National University of Life and Environmental Sciences of Ukraine

ADVANCED IDENTIFICATION METHODS OF THE FUNCTIONAL FEATURES OF MICROBIOME OF WINTER WHEAT RHIZOSPHERE

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Relevance of the work

- Modern scientific researches aimed at studying the mechanisms of formation and quantitative analysis of plant-microbial interactions allow us to study the formation and ecological role of the cenoses in agrolandscapes.
- Rhizosphere formation is an integral basis for homeostasis of plant ontogenesis.
- The biological and functional features of the microbial communities of the rhizosphere are evaluated according to their effective interaction in plant-microbial systems.
- Functionally important for the rhizosphere microorganisms determine the optimal development of plants by at least 70% .
- The study of biological functional features of plant-microbial interactions, groups of microorganisms, the orientation of nutrition processes, in particular the biological transformation of carbon and nitrogen in the soil is of particular importance.

Fig. 1. Plant-microbial interactions

The purpose of the research

- The **purpose of the research** was to study the functional features of the formation of the rhizosphere microbiome in winter wheat agrophytocenosis.

Object and subject of the research

- **Object of the research:** microbial biological processes in the rhizosphere of winter wheat.
- **Subject of the research:** dominant groups of microorganisms, features of orientation of processes of biological transformation of carbon and nitrogen in soil, functional characteristics of microbiome formation of winter wheat rhizosphere.

Research objectives

1. To investigate the features of microbiome formation in winter wheat rhizosphere in relation to biological features of winter wheat culture.
2. To identify functionally significant dominant microorganisms of the rhizosphere.
3. To investigate the influence and interaction of microbial agents and plants at different stages of vegetative development.
4. To determine the orientation of the processes of biological transformation of carbon and nitrogen in the soil.

Research methods

- **Field methods** – on-field experiments and soil sampling
- **Laboratory methods** - cultivation and obtaining of pure cultures and further cultivation of obtained isolates; analysis of biochemical properties of obtained isolates
- **Statistical Methods** – calculations and analysis of results in Microsoft Office Excel

Fig. 2. Scheme of methods used for the thesis work

Determination of the number of microorganisms in different agricultural systems in the ontogeny of winter wheat

