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EXPERIMENTAL ARTICLES

The Structure of Bacterial Complexes in the Protva River Floodplain

A. V. Golovchenko[1], T. G. Dobrovol'skaya[2], M. S. Fedoritenko[2], N. G. Dobrovol'skaya[3], and D. G. Zvyagintsev[2]

[1] *Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr. 33, Moscow, 117071 Russia*

[2] *Faculty of Soil Science, Moscow State University, Vorob'evy gory, Moscow, 119899 Russia*

[3] *Faculty of Geography, Moscow State University, Vorob'evy gory, Moscow, 119899 Russia*

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Abstract—The synecological analysis of bacterial communities from the Protva River floodplain biogeocenosis showed that all of their horizons contain spirilla, which are typical hydrobionts, and pigmented coryneform bacteria associated with the herbaceous plants of the floodplain meadows. The alluvial meadow soils of the inundated regions of the floodplain differ from the unflooded regions of the floodplain in that they have a more diverse bacterial population that is continuously distributed over the soil profile.

Key words: structure of bacterial complexes, floodplain biogeocenosis, monitoring, synecological indices.

River floodplains make up as little as 3% of the land, but their importance to human life is so great that they have been the subject of investigation by geographers, geologists, botanists, soil scientists, and other researchers for more than a century [1]. As G.V. Dobrovol'skii wrote, "River floodplain landscapes are densely populated and have high activities of geochemical and soil forming processes. This implies the enhanced biological recycling of chemical elements, the metabolic activity of soil-inhabiting animals and microorganisms, the high intensity of various chemical and biochemical processes, and the natural fertility of floodplain soils" [2]. The sustainable use of floodplain soils requires the combined efforts of various scientists, including microbiologists. Most of the relevant microbiological studies were performed between the 1960s and the 1980s, with emphasis on the numerical composition of soil bacterial communities at the level of physiological groups and genera [3–8].

The present work was aimed at studying the taxonomic composition of saprotrophic bacterial complexes in the floodplain soil horizons with the use of synecological indices.

MATERIALS AND METHODS

The study was performed at the Borovsk Training and Research Station of the Moscow State University in the Kaluga region, which has a moderate continental climate with an average annual precipitation of about 600 mm, a January average temperature of –10.3°C, and a July average temperature of 17.5°C.

The areas of the Protva River floodplain under investigation were a low floodplain inundated during the periods of flooding and a high, unflooded part of the river bank. These areas of the floodplain represent alluvial soils covered with grass–forb–legume meadows. The humic horizons of the soils are high in the organic carbon content and have neutral pH.

Samples were collected in summer, winter, and autumn. Substrates were analyzed in the following spatial and successional series: the leaves, stalks, and flowers of live herbaceous plants; their dead parts; plant waste; and soil horizons to a depth of 1 m.

The samples in amounts of 1 g were placed in flasks with 100 ml of distilled water and suspended using a UZDN-1 ultrasonic disintegrator (22 kHz; 0.44 A; 2-min exposure). The bacteria were enumerated using the agar medium with glucose, peptone, and yeast extract described by Dobrovol'skaya *et al.* [9]. Agar plates were inoculated, in 5 replicates, with the appropriate dilutions of analyzed soil suspensions and incubated at 20°C for 2–3 weeks. Then, the abundance of particular taxonomic bacterial groups was determined as the number of colonies of the particular morphotype expressed as colony-forming units (CFU) per 1 g of soil. Three to five species from each morphotype of the colonies were isolated in pure cultures and identified at a generic level based on their morphological, cultural, and chemotaxonomic characteristics as recommended in the handbook [10].

All the results presented in this paper refer to the group of saprotrophic aerobic and facultatively anaero-

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bic bacteria able to grow on the aforementioned glucose–peptone–yeast extract medium.

The occurrence rate of a given taxonomic bacterial group was defined as the ratio of the number of samples in which the members of this group were detected to the total number of analyzed samples. The domination rate of the bacterial group was defined as the ratio of the number of samples in which the relative abundance of this group made up more than 30% to the total number of analyzed samples.

