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Change of microbiological properties of disturbed permafrost soils during their restoration under the influence of fertilizers

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The extent of microbiological processes in soil is determined not only by the number of microorganisms, but mainly by their activity. Therefore, it is extremely relevant to study the influence of various organic, mineral fertilizers, lime used for the cultivation of gley-podzolic soils of the Far North, on the change of intensity and development of the most important microbiological processes in these soils. In its natural state, soils are a multi-component system consisting of many heterogeneous microenvironments inhabited by microorganisms with different food chains. In anthropogenic activities, and in particular, in the introduction of organic and mineral fertilizers, the environmental conditions in soils change significantly, leading to a significant adjustment in taxonomic structure of microbial cenoses and their functional activities. Particularities of soil-climatic conditions also significantly affect species composition and features of functioning of microbocenoses in soils. Therefore, when choosing the most effective methods of soil cultivation in each particular climate zone, a lot of attention is paid to their biological assessment. The studies show that fertilizer and lime has a positive effect on the prokaryote complex of soil microorganisms of everfrozen soils.

Keywords: Everfrozen soils; Fertilizers; Peat; Manure; Lime; Bacteria; Fungi; Soil enzymes

Introduction

Bacteria make up 80-90% of the total number of microorganisms determined by the method of crops in tundra and forest-tundra soils. However, the biomass of fungi taken into account by direct fluorescent microscopy is 3 to 4 times superior in the organic horizon to bacterial biomass determined by the same method. Bacterial development depends primarily on the presence of available organic substrate and wetting conditions, and these factors in tundra are rarely at the minimum.

Actinomycetes in tundra soils are extremely small. Their development is limited by the acidic reaction of soil solutions and lack of oxygen in overhumidified soils. Most actinomycetes are nitrophils. It is found in quantities from 1 to 10 thousand in 1 g of soil in the upper well-aerated horizons (Evdokimova & Mozgova, 2001). The development of fungi differs significantly in different Arctic soils. The length of fungal mycelium decreases when soil pH increases and increases at higher humidity and temperature levels. There is very little data on productivity and production of microscopic fungi in tundra soils. It is calculated that in Alaskan tundras, the biomass of fungi resumes 2-6 times per season (Bunnell et al, 1980).

Among the unicellular fungi flora, the yeast cells are characteristic for acidic soils. In typical Taimyr tundras, their numbers range from 1.6x103 cells/g soil (Babyeva & Chernov, 1982). Fungi show the greatest adaptation to low temperatures vs. all groups of soil microorganisms. They are carrying out the process of destruction of plant residues in Arctic soils and are extremely important in the functioning of Arctic ecosystems (Evdokimova & Mozgova, 2001). The minimum temperature of pulp decomposition lies between 6 and 8°C, but fungi do not stop this process and at temperatures below 5°C (Bunnell et al., 1980). Various agronomic activities, including fertilization, have a significant impact on the complex of soil microorganisms (Marfenina, 1994; Polyanskaya et al., 1997). There is extensive papers on changes in the species composition of microorganisms under the influence of anthropogenic effects on soil (Marfenina & Mirchinc, 1988).

It is known that one of the most important soil regimes affecting the efficiency of mineral fertilizers is the microbiological regime. Thus, a number of authors point to the stimulating effect of mineral fertilizers on microflora (Geller & Yuspe, 1954; Wozniakovskaya, 1955; Stepanova, 1959). E.N.Mishustin (1972; 1975) notes the beneficial effect of small and oppressive effects of large quantities of mineral fertilizers on the number of microorganisms. The use of mineral fertilizers tends to increase the number of bacteria, actinomycetes and fungi in soils. (Kautskaya,1982). However, there are many studies in which there has been no increase in the total number of microorganisms in the soil (Vyblov, 1979), and sometimes there has been a decrease under the influence of mineral fertilizers (Goloborodko & Iutinskaya, 1978). The growth of microorganisms is associated with enrichment of fertilized soils by elements of mineral nutrition, increasing the size of exo-osmosis and root fall of plants during the growing season and a large number of plant residues after harvesting crops on fertilized plots (Mishustin, 1975). Studies of recent years carried out on various soils found that high doses of mineral fertilizers reliably reduces the total number of microorganisms. Therefore, the relevant but poorly developed direction remains the identification of norms of mineral fertilizers and the duration of their usage, at which there is no disruption of structure and functioning of the complex of soil microorganisms.

Thus, there are extensive papers on the impact on soil microbiological activity, in a complex of agronomic measures, mineral and various organic fertilizers. On the quantitative side, that is on the change in total and biomass, there is much less reliable data here. In most cases, the accounting of microorganisms was carried out by seeding on dense nutritional environments. Meanwhile, there are modern methods for direct microscopic accounting of soil microorganisms that allow for very representative results. Much work on assessing the impact of anthropogenic factors on the content of microorganisms in soil with the help of modern accounting methods has already been started (Zvyagintsev, 2005). It is very interesting and useful to determine the number of microorganisms and their biomass using these methods in the gley-podzolic soils of the far north.

Soils of many individual regions of the Russian Federation are very poorly studied, but even against this background, soils of the Far North are a “white spot”. However, justified recommendations on economic development of various territories are possible only on the basis of comprehensive ecological research.

