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BIOLOGICAL AND AGROCHEMICAL PROPERTIES OF THE MEADOW-CHERNOZEM SOIL OF OMSK IRTYSH REGION AND FODDER CROP PRODUCTIVITY AS INFLUENCED BY MINERAL FERTILIZERS

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Abstract

The world experience indicates that both natural factors (soil fertility, plant biopotential, etc.) and agrogenic effects (fertilizers, farming systems, etc.) promote sustainable growing crops. Mineral fertilizers have an impact on the abundance, activity and diversity of soil microflora by increasing the productivity of the system, the return of plant residues and the content of organic matter in the soil. Here we show that as a result of the systematic application of mineral fertilizers, the biological state of meadow-chernozem soil remains favorable for a number of microbiological indicators and for an increase in fodder crop yields. Our purpose was to assess the effects and relationship of application of fertilizers on soil microbiological and agrochemical parameters and, as a result, on crop productivity. The tests were carried out in 2020-2022 in the forest-steppe zone of the South Western Siberia, Omsk region (55.04192°N, 73.46504°E) in a stationary field experiment. The content of mobile phosphorus in the soil according to Chirikov is medium. In eight-field grain-grass crop rotation, a mix of perennial grases *Dбctylis glomerбta* L. with *Onobrэchis arenбria* (Kit.) DC. and annual sorghum-sudank hybrid grass *Sorghum* × *drummondii* (Steud.) Millsp. & Chase were grown. The number of different physiological groups of microorganisms, enzymatic activity, the content of nitrates, the nitrification ability of the arable soil layer, and crop yields were assessed. We revealed that the growth of agronomically valuable groups under annual grasses is more intensive than under perennial grasses. Optimized mineral nutrition ($N_{60}P_{60}$) of the mixed grass stand stimulated mostly the growth of the phosphate-mobilizing microorganisms and soil micromycetes (by 118 and 122 % compared to control), under the sorghum Sudangrass hybrid, the number of amylolytic and oligonitrophilic microbiota significantly increased by 57-90 % vs. control, respectively. The analysis of changes in the total microbial number showed the stimulating effect of mineral fertilizers on the soil microbocenosis under agricultural crops. The use of mineral fertilizers affected the total microbial numbers equally under perennial and annual grasses, with 51-52 % increase vs. the control without fertilizers. It was revealed that the long-term use of mineral fertilizers negatively affects the activity of catalase, the redox enzyme. The decrease vs. the control was up to 14 % depending on the culture. The activity of hydrolytic enzymes urease and invertase remained not significantly affected. Observations of the meadow-chernozem soil nitrate regime showed that mineral fertilizers used at a dosage of $N_{60}P_{60}$ in sorghum-Sudangrass hybrid and grass mixture crops increased the soil nitrate nitrogen content during the growing season by two times or more compared to variants without fertilizers. In our research, the yield of perennial grasses over the years was 3.84-4.57 t/ha DM in the control and 4.82-4.89 t/ha DM upon fertilization. The studied technology of mineral fertilizer application significantly increases the yield of sorghum-Sudangrass (by 1.65 t/ha DM, or by 39 % vs. control. Crop yields had the strongest direct correlation with the microbiota of the nitrogen cycle, the amylolytic and proteolytic microorganisms (r = 0.98 and r = 0.85, respectively, $p < 0,05$).

Keywords: soil microorganisms, enzymatic activity, mineral fertilizers, nitrate nitrogen, nitrification ability, crop rotation, perennial grasses, sorghum-Sudangrass hybrid, yield

Sorghum crops are among the leaders in global agricultural production [1]. The area of their cultivation has a steady tendency to expand due to high and stable productivity, efficient use of insolation and photosynthetic resources, and drought resistance [2, 3]. Perennial grasses, in addition to their nutritional benefits, enrich the soil with organic matter and improve water-physical, agrochemical and biological properties of the soil [4].

The role of biological factors in maintaining the health and productivity of plants, as well as sustainable agrocenoses, is currently receiving much attention [5]. Microorganisms are the most numerous participans of of biology life of the soil, which directly affects the stability of the agroecosystem [6].

Numerous organisms with active metabolism inhabit the soil [7-9]. Microbial cenosis forms the basis of the fertility of all soil ecosystems [10, 11]. Counts and diversity of microorganisms are indicators, assessing the impact of various agricultural practices on ecosystems. Microbial activity is susceptible to different types of fertilizers and plant protection products, soil tillage methods, or exposure to root exudates of the cultivated plant [12, 13].

Studying the structure of ecological-trophic groups of soil microorganisms, which are indicative of the state of microbiocenosis, is necessary to determine the balance of mineralization and synthesis of organic substrates [14].

World experience indicates the need to use natural (soil fertility, climate, landscape, plant biopotential) and agrogenic (fertilizers and plant protection products, modern farming systems) factors in crop growing [15].

The effect of mineral fertilizers is not limited to the direct impact on the production process of the plant. They also influence the number, activity, and diversity of soil organisms. Mineral fertilizers can intensify processes in the soil biological system. Its productivity enhances, crop residue return increases, and soil organic matter content rises [16]. In moderate doses, fertilizers activate the vital activity of various groups of microorganisms. Populations of soil aerobic and anaerobic nitrogen fixers, ammonifiers, cellulose decomposers, actinomycetes and microfungi become more abundant [17, 18]. However, excessive activation of soil microbiota can lead to negative environmental consequences, e.g., deterioration of the biological and physicochemical properties of soils, humus mineralization, increased gaseous nitrogen losses due to denitrification and nitrification, accumulation of nitrates in soil, plants, groundwater, destruction of the ozone screen of the stratosphere [19, 20]. Long-term use of increased doses of mineral fertilizers can intensify growth of toxin-producing microbes [21]. A number of studies have shown the negative effect of mineral fertilizers on the microbiological activity of the soil, which is explained by acidification and a decrease in the reserves of biologically available carbon [22].

Changes in the enzymatic activity of soils are indicators of the influence of various agricultural practices on the soil biological processes [23, 24]. The relationship between soil fertility and enzymatic activity established in many studies allows us to compare the effectiveness of agricultural practices, soil fertility in general, and changes in soil properties during various anthropogenic and natural processes in the ecosystem by the soil enzymatic activity [25].

