

ON THE CENTENNIAL ANNIVERSARY
OF THE BIRTH OF G.V. DOBROVOL'SKII

The Role of Microorganisms in the Ecological Functions of Soils

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Received March 18, 2015

Abstract—The results of long-term investigations performed by researchers from the Department of Soil Biology at the Faculty of Soil Science of Moscow State University into one of the major functions of soil microorganisms—sustenance of the turnover of matter and energy in the biosphere—are discussed. Data on the population densities of soil microbes and on the microbial biomass in different types of soils are presented. The systemic approach has been applied to study the structural-functional organization of the soil microbial communities. The role of eukaryotic and prokaryotic microorganisms in the carbon and nitrogen cycles is elucidated. It is argued that the high population density and diversity of microorganisms are necessary to maintain the turnover of chemical elements in terrestrial ecosystems. The viability of microbes stored in the soils is important. New data on the preservation and survival of bacteria in nanoforms are presented. It is shown that peatlands and paleosols are natural banks, where microbes can be preserved in a viable state for tens of thousands years.

Keywords: microbial biomass, microbial diversity, nanoforms, pool of microorganisms, structural-functional arrangement, environmental functions, bacteria, fungi

DOI: 10.1134/S1064229315090033

INTRODUCTION

“Soil systems are complex multicomponent formations with the high spatial and temporal variability, so the study of their life and functioning is a labor-consuming problem; to gain success, cooperative efforts of many like-minded specialists are required” [9]. Soil microbiologists surely belong to these like-minded specialists, because one of the central problems of ecology—the organization and stability of biological systems—cannot be solved without the data on the numbers and structural-functional organization of soil microbial communities. It should be remembered that up to 60–90% of the biomass on our planet is composed by microorganisms that mainly dwell in soils.

THE POOL AND STRUCTURE OF MICROBIAL BIOMASS IN THE SOILS

Estimate of population density of soil microorganisms is of primary importance for understanding the microbiological processes proceeding in the soil. The concepts of population density and biomass of microorganisms changed as new advanced methods were developed. Forest litter, sod, and upper humus horizons, where population density of bacteria is 5–10 times greater than their population density in mineral horizons, are biotopes with maximal concentration of bacterial cells in many studied soils. Biomass reserves and structure were calculated on the basis of obtained data on the population densities of the main groups of microorganisms (table). Maximum biomass was recorded in the horizons, where population den-

Stores of microbial biomass (kg/ha (%)) in some zonal soil types

Soil	Total biomass	Bacteria	Mycelium		Spores of fungi
			actinomycetic	fungal	
Gray forest	5670	661 (11.6)	38 (0.8)	3127 (55.1)	1845 (32.5)
Soddy-podzolic	13946	195 (1.4)	27 (0.2)	11914 (85.4)	1811 (13.0)
Ordinary chernozem	15123	421 (2.8)	66 (0.4)	13282 (87.8)	1353 (9.0)
Chestnut	31830	280 (0.8)	41 (0.1)	28689 (90.1)	2821 (8.9)

sity of fungi was great (as in chernozem or chestnut soil), the thickness of soil horizon B2 was great (as in gray forest or soddy-podzolic soil), or both factors were combined as in mountain cinnamonic soil. Prokaryotic biomass in most soil types comprises no more than 3%, excluding gray forest soil, in which the portion of prokaryotes was 4 times greater (12%). The values of total microbial biomass vary from several tons (in gray forest soil) to tens of tons (in chernozem, soddy-podzolic, and chestnut soil). Most biomass falls on the fungal mycelium (55–90%), and only 8 to 33% are presented by spores [28, 29].

Hence, the soil microbial community is absolutely dominated by fungi in the biomass, and this is the group which fulfills the destruction of plant material. It is borne in a commonly found deficiency of fixed nitrogen in soil despite a great amount of nitrogen-fixing bacteria. Fungi do the main share of the work on plant residues destruction, but they do not fix nitrogen.

