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EXPERIMENTAL ARTICLES

Evaluation of Bacterial Diversity in Soil Microcosms at Different Moisture Contents

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Abstract—The succession analysis of bacterial diversity in the A horizons (rich in organic matter) of three contrasting types of soil—burozem, soddy gley soil, and chernozem—showed that the bacterial diversity of soil microcosms in humid regions can be adequately evaluated only if soil samples are incubated at different soil moisture contents. A complete account of actinobacteria and proteobacteria requires the levels of soil moisture corresponding to the maximum capillary–sorption moisture and capillary moisture, respectively. The bacterial diversity, whose value was maximum on the 40th day of succession, was higher in soddy gley soil than in burozem. The taxonomic structures of the bacterial communities of these two types of soil were different. After wetting chernozem samples from arid regions, the soil bacterial community changed insignificantly with time and drastically differed from that of soils from humid regions. The difference in the bacterial diversity of soils was the most distinct when it was evaluated by measuring the proportion between proteobacteria and actinobacteria.

Key words: bacterial diversity, succession analysis, soil moisture, soil microcosms, model experiments.

The bacterial diversity of soils can be adequately evaluated in terms of the succession analysis of soil microcosms, which allows the taxonomic structure of soil microbial communities to be determined in dynamics. In this case, soil samples are kept under different conditions in order to evaluate the soil bacterial diversity adequately and to detect the bacterial community that is adapted to specific conditions of a given type of soil. In our earlier laboratory studies, fresh soil samples were subjected to succession analysis at a constant soil moisture content equal to 60% of that observed under field conditions [1, 2]. Those studies did not reveal considerable differences in the taxonomic structure of soil bacterial communities in the course of time. A critical examination of the results of those studies prompted us to change experimental conditions used for the succession analysis of soils.

The aim of the present study was to evaluate bacterial diversity in contrasting types of soil under conditions when microbial succession in the preliminarily air-dried soil samples was initiated by wetting them to different moisture contents.

MATERIALS AND METHODS

Experiments were carried out with soil samples taken from the A horizons (rich in organic matter) of three types of soil contrasting in their hydrological characteristics and phytocenosis: burozem (the Ahf horizon) and soddy gley soil (the A1 horizon) from the Central State Forest Biosphere Reserve (CSFBR) in the Tver oblast and chernozem (the A horizon) from the Rostov oblast. The burozem was sampled beneath a spruce forest. It was formed on loamy morainic slopes and had an acidic pH, sandy granulometric composition, and 4.5% humus. This soil was characterized by intense intrasoil drainage. The soddy gleisolic, weakly loamy soil was sampled beneath an alder grove dominated by the growth of spireas. It was formed on light loam underlain by morainic carbonate rock. This soil had a neutral pH and a humus content of 6.8%. The south chernozem was sampled at an arid watershed overgrown by grasses and forbs. It had a loamy granulometric composition, slightly alkaline pH, and 7.7% humus.

Soil samples were air-dried and then wetted to two moisture contents, maximum capillary–sorption moisture (MCSM) and capillary moisture (CM). Wet soil samples were placed in petri dishes and incubated in a desiccator to maintain the soil moisture content at constant levels corresponding to MCSM and CM. After 0, 10, 20, 40, 70, 100, and 170 days of incubation, or succession, 1-g aliquots of the incubated soils were subjected to microbiological analysis. Data on the 0th day of succession refer to the air-dry soil.

The numerical and the taxonomic composition of soil bacterial complexes were determined by the culture method [3]. Bacterial cells were desorbed from soil samples by dispersing them in a UZDN-1 ultrasonic disintegrator and plated, in 10 replicates, onto glucose–

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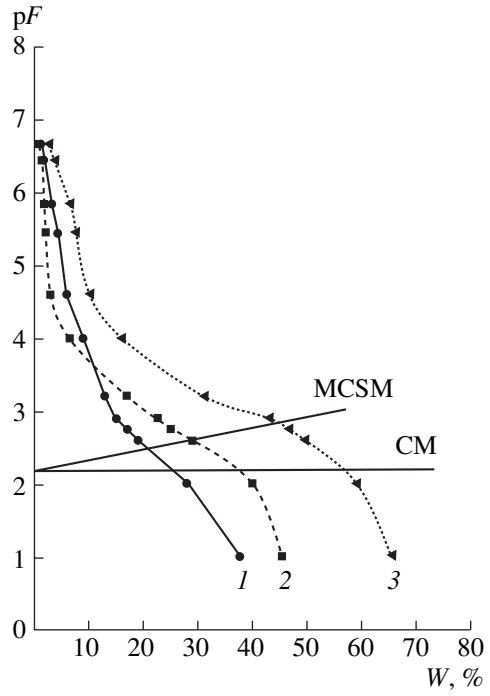

