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BIOTA'S EVOLUTION OF GRAY FOREST SOILS IN THE CENTRAL ZONE OF THE REPUBLIC OF MOLDOVA

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Abstract

The biota status of virgin and arable gray forest soils in the central zone of the Republic of Moldova has been investigated statistically. Sampling was carried out in profiles per soil horizons and from 0-30 cm layer separately. Database of invertebrates, microorganisms and enzymatic activities has been formed. The current status of biota of the old-arable gray forest soil is characterized by the significant reduction in comparison with the level of the 1960s and with soil's standards that are in conditions of natural ecosystems. The highest values of biota's abundance were registered in virgin soils in the forest with the exception of humus-mineralizing microorganisms. Number of invertebrates was 169.3-222.3 ex m^{-2}, Lumbricidae family - 63.6-102.4 ex m^{-2} and microbial biomass - 686.9-1065.1 µ g C g^{-1} soil respectively. Biological indices in soil profiles decreased with the depth and depended of the form of farming system. The negative effects on biota and humus status have been observed as a result of the long-term land management practices without organic fertilizers. The growth of humus-mineralizing microorganisms has been noticed. Annual losses due to mineralization processes account for 0.01%. Trends and regression equations describing the growth of humus-mineralizing microorganisms and the fall of humus content in old-arable gray forest soils have been calculated.

***Key words**: biota, humus, gray forest soil, mineralization process, land management.*

INTRODUCTION

Soil biota should be considered as a component of the integrated management of natural resources. Microbial soil quality indicators are widely used in the ecological management of Europe's forests (Raubuch and Beese, 1995). In Canada, the forest certification process in environmentally sustainable forest management is associated with applying of microbial soil quality indicators (Staddon et al., 1999). Database of soil biota-standards is essential for determination of the mechanism of biota's natural resistance to changes of the fluctuation of climatic conditions and research of different aspects of biodiversity, degradation processes diagnostics and ecological certification of soils (Senicovscaia et al., 2012). Soil biological indicators contain the information about the landscape stability and environmental benefits for the agricultural politics at the national level. The use of soil bio-indication as an integrated monitoring tool for soil degradation might serve as a prospect solution (Ananieva et al., 2002).

The process of destruction of natural ecosystems and intensification the biological degradation of soils are interrelated. Investigations of soil biota in conditions of natural standard sites that are in equilibrium, preserving all basic parameters and have not lost their ecological and genetic links with components of the landscape are very important. At that point, the ideal research object is biota of gray forest soils (grayzems) located in the central zone of the Republic of Moldova. Evolution of the biota of these soils is interesting also from the point of view of the national soil quality standards.

The purpose of the research was to determine the influence of different land management practices on soil biota's state in gray forest soils aiming to develop the scale parameters of their stability for the national soil quality standards.

MATERIALS AND METHODS

The experimental site is located in the central zone of the Republic of Moldova, in the wooded steppe of the central - Moldovan forest province, in the district No. 8 of gray forest soils and leached chernozems of the wooded steppe of hilly Kodru Forests, in the Ivancha village, Orhei region (Figure 1). Biota's state in the gray forest soil in the condition of long-term arable has been investigated in comparison with virgin soils in the old-growth (primary) forest in conditions of natural ecosystems. The soil is a gray forest soil.

Sampling for microbiological and enzymatic indicators was carried out in 2 profiles per soil horizons to a depth of 140 cm in 2005 and from 0-30 cm layer separately during 1988-1998 and 2005-2010. Additionally, some microbiological indicators were compared with the level of the 1960s. Invertebrates sampling was carried out from 14 soil semi-profiles to a depth of 60 cm in 2009 and 2010. Furthermore the database of invertebrates for the period 1991-2006 has been used (Demchenko, 2006). Thus, the database of soil biological indicators covers the period between 1958 and 2010.

