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Agrotechnology for Snapshots Soil Health with Bacteria

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Abstract

Taxonomic structure of bacterial microbiome of sod-podzolic soil (Gatchina district of Leningrad region, Russia) with varying degrees of moisture were studied using pyrosequencing of 16sRNA gene. *Proteobacteria* were revealed mostly in drought conditions and may be used as indicator microorganisms. The fractal analysis of the data showed that the index of the biosystem determinism of the frequencies of soil microbial communities decreased with drought (from 0.73 to 0.65), since decreases the number of variations of taxonomic units, due to which a smaller number of biosystem groups were formed.

Keywords: soil microbiome, soil health, drought, fractal portrait of microbial community, index of biosystem determinism

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1. Introduction

Soil is the largest depositary of microbial communities of both the prokaryotic and fungal complex. Different types and facies subtypes currently known in the soil hierarchy [10, 12], to one degree or another, affect differences in the frequency-taxonomic structure of microorganisms and indicators of alpha and beta biodiversity. Frequency-taxonomic bonitet is determined by the combination of all the properties of a particular soil: physical, water-physical, physico-chemical, genesis, particle size distribution and aggregate composition, as well as previous microbiological biochemical processes. In addition, when studying the microbial successions of the rhizosphere of the soil, an abundance of plant formations and the composition of their root secretions (exometabolites), mainly low molecular weight organic acids, can also be an important factor [7, 8, 17, 18, 22].

It is known that drought is one of the main causes of crop shortages worldwide. It can also negatively affect the structure of microbial communities. At the same time,

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soil microflora, as the system most sensitive to any changes in biogeocenose, in this aspect acts as an indicator of the state of fertility and ``*soil health*'' [1, 16, 19, 20]. Different numerical taxonomy groups of microorganisms participating in the conversion of organic substrates. Therefore, indicator groups can serve not only major fils, as it considered in present time, but also minor. It is the balanced frequency and taxonomic diversity of microorganisms that determines the self-cleaning (sanitation) and suppressive activity of soil microbial community [1, 19].

The cultivation of microorganisms in selective media allows studying about 1--20 % of the total number of microorganisms in the soil. The remaining 80--99 % of the populations was not cultivated on known selective media [4]. Therefore, due to the appearance and active development over the past 20 years in the field of molecular ecology of the latest molecular genetic methods, in particular, high-performance pyrosequencing technology [9, 13, 14], microbiology has gained the opportunity to analyze structural changes in soil microbial communities in their full genomic biodiversity -- a microbiome reflecting the entire pool of microorganisms.

Nowadays, a large proportion of the costs in scientific research is associated not with the actual sequencing process, but with the analysis of the data obtained.

About 60 % of the soil microbiome is associated with the organo-mineral humus horizon of the soil, which adsorbs most of the microbial cells on the surface of the aggregate solid phase [6, 15]. The relationship of the structural rearrangements of microbial communities with external conditions and the use of agricultural technologies has not been studied much and can lead to a complete rethinking of the entire modern scientific paradigm in soil microbiology [12].

For the most complete disclosure of the ecological and service functions of the soil microbiome, some researchers propose combining the methods of metagenomics (to assess the phylogenetic diversity of microorganisms), with the analysis of biomarkers (to determine the functional diversity) and measurements of enzymatic activity (to assess the actual functionality of soils) [3].

Since the soil is a labile environment, capable of continuously and dramatically changing over the short-term (seasonal, annual) and longer (tens and hundreds of years) time periods, the prospect of this direction, subject to large-scale and long-term ``chrono-screening'' of the main soil types, taking into account changes in environmental factors. It may be the creation of the world's largest interactive (visual) geographic information database to develop effective algorithms and multi-factor forecasting models and Maintaining the existing level of fertility in adaptive-landscape farming systems of one or another locality of the region [21]. Of course, such a large-scale work cannot be carried out without the close cooperation of specialists from various fields of science [5, 11].

2. Methods and Equipment

The selection of soil samples was carried out in the summer of 2019 from sod-podzolic soil in the agricultural field, near the village of Menkovo (Figure 1). Localization sampling points were carried out using the GPS system. In total, five soil samples were selected.

