

Monitoring Soil Bacteria with Community-Level Physiological Profiles Using Biolog™ ECO-Plates in the Republic of Tatarstan (Russia)

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Abstract. Conservation of soil fertility is one of the most important tasks of the present time. As microorganisms are among the key factors in forming soil fertility, monitoring their state in natural and anthropogenically changed soils is an important component of compulsory environmental monitoring. Modern methods make it possible to evaluate the diversity and the functions of soil microorganisms, however, unfortunately, not all the soils are analyzed with their help up to the present moment. The present investigation is aimed to evaluate the functional diversity of five natural soil samples in the Republic of Tatarstan (belonging to sod-podzol, sod-carbonate, alluvial, and gray types) using the method of Biolog EcoPlate according to the index of average well color development, alpha-biodiversity Shannon index (H), amount of substrates consumed ®, and strategy of consumption of various carbon substrate groups. It was shown that the highest AWCD index was found in sample No 3 – alluvial soil type (3.159 ± 0.460), the lowest one – in sample No 5 – gray soil type (0.572 ± 0.230). Correlation of biological activity of microorganisms with organic matter content in soil was shown.

1. Introduction

One of the main tasks of modern environmental management is the preservation of soil diversity. To make scientific researches in the area of ecologization and anthropogenic landscape recovery effective it is necessary to examine natural soils that have been preserved [1]. Microorganisms play the most important part in soil formation, they are the basic agents of the circulation of elements [2, 3]. The scale of soil microbiological processes influence on the biosphere can be compared only with photosynthesis [4]. In soil science physiological groups of microorganisms being certain indicators of biochemical processes in soil, which are important for its fertility and the productivity of agricultural crops, are of great interest [5]. In 1991 J.L. Garland and A.L. Mills [6] offered to use Biolog system to evaluate functional diversity and developed the main approaches to the interpretation of carbon-bearing substrate consumption ranges received (SCR) using the methods of multivariate statistics. These methods have been widely recognized as an instrument of soil content monitoring [7, 8] to compare the functional diversity of microbial communities both in polluted and unpolluted soils [9, 10]. Thus, the method of Biolog Eco Plate was used in examining soils in the Netherlands, in Europe [8], in Hungary [11], in Poland [12], and in People's Republic of China [13].

In the territory of Russia the method of multisubstrate testing «Ecolog», the composition of which is similar to Biolog Ecoplate, was used to estimate the physiological profiles of bacterial communities in the territories of taiga forests in the Komi Republic, and the Republic of Bashkortostan [14, 15]. However, the information on the SCR for soil microorganisms in the Republic of Tatarstan is very poor.

The objective of the present investigation was to estimate the quality of soils typical for the Republic of Tatarstan (belonging to sod-podzol, sod-carbonate, alluvial, and gray types) with the help of Biolog EcoPlate method.

2. Materials and Methods

Soil samples were selected from the topsoil (0 to 20 cm) in the following sites: No 1 sod-podzol soil (55°42'32.58"N, 49°23'5.28"E), sample No 2 sod-carbonate soil (55°45'15.08"N, 49°18'45.83"E), sample No 3 alluvial soil (55°54'55.8"N, 49°12'49.3"E), sample No 4 alluvial soil (55°91.77'.28.7"N, 49°21'98"E), sample No 5 gray soil (55°75'39.85"N, 49°86.48'61"E).

Initial characteristics of the investigated soils were determined according to standard methods: electrical conductivity and pH as per ISO 26423–85 [16], total nitrogen content (N_{total}) as per ISO 11261:1995 [17], humus content – using the translation method of organic carbon concentration, determined as per ISO 14235:1998 [18]. Grain size measurement was carried out using the method of laser diffraction with the help of Microtrac Bluewave device, in accord with ISO 13320:2009 [19].

To estimate the functional diversity of soil microbial communities Biolog system (Biolog, Int) was used. For this soil extract was previously prepared (1:40) for 30 min. Soil extract was dug into the holes of Biolog EcoPlate plates containing one of low-molecular carbon-bearing substrates (Table 1) in triplicate and tetrazolium salt as an indicator of the substrate consumption.

