

## **BIOLOGICAL ACTIVITY OF SOIL DEPENDING ON FERTILIZER SYSTEMS**

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### **Abstract**

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Soil microbial flora has an important role in the life of plants, providing them with nutrients and other factors. The research was aimed at studying the effect of the long-time fertilizer application on the number and composition of the microbial flora in the soil, involved in the synthesis and decomposition of organic matter. Studies were conducted in the V<sup>th</sup> field rotation with a crop alternation: fallow land, winter rye, summer wheat with clover under sow, clover of the first year of use, clover of the second year of use, barley, potato and oat. We studied the effectiveness of various systems of fertilizers (organic, mineral, organic and mineral). Soil of experimental field was sod-podzolic. The use of arable land without fertilizers during five crop rotations (40 years) has led to a significant reduction of humus content, exchangeable potassium, and soil acidification. The use of mineral fertilizer systems also acidifies the soil. Joint application of manure and fertilizers led to an increase humus content in the soil. All the studied fertilizer systems increased the content of mobile phosphorus and exchangeable potassium and significantly changed its biological state of soil. Long-time use of fertilizer systems, compared with the control, helped to increase the total number of microorganisms, nitrogen-fixing and nitrifying bacteria, nitrification and cellulolytic activity of soil and carbon dioxide production. On the basis of conducted studies we have established the feasibility of long-time use of organic and mineral fertilizer system with saturation of arable land with manure of 10 t/ha and with an equivalent amount of mineral fertilizers for improvement of bacteria activity involved in nitrogen regime, soil respiration, processes of decomposition and the replenishment of organic matter that increase soil fertility.

*Key words:* fertilizer, nitrogen-fixing bacteria, nitrifying bacteria, soil respiration, cellulolytic activity

### **Introduction**

The basic task of modern agriculture is conservation and improvement of soil fertility, its environmental cleanliness, as the main wealth of any state, the material basis of human existence on the planet (Lobkov and Plygun, 2012). As a rule, without the fertilizer use even on cultivated soils there is a significant incompleteness in the field crops harvest, and depletion of soils with available forms of nutrients (Ailincăi et al., 2012; Dasci and Comakli, 2011).

The material basis of soil fertility is a complex of soil components grouped according to some authors (Kiriushin,

2011) in three groups namely biological – the content and composition of the microbial flora, nitrifying and nitrogen-fixing activity of soil, CO<sub>2</sub> emission intensity and decay of cellulose in the soil; agrochemical – humus, pH, indicators of soil-absorbing complex, the content and forms of elements of plant nutrition and agrophysical – mechanical composition, structure and the formation of the arable layer, humus horizon thickness, density, porosity, moisture reserves, air, thermal properties and their modes.

Microorganisms are the powerful converters of the environment, the most active geochemical factor causing the most intense migration of chemical elements in the bio-

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sphere (Maksimovich and Hmurchik, 2012; Schulz et al., 2013; Musaev, 2014).

Fertilizers being effective means of increasing productivity of agrophytocenosis change the direction and intensity of a number of processes in the soil. Numerous studies have found that the use of fertilizers affects not only the agrochemical, agrophysical properties of the soil, but also changes the number of bacteria, actinomycetes and fungi (Dzyuin and Dzyuin, 2014). The increase in number of microorganisms at fertilization is associated with enrichment of fertilized soils with nutrients, increasing the size of exosmosis and root litter of plants during the growing season and plenty of plant residues after harvesting of crops. At the same time, mineral fertilizers do not always have a stimulating effect on the number of soil microorganisms. Typically, the degree of impact of different doses and types of fertilizers on microbial flora depends on many factors including the physical and chemical characteristics of the investigated soil, moisture conditions, soil temperature, cultivated crops and other related factors.

It should be noted that long-time experiments more objectively reflect the fertilizer effect on soil fertility, direction of agrochemical, agrophysical and microbiological process changes.

The purpose of the research is to establish the effect of fertilizer systems on indicator changes of biological activity of the soil in the long-time experiment (determine the total number of microorganisms in the sod-podzolic soil, explore the qualitative composition of microflora).

