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MOLECULAR BIOLOGICAL AND MICROBIOLOGICAL CHARACTERISATION OF MICROBIAL COMMUNITIES OF SOIL ECOSYSTEMS UNDERGOING OLIGOTROPHICATION FOR THE DEVELOPMENT OF WAYS TO MANAGE MICROBIAL COMMUNITIES BY THEIR TROPHICS

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Abstract

Indicators of the number and activity of the microbial community (MC) of the soil ecosystem (SE), which underwent eutrophication due to the annual application of mineral or organic substances from 2011 y. to 2019 y., and then from 2019 y. to 2024 y. after stopping the additions of substances the oligotrophication of SE have occurring. Comparative analysis of basal respiration (BR) and substrate-induced respiration (SIR) and determination of the number of ribosomal copies of genes of bacteria, archaea and micromycetes (quantitative PCR) in soil samples, and 2024 y. revealed significant differences in these indicators due to of the changes in the trophic status of the soil ecosystem (SE). The eutrophication of soil ecosystem as the result of the introduction of minerals substances led to a decrease in respiratory activity (Table 1.) (Fig. 1) and the number of ribosomal copies genes (Fig. 2), while the introduction of organic substances significantly increased these indicators. In general, the results of the oligotrophication process, the determined parameters as indicators the oligotrophication become close to those in the control soil variant. The methods used to assess the consequences of oligotrophication of SE showed their sensitivity, and the results obtained make it possible to assess the processes that occurred in the microbial community when the trophic status of SE changed. Overall, the approach may prove useful for the development of methods for managing soil microbial communities.

Keywords: oligotrophication, soil ecosystem, microbial community, substrate-induced respiration, basal respiration.

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Introduction

Oligotrophication is the process of transforming soil ecosystems, including agroecosystems, into a state characterized by a low concentration of available biophilic elements, but a high total content of these and other elements, as well as a high diversity and abundance of saprotrophic microbiota (Semenov et al., 2011; Semenov et al., 2023; Senechkin et al., 2010). The process is aimed at creating resilient soil ecosystems that are more resistant to disturbances and more effectively suppress phytopathogenic and other parasitic microorganisms due to the dominance of saprotrophs (Semenov et al., 2016). Ecosystem management through their trophic status (oligotrophication) is an important process that affects biological diversity and ecosystem resilience to natural and anthropogenic impacts (Semenov et al., 2011; van Bruggen and Semenov, 2000**).**

The purpose of the research was to identify the reaction of the soil microbial community to changes in the trophic status of the soil ecosystem in the form of long-term oligotrophication.

Soil samples were taken from many years of microfield experiment, which was carried out in plastic bottomless vessels with an area of 0.25 m^2 (0.5 × 0.5 ×0,3 м), on the territory of the Institute of Physicochemical and Biological Problems of Soil Science of the Russian Academy of Sciences IPChBPoSS RAS, Pushchino (54°8308′ N, 37°6052′ E). The vessels were filled with arable gray forest soil of medium loamy granulometric composition (*Luvic Retic Greyzemic Phaeozems* (*Loamic*)) from the unfertilized massif of the former Experimental Station of the Institute. The average physical and chemical characteristics of the soil were as follows: pH KCl – 4.96 ± 0.16; $Carbon_{org}$ and N_{total} (dry burning) – 0.97 ± 0.03 and 0.095 ± 0.001% of the soil weight, respectively; N_{min} (according to Kudeyarov), mobile P_2O_5 and K_2O (according to Kirsanov) – 19.8 ± 0.4; 88.2 ± 10.6 and 73.3 ± 1.8 mg/kg of air-dry soil, respectively, the content of physical clay is 32 ± 1%.

Initially, the soils were subjected to experimental eutrophication from 2011 to 2019, due to the annual application of mineral (urea, double superphosphate and potassium sulfate), and organic substances (cattle manure), and since 2019 y. the application has been stopped (oligotrophication). The following experimental options were analyzed: control (without application) and four options with mineral substances in the form of NPK. In the first variant, designated N1P1K1, the concentrations of the elements corresponded to (kg/ha): N90P75K100, subsequent variants, contained (kg/ha): N180P150K200 (N2P2K2); N270P225K300 (N3P3K3); N360P300K400 (N4P4K4). Variants with organic substances (designated as Org. substances) contained (t/ha): 25; 50; 75; 100 (Semenov et al., 2023).

