ISSN 0026-2617, Microbiology, 2008, Vol. 77, No. 3, pp. 356–364. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © N.D. Ananyeva, L.M. Polyanskaya, E.A. Susyan, I.V. Vasenkina, S. Wirth, D.G. Zvyagintsev, 2008, published in Mikrobiologiya, 2008, Vol. 77, No. 3, pp. 404–412.

EXPERIMENTAL ARTICLES

Comparative Assessment of Soil Microbial Biomass Determined by the Methods of Direct Microscopy and Substrate-Induced Respiration

N. D. Ananyeva[a,1], L. M. Polyanskaya[b], E. A. Susyan[a], I. V. Vasenkina[b], S. Wirth[c], and D. G. Zvyagintsev[b]

[a] *Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, ul. Institutskaya 2, Pushchino, Moscow oblast, 142290 Russia*

[b] *Moscow State University, Faculty of Soil Science, Vorobyevy Gory 1, 119992 Moscow*

[c] *ZALF, Leibniz-Centre for Agricultural Landscape Research, Institute of Landscape Matter Dynamics, Muncheberg, Germany*

Received August 26, 2007

Abstract—The content of microbial biomass (MB) was determined in samples of gray forest, chestnut, and tundra soils with different physicochemical properties (0.4–22.7% C_{org}; 8.4–26.8% silt particles; pH 4.3–8.4) by the methods of substrate-induced respiration (MB_{SIR}) and direct microscopy (MB_M). The samples of two upper soil layers, 0–5 and 5–10 cm (without plant litter), from different ecosystems (forest, forest shelter belt, meadow, fallow, and arable) and elements of relief of interfluvial tundra (block/upper land plateau, depression between blocks) have been analyzed. The content of microbial biomass in the 0–5-cm soil layer was 216–8134 and 348–7513 μg C/g soil as measured by the methods of substrate-induced respiration and direct microscopy, respectively. The MB_{SIR} and MB_M values closely correlated with each other: r = 0.90 and 0.74 for 0–5 and 5–10 cm, respectively. The average MB_{SIR}/MB_M ratio was 90 and 60% for 0–5 and 5–10 cm, respectively. The portion of microbial carbon in total organic soil carbon was, on average, 4 and 3% (SIR) and 5 and 7% (direct microscopy) for 0–5 and 5–10 cm, respectively. Possible reasons for the differences between MB_{SIR} and MB_M values in the soils under study are discussed.

Key words: soil, microbial biomass, direct microscopy, substrate-induced respiration.

DOI: 10.1134/S0026261708030168

Microbial biomass is an important living and labile soil component. Its reserves, activity, and structure are key characteristics in ecological studies. Soil microbial biomass is determined by direct microscopic and indirect (biochemical, physiological) methods. The former are used to determine the quantity and contents of microorganisms (microscopy); the latter, to estimate their activity, e.g., respiratory activity, after fumigation or introduction of an additional substrate (substrate-induced respiration, SIR). Researchers place high emphasis on the results of assessment of soil microbial biomass by these two groups of methods [1–4].

Some works have shown that the microbial biomass values obtained by direct microscopy and SIR closely and positively correlate with each other [4, 5]. It has also been established that the fungi/bacteria ratios determined by direct microscopy and selective inhibition of SIR are close on plant residues [6] and in soil [4]. However, some researchers also mention the differences between the values of soil microbial biomass determined by "direct" and "indirect" methods. For example, microbial biomass measured by the methods of fumigation [7] or SIR [8, 9] was much greater than the value obtained by direct microscopy. Other authors note that microbial biomass values obtained by the fumigation method, on the contrary, were less than those obtained by direct microscopy [1, 2, 10]. In this line of research, direct microscopy has been carried out mainly with agar [1, 2, 8, 10] or membrane [9] films and the cells were stained by dianiline blue [1, 2, 10], fluorescein diacetate [7], or calcofluor white [9].

The method of fluorescence microscopy, which has been used in the present work, makes it possible to determine the length of mycelium of fungi and actinomycetes, the quantity of bacterial and fungal spores, and to measure the diameter of mycelium and fungal spores in the soil suspension on a glass mount stained with calcofluor white (for eukaryotes) or acridine orange (for prokaryotes) [12, 13]. Moreover, the method of fluorescence microscopy has been used to