Fig. 1. The main hydrophysical characteristic of (*1*) chernozem, (*2*) burozem, and (*3*) soddy gley soil. pF = log|P|, where pF is the soil moisture potential and P is the soil moisture pressure expressed in cm of water column. W (expressed as a percentage) is the total soil moisture content, MCSM is the maximum capillary–sorption moisture, and CM is the capillary moisture.

peptone–yeast extract agar [4]. To inhibit fungal growth, the agar medium was supplemented with 100 mg/l nystatin. The grown colonies were enumerated after the incubation of the plates for 2–4 weeks. The results were expressed in colony-forming units (CFU) per g dry soil (total bacterial count). In addition, different colonial morphotypes were counted on each of the agar plates and the results were presented as a percentage of the total number of the colonies grown. Three to five colonies of each morphotype were isolated in pure cultures and the strains thus obtained were identified on the basis of micromorphological (the shape and size of cells, their motility, the type of developmental cycle, mycelial characteristics, cell division, and spore formation), physiological, and biochemical features using the identification criteria of *Bergey's Manual* [5, 6]. The collection of pure cultures obtained from the soil samples amounted to 100 entities.

RESULTS AND DISCUSSION

The main hydrophysical characteristic (MHC) of soils (Fig. 1) was determined in a range of low soil moisture pressures by the static adsorption method above saturated salt solutions and in a range of high moisture pressures using a probe capillarimeter [7]. Some relevant hydrophysical characteristics of soils presented in Table 1 were calculated from the MHC. The dependence of the capillary–sorption pressure of soil moisture on its content allowed some important hydrological soil parameters to be determined. In the present work, we used two parameters: maximum capillary–sorption moisture (MCSM) and capillary moisture (CM).

In the range of low soil moisture pressures, the soils ranked, in order of decreasing pressures, as soddy gley soil > chernozem > burozem. Accordingly, the effective specific surface of soddy gley soil was two times greater than that of chernozem and 2.5 times greater than that of burozem (Table 1). It is known that the higher the specific surface of a soil, the more moisture, nutrients, and microorganisms can be kept by this soil. In the range of high soil moisture pressures, where soil moisture is mainly kept due to the action of capillary forces, the soils ranked in a different order: soddy gley soil > burozem > chernozem. It can be seen that the physical properties of soddy gley soil markedly differed from those of burozem and chernozem.

Microbiological analysis showed that the initiation of microbial succession in soils by wetting them led to a rise in the soil bacterial population within the first 10 days of incubation of all soils, irrespective of their moisture contents. In soddy gley soil, the total bacterial population increased by a factor of 50 (from 5 to 250 million CFU/g soil), whereas the total bacterial population of chernozem and burozem increased by a factor of 10 (from 10 to 100 million CFU/g soil). The more profound increase in the bacterial population of

Table 1. Some hydrologic characteristics of soils

Soil	Specific surface, m^2/g soil	Moisture capacity, %	Hydrologic constant	Total moisture content, %	Soil moisture pressure, MPa	Moisture film thickness, μm
Chernozem	54	37.5	MCSM	21.5	−0.0223	6.7
			CM	24.5	−0.0147	10.2
Burozem	40	46	MCSM	30	−0.0316	4.7
			CM	38	−0.0147	10.2
Soddy gley soil	110	65.5	MCSM	45	−0.0447	3.3
			CM	57	−0.0147	10.2

soddy gley soil may be due to the higher specific surface of this type of soil.

The wetting of soils influenced their bacterial diversity. In the soddy gley soil and burozem samples taken from the humid regions, the bacterial diversity gradually increased in the course of succession. The maximum values of the Shannon diversity index were attained on the 40th day of succession induced by the soil moisture content corresponding to the capillary moisture capacity. The Shannon index was higher for soddy gley soil than for burozem. Conversely, after wetting the chernozem samples taken from the arid region, the bacterial diversity gradually decreased. The decrease was steeper at a high soil moisture content corresponding to the capillary moisture capacity. These observations were confirmed in factorial experiments with three variables—succession stage, soil type, and moisture content (Table 2). The Shannon diversity index depended on all three variables, which ranked, in order of decreasing significance, in the succession stage > soil type > moisture content. These data proved the adequacy of succession analysis in providing for a more complete account of bacterial diversity in soils.

