

## MICROBIAL BIOMASS IN SOILS OF THE REPUBLIC OF MOLDOVA: ESTIMATION AND RESTORATION

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### Abstract

The content, reserves and profile distributions of microbial biomass in the different soil types and subtypes of the Republic of Moldova have been investigated in the connection of the soil carbon sequestration and environmental protection. Sampling was carried out in 11 profiles per soil horizons to a depth of 170 - 200 cm and from 0 - 30 cm layer separately. Database was evaluated statistically by the variance and correlation analysis. The negative effects on soil microbial biomass were observed as a result of erosion processes and long-term land management practices without of organic fertilizers. The content and reserves of microbial carbon in soil profiles decreased with its depth. The reserves of microbial biomass in virgin and fallow soils were 5.9-12.7 t ha<sup>-1</sup>, in arable soils with the normal profile – 3.6-7.2 t ha<sup>-1</sup>, in eroded arable soils – 1.6-1.9 t ha<sup>-1</sup> in the 0 - 100 cm layer. The microbial biomass was connected with the humus content and amounts of agronomic valuable aggregates. Correlation coefficients constitute 0.58-0.97 and 0.86 respectively. The significance of microorganisms in the formation of the water-stable structure in soils is discussed. The organic farming system with the application of 50 t ha<sup>-1</sup> of manure and green manure crops returns the organic matter to the soil and creates conditions for the carbon stock. The microbial carbon content in the arable layer of the leached and ordinary chernozem increases by 1.5 times. The use of organic fertilizers has been recommended for the restoration of the microbial communities and improvement of carbon fluxes into degraded soils.

**Key words:** microbial biomass, soil, carbon, humus

Soil microbial community plays an exceptional role in organic matter transformations, nutrient cycles, the support of soil stability and the ecological aspects of sustainability of soil fertility [Zvyagintsev D., Dobrovolskaya T., et al., 2003; Inubushi K. et al., 2005]. Microbial carbon acts in soil as a “living engine”, realizing the global turnover of carbon and other elements, maintaining the soil fertility and protecting the soil from the contamination and degradation. Soil microbial biomass represents 1-5 % of the total carbon content, is estimated in arable soils of the Republic of Moldova as 3.6-7.2 t ha<sup>-1</sup> without calculation of the turnover rate and numbers of microorganisms generations [Senicovscaia I., and oth., 2012]. The index of the soil microbial biomass (or microbial carbon) are suggested for the estimation of soil quality [Kennedy A., Papendick R., 1995; Hargreaves, P., Brookes, P., et al., 2003] as well as for the stability of agricultural and natural ecosystems in conditions of anthropogenic impacts [Bending, G.D., Turner, M.K., et al., 2004;]. The objective was to obtain an indication of the microbial biomass status of the zonal soils and to assess the impact of the

long-term use of soils and erosion processes on the microbial carbon in soils from different regions and various management systems in the connection of the soil carbon sequestration and environmental protection.

### MATERIAL AND METHOD

Our comparative study has been performed in different zones of the Republic of Moldova. The content, reserves and profile distributions of microbial biomass of zonal arable soils with the normal profile in the condition of long-term field experiments were investigated in comparison with the undisturbed soils in natural ecosystems. Additionally, the microbial biomass parameters of one of the zonal soils (ordinary chernozem) were compared with soils of different degrees of erosion. Investigations were performed on the typical, leached, ordinary, xerophyte-forest chernozem and the gray forest soil. Sampling was carried out in 11 profiles per soil horizons to a depth of 170 - 200 cm and from 0 - 30 cm layer separately. The database of the soil microbial biomass indicator covers the period between 1986 and 2011. Soil samples were also collected from

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sites with the organic fertilizers management (farm manure, green manure) from 0-25 cm layer.