STRUCTURAL-FUNCTIONAL ARRANGEMENT OF SOIL MICROBIAL COMMUNITIES

Systemic approach is required to understand and assess how microbial communities are arranged in space and in time. Such an approach was developed by the staff of Soil Biology Department [6, 15]. It includes vertical-layer, successional, locus-oriented, and geographical methodical procedures for studying the structural-functional arrangement of microbial communities in terrestrial ecosystems.

Using the discussed approaches for studying the ecology of natural microbial communities allowed the revealing of some particular regularities in the structural-functional arrangement of soil microbial communities. It was demonstrated that microorganisms are found in all layers of terrestrial ecosystems; their distribution is nonuniform; both population density and taxonomic composition of microbial communities vary from layer to layer; the position of an organism in the vertical line is not accidental, as this position correlates with its functions and type of ecological strategy. The main spheres of microbial life in different geographical zones are concentrated in different layers, and the set of dominants in these layers characterizes the type of ecosystem and reflects the specific features of a natural zone.

Vertical-layer and geographical approaches were developed most thoroughly by the example of yeast fungi, for which especially abundant data were accumulated on their distribution in biogeocenoses of different natural zones. It was shown that the structure of yeast communities varied significantly in the series of substrates that corresponded to different stages of plant residue destruction, as well as along the latitudinal gradient. The availability of simple sugars decreases in the course of plant residue destruction, so the environment becomes more and more extreme for

yeasts as typical copiotrophs. This is reflected in the decrease of their total abundance and species diversity. At the same time, the ratio between the relative abundances of dominating eurybiontic species and greater taxonomic groups significantly changes in the zonal series. In addition to the above, relationships are found between particular adaptive features of yeast fungi and the most important environmental factors: frequencies of thermotolerant species increases from the north to the south, and frequencies of psychrophilic species increases from the south to the north; polytropy of soil yeast communities is higher in high latitudes, where the degree of mineralization of organic matter is low; the species widely distributed in the areas of climatic pessimum demonstrate the trend towards the formation of resting structures.

Latitudinal-zonal trends were found almost for all integral parameters of the structure of yeast communities in soils and on plant substrates. Mean values of total population density of yeasts, species diversity, and parameters of ecological structure of yeast communities vary, essentially depending on the latitude. These are trends, i.e. regular increase or decrease in wide latitudinal ranges, rather than significant changes. Most parameters of yeast population density and diversity vary in latitudinal range in the same way: they give maximum values most often in the boreal zone and the decrease in the areas of climatic pessimum, i.e. in Arctic and in deserts, demonstrating the law of optimum. In other words, yeasts at large have the same stance towards global environmental factors as many other large taxonomic and ecological groups of plants and animals. Hence, yeasts being a very small and specialized group of fungi can be a representative and efficient model to study fundamental problems of synecology and biogeography.

Specific loci—the habitats of peculiar microorganisms which are not practically found in such “background” substrates as plants, plant residues, and soils, representing the vertical-layer or spatial-successional series—exist of course in every type of ecosystem. The examples of such loci can be presented by soil neoformations, excrements and intestines of soil invertebrates, ant nests, algal accretions, etc.

The population density and taxonomic structure of microbial communities vary not only in space, but also in time. Temporal changes in microbial complex, which are described as microbial successions, are not accidental, and are caused not only by external effects. They are determined also by internal events in natural substrates. Biogeocenotic horizons are visual images, “still photographs”, of particular succession stages of microorganisms, formed in the course of plant residues decomposition. The study of microbial succession under stable model conditions enables revealing the dominants at all stages of succession and determining their environmental functions.

All environmental functions of soil microorganisms are so diverse and numerous that they are

included into every of 16 types of soil biogeocenotic functions [15, 16]. We shall discuss in this review one of primary functions of soil microbial communities, the transformation of carbon and nitrogen, being performed by both eukaryotic and prokaryotic microorganisms.

