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EXPERIMENTAL ARTICLES

New Criteria for the Evaluation of Soil Bacterial Complexes

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Abstract—The initial concentration of prokaryotic microorganisms, the type of their growth, doubling time, and the growth dynamics of bacteria and actinomycetes in three types of soil (meadow, chestnut, and soddy forest) were evaluated by the luminescence microscopic analysis of soil samples incubated in a humid chamber for 1 day. Soddy forest and chestnut soils differed in most of the parameters analyzed. Meadow soil was close to soddy forest soil in some parameters and to chestnut soil in other parameters. All soil suspensions exhibited high growth rates of bacteria and actinomycetes, indicating that the fraction of viable microorganisms in the soils was high.

Key words: growth rate of prokaryotic microorganisms, soil suspensions, viability, luminescence microscopy.

The reliable differentiation of dead and living microbial cells is one of the topical problems of microbiology, since the differential count of viable and nonviable cells even in pure cultures is the subject of debate [1–8]. Still more questionable are the existing methods of microbiological investigation of soil samples [9, 10]. The data available in the literature indicate that only 20 to 80% of the cells stained with acridine orange are alive. The direct evaluation of microbial growth seems to be the most promising method for the determination of the number of viable cells.

The aim of the present work was to estimate the growth of prokaryotic microorganisms in soil suspensions by luminescence microscopy. The main growth parameters to be estimated were the initial concentration of prokaryotic microorganisms and their growth rates in the humus and mineral horizons of the soils, by which are meant hereafter the concentration and the growth rate of bacterial cells and actinomycete mycelium in the soil suspensions.

MATERIALS AND METHODS

Analyzed were the upper humus horizons and the lower mineral horizons of (1) soil beneath a typical meadow in the first floodplain terrace of the Selenga River, (2) chestnut soil beneath southern mountain and ridge slopes overgrown with dry-steppe vegetation, and (2) soddy soil beneath northern mountain slopes overgrown with pine forests and forest forbs in the Selenga region of Buryatia.

The number of prokaryotic microorganisms in the soils was determined using soil suspensions prepared by the standard method [11]. To determine the viability of bacteria and actinomycete mycelium, the soil suspension preparations were placed in a humid chamber and incubated at 28°C at an air humidity of 100% for 6, 10, and 24 h. Then the preparations were dried in the air, fixed, and stained with acridine orange. The control soil suspensions were fixed and stained as prepared. Such a procedure allows the germination rate of bacteria and actinomycete propagules in soil suspensions to be determined.

The specific growth rate of soil microorganisms capable of growing under the experimental conditions used was calculated by the formula: $\mu = N_1 - N/(t_1 - t)N$, where N is the initial number of bacterial cells, N_1 is their number after incubation, t is the time of the onset of the experiment, and t_1 is the time of the experiment, end.

In the experiments described, standard deviation did not exceed 5% for the number of bacteria and 10% for the amount of actinomycete mycelium.

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RESULTS AND DISCUSSION

The initial population of bacterial cells in the humus horizons of all types of soil reached billions of cells per g soil and was 2 to 3 times greater than in the mineral soil horizons. In the upper soil horizons, bacteria comprised from 3 billion cells/g in the chestnut soil to 4 billion cells/g in the meadow soil and 6 billion cells/g in the soddy forest soil. In the bacterial content of their mineral horizons, the soils ranked, in descending order, as the meadow soil, the soddy forest soil, and the chestnut soil. The difference between the bacterial populations of the humus and mineral soil horizons of the meadow soil was less than in the case of the chestnut soil and the soddy forest soil (Fig. 1).

The initial length of the actinomycete mycelium in the soils amounted to hundreds of meters per g soil. The maximum mycelium length was found in the meadow soil (600 m/g), whereas in the humus and mineral horizons of the two other types of soil, it was 1.5–2 times shorter (350–400 and 230–250 m/g, respectively) (Fig. 1).

Thus, the horizons of the soils under study differed in both initial bacterial population and actinomycete mycelium length. The concentration of bacteria and actinomycetes was higher in the meadow and soddy forest soils below the phytocenoses that were characterized by more favorable conditions for microbial populations.

