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RESTORATION OF BIOTA IN ARABLE CHERNOZEMS BY GREEN MANURING AND PERENNIAL GRASSES

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Abstract

The status of biota of old-arable chernozems in conditions of the green manure and perennial grasses applications has been evaluated statistically. Two experimental sites located in the central and southern zone of the Republic of Moldova have been tested by biological indicators during 2010-2012. The application of vetch as a green manure and grasses-and-legumes 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 effect of green manure was manifested in the increase of the share of Lumbricidae family in the total number of invertebrates by 12.1-20.8% and the microbial biomass productivity of 1.4 to 1.5 times. The use of the mixture of ryegrass + lucerne during 3-5 years led to the growth of the number of invertebrates and Lumbricidae family by 2.5 and 3.0, their biomass - by 1.6-2.0 times in comparison with the control plot. Invertebrates' diversity has improved. The microbial biomass content and dehydrogenase activity in the 0-25 cm layer were increased in average by 1.4 times. The microbial biomass accumulated in the soil in amounts of 494.6-830.1 kg ha^{-1} or an average of 132.5 kg ha^{-1} annually. The population growth of worms reached of 74.4 ex m^{-2} (744.000 ex ha^{-1}), or 14.9 ex m^{-2} (149.000 ex ha^{-1}) annually. An interconnection of biological parameters of the soil quality is under discussion.

Key words: *soil biota, restoration, green manure, perennial grass.*

INTRODUCTION

The intensification of the anthropogenic soil degradation and desertification in the Republic of Moldova has caused the destruction of the soil biota habitat. The biological degradation accompanies all types of the soil degradation, and in some cases even amplifies their (Senicovscaia et al., 2012). The edaphic fauna and microorganisms in degraded soil are under stress for a long time and require to be restored. Soil biota needs an easily available carbon with a simultaneous optimization of moisture, aeration, chemical and physical parameters of habitat. This may be achieved by using environmentally friendly agricultural technologies based on the utilization green manure crops, perennial grasses and organic fertilizers. These methods create the basis for solving the problem of the soil resistant formation with a high level of biodiversity and metabolic activity. This in turn, will increase the flow of carbon and nitrogen through the soil biomass, soil quality restoration, maintaining fertility potential and obtaining of ecologically pure products.

The purpose of the research was to determine the influence of different restoration management practices on the biological properties of soils, to develop scale parameters of the soil biota stability and to suggest methods for the biota's restoration of soils, degraded as the result of the long-term agricultural utilization.

MATERIALS AND METHODS

Experimental sites. Two experimental sites located in different zones of the Republic of Moldova have been tested. Various techniques of restoring the soil biota in the conditions of field experiments have been analyzed (Cerbari et al., 2012; Cerbari and Ciolacu, 2012).

The first site was located in the center of the country, in the Ivancha village, Orhei region (Figure 1). The long-term arable soil with crop rotation without fertilizers (control), farmyard manure and green manure treatments, grass mixture (ryegrass + lucerne and ryegrass + sainfoin) cultivation were tested. Farmyard manure was introduced in the dose of 50 t ha^{-1} in2010. Vetch was planted twice in 2009 and in 2010 and its green mass in the amount of 19 t

ha^{-1} was plowed under disc in 2010. Plots under mixtures of perennial grasses (ryegrass + lucerne) were founded in the autumn of 2007. The mixture of ryegrass + sainfoin was seeded in the autumn of 2010.
The soil is the leached chernozem with humus content of 3.43% and pH = 6.6 in the 0-25 cm layer.

Figure 1. Experimental plots in the central zone of the Republic of Moldova (Ivancha village, Orhei region, May 2012)

The second site was located in the southern zone, in the Tartaul de Salchie village, Cahul region (Figure 2). These were plots with long-term arable (control), sheep manure and green manure treatments, mixture of ryegrass and lucerne and mixture of ryegrass and sainfoin.
Sheep manure was introduced in the dose of 50 t ha^{-1} in September 2009. Manure management plots served for the comparison. Vetch was used once as green manure.Vetch was planted in the autumn of 2009 and its green mass in the amount of 28 t ha^{-1} was plowed under disc in July 2010. Plots under mixtures of perennial grasses (ryegrass + lucerne and ryegrass + sainfoin) were founded in the spring of 2010.
The soil is the ordinary chernozem with humus content of 3.16% and pH = 7.1 in the 0-25 cm layer (Cerbari et al., 2012).
Soil samples were collected from the 0-25 cm layer of the experimental plots during 2010-2012.

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 (Gilyarov and Striganova, 1987). The identification of invertebrate's diversity at the level of families was carried out with the implementation of Gilyarov and Striganova's method (1987).