CARBON CYCLE IN THE SOILS

The experiments performed suggest an important part of microbial processes of carbon cycle in the formation of recent atmosphere composition and emission and sink of greenhouse gases in different ecosystems. Gas exchange between soil and atmosphere runs most actively in soils with a high content of organic carbon, which is determined by significant microbial diversity and active process of microbial transformation of CO₂ and CH₄ in soil by corresponding physiological groups of microorganisms.

The data on the intensity of production and adsorption of carbon dioxide and methane in soils of the main bioclimatic zones in the European part of Russia suggest that the microbial potential of absorption of greenhouse gases in zonal soils exceed as a rule the extent of their production.

The increasing flux of greenhouse gases from soils is the result of disturbance of dynamic equilibrium between the processes of their production and absorption under the influence of environmental factors and anthropogenic effects on the soil: application of mineral and organic fertilizers; artificial irrigation and often connected with it destruction of soil aggregates and development of soil salinization; application of crop-protection agents (herbicides); accumulation of heavy metals and radionuclides; acid precipitations and other factors.

Specific character of organic matter oxidation was found in peat soils, where predominant methane production was found, and production of carbon dioxide was the result of ground water table drawdown. Taking into consideration the trend towards land aridization and the increase of the areas subjected to artificial drainage, we can consider that these processes (additionally to known negative environmental results) will promote the increased emission of CO₂ from soil to atmosphere. Maximum emission of CO₂ is observed under moisture close to field moisture capacity (–0.1 atm). The intense emission of carbon dioxide and methane occurs under the changing water regime of soil (drying–moistening).

Aggregate composition of the soil has a significant influence on the carbon cycle: as aggregate size increases, the rate of organic matter decreases. Destruction of soil structure and soil dispersion promote formation of oxidative conditions, increase of CO₂ emission, and the increase of its portion in the flux of greenhouse gases from soil to atmosphere [33].

NITROGEN CYCLE IN SOILS

Not one of the parameters of the soil environmental status is as connected with soil as nitrogen. It is true that appearance in primary (Precambrian) fine earth of such inherent and important function as fertility (not only in the narrow “agronomical” sense, but in much broader general biological sense as essential component for all living beings) was determined by the appearance in its composition of available nitrogen, supposedly in the form of ammonium compounds. Thereafter other nitrogen compounds appeared and were accumulated in the course of their microbiological transformation and, hence, formed the nitrogen pool of soil. So it is not by coincidence that the parameters of activity of the main chains of the nitrogen cycle, namely, nitrogen fixation, nitrification, and denitrification, are widely used for the assessment of the ecological status of soil [10]. According to the present-day ideas, these processes are performed by most microbial populations of the soil and reflect rather completely real (actual, but not potential) physiological-biochemical status. The analyses are carried out using the methods of gas chromatography with high sensitivity and rapidity, allowing analyzing the samples of great volumes (replications), and this allows performing full statistical treatment of the results and judging about ecological status of the soil.

Nitrogen fixation. The greatest paradox of life is that each and every organism on the Earth, being in permanent need of available nitrogen compounds and having no way to lay nitrogen in stock, is in the ocean of molecular nitrogen, which not simply surround, but figuratively impregnates it. Only nitrogen-fixing bacteria (diazotrophs) can provide not only themselves, but the whole biosphere with “biological nitrogen” (in the form of amino acids, amino sugars, proteins, etc.) and, just as important, to lay it in stock, converting into soil organic matter humus and realizing in doing so the fertility, the most important environmental property of soil. Nitrogen fixation and assimilation is comparable in terms of the scale and importance for wildlife only with the other global process, photosynthesis. It was considered for a long time [1] that nitrogen fixation is inherent only to a narrow range of microorganisms (*Azotobacter*, *Clostridium*, and *Rhizobium*). There were attempts to assess the biological activity of soil by their population density, and to use this parameter to characterize the environmental functions of soil. It has been proved by now that most prokaryotic microorganisms can fix nitrogen: eubacteria and archaea, chemolithotrophs, phototrophs and heterotrophs, aerobes, microaerophiles and anaerobes, trichome, budding, and mycelial, Gram-positive, and Gram-negative ones [34]. Moreover, it was found that it is nitrogen fixation, which in the world of prokaryotes is the most widely spread physiological-biochemical property. Eukaryotes, fungi, plants, and animals were excluded with finality from nitrogen fixers. Eukaryotes have no capability for nitrogen fixation

