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INFLUENCE OF *NO-TILLAGE* PRACTICES AND GREEN MANURE TREATMENT ON SOIL BIOTA OF THE ORDINARY CHERNOZEM IN THE SOUTHERN ZONE OF THE REPUBLIC OF MOLDOVA

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Abstract

Statistical parameters of the biological properties of ordinary chernozem under different land management in the southern zone of the Republic of Moldova have been elaborated. The number of Lumbricidae family and total invertebrates in conditions of no-tillage and vetch as a green manure increased on average by 3.0-6.0 and by 2.2-2.5 times respectively compared with plowing. The base mass of invertebrates and earthworms in the ordinary chernozem under arable is concentrated in the 0-10 cm layer, in conditions of no-tillage and green manure treatment - in the 0-20 and 0-30 cm layer. The application of conservative tillage and green fertilizers led to the restoration of the total number of invertebrates and the Lumbricidae family, the annual growth rate is of 36.1 and 12.4 ex m^{-2} accordingly. The positive action of no-tillage and vetch as a green fertilizer on biota is manifested by the growth of microbial biomass on average by 13.9% and activation of dehydrogenase, polyphenoloxidase and peroxidase by 15.3%, 28.9% and 6.4%. Soil biological parameters did not reach the level of the soil under natural vegetation.

Key words: *biota, parameters, ordinary chernozem, no-tillage, green manure.*

INTRODUCTION

The soil cover of the Republic of Moldova is subjected to processes of desertification and anthropic degradation (Cerbari et al., 2010). The biological degradation of arable soils is interconnected with the dehumification processes, compaction and destruction of the soil structure. The current state of the biota in the arable soils of the Republic of Moldova is characterized by a significant reduction of abundance, biomass, activity and diversity, as compared to the soils of standard natural ecosystems (Senicovscaia et al., 2012). The values of most biological indicators in zonal soils decrease in the following sequence: natural and long-term fallow land → arable land under ryegrass and the organic farming system with the incorporation of manure and crop residues → arable land without fertilizers.
A major reason for the deterioration of soil biological properties and for the decrease of humus content under arable agriculture is annual tillage, which aerates the soil and breaks up aggregates where microbes are living. Previous research has demonstrated that the conventional tillage system leads to structural aggregates destruction in the soil, and therefore of biota' habitat (Senicovscaia et al., 2008). Annual plowing destroys agronomically valuable aggregates in the top layer of chernozems and their hardening in big sized soil particles in underlying 10-20 cm layer, thereby forming the soil compaction. The microbial biomass content in soil aggregates decreases according to the same regularity (Lungu et al., 2017).
The negative effects of conventional tillage have led to necessity of application of alternative soil management practices. These techniques are known as *"conserving/preserving tillage"*. It should be noted that the concept of conservation processing includes many processes from direct seeding and *no-tillage* to the deep cultivation without turning of soil layers. The implementation of soil tillage technologies should be based on the detailed knowledge of characteristics of soil. Necessarily should be taken into account such important factor as soil biological properties in cases when soil conservation tillage is applied.
Previous studies have demonstrated the effectiveness of *no-tillage* technology in

conditions of the northern zone of the Republic of Moldova (Senicovscaia et al., 2015).

The way to support the functioning of soil biota, to increase the level of biodiversity and resistance of soils used for a long time as arable is the cultivation of leguminous cultures as a green manure (Senicovscaia, 2013). This practice has several positive aspects from the microbiological point of view. It provides nutrient-rich organic matter for the microbial community which easily converts organically bound nutrients in plant residues to easily available nutrient form to the crops. Green manure application enhances the biodiversity of soil microorganisms. Green manuring with legumes has added advantage due to the capacity of legumes to fix atmospheric nitrogen and to decompose easily. Presumably this technique will improve the functioning of edaphic fauna.

In connection with foregoing, the evaluation of the biota recovery process in degraded chernozems, including *no-tillage* soil conservative system and the application of leguminous crops as a green fertilizer, is an actual problem.

Such research corresponds to the tasks of soil bio-testing for biodiversity conservation and development of soil quality standards.

