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Population Density and Total Biomass of Microbial Communities in Chestnut Soils and Solonetzes of the Dry Steppe Zone in the Lower Volga Region

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Abstract—The population density and total biomass of microbial communities were determined in chest nut soils and solonetzes of the dry steppe zone in the Lower Volga region with the use of the methods of sequential fractionation of the soil and direct counting. The mean weighted values of the population den sity of the microbial communities in the soil profiles $(A1 + B1 + B2$ horizons) in the studied soils varied within 3.8–8.0 \times 10¹¹ cells/g of soil. The total microbial biomass in the soils of the Privolzhskaya Upland reached 0.9–2.4 mg C/g of soil; in the soils of the Ergeni Upland, it was 20 to 75% lower. The microbial cells in the soils of the Privolzhskaya Upland were larger than those in the soils of the Ergeni Upland. Sequential fractionation of the soil prior to direct counting contributed to the more complete assessment of the population density of the microbial communities.

Keywords: biological activity, luminescent microscopy, sequential fractionation **DOI:** 10.1134/S1064229315010093

INTRODUCTION

The evaluation of the population density and biom ass of soil microbial communities participating in diverse biological processes is important for the com parative analysis of the biological activity of soils in the dry steppe zone [7]. It is known that about 70% of the total number of soil microbes are in the dormant state [21]. The active and dormant cells are characterized by different degrees of adsorption on the soil particles. The distribution patterns of the microbial community in dif ferent soil loci are different. Winogradsky suggested that soil fractionation should be applied for the more ade quate assessment of the numbers of soil microorganisms [13]. For example, upon separation of a peat soil into two fractions [18], the total number of bacteria stained with acridine orange in these two fractions comprised 14.8×10^{11} cells/g. Panikov [20] separated a peat soil into five fractions; the total number of bacterial cells determined in them reached 3.6×10^{12} , which was two orders of magnitude higher than the number of bacteria determined in this soil without fractionation. Note that the last of the obtained fractions contained 95% of the total number of microbial cells in the five fractions. The total number of microbial cells determined in four frac tions [6] from a chestnut soil using DAPI stain reached $2.6-3.9 \times 10^{11}$ cells/g [9] and did not differ much from the total number of microbial cells determined in the microbial fraction and the soil residue [10, 14], which comprised $3.3-4.1 \times 10^{11}$ cells/g [4, 11]. After activation of the same samples with β-indole-3-acetic acid, the population density of microorganisms reached $2 \times$ 10^{11} CFU/g of soil [4]. These data are in agreement with the earlier obtained data on the number of microorgan isms calculated from the amounts of soil phospholipids in the chestnut soils and solonetzes [15]. Taking into account the coefficient suggested by Balkwill with coauthors [16, 17], the number of living microbial cells comprised $0.67-1.21 \times 10^{10}$ cells/g. In the case of operating with data on the average weight of the cells isolated from the chestnut soil (without taking into account their outer organomineral layer [11]), the number of liv ing cells increased by an order of magnitude (up to $0.75-1.36 \times 10^{11}$ cells/g). In sediments, the number of microbial cells as calculated from their biomass estimated on the basis of data on the content of soil phos pholipids reached $1.7-2.5 \times 10^9$ cells/g and exceeded the number of cells obtained by direct counting by 2.7– 22 times [19]. In the soils without fractionation, the number of undamaged bacterial cells (without fungal spores) as estimated by direct counting using acridine orange [2, 3] varied from 0.5×10^5 cell/g (685 \times 10^{12} cells/m³) in the gray forest soil to 1.2×10^9 cells/g $(1740 \times 10^{12} \text{ cells/m}^3)$ in the chernozem. The number of bacteria stained with L7012 (LIVE/DEAD) varied from 12.9×10^9 cells/g (loamy mountain meadow soil)

[†] Deceased.

to 1.3×10^9 cells/g (light loamy soddy-podzolic soil) [12]. Thus, soil fractionation before direct counting of the microbial cells favors the more complete assessment of the total microbial pool.

The aim of our study was to perform a comparative assessment of the population densities and biomasses of the microbial communities in chestnut soils and solonetzes from the dry steppe zone of the Lower Volga region using the methods of sequential soil fraction ation and direct counting of microbes.