Table 1. Carbon substrates in Biolog ECO MicroPlates (Biolog, Ing).

Groups of Substrates	Substrates
Polymers	a-cyclodextrin
	glycogen
	Tween 40
	Tween 80
Carbohydrates	D-cellobiose
	i-erythritol
	D-galactonic acid g-lactone
	N-acetyl-D-glucosamine
	glucose-1-phosphate
	b-methyl-D-glucoside
	D,L-a-glycerol phosphate
	a-D-lactose
	D-mannitol
	D-xylose
Carboxylic acids	g-hydroxybutyric acid
	a- ketobutyric acid
	D-galacturonic acid
	D-glucosaminic acid
	itaconic acid
	D-malic acid
	pyruvatic acid methyl ester
Amino acids	L-arginine
	L-asparagine
	glycyl-L-glutamic acid
	L-phenylalanine
	L-serine
Amines	L-threonine
	phenyl ethylamine
Phenolic compounds	putrescine
	2-hydroxybenzoic acid

4-hydroxybenzoic acid

Substrate consumption rate was estimated with the help of a plan-table photometer Thermo Scientific Multiskan FC at 595 nm after the incubation in darkness at 25°C during 24 and 168 hours.

With the help of the results received the following parameters were calculated: ACWD (average well color development), alpha-biodiversity Shannon index, amount of substrates consumed by microorganisms (R), types of the substrates consumed. The formulae used to calculate the indices are presented in Table 2.

Table 2. Formulae for the calculation of average well color development (AWCD), shannon diversity (H), and index of intensity of utilization of substrates (R).

Index	Formula	Definitions
The average well color development	$AWCD = \sum \frac{A_i - A_0}{31}$	A_i – is the optical density within each well; A_0 – is the absorbance value of the control well
Shannon diversity	$H = -\sum p_i * \ln(p_i)$ $p_i = \frac{A_i}{\sum A}$	p_i - proportional color development of the well over total color development of all wells of a plate
Index of intensity of utilization of substrates	R=1, if is the optical density i wells > 0.25 R=0, if is the optical density i wells < 0.25	R - Index of intensity of utilization of substrates

Statistical processing was carried out using the statistical analysis procedures of Microsoft Excel application program package.

3. Results and discussion

According to Soil Atlas of Tatarstan the following soil types are typical for the Republic: sod-podzol soils (17.0%), gray forest soils (32.4%), sod-carbonate soils (3.1%), black soils (39.7%), black meadow soils (2.5%), brown and gray soils (7.1%), bog soils (1.0%), floodplain soils (4.1%), alkali soils (0.1%), and alkaline soils (0.1%). The first three types, as well as two azonal alluvial soil types were analyzed in this study. To achieve homogeneous data when determining the functional profile of microbial communities sampling was carried out in equal temporary and weather conditions. The characteristics of soil samples selected are presented in Table 3.

Table 3. Characteristics of soil samples used in the study.

Soil Sample	pH	Humus, %	Ntot, mg g ⁻¹	Sand (%)	Silt (%)	Clay (%)
1	6.02±0.02	1.1±10.2	0.77±0.02	17.30±0.05	81.39±0.05	1.31±0.05
2	8.33±0.03	2.0±8.9	1.35±0.01	22.50±0.05	73.27±0.05	4.23±0.05
3	6.56±0.01	3.6±11.3	0.91±0.02	25.41±0.05	72.87±0.05	1.72±0.05
4	6.64±0.04	2.4±14.1	0.40±0.01	22.34±0.05	73.61±0.05	4.05±0.05
5	6.51±0.03	1.4±10.1	0.59±0.01	26.64±0.05	70.08±0.05	3.28±0.05

Table 3 shows that pH of water extracts of soil samples No 1, 3, 4, and 5 is neutral, and pH of water extract of soil sample No 2 is weakly alkaline [20]. The analysis revealed differences in humus and total nitrogen content: the highest humus content was found in sample No 3 (3.6±11.3), the lowest – in sample No 1 (1.1±10.2), the highest total nitrogen content was found in sample No 2 (1.35±0.01), the lowest – in sample No 4 (0.40±0.01). Grain-size analysis of the samples selected has shown practical absence of differences in fraction content in the samples. In all the samples selected the

fraction of dust prevails varying from (70.08 ± 0.05) for sample No 5 to (81.39 ± 0.05) for sample No 1, which classifies all the soil samples selected as heavy clays according to their grain-size distribution [20].

