ESTIMATION OF DNA QUANTITY IN DIFFERENT GROUPS OF MICROORGANISMS WITHIN GENETIC HORIZONS OF THE DARK-GRAY SOIL

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Molecular-biological methods permitted to study the structure of the microbial community in the profile of dark-gray soil (Luvic Retic Greyzemic Phaeozem) in Kashira district of the Moscow region. Microorganisms playing an important role in transformation of the soil organic matter are mainly concentrated in the topsoil and the major microbiological studies are related to this part of the soil profile. However, the study of the microbial community in the lower soil horizons is not only of theoretical but also practical interest in view of increasing the intensity of erosion processes. The method of quantitative polymerase chain reaction was used to estimate the DNA quantity of bacteria, archaea and micromycetes in horizons of the above soil. The material of mole passage on a depth 80 cm was also studied. The highest DNA quantity of bacteria and archaea was found in the upper humus-accumulative horizon (9.6×10^8 and 9×10^7 copy/g of soil respectively). Its quantity was decreased downwards the profile, what is connected with changes in the physic-chemical conditions of soil. DNA of micromycetes was evenly distributed throughout the soil profile ($5.4–9.4 \times \times 10^7$ copy/g). In the mole passage material the DNA content of different microorganism groups was close to that in lower mineral soil horizons. This may be explained by water infiltration through the mole passage material accompanying by eluviation of microorganisms in the period of soil wetting. The factors affecting the DNA amount of microorganisms are elementary soil processes including the biogenic-accumulative process in the upper soil horizons, clay-illuvial and humus-illuvial processes in the lower horizons of the dark-gray soil.

Keywords: Polymerase chain reaction, profile distribution of microorganisms, bacteria, micromycetes.

INTRODUCTION

According to the State register of soil resources in Russia (2014) the gray and dark-gray forest soils occupy 41 million hectares. This is a zonal soil type developed under broad-leaved forests within the forest-steppe zone. Geographically, they are found in the zone of the moderate continental climate, in which the close ratio between the precipitation and evaporation (the moistening coefficient is about 1) is conducive to use these soils in agriculture. The gray forest soils occupy 11.8% from the total area of agricultural lands and 14.9% of the area under crops. The texture-differentiated profile of soils is developed because of a complicated combination of elementary soil processes embracing the whole profile or its part in dependence on relief and climate [10].The gray soils of natural cenoses are well structured [3]. The dark-gray forest soils reveal intensive humus formation and biogenic accumulation of ashy elements. The input of organic residues to soil located near the southern boundary of the zone where the gray forest soils are widely spread becomes equal to the decomposition rate of leaf debris, thus resulting in the absence of the forest litter. The humus migration in the kind of organomineral compounds leads to increasing the thickness of the organomineral horizon being as a cause for the formation of the second humus horizon. However, there is a hypothesis that the second humus horizon is relic by origin. [5]. Processes of lessive, illitization and claying are favorable for the formation of the eluvial horizon and some peculiar features of morphology in lower horizons.

The activity of microorganisms is closely associated with the intensity of biochemical, oxidation-reduction processes, water and air regimes as well as with the destruction and transformation of minerals. The microbiological processes in forest soils are highly affected by the favorable heat regime and water-physical properties. The temperature $+15^{o}C$ is seldom observed at a depth of 40 cm, $+20^{o}C$ is absent even in surface horizons during the vegetation period. The temperature fluctuation in daily cycle doesn't exceed $15^{o}C$ within the topsoil of the gray forest soil to be favorable for the population of microorganisms and the rate of their metabolism.

The microbial community plays a primary role in soil formation processes and organic matter transformation that is why the great amount of microorganisms is concentrated in the upper part of the soil

profile. Microbiological processes in mineral horizons are not so active. However, the study of the microbial community in the lower part of the profile is not only of theoretical but also practical interest in view of manifesting the erosion processes and mineral horizon exposition on the surface. Moreover, there are data about the uniform distribution of the microbial biomass throughout the profile of gray forest soils [9].

Classical methods of soil microbiology are performed to study the biomass and structure of the microbial community in soil. Among them are such methods as substrate-inducted respiration, fumigation, luminescence microscopy, seeding on nutrient medium [4]. At present, new methods based upon the analysis of DNA microorganisms extracted from the soil are widely adopted. They are rather sensitive and informative. Besides, the uncultivated forms of microorganisms can be taken into account only by molecular-biological methods [8].