[1] Corresponding author; e-mail: ananyeva@rambler.ru

Table 1. Characteristics of soils under study

Soil	Region (site)	Ecosystem (element of relief*)	Vegetation	Layer (cm)	C_{org}, %	pH_{water}	Soil texture (particles/mm), %		
							Sand (1–0.05)	Clay (0.05–0.001)	Silt (<0.001)
Tundra gley (1)	Vorkuta (Tal'nik)	Dbb*, Block	Shrub Moss, low shrub	0–5 0–5	22.65 13.25	5.75 4.3	N.d.** N.d.	N.d. N.d.	N.d. N.d.
Gray forest (2)	Moscow (Pushchino)	Forest	Aspen, birch	0–5 5–10	2.42 1.31	5.95 5.5	18.80 12.68	70.80 74.12	10.40 13.20
		Meadow	Cereal–grass	0–5 5–10	1.76 1.62	6.6 6.85	31.20 13.60	56.64 75.20	12.16 11.20
		Arable	Wheat	0–5 5–10	0.96 0.92	5.85 6.05	8.40 17.60	78.00 65.04	13.60 17.36
Dark chestnut (3)	Volgograd (Netkachevo)	Forest shelter belt	Oak	0–5 5–10	1.55 0.73	6.45 6.3	65.20 81.48	25.20 9.72	9.60 8.80
		Young fallow	Weeds	0–5 5–10	0.49 0.36	6.05 6.1	76.40 76.80	15.20 14.00	8.40 9.20
		Fallow	Mustard	0–5 5–10	1.03 0.96	6.3 6.4	75.60 76.80	13.20 12.00	11.20 11.20
Chestnut (4)	Volgograd (Plemkhoz)	Forest shelter belt	Elm	0–5 5–10	1.57 1.49	7.7 8.4	38.40 39.60	38.20 38.20	23.40 22.20
		Fallow	Fescue, wormwood	0–5 5–10	1.99 1.10	6.25 6.9	42.88 36.40	36.12 38.92	21.00 24.68
		Arable	Sunflower	0–5 5–10	0.96 0.92	8.2 8.4	38.40 43.20	34.80 30.00	26.80 26.80

* Dbb, depression between blocks.

** N.d., not determined

obtain extensive knowledge about the microbial pool of soils of our country [13]. The method of substrate-induced respiration (physiological method), assessing the state of microbial biomass carbon in different soils, is widely used by researchers of many foreign countries [3–5, 8, 11].

The goal of the present work was to give comparative estimation of soil microbial biomass (MB) values measured by the methods of direct fluorescence microscopy and substrate-induced respiration in: (a) different types of soils and ecosystems and (b) two upper soil layers (0–5 and 5–10 cm). The study was also focused on establishment of the correlation between MB values obtained by different methods as well as between MB and physicochemical characteristics of soil.

MATERIALS AND METHODS

Selection and preparation of soil samples. Samples of tundra, gray forest, and chestnut soils from different ecosystems were taken from the upper layers (0–5 and 5–10 cm); the litter layer was not analyzed (Table 1). A mixed soil sample (at natural moisture) of each ecosystem was sieved through a sieve with cell diameter 2 mm, placed into a polyethylene bag with a cotton stopper (for gas exchange), and stored in a refrigerator before application in the experiments.

The content of organic soil carbon was determined by dichromate digestion, pH was measured in water suspensions (soil : water ratio = 1 : 2.5), and texture composition was determined by weighing with sodium pyrophosphate.

Preincubation of samples. Prior to the analysis, soil samples (0.5 kg) were moistened up to 50–60% of the water holding capacity and incubated at 22°C for 7 days in polyethylene bags with gas exchange. Weighed soil samples for determination of substrate-induced respiration and direct microscopy were taken from preincubated samples. Measurements of soil microbial biomass by the two methods were made simultaneously.

Substrate-induced respiration (SIR) of soil was assessed by the rate of initial maximal respiration of microorganisms after soil enrichment with an additional carbon and energy source (glucose). Weighed soil samples (1 g) were placed into a flask (15 ml); glucose solution (0.1 ml; final concentration, 10 mg/g soil) was added; and the flask was sealed hermetically. The enriched soil sample was incubated (for three to five hours at 22°C); then, an air sample was taken from the

flask and analyzed in a gas chromatograph. The time of the onset of the experiment and of gas sampling was recorded. Preliminary experiments showed that the introduction of 10 mg of glucose per 1 g of tested soils provided the highest rate of SIR after 4 h of their incubation. The SIR rate was expressed in μg CO_2-C g^{-1} dry soil h^{-1}. SIR was determined in five repeats.

Microbial biomass was calculated using the formula: C_{mic} (μg C g^{-1} soil) = (μl CO_2 g^{-1} dry soil h^{-1}) 40.04 + 0.37 [11].

Direct microscopy. The total quantity of microorganisms in soil was measured by the method of fluorescence microscopy. Soil suspension was prepared by placing 1 g of soil (3 repeats for each soil sample) into 100 ml of water and dispersing by ultrasound (22 kHz, 0.44 A, 2 min). Then, the aliquots of 0.01 and 0.02 ml were taken from the suspension for counting prokaryotic and eukaryotic cells, respectively (4 repeats for each group of microorganisms) and spread by a loop over the area of 4 cm^2 of glass microscope slides. The specimens were then air-dried and fixed (2–3 s) in the flame of a burner. The preparations were stained with acridine orange solution (1 : 10000, 2–3 min) for counting the bacteria and mycelium of actinomycetes and with calcofluor white (15 min) for counting the spores and mycelium of fungi.

The quantity of cells (mycelium) in 1 g of soil was determined using the formula $N = S_1an/VS_2C$, where N is the number of cells (length of mycelium, μm) in 1 g of soil; S_1 is the area of specimen, μm^2; a is the cell number (length of mycelium, μm) in one field of vision (average of 4 specimens); n is dilution of soil suspension, ml; V is the volume of a drop placed onto glass, ml; S_2 is the area of the field of vision of the microscope, μm^2; and C is the weight of the dry soil sample, g.

At counting, 20 fields of vision of a specimen were examined for bacteria and 50 fields of vision were examined for the mycelium of actinomycetes and fungi as well as fungal spores. The quantity of bacteria, fungal spores, and the lengths of fungal/actinomycete mycelium in 1 g of soil were recorded as the mean values obtained by examination of 12 microscope slides.

