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The Influence of Biomodified Fertilizers on the Productivity of Crops and Biological Properties of Soddy-Podzolic Soils

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ABSTRACT

The problem of preserving the ecological balance of agrocenoses while using mineral fertilizers is one of the most important in modern agriculture. The use of new types of organomineral fertilizers (OMF), the pellets of which are modified by various types of biopreparations (BP), is one of the ways to solve it. The studies were conducted in 2013-2017 on the experimental field of the Vologda State Dairy Farming Academy by N.V. Vereshchagin (Russia, Molochnoe). In the field experiments, the spore forms of the rhizosphere bacteria Bacillus subtilis Ch-13 (biopreparation BisolbiFit), Bacillus mucilaginosus (Phosphatovit), and the consortium based on these two strains (phosphoActiv) were used as modifiers of OMF. The first experiment consisted of four variants: (1) control (without fertilizers), (2) BP BisolbiFit, (3) OMF, (4) OMF + BP BisolbiFit (on spring wheat and flax); the second experiment consisted of six variants: (1) control (without fertilizers), (2) NPK, (3) OMF, (4) OMF + BP BisolbiFit, (5) OMF + BP Phosphatovit, (6) OMF + BP phosphoActiv with pHKCl 5.1 - 5.2 and 5.8 – 6.0 (vetch-oat mixture). The method of high-throughput sequencing of 16S rRNA gene with real-time detection was used to study the effect of fertilizers on the taxonomic structure of the prokaryotic community of sod-podzolic sandy loam soil (Albic Retisol (Loamic, Aric, Cutanic, Differentic, Ochric)). Results: The biological modification of pellets of organomineral fertilizers by biopreparation BisolbiFit contributed to the growth of the yield of flax straw by 5.3%, wheat grain - by 5.1%, green weight of the vetch-oat mixture by 10.6%. The greatest amount of bacterial DNA was observed in variants with the addition of OMF on the background of liming (modifier - BisolbiFit). In control and NPK variants (without liming), the number of bacteria was the smallest. Distribution of the bacterial community at the level of phyla showed that the greatest proportion of Actinobacteria (more than 20%) was noted in variants with the introduction of OMF and OMF + BisolbiFit. This indicates that the use of organomineral fertilizers positively affects their development. 1)The biological modification of pellets of organomineral fertilizers by the spore forms of rhizosphere bacteria Bacillus subtilis Ch-13 and Bacillus mucilaginosus contributed to the growth of the yield of flax by 5.3% (flax straw), wheat grain - by 5.1%, green weight of the vetch-oat mixture by 10.6%. 2)The results of the work indicate a close relationship of the taxonomic structure of the soil microbocenosis with the liming and application of fertilizers. 3)Based on the analysis of the metagenome of the prokaryotic community, it is possible to evaluate "soil health" when introducing various types of fertilizers.

Keywords: Sod-Podzolic Soil, Organomineral Fertilizers, Biological Modification, Bisolbifit, Soil Microbocenosis, Taxonomic Structure

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INTRODUCTION

The problem of preserving the ecological balance of agrocenoses is one of the most important components of sustainable development of agriculture [1, 2]. As the experience of world agriculture shows, the level of productivity of cultivated crops and soil fertility is directly dependent on the amount of fertilizers used [3-5]. In the Northern and North-Western regions of Russia, soils are characterized by low natural soil fertility, so the use of fertilizers on such soils gives a high effect [6].

The yield and quality of agricultural products are reduced due to a decrease in the volume of application of mineral and organic fertilizers [7]. As a consequence, the search for additional sources of increasing their effectiveness is relevant [8, 9].

One of the ways to solve this problem is the use of microbial preparations. They are applied to the pellets of mineral fertilizers. Microorganisms, which are part of biological preparations, increase the use of fertilizer by the plants, the availability of phosphorus and potassium in the soil [10, 11].

Application of biopreparations to fertilizer pellets forms a kind of "biocapsule", which performs several functions at once: fertilizing, stimulating, protective. A significant increase in the yield of agricultural crops and the payback of mineral fertilizers is provided by such a set of useful properties [12].

A number of authors for many years studied the use of microbiological preparations in pure form and in combination with mineral fertilizers for inoculation of planting material [13-15].

Modification of fertilizers increases the utilization rate of plants from fertilizers: nitrogen by 20-50%, phosphorus by 20-60%, potassium by 10-40%; promotes yield growth and obtains better quality of crop production, and also allows increasing the payback of fertilizers with additional yields [16, 17].