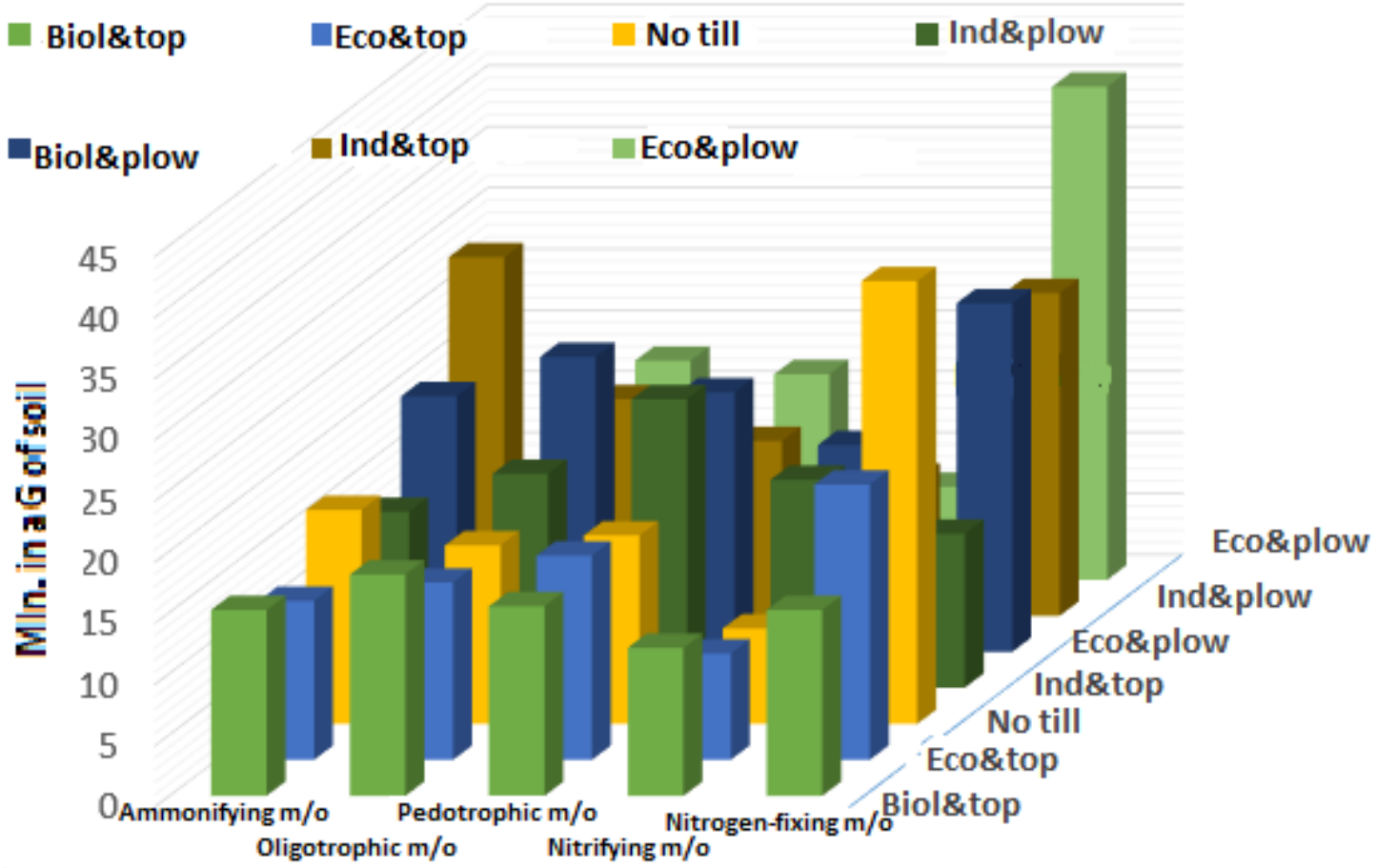


Fig. 3. The number of microorganisms of the basic physiological groups of typical black soils in the flowering phase of winter wheat

