

Features of the distribution of free-living nitrogen-fixing bacteria in anthropogenic soil (near the Tbilisi Sea) of eastern Georgia

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ABSTRACT

The quantitative composition of microorganisms distributed in the anthropogenic type soil of Eastern Georgia (near the Tbilisi Sea), the share of nitrogen-fixing microorganisms in the microbial association, the features of their distribution and the correlation of this distribution with some physiological groups of microorganisms have been studied. It has been established that the qualitative and quantitative composition of nitrogen-fixing microorganisms changes depending on the soil profile (depth). Microbiological analysis over the five years of the study period showed that the anthropogenic type soil of Eastern Georgia is abundantly represented by nitrogen-fixing microorganisms of the genus *Beijerinckia*; at the same time, there is a decrease in the number of nitrifiers in the soil and an increase in the number of anaerobic bacteria.

Keywords: Nitrogen-fixing microorganisms, anaerobes, nitrifiers, anthropogenic soil.

Introduction

Soil is a complex and dynamic ecosystem in which many physiological groups of microorganisms are widely distributed. They play an important role in the metabolism, degradation of organic and synthetic substances, and in the maintenance of ecosystem functions in general [1]. However, anthropogenic impacts on soil, such as agriculture, urbanization, industrial activities, and recycling, often lead to the disruption of the soil microbial structure [2].

Anthropogenic soils are defined as soils that have been directly or indirectly affected by human intervention. Such changes may include nutrient enrichment, compaction, chemical pollution or land cover change. Under these conditions, the natural soil microbiota often shifts towards new ratios, which can have positive or negative effects on soil fertility and ecosystem properties [3]. Therefore, the response of microorganisms to temporal dynamics under anthropogenic impacts is crucial for ecological rehabilitation and sustainable management.

Microbial structure: baseline and indicators of change

The soil microbial community is mainly composed of bacteria, archaea, fungi, and protozoa. Among them, bacteria and fungi dominate quantitatively and functionally. These communities are not static—they constantly respond to environmental

changes such as moisture, organic carbon content, and vegetation cover. In natural soils, the main factors determining microbial structure are pH, nutrient supply, and humus [4], while in anthropogenic soils, fertilizers, herbicides, and heavy metal pollution are dominant factors.

Many studies have shown that long-term anthropogenic farming leads to microbial succession, in which copiotrophic groups (e.g., Proteobacteria, Bacteroidetes) predominate in nutrient-rich conditions, while oligotrophic bacteria (e.g., Acidobacteria) predominate in less fertile environments [5] [6]. These changes affect both biodiversity and microbial functional activity.

Timescales: From short-term changes to long-term stability

Microbes respond to anthropogenic disturbances on different timescales. In the short term (a few months to 2–3 years), microbial diversity often declines dramatically as a result of physical soil disturbance and chemical stress [7]. However, in the long term, stabilization or ecological adaptation is possible, depending on the type and intensity of the impact. For example, the Rothamsted fertilizer experiments in the UK have shown that the distribution and functional gene structure of nitrifiers and denitrifiers in soils change significantly over decades. Similarly, chronic pollution in urban soils leads to the dominance of metal-resistant microbes and a decrease in functional diversity [8]. Microbial responses to disturbance include both resistance and resilience, although the success of these mechanisms depends on environmental conditions and recovery capacity.

Functional consequences: element cycles and ecosystem services

Microbial structural changes are often accompanied by changes in functional properties. Microbes play important roles in the nitrogen, phosphorus, and carbon cycles [9]. For example, long-term monoculture reduces the abundance of nitrogen-fixing bacteria (*Bradyrhizobium*, *Azotobacter*) and increases the abundance of denitrifiers, leading to nitrogen loss [10].

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In addition, some anthropogenic factors, such as pesticides and microplastics, promote the spread of mobile genes in microbial communities, leading to the emergence of antibiotic-resistant strains and disruption of natural functions.

Anthropogenic Soil Categories and Microbial Responses

Anthropogenic soils are diverse—urban, agricultural, industrial, and mining soils differ both physically and biologically. Urban soils often exhibit compaction, alkalinity, and organic carbon deficiency, which reduce microbial activity [11]. In mining contamination zones, microbial biomass is dramatically reduced, although metal-resistant microbial consortia develop over time [12].

In agricultural soils, microbial cycling often depends on seasonal dynamics, crop rotation, and fertilizer use. Organic farms are sometimes noted for a more stable microbial structure [13].

Temporal monitoring and genomic methods

The use of modern genomic methods (e.g. 16S rRNA sequencing, metagenomics) has made it possible to monitor microbial changes over time. Long-term studies have shown that anthropogenic soils require years of restoration—sometimes decades.

For example, a 20-year study of a coal mine rehabilitation site showed a slowly increasing microbial diversity and convergence to natural soils, although complete recovery was not observed.

Ecological restoration perspective

Understanding microbial dynamics allows for effective soil restoration planning. Microbial bioindicators can be used to diagnose soil health. Different interventions (e.g., bioaugmentation, organic composting, phytoremediation) can be selected based on microbial heritability analysis. Also, the integration of microbial data into soil quality indicators will contribute to the formation of environmental policy and the sustainable development of urban spaces.

Therefore, the goal of our research was to study the physiological groups of individual microorganisms distributed in the anthropogenic type soil of Eastern Georgia, including free-living nitrogen-fixing microorganisms, to analyze the dynamics of their quantitative change over the years and to determine the optimal conditions for the distribution of microorganisms.

Materials and methods

The object of the study was the anthropogenic type soil of Eastern Georgia (the area adjacent to the Tbilisi Sea).

