Effect of irrigation with saline water on the ordinary chernozem microbial community state

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Abstract: *The effect of irrigation with saline waters on the soil microbial community structure and functioning were studied. Our research was carried out in a long-term stationary field experiment. We identified the biological indices of irrigated soil and its unirrigated analogue. We studied indicators such as: the number of microorganisms belonging to different ecological- functional groups, invertase activity, and cellulose-destroying capacity of soils. To assess the functional state of the microbial community, the following indicators were calculated: oligotrophy and mineralization indices, the total biological index, and the soil biological degradation index. The direction and the degree of change in the biological properties of ordinary chernozem influenced by of irrigation with saline waters have been identified. Irrigation with saline water (total mineralization from 1.2 to 2.2 g/l) of ordinary chernozem (Odessa region, Ukraine) in an intensive mode lasting 12–13 years led to a noticeable suppression of the soil microbial community, a decrease in the number of microorganisms of the main groups on average by 30–40%, increased mineralization processes. The use of land reclamation measures (such as the annual application of phosphogypsum in a dose (3 t/ha) and/or comprehensive measures (3 t/ha of phosphogypsum annually +* $N_{150}R_{90}K_{60}$ *+ 18 t of manure per hectare of the crop rotation area) enable the regulation of soil biodynamic processes and partial or complete prevention of biological degradation.*

Keywords: *Soil microbial community, microbiological indicators, soil invertase activity, irrigation with saline water, ordinary chernozem.*

I. Introduction

*Influence of irrigation on fundamental soil processes***.** Irrigation is greatly affects on soils and removes it from the natural systemic balance in order to further stabilization of soil properties for making a higher level of productivity. Additional moisture, in addition to natural moisture, primarily affects the water-salt regime of irrigated soils, which determines the direction and changes in the biological rhythm of soils. As a result, this causes the transformation of the main soil components - organic and mineral, and also affects the soil formation processes deeper into the profile (Baliuk et al., 2017). A long-term study found that irrigation accelerates anthropogenic soil evolution. The variability of the acquired soils qualities in space and time are determined by the reaction of soils regimes in response to the quality of irrigated water and changes of hydrothermal conditions (Baliuk, 2001; Baliuk et al., 2014 and 2017; Bouaroudj et al., 2019; Mora et al., 2017).

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Using water of limited suitability for irrigation and, especially, water not suitable for irrigation according to agronomic and environmental criteria, caused the development of degradation processes: flooding, salinity, alkalinity, deterioration of agrophysical and hydrophysical properties, depletion in major and trace nutrients, and pollution (Baliuk et al., 2014, Strizhenok and Korelskiy, 2016, Minhas et al., 2019).

Influence of salinity on the soil microbial communities condition of chernozems. At this time, changes in the basic properties of chernozems, such as chemical, physicochemical, and agrophysical, that are caused by irrigation with saline waters has been studied quite thoroughly (Hillel et al., 2008), while the data on the effect of irrigation with adverse quality waters on the their biological properties remains insufficient. However, this data is not sufficient for an assessment of the current state of chernozems irrigated with saline waters, because it unilaterally reflects the essence of the processes occurring in secondary alkaline soils and secondarily saline soils under the irrigation with saline water. For a clear understanding of the functioning of soils nature, it is necessary to know the role of soil microbial communities in soils formation and the occurring processes, as well as responding of soils microbial communities to various influences of agricultural use.

Microbiological indicators ensure early diagnostics and allow estimating the anthropogenic burden influence on the biological properties of soils (Entry et al., 2008). Soil microbial communities, microbiological and biochemical processes are sensitive indicators. They are rapidly respond to any changes in the environment, soils regimes and properties and changes its structural and functional organization according to it. This phenomenon enables the identification of the trends of soils property changes before degradation symptoms manifest themselves. Therefore, for a valid assessment of the condition of irrigated soils, to determine the degree of their degradation, biological criteria must also be taken into account.

Nowadays, various microbiological and biochemical indicators for assessment of soil quality or soil biological degradation degree are used (Oldeman et al., 1990; Sims, 1990; Sparling, 1997; Mishra and Dhar, 2004;Bloem et al., 2006; Ritz et al., 2009; Blagodatskaya and Kuzyakov, 2013; Görgün and Yilmaz, 2014; Veum et al., 2014; Schloter et al., 2018).