The purpose of the research was to determine the influence of *no-tillage* practices and green manure application on biota of the ordinary chernozem for the improvement of its soil quality and environmental certifications.

MATERIALS AND METHODS

Site. The experimental site is located in the southern zone of the Republic of Moldova, in Natcubii-Agro SRL, Larga Noua village, Cahul region. Agricultural lands are located on the quasi-horizontal surface of the highest terraces of the Prut River. The absolute altitude of the site is about 120-121m.

The technology of *no-tillage* has been implemented on the area of about 2.000 hectares during 2 years before the application of green manure treatment.

Vetch was used twice as a green manure. Vetch was planted in the September of 2014 and its green mass in the amount of 26 t ha^{-1} was plowed under disc in May, 12, 2015 (Figure 1). On the same plot, a vetch was sown again, and its green mass was introduced in the amount of 17 t ha^{-1} into the soil by disking in September, 30, 2015. The experimental plot occupied 0.15 ha.

Figure 1. Field with a vetch in the southern zone of the Republic of Moldova, in Natcubii-Agro SRL, Larga Noua village, Cahul region

Land management practices with application of *no-tillage* with green manure treatments (plot 3) have been compared with the long-term conventional tillage with plowing to a depth of 25-27 cm (plot 1) and 55-year-old fallow plot.

Soil. The soil is the ordinary chernozem, subjected to deep plowing 40 years ago and used for plowing in the next 20 years. The profile of the arable chernozem demonstrates the strong compaction of the arable layer in depths of 5-20 cm and massive structure (Figure 2, a). As a result of the incorporation into the soil of the green mass of vetch (Figure 2, b), the physical quality of the layer 0-20 cm has evolved from the unfavorable to very favorable. The soil penetration resistance has become low and extremely low and the structure turned out to be agronomically favorable.

a b

Figure 2. The profiles of ordinary chernozem in conditions of long-term conventional tillage (a) and *no-tillage* with green manure treatments (b)

The initial values of the humus content in the soil of plot 1 constitute 2.43 %, pH = 7.9, the content of mobile phosphorus - 1.09 mg 100 g^{-1} soil, mobile potassium - 17.7 mg 100 g^{-1} soil, nitrates ($N-NO_3$) - 0.24 mg 100 g^{-1} soil in the 0-35 cm layer. The humus content in the soil of plot 3 before green manuring constitutes 2.49%, pH = 8.0, the content of mobile phosphorus - 1.47 mg 100 g^{-1} soil, mobile potassium - 19.7 mg 100 g^{-1} soil, nitrates ($N-NO_3$) - 0.24 mg 100 g^{-1} soil in the 0-35 cm layer.

Soil samples were collected from the 0-10, 10-20 and 20-30 cm layers of the experimental plots during 2015-2018.

Status of invertebrates was identified from the test cuts by manual sampling of the soil layers to the depth of soil fauna occurrence. The diversity at the family level was categorised according to Gilyarov and Striganova (1987).

Microbiological properties. The microbial biomass carbon was measured by the rehydration method (the difference between C extracted with 0.5 M K_2SO_4 from fresh soil samples and from soil dried at 65-70°C for 24 h (Blagodatsky et al., 1987). K_2SO_4 - extractable organic 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 "СФ-103" spectrophotometer. To estimate the contribution of microbial carbon to the total content carbon, the ratio between the carbon of the microbial biomass and the total carbon expressed in % was used (Kennedy & Papendick, 1995). Reserves of microbial biomass have been calculated taking into account the carbon content of the microbial cell and the bulk density of soils.

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 dehydrogenase activity was determined by the colorimetric technique on the basis of triphenylformazan (TPF) presence from TTC (2,3,5-triphenyltetrazolium chloride) added to the soil (Haziev, 2005). The polyphenoloxidase and peroxidase activities were determined by the colorimetric technique using hydroquinone as a substrate (Karyagina & Mikhailovskaya, 1986).