Materials and Methods

Study of biological regime of soils of the Far North during their cultivation by applying different organic, mineral fertilizers and lime were carried out using various methods to assess changes in microflora composition, critical microbiological and biochemical processes. Qualitative and quantitative analysis of microflora was carried out according to methods generally accepted in soil microbiology (Large workshop on microbiology, 1962; Methods of soil microbiology, 1991). To estimate the total number of ammonifying bacteria meat-pepton agar (MPA) was used; bacteria assimilating mineral nitrogen and actinomycetes — starch-ammonia agar (SAA); fungi — Chapek's environment; cellulozodegrading microorganisms — Gatchinson's environment. The total number of microorganisms was determined at the Department of Soil Biology of Moscow State University using the method of luminescent microscopy. Agents for counting the number of microorganisms were prepared in accordance with the guidance “Methods of soil microbiology and biochemistry” (1991).

Calculation of the number of cells (mycelium) in 1 g of soil was performed using the formula:

$$N= S_1 \times a \times n / v \times S_2 \times C,$$

Where N - number of cells (mycelium length, μm) per 1 g of soil; S_1 - area of agent (μm^2); a - number of cells (mycelium length, μm) in one field of view (averaging is done for all agents); n - index of soil suspension breeding (ml); v -volume of the drop applied to the glass (ml); S_2 -area of view of the microscope (μm^2); C - soil sample weight (g).

For bacterial numbers, the mean quadratic deviation (b p-1) did not exceed 5%, for mycelium and spores of fungi, as well as actinomycete mycelium - 10%. Biomass calculations were carried out, considering that the biomass of dry matter for one bacterial cell of 0.1 μm^3 is $2 \cdot 10^{-14}$g, 1m actinomycete mycelium with a diameter of 0.5 μm - $3.9 \cdot 10^{-8}$g (Kozhevin et al.; 1979, 1989). Taking into account the measured diameter of spores and mycelium of fungi, real biomass was calculated according to the formula: for spores — $0.0836g^{3} \cdot 10^{-11}$g, for mycelium -$0.628g^{2} \cdot 10^{-6}$g (Polyanskaya, 1998; 2013). Biomass data were expressed in mg/g dry organic matter.

The potential ability of the soil to form nitrates (nitrification capacity) was judged by the amount of nitrate nitrogen formed after a month of soil composting with ammonium sulfate (optimal humidity and temperature modes). Sterile soil served as a reference. Nitrate nitrogen was determined by disulfophenol method. The determination of urea activity is based on the accounting of ammonia formed during enzymatic hydrolysis of soil urea. The amount of ammonia nitrogen formed during composting of soil with urea was determined by colorometric method according to Nessler (Arinushkina,1970).

The activity of catalase was determined by a gasometric method based on the accounting of the amount of oxygen released as a result of soil catalytic action on hydrogen peroxide (Soil microbiology, 1991). Nitrogenase activity of the soil was determined using the acetylene method on the Chrom-4 gas chramotograph with flame-ionization detector. For the separation of gases ASK selica-gel with a particle size of 0.25 to 0.5 mm eas used, which was filled into a metal column with a length of 1.2 m and a diameter of 3 mm. Nitrogen was used as a carrier gas, the constant current rate was 42 ml/min. Phosphatase activity of the soil was determined by V.V.Kotelev (Smirnov & Kotelev, 1971), the activity of invertase - according to A.I. Chunderova (1973).

Results and Discussion

The data we obtained indicate a positive impact on soil microbocenosis of all kinds and doses of fertilizers.

Manure increased the number of ammonifying bacteria, taken into account on MPA, by 1.9-2.5 times. At the same time, it should be noted that the dose of it was not essential for this group of microorganisms. The increase from 120 to 480 t/ha was not accompanied by an increase in the number of bacteria using nitrogen organic compounds. A much greater stimulating influence on the development of bacteria-ammonifiers showed the application of full mineral fertilizer. Their numbers increased in the second year of experiment compared to the option without fertilizers by 2.7 times, compared with manure - by 1.4 times, in the third year of experiment - by 5.7 and 2.3 times respectively.

The joint application of manure and mineral fertilizers was favorable for this group of microorganisms. At the same time, unlike the application of pure organic fertilizer, the increase in the dosage of manure in the organo-mineral system of fertilizers was accompanied by an increase in the number of bacteria counted on the MPA. The most favorable for this physiological group of organisms was the application of a maximum dose of manure (480 t/ha) together with standard yearly mineral fertilizers. The introduced organic mineral fertilizers had a positive influence on the group of autotrophic bacteria that consume nitrogen of mineral compounds and take into account on SAA, which manifested in a sharp increasing their numbers.

The greatest stimulating influence showed manure. Unlike ammonifying bacteria, SAA responded positively to an increase in its dose. This was especially evident in the second year of the experiment. With the increase in the dosage of manure from 120 to 480 t/ha, the number of bacteria on SAA also increased and reached the maximum value (22650 kos/g) at the dose of organic fertilizer of 480 t/ha. In the third year, the most favorable dose of manure was 120 and 240 t/ha.

The application of mineral fertilizers alone increased the number of autotrophic bacteria less than the application of manure.

With co-introduction of manure and mineral fertilizers, the number of bacteria on SAA also increased, but was lower than when introducing the manure alone. In the second year of the experiment, the maximum number of bacteria was observed at a dosage of 480 t/ha in the organic-mineral system of fertilizers.