For the dry biomasses of a bacterial cell (0.1 μm^3) and 1 m of actinomycete mycelium (0.5 μm in diameter), the values 2×10^{-14} g and 3.9×10^{-8} g, respectively, were accepted. The diameter of fungal hyphae and spores was determined using the microscope eyepiece micrometer. The biomass was calculated with allowance for the biovolume of a cylinder (L, length; r, radius) for fungal mycelium and that of a sphere (A, number of spores; r, radius) for a fungal spore using the formulas: $0.628L\ r^2 \times 10^{-6}$ g and $0.0836Ar^3 \times 10^{-11}$ g, respectively. Specific weight (density) of the fungal mycelium and spores was taken to be equal to 1 g/cm^3. For calculation of microbial biomass, the water and carbon contents in the cells of soil microorganisms were taken as 80 and 50% [12, 13] and 85 and 45%, respectively [8].

Statistical data processing was performed using the Statistica 6.0 software package (StatSoft Inc., Tulsa, USA). Standard deviation (std. dev.) was calculated for the mean values. For the total values of microbial biomass (direct microscopy), std. dev. was calculated as an error of functions of random values.

RESULTS

The tested soils had a wide range of organic matter (0.4–22.6%) and silt (8.4–26.8%) content and pH values (4.3–8.4) (Table 1). The highest and the lowest quantities of organic matter were revealed in the soil of interfluvial tundra and in the dark chestnut soil of young fallow, respectively. The highest and the lowest values of microbial biomass determined by the SIR method (MB_{SIR}) were revealed in the tundra soil of a depression between blocks (8134 μg C g^{-1} soil) and in the dark chestnut soil of young fallow (163 μg C g^{-1} soil), respectively (Fig. 1). In the depression between blocks, the MB_{SIR} value was almost sixfold higher than on the elevated element of relief (block). In the natural ecosystems of gray forest (forest), dark chestnut (forest shelter belt), and chestnut (fallow) soils, MB_{SIR} (0–5 cm) was 3.9, 3.0, and 3.6 times higher than in the arable analogs, respectively. The lower layer of the tested soils (5–10 cm) had much lower MB_{SIR} values as compared with the upper one. In natural cenoses, MB_{SIR} of the upper layer was higher than in the corresponding lower layer: 1.4 (meadow) and 2.4 (forest) times for gray forest soil; 1.7 (forest shelter belt) and 3.5 (young fallow) times for dark chestnut soil; 2.4 (forest shelter belt) and 2.8 (fallow) times for chestnut soil. In the upper layer of arables, MB_{SIR} was only 1.6, 1.0, and 1.6 times higher than in the lower layer for gray forest, dark chestnut, and chestnut soil, respectively.

The main characteristics of the components of soil microbial biomass determined by direct microscopy are listed in Table 2. The length of fungal mycelium in soils of natural ecosystems was 260 (young fallow) to 4830 m/g soil (depression between blocks) in the 0–5-cm layer. In tundra soil, a significant portion of fungal mycelium belonged to basidiomycetes (51 and 24% of the total mycelium length in the depression between blocks and in the block, respectively), which are associated with plant mycorrhiza. In the studied soils, the diameter of hyphae was 5.1–5.3 μm for basidiomycetes and below 5 μm (3.75–4.15 μm) for buckleless fungi. The contents of fungal spores varied in the range of $5–25 \times 10^6$ per gram, their diameter being more than 5 μm (5.35–6.50 μm). Prokaryotic microorganisms amounted to $1.4–13.9 \times 10^9$ cells/g and 131–412 m/g of soil for bacteria and actinomycetes, respectively. The soils of natural ecosystems contained more fungal mycelium than the corresponding arables.

Table 3 shows the values of dry microbial biomass (MB_M) with allowance for 80% water content in cells. The highest and lowest MB_M values (dry matter) were revealed in tundra soil (15027 μg/g) and in dark chest-

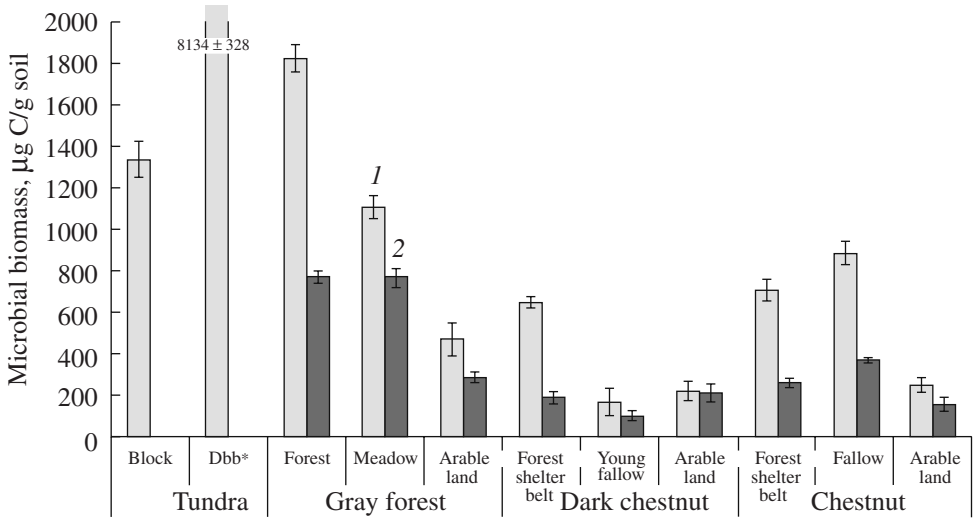

Fig. 1. Microbial biomass of tundra, gray forest, dark chestnut, and chestnut soils of different ecosystems measured by the method of substrate-induced respiration. *1*, 0–5 cm; *2*, 5–10 cm. Dbb, depression between blocks.