To quantify the groups of depolymerizing bacteria, soil dilutions were inoculated into media containing carboxymethylcellulose (CMC) (to detect cellulolytic bacteria), starch (to detect bacteria with amylolytic activity), egg yolk (to detect bacteria with lecithinase, lipase, and protease activities), proteobacteria (to detect bacteria with bacteriolytic activity), and yeast (to detect bacteria capable of the yeast lysis) [11]. For this purpose, the $1 : 10^1$ to $1 : 10^6$ soil dilutions (each taken in 6 replicates) were placed, in an amount of 0.1 ml, in the replicator wells containing agar media with different polymeric substrates. The most likely values of the population density of particular bacterial groups were calculated knowing the volume of the added soil dilution and the degree of these dilutions. To determine the cellulase activity of colonies grown on the CMC-containing agar placed in the replicator wells, they were filled with a solution of Congo Red. The reaction was considered to be positive if the colorless zones of the CMC hydrolysis were observed around the colonies against the background of the CMC-containing agar, which turned red as a result of staining with Congo Red [12]. The ability of bacteria to hydrolyze starch, lipids, proteins, and lecithin, and to lyse bacterial and yeast cells was evaluated by the routine methods [13].

Specimens for microscopy were prepared from the colonies grown on the soil replicas.

The pure cultures obtained with the use of microscopy were identified as in the case of those that were obtained with the culture method.

RESULTS AND DISCUSSION

Factorial analysis with two variables (the season of sampling and the type of substrate) showed that the bacterial population of the floodplain biogeocenosis (BGC) depends both on the position of the analyzed sample in the spatial–successional series (i.e., on the type of substrate) and on the season of sampling (either summer or autumn) (Table 1).

Depending on the position of the sample in a vertical profile from the green parts of plants to the mineral soil horizons, the bacterial population density of the floodplain BGC varied from 10^6 CFU/g sample in the BC horizon to 10^{10} CFU/g sample in plant waste (Fig. 1). Therefore, the difference in the bacterial population densities of the upper and lower horizons of the alluvial meadow soils reaches 4 orders of magnitude. Such vertical distribution of saprotrophic bacteria is typical of most soils analyzed by the culture technique [14].

Table 1. Effect of (1) the type of substrate and (2) sampling season on the population of saprotrophic bacteria in the floodplain biogeocenosis according to the data of factorial analysis

Variation of	The number of degrees of freedom	Variance	Fisher criterion value	Significance level
1	6	3.17	7.36	0.0002
2	1	5.46	12.69	0.0018
1 + 2	6	3.33	7.74	0.0002
Random	21	0.43		

Seasonal variations in the bacterial population of the plant phyllosphere were wider than in the population of soil horizons. For instance, the bacterial population on all parts of plants was 1 to 2 orders of magnitude denser in autumn than in summer, while the bacterial population of the A1 soil horizon in summer was only one order of magnitude greater than in autumn.

The bacterial population of the upper humic horizons of the floodplain soils turned out to be one order of magnitude larger in winter than in summer, while such seasonal variations were not observed in the mineral soil horizons. According to Zvyagintsev, the elevated content of bacteria in frozen soils is explained by a better desorption of bacterial cells from the frozen soil particles [15]. Alternatively, Rakhno *et al.* [16] explained this fact by the breaking of bacterial cells and the slowing down of their death rate in winter.

The Shannon index of bacterial diversity was found to be dependent on the type of substrate but not on the season of the sampling. The Shannon index increased in the direction from the phyllosphere to the rhizosphere. Namely, it decreased from a value of 2.9 in the

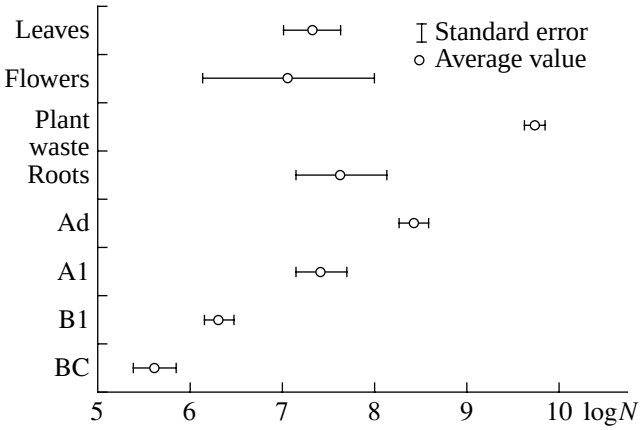

Fig. 1. The average number of bacteria, *N*, on various substrates in the floodplain biogeocenosis. *N* is expressed in CFU/g sample.

