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BIOTA OF DEGRADED SOILS AND METHODS FOR ITS RESTORATION

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Abstract

In the present paper we discuss different aspects of the soil biota functioning in the current farming system of the Republic of Moldova. The natural stability of the biota in virgin, fallow and arable soils has been estimated, the assessment system of the sustainability of invertebrates and microorganisms of soils to the anthropogenic impacts has been developed. The zones of homeostasis have been determined and criteria of the resistance of soil biota standards have been elaborated. The modifications of the biological properties of soils have been established, as a result of their long-term arable use and the application of high doses of mineral fertilizers. More information about the biological soil degradation has been acquired, as a result of dehumification processes and aggregates destroying the anthropogenic nature. The system of fertilizers application on soils with a normal profile has been substantiated from the biological point of view. The method of green manure application has been applied in order to restore the biota of degraded soils and to improve soil quality and the environment.

Key words: *degradation, green manure, homeostasis, soil biota*

INTRODUCTION

Biota, an essential component of soils and ecosystems as a whole, participates in the processes of pedogenesis, water-stable structure formation, self-purification from contaminants, nutrient cycling, energy cycle, and maintains homeostasis [3, 4, 9]. Ecologically, soil biota is responsible for regulating several critical functions in soil. Soil organisms contribute a wide range of essential services to the sustainable functioning of all ecosystems by acting as regulators of the dynamics of soil organic matter, soil carbon sequestration and greenhouse gas emission; modifying the soil physical structure and water regime; enhancing the amount and efficiency of nutrient acquisition by vegetation; and enhancing plant health [4, 6, 9]. The main role of the soil biota relates to the organic matter mineralization and the conservation of resources that have been formed within the limits of the ecosystem. In 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, equilibrium is disturbed, mineralization processes are predominant. 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 [9]. The changes in biological properties may indicate the risk of likely soil degradation as a result of human activity. In this respect, the use of soil bio-indication as an integrated monitoring tool for soil degradation might serve as a prospect solution.

The current state of the soil cover in the Republic of Moldova is characterized by the intensification of the processes of degradation and desertification [10]. The total plowing of the land in the 1950s and 1960s, and an intensive use of chemicals in the years that followed, rendered the most powerful effect on the natural stability of soils. 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 are many examples which prove the negative effects of not using organic fertilizers on the humus content and soil physical properties [10]. At the same time, the massive diffusion and the excessive application of chemical fertilizers and xenobiotic compounds in agriculture resulted in the fragmentation and simplification of soil habitats. There has been a significant deterioration of the conditions needed for the vital activity of soil invertebrates and microorganisms.

At the scale of an agricultural ecosystem, the preservation of soil functions and services depends upon the preservation of soil biota. 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 degraded 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 an array of substrates capable of supporting diverse soil organisms. In Moldova, crop residues, green manures, animal wastes and composts can be used as organic matter.

The purpose of the research was to determine the influence of different management practices on the biological properties of soils, to develop scale parameters of the soil biota stability and to establish the green manure method for the biota restoration of the soils that have been degraded as the result of long-term agricultural use.

MATERIAL AND METHOD

Experimental site. Three experimental sites located in different zones of the Republic of Moldova have been tested. Various ways of treatment-utilization of the soil 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 3 plots: fallow land (60-year-old), fallow land (10-23-year-old) and long-term arable land (management with no fertilizer).

The second site was located in the center of the country, in the Ivancha village, Orhei region (Photo 1). The natural land under forests, the long-term arable land with crop rotation without fertilizers, mineral fertilizer systems ($N_{60}P_{60}K_{60}$ and $N_{90\text{-}300}P_{60}K_{60}$), and organic manure with crop residue treatments were tested. Mineral fertilizers were applied during 1965-1995 and 2006. Crop residues were plowed annually; farmyard manure was introduced in the dose of 60 t ha^{-1} in 1991, 1996 and 2005.