To identify the response of the soil microbial community to oligotrophication, available, widely used methods were used, which reflect the functional properties and numerical characteristics of MC in SE. As a functional characteristic of MC, the respiratory activity of soils was chosen, which reflects the activity of the dominant aerobic microbial community For this purpose, an aqueous solution of glucose (10 mg/g of soil) was added, and CO2 was determined by gas chromatography according to the data of the work (Semenov et al., 2013)

To determine the quantitative characteristics of MC (bacteria, archaea, micromycetes), the method of quantitative real-time PCR (qPCR) was chosen in accordance with the conditions described in the works (Semenov et al., 2019; Semenov et al., 2021).

Microbial activity, defined as “substrateinduced respiration (SIR), decreased in the variants with mineral application at the end of eutrophication (2019 y.) and the higher the concentration of mineral additives applied, the lower the SIR. After five years of oligotrophication in the variants with mineral elements, recovery of the SIRs to a level comparable to the control one was observed (Fig. 1). In the areas subjected to eutrophication due to the introduction of organic substances, at the time of its completion (2019 y.) there was a significant increase in the intensity of SIR, but after five years of oligotrophication, the level of SIRs decreased (Fig. 1), remaining above the control values, which can be explained by the accumulation of organic substances and an increase in the amount of microorganisms.

The results of determining the basal respiration (BR) demonstrate that long-term eutrophication of soils with mineral additives did not lead to its significant changes (Table 1.), but because of oligotrophication, there is an increase in BR in soils subjected to mineral eutrophication compared to 2019. However, after five years of oligotrophication, the BR values in these sites decreased to the control level (Table 1.).

Quantitative analysis of microbial populations as determination of the number of ribosomal copies of genes of bacteria, archaea and micromycetes obtained by the qPCR method at the time of completion of eutrophication with mineral elements showed that with an increase in the concentration of mineral elements, the "abundance" of bacteria and archaea decreased (Fig. 2). However, after five years of oligotrophication of these sites, the number of ribosomal copies of archaeal genes increased significantly, exceeding the control parameters, while the number of ribosomal copies of bacterial genes remained approximately at the same level (Fig. 2). Determination of the same indicators during eutrophication of soils with organic substances showed a significant increase in the number of ribosomal copies archaea and bacteria genes as the concentration of applied organic substances increases (Fig. 2). After five years of oligotrophication, there is a slight decrease in the number of ribosomal copies of archaeal genes and a significant decrease in the number of copies of bacterial genes.

The results of the PCR analysis of micromycetes showed similar results to mineral and organic eutrophication and subsequent oligotrophication as in bacteria (Fig. 2). Oligotrophication of soils has practically restored the number of micromycete gene ribosomal copies to the control level.

Thus, the methods used to identify and determine the results of oligotrophication in SE make it possible to record changes in soil ecosystems. Apparently, the method of controlling microbial communities through the trophic status of SE (oligotrophication of SE) can be considered as applicable.

TABLE

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Table 1. The numerical value of the database at the end of eutrophication (2019 y.) and after five years of oligotrophication (2024 y.)

Year	2019 y.		2024 y.	
Indicators	BR, µg C-CO2/g soil/h	st. deviation	BR, µg C-CO2/g soil/h	st. deviation
Control	0,303	0,025	0,452	0,168
N1P1K1	0,336	0,037	0,356	0,110
N2P2K2	0,284	0,010	0,832	0,336
N3P3K3	0,322	0,035	0,477	0,043
N4P4K4	0,325	0,046	0,400	0,043
Organic substances 25 t/ha	0,640	0,038	0,699	0,513
Organic substances 50 t/ha	0,797	0,031	0,332	0,082
Organic substances 75 t/ha	1,814	0,403	0,431	0,021
Organic substances 100 t/ha	2,203	0,175	0,409	0,057