and form with prokaryotes—diazotrophs diverse communities with one common feature, namely, close coupling of the biogeochemical cycles of nitrogen and carbon, which in turn is clearly reflected in the environmental functions of soils populated by these organisms. Maximum productivity of nitrogen fixation is observed in legume—rhizobium symbioses, reaching 350 kg of fixed nitrogen per hectare over a growing period. Symbioses of some nonleguminous plants (alder, sea buckthorn, raspberry, etc.) with actinomycetes from the *Frankia* genus have approximately a similar productivity. The symbioses of cyanobacterium from the *Nostoc* genus and water fern azolla are abundant in tropical and subtropical regions. Associative nitrogen fixation is most widely spread in nature and runs in the rhizosphere and phyllosphere of all plants. Though its productivity is lower and averaged 50–100 kg/ha per season, it plays the key role in the nitrogen budget of most terrestrial ecosystems, and this is reflected in the environmental functions of the soil. The conclusion was drawn on the basis of presented data that nitrogen is not the limiting factor in natural biogeocenoses, because all links of nitrogen biogeochemical cycle are well-balanced. Alternatively, it is strongly disturbed due to regular treatment of soil, crop rotations, and removal of essential amount of nitrogen with yield.

Nitrification. The process of nitrification (oxidation of ammonium compounds into nitrites and nitrates), which is carried out by two fundamentally different groups of microorganisms, autotrophic and heterotrophic nitrifiers, is an important and highly-informative parameter of the ecological status of the soil. The key role in nitrification process was attributed to the first group for a long time, and the activity of heterotrophic nitrifiers as the source of oxidized nitrogen was not even considered. Recently, we succeeded in revealing the leading role of the second group, especially in soils of the podzolic zone, due to new methods for studying the nitrogen fixation (inhibitor analysis). One more type of nitrification, ammonium oxidation at the expense of nitrates under anaerobic conditions (anammox process), was found also recently. Irrespective of mechanisms, the nitrification process is used widely to characterize the ecological status of soil.

Denitrification. In the course of denitrification, oxidized nitrogen compounds turn into nitrous oxide (N_2O), or molecular nitrogen (N_2), and the activities of these processes are used as an integrated parameter of ecological status of the soil. According to recent ideas, denitrification as well as nitrogen fixation is carried out by the representatives of most groups of prokaryotic microorganisms. Formation of nitrous oxide is typical for soils with pH values below seven, structureless, salinized, and contaminated with heavy metals, pesticides, etc. Nitrous oxide (“laughing gas”) is known as one of the main factors of ozone screen destruction in the Earth’s troposphere, and, therefore,

its emission from the soil is closely connected with ecological status of the soil.

THE ROLE OF FUNGI IN ORGANIC MATTER TRANSFORMATION IN SOIL

We understand that under soil fungi there are fungi which are permanently and regularly isolated from soil and from different substrates in the soil. According to the present-day ideas, fungi produce maximal microbial biomass among soil inhabitants. They can comprise 50–90% of total biomass of microorganisms in the soil. Fungi are preserved in soil in the form of spore pool, spores germinate under favorable conditions with germ tubes, and mycelium develops actively. It is the mycelial type of structure and development of fungal colonies in the form of repeated mycelial modules which allows fungi to distribute actively in the soil and colonize different organic substrates. The content of fungi in the litter of moist soils enriched with organic matter can reach a thousand meters of mycelium per gram of substrate [25].