Figure 1. Fragments of natural and agricultural landscapes located in the central zone of the Republic of Moldova

Status of invertebrates was identified from test cuts by manually sampling the soil layers to the depth of soil fauna occurrence (Gilyarov, 1965). Diversity at the family level and classification according to feeding habits were categorised according to Gilyarov and Striganova (1987).

Microbiological properties. The microbial biomass carbon (C) was measured by the rehydration method (the difference between C extracted with 0.5M K_2SO_4 from fresh soil samples and from soil dried at 65-70^oC for 24h (Blagodatsky et al., 1987). Reserves of MB have been calculated taking into account the carbon content of the microbial cell and the bulk density of soils. Counts of culturable microorganisms (heterotrophic bacteria, humus-mineralizing microorganisms, actinomycetes, bacteria from the family of the *Azotobacter* and fungi) were obtained on agar plates (Zvyagintsev, 1991).

Enzymatic activity. The potential enzymatic activity was determined in samples of the air-dry soil. The urease activity was measured by estimating the ammonium released on incubation of soil with buffered urea solution by colorimetrical procedure (Haziev, 2005). The catalase activity was determined by the volumetric method by the rate of hydrogen peroxide's decomposition during its interaction with the soil and by the volume of released oxygen (Galstyan, 1978). The dehydrogenase activity was determined by the colorimetric technique on the basis of triphenylformazan (TPF) presence from TTC (2,3,5-triphenyltetrazolium chloride) added to soil (Haziev, 2005). The polyphenoloxidase and peroxidase activities were determined by the colorimetric technique using hydroquinone as a substrate (Karyagina and Mikhailovskaya, 1986).

Soil chemical properties. Organic carbon was determined by dichromate oxidation; the humus content was estimated using the coefficient 1.724 (Arinushkina, 1970).

Soil biological indices were evaluated by analysis of variance and correlation. Statistical parameters of soil invertebrates were calculated taking into account the depth of soil fauna occurrence, microorganisms and enzymes - for the layer of 0-30 cm.

RESULTS AND DISCUSSIONS

Comparison of biota in virgin and arable gray forest soils. The number of invertebrates in the virgin gray forest soil amounts to 169.3-222.3 ex m^{-2}, *Lumbricidae* family - to 63.6-102.4 ex m^{-2}, and its biomass - to 29.1-64.7 and 23.9-59.1 g m^{-2} accordingly (Table 1). The number of saprophagous constitutes of 90.4-127.8 ex m^{-2} that is 53.4-57.5% of the total number of invertebrates. The share of earthworms in the total abundance of invertebrates in the soil of natural ecosystems constitutes of 37.6-46.1% and their biomass - 82.1-91.3%. The average weight of one exemplar of *Lumbricidae* family in the virgin gray forest soil constitutes 0.5 g. Indices of invertebrates' number and biomass in the virgin soil are characterized by the medium and considerable variability (27-67%). Virgin soils are characterized by a high diversity of invertebrates. In addition to the *Lumbricidae* family species from the families of *Formicidae*, *Enchytraeidae*, *Elateridae*, *Carabidae, Scarabaeidae, Araneae, Apidae, Pieridae, Cerambycidae, Scutelleridae, Tenebrionidae, Coccinellidae* and other have been found in the virgin soil.

Table 1. Statistical parameters of biota in the gray forest soil under different land management in the Central zone of the Republic of Moldova