OBJECTS AND METHODS

The studied area belongs to the Volga–Don inter fluve in the zone of dry steppes (the subzone of chest nut soils) in Volgograd oblast. It is characterized by a moderately continental climate.

The Salomatino site is the northernmost site on the Privolzhskaya Upland 5 km to the north of the village of Salomatino in the Kamyshin district. This is a flat watershed surface slightly inclined towards the Ilovlya River (left tributary of the Don River) valley. The sur face deposits consist of two layers: the layer of loess like sandy loam (50–60 cm) and the underlying layer of colluvial gleyed and ferruginated loamy sandy and loamy sediments with inclusions of gravels. The mean annual precipitation is about 400 mm. Forb–fescue– feather grass (in the areas with chestnut soils) and fes cue–feather grass (in the areas with solonetzes) vege tation associations predominate; their projective cov ers reach 90 and 50%, respectively. A chestnut soil (pit D-651) and a solonetz (pit D-648) were studied on the divide between the Ilovlya and Bol'shaya Kazanka riv ers (the Don River basin).

The Kachalino site is found on the Privolzhskaya Upland 90 km to the south of the Salomatino site and 10 km to the southeast of the village of Kachalino in Ilovlya district in the basin of the Sakarka River (left tributary of the Don River). In this area, the parent materials on the interfluves are represented by saline and calcareous loess-like loams. The mean annual precipitation is 380 mm. A wormwood–fescue associ ation with a projective cover of 50–60% predominates. A virgin chestnut soil (pit D-643) and a solonetz (pit D-691) were studied on the north-facing watershed slope, and a virgin chestnut soil (pit D-749) was stud ied on the south-facing slope.

The Aksai site is found in the northern part of the Ergeni Upland 140 km to the south of the Kachalino site and 8 km to the southeast of the village of Aksai in Oktyabr'skii district. This is the dry-steppe zone near its boundary with the desert-steppe zone. The site occu pies an interfluve surface between two ravines; surface deposits consist of loess-like saline and calcareous loams. The mean annual precipitation is about 360 mm. Wormwood–fescue and herbaceous–wormwood asso ciations predominate in the areas of chestnut soils and solonetzes, respectively; their projective cover reaches 90 and 40%, respectively. At this site, we studied chestnut soils under virgin vegetation (pit D-678), long-term fallow (pit D-677), and a plowed field with Sudan grass (pit D-671).

At all the plots, the groundwater is found at a depth of more than 10 m and does not affect the modern pedogenesis. Some data on the soil properties are given in Table 1.

Methods. Soil samples were taken from the genetic horizons observing sterile conditions. The method of direct counting of microorganisms in the soils without their preliminary fractionation included the following stages. Soil samples (1 g) were stirred in 10 mL of a 0.5% solution of sodium pyrophosphate and treated with ultrasound impulses (30 s) with pauses (30 s) between them [22] with an ultrasonic generator (UZG 13-0.1/22) at 50 W power and 22 KHz fre quency. To evaluate the effect of the duration of the ultrasonic treatment on the obtained counts of microbes, the soil samples were treated with two, four, and six impulses (so that the total duration was 1, 2, and 3 min, respectively). Then, the soil suspensions were diluted to the optimum, and microslides were prepared from them via application of $10 \mu L$ of the diluted suspension onto microscopic glasses (side 24 mm). The slides were dried, flamed, and stained with DAPI $(5 \mu g/mL)$ during 15–20 min; then, the microbial cells were counted under a luminescent microscope (Biomed YX1) at magnification of 10×100 .

The method of sequential fractionation of the soils included the following stages. Weighed portions of soil samples (6 g; weight of the native samples) were stirred in 60 mL 0.5% sodium pyrophosphate and treated with two ultrasonic impulses as described above. The soil suspensions were diluted to 200 mL. Extracts with microbial cells were separated from the soil residue via centrifuging at 1000 g for 30 min at 4°C. A sodium pyrophosphate (200 mL) solution was added to the soil precipitates, and the above described procedure was repeated. This extraction was repeated three times. Microslides were prepared from the obtained fractions (extracts and residues) as described above.

