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EXPERIMENTAL ARTICLES

The Effect of Tillage and Mesorelief on the Structure of Soil Microbial Cenoses

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Abstract—The investigation of soil microbial cenoses in cultivated catenas and in virgin soils at the foot of catenas showed that the structure of these microbiocenoses depends on the type of the vegetation cover, the characteristics of mesorelief, and the degree of soil tillage. The microbiocenoses were found to be dominated by the fungal mycelium. The proportion of bacteria and fungal spores was higher and the seasonal variations in the soil microbial communities were more distinct in the cultivated than in the virgin swamp and forest soils. The type of mesorelief was found to appreciably influence microbial populations in the top humus-rich horizons of the cultivated soils and not in the mineral soil horizons.

Key words: microbial biomass, the structure of microbial biomass, tilled and virgin soils, mesorelief.

The microbial communities of various soils in Russia and the former Soviet Republics have been subject to extensive investigation in recent years at the Department of Soil Biology of the Faculty of Soil Science of the Moscow State University [1–6]. A special emphasis in these studies was placed on the soils of reserves and other natural biotopes (forests, meadows, floodplains, steppes, and marshes) as standards of virgin soils. At the same time, tilled soils are much less studied in this respect [7, 8], although the comparative characteristics of the virgin and tilled soil biotopes are of great theoretical and practical interest. A great deal of accumulated information on the microbiological characteristics of zonal soil types allows the description of the anthropogenic impact on soil microbial communities and the microbiological monitoring of arable lands.

Relief is an important factor that influences soil forming processes and the soil characteristics. However, soil microbiologists deal primarily with the effect of macrorelief on soil characteristics [9–11], whereas the effect of mesorelief is poorly studied [12, 13]. In our recent studies of the effect of soil tillage on the soil microbiota, the influence of the relief was not taken into account [7, 8], although extended flat lands are actually scarce.

The aim of the present work was to study the consequences of the plowing of catenas, which are the typical landscapes of European Russia. Namely, the effect of mesorelief on the microbial communities of natural biotopes and cultivated soddy podzolic and gray forest soils in the Vladimir region was studied.

MATERIALS AND METHODS

Samples were collected during June and September 1997 in the Vladimir region along the north–south horizontal transect of a catena dominated by soddy podzolic soils and along the northeast–southwest transect of another catena dominated by gray forest soils (Fig. 1).

The first catena was dominated by a soddy, heavily podzolized, weakly gleysolic, sandy soil that was underlain by a morainic clay loam. The cultivated soil of the test field from the All-Russia Research Institute of Organic Manures was fertilized with dolomite meal (100 kg/hectare), manure (10 tons/hectare), and $N_{50}P_{25}K_{60}$.

Section 45–97 was located at the foot of the northern slope of the catena with a soddy, weakly podzolized, sandy alluvial soil subject to crop rotation.

Section 46–97 was located in the lower part of the northern slope with a slightly eroded, soddy, heavily podzolized, weakly gleysolic, sandy soil underlain, at a depth of 37 cm, by the morainic clay loam. The soil was subject to crop rotation.

Section 47–97 was located at the watershed plateau of the catena with the soddy, heavily podzolized, weakly gleysolic, sandy soil underlain, at a depth of 58 cm, by the morainic clay loam. The soil was subject to crop rotation.

Section 49–97 was located in the lower part of the southern slope with the slightly eroded, soddy, heavily podzolized, weakly gleysolic, sandy soil underlain, at a depth of 50 cm, by the morainic clay loam. The soil was subject to crop rotation.

Section 50–97 was located at the foot of the southern slope with the soddy, heavily podzolized, sandy alluvial soil subject to crop rotation. The underlying moreinic clay loam was located at a depth of more than 200 cm.

Section 51–97 was located at the slope swampy tail with an alluvial muddy, humus-rich, gleysolic soil underlain by buried peat.

Section 52–97 was located in the lower part of the southern slope covered by a pine forest dominated by true mosses. The soddy podzolic, sandy, illuvial, ferruginous soil was weakly differentiated.

The second catena was dominated by a grayish, weakly gleysolic, moderately loamy, forest soil underlain by a blanket of loam.

