Monitoring and recovery of the soil biota in conditions of the degradation processes intensification in the Republic of Moldova

Senicovscaia, Irina

“Nicolae Dimo” Institute of Pedology, Agrochemistry and Soil Protection, Kishinev, Republic of Moldova

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MONITORING AND RECOVERY OF THE SOIL BIOTA IN CONDITIONS OF THE DEGRADATION PROCESSES INTENSIFICATION IN THE REPUBLIC OF MOLDOVA

IRINA SENICOVSCAIA[1]

Abstract. *Statistical parameters, criteria and scales of the soil biota stability to the natural and anthropogenic impact in the Republic of Moldova has been developed. The modifications of the biological properties of soils as a result of their long-term arable use and the impacts of erosion processes have been established. The zones of homeostasis (natural stability) of the biota of different soils have been determined for the first time at the national level. The current status of biota of arable soils is characterized by the significant reduction in comparison with soil's standards that are in conditions of natural ecosystems and with the level of the 1960s. The scales of soil biological indicators for the evaluation of the degree of chernozems' degradation and its environmental certifications have been developed. The application of no-tillage technology on the soils of the Republic of Moldova has been discussed.*

Key-words: *soil biota, degradation, natural stability, recovery, quality standards.*

INTRODUCTION

Soil global degradation has acquired a catastrophic character and is one of the main causes of global environmental crisis [6]. The current state of the soil cover in the Republic of Moldova is characterized by the intensification of the processes of degradation and desertification [2]. The decline in the organic matter content and the compaction of the arable soils with the normal profile is one of the main manifestations of the degradation processes in agricultural lands. There has been a significant deterioration of the conditions needed for the vital activity of soil invertebrates and microorganisms.

In the balanced ecosystems processes of microbial decomposition of organic matter and its synthesis are closely linked with the growth of plants that provides the stable existence of undisturbed ecosystems during long periods of time. In degraded ecosystems the equilibrium is disturbed, mineralization processes are predominant in them. The stable deviation of biota indices from the equilibrium state of the parameter values either displaying an increase or decline indicates essential ecological changes or the destruction of soil ecosystem [4, 12 and 13]. The changes in biological properties may indicate the risk of likely soil degradation as a result of human activity. The basic criterion to evaluation the stability of soil biota to anthropic influence is the capacity to preserve and maintain the dynamic equilibrium or homeostasis [11 and 12]. In this respect, the use of soil bio-indication as an integrated monitoring tool for soil degradation might serve as a prospect solution [1].

The purpose of the research was to determine the effect of different anthropogenic and natural impacts on biota's state in zonal soils aiming to develop the scale parameters of their stability for the national soil quality standards and to estimate the effect of *no-tillage* technology on the biota's restoration of degraded soils.

MATERIAL AND METHODS

Experimental sites and soils. Our comparative study has been performed in three zones of the Republic of Moldova. Land management practices in the condition of long-term field experiments have been analyzed.

The first site was in the north, on the long-term field experiments of the Research Institute for Field Crops "Selectia" (Beltsy). It had 3 plots: fallow land (10-23-year-old), the long-term arable land with crop rotation (management without fertilizers) and black fallow established for 30-43 years. The soil was the typical chernozem with humus content of 5.06 % under lea, 4.17 % under arable

[1] Assistant of Professor, Ph.D, "Nicolae Dimo" Institute of Pedology, Agrochemistry and Soil Protection, Kishinev, Republic of Moldova, irina_sen@mail ru

land and 3.54 % under black fallow land. Soil samples were collected from the 0-30cm layer of the experimental plots during 1994-1998 and 2006-2007.

The second site was located in the center of the country, in the Ivancha village, Orhei region. The natural land under fallow (40-60-year-old), forests and the long-term arable land with crop rotation without fertilizers were tested. Soils were the leached chernozem with humus content of 3.42 % and pH = 6.9 and the gray forest soil with humus content of 2.32 % and pH = 6.6 in the 0-30 cm layer. Soil samples were collected during 1958-2006.

The third site was located on the territory of ”SoroAgro” SRL in the Rediu Mare village, Dondusheni region. Technology of *no-tillage* has been implemented for 2 years at the demonstration plot with the area of 106 ha. The area of control plot with conventional tillage (plowing to a depth of 25-27 cm) consists of 109 ha. The soil was presented by the deep heavy loamy leached chernozem with humus content of 3.71 % and pH = 6.66 in the 0-40 cm layer. The site has been tested in 2014.

