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Biological indicators in the environmental monitoring of gray forest soil of agrosystems

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ABSTRACT
The objective was to monitor the biological indicators to assess the ecological sustainability of gray forest soil, due to the different level of agrotechnical burden on agricultural landscapes. During 10 years of research (2011-2020) the number of bacteria using various forms of nitrogen was continuously decreasing on the background of mineral intensification. The total average pool of active microflora on organomineral and mineral backgrounds in moulboard plowing was 20.1 and 16.1 million CFU g^{-1} of soil, respectively, during the period of observations. In non-moldboard plowing, the same average pool was 18.8 and 15.9 million CFU g^{-1}. The minimum pool was maintained on a high-intensity mineral background in moldboard plowing, amounting to 14.5 million CFU g^{-1} of soil. The decrease in the total bacterial number in the soil of mineral backgrounds indicates a deterioration in their ecological stability. The calculated ecological and trophic indices indicate less ecologically stable low-intensity mineral background in moldboard plowing. This variant showed relatively low values of humus accumulation coefficients (Ch = 0.39) and transformation of organic residues into soil organic matter (Ct = 5.4) established at the highest mineralization coefficient (Cm = 1.57). In this soil, mineralization of organic matter prevails, which reduces its fertility and environmental sustainability. On this background, the activity of the studied enzymes was lower than in other variants and in the soil deposits. A set of micromycetes has shown to grow on mineral intensification backgrounds, especially on a high-intensity mineral background, where moldboard plowing was used as the main treatment. The lowest phytotoxicity was noted on a high-intensity organic-mineral intensification background –21.4%. Both microbiological and biochemical parameters represents an informative diagnostic feature of the ecological state of agrolandscapes. They ensure an objective assessment of the efficiency and degree of agrotechnical burden, predicting a decrease in environmental sustainability on mineral intensification backgrounds.

Keywords: Microbiological monitoring, Ecological sustainability, Agricultural landscapes, Microbial pool, Gray forest soil.
Article type: Research Article.

INTRODUCTION
A comprehensive assessment of modern agricultural activity has become a powerful ecological factor that affects the nature of the soil-forming process and sometimes irreversibly changes both the soil properties and the associated ecosystems. Its scale of the consequences is comparable only with global climate change (Lal 1998). Intensive plowing, regular crop rejection, and massive use of chemical agents have caused serious disfunction of the soil system, including maintenance of the balance of important atmospheric gases (carbon dioxide, nitrogen oxides, and methane) (Mosier 1998). A decrease in crop productivity indicates a loss of stability in the

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agroecosystem. However, this is already the final stage of its reaction to agrotechnical impact, which was preceded by changes in other indicators, such as physical and chemical properties of the soil, balance of biochemical processes, phytotoxic activity of soil microbial communities, etc. (Ananieva *et al.* 2002). Scientific and methodological approaches to environmental regulation of soil quality are developing quite actively. However, there are few methods for assessing and regulating the anthropogenic load for the formation of ecologically balanced agricultural landscapes. The phrase "environmental regulation", especially in the context of chemical and radiation pollution of the environment and an alternative to hygienic regulation, was first mentioned in the domestic scientific literature in the early 70s of the 20th century. Currently, Russia has no officially approved or at least completely generally accepted guidelines for environmental regulation of man-made loads on terrestrial ecosystems. Rationing of loads on agroecosystems is also one of the most important, complex, and underdeveloped environmental problems of agricultural production. There is still neither official regulatory and methodological documents for determining the permissible loads on agroecosystems nor a justified rationing methods. Agrolandscape is the main component of the agrosystem. An ecologically balanced agricultural landscape should be ecologically sustainable.

This is expressed in the maintainability of the formed structure and specified productive, social functions under the influence of external factors, while preserving the biosphere. To ensure the ecological sustainability of the agricultural landscape, it is necessary to regulate technological burdens and set certain parameters for production (Masiutenko *et al.* 2014) The general form of the solution to the standardization problem does not cause difficulties: the ultimate load is the maximum load that does not cause a disbalance of the system within the states specified according to certain criteria for a given period of time. However, solving the problem in this general form implies the presence of almost complete knowledge of the functioning of the ecosystem.