Section 53–97 was located at the foot of the southwestern slope of the catena with an alluvial, gray, weakly gleysolic, moderately loamy, forest soil underlain by a blanket of loam. The soil contained the second humus-rich horizon and was subject to crop rotation.

Section 54–97 was located in the lower part of the southwestern slope with a weakly alluvial, grayish, moderately loamy, forest soil underlain by a blanket of loam. The soil was subject to crop rotation.

Section 55–97 was located at the watershed plateau covered by a broad-leaved forest. The gray, moderately loamy, forest soil was underlain by a blanket of loam.

Section 56–97 was located at the watershed plateau with the gray, moderately loamy, forest soil underlain by a blanket of loam. The soil was subject to crop rotation.

Section 57–97 was located in the lower part of the northeastern slope of the catena with the gray, moderately loamy, forest soil underlain by a blanket of loam. The soil was subject to crop rotation.

Section 58–97 was located at the foot of the northeastern slope with the gray, weakly gleysolic, moderately loamy, forest soil underlain by a blanket of loam. The soil had the second humus-rich horizon and was subject to crop rotation.

Section 59–97 was located at the foot of the northeastern slope at the bottom of a gully. The alluvial, moderately loamy soil was underlain by the blanket loam and was covered by a water meadow.

The preparation of soil samples for microbiological analysis. The soil samples were sonicated using a UZDN-1 ultrasonic disintegrator (22 kHz; 0.44 A; 2-min exposure) as described by Zvyagintsev [14].

The total number of microorganisms was determined by luminescence microscopy using specimens prepared by the routine methods [15]. Namely, soil suspensions were placed, using micropitettes, onto thoroughly degreased slides in amounts of 0.01 and 0.02 ml for the enumeration of bacteria and fungi, respectively. The suspension was spread with a loop over a slide area

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Fig. 1. Schematic representation of sections on two types of soils. N, north; NE, northeast; S, south; and SW, southwest. Catena sectors: E, eluvial; T^e , transeluvial; T^a , transaccumulative; and Saq, superaquatic.

of about 4 cm^2 , completely dried, and fixed in the flame of a burner. Each soil sample was used to prepare 12 replicate specimens. To enumerate bacterial cells and to evaluate the actinomycete mycelium, the specimens were stained with a 1 : 10000 acridine orange solution for 2–3 min. To enumerate fungal spores and to evaluate the fungal mycelium, the specimens were stained with calcofluor white for 15 min [16].

The amount of cells or the mycelium per 1 g of soil was calculated using the formula $N = S_1 an/vS_2 c$, where N is the number of cells (or the mycelium length expressed in μ m) per 1 g of soil, S_1 is the specimen area expressed in μ m², a is the number of cells (or the mycelium length in μ m) per one microscopic field averaged over all specimens examined, n is the dilution factor of the soil suspension expressed in ml, v is the volume of the drop (in ml) placed on the slide, S_2 is the area of the microscopic field in μ m², and c is the weight of the soil sample used for the preparation of the soil suspension.

The standard deviation (δ_{n-1}) of the number of bacteria did not exceed 5% and the standard deviations of the amounts of the mycelia and spores were within 10%. The biomass was calculated taking into account that the dry biomass of one bacterial cell 0.1 µm³ in volume is 2×10^{-14} g and that the dry biomass of 1 m of the actinomycete mycelium 0.5 µm in diameter is 3.9×10^{-8} g [17]. The actual biomasses of fungal spores and 1 m of the fungal mycelium were calculated by the formulas $0.0836r^3 \times 10^{-11}$ g and $0.628r^2 \times 10^{-6}$ g, respectively [18].



Fig. 2. Vertical distribution of bacteria in the soddy podzolic soils in summer and in autumn.

RESULTS AND DISCUSSION

In summer, the content of bacteria in the upper horizons of the soddy podzolic soil was higher than in the lower horizons. This general tendency was more distinct in the soils of the southern slope than of the northern slope (Fig. 2). The most profound changes in the vertical distribution of soil microorganisms were observed in the swampy region at the foot of the slope located close to the gully and in the forested flat region.

In autumn, the vertical distribution of microorganisms in the southern slope soils was more uniform than in summer. The bacterial population of the upper soil horizons of the forested flat regions was at a maximum.

