CONTRIBUTION OF SOIL BIOTA TO THE STABILITY OF DEGRADED SOILS IN THE REPUBLIC OF MOLDOVA

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Abstract

The biota of soils degraded as a result of a long-term arable use has been investigated statistically in the context of improving the soil stability and quality. The database of invertebrates, microorganisms and enzymatic activities of different zonal soils in the long-term field experiments has been developed from a viewpoint of the operative evaluation of the degradation processes and ecological effectiveness of the land management. The long use of soils in agricultural production led to the imbalance between the processes of decomposition and humus formation and promoted the decrease of soil biota stability and degradation. The current status of the biota of arable soils of the Republic of Moldova is characterized by the significant reduction in the abundance, biomass, activity and diversity in comparison with soil's standards that are in conditions of natural ecosystems. The highest values of invertebrates' abundance were registered in the soils with a normal profile under natural vegetation. Soil microbial biomass increased from 244.3-318.4 μ g C g^{-1} soil in arable soils to 355.8-876.0 μ g C g^{-1} soil in virgin and fallow soils. A land management with the involvement of areas with natural vegetation in a crop rotation system, organic fertilizers and perennial grasses created conditions for the improvement of the biota's vital activity in degraded soils. The recovery rate of the population of *Lumbricidae* family reaches of 3-5.6 worms m^{-2} per year. Annual increase in the content of microbial biomass in the typical chernozem can be up to 81.3 kg C ha^{-1} in the layer of the 0-50 cm. The organic farming system greatly improves the enzymatic and humus status of the old-arable soils, but does not restore the biodiversity of invertebrates.

Key words: soil biota, degradation, stability, land management, organic fertilizers.

The degradation of soils in the Republic of Moldova is the most critical, threatening problem for agriculture, for environment and people's habitat. 56.4% of agricultural lands of the RM are degraded (Andries, S., et al., 2004). The total plowing of the land in 1950-1960 years and intensive use of chemicals in the years that followed rendered the most powerful effect on the natural stability of soils. At the same time, the massive diffusion and the excessive application of chemical fertilizers in the agriculture resulted in the fragmentation and simplification of soil habitats.

Dehymification of the arable soils is one of the main forms of soil degradation in the Republic of Moldova. The area of arable soils reaches 854 900 ha (Andries, S., et al., 2004). The decrease of productivity of arable soils as a result of mineralization processes amounts to 10%, their compaction – to 10%. 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. The uncompensated loss of humus, caused by its mineralization, constitutes more than 700 kg ha^{-1} $year^{-1}$. The main cause of the reduction of the organic matter content is its mineralization as a result of intensive soil tillage.

So, the long use of soils in agricultural production led to the imbalance between the processes of decomposition and humus formation and promoted the decrease of soil biota stability and degradation. There has been a significant deterioration of the conditions needed for the vital activity of soil invertebrates and microorganisms (Senicovscaia, I., 2012; Senicovscaia, I., Marinescu, C., et. al., 2012).

To stop the degradation process and to restore the biological soil functions, it is necessary to carry out a set of measures aimed at increasing the carbon sink in old-arable soils. The application of rich and varied sources of organic matter not only supplies plant nutrients, but also helps to increase below-ground biodiversity by providing array of substrates capable of supporting diverse soil organisms. In Moldova crop residues, animal wastes and perennial grasses can be used as sources of organic matter.

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

MATERIAL AND METHOD

Four experimental sites located in different zones of the Republic of Moldova have been tested. Various kinds of the soil degradation and 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 of Field Crops "Selectia" (Beltsy). It had 2 plots: fallow land (10-23-year-old) and the long-term arable land with crop rotation (management without fertilizers). The soil was the typical chernozem.

The second site was located in the center of the country, on the long-term field experiments of the "Nikolae Dimo" Institute of Pedology, Agrochemistry and Soil Protection 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 presented by the leached chernozem and the gray forest soil.