The formation of enzyme potential largely depends on the agrochemical properties of soils. Their optimal parameters provide favorable conditions for the development of microorganisms and plants, and as a result, the natural supply of the soil with enzymes increases due to the formation of a larger mass of microbes and their higher physiological activity [26].

It has been reported that the application of high doses of mineral nitrogen-phosphorus fertilizers has a positive effect on the soil agrochemical parameters soil and increases enzyme activity [27]. However, other studies have established a negative effect of anions of mineral fertilizers on the activity of the redox enzyme catalase [28] and the inhibitory effect of chemicalization agents on the activity of urease in early development of wheat plants, while the phosphatase and invertase activity of chernozem practically did not change in dynamics [29].

In the microorganisms—soil—plants system, mineral fertilizers pose a direct positive effect on the productivity of agrocenoses and a stimulating effect on the microbial community, thus affecting soil fertility which, in turn, also affects plant productivity.

It can be stated that in the scientific literature there is data on the absence, positive and negative effects of fertilizers on the soil enzymatic activity. Little is known about the consequences of prolonged use of mineral fertilizers for enzymatic activity in the agricultural landscapes of the Omsk region, since in Siberia (and in Russia as a whole) a limited number of long-term stationary experiments have been preserved in which such information can be obtained.

Meadow-chernozem soil has been fertilized for a long time in field experiments in a crop rotation system, and in this regard, the tolerance of microbial communities to fertilizers and changes in the soil biochemical and agrochemical parameters, directly affecting crop productivity are of special interest. There is still little research into the biological activity of soil during long-term (more than 40 years) agricultural use in the conditions of the Omsk Irtysh region. [24, 30, 31].

The impact of mineral fertilizers on the activity of ecological trophic groups of soil microbiota and enzymatic activity still remains relevant and important problem that requires permanent monitoring.

In this work, we established that intensive crop cultivation technology in crop rotation increases activity of the microbial cenosis of meadow-chernozem soil. This has a positive effect on the yield of perennial grasses and sorghum-Sudangrass hybrid. There are no significant changes in the activity of hydrolytic enzymes, but a decrease in catalase activity occurs.

Here, we aimed to assess changes in the biological properties, namely, in microbial pool activity and enzymatic activity, and agrochemical properties of meadow-chernozem soil under application of mineral fertilizers in a long-term (more than 40 years) stationary experiment, and the impact of these changes on productivity of annual and perennial herbal agrocenoses.

Materials and methods. The research was carried out in 2020-2022 in an eight-field crop rotation based on a stationary experiment (established in 1978). Perennial grasses, the mix of *Dactylis glomerata* L. with *Onobrychis arenaria* (Kit.) DC., were sown in 2020. The choice of the mixture is due to the nutritional value of the crops. An annual grass was sorghum-Sudangrass hybrid *Sorghum* × *drummondii* (Steud.) Millsp. & Chase.

The experimental site is located in the southern forest-steppe zone (Omsk Province, Omsk District, 55.04192°N, 73.46504°E). The territory belongs to the Priomskaya Plain, part of the Barabinskaya Neogene Plain.

In the two-factor experiment design a crop (factor A) was perennial grasses or annual grass (sorghum-Sudangrass hybrid); nitrogen-phosphorus fertilizer (factor B) options were without fertilizers (control), annual pre-sowing application of $N_{60}P_{60}$. For fertilization, ammonium nitrate (grade B, KAO Azot, Russia) and ammophos (Balakovo branch of JSC Apatit, Russia) were used. Mineral fertilizers were applied with a disk seeder SZ-3.6 (Russia) before sowing, followed by pre-sowing soil cultivation and the sowing. From the second year of life of perennial grasses, fertilizers were applied with a seeder as top dressing in the spring. The seeding rate for sainfoin is 6 million seeds/ha, for cocksfoot grass are 8 million seeds/ha (sowing every 0.45 m), for sorghum-Sudangrass hybrid 20 kg/ha (1 million seeds/ha), sowing time is the second decade of May. In each variant, the plot area was 360 m^2, each year the experiment was carried out in triplicate.

The soil samples for microbiological studies were collected in sterile parchment bags 3 times in the main stage of plant development, for perennials cocksfoot grass at tillering, heading, filling, for perennials sainfoin at stemming, budding, flowering, for annual sorghum-Sudangrass hybrid at tillering, stemming, and milky ripeness. Each bulk soil sample was formed from four drilling to a 0-20 cm depth. Identification and quantification of microbial groups were carried out on meat peptone agar (MPA) for proteolytic bacteria, on starch-ammonia agar (SAA) for amylolytics, on Mishustina's medium for oligonitrophils, on Muromtsev's medium for phosphate-mobilizers, on Hutchinson's medium for cellulose decomposers, on aqueous leached agar added with double ammonium-magnesium salt of phosphoric acid for nitrifiers, on acidified Czapek medium for soil fungi [32]. The counts of soil bacteria, actinomycetes and micromycetes was expressed in colony-forming units (CFU) per 1 g of absolutely dry soil. Micromycetes were identified to genus based on morphological and cultural properties [33].

The soil enzymatic activity analysis was carried out in air-dry samples, for invertase according to Kuprevich, for urease according to Hoffmann, for catalase gasometrically [34].

Nitrate nitrogen in the soil was determined by the Grandval-Lage method/ To evaluate the results, scales of soil nitrate nitrogen supply generally accepted in agrochemistry were used [35, 36]. The nitrification capacity of the soil was determined according to Kravkov with 21-day incubation [37].

Accounting for green mass yield, calculated for DM separately for each cutting according to the botanical composition, was carried out manually from 1 m^2 area with 3 repetitions [38].

Statistical processing was performed in Microsoft Excel (Statistica 10.0, StatSoft, Inc., USA). The influence of factors was assessed by analysis of variance at $p < 0.05$ [39]. Mean values (*M*), standard error of means (±SEM), and Pearson correlation coefficients (*r*) were calculated. The least significant differences at a 5% significance level (LSD_{05}) were assessed [39] using the Microsoft Excel statistical software package.