The amount of the actinomycete mycelium was also greater in the upper than in the lower soil horizons, seasonal differences being largest in the watershed soils. This was probably due to the low moisture content of the upper horizons of the watershed soils in summer. The content of the actinomycete mycelium was maximum in the forest litter in autumn. Earlier, similar data were obtained in studies of the reserve forest soils.

The content of the fungal mycelium in all the horizons of the cultivated soils was lower than in the virgin soils (Fig. 3). In summer, the maximum content of the fungal mycelium was observed on the watershed plateau, at the foot of the southern slope, and in the swampy soil. In autumn, the amount of the mycelium in the forest litter considerably increased.

Thus, the effect of mesorelief on the content of bacterial cells and the fungal mycelium in the soddy podzolic soil was more profound in summer than in autumn. The amount of fungal spores in the cultivated soils was greater than in the forest soils.

The bacterial population of the gray forest soil was denser on the watershed plateau and at the foots of both slopes than in their steep portions (Fig. 4), reaching a maximum in the water meadow and forest soils. Soil bacteria were more abundant in autumn than in summer. In the southwestern slope soils, the bacterial population of the upper soil horizons was dense, but it sharply decreased in a downward direction.

As with bacteria, the content of the actinomycete mycelium in soils was greater on the watershed plateau and at the foots of both slopes than in their steep portions, although the seasonal changes in the bacterial population were less pronounced. At the same time, profound seasonal changes were observed in the upper horizons of the water meadow soil, where the amount of the actinomycete mycelium was considerably greater in summer than in autumn, and in the forest litter, where this parameter was essentially higher in autumn than in summer.



Fig. 3. Vertical distribution of fungal mycelium in the soddy podzolic soils in summer and in autumn.

In summer, the fungal mycelium was more abundant at the foots of both slopes than on the watershed plateau and in the steep portions of the slopes (Fig. 5). In autumn, the content of the fungal mycelium in the upper catenal horizons and in the water meadow soil decreased. At the same time, in the forest litter, the fungal mycelium was more plentiful in autumn than in summer.

The number of fungal spores at the foot of the southwestern slope, in its steep portions, and on the watershed plateau was larger in autumn than in summer. In autumn, the number of fungal spores decreased in the lower horizons of the water meadow soil and increased in the forest litter.

The total biomass of soil microorganisms (expressed in tons/hectare) in all the habitats studied is shown in Fig. 6, I and II. It can be seen that the contrasted habitats considerably differ in the microbial biomass. Both catenas exhibited the maximum biomasses in the forest, swampy, and water meadow soils. In the soddy podzolic forest soil, the maximum microbial biomass was observed in autumn. Conversely, in the gray forest, waterlogged, and water meadow soils, the maximum microbial biomass was observed in summer.

Seasonal changes in the microbial biomass were most distinct in the cultivated soddy podzolic soil, with the biomass being greater in summer on both the southern and the northern slopes of the catena.

In the cultivated gray forest soil, the total microbial biomass at the foots and on the slopes was greater in summer, whereas on the watershed plateau, it was greater in autumn.

It should be noted that the total microbial biomass in soils is mainly determined by the content of fungi. In view of this, data on the biomass of soil prokaryotic bacteria are presented separately (Fig. 6, III and IV). The maximum prokaryotic biomass was observed in the waterlogged and forest soils. In the forest soils, this parameter was greater in autumn than in summer, whereas in the swampy and water meadow soils, it was greater in summer than in autumn. In the cultivated soddy podzolic, the prokaryotic biomass was greater at the southern than at the northern slope. At the northern slope, seasonal variations in this biomass were small. At the southern slope, the prokaryotic biomass was heavier in summer. In the cultivated gray forest soil, the prokaryotic biomass was maximum at the northeastern slope in summer.

According to the earlier observations, the prokaryotic biomass comprises no more than 1% of the total microbial biomass of the forest reserve soils [18, 19] and from 3 to 5% of the total microbial biomass of cul-

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Fig. 4. Vertical distribution of bacteria in the gray forest soils in summer and in autumn.



Fig. 5. Vertical distribution of fungal mycelium in the gray forest soils in summer and in autumn.



