A COMPREHENSIVE ANALYSIS OF THE MICROBIOME IN THE COMPLETE PROFILE OF VIRGIN LIGHT-COLORED SOLONETZ SOIL AT THE TERRITORY OF DZHANYBEK RESEARCH STATION

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Under study was the microbiome structure in virgin light-colored hydromorphized solonetz soil by using molecular-genetic (analysis of prokaryotic metagenome) and cultural methods. It is shown that the prokaryotic diversity (according to OTU amount, Shennon index and Chao1) is gradually decreasing downwards the soil profile. Common features of microbiomes are found to be in the solonetz horizon and the lower part of the profile (low biodiversity, some *Proteobacteria* are dominant). A higher share (almost 20% of the community) of *Archaea* from the group of *Thaumarchaeota* is observed in the horizon overlying the solonetz one.

Keywords: solonetz soil, metagenome, soil microorganisms, pyrosequencing, 16S pPHK, Archaea, biodiversity.

INTRODUCTION

Microbiological complexes of arid soils including saline ones have been so far examined insufficiently and some problems relating to the diversity, functioning and variability of microbial communities in arid soils are still far from being understood. As a rule, the uppermost soil horizons characterizing by main biochemical processes associated with the organic matter transformation are of interest for researchers [5, 10]. However, the lower horizons of arid soils which are poor in the organic matter and frequently salt-affected need to be thoroughly studied from the viewpoint of identifying the factors determining the structure of microbial communities in soil. In literature there is no information on the analysis of microbiome along the profile of arid soils to the depth of 150 cm. In view of this, an attempt was undertaken to study the microbiome in the profile of virgin solonetz soil at the territo-

ry of Dzhanybek Research Station of Forestry Institute of Russian Academy of Sciences.

This paper presents a comparative analysis of microbial communities along the profile of virgin light-colored hydrometamorphized solonchakous solonetz by using traditional methods of seeding on nutrient media and molecular-biological methods – analysis of prokaryotic metagenome.

OBJECTS OF RESEARCH AND METHODS

Dzhanybek Research Station is located within the subboreal belt in the northern part of the Pre-Caspian lowland in the Volga-Ural interfluve area elevated by 25–28 m. The low precipitation (250–350 mm) and the higher evaporation (about 1000 mm) are characteristic of the semi-arid climate. The territory under study is a closed plain confined to brown loess-like carbonate heavy loams underlying by clay with loam-sandy and sandy interlayers at a depth lower than 15 m [6]. The groundwater level is 4–5 m [7].

The object of research is a solonetz profile (pit 7M-13) at the virgin territory of Dzhanybek Research Station (49.39943^{o} N, 46.81062^{o} E). The vegetation is represented by *Poa bulbosa, Lepidium perfolliatum, Artemisia australica, Kochia prostrate;* the soil is effervescent at the depth of 15 cm; the groundwater level – 4.74 m. The morphological, micromorphological and chemical properties of this soil profile have been studied and published earlier [3]. In the course of our studies the following soil horizons were described:

AYEL, 0–7(10) cm – light-pale-gray, structure is changing along the profile, being flake and lens-like in the upper part and crumby downwards the profile; dry, light-loamy fine-silt, slightly compacted; the plant roots in the kind of rosettes, gradual transition, wavy boundary.

BSN, 7(10)–14 cm – pale-whitish sites in the upper part of dense prismatic soil particles; dry; abundant plant roots of different size; porous, medium loamy; fine iron coatings and patches; transition according to density, coloring and structure.

BSNbc, 14–24(26) cm – coffee-like in color, very dense; prismatic structure; heavy loamy, fresh; gradual transition according to coloring and density.

BSNs,bc, 24(26)–33(36) cm – brown; coffee-like coatings at the edges of soil particles; prismatic structure; dense to a lesser extent; medium

loamy, fresh; gypsum-bearing veins and fine carbonate spots; transition according to occurrence of a great amount of salt pedofeatures.

NCAs, 33(36)–57 cm – uneven coloring: in some sites a great amount of white and pale spots and patches against the brown background – gypsiferous and carbonate pedofeatures; dense to a lesser extent and more fresh as compared to the overlying horizon; a classical pseudosandy medium loamy horizon [6], instable lumpy structure; transition according to coloring and the decreased amount of salt pedofeatures.