The fourth site was located in the southern zone, in the Ursoaia village of the Lebedenco district, Cahul region. The soil of the site is an ordinary chernozem. Chernozems with different degrees of degradation caused by (slightly, moderately and severely eroded) erosion processes have been compared to the chernozem with a normal profile and the eroded soil which was under 58-years-old fallow. Soil samples were collected from the 0-30 cm layer of the experimental plots during the period of time between 1997 and 2008.

Status of invertebrates. The state of invertebrates was identified from test cuts by manually sampling the soil layers to the depth of soil fauna occurrence applying Gilyarov and Striganova’s method [7].

Microbiological properties. The microbial biomass carbon was measured by the rehydratation method based on the difference between carbon extracted with 0.5 M K_2SO_4 from fresh soil samples and from soil dried at 65-70ºC for 24h, with K_c coefficient of 0.25 [5]. Counts of microorganisms (bacteria, fungi and humus-mineralizing microorganisms) were obtained on agar plates [14].

Enzymatic activity. The urease activity was measured by estimating the ammonium released on incubation of soil with buffered urea solution by colorimetrical procedures [8]. 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 air-dry basis of soil [8]. The polyphenoloxidase and peroxidase activities were determined by the colorimetric technique with the use of hydroquinone as a substrate [9].

Chemical properties. Organic C was analyzed by the dichromate oxidation method [3]. The humus content was calculated using the coefficient of 1.724. Humus reserves were calculated taking into account the bulk density of soils.

Statistical analysis. Biological indices were evaluated statistically using the variation and correlation analysis. Statistical parameters of the state of soil invertebrates were calculated taking into account the depth of soil fauna occurrence, microbes and enzymes – for the layer of 0-30 cm.

RESULTS AND DISCUSSIONS

Evolution of the soil biota and humus content in arable soils. During the 50 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 0.01-0.02%. 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 1).

The current state of microorganisms of arable soils in the conditions of agricultural ecosystems of the Republic of Moldova is characterized by a decline in the number and activity compared to the level registered in 1960s (Figure 2). 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. The long-term use of plowing on soils with the whole profile leads to the decrease of microbial biomass by 1.6-3.6 times in the 0-30 cm layer. Profiles of the soil are covered by the degradation process as in the upper horizons, and as a whole. The reserves of microbial biomass in virgin and fallow soils constitute 5.9-12.7 t ha-1, in arable soils with the normal profile – 3.6-7.2 t ha-1, in eroded arable soils – 1.6-1.9 t ha-1 in the 0 - 100 cm layer. A characteristic feature of long-term dynamics of arable is a significant decrease in the number of bacteria and fungi. The reduction of the biochemical potential and diminution in the size of homeostasis zone of soil invertebrates and microorganisms result in the attenuation of natural soil stability. The size of the homeostasis' zone and natural stability of the biota in arable soils decline respectively: typical chernozem → leached chernozem → gray forest soil.