Thus, when introducing organic and mineral fertilizers during cultivation of gley-podzolic soils of the Far North, there is a fundamental transformation of the arable horizon, and its biological properties greatly improve. The introduced fertilizers showed positive influence on the prokaryote complex of soil microorganisms. However, its different physiological groups reacted to them ambiguously. Bacteria that perform the initial stages of mineralization of soil organic matter (on MPA) developed better when introducing mineral and organic-mineral fertilizers, whereas bacteria that use Nitrogen of mineral compounds for their activity (on SAA) developed better when introducing manure. The highest level of mineralization of soil organic matter was shown by variants with manure at doses of 120 and 240 t/ha (3.94 and 3.87 respectively), indicating here higher rates of microbiological processes of soil organic matter decomposition. Manure in all studied doses had a positive stimulating effect on the number of actinomycetes. At the same time, the increase in the dose of organic fertilizer was accompanied by an increase in the amount of soil actinomycetes and amounted to 300 thousand./g of soil at a manure dosage of 120 t/ha, 450 - at 240 t/ha and 570 - at a maximum dose of 480 t/ha. In the reference sample their number was 170 thousand per g of soil.

The dependence between dosages of manure (x, t/ha) and the development of microflora in the gley-podzolic soil of the Far North can be traced according to correlation coefficients and regression equation (1):

$$\text{Actinomycetes, thous/g} = 198 + 0.831 \cdot x; \qquad r = 0{,}98; \quad (1)$$

Mineral fertilizers had a negative effect on this group of soil microorganisms, reducing their number by 1.7 times. A combination of manure and mineral fertilizers had a similar effect. The number of actinomycetes was lower by 70% than in the reference - without fertilizers - sample. The opposite effect had the applied fertilizers on soil microscopic fungi. The number of fungi microflora was maximum at a dosage of 120 t/ha combined with NPK, in all other variants it was equal to or less than in the reference sample.

The ability of the soil to form nitrates - the nitrification capacity of the soil - is associated with the activity of nitrifying bacteria, which are mainly autotrophic microorganisms transforming mineral soil compounds.

The dependence between dosages of manure in soil (x, t/ha) and nitrification process — N-NO_3content in soil has a high correlation coefficient and is expressed in equation (2):

$$\text{N-NO}_3\text{, mg/kg} = 5.2 + 0.196 \cdot x; \qquad r = 0.95; \quad (2)$$

Mineral fertilizers had a significant influence on the activation of their life in the soil of experimental samples. Adding them at a dose of $N_{120}P_{90}K_{120}$increased the nitrification capacity of the studied soil by 8 times (Table 1). However, the most favourable combination of $N_{120}P_{90}K_{120}$ with manure. The maximum activity of nitrifiers is achieved by applying 480 t/ha of manure together with mineral fertilizers. The nitrification capacity in these samples exceeded the reference sample by 32 times and compared to NPK alone by 3.9 times. Manure stimulated nitrate formation in the studied soil at doses of 240 and 480 t/ha, but significantly less than NPK and especially manure + NPK. Thus, the organic-mineral and mineral background of fertilizers was the most favorable for the nitrification process.

Table 1. Soil biochemical activity when introducing manure and mineral fertilizers.

Experiment samples	Nitrification capacity, N-NO_3, mg/kg	Invertase, mg of sugar in 1 g of soil	Phosphatase, μg/g, Phenolphthalein, 24 hours
No manure	19	44	0.15
$N_{120}P_{90}K_{120}$	153	78	0.10
Manure 120 t/ha	17	53	0.25
Manure 120 t/ha+ $N_{120}P_{90}K_{120}$	236	78	0.10
Manure 240 t/ha	42	68	0.29
Manure 240 t/ha+ $N_{120}P_{90}K_{120}$	76	53	0.34
Manure 480 t/ha	107	68	0.60
Manure 480 t/ha+ $N_{120}P_{90}K_{120}$	597	124	0.96

Soil enzymatic activity is generally considered as a set of processes catalyzed by extracellular and intracellular enzymes of soil biota (Zvyagintsev, 2005). In the soil accumulates a certain “pool” of enzymes, the qualitative and quantitative composition of which is typical for this type of soil, whyle the activity of enzymatic processes depends on specific conditions: presence and concentration of substrate, temperature, humidity, pH, etc. The enzymatic complex of the studied soil, and hence the activity of microbiological processes carried out by it, are significantly activated during the cultivation of gley-podzolic soils of the Extreme North through the application of organic and mineral fertilizers. Transformation in the soil of disaccharides, carried out by invertase, is activated when a full mineral fertilizer is applied, as well as when combined with manure at a dose of 120 t/ha if compared to the original soil. Organic fertilizer alone stimulates the conversion of sugars to a lesser degree. The invertase activity had the highest importance at a maximum dosage of manure of 480 t/ha in combination with NPK, which is due to the larger accumulation of organic matter here and the presence of a sufficient quantity easily accessible food supplies for microorganisms delivered by mineral fertilizers.