BCs, 57–100 cm – yellowish-brown; more wet and compact to a lesser extent; lumpy structure with rare rounded patches comprising fine gypsum crystals of 1 cm in diameter; medium loamy; wave boundary; transition according to decreasing the amount of salt patches.

BC, 100–150 cm – light-yellowish-pale; wet medium loamy; instable lumpy structure; rare rounded salt patches.

The soil under study is classified as a virgin light-colored hydromorphized solonetz (Soil Classification of Russia, 2004) and Episalic Solonetz (WRB, 2006).

The soil samples taken in the middle-profile genetic horizons were used for the molecular-biological analysis and storage at –70°C. Their preparation included the following procedures. DNA isolation from 0.5 g of soil after mechanical destruction with the use of small glass balls in the extracting buffer solution containing 350 pL of solution A (sodium-phosphate buffer – 200 mM, isocianate guanidine – 240 mM, pH – 7.0), 350 pL of solution B (Tris-HCl -500 mM, SDS – 1%, pH – 7.0) and 400 pL of phenol with addition of chloroform (1 : 1). The homogenizer Precellys 24 (Berlin Technologies, France) was used to destruct the samples over 40 s at the maximum power (6500 rev/min). The obtained suspension was centrifuged at 16000 rev/min for 5 min, the water phase was separated and subjected to repeated extraction with chloroform. The DNA precipitation was performed by adding equal volumes of isopropyl alcohol. After centrifuging, the precipitate was with 70% ethanol and dissolved in water at 65°C over 5–10 min. The purification of DNA was performed by the method of electrophoresis in 1% agaric gel with further isolation of DNA from the gel by the sorption on silicon oxide [1].

The purified DNA was used as a matrix in the PCR reaction together with a pair of universal primers for the variable site V4 of 16SpPHK-F515 (GTGCCAGC-MGCCGGTAA) and R806 (GGACTACVSGGG-TATCTAAT) [8]. Primers were used with oligo-

nucleotide identifiers for each of the samples and sequences required for pyrosequencing according to the protocol of Roche firm (Switzerland). The sample preparation for sequencing was performed on GS Junior device following the standard recommendations.

About 2000 sequences were obtained for every sample. QIIME allowed processing of the obtained data [9]. The quality, filtration of sequences (nucleotide sequences), combination of sequences into operation taxonomic units (OTU) were tested with the use of 97% similarity level; the OTU taxonomic position – by using the database RDP available on the internet (http://rdp.cme.msu.edu). The taxonomic structure of every microbial community was estimated according to OTU shares referred to different taxones.

Several indices were used to determine the diversity of prokaryotic communities in different soil horizons: the amount of OTUs (S_{obs} – analog of species abundance), Shennon index ($H = \Sigma^{s}_{i=1} p_i \log p_i$, where p_i – a share of species abundance, i) and Chao1 index capable to estimate the actual OTU amount in the community (Chao1 = $S_{obs} + a^2$, where S_{obs} – a number of OTU, a – a number of OTU containing one sequence, b – a number of OTU containing two sequences).

The storage of samples for cultural study was at +4°C. The seeding on nutrient media was in the following composition: KAA: $(NH_4)_2SO_4$ – 2 g, K_2HPO_4 – 1 g, $MgSO_4$ – 1 g, NaCl –1 g, $CaCO_3$ – 3 g, starch – 10 g, agar – 20 g; Chapek medium – KH_2PO_4 – 1 g, $MgSO_4$ – 0.5 g, KCl – 0.5 g, $FeSO_4$ – 0.01 g, $NaNO_3$ – 3 g, glucose or saccharose – 30 g, lactic acid – 4 ml, agar – 20 g; Eshbi medium – KH_2PO_4 – 0.2 g, $MgSO_4$ – 0.2 g, NaCl – 0.2 g, $CaCO_3$ – 3 g, K_2SO_4 – 0.1 g, sugar (mannite) – 10–15 g, agar – 20 g; Vinogradskiy medium – K_2HPO_4 – 0.5 g, $MgSO_4$ – 0.5 g, NaCl, $FeSO_4$, $MnSO_4$. $CaCO_3$ – trace amounts; AMI (agar meat infusion) – meat broth – 1 l, peptone – 10 g, NaCl – 5 g, agar – 2–2.5%, pH = 7.6–7.8. After incubation from 3 to 14 days in dependence on the used nutrient medium the colony-forming units (CFU) were calculated for 1 g of soil.