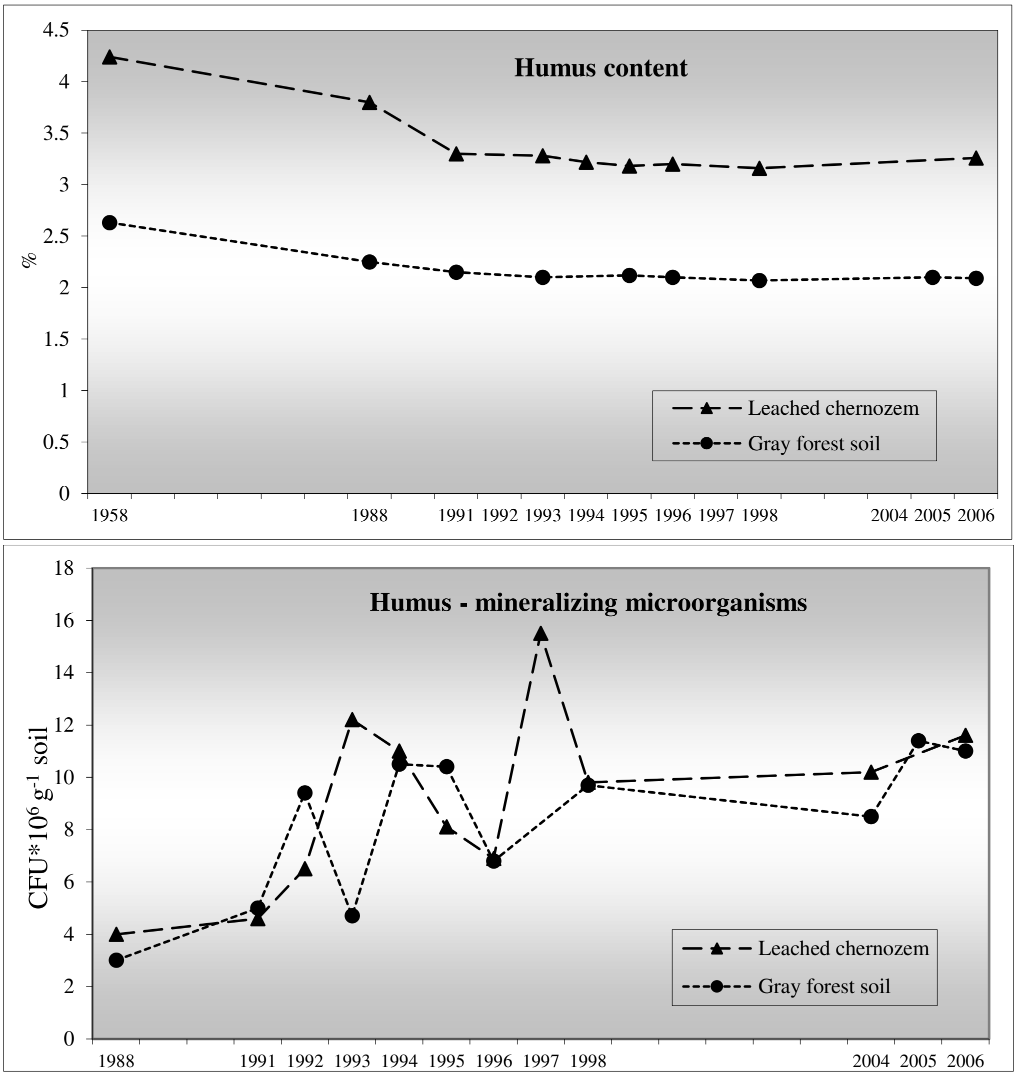

Figure 1. Dynamics of the humus and humus-mineralizing microorganisms' content in arable soils of the Republic of Moldova (0-30 cm)

For the arable soils which were used for a long time under arable it is characteristic the migration of invertebrates into the underlying layers. Layer from 0 to 10 cm practically does not contain invertebrates.

The level of enzymatic activity of modern arable soils for most indicators below in 40-70% compared with the level of the sixties of last century.

There is a tendency to reduce the activity of dehydrogenase and polyphenoloxidase - an enzyme participating in the transformation of organic compounds to humus components, and increase in activity of peroxidase. This fact indicates about the intensification of mineralization processes of organic matter in arable soils.