The number of microbial cells in the soil without fractionation and in separate fractions (extracts and residues) was calculated according to the following equation:

$$
N = a \times 37086.5 \times 100 \times V_0 X/m,
$$

where *N* is the number of cells (in the whole soil sam ple or in separate fractions), *а* is the average number of cells in one vision field, 37086.5 is the number of vision fields on the slide $(24 \times 24 \text{ mm})$, 100 is the number of 10 - μ L aliquots in 1 mL, V_0 is the initial volume of the soil suspension without fractionation (or the suspensions of separate fractions), *X* is the dilution (without accounting for the initial volume), and *m* is the weighed soil sample.

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Horizon; depth, cm	C_{org} , %	$pH_{H,0}$	CaCO ₃ , $%$	Fraction content, %		
				< 0.001 mm	< 0.01 mm	
Privolzhskaya Upland Salomatino site Chestnut soil, pit D-648						
Asod, $0-11$	1.6	6.7		$\,8\,$	26	
A $1, 11-30$	0.8	6.5		13	28	
$B1, 30 - 53$	0.6	7.3		31	40	
$B2, 53 - 65$	0.4	8.2	0.9	18	27	
Solonetz, pit D-651						
A $1, 0-13$	0.8	6.7		$\boldsymbol{7}$	28	
$B1, 13 - 30$	0.9	8.6	2.2	41	56	
B _{2ca} , 30-45	0.4	9.0	6.4	30	46	
Kachalino site, northern slope Chestnut soil, pit D-643						
A $1, 0-16$	1.6	7.1		26	41	
$B1, 16 - 37$	1.6	7.6	1.7	30	46	
B _{2ca} , 37-54	1.2	8.2	9.4	31	49	
Solonetz, pit D-691						
A $1, 0-14$	2.1	7.0		15	33	
B1, 14-45	0.9	7.6	1.4	30	51	
B _{2ca} , 45-56	0.7	8.6	7.0	25	49	
Kachalino site, southern slope Chestnut soil, pit D-749						
$A1, 0-12$	1.3	6.7	0.5	$\,8\,$	22	
$B1, 12-32$	0.7	7.1	1.0	21	35	
B _{2ca} , 32-43	0.5	8.3	6.8	19	36	
Ergeni Upland Aksai site Virgin chestnut soil, pit D-678						
A $1, 0-18$	$1.1\,$	8.1	1.3	22	39	
B1, 18-29	$0.7\,$	9.1	4.5	$30\,$	48	
B2ca, 29-60	$0.1\,$	$10.0\,$	13.8	32	47	
Fallow chestnut soil, pit D-677						
$A1, 0-17$	$1.0\,$	7.7	0.6	$20\,$	38	
$B1, 17-32$	$\rm 0.8$	8.9	4.8	35	50	
B2, 32-44	0.4	8.9	10.9	30	50	
Plowed chestnut soil, pit D-671						
$Ap, 0-28$	0.7	7.0	0.6	18	36	
$B1, 28 - 40$	$0.6\,$	7.0	1.6	26	41	
B2, 40-48	$0.3\,$	8.5	$4.8\,$	19	31	

Table 1. Properties of chestnut soils and solonetzes on the Privolzhskaya and Ergeni uplands

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The population density of the microbial communi ties was determined according to the following equa tion:

$$
N_{\text{(extract + residue)}} = N_{\text{(extract)}} + N_{\text{(residue)}}.
$$

We also determined the coefficient extraction K:

$$
K = N_{\text{(extract)}} / N_{\text{(extract + residue)}}.
$$

The extracts of the microbial fraction were settled via centrifuging at 5000 g and lyophilized. The organic carbon content in them was determined by the wet combustion method with a spectrometric ending [1]. The carbon of the total microbial biomass $C_{(TMB)}$ was calculated as follows:

$$
C_{(TMB)} = C_{(MF)}/K,
$$

where $C_{(MF)}$ is the concentration of organic carbon in the extracted microbial fraction, and K is the coefficient of extraction. The microbial extracts were stored at 4°C. The experiments were performed in triplicate. The statistical treatment of the data was performed by standard methods [5].

RESULTS

Data on some properties of the studied chestnut soils and solonetzes from the Privolzhskaya and Ergeni uplands are presented in Table 1.