The dependence between the application of manure doses and the invertase content (mg of sugar in 1 g of soil) in soil is expressed by the regression equation (3):

$$\text{Invertase, mg sugar in 1 g of soil} = 42.69 - 0.0002 \cdot x^2 + 0.136 \cdot x; \qquad R^2 = 0{,}95; \quad (3)$$

The soil samples fertilized by manure are characterized by a higher level of phosphatase activity compared to the original soil and especially with the sample where the mineral fertilizer was introduced alone, while the increase in the dose of organic fertilizer increses the activity of phosphineralizing bacteria, reaching a maximum value (0.60) at a dosage of 480 t/ha. The processes of mineralization of organic-phosphorus compounds of soils proceeded more actively at the maximum dosage of manure (x, t/ha) with the introduction of full mineral fertilizer (equation 4) in carbon-containing organic soil compounds:

$$\text{Phosphatase} = 0.128 + 0.0009 \cdot x; \quad r = 0.98; \quad (4)$$

The catalase activity increased when manure was introduced, and the increase in the dose of the latter was accompanied by the activation of the process of decomposition of hydrogen peroxide formed in the soil (Table 2, equation 5).

$$\text{Catalase, } O_2\ cm^3\ /g \text{ per 1 min} = -1E — 0.6 \cdot x^2 + 0.0032 \cdot x + 0.234; \quad R^2 = 0.87; \quad (5)$$

However, the combination of organic fertilizer with NPK was most favorable, causing an increase in catalase activity to a greater extent than the organic fertilizer introduced alone. The activity of the enzyme involved in the humus generation process increased drastically at the maximum dosage of manure and especially when combined with NPK. The amount of oxygen generated by hydrogen peroxide decomposition increased vs reference by 9.6 times and by 8.9 times compared to mineral fertilizer introduced alone. There is a correlation between dosages of manure (x, t/ha) and humus formation in soil (equation 6):

$$\text{Humus,\%} = 1.096 + 0.0015 \cdot x; \quad r = 0.95; \quad (6)$$

Table 2. Influence of forest tundra soil cultivation on soil enzyme activity.

Experiment samples	Humus, %	Catalase, O_2 cm^3 /g for 1 min	Polyphenol oxidase	Peroxidase	C, %
			mg 1,4-p-benzoquinine per 10 g of soil for 30 min		
No manure	1.07	0.34	5.90	44.17	12.5
$N_{120}P_{90}K_{120}$	1.14	0.37	4.13	47.30	7.3
Manure 120 t/ha	1.21	0.32	3.06	56.22	5.4
Manure 120 t/ha+ $N_{120}P_{90}K_{120}$	1.83	0.93	4.52	52.77	8.6
Manure 240 t/ha	1.59	1.15	4.09	56.22	7.3
Manure 240 t/ha+ $N_{120}P_{90}K_{120}$	1.10	1.07	3.60	54.17	6.6
Manure 480 t/ha	1.74	1.48	3.42	31.41	10.9
Manure 480 t/ha+ $N_{120}P_{90}K_{120}$	2.14	3.28	3.12	30.43	10.2

There is no positive correlation of humus content in soil with the activity of polyphenol oxidase involved in the synthesis of humus molecules. Although humus content in the studied soil increased when manure was introduced and combined with NPK, the activity of this enzyme went down. The maximum value is marked on the reference sample. This appears to be due to the low soil acidity values of the studied experiment samples. Polyphenol oxidase catalyzes the oxidation of polyphenols into quinones. The enzyme optimum lies in the pH range of 6.0-7.0. As the pH decreases to 5.0, the copper ion pinching and the enzyme activity drop begins. At lower soil acidity values, a number of non-specific phenol oxidation products are produced, which may be the reason for changes in the qualitative composition of humus in acidic soils. With increasing fertility of the studied soil, peroxidase activity fell. The best conditions for decomposition processes of humus substances, for which peroxidase is responsible, were at a dosage of manure of 120 and 240 t/ha both at separate application and in combination with mineral fertilizer.

In this regard, the correlation between humus content in soil and its conditional accumulation coefficient obtained in determining the ratio of polyphenol oxidase and peroxidase activity of soil (Chunderova, 1970). In our experiment, the conditional coefficient of humus accumulation under the influence of large doses of manure and mineral fertilizers decreased compared to reference sample (Table 2). However, when applying of the highest dosage - 480 t/ha compared to the dose of 120 t/ha, the coefficient “C” was higher by 2 times. Consequently, activation of some redox reactions (catalase activity) and reduction of activity of others (peroxidase activity) when applying manure and its combination with mineral fertilizers resulted in an increase in humus content in the studied soil compared to the initial levels. Thus, the high level of activity of both hydrolases and oxidoreductases taking part in the transformation of carbon - and phosphorus-containing organic compounds and redox reactions of synthesis and decomposition of humus substances, established through the cultivation of gley-podzolic soils of the Far North by a one-time introduction of large doses of manure and annual mineral fertilizers created favorable background for the formation of a high crop yield.

Similar to manure, the use of large single doses of peat as organic fertilizer and the combination of it with annual mineral fertilizers during the cultivation of surface-podzolic eluvial-gley soils led to a change in its microbial complex, manifested in the increase in the number of microflora of the studied physiological groups and the activation of microbiological processes.

When applying peat in increasing doses, the number of ammonifiers increased accordingly.

Their greater value is noted at a dose of peat 480 t/ha. However, the activation of this group of microorganisms occurs greater when mineral fertilizer is introduced alone. The number of microorganisms involved in the transformation of organic nitrogen-containing substances of soil increased with $N_{120}P_{90}K_{120}$, compared to the sample - without fertilizers in 4.1 times, compared to the dose of peat of 120 t/ha - in 1.3 times, 240 t/ha - in 1.7 times, 480 t/ha - 2.3 times and 2.2 times at a dose of peat of 720 t/ha. The most favorable for the development of this ecological-trophic group of microorganisms was the combination of increasing doses of peat and annually introduced mineral fertilizers and especially the minimum dose of peat pf 120 t/ha.