Index	Virgin gray forest soil						Arable gray forest soil					
	min	max	mean value	V,%	confidence interval ($P \leq 0.05$)	n	min	max	mean value	V,%	confidence interval ($P \leq 0.05$)	n
Invertebrates												
Number of invertebrates, ex m^{-2}	96.0	248.0	195.8	27	169.3-222.3	12	25.0	192.0	63.8	74	46.6-81.0	22
Biomass of invertebrates, g m^{-2}	16.4	100.8	46.9	60	29.1-64.7	12	4.5	9.8	7.6	27	6.5-8.6	12
Number of *Lumbricidae* fam., ex m^{-2}	40.0	144.0	83.0	37	63.6-102.4	12	14.0	104.0	43.2	71	32.0-54.4	22
Biomass of *Lumbricidae* fam., g m^{-2}	7.4	90.8	41.5	67	23.9-59.1	12	4.3	9.6	6.8	27	5.8-7.7	12
Microorganisms (0-30 cm)												
Microbial biomass, µ g C g^{-1} soil	529.0	1105.5	876.0	26	686.9-1065.1	8	119.6	331.2	244.3	28	209.9-278.7	18
Heterotrophic bacteria, CFU g^{-1}soil*10^6	4.1	9.9	5.9	36	4.8-8.0	15	2.3	4.8	3.3	10	3.3-3.4	33
Humus-mineralizing microorganisms, CFU g^{-1} soil*10^6	1.2	3.6	1.9	46	1.1-2.8	15	5.0	11.4	8.9	15	8.6-9.2	33
Actinomycetes, CFU g^{-1} soil*10^6	0.9	4.3	2.5	42	1.4-3.6	15	0.9	3.1	2.4	10	2.38-2.42	33
Fungi, CFUg^{-1} soil*10^3	92.0	130.0	110.0	11	98.1-121.9	15	24.0	70.0	40.5	30	33.5-47.6	33
Azotobacter gen., CFUg^{-1} soil	0	0	0	0	0	15	0	52.0	8.1	26	1.6-14.5	33
Enzyme activity (0-30 cm)												
Urease, mg NH_3 10 g $^{-1}$soil 24 h^{-1}	6.2	9.9	8.1	18	5.4-9.7	6	0.3	2.5	1.4	51	0.9-1.9	10
Catalase,	2.1	5.8	3.9	36	3.1-4.7	14	0.8	3.5	2.2	39	1.8-2.6	22
Dehydrogenase, mg TPF 10g^{-1} soil 24h^{-1}	2.00	2.76	2.40	11	2.13-2.67	6	0.25	1.50	0.74	42	0.59-0.89	20
Polyphenoloxidase, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	1.5	8.5	4.1	70	1.1-7.1	6	1.0	4.5	2.3	41	1.9-2.7	22
Peroxidase, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	22.5	32.5	27.8	15	23.4-32.2	6	14.0	31.0	25.7	20	23.4-28.0	22

The soil in conditions of natural ecosystems contains 12 families of invertebrates. About 15 species of invertebrates have been identified in the virgin gray forest soil. The following species have been detected in the virgin gray forest soil: *Lumbricus terrestris, Apporectodea roseus, Glomeris marginata, Diplopoda De Blainville, Dorcadion fulvum, Eurygaster maura, Bombus terrestris, Carabus convexus, Scolopendra cingulata, Coccinella septempunctata, Galeruca tanaceti, Blaps mucronata, Formica rufa* et al. Saprophagous prevail in the composition of the edaphic fauna in the virgin soil. They comprise 88.6% of the total number of invertebrates in the virgin gray forest soil.

The total biomass of microorganisms in the soil under forest ranges from 529.0 to 1105.5 μ g C g^{-1} soil in the 0-30 cm layer. It is much greater than its abundance in arable soils. A similar trend has been noticed in the number of the heterotrophic bacteria and fungi. But the number of the humus-mineralizing microorganisms and actinomycetes in most cases is much lower than in the arable soil. *Azotobacter* genus in the gray forest soil is found out occasionally in conditions of the arable management.

The current status of the biota in the arable gray forest soil is characterized by the significant reduction in the abundance, biomass and activity in comparison with soil's standards that are in conditions of natural ecosystems (Table 1). Indices of the number and biomass of invertebrates and earthworms decreased in the arable soil by 3.1-6.2 and 1.9-6.1 times respectively in comparison with the virgin soil. Arable gray forest soil contains only 2-5 families of invertebrates. Species from the *Lumbricidae, Scarabaeidae, Diplopoda* and *Araneae* families prevail in the faunal samples.