## Materials and Methods

The experimental field is situated in Eastern part of Russian plain near the city Perm. Climate is moderate continental (moderate cold), the average annual air temperature is 2.1°C, average annual precipitation is 584 mm, evaporation is 339 mm, water that can penetrate is 245 mm, average air humidity is 75%.

The research was held in long-time experiment. Following systems of fertilizers were studied: organic (saturation of hectare of arable land with manure of 10 t/ha per year, the actual dose – 80 t/ha); mineral, in which doses of fertilizers are calculated by the equivalent content of nutrients in the manure; organic and mineral, where the mineral fertilizers with manure saturation is 5, 10 t/ha per year (in fact – 40, 80 t/ha) equivalent to the content of nutrients in the manure are applied.

Experiment scheme: 1. without fertilizers; 2. manure – 80 t/ha (in composition of manure – 1612 kg/ha N, 996 kg/ha P<sub>2</sub>O<sub>5</sub>, 2076 kg/ha K<sub>2</sub>O); 3. NPK equivalent to manure –

80 t/ha (in composition of mineral fertilizers – 1612 kg/ha N, 996 kg/ha P<sub>2</sub>O<sub>5</sub>, 2076 kg/ha K<sub>2</sub>O; 4. manure 40 t/ha + NPK equivalent to manure (in composition of manure – 806 kg/ha N, 498 kg/ha P<sub>2</sub>O<sub>5</sub>, 1038 kg/ha K<sub>2</sub>O + in composition of mineral fertilizers – 806 kg/ha N, 498 kg/ha P<sub>2</sub>O<sub>5</sub>, 1038 kg/ha K<sub>2</sub>O); 5. manure 80 t/ha + NPK equivalent to manure (in composition of manure – 1612 kg/ha N, 996 kg/ha P<sub>2</sub>O<sub>5</sub>, 2076 kg/ha K<sub>2</sub>O + in composition of mineral fertilizers – 1612 kg/ha N, 996 kg/ha P<sub>2</sub>O<sub>5</sub>, 2076 kg/ha K<sub>2</sub>O). Amount of nutrients specified in the sum during five crop rotations (40 years).

Replication of variants is quadruple, placement is randomized. Investigations were carried out in the field crop rotation with crop alternation: fallow land, winter rye, summer wheat with clover under sow, clover of the first year of use, clover of the second year of use, barley, potato and oat.

The research was carried out on sod-podzolic soil (according to a classification WRB Umbric Albeluvisols Abruptic). In the soil texture the large dust particles (28-33%), fine sand (17-21%) and silt (14-19%) prevailed. Before carrying out the experiment the humus content was in the range of 2.1-2.2%, pH<sub>KCl</sub> was of 5.4-5.5, P<sub>2</sub>O<sub>5</sub> 125 and K<sub>2</sub>O 170 mg/kg soil.

Chemical analyses of the soil according to adopted in Russia methods and standards. Microbiological properties of the soil were studied by a complex of methods. Nitrifying capacity of the soil was studied by the Kravkov method in modification of Bolotina and Abramova (Agrochemical methods of soil research, 1975). The amount of produced carbon dioxide was defined by Aksenov and Bankina method (1986) by composting the soil at a temperature of 28°C, humidity of 60% of total moisture capacity, with fixing of the CO<sub>2</sub> allocation on a gas chronometer CHROM-5. The degree of cellulose decomposition by the method of “applications”.

Influence of fertilizer systems on agrochemical and microbiological indicators of soil were evaluated by standard analysis of variance (ANOVA) and correlation analysis (Little and Hills, 1981). Significance of differences in the degree of features variation was set with the help of the criterion F, the significance assess of the difference between the average - at the least significant difference (LSD) at a probability level of  $p \leq 0.05$  with the program SPSS (V. 18) using.

## Results

After 5 crop rotations the significant changes in soil have appeared. The use of arable land without fertilizers has led to a significant reduction of humus content, exchangeable potassium and soil acidification (Table 1).