The microbial biomass C was measured by the rehydration method based on the difference between C extracted with 0.5 M  $K_2SO_4$  from dried soil at 65-70°C within 24 h and fresh soil samples with  $K_c$  coefficient of 0.25 [Blagodatsky S., Blagodatskaya E. et al., 1987].  $K_2SO_4$  – extractable organic C concentrations in the dried and fresh soil samples were simultaneously measured by dichromate oxidation. The quantity of  $K_2SO_4$ -extractable C was determined at 590 nm with "CΦ-46" (Russia) and "Specol-221" (Germany) spectrophotometers. Stocks of microbial biomass have been calculated taking into account the carbon content of the microbial cell and the bulk density of soils [Senicovscaia I., 2001; Senicovscaia I., et. al., 2012]. Organic C was analyzed by the dichromate oxidation method [Ariushkina E., 1970]. The humus content was calculated using the coefficient of 1.724. Humus reserves were calculated taking into account the bulk density of soils. The microbial biomass index was evaluated statistically by the variance and correlation analysis.

## RESULTS AND DISCUSSIONS

Microorganisms of virgin and fallow soils exist in conditions of the high supply of the organic matter and its conservation within the limits of the ecosystem. As a consequence, soils in conditions of the natural ecosystems are characterized by a higher biomass of soil microorganisms in comparison with arable soils which are as a normal profile as eroded (fig. 1; 2). Microorganisms in natural soils are concentrated in the 0-60 cm layer (78-83 %), the biomass index decrease sharply in the soil profile to a depth of 30-50 cm.

In arable soils the base mass of microbes is concentrated in the 0-30 cm layer, while in the arable eroded soils – in the layer 0-10 cm. The highest level of the microbial biomass and organic carbon content have been determined in the  $A_1$  horizon of the virgin and fallow soils and whereas the lowest – in the BC and C horizons of all profiles. The quantity of the microbial biomass reaches in the virgin gray forest soil to 1631.1  $\mu g C g^{-1}$  soil, in the 40-year-old fallow typical chernozem – to 979.5  $\mu g C g^{-1}$  soil (fig. 1). Arable soils are characterized by the gradual decrease in the biomass with the depth as compared to soils of natural phytocenoses.

The reserves of the microbial biomass in 0-100 cm layer of natural soils with the normal profile constitute from 6.3 to 12.7, of eroded soil under fallow – 4.3 t dry matter  $ha^{-1}$  (fig. 2). The long-term use of plowing leads to the decrease of

the content and reserves of microbial biomass in arable soils as in the upper horizons, and as a whole in the soil profile. Profiles of the soil is covered by the degradation process as a whole. The reserves of the microbial biomass in the 0-100 cm layer of the modern arable soils is declined to the level of 3.6 - 7.2 t dry matter  $ha^{-1}$ .

The soil microbial biomass content decreased on average from 415.6-876.0 to 244.3-318.4  $\mu g C g^{-1}$  soil as a result of the long-term arable land management without the application of organic fertilizers (tab.1). The content of the microbial carbon in soils affected by the long-term arable use (layer 0-30 cm), lower in 1,2-3,6 times compared with soil-standards. This regularity is observed on the mean values of indicators as well as their confidence intervals. Confidence intervals of the microbial biomass of arable chernozems are approximately equal but differ from those the arable gray forest soil statistically. In the most cases, there is a high variability of the microbial biomass index, because the soil is characterized by the heterogeneity of habitats and the patchy distribution of microorganisms in the soil.

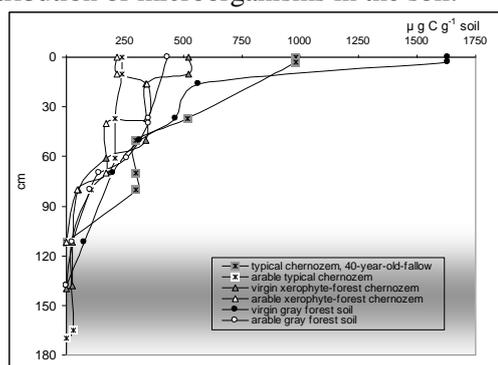


Figure 1. The profile distribution of microorganisms in the virgin, fallow and arable soils