Figure 2 illustrates the growth of bacterial populations in the soil suspensions placed in the humid chamber. It can be seen that bacterial growth was linear in the chestnut soil and exponential in the meadow and soddy forest soils. The doubling time of bacteria in the humus horizons of the meadow and soddy forest soils was 10 h, whereas more than 24 h in the humus horizons of the chestnut soil. After incubation for 10 h, bacterial growth slowed down, likely due to competition between bacterial cells. The doubling time of bacteria in the mineral horizons of all types of soil was 6 h.

According to the specific growth rate of soil bacteria in the humid chamber, which was measured in the time intervals of 0–6, 6–10, and 10–24 h of growth, the soils under study fell into two groups (Fig. 3). The first group included the soddy forest soil, which was characterized by similar specific growth rates of soil microorganisms in its humus horizons throughout the incubation period and by the highest specific growth rates in its mineral horizons in the late incubation terms (6–10 and 10–24 h). The second group comprised two soils, the chestnut and meadow soils, which exhibited the maximum specific growth rates of soil microorganisms in their humus and mineral horizons in the early terms of incubation. Then the specific growth rate of bacteria in the humus horizons of these soils decreased, the decrease being steeper in the meadow soil. Unlike the humus horizons, the mineral horizons showed a decrease in the bacterial growth rate only in the early terms of incubation (6–10 h). Then the bacterial growth rate began to rise but did not reach the values typical of the humus horizons. These data indicate that the dominant bacterial populations changed in the course of succession due to, for instance, the germination of resting bacterial forms.

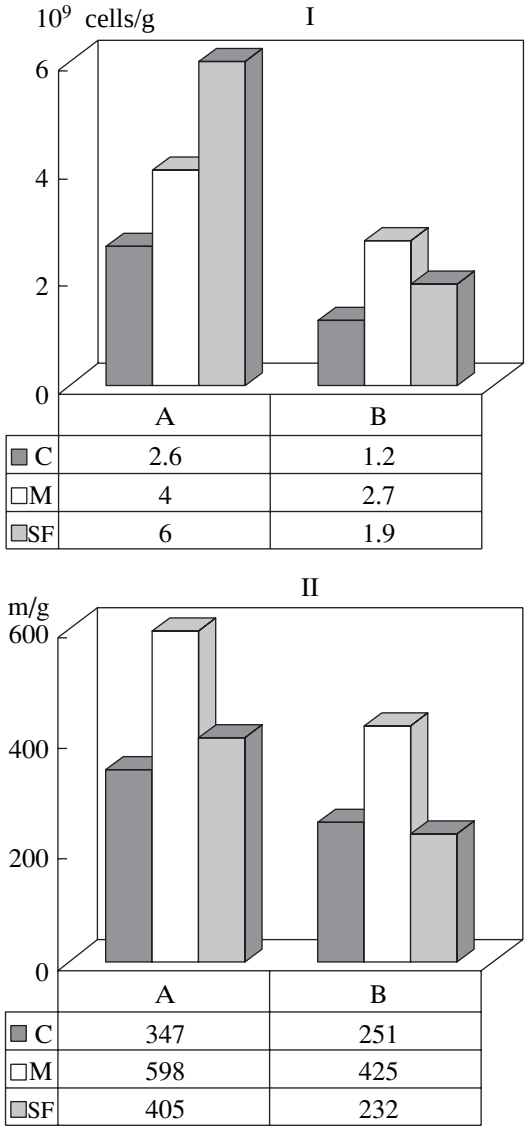

Fig. 1. (I) Concentration of bacteria and (II) the actinomycete mycelium length in (A) the humus and (B) mineral horizons of (C) the chestnut, (M) meadow, and (SF) soddy forest soils.