**Table 1**

**The influence of fertilization systems on soil chemical properties. The end of 5<sup>th</sup> rotation (2008–2009), average for two replications in time, 0–20 cm**

Treatment	Humus, %	pH <sub>KCl</sub>	S	Ha	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
			mmol/100 g of soil		mg/1000 g of soil	
1. No fertilizers	2.12	4.9	19.2	3.07	173	163
2. Manure 80 t ha <sup>-1</sup>	2.22	5.0	20.1	2.83	208	222
3. NPK rates equivalent manure 80 t ha <sup>-1</sup>	2.23	4.8	18.8	3.81	197	251
4. Manure 40 t ha <sup>-1</sup> + NPK rates equivalent manure 40 t ha <sup>-1</sup>	2.35	4.9	18.5	3.26	201	268
5. Manure 80 t ha <sup>-1</sup> + NPK equivalent rates	2.46	5.1	19.6	2.95	248	339
LSD <sub>05</sub> *	0.23	0.3	1.3	0.71	60	41

\* LSD<sub>05</sub> - least significant difference

The saturation of arable land with manure 10 t/ha (variant 2) and mineral fertilization in a dose equivalent to the content of NPK in manure (variant 3) contributed to the preservation of humus content of the soil. Mineral fertilizer system has led to an increase in hydrolytic acidity of soil and reduction of the degree of base saturation. Joint application of manure and mineral fertilizers contributed to the humus accumulation. All the fertilizer systems contributed to improvement of the content of mobile phosphorus and exchangeable potassium in the soil.

Long-time use of mineral and organic fertilizers increased the total number of microorganisms from 450 thousand in unfertilized soil to 2500 thousand per 1 g. (Table 2).

The minimum number of microorganisms was observed in unfertilized soil - 450 thousand per 1 g. According to the scale of assessment of the soil enrichment with microorganisms proposed by Zvyagintsev (1978), this soil belongs to the very poor type. The total number of microorganisms was increasing with the increasing of doses of applied fertilizers, especially organic. Organic and mineral fertilizer system with saturation of arable land with manure of 10 t/ha,

and mineral fertilizing of an equivalent amount (variant 5) increased the concentration of microorganisms in 5.5 times (2500 thousand per 1 g. of soil) – the soil became middle-concentrated.

The fertilizer use influenced the qualitative composition of microbial flora. All the fertilizer systems have increased the content of nitrogen-fixing bacteria in 10-13 times in comparison with unfertilized soil. The mineral system was inferior to the organic and organic and mineral systems in accumulation of this group of bacteria, the number of which amounted to 150, 200 and 200 thousand per 1g of the soil respectively.

Correlation analysis revealed a close relationship between the content of nitrogen-fixing microorganisms and humus content, humic acid and available phosphorus content, as it is evidenced by the correlation coefficients shown in Table 3.

One of indicators of the biological activity of the soil is the presence of nitrifying bacteria in it. The maximum number of this group of bacteria has been found in the organic fertilizer system with the arable land saturation with manure

**Table 2**

**Effect of fertilizer systems on indicator changes of biological activity of the soil, the end of the 5<sup>th</sup> rotation**

Treatment	General number of microorganisms	Nitrogen-fixing bacteria	Nitrifying bacteria	Nitrifying ability, mg of N-NO <sub>3</sub> /kg/14 days	Production of C- CO <sub>2</sub> , mg/m <sup>2</sup> /h	Linen decomposition, % month
	Thousand per 1g of soil					
1. No fertilizers	450	15	1.5	36.5	288	20.9
2. Manure 80 t ha <sup>-1</sup>	950	200	45	43.1	362	23.2
3. NPK rates equivalent Manure 80 t ha <sup>-1</sup>	1500	150	15	49.5	302	23.6
4. Manure 40 t ha <sup>-1</sup> + NPK rates equivalent Manure 40 t ha <sup>-1</sup>	2000	200	20	53.6	340	29.3
5. Manure 80 t ha <sup>-1</sup> + NPK equivalent rates	2500	200	20	64.7	382	35.4
LSD <sub>05</sub>	184	13	3	4.9	17	4.7

**Table 3****The coefficients of correlation ( $r^2$ ) between the biological activity and agrochemical characteristics of the soil**