RESULTS AND DISCUSSION

The analysis of diversity indices showed gradual decreasing the abundant amount of prokaryotic community with the depth (Fig. 1). In the lower part of profile (BC horizon) the species abundance of com-

munities estimated by Chao1 index made up 130 species (OTU). Against the background of gradual decreasing downwards the profile the biodiversity was also sharply declined in the BSNbc horizon (Shennon index was changed from 7.4 to 5.0, the amount of OTU – in the range from 560 to 253 as compared to the BSN horizon). It is known that the solonetz horizons have unfavorable properties for the growth and development of natural vegetation and diversity of its species as well as cultural crops [6]. Having analyzed the data about the diversity indices, it was established that the conditions of the solonetz horizon are also unfavorable for the major part of microbial communities. These data are of interest with account of the fact that the humus content and soil-pH as the main factors affecting the structure of soil microbiome in the BSNbc horizon are almost similar to those in the BSN horizon (Table). Probably, in this case a small diversity of microbiota is connected with the influence of the other factors, for instance, the increased content of exchangeable sodium or the high density in the BSNbc horizon.

The taxonomic structure of prokaryotic microbiomes in different soil horizons at the phylum level is shown in Fig. 2. It is worth empha-

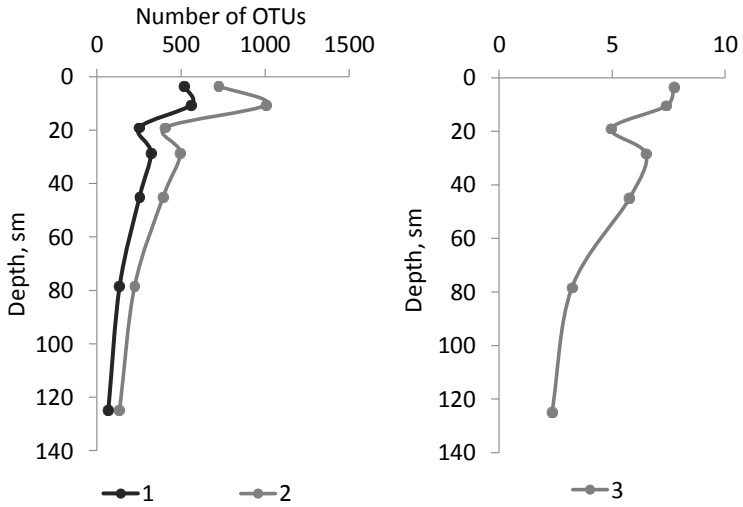

Fig. 1. Values of the biodiversity indices: 1 – the amount of OTU, 2 – Chao1 index (approximate amount of OTU in the community), 3 – Shennon index in genetic horizons within the profile of virgin light-colored solonetz soil.

Results of chemical analysis of virgin light-colored solonetz soil

Depth, cm	pH H_2O	Humus, %	Exchangeable base mmol-equ/100 g					Na^+	Mg^{2+}
			Ca^{2+}	Mg^{2+}	Na^+	K^+	sum	% of the sum	
0–7	7.17	1.51	5.36	2.64	0.69	0.83	9.52	7.25	27.73
7–14	8.11	1.09	4.68	5.36	5.82	0.39	16.25	35.82	32.98
14–24	8.14	1.82	2.48	9.65	10.11	0.52	22.76	44.42	42.40
24–33	8.34	–	1.60	10.42	8.94	0.52	21.48	41.62	48.51
33–57	8.61	–	2.27	6.92	7.09	0.44	16.72	42.40	41.39
57–100	8.86	–	2.60	5.12	6.62	0.35	14.69	45.06	34.85
100–141	8.77	–	2.37	5.82	6.68	0.36	15.23	43.86	38.21
140–160	8.85	–	2.55	6.13	7.07	0.35	16.10	43.91	38.07