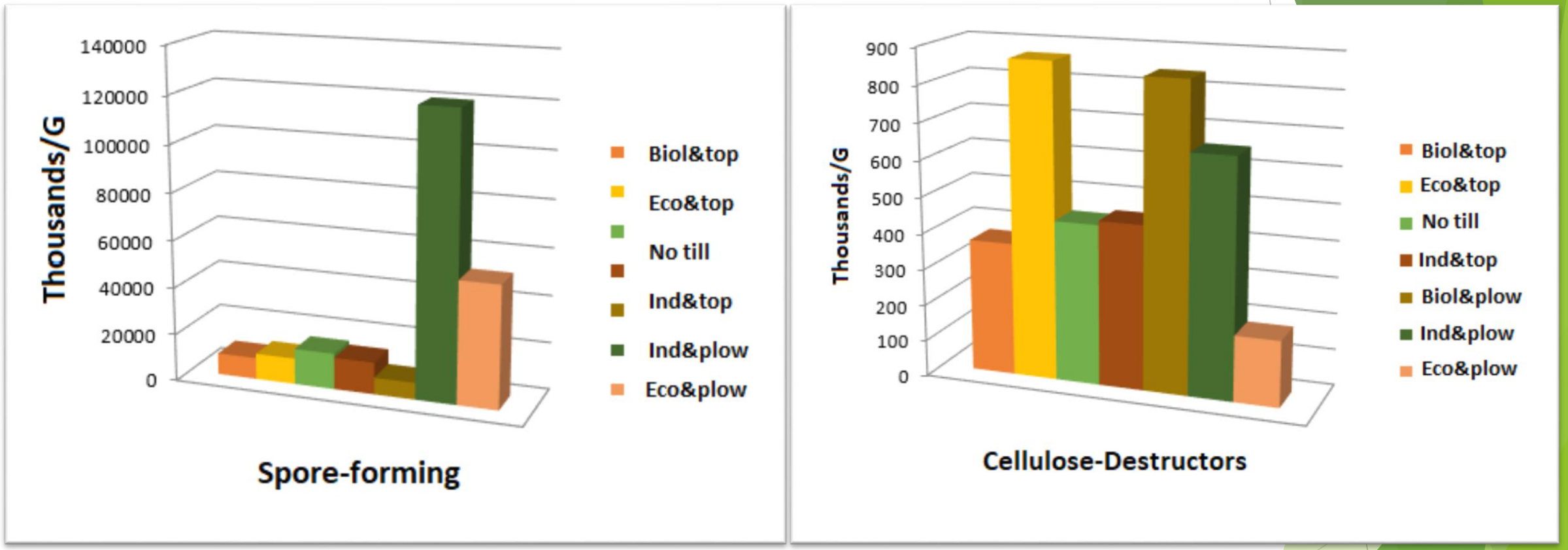


Fig. 4. The number of spore-forming (left) and cellulose-destructing microorganisms of the rhizosphere in the flowering phase of winter wheat