The third site was located in the southern zone, in the Tartaul de Salchie village of the Cahul region. The state of biota in the conditions of arable land was investigated in comparison with the 58-year-old fallow land. The soil was the ordinary chernozem.

The fourth site was also in the southern zone of the country, in the Baurchi-Moldoveni village, Cahul region. It had 2 plots: virgin and arable land. The soil was the xerophyte-forest chernozem.

Soil samples were collected from the 0-30 cm layer on the experimental plots during the period of time between 1988 and 2014. Soil samples were also collected from sites with the organic fertilizers management (farmyard manure), plants residues and perennial grasses from 0-25 cm layer.

Status of invertebrates. The state of invertebrates was identified from test cuts by manual sampling of soil layers to the depth of soil fauna occurrence with application of Gilyarov and Striganova's method (Gilyarov, M.S. and Striganova, B.R., 1987). The identification of invertebrate's diversity at the rate of families and their classification according to nutrition were carried out by Gilyarov and Striganova's method (Gilyarov, M.S. and Striganova, B.R., 1987).

Microbiological properties. The microbial biomass C was measured by the rehydratation method based on the difference between C extracted with 0.5 M K_2SO_4 from dried soil at 65-70^{0}C within 24 h and fresh soil samples with K_c coefficient of 0.25 (Blagodatsky, S.A., et al., 1987). Reserves of microbial biomass have been calculated taking into account the carbon content of the microbial cell and the bulk density of soils (Senicovscaia I., et. al., 2012). Counts of microorganisms (heterotrophic bacteria, humus-mineralizing microorganisms and fungi) were obtained on agar plates (Zvyagintsev, D.G., 1991).

Enzymatic activities. The urease activity was measured by estimating the ammonium released on incubation of soil with buffered urea solution by colorimetrical procedure (Haziev, 2005). 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 (Haziev, 2005). The polyphenoloxidase activities were determined by the colorimetric technique on the basis of the oxidation of phenolic compounds to quinones with the use of hydroquinone as a substrate (Karyagina and Mikhailovskaya, 1986).

Soil chemical properties. Organic C was analyzed by the dichromate oxidation method (Arinushkina E.V., 1970). The humus content was calculated using the coefficient of 1.724.

The soil biological indices were evaluated statistically using the variation analysis.

REZULTS AND DICUSSIONS

Difference in the biota' state between long-term arable soils and soils under natural vegetation. Soil biota of agricultural ecosystems is substantially different from the soil biota, living under natural ecosystems. The abundance and biomass of the biota in the soils of arable land statistically significantly lower than in soils under the natural vegetation (*figure 1, 2*).