The main difficulty lies in the absence of methods for bringing the entire set of possible loads on a certain territory into a comparable form. In addition, the question of finding criteria to assess the effectiveness of soil-protective agricultural technologies and to be used for environmental monitoring of the state of the soil environment is highly relevant (Tereshchenko & Bubina 2009; Masiutenko *et al.* 2014; Gholoubi *et al.* 2019; Bagheri et al. 2020; Mirsaleh Gilani et al. 2020; Dalir *et al.* 2021). All the variety of existing approaches and concepts in environmental monitoring arises from the concretization of the general problem. We have significantly limited the scope of the concept of "environmental monitoring", considering only its "biological" component. The main habitat of the biota (both by number of species and biomass) in the modern biosphere is soil, which plays a key role in the transformation of all terrestrial ecosystems. Therefore, biological assessment of various agroecosystems is one of the main elements of the overall environmental monitoring. The objective was to monitor the biological indicators to assess the ecological sustainability of gray forest soil, due to the different level of agrotechnical burden on agricultural landscapes. Both domestic and foreign literature review has shown most of the microbiological criteria for the ecological sustainability of soils to be based on the species diversity of the microbial community and the degree of interrelation between the elements of the community (Schimel *et al.* 1999; Dobrovolskaia *et al.* 2015). Another widely used indicators are total biomass of microorganisms, respiration rate, enzyme activity, and metabolic coefficient qCO_2 (Polianskaia *et al.* 2016; Katsairou *et al.* 2010). In our study we used soil biological activity as a diagnostic indicator for standardizing the level of man-made load on agricultural landscapes. We studied the long-term agrotechnical impact on the biological activity of the soil, evaluating the possible use of biotests (indicators of enzymatic activity, the number of various physiological groups of microflora, microbiological coefficients, test plants) as parameters of ecosystems in environmental monitoring. We tried to combine consideration of theoretical and methodological issues with the analysis of experimental data on the reaction of soil microbiocenosis to the levels of agrotechnical burden in agricultural systems of gray forest soil (Zinchencko 2014; Zinchenko *et al.* 2018). The results of ten-year research (2011-2020) served as the bases for our study. The annual biological monitoring of the gray forest soil of agricultural systems was conducted to assess the degree of agrotechnical burden on soils and to identify areas of high ecological risk, which makes it possible to further normalize the level of anthropogenic impact on agricultural landscapes of gray forest soil.

MATERIALS AND METHODS

The aim of the present study was gray forest medium loamy soil of a stationary field experiment launched to examine and improve adaptive landscape farming systems in the Vladimirsky high plane. The experimental work was carried out during the first establishment of the experiment in 1996 in the agriculture department. The humus

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content in the arable layer (0-20 cm) of gray forest soil varied from 3.9 to 4.2% (according to Tyurin), the content of mobile phosphorus supply (according to Kirsanov) and exchangeable potassium (according to Maslova) was 100-150 mg kg^{-1} and 100-120 mg kg^{-1} soil, respectively, pH_{kcl} was 5.9-6.1. The studied variants of the experiment are shown in Table 1.

Table 1. Intensification backgrounds under study.

Intensification background	The total doses of fertilizers applied during crop rotation
Null (N)	Manure 40 t ha^{-1}, once per crop rotation
Intensive (I)	
Intensive mineral (IM)	N_{350} P_{220} K_{390}
Highly intensive mineral (HIM)	N_{480} P_{280} K_{575}
Intensive organomineral (IOM)	N_{310} P_{150} K_{310} + manure 60 t ha^{-1}, once per crop rotation
Highly intensive organomineral (HIOM)	N_{430} P_{160} K_{360}+ manure 80 t ha^{-1}, once per crop rotation

This scheme was studied using two methods of basic soil cultivation: moldboard plowing to a depth of 20-22 cm (MP) and energy-saving non-moldboard plowing to a depth of 10-12 cm (NMP). Manure was applied once per crop rotation; mineral fertilizers were applied annually. Soil samples were taken 3 times during the growing season from a layer of 0-20 cm for microbiological and biochemical analysis. Calendar terms of sampling were May 10-14, July 8-15, and September 2-10. The concentration of organic nitrogen fixing bacteria in soil was determined (inoculation of meat-peptone agar (MPA)); mineral nitrogen fixing bacteria (inoculation of starch ammonia agar (SAA)); actinomycetes (Krasilnikov's medium); the amount of oligonitrophilic (diazotrophic) grouping (Ashby's medium); micromycetes (Czapek's medium); contamination of the soil with azotobacter (the method of soil lumps fouling on Ashby's medium (Tepper *et al.* 1987).