This paper is aimed to analyze DNA of bacteria, archaea and micromycetes extracted from genetic horizons of the natural dark-gray soil and to study the dependence of their distribution along the profile on the soil chemical properties.

OBJECTS AND METHODS

The samples of the natural dark-gray forest soil were taken at the territory of experimental station of the Dokuchaev Soil Science Institute in June 2014. (the Moscow region, "Bogoslovskoe". 54°46'37.52"N and 38°01'55.34"E). The vegetation is represented by oak (Quercus robur), linden (*Tilia cordata*), birch (*Betula sp*.), aspen (*Populus tremola*) in the forest as well as by *Corylus avellana, Sorbus aucuparia, Galeobdolon luteum, Glechoma hederacea, Geranium pratense, Aegopodium podagraria* in the grass cover. The description of this soil profile with the second humus horizon is the following.

AU – dark-gray with brownish hue, fresh, heavy loamy; there are layers at a depth of 0–15, 15–30, 30–40 cm.

0–15 cm – friable, crumby-granular, abundant roots, active soil fauna (earthworms, mouses), many coprolites, transition according to structure;

15–30 cm – dark-gray with brownish hue, heterogeneous in density and structure, crumby-powder-like, compacted in some places, zoogenic zones with the coprogenic structure are met, very many fine roots to 2 cm in size, crotovinas, transition according to density and structure;

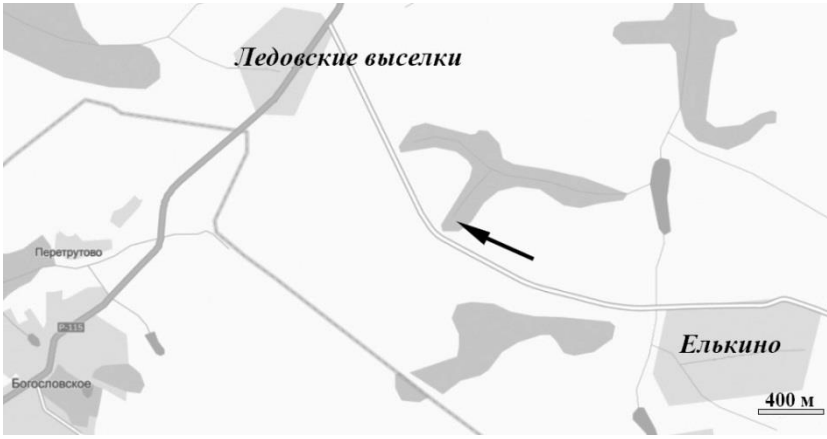

Fig. 1. Location scheme of the gray forest soil profile

30–40 cm – interrupted, dark-gray with brown hue and whitish skeletons, compacted, crumby-powder-like, sometimes angular-platy, transition according to structure, coloring and presence of skeletons.
BEL (hh), 40–55(60) cm – dark-gray, whitish, brown; heavy loamy, dense, porous, abundant earthworms; nutty, platy in some places; there are dark-colored humus cutans and abundant whitish skeletons; the lower boundary is tongued, wavy, transition according to color and structure;
BT1, 55(60)–85 cm – brown with gray clay-humus cutans and rare whitish skeletons, dense, moist, at a depth of 80 cm there is a crotovina filled up by a dark-colored material; nutty-prismatic, prisms of vertical orientation, the structure is identical to that in the BEL horizon;
BT2, 85–110 cm – brown with gray tongues, moist, heavy loamy to clayey, blocky-prismatic, many humus patches, few roots;
BT3, 110–140 cm – identical to the overlying horizon, there are ortsteins, blocky, gradual transition, humus patches along root channels and frost fissures are met;
BC, > 140 cm – dark-brown, dense, heavy loess-like loam.

According to a complex of features the soil is classified as a dark-gray forest soil with the second humus horizon (Classification of Soils in Russia, 2004), in WRB (2014) as Luvic Retic Greyzemic Phaeozem (Loamic).

Soil samples for the microbiological analysis were taken in central part of every genetic horizon. The results of chemical analyses ob-

tained in the laboratory of the V.V. Dokuchaev Soil Science Institute are presented in Table 1.