It has been shown that salinity reduces microbial biomass and microbial activity, as well as changes the microbial community structure (Garcia and Hernandes, 1996; Rietz and Haynes, 2003; Wichern et al., 2006; Wong et al., 2008; Muscolo et al., 2011; Andronov et al., 2012; Elmajdoub and Marschner, 2013; Yan and Marschner, 2012, 2013; Yan et al., 2015). Secondary salinization is one of the main problems in irrigated agriculture. (Muscolo et al., 2011). Irrigation with saline waters often causes the soil (alkalinization) sodicity also. Soils microbial communities changes under the secondary salinization have been studied to a lesser extent.

The purpose of study - to find out the transformation (reorganization) of the microbial community state of ordinary chernozem under the irrigation with saline water, determine the direction and of change in the biological properties of soils, and also to identify the effect of agro-reclamation measures on the restoration structure and functioning of microbial community.

II. Materials and methods

2.1. Study site

The study site was located in Steppe zone, Danube-Dniester irrigation system (Odesa Region, Ukrine). Geomorphologically the territory belongs to the Pontic Black Sea coastal lowland. According to agroclimatic zoning, the territory belongs to the south-steppe subzone of the Atlantic-continental steppe climatic region, which is characterized by long hot summers and short warm winters. An average annual temperature is +10°C, sum of temperatures above +10°C is 3280, precipitation from April to October 127 mm, annual average precipitation is 340 mm.

We studied the influence of irrigation with saline waters on soil properties and microbial cenosis of ordinary chernozem. Soil has pH 7.4 (±0.1), content of toxic salts 0, 14 mEq/100 g of soil, $CaCO_3$ – 1.5-2.0%, Ca/Na in a water extraction 5.6, content of $Na^+ + K^+$ from the sum of absorbed bases 3.0%, average humus (0-25 cm) 3.0%.

The source for irrigation is Sasyk reservoir. On the average, the total mineralization of the irrigation water during the years of irrigation ranged from 1.2 - 2.2 g/l, pH 7.3 - 8.5, the content of chlorine anions was 12.0 - 19.4 meq/l, the content of $Na^+ + K^+$was 10.0 - 18.0 meq/l. Irrigated water is partially suitable for irrigation (class 2) due to the risk of alkalization and secondary salinization and unsuitable (class 3) due to the danger (hazard) of secondary sodicity; the intensive mode lasted for 13 years. During the experiment, irrigation was carried out using a sprinkler.

Studies were carried out in a scientific and production experiment. Crop rotation used in the experiment included seven fields as well as sowing perennial legumes. Alternation of crops was as follows: winter barley with the sowing of alfalfa – alfalfa – alfalfa – winter wheat – fodder beet – corn for grain – corn in the phase of milky-wax ripeness (for silage). The variants under study were as follows: irrigation, control; irrigation, phosphogypsum (3 t/ha, annually); irrigation, package of measures (phosphogypsum – 3 t/ha annually, $N_{150}P_{90}K_{60}$ (300 kg of active substance per ha), and manure, 18 t per hectare of the crop rotation area).

The area of the experimental plot was 0.3 ha, the iteration was 3 times. As a protective band for the experiment, we used an unirrigated control plot, which was sown and treated similarly.

2.2. Sampling

A soil profile pit was dug out on each investigated experimental plot andthe morphological structure of the profile was characterized. From each soil pit, we took mixed soil samples of each genetic horizon with account of their boundaries. In addition, we laid three wells on each fieldsite.In the wells, soil samples were picked from the layers at 0 - 25, 25 - 50, 50 - 75, and 75 - 100 cm. The depth of soil sampling for microbiological examination was within the topsoil layer at 0 - 25 cm. The volume mass density was determined by the cutting ring method and based on the structural aggregate composition (content of air-dry aggregates) by screening. Transportation, storage, and preparation of samples for further research in the laboratory was carried out according to the methodological framework existing in Ukraine. Soil samples were drying at 105 °C for 24 h for determination moisture content.

2.3. Chemical analysis

To study the chemical composition and physicochemical properties of the soils, we performed analytical work at the certified Laboratory of Instrumental Soil Research Methods of the National Scientific Centre “Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky.” The analytical measurements was carried out under the current State Standards of Ukrain. We analyzed the chemical ionic composition of the aqueous extract (1:5), the content of exchange-adsorbed cations by the Schollenberger method (modified according to the NSS ISSAR, pH potentiometric method). The maximum value of relative standard deviation (by a three-replicate analysis) of an individual sample was less than 10%.