nut soil (697 μg/g), respectively. The biomass of fungal spores was 3.2 to 26.5% of MB_M; its highest portion (20–26% MB_M) was found in the dark chestnut soil of young fallow and arables. In arable soils, the fungal spore biomass made up a larger portion of total biomass (10–26% MB_M) as compared with the soils of natural cenoses (3.2–12.1% MB_M). Fungal mycelium constituted a significant part of MB_M (73–96% and 69–94% in the 0–5 and 5–10 cm layers, respectively). In the arable gray forest, dark chestnut, and chestnut soils (0–5 cm), the fungal mycelium biomass was 7.5, 4.0, and 2.6 times less than in the forest and forest shelter belts, respectively.

Microbial biomass determined by direct microscopy was calculated on the basis of different content of water (80 and 85%) and carbon (45 and 50%) in the cells (Table 3). At the higher content of water (85%) and lower content of carbon (45%), the obtained values of microbial biomass were lower (by ~30% as compared with respective water and carbon contents of 80 and 50%). The MB_M carbon content was also calculated without taking the contribution of fungal spores into consideration. The values of microbial biomass carbon obtained by the methods of SIR (C_{mic}-MB_{SIR}) and direct microscopy (C_{mic}-MB_M) are compared in Table 4. The ratio of C_{mic}-MB_{SIR}/C_{mic}-MB_M in the tested soils was 36–108% and 14–57% in the 0–5 and 5–10 cm layers, respectively (80% H_2O and 50% C). Calculation of microbial biomass assuming higher water (85%) and lower carbon (45%) content resulted in the C_{mic}-MB_{SIR}/C_{mic}-MB_M ratios increase to 53–160% and 21–85% for 0–5 and 5–10 cm, respectively. The MB_{SIR} and MB_M values were closer when the values of 85% H_2O and 45% C in the cells were used for calculation (on average, MB_{SIR}/MB_M = 88% for 0–5 cm).

It should be noted that microbial biomass determined by the SIR method obviously does not include the contribution of fungal spores. The biomass of spores in tested soils exceeded the biomass of prokaryotic microorganisms (Table 3). The C_{mic}-MB_M value without fungal spores was calculated (Table 4). The portion of C_{mic}-MB_{SIR} in C_{mic}-MB_M (without spores) significantly increased: to 39–112% and 15–72% for 0–5 and 5–10 cm, respectively (C_{mic} was calculated assuming 80% water and 50% C content). As a result of calculation of C_{mic}-MB_M with higher content of water and lower content of carbon, the MB_{SIR}/MB_M ratio increased to 58–166% and 23–106% in the 0–5 and 5–10 cm layers, respectively. On average, the biomass carbon values measured by the method of SIR were 88 and 58% of the value determined by direct microscopy (85%H_2O, 45% C, without spores).

The ratio of the carbon content of microbial biomass measured by the two methods to the total soil organic carbon was calculated. The highest content of microbial carbon (the SIR method) in C_{org} was revealed in gray forest soil: 4.8–7.5% for 0–5 cm (Table 4). The lowest portion of C_{mic} in C_{org} (1%) was in the tundra soil of the block. In arable soils, microbial carbon was shown to constitute a lesser portion of the total organic carbon as compared with natural analogs. In the upper soil layer, the content of C_{mic}-MB_{SIR} was generally 1.3–2.6 times higher than in the lower layer.

The fraction of microbial carbon in total organic carbon, according to the results of direct microscopy, was as a whole greater than the value determined by SIR (Table 4). The value of C_{mic}-MB_M/C_{org} in tested soils (0–5 cm) was 1.9–13.7% with spores and a little less (1.7–12.9%) without spores. In the 5–10 cm layer, the C_{mic}-MB_M/C_{org} ratio was on average 2.0–2.2% higher than in the 0–5 cm layer. In arable soils, this

Table 2. The length of mycelium of fungi and actinomycetes, the quantity of bacteria and fungal spores, and the mean diameter of hyphae and fungal spores in soils