Similarly to microorganisms growing on MPA, the number of bacteria using mineral forms of nitrogen (on SAA) has changed. The most successful for them was the application of mineral fertilizers alone and their combination with a dose of peat of 240 and 720 t/ha. The quantity of actinomycetes was positively influenced by mineral fertilizers and peat in a dose of 480 and 720 t/ha, as well as their combination. The largest number of these microorganisms is noted in the sample with a dose of peat of 720 t/ha + $N_{120}P_{90}K_{120}$.

The applied fertilizer complex had no significant influence on microscopic fungi. There is only a slight increase in their number with the introduction of organic fertilizers. Only a dose of 240 t/ha of peat had a stimulating effect.

Thus, peat, like manure, contributed to an increase in the number of all studied ecological-trophic groups of microorganisms, most significantly in large doses of 480 and 720 t/ha, bringing the studied soil according in its level of biological activity to medium and well cultivated sod-podzolic soils.

The biochemical activity of the original gley-podzolic soil when introducing peat as an organic fertilizer was changed as follows.

The nitrification capacity did not change when peat was applied alone in doses of 120-240 t/ha. Only the maximum dose of it (480-720 t/ha) activated the nitrate process by 4.6 times compared to the reference sample. The use of mineral fertilizer alone in doses of $N_{120}P_{90}K_{120}$increased the ability of nitrifying bacteria to generate nitrates by 5.7 times compared to the reference sample and the sample with peat at doses of 120, 240 and 480 t/ha. The use of mineral fertilizers on the samples with peat did not change the nitrate formation. And only the use of the highest dose of peat 720 t/ha + NPK dramatically increased nitrification capacity - $N\text{-}NO_3$ increased to 687 mg/kg. Systematic application of mineral fertilizers against the background of large doses of peat did little to change the activity of enzymes in the hydrolase class (Table 3).

Table 3. Soil biochemical activity when introducing peat and mineral fertilizers.

Experiment samples	Nitrification capacity, $N\text{-}NO_3$, mg/kg	Invertase, mg of sugar in 1 g of soil	Phosphatase, µg/g, Phenolphthalein. 24 hours
No peat	39	89	0.37
$N_{120}P_{90}K_{120}$	223	93	0.69
peat 120 t/ha	35	35	0.47
peat 120 t/ha+ $N_{120}P_{90}K_{120}$	253	78	0.34
peat 240 t/ha	39	84	0.47
peat 240 t/ha + $N_{120}P_{90}K_{120}$	155	89	0.37
peat 480 t/ha	45	59	0.59
peat 480 t/ha+ $N_{120}P_{90}K_{120}$	301	74	0.49
peat 720 t/ha	181	163	0.63
peat 720 t/ha+ $N_{120}P_{90}K_{120}$	687	148	0.62

The invertase and phosphatase activity increased significantly only at the maximum dose of peat both separately and in combination with NPK. At the same time, phosphatase activity increased sharply with annual introduction of mineral fertilizers.

The general nature of alteration of redox enzymes when applying peat and NPK was similar to experience with manure. However, the use of peat as an organic fertilizer increased catalase activity to a significantly greater extent than manure application (x, t/ha) and correlated with humus (equations 6 and 7):

$$\text{Humus\%} = 2E - 0.5 \cdot x^2 + 0.0054 \cdot x + 2.32; \qquad R^2 = 0{,}97; \quad (6)$$

$$\text{Catalase, } O_2 \text{ cm}^3\text{/g per 1 min} = 1.30 + 0.0065 \cdot x; \qquad r = 0.96; \quad (7)$$

The increase of the latter with an increase in the dose of peat was accompanied by a sharp increase in the amount of oxygen released from the soil. Thus, the activity of catalase at the dose of peat 120 t/ha was equal to 2.58 cm^3/g. O_2, at 240 t/ha - 3.08, at 480 t/ha - 4.87 and at 720 t/ha - 5.51, whereas on the reference sample this value was 0.58 cm^3g O_2. I.e. increased compared to reference by 4.4; 5.3; 8.4; 9.5 times respectively. The joint introduction of peat at increasing doses with NPK also significantly increased catalase activity compared to the reference samle and sample with NPK only, but was significantly inferior in its values to the samples with peat (Table 4). The correlation of polyphenol oxidase content with humus content and the conditional humus accumulation coefficient has not been found. The enzyme activity was lower than reference when applying peat and especially peat with NPK, which appears to be associated with increased acidity in these experiment samples. Peroxidase activity was reversed and increased with reduced soil fertility.

Thus, a direct link to humus catalase content and reverse for peroxidase has been established as a result of the study. There is no correlation with humus content of polyphenol oxidase activity and thus humus accumulation coefficient, which appears to be associated with low soil pH value when applied peat and peat with NPK. Therefore, at the beginning of the experiment, manure and peat used in increasing doses in the cultivation of gley-podzolic soils of the Far North increased its biological activity. The number of both prokaryote and eukaryote groups of soil has increased, as well as the activity of microbiological processes of transformation of soil organic matter. The most significantly noted activation is observed on samples with high - 480 and 720 t/ha - doses of organic fertilizers.