Examination of functional orientation of microorganisms

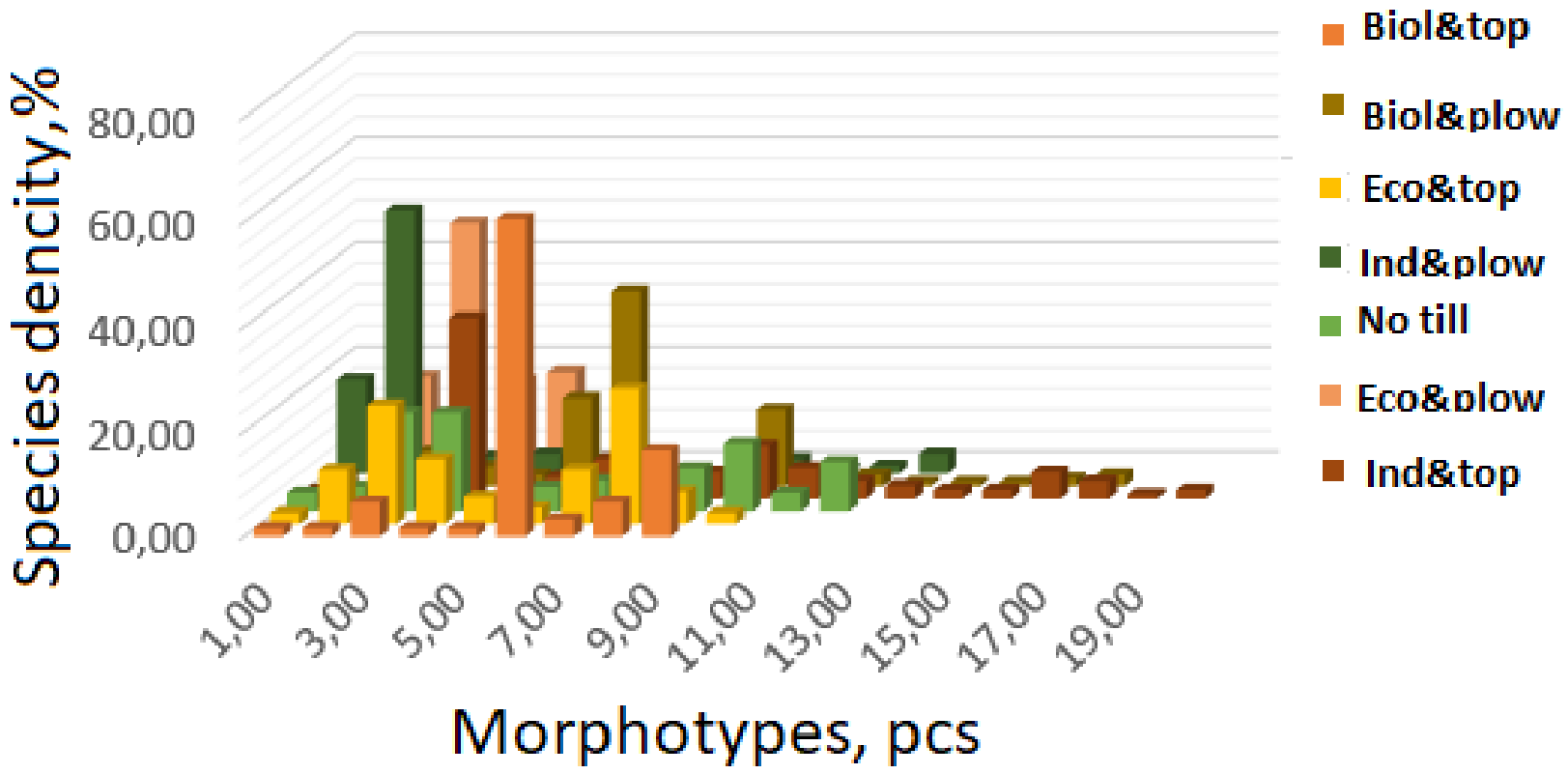
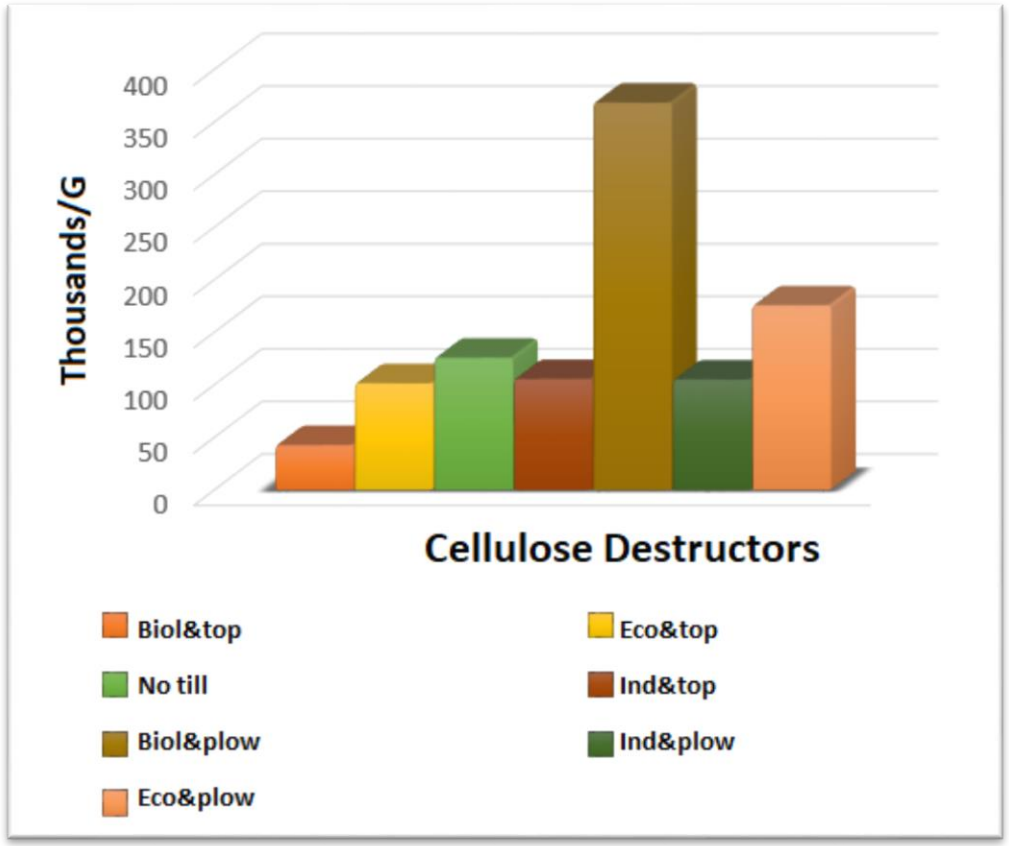


