https://doi.org/10.26898/0370-8799-2023-4-1
УДК: 631.4:630*114.68

Тип статьи: оригинальная
Type of article: original

МИКРОБИОЛОГИЧЕСКАЯ АКТИВНОСТЬ ЧЕРНОЗЕМА В ЗАВИСИМОСТИ ОТ ХАРАКТЕРА ИСПОЛЬЗОВАНИЯ И РАЗМЕРА ПОЧВЕННЫХ АГРЕГАТОВ

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Представлены результаты исследований по изучению изменения биологической и ферментативной активности почвы в зависимости от антропогенного влияния и размера почвенных агрегатов. Работа выполнена в 2015–2021 гг. в условиях юго-востока Центрального Черноземья. Почва опытного участка – чернозем обыкновенный (сегрегационный) среднемощный среднегумусный тяжелосуглинистый. Объекты исследований – пашня с 1952 г. и залежь, используемая под сенокос с 1882 г. Показано количественное изменение отдельных групп микроорганизмов, определяемое характером воздействия на почвенный покров. Общее количество микроорганизмов как на пашне, так и в черноземах под естественной растительностью было на одном уровне – 40,0 и 39,3 млн КОЕ соответственно. Отмечены существенные различия биологической активности черноземов по отдельным группам структуры микробного ценоза. Выявлено увеличение количества бактерий аммонификаторов в пахотном аналоге в среднем на 30,7%, микромицетов – на 4,4, целлюлозолитиков – на 46,4, нитрификаторов – на 46,9, расчетного коэффициента гумуфикации – на 45,4%. По другим компонентам микробного ценоза отмечено преимущество залежных степных почв: актиномицетов на 18,5%, минерализаторов гумуса на 11,8%. Показано увеличение активности большинства групп микроорганизмов в структурных отдельностях меньшего размера – 1–5 мм. В более крупных мезоагрегатах численность КОЕ снижается. Наиболее рельефно данная закономерность прослеживается в агрогенно измененных почвах. В почвах, занятых естественной растительностью, отмечена более высокая активность биохимических процессов.

Ключевые слова: чернозем, залежь, пашня, почвенные агрегаты, микробиологическая активность, ферментативная активность

MICROBIOLOGICAL ACTIVITY OF CHERNOZEM DEPENDING ON THE USE AND SIZE OF SOIL AGGREGATES

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The results of the studies of changes in biological and enzymatic activity of soils depending on the anthropogenic influence and the size of soil aggregates are presented. The work was carried out in 2015-2021 in the conditions of the south-east of the Central Chernozem region. The soil of the experimental site is ordinary (segregational) medium–sized medium-humus heavy loamy chernozem. The objects of research are long–used arable land and the layland used for haying since 1882. The quantitative change of individual groups of microorganisms, determined by the nature of the impact on the soil cover, is shown. The total number of microorganisms in both arable land and chernozems under natural vegetation was at the same level of 40.0 and 39.3 million CFU, respectively. Significant differences in the biological activity of chernozems by individual groups of microbial cenosis structure were noted. An increase in the number of ammonification bacteria on the arable analogue was revealed by an average of 30.7%; micromycetes by 4.4%; cellulolytics by 46.4%; nitrifiers by 46.9%; calculated humification coefficient by 45.4%. According to other components of microbial cenosis, the advantage of layland steppe soils is noted: actinomycetes by 18.5%; humus mineralizers by 11.8%. An increase in the activity of most groups of microorganisms was shown in the smaller structural units of 1-5 mm. In larger mesoaggregates, CFU numbers tend to decrease. This pattern is most clearly traced in agrogenically altered soils. In the soils occupied by natural vegetation, there is a higher activity of biochemical processes.