Results. The soil of the experimental plot is meadow-chernozem, medium-thick, medium-humic, heavy loamy, with a humus content in the 0-0.2 m layer of approximately 7.0%, $pH_{aq.}$ 7.2; the thickness of the humus horizon A is 0.45 m. The surface soil is heavy loam with 40-46% physical clay. The profile has a 4-5-membered structure and is typical for the southern forest-steppe soil-climatic zone. Water permeability, determined 1 month after flat-cut loosening in the fall, is characterized as good. The density of the arable layer is 1.07-1.14 g/cm^3. The phosphorus content in the 0-0.2 m layer in the control is medium (less than 100 mg/kg), the supply of exchangeable potassium is very high (more than 180 mg/kg) regardless of the agricultural background [40].

The summer of 2020 was dry, with HTC = 0.69 from May to August vs. a norm of 1.0. In June, there were rains only in the third decade, 44 mm of precipitation vs. a norm of 51 mm. August was favorable in terms of precipitation. The 2021 season can generally be recognized dry and hot. Precipitation fell unevenly and was torrential in nature. May and the first half of June, the first and third decades of July, and the first half of August were particularly hot and dry, for May-August, HTC = 0.7. The growing season of 2022 was insufficient in moisture and unfavorable for the growth and development of crops in the experiment, on average for May-August, HTC = 0.81.

The aridity of the growing seasons resulted in small reserves of available moisture in the soil. Thus, in 2020, when sowing perennial grasses, June moisture reserves did not exceed 73% of the lowest moisture capacity (LMC) in both the arable and meter layers of soil, decreasing in July to critical values close to wilting moisture. Similar changes occurred in sowings of the sorghum-Sudangrass hybrid. Due to autumn-winter precipitation, moisture was replenished and the next year in June it amounted to more than 80% of the moisture content, which is sufficient for normal growth and development of plants. However, reserves further decreased to 55% of LMC in both arable and meter layers, regardless of the crop. The year 2022 was no exception. Throughout the growing season, drying of the soil profile occurred except for the arable horizon in July after precipitation, which increased the level of moisture to 86% LMC.

Studies by Chinese colleagues conducted at the Shenyang experimental station (Northeast China) showed that long-term application of mineral fertilizers has no does not have a significant effect on the soil microbiological activity [41]. A significant body of information by American researchers revealed a negative effect of mineral fertilizers on growth in the microbial community, which is explained by their acidifying effect and the deficiency of available carbon sources after the initial increase in mineralization activity [42].

In our experiement, we used a dose of $N_{60}P_{60}$ fertilizer calculated to ensure a positive balance of nutrients. In our studies in 2020 on perennial grasses, the application of $N_{60}P_{60}$ statistically significantly ($p < 0.05$) stimulated an increase in the number of phosphate-mobilizing microorganisms by 122% compared to the control, cellulolytics and microfungi that destruct organic matter in the soil by 51 and 128%, respectively (Table 1).

1. Composition of microbial community (CFU/g) **of the meadow-chernozem soil as affected by the fertilizers and crops** (for each group, n = 9, M±SEM; Omsk ASC, Omsk, 2020-2022)

Microbial group	Perennial grasses		Sorghum-Sudangrass hybrid		LSD_{05}	LSD_{05}
	control	$N_{60}P_{60}$	control	$N_{60}P_{60}$	A, B	AB
		In 2020				
Proteolytics, ×10^6	46.6±24.7	56.2±22.4	35.8±11.4	47.2±9.6	19.9	28.4
Amylolytics, ×10^6	13.1±5.0	17.7±5.0	12.6±6.9	21.3±7.6*	5.8	8.2
Oligonitrophils, ×10^6	221.5±8.1	199.4±13.2	167.9±5.6	340.4±38.4*	115.3	163.0
Phosphate mobilizing, ×10^6	179.8±5.1	398.9±16.5*	223.7±14.5	229.3±36.3	77.1	109.1
Microfungi, ×10^3	103.0±16.9	236.1±17.7*	144.4±32.2	103.7±27.2	44.4	62.7
Nitrifiers, ×10^3	1.65±0.39	1.76±0.18	1.57±0.31	1.81±0.49	0.68	0.97
Cellulose decomposers, ×10^3	85.2±31.3	128.3±25.4	72.8±3.2	129.2±9.0*	46.4	65.5
Total, ×10^6	461.3±18.4	672.6±15.0*	440.2±29.2	638.5±89.3*	188.2	111.2
		In 2021				
Proteolytics, ×10^6	22.8±4.0	28.0±4.4	27.8±4.7	39.1±8.9*	9.3	13.2
Amylolytics, ×10^6	16.9±4.3	28.6±7.9*	24.3±8.5	34.8±5.1*	11.3	16.0
Oligonitrophils, ×10^6	35.4±3.9	58.4±7.6*	58.6±16.5	119.3±24.3*	33.7	47.7
Phosphate mobilizing, ×10^6	32.3±10.6	49.5±6.6	65.6±27.1	108.8±7.1*	27.4	38.8
Microfungi, ×10^3	26.2±6.3	78.1±55.2*	25.2±3.9	39.2±1.7	32.7	39.8
Nitrifiers, ×10^3	0.55±0.04	0.58±0.09	0.75±0.19	1.11±0.60	0.72	1.02
Cellulose decomposers, ×10^3	61.5±21.9	88.7±34.8	71.1±20.6	80.4±28.31	38.2	54.0
Total, ×10^6	107.5±17.2	164.8±11.6	176.4±50.4	302.2±36.1*	66.0	93.3
		In 2022				
Proteolytics, ×10^6	25.0±7.1	33,0±7,1*	26,5±4,3	31,1±9,3	7,0	10,0
Amylolytics, ×10^6	21.2±5.9	26,4±6,4	22,6±6,3	35,1±11,5*	10,8	15,1
Oligonitrophils, ×10^6	63.9±23.6	92,2±34,8	70,4±30,9	100,2±54,2	62,5	88,3
Phosphate mobilizing, ×10^6	39.2±11.7	97,5±32,1*	47,7±11,9	87,9±44,7	45,4	64,2
Microfungi, ×10^3	35.0±10.3	46,8±7,5	31,5±3,3	49,2±8,3	21,6	30,5
Nitrifiers, ×10^3	1.70±0.44	1,40±0,21	1,70±0,61	1,50±0,23	0,79	1,12
Cellulose decomposers, ×10^3	63.7±14.5	116,1±10,8*	55,8±17,4	81,4±4,5*	20,6	29,2
Total, ×10^6	149.3±47.6	249,3±79,3	167,27±49,4	254,47±89,5	118,5	167,5

Note. Perennial grasses are a cocksfoot grass *Dactylis glomerata* mix with sainfoin *Onobrychis arenaria*. $LSD_{05}A$ corresponds to the crop factor, $LSD_{05}B$ to the fertilizer application, $LSD_{05}AB$ to the interaction crop × fertilizers; n is the number of measurements.
* Differences with the corresponding control are statistically significant at $p < 0.05$.