Figure 4 shows the dynamics of the length and the specific growth rate of the actinomycete mycelium in soil suspension in different incubation terms. The initial length of the mycelium increased only in the soddy forest soil, the maximum specific growth rate of actinomycetes being observed in the interval 6–10 h (0.13 and 0.09 h^{-1} in the humus and mineral horizons, respec-

Differentiating characteristics of soils

Parameter	Chestnut soil	Meadow soil	Soddy forest soil
Initial concentration of prokaryotes	Low	High	High
Bacterial growth in humus and mineral horizons	Linear	Exponential	Exponential
Doubling time of bacteria in humus horizons	More than 24 h	10 h	10 h
Dynamics of bacterial growth in humus horizons	Maximum growth rate in the interval 0–6 h and then gradual decrease	Maximum growth rate in the interval 0–6 h and then drastic decrease	Equal growth rates throughout the incubation period
Dynamics of bacterial growth in mineral horizons	Maximum growth rate in the interval 0–6 h, decrease in the interval 6–10 h, and increase in the interval 10–24 h	Maximum growth rate in the interval 0–6 h, decrease in the interval 6–10 h, and increase in the interval 10–24 h	Maximum growth rate in the interval 0–6 h and decrease in the intervals 6–10 and 10–24 h
Dynamics of actinomycete mycelial growth	Growth only in the interval 10–24 h	Growth only in the interval 10–24 h	Growth in all intervals with the maximum growth in the interval 6–10 h
Growth of prokaryotes in the interval 0–24 h	Slow	Medium	Fast
Difference in the specific growth rates of prokaryotes in the humus and mineral horizons during all cultivation periods	Low	Moderate	High

tively). In the meadow and chestnut soils, mycelial growth was detected only in the interval 10–24 h. The humus and mineral horizons of these soils did not differ in mycelial growth rate.

The distributions between the soils investigated were most pronounced if the growth rates of prokaryotic microorganisms were analyzed in the time interval 0–24 h (Fig. 5).

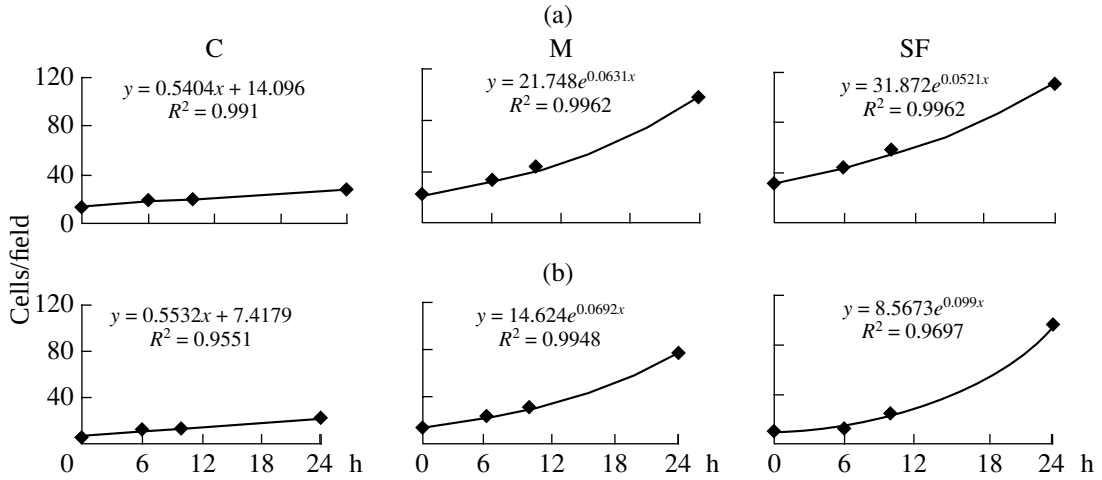

Fig. 2. Growth of bacterial cells in the soil suspensions from (a) the humus and (b) mineral horizons of (C) the chestnut, (M) meadow, and (SF) soddy forest soils incubated in the humid chamber.

