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Development of methods for managing microbial communities in the soil ecosystem and their trophy

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ABSTRACT

The effects of changes in the trophism of the soil ecosystem (SE) as a result of changes in the concentrations of organic and mineral substances in the soil were studied by determining the abundance and activity of the microbial community (MC) in the soil ecosystem. A quantitative assessment of the number of microorganisms was carried out using the qPCR method and the activity of the MC by measuring basal respiration (BR) and substrate-induced respiration (SIR) in soil samples. Soil samples were collected from different sites subjected to experimental eutrophication from 2011 to 2019 by annual addition of organic matter or minerals to the corresponding sites, and then oligotrophication - cessation of all application from 2019 to the present. The methods used to assess the effects of oligotrophication showed the sensitivity of the methods, and the results obtained are clear and suitable for further development of MC management methods.

Keywords: *oligotrophication, soil ecosystems, microbial communities, substrate-induced respiration, basal respiration, qPCR.*

INTRODUCTION

Oligotrophication is the process of transformation of soil ecosystems (SE), including agroecosystems, into a state characterized by a low concentration of available, easily assimilable biophilic elements (such as nitrogen, phosphorus, potassium, and even carbon), but a high total content of these and other elements, as well as high biodiversity and abundance of saprotrophic microbiota (Semenov, 1991; Semenov et al., 2011; Senechkin et al., 2010; Van Bruggen and Semenov, 2000). The process is aimed at creating sustainable and healthy soil ecosystems that are more resistant to disruptive influences and more effectively competitively suppress phytopathogenic and other parasitic microorganisms due to the dominance of saprotrophs (Semenov et al., 2016). Management of ecosystems, including soil ecosystems, and the conditions of their trophic state (for example, oligotrophication) is an important but complex process that affects biological diversity, i.e., management of ecosystem services “through” biodiversity and changes in the stability of ecosystems to natural, climatic and anthropogenic impacts (Semenov et al., 2011).

The study is aimed at assessing the impact of changes in the trophic state - trophism of the SE by exposure to organic or mineral substances on microbial communities (MC) in the SE, which will somehow be reflected in some microbiological indicators of MC activity. The study consisted of a quantitative assessment in experimental variants of the number of bacteria, archaea and micromycetes by the qPCR method and an assessment of MC activity in the form of basal respiration (BR) and substrate-induced respiration (SIR) in soil samples.

MATERIALS AND METHODS

Soil samples from different soil areas subjected to experimental oligotrophication were collected for the analysis. These soils were initially subjected to experimental eutrophication from 2011. to 2019., four variants of mineral eutrophication and four variants of organic eutrophication. Since 2019, i.e. 5 years ago, the introduction of organic matter and mineral elements into these SEs was stopped, and thus mineral oligotrophication was carried out in soil variants with mineral eutrophication and mineral-organic oligotrophication in soil variants with organic eutrophication.

The types of organic matter and mineral elements introduced into the soil annually and their dosages were as follows: control (without introducing organic matter and mineral elements); dosages of introduced mineral elements: N1P1K1 (N90P75K100); N2P2K2 (N180P150K200); N3P3K3 (N270P225K300); N4P4K4 (N360P300K400); dosages of introduced organic matter: 25 t ha^{-1}; 50 t ha^{-1}; 75 t ha^{-1}; 100 t ha^{-1} (Semenov et al., 2023).

To determine substrate-induced respiration (SIR), soil samples were initiated with glucose (10 mg/g soil), the intensity of CO_2 emission was determined gas chromatographically with subsequent calculation of C-CO_2. To determine basal respiration (BR), soil samples were initiated by adding water, CO_2 was also determined gas chromatographically with subsequent calculation of C-CO_2. To conduct qPCR, DNA was isolated from soil samples, sequencing was performed, with obtaining quantitative values of the number of archaea, bacteria, fungi in the studied variants.

RESULTS AND DISCUSSION

Microbial activity, defined as SIR, has been decreasing since the end of the eutrophication period (2019) in variants with the addition of mineral elements (Figure 1). The decrease was proportional to the increase in the applied concentrations of mineral additives (during eutrophication). At the same time, in soils subjected to five-year oligotrophication (until 2024) initially with high doses of mineral additives, SIR was restored to the initial level, comparable with the control indicator.