The increase in the number of biota that decomposes organic compounds in the soil when fertilizers are used is associated with its enrichment with mineral nutrition elements and an increase in plant mass during the growing season [43, 44]. Plant residues are a source of nutrition and energy for microorganisms and a comfortable space for colonization. Residues contain a complex nutrient and energy substrate for most microorganisms, the main source of soluble low molecular weight organic substances. Elements of mineral nutrition that enter the soil with fertilizers, in particular nitrogen, are especially important for the active life of microorganisms and the decomposition of organic matter [45-47].

Improving the mineral nutrition of sorghum-Sudangrass hybrid plants had a positive effect on the agronomically valuable microbiota. The number of copiotrophs (proteolytics and amylolytics) increased by 32 and 69%, respectively ($p < 0.05$) compared to the control. More than 52 and 87% effects, respectively, were due to the significant influence of fertilizers. Studies by Polish scientists have revealed a similar pattern of growth in the number of biota secreting amylase with the use of fertilizers [48].

In the microbial community we studied, oligonitrophils are the most common group. Oligonitrophilic microbiota that plays a major role in preserving and replenishing nitrogen reserves in the soil, and cellulose-degrading microorganisms responded to a greater extent to the $N_{60}P_{60}$ application by changing their numbers with statistically significant increase by 103 and 79%, respectively, compared to the control ($p < 0.05$). According to N. Roljević et al. [49], mineral fertilizers stimulated this microbiota with a 14.6 to 37.7% increase compared to the control. Differences in the absolute CFU values in our studies and those of our colleagues are due to different types of soils and, particularly, different enrichment in humus. It is known that richer and cultivated soils are highly biogenic [50], in our experiement, meadow-chernozem soil had a humus content of 7.0%.

The fertilizers provided a significant ($p < 0.05$) and almost equal increase in the total number of microbiota we found under annual and perennial crops, by 45 and 46%, respectively, compared to the control (see Table 1).

In 2021 which was characterized by a deficit of atmospheric precipitation coupled wirh extremely high air temperatures during the growing season, the increase in the number of soil microbiota was less than in the previous year 2020, due to unfavorable environmental factors.

The highest abundance of microorganisms in the control and treatment was found under annual grass. With a high number of bacteria upon applying fertilizers, the number of microfungi in the soil under the sorghum-Sudangrass hybrid decreased. Apparently, the growth of micromycetes was suppressed by the high number of bacteria. This may be due to the well-developed root system of the sorghum-Sudangrass hybrid and its specific root secretions (see Table 1).

The use of mineral fertilizers had a positive effect on various groups of microorganisms. Thus, in the soil under perennial and annual grasses, the number of proteolytic microorganisms increased by 23 and 41%, of bacteria exhibiting amylolytic activity by 69 and 43%, oligonitrophils which play an important role in the nitrogen cycle in nature, in particular in supplying plants with available nitrogen by 65 and 104%.

Microfungi in the soil play the role of saprophytes, reducers, and symbionts. Their contribution to the yield is enormous. They participate in decomposition of complex organic compounds, enter into symbiosis with plants, produce pigments, antibiotics, biologically active compounds and form soil structure [51].

In our studies, the number of soil microfungi under perennial grasses increased by almost 200%, that is, 3 times. Scientific publications on this issue indicate that the use of chemical agents does not always have a clear effect on this group of microorganisms. The authors of the studies point to both the inhibitory and stimulating effects of such agricultural practices on the growth of microfungi

[52, 53]. In addition, this effect may be due to less competition between these microorganisms and bacteria. It should be noted that more intensive growth of these microorganisms in the soil may be an unfavorable phenomenon posing a risk of proliferation of toxicogenic or pathogenic species [54].

It is worth noting a significant increase in the number of phosphate-mobilizing and nitrifying microorganisms involved in plant nutrition that we rebealed under sorghum-Sudangrass hybrid upon fertilization, by 66 and 48%, respectively, vs. the control.

The total number of detectable microorganisms, as influenced by the fertilizers, increased compared to control, in the soil under perennial grasses by 53%, under sorghum crops by 71%.

In 2022, the use of $N_{60}P_{60}$ contributed, depending on the type of crop, to an increase in the number of proteolytic bacteria decomposing organic nitrogen-containing compounds by 17 and 32%, amylolytic microflora by 24 and 55% ($p < 0.05$) vs. the control, with 67% influence of factor B (fertilizers) (see Table 1).

The number of oligonitrophilic microorganisms, as well as phosphate-mobilizing bacteria, statistically significantly ($p < 0.05$) increased under the sowing of perennial grasses by 46 and 148%, respectively, under the sorghum-Sudangrass hybrid by 43 and 85%, with 94 and 96% influence of factor B (fertilizers). Changes in the number of microorganisms decomposing organic residues under perennial and annual grasses were significant ($p < 0.05$). With the use of fertilizers, the number of cellulolytics increased by 84 and 47%, respectively, and soil micromycetes by 31 and 58%.

The use of fertilizers provides an increase in the total number of detectable microflora in the soil under perennial grasses by 67%, under sorghum crop by 52% vs. the control.

The growing seasons during the experiment differed in heat and moisture supply, which made it possible to more fully assess the impact of the studied agricultural practice. On average, over 3 years of research, long-term moderate use of mineral fertilizers ($N_{60}P_{60}$) in a stationary experiment stimulated several ecological trophic groups of soil microorganisms, that is, the trend in their activity remained over the years. In perennial grasses upon application of fertilizers, the number of phosphate-mobilizing microorganisms and soil micromycetes increased to the greatest extent, by 118 and 122% vs. the control ($p < 0.05$), with 77 and 44% influence of factor B (fertilizers). Under sorghum-Sudangrass hybrid, the counts of amylolytics and oligonitrophilics increased significantly by 57-90% compared to the control, wirh 77 and 48% influence of factor B (fertilizers) (Table 2).