We compared the numbers of microorganisms determined in the whole soil samples without frac tionation at different durations of ultrasonic treat ment and in the sum of the fractions isolated from the same samples using the method of sequential frac tionation (Table 2). No significant changes in the population density of the microbial communities were observed upon an increase in the duration of the ultrasonic treatment from 1 to 3 min. The total num ber of cells determined in the soil samples without fractionation comprised $0.41-0.88 \times 10^{11}$ cells/g of soil and was 8–15 times lower than the total number of cells determined in the sum of fractions isolated from the same soil. Hence, the sequential fraction ation of the soil before direct counting of the micro bial cells allows us to obtain a more complete estimate of the population density of the soil microbial com munity. The subsequent experiments and calculations were performed with the use preliminary fraction ation of soils by this method before direct counting of the soil microorganisms.

The population density and total biomass of microor ganisms (Table 3) indicate that the population density of the microbial communities in the chestnut soil from the Salomatino site (pit D-648) varied within 3.2– 4.5×10^{11} cells/g of soil; in the B1 horizon, it was 35% higher than in the other horizons, in which its values did not differ significantly. The total microbial biomass reached 1.7– 1.9 mg C/g of soil in the humus horizon and was 50–70% lower in the B2 horizon. The popula tion density of the microbial communities in the solo netz from the same site (pit D-651) comprised $3.1 \times$ 10^{11} cells/g of soil in the A1 horizon; its values in the B1

Table 2. Comparison of the numbers of soil microorgan isms determined in the whole soil sample and in the sum of the soil fractions

and B2 horizons did not differ significantly and were 45 to 50% higher than those in the A1 horizon. The total microbial biomass in this solonetz varied from 1.6 to 3.6 mg C/g of soil and was two times higher in the solo netzic horizon than in the A1 and B2 horizons.

The population density of soil microorganisms at the Kachalino site varied within $+6.0-9.4 \times 10^{11}$ cells/g of soil. Its values in the humus horizons of the chestnut soil and solonetz on the north-facing watershed slope (pits D-643 and D-691) did not differ significantly and were 40–45% lower than those in the B2 horizon. The total microbial biomass in the chestnut soil (pit D-643) com prised 2.2–2.7 mg C/g of soil and did not differ signifi cantly in the soil profile. The total microbial biomass in the solonetz (pit D-691) varied from 1.3 to 2.8 mg C/g of soil with the maximum value in the B1 horizon. The numbers of soil microorganisms in the profile of the chestnut soil on the south-facing slope (pit D-749) were approximately the same; the total microbial biomass in the A1 horizon was 3.7 times higher than that in the B1 and B2 horizons.

In general, the population densities of the microbial communities in the soils from the Kachalino site were about two times higher than those in the soils of the Salomatino site. The values of the total microbial biom ass in the studied soils on the Privolzhskaya Upland var ied within the same limits (1.0 to 3.7 mg C/g of soil) in all these soils.

The population density of the microbial communi ties in the virgin chestnut soil from the Aksai site (pit D-678) comprised $5.0-7.0 \times 10^{11}$ cells/g of soil and differed significantly only in the B1 and B2 hori zons; the total microbial biomass virtually did not vary in the soil profile and was about 1.0 mg C/g of soil. In the chestnut soil under fallow (pit D-677), the popu lation density of the microbial communities com prised $6.5-10.7 \times 10^{11}$ cells/g of soil, and the total biomass reached $1.6-2.5$ mg C/g of soil. The population density of the microbial communities in the plowed chestnut soil comprised $2.9-4.7 \times 10^{11}$ cells/g of soil with a maximum in the B1 horizon. The total micro bial biomass varied from 1.2 to 1.6 mg C/g of soil and did not differ much in the lower horizons.