The third site was located in the southern area, in the Tartaul de Salchie village, Cahul region. These were plots with a long-term arable land and green manure treatments. Vetch was used once as green manure.

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

Soils. Investigations were performed on the typical, leached, ordinary chernozem and the gray forest soil. The database of soil biological indicators covered the period between 1986 and 2011.

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 [12].

Microbiological properties. Microbial biomass C (MB) was measured by the rehydratation method [5]. Counts of microorganisms (heterotrophic bacteria, fungi, humus-mineralizing microorganisms) were obtained on agar plates [11].

Enzymatic activity. The (potential) urease activity was measured by estimating the ammonium released on incubation of soil with buffered urea solution by colorimetrical procedure [7]. The (potential) 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 [7]. The (potential) polyphenoloxidase activity was determined by the colorimetric technique with the use of hydroquinone as a substrate [8].

Soil chemical properties. The humus content was analyzed by the dichromate oxidation method [1].

The 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, microorganisms and enzymes - for the layer of 0-30 cm.

RESULTS AND DISCUSSIONS

Biota of the soils degraded as a result of a long arable land-use. The current state of the soil biota in the Republic of Moldova is the result of a long-term influence of human activity, through agricultural land management. Conventional tillage practices are generally unfavorable to the edaphic fauna, heterotrophic bacteria and various fungi (Table 1). Indeed, many of these organisms are extremely sensitive to perturbations of the soil. Indices of the number of invertebrates and *Lumbricidae* family decreased in arable soils by 2.4-3.1 and 1.9-2.5 times respectively in comparison with virgin and fallow soils under conditions of natural ecosystems. More significant changes were registered in the biomass of invertebrates and the *Lumbricidae* family. That index reduced by 4.6-6.2 and 3.7-6.1 times, respectively. According to the average statistical data, the weight of one individual of the earthworm in the soil under arable management practices was 0.16-0.18 g, which was 1.5-3.1 times lower than in the virgin and fallow land.

Soil microbial biomass decreased on average from 355.8-876.0 to 244.3-318.4 μg C g^{-1} soil as a result of a long-term arable land management without the application of organic fertilizers. A similar trend in decrease was noticed in the number of heterotrophic bacteria and fungi. Activities of soil enzymes reduced: urease - by 2.8-5.8, dehydrogenase - by 1.5-3.2 times, polyphenoloxidase - to 8.1-43.9%.

The long-term use of soil under arable conditions affected the structure of soil microbial communities. The ratio between bacteria and fungi increased from 98 to 139 in the typical chernozem and from 64 to 82 in the gray forest soil. There was a 2.5-4.7 - fold increase in the number of humus-mineralizing microorganisms. More intensive land-use involving soil tillage stimulated the microbial decomposition of organic matter and tended to result in a decrease in the microbial carbon pool and ultimately in a decrease in the humus content (Table 1).

The biological indices of investigated soils were characterized by the medium and considerable variability. There was a tendency of increase in the variation coefficient from the virgin and fallow soils to the arable soils on some indicators. Taking into consideration the decrease in the total level and increase in the amplitude of the biological parameters oscillations in degraded soils, this testified the decline of soil ecological resistance to anthropogenic impacts. The biological parameters of soils were grouped according to the humus content. The level and size of homeostasis zones, and therefore the biota stability, reached the maximum levels in the natural and fallow soil.

The long-term plowing led to the destruction of water-stable soil structure. The content of agronomic valuable aggregates (Σ10-0.25 mm) in the 0-25 cm layer of the typical chernozem decreased from 89.4-95.9% in fallow soils to 59.2% in the arable soils, the content of particles >10 mm and <0.25 mm increased from 4.2-5.4% to 40.8% respectively. The amount of water-stable aggregates declined from 79.6-80.2% to 65.9%, the structure coefficient went down from 30.9 to 1.5-9.5 [2].