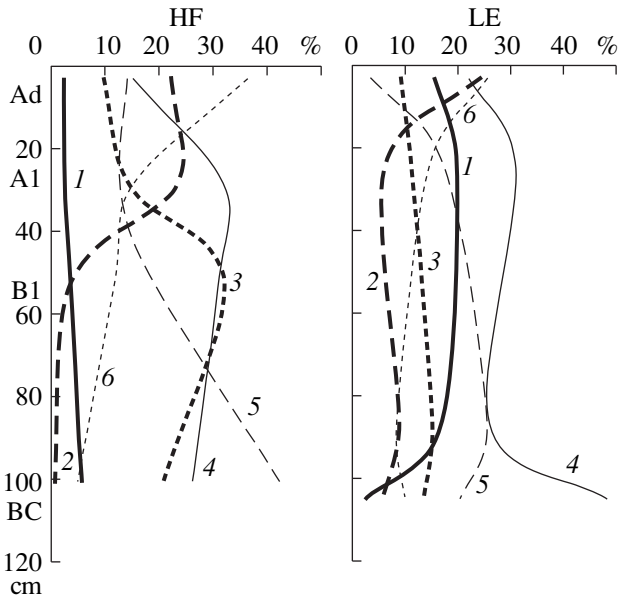

Fig. 2. Vertical distribution of different bacterial taxa in the high floodplain (HF) and low floodplain (LF) soils: *1*, spirilla; *2*, gliding bacteria (myxobacteria and cytophages); *3*, bacilli; *4*, streptomycetes; *5*, arthrobacters; and *6*, other coryneform bacteria.

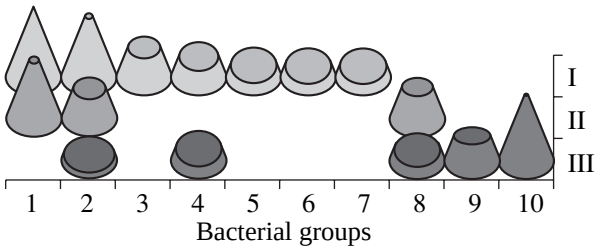

Fig. 3. The domination rate of different bacterial taxa in the (I) phyllosphere, (II) rhizosphere, and (III) soil of the floodplain biogeocenosis: (1) yellow coryneform bacteria; (2) myxobacteria; (3) rhodococci; (4) spirilla; (5) cytophages–flavobacteria; (6) azospirilla; (7) other gram-negative bacteria; (8) arthrobacters; (9) bacilli; and (10) streptomycetes.

Ad turf horizon to 2.2 in the B horizon and 1.8 in the BC horizon.

The bacterial diversity was greater in the inundated regions of the floodplain, except for the turfs of the inundated and unflooded regions where the index was the same (and maximum).

The distribution of bacterial groups over the soil profile greatly differed in these two regions of the floodplain (Fig. 2). In the high floodplain soils, the bacterial distribution was discrete. The fraction of bacteria of the genera *Arthrobacter*, *Bacillus*, and *Streptomyces*, which are typical pedobionts, increased with depth, while the fraction of the litter bacteria (gliding and pigmented coryneform bacteria) decreased with depth. The relative abundance of spirilla, which are typical hydrobionts, was low and varied little over the soil profile. It should be noted that the vertical distribution of bacteria in the watershed soils, which, like the high floodplain soils, are not subject to flood, is similar.

In the low floodplain soils, bacteria were distributed more uniformly, which was probably due to the washing of bacteria from plants and upper soil horizons with the flood water and their penetration into lower soil horizons. The content of spirilla in the inundated floodplain soils was high. The relatively uniform vertical distribution of most bacteria and the high content of spirilla are also typical of peat soils. In the latter case, however, such bacterial distribution is primarily due to the organogenic nature of peats [17], while in the alluvial soils, which are regularly inundated and are well permeable to water, it is primarily due to the easy downward penetration of bacteria.