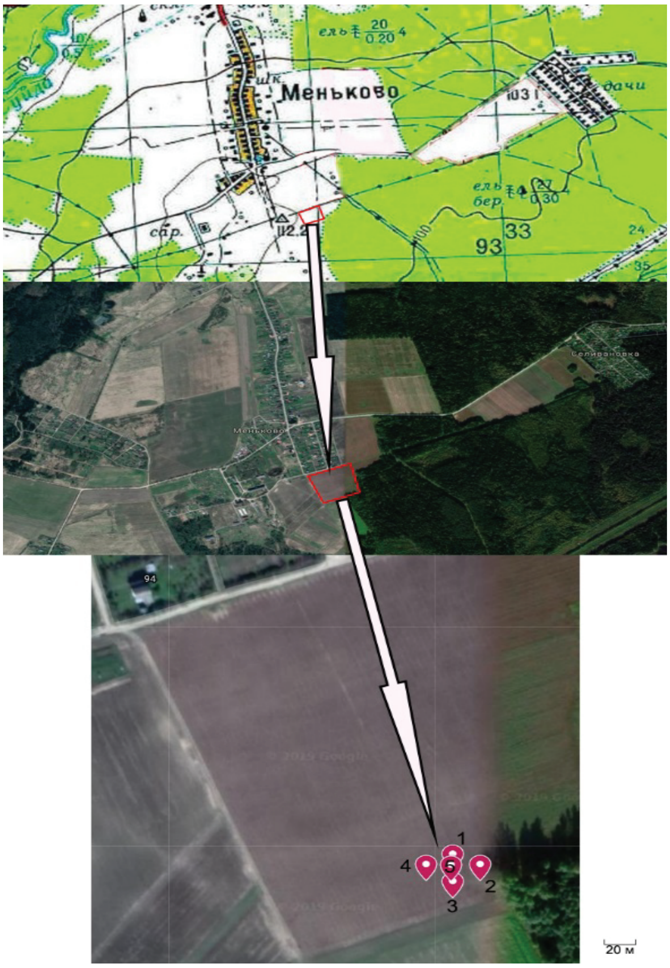

Figure 1: Land with sampling points: T1 -- 59°25'06.1"N 30°02'12.5"E; T2 -- 59°25'05.9"N 30°02'13.5"E; T3 -- 59°25'05.5"N 30°02'12.5"E; T4 -- 59°25'05.9"N 30°02'11.5"E; T5 -- 59°25'05.9"N 30°02'12.4"E.

The samples were taken from the arable horizon A1, at a depth of 10 cm. Each sample was sieved through a sieve with a diameter of 3 mm and brought to an air-dry state so that the total weight of each of them was 5.0 kg. After that, 2 equal shares in a volume of 2.5 kg were taken from each sample and placed in vessels without drainage. Then the soil was brought with water to varying degrees of moisture (normal -- 60 % and drought -- 30 % PPV). In this state, the system was maintained using the weight method for one month with daily monitoring.

After 30 days of incubation, from each vessel were taken 0.5 g samples to isolate bacterial DNA, purify it from humic and fulvic acids, perform PCR analysis and subsequent sequencing.

Computer processing of the resulting nucleotide sequences by sequencing was performed according to the guidelines in the Ribosome Database Project (RDP) application. The sequences were then classified into OTU (Operational Taxonomic Unit) using a 97 % similarity criterion. The taxonomic analysis of the nucleotide sequences of amplicon libraries was carried out using the QIIME program.

Statistical processing of experimental data was carried out using the data analysis program R 3.1.1. ANOVA analysis of variance modules was used taking into account intergroup differences using the Tukey post hoc test criterion.

3. Results

The studies revealed that under the conditions of artificially created drought as a result of adaptation processes in the structure of the microbial community among the remaining taxonomic diversity, bacteria of certain species begin to prevail. Thus, for example, phylloids *Actinobacteria, Acidobacteria* and *Gemmatimonadetes* had a large share in the normal water regime of irrigation -- 23.19; 10.52 and 5.65 %. In the soil microbiome during drought, they amounted to 8.86; 2.53; and 1.61 %, respectively. Fila *Proteobacteria*, had a lower representation under normal conditions (41.39 %), and the largest share (77.74 %) under the influence of drought. Maybe, this can be associated with different dynamics of nutrients in the soil. In the Actinobacteria fillet, a large share was occupied by the genera of microorganisms from the class *Thermoleophilia, Acidimicrobiia, Solibacteres*. In addition several fil has been distinguished, which in the community accounted for about 1.1--1.6 % -- these are *Crenarcheota, Verrucomicrobia, Bacteroidetes*. The number of microorganisms from the remaining phyla in the communities did not reach 1.0 %.