Soil	Ecosystem (element of relief)	Layer (cm)	Length of mycelium of fungi and actinomycetes, numbers of bacteria and fungal spores (average in 1 g of soil ±SD*)					
			Fungal mycelium		Fungal spores		Bacteria (10^9/g)	Actinomycetes (m/g)
			Length (m/g)	Diameter (μm)	10^6/g	Diameter (μm)		
1	Dbb	0–5	2368 ± 188 2462 ± 240 (Bm**)	3.40 5.10	16.8 ± 1.8	6.50	9.3 ± 1.3	184 ± 26
	Block/upper land (bul)	0–5	1777 ± 123 573 ± 47 (Bm**)	3.80 5.30	19.0 ± 1.2	6.0	13.9 ± 0.69	412 ± 45
2	Forest (fr)	0–5	4228 ± 670	3.70	25.2 ± 3.4	5.95	7.85 ± 0.43	248 ± 19
		5–10	4330 ± 460		22.5 ± 2.7		4.25 ± 0.45	194 ± 25
	Meadow(md)	0–5	1318 ± 121	4.10	6.48 ± 0.96	5.85	7.50 ± 0.34	351 ± 30
		5–10	1189 ± 93		5.28 ± 0.96		3.60 ± 0.41	168 ± 33
	Arable (al)	0–5	537 ± 85	3.90	8.2 ± 0.8	5.70	3.45 ± 0.18	236 ± 50
		5–10	670 ± 65		9.7 ± 1.4		4.60 ± 0.33	291 ± 32
3	Forest shelter belt (fsb)	0–5	1199 ± 168	4.05	15.0 ± 1.8	5.35	2.60 ± 0.26	212 ± 25
		5–10	558 ± 162		10.8 ± 1.2		2.00 ± 0.30	164 ± 27
	Young fallow (yf)	0–5	260 ± 32	4.05	11.6 ± 1.0	5.55	1.40 ± 0.45	304 ± 27
		5–10	189 ± 23		9.1 ± 1.6		1.70 ± 0.14	347 ± 37
	Arable (al)	0–5	316 ± 10	3.80	11.9 ± 1.0	5.40	1.80 ± 0.16	191 ± 23
		5–10	237 ± 65		12.6 ± 1.6		1.50 ± 0.19	170 ± 26
4	Forest shelter belt (fsb)	0–5	1191 ± 119	4.15	13.0 ± 1.3	6.0	4.00 ± 0.30	247 ± 20
		5–10	1213 ± 127		10.8 ± 1.6		2.60 ± 0.10	197 ± 18
	Fallow (f)	0–5	603 ± 69	4.15	9.4 ± 0.9	5.45	2.10 ± 0.10	239 ± 18
		5–10	399 ± 60		9.2 ± 0.7		2.10 ± 0.20	131 ± 18
	Arable (al)	0–5	497 ± 49	3.75	8.3 ± 0.9	5.65	2.70 ± 0.20	197 ± 18
		5–10	290 ± 79		67.1 ± 0.7		2.00 ± 0.10	160 ± 22

Note: See designations in Table 1.
* SD, standard deviation.
** Bm, basidiomycetes.

value was 2.7, 2.8, and 1.7 times less than in the natural analogs under wood vegetation of gray forest, dark-chestnut, and chestnut soils (0–5 cm), respectively.

Figure 2 shows the regression dependence between MB values determined by the SIR method and direct microscopy. The coefficient of determination between the series of these values is higher for the upper soil layer ($R^2 = 0.88$) than for the lower layer ($R^2 = 0.67$). Coefficients of correlation (r) between microbial biomass and physicochemical properties of soil (C_{org}, pH, silt content) are presented in Table 5. The closest correlation was revealed between the contents of MB_{SIR} and C_{org} ($r = 0.90$ and 0.74 for 0–5 and 5–10 cm, respectively) and between MB_{SIR} and MB_M/without spores ($r = 0.90$ for 0–5 cm); however, in the lower layer this correlation was not so pronounced ($r = 0.78$). The correlation between MB_M (with and without spores) and C_{org} was close as well, but the r value was less than for MB_{SIR} and C_{org}. Correlation was also established between MB_{SIR}, MB_M, and the content of silt particles ($r = 0.70$) only for the upper layer of tested soils. The pH values had a weak negative correlation with microbial biomass values.

DISCUSSION

Direct microscopy is a unique method for determination of the total microbial pool in soil. Moreover, it allows clear differentiation of prokaryotes and eukaryotes as well as their components (mycelium and

Table 3. Microbial biomass (MB) of the groups of microorganisms and the content of MB carbon at different values of water and carbon content in the cells (direct microscopy)