FIGURE CAPTIONS

Fig. 1. Intensity of substrate-induced respiration for C-CO2 in 2019 y. and 2024 y., where 1 is control (without application); 2 - N90P75K100 (N1P1K1); 3 - N180P150K200 (N2P2K2); 4 - N270P225K300 (N3P3K3); 5 - N360P300K400 (N4P4K4); 6 – Organic substances 25 t/ha; 7 – Organic substances 50 t/ha; 8 – Organic substances 75 t/ha; 9 – Organic substances 100 t/ha.

Fig. 2. Dynamics of the number of ribosomal copies of genes of archaea (I), bacteria (II) and micromycetes (III) in soil samples in 2019 y. and 2024y., where 1 is control (without application); 2 - N90P75K100 (N1P1K1); 3 - N180P150K200 (N2P2K2); 4 - N270P225K300 (N3P3K3); 5 - N360P300K400 (N4P4K4); 6 – Organic substances 25 t/ha; 7 – Organic substances 50 t/ha; 8 – Organic substances 75 t/ha; 9 – Organic substances 100 t/ha. Y-axis: Number of ribosomal copies of genes (logarithmic scale, copies/g of soil) determined by qPCR. X-axis: Tillage options.

FIGURES

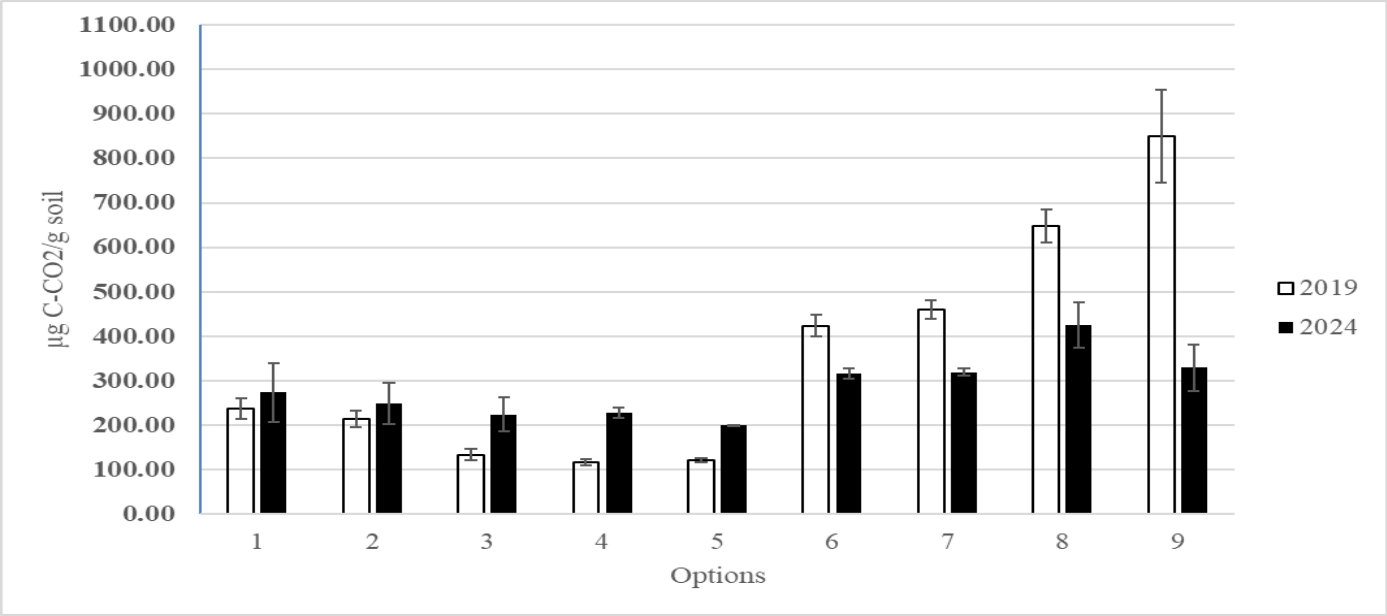

Fig. 1

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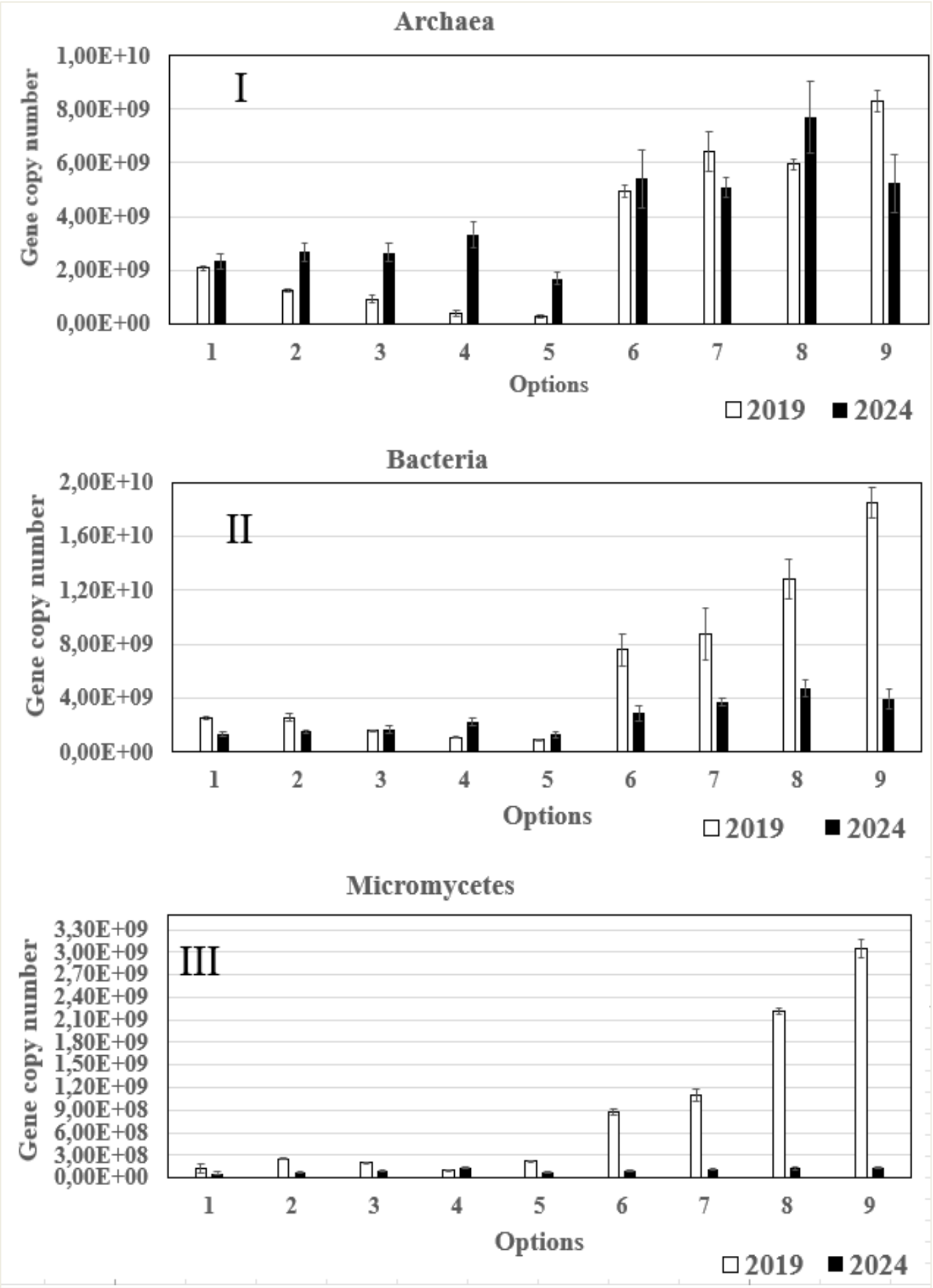

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