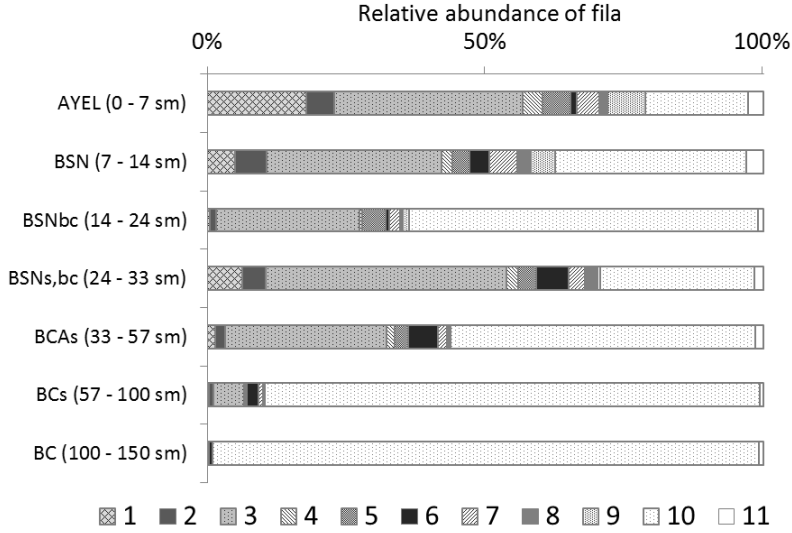

Fig. 2. Taxonomic structure of microbiomes in soil horizons of virgin light-colored solonetz soil, the share of sequences referred to phylumes: 1 – *Thaumarchaeota*, 2 – *Acidobacteria*, 3 – *Actinobacteria*, 4 – *Bacteroidetes*, 5 – *Chloroflexi*, 6 – *Firmicutes*, 7 – *Gemmatimonadetes*, 8 – *Planctomycetes*, 9 – *Verrucomicrobia*, 10 – *Proteobacteria*, 11 – others.

sizing that the major portion of sequences (almost 18% in the topsoil horizon) is regarded to the group of *Thoumararchaeota Archaea*. The representatives of this *Archaea* group are considered as active ammonium oxidizers, which are often present in soil, but they are determined as minor components of the prokaryotic community and don't form a considerable part in the soil microbiome as compared to the solonetz under study.

It was possible to observe the reverse linkage between the biodiversity and portions of *Proteobacteria* representatives – the smaller is the taxonomic diversity of the community, the higher is its share in *Proteobacteria*. In the lower horizon, where the species composition makes up 67 OTU, about 98% of sequences are referred to *Proteobacteria*. Probably, some representatives of this phylum in the given soil are capable to be survived under unfavorable conditions (the low content of organic substances, the increased content of salts), where they seem dominant due to decreasing the population of the other microorganisms. As seen from Fig.3, the share of some prokaryotic species is highly dependent on the depth of genetic horizons. The share of *Candidatus Nitrososphaera (Archaea)*, *Streptomyces*, *Bacillus* and