Fig. 6. (I and II) The total microbial biomass and (III and IV) the prokaryotic biomass of (I and III) soddy podzolic and (II and IV) gray forest soils: 45, the foot of the northern slope; 46, the lower one-third portion of the northern slope; 47, the watershed plateau; 50, the foot of the southern slope; 51, waterlogged soil; 52, forested soddy podzolic soil; 58, the foot of the northeastern slope; 57, the lower one-third portion of the northeastern slope; 56; the watershed plateau; 54, the lower one-third portion of the northwestern slope; 59, water meadow soil; and 55, wooded gray forest soil.

tivated soils [6, 7]. In the present study, the portion of prokaryotic bacteria in the total microbial biomass was found to vary from 7 to 25% (Fig. 7, I and II). The portion of prokaryotes in the cultivated soddy podzolic soil increased from the watershed plateau to the catena foots and was maximum on the watershed plateau and the foots of the second catena. In both catenas, the portion of prokaryotes was larger in autumn than in summer, which was due to a decrease in the relative amount of the fungal biomass.

The relative amount of fungal spores in the cultivated soils of both catenas varied from 15 to 30-50% (Fig. 7, III and IV) and was greater than in the water-logged and forest soils (5–10%).

Thus, environmental conditions (the type of vegetation, mesorelief, and the degree of soil tillage) can appreciably influence the soil microbial complex [20–22]. The seasonal dynamics of the complex was distinct in the waterlogged soils, where the bacterial population was maximum in summer and minimum in autumn, and in the forest soils, where the fungal population was maximum in autumn and minimum in summer. Seasonal variations in the microflora of cultivated soils were less profound than in the microflora of virgin soils.

Forest soils were dominated by the eukaryotic biomass (primarily the fungal mycelium), whereas the fraction of the prokaryotic biomass was small. In the cultivated soils, this fraction was larger (from 7 to 25%), and the eukaryotic biomass was dominated by the fungal spores.

The effect of mesorelief on the microbial complex of the soddy podzolic soil was more profound than its effect on the microbial complex of the gray forest soil. The mesorelief was found to exert the greatest effect on the prokaryotic complex of the upper humus-rich hori-

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Fig. 7. (I and II) The fraction of prokaryotes in the total microbial biomass and (III and IV) the fraction of fungal spores in the eukaryotic biomass of (I and III) soddy podzolic and (II and IV) gray forest soils.

zons of the cultivated soils, whereas its effect on the microbial complex of the mineral soil horizons was negligible.

REFERENCES

- Golovchenko, A.V., Polyanskaya, L.M., Dobrovol'skaya, T.G., Vasil'eva, L.V., Chernov, I.Yu., and Zvyagintsev, D.G., The Spatial Distribution and Structure of Microbial Complexes in Swampy Forest Ecosystems, *Pochvovedenie*, 1993, no. 10, pp. 78–89.
- Polyanskaya, L.M., Pavlova, O.S., Dobrovol'skaya, T.G., Lysak, L.V., and Zvyagintsev, D.G., Microbial Complexes in the Oka Reserve Biogeocenoses, *Mikrobiologiya*, 1995, vol. 64, no. 4, pp. 540–547.
- Dobrovol'skaya, T.G., Pavlova, O.S., Polyanskaya, L.M., and Zvyagintsev, D.G., Bacterial Complexes in the Forest Biogeocenoses of the Oka Reserve: Spatial Structure and Taxonomic Diversity, *Mikrobiologiya*, 1995, vol. 64, no. 6, pp. 829–833.
- Golovchenko, A.V. and Polyanskaya, L.M., Seasonal Changes in the Vertical Distribution of Microorganisms in Soil, *Pochvovedenie*, 1996, no. 10, pp. 1227–1233.
- Zvyagintsev, D.G., Dobrovol'skaya, T.G., Bab'eva, I.P., Zenova, G.M., Lysak, L.V., Polyanskaya, L.M., and Chernov, I.Yu., The Structural and Functional Organization of Microbial Communities, in *Ekologiya v Rossii na rubezhe XXI veka: nazemnye ekosistemy*) (Ecology in

Russia at the Threshold of the 21th Century: Terrestrial Ecosystems), Moscow: Nauchnyi Mir, 1999, pp. 147–180.