Soil*	Ecosystem*	Layer (cm)	Dry microbial biomass (80% H_2O), μg/g soil (average ± SD*)					MB carbon, μg C/g soil			
								80% H_2O, 50% C		85% H_2O, 45% C	
			Fungal mycelium	Fungal spores	Bacteria	Actinomycetes	Total	Spores included	Spores excluded	Spores included	Spores excluded
1	Dbb	0–5	4298 ± 250 10054 ± 930	482 ± 50	186 ± 30	7 ± 1	15027 ± 966	7513	7272	5072	4909
	Bul	0–5	4029 ± 850 2527 ± 620	429 ± 60	278 ± 60	16 ± 2	7279 ± 1080	3639	3425	2457	2312
2	Fr	0–5	9087 ± 1200	555 ± 60	157 ± 8	10 ± 1	9809 ± 1202	4904	4627	3310	3123
		5–10	9307 ± 1160	495 ± 74	85 ± 9	8 ± 1	9894 ± 1162	4947	4700	3339	3172
	Md	0–5	3478 ± 300	136 ± 20	150 ± 7	14 ± 11	3778 ± 302	1889	1821	1275	1229
		5–10	3138 ± 260	110 ± 18	72 ± 8	7 ± 1	3327 ± 262	1663	1608	1123	1086
	Al	0–5	1282 ± 230	159 ± 15	69 ± 4	9 ± 2	1519 ± 212	760	680	513	459
		5–10	1600 ± 210	188 ± 29	92 ± 6	11 ± 1	1891 ± 232	946	852	638	575
3	Fsb	0–5	3088 ± 470	240 ± 30	52 ± 5	8 ± 1	3388 ± 472	1694	1574	1143	1062
		5–10	1437 ± 400	173 ± 30	40 ± 6	6 ± 1	1656 ± 402	828	742	559	501
	Yf	0–5	670 ± 80	207 ± 15	28 ± 9	12 ± 1	917 ± 82	458	355	309	239
		5–10	487 ± 60	163 ± 3	34 ± 3	14 ± 1	697 ± 60	348	267	235	180
	Al	0–5	716 ± 200	196 ± 16	36 ± 3	7 ± 1	956 ± 202	478	380	323	256
		5–10	537 ± 150	207 ± 26	30 ± 4	7 ± 1	781 ± 152	391	287	264	194
4	Fsb	0–5	3220 ± 330	293 ± 38	80 ± 6	10 ± 1	3603 ± 332	1802	1655	1216	1117
		5–10	3280 ± 320	244 ± 40	52 ± 2	8 ± 1	3583 ± 322	1792	1670	1209	1127
	F	0–5	1630 ± 160	159 ± 15	42 ± 2	9 ± 1	1840 ± 132	920	841	621	567
		5–10	1079 ± 130	156 ± 12	42 ± 4	5 ± 1	1281 ± 162	641	563	433	380
	Al	0–5	1097 ± 120	156 ± 16	54 ± 4	8 ± 1	1315 ± 122	658	579	444	391
		5–10	640 ± 210	134 ± 18	40 ± 2	6 ± 1	820 ± 212	410	343	277	232

* See designations in Tables 1 and 2.

spores). Substrate-induced respiration registers the response of microorganisms after soil enrichment with glucose. The rate of SIR multiplied by an experimentally established value (40.04) is converted into total microbial biomass expressed in the units of microbial carbon [11].

In the studied soils, MB_{SIR} values were as a whole less than the values obtained by direct microscopy, and the differences between them were smallest when values of 85% water and 45% carbon content in the cells were assumed (Table 4). The MB_{SIR} fraction of MB_M was on average almost 90 and 60% in the 0–5 and 5–10 cm layers, respectively. In other words, MB values measured by the methods of direct microscopy and SIR were closer in the upper layer of soils with contrasting physicochemical properties than in their lower layer. One of the reasons for this fact may be associated with different metabolic states of fungi in the tested soil layers (substrates). It was shown, for example, that the inhibition of fungal respiration by cycloheximide was only 12% of the total SIR in the forest soil containing 313 ± 140 m of fungal mycelium/g soil [14]. Besides this, high correlation was shown between selective inhibition of SIR by cycloheximide and the content of active fungal biomass revealed by fluorescein diacetate dye [8]. It was also shown that about 60% of fungal biomass in the soil of prairies had empty hyphae [15].

Some researchers point out that the method of direct microscopy tends to overestimate, first of all, the content of fungi in soil. A significant error in cell counting (up to 100%) may be introduced also by the subjectivity of an observer [8, 16] and selection of dye. Calcofluor, for example, stains fungal mycelium independently of its metabolic state [15]. There is information that only about a half of the total microbial biomass in soil is in an active state [7, 17]. Besides, it has been shown that the MB value measured by direct microscopy (brown fungal hyphae not stained with aniline blue were taken into account) is approximately twice as high as the value obtained by the fumigation–incubation method [10].

Table 4. The ratio of microbial biomass carbon (C_{mic}) determined by the methods of substrate-induced respiration (MB_{SIR}) and microscopy (MB_M) and the content of microbial carbon (C_{mic}, in % of C_{org}) in studied soils

Soil*	Ecosystem*	Ratio, % (calculation**)				C_{mic}/C_{org}, %			
		C_{mic}-MB_{SIR}/C_{mic}-MB_M, with spores		C_{mic}-MB_{SIR}/C_{mic}-MB_M, without spores		SIR		Microscopy (85% H_2O, 45% C) (with spores/without spores)	
		0–5 cm	5–10 cm	0–5 cm	5–10 cm	0–5 cm	5–10 cm	0–5 cm	5–10 cm
1	Dbb	108 (160)	N.d.	112 (166)	N.d.	3.6	N.d.	2.2/2.2	N.d.
	Bul	37 (54)	N.d.	39 (58)	N.d.	1.0	N.d.	1.9/1.7	N.d.
2	Fr	37 (55)	16 (23)	39 (58)	16 (24)	7.5	5.9	13.7/12.9	25.5/24.2
	Md	58 (87)	46 (68)	61 (90)	47 (70)	6.3	4.7	7.2/7.0	6.9/6.7
	Al	61 (91)	30 (44)	68 (101)	33 (49)	4.8	3.1	5.3/4.8	6.9/6.2
3	Fsb	38 (56)	22 (33)	41 (61)	25 (37)	4.2	2.5	7.4/6.9	7.7/6.9
	Yf	36 (53)	27 (40)	46 (68)	36 (53)	3.3	2.6	6.3/4.9	6.5/5.0
	Al	45 (67)	53 (78)	57 (84)	72 (106)	2.1	2.1	3.1/2.5	2.7/2.0
4	Fsb	39 (58)	14 (21)	43 (63)	15 (23)	4.5	1.7	7.7/7.1	8.1/7.6
	F	96 (142)	57 (85)	105 (155)	65 (97)	4.4	3.3	3.1/2.9	3.9/3.5
	Al	38(56)	37 (55)	43 (63)	44 (66)	2.6	1.7	4.6/4.1	3.0/2.5
Average		54 (80)	34 (50)	60 (88)	39 (58)	4.0	3.1	5.7/5.2	7.9/7.2

* See Table 1.