The taxonomic structure of the bacterial complex of the air-dry burozem was characterized by a prevalence of bacilli, whereas actinobacteria and myxobacteria were detected in minor amounts (Fig. 2). After 10 days of succession, the relative number of bacilli decreased and that of proteobacteria (*Azotobacter, Aquaspirillum,* and *Alcaligenes*) increased. As a result, the proportions between different bacterial taxa in the soil bacterial complex became more uniform.

The bacterial diversity of burozem was at a maximum on the 40th day of succession. At a lower soil moisture content corresponding to MCSM, when soil moisture forms thin films, the abundance and diversity of gram-positive bacteria (the genera *Bacillus*, *Arthrobacter, Rhodococcus, Cellulomonas, Micrococcus*, and *Streptomyces*) were greater than those of gram-negative bacteria. Conversely, at a higher soil moisture content corresponding to CM, when soil moisture forms thick films (Table 1), the abundance and diversity of gram-negative bacteria (*Myxobacterales* and the genera *Azotobacter, Aquaspirillum, Alcaligenes, Cytophaga*, and *Flavobacterium*) and dissipotrophic proteobacteria were greater than those of gram-positive bacteria. The account of bacteria at two different values of the soil moisture content after the preliminary desiccation of soil samples allowed the estimated bacterial diversity in burozem to be increased by approximately three times.

The dynamics of bacterial complexes in soddy gley soil at the two soil moisture contents is presented in Fig. 3. It can be seen that, like the air-dry burozem, the air-dry soddy gley soil was dominated by bacilli (50%), whereas actinobacteria and myxobacteria comprised 5–20% of the total. After 10 days of succession induced by wetting, the bacterial diversity began to rise (due to an increase in the fraction of gram-negative bacteria) and attained a maximum by the 40th day of succession. As in the case of burozem, the bacterial diversity increased threefold. The soil moisture content corresponding to MCSM (when moisture films are thin) enhanced the evaluated diversity and abundance of actinobacteria (detected were the genera *Cellulomonas, Nocardioides, Promicromonospora*, and *Micrococcus*). The soil moisture content corresponding to CM (when moisture films are thick) enhanced the evaluated diversity and abundance of proteobacteria. On day 40 of succession, the proteobacteria were represented by *Myxobacterales*, dissipotrophic proteobacteria, and the genera *Pseudomonas, Azotobacter, Aquaspirillum, Alcaligenes, Cytophaga*, and *Flavobacterium*. After 70 days of succession, the bacterial diversity tended to decline. It should be noted that most of the dissipotrophic proteobacteria were isolated between the 40th and 70th day of succession. Some of the dissipotrophic isolates represented rare and, presumably, new species. In total, the succession induced by creating and maintaining two different soil moisture contents allowed 11 new bacterial taxa to be detected in soddy gley soil.

The fact that the bacterial diversity in soil samples is maximum after 30–40 days of succession induced by soil wetting was also reported by other authors [8, 9]. The increase in the abundance and diversity of bacteria during this period may be related to the resuscitation of dormant bacteria [10] and to the bacterial utilization of fungal autolysis products, since the bacterial population in soils attains a maximum after the death of soil fungi [11]. It is obvious that the most favorable conditions for the development of soil bacteria are created after 40 days of succession induced by soil wetting.

As opposed to the bacterial diversity in soddy gley soil and burozem, the bacterial diversity in chernozem changed insignificantly after soil wetting (Fig. 4). Another specific feature of the bacterial complex of chernozem was that it did not not contain dominant bacterial species. The bacteria that were isolated from the air-dry chernozem represented mainly actinobacte-

Table 2. Effect of (1) soil type, (2) moisture content, and (3) succession stage on the evaluated bacterial diversity according to the results of factorial experiments

Variables	Degrees of freedom	Variance	Fisher criterion*
1	2	0.24795	8.31760
2	1	0.09482	3.18099
3	1	0.46738	15.6783
12	2	0.64315	21.5744
13	2	1.00268	33.6346
23	1	0.17951	6.02177
123	2	0.15316	5.13789
At random	53	0.02981	

* Fisher criterion was calculated for significance $\alpha < 0.05$.