Keywords: chernozem, layland, arable land, soil aggregates, microbiological activity, enzymatic activity

Для цитирования: *Чевердин Ю.И., Киценко В.П., Чевердин А.Ю.* Микробиологическая активность чернозема в зависимости от характера использования и размера почвенных агрегатов // Сибирский вестник сельскохозяйственной науки. 2023. Т. 53. № 4. С. 5–12. https://doi.org/10.26898/0370-8799-2023-4-1

For citation: Cheverdin Yu.I., Kitsenko V.P., Cheverdin A.Yu. Microbiological activity of chernozem depending on the use and size of soil aggregates. *Sibirskii vestnik sel'skokhozyaistvennoi nauki = Siberian Herald of Agricultural Science*, 2022, vol. 53, no. 4, pp. 5–12. https://doi.org/10.26898/0370-8799-2023-4-1

Конфликт интересов
Авторы заявляют об отсутствии конфликта интересов.
Conflict of interest
The authors declare no conflict of interest.

INTRODUCTION

Chernozems, which are the predominant background soils of the Central Chernozem Zone, possess high natural fertility. Prolonged agricultural use leads to changes in their properties and quality, primarily affecting the agrochemical and agrophysical parameters of the plow layer of soil. As a result of intensive agrogenic impact, the bulk density, structural and granulometric composition, microaggregate condition, biological activity, tillable land productivity, and other indicators undergo significant transformations [1–5].

Higher crop yields are achieved under optimal soil density conditions [6]. Soil fertility regulation affects multiple aspects. The application of mineral fertilizers [7] is a radical and effective means of enhancing plant nutrition. Their use reduces the negative risks associated with drought, which is particularly relevant in the steppe regions of Russia with insufficient moisture [8].

Physical indicators, in turn, determine the direction and flow of soil processes responsible for the chemical, physicochemical, and microbiological aspects of fertility. Increased soil density can act as a restraining factor on their biological activity [9, 10]. The structural-aggregate condition is closely related to the soil's humus content [11]. Several authors have noted changes in microbial activity and fertility indicators depending on the soil's structural condition [12, 13].

The purpose of this study is to investigate the changes in the microbial cenosis structure of chernozem soil depending on the size of soil aggregates as a result of anthropogenic impact.

MATERIAL AND METHODS

The research was conducted at the Voronezh Federal Agricultural Scientific Centre named after V. V. Dokuchaev from 2015 to 2022. The study objects were a natural soil deposit formed in 1882 and a cultivated plot established in 1952. The soil deposit area has been annually used for haymaking over the course of 140 years, while the cultivated plot follows a grain-fallow crop rotation. The soil was plowed to a depth of 20-22 cm. The soil type is medium-loamy ordinary chernozem on heavy carbonate clay loams. The organic matter content is 10.2% in the deposit and 6.7% in the cultivated plot, with a pH of the soil extract ranging from 6.8 to 7.2. The exchangeable calcium content is 30-32 mmol (eq.)/100 g of soil, and magnesium content is 5-7 mmol (eq.)/100 g. Favorable moisture conditions prevailed during the years of the study, with annual precipitation exceeding 500 mm, compared to the long-term average of 430 mm.

Microbiological activity was determined using the following method[1]; ammonifying bacteria were cultivated on the meat-peptone agar (MPA), bacteria utilizing mineral forms of nitrogen and actinomycetes were cultivated on starch-ammonium agar (SAA). Soil plates were used to assess the quantity of nitrogen-fixing bacteria. The population of nitrifying bacteria was estimated through cultivation on alkaline agar, while humus mineralizers were assessed on nitrate agar.

Soil enzymatic activity was determined as follows: catalase activity was measured using the Johnson and Temple method, invertase activity was measured using the V.F. Kuprevich method, urease activity was measured using the A.Sh. Galstyan method modified by F.Kh. Khaziev on a photoelectrocolorimeter KFK-2-UHL4.2, and phosphatase activity was measured using the F.Kh. Khaziev method on KFK-2-UHL4.2[2].

The structure of the microbial cenosis was determined using fresh soil samples. In the field conditions, soil samples were dispersed into fractions of 1-2, 2-3, 3-5, 5-10, and more than 10 mm.