Figure 2. Experimental plots in the south zone of the Republic of Moldova (Tartaul de Salchie village, Cahul region, May 2011)

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 65-70°C for 24h, with K_c coefficient of 0.25 (Blagodatsky et al., 1987). K_2SO_4 - extractable carbon concentrations in the dried and fresh soil samples were measured simultaneously by dichromate oxidation; K_2SO_4-extractable carbon was determined at 590 nm using a CΦ 46 spectrophotometer.
Counts of microorganisms (heterotrophic bacteria, actinomycetes, fungi, polysaccharides - forming microorganisms) were obtained on agar plates (Zvyagintsev 1991, Skvortsova, 1981).

Enzymatic activity. 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 (Haziev, 2005). The (potential) polyphenoloxidase and peroxidase activities were determined colorimetrically using hydroquinone as a substrate (Karyagina and Mikhailovskaya, 1986).
The biological indices were evaluated statistically using the variation 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 0-25 cm.

RESULTS AND DISCUSSIONS

Invertebrates. The use of manure and green manure management led to the increase of all zoological indices in chernozems (Figures 1 and 2).

In conditions of the farmyard manure application in the leached chernozem the number of invertebrates increased on average from 55.0 to 94.0 ex m^{-2}, the number of *Lumbricidae* family – from 38.0 to 66.0 ex m^{-2}. A similar regularity was recorded in the ordinary chernozem when using the sheep manure. Invertebrates' population increased from 48.1 to 82.6 ex m^{-2}, the number of fam. Lumbricidae - from 25.6 to 51.1 ex m^{-2}.

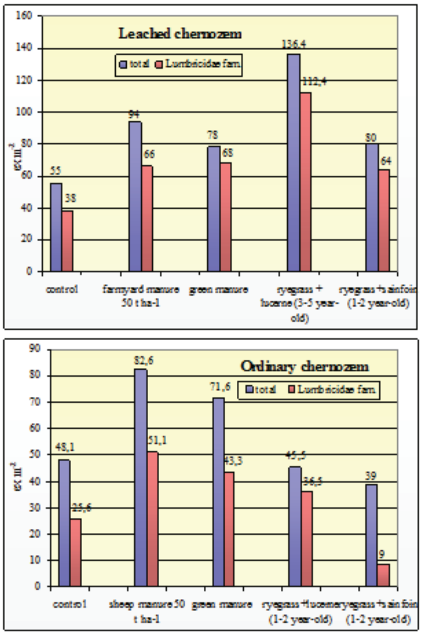

Figure 3. The influence of the manure, green manure application and perennial grasses on the number of invertebrates in chernozems (mean values, n = 6-12, P≤0.05)

The biomass of invertebrates increased of 1.4-1.5 times in both soils, biomass of *Lumbricidae* family – of 2.0 times, only in the leached chernozem (Figure 4). The family of *Lumbricidae* occupies a dominant position in the edaphic fauna. According to the statistical parameters its share in the total number of invertebrates increased from 69.1% to 70.2% in the leached chernozem, and from 53.2% to 61.9% in the ordinary chernozem when applying the manure management.

The favorable effect of the green manure management on invertebrates in chernozems has been noted both as the average values of indicators and as the confidence intervals. The number of invertebrates in the chernozem leached increased from 55.0 to 78.0 ex m^{-2}, in the ordinary chernozem – from 48.1 to 71.6 ex m^{-2}, the number of worms – from 38.0 to 68.0 ex m^{-2} and from 25.6 to 43.3 ex m^{-2} respectively. The biomass of invertebrates and *Lumbricidae* family remained practically unchanged in the leached chernozem, while these indices grew from 7.6 to 10.4 and from 6.2 gm^{-2} to 7.8 gm^{-2} in the ordinary chernozem. The share of worms in the total population increased from 69.1% to 87.2% in the leached chernozem, and from 53.2% to 60.5% in the ordinary chernozem.

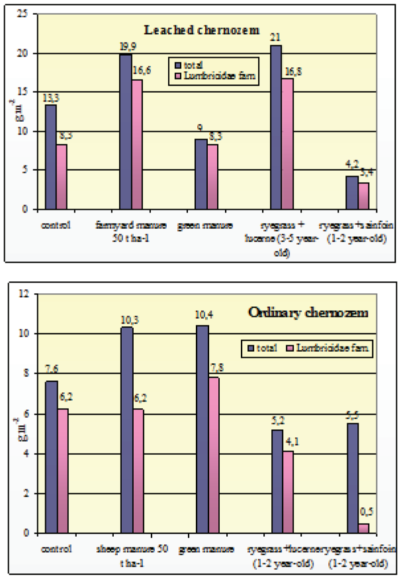

Figure 4. The influence of the manure, green manure application and perennial grasses on the biomass of invertebrates in chernozems (mean values, n = 6-12, P≤0.05)

The application of perennial legume-cereal grass mixtures led to the restoration of the total number of invertebrates and the *Lumbricidae* family. Statistically significant growth of soil zoological indicators has been registered after the third year of investigations on plots with grass mixtures (Figure 5). The number of invertebrates in 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. Their number in the leached chernozem increased by 3.0, and biomass – by 2.0 times. The population growth over 5 years constitutes 74.4 ex m^{-2} (744.000 ex ha^{-1}) or 14.9 ex m^{-2} (149.000 ex ha^{-1}) annually.