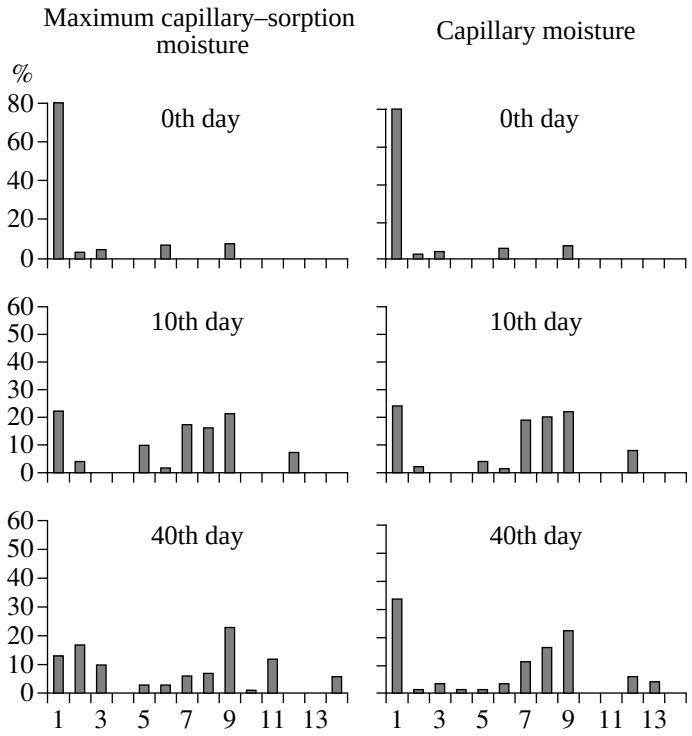

Fig. 2. Dynamics of bacterial complexes in burozem during the succession induced by soil wetting: 1, *Bacillus;* 2, *Rhodococcus*; 3, *Arthrobacter*; 4, *Cellulomonas*; 5, *Micrococcus*; 6, *Streptomyces*; 7, *Azotobacter*; 8, *Aquaspirillum*; 9, myxobacteria; 10, *Cytophaga;* 11, *Flavobacterium*; 12, *Alcaligenes*; 13, dissipotrophs; and 14, *Azospirillum.*

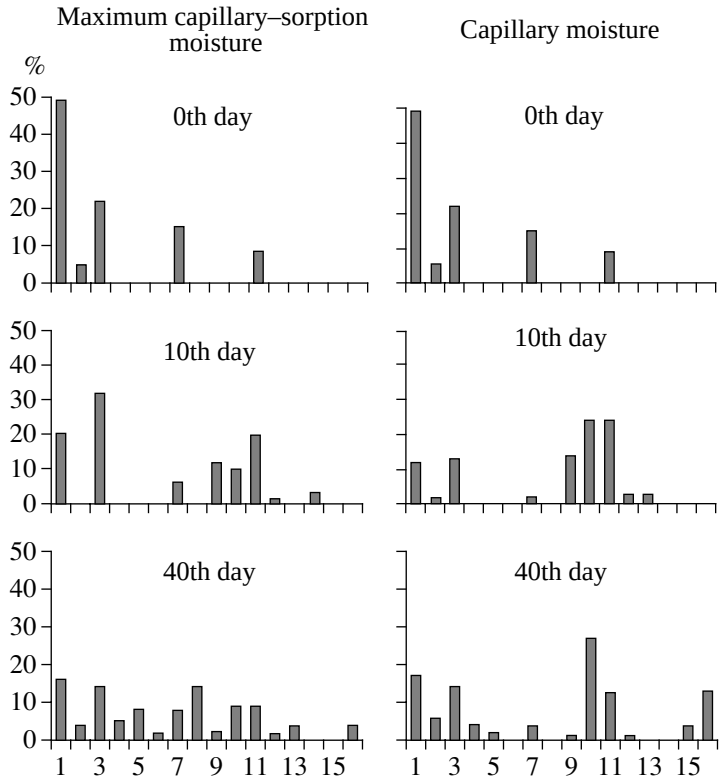

Fig. 3. Dynamics of bacterial complexes in soddy gley soil during the succession induced by soil wetting: 1, *Bacillus*; 2, *Rhodococcus*; 3, *Arthrobacter*; 4, *Cellulomonas*; 5, *Nocardioides*; 6, *Micrococcus*; 7, *Streptomyces*; 8, *Promicromonospora*; 9, *Azotobacter*; 10, *Aquaspirillum;* 11, myxobacteria; 12, *Cytophaga*; 13, *Alcaligenes*; 14, *Flavobacterium*; 15, *Pseudomonas*; and 16, dissipotrophs.