Maintaining the functioning of microorganisms and their biomass's reserves in soils of agro ecosystems is determined by amounts of crop residues from the crop rotation, entering into the soil in conditions of arable land. The total number of fungi and heterotrophic bacteria in the gray forest soil under arable land is significantly lower than in the virgin soil, including extinct groups typical for litter. Bacteria are dominated in the structure of the microbial community; the share of fungi is reduced. *Penicillium, Mucor, Trichoderma* and *Fusarium* genus are predominated among the fungi.

The total microbial biomass content decreased on average from 876.0 to 244.3 μ g C g^{-1} soil as a result of the long-term arable land management without the application of organic fertilizers. The content of the microbial carbon in soils affected by the long-term arable use, is lower in 3.6 times compared with the soil-standard. This regularity is observed on the mean values of indicators as well as on their confidence intervals.

The characteristic feature of microbial communities of arable gray forest soils is the high content of the humus-mineralizing microorganisms and the low enzyme activity. In the most cases, there is a high variability of microbiological and enzymatic indices, the reason is that soil is characterized by the heterogeneity of habitats and the patchy distribution of microorganisms in the soil.

More intensive land-use involving soil tillage stimulates the microbial decomposition of organic matter and tends to result in a decrease in the humus content in the arable soil. The soil layer of 0-10 cm is exposed by the highest mineralization. The humus content in the 0-30 cm layer constitutes in average 3.61% in the virgin soil and 2.32% in the arable soil (Table 2).

Table 2. Humus content* in the gray forest soil under different land management (%)

Depth, cm	Virgin land	Arable land
0-10	6.83	2.28
10-20	2.38	2.37
20-30	1.62	2.31
0-30	3.61	2.32

*mean values (n=10 for each layer)

Strong positive correlation links were found between the abundance of invertebrates and humus content in the 0-30 cm layer. The correlation coefficient (R^2) between the total number of invertebrates and humus content constitutes R^2 = 0.76; between the total biomass of invertebrates and humus content - R^2 = 0.85; between the biomass of *Lumbricidae* family and humus content - R^2 = 0.70 respectively.

Profile distribution of biota in the gray forest soil. A characteristic feature of virgin gray forest soils is the high concentration of invertebrates (91.8%) and *Lumbricidae* family (78.7%) in the upper layers of soils and in the litter (Figure 2). The migration of invertebrates into the underlying layers it is typical for the arable soils which were used for a long time under arable. Layer from 0 to 10 cm practically does not contain invertebrates.

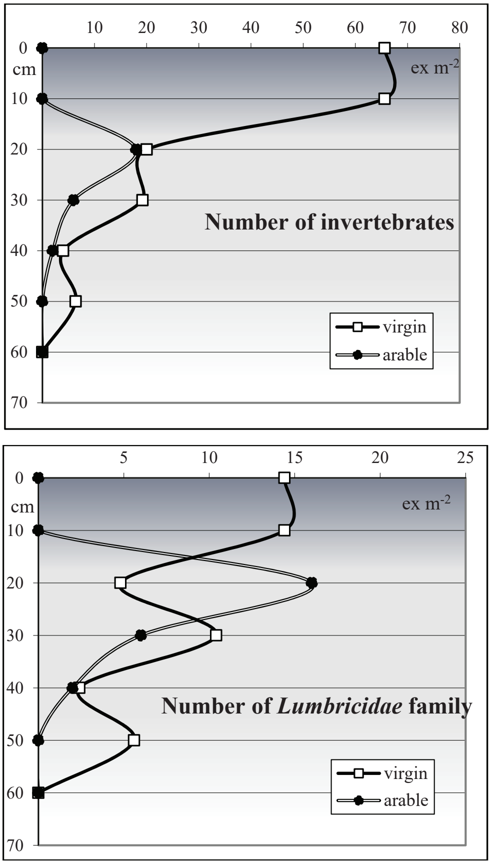

Figure 2. The profile distribution of invertebrates and *Lumbricidae* family in virgin and arable gray forest soils (mean values, data base of 2008-2009)

The highest level of the microbial biomass has been determined in the A_1 horizon of the virgin soils and whereas the lowest - in the BC and C horizons of both profiles (Figure 3). Microorganisms in virgin gray forest soils are concentrated in the 0-50 cm layer (81.8%), the biomass index decreases sharply in the soil profile to a depth of 30-50 cm. The concentration of microorganisms in the top layer reaches 1631.1 μ C g^{-1} soil. The reserves of the microbial biomass in 0-100 cm layer of virgin gray forest soil constitute 12.7 t dry matter ha^{-1}.