Objective: to study the effect of new types of biomodified fertilizers on the yield of agricultural crops, the quality and payback of fertilizers.

MATERIALS AND METHODS

Site description and experimental design

- **Experiments with flax and spring wheat**

The studies were conducted on the experimental field of the Vologda State Dairy Farming Academy by N.V. Vereshchagin which is located in the village of Marfino, Vologda district in 2013-2017.

Schemes of field experiments with flax and spring wheat included the following variants: 1 - control (without fertilizers); 2 – biopreparation BisolbiFit based on the *Bacillus subtilis* strain Ch-13 (BP); 3 - organomineral fertilizer (OMF) – 2.5 quintal/ha; 4 – OMF modified with BisolbiFit - 2.5 quintal/ha.

Organomineral fertilizer (OMF) of the brand "Universal", produced by JSC "Buysky Chemical Plant" was used in the third variant. It contains: organic matter (SOM) - 40%, nitrogen - 7%, phosphorus - 8%, potassium - 8%, magnesium - 1.5%, and trace elements (boron, copper, zinc, and manganese 0.01-0.02%). In the 4th variant, the granules of OMF were processed by BP BiolobiFit prior to sowing.

Repeatability is threefold; the registration area of the plots is 3.6 m^2. Fertilizers were added to the row, according to the scheme of the experiment.

During the vegetation period, an integrated protection system was applied taking into account the harmfulness thresholds [18].

- **Experiment with vetch-oat mixture**

The experiment was carried out in 2015 - 2017 at two levels of acidity at pH 5.1 and 5.9 (in 1M KCl extract) in 3-fold repeatability. The area of the plots was 100 m^2. The scheme of the experiment included 6 variants: 1 - control (without fertilizers); 2 - $N_{30}P_{30}K_{30}$; 3 - OMF ($N_{30}P_{30}K_{35}$); 4 - OMF + BisolbiFit (*Bacillus subtilis*); OMF + Phosphatovit (*Bacillus mucilaginosus*); 6 – OMF + phosphoActiv (consortium of microorganisms: *Bacillus subtilis + Bacillus mucilaginosus*).

Soil sampling and analyses

The soil of the experimental plot is medium-cultivated, sod-podzolic, sandy loam, characterized by average humus content, weakly acidic reaction of the soil medium, very high supply of mobile phosphorus, and elevated potassium content (Table 1).

Soil samples were taken from the arable layer of soil (0 - 20 cm) from each plot before the experiment was laid, and then every fall after harvesting. The mixed sample consisted of 20 individual samples taken with the help of a drill bit. After drying, the soil was sieved through a 2 mm mesh screen and analyzed by the following methods: pH - potentiometrically in salt extract (1 mol l^{-1} KCl solution with soil: solution ratio of 1:2.5). The mobile compounds of phosphorus and potassium were extracted with a hydrochloric acid extract (0.2 mol l^{-1} HCl solution with soil: solution ratio of 1:5). In the resulting extract, the phosphorus content was determined colorimetrically (modification of the Deniges' method, based on the preparation of a complex phosphoric acid compound with molybdenum oxides in the presence of tin chloride); potassium content - on a fiery photometer. The humus content (soil organic carbon - SOC) was determined by the method of wet soil ashing with chrome mixture (0.4 M $K_2Cr_2O_7$ in H_2SO_4 diluted with water in a ratio of 1:1) by a coefficient of 1.724, suggesting that humus contains 58% of SOC [19].

Soil microbial community structure

Determination of the microorganisms' number was carried out by PCR (polymerase chain reaction) with real-time detection, which allows the identification of microorganisms without their cultivation. The rationing was carried out according to the number of operons of the ribosomal 16S rRNA gene per 1 g of soil. This parameter was used for a comparative assessment of the number of microorganisms in different soil samples [20, 21]. The taxonomic structure of the prokaryotic community (archaea, bacteria) was analyzed at the level of families and expressed in relative percentages. The threshold of significance of the factor influence on the soil microbiome was determined to be 0.05%. Analyzes were performed in the Center for Collective Use on the scientific equipment "Genomic Technologies, Proteomics and Cell Biology" of the FSBSI "All-Russia Research Institute for Agricultural Microbiology".

