature regime and the amount of precipitation. However, they were close to typical on average over the years of monitoring for the southeast of the Central Black-Earth Region, which made it possible to fairly objectively assess the effect of the studied factors.

RESULTS AND DISCUSSION

We studied the development dynamics of the most agronomically important groups of microorganisms associated with the nitrogen cycle, the nitrifying bacteria, and Azotobacter.

The physiological characteristics of various species of nitrifying bacteria and their relationships with various environmental factors made it possible to consider that the nitrogen regime and soil fertility are determined by the development of these microorganisms, since they take an active part in the mobilization of soil nitrogen, oxidizing ammonium salts to nitric acid salts, which are involved in the mineral nutrition of plants.

Nitrifying bacteria are very sensitive to the environmental conditions; to the water, air, nutrient, and thermal regimes; and to the content of soil organic matter [24–26]. Since the oxidation of ammonia to nitrous and then to nitric acid occurs with the participation of molecular oxygen, a change in the physical properties of the soil and, especially, aeration has an indirect but significant effect on the intensity of development of nitrifying bacteria.

The results obtained showed that the intensity of the development of this group of microorganisms depended on the hydrothermal conditions of the growing season, the crop culture, and tillage type (Fig. 1). Since there is significant variability in the nitrification activity of the soil over the years, primarily due to differences in agrometeorological conditions and the crop rotation, the effect of the studied types of tillage manifested itself in the form of a trend. In the soil and climatic conditions of the southeast of the Central Black Earth Region, a higher moisture supply during the growing season reduced the intensity of the development of soil nitrifying bacteria, as evidenced by the correlation coefficient. In particular, in the plowland soil, the correlation coefficient between the hydrothermal coefficient of the growing season and the activity of nitrifying bacteria was $r = -0.85$, the surface plowing resulted in $r = -0.42$, while no-tillage showed the least close correlation, $r = -0.32$.

The effect of the studied tillage techniques on the nitrification activity in the ordinary chernozem was manifested in the form of a trend. The average annual number of nitrifying microorganisms was hardly affected by the tillage types and was in the range of 381–395 CFU/g of absolutely dry soil and slightly changed with the time of No-till technique application, as evidenced by the trend lines and regression equations. This can be explained by the good agrophysical properties of the soil at the experimental plots.

The most stable nitrification processes were found in the cultivated soil, for which the coefficient of variation of this trait was $V = 10.5–9.2\%$, while the development of nitrifying bacteria in the soils under the completely reduced tillage was more influenced by the external environment and weather conditions and was less stable: the coefficient of variation was $V = 13\%$ in this case.

Analysis of the dynamics of changes in the nitrification activity of chernozem over time, i.e., taking into account the increasing effect of tillage, showed that the development patterns of nitrifying bacteria changed significantly in the first 4 years after the transition to surface plowing and No-tillage techniques. However, starting from the fifth year, the intensity of development of this group of microorganisms was less affected by the tillage type, and was determined to a greater extent by the hydrothermal conditions of the growing season and agrocenosis.

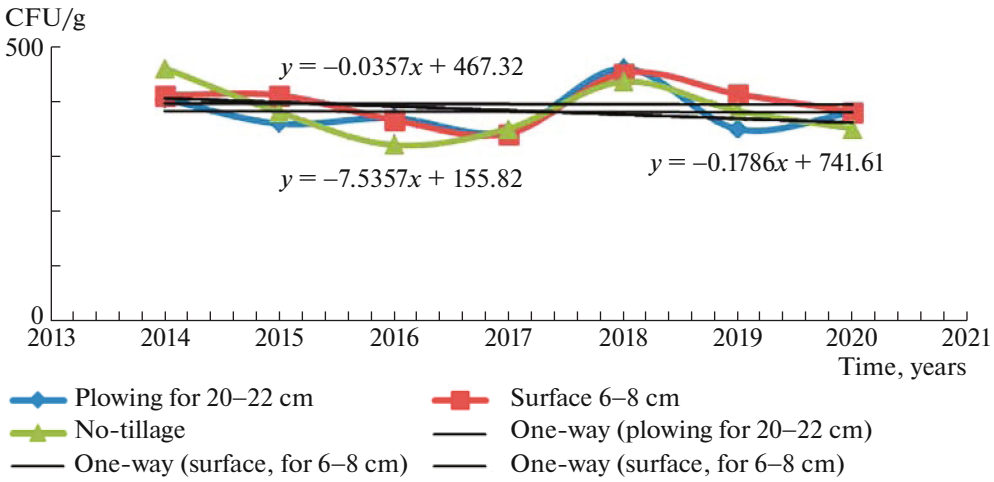

Fig. 1. Development of nitrifying bacteria in the 0–20-cm layer of soil under various tillage methods in grain-crop rotation.