In arable soils the base mass of microbes is concentrated in the 0-30 cm layer. Arable soils are characterized by the gradual decrease of the biomass with the depth as compared to soils of natural ecosystems.

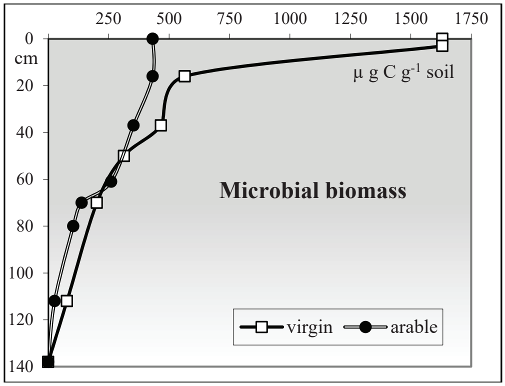

Figure 3. The profile distribution of microbial biomass in virgin and arable gray forest soils

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. A profile 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 arable gray forest soils is declined to the level of 7.9 t dry matter ha^{-1}.

The highest level of enzyme activities has been determined in the upper layers of virgin soil and whereas the lowest - at the depth of 60-120 cm in both profiles (Figure 4).

The activity of urease reaches in the virgin gray forest soil to 19.7 mg NH_3 10 g^{-1} soil 24 h^{-1}. The similar trend has been determined also for catalase and dehydrogenase activities. Arable soils are characterized by the gradual decrease in the enzymes activity with the depth as compared to virgin soils.

So, the profile distribution of soil biota in virgin soil is a classic phenomenon, when a major amount of biota is concentrated in the top layer. This is the result of the fact that the main mass of leaf litter is concentrated in the upper soil layers.

The distribution of the biota in the profile of old-arable soil is more uniform. This agrees with the fact that first three horizons of arable forest soils were mixed in one as a result of use in agriculture (Lungu, 2015).

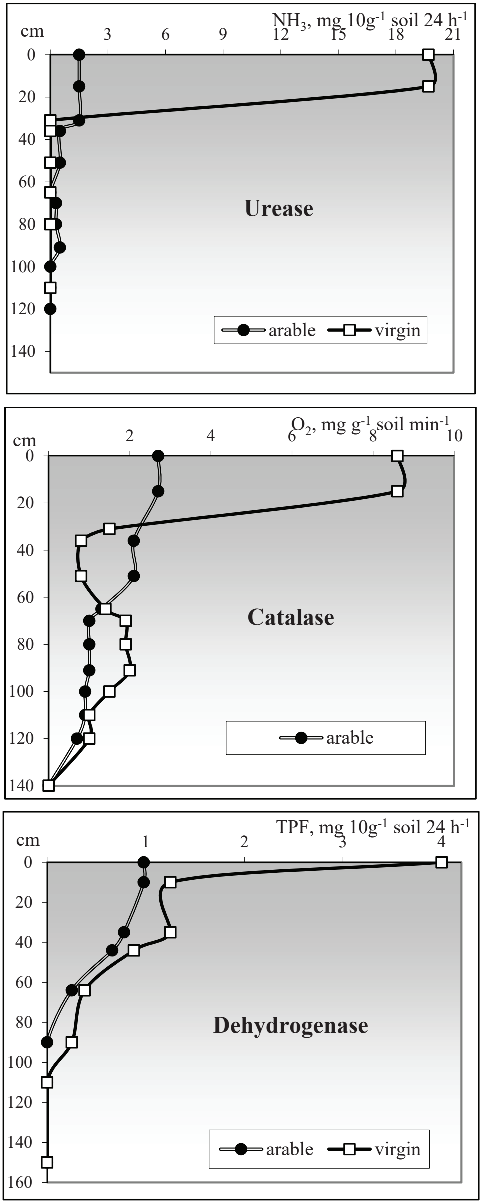

Figure 4. The profile distribution of enzymes in virgin and arable gray forest soils