Fig. 5. The influence of agricultural systems and soil cultivation on qualitative composition of typical soil prokaryotes (flowering phase of winter wheat)



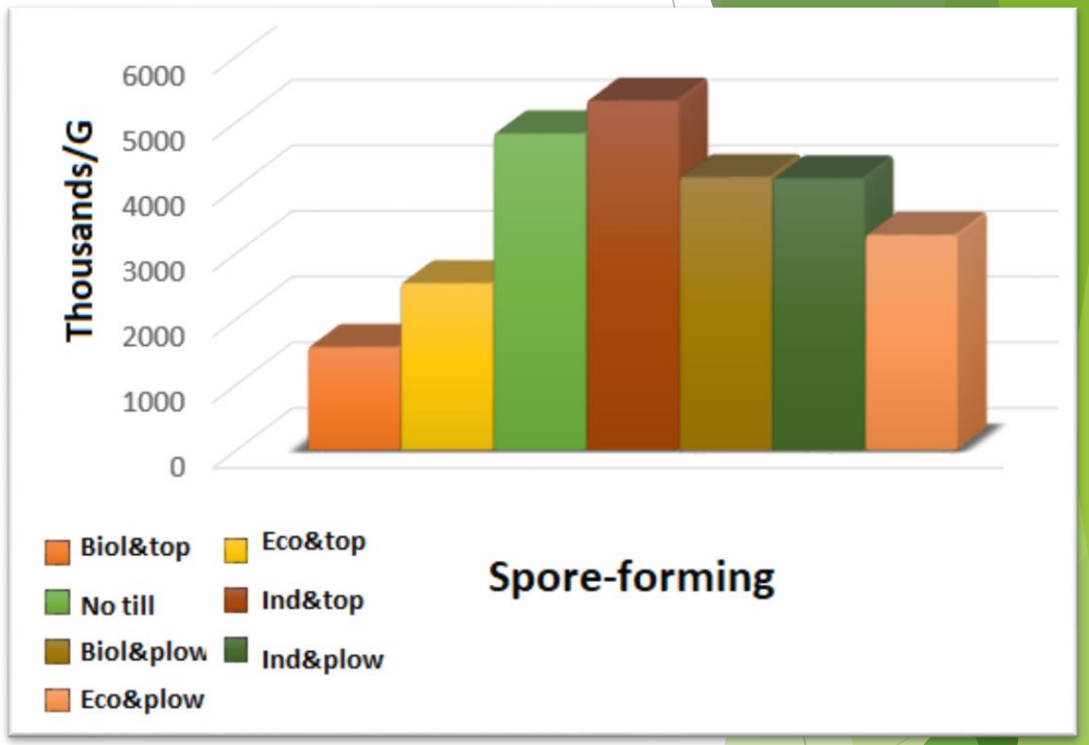


Fig. 6. The number of microorganisms of the basic physiological groups of typical black soils in the phase of waxy maturity of winter wheat

