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SOIL BIOLOGY

Bacterial Complexes of a High Moor Related to Different Elements of Microrelief

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Abstract—The analysis of bacterial complexes, including the number, taxonomic composition, physiological state, and proportion of ecological trophic groups was performed in a high moorland related to different elements of the microrelief. The abundance of bacteria, their ability for hydrolysis of polymers and the share of *r*-strategists were found to be higher in the sphagnum hillocks than on the flat surfaces. The total prokaryote biomass was 4 times greater in the sphagnum samples from microhighs (hillocks). On these elements of the microrelief, the density of actinomycetal mycelium was higher. Bacteria of the hydrolytic complex (*Cytophaga* and *Chitinophaga* genera) were found only in microhigh samples.

Keywords: peat, microrelief, bacteria, biomass, ecological and trophic groups, physiological state

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INTRODUCTION

In Russia, high moors predominate in the area and peat stocks over other bog types. Having a high microbial biomass, they are characterized by the low rate of peat mineralization. The analysis of reasons for the slow decomposition of high moor peat was described in the monograph "The function of the microbial complexes in high-moor peat—an analysis of reasons for slow peat decomposition" [10]. The microbial communities of high moors have been studied by both Russian and foreign researchers [2–4, 14, 17]. One should pay attention to a little studied feature of the high moor peatland surface developed in the course of peatland formation. This feature is the formation of various forms of the microrelief: microhighs—hillocks, flat areas, and microlows. Soil scientists described and studied some peat properties and carbon emissions from peat in these elements of the microrelief, but in works of microbiologists, the influence of the microrelief of high moors on the abundance and diversity of microorganisms has been poorly investigated.

The aim of this work was to study the number, taxonomic composition, and physiological state of bacterial communities as related to the microrelief of a high moor peatland.

OBJECTS AND METHODS

The oligotrophic residual– eutrophic peat soil (Dystric Fibric Histosol) under a dwarf shrub–cotton grass–sphagnum pine forest in the permanent sample area of the Zapadnaya Dvina Forest-Bog Station of the Institute of Forestry of the Russian Academy of Sciences (Tver oblast, 56°09′ N, 32°10′ E и 56°09′ N, 32°08′ E) was the object for studies. The peatland investigated was composed of high moor, transitional, and low moor peat underlain by sands that were gleyed in different degree. The degree of the peat decomposition depended on its composition and varied from 14 to 40% within the profile.

A levelling survey of the peatland was made by squares 2 × 2 m, which allowed estimating the areas of microhighs, microlows, and flat areas. The area of microhighs was found to occupy 52%, flat areas 31%, and microhighs (hillocks) 17% of the total studied area [1]. In this work, only flat areas and hillocks were studied. Samples of *Sphagnum magellanicum*, its weakly decomposed residues, and samples from the peat horizons were taken in five hillocks and five flat plots in late August, 2014 when the air and soil temperatures were high and precipitation was low. The whole 50-cm thickness investigated was represented by the high moor peat decomposed by 14–16%.

The number and biomass of prokaryote microorganisms were determined using a direct method of fluorescent microscopy [7]. Soil samples (1 g) were put into flasks with 100 mL of distilled water. The suspension obtained was treated on a Bandelin Sonopuls HD 2070 (Germany) ultrasound dispersant for 2 min to desorb cells. Using a micropipette, 0.01 mL of the suspension was distributed evenly over the area of 4 cm^2 on a defatted glass. Six preparations were made for each sample of peat, green part of sphagnum and its

† Deceased.

residues. Twenty fields were examined for counting bacteria and 50 fields to count actinomycetal mycelium. Then, the preparations were dried at room temperature and fixed by weak heating on a gas burner. The prepared samples were stained with an aqueous solution of acridine orange. An excess of the stain was removed by washing. The stained preparations were dried at room temperature and examined using a Lyumamiz fluorescence microscope (light filters ZhS-19, ZnS-18, objective ×90 L, oculars ×4 or ×5). In the preparations, some bacterial cells were counted, and the length of residues of actinomycetal mycelium was measured [μm].