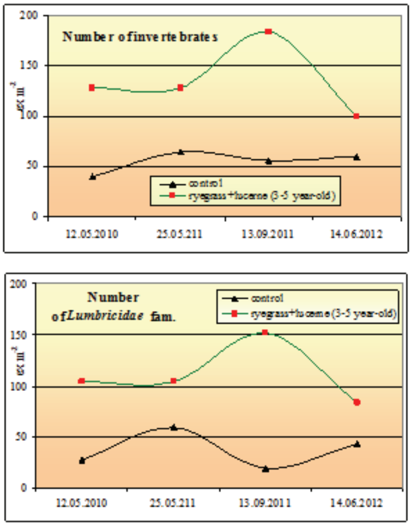

Figure 5. Restoration of invertebrates and Lumbricidae family in the leached chernozem by the perennial grasses' application

The application of grass cultivation improved the invertebrates' diversity in the leached chernozem after 5 years. The grass cultivated soil is characterized by a greater diversity of invertebrates. In addition to the *Lumbricidae* family, species of the *Formicidae, Gloremidae, 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 represents is observed. In general, the soil under grass mixture with ryegrass and lucerne contains 5 families of invertebrates, while the soil controls – only 3 (Table 1).

Table 1. Diversity of invertebrates in the leached chernozem after 5 years of the cultivation of grass mixtures ryegrass + lucerne (14.06.2012)

Variant	Family	Number of invertebrates, ex m^{-2}	
		families	total
Control	*Lumbricidae*	44	60
	Gloremidae	8	
	Coccinelidae	8	
Ryegrass+lucerne (3-5 year old)	*Lumbricidae*	84	100
	Gloremidae	4	
	Coccinelidae	8	
	Carabidae	4	
	Formicidae	+++	

Microorganisms. The utilization of manure and green manure contributed to the increase of the microbial carbon content from 275.8 to 350.9-384.6 µg C g^{-1} soil in the leached chernozem and from 216.2 to 299.4-313.5 µg C g $soil^{-1}$ in the ordinary chernozem (Figure 6).

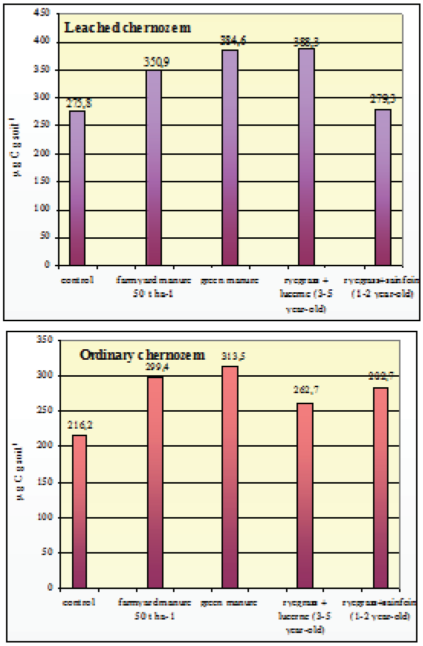

Figure 6. Microbial biomass in chernozems in conditions of the application of different restoration methods (mean values, n = 9-15, P ≤ 0.05)

Reproduction and stimulation of the growth of bacteria and fungi were observed. The number of polysaccharides-forming microorganisms, which play an important role in the formation of water-stable soil structure and the retention

of moisture in arable layers, increased by 1.3-1.8 times in the application of manure. The effect of green manure crops to polysaccharides-forming microorganisms was significant only in the leached chernozem, their numbers increased by 1.4 times.
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.
The grass cultivation during 1-2 years stimulates the restoration of bacteria and actinomycetes in the leached chernozem, fungi – in the leached chernozem and ordinary chernozem. Microbial biomass increases significantly only in the ordinary chernozem (Figure 6, Table 2).
The process of natural recovery of the soil biota composition and activity in degraded soils is slow. Therefore, statistically valid changes in the restoration of the microbial community were registered for 3-5 years cultivation of ryegrass and lucerne. The mixture of ryegrass with lucerne contributes to increase the microbial biomass from 231.8 to 319.8 μg C g^{-1} soil to 316.8 to 459.8 μg C g^{-1} soil, stimulates the recovery of bacteria, actinomycetes and fungi.
Enzymatic activity. The tendency to activate the enzymes in fertilized with manure and green manure chernozems has been identified. Dehydrogenase activity in the leached chernozem increased on average of 1.4 times under the influence of farmyard manure. Some growth of the activity of polyphenoloxidase in the ordinary chernozem with sheep manure application was observed.