2. Microbial abundance (CFU/g) **in the meadow-chernozem soil as affected by the fertilizers and crops** (for each group, n = 9, M±SEM; Omsk ASC, Omsk, 2020-2022)

Microbial group	Perennial grasses		Sorghum-Sudangrass hybrid		LSD_{05} A, B	LSD_{05} AB
	control	$N_{60}P_{60}$	control	$N_{60}P_{60}$		
Proteolytics, $\times 10^6$	31.4±5.4	39.0±4.9	30.0±4.1	39.1±2.6*	7.8	11.0
Amylolytics, $\times 10^6$	17.0±4.3	24.2±6.3*	19.8±6.6	30.4±4.9*	3.4	4.7
Oligonitrophils, $\times 10^6$	106.9±54.1	116.6±28.1	98.9±38.4	186.6±64.8*	49.2	69.5
Phosphate mobilizing, $\times 10^6$	83.7±47.2	181.9±102.1*	112.3±65.9	142.0±36.0	77.1	109.0
Microfungi, $\times 10^3$	54.7±25.2	120.3±65.1*	66.9±25.6	64.0±15.9	44.4	62.8
Nitrifiers, $\times 10^3$	1.30±0.02	1.25±0.06	1.34±0.10	1.47±0.25	0.39	0.56
Cellulose dcomposers, $\times 10^3$	70.1±013.8	111.0±9.6*	66.5±4.7	97.0±5.5*	18.2	25.7
Total, $\times 10^6$	239.3±96.7	362.2±98.4*	261.2±103.4	398.3±98.3*	78.6	111.1

N o t e. Perennial grasses are a cocksfoot grass *Dactylis glomerata* mix with sainfoin *Onobrychis arenaria*. LSD_{05}A corresponds to the crop factor, LSD_{05}B to the fertilizer application, LSD_{05}AB to the interaction crop × fertilizers; *n* is the number of measurements.
* Differences with the corresponding control are statistically significant at $p < 0.05$.

Research by A.V. Kurakova [44] found that an increase in the number of soil microfungi may resulted from the soil acidification with salt anions during long-term application of fertilizers. Since the main function of soil microfungi is the decomposition of plant organic residues, it can be assumed that when using fertilizers this process occurs more intensely due to more substrate. We identified microscopic fungi belonging to six genera, the *Penicillium*, *Aspergillus*, *Mucor*, *Fusarium*, *Trichoderma* and *Alternaria* (Fig. 1).

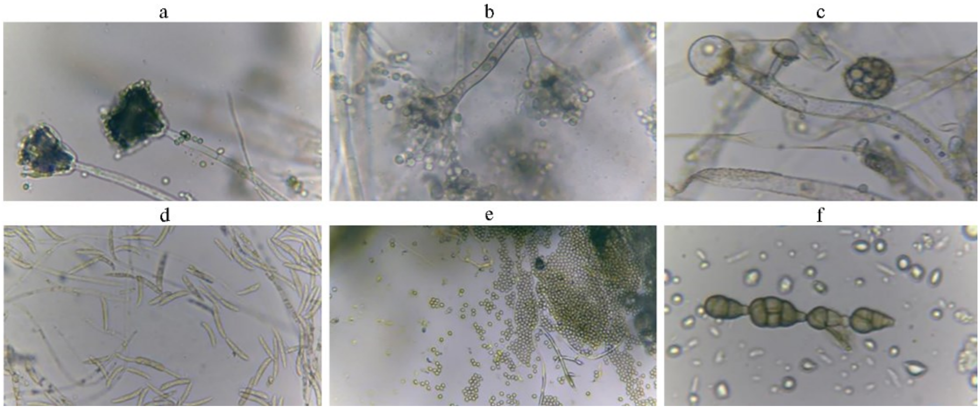

Fig. 1. Micromycetes isolated from the meadow-chernozem soil: a — *Penicillium* ssp., b — *Aspergillus* ssp., c — *Mucor* ssp., d — *Fuzarium* ssp., e — *Trichoderma* ssp., f — *Alternaria* ssp. (a microscope TS 2000, Biolab, Russia, ×200; Omsk ASC, Omsk, 2020-2022).

Facultative saprophytes which include representatives of the genera *Fusarium* and *Alternaria*, are widespread regardless of the precursor crop, they cause leaf damage and root rot and are able to survive in winter on plant debris. Members of the genera *Aspergillus* and *Penicillium*, according to the publication [55], are classified as epiphytes that use exclusively the waste products of the plant, without causing harm to it, but worsening the crop quality.

It should be noted that the lack of growth in the number of nitrifying bacteria under perennial grasses is possibly due to low soil moisture. This is consistent with the research [56] in which a negative effect of low soil moisture on this microbial group was also revealed.

Analysis of changes in the total number of detectable microflora showed the stimulating effect of mineral fertilizers on the state of soil microbiocenosis under agricultural crops. In terms of microbial populations, the effects during the years of observation, varied to a greater or lesser extent depending on the crop. However, on average for 2020-2022, optimization of mineral nutrition increased the total microbial pool in the soil under perennial and annual grasses equally, by 51-52% compared to the control. The highest abundance of soil microorganisms was found under sorghum-Sudangrass, from 261×10^6 to 398×10^6 CFU/g vs. 239×10^6 to 362×10^6 CFU/g under perennial grasses.

The activity of soil enzymes can be used to assess the intensity of biological processes. In our experiment, the use of mineral fertilizers reduced catalase activity under perennial grasses by 14% and under sorghum by 11%. Similar studies in stationary experiments showed that as the time of using mineral fertilizers and the soil $N\text{-}NO_3$ content increse, the activity of this enzyme decreases [23, 28]. F.H. Khaziev [26] reported a negative correlation between the activity of catalase and the content of nitrate nitrogen in the soil. We also found an inverse in correlation between the activity of catalase and the soil content of nitrate nitrogen ($r = -0.85 \pm 0.21$, $p < 0.05$) due to which there was a decrease in enzyme activity when applying fertilizers. This is related to the duration of fertilizer application, as it was reported that during the first crop rotations this trend was not observed (31).