The number of bacterial cells in 1 g of a sample was determined by the equation

$$N = S_1an/vS_2c,$$

where N is the number of bacterial cells in 1 g of the sample; S_1 is the area of the preparation, μm^2; a is the average number of bacteria in the field; n is the index of suspension dilution, mL; v is the volume of the drop applied, mL; S_2 is the area of the microscope field, μm^2; c—the mass of the sample, g. The specific bacterial mass was accepted equal to 1 g/cm^3; the water content in cells is 80%. The length of actinomycetal mycelium was determined according to the following equation:

$$N_1 = S_1an/vS_2c \times 10^6,$$

where N_1 is the length of actinomycetal mycelium in 1 g of the sample, μm; S_1 is the area of the preparation, μm^2; a is the mean length of residues of actinomycetal mycelium in the field, μm; n is the index of suspension dilution, mL; v is the volume of the drop applied, mL; S_2 is the area of the microscope field, μm^2; c—the mass of the sample, g.

The number of bacterial cells and the length of actinomycetal mycelium were calculated on a unit mass of dry sample. Moisture of the samples was measured by drying at 105°C for 6 h.

The prokaryote biomass was calculated taking into account that the biomass of dry substance for one bacterial cell with the volume of 0.1 μ^3 is 2×10^{-14} g and the biomass of 1 m of actinomycetal mycelium with the diameter of 0.5 μm is 3.9×10^{-8} g [6, 9].

The number and taxonomic composition of bacteria from the saprotrophic complex were determined by the inoculation on glucose–peptone–yeast medium (GPY). In order to inhibit fungi, 50 mg of nystatin was added per 0.5 l of the medium. A suspension remained after the preparation of the samples was used for fluorescent microscopy. The inoculation was carried out in five replicates from experimentally selected dilutions. The preparations were incubated at room temperature for 2–3 weeks. The total number of bacteria was expressed in colony-forming units (CFU) per 1 g of dry sample. The colonies of bacteria of different taxonomic groups were counted. The main representatives of bacteria were identified as a separate culture. The isolated strains were identified to genus level basing on the morphological, cultural, and chemotaxonomic characteristics [5, 8]. The dominant taxa of bacteria were determined basing on sequencing the nucleotide sequences of 16S rDNA gene using the BLAST program [13].

The term "ecophysiological characterization of bacterial community" means the physiological diversity of a bacterial complex, the physiological state of bacteria (assessed by the metabolic readiness to growth on polymers), and the predominant ecological strategy among members of the community. The mentioned parameters were found using a complex structural–functional method, which permits to characterize a microbial community *in situ* according to the growth of the community *ex situ* (on selective liquid nutrient media) [12]. As the methodology needs sterility in performing the analysis, a control variant of the test for sterility of nutrient media was provided. The homogenization and desorption of microorganisms from the substrates investigated was carried out by the treatment of the water suspension (1:10) for 20 min using a "Vortex" shaker ("Multi Reax", "Heidolph" Company) at 2000 rpm. In order to inhibit the growth of fungi, 0.05% nystatin was introduced to the suspension. An excess of the substrates (moss residues, moss, and peat) were removed by centrifugation at 3200*g* for 5 min. The concentration and composition of the microorganisms cultivated in the initial substrate were detected by the inoculation of the supernatant on the GPY-medium. The supernatant (100 μL) was added to cells of a 96-well flat-bottomed plate with a cover. A set of different liquid nutrient media was used. The hydrolytic bacterial complex was investigated, therefore, the following polymers were used as organic components: nucleic acid, casein and keratin, Tween 20 (a fat analogue), and polysaccharides: dextran, inulin, chitin, carboxymethylcellulose (CMC), xylan, starch, and pectin. Chitin, keratin, pectin, casein, and starch were sterilized in an autoclave (0.5 atm, 20 min). The other substrates were filtered through membrane filters with a diameter of 0.22 μm. The control variant was without polymers to assess the growth only on soil organic matter. To prevent water evaporation from the cells, the plate was closed from its sides with parafilm. The plate was incubated with periodic shaking on an automated "Sunrise" ("Tecan" Company) enzyme-immunoassay analyzer, which registers the growth of microorganism based on the optical density (at 620 nm). The analyzer was placed into a climatic chamber to keep the constant temperature (25ºC) during the incubation of the plate. The composition of associations developing on polymers was investigated by plating on the GPY medium for 68, 100, and 204 h. Using the data on the inoculation from the plate cells; a calibrating equation was constructed to calculate the optical density on the concentration of bacterial cells. A complex model developed for a pure culture [14] described the growth of a periodic mixed culture in the cells. In this work, the phase of pre-