Table 1 shows all the minor fils, which were isolated in variants under normal and under drought conditions.

When considering the community at the genus level, representatives of *Alphaproteobacteria* dominated in the *Proteobacteria* fillet (their content ranged from 14.0--39.7 %), *Sphingomonas sp., Pseudomonas sp.*, as well as the representatives of the *Proteobacteria* class that were not identifiable before the genus.

TABLE 1: Percentage of minor fils bacteria in soil samples in variants under norm and under drought.

Class-level taxonomic unit of bacteria
k__Bacteria;p__Acidobacteria;c__Acidobacteria-5;o__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Micrococcales
k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__FAC88
k__Bacteria;p__Planctomycetes;c__OM190;o__CL500-15
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales

Visually, the results of comparing the taxonomic structure of the experimental options at the level of classes and orders are shown in Figure 2.

To calculate the indexes of biosystem determination of the frequencies (BDF) of soil microbial community, were adjusted fractal portraits [23], which can be used as universal snapshots of soil health (Figure 3). It was shown that the BDF decreases with drought from 0.73 to 0.65, since the number of variations of taxonomic units decreased, in the formation of which all the studied bacteria were involved. Mineral fertilizer use during drought can effect on indices BDF.

4. Discussion

It should be noted that these results are still preliminary and reflect changes in the structure of the rhizosphere community only during one summer season and cannot be interpreted as the only true and reliable for subsequent years. Therefore, in order to obtain a more verified and representative sample, it is necessary to continue further long-term studies related to the setting up of experiments with stable reproduction of the stressor and all other related conditions from year to year.

5. Conclusion

In order to search for possible variables that determine the structure of microbial diversity depending on fluctuations in environmental parameters, also can be applied spectral analysis of derivation steps and searching for bifurcation points and attractors, arising in the system thanks to the invasion theory of fractal chaos [2]. Indirect relationships between environmental situations in soils and frequency-taxonomic molecular genetic data of soil microbial communities can be established using fractal portraits of microbial communities and fractal models of soil microbial community. To compare the