On average, over the years of our research, when applying mineral fertilizers to perennial grasses and sorghum-Sudangrass hybrid, the activity of the hydrolytic enzymes ureased while activity of invertase did not change significantly and we did not find a negative effect of mineral fertilizers on the activity of these enzymes (Table 3). According to the D.G. Zvyagintsev scale [20] proposed to assess the enrichment of soils with enzymes, the studied meadow-chernozemic soil for catalase activity is classified as poor (1.0-3.0), for urease activity as very poor (< 3.0), and for invertase as medium enriched (15.0-50.0).

3. Enzimatic activity of the meadow-chernozem soil as affected by the fertilizers and crops (for each variant, n = 9, M±SEM; Omsk ASC, Omsk, 2020-2022)

Treatment	Catalase, O_2 $cm^3 \cdot min^{-1} \cdot g^{-1}$	Urease, mg NH_3/g	Invertase, mg invert sugar/g
	Perennial grasses		
Control	2.026±0.099	0.665±0.012	17.89±0.99
$N_{60}P_{60}$	1.776±0.100	0.654±0.019	18.43±0.97
	Sorghum-Sudangrass hybrid		
Control	1.880±0.090	0.678±0.059	18.47±0.33
$N_{60}P_{60}$	1.690±0.090	0.699±0.072	18.41±1.53
LSD_{05}A, B*	0.321	0.832	1.6
LSD_{05}AB	0.139	0.361	0.7

N o t e. Perennial grasses are a cocksfoot grass *Dactylis glomerata* mix with sainfoin *Onobrychis arenaria*. LSD_{05}A corresponds to the crop factor, LSD_{05}B to the fertilizer application, LSD_{05}AB to the interaction crop × fertilizers; n is the number of measurements.
* Differences with the corresponding control are statistically significant at $p < 0.05$.

In long-term studies on the regime of mineral nitrogen compounds in chernozems, the dependence of the supply of field crops with nitrogen on the amount of its nitrate form ($N-NO_3$) was established, which makes it possible to recognize this parameter as a diagnostic indicator [57, 58]. Our observations showed that during the tillering phase (June), under sorghum-Sudangrass hybrid and perennial grasses, the $N-NO_3$ content in the arable layer (0-20 cm) upon fertilization was high (> 20 mg/kg) according to the scale used [36], with 48% influence of factor B (fertilizers) (Table 4).

4. N-NO3 content (mg/kg) **in the meadow-chernozem soil as affected by the fertilizers and crops** (a 0-20 cm layer; for each variant, n = 9, M±SEM; Omsk ASC, Omsk, 2020-2022)

Crop (factor A)	$N_{60}P_{60}$ (factor B)	Initial level				Post-nitrification level			
		June	July	August	average	June	July	August	average
Perennial grasses	Control	12.0±5.6	2.3±1.72	0.6±0.4	5.0±3.5	54.0±13.9	30.1±1.8	19.1±4.6	34.4±10.3
	$N_{60}P_{60}$	27.4±9.9	10.0±5.8	4.0±3.5	13.8±7.0*	69.0±14.7	42.2±8.7	25.4±6.0	45.5±12.7
Sorghum-Sudangrass hybrid	Control	26.2±1.2	9.6±2.8	11.9±11.2	15.9±5.2	69.5±3.4	44.7±6.6	36.3±12.5	50.2±10.0
	$N_{60}P_{60}$	59.7±2.6*	37.2±20.4*	24.0±11.5*	40.3±10.4*	159.6*±31.9	71.0±16.2	53.4±10.0	94.7±32.9*
LSD_{05} A, B		8.0				27.3			
LSD_{05} AB		11.3				38.6			

N o t e. Perennial grasses are a cocksfoot grass *Dactylis glomerata* mix with sainfoin *Onobrychis arenaria*. LSD_{05}A corresponds to the crop factor, LSD_{05}B to the fertilizer application, LSD_{05}AB to the interaction crop × fertilizers; n is the number of measurements.
* Differences with the corresponding control are statistically significant at $p < 0.05$.

During the growing season, a decrease in the amount of nitrogen and nitrates in the soil occurred, mainly due to removal by crops. The use of mineral fertilizers in crops of perennial and annual grasses at a dose of $N_{60}P_{60}$ increased the content of the element on average during the growing season by two or more times vs. unfertilized sowings.

Soil microorganisms are also responsible for processes associated with the circulation of nitrogen, the most important nutrient for plant nutrition. Nitrification is one of the key microbiological processes affecting crop yields [59].

Quantitative changes in soil microbiota affected the mobilization of nutrients. Differences in the abundance and activity of proteolytic and nitrifying microorganisms determine the ability to provide plants with nitrogen nutrition, which can be seen in the soil's ability to accumulate nitrogen under favorable conditions [60]. The accumulation of nitrogen and nitrates during soil composting in the experiment was highest in June-July. Under sowing of perennial grasses and sorghum upon fertilization, the $N\text{-}NO_3$ content increased by 130% with 37% influence of factor B (fertilizers). In August, this indicator decreased which was associated with a decrease in the amount of easily mobilized nitrogen-containing compounds in the soil towards the end of the plant growing season [61].

Based on the results of a laboratory assessmrnt of the nitrification activity of soils under favorable heat and moisture supply, the accumulation of nitrogen and nitrates in sorghum-Sudangrass hybrid crops was more intense compared to perennial grasses. On average, the nitrification capacity of soil over the years of research upon the use of fertilizers exceeded the control values under perennial grasses and sorghum-Sudangrass hybrid by 8 and 59%, respectively (at $p < 0.05$) due to increased mineralization. Polish scientists made similar conclusions. Their experiment was carried out at the University of Life Sciences in Lublin (Poland), and it was shown that nitrification was more intense when fertilizers were used [62].

During the growing season in July and August, the accumulation of nitrogen and nitrates slowed down which can be explained by the consumption of easily mobilized organic compounds in mineralization. During the years of research, the the sowing of sorghum crop stood out as having the greatest nitrification capacity (Fig. 2).