Table 1. The number of bacteria, the length of actinomycete mycelium (AM), and their biomass in the high moor peat related to different elements of the microrelief

Peat layers, cm	Number of bacteria, $\times 10^9$ cells/g	Length of AM, m/g	Total biomass of bacteria and AM, mg/g	Share in biomass, %	
				bacteria	AM
Microhighs					
0–3 (green part of moss*)	20 ± 2	247	0.4	98	2
3–6 (moss residues)	40 ± 3	600	0.8	97	3
6–10	51 ± 3	675	1.1	97	3
10–20	80 ± 10	811	1.6	98	2
20–35	94 ± 9	1041	1.9	98	2
35–50	69 ± 7	1458	1.4	96	4
Flat areas					
0–3(green part of moss*)	7 ± 0.5	0	0.1	100	0
3–6 (moss residues)	9 ± 1	172	0.2	96	4
6–10	63 ± 6	487	1.3	99	1
10–20	96 ± 8	1186	2.0	98	2
20–35	93 ± 10	2714	2.0	95	5
35–50	105 ± 11	2331	2.2	96	4

* *Sphagnum magellanicum*.

paring microorganisms for growth (lag-phase) and the phase of exponential growth were described theoretically. The following mathematical model describes these phases—$x(t) = x_0(1 - \rho_0 + \rho_0 e^{\mu_m t})$,where $x(t)$ is the concentration of cells, CFU/ml at the time t, h; x_0 is the initial concentration of cells, CFU/mL; ρ_0 is the value characterizing the physiological state of the growing culture at the time zero, and μ_m is the maximal specific rate of the growth of microorganisms, h^{-1}.

The physiological diversity (PD, %) of the hydrolytic bacterial complex was determined as PD = n_{growth}/n_{total} 100, where n_{growth} is the number of media, on which the growth of bacteria is recorded; n_{total} is the total number of media used in the experiment. The physiological diversity depends on the initial dilution of the suspension and mass of the substrate. In our experiment, the lower detection threshold of bacteria capable to grow on any polymer was 100 cells/g of the initial substrate.

The metabolic readiness to the growth of bacterial associations](γ) was calculated by the equation: $\gamma = 100 + \ln(\rho_0)$. Our hypothesis suggests that the share of associations growing on polymers reflects a real metabolic readiness of bacteria to the consumption of polymers *in situ*. The greater are the γ values, the higher is the readiness of bacteria to growth.

The predominant ecological strategy of the bacterial complex is determined according to the median value μ_m of the mixed bacterial cultures growing on all the polymers. We suggest that the share of fast-growing associations on polymers reflects the share of bacteria—*r*-strategists *in situ*.

RESULTS AND DISCUSSION

The number of bacteria in sphagnum from flat areas determined by direct count amounted to 7×10^9 cell/g in the green part of plants and 9×10^9 cells/g in the moss residues. The bacterial abundance in the peatland increased drastically to 63×10^9 cells/g in the 6- to 10-cm layer and to $(93–105) \times 10^9$ cells/g in the 10- to 50-cm layer (Table 1).

The abundance of bacteria in the green part of sphagnum from hillocks was 20×10^9 cells/g. In the sphagnum moss residues, the number of bacteria was twice greater. In the microhighs, downward the vertical gradient, the abundance increased up to the maximum (94×10^9 cells/g) at their base.

The comparison of the number and distribution of bacteria in the microhighs and flat areas showed that they were similar. The differences in these parameters were manifested only in the sphagnum sod. In the microhighs, the density of bacterial populations in the green part and residues of sphagnum was 3 and 4 times higher, respectively, than their density in the same parts of moss from the flat areas.

These data were in agreement with the elevated CO_2 emission from the microhighs, which 2–5 times exceeded that from the flat areas and microlows [1]. In addition, taking into account that the life of mosses is longer on microhighs than on flat areas, one can say

Table 2. The taxonomic position of dominating genera of proteobacteria in the high moor peat

Genus	Class	Order	Family
Pseudomonas	γ–Proteobacteria	Pseudomonadales	Pseudomonadaceae
Acinetobacter	"	"	Moraxellaceae
Dyella	"	"	Xanthomonadaceae
Burkholderia	β–Proteobacteria	Burkholderiales	Burkholderiaceae

that the microhighs are more stable in time. All this along with the higher oxygen concentration in the microhighs compared to that in the flat areas testifies to the favorable conditions for the development and functioning of microorganisms in the hillocks. To talk about the various degrees of peat decomposition on different elements of the microrelief in the high moor peatland is almost impossible taking into account the low rate of high-moor peat decomposition as a whole.