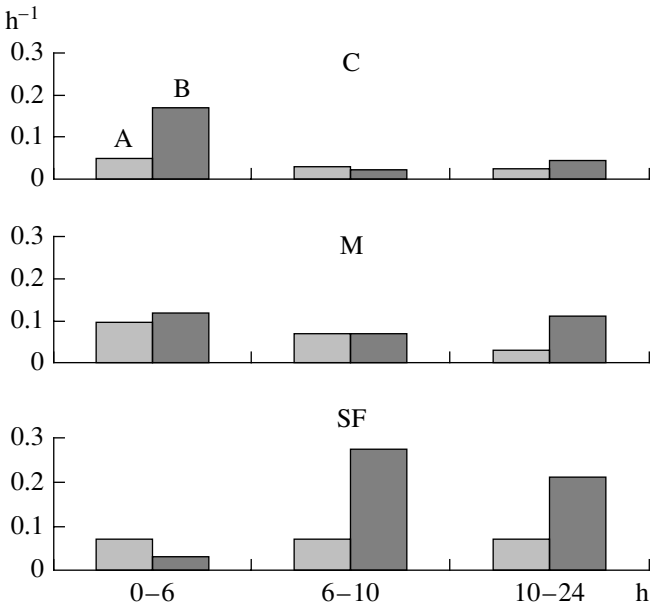

Fig. 3. Specific growth rates of bacterial cells in the soil suspensions from (A) the humus and (B) mineral horizons of (C) the chestnut, (M) meadow, and (SF) soddy forest soils incubated in the humid chamber.

In general, the chestnut soil was characterized by the minimum specific growth rates of bacteria and actinomycete mycelium, the growth rate in the mineral and humus horizons being different for the bacteria and equal for the mycelium. The soddy forest soil was characterized by high specific growth rates of bacteria and the actinomycete mycelium, which greatly differ in the mineral and humus horizons. The meadow soil was close to the soddy forest soil in the high growth rate of bacteria in different soil horizons and to the chestnut soil in the minimal and almost equal specific growth rates of the actinomycete mycelium in the humus and mineral horizons.

Thus, the humus and mineral horizons of the chestnut, meadow, and soddy forest soils differed in the initial concentration of prokaryotic microorganisms, the type of their growth (either linear or exponential), doubling time, and the growth dynamics of bacteria and actinomycetes (see table). The soddy forest and chestnut soils differed in most of the parameters analyzed. The meadow soil was close to the soddy forest soil in some parameters and to the chestnut soil in other parameters.

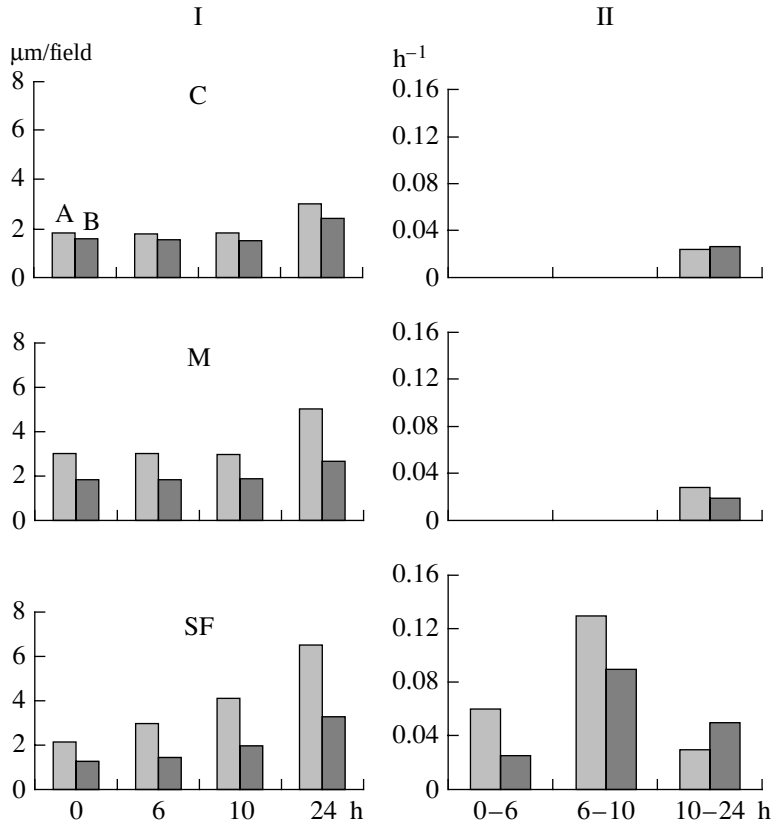

Fig. 4. Dynamics of (I) the length and (II) the growth rate of the actinomycete mycelium in the soil suspensions from (A) the humus and (B) mineral horizons of (C) the chestnut, (M) meadow, and (SF) soddy forest soils incubated in the humid chamber.