At the following step soil extracts were prepared and analyzed using Biolog EcoPlate method. It was shown that during the first 7 days of the investigation average well color development (AWCD) has grown in all the samples selected – by 1.4 on average. The growth of average well color development index shows that microbial community metabolic activity in relation to carbon substrates under analysis is high. The highest AWCD index was found in sample No 3 (3.159 ± 0.460), the lowest one – in sample No 5 (0.572 ± 0.230). The values received correspond with the data obtained by other authors [21, 22]. Likewise, the highest R value showing the number of substrates consumed by the community microorganisms was determined in sample No 3 (31), the lowest one – in sample No 5 [20]. The correlation between the indices of ACWD and R was found by other authors as well [21, 22]. The highest values of ACWD and R in sample No 3 are most likely connected with high humus content in it as compared to the other samples. Thus, in sample No 3 humus content was 3.6%, in the other samples it varied from 0.4 % to 2.4 % (Figure 1).

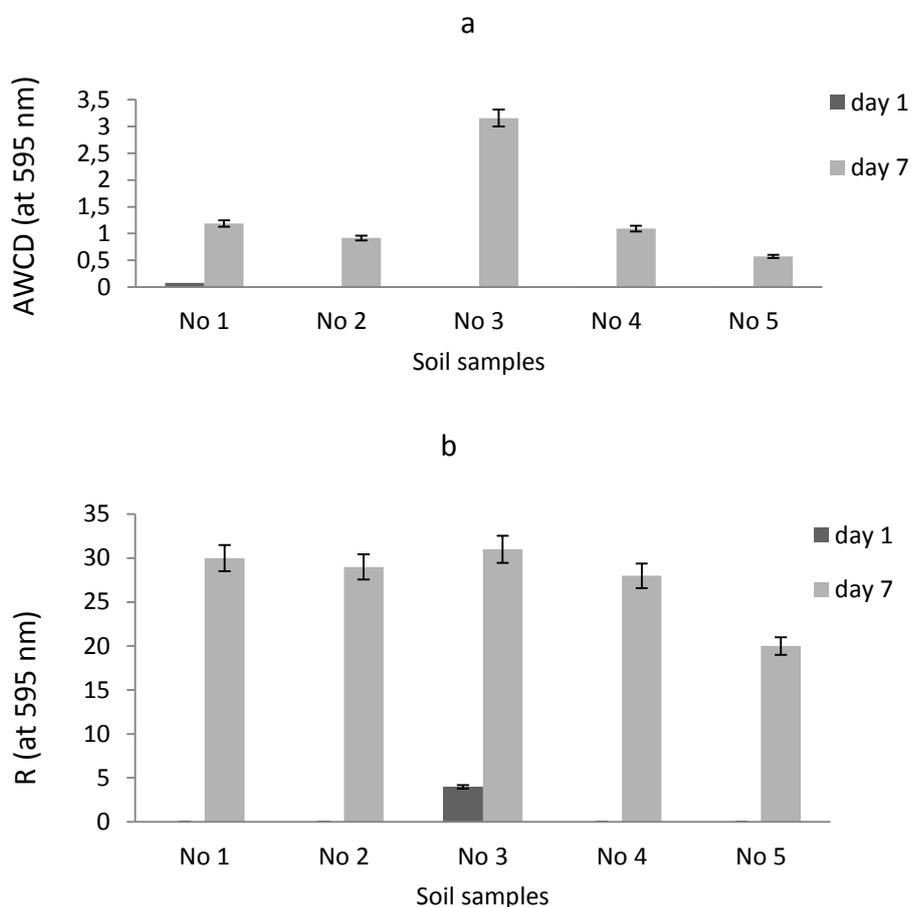


Figure 1. Average well color development AWCD (a) and index of intensity of utilization of substrates R (b) of five soil samples selected in Tatarstan, estimated on day 1 and day 7 of incubation.