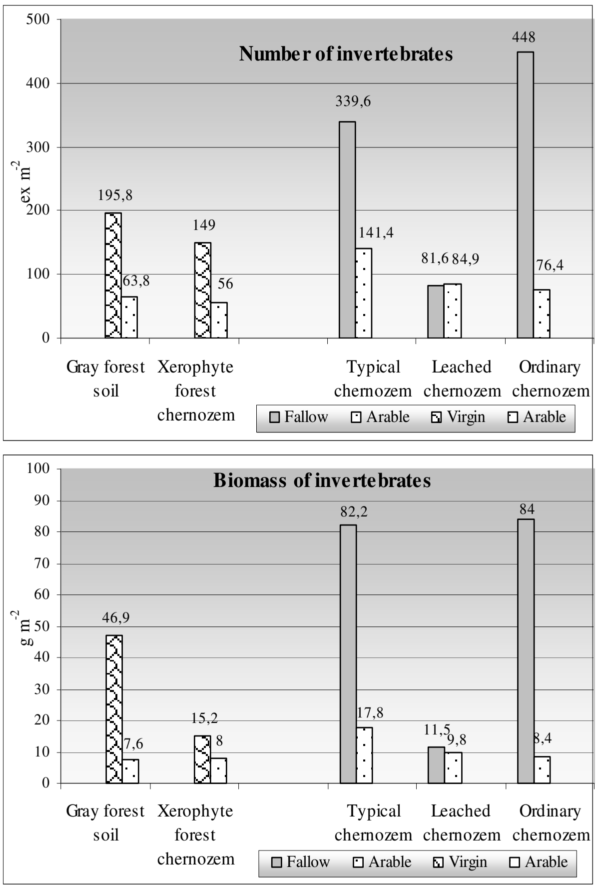

Figure 1.**The total number and biomass of invertebrates in soils of the arable, fallow and virgin land (mean values, n=3-32)**

Conventional tillage practices are generally unfavorable to the edaphic fauna. Indices of the number of invertebrates and *Lumbricidae* family decreased in arable soils by 2.4-5.9 and 1.9-7.4 times respectively in comparison with virgin and fallow soils that are in conditions of natural ecosystems. More significant changes have been registered in the biomass of invertebrates and the *Lumbricidae* family. This index reduced by 1.2-10.0 and 1.3-10.3 times respectively. According to the average statistical data, the weight of one individual of the earthworm in the soil under arable management practices is 0.16-0.18 g, while in the fallow and virgin soils this index is 0.22-0.50 g.

The fauna is specific for every soil. 16 families, 9 genera and 23 invertebrate species were determined in examined soils. The *Lumbricidae* family is presented in all types of soil. In addition to the *Lumbricidae* family species from the families of *Formicidae*, *Arthropoda, Carabidae, Araneidae, Apidae, Forficulidae, Pieridae, Pentatomidae, Coccinelidae* and other have been found in soils of natural ecosystems. The distinctive feature of arable soils is the sharp decline of invertebrates' diversity. In general, the soil under natural vegetation contains 5-12 families of invertebrates; while in arable soils can be found only 2-5.

Soil microbial biomass decreased on average from 355.8-876.0 μg C g^{-1} soil in virgin and fallow soils to 244.3-318.4 μg C g^{-1} soil in arable soils as a result of a long-term arable land management without the application of organic fertilizers (*figure 2*). The level of microbial biomass in all arable soils was similar. Thus, the difference between soils in this indicator was offset. A similar trend in decrease has been noticed in the number of the heterotrophic bacteria and fungi (*table 1*).

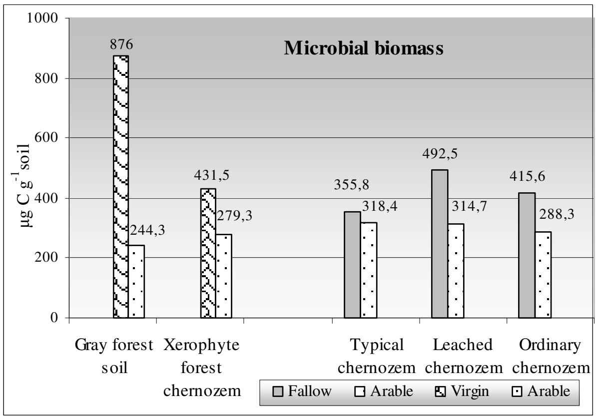

Figure 2. **The microbial biomass content in soils of the arable, fallow and virgin land (mean values, n=3-33, 0-30 cm)**

The long-term use of soil under arable affected the structure of soil microbial communities. The abundance of heterotrophic bacteria in the gray forest soil, typical and leached chernozems decreased in 1.2-1.8 times. Their number in the xerophyte forest and ordinary chernozem remained practically constant as a result of plowing. The most significant changes have taken place in the complex of soil fungi. Their number has fallen by 1.4-2.7 times. The ratio between bacteria and fungi decreased also from 82-142 in virgin and fallow soils to 54-98 in arable soils. There was 1.9-4.7 – fold increase in the number of humus-mineralizing microorganisms in all soils in conditions of the long-term arable land management.