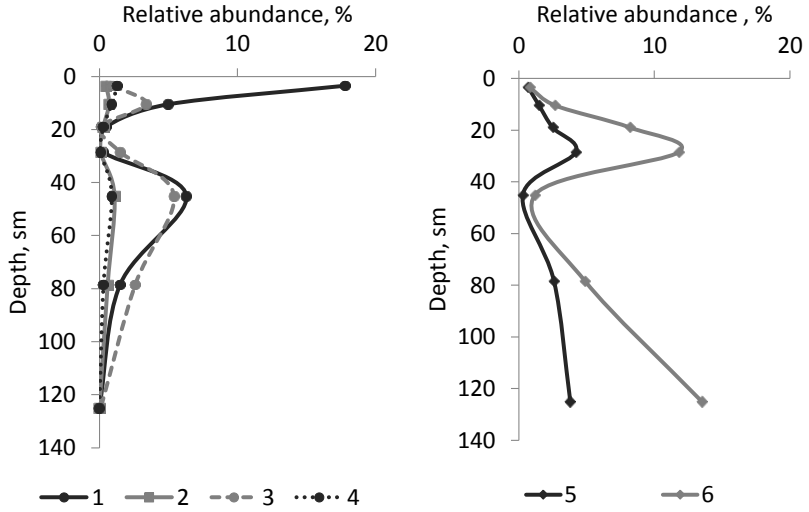

Fig. 3. Distribution of some prokaryotic species along the soil profile: 1 – *Candidatus Nitrososphaera*, 2 – *Streptomyces*, 3 – *Bacillus*, 4 – *Rhodoplanes*, 5 – *Sphingomonas*, 6 – *Pseudomonas.*

Rhodoplanes corresponds to general diversity – the minimum in the BSNbc horizon and in the lower part of the profile. On the contrary, the share of *Springomonas* and *Pseudomonas* as regarded to *Proteobacteria* shows quite the other dependence – the maximum in the BSNbc and BC horizons, whereas their values are minimal in horizons with the increased biodiversity. The population of cultivated groups of microorganisms is shown in Fig. 4. Some ecologic-trophical groups are present only in the topsoil horizons of solonetz soil (about 50%), in the lower horizons they were not found by method of seeding on nutrient media. This is amilolytics and mycelium organisms (fungi and actinomycetes), the functions of which in soil are associated with the destruction of organic polymers that are rather rare in deep soil horizons. It is worthy of note that the results of seeding method revealed no decreasing the population of microorganisms in the solonetz horizon as compared to data of metagenomic analysis. It is evident that the nutrient media cultivate

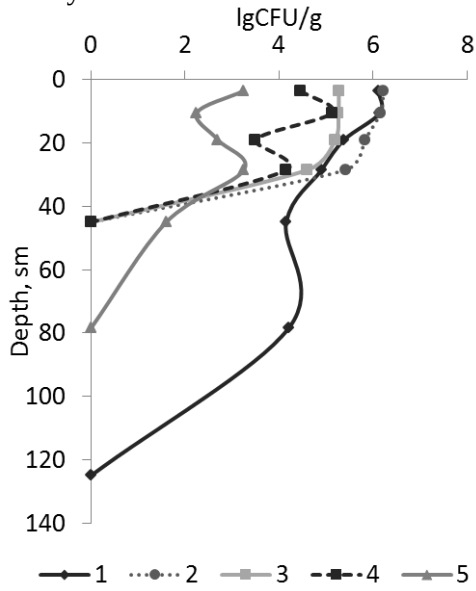

Fig. 4. Population of cultivated microorganisms (lgKOE/g soil) along the profile of virgin light-colored solonetz soil: 1 – ammonifiers, 2 – amilolytic (KAA), 3 – actinomycetes (KAA), 4 – micromycetes (Chapek medium), 5 – anaerobic nitrogen fixers or oligonitrophilous microorganisms (Vinogradskiy medium).

only a small part of soil microbiota but the great amount of rare taxons accounting for a considerable part of microbial diversity cannot be determined with the use of this method. Besides, in horizons with abundant diversity and population of microorganisms KOE can be represented not by cells and spores, they reveal the micro-colonies what additionally decreases their amount.

CONCLUSION

The study of the microbial complex in the complete profile of virgin light-colored solonetz was carried out for the first time by using molecular-biological methods. It was established that the diversity of the prokaryotic complex is gradually changing with depth being sharply declined in the solonetz BSNbc horizon characterizing by the increased density and the high content of exchangeable sodium. There is a reverse linkage between general diversity of the prokaryotic complex and the portion of *Proteobacteria* representatives including *Springomonas* and *Pseudomonas.* In horizons with the lower biodiversity (BSNbc, BCs, BC) these taxons are dominant. In horizons with the higher biodiversity there are representatives from the group of *Thaumarchaeota (Archaea)*, the share of which in the topsoil horizon reached 18% of the total prokaryotic complex. The differences in the diversity and structure of microbiome in soil horizons are probably conditioned by the content of the organic matter and the salt composition.

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