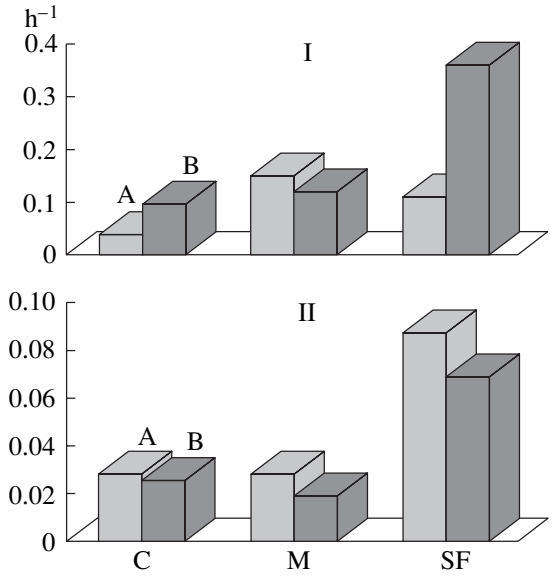

Fig. 5. Specific growth rates of (I) bacterial cells and (II) the actinomycete mycelium in the soil suspensions from (A) the humus and (B) mineral horizons of (C) the chestnut, (M) meadow, and (SF) soddy forest soils incubated in the humid chamber for 24 h.

Taking into account indirect evidence that a considerable portion of the detected prokaryotes is viable, the observed difference in the microbial growth rate may reflect the difference in the taxonomic structure of the soil microbial complexes. The growth rates measured in the above experiments can be considered to be very high.

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REFERENCES

1. Meisel', M.N. and Zavarzina, N.B. Investigation of Living Microbial Cells by Fluorescence Microscopy, *Mikrobiologiya*, 1947, vol. 16, no. 5, pp. 394–402.
2. Razumovskaya, Z.G. and Osipova, I.V., On the Ratio between Dead and Live Cells in a Growing *Acetobacter melanogenum* Culture, *Mikrobiologiya*, 1958, vol. 27, no. 6, pp. 727–731.
3. Babiuk, L.A. and Paul, E.A., The Use of Fluorescein Isothiocyanate in the Determination of the Bacterial Biomass of Grassland Soil, *Can. J. Microbiol.*, 1970, vol. 16, pp. 57–62.
4. Coleman, A., Enhanced Detection of Bacteria in Natural Environments by Fluorochrome Staining of DNA, *Limnol. Oceanogr.*, 1980, vol. 25, pp. 948–951.
5. Lusta, K.A. and Fikhte, B.A., *Metody opredeleniya zhiznesposobnosti mikroorganizmov* (Methods for Determining the Viability of Microorganisms), Eroshin, V.K., Ed., Pushchino: Nauchn. Tsentr Biol. Issl. Akad. Nauk SSSR, 1990.
6. Chrzanowski, T.H., Crotty, R.D., Hubbard, J.G., and Welch, R.P., Applicability of the Fluorescein Diacetate Method of Detecting Active Bacteria in Freshwater, *Microb. Ecol.*, 1984, vol. 10, pp. 179–185.
7. Fry, J.C., Direct Methods of Biomass Estimation, *Methods in Microbiology*, Grigorova, R. and Norris, Y.K., Eds., London: Academic, 1990, vol. 22, pp. 41–81.
8. Lundgren, B., Fluorescein Diacetate as a Stain of Metabolically Active Bacteria in Soil, *Oikos*, 1981, vol. 36, pp. 17–22.
9. Polyanskaya, L.M., Golovchenko, A.V., and Zvyagintsev, D.G., Determination of the Viability of Fungal Spores and Mycelium in Soil, *Mikrobiologiya*, 1998, vol. 67, pp. 832–836.
10. Kjoller, A. and Struwe, S., Microfungi in Ecosystems: Fungal Occurrence and Activity in Litter and Soil, *Oikos*, 1982, vol. 39, pp. 389–422.
11. *Metody pochvennoi mikrobiologii i biokhimii* (Methods of Soil Microbiology and Biochemistry), Zvyagintsev, D.G., Ed., Moscow: Mosk. Gos. Univ., 1991.