Fungi have the adsorptive type of nutrition, i.e. they can absorb nutrients through the entire surface of their body and have thus the most important effect on the transformation of substances and on soil structure. According to our rough estimate, the area of such surface, for example in soddy-podzolic soil, accounts for several cm^2 per one gram of soil. Soils are peculiar by their great diversity of fungi; representatives of Zygomycota, Glomeromycota, Ascomycota, Basidiomycota, and Mitisporicfungi phyla. The total number of genera or the much fewer species of soil-inhabiting fungi is yet known. The number of cultivated species of fungi can reach thousands by the most conservative estimate [40]. Using modern methods of molecular biology for identification of fungi in soil significantly increases this value at the expense of revealing fungal groups (mostly of basidial fungi), which were not formerly accounted with the cultural methods. Fungi perform diverse functions in soils, namely destruction of organic substances, transformation of mineral elements, and transport of nutrients from soils to plants; they participate in mutualistic symbiosis (mycorrhiza, lichens) and in trophic chains of soil invertebrate animals, in the production of secondary metabolites, detoxification of soils, synthesis of humic acids, structure formation, etc. [39].

Fungi play the key role in the processes of organic substances destruction. They participate in the transformation of the most diverse groups of organic substances in nature: mono- and disaccharides, polysaccharides (hemicellulose, cellulose, starch, and chitin), organic acids, alcohols, wax, paraffins, cyclic compounds (for example, phenols), and complex natural polymers (lignin and humic substances). Different ecological-trophic groups of fungi were classified on the basis of their capability for assimilating particular

organic substances: saccharolytic, cellulose-decomposing, keratinolytic, lignin-decomposing, etc.

In nature, organic substances, namely plant residue falling on the soil surface, are decomposed in the process of succession: successive change of different groups of fungi. Classical pattern of succession during litter destruction looks as follows: development of fungi consuming sugars, then active hydrolytic fungi decomposing cellulose, which can develop in parallel with quickly growing copiotrophs, then slowly growing cellulose-decomposing and lignin-decomposing fungi appear. As the succession runs and the products of organic substances destruction come to the soil, the groups can develop which consume more simple organic substances. Fungi, being satisfied with a little content of available sugars and possessing peculiar kinetic parameters, associate themselves with primary destructors of particular substrate at all stages of destruction. These are oligotrophic fungi, or so called "microflora of dispersion" [13]. The relationship of fungi from different genera and species with certain stages of succession is observed. The representatives of zygomycetes participate actively in the destruction of sugars at the first stages, and ascomycetes participate in cellulose decomposition. The capability for lignin destruction is the uncanny ability of fungal organisms. Only basidial fungi have this ability in nature. Complete destruction of tree residues in nature would be impossible without their participation.

Production of physiologically active substances, which can affect the development of other organisms in the soil, is one of most important environmental functions of fungi. When transforming the organic substrates, fungi can release into the environment antibiotics, toxins, and growth-promoting substances, and can have inhibiting or stimulating effects on soil bacteria, other fungi, invertebrate animals, and plants.

PROKARYOTIC HYDROLYTIC COMPLEXES OF SOIL

Integrated study of hydrolytic prokaryotic complexes formed depending on the structure of biogeocenosis and environmental factors is actively performed at Soil Biology Department [7, 17, 37]. Functional importance of hydrolytic prokaryotic microbial complexes in terrestrial ecosystems was determined on the basis of ecophysiological criteria, and the degree of their tolerance to extreme parameters of environmental factors was determined [23]. Alternate version was developed of fluorescence in situ hybridization (FISH), which provided the possibility to evaluate the population density and the phylogenetic composition of metabolically active bacterial hydrolytic complexes in vertical layers of terrestrial ecosystems. Population density of metabolically active cells of hydrolytic complexes comprises one third of population density of all prokaryotic organisms in above-ground, ground, and soil layers of biogeocenoses. The differences were found

in the phylogenetic structure of metabolically active hydrolytic prokaryotic complexes in spatial-successional series. If the destruction of biopolymers in the above-ground layer is performed mostly by the group of proteobacteria (α - and β -), so the portion of firmicutes and actinobacteria increases in the hydrolytic complexes of all studied soil types. Hence, specific hydrolytic prokaryotic complex is formed in every layer.