Table 4. Effect of culturation of Gley-Podzolic Soil of Forest-Tundra on the activity of soil enzymes.

Experiment samples	Humus, %	Catalase, O_2 cm^3 /g per 1 min.	Polyphenol oxidase	Peroxidase	C, %
			mg 1,4-p-benzoquinine per 10 g of soil for 30 min		
No manure	2.07	0.58	5.51	46.92	12
$N_{120}P_{90}K_{120}$	1.93	0.48	5.33	46.11	12
Manure 120 t/ha	1.98	2.58	4.11	39.12	11
Manure 120 t/ha+ $N_{120}P_{90}K_{120}$	1.97	1.76	3.05	44.20	7
Manure 240 t/ha	3.02	3.08	4.11	37.11	11
Manure 240 t/ha+ $N_{120}P_{90}K_{120}$	2.95	0.56	2.95	40.00	7
Manure 480 t/ha	3.90	4.87	5.12	40.00	13
Manure 480 t/ha+ $N_{120}P_{90}K_{120}$	4.47	2.12	2.96	29.45	10
peat 720 t/ha	9.86	5.51	3.77	25.13	15
peat 720 t/ha+ $N_{120}P_{90}K_{120}$	6.76	3.67	3.52	29.45	12

The best microbiological background to improve soil fertility and agricultural productivity was established using a combination of organic and annually introduced mineral fertilizers. By the level of biological activity, the initial soil on options with large doses of organic fertilizers in combination with NPK approached well-cultivated sod-podzolic soils. Mineral fertilizers also increase the microbiological activity of the original soil, especially the number of all ecological-trophic groups of microorganisms and the nitrification capacity of the soil and in the level biological activity occupy an intermediate position between organic and organic-mineral fertilizers.

The noted activation of soil microbocenosis facilitated the translation of nutrients that were difficult for plants and microorganisms to reach into easily accessible, increasing the fertility level of gley-podzolic soil and crop yields. In the ninth year of experiment, the determination of the number and biomass of major taxonomic groups of soil microorganisms was repeated, but using the method of fluorescence microscopy. Changes in the number of microorganisms and their biomass depending on the fertilizers used in the experiment were ambiguous.

The number of bacteria in the soil without manure was low and amounted to 1.2 billion cells/g of soil. The introduction of manure resulted in an increase in bacterial numbers by about 1.2 to 2 times, with the largest number recorded when manure was applied at a dose of 240 t/ha (2.2 billion cl/g). When the manure dose increased by another 2 times, the cell number even decreased slightly, but was higher than in the control sample without manure. The application of a full mineral fertilizer resulted in an increase in the number of bacteria in the soil by a factor of 1.5 compared to the soil without fertilizer. The introduction of NPK on the background of manure (120 t/ha) led to an increase in the number of bacteria by 3 times and amounted to 3.5 billion calls/g. Further increase in manure dosage on the background of NPK application resulted in a decrease in bacterial numbers compared to the soil where manure was applied at low dosage. However, here the number was slightly higher than in the version with the introduction of NPK without manure. The mycelium content of actinomycetes in the soil without manure application was about 100 m/g. The application of manure increased the length of actinomycetes mycelium by 1.4 to 1.8 times compared to reference, with the greatest length of actinomycete mycelium recorded in the soil of the variant where manure was introduced in a dose of 480 t/ha.

The introduction of NPK contributed to a dramatic increase in the length of actinomycete mycelium. The combination of NPK with manure proved less favorable to actinomycetes than pure NPK. In these samples, the mycelium length was approximately 200 m/g regardless of the manure dose.

The number of fungi spores was maximum in the version with the introduction of 480 t/ha of manure and was 7.2 million spores/g. In all other variants it almost did not change: 4.3-5.3 million spores/g. The introduction of the NPK resulted in an increase in the number of spores, but the combination of NPK and manure was not accompanied by a further increase in numbers, but at the maximum dose of manure (480 t/ha) the numbers fungi spores were equal in the reference sample (without the application of organic and mineral fertilizer). The length of fungi mycelium was maximum in the variant with the application of manure at a dose of 240 t/ha, being about 900 m/g. Further increase in the manure application dose reduced the mycelium content to a minimum value of 320 m/g. The introduction of NPK increased the content of fungal mycelium by 1.5 times compared to reference (350 m/g and 570 m/g respectively). At the same time, the combination of NPK and manure did not lead to a significant increase in the content of fungal mycelium, and in the version with dosage of manure of 480 t/ha together with NPK, the mycelium content of fungi was comparable with the reference where no fertilizer was introduced. On a sample of the experiment, where peat was applied as a fertilizer different doses and peat and NPK together. As can be seen from the figure, the maximum number of bacteria was recorded in the reference version without fertilizer and was 2.2 billion cells/g of soil. The application of peat was not conducive to the development of bacteria, and at the maximum dose (720 t/ha) the bacterial population decreased to a value of 1.5 billion cl/g.