High microbial counts and diversity are usually attributed to rich soils. Thus, in the investigations carried out by T.G. Dobrovolskaya et al. [23] and V.A. Kovda et al. [24] tight direct correlation between the biological activity of microorganisms and organic matter content in soil was shown. In the

study carried out by high metabolic activity of soil microflora is connected with the influence of the environment, such as the type of litter and the availability of organic matters [13].

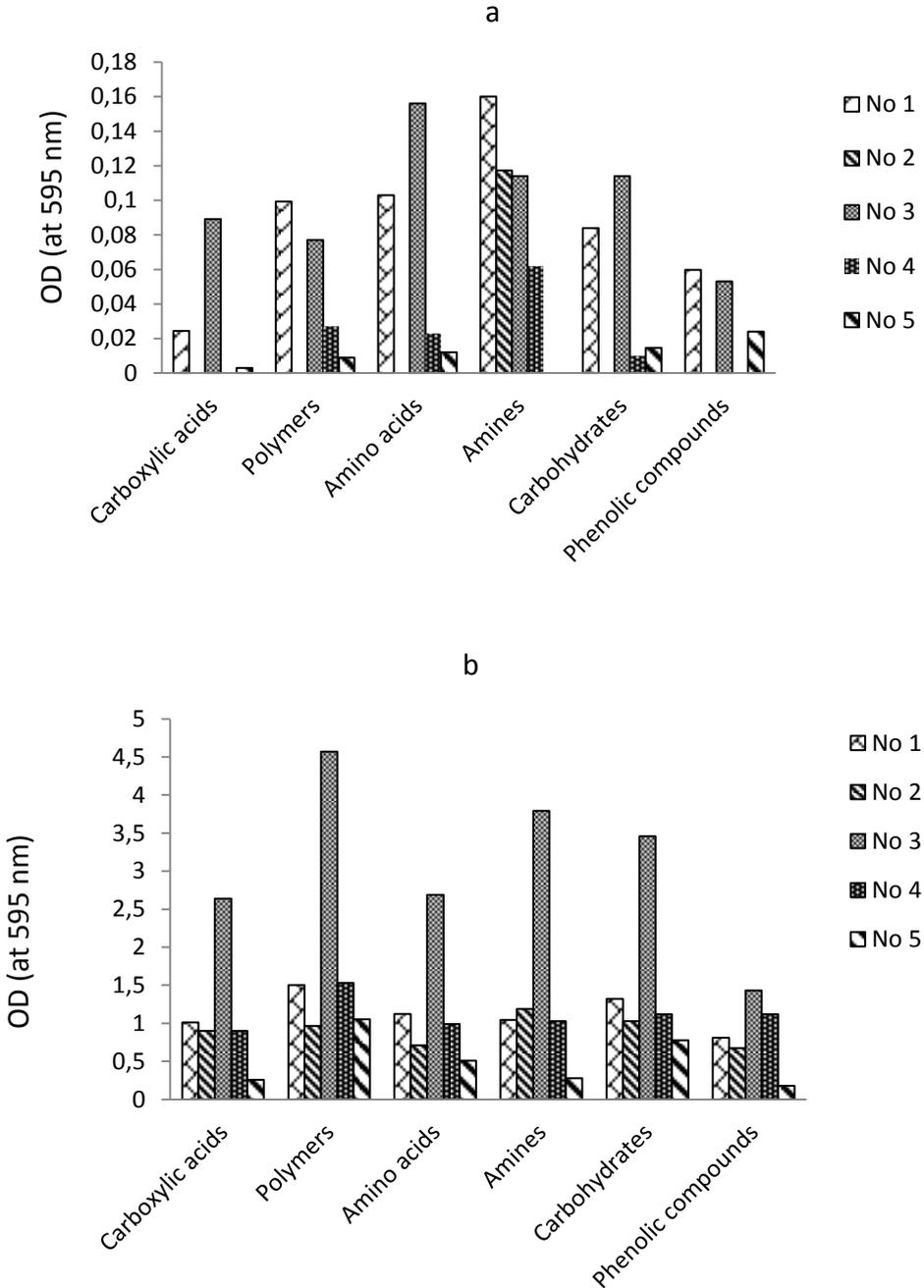


Figure 2. Level of consumption of substrates by microbial communities of soils, sampled in the republic of Tatarstan, estimated on day 1 (a) and day 7 (b) of incubation.