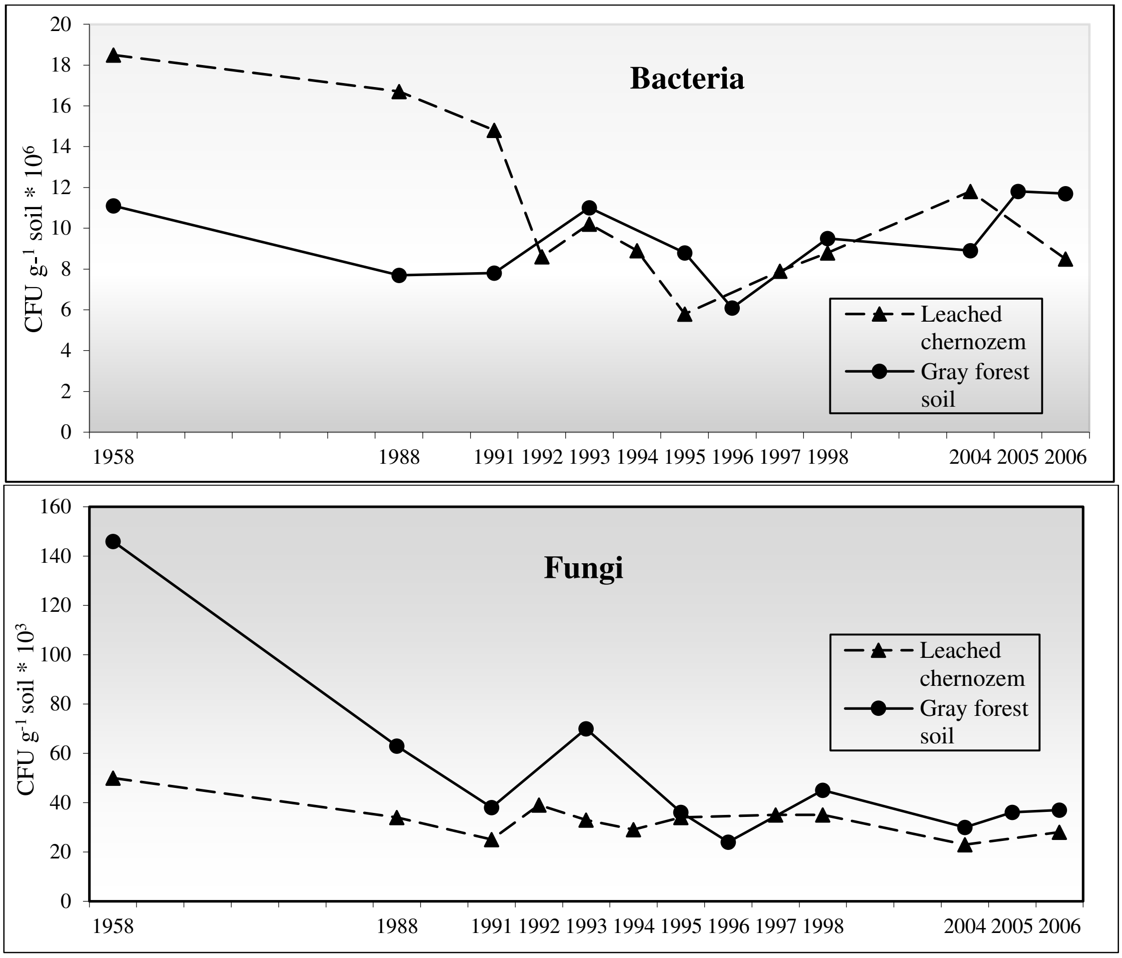

Figure 2. Dynamics of bacteria and fungi in arable soils of the Republic of Moldova (0-30 cm)

Evaluation criteria for biota's stability of zonal soil. Biota of virgin and fallow soils exists in conditions of the high supply of the organic matter and the conservation of resources that have been formed within the limits of the ecosystem. The soil biota of natural ecosystems is characterized by the highest resistance. The virgin and multiannual fallow soils are the place of storage and reproduction of different species of invertebrates and microorganisms, and also they have high levels of biomass and enzyme activity [11]. The values of most biological indicators in zonal soils decrease in the following sequence: natural and long-term fallow land → arable land without fertilizers → arable eroded land. The biological parameters of soils have been grouped according to the humus content. To assess the stability and degradation of arable soils and to carry out the ecological certification we suggest a set of faunal, microbiological and enzymatic criteria. The database of the biota's state of virgin (fallow) soils has a practical importance as the natural standard for the operative evaluation of degradation processes and ecological effectiveness of the land management (Table 1 and 2).

Table 1. Scale of biological parameters for the typical chernozem

Degree of stability	**Humus content, %**	**Invertebrates**		**MB, μ g C g^{-1} soil**	**Humus-mineralising microorganisms, CFU g^{-1} soil*10^{6}**	**Dehydrogenase, mg TPF 10g^{-1} soil 24h^{-1}**
		number, ex m^{-2}	biomass, g m^{-2}			
Very high	>5.1	>395	>35	>487	4.7-5.4	>3.6
High	4.9-5.1	317-395	24-35	275-487	5.4-7.5	2.2-3.6

Moderate	4.4-4.7	118-165	12-23	266-371	15.2-17.3	1.6-2.3
Low	3.5-3.7	0	0	82-136	2.6-4.7	0.1-0.4

Table 2. The evaluation criteria of biota's stability of the gray forest soil and the leached chernozem