Seasonal variations in the composition of bacterial communities in the inundated regions of the floodplain soils were low. In the high floodplain soils, however, some taxonomic groups of bacteria were subject to seasonal changes: (1) arthrobacters dominated in summer (up to 38% of the total bacterial population); (2) the hydrolytic bacilli, myxobacteria, and cytophages dominated in autumn; (3) spirilla were detected only in the samples that were saturated in winter, when the high floodplain soils were saturated due to heavy rains in autumn and snow in winter; and (4) the relative abundance of streptomycetes increased from 22% in summer to 38% in winter.

Analysis with the use of synecological indices (the occurrence and the domination rates of bacterial groups) allowed us to reveal the general and differentiating taxonomic characteristics of bacterial communities inhabiting the different horizons of the floodplain (Table 2).

General characteristics. Pigmented coryneform bacteria and myxobacteria were usually associated with all the substrates of the floodplain BGC.

Differentiating characteristics. The plant phyllosphere was dominated by brightly colored gliding and coryneform bacteria, whereas spirilla and azospirilla were detected in 50% of samples analyzed. Plant waste and roots were intermediate between the phyllosphere and soil horizons in the occurrence rate of various bacterial groups, the plant waste being closer to plant substrates in the value of this diversity index, whereas the roots being closer to the soil horizons. The occurrence rate of various bacterial groups was maximum in the Ad and A1 humic soil horizons, where both the phyllosphere bacteria and the typical pedobionts (streptomycetes, bacilli, and arthrobacters) were detected. The mineral horizons of the floodplain soils were distinguished by the presence of azotobacters and a low occurrence rate of gliding bacteria and pigmented coryneform bacteria.

Table 2. The occurrence rate (OR) of various bacterial groups on different substrates in the floodplain biogeocenosis

Substrate	Spirilla	Myxobacteria	Cytophages–flavobacteria	Azotobacters	Azospirilla	Bacilli	Streptomycetes	Arthrobacters	Yellow coryneform bacteria	Rhodococci
Leaves and stalks	II	I	I	IV	II	IV	III	IV	I	I
Flowers	III	I	II	IV	II	IV	III	II	I	III
Plant waste	I	I	I	IV	IV	IV	III	IV	I	III
Rhizosphere	IV	I	I	IV	IV	II	I	I	I	II
Ad	I	I	I	IV	III	I	I	II	I	I
A1	I	I	II	IV	IV	I	I	I	I	I
B1C	I	II	III	II	IV	I	I	I	II	I

I
II
III
IV

Note: I, OR = 75–100%; II, OR = 33–67%; III, OR = 8–17%; and IV, OR = 0%.

The domination rate turned out to be a more informative synecological index than the occurrence rate (Fig. 3). The spectrum of dominant bacterial groups was the widest in the phyllosphere, where the representatives of seven taxonomic bacterial groups were detected, among them pigmented coryneform bacteria of the genera *Arthrobacter*, *Cellulomonas, Promicromonospora*, and *Rhodococcus* and myxobacteria. The rhizosphere was dominated by three bacterial groups: yellow coryneform bacteria and myxobacteria (also typical of the phyllosphere) and arthrobacters (also typical of mineral soil horizons). It should be noted that the soil bacterial complex included five bacterial taxa, dominated by bacilli and streptomycetes, in comparison with seven taxa detected in the epiphytic bacterial complex.

Since the floodplain ecosystems border on aquatic ecosystems, the latter were also analyzed. The phyllosphere and the rhizosphere of aquatic plants, river water, and bottom sediments were found to be dominated by spirilla in winter and gliding bacteria (cytophages and myxobacteria) in autumn. The fraction of other bacterial groups did not exceed 20% of the total. In the river water, bacterial diversity was considerably narrower than that on aquatic plants and in bottom sediments.

The facultatively anaerobic bacteria of the family *Vibrionaceae* were dominant on the leaves and stalks of aquatic plants, subdominant on plant roots, and minor in the bottom sediments. The populations of spirilla and rhodococci on aquatic plants were greater than on the plants of the floodplain BGC. The relative abundance of benthic bacteria (myxobacteria and cytophages) in the bottom sediments was as high as 50% of the total number of the detected bacterial taxa.