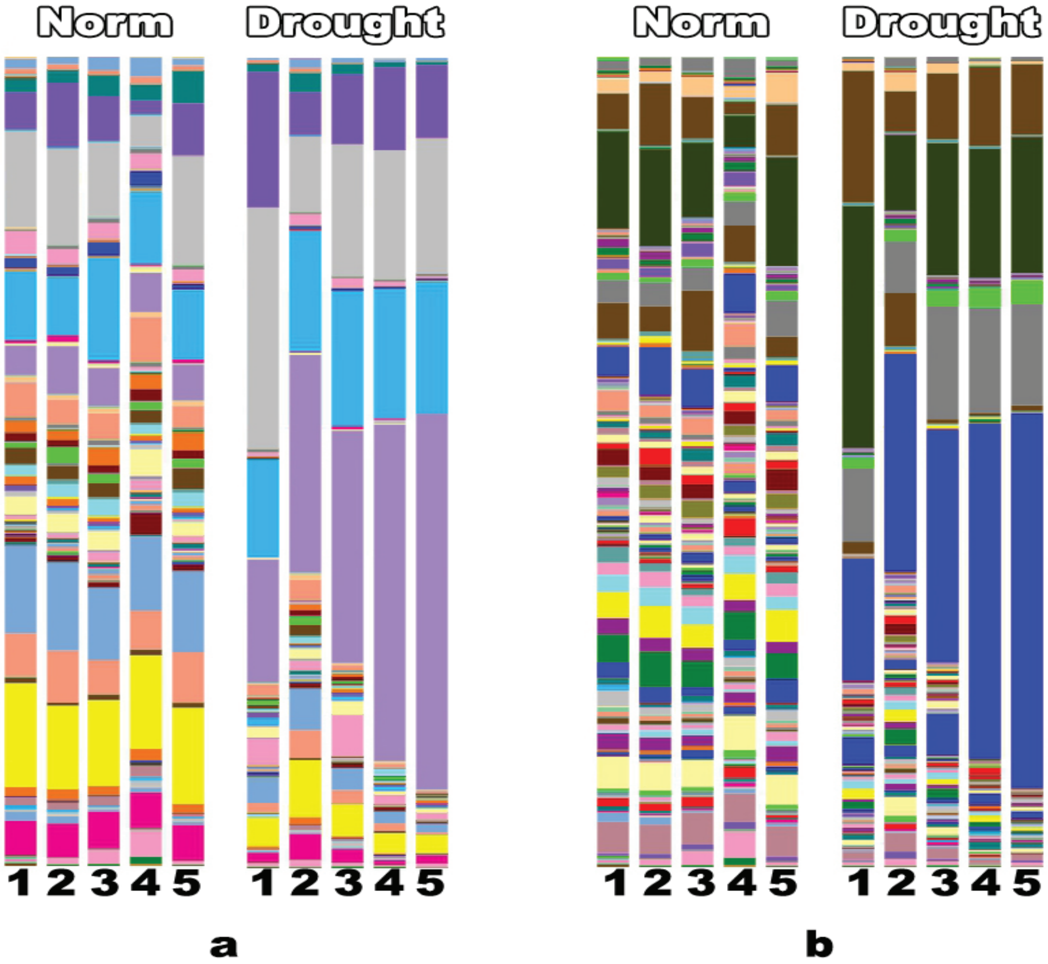

Figure 2: Taxonomic structure of prokaryotic communities at the class level (a) and at the level of orders (b).

composition of the identified biosystem groups with taxonomic data, we can learn about the prevailing biochemical processes occurring in soils and outline ways to preserve and maintain the soil cover bonitet. The fractal portraits can serve a ``snapshots'' soil health that can be included in agrosmart-technology.

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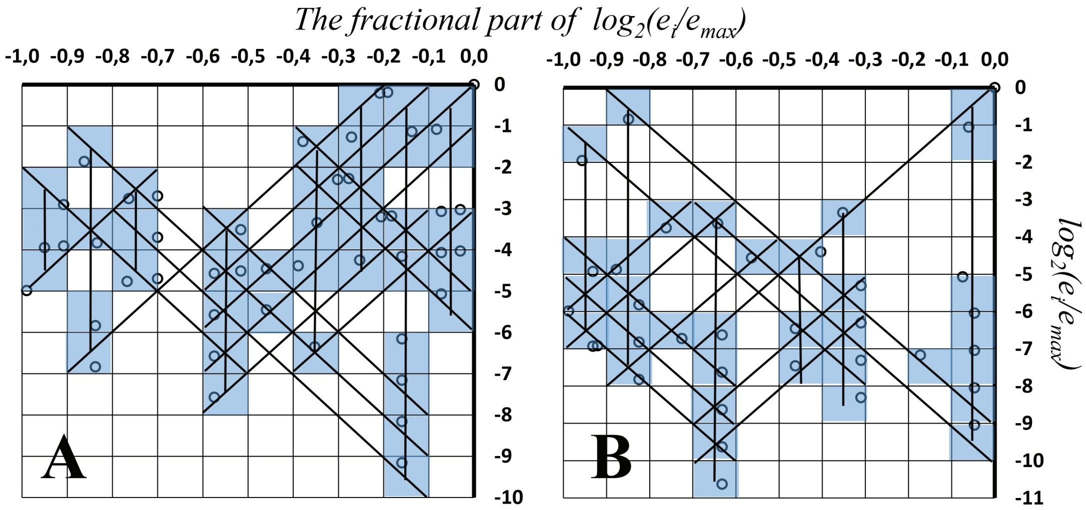

Figure 3: Fractal portraits of frequency-taxonomic data of soil microbial communities: norm (A) -- BDF = 0.73, drought (B) -- BDF = 0.65. Note: e_i and e_{max} are the frequency of occurrence of a taxonomic unit of bacteria at the level of classes (i) and the maximum frequency of occurrence among soil microbial communities. Lines highlight fractal biosystem groups of soil microbiota.

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Conflict of Interest

The authors have not conflict of interest to declare.

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