In areas subjected to eutrophication due to the introduction of organic matter, there was, at the time of completion of eutrophication (2019), a significant increase in the intensity of SIR, but during the five-year oligotrophication, the level of SIR decreased, but it remained above the control values, which can be explained by the accumulation of organic matter in these areas and an increase in the number of microorganisms.

The results of the BR show that long-term eutrophication of soils with mineral additives did not lead to any changes in BR, but as a result of oligotrophication, some increase in BR is observed in soils subjected to mineral eutrophication (Table 1). The sites subjected to eutrophication due to organic matter showed a huge increase in BR, correlating with the increase in the concentration of organic additives, as was observed when determining the SIR. However, after five years of oligotrophication, the BR values decreased to the control level, which was not observed when determining the SIR.

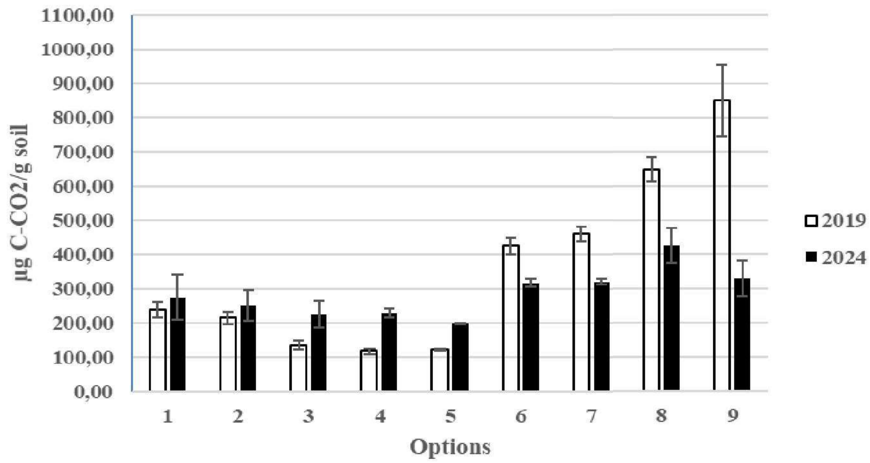

Figure 1. The C-CO_2 SIR ratio in 2019. and 2024: 1) control, without adding substances; 2) N1P1K1 (N90P75K100); 3) N2P2K2 (N180P150K200); 4) N3P3K3 (N270P225K300); 5) N4P4K4 (N360P300K400); 6) organic matter, 25 t ha^{-1}; 7) org. matter, 50 t ha^{-1}; 8) org. matter, 75 t ha^{-1}; 9) org. matter, 100 t ha^{-1}

Table 1. Numerical value of BD at the time of completion of eutrophication (2019) and after five years of olgitrophication (2024).

Years / Indicators	2019		2024	
	BR (µg C-CO_2/g soil)	**st. dev.**	**BR (µg C-CO_2/g soil)**	**st. dev.**
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
Org. matter. 25 t ha^{-1}	0,640	0,038	0,699	0,513
Org. matter. 50 t ha^{-1}	0,797	0,031	0,332	0,082
Org. matter. 75 t ha^{-1}	1,814	0,403	0,431	0,021
Org. matter. 100 t ha^{-1}	2,203	0,175	0,409	0,057

Quantitative analysis of microbial populations (archaea, bacteria) obtained by the qPCR method at the end of eutrophication in variants with mineral elements revealed that the number of bacteria and archaea decreased with an increase in the concentration of mineral elements (Figure 2). However, after five years of oligotrophication of these areas, the number of archaea increases significantly, exceeding the control indicators, while the number of bacteria remains at approximately the same level. Determination of the number of archaea and bacteria during eutrophication of soils with organic matter showed a significant increase in the number of archaea, as well as bacteria, with an increase in the concentration of introduced organic matter. After five years of oligotrophication, a slight decrease in the number of archaea is noted, but a significant decrease in the number of bacteria.