Table 1. Statistical parameters of the biota status in zonal soils under different land management ($P \leq 0.05$)

Index	Typical chernozem						Gray forest soil					
	Fallow land			Arable land (no fertilizer)			Virgin land			Arable land (no fertilizer)		
	mean values	confidence intervals	V, %	mean values	confidence intervals	V, %	mean values	confidence intervals	V, %	mean values	confidence intervals	V, %
Invertebrates (n = 6-22)												
Number of invertebrates, ex m^{-2}	339.6	279.3-399.9	17	141.4	118.2-164.6	40	195.8	169.3-222.3	27	63.8	46.6-81.0	74
Biomass of invertebrates, g m^{-2}	82.2	32.0-132.4	58	17.8	12.3-23.4	68	46.9	29.1-64.7	60	7.6	6.5-8.6	27
Number of *Lumbricidae fam.,* ex m^{-2}	227.3	172.4-282.2	23	91.1	73.5-108.7	47	83.0	63.6-102.4	37	43.2	32.0-54.4	71
Biomass of *Lumbricidae fam.,* g m^{-2}	61.2	2.7-119.7	92	16.5	11.3-21.7	72	41.5	23.9-59.1	67	6.8	5.8-7.7	27
Microorganisms (n = 8-33)												
Microbial biomass, μ g C g^{-1} soil	355.8	267.5-444.1	35	318.4	265.9-370.9	26	876.0	686.9-1065.1	26	244.3	209.9-278.7	28
Heterotrophic bacteria, CFU g^{-1}soil*10^{6}	6.3	5.4-7.2	30	5.2	4.7-5.7	24	5.9	4.8-8.0	36	3.3	3.3-3.4	10
Humus-mineralizing microorganisms, CFU g^{-1} soil*10^{6}	6.5	5.4-7.5	36	16.2	15.2-17.3	14	1.9	1.05-2.75	46	8.9	8.6-9.2	15
Fungi, CFU g^{-1} soil*10^{3}	64.6	59.0-70.2	19	37.4	34.6-40.3	17	110.0	98.1-121.9	11	40.5	33.5-47.6	30
Enzyme activity (n = 3-22)												
Urease, mg NH_3 10 g^{-1}soil 24 h^{-1}	12.5	10.0-15.0	20	4.5	3.4-5.6	10	8.1	5.4-9.7	18	1.4	0.9-1.9	51
Dehydrogenase, mg TPF 10g^{-1} soil 24h^{-1}	2.92	2.22-3.62	38	1.94	1.59-2.29	31	2.40	2.13-2.67	11	0.74	0.59-0.89	42
Polyphenoloxidase, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	7.4	4.1-10.7	63	6.8	4.7-8.9	53	4.1	1.1-7.1	70	2.3	1.9-2.7	41
Humus content, %		4.9-5.1			4.4-4.7			4.0-5.7			2.1-2.4	

[1]CFU – colony forming units

The reduction of organic matter, the destruction of soil structure and the significant deterioration of the biological parameters of arable soils represent interconnected and interdependent processes. The correlation coefficient (R^2) between the biomass and number of microorganisms and humus content in the typical chernozem under arable constituted 0.58 - 0.90, in conditions of 15-old-years fallow 0.76 - 0.95, of 60-old-years fallow – 0.74 - 0.94.

Strong positive correlation links were found between the abundance of microorganisms and the amount of agronomic valuable aggregates, R^2 = 0.86 - 0.92. Microbial biomass in the typical chernozem was localized partly in fractions of 5-3, 3-2, 2-1 mm and 7-5 mm fraction. The link between the microbial complex and the amount of fractions >10 mm and <0.25 mm was strongly negative, R^2 = -0.86 – (-0.92). Water-stable aggregates formation was mainly influenced by the presence of microscopic fungi in the soil (R^2 = 0.79).