Evolution of the biota and humus status in arable gray forest soils. The current state of biota of arable gray forest soils in the conditions of agricultural ecosystems of the Republic of Moldova is characterized by a decline of biota's abundance and activity compared to the level registered in 1960s (Senicovscaia, 2015). The biological degradation of arable soils is interconnected with the dehumification processes, compaction and destruction of the soil structure. A major reason for the deterioration of soil biological properties 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 selection process of species that can survive in conditions of a lower organic matter content and deterioration of physicochemical parameters of soil systems is taking place among the microorganisms.

A characteristic feature of long-term dynamics of arable gray forest soils is a significant decrease in the number of fungi (Figure 5). The time trend of the fungi is described by the polynomial function and reveals the strong link (R^2 = 0.87). Statistically significant changes in the number of heterotrophic bacteria have not been fixed.

During the 52 years of the utilization of arable soils the humus content decreased on average by 20-25% from its initial level. Annual losses due to mineralization processes account for 0.01%. Mineralization processes are dominated in soils, degraded as a result of long-term arable use. The growth of humus-mineralizing microorganisms has been noticed (Figure 6).

The database of humus-mineralizing microorganisms and the humus content was processed separately by the correlation and regression analysis during periods of observations (Figure 6). Regression equations with the highest correlation coefficients were chosen from all of the regression equations. Humus-mineralizing microorganisms' trend is described by the power function. Trend has the high correlation coefficients: 0.69.

Trend of the humus content is described by the polynomial function. Correlation coefficient constitutes 0.89. These results indicate that the humus content was closely linked to the content of humus-mineralizing microorganisms in soil.

The intensification of mineralization processes in arable gray forest soils leads to a steady decline in the humus content and reserves.

The temporary long-term variability of the enzymatic complex of arable gray forest soils managed to trace to the catalase activity (Figure7). Soil has lost about 50% of their catalase activity in comparison with the initial level as a result of 52 years of arable use.

It was found that catalase activity is characterized by a trend of decreasing activity described by the polynomial equation. Trend has the high correlation coefficients: 0.63.