2.4. Microbiological analysis

The number of microorganisms was determined by classic methods of soil microbiology (Zvyahintsev et al., 1980; ISO 7847:2016). Soil suspension of appropriate dilution was plated on selective nutrient media. Organic nitrogen-assimilating bacteria were cultured on meat-and-peptone agar (MPA), mineral nitrogen-assimilating bacteria and actinomycetes on starch-ammonia agar (SAA), fungi on theVaxman's medium, olygotrophic microorganisms on starvation agar. Plating was replicated four times with $1{:}10^4$ dilution. Petri's dishes were incubated during 5 days at a temperature 28 °C.

2.5. Enzyme activities

Invertases activity was determined by the photocolorimetric method according to Galstyan by the Galstyan's method (Haziev, 1976). A sample of 5 g of air-dry soil was placed in flasks with a volume of 50 ml. Then 10 ml of 5 % saccharose, 10 ml of acetate buffer (pH 4.7)), and 3–5 drops of toluene were added to flasks with soil. The contents of the flasks were mixed and the flasks were closed with cork stoppers and placed in a thermostat, where they were incubated at 37 C for 24 h. Further the soil suspension was filtered. 6 ml of the filtrate were placed in tubes, 3 ml of Rochelle salt and 3 ml of 10 % $CuSO_4$ were added, and the tubes were boiled in a water bath for 10 min. After cooling in cold water, the contents of the tubes were centrifuged at 3000 rpm for 5 min. The centrifugate was colorimetered on a photoelectrocolorimeter in cuvettes 1 cm wide at a wavelength of 590 nm

2.6. Cellulose-destroying capacity

The cellulose-destroying capacity of the soil was determined in laboratory by decreasing the mass of the cotton plates (Popova et al., 1987). The procedure was iterated 3 times.

2.7. Calculated indicators

We calculated the following indicators that characterize the functional state of the soil microbial community: the olygotrophy index according to Aristovskaya and Khudiakova (1977) and the mineralization index by the Mishustin (1975) method; the integral biological indices such asthe total biological index (TBI) using method of relative values (Azzi,1959) and the of biological degradation index (BDI) (Snakin et al., 1992, Naydyonova, Baliuk, 2014) The experimental data obtained in the course of our research has been processed using the Statistica 7.0 standard program package (Hrechanina, Isayeva, Kolesnikova, Isakova, 2019).

III. Results

During the years of irrigation, the following changes in the chemical, physicochemical, and physical properties took place in these soils (Table 1). The salt composition changed from sulphate-hydrocarbonate-calcium to chloride-hydrocarbonate-calcium-sodium and it caused increasing of toxic salts content (0 - 50 cm layer) to 0.66 - 0.69 %. The irrigated soils alkalinized; the content of absorbed $Na^+ + K^+$ increased from 3 % to 7 - 8 % of the sum of absorbed cations (soil alkalinity). Soil condensation took place, which is indicated by an increase in the bulk density from 1.3 to 1.4 - 1.45 g/cm^3, and the structural and physical state deteriorated (the amount of agronomically valuable aggregates reduced from 80 to 61 - 66 % (Table 1).

Table 1. Effect of chemical melioration and complex of measures on the properties of ordinary chernozem

Variants of experiment	Parameters of soil properties (0 - 25 cm; *0 - 50 cm)							
	content of toxic salts, eq.Cl^-, mEq/100 g of soil*	Ca^{2+}/Na^+ in a water extraction*	absorbed Na^+, mEq/100 g of soil	Na^++K^+, % from the sum of absorbed bases	pH water extraction	HCO^{-3}-Ca^{2+}, mEq/100 g of soil	content of air-dry aggregates (size 0.25 - 10 mm, %)	Volumetric mass density, g/cm^3
Rainfed control	0.14	5.6	0.4	3.0	7.4	0	82.8	1.35
Irrigated control	0.69	0.3	2.1	8.2	7.4	0.09	61.2	1.45
Irrigation+ phosphogypsum, 3 t/ha annually	0.66	0.5	1.7	7.0	7.5	0.13	66.0	1.38
Irrigation +complex of measures	0.69	1.9	1.3	7.0	7.2	0.05	77.6	1.33

In the soil samples of irrigated control sites, the number of bacteria utilizing organic nitrogen was reduced on average by 2 times compared with non-irrigated control sites. The number of bacteria assimilating mineral nitrogen was reduced by 30 - 44%, the number of actinomycetes by 28 - 36%, the number of fungi by 20 - 46%, the number of oligotrophs by 31 - 46%, and the number of eutrophs was by 37 - 47% lower, respectively, depending on the year of observation (Table 2). Introduction of phosphogypsum into the soil increased the number of microorganisms. Phosphogypsum dumps significantly influence the biosphere (Lutskiy et al., 2018). A package of ameliorative measures (phosphogypsum + NPK + manure) also increases the population of microorganisms to the level of unirrigated soil.