Horizon; depth, cm	Number of soil microorganisms, $\times 10^{11}$ cells/g of soil	Total microbial biomass, $mg C/g$ of soil				
Privolzhskaya Upland Salomatino site Chestnut soil, pit D-648						
Asod, $0-11$	3.2 ± 0.0	1.9 ± 0.02				
A $1, 11-30$	3.5 ± 0.5	1.7 ± 0.10				
$B1, 30 - 53$	4.5 ± 0.4	1.7 ± 0.08				
$B2, 53 - 65$	3.5 ± 0.4	1.1 ± 0.30				
Solonetz, pit D-651						
$A1, 0-13$	3.1 ± 0.1	1.6 ± 0.30				
$B1, 13 - 30$	4.5 ± 0.2	3.6 ± 0.63				
B _{2ca} , 30-45	4.7 ± 0.1	1.7 ± 0.27				
Kachalino site, northern slope Chestnut soil, pit D-643						
$A1, 0-16$	6.5 ± 0.3	2.2 ± 0.25				
$B1, 16 - 37$	6.2 ± 0.2	2.7 ± 0.29				
B _{2ca} , 37-54	8.9 ± 0.3	2.3 ± 0.27				
Solonetz, pit D-691						
A $1, 0-14$	6.1 ± 0.7	1.3 ± 0.14				
$B1, 14-45$	6.8 ± 1.1	2.8 ± 0.31				
$B2ca, 45-56$	9.4 ± 1.0	2.0 ± 0.10				
Kachalino site, southern slope Chestnut soil, pit D-749						
$A1, 0-12$	6.3 ± 0.5	3.7 ± 0.85				
$B1, 12-32$	7.4 ± 0.8	1.0 ± 0.10				
B _{2ca} , 32–43	6.0 ± 0.5	1.0 ± 0.01				
Ergeni Upland Aksai site Virgin chestnut soil, pit D-678						
$A1, 0-17$	5.9 ± 0.8	1.0 ± 0.05				
$B1, 17-34$	7.0 ± 0.4	1.1 ± 0.50				
B2ca, $34-60$	5.0 ± 0.5 0.8 ± 0.21					
Fallow chestnut soil, pit D-677						
$A1, 0-17$	6.5 ± 0.7	1.6 ± 0.15				
$B1, 17-32$	10.7 ± 1.0	2.5 ± 0.17				
$B2, 32-44$	6.7 ± 0.3 1.9 ± 0.24					
Arable chestnut soil, pit D-671						
$Ap, 0-28$	4.0 ± 0.3	1.6 ± 0.18				
$B1, 28 - 40$	4.7 \pm 0.2	1.2 ± 0.05				
$B2, 40 - 48$	2.9 ± 0.3	1.3 ± 0.20				

Table 3. Population density and total biomass of microbial communities in chestnut soils and solonetzes of the Priv olzhskaya and Ergeni uplands

Data on the population density of the microbial communities and the total microbial biomass in chest nut soils and solonetzes on the Privolzhskaya and Ergeni uplands as estimated by the mean weighted val ues for the A1, B1, and B2 horizons are presented in Fig. 1. Population densities of microbial communities in the chestnut soil and solonetz from the Salomatino site (Fig. 1, *1*; pits D-648 and D-651) reached 3.8– 4.1×10^{11} cells/g of soil and did not differ significantly. In the chestnut soils and solonetzes from the Kachalino site (pits D-643, D-691, and D-749), the population densities of the microbial communities also did not differ significantly from one another and were 80% higher than those in the soils from the Salo matino site. Among the soils from the Ergeni Upland (Aksai site, (Fig. 1, *2*), the chestnut soil under fallow (pit D-677) was characterized by the maximum popu lation density of the microbial communities $(8.0 \times 10^{11}$ cells/g of soil). The mean weighted value of the popula tion density of the microbial communities in the virgin chestnut soil (pit D-678) was 1.5 times lower than that in the fallow soil; in the plowed soil (pit D-671), it was 20% lower than that in the virgin chestnut soil.

The total microbial biomass in the chestnut soil from the Salomatino site (Fig. 1, *3*) constituted 1.6 mg C/g of soil and was 1.5 times lower than that in the solonetz. The total microbial biomasses in the chestnut soil and solonetz on the north-facing slope at the Kachalino site (pits D-643 and D-691) did not differ significantly and comprised 2.2–2.4 mg C/g of soil. In the chestnut soil from the south-facing slope (pit D-749), it was 40% lower. The mean weighted value of the total microbial biomass in the chestnut soils from the Ergeni Upland (Fig. 1, *4*) comprised 0.9–1.9 mg C/g of soil. The max imum value was found in the soil under fallow; the val ues of the total microbial biomass in the plowed and vir gin chestnut soils were by 1.5 and 2 times lower, respec tively. Thus, the mean weighted values of the population density of the microbial communities in the soils of the Privolzhskaya Upland did not differ significantly within the same key sites. In the soils from the Kachalino key site, they were 80% higher than those in the soils of the Salomatino site. The differences in the total microbial biomass in the soils of the Privolzhskaya Upland could be up to 50% within the same key site. The population density of the microbial communities in the soils of the Ergeni Upland (the Aksai site) increased by two times in the fallow–virgin plot–plowed field sequence, whereas the maximum microbial biomass was determined in the virgin chestnut soil.