The activity of soil enzymes was diagnosed using classical methods: catalase activity was determined by a gasometric method based on measuring the hydrogen peroxide decomposition rate (A.Sh. Galstian's method); polyphenol oxidase (PPO) and peroxidase (PD) activity was determined based on the method of iodometric titration of the reaction mixture containing pyrocatechol as a substrate after interaction with the soil suspension (K.A. Kozlov's method); urease activity was determined based on the amount of ammonia nitrogen formed during the enzymatic hydrolysis of urea under optimal conditions (T.V. Shcherbakova/s method); invertase activity was determined by iodometric measurement of reducing sugars by titration with hyposulfite (I.N. Remeiko, S.M. Malinovskaya); total phosphatase activity was determined using the method of I.T. Geller, K.E. Ginzburg, based on the quantitative assessment of inorganic phosphorus, formed during the cleavage of organic phosphorus compounds under the action of phosphatases. The total phytotoxicity of soil microfungi in the soil of studied intensification backgrounds was determined using A.A. Danilova's method (Danilova 2006). The results were statistically processed in Statistic 6.

RESULTS

The obtained data substantiate the system of comprehensive monitoring of gray forest soils in the agrarian territories of the Upper Volga region. It includes a database on the quantitative and qualitative composition of the main agronomically significant ecological and trophic groups of microorganisms; microbiological indices characterizing the intensity and direction of the transformation processes of soil organic matter; the total level of the enzymatic pool based on the activity of individual enzymes; determination of the phytotoxicological effect caused by a set of micromycetes. Analysis of microbiological monitoring data made it possible to assess the agroecological state of agricultural systems of gray forest soil. Microbocenoses are both the most active and dynamic structural unit of the ecosystem. Therefore, the total number of the pool of agronomically significant ecological trophic groups of microorganisms in the gray forest soil was calculated from the sum of viable cells of ammonifiers, immobilizers of mineral nitrogen, oligonitrophils (diazotrophs), micromycetes, and actinomycetes. The size of the active microflora pool was influenced by the changing abiotic conditions of the study years and agrotechnical factors. However, there was a stable reliable pattern of a decrease in the total microbial count on mineral intensification backgrounds treated mainly by moldboard plowing (Table 2). During 10 years of research (2011-2020) the number of bacteria using various forms of nitrogen was continuously decreasing on the background of mineral intensification. Their total number (MPA + SAA) on an intensive and highly intensive mineral background was in the range of 7.8 million CFU g^{-1} of soil, which is statistically lower than on organic

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mineral backgrounds (11 million CFU g^{-1} g of soil). The total average pool of active microflora on organomineral and mineral backgrounds in moldboard plowing was 20.1 and 16.1 million CFU g^{-1} of soil, respectively, during the period of observations. In non-moldboard plowing, the same average pool was 18.8 and 15.9 million CFU g^{-1} . Manure at a dose of 40, 60, and 80 t ha^{-1} had a positive effect on the development of the microbial complex of gray forest soil. The minimum pool was preserved on a highly intensive mineral background in moldboard plowing, with the average amount of the studied ecological-trophic groups of microorganisms amounting to 14.5 million CFU g^{-1} of soil.

Table 2. Microbiological and biochemical assessment of the biological state of gray forest soil for 2011-2020.

Background	**Total bacteria mil. CFU g^{-1}**	**MPA+ SAA, mil. CFU g^{-1}**	**Cm**	**Ct**	**Ch**	**Average enz. activity (%)**	**Micromycete toxicity (%)**
			Modboard plowing				
N	20.6	9.71	1.14	10.50	0.55	92	35.7
I	21.4	10.30	0.97	11.41	0.96	116	26.2
IM	17.7	8.49	1.18	8.46	0.69	102	40.1
HIM	14.5	7.14	1.57	5.44	0.39	98	45.8
IOM	18.2	8.79	1.05	11.45	0.75	111	38.1
HIOM	20.3	10.72	0.94	13.80	0.74	106	21.4
			Non-moldboard plowing				
N	16.8	9.10	1.0	11.45	0.51	104	28.6
I	16.7	9.05	0.93	13.1	0.77	115	26.2
IM	15.6	7.61	1.05	8.15	0.58	101	31.0
HIM	16.2	7.98	1.30	8.70	0.46	105	33.3
IOM	22.4	11.51	0.92	14.53	0.85	121	26.2
HIOM	19.2	10.93	0.80	17.89	0.81	111	26.2