Data analyses

The increase in yields from various types of fertilizers is calculated for absolute control (without fertilizers). The statistical processing of the results was carried out using the STATVIUA.EXE program. The significance of the differences between the variants was determined using the LSD criterion, using one and two-way analysis of variance (ANOVA), wherein the differences between the variants were considered significant at $p < 0.05$ [22].

RESULTS AND DISCUSSION

Effect of biomodified organomineral fertilizers on the yield of flax and spring wheat

The use of OMF ensured the yield of flax and wheat almost throughout all the years of research (Table 2).

At the same time, the introduction of BP Bisolbifit into the soil did not significantly affect the yield of flax straw in 2013-2014. Throughout the studies, there was a tendency to increase yields from the action of OMF modified by BisolbiFit biopreparation, where the increments were from 0.59 to 0.72 t/ha.

In 2014-2015, the use of fertilizers had a positive effect on the growth of seed yield and their completeness (Table 2). The trend in yield growth in both years of research was noted in variants using OMF and biomodified OMF. The addition to the control in 2014 was 0.07 t/ha in variant 3; in the fourth variant - 0.09 t/ha; in 2015 - 0.05 t/ha and 0.08 t/ha, respectively.

The slight trend of flax seeds yield growth in 2013 was noted in variants with BP, OMF and modified OMF. It can be assumed that the decrease in the yield of flax seeds was due to the unusual flowering of flax, i.e., the first wave of flowering was noted in July, and in the beginning of August a repeated flowering of plants against the background of seed formation was observed [23].

The use of BP on spring wheat based on the *Bacillus subtilis* strain Ch-13 in 2013 significantly increased grain yields compared to the control by 0.18 t/ha (2nd variant). Modification of granules of OMF with "BisolbiFit" provided the greatest increase in the yield of grain, which was 0.84 t/ha.

In 2014-2015, the same patterns were preserved. During all the years of research, the effect of all types of fertilizers provided significant increases. At the same time, it should be noted that the modification (processing) of OMF fertilizer pellets with a biological preparation produced a more significant yield increase (0.18 - 0.22 t/ha), compared to OMF, pellets of which did not undergo BP modification.

The influence of biomodified organomineral fertilizers on the yield of the green mass of vetch-oat mixture

The results of the effect of different brands of OMF and BP on the yield of the green mass of vetch-oat mixture are presented in Table 3.

The studied fertilizers significantly increased the yield of the green mass of the vetch-oat mixture in relation to the control by 32.2-46.1%. In 2015-2017, a reliable increase (2.6 - 4.1 t/ha to the 3rd variant) was obtained from the biological modification of the OMF pellets by BP "Bisolbifit" and "Phosphatovit" [24].

Reducing soil acidity (pH_{KCl}) from 5.1 to 5.9 increased the efficiency of fertilizers by an average of 8 - 12%. On not limed soil, the increase in the yield of green mass in excess of 10 t/ha (to the corresponding control) was obtained only with the introduction of OMF with BisolbiFit and on a limed background – with all biomodified OMF that were used.

The influence of biomodified organomineral fertilizers on the taxonomic composition of the prokaryotic community of soil

The total number of bacterial DNA according to the variants of the experiment was 1.74-2.78 • 1010 copies/g soil. The largest number of microorganisms was observed in variants with the introduction of biomodified OMF on the background of liming (Fig. 1).

The smallest number of bacteria was noted in the variant with the use of mineral fertilizer without liming, and also without fertilization (variant 1).

Based on the research, the dominant phylums the proportion of which was significantly higher than the others were found (Fig 2). They are: *Acidobacteria* (7.1 – 11.5%), *Actinobacteria* (13.6 – 20.4%), *Bacteroidetes* (7.2 - 19.3%), *Proteobacteri*a (45.3 – 56.2%), *Verrucomicrobia* (4.3 – 10.3%).

The largest share of *Actinobacteria* (more than 20%) was noted in variants with the introduction of OMF and OMF with BisolbiFit, which indicates that the application of these fertilizers positively affects their development. According to modern ideas, *Actinobacteria* are able to actively decompose organic matter, being participants of the carbon cycle.