The variations in the population density of the microbial communities differed from the variations in the total microbial biomass in the soil profiles and in the different soils. On the basis of the data on the num bers and biomass of the soil microbes, the average vol umes of microbial cells in the profiles of the chestnut soils and solonetzes of the Privolzhskaya and Ergeni uplands were estimated (Fig. 2). In the chestnut soil from the Salomatino site (Fig. 2, *1*, pit D-648), the

Fig. 1. Mean weighted values of the population density of the microbial community and the total microbial biomass in chestnut soils and solonetzes of the (*1*, *3*) Privolzhskaya and (*2*, *4*) Ergeni uplands.

average volumes of microbial cells decreased down the soil profile; in the solonetz (pit D-651), the largest vol ume of the cells was found in the B1 horizon. In the chestnut soil and solonetz on the north-facing slope at the Kachalino site (Fig. 2, *2*, pits D-643 and D-691), the largest cells were also in the B1 horizon; in the chestnut soil on the south-facing slope (pit D-749), the largest volume of microbial cells was in the humus (A1) horizon. On the Ergeni Upland, the volumes of microbial cells in the chestnut soils under fallow and under virgin vegetation (Fig. 2, pits D-677 and D-678) varied insignificantly. In the chestnut soil of the plowed field (pit D-671), the microbial cells in the Ap and B2 horizons were 2.5–3.0 times larger than those in the analogous horizons of the virgin chestnut soil; in the B1 horizon, they were 1.5 times larger those in the analogous horizons of the virgin chestnut soil. The microbial cells in the soils of the Privolzhskaya Upland were generally larger than those in the soils of the Ergeni Upland. We consider that the more favorable conditions for microorganisms in the soils of the Priv olzhskaya Upland are related to the geographic posi tions of the analyzed soils.

Fig. 2. Average volumes of microbial cells in the chestnut soils and solonetzes of the (*1*, *2*) Privolzhskaya and (*3*) Ergeni uplands.

DISCUSSION

The variations in the population density and biom ass of the microbial cells represent deviations from certain minimums of the microbial pools typical of a given soil. The number of microorganisms supported by the soil in the periods most unfavorable for their development can be considered the microbial pool [8]. The samples of studied chestnut soils and solonetzes were taken in droughty seasons unfavorable for the development of microbial communities, and this gives us grounds for taking the values of their population density and total biomass as parameters of the soil microbial pools. Data on the numbers and total biom ass of microbial cells in different soils were compared with due account for the content of organic carbon and clay particles in these soils (Fig. 3). The latter parameter is of special interest, because most of the microorganisms are concentrated around soil particles in the organomineral gel [8]. The values of the popula tion density and biomass of the microbial communi-

Fig. 3. Deviations in the contents of (*1*) clay particles and (*2*) organic carbon, (*3*) the population density of microbial communities, and (*4*) the total microbial biomass in chest nut soils and solonetzes of the Privolzhskaya and Ergeni uplands from reference (zero) values in pits D-648, D-643, and D-678, %..

ties and the contents of organic carbon and clay parti cles in the separate horizons of the virgin chestnut soils (pit D-648, Salomatino site; D-643, Kachalino site; and D-678, Aksai site) were taken as reference (zero) values without regard for the difference between them. For other soils, a comparative analysis of the variations in the parameters of the microbial pools and in the organic matter and clay contents was performed. For the B2 horizons, the variations in the organic carbon content were not taken into account because of the small absolute values (in relative values, the differ ences between the soils reached 200–300%).