Index	**Gray forest soil (homeostasis zone)**		**Leached chernozem (homeostasis zone)**
	virgin	**arable**	**arable**
Invertebrates			
Total number, ex m^{-2}	169 - 222	47 - 81	75 - 95
Number of *Lumbricidae* family, ex m^{-2}	64 - 102	32 - 54	52 - 71
Biomass of *Lumbricidae* family, g m^{-2}	24 - 59	4 - 14	8 - 11
Microorganisms			
Microbial biomass, µg C g^{-1} soil	687 - 1065	210- 279	287 - 343
Humus-mineralizing microorganisms, CFU g^{-1} soil*10^{6}	1.1 – 2.8	8.6 – 9.2	7.8 – 11.4
Fungi, CFU g^{-1} soil*10^{3}	98 - 122	34-48	27– 34
Enzymatic activity			
Urease, mg NH_3 10 g^{-1} soil 24 h^{-1}	5.4 – 9.7	0.9 – 1.9	2.1 – 4.1
Dehydrogenase, mg TPF 10 g^{-1} soil 24 h^{-1}	2.1 – 2.7	0.6 – 0.9	1.2 – 1.7
Polyphenoloxidase,mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	1.1 – 7.1	1.9 – 2.7	3.4 – 4.4
Humus content, %	4.0 – 5.7	2.1 – 2.4	3.2 – 3.8

Impact of *no-tillage* technology on the soil biota. The application of *no-tillage* technology has a positive effect on the biota in the leached chernozem. The rise of the total number of invertebrates and earthworms from 33.3 to 136.0 ex m^{-2} and their biomass from 1.7 to 23.9 g m^{-2} in conditions of *no-tillage* has been observed.

The dominant position in the composition of soil fauna occupies *Lumbricidae* family in *no-tillage* technology. Their share in the total number of invertebrates increases from 84.9% in the plot with conventional tillage to 92.2% in conditions of no-tillage technology. A similar tendency has been established in the indicators of biomass. Their share in the total biomass of invertebrates rises from 71.8% in the plot with plowing to 93.3% in conditions of *no-tillage* technology.

In the leached chernozem in conditions of conventional tillage earthworms are concentrated in the layer of 30-50 cm. On the contrary in the soil under *no-tillage* a major amount of invertebrates (92.6-100.0%) is located in the layer of 0-40 cm. The number of *Lumbricidae* family decreases gradually in the soil profile to a depth of 50 cm.

In the fauna of the leached chernozem *Lumbricidae* family occupies a dominant position also, their numbers amounts to 85.0-100.0%. The average weight of *Lumbricidae* family's representatives increased from 0.05 g on the plot with arable to 0.18 g on the plot with *no-tillage*. *Lumbricus terrestris* is the most typical specie of earthworms in the leached chernozem. *Allolobophora rosea* has been encountered in single copies.

Saprophagous predominated in both methods of tillage in investigated chernozems. The contribution of saprophagous to the total number of invertebrates in the leached chernozem is significant and constitutes 85.0-100.0% for both types of soil tillage. Invertebrates with mixed type of nutrition (*Formicidae* family) are significant in the plot with *no-tillage*. Ecological pyramids are incomplete, but the soil under *no-tillage* is characterized by greater stability compared to the conventional tillage due to larger number of saprophagous.

The increasing trend of microbial carbon content and the ratio between microbial and total carbon content have been recorded in the leached chernozem in conditions of *no-tillage* technology. There has also been indicated the increase of microbial biomass reserves under the application of *no-*

tillage. Reserves of microorganisms' biomass in the 0-40 cm layer are 4796.6-6449.9 kg ha^{-1} in the plot with plowing and 5649.4-6563.9 kg ha^{-1} in the plot with *no-tillage*.

Favorable conditions for the functioning of enzymes in soil under conditions of *no-tillage* have been found. The most powerful impact of the conservation technology on soil enzymes was recorded in the 20-40 cm layer.

Impact of erosion on biota's state. The humus loss as a result of erosion causes a sharp decline in the abundance, biomass and activities of soil biota. Indices of invertebrates' activities decreased in slightly, moderately and severely eroded soils by 15-30%, 31-62%, 63-78% respectively in comparison with the chernozem with a normal profile. The number of saprophagous decreased by 1.5 - 4, predators – by 2-3. An increase in phytophagous activity has also been observed on the basis of their absolute values. Their number may reach 40-48% of the total number of invertebrates. The ratio between saprophagous and phytophagous amounts in the ordinary chernozem with normal profile to 7.3, slightly eroded – 3.4, moderately eroded – 1.3, severely eroded – 1.0.