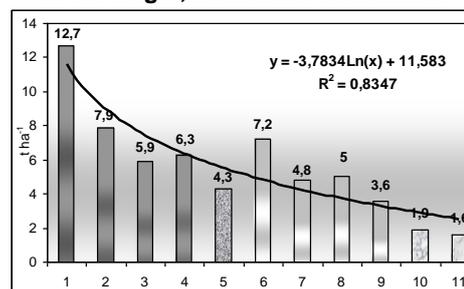


Figure 2. Reserves of microbial biomass in soils of natural and agricultural ecosystems (0-100 cm): 1– virgin gray forest soil (Orhei reg.); 2–typical chernozem, 40-year-old-fallow (Drochia reg.); 3–typical chernozem, 60-year-old-fallow (Beltsy t.); 4–virgin xerophyte-forest chernozem (Cahul reg.); 5–moderately eroded ordinary chernozem, 58-year-old-fallow (Cahul reg.); 6–arable leached chernozem (Orhei reg.); 7– arable typical chernozem (Drochia reg.); 8–arable xerophyte-forest chernozem (Cahul reg.); 9–arable ordinary chernozem (Cahul reg.); 10–moderately eroded arable ordinary chernozem (Cahul reg.); 11–severely eroded arable ordinary chernozem (Cahul reg.)

Table 1

**Statistical parameters of the microbial biomass content ( $\mu\text{g C g}^{-1}$  soil) in zonal soils of the Republic of Moldova under different land management (0-30 cm)**

Soil	Land use	n	min	max	Mean value	V, %	$S_x$	Confidence interval (P ≤ 0.05)
Typical chernozem	Fallow land (10-23-year-old)	10	244.8	584.3	355.8	35	39.1	267.5-444.1
	Arable land	12	238,6	520,8	318,4	26	23.9	265,9-370,9
Leached chernozem	Arable land	24	191,0	434,0	314,7	21	13.5	286,8-342,6
Ordinary chernozem	Fallow land (55-year-old)	6	389.31	452	415.6	8	13.6	380.7-450.5
	Arable land	20	176,9	360,9	288,3	17	10.8	265,8-310,8
Gray forest soil	Virgin land	8	529.0	1105.5	876.0	26	79.7	686.9-1065.1
	Arable land	18	119,6	331,2	244,3	28	16.3	209,9-278,7

The content of microbial biomass in the severely eroded chernozem amounts in average to  $110.0 \mu\text{g C g}^{-1}$  soil, which is 2.6 times less than in the soil with normal profile (tab. 2). A similar trend in decrease has been noticed in the confidence intervals, minimal and maximal values of this index. The variation coefficient of the microbial biomass index increased from 16.8% to 46.3%, which indicates that the equilibrium and

natural resistance of the soil microbial association decreases being higher in the chernozem with a normal profile and lower in the severely eroded soil. These negative changes reflect the catastrophic effect of erosion processes on soil microorganisms, destruction and loss of the most valuable compounds of soil organic matter – the microbial carbon.

Table 2

**Statistical parameters of the microbial biomass content ( $\mu\text{g C g}^{-1}$  soil) in eroded soils of the Republic of Moldova under different land management (0-30 cm)**

Land use	Degree of erosion	n	min	max	Mean value	V, %	$S_x$	Confidence interval (P ≤ 0.05)
Fallow land	Moderately eroded	13	376,6	496,1	445,5	10,6	13,1	417,0-474,0
Arable land	Normal profile (standard)	20	176,9	360,9	288,3	16,8	10,8	265,8-310,8
	Slightly eroded	14	112,6	336,9	224,6	31,6	19,0	183,6-265,6
	Moderately eroded	12	74,8	264,5	159,4	36,1	16,6	122,9-195,9
	Severely eroded	25	0	178,1	110,0	46,3	10,2	89,0-131,0

The application of fallow contributes to the increase of the microbial biomass and humus stocks in eroded chernozem. The microbial biomass content in 0-30 cm layer increased by 2.5, humus content - from 1.57% to 3.14% that represents an increase of 0.027% on average per year. The reserves of the microbial biomass in 0-170 cm layer increased from 1.6 to 4.3 t dry matter  $\text{ha}^{-1}$  (fig. 2). The microbial biomass accumulation in the soil was registered in amount of  $2.63 \text{ t ha}^{-1}$  which represents an average of  $45.4 \text{ kg ha}^{-1}$  per year. Humus reserves in the 0-100 cm soil layer increased from  $74.4 \text{ t ha}^{-1}$  to  $192.8 \text{ t ha}^{-1}$ ; the annual growth rate was of  $2 \text{ t ha}^{-1}$ . The transition of the eroded chernozem from the category of arable land to the category of fallow land increases microbial carbon stocks and carbon sequestration. The microbial biomass is connected with the humus content. The correlation coefficient ( $R^2$ ) between the biomass and humus content in the typical chernozem under arable constitutes 0.58, in conditions of 15-old-years fallow 0.76, of 60-old-years fallow 0.94. The profile distribution