The Salomatino site. The content of clay particles in the A1 horizon of the solonetz (pit D-651) was 50% lower than in the A1 horizon of the chestnut soil (pit D-648), and the contents of organic carbon in these soils were similar. The population density and total biomass of the microbial communities in the A1 horizons of the solonetz and chestnut soil did not differ significantly. The population density of the micro bial communities in the B1 horizons of the solonetz (pit D-651) and chestnut soil (pit D-648) did not dif fer significantly, but the total microbial biomass in this horizon of the solonetz was two times higher than that in the chestnut soil. This could be due to the higher contents of both clay particles and organic carbon in the B1 horizon of the solonetz. The population density and total biomass of the microbial communities in the B2 horizon of the solonetz (pit D-651) were 35 and 55% higher than those in the same horizon of the

chestnut soil (pit D-648), respectively. This could be due to a higher content of clay particles in the B2 hori zon of the solonetz.

The Kachalino site. The content of clay particles in the A1 horizon of the solonetz (pit D-691) was 40% lower than that in the chestnut soil (pit D-643), whereas the content of organic carbon in the solonetz was 30% higher. The difference between the popula tion densities of the microbial communities in the A1 horizons of these soils was uncertain, and the total biomass in the solonetz was 40% lower than that in the chestnut soil. The contents of clay particles in the B1 horizons of these soils were close to one another, and the organic carbon content in the B1 horizon of the solonetz was 45% lower than that in the B1 horizon of the chestnut soil. The population density and total biomass of the microbial communities in the B1 hori zons of these soils did not differ much. The difference between the contents of clay particles in the B2 hori zons of the chestnut soil (pit D-643) and the solonetz (pit D-691) was not so pronounced as in the A1 hori zons and reached about 20%. The population density and total biomass of the microbial communities in the B2 horizons of the chestnut soil and solonetz did not differ significantly.

Thus, the population density of the microbial com munities in the profiles of chestnut soils and solo netzes on the north-facing interfluve surface virtually did not differ from one another, and the values of the total microbial biomass differed only in the A1 hori zon. The higher (by 40%) microbial biomass in the A1 horizon of the chestnut soil in comparison with the solonetz was apparently related to the higher content of clay particles accumulating organic matter available for the microorganisms in the chestnut soil. The microbial parameters of these two soils did not differ in their B1 and B2 horizons. This allows us to suppose that some differences in the physicochemical proper ties of the chestnut soil and solonetz did not have a decisive effect on the microbial parameters of these soils. It is probable that some other factors, including the climatic conditions, microrelief, slope aspect, etc., exerted a more pronounced impact on the soil micro bial parameters.

The content of clay particles in the A1 horizon of the chestnut soil on the south-facing slope (pit D-749) was 70% lower, and the content of organic carbon was 20% lower than those in the A1 horizon of the chestnut soil on the north-facing slope (pit D-643). The popu lation densities of the microbial communities in these horizons did not differ significantly, whereas the total microbial biomass in the A1 horizon of the soil on the north-facing slope (pit D-749) was 70% higher than that in the A1 horizon of the soil on the south-facing slope (pit D-643). The contents of clay particles and organic carbon in the B1 horizon of the soil on the south-facing slope were, respectively, 30 and 60% lower than those in the B1 horizon of the soil on the north-facing slope. The total microbial biomass in the

B1 horizon of the chestnut soil on the south-facing slope was 60% lower, whereas the population density of the microbial community was 20% higher than those in the B1 horizon of the chestnut soil on the north-facing slope. In the B2 horizon, the content of clay particles was 40% lower, the population density of the microbial community was 30% higher, and the total microbial biomass was 60% lower in pit D-749 than in pit D-643.

Thus, the comparison of the chestnut soils on the northern and southern slopes showed that the differ ences in the microbial biomass of these soils in the A1 horizon are not related to the content of clay parti cles. It is probable that slope aspect and specific char acteristics of the erosional–accumulative processes at the microlevel are more important factors affecting the microbial biomass in the A1 horizon. In the B1 horizon of the soil on the north-facing slope, the population density of the microbial community is 20% higher, and the total microbial biomass is 60% lower than those in the B1 horizon of the soil on the south-facing slope. Thus, the microbial cells in this horizon on the north facing slope are smaller in size than those on the south facing slope. The low total microbial biomass in the lower horizons of the chestnut soil in pit D-749 (as compared with the chestnut soil in pit D-643) can be related to the lower contents of organic carbon (in the B1 horizon) and clay particles, which retain organic substances available for the microorganisms.