Thus, the bacterial complexes of aquatic plants, river water, and bottom sediments differ from the bacterial complexes of the floodplain plants and soils in that they exhibit lower bacterial diversity, a higher abundance of spirilla, and the presence of facultatively anaerobic proteobacteria.

To evaluate the population of hydrolytic bacteria in the humic horizons of the floodplain soils, we attempted to detect bacteria able to degrade lecithin, Tween, protein, starch, and CMC and to lyse yeast and bacterial cells.

In autumn, bacteria with the protease, lipase, and lecithinase activities were dominant in the Ad (10^6 CFU/g) and the A1 (10^4 CFU/g) soil horizons. Microscopic analysis showed that these bacteria are the spore-forming bacilli *Bac. cereus* and *Bac. cereus* var. *mycoides*. The second abundant bacterial group included gliding myxobacteria and cytophages with amylolytic activity and the third abundant bacterial group represented streptomycetes. Myxobacteria, bacilli, and streptomycetes with the ability to hydrolyze CMC and to lyse bacterial and yeast cells were the least abundant: their number varied from 10^3 CFU/g in the A1 horizon to 10^4 CFU/g in the Ad horizon (Fig. 4).

In winter, the population density of hydrolytic bacteria, including those with the CMCase activity, increased by a factor of 3–5 in the turf horizon and by a factor of 2–3 in the humus-accumulating horizons. At the same time, the population density of amylolytic and lipolytic bacteria in winter decreased by two orders of magnitude in the Ad horizon and by 5 times in the A1 horizon in comparison with autumn, when the population of these bacteria was at a maximum.