of the microbial biomass demonstrated the close linear connection with the humus content in soils, the correlation coefficient reached 0.97. Strong positive correlation links were found between the microbial biomass and the amount of agronomic valuable aggregates,  $R^2 = 0.86$  (tab. 3). Microbial biomass in the typical chernozem is localized in fractions of 5-3, 3-2, 2-1 mm and partly in 7-5 mm fraction. The link between the microbial complex and the amount of fractions  $>10 \text{ mm}$  and  $<0.25 \text{ mm}$  is strongly negative,  $R^2 = -0.86$ . Water-stable aggregates formation was mainly influenced by the presence of microbial biomass in 5-3, 3-2, 2-1 mm fractions ( $R^2 = 0.51-61$ ). The correlation with the structure coefficient was strong –  $R^2 = 0.74$ . Carbon is usually the growth limiting factor for soil microorganisms. The restoration of the microbial biomass in degraded soils is possible on the base of farming conservation with the application of organic fertilizers. The

favorable effect of the farmyard manure and green manure management on the microbial biomass in the ordinary and leached

chernozem has been noted both as the average values of indicators and as the confidence intervals.

Table 3

The correlation coefficients between the content of microbial biomass and the aggregates content in the typical chernozem under different land management (0-25 cm)

Aggregates content with the diameter (mm)									Σ 10-0.25	Σ >10+<0.25	Ks
>10	10-7	7-5	5-3	3-2	2-1	1-0.5	0.5-0.25	<0.25			
Dry sieving											
-0.85	-0.16	0.56	0.90	0.82	0.81	-0.13	0.11	0.19	0.86	-0.86	0.74
Wet sieving											
-	-	-	0.51	0.54	0.61	-0.31	-0.72	-0.78	0.78	-0.78	-

The use of organic fertilizers increased the microbial biomass content from 212.6-254.3 to 300.9-371.8  $\mu\text{g C g}^{-1}$  soil in the 0-25 cm layer (fig. 3) and the humus content by 0.2%.

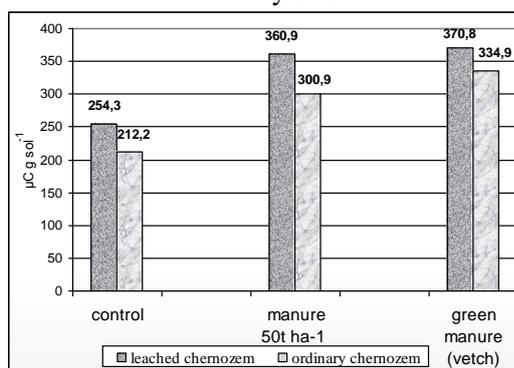


Figure 3. The influence of organic fertilizers on the microbial biomass content (mean values, n = 6-9)

## CONCLUSIONS

The long-term arable use of soils and erosion processes leads to the decline of the microbial biomass content and its reserves in the main zonal soil of the Republic of Moldova. A major reason for the reduction of the amount of the soil microbial carbon and for the decline of humus content under arable agriculture is annual tillage, which aerates the soil and breaks up aggregates where microbes are living. The catastrophic loss of the biomass of microorganisms represents a particularity of eroded chernozems and is one of the typical manifestations of the erosion process. The results have proved that the interaction between microbial components, the soil structure and humus status is closer in soils of natural ecosystems, especially in the layer of 0-25 cm. As a result, their resistance to natural and anthropogenic negative impacts is higher than that of the soils in agricultural ecosystems. The rupture and the attenuation of relations between the biotic and abiotic components of soils lead to the decrease in their natural stability and the development of degradation processes.

The organic farming system with the application of 50 t ha<sup>-1</sup> of manure and green manure crops returns the organic matter to the soil and creates conditions for the carbon stock. The microbial carbon content in the arable layer of the leached and ordinary chernozem increases by 1.5 times. The use of organic fertilizers has been recommended for the restoration of the microbial communities and improvement of carbon fluxes into degraded soils.

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