Table 2. Number of microorganisms belonging to basic ecological-trophic, taxonomical and physiological groups

Variants of experiment	Soil moisture content, %	Nitrogen assimilating micro-organisms, million CFU/g		Actinomycetes, million CFU/g	Fungi, thousands CFU/g	Olygotrophs, million CFU/g	Eutrophs, million CFU/g
			m				
After 12 years irrigation (crop – barley with the sowing of alfalfa)							
Without irrigation, control	17.39	4.16	32.56	11.04	42.51	45.58	46.76
Irrigation, control	17.91	.26	18.28	7,10	22.97	31.60	24.56
Irrigation, phospho-gypsum	17.35	2.72	30.41	9.44	32.99	42.75	43.16
Irrigation, complex of measures	17.52	4.62	25.19	9.07	56.17	52.46	39.87
LS	–		5.	3.03	3.	10.3	–
After 13 years irrigation (crop – winter wheat)							
Without irrigation, control	23.10	5.08	25.25	10.27	41.36	8.03	40.37
Irrigation,	23.26	.69	17.79	7.38	33.29	4.31	25.51

control							
Irrigation, phospho-gypsum	24.19	3.15	30.84	8.18	35.02	19.85	44.03
Irrigation, complex of measures	26.54	3.95	30.59	8.21	35.16	19.89	44.58
LS	–	[illegible]	4.	2.32	10	1.34	–

There have been also changes in the functional state of the microbial complex. An increase in the mineralization potential and the oligotrophy index of irrigated soil indicates a deterioration of the trophic regime in the soil. In addition there was a decrease in biochemical activity in irrigated soil. Invertase activity and cellulose destroying capacity of irrigated soil is much lower than that of similar non-irrigated soil (Table 3).

Table 3. Indicators, which characterizing the functional state of microbial community, and invertase activity of ordinary chernozem

Variants of experiment	Olygotrophy index		Mineralizationindex		Invertase activity, mg glucose/g soil for 24 hours	Cellulose destroying capacity, loss of mass, %
Duration of irrigation, years					12	12
Without irrigation, control					7.62	30.25
Irrigation, control					5.89	16.11
Irrigation, phosphogypsum					6.61	18.92
Irrigation, complex of					8.85	31.84
$LSD_{0,05}$					0.48	5.66

The TBI value of the irrigated control soil sample is significantly lower than that of the non-irrigated plot (Table 4). The TBI value growth in the reclaimed soil samples reflects the positive effect of reclamation on the soil microflora. Complex ameliorative measures showed the best efficiency. The BDI values show an average degree of degradation changes in the microbial communities of irrigated soil, a weak degree of biological degradation in case of phosphogypsum introduction, and absence negative changes in case of taking comprehensive measures (BDI<10).

Table 4. Assessment of intensive irrigation with saline water impact on the microbial community of ordinary chernozem and efficiency of agroamelioration measures application

Variants of experiment	Integral biological indices			
	TBI, %		BDI, %	
Duration of irrigation, years	2	3	2	3
Without irrigation, control	2	4		
Irrigation, control	3	7	42	35
Irrigation, phosphogypsum	1	0	12	10
Irrigation, complex of measures	2	1	8	

IV. Discussion

During the irrigation with saline water both are possible: low toxic salts accumulation in 0–25 cm and 25–50 cm layers or preservation of the total amount of salts, but transformation of their composition, which caused increasing of toxic ions content, primarily sodium (Baliuk et al., 2017). In the investigated object, the salt regime is of seasonal-reversible type, which manifests itself in the accumulation of salts, introduced with irrigation water during the irrigation season, in the root layer of irrigated soils, and in temporary salinization of this layer during the non-vegetation period under the influence of atmospheric precipitation.