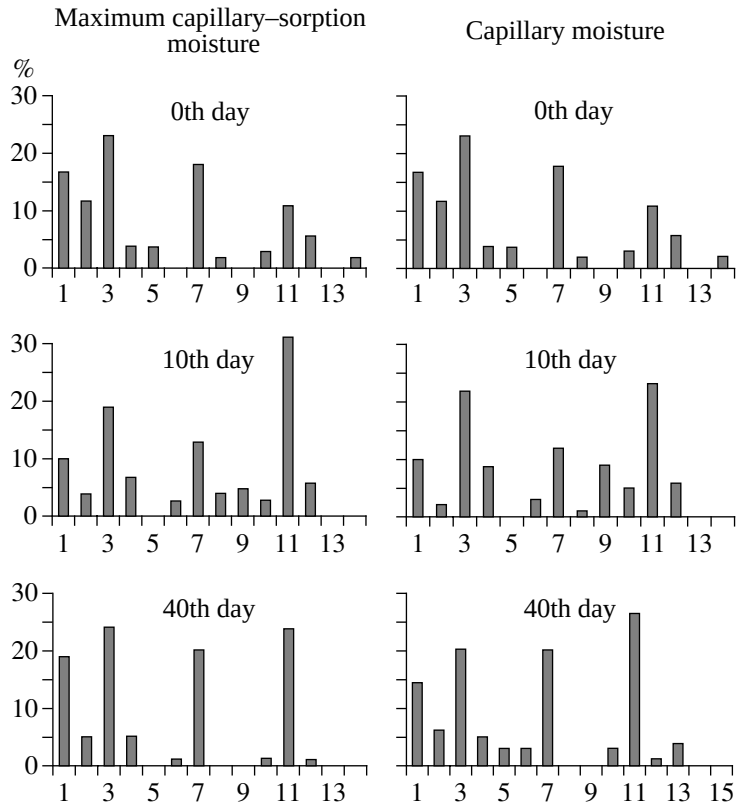

Fig. 4. Dynamics of bacterial complexes in chernozem during the succession induced by soil wetting: 1, *Bacillus;* 2, *Rhodococcus*; 3, *Arthrobacter*; 4, *Cellulomonas*; 5, *Nocardioides*; 6, *Micrococcus*; 7, *Streptomyces*; 8, *Promicromonospora*; 9, *Curtobacterium*; 10, *Aquaspirillum*; 11, myxobacteria; 12, *Cytophaga*; 13, *Flavobacterium*; and 14, *Beijerinckia.*

ria of the genera *Arthrobacter*, *Rhodococcus*, *Cellulomonas*, *Nocardioides*, *Promicromonospora*, and *Streptomyces*, as well as bacilli and myxobacteria. The fraction of bacteria of the genera *Cytophaga*, *Flavobacterium*, and *Beijerinckia* was low. The wetting of chernozem not only failed to augment its bacterial diversity but even slightly decreased it. This was possibly due to the prevalence of desiccation-resistant actinobacteria in this arid type of soil. Furthermore, the bacterial diversity of wet chernozem was greater when its moisture content was maintained at the level corresponding to MCSM. Therefore, the moisture content of soil typical of natural conditions and determining the soil moisture capacity and the adsorptive ability of soil aggregates correlates with the abundance and taxonomic composition of soil bacterial complexes.

Let us determine what indices characterizing the structure of soil bacterial complexes can best reflect their adaptation to the aeration and hydrologic regime of soils.

As was established earlier, the adaptation of a bacterial community to a given soil can be most adequately characterized by a proportion between large bacterial taxa, such as proteobacteria and actinobacteria [12].

The proteobacteria-to-actinobacteria ratio turned out to be drastically different for burozem and chernozem soils, which greatly differ in their hydrologic properties (Table 3). This ratio varied from 2 to 4 for burozem and was equal to 1/2 for chernozem, indicating that, irrespective of the soil moisture content, burozem was dominated by gram-negative bacteria and chernozem was dominated by gram-positive bacteria. At the same time, soddy gley soil was dominated by gram-negative bacteria at the moisture content corresponding to CM and by gram-positive bacteria at the moisture content corresponding to MCSM (Fig. 5). The prevalence of proteobacteria is most likely due to the repeated flooding of the gleisolic horizon of this soil, whereas the development of actinobacteria may be promoted by the neutral pH of this soil and the presence of