The introduction of NPK reduced the number of bacteria in this soil. The combination of peat and NPK also did not favor the bacterial complex: the bacterial population in the experiment with NPK was 1.5-3 times lower compared to the variants where only peat was introduced. The maximum number of bacteria in this experiment was observed when combined NPK with peat at a dosage of 240 t/ha - 1.7 billion cells/g, but here it was lower than in reference (no peat -2,2 billion cells/g). The length of the actinomycete mycelium was at maximum in the reference. The application of peat oppressed the actinomycete complex, especially at high doses. The least favorable for actinomycetes was a variant with the introduction of peat at a dose of 720 t/ha, in which the mycelium length of actinomycetes decreased to 145 m/g. The introduction of NPK resulted in a 1.5 fold reduction in the length of actinomycete mycelium compared to the reference. In this experiment, the combination of MPK with low doses of peat increased the content of actinomycete mycelium in the soil, however, when peat doses increased to 480-720 t/ha, the lowest levels of actinomycete mycelium (90-100 m/g) were observed. The number of fungal spores was weakly dependent on the dose of peat application. The maximum number of spores was recorded in the sample with a dose of peat 480 t/ha.

The NPK introduction increased the number of spores compared to the reference where no fertilizer was introduced. However, the co-introduction of peat and NPK reduced the number of spores compared to the pure NPK variant. With increased doses of peat application in this experiment, the content of the spores in the soil has been consistently reduced.

The length of mushroom mycelium in the variant without fertilizer was 480 m/g. The application of peat at doses of 120 and 240 t/ha resulted in an increase in the length of mushroom mycelium - over 1000 m/g, further increase in the dose of peat application reduced the length of fungi mycelium to reference values. The introduction of NPK led to an increase in the content of fungal mycelium by 2.5 times compared to reference - to a value of 1200 m/g, with co-introduction of NRK and peat at a dose of 120 t/ha the content of fungal mycelium remained high. However, increased doses of peat application resulted in a consistent decrease in mycelium content in this experiment. At the same time, the content of fungi mycelium in the experiment with the introduction of MPK was 2-2.5 times higher than in the experiment where NPK was not introduced. The application of manure stimulated the development of microorganisms, but the application of its maximum dose proved unfavorable. The use of mineral fertilizer significantly increased microbial biomass content, but the combination of NPK with manure proved less favorable to microorganisms than the application of pure NPK. At the same time, in the samples with joint application of manure and NPK against the background of decrease of biomass of fungi in the soil the biomass content of prokaryote microorganisms significantly increased and its share in total microbial biomass grew as well.

The application of peat in moderate doses increased the microbial biomass content in the soil. However, exceeding a certain deposit limit had the opposite effect. The introduction of NPK has had a positive impact on the microbial biomass content in this soil. Most favorable for microorganisms was the option with joint application of NPK and peat at a dose of 240 t/ha, but further increase in the dose had an oppressive effect on the microbial complex. In this soil, the introduction of NPK and the co-introduction of NPK and peat did not stimulate the development of prokaryote microorganisms, but contributed to the increase in the biomass content of fungi. Thus, both the introduction of manure and the introduction of peat stimulated the eukaryote complex dominant in the soil. But this effect was observed up to certain threshold values of the introduction, exceeding which the opposite effect was observed. The application of manure also favored prokaryote microorganisms, and the application of peat had no effect on their content in the soil, which may be associated with less readily organics available in peat.

The co-introduction of manure and NPK stimulated to greater extent the prokaryote than the eukaryote complex. For the latter, the separate application of organic and mineral fertilizers was more favorable. In the experiment with peat, the co-application of mineral and organic fertilizers was more favourable to fungi than separate introduction, and for bacteria, none of the variants noted positive impact. Characteristically, in the first soil studied, the application of pure NPK stimulated the prokaryote complex, and in the second - oppressed: it should be associated with agrochemical features of the habitat.

Conclusion

The conducted studies showed that the biogenicity of the original soil increased with the introduction of all fertilizers used in the experiment. However, the most favorable biological mode of the soil was created with joint introduction of mineral fertilizers in the dose N120P90K90, manure in doses 60 and 120 t/ha and lime in a dose of 1 g.k., this combination increased soil cellulozolytic and nitrification capacity, urea activity, ammonifying, nitrifying and cellulozodegrading microorganisms. It reduced the total number of mouldy fungi, increasing the number of genera Mucor and Dicocum. The introduction of pure lime to the soil at a dose of 1 g.k favours the high supply of nitrogen metabolism enzymes, which can serve as an important diagnostic indicator of mobilization intensity pf soil nitrogen. All of this eventually contributed to the increase in fertility of the original soil and had an impact on the growth, development, and yield of spring rapeseed. When applying organic and mineral fertilizers during the cultivation of surface-podzolic eluvial-gley soils of the Far North, there is a fundamental transformation of the arable horizon and significant improvement its biological properties. The introduced fertilizers showed positive influence on the prokaryote complex of soil microorganisms. However, its different physiological groups reacted to them ambiguously. Bacteria that perform the initial stages of mineralization of soil organic matter (on MPA) developed better when introducing mineral and organic-mineral fertilizers, whereas bacteria that use Nitrogen of mineral compounds for their activity (on SAA) developed better when introducing manure. The highest level of mineralization of soil organic matter was shown by variants with manure at doses of 120 and 240 t/ha (3.94 and 3.87 respectively), indicating here higher rates of microbiological processes of soil organic matter decomposition.