Research has established that the introduction of phosphogypsum, both separately and as part of complex measures, has virtually no effect on the content of toxic salts in the root-containing layer, pH of soil solution, and toxic alkalinity (which approaches zero),as opposed to the irrigated control sample (Table 1). Salinity of the arable layer decreases slightly under the influence of chemical melioration butremains within the medium gradation degree (Isayeva, 2014).

In this case, the content of absorbed sodium in the SAC sample with the set of measures is lower than the one with a single phosphogypsum option, which can be explained by the addition of calcium with organic and mineral fertilizers and a decrease in the soil solution pH (up to 6.6) and also contributed to the penetration of Ca^{2+} at SAC. At the same time, the salinity level remains nominally moderate due to the high content of K^+ in SAC. Researchers, who have compared the absorption of sodium and potassium at SAC have noted that absorption of potassium from water extracts is much faster, its absolute values higher than sodium's (Kanwar, 1969, Baliuk, 2001). Potassium from organic and mineral fertilizers absorbed at SAC firstly. At the same time, potassium prevails sodium in 4-4.5 times in chemical composition of studied soils. It confirms possibility of potassium's transition from clay minerals from non-exchangeable form to exchangeable.

The parameters of the agro-physical state of the soil due to the application of the set of techniques are restored to the level of the non-irrigated control sample. The accumulation of soil colloids, due to the high content of NPK, organic fertilizers, and water-soluble salts, plays a certain role in this case. Only phosphogypsum affects these indicators to a somewhat lesser degree.

Chemical reclamation of irrigated soil with phosphogypsum is not capable to eliminating irrigation salinization completely, but only slightly decreases the salinity degree. Among the possible reasons is the insufficient solubility of phosphogypsum, which does not allow reaching such a concentration of Ca^{+} cations in the soil solution to prevent sodium absorption at SAC; the rapid passivity in the soil of water-soluble calcium from phosphogypsum, due to the formation of poorly soluble the rapid passivity of water-soluble calcium from phosphogypsumin in soil, due to formation of poorly soluble $CaCO_3$; the impossibility to remove completely the sodium sulfate (product of exchange between SAC and calcium from phosphogypsum) beyond the root layer, which slows down and stops the soil desalination process (Kanwar, 1969, Baliuk, 2001).

Research has shown that irrigation with mineralized water upsets the ecosystem's equilibrium state and induces the formation of microbial community with new parameters. The changes innumber of microorganisms, violation of structure and functioning of soil microbial community corresponded to transformation of the chemical, physicochemical, and agrophysical properties of soil caused by irrigation with saline water.

Intensive irrigation of ordinary chernozem with saline water for 13 years resulted in extensive negative deviations of structure and functioning of microbial community, enhancing its mineralization function. Agroameliorative measures allow to regulate microbiological and biochemical processes in the soil, partly or fully eliminating the phenomena of biological degradation.

The use of phosphogypsum and comprehensive reclamation measures (phosphogypsum + NPK + manure) increases the biogenicity of the soil, corrects the biological parameters, bringing them closer to the level of non-irrigated soil. In the sample plot, exposed to complex ameliorative measures, the tension of mineralization processes decreases.The mineralization index of soil from this sample plot is lower than in the irrigated control plot.

According to the values of TBI and BDI for soil from plot where the complex agroameliorative measures were applied, it is more effective to positively influence the on microflora's abundance, invertase activity, and the cellulose destroying capacity of soil than by introducing phosphogypsum only.

V. Conclusion

Thus, long-term irrigation with saline water leads to negative deviations of the microbial community parameters irrigated ordinary chernozem from its non-irrigated analogue. As a result of irrigation, soil sodicity also develops, which led to a decrease in the number of microorganisms and the biochemical activity of the soil. Complex of agroameliorative - measures (phosphogypsum + NPK + manure) effectively eliminate degradation manifestations.

It would be expedient to include the biological indicators, which we used, in the system of indicators that are used in the ecological monitoring of irrigated soils, and also to use them for ameliorative survey of irrigated soils. Microbiological and biochemical indicators will make it possible to more accurately, more fully and objectively assess the condition of irrigated soils, the direction and degree of change in microbiological processes in irrigated soils, as well as evaluate the effectiveness of agroameliorative measures to eliminate degradation phenomena and increase the fertility of irrigated chernozems.

Conflicts of interest: The authors declare to have no conflict of interest.

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