Proteobacteria accounts for more than half of the total microbial diversity in sod-podzolic soils. These include nitrifying and nitrogen-fixing bacteria that participate in the nitrogen cycle of the soil. Among the representatives of alpha proteobacteria (*Alphaproteobacteria* constitute 45% of the total number of proteobacteria) in the arable soil under the vetch-oat mixture, representatives of free-living and symbiotic nitrogen-fixing bacteria of the genera *Agrobacterium* and *Rhizobium*, as well as *Rhodoplanes* and *Kaistobacter* prevailed. The share of beta proteobacteria is also significant and is 37%. The most numerous is the family of *Comamonadaceae*, unidentified to the families of the orders *Ellin 6067* and *SC-I-84*. The number of *Deltaproteobacteria* and *Gammaproteobacteria* is much lower and is 7 and 11%, respectively. The most representative is the delta-bacteria of the family *Haliangiaceae* of the order *Myxococcales* and *Syntrophobacteraceae* of the order *Syntrophobacterales*. Among gamma proteobacteria, the families *Sinobacteraceae* and *Xanthomonadaceae* are distinguished [25].

The distribution of phylums according to the variants of the experiment shows an extremely complex structure of the community, the share participation of which can vary significantly, regardless of the studied factors [26].

CONCLUSION

This work is one of the first steps in understanding the close relationship of the taxonomic structure of the soil microbocenosis with such exogenous factors as liming of soil, application of fertilizers and evaluation of "soil health" during different uses of arable land. Based on the analysis of the metagenome of the prokaryotic community using high-throughput sequencing of the 16S rRNA gene in various variants of the stationary experiment in comparison with the set-aside lands, microorganisms-indicators of the directivity of soil-biological processes in agrocenosis were revealed. It is shown that the modification of fertilizer pellets with biological preparations promotes an increase in the yield of flax fiber, spring wheat and vetch-oat mixture. All this serves as a basis for obtaining sustainable crop yields for a long time and maintaining soil fertility [27].

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Table 1. Agrochemical soil indicators by years of research

Years	Humus	pH	P_2O_5	K_2O
	%	units	mg/kg	mg/kg
2013	3.3	5.2	285	100
2014	2.9	5.0	272	146
2015	3.2	5.2	261	125
2016	3.0	5.5	238	120
2017	2.9	5.2	225	115

Table 2. The yield of flax and spring wheat with the application of biopreparation BisolbiFit and organomineral fertilizer (OMF), t/ha

Variants	Years			Average for 3 years	Increase to control		Increase from modification	
	2013	2014	2015		t/ha	%	t/ha	%
Flax straw								
1. Control (without fertilizers)	5.05	2.95	3.03	3.68	-	-	-	-
2. BP (BisolbiFit)	5.33	3.08	3.25	3.89	+0.21	5.7	-	-
3. OMF (2.5 quintal/ha)	5.57	3.35	3.47	4.13	+0.45	12.2	-	-
4. modified OMF (2.5 quintal/ha)	5.64	3.66	3.75	4.35	+0.67	18.2	+0.22	5.3
HCP_{05}	0.38	0.20	0.21	-	-	-	-	-
Flax seeds								
1. Control (without fertilizers)	0.60	0.52	0.55	0.56	-	-	-	-
2 BP (BisolbiFit)	0.62	0.54	0.57	0.58	+0.02	3.6	-	-
3. OMF (2.5 quintal/ha)	0.63	0.59	0.60	0.61	+0.05	8.9	-	-
4. modified OMF (2.5 quintal/ha)	0.63	0.61	0.63	0.62	0.06	10.7	+0.01	1.6
HCP_{05}	$F_{actual}<F_{05}$	0.04	0.02	-	-	-	-	-
Spring wheat grain								
1. Control (without fertilizers)	2.85	3.17	2.94	2.99	-	-	-	-
2. BP (BisolbiFit)	3.03	3.37	3.16	3.19	+0.20	6.7	-	-
3. OMF (2.5 quintal/ha)	3.48	4.03	4.16	3.89	+0.90	30.1	-	-
4. modified OMF (2.5 quintal/ha)	3.69	4.21	4.38	4.09	+1.10	36.8	0.2	5.1
HCP_{05}	0.11	0.14	0.14	-	-	-	-	-

Table 3. Effect of fertilizers on the yield of green mass of vetch-oat mixture, t/ha