Soil biological indices were evaluated by analysis of variance. 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

Invertebrates. Application of *no-tillage* with vetch as a green fertilizer has a favorable effect on the edaphic fauna of the ordinary chernozem. This effect has been noted both by the average values of indicators and by the confidence intervals. The number of invertebrates increased on average from 21.7 to 130.1 ex. m^{-2}, the number of earthworms - from 18.5 to 55.6 ex m^{-2} in comparison with the plot with arable tillage. The biomass of invertebrates rose from 4.4 to 10.8 ex m^{-2}, the biomass of *Lumbricidae* family - from 4.3 to 9.3 ex m^{-2} (Table 1).

The dominant position in the complex of invertebrates occupies the *Lumbricidae* family. The share of earthworms in the total abundance of invertebrates in the arable soil constitutes 85.3 % and their biomass - 97.7%. The share of *Lumbricidae* family in the total population on the plot with conservation tillage and application of green manure constitutes 42.7%, their biomass - 86.1%. These data indicate that when *no-tillage* and green fertilizer were used, other species appear in the complex of edaphic fauna of the ordinary chernozem.

The average weight of one exemplar of *Lumbricidae* family in the arable soil constitutes from its total absence to 0.24 g, on the plot with *no-tillage* and green manure - 0.20-0.33 g. The base mass of invertebrates (80.0%) and fam. *Lumbricidae* (88.0%) in the ordinary chernozem under arable is concentrated in the 0-10 cm layer according to the average data. When green fertilizers were applied together with the *no-tillage* soil conservation system, the most invertebrates (93.9%) are concentrated in the 0-20 cm layer and earthworms (93.2%) - in the 0-30 cm layer (Figures 2, 3). Chernozem in conditions of *no-tillage* and green manure management is characterized by an active thick layer of soil.

Table 1. Statistical parameters of biota in the ordinary chernozem under different land management in the southern zone of the Republic of Moldova (2015-2018)

Index	Plot 1, arable 25-27 cm					Plot 3, *no-tillage* and green manure					55-year-old fallow plot
	mean value	min	max	V, %	confidence interval ($P \leq 0.05$)	mean value	min	max	V, %	confidence interval ($P \leq 0.05$)	mean value (n=3)
Invertebrates (n=7)											
Number of invertebrates, ex m^{-2}	21.7	0	48.0	75.9	6.5-36.9	130.1	32.0	272.0	78.6	35.4-224.8	448.0
Number of *Lumbricidae* fam., ex m^{-2}	18.5	0	40.0	83.5	4.2-32.8	55.6	16.0	96.0	54.3	27.6-83.6	340.0
Biomass of invertebrates, g m^{-2}	4.4	0	10.0	92.4	0.6-8.2	10.8	7.0	19.2	41.1	6.7-14.9	84.0
Biomass of *Lumbricidae* fam., g m^{-2}	4.3	0	9.6	93.2	0.6-8.0	9.3	5.2	18.8	54.6	4.6-14.0	74.8
Microorganisms (n=33-45)											
Microbial biomass, µg C g^{-1} soil	204.1	144.1	258.7	16.6	192.1-216.1	232.4	126.9	486.9	43.9	201.8-263.0	415.6
Enzyme activity (n=21-45)											
Urease, mg NH_3 10 g^{-1}soil 24 h^{-1}	3.9	2.9	5.7	22.6	3.3-4.5	3.8	2.8	5.3	17.4	3.5-4.1	5.5
Dehydrogenase, mg TPF 10 g^{-1} soil 24h^{-1}	1.57	0.95	2.35	23.9	1.43-1.71	1.81	0.96	3.53	34.7	1.63-1.99	2.79
Polyphenoloxidase, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	18.0	6.5	28.0	35.6	15.7-20.3	23.2	12.5	31.0	25.0	21.5-24.9	20.1
Peroxidase, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	28.3	23.0	34.0	11.1	27.1-29.5	30.1	24.5	36.0	11.2	29.0-31.2	31.3