RESULTS AND DISCUSSION

The results of the conducted research indicate significant differences in the structure of the microbial community depending on the size of soil particles. The most noticeable differences are characteristic of arable soils. The maximum total microbial population is observed in aggregates with a minimum size of 1-2 mm - 43.1 million CFU (see Table 1). Increasing the size of soil particles to 10 mm and larger leads to a decrease in the total microbial population to 37.0 million CFU.

In chernozem soil deposits used for hayfields, the total number of microorganisms per fraction varied to a lesser extent, ranging from 38.2 to 40.8 million CFU. The exception

Табл. 1. Структура микробного ценоза (2015–2021 гг.)
Table 1. Structure of microbial cenosis (2015–2021)

Option	Soil particle size, mm	Total number	MPA	SAA	Actinomycetes	Humus mineralizers	Micromycetes	Cellulosolithics	Nitrifying agents	Humification coefficient Hl
		mln CFU					thous. CFU			
Arable land since 1952	1–2	43,1	10,3	17,9	2,69	12,1	24,8	64,7	0,47	16,4
	2–3	41,7	10,4	18,1	2,84	10,3	24,2	65,1	0,47	16,6
	3–5	36,1	8,7	15,5	2,29	9,5	24,4	61,3	0,47	13,7
	5–10	42,1	10,5	17,3	2,99	11,3	26,0	57,5	0,45	17,1
	>10	37,0	9,2	17,0	2,94	7,9	30,0	68,5	0,50	14,7
Layland since 1882	1–2	38,2	7,7	17,4	3,09	10,1	24,0	36,9	0,34	11,0
	2–3	40,8	7,0	18,1	3,48	12,3	25,5	43,2	0,33	9,7
	3–5	40,1	7,4	18,3	3,20	11,3	23,7	44,2	0,30	10,4
	5–10	37,8	7,8	15,9	2,93	11,2	23,5	45,5	0,34	11,7
	>10	39,4	7,6	15,9	3,59	12,2	27,1	46,8	0,32	11,2
$LSD_{0,95}$		0,25	0,12	0,32	0,08	0,51	0,22	0,92	0,03	

[1] *Tepper E.Z., Shilnikova V.K., Pereverzeva G.I.* Microbiology Practicum. Moscow: Drofa, 2005. 256 p.
[2] *Khaziev F.H.* Methods of soil enzymology. Moscow: Nauka, 2005. 252 p.

was particles ranging from 5 to 10 mm, which had a population of 37.8 million CFU.

It is necessary to note a general pattern - an increase in all groups of the microbial cenosis structure in smaller soil aggregates. Microorganisms that grow on meat-peptone agar (MPA) made a significant contribution to the immobilization of organic carbon. Higher numbers of these microorganisms were observed in agrogenically transformed soils, ranging from 8.7 to 10.5 million CFU/g of soil (see Table 1). In the uncultivated section of ordinary chernozem, the number of ammonifiers varied within narrower limits, from 7.0 to 7.8 million CFU. Similar values were observed for soil aggregates regardless of their size.

Microorganisms that grow on starch-ammonium agar (SAA) actively participated in the reutilization of mineral forms of nitrogen. Higher activity of amylolytic microorganisms was observed in mesoaggregates with a size of 1-5 mm, both in the deposits and in the plowed analog. The number of microorganisms growing on SAA under natural vegetation varied within the range of 17.4-18.3 million CFU. Similar values were observed in the plowed field, ranging from 17.9 to 18.1 million CFU. It is worth noting a lower activity of this group of microorganisms in soil aggregates with an increase in their size.

Transformation coefficient of organic matter, Pm = (MPA + SAA) × (MPA/SAA), reflecting humus accumulation processes, had higher values in anthropogenically influenced chernozem soils, ranging from 13.7 to 17.1. In hayfield deposits, the values of the Pm coefficient were significantly lower, ranging from 9.7 to 11.2. Higher values of this coefficient are characteristic of agriculturally valuable fractions. Thus, plowing the soil and optimizing its physical condition with a dominance of smaller-sized fractions enhance humus accumulation processes.