Indicators	Humus	HA	HA-1	pH <sub>KCl</sub>	P <sub>2</sub> O <sub>5</sub>
1. Total number of microorganisms	0.9±0.02	0.9±0.01	0.7±0.08	0.4±0.25	0.8±0.15
2. Nitrogen-fixing bacteria	0.7±0.03	0.9±0.02	0.6±0.11	0.4±0.29	0.7±0.21
Nitrifying bacteria	0.2±0.54	0.6±0.19	0.3±0.31	0.4±0.31	0.4±0.31
3. Nitrifying capacity	0.9±0.15	0.6±0.21	0.8±0.16	0.5±0.19	0.8±0.14
4. Nitrifying ability, mg /kg/day	0.7±0.19	0.8±0.12	0.6±0.18	0.8±0.12	0.8±0.18
5. Cellulolytic ability	0.8±0.17	0.6±0.23	0.6±0.17	0.3±0.28	0.7±0.15

The significance of the correlation coefficients was tested by Student's test with a confidence correlation level  $P \leq 0,05$

of 10 t/ha per year (variant 5 of Table 2) - that is 30 times higher than in unfertilized soil. The use of mineral and organic and mineral fertilizer systems increased the number of nitrifying bacteria in comparison with controls in 10-13 times.

Long-time use of organic and mineral fertilizer system with manure of 10 t/ha per year, and the equivalent quantity of mineral fertilizer ensure the maximum capacity of the soil to accumulate nitrates, which are almost twice higher than in unfertilized soil.

In our experiment, the minimum level of carbon dioxide production was observed in the control variant (Table 2). The low amount of fresh organic matter in the unfertilized soil reduced not only the total number of microorganisms, but also the production of carbon dioxide from the soil surface. Long-time use of mineral fertilizer system a little increased the C-CO<sub>2</sub> emission according to a control variant. Systematic manure application enhances this process. The maximum amount of carbon dioxide was allocated by the soil while using the organic and mineral fertilizer system with manure saturation of 10 t/ha per year and NPK in equivalent amounts.

The study of the intensity of cellulose degradation under the crops of V-th crop rotation showed that with organic and mineral fertilizer systems application the process was more active (Table 2). Cellulolytic activity of soil in this variants was 29.3-35.4 against 20.9 in control,  $LSD_{05}=4.7$ . The intensity of cellulose degradation was closely correlated with the content of humus and mobile phosphorus in the soil (Table 3).

## Discussion

In our experiment, application of organic fertilizers in the dose of 80 t/ha the equivalent amount of mineral fertilizers increased the number of microorganisms in the soil, but the biggest increase occurred in the number of cases with

the joint application of higher doses of fertilizers. The total number of microorganisms in the experiment was increasing with increasing of humus and humic acids in the soil. A close correlation between these indicators was revealed (Table 3).

Typically, organic fertilizers, plant residues, root exudates of plants are the nutrition sources for soil microorganisms and energy for biochemical processes. Studies of Iovieno et al. (2009), Nakho and Dkhar (2010) showed the importance of organic fertilizers in increasing of the total number of microorganisms. In the research of Geisseler and Scow (2014) the long-time use of fertilizers led to a significant increase of carbon of microbial biomass. Naher et al. (2013) showed that the application of all-nutrient mineral fertilizer (NPKSZn) contributes to the increase of total number of microorganisms in comparison with a variant without fertilizers. Absence of any one element (N or P or K) reduced the number microbial population. The combination of organic fertilizers with mineral in the studies of Lazcano et al. (2013), Zinchenko et al. (2014) significantly increased the number of soil microorganisms.

Typically, nitrogen-fixing bacteria grow well in the fertile, provided with organic matter, phosphorus and moisture in soils with pH of 5.5-5.8. Improving of the soil fertility in the experiment caused by the use of organic fertilizers, contributed to the increase in number of nitrogen-fixing bacteria. However, the use of mineral fertilizer system has led to soil acidification, which negatively affected the number of nitrogen-fixing bacteria, in comparison with the organic and organic-mineral fertilizer system.

According to Zvyagintsev et al. (2005) in nutrient-poor acidic podsol soils (to which the analyzed soil belongs) the nitrifying bacteria are in a small amount or absent at all. In nature, nitrifying bacteria exist where ammonia is formed under the condition of an abundance of organic matter. Obviously, a fresh organic matter of manure contributed to intensive nitrifiers growth in the experiment (80 t/ha of mature). It should be taken into account that the growth and metabolism

of autotrophic nitrifying bacteria proceeds optimally only in the pH range from 7 to 8. The pH range, in which there is complete nitrification from ammonia to nitrate, is very narrow, as both free ammonia (with high pH value) and nitric acid (with low pH value) are toxic for nitrifier. Therefore, raising pH soil is accompanied by an increase in the number of nitrifying bacteria (Russell, 2013).