In all the samples studied, actinomycetal mycelium occurred, which is uncommon for the prokaryote complex of high moors. Evidently, the sampling was made in the period favorable for the development of actinomycetal mycelium. Just as for bacteria, the higher density of actinomycetal mycelium was revealed for sphagnum growing on the hillocks but not in the flat areas. In the 10- to 50-cm layer of the flat area, the prokaryote mycelium was somewhat longer in the microhighs.

The total prokaryote biomass in the investigated samples varied from 0.1 to 2.2 mg/g of substrate. In the hillocks, the biomass of the sphagnum green parts was 0.4 mg/g and the mass of its residues was 0.8 mg/g, which was 4 times greater than in the sphagnum on the flat areas. In the other layers of all the objects, the prokaryote biomass varied within the narrow range—2–3 mg/g. Bacteria predominated in the biomass of all the samples. The share of actinomycetal mycelium was not more than 5%.

The number of bacteria in the saprotrophic complex ((2–4) × 10^6 CFU/g of substrate) of the microhighs determined by inoculation was higher by 1–2 orders of magnitude than their abundance on the flat areas.

Proteobacteria predominated in the bacterial communities of sphagnum growing on the hillocks. Bacteria of the hydrolytic complex could be found moving downward the hillocks. Bacilli and actinomycetes represented them. Their portion varied from 2 to 20%. On the flat area, under the dominance of proteobacteria, the share of hydrolytics in the 3- to 20-cm layer reached 40%. In the layers of 20–35 and 35–50 cm, bacteria of the hydrolytic complex were not found.

The generic composition of proteobacteria was determined. The dominants among proteobacteria were represented by four genera: *Pseudomonas*, *Acinetobacter, Dyella,* and *Burkholderia*. Representatives of the first three genera are referred to the class of γ-Proteobacteria, the order of Pseudomonadales, but to different families. The *Burkholderia* belong to the β-Proteobacteria class (Table 2). Some of these bacteria (*Pseudomonas, Burkholderia*) are considered as typical representatives of sphagnum bogs; they were mentioned in works of many Russian and foreign specialists. There was no information on isolation of the *Acinetobacter* and *Dyella* bacteria.

Bacteria of the enumerated genera are aerobic and belong to the ecological and trophic group of copiotrophs that are incapable for hydrolysis of complex polymers. However, it should be taken into account that in the sphagnum composition, in addition to complex polymers, there is a large fraction of organic matter readily available for microbes. Easy hydrolysable and water-soluble compounds amount to 42–52%. The share of carbohydrates is 40–80% of the organic mass of plants. Sphagnum moss is composed of many various organic acids (oxalic, malic, succinate, citric and others) [15].

Let us consider the distribution of bacteria from different genera within the profile of the high moor related to the microrelief. Representatives of the *Acinetobacter* genus predominated in the sphagnum

Table 3. The distribution of bacteria from different genera along the profile of the high moor peat

Peat layer, cm	Genus	Share, %
Hillocks		
0–3 (green part of sphagnum)	*Pseudomonas*	70
3–6 (sphagnum residues)	*Acinetobacter*	85
6–10	*Burkholderia*	65
10–20	"	85
20–35	"	88
35–50	"	78
Flat areas		
0–3 (green part of sphagnum)	*Dyella*	59
3–6 (sphagnum residues)	*Burkholderia*	15
3–6 (sphagnum residues)	*Acinetobacter*	7
6–10	"	7
10–20	"	21
20–35	"	31
35–50	"	37