New function was found of mycelial forms of actinobacteria in the development of hydrolytic complex. Respiration of the complex in a wide range of values (moisture, organic matter input, succession) can be essentially controlled by actinomycetes, the role of which is determined not so much by their direct hydrolytic activity as by their regulatory function connected apparently with the production of biologically active substances [23].

SOIL AS NATURAL POOL OF MICROORGANISMS

The protective function of soil, i.e. preservation in viable state of different surviving stages of microorganisms, is of great importance for conservation of biodiversity. Soil is also the source of discovering and describing numerous unknown forms of life. For example, the phenomenon of uncultivated microorganisms suggests that true diversity of soil biota is significantly higher than current estimates. Determination of soil conditions promoting the formation and conservation of biodiversity and particularities of soil biota formation in different soil loci is the necessary task of further investigations [38].

Peatlands and paleosols, in which viable microbes are preserved during hundreds, thousands, and even tens of thousands years, are the peculiar long-term pools keeping the microorganisms.

The problem of wetland conservation as the reservoirs for accumulation great amounts of carbon is connected directly with the problem of conservation the microbial diversity on the planet. Recent bogs in Northern Eurasia were formed during the Holocene. The age of the most ancient of them does not exceed 12000 years [36]. If we consider the whole profile of peat deposit, it is instructive to present the data on the age of every horizon determined with the radiocarbon method. For example, it was found in the dynamics of peat accumulation in the Bakchar Bog (southern taiga in West Siberia) that at the oligotrophic stage of development, the layer 0–10 cm has an age of 5 years, 10–38 cm 15 years, 38–52 cm 34 years, and 58–72 cm 318 years. The lowest layer, 150–160 cm, has the age more than 1000 years [30].

The reserves of microbial biomass in peat deposits comprise in the meter-layer several t/ha and tens of t/ha per the whole profile. Fungal component predominates in the structure of microbial biomass in most analyzed peatlands. It is presented by mycelium in the upper layers and mostly by spores of fungi and

yeast-like cells in the lower ones. The portion of not only spores, but also of bacterial cells, becomes high in the lower horizons [2, 3, 7].

The study of a peatbog as of a soil body with a special profile allowed revealing the following regularities in the vertical distribution of microorganisms:

(a) bacteria, actinomycetic mycelium, spores of fungi, and yeast-like cells were found throughout the whole profile. Their population density in the deep layers could be the same or even greater than in the upper ones. Fungal mycelium was found mostly in the upper one-meter layer; its density decreased down the soil profile;

(b) seasonal dynamics of the parameters of microorganisms' abundance was observed not only in the upper, but also in the lower layers of peatbogs, and this brings us to the assumption about the real reproduction of the part of microbial complex at these depths;

(c) all layers of peatlands have the potential activity of nitrogen fixation and denitrification;

(d) microorganisms can be in a viable state in the whole profile of a peatbog, and this is confirmed by their growth on nutrient media when inoculated from different depths down to the parent rock;

(e) bacteria of hydrolytic complex, potentially capable of destructing polymers, were found in all seasons and at all depths of peatlands.

Presented facts demolish the view about the "sterility" of the lower layers in the peat deposit and can be an additional argument for including the term "peat soil" to the whole layer of peat deposit irrespective of its thickness [2].