As in the experimental soil by the end of V-th crop rotation the acid reaction of the soil environment has developed, the use of mineral and organic and mineral fertilizer systems did not contribute to the growth of the number of nitrifying bacteria.

Nitrifying capacity of the soil reflects its potential abilities in the accumulation of mineral nitrogen. The nitrification process is defined out by specific microorganisms nitrifying with high standards to the conditions of existence. This suggests that the level of their life is objective indicator of soil fertility degree (Lykov et al., 2004).

The high level of nitrifying capacity of the soil in our experiment corresponds to a higher content of easy mineralizing organic matter in the soil - humic acids of the first fraction (HA-1) between these indicators there is a close correlation connection (Table 3). It should be noted that increasing of the nitrifying capacity of the soil at all fertilized variants is mentioned, but the organic fertilizer system was inferior to the mineral and organic and mineral, possibly, because of immobilization of nitrate by microorganisms, which number in the manure application has increased significantly.

The intensity of carbon dioxide emissions is used as an indicator of biological activity of microorganisms and stability of soil carbon to the mineralization (Mishustin, 1972). Among the studied fertilizer systems, organic fertilizer system had advantages in effect on the soil respiration indicators (Table 2). This indicator was in close positive connection with the content of organic matter in the soil (Table 3). Similar results were obtained in the experiment of Dolgoprudnaia Agrochemical Experimental Station (Lykov et al., 2004), the experiment of the Moscow State University (Mineev, 2004).

Influence of mineral fertilizers on soil respiration in the experiment was not statistically proved. Studies of Bhattacharya et al. (2013) found that the use of mineral fertilizers in the recommended doses improves the soil respiration. Grishin (2001) showed that the fertilizers applied annually in the low and medium doses have increased the amount of carbon dioxide in comparison with the control variant, and high doses have decreased its production. Therefore, the effect of fertilizers on the C-CO<sub>2</sub> emissions is ambiguous.

The C-CO<sub>2</sub> emissions were significantly affected by the reaction of the soil environment. Reduction of soil acidity caused an increase in the intensity of the "soil respiration".

A close correlation between these indicators was revealed (Table 3).

Role in the decomposition of organic matter in the soil, especially fresh plant residues was played by cellulose-decomposing microorganisms whose activity is determined by the degree of cellulose (linen) degradation. The degree of cellulose (linen) degradation allows estimating the presence of mineral nitrogen in it and mobilization opportunities of soil in relation to this element.

Application of organic and mineral fertilizer systems in the experiment stimulated the development of the root system of plants, increasing the supply of organic mass and, consequently, had a positive effect on the number and activity of the cellulolytic microbial flora.

In the scientific literature there are different views on the effect of fertilizers on that indicator of biological activity of the soil, as microbial flora which can degrade cellulose, is extremely diverse. It is both aerobic and anaerobic, both mesophilic and thermophilic bacteria, various fungi and some protozoa. While changing soil conditions there is a replacement of one group of cellulose-decomposing microorganisms by the other (Titova and Kozlov, 2012).

The positive effect of mineral fertilization on the activity of cellulolytic microbial flora is analyzed in the studies of Zinchenko (2011), Poddymkina (2004). Lykov et al. (2001) noted that the organic and mineral fertilizers with liming contributed to the intensification of cellulose degradation, which is connected with an increase in the number of the bacterial part of microorganisms, and without soil liming that is connected with the fungi intensification.

## Conclusion

The use of organic, mineral and organic and mineral fertilizer systems on sod-podzolic soil has substantially changed its biological condition. Organic and mineral fertilizer system with saturation with manure of 10 t/ha contributed to biogenic rise of sod-podzolic soil. The total number of microorganisms has increased in 5.5 times according to the unfertilized soil; the number of nitrogen-fixing and nitrifying bacteria has significantly increased. Nitrifying capacity of the soil, the production of carbon dioxide, the degradation of linen have reached the maximum values.

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