Activation of some redox reactions (catalase activity) and reduction of others (peroscidase activity) when applying manure and its combination with mineral fertilizers led to increase in humus content in the studied soil compared to the original. A direct correlation with humus content — catalase and reverse peroxidase has been established. There is no correlation with humus content of polyphenolaxidase activity and hence the coefficient of humus accumulation. Both the introduction of manure and the introduction of peat stimulated the eukaryote complex dominant in the soil. The co-introduction of manure and NPK stimulated to greater extent the prokaryote than the eukaryote complex. Phosphotase activity increased on variants with mineral as well as organic fertilizers, introduced separately. Liming reduced phosphotase activity. Co-application of all the fertilizers studied had no significant effect on phosphotase. The biogenicity of the original soil increases when all studied fertilizers are applied. However, the most favorable biological mode of the soil was created with joint introduction of mineral fertilizers in the dose N120P90K90, manure in doses 60 and 120 t/ha and lime in a dose of 1 g.k., this combination increased soil cellulozolytic and nitrification capacity, urea activity, ammonifying, nitrifying and cellulozodegrading microorganisms. It reduced the total number of mouldy fungi, increasing the number of genera Mucor and Dicocum. The introduction of pure lime to the soil at a dose of 1 g.k favors the creation of a high supply of nitrogen metabolism enzymes, which can serve as an important diagnostic indicator of mobilization intensity pf soil nitrogen.

All of this eventually contributed to the increase in fertility of the original soil and had an impact on the growth, development, and yield of forage crops.

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References

Arinushkina E.V. (1970). Manual for the chemical analysis of soils. Moscow: Publishing house Mosk. University.

Babeva I.P., Chernov I.Yu. (1982). Yeast in the tundra soils of Taimyr. Soil Science, 10, 60-64.

Bunnell F.I., Miller A.K, Flanagan P.W & Bennoit K.F. (1980). The microflora: composition, biomass and environmental relation. An arctic ecosystem: (The coastal tundra at Barrow, Alaska). Stroudsburg (Pennsylvania).

Chunderova A.I. (1973). Enzymatic activity of soddy-podzolic soils of the North-West zone: abstract of Ph.D. dis. Dr. Biol. sciences . Tallinn.

Evdokimova G.A., Mozgova N.P. (2001). Microorganisms of tundra and forest podzols of the Kola North. Ed. KSC RAS, Apatity.

Geller I.A., Yuspe F.B. (1954). On the influence of mineral fertilizers on the microbiological processes of the soil. Microbiology, 23(4), 451-453.

Goloborodko S.P., Iutinskaya G.A. (1978).Influence of irrigation and intensive fertilization of a cultivated pasture on its productivity and the number of microorganisms in the conditions of the south of the Ukrainian SSR. Dokl. TSKHA, 239, 121-124.

Great Workshop on Microbiology (1962). Ed. G. L. Seliber. Moscow. Vyshshaya shkola.

Kautskaya L.B. (1982). Some microbiological indicators of powerful, weakly leached chernozem with prolonged use of fertilizers. Microorganisms as a component of biocenosis. All-Union materials. symposium. (Alma-Ata, Sept. 1982). Alma-Ata.

Kozhevin P.A. (1989) Microbial populations in nature. Publishing house of Moscow State University.

Kozhevin P.A., Polyanskaya L.M. & Zvyagintsev D.G. (1979). Dynamics of the development of various microorganisms in the soil. Microbiology, 48(4), 490–494.

Marfenina O.E. (1994). Mycological monitoring of soils: opportunities and prospects. Pochvovedenie. Moscow.

Marfenina O.E., Mirchink T.G. (1988). Microscopic fungi under anthropogenic impact on the soil. Soil science, 9, 107-112.

Methods of soil microbiology and biochemistry. (1991). V. I. Aseeva, I. P. Babieva, B. A. Byzov (Eds.). Moscow. Moscow State University Publishing House.

Mishustin E.N. (1972). Microorganisms and agricultural productivity. Moscow.

Mishustin, E.N. (1975). Association of soil microorganisms. Moscow. Nauka.

Pectolytic enzymes of microorganisms. (1971). V.I.Smirnov, V.V. Kotelev. Academy of Sciences of the MSSR. Dept. microbiology. Chisinau.

Polyanskaya L.M., Golovchenko A.V. & Zvyagintsev D.G. (1998) Determination of the viability of spores and mycelium of fungi in the soil. Microbiology, 67(6), 832-836.

Polyanskaya L.M., Lukin S.M. & Zvyagintsev D.G. (1997) Changes in the composition of microbial biomass in soil during cultivation Pochvovedenie, 2, 206-212.

Polyanskaya L.M., R.B. Gorodnichev, & D.G. Zvyagintsev (2013). Sizes of bacteria cells in soils, determined by the method of "cascade" filtration. Izvestia RAN. Biological series, 2, 4. DOI: 10.7868 / S00023329130101284–151

Stepanova L.N. (1959). Influence of long-term use of mineral fertilizers on the content of toxic bacteria in sod-podzolic soils. Scientific. doc. higher. schools. Biol. Science, 3, 243-247.

Voznyakovskaya Yu.M., Kulikova A.S. (1955). Microflora accompanying mycorrhiza. Tr. conf. on plant mycotrophy. Ed. Academy of Sciences of the USSR. Moscow.

Vyblov N.F. (1979). The influence of fertilizers on the microflora of gray forest soils in Gorny Altai. Microbial associations and their functioning in the soils of Western Siberia.Novosibirsk.

Zvyagintsev D.G., Babieva I.P. & Zenova G.M. (2005). Biology of soils. Moscow: Publishing house of Moscow State University.

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