** Dry biomass carbon was calculated assuming 80% H_2O, 50% C (85% H_2O, 45% C).

MB_M in the tested soils has been calculated with allowance for different contents of water (80 and 85%) and carbon (45 and 50%) in microbial cells. In many works by Russian researchers, soil microbial biomass (direct counting in a fluorescence microscope) is calculated at a rate of 80% water and 50% carbon content [12]. In tundra soil (depression between blocks), the MB_{SIR} value was even higher than the value determined by direct microscopy (Table 4). It may be due to the high content of organic matter and fungal mycelium in this soil as compared with others, which seems to be the reason of MB underestimation by direct microscopy. Previously it has been shown that high dilutions of soil suspension should be used for direct counting of fungal mycelium in soils rich in organic matter [8] or rhizosphere soil [18]. On the other hand, the temperature of incubation and SIR measurement for tundra soil was higher than in natural conditions, which could also favor an increase of the SIR rate and, as a consequence, of the MB_{SIR} value. Moreover, basal respiration of the studied soils measured in pre-incubated samples (7 days, 22°C) was the highest for tundra soil (data not shown).

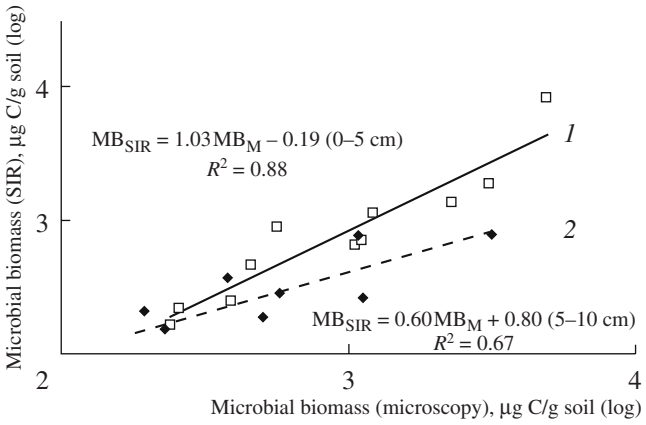

Fig. 2. Linear regression between microbial biomass (MB) values obtained by the methods of direct microscopy (MB_M without spores, 85% water and 45% C) and substrate-induced respiration (MB_{SIR}) in tundra, gray forest, dark chestnut, and chestnut soils of natural and arable ecosystems: *1*, 0–5 cm; *2*, 5–10 cm.

MB_{SIR} values diagnosed the tested soil layers more clearly; the upper 5-cm layer contained more microbial biomass than the corresponding lower layer. This conforms to the observations of other authors [3, 19]. It should also be noted that the difference in microbial biomass values for the tested layers revealed by direct microscopy was not so distinct.

Table 5. Coefficient of correlation (r) between microbial biomass (MB) values determined by the methods of SIR (MB_{SIR}) and microscopy (MB_M, 85% water and 45% C) and physicochemical properties of soils (coefficients given in italics are significant at $p < 0.05$; $n = 11$ and 9 for the layers of 0–5 and 5–10 cm, respectively

	C_{org}	pH	Silt	MB_{SIR}	MB_M	MB_M*
	0–5 cm					
MB_{SIR}	*0.90*	–0.27	*0.70*	–	*0.89*	*0.90*
MB_M	*0.85*	–0.41	*0.70*	*0.89*	–	*1.00*
MB_M*	*0.85*	–0.40	*0.70*	*0.90*	*1.00*	–
	5–10 cm					
MB_{SIR}	*0.74*	–0.30	–0.16	–	*0.77*	*0.78*
MB_M	0.53	–0.33	–0.13	*0.77*	–	*1.00*
MB_M*	0.54	–0.32	–0.13	*0.78*	*1.00*	–

* Without taking into account the fungal spore biomass.

In the studied soils, the fraction of microbial biomass carbon in total organic carbon was, on average, 4 and 3% (SIR) for 0–5 and 5–10 cm, respectively, and about twice as high according to direct microscopy (85% H_2O, 45% C, without spores) (Table 4). This may be due to the fact that calcofluor stains both metabolically active and inactive fungal hyphae [15].

For the studied soils, close correlation has been found between microbial biomass values determined by the methods of SIR and direct microscopy. Other researchers also note a reliable correlation between MB values determined by the methods of fumigation–incubation [1] or SIR [4–6] and direct microscopy. However, there is also information about the absence of such correlation [7, 20].

Thus, microbial biomass values for different soils measured by the methods of direct fluorescence microscopy and SIR were as a whole close for the upper layer (0–5 cm) of tested soils. In the lower soil layer (5–10 cm), the differences between the measured values were probably determined by different activity, first of all, of fungal mycelium.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research, projects no. 07-04-00628 and 07-04-00631, and by the scientific school grant no. 3096.2006.4.