The total loss of invertebrates amounts to 30.9%, 49.9% and 68.4%. Erosional loss of *Lumbricidae* and *Enchytraeidae* families is quite significant and constitutes 31.7% and 79.5% in slightly eroded chernozems, 62.7% and 100.0% in moderately eroded chernozems, 66.0% and 100.0% in severely eroded chernozems. Some species from the *Formicidae*, *Curculionidae*, *Carabidae* family are not encountered in eroded soils.

The content of microbial biomass in the severely eroded chernozem amounts in average to 110.0 μ g C g^{-1} soil, which is 2.6 times less than in the soil with normal profile. 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. The losses of the microbial biomass as a result of erosion processes ranges from 213.6 to 496.2 kg ha^{-1} in slightly eroded, from 609.6 to 866.8 kg ha^{-1} in moderately eroded and from 1025.5 to 1070.5 kg ha^{-1} in severely eroded chernozems. The share of microbial carbon in the organic content falls from 1.66 % for soils with normal profile to 1.16% for severely eroded soils. A similar trend in decrease has been noticed in the confidence intervals of this index. 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.

The erosion degradation is manifested in the extreme loss of systematic groups of microorganisms and in the considerable reduction of their biodiversity. Diversity of the *Bacillus* family reduces from 14 to 7-10 species. Sorensen's indicator decreases when the erosion degrees increase from 1.0 to 0.66 and Shannon's diversity index – from 4.18 to 2.05 accordingly. The diversity of fungi species narrows; plant pathogen species of *Penicillium funiculosum, Penicillium purpurogenum, Aspergillus ochraceus* accumulates. *Trichoderma lignorum* species disappear completely in the severely eroded chernozem. The simplification of the actinomycetes species diversity and the accumulation of *Streptomyces* genus occur in eroded soils.

Eroded soils have a low enzymatic activity. The urease activity decreases by 17.5, dehydrogenase activity – by 2.3, polyphenoloxidase and peroxidase activities – from 1.4 to 1.5 in the severely eroded soil compared to the chernozem with normal profile. The losses of the urease activity constitute 68.6-94.3%, dehydrogenase – 19.1-55.6%, polyphenoloxidase – 6.5-32.8% and peroxidase - 10.9 to 28.7% depending on the degree of erosion. The declines in the enzymatic activity caused by erosion represents a complicated process conditioned by the deterioration of physical, chemical and physico-chemical properties of eroded soils, destruction of organic compounds, abrupt reduction in the microorganisms' biomass. When a mass of soil layers are washed away, eroded soils are not only losing the enzymes but also the conditions for their synthesis, immobilization and stabilization in remaining horizons are deteriorating.

Recovery of the biota and humus status under fallow. The eroded chernozem in conditions of a long-term fallow (58-year-old) is characterized by a higher number, biomass, activity and diversity of soil invertebrates in comparison with arable chernozems which are as eroded as a normal profile. Invertebrates (95%) are concentrated in the upper layer, the number and biomass indices decrease sharply in the soil profile to a depth of 20 cm. In arable eroded soils the base mass of invertebrates is concentrated in the 0-10 cm layer, while in the arable soil with the normal profile – in the layer 0-30 cm.

The volume of invertebrates' biomass accumulated during a 58 years period constitutes 507 kg ha^{-1} or 8.7 kg ha^{-1} annually, biomass of the *Lumbricidae* family – 464 kg ha^{-1} or 8.0 kg ha^{-1} annually. The application of the natural cover of wild grass, led to the restoration of the total number of invertebrates and the *Lumbricidae* family, the annual growth rate is of 4 and 3 ex m^{-2} accordingly.

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 ha^{-1}. The microbial biomass accumulation in the soil was registered in amount of 2.63 t ha^{-1} which represents an average of 45.4 kg ha^{-1} per year. Humus reserves in the 0-100 cm soil layer increased from 74.4 t ha^{-1} to 192.8 t ha^{-1}; the annual growth rate was of 2 t ha^{-1}.

The biological parameters of soils have been grouped according to the humus content. Five of twenty indicators have been selected for the estimation scale. A scale has been developed to estimate the biota' stability as a result of the erosion processes impact on the ordinary chernozem and its ecological certification (Table 3). The scale of the biological parameters can also be used to assess the quality of eroded soil.