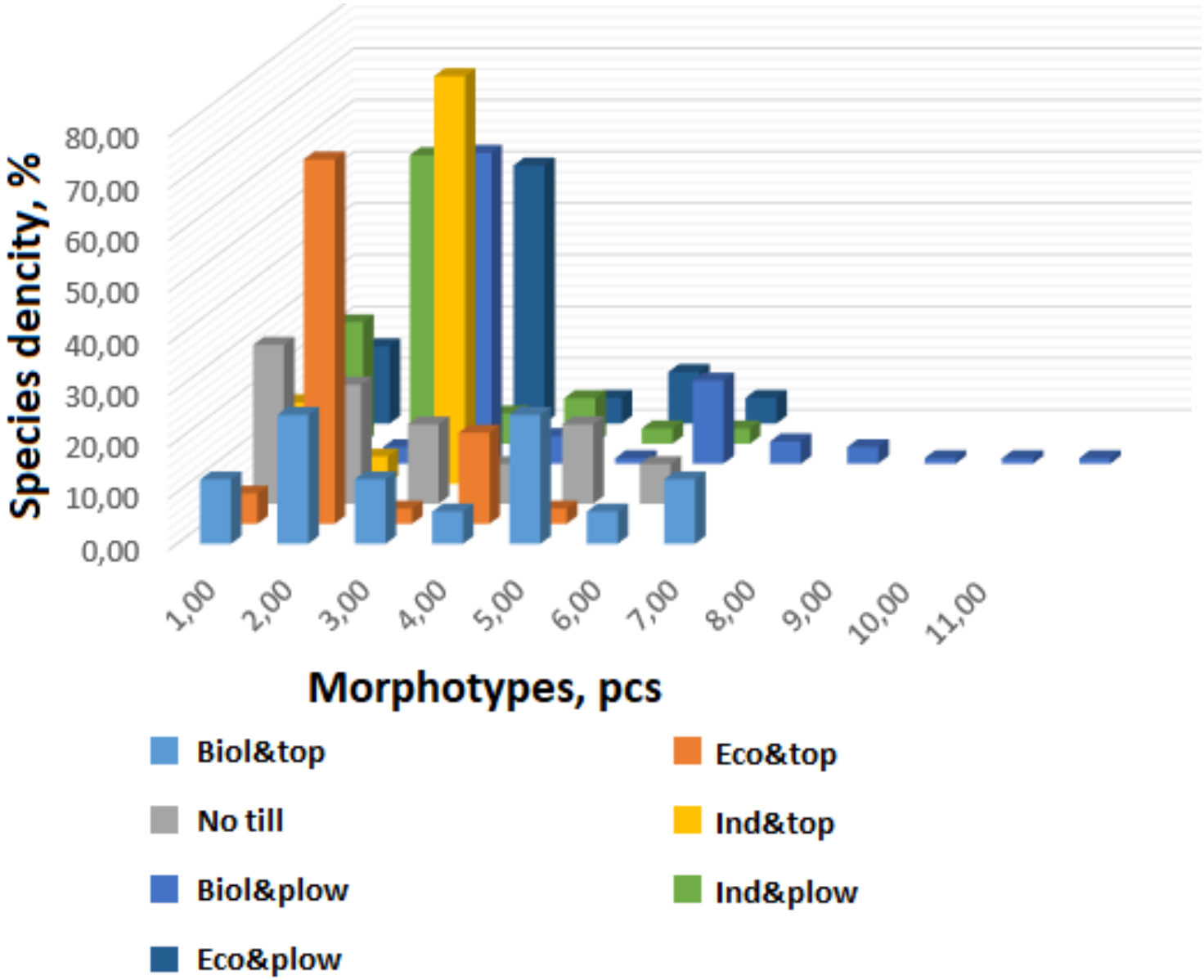


Fig. 7. The influence of agricultural methods and cultivation of soil on the qualitative prokaryote composition of the rhizosphere during the waxy maturity phase of winter wheat

Estimation of ecological indexes of microbiota formation of winter wheat rhizosphere

Agricultural System	Soil cultivation	Flowering stage		Maturing stage	
		Indexes			
		Shannon's	Simpson's	Shannon's	Simpson's
Industrial	top	1,05	0,15	0,27	0,65
	plow	0,74	0,30	0,54	0,38
Biological	top	0,58	0,41	0,79	0,18
	plow	0,90	0,20	0,62	0,18
Ecological	top	0,87	0,16	0,40	0,53
	plow	0,61	0,29	0,63	0,31
No till		0,99	0,12	0,73	0,21

Table 1. Indexes of microbiota formation of winter wheat rhizosphere during different stages of ontogeny