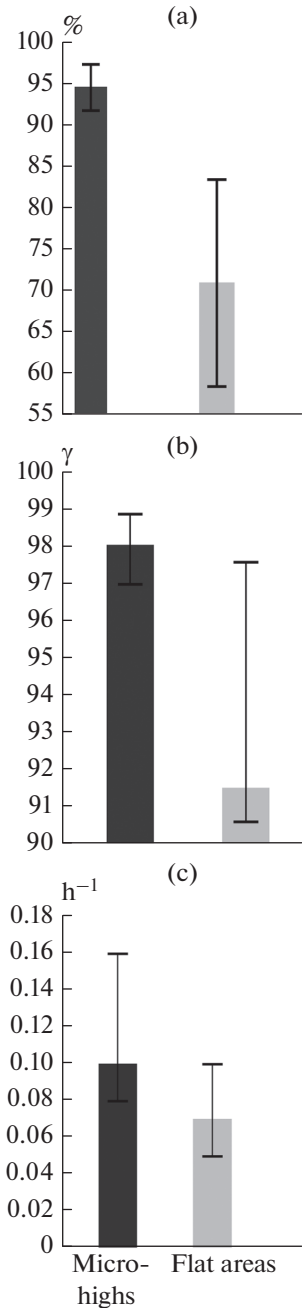

Fig. 1. The physiological diversity of the complex of hydrolytic bacteria (a), metabolic readiness to growth (b), and maximum specific growth rate (c) in members of the bacterial block in situ (in sphagnum residues).

residues on the microhighs; their share on the flat areas increased with depth from 7 to 37%. Representatives of the *Burkholderia* genus were found in the peat layers of the microhighs; their portion was high and accounted for 65–88% of the total number of proteobacteria. In the flat area, these bacteria occurred only in the sphagnum residues; their share did not exceed 15%. Representatives of the *Dyella* and *Pseudomonas* genera were found only on the green parts of sphagnum (Table 3). The taxonomic composition of the isolated proteobacteria on the generic level is considered below. Representatives of the *Pseudomonas, Flavobacterium, Burkholderia*, and *Dyella* genera as endophytic bacteria were also isolated from sphagnum by Shcherbakov [11]. These bacteria form specific microniches within tissues, and this author considers them as characteristic inhabitants of sphagnum gametophytes. It should be noted that some works [18] inform that *Burkholderia* bacteria were found in soils, including peat ones, and in the mycosphere colonized by the saprotrophic microscopic sp. fungus. The *Burkholderia terrae* BS001 strain was isolated among few bacteria capable for migration on the growing mycelium of *Lyophyllum* sp. Karsten. Recently, it was found that this ability to migrate was not limited only to one species, it extends to other species of the *Burkholderia* genus [16]. Thus, considering the great number of fungi in living sphagnum and its residues, one can suggest positive interactions between fungi and bacteria of the *Burkholderia*, and *Dyella* genera isolated from the peat soils. The authors [18] studying these interactions noted the absence of antagonistic relations between proteobacteria and fungi. In addition, *Burkholderia terrae* bacteria associated with fungi were found to protect fungi against antagonistic agents.

The more diverse physiologically bacterial hydrolytic complex (its growth was noted on all the polymers) was revealed in sphagnum of the hillocks (figure). In the sphagnum samples from the flat areas, the growth of bacteria on difficultly decomposable polymers—chitin and cellulose, as well as on insulin (a reserve carbohydrate mainly in Compositae plants) and keratin (a difficultly decomposable animal protein) was not registered. The obtained data testify to the potential ability of the bacterial hydrolytic complex to use a wider spectrum of polymers in the microhighs than on the flat areas. This may be related to the fact that microorganisms dwelling in hillocks contact various polymers of higher plants, including herbaceous ones, which contain cellulose and chitin in their cells. From the cells with media containing cellulose and chitin, we succeeded to isolate bacteria in pure cultures and to identify their genus. Representatives of the genus predominated on cellulose; those of *Chitinophaga* were dominants on chitin.

The complex method applied showed that the metabolic readiness of bacteria to the consumption of polymers *in situ* and the share of bacteria—*r-strategists* was higher in sphagnum of the microhighs that in sphagnum from the flat areas.

CONCLUSIONS

The indices of the prokaryote complex abundance determined by the direct fluorescence—microscopic method were 3–4 times higher in the sphagnum mats than in the flat areas. The number of bacteria in the

hillocks detected by plating was also greater by two orders of magnitude.

In the high moor peat, proteobacteria predominated. Among them, copiotrophs and oligotrophs were dominants. These bacteria are incapable for hydrolysis of complex polymers, but they grow on sugars, organic acids, and amino acids.

The complex structural–functional method used allowed establishing that the bacterial communities of sphagnum growing in hillocks were more adapted to the use of complex polymers (chitin, cellulose, keratin).

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Translated by L. Kholopova

SPELL: 1. inulin