The Aksai site. The contents of clay particles and organic matter in the A1 horizon of the chestnut soil of the fallow plot (pit D-677) were about 10% lower than those in the virgin chestnut soil (pit D-678). The pop ulation densities of microbial communities in this horizon did not differ significantly, and the total microbial biomass in the fallow soil was 60% higher than that in the virgin soil. The contents of clay parti cles and organic carbon in the B1 horizon of the fallow chestnut soil were 15% higher than those in the B1 horizon of the virgin chestnut soil. The population density of the microbial community and the total microbial biomass in the B1 horizon of the fallow soil were 1.5 and 2.5 times higher, respectively, than those in the B1 horizon of the virgin soil. The contents of clay particles in the B2 horizons of these soils were approximately similar, whereas the population density of the microbial community was 35% higher in the fal low soil. The total microbial biomass in the B2 horizon of the fallow soil was 2.3 times higher than that in the virgin soil.

The contents of clay particles and organic carbon in the A1 horizon of the plowed chestnut soil (pit D-671) were 20 and 40% lower than those in the virgin chestnut soil, respectively. The population density of the micro bial community in the A1 horizon of the plowed soil was 30% lower, and the total biomass was 60% higher than those in the virgin soil. The contents of clay particles and organic carbon in the B1 horizon of the plowed soil were 15% lower, the population density of the microbial community was 35% lower, and the total biomass was 10% higher than those in the corresponding horizon of the virgin soil. The content of clay particles and the population density of the microbial community in the B2 horizon of the plowed soil were 40% lower and the total microbial biomass was 60% higher than those in the B2 horizon of the virgin chestnut soil.

Thus, the population density of the microbial community in the profile of the plowed chestnut soil was lower and the microbial biomass was higher than those in the profile of virgin chestnut soil. It can be supposed that the size of the microbial cells in the plowed soil was larger than that in the virgin soil, which could be due to the disturbance of the solonetzic (B1) horizon during the soil tillage with its admixture into the plow layer with more favorable conditions for the microbial cenoses.

CONCLUSIONS

(1) The sequential fractionation of soil prior to direct counting of microbes makes it possible to obtain a more complete estimate of the population density of the soil microbial communities.

(2) The mean weighted population density of the microbial communities in the $AI + B1 + B2$ horizons of the studied soils varied from 3.8 to 8.0×10^{11} cells/g of soil. In the chestnut soils and solonetzes of the Priv olzhskaya Upland at the Kachalino site, it was 80% higher than at the Salomatino site. In the chestnut soils of the Ergeni Upland, it decreased by two times in the fallow–virgin–plowed soil sequence.

(3) The mean weighted values of the total microbial biomass in the chestnut soils and solonetzes from the Privolzhskaya Upland comprised 1.6–2.4 mg C/g of soil. At the Salomatino site, it was 1.5 times higher in the solonetz than in the chestnut soil. At the Kachalino site, it did not differ significantly in the chestnut soil and solonetz on the northern slope and decreased by about 40% in the chestnut soil on the southern slope. In the chestnut soils of the Ergeni Upland, it varied within $0.9-1.9$ mg C/g of soil with a maximum in the fallow soil. The values of the total microbial biomass in the plowed and virgin soils were 1.5 and 2 times lower, respectively. In general, this parameter in the chestnut soils of the Privolzhskaya Upland was 20–75% higher than that in the chestnut soils of the Ergeni Upland.

(4) The microbial cells in the soils of the Privolzhs kaya Upland were larger than those in the soils of the Ergeni Upland; among the latter, the largest cells were in the plowed chestnut soils, which could be due to the admixture of the former solonetzic horizon (B1) into the plow layer with more favorable conditions for the development of microbial cenoses.

(5) The differences in the numbers of microbial cells between the soils of particular key plots on the Privolzhskaya Upland were statistically insignificant, and the changes in the total microbial biomass were in agreement with the changes in the clay content. On the Ergeni Upland, the maximum population density and total biomass of the microbial community were found in the chestnut soil of the fallow plot.

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