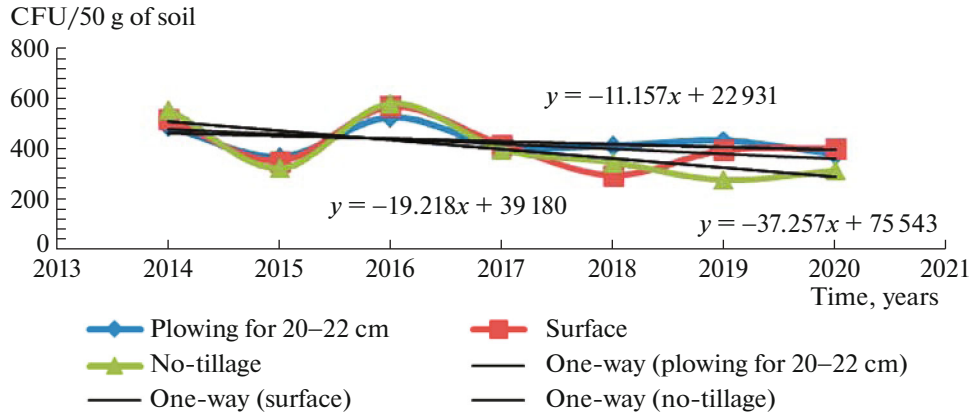

Fig. 2. Development of *Azotobacter* colonies in the 0–20-cm layer under various tillage methods in grain-crop rotation.

An important indicator of soil fertility and the favorable course of soil biological processes is the intensity of development of nitrogen-fixing bacteria. Of particular importance among such bacteria belongs to the representatives of the genus *Azotobacter*, because these bacteria are able to assimilate molecular nitrogen directly from the air, converting it into accessible forms for other living organisms. *Azotobacter* representatives react negatively to the deterioration of the valuable agronomic properties of the soil and are especially sensitive to a lack of moisture. The active development of this group of microorganisms in the soil is one of the indicators of its fertility and ecological well-being [27, 28].

The results of the studies and statistical processing of data on the development of *Azotobacter* showed that the hydrothermal regime of the growing season had an insignificant effect on the development of this group of microorganisms in the soil (Fig. 2). The correlation between the abundance of *Azotobacter* and the hydrothermal coefficient was inverse and very weak. The correlation coefficient had a value of $r = -0.16$ in the variant with traditional plowing and –0.07 in the No-Till variant. In the variant with the surface plowing, the relationship between the hydrothermal coefficient of the growing season and the development of *Azotobacter* was direct and weak: $r = 0.23$.

The influence of the studied techniques of soil tillage reduction on the development of *Azotobacter* manifested itself as a decrease in the activity of the microorganism, which increased with the duration of the applied tillage. Over the years of the study, the maximum amount of *Azotobacter* representatives were determined for the traditional tillage variant and amounted to 426.79 CFU/50 g of soil, while the minimum was for the No-Till variant (395 CFU/50 g of soil). The number of *Azotobacter* in the variant with the surface plowing of the ordinary chernozem occupied an intermediate position and amounted to 417 CFU/50 g of soil. The most stable development of

Azotobacter in ordinary chernozem occurred in the variant of traditional tillage. The coefficient of variation for the abundance of Azotobacter over the years on the experimental plot with traditional tillage was $V = 13.4$, while it was 22.5 with surface plowing and 30.4% with no-tillage.

At the same time, the strongest relationship between the content of nitrate nitrogen in the soil and the intensity of the development of *Azotobacter* was determined for the soil under No-tillage treatment, $r = 0.66$. In cultivated soil, *Azotobacter* had less effect on the supply of soil with nitrate nitrogen. The relationship between these indicators was less significant, and the correlation coefficient was in the range of 0.41–0.45. More than that, the correlation coefficient calculated for the number of nitrifying bacteria and the supply of soil with nitrate nitrogen was maximal for the soil under traditional tillage, $r = 0.54$, which indicated the optimization of conditions for the development of nitrifying bacteria under this type of tillage.

Azotobacter developmental dynamics in the No-Till ordinary chernozems showed the reverse pattern compared to the development of nitrifying bacteria. In the first years of the experiment, the intensity of the development of Azotobacter colonies differed insignificantly depending on the tillage type. However, starting from the fourth year of the systematic use of surface plowing and direct seeding, there is a clear trend towards a decrease in the number of Azotobacter colonies in the No-tillage variant.

CONCLUSIONS

Thus, monitoring the development of groups of microorganisms associated with the nitrogen cycle in ordinary chernozems under the conditions of reduced tillage in a grain-row crop rotation in the southeast of the Central Black Earth Region, showed a general trend towards the decrease of activity of these microorganisms in soils associated with the increasing level of the tillage reduction. The most significant depression of microbiological processes, associated with the development of both nitrifying bacteria and Azotobacter colonies, was determined in the No-tillage variant, the one with direct seeding.

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