Conditions unfavorable for fungi in the deep layers of peat deposits block their participation in the processes of destruction of organic substances and promote further accumulation and conservation in the peatlands of plant residues, as well as in microbial biomass.

As aeration down peat deposit becomes worse, fungi give place to bacteria, the density of which can be similar to that in the upper horizons, or can gradually decrease down the profile. At that, bacteria work under relatively narrow and specific conditions. Functions of bacteria in the highmoor peatlands are very diverse. Some of them are performed only by prokaryotes (nitrogen fixation, methane production and oxidation, and sulfate reduction); production of antibiotics, inhibiting growth of pathogenic fungi by bacteria inhabiting the high moors, protects sphagnum mosses from diseases. Transformation of different polysaccharides, sugars, organic acids, and phenols, among others, seems to be the most important functions of microbes. Degradation of toxic phenolic compounds is one of most important functions of bacteria in bog ecosystems, so that functioning of hydrolytic microorganisms becomes possible in some loci [4, 8].

However, low rate of plant residue destruction in bog ecosystems resulting in peat accumulation forces us to think of which factors limit the functioning of

viable microorganisms in deep layers of peat bed. The analysis of these factors is given in a comprehensive monograph [35] and in several papers [3, 7].

The study by the team of scientists headed by D.G. Zvyagintsev and D.A. Gilichinskii showed that microbial communities are preserved for a very long time and maintain their initial features in permafrost of different ages from the Arctic and Antarctic regions [14].

Characteristics of microbial communities in buried paleosols are indicative of the biospheric processes in the earlier stages of the Holocene [5]. Active strains of hydrolytic microorganisms obtained from the ancient soils can be used in biotechnology. In this relation, hydrolytic complexes of soil microbes attract special attention. It was found that fungal communities in the buried Late Pleistocene and Holocene paleosols are represented by their spores rather than by mycelium, and the activation of fungal destroyers is not pronounced [24]. The structure and specific features of microbial complexes in chestnut paleosols under burial mounds of different ages (ca. 4500 and 3500 yrs) and in their recent analogues were studied. It was demonstrated that the potential activity of hydrolytic microbes in the buried paleosols was higher than that in the recent soils. The FISH method showed that the portion of metabolically active cells of prokaryotes in the paleosol (after application of the growth-initiating polysaccharide) reached 50% of the total isolated prokaryotic community. The response of the microbial community of the paleosols to the introduction of polysaccharide was more active than that of the microbial community of the recent soils. The difference in phylogenetic structures of prokaryotic metabolically active hydrolytic complexes of buried and recent soils was determined. Hydrolytic complex in the recent soil is more diverse in comparison with that of paleosols, in which the groups of Actinobacteria (both unicellular and mycelial representatives) and Proteobacteria acted as hydrolytic dominants [41].

Botanical gardens play an important role in preservation of the microbial pool under urban conditions. For example, great diversity was demonstrated for the bacterial complexes in the lower part of the profile of recreazems and culturozems in the territories of botanical gardens. This emphasized the important environmental function of botanical gardens performing preservation not only of plants, but also of bacterial gene pool [31].

NANOFORMS OF BACTERIA AS THE TECHNIQUE OF SURVIVAL AND LONG PRESERVATION IN SOIL

Recent concept on microorganisms' survival in natural environments stipulates their transformation to the so-called "not cultivated, but viable state". The decrease of cell size and high resistance of cells to physical and chemical effects is the distinctive feature

of such state. The decrease of cell size results in the appearance of ultramicroforms of bacteria (nanofoms). The phenomenon of nanotransformation in natural environments (in water and soil), as well as in pure cultures, was found for some bacteria as far back as in the last century [1, 12, 27, 19].

Small prokaryotic organisms with linear size less than 0.2 μm (some authors consider less than 0.4 μm) are classified recently as nanofoms. Other terms are used additionally to describe nanofoms in scientific literature: ultramicrobacteria, nanobacteria, dwarf cells, filterable forms of prokaryotes, *l*-forms [11]. Nanofoms of bacteria have drawn close attention of microbiologists, soil scientists, hydrobiologists, geologists, and medical workers in last the decade because they were found in soil, on the surface of rocks and minerals, and within Mars meteorites [11, 26, 42].