Pure cultures of dominating microorganisms

Fig. 8. Obtained pure cultures of microorganisms

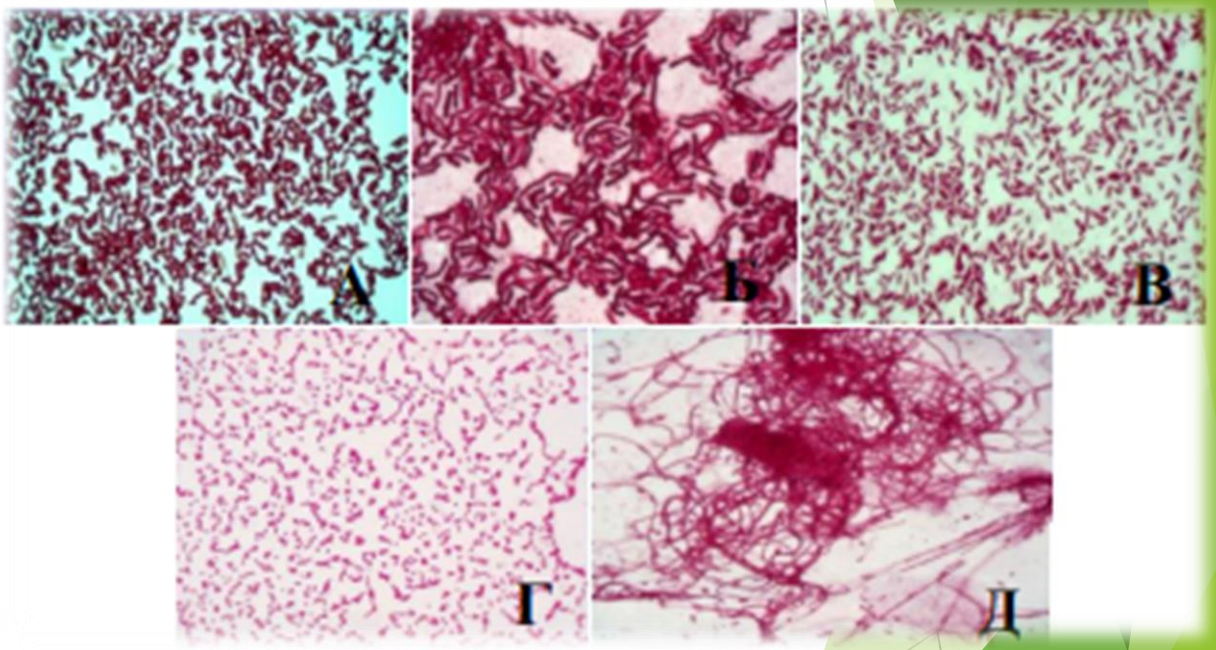


Fig. 9. Microscopic examination of pure cultures
A – “GPA-1”, B - “GPA-3”, C - “GPA-6”, D - “GPA-8”, E - “GPA-10”

Determination of sources of carbon nutrition of microorganisms

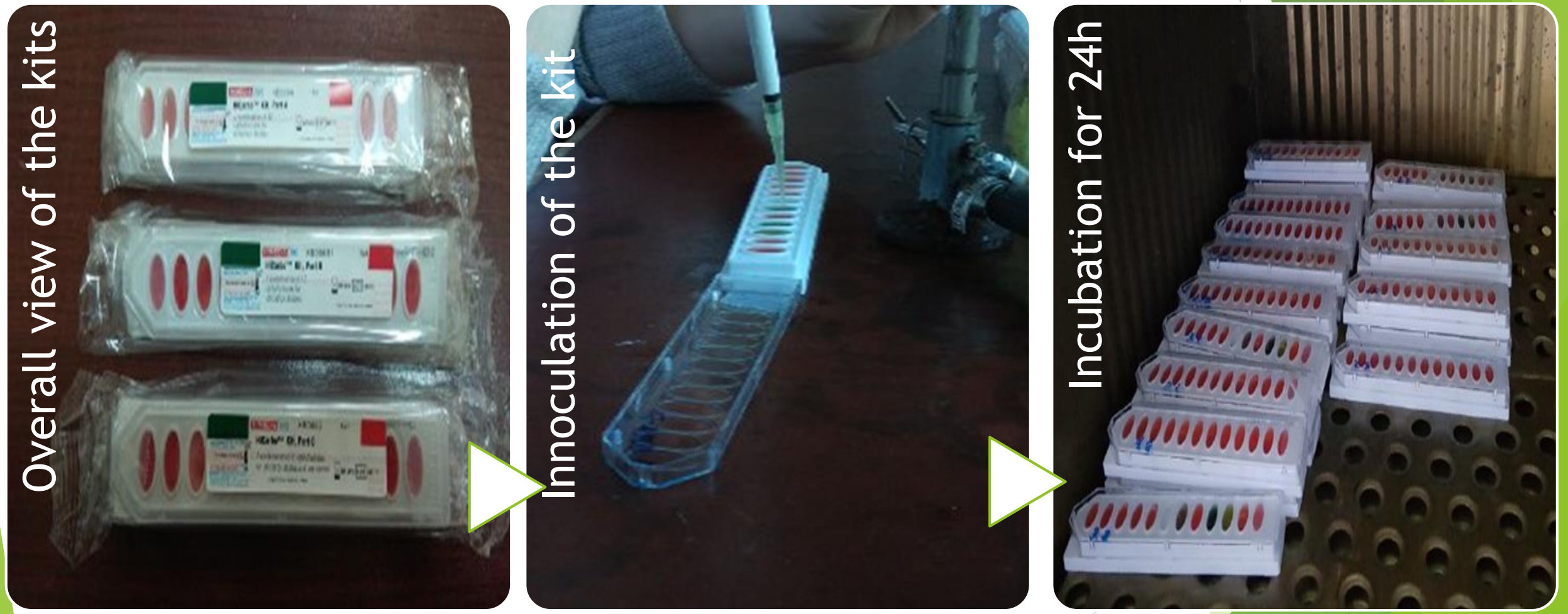


Fig. 10. Steps of biochemical analysis of obtained isolates using KB009 TM HiCarb