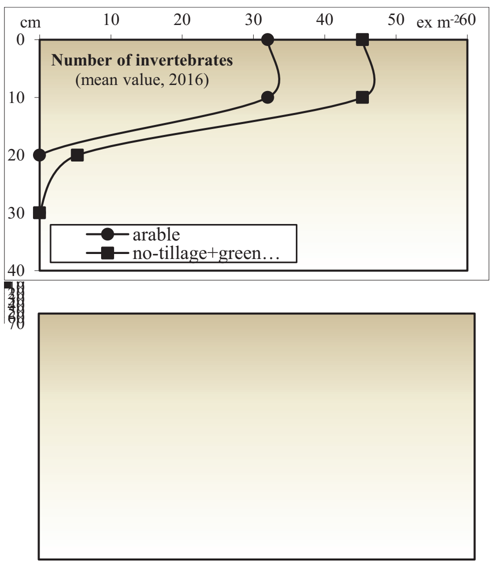

Figure 2. Profile distribution of invertebrates in the

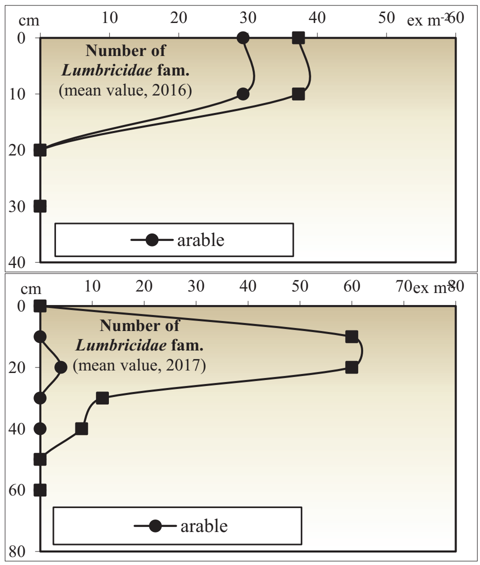

Figure 3. Profile distribution of earthworms in the ordinary chernozem under different land management

Ordinary chernozem in conditions of *no-tillage* and green manure is characterized by a high diversity of invertebrates in comparison with the soil under arable.

This soil contains 4-8 families of invertebrates, whereas under *arable* only 3-5 families depending on the year of sampling (Table 2).

Lumbricidae family is prevalent in soils with both types of tillage. *Lumbricus terrestris* was a typical representative of *Lumbricidae* family. In addition to *Lumbricidae* family, other species of the *Enchytraeidae, Elateridae, Julidae, Araneidae, Carabidae, Coccinellidae* and *Formicidae* families in faunal samples have been identified.

Table 2. Biodiversity of invertebrates (ex m^{-2}) in the ordinary chernozem under different land management (mean value, 16.05.2018)

Invertebrate family	Plot 1, arable 25-27 cm	Plot 3, *no-tillage* and green manure
Lumbricidae	34.6	24.0
Enchytraeidae	0	5.3
Elateridae	5.3	2.6
Julidae	5.3	8.0
Araneidae	0	2.7
Scarabaeidae	10.7	0
Carabidae	2.7	2.7
Coccinellidae	0	8.0
Geophilidae	0	0
Formicidae	0	+
Specii neidentificate	5.4	0

Indices of invertebrates' number and biomass in the soil of both plots are characterized by the strong variability. There is a tendency of decrease of the variation coefficient from the arable soil (75.9-93.2 %) to the soil under *no-tillage* and green manure (41.1-78.6 %) by faunal indicators. Taking into consideration the increase in the total level and decrease in the amplitude of the biological parameters oscillations in soil with *no-tillage* and green manure, this testifies to the enhance of soil's ecological stability against anthropogenic impacts.

Microorganisms. The application of *no-tillage* technologies and vetch stimulates the restoration of microorganisms in the ordinary chernozem. According to statistical data, the use of *no-tillage* with green manure lead to the slight increase of microbial biomass content on average from 204.1 to 232.4 μg C g^{-1} soil (Table 1). Indices of microbial biomass in the soil of both plots are characterized by the medium and strong variability.