Research performed in Soil Biology Department (2007–2015) provides evidence of wide distribution of nanofoms of bacteria in soils. Population density of nanofoms in studied soils of Russia (soddy-podzolic, gray forest, chernozem, raised peaty bog, and mountain soils of the Caucasus) was very high and comprised tens and hundreds of million cells per gram of soil. The portion of nanofoms of bacteria reached 5–10% of the number of cells accounted with direct microscopic method [21, 22]. The study of prokaryotic communities of cryptogamous wastelands in East Antarctic revealed a significant population density (tens of million cells per 1 g) and portion of nanofoms of bacteria (70–80% of total number of isolated cells) in soils [18]. High population density of nanofoms of bacteria was recorded in urban soils contaminated with petroleum products and heavy metals, up to 100 million cells per 1 g, and their portion reached 15% [20].

Taxonomic position of nanofoms of bacteria from chernozem, mountain meadow soil, highmoor peatland, and some soils of Antarctic was studied with the help of FISH method. Representatives of Archaea (10–16%) and Bacteria (26–35%) domains were found among these forms of bacteria. Gram-negative bacteria from the Proteobacteria (classes Alpha-, Beta-, Gamma-, and Deltaproteobacteria) and Cytophaga phyla dominate the Bacteria domain. The portion of not identified cells from nanofoms of bacteria was greater than that from bacteria of common size (up to 55%), and this attests to the presence among these forms of bacteria of unknown or poorly studied taxa.

Soil loci, iron-manganic nodules, were found in soddy-podzolic and soddy-meadow soils; a significant part of the bacteria there (up to 40%) was presented by nanofoms, and poorly studied Acidobacteria and Planctomycetes phyla were found among them [20–22]. These results allow the assumption that these ultrafine forms of prokaryotes participate in the process of iron–manganic nodules formation. High viability is their distinctive feature. The portion of viable cells is always greater among the nanofoms of bacteria

(75–95%), in comparison with this parameter (60–65%) for bacteria of common size [18, 21, 22]. Electron-microscopic study of nanofoms of bacteria in soils and underlying deposits carried out with the methods of scanning and transmission microscopy also suggests high viability of such cells: the presence of a dividing cell was found; ultrastructural characteristics of some cells were similar to those of dormant forms [32].

Environmental meaning of transition of prokaryotes to the state of nanofoms can be considered as a manifestation of life strategy for the survival of microorganisms upon the occurrence of unfavorable conditions (temperature, moisture, deficiency of life space in soil neoformations, etc.). It is apparent that nanofoms of bacteria represent a significant, but least understood, part of soil bacterial diversity, because there is among them a great portion of not identified cells additionally to the known taxa. The study of true nanobacteria preserving their small size during the whole life cycle deserves special attention.

CONCLUSIONS

Microorganisms perform the long-range and very significant role in the environmental functions of soils. They are critical in climax ecosystems in supporting homeostasis by some chemical and physicochemical parameters. This is achieved owing to the great biomass of microorganisms and their taxonomic diversity. As a rule, microbes can perform processes in two opposite directions, i.e. they always bring the system into the equilibrium state. The reserve dormant pool composed of very diverse microbes is always in soils for greater system stability. These can be nanofoms, spores, not cultivated forms, and latent forms. They pass into active state upon the disturbance of homeostasis. Agriculture with application of high rates of mineral fertilizers and some toxic chemicals causes irreversible disturbance of homeostasis and soil degradation. Every soil type has its own ultimate resistance.

ACKNOWLEDGMENTS

This work was supported by the Russian Scientific Foundation, project no. 14-50-00029.

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Translated by T. Chicheva