Table 1

Microbiological and enzymatic characteristics of arable, fallow and virgin soils in the 0-30 cm layer (mean values, n = 3-33)

Index	Gray forest soil		Xerophyte forest chernozem		Typical chernozem		Leached chernozem		Ordinary chernozem	
	Virgin land	Arable land	Virgin land	Arable land	Fallow land	Arable land	Fallow land	Arable land	Fallow land	Arable land
Heterotrophic bacteria, CFU g^{-1}soil*10^6	5.9	3.3	4.5	4.5	6.3	5.2	5.4	4.3	4.7	4.5
Humus-mineralizing microorganisms, CFU g^{-1} soil*10^6	1.9	8.9	4.7	9.1	6.5	16.2	2.7	9.6	5.5	11.9
Fungi, CFUg^{-1} soil*10^3	110.0	40.5	55.9	40.8	64.6	37.4	60.0	30.2	53.6	35.0
Bacteria/Fungi	54	82	81	110	98	139	90	142	88	129
Urease, mg NH_3 10 g $^{-1}$soil 24 h^{-1}	8.1	1.4	3.9	2.0	12.5	4.5	8.6	3.1	5.5	3.5
Dehydrogenase, mg TPF 10g^{-1} soil 24h^{-1}	2.40	0.74	2.35	1.0	2.92	1.94	2.31	1.47	2.79	1.78
Polyphenoloxidase, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	4.1	2.3	4.0	3.2	7.4	6.8	5.5	3.9	20.1	18.6
Humus content, %	4.0-5.7	2.1-2.4	7.63	2.99	4.9-5.1	4.4-4.7	3.7-4.6	3.2-3.8	3.8-4.0	2.9-3.2

The characteristic feature of arable soils is the low enzyme activity. The enzymatic activity in arable soils on the mean values significantly lower than in fallow soils and soils of forest ecosystems (*table 1*). Indices of the urease activity have been decreased in arable soils by 1.6-5.8 in comparison with virgin and fallow soils that are in conditions of natural ecosystems. The dehydrogenase activity in arable soils have been inhibited by 1.5-3.2 times. Less significant changes have been registered in the polyphenoloxidase activity.

The humus content (confidence intervals, $P \leq 0.05$) in virgin and fallow soils constitutes: 4.9-5.1 % in the typical chernozem, 3.7-4.6 % in the leached chernozem, 3.8-4.0 % in the ordinary chernozem and 4.0-5.7 % in the gray forest soil. The index of humus content in the xerophyte forest chernozem has a small number of measurements. However, the organic carbon content in this soil constitutes 4.43 % (humus content 7.63 %). 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. The humus content in arable soils constitutes: 4.4-4.7% in the typical chernozem, 3.2-3.8 % in the leached chernozem, 2.9-3.2 % in the ordinary chernozem, 2.99 % in the xerophyte forest chernozem and 2.1-2.4 % in the gray forest soil.

Recovery of the biota in long-term arable soils in conditions of the land management with farmyard manure and plants residues. The biota in the long-term arable soil has been under stress for a long time and requires to be restored. The process of natural recovery of soil biota composition and activity in agricultural lands has been slow. The biomass of biota is restored quicker, its diversity and enzymatic activity – to a lesser extent. The recovery rate of the population of *Lumbricidae* family reaches of 3.0-5.6 worms m^{-2} per year by the use of soils under recreation. Annual increase in the content of microbial biomass in the typical chernozem can be up to 81.3 kg C ha^{-1} in the layer of the 0-50 cm.

The manure application with plant residues additives and restores the biota of old arable soils to the homeostasis zone (*table 2*). The number of invertebrates increased from 54.8-175.6 to 85.0-214.7 ex m^{-2}, the number of saprophagous – by 1.3-1.7 times, the biomass of *Lumbricidae* family – by 1.7-2.3 times. The exception is the biomass of earthworms in the ordinary chernozem, where this indicator remained practically unchanged.