REFERENCES

1. Jenkinson, D.S., Powlson, D.S., and Wedderburn, R.W., The Effects of Biocidal Treatments on Metabolism in Soil. III. The Relationship Between Biovolume Measured by Optical Microscopy, and the Flush of Decomposition Caused by Fumigation, *Soil Biol. Biochem.*, 1976, vol. 8, no. 2, pp. 189–202.
2. Brookes, P.C., Heijnen, C.E., McGrath, S.P., and Vance, E.D., Soil Microbial Biomass Estimates in Soils Contaminated with Metals, *Soil Biol. Biochem.,* 1986, vol. 18, no. 4, pp. 383–388.
3. Beck, T., Joergensen, R.G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H.R., and Scheu, S., An Inter-Labaratory Comparison of Ten Different Ways of Measuring Soil Microbial Biomass, *Soil Biol. Biochem.*, 1997, vol. 29, no. 7, pp. 1023–1032.
4. Lin, Q. and Brookes, P.C., Comparison of Substrate-Induced Respiration, Selective Inhibition and Biovolume Measurements of Microbial Biomass and Its Community Structure in Unamended, Reygrass-Amended, Fumigated and Pesticide-Treated Soils, *Soil Biol. Biochem.*, 1999, vol. 31, no. 14, pp. 1999–2014.
5. West, A.W. and Sparling, G.P., Modification to Substrate-Induced Respiration Method to Permit Measurement of Microbial Biomass of Different Water Contents, *J. Microbiol. Methods*, 1986, vol. 5, pp. 177–189.
6. Neely, C.L., Beare, V.H., Hargrove, W.L., and Coleman, D.C., Relationship between Fungal and Bacterial Substrate-Induced Respiration, Biomass and Plant Residue Decomposition, *Soil Biol. Biochem.*, 1991, vol. 23, no. 10, pp. 947–954.
7. Ingham, E.R. and Horton, K.A., Bacterial, Fungal and Protozoan Responses to Chloroform Fumigation in Stored Soil, *Soil Biol. Biochem.*, 1987, vol. 19, no. 5, pp. 545–550.
8. Domsch, K.H., Beck, Th., Anderson, J.P.E., Soderstom, B., Parkinson, D., and Trolldenier, G., A Comparison of Methods for Soil Microbial Population and Biomass Studies, *Z. Pflanzenernaehr. Bodenkd.*, 1979, no. 142, pp. 520–533.
9. Kaiser, E.A., Muller, T., Joergensen, R.G., Insam, H., and Heinemeyer, O., Evaluation of Methods to Estimate the Soil Microbial Biomass and the Relationship with Soil Texture and Organic Matter, *Soil Biol. Biochem.*, 1992, vol. 24, pp. 675–683.
10. Schnurer, J., Clarholm, M., and Rosswall, T., Microbial Biomass and Activity in An Agricultural Soil with Different Organic Carbon Contents, *Soil Biol. Biochem.*, 1985, vol. 17, no. 6, pp. 611–618.
11. Anderson, J.P.E. and Domsch, K.H., A Physiological Method for the Quantitative Measurement of Microbial Biomass in Soils, *Soil Biol. Biochem.*, 1978, vol. 10, no. 3, pp. 215–221.
12. Kozhevin, P.A., *Mikrobnye populyatsii v prirode* (Microbial Populations in Nature), Moscow: Mosk. Gos. Univ., 1989.
13. Polyanskaya, L.M., Golovchenko, A.V., and Zvyagintsev, D.G., Microbial Biomass in Soils, *Doklady AN,* 1995, vol. 344, no. 6, pp. 846–848.
14. Velvis, H., Evaluation of the Selective Respiratory Inhibition Method for Measuring the Ratio of Fungal : Bacterial Activity in Acid Agricultural Soils, *Biol. Fertil. Soils*, 1997, vol. 25, pp. 354–360.
15. Stahl, P.D. and Parkin, T.B., Relationship of Soil Egrosterol Concentration and Fungal Biomass, *Soil Biol. Biochem.*, 1996, vol. 28, pp. 845–855.

16. Stahl, P.D., Parkin, T.B., and Eash, N.S., Sources of Error in Direct Microscopic Methods for Estimation of Fungal Biomass in Soil, *Soil Biol. Biochem.*, 1995, vol. 27, pp. 1091–1097.
17. Ingham, E.R. and Klein, D.A., Soil Fungi: Relationships between Hyphal Activity and Staining with Fluorescein Diacetate, *Soil Biol. Biochem.*, 1984, vol. 16, pp. 273–278.
18. Polyanskaya, L.M., Orazova, M.K., Burkanova, O.A., and Zvyagintsev, D.G., Microsampling of Rhizosphere Soil and Laboratory Artifacts, *Mikrobiologiya*, 2000, vol. 69, no. 4, pp. 586–589 [*Microbiology* (Engl. Transl.), vol. 69, no. 4, pp. 486–489].
19. Evdokimova, G.A. and Mozgova, N.P., Microflora of Tundra Soils Of the Kola Peninsula, *Pochvovedenie*, 1995, no. 12, pp. 1487–1497.
20. Wardle, D.A., Yeates, G.W., Watson, R.N., and Nicholson, K.S., Response of Soil Microbial Biomass and Plant Litter Decomposition to Weed Management Strategies in Maize and Asparagus Ecosystems, *Soil Biol. Biochem.*, 1993, vol. 25, pp. 857–868.