KB009 Hicarbo Kit Test Results

Fig. 11 . GPA-1 test results

Fig. 12 . GPA-2 test results

KB009 Hicarbo Kit Test Results

Fig. 13 . GPA-3 test results

Fig. 14 . GPA-4 test results

Table 2. KB009 Hicarbo Kit Test Results

№	Test	GPA-1	GPA -2	GPA-3	GPA-4	GPA-5	GPA-6	GPA-7	GPA-8	GPA-9	GPA-10
1	Lactose		+								
2	Xylose	+	+			+	+	+			
3	Maltose		+			+					+
4	Fructose					+				+	
5	Dextrose	+	+						+		
6	Galactose	+	+				+				
7	Raffinose								+		
8	Trihalose										+
9	Melibiose	+	+	+				+			
10	Saccharose			+				+			
11	L-arabinose		+				+				
12	Mannose		+								

№	Test	GPA-1	GPA -2	GPA-3	GPA-4	GPA-5	GPA-6	GPA-7	GPA-8	GPA-9	GPA-10
13	Inulin										
14	Na-gluconate								+	+	
15	Glycerol										
16	Salicin										
17	Dulcit										
18	Inositol										
19	Sorbitol										+
20	Mannitol							+	+	+	
21	Adonitol										+
22	Arabitol						+				
23	erythritol									+	
24	α-methyl-						+			+	+

№	Test	GPA-1	GPA -2	GPA-3	GPA-4	GPA-5	GPA-6	GPA-7	GPA-8	GPA-9	GPA-10
25	Ramnosa				+	+	+			+	
26	Celopeis		+		+						
27	Maleotozes				+						+
28	α-methyl-D-mannoside					+			+		
29	Xylitol										+
30	ONPG			+			+	+		+	
31	Esculin	+	+	+	+						
32	D-arabinose	+					+	+		+	
33	Citrate	+	+	+				+	+		
34	Malonat	+	+	+	+		+	+		+	
35	Sorbose					+					+

Conclusions

1. The method of cultivating microorganisms and the allocation of pure cultures of rhizospheric microorganisms has been worked out. It was established that for the most effective obtaining of individual colonies it is expedient to allocate clean lines of microorganisms by the Koch method.
2. The selection of dominant microorganisms of the rhizosphere of winter wheat Triticum aestivum has been carried out and the morphology of isolated isolates has been described. In the flowering phase of winter wheat, in general, 85 morphotypes were described. According to the indicators, the following groups of microorganisms were identified: dominant (> 10%), subdominant (5-10%), frequent (1-5%), rare (<1%). Among them, the dominant – 21, sub-dominant – 14, which frequent - 40 and random - 8 pieces. Significant influence on the structure of the distribution of various groups of detected morphotypes had a system of agriculture and soil cultivation.
3. It was revealed that the various stages of wheat ontogenesis, the quantitative and qualitative composition of morphotypes of microorganisms is changing. Thus, at the end of the vegetation there was a decrease in the number of detected morphotypes. Number of detected morphotypes - 44 pcs. Among them there were 20 units, sub-dominant ones - 20 units, and those that often occur - 14 units.
4. The carbon nutrition sources of dominant morphotypes of winter wheat rhizosphere were studied using different sources of carbon with the help of the innovative universal system KB009 TM HiCarbo Kit. Ten isolates of rhizospheric microorganisms were tested, and carbon sources of these cultures were detected. The obtained results indicate that the received microorganisms have different sources of nutrition, which may indicate a different type and species affiliation.

Approbation of research results

- Milantieva T. S., Patyka M. V. PLANT-MICROBIAL INTERACTIONS AND BIOTECHNOLOGICAL ASPECTS OF THEIR FORMATION/Biotechnology: accomplishments and hopes: book of abstracts: VI International Scientific-Practical conference, dedicated to 120 years of NULES of Ukraine (14-16 Nov 2017, Kyiv) – Komprint
- Milantieva T. S., Patyka M. V. PLANT-MICROBIAL INTERACTION AS A BASIS OF BIOTECHNOLOGICAL RESEARCH/Shevchenkivska vesna: Bioscience advances:book of abstracts: XVI International Scientific Conference of Students & Young scientists (24-27 April, 2018, Kyiv) – p. 103

Thank you for attention!