The results proved that the interaction between microbial components (and biota as a whole), soil structure and soil fertility status was 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 was higher than that of the soils in agricultural ecosystems. The rupture and the attenuation of relations between the biotic and abiotic components of soils led to the decrease in their natural stability and the development of degradation processes.

The soil biota degraded as a result of the impact of mineral fertilizers application. A long-term soil management practice with high doses of inorganic fertilizers application determined the appearance of negative changes in the complex of biota of leached chernozem and gray forest soils, reducing their stability and deteriorating their quality (Table 2). As a result of arable layer acidification (pH is reduced by 0.15-0.35) and increased hydrolytic acidity (at 0.55-1.95 me 100 g^{-1}), the total number of invertebrates in the leached chernozem reduced by 1.9 times, biomass - 2.1 times, mainly due to the reduction of saprophagous by 4-5 times. The reproduction of phytophagous larvae (for example, *Melolontha melolontha)* was observed, their numbers increased by 9.5 times. The number of *Limbricidae* family declined to 1.5-2.5, their biomass – of 1.8-1.9 times in both soils (Table 2). Invertebrates migrated in the subsurface soil layers.

The total microbial biomass changed insignificantly, but the limits of its oscillations on the plots with $N_{90\text{-}300}P_{60}K_{60}$ were more essential in comparison with plots without fertilizers. The increase in the abundance of microscopic fungi by 2.3 times under application of mineral fertilizers in high doses the nitrogen was observed. The number of phytotoxic species grew by 23-33%.

Table 2. Biota degradation in long-term use of high doses of mineral fertilizers (n = 3-34, $P \leq 0.05$)

Index	Leached chernozem		Gray forest soil	
	no fertilizer	$N_{90\text{-}300}P_{60}K_{60}$	no fertilizer	$N_{240}P_{60}K_{60}$
Number of *Lumbricidae fam.*, ex m^{-2}	108 ± 22	44 ± 7	38±6	26±4
Biomass of *Lumbricidae fam.*, g m^{-2}	8.3 ± 3.5	4.7 ± 0.9	6.8±1.0	3.6±3.1
Microbial biomass, μ g C g^{-1} soil	315±28	323±59	244±34	208±77
Fungi, CFU g^{-1} soil*10^3	30.2±3.3	69.8±10.9	40.5±7.0	94.2±27.8
Dehydrogenase, mg TPF $10g^{-1}$ soil $24h^{-1}$	1.47±0.24	1.19±0.27	0.74±0.15	0.68±0.42
Polyphenoloxidase, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	3.9±0.5	2.6±0.5	2.2±0.5	1.5±0.5

The long-lasting application of mineral fertilizers led to decreases in dehydrogenase activity in average by 8-19% and polyphenoloxidase activity by 1.5 times compared to unfertilized control plots. Enzyme activities were lower than the optimum level, providing the soil system stability. Negative shifts in the state of enzymes were accompanied by an increase in the soil acidity, disturbance in the humification–mineralization equilibrium and by soil degradation on the whole. Enzyme activities under mineral system with maximum doses were suppressed even in 10 years after the cessation of inorganic fertilizers use. The use of high doses of nitrogen fertilizers had a long aftereffect and persisted for some indicators on the organic fertilizers backgrounds.

The restoration of the degraded soil biota by using organic amendments and green manures. The biota in degraded soil was under stress for a long time and required restoration. The process of natural recovery of the soil biota composition and activity in agricultural lands with the mineral fertilizers after-effect was slow. The biomass of biota was restored quicker, its diversity and enzymatic activity – to a lesser extent.

The manure application with plant residues additives and $N_{60}P_{60}K_{60}$ restored the biota of old arable soils to the homeostasis zone (Table 3). The decline of humus-mineralizing microorganisms and the activation of enzymes was registered.