Humus mineralizers had higher population numbers in the chernozem soil deposits, ranging from 10.1 to 12.3 million CFU. The plowed analogs slightly lagged behind in this regard. Higher values are characteristic of agriculturally valuable aggregate fractions, ranging from 9.5 to 12.1 million CFU. It should be emphasized that bacteria responsible for organic matter mineralization had the highest activity in arable soils within the fractions of the smallest size (1-2 mm), gradually decreasing with an increase in aggregate size. In deposit soils, on the other hand, a decrease in activity was observed with an increase in soil particle size.

Nitrogen nitrifiers play a significant role in soil fertility stabilization. In hayfield soils, their baseline activity was slightly lower compared to plowed analogs. The number of nitrifiers in hayfield deposits ranged from 0.30 to 0.34 thousand CFU. Plowing, due to the activation of microbiological processes, increased their numbers to 0.45-0.50 thousand CFU. In plowed fields, the activity of nitrifiers was similar between fractions, with the exception of the dense part of structural units with the highest quantity. In deposit analogs, the activity of nitrifiers was nearly independent of particle size, showing minimal fluctuations around the average value.

Higher content of actinomycetes was found in deposit soils, ranging from 3.09 to 3.59 million CFU. Plowing led to a noticeable decrease in this group of microorganisms, to 2.29-2.99 million CFU. In this case, there was no clear regularity in the change of actinomycete activity depending on the size of mesoaggregates. In plowed fields, the maximum content of microorganisms was observed in aggregates sized 5-10 mm and larger, ranging from 2.94 to 2.99 million CFU. In chernozem deposits, no clear regularity was identified.

Bacteria involved in cellulose decomposition play an important role as cellulolytic bacteria. Higher total baseline values were characteristic of plowed soils, ranging from 57.5 to 68.5 thousand CFU. In deposit soils, their activity was significantly lower, ranging from 36.9 to 46.8 thousand CFU. The activity of cellulolytic bacteria varied depending on the size of soil aggregates and showed peculiarities across different land types. In plowed fields, a higher population of cellulolytic bacteria was observed in aggregates sized 1-3 mm and in the dense part of soil fractions larger than 10 mm. In deposit soils, the activity of cellulolytic bacteria increased with an increase in aggregate size.

The fungal microflora belongs to the group of microorganisms responsible for the deep decomposition of organic matter. Higher levels of fungal population were observed in arable chernozem soils, ranging from 24.2 to 30.0 thousand CFU (colony-forming units) per gram of soil. In comparison, the activity of fungi in residual soils was lower, ranging from 23.7 to 27.1 thousand CFU. Similar fungal population densities were found in the agriculturally valuable fractions of both residual and arable soils, with the highest activity observed in structural aggregates.

Enzymatic activity is an important soil property. Changes in the activity of urease, phosphatase, invertase, and catalase were evaluated.

Catalase activity was higher in the long-fallow chernozem soils, ranging from 5260 to 6238 mg NH_3/100 g soil (see Table 2). Tillage practices reduced catalase activity to 4600-4812 mg NH_3. Overall, there was a general decrease in catalase activity with increasing size of soil particles.

A similar trend was observed for phosphatase activity. In layland soils, phosphatase activity was observed at levels of 122.5-160.0 mg glucose/100 g soil, while in arable chernozem soils, it ranged from 44.0 to 92.5 mg glucose/100 g soil. Higher values were found with increasing size of mesoaggregates.

Urease refers to enzymes involved in the transformation of proteins. The ammonia generated through urease activity contributes to soil fertility. Mowing layland soils exhibited higher urease activity, ranging from 268 to 354 mg phenolphthalein/100 g soil. Tillage practices results in a significant reduction in urease activity, reaching 148-174 mg phenolphthalein/100 g soil. Increased urease concentrations were observed in larger linear-size aggregates in both layland and arable soils.