*Isolation of DNA from soil samples. F*rosen soil samples (0.2 g) were mechanically destructed with the use of glass balls in the following extracting buffer solution: 350 pL of solution A (sodium-phosphate buffer – 200 mM; quanidine isothiocyanate, 240 mM; pH 7.0), 350 pL of solution B (Tris-HCl, 500 mM; SDS, 1 wt. %; pH 7.0) and 400 pL of a mixture of phenol with chloroform (1 : 1). The destruction of samples was performed for 40 s on Precellys 24homogenizer (Berlin Technologies, France) at the maximum power (6500 rpm (680 rad/s) using 3D rotation. The obtained suspension was centrifuged at 16000 rpm for 5 min. The water phase was separated and subjected to repeated extraction with chloroform. Then, the DNA precipitation was performed by adding equal volumes of isopropyl alcohol. After centrifuging the precipitate was washed with 70% ethanol and dissolved in water at 65°C for 5-10 min. The purification of DNA was performed by the method of electrophoresis in 1% agaric gel with further isolation of DNA from the gel by the sorption on silicon oxide [1].

The method of quantitative polymerase chain reaction was used to give a quantitative assessment of DNA in bacteria, archaea and fungi. To detect the concentration of purified DNA, Cycler (Biorad) amplificator was applied with measuring the fluorescence intensity of the reaction mixture in every cycle. The reaction mixture was prepared

Chemical properties of the dark-gray soil with the second humus horizon

Horizon	Depth, cm	Humus	N total	pH		Exchangeble		P mob	K exch
				H_2O	HCl	Ca^{2+}	Mg^{2+}		
		%				meq/100g			
AU	0–15	6.36	0.323	5.49	4.49	9.53	1.56	4.08	20.97
	15–40	3.83	0.185	5.14	3.95	7.98	0.97	4.36	11.55
BEL	40–55(60)	2.88	0.151	5.52	4.35	10.65	1.23	8.86	10.27
BT1	55(60)–85	Not		5.65	4.07	11.12	2.05	10.26	18.95
BT2	85–110	»		5.86	4.07	10.69	2.75	10.26	18.63
BT3	110–140	»		5.99	4.22	12.17	3.74	13.92	23.36

from SuperMix EvaMix EvaMix Biorad (concentrated buffer with desoxyribonucleotides, polymerase Sso7d-fusion, MgCl2, EvaGreen dye and stabilizers). The dependence between the fluorescence intensity and logarithm of DNA concentration in standard solutions was estimated to determine the DNA concentration by means of the software CFX Manager. As a standard for solutions of concentrated fragments were used for bacteria – *Escherichia coli*, for archaea – FG-07 *Halobacterium salinarum* and for fungi – *Saccharomyces cerevisae* Meyen IB-D11606. Then, the DNA concentration of conservative sites in bacteria, archaea (site 16sDNA) and fungi (site 18sDNA) was recalculated into an amount of copies per 1g of soil by the following equation:

$$A = 4500Q,$$

where Q – the DNA concentration in the solution calculated by CFX-Manager; A – amount of copies of the DNA conservative site per 1 g soil; 4500 – calculation coefficient with account of the initial sample and the DNA extract isolated from the soil.

The obtained data permit to draw a conclusion about the population of microorganisms in soil horizons. An amount of target fragments in genomes of bacteria, fungi and archaea is varying, however the distribution of their population in different soil horizons remains unchanged [1].

RESULTS AND DISCUSSION

The bacterial DNA amount is found to be the greatest at a depth of 15 cm in the AU horizon; in the lower part of this horizon it is decreased by 4 times (2.5×10^8), being declined downwards to 8.1×10^7 in the BC horizon (Fig. 2). The identical picture is observed for the DNA amount of archaea; it is ranged from 9×10^7 to 6×10^6.The distribution of its amount in the AU horizon is uneven: the maximum is at a depth of 30-40 cm, what is conditioned by the increased soil heterogeneity within this horizon. The specific feature of the studied soil is the exceeding bacterial DNA amount in the AU horizon in comparison with archaeal and micromycetes' DNA. The French researchers indicated quite another ratio between the DNA amount of fungi and bacteria (about 1 : 1) in clay soils used in agriculture [14]. In the studied soil the amount of microorganisms is sharply decreased downwards the profile. The DNA amount is also declined due to decrease in the organic matter