Table 2. Recovery of the biota of long-term arable soils in conditions of the land management with farmyard manure, green manure and perennial grasses (confidence intervals, P ≤ 0.05)

Variant	Number of invertebrates, ex m^{-2}		Biomass of invertebrates, gm^{-2}		Microbial biomass, µg C g^{-1} soil	Polysaccharides-forming microorganisms, CFU g^{-1}soil*10^6	Dehydrogenase, mg TPF $10g^{-1}$ soil $24h^{-1}$	PPO, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}
	total	*Lumbricidae* family	total	*Lumbricidae* family				
Leached chernozem (n=6-12)								
Control	35-75	20-56	3.5-23.1	0-17.2	214-320	1.8-2.8	0.5-1.8	4.3-6.1
Farmyard manure 50 t ha^{-1}	40-148	23-109	6.7-33.1	4.5-28.7	328-374	1.2-4.8	1.1-2.1	4.7-6.7
Green manure (vetch)	44-112	32-104	3.2-14.8	2.4-14.2	331-439	2.0-4.4	0.8-1.4	4.2-6.2
Ryegrass+lucerne (3-5 year-old)	103-170	83-142	12.5-29.5	8.8-24.8	317-460	2.2-2.8	1.4-1.8	4.1-5.5
Ryegrass+sainfoin (1-2 year-old)	15-145	6-123	1.4-7.1	0.6-6.3	237-319	2.2-2.8	1.0-1.6	3.9-5.7
Ordinary chernozem (n=6-15)								
Control	32-65	13-38	3.0-12.2	1.4-11.0	206-227	1.4-2.2	0.9-2.1	7.8-14.0
Sheep manure 50 t ha^{-1}	45-120	31-71	4.6-16.0	3.6-8.8	243-356	2.0-4.4	1.2-1.7	9.1-13.1
Green manure (vetch)	36-108	12-75	3.3-17.5	1.1-14.5	283-344	1.4-2.0	1.3-1.8	6.7-11.3
Ryegrass+lucerne (1-2 year-old)	12-79	8-65	1.7-8.7	0.5-7.7	229-297	1.0-2.6	1.8-2.3	6.6-12.5
Ryegrass+sainfoin (1-2 year-old)	16-62	1,4-17	0.2-10.8	0-1.9	249-317	1.4-2.8	1.7-2.2	7.2-13.0

[1] CFU – colony forming units
[2] PPO – polyphenoloxidase

The downward trend of the peroxidase activity was registered in both soils. This shows that the intensity of the decomposition of humus compounds in the soil amended with manure and green manure decreases. Ratio between polyphenoloxidase and peroxidase
activities characterizing the process of humus accumulation increases from 0.19 in the control

plot to 0.22-0.23 in the plot with application of manure and green manure on the leached chernozem.

The use of the mixture of perennial grasses had a stimulating effect regarding to the dehydrogenase in both soils. Its activity has 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%. In contrast, activities of polyphenoloxidase and peroxidase have not been changed statistically significant.

It should be noted that the indicators of edaphic fauna and microbial biomass responded more quickly to the application of biological methods of the soil quality restoration in comparison with the number of microorganisms and enzymatic activity indicators. The biomass of biota is restored quicker, its diversity and enzymatic activity – to a lesser extent.

CONCLUSIONS

The application of organic fertilizer in the forms of farmyard and sheep manure, green manure by vetch according to most biological indices contributes to the regeneration of the biota in chernozems, which was degraded as a result of the long-term utilization in conditions of the Republic of Moldova. The common characteristic of these methods is the growth of the number and biomass of invertebrates and microorganisms, the multiplication and development of the young generation of *Lumbricidae* family, increasing the number of polysaccharides-forming microorganisms.

The positive action of farmyard and sheep manures on the soil biota manifests through the activation of polysaccharides-forming microorganisms by 1.3-1.8 times and the intensification of polyphenoloxidase activity.

The effect of green manure on the soil biota manifests by the increase of the share of *Lumbricidae* family in the total number of invertebrates by 12.1-20.8% and the microbial biomass productivity of 1.4 to 1.5 times.

The effective restoration of the biota in degraded arable chernozems occurs as a result of the cultivation of perennial grasses. The use of the mixture of ryegrass + lucerne during 3-5 years led to the growth of the number of invertebrates and *Lumbricidae* family by 2.5 and 3.0, their biomass - by 1.6-2.0 times in comparison with the control plot. The microbial biomass accumulates in the soil in amounts of 132.5 kg ha^{-1} annually. The annual population growth of worms reaches of 14.9 ex m^{-2}.

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