Enzymes belonging to the class of oxidoreductases, such as catalase, actively participate in the transformation of humic substances. Our data showed higher catalase activity in arable chernozem soils, ranging from 20.45 to 23.75 O_2 in 3 minutes. In layland steppe soils, catalase activity was slightly lower, ranging from 18.75 to 20.30 O_2 in 3 minutes. There was a weak trend of decreasing catalase activity with increasing size of soil particles, particularly evident in the layland chernozem soils.

Physical parameters of soil structure are important indicators of soil fertility. Agrogenic influences can alter the structural-aggregate state of chernozem soils. The size of soil aggregates is a key factor determining the abundance and intensity of soil biological processes[3].

Табл. 2. Ферментативная активность почвы
Table 2. Soil enzymatic activity

Option	Soil particle size, mm	Invertase, mg NH_3/100 g of soil	Urease, mg of phenolphthalein/100 g of soil	Phosphatase, mg of glucose/100 g of soil	Catalase, O_2 in 3 min
Arable land since 1952	1–2	4812	148	44,0	22,25
	2–3	4910	152	47,5	20,75
	3–5	4600	152	66,0	20,45
	5–10	4750	174	90,0	23,75
	>10	4812	160	92,5	23,05
Layland since 1882	1–2	6238	268	122,5	20,3
	2–3	6180	332	137,5	19,85
	3–5	5710	334	145,0	19,95
	5–10	5600	354	160,0	19,3
	>10	5260	276	147,5	18,75

[3] *Vasilenko E.S., Kutovaya O.V., Tkhakahova A.K., Martynov A.S.* Change in the number of microorganisms depending on the size of aggregates of humus horizon of migration-micellar chernozem // Bulletin of the V.V. Dokuchaev Soil Institute. 2014. N 73. pp. 150-173.

Transformation of complex organic compounds occurs more intensively in small aggregates due to the higher microbial population involved in the carbon cycle.

Aggregates of small size fractions are characterized by high diversity rates[4]. Increasing linear dimensions of structural units lead to increased nitrogen oxide emissions, anaerobic conditions, and negative effects on biological processes[5]. The reason for the formation of anaerobiosis in the aggregate is the physical processes of swelling - shrinkage of the aggregates, which contribute to the almost constant maintenance of a lack of air in the aggregates[6].

CONCLUSIONS

1. Tillage and intensive agricultural use of chernozem soils lead to changes in the physical parameters of soil fertility and the direction of soil biological and biochemical processes. Biological activity is directly related to the nature of land use. The highest number of major groups of microorganisms is observed in structural units of mesoaggregates, which are considered agriculturally valuable fractions. As the size of soil particles increases, the activity of soil microflora significantly decreases. The application of agronomic practices aimed at improving the structural condition of arable soils and optimizing the composition ratio of chernozem mesoaggregates during tillage can serve as a means of improving soil fertility.

2. The number of ammonifiers increases in structural units of smaller size (1-3 mm) in agrogenically transformed soils. In this regard, the layland soil variant is more conservative with a lower population of the considered bacteria regardless of the fractions size.

3. The maximum population of soil amylolytic bacteria (SAA) is characteristic of the aggregates ranging from 1 to 5 mm. Both in arable chernozem soils and in layland soils, their sizes were approximately the same order of magnitude.

4. Significantly higher numbers of actinomycetes are observed in layland soils. The highest values are characteristic of the clumpy part of the aggregates. It can be assumed that in cultivated soils, certain processes occur more rapidly.

5. Regarding the quantity of humus mineralizers, structural fractions in the 1-3 mm range have an advantage in cultivated soils. In layland soils, no differences were found depending on the fraction size, indicating the similarity of mineralization processes in all fractions.

6. The maximum number of microfungi is found in the clumpy part of the aggregates. This is due to the high sensitivity of soil fungal microflora to aeration.

7. Phosphatase, invertase, and urease show higher activity in the soils occupied by natural vegetation. Catalase activity is higher in agricultural soil ecosystems.

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Дата поступления статьи / Received by the editors 14.10.2022
Дата принятия к публикации / Accepted for publication 13.12.2022
Дата публикации / Published 22.05.2023