- Golovchenko, A.V. and Polyanskaya, L.M., Annual Microbial Successions in the Southern Taiga Soils with Reference to the Central State Forest Biosphere Reserve, *Pochvovedenie*, 2000, no. 4, pp. 471–477.
- Polyanskaya, L.M., Lukin, S.M., and Zvyagintsev, D.G., Changes in the Composition of the Microbial Biomass of Soil Induced by Its Cultivation, *Pochvovedenie*, 1997, no. 2, pp. 206–212.
- Zvyagintsev, D.G., Polyanskaya, L.M., Gonchikov, G.G., and Korsunov, V.M., Microbial Biomass in the Transbaikal Soils, *Pochvovedenie*, 1999, no. 9, pp. 1132– 1139.
- Timofeev, V.A., Aerobic Microflora in the Chu Valley Soils, in *Mikroflora pochv Yuzhnoi chasti SSSR* (Soil Microflora in the Southern Regions of the USSR), Moscow: Nauka, 1966, pp. 118–148.
- Goguadze, V.D., Microbiological Characteristics of Red Soils in Georgia, in *Mikroflora pochv Yuzhnoi chasti* SSSR (Soil Microflora in the Southern Regions of the USSR), Moscow: Nauka, 1966, pp. 246–263.
- Polyanskaya, L.M., Sveshnikova, A.A., Vladychenskii, A.S., and Zvyagintsev, D.G., Microbial Biomass in the Brown and Black–Brown Soils of the Southwestern Tien Shan, *Mikrobiologiya*, 1995, vol. 64, no. 4, pp. 558–566.
- Lysak, L.V. and Dobrovol'skaya, T.G., Bacteria in the Tundra Soil of the Taymyr Peninsula, *Pochvovedenie*, 1982, no. 9, pp. 74–78.

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- 13. Dobrovol'skaya, T.G. and Lysak, L.V., Coryneform Bacteria in the Bacterial Cenoses of Some Dry Subtropical Soils, *Pochvovedenie*, 1986, no. 2, pp. 81–85.
- Zvyagintsev, D.G., Preparatory Ultrasonic Treatment of Soils for the Enumeration of Microorganisms, *Vestn. Mosk. Univ., Ser. 6: Biol. Pochvoved.*, 1968, no. 3, pp. 127–129.
- Metody pochvennoi mikrobiologii i biokhimii (Methods in Soil Microbiology and Biochemistry), Zvyagintsev, D.G., Ed., Moscow: Mosk. Gos. Univ., 1991.
- Polyanskaya, L.M., Direct Microscopic Evaluation of Fungal Spores and Mycelium in Soil, *Tez. konf. "Izuchenie gribov v biogeotsenozakh* (Proc. Conf. "Investigation of Fungi in Biogeocenoses"), Sverdlovsk, 1988, p. 30.
- 17. Kozhevin, P.A., Polyanskaya, L.M., and Zvyagintsev, D.G., Dynamics of the Development of Various Microorgan-

isms in Soil, Mikrobiologiya, 1979, vol. 48, pp. 490-494.

- Polyanskaya, L.M., Microbial Succession in Soil, *Doctoral (Biol.) Dissertation*, Moscow: Moscow State Univ., 1996.
- 19. Polyanskaya, L.M., Geidebrekht, V.V., Stepanov, A.L., and Zvyagintsev, D.G., Zonal Distribution of the Number and Biomass of Microorganisms in Soils, *Pochvovedenie*, 1995, no. 3, pp. 322–328.
- 20. Baath, E. and Soderström, B., Seasonal and Spatial Variations in Fungal Biomass in Forest Soil, *Soil Biol. Biochem.*, 1982, no. 14, pp. 3353–358.
- 21. Kjoller, A. and Struwe, S., Microfungi in Ecosystems: Fungal Occurrence and Activity in Litter and Soil, *Oikos*, 1982, vol. 39, pp. 389–422.
- 22. Naumova, N.B., Microbial Biomass in Virgin Siberian Soils, *Sib. Ekol. Zh.*, 1994, no. 3, pp. 275–281.