№	Variant (B-fertilizers)	Years			Average for 3 years				
		2015	2016	2017	quintal/ha	Increase to control quintal/ha	%	Increase to $N_{30}P_{30}K_{30}$ quintal/ha	%
A_1 – without liming (pH_{KCl}) – 5.1									
1	Control (without fertilizers)	19.9	24.8	25.0	23.2	-	-	-	-
2	$N_{30}P_{30}K_{30}$	27.5	30.4	34.9	30.9	7.7	33.2	-	-
3	OMF ($N_{30}P_{30}K_{35}$)	29.2	30.9	34.9	31.7	8.4	36.3	0.7	2.3
4	OMF + BisolbiFit	31.4	34.2	35.6	33.7	10.5	45.3	2.8	9.0
5	OMF + Phosphatovit	31.1	32.7	35.4	33.1	9.8	42.4	2.1	6.9
6	OMF + PhosphoActiv	30.4	30.7	33.9	31.7	8.4	36.3	0.7	2.3
	Average for A_1	28.2	30.6	33.3	30.7	-	-	-	-
A_2 – with liming (pH_{KCl}) – 5.9									
1	Control (without fertilizers)	20.5	27.0	31.9	26.5	-	-	-	-
2	$N_{30}P_{30}K_{30}$	30.8	32.8	40.3	34.6	8.2	30.8	-	-
3	OMF ($N_{30}P_{30}K_{35}$)	31.7	32.8	40.5	35.0	8.5	32.2	0.4	1.0
4	OMF + BisolbiFit	35.0	37.8	43.3	38.7	12.2	46.1	4.0	11.7
5	OMF + Phosphatovit	34.6	35.1	41.7	37.1	10.6	40.2	2.5	7.2
6	OMF + PhosphoActiv	33.9	33.7	43.2	36.9	10.4	39.5	2.3	6.6
	Average for A_2	31.1	33.2	40.1	34.8	-	-	-	-
	HCP_{05} for factor A	1.3	0.9	1.8	-	-	-	-	-
	HCP_{05} for factor B	2.3	1.5	3.1	-	-	-	-	-
	HCP_{05} private differences	3.3	2.2	4.4	-	-	-	-	-

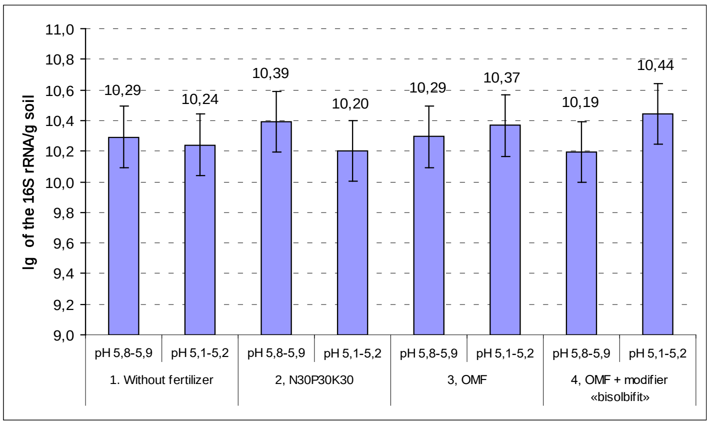

Fig. 1. The amount of bacterial DNA according to the variants of the stationary field experiment prior to harvesting the vetch-oat mixture

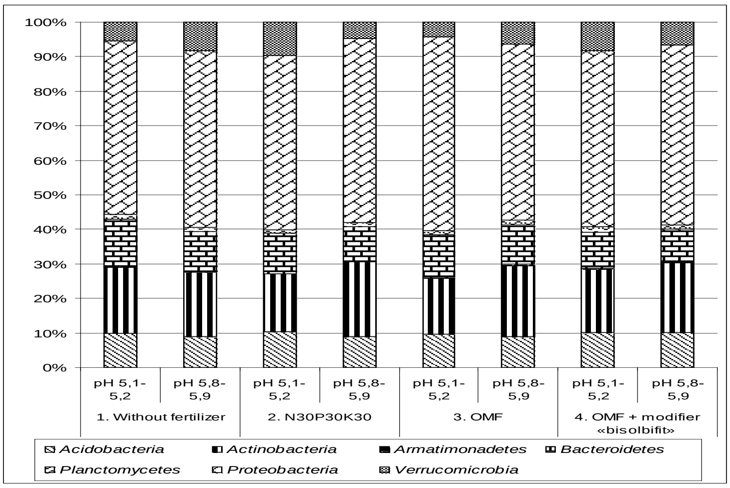

Fig. 2. Taxonomic structure of prevailing soil bacteria at the level of the phylums according to the variants of the experiment, in % of the population