The main changes have been occurred in the soil layer of 0-20 cm. Microbial biomass increased from 219.3 μg C g^{-1} soil in the arable soil to 276.3 μg C g^{-1} soil in conditions of *no-tillage* and green manure (Table 3).

The share of microbial carbon in the total carbon in the plot under arable constitutes on average 1.55% and in the plot with *no-tillage* and green manure - 1.67%.

Table 3. The content and reserves of microbial biomass in the ordinary chernozem in conditions of different land management

Plot, variant	Depth, cm	Microbial biomass, μ g C g^{-1} soil	C_{MB}/C_{org}, %	Reserves of MB, kg ha^{-1}	
				in layers	in the 0-20 cm layer
25.04.2016					
Plot 1, arable 25-27 cm	0-10	235.1	1.66	573.6	1147.3
	10-20	235.1	1.66	573.7	
Plot 3, *no-tillage* and green manure	0-10	310.2	1.85	732.1	1414.3
	10-20	281.9	1.71	682.2	
29.05.2017					
Plot 1, arable 25-27 cm	0-10	216.4	1.52	528.0	1056.0
	10-20	216.4	1.52	528.0	
Plot 3, *no-tillage* and green manure	0-10	225.0	1.34	558.0	1240.9
	10-20	271.0	1.61	682.9	
16.05.2018					
Plot 1, arable 25-27 cm	0-10	194.2	1.35	431.1	1012.8
	10-20	218.7	1.56	581.7	
Plot 3, *no-tillage* and green manure	0-10	397.9	2.41	970.9	1409.7
	10-20	171.4	1.09	438.8	

The application of *no-tillage* contributes to the increase of microbial biomass reserves from 1072.0 to 1355.0 kg ha^{-1} in the 0-20 cm layer. Reserves of microorganisms' biomass in the 0-30 cm layer are 1492.7-1862.7 kg ha^{-1} in the plot with plowing and 1678.7-2012.2 kg ha^{-1} in the plot with *no-tillage* and green manure.

Enzymatic activity. A soil management with the application of *no-tillage* and the introduction of green fertilizers created favorable conditions for the improvement of the enzymatic activity in the soil. Dehydrogenase activity in the ordinary chernozem increased on average by 1.2 times and polyphenoloxidase activity - by 1.3 times under the influence of *no-tillage* and green fertilizers (Table 1). In contrast, activities of urease and peroxidase on average have not shown statistically significant changes. Although in some periods the activity of urease and peroxidase on the plot with conservation technology was significantly higher than on the plot with arable tillage. The strongest impact on enzymes was recorded in the 0-20 cm layer.

Urease, dehydrogenase and peroxidase indices are characterized by the medium variability, polyphenoloxidase - by the strong variability.

The variation coefficients of the urease and polyphenoloxidase activities in the chernozem under conservation tillage and green manure are lower than in the arable chernozem.

CONCLUSIONS

The application of *no-tillage* practices with the introduction of green fertilizers in the southern zone of the Republic of Moldova improves the conditions for functioning of biota in the ordinary chernozem at the level of high values of parameters. The number of invertebrates increases on average by 6.0 times, *Lumbricidae* family - by 3.0 times, biomass - by 2.5 and 2.2 times as compared with conventional tillage. The annual growth rate of invertebrates and earthworms constitutes 36.1 and 12.4 ex m^{-2} accordingly. Biomass of microorganisms increases on average by 13.9%, dehydrogenase, polyphenoloxidase and peroxidase activities - by 15.3%, 28.9% and 6.4% in comparison with the arable plot. The base mass of invertebrates and fam. *Lumbricidae* in conditions of *no-tillage* and green manure treatment is concentrated in the 0-20 and 0-30 cm layers, maximum values of microbial biomass and enzyme activity have been recorded in the soil layer of 0-20 cm. Biological indications show a tendency to decrease the coefficient of variation from the arable soil to the soil under application of *no-tillage* with vetch as a green fertilizer. This indicates an improvement of soil's ecological stability to anthropogenic impacts and increases the homogeneity of the topsoil, although biological parameters do not reach the level of soils under the natural vegetation.

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