Note: Cm is the coefficient of mineralization (SAA/MPA); Ct - coefficient of transformation of organic residues ((SAA + MPA)·MPA/SAA); Ch is the coefficient of humus accumulation (PPO/PD).

Thus, soil biota is an informative diagnostic component in monitoring the ecological state of agricultural landscapes, sensitive to agrotechnical burden. According to a number of researchers, only the functional characteristics of the soil microbial community fully reflect the degree of soil resistance to anthropogenic impact (Dighton 1997; Griffiths *et al*. 1999). A decrease in the total microbial count in the soil of mineral backgrounds makes it possible to predict a decrease in their ecological sustainability. As the resulting ecological-trophic indices have shown, the highly intensive mineral background in the moldboard plowing is less ecologically stable low-intensity mineral background in moldboard plowing. This variant showed relatively low values of humus accumulation coefficients (Ch = 0.39) and transformation of organic residues into soil organic matter (Ct = 5.4) established at the highest mineralization coefficient (Cm = 1.57). In this soil, mineralization of organic matter prevails, which reduces its fertility and environmental sustainability. Moldboard plowing intensifies the mineralization of soil organic matter, while non-moldboard plowing, on the contrary, reduces the intensity of these processes and provides an increase in the synthesis of humic compounds. Mineralization coefficients close to 1 were determined on organomineral backgrounds, which characterizes the relative balance of the processes of microbiological mineralization and the synthesis of soil organic matter. At the same time, these backgrounds had relatively higher transformation coefficients of organic matter and the coefficients of humus accumulation (Cm and Kh), which shows the nature of microbiological and biochemical processes aimed at a more active synthesis of soil organic matter. Microbiological indices make it possible to assess the nature and intensity of transformation of organic substances in the soil of agricultural landscapes and to identify the clearest risk zones in the ecological state and soil fertility. The proposed biochemical indicators of the activity of mineralization and humification of organic substances can be used to substantiate the methods of ecologically optimal and agrotechnically justified impact on the soil, biological regulation of the anthropogenic load on the soil system. The average index of the enzymatic activity of the studied backgrounds exceeded or was at the level of natural soil analogs (perennial fallow lands), the enzymatic activity of which was taken as 100%. The lowest pool of enzymatic activity (98%) was in the high-intensity mineral background in moldboard plowing. This variant had lower activity of the studied enzymes (catalase, polyphenol oxidase, peroxidase, invertase, urease, phosphatase) than other variants and the fallow soil. The toxicity of the micromycete complex used as a biodiagnostic indicator made it possible to reveal

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an increase in its activity on mineral intensification backgrounds, especially on a high-intensity mineral background, where moldboard plowing was used as the main treatment. The lowest phytotoxicity was noted on a high-intensity organic-mineral intensification background – 21.4%.

CONCLUSION

Thus, a set of microbiological and biochemical indicators is an informative diagnostic sign of the ecological state of agricultural landscapes. It provides an objective assessment of the efficiency and level of agrotechnical load in the system of adaptive landscape farming. A decrease in the total number of microflora, changes in the taxonomic structure of microbial associations, their biochemical and functional activity, with long-term use of the mineral fertilizer system, makes it possible to predict a decrease in environmental stability on mineral intensification backgrounds compared to organomineral ones. The response of microorganisms to the long-term use of high doses of mineral fertilizers made it possible to identify the most unbalanced ecological zone, which has developed on a high-intensity mineral background in moldboard plowing. As the data of microbiological and biochemical monitoring of the ecological state of gray forest soils of agricultural landscapes has shown, the organomineral fertilizer system turned out to be the most feasible, focused on the use of manure once per crop rotation at a dose of 40 and 60 t ha^{-1} on the background of the annual use of average doses of mineral fertilizers.

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