The results of the analysis of micromycetes by the qPCR method showed an analogy in the reaction to mineral eutrophication and subsequent oligotrophication with bacteria, as well as to organic eutrophication. Oligotrophication of soils, regardless of the method of their eutrophication (mineral, organic), practically restored the indicators of the number of micromycetes to the control level (Figure 2).

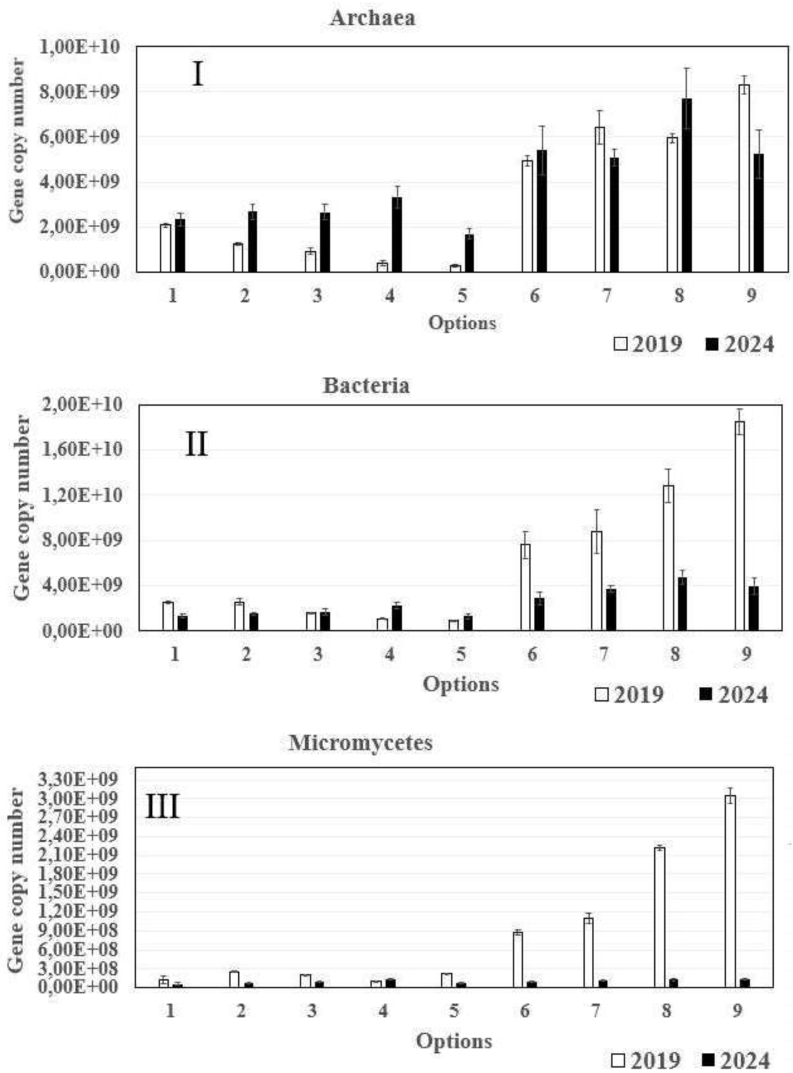

Figure 2. The ratio of the numbers of archaea (I), bacteria (II) and micromycetes (III) in soil samples in 2019 and 2024, where: 1) control, without adding substances; 2) N1P1K1 (N90P75K100); 3) N2P2K2 (N180P150K200); 4) N3P3K3 (N270P225K300); 5) N4P4K4 (N360P300K400); 6) organic matter, 25 t ha^{-1}; 7) org. matter, 50 t ha^{-1}; 8) org. matter, 75 t ha^{-1}; 9) org. matter, 100 t ha^{-1}. The Y-axis shows the number of microorganisms according to the numerical values shown in the figures, and the X-axis shows the SE variants

CONCLUSION

The application of the qPCR method for quantitative assessment of the number of microorganisms, as well as the measurement of basal respiration (BR) and substrate-induced respiration (SIR) to assess the activity of the microbial community, can be justifiably used to monitor the effects of changes in the trophism of the soil ecosystem (SE) resulting from changes in the concentrations of organic and mineral substances in the soil.

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