Table 3. The estimation scale of biota' stability of the ordinary chernozem following the impact of erosion processes

Degree of stability	**Humus content, %**	***Lumbricidae* family**		**Microbial biomass*, µg C g^{-1} soil**	**Dehydro-genase, mg TPF 10 g^{-1} soil 24h^{-1}**	**Urease, mg NH_3 10 g^{-1} soil 24 h^{-1}**
		number, ex m^{-2}	biomass, g m^{-2}			
Very high	>3.2	>50	>8.6	417-474	>2.2	>4.0
High	2.9-3.2	43-50	6.0-8.6	266-311	1.3-2.2	3.0-4.0
Moderate	2.4-2.8	35-42	4.2-5.6	184-265	0.9-2.0	0.5-1.7
Low	1.8-2.3	17-21	3.6-4.6	123-196	0.7-1.8	0.2-0.8
Very low	<1.5	10-15	2.7-3.5	<123	<0.7	<0.2

The degree of the soil stability decreases with the raising of the level of its degradation. The size of homeostasis zones and biota's natural resistance in the ordinary chernozem decreases consecutively: chernozem under fallow → chernozem with the normal profile → slightly eroded chernozem → moderately eroded chernozem → severely eroded chernozem.

The level and size of homeostasis zones and therefore the biota stability reached the maximum levels in the fallow soil. Severely eroded chernozems have the lowest resistance.

CONCLUSIONS

The current state of the biota of arable soils in the conditions of agricultural ecosystems of the Republic of Moldova is characterized by a decline in the number, biomass, activity and diversity compared to the virgin and fallow soils of natural ecosystems and with the level of 1960 years. Mineralization processes are dominated in soils, degraded as a result of long-term arable use.

The invertebrates and microorganisms homeostasis is determined by the humus content. The level and size of the homeostasis' zones and consequently the biota' stability for each criterion were highest in the soil of natural ecosystems. The exception is the number of humus-mineralizing microorganisms. The arable soil has the lower stability.

The reduction of the biochemical potential and decrease of the size of the zone of homeostasis of soil invertebrates and microbes leads to the decline of the natural soil stability. To assess the resistance and degradation of arable soils and to realize of ecological certification we propose a set of faunistic, microbiological and enzymatic criteria.

The application of conservation *no-tillage* practices in conditions of the northern zone of the Republic of Moldova has the positive effect on soil biota. The effect of *no-tillage* on the soil biota manifests by the growth of the number of invertebrates, reserves of microbial biomass, the multiplication and development of the *Lumbricidae* family, increase enzymatic activity. The number of *Lumbricidae* family in the leached chernozem in average was 4.1 times higher compared with the arable plot, the biomass – 14.1 times respectively. The weight of one exemplar of earthworms in chernozems was 3.6 times higher and constituted 0.18g. The characteristic feature of the chernozem with *no-tillage* application is concentration of earthworms in the layer of 0-40 cm and the higher share of saprophagous in the total population of soil invertebrates.

Erosion is disastrous for the soil's habitat: invertebrates and microorganisms. The current state of the eroded chernozems biota is characterized by a decline in indices of numbers, biomass, activities and diversity compared to the soils with a normal profile. The biological indicators of chernozem depend on the degree of erosion. The growth of variation coefficients once the erosion degree increases indicates the reduction of the soil's biota stability.

The catastrophic loss of biomass, of the edaphic fauna diversity and microorganisms represent a particularity of eroded chernozems. The considerable loss of enzymes and their inactivation is one of the typical manifestations of the erosion process. A scale of biological parameters has been proposed to assess the stability of biota of the ordinary chernozem and to develop the national soil quality standards.

Multiannual fallow soils under natural vegetation are a source of the conservation and reproduction of different species of invertebrates and microorganisms; they have a high level of biomass and enzyme activity. The transition of the eroded chernozem from the category of arable land to the category of fallow land promotes regeneration of soil invertebrates, increases microbial carbon stocks and carbon sequestration. This procedure is based on the gradual restoration of natural activities of invertebrates and microorganisms in the soil at the expense of root exudates and vegetal residues of sod-forming grass. The humification processes intensified as a result of the interaction between the root systems and the soil biota.

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