To compare the strategy of substrate consumption by the microbiomes contained in the soils under analysis, the matters contained in a Biolog EcoPlate plate were classified into 6 main groups: amino acids, carbohydrates, carboxylic acids, polymers, amines and amides and phenol compounds. The

analysis of intensity of utilization of substrates belonging to the indicated groups helped to find out that the microorganisms in sample No 3 actively utilize all the types of substrates, the microorganisms in samples No 1, 2 and 4 show average intensity of utilization in respect to 5 types of substrates (except for phenol compounds), and microorganisms in sample No 5 show the lowest level of utilization intensity (Figure 2). The low level of phenol substrate consumption is probably connected with the complexity of phenol substrate chemical structure and they require more time for decomposition [11].

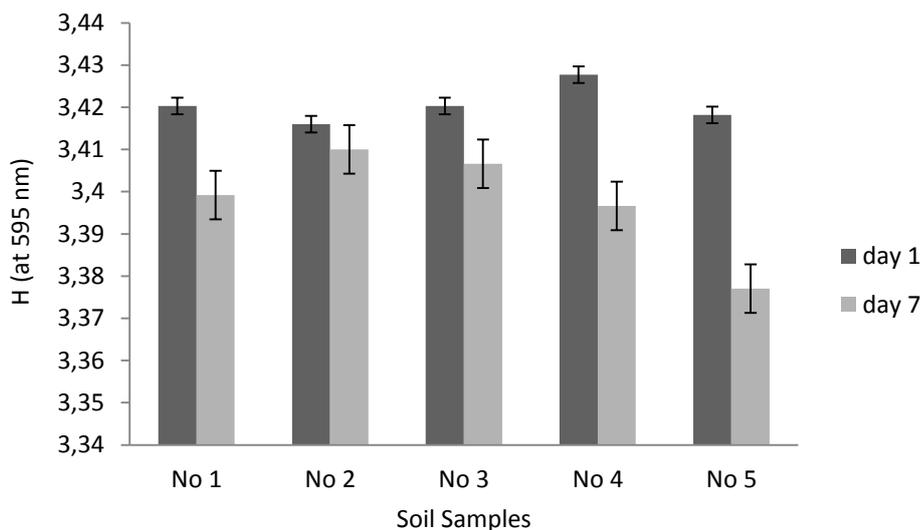


Figure 3. The Shannon index of five soil samples selected in Tatarstan, evaluated on the 1st day and the 7th day of incubation.

Shannon index showing the level of alpha-diversity in the community was analyzed on day 1 and day 7 of the experiment (Figure 3). In all the samples it practically had not changed during the time indicated, which probably shows that all the species in the community were active at the moment of sampling, there were no dormant species [25]. Such state of the community is most likely determined by favorable abiotic factor conditions – sampling was carried out in July 2017 at the average temperatures of 23°C to 25°C and the humidity of 75% to 80%. It is interesting to note that in samples 1 to 4 Shannon index was practically the same – 3.41 points. In sample No 5 it was slightly lower (3.38 points), which is probably connected with relatively low humus content in the sample. In whole, the data correlate with the data obtained by other authors [11, 26].

Conclusions

In the present investigation physiological profiles of natural soils sampled in the Republic of Tatarstan were determined for the first time. Average well color development index, alpha-biodiversity Shannon index, substrate consumption index, strategies of consumption for different carbon substrate groups determined using Biolog Ecoplate method, show that the biological activity of microbial communities is higher in soil sample No 3 out of the 5 soil samples selected. The interrelation of biological activity and organic matter content in soil was also revealed – the highest humus content was found in sample No 3.

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