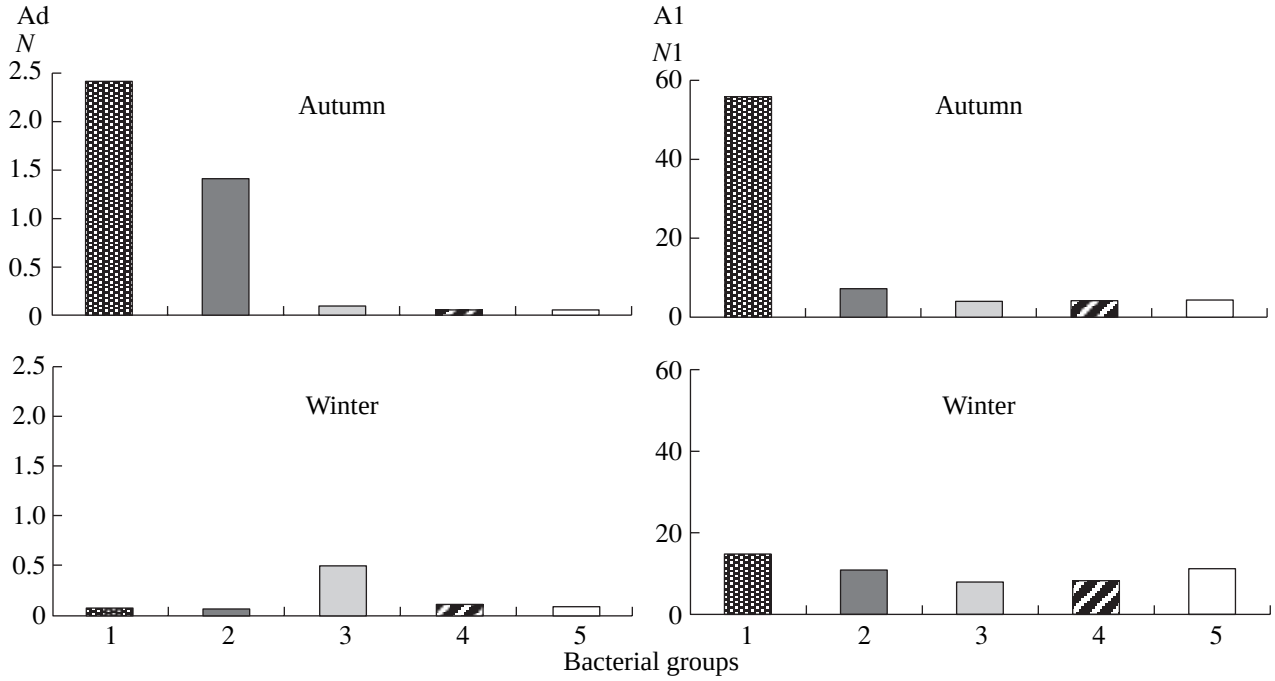

Fig. 4. The number of hydrolytic bacteria in the humic horizons of the floodplain soils: (1) proteolytic and lipolytic bacteria; (2) amylolytic bacteria; (3) bacteria able to lyse yeast cells; (4) bacteriolytic microorganisms; and (5) cellulolytic bacteria. *N* and *N1* are expressed in 10^6 and 10^3 CFU/g sample, respectively.

Thus, analysis of the synecological indices, such as the total number of bacteria and their mean diversity, as well as the relative abundance, the occurrence rate, the domination rate, and the spatial and temporal variabilities of different taxonomic bacterial groups, allowed us to reveal both the general characteristics of the floodplain bacterial complexes and their differentiating characteristics.

The general characteristics of the bacterial complexes of the floodplain BGC are as follows: (1) the maximum population of bacteria in plant waste; (2) the maximum bacterial diversity in the floodplain turf; and (3) the presence of spirilla (typical hydrobionts) and pigmented coryneform bacteria associated with herbaceous plants in all floodplain horizons.

The bacterial communities of the low floodplain differ from those of the high floodplain in exhibiting (1) a higher value of the Shannon index, (2) a higher fraction of hydrobionts, (3) a continuous distribution of particular taxonomic groups of bacteria, and (4) seasonal variations in the taxonomic composition of bacterial complexes (in the high, unflooded floodplain regions, seasonal variations were observed only for some bacterial groups).

The vertical distribution of bacterial groups in the floodplain does not differ from that in forests: the bacterial population density increases in the direction from the plant phyllosphere to the soil horizons in which the digestion of plant residues occurs (plant waste, turf, and litter) and then decreases in a downward direction due to the diminishing content of plant roots and organic matter in the soil.

It should be noted that the bacterial population of the phyllosphere of the floodplain BGC is the same as that of marshy BGC [17] and is an order of magnitude smaller than that of the forest BGC [18]. The turf horizon of alluvial soils is less populated by bacteria than the forest litter formed both mesomorphically and hydromorphically [17–19].

Like forest and marshy biotopes [17, 18], the floodplain biotope exhibits seasonal variations in the bacterial population of its horizons. The bacterial population is maximum in autumn, due to the fall and decomposition of plant residues. During the autumn period, the taxonomic structure of the floodplain bacterial complexes changes toward the prevalence of hydrolytic bacteria (myxobacteria, cytophages, streptomycetes, bacilli, and cellulomonads). Seasonal changes are most pronounced in the high, unflooded regions of the floodplain, whereas in the low floodplain soils, seasonal changes are not well pronounced.

As opposed to the forest and marshy biogeocenoses, where continuous bacterial distribution is due to proteobacteria, the uniform vertical distribution of bacteria in the floodplain biotope is due to pigmented coryneform bacteria, whose relative number is more than 30% in the phytosphere, more than 20% in the turf and plant waste, and more than 10% in the soil horizons. It should be noted that the occurrence rate of these bacteria is very high (75–100%) in all the floodplain horizons

except for illuvial soil horizons, where their occurrence rate is from 30 to 67%.

A comparison of the hydrolytic bacterial complexes of white podzolic, peaty, and soddy gley soils [20] with those of the floodplain soils showed a similarity of the soddy gley and the floodplain soils in the relative abundance and taxonomic composition of bacteria involved in the decomposition of plant polymers. The similarity is obviously due to the close conditions of the formation of these two types of soils, both of which are formed on carbonate rocks beneath a thick herbaceous cover. The optimum conditions that are created in the humic horizons of soddy gleisolic and floodplain soils promote the development of hydrolytic bacteria and, hence, the bacterial degradation of plant polymers.

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