We collected soil samples using the envelope method. In the experiments, we determined soil moisture (calculated as the number of microorganisms per 1 g of dry soil) and pH (we used an ionomer, brand иономер-130.)

To calculate the number of microorganisms, we made dilutions of soil samples (decimal dilution method) [14].

For the cultivation of the isolated microorganisms, we used the following nutrient media:

Ash, ingredients; g / l. Mannitol-20; K_2HPO_4 - 0.2; $MgSO_4$ - 0.2; NaCl -0.2, K 2SO 4-0.1; , $CaCO_3$ 5.0 ; Agar 15.0 ; Final pH (cultivation temperature 25°C) 7.4±0.2

Beijerinckia area, ingredients: g/l. KH_2PO_4 - 0.8, Sucrose- 20.0, K_2HPO_4 - 0.20 , $MgSO_4$ 0.50; $FeCl_3$ - 0.10, Na_2MoO_4 -0.005 , Agar- 15.0, Final pH (cultivation temperature 25°C) 6.5±0.2

Nitrogen-free, glucose-enriched agar (NFGMM)*, ingredients: g/l: KH_2PO_4 1.0, $CaCl_2$ 1.0, $MgSO_4 \cdot 7H_2O$ 0.25, ; NaCl 0.5 ; $FeSO_4 \cdot 7H_2O$ 0.01, $MnSO_4 \cdot H_2O$ 0.01, Na_2MoO_4 0.01, Glucose 7.0, Agar 20.0.

Burks's Area, Ingredients: g/l. $MgSO_4$ - 0.2 , K_2HPO_4 -0.8 , KH_2PO_4 - 0.200 , $CaSO_4$ - 0.130, $FeCl_3$ - 0.00145, Na_2MoO_4 - 0.000253, Sucrose- 20.0

Azospirillum area, ingredients: g/l $C_4H_6O_5$ -5.0, K_2HPO_4 -0.500 , $FeSO_4$ -0.500 , $MnSO_4 \cdot H_2O$ -0.010, $MgSO_4$ -0.200, NaCl -0.100, Brothymol blue- 0.002, Na_2MoO_4 - 0.002, $CaCl_2$ -0.020, Agar 1.750, KOH 4.0 Final pH (Cultivation temperature 25°C) 6.8±0.2 We used the Vinogradsky area for the cultivation of nitrifiers. , were also used : Actinomycete Isolation Agar, nutrient medium, Czapek medium, Nutrient Broth agar, To identify the microbe, we tested the isolated cultures for enzymatic activity: urease, amylase, oxidase, catalase, and other biochemical tests.

Results and judgment

The quantitative and qualitative composition of a number of physiological groups (Table 1) and free-living nitrogen-fixing microorganisms (Table 2) distributed in the anthropogenic type of soil of Eastern Georgia was studied; the acidity, salinity, and humidity of the soil (profile 5 cm, 10 cm, and 20 cm) were also determined.

The results of the study are given in Tables №1, 2

Table 1. Quantitative composition of some physiological groups of microorganisms in anthropogenic soil

Physiological group	Quantity	Percentage share
Ammonifier	20,836,666	94,07%
Amylolytic bacteria	1,289,450	5,8%
Anaerobes	13,333	0,060%
Nitrifiers	6,260	0,02%
Actinomycetes	323	0,001%
Microscopic fungi	2,163	0,009%
Total number of microbes	22,148,195	100%

Analysis of the results presented in the table shows that in the studied soil, aminoferators are dominant among microorganisms (94.07%), amylytic bacteria are much less numerous (5.8%), and anaerobes, nitrifiers, actinomycetes, and microscopic fungi are present in insignificant quantities (0.060%, 0.02%, 0.001%, 0.009%, respectively).

Soil sampling depths	20-25 cm	10 -15cm	5-10 cm
pH	6	6.5	6.8
moisture	10%	8%	4%
Salinity	0,10%	0,8%	0,12%
Beijerinckia	375000	3650	1200
Azotobacter	0	260	8670
Derxia	0	0	430
Azospirillum	0	0	1670

Table 2. Analysis of the results shows that in the studied anthropogenic type of soil (in terms of depth), under conditions of relatively high humidity and weak acidity (pH) the dominant nitrogen-fixing microorganisms is the nitrogen-fixing Beijerinckia, which is distributed throughout the entire profile of the test soil, while, as can be seen from the table, their number increases with depth and reaches a maximum at a depth of 20-25 cm of the soil. In the upper (5-10 cm) and middle layers (15-10 cm) of the test soil, the nitrogen-fixing Azotobacter was identified, the number of which in the upper soil layer exceeds the number of other nitrogen-fixing microorganisms identified in this soil (Beijerinckia, Derxia, Azospirillum). According to the overall soil profile, nitrogen fixers of the genus Derxia are much less abundant than other nitrogen-fixing microorganisms and are found only in the top layer of soil (5-10 cm). Also, only nitrogen fixers of the genus Azospirillum have been identified in

this layer, but in much greater numbers than the genera *Derxia* and *Beijerinckia* (behind the number of *Azotobacter*).

Conclusions

1. The anthropogenic soil of Eastern Georgia (adjacent to the Tbilisi Sea) is richly represented by aminofixing microorganisms (94.07% of the total number of microorganisms).

2. The following free-living nitrogen-fixing microorganisms are widespread in the anthropogenic soil of Eastern Georgia: genus *Beijerinckia*, *Azotobacter*, *Dexsia*, *Zosporillum*. *Beijerinckia* is dominant.

3 genera, *Derxia* and *Azospirillum* are widespread only in the upper layer of the studied soil (5-10 cm), while microorganisms of the genus *Azotobacter* are not found in the lower layer of the soil (20-25 cm), the genus *Beijerinckia* is found throughout the entire profile of the studied soil

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