Table 3. The proteobacteria-to-actinobacteria ratio for various soils

	Soddy gley soil	Burozem	Chernozem
Capillary–sorption moisture capacity	1/2	2/1	1/2
Capillary moisture capacity	2/1	4/1	1/2
Mean for a given soil	2/2	3/1	1/2

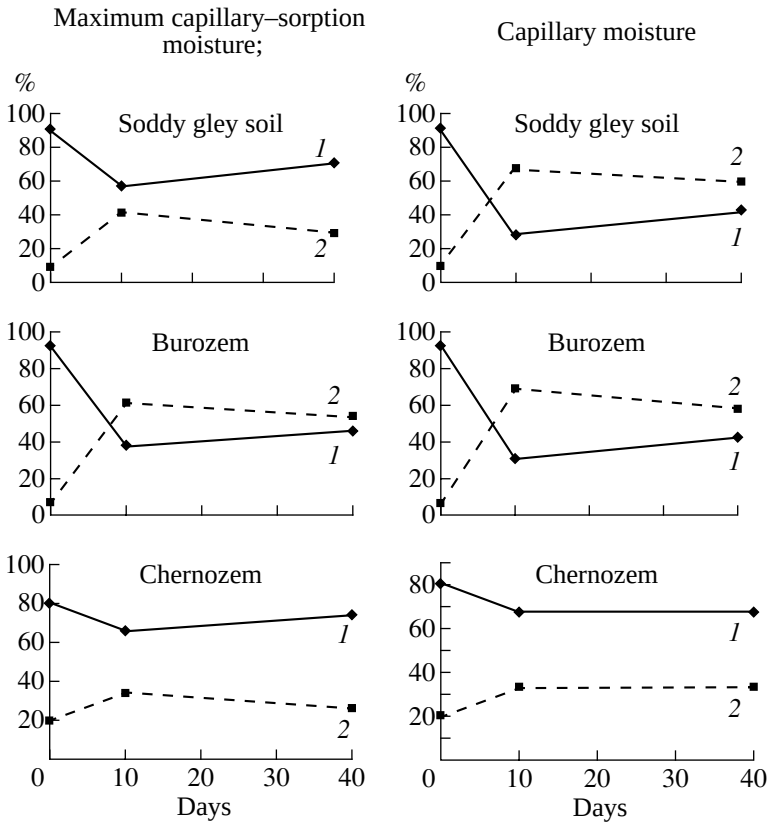

Fig. 5. Dynamics of (*1*) gram-positive and (*2*) gram-negative bacteria at different soil moisture contents.

leaves and grasses, with which coryneform bacteria are associated, in its litter.

Factorial analysis with three variables showed that the proportion of proteobacteria and actinobacteria is primarily determined by the soil type (Table 4). The effect of each of the variables studied was more profound than any of their combinations.

The bacterial diversity of soddy gley soil was greater than that of burozem and chernozem, whereas the populations of fungi and actinomycetes in this soil were low. These data suggest that bacteria may perform the functions of fungi and actinomycetes in soddy gley soil.

Table 4. Effect of (1) soil type, (2) moisture content, and (3) succession stage on the proteobacteria-to-actinobacteria ratio according to the results of factorial experiments

Variables	Degrees of freedom	Variance	Fisher criterion*
1	2	7.35340	60.9394
2	1	5.02337	41.6299
3	1	5.24936	43.5026
12	2	3.10448	25.7275
13	2	0.83206	6.89553
23	1	0.29350	2.43236
123	2	0.56799	4.70709
At random	53	0.12066	

* Fisher criterion was calculated for significance $\alpha < 0.05$.

The analysis of the data published previously [1, 2] and those presented in the given paper allows us to give the following methodological recommendations designed to improve the succession analysis of soils: (1) For an adequate evaluation of bacterial diversity in the soils of humid regions, soil samples should be preliminarily air-dried and the air-dry soil samples should not be stored for more than two months, since longer storage will decrease the evaluated bacterial diversity [2]. (2) For an adequate account of actinobacteria and proteobacteria, soil samples should be incubated at low and high moisture contents corresponding, for instance, to the maximum capillary–sorption soil moisture capacity and capillary soil moisture capacity, respectively.

The question may arise: Why is the desiccation of soil samples a necessary step for an adequate evaluation of the taxonomic composition of the bacterial complexes of soil? The reason for this may lie in the fact that prior to study a soil occurs at an unknown succession stage and it should first be dried and then wetted to initiate succession under controlled conditions.

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