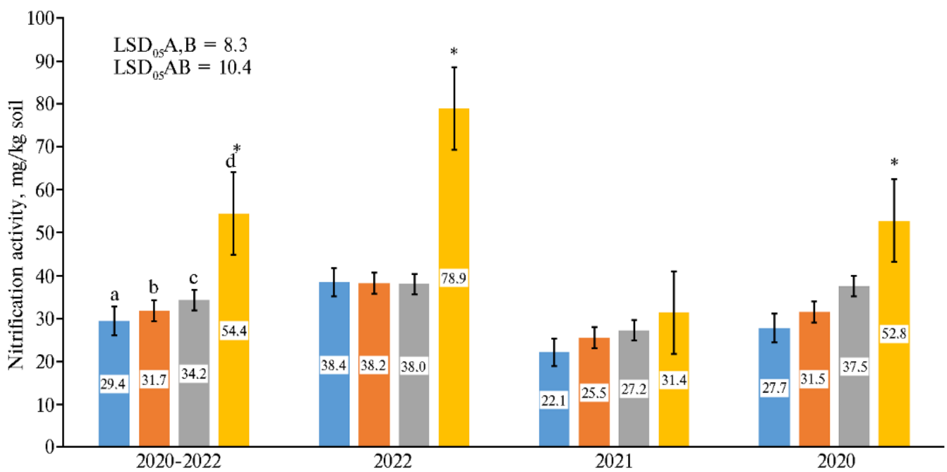

Fig. 2. Nitrification activity of the meadow-chernozem soil as affected by the fertilizers and crops: a — perennial grasses (control), b — perennial grasses ($N_{60}P_{60}$), c — Sorghum-Sudangrass hybrid (control), г — Sorghum-Sudangrass hybrid ($N_{60}P_{60}$) (for each variant, *n* = 9, *M*±SEM; Omsk ASC, Omsk, 2020-2022).
* Differences with the corresponding control are statistically significant at $p < 0.05$.

The prevailing weather conditions, moisture supply and mineral nutrition regimes during the years of observation had a positive effect on the productivity of agricultural crops. Perennial grasses were not mowed in the first year of life (in 2020). In subsequent years, the components of the grass mixture behaved differently. The cocksfoot grass component predominated in the grass mixture. V.I. Chernyavsky [4] noted that the loss of leguminous grasses occurs most quickly when grown with cocksfoot grass. In the future, the application of nitrogen fertilizers under the grass mixture can lead to the displacement of the legume crop and a significant reduction in its share in the botanical composition. Our studies have established a strong negative correlation between the mass of orchard grass plants and legumes. The share of sainfoin in the second year of life was 41-50%, in the third up to 47%. The yield of cocksfoot grass mixed with sainfoin was 3.84-4.57 t/ha DM in the control and 4.82-4.89 t/ha when fertilizers were used (Table 5).

5. Crop yield (t/ha DM) **on the meadow-chernozem soil as affected by the fertilizers** (for each variant, n = 9, M±SEM; Omsk ASC, Omsk)

Crop (factor A)	$N_{60}P_{60}$ (factor B)	Year			
		2020	2021	2022	average
Perennial grasses	Control		4.57±0.04	3.84±0.19	4,20±0,18
	$N_{60}P_{60}$		4.82±0.10	4.89±0.38*	4,86±0,18*
Sorghum-Sudangrass hybrid	Control	6.55±0.80	2.96±0.28	3.10±0.49	4,21±0,66
	$N_{60}P_{60}$	7.24±0.86	6.67±0.32	3.64±0.34	5,85±0,62*
LSD_{05} A, B			0.52	0.85	0.59
LSD_{05} AB			0.74	1.19	0.84

N o t e. Perennial grasses are a cocksfoot grass *Dactylis glomerata* mix with sainfoin *Onobrychis arenaria*. LSD_{05}A corresponds to the crop factor, LSD_{05}B to the fertilizer application, LSD_{05}AB to the interaction crop × fertilizers; n is the number of measurements.
* Differences with the corresponding control are statistically significant at $p < 0.05$.

The yield of sorghum-Sudangrass hybrid during the years of research varied from 2.96 to 6.65 t/ha DM in the control and from 3.64 to 7.24 t/ha DM when using nitrogen-phosphorus fertilizers, which is associated with better plant supply with elements of mineral nutrition. On average for 2020-2022, the use of fertilizer at a dose of $N_{60}P_{60}$ contributed to a significant increase in the yield of sorghum-Sudangrass hybrid by 1.65 t/ha DM, or by 39%, compared to the control. The results we obtained are generally consistent with the studies of African colleagues who noted an increase by 47-98% vs. the control in the yield of sorghum when using mineral fertilizers [63, 64].

The importance of microorganisms is demonstrated by their ability to improve soil composition, stimulate plant growth, and increase crop yields [65-68]. In our experiement, 2020 was generally dry. However, when during the critical period of plant development (the third decade of June) the precipitations was 78% higher than the long-term average, the number of soil microorganisms determined on agar media was the greatest compared to 2021 and 2022, and the yield of the sorghum-Sudangrass hybrid was 2.1-2.2 times higher than in 2021 and 2022.

6. Correlations (*r*) between the counts of ecological trophic microbial groups in the meadow-chernozem soil under crops (for each group, n = 9, M±SEM; Omsk ASC, Omsk, 2020-2022)

Microbial group	1	2	3	4	5	6	7
Proteolytics (1)		0.85	0.71	0.84	0.58	0.96	0.38
Amylolytics (2)	0.85		0.91	0.66	0.23	0.72	0.09
Oligonitrophils (3)	0.71	0.91		0.32	0.14	0.49	0.38
Phosphate mobilizing (4)	0.84	0.66	0.32		0.88	0.93	–0.65
Microfungi (5)	0.58	0.23	0.14	0.88		0.78	0.92
Nitrifiers (6)	0.96	0.72	0.49	0.93	0.78		–0.61
Cellulose decomposers (7)	0.38	0.09	0.38	–0.65	0.92	–0.61	

N o t e. For the crops of perennial grasses (a cocksfoot grass *Dactylis glomerata* mix with sainfoin *Onobrychis arenaria*) and Sorghum-Sudangrass hybrid, n is the number of measurements; r values are statistically significant at $p < 0.05$.