Table 2

Biota in long-term arable soils in conditions of the land management with farmyard manure and plants residues (mean values)

Variant	Number of invertebrates, ex m^{-2}	Biomass of *Lumbricidae* family, g m^{-2}	Saprophagous, ex m^{-2}	Microbial biomass, µ g C g^{-1} soil	Dehydro-genase, mg TPF 10 g^{-1} soil 24 h^{-1}	Polyphenol-oxidase, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}
Typical chernozem (n = 6-8)						
Control	175.6	14.7	122.3	298.4	2.32	9.2
Manure 60 t ha^{-1}	214.7	33.7	157.3	324.3	2.69	15.5
Leached chernozem (n = 8-34)						
Control	76.0	7.0	56.0	314.7	1.47	3.9
Manure 60 t ha^{-1}+ plant residues	85.0	12.0	73.0	362.0	1.99	5.9
Gray forest soil (n = 6-33)						
Control	96.0	7.6	74.7	244.3	0.74	2.3
Manure 60 t ha^{-1}+ plant residues	133.3	15.1	96.0	302.4	1.40	5.0
Ordinary chernozem (n = 9)						
Control	54.8	7.8	42.2	212.6	1.34	7.8
Manure 50 t ha^{-1}	94.1	7.0	72.3	300.9	1.28	8.6

The growth of the biomass of microorganisms by 8,7-41,5 % and the activation of enzymes have been registered.

Biota' state of the long-arable chernozems in conditions of the land management with perennial grasses. The effective restoration of the biota in degraded arable chernozems occurs as a result of the cultivation of perennial grasses. The application of perennial legume-cereal grass mixtures led to the restoration of the total number of invertebrates and the *Lumbricidae* family (*table 3*). The biomass of populations under grass mixture (1-2-year-old) was smaller than those under traditional arable management, but the number of invertebrates sometimes was significant. This demonstrates the reproduction of young invertebrates' populations and the occurrence of the initial stage of successional changes. Statistically significant growth of zoological indicators has been registered after the third year of investigations on plots with grass mixtures of ryegrass and lucerne. The number of invertebrates on average was 2.5 times higher compared with the control plot, the total biomass – 1.6 times respectively. This method is especially effective for restoring the *Lumbricidae* family. Earthworm populations in the grass cultivation by ryegrass and lucerne during 3-6 years were significantly larger than those in the arable management, both in terms of earthworm abundance and biomass. Their number in the leached chernozem increased by 3.0 and biomass – by 2.0 times. The population growth over 6 years constitutes 74.4 ex m^{-2} or 14.9 ex m^{-2} annually.

The diversity of invertebrates was different, depended on the agricultural management. The grass cultivated soil is characterized by a greater diversity of invertebrates. In addition to the *Lumbricidae* family, species of the *Formicidae, Gloremidae, Scarabaeidae, Elateridae, Geophilidae, Araneae, Coccinelidae* and *Carabidae* families were found. *Lumbricus terrestris* and *Allolobophora terrestris* species are the most typical representatives of the *Lumbricidae* family in the chernozem under perennial grasses. The abundant presence of the *Formicidae* family representatives is observed. In general, the soil under grass mixture with ryegrass and lucerne contains 5-6 families of invertebrates, while the soil under arable only 2-4 families of edaphic fauna.

The use of phytorestoration procedures with legume-cereal grass mixtures creates advantageous conditions for the existence and functioning of autochthonous microorganisms and activates the internal reserves of microbiological systems of degraded soils. Microbial biomass in chernozems increases significantly according to the average data. The maximum values have been registered in the leached chernozem with the application of the mixture of ryegrass and lucerne grass mixtures during 3-6 years. The microbial biomass is accumulated in soil in amounts of 132.5 kg ha^{-1} annually.