Table 3. Influence of the organic and mineral fertilizers system on the biota restoration in the long-term arable soils

Variant	Number of *Lumbricidae fam.*, ex m^{-2}	MB, μ g C g^{-1} soil	Humus-mineral. microorganis. CFU g^{-1} soil * 10^6	Dehydro-genase, mg TPF $10g^{-1}$ soil 24 h^{-1}	Polyphe-noloxidase, mg 1,4-p-benzoquino-ne 10 g^{-1} soil 30 min^{-1}
Leached chernozem					
No fertilizer	52 – 71	315±28	9.6±1.8	1.47±0.24	3.9±0.5
Fond	44 – 72	362±52	7.9±2.0	1.99±0.33	5.9±1.2
Fond + $N_{60}P_{60}K_{60}$	50 – 76	395±56	7.1±1.7	1.86±0.40	4.4±1.5
Gray forest soil					
No fertilizer	32 – 54	244±34	8.9±0.3	0.74±0.15	2.4±0.7
Fond	16 – 72	302±49	6.0±1.4	1.40±0.76	5.0±0.5
Fond + $N_{60}P_{60}K_{60}$	36 – 74	276±23	6.7±0.9	1.45±0.08	2.8±0.5

[1] Fond: plant residues + farmyard manure 60 t ha^{-1}

The favorable effect of green manure management on invertebrates in the ordinary chernozem was noted, both as the average values of indicators and as the confidence intervals.

The number of invertebrates increased from 54.8 to 86.9 ex m^{-2}, the number of *Lumbricidae* family – from 30.1 ex m^{-2} to 56.8 ex m^{-2} respectively (Fig. 1).The biomass of invertebrates remained practically unchanged, while the biomass of *Lumbricidae* family increased by 1.4 times. The share of *Lumbricidae* family in the total population increased from 54.9% to 65.4%. The maximum number of invertebrates and earthworms in the soil was registered in the spring of 2010, the minimum - in the autumn of 2011.

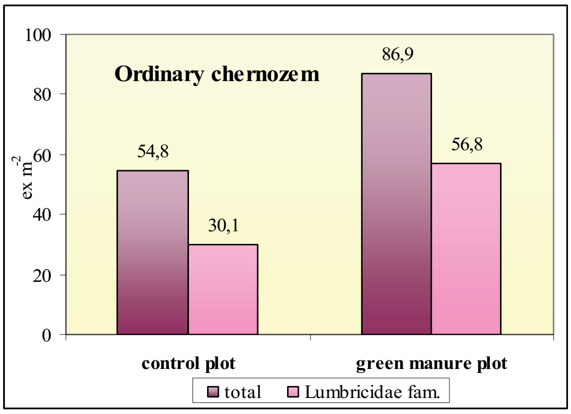

Fig. 1. Effect of green manure on the abundance of soil invertebrates in ordinary chernozem (mean values, n=7-9, $P \leq 0.05$)

This was not only due to the reduction of the fresh organic matter in the soil but also the decrease of moisture content in the root layer (0-40 cm) from 20.3% in spring to 9.3% in autumn in the control plot and from 21.9% to 10.8 % in the green manure plot accordingly. The use of green manure method increased the microbial biomass content from 212.6 to 300.9-334.9 μ g C g^{-1} sol. A stimulation of bacteria growth, microorganisms forming polysaccharides and fungi were observed.

CONCLUSIONS

The long-term arable use of soils leads to its degradation. The biological degradation of arable soils is interconnected with the dehumification processes, compaction and destruction of 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. In the arable soil humus-destroying microorganisms dominate. 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 land with mineral fertilization of N_{90-300}. 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 restoration of biota is based on the change in the existing balance of carbon in degraded soils of agricultural ecosystems. Application of organic fertilizers in the form of farmyard manure and the annual addition into degraded soils of crop residues with $N_{60}P_{60}K_{60}$ helps to prevent ecological violations in the state of soil biota, to restore individual species and populations of invertebrates and microorganisms, stabilize and improve the enzymatic activity. The green manure use restores the biota functioning to the zone of homeostasis and increases the stability of soils to the degradation.

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