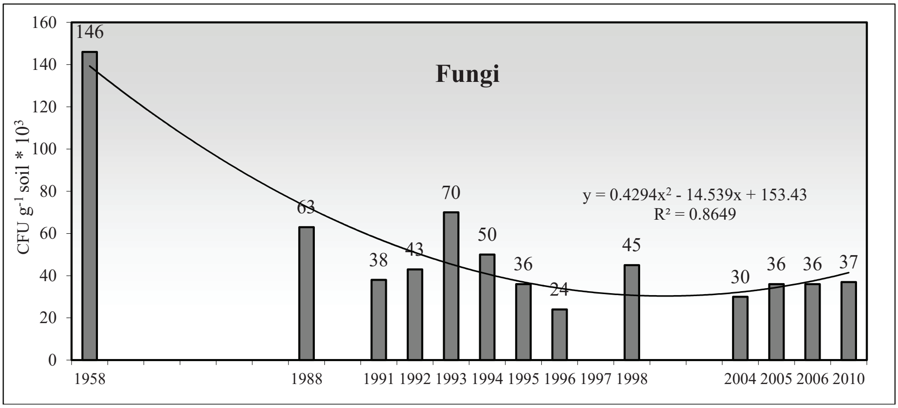

Figure 5. Dynamics of the fungi's content in arable gray forest soils

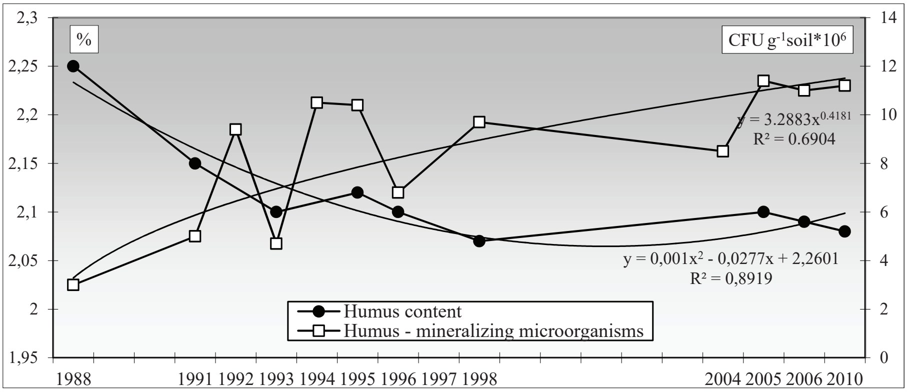

Figure 6. Dynamics of the humus and humus-mineralizing microorganisms' content in arable gray forest soils

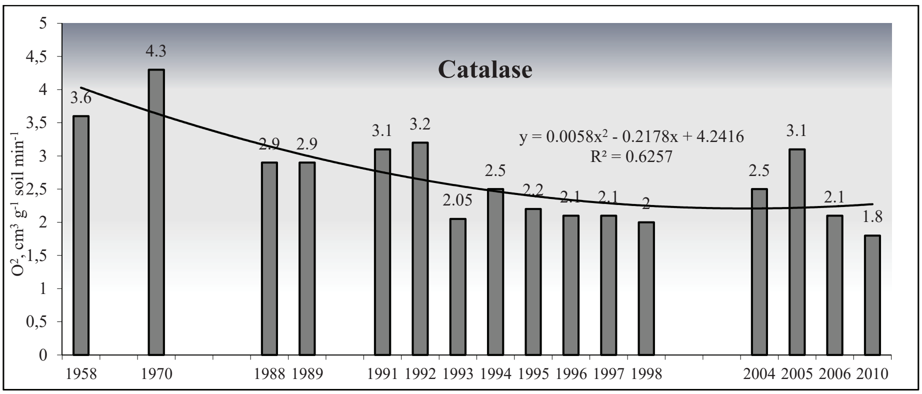

Figure 7. Dynamics of the catalase activity in arable gray forest soils

CONCLUSIONS

Virgin gray forest soils are ideal standards in regard to the composition, biomass and activity of the biota. The soil biota's composition in natural ecosystems is complex and diverse. Forests are habitat and source of conservation and reproduction of the edaphic fauna and soil microorganisms.

Biological indices in soil profiles decreased with the depth and depended of the form of farming system. The highest values of biota's abundance were registered in the A_1 horizon of virgin soils with the exception of humus-mineralizing microorganisms. Number of invertebrates was 169.3-222.3 ex m^{-2}, *Lumbricidae* family - 63.6-102.4 ex m^{-2} and microbial biomass - 686.9-1065.1 μ g C g^{-1} soil respectively. The topsoil is a unique locus with a high enzymatic activity.

The agricultural management without application of organic fertilizers leads to soil degradation. This reflects in deterioration of soil biological properties and in reduction of humus content in soil. In the arable soil humus-mineralizing microorganisms dominate.

The evolution of biota in the old-arable gray forest soil is characterized by the significant reduction in the number, biomass, activity and diversity in comparison with the level of the 1960s and with soil's standards that are in conditions of natural ecosystems. Time trends are described by the power and polynomial function with high correlation coefficients.

Statistical parameters of the biota's state of virgin and arable gray forest soils have a practical importance for the operative estimation of effectiveness the degradation processes and agricultural practices for the conservation of soil quality.

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