Table 3

Influence of perennial grasses on the biological properties of the long-arable chernozems

Variant	Total number of invertebrates, ex m^{-2}	*Number of earthworms*, ex m^{-2}	Biomass of *Lumbricidae* fam., g m^{-2}	Microbial biomass, µg C g^{-1} soil	Polysaccharides-forming micro-organisms, CFU*10^6 g^{-1} soil	Dehydrogenase, TPF, mg 10 g^{-1} soil 24 h^{-1}	Urease, NH3, mg 10 g^{-1} soil 24 h^{-1}
Leached chernozem (n = 9-15)							
Control (arable)	34-69	22-52	0-15.2	256-341	1.8-3.6	0.9-1.2	4.0-5.0
Ryegrass+lucerne (3-6 years)	115-169	96-145	13.3-28.1	355-492	1.1-13.1	1.4-1.7	4.9-5.9
Ryegrass+sainfoin (1-3 years)	24-107	19-91	2.4-4.5	266-371	1.3-10.7	1.1-1.6	3.8-5.4
Ordinary chernozem (n = 12-18)							
Control (arable)	32-65	13-38	1.4-11.0	194-226	1.5-2.1	1.3-1.7	3.5-4.1
Ryegrass +lucerna (1-2 years)	33-79	23-59	2.8-12.2	229-297	1.0-2.6	1.8-2.3	3.6-4.4
Ryegrass+sainfoin (1-2 years)	33-71	9-37	0-10.2	249-317	1.4-2.8	1.7-2.2	3.4-4.4

The use of the mixture of perennial grasses had a stimulating effect regarding to the dehydrogenase in both soils (*table 3*). Dehydrogenase activity increased on average by 1.4 times in the conditions of the ryegrass and lucerne mixture application. The cultivation of ryegrass and sainfoin mixture led to the stimulation of the dehydrogenase activity in the leached chernozem by 14%, in the ordinary chernozem – by 30% respectively and the increase of the humus content by 0.2-0.3 %.

CONCLUSIONS

The long use of soils in agricultural production led to the imbalance between the processes of decomposition and humus formation and promoted the decrease of soil biota stability and degradation. The current status of the biota of arable soils of the Republic of Moldova is characterized by the significant reduction in the abundance, biomass, activity and diversity in comparison with soil's standards that are in conditions of natural ecosystems. The highest values of biota's abundance were registered in the soils with a normal profile under natural vegetation. The values of most biological indicators in zonal soils decrease in the following sequence: natural and long-term fallow land → land with perennial grasses → arable land under organic system with farming manure and incorporation of crop residues → arable unfertilized land. In the arable soil humus-mineralizing microorganisms dominate. 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.

Virgin and multiannual fallow soils under natural vegetation are the source of conservation and reproduction of different species of invertebrates and microorganisms; they have a high level of biomass and enzyme activity. A stable state of the biota is provided by the humus content in the level of 4.0-6.0 % in the 0-30 cm layer. A soil management with the involvement of areas with natural vegetation in a crop rotation system created conditions for the improvement of the biota's vital activity in the soil which degraded as a result of a long-term arable use. The database of the biota's state of virgin and fallow soils has a practical importance as the natural standard for the operative evaluation of degradation processes and ecological effectiveness of the land management.

Application of organic fertilizers in the form of farmyard manure, the annual addition into degraded soils of crop residues and perennial grass mixtures help to prevent ecological violations in the state of soil biota, to restore individual species and populations of invertebrates and microorganisms, to stabilize and to improve the enzymatic activity. Perennial grass mixtures can be used to create oases and migration corridors for the soil biota as well as the locus with a high content of the microbial biomass and the enzymatic activity. The restoration of the lost components of the soil biota in the arable chernozems is a long process. Soil biological parameters (by some indicators) have reached the level of soils under natural vegetation. The organic farming system greatly improves the status of biota and fertility of old-arable soils, but does not restore completely the biodiversity of invertebrates.

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