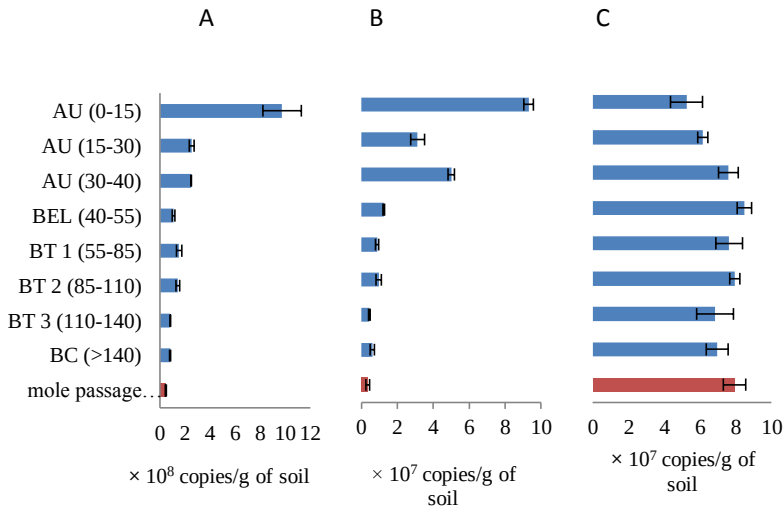

Fig. 2. DNA amount of bacteria (A), archaea (B) and micromycetes (C), its distribution with the depth and in horizons of the dark gray soil.

content and changes in the chemical composition and physical properties. The DNA amount of bacteria and archaea is sharply decreased with the depth. The DNA of microorganisms in lower soil horizons is not always connected with their biological activity; it can be also affected by the inactive extracellular DNA from the upper soil horizons [11]. The DNA of fungi is distributed without any differences between the horizons being varied in the range of 5–9.4 × 10^7. It is known that the main fungi biomass is concentrated in the litter and the topsoil [13]. However, the fungi spores are evenly distributed throughout all the mineral horizons of the gray forest soil [9]. It should be explained by a trophic link between the micromycetes and the root mass of woody plants, the absence of competition for the trophic resources in lower soil horizons and the possible fungi DNA conservation owing to a lower intensity of biological processes.

The DNA amount was also studied in the mole passage material at a depth of 80 cm (BT1 horizon). Despite its morphological similarity with the material of upper horizons (dark-gray color, crumby-granular structure) the DNA amount of bacteria, archaea and fungi is rather low

being equaled to that in lower mineral horizons of the parent material (bacteria – 4.6×10^7, archaea – 3.6×10^6, micromycetes – 7.9×10^7).

Thus, it is possible to speak about a relatively higher content of bacteria and archaea in upper soil horizons and an even distribution of micromycetes along the profile of the studied soil. It was expected that the distribution of the DNA amount (bacteria and arches) in the upper part of the soil profile seems to be similar to that in the content of humus and nitrogen. The upper horizons are characterized by the increased biogenic processes at the expense of the organic matter transformation connected with the activity of microorganisms (mineralization of plant residues, synthesis of humus substances, biogenic accumulation of micro-and macroelements). The lower part of the soil profile reveals an opposite dependence between the DNA amount of arches and the content of exchangeable bases, mobile K_2O and soil-pH conditioned by ecological peculiarities of this group (extreme- and acidofility). The factors affecting the DNA amount of microorganisms in lower mineral and upper organogenic horizons are quite different due to elementary soil processes taking place in genetic horizons of the gray forest soil. In the upper horizons the biogenic-accumulative processes proceed simultaneously including decomposition of plant and animal residues, the humus formation and accumulation of humus substances as well as the humus mineralization and its movement downwards the profile. The formation of lower horizons is conditioned by the clay-illuvial (lessive) and humus-illuvial processes that determine morphological peculiarities of these horizons – the formation of clay and humus cutans at edges of structural particles and the second humus horizon.

CONCLUSION

The methods of molecular biology permitted for the first time to obtain quantitative indices of microorganism population along the profile of the dark-gray soil. The upper part of the humus-accumulative (0–15 cm) AU horizon is the most biogenic and reveals a significant DNA amount of bacteria and archaea (9.6×10^8 and 9×10^7). The DNA of micromycetes is evenly distributed throughout the profile ranging from 5×10^7 to 9.4×10^7. The increased biological activity and the DNA amount of the procaryotic group of microorganisms in upper

horizons are highly dependent on soil-biological and biochemical processes of the organic matter transformation.

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