We assessed both the relationship between the abundance of the studied groups of microorganisms and their relationship with the productivity of the crops. Examining patterns of interdependence between various ecological and trophic groups of soil microorganisms, we revealed a strong positive correlation between the number of saprotrophic bacteria isolated on meat peptone agar and amylolytics, oligonitrophilics, phosphate mobilizrs, and cellulose decomposers. This occurs since the main types of interaction between microorganisms in the biocenosis are reduced to trophic and metabolic connections via excretion of metabolic products, physiologically active substances, etc. (Table 6). Nitrifying bacteria occupy their own ecological niche and are more dependent on environmental conditions.

The yield of forage crops had a strong correlation with the microbiota of the nitrogen cycle. These groups are amylolytic microorganisms that consume mineral forms of nitrogen (NH_3) and serve as an indicator of the intensity of mineralization processes in the soil, proteolytic microorganisms that assimilate organic nitrogen, and cellulose-degrading microorganisms acting as decomposers in the trophic chain of soil ecosystems (*r* 0.98 and –0.93; 0.83 and 0.94; 0.99 and 0.82, respectively, p < 0.05) (Table 7).

7. Crop yields correlations (*r*) with the counts of ecological trophic microbial groups, nitrification capacity and N-NO_3 accumulation (*M*±SEM) in the meadow-chernozem soil (for each group, *n* = 9,; Omsk ASC, Omsk, 2020-2022)

Parameter	Microbial counts, CFU/g	*r*	±*Sr*	t_o	t_t
	Perennial grasses				
Proteolytic microbiota, $\times 10^6$	39.0±4.9	0.83*	0.39	1.62	2.45
Amylolytic microbiota, $\times 10^6$	24.2±6.3*	0.98*	0.10	9.85	2.45
Oligonitrophils, $\times 10^6$	116.6±28.1	–0.16	0.49	–0.32	2.45
Phosphate mobilizing microbiota, $\times 10^6$	181.9±102.1*	0.58	0.41	1.42	2.45
Microfungi, $\times 10^3$	120.3±65.1*	0.38	0.46	0.82	2.45
Nitrifiers, $\times 10^3$	1.25±0.06	0.23	0.49	0.47	2.45
Cellulose-destroying microbiota, $\times 10^3$	111.0±9.6*	0.99*	0.07	14.04	2.45
Total counts, $\times 10^6$	362.2±98.4*	0.53	0.42	1.25	2.45
Nitrification capacity, mg/kg dry soil	31.7±5.8	0.73	0.34	2.14	2.45
N-NO_3 conent, mg/kg dry soil	13.8±7.0*	0.99*	0.07	14.04	2.45
	Sorghum-Sudangrass hybrid				
Proteolytic microbiota, $\times 10^6$	39.1±2.6*	0.94*	0.17	5.51	2.45
Amylolytic microbiota, $\times 10^6$	30.4±4.9*	–0.93*	0.50	–0.06	2.45
Oligonitrophils, $\times 10^6$	186.6±64.8*	0.97*	0.12	7.98	2.45
Phosphate mobilizing microbiota, $\times 10^6$	142.0±36.0	0.98*	0.10	9.85	2.45
Microfungi, $\times 10^3$	64.0±15.9	0.58	0.41	1.42	2.45
Nitrifiers, $\times 10^3$	1.47±0.25	0.10	0.50	0.20	2.45
Cellulose-destroying microbiota, $\times 10^3$	97.0±5.5*	0.82*	0.35	2.08	2.45
Total counts, $\times 10^6$	398.3±98.3*	0.98*	0.10	9.85	2.45
Nitrification capacity, mg/kg dry soil	54.4±22.8	–0.30	0.48	–0.63	2.45
N-NO_3 conent, mg/kg dry soil	40.3±10.4*	0.83*	0.25	3.33	2.45

Note. Perennial grasses are a cocksfoot grass *Dactylis glomerata* mix with sainfoin *Onobrychis arenaria*. Correlations were calculated for yield upon fertilizer application, 4.86 t/ha for perennial grasses and 5.85 t/ha for Sorghum-Sudangrass hybrid (with statistically significant difference vs. the corresponding control), *n* is the number of measurements.

* The *r*-values are statistically significant at p < 0.05.

For the abundance of phosphate-mobilizing microflora, medium correlation was found with the productivity of perennial grasses (*r* = 0.58) and strong correlation with the productivity of sorghum-Sudangrass hybrid (*r* = 0.98, p < 0.05) which is explained by the participation of this microbial group directly in providing plants phosphorus. The content of available soil nitrogen which characterizes the potential reserves of its organic form, turning into mineral compounds under favorable conditions, is important for the formation of productivity (*r* = 0.99 and *r* = 0.83, respectively; p < 0.05).

Thus, fertilizers at a dose of $N_{60}P_{60}$ applied to meadow-chernozemic soil under perennial grasses mostly stimulated reproduction of phosphate-mobilizing microorganisms and soil micromycetes, by 118 and 122% (p < 0.05). Under the sorghum-Sudangrass hybrid, the abundance of amylolytic and oligonitrophilic microbiota significantly increased, by 57 and 90% (p < 0.05). The total number of microorganisms increased equally under perennial grasses and under sorghum-Sudangrass hybrid, by 51-52% (p < 0.05) tthat enhanced mineralization of plant residues and had a positive effect on soil fertility. The use of mineral fertilizers reduced the activity of catalase in the soil by 14% (p < 0.05), but did not have a significant effect on the hydrolytic enzymes urease and invertase. Under the influence of fertilizers, the content of nitrate nitrogen during the growing season increased on average by 2 times or more. The high biological activity of the soil dus to addition of $N_{60}P_{60}$ positively influenced productivity of perennial grasses and sorghum-Sudangrass hybrid, the yields were 4.82-4.89 and 3.64-7.24 t/ha DM, respectively, vs. 3.84-4.57 and 2.96-6.65 t/ha DM in control. We revealed a close correlation between the yield of crops and the abundance of soil amylolytic, proteolytic and cellulose-degrading microbiota, *r* values were 0.98 and -0.93, 0.83 and 0.94, 0.99 and 0.82, respectively (p < 0 .05). Our results confirm the importance of mineral fertilizers in optimizing soil health and stimulating the growth of beneficial microorganisms, which ultimately slows down the decomposition of soil organic matter.

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