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Influence of crop species on quantity and physiological activity of rhizosphere microorganisms

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Goal. Study of the number and physiological activity of the main trophic and systematic groups of microorganisms. **Results.** We registered that the rhizosphere soil under soybean plants had the maximum number of microorganisms associated with the transshipment in the root secretions of legume amino acids and amines. When soybean is grown on an intensive agricultural background, denitrifiers' physiological and biochemical activity is maximal. Among the studied cultures, their abundance is also maximal. In the corn rhizosphere, a minimal number of denitrifiers with a low physiological and biochemical activity are 8.2 times less than the physiological-biochemical activity of the row-spacing microorganisms that indicates the synthesis of substances with inhibitory properties of this culture relative to the denitrification process's pathogens. In the soil with extensive farming, the Azotobacter number considerably exceeds the indices of intensive-farming agrozem: when growing corn - in 3.26 times, wheat – 4.28, flax – 2.08, soya – 3.29, row-spacing – 4.05 times (organic and mineral fertilizers have not been introduced since 1987). This indicates the impossibility of using this indicator, as a diagnostic indicator, to sufficient soil fertility level. The number of polysaccharides synthesizing microorganisms in most crops' rhizosphere is higher when grown on an intensive agricultural background, except for winter wheat, in the rhizosphere of which the higher abundance of polysaccharide synthesizing microorganisms is shown on an extensive agricultural background. **Conclusions.** We established that the number and biochemical activity of microorganisms in soil depend on the type of crop and varies when growing plants on agricultural backgrounds of different intensity. We registered that in the rhizosphere of leguminous plants, the mineralization of humus slows down compared to cereal crops. The intensity of consumption of organic matter and humus is higher when growing the studied crops on an extensive agricultural background, the activity of mineralization of nitrogen compounds, on the contrary, when growing on an intensive agricultural background. The soil of soybean rows characterizes the minimum phytotoxicity, and the maximum is the soil of row-spacing and rhizosphere of wheat. Thus, we confirmed long-standing observations concerning the greater phytotoxicity of the rhizosphere with cereals than legumes.
Keywords: microorganisms, ecotrophical groups, nitrogen mineralization, humus mineralization rate, soil phytotoxicity, maize, wheat, soybean, agricultural background.

Introduction

It has been experimentally determined that plants form a functional, taxonomic and spatial structure of the root zone's microbial community that influences plants' growth and productivity (Kravchenko, 2000; Meretskaya, 1999; Jacoby, 2020, 2021). Compositions of root secretions of different crops and plant species vary substantially (Ivanov, 1973; Kuzmicheva, 2014; Shaposhnikov et al., 2016). Thus it was found that the root secretions of corn contain carbohydrates (glucose, fructose, sucrose), amino acids (aspartic and glutamic); amino acids and amines prevail in the root secretions of legume; organic acids (oxalic, malic, amber), and phenolic compounds (coumarin, ferulic, syringic acids) - in the root secretions of cereals (Ivanov, 1973). Differences in quantitative and qualitative compositions of root secretions influence the growth of certain ecotrophical and functional groups of microorganisms in the root area (Kravchenko, 2000; Meretskaya, 1999).

The rhizosphere of plants is a dynamic environment in which many factors and soil microbiota are being adapted to the conditions that plants create in the phase of active growth (Canarini et al., 2019; Korenblum et al., 2020). In this regard, the parameters of the integration processes in microbial cenosis of the rhizosphere are an essential indicator of soil condition change at the cultivation of various crops and can be the basis for developing their regulation. Influence of crop growing technologies on the composition and quantity of root exudates that determine the direction and intensity of microbial processes in the rhizosphere remains insufficiently studied. So the research of the influence of crop species on quantity and physiological and biochemical activity and the ratio of microorganisms of main functional and taxonomic groups of rhizosphere microbial cenosis in the conditions of intensive and extensive farming was begun.

Materials and methods

The studies were conducted in a field stationary experiment of the Department of Adaptive Intensive Technologies of Cereals and Maize of the National Scientific Center "Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine" in dark-gray podzolic soil in the Northern Forest-Steppe (experimental farm "Chabany" Kyiv-Svyatoshinsky district, Kyiv region). Field 1 had crop rotation with the saturation of mineral fertilizers in $N_{96}P_{108}K_{112,5}$ on the background of crop by-products plowing (intensive crop farming); Filed 2 was the control, field crop rotation without application of mineral and organic fertilizers from 1987 (extensive crop farming). The investigated crops were grown in 2 grain-cultivated crop rotations: 1) flax, winter wheat, soybeans, spring wheat; 2) peas, winter wheat, maize, oats. The samples for research were taken from the rhizosphere of plants in phases: maize - in the early fruiting, wheat - in the milky wax ripeness, flax - in the early ripening, soybeans - at the end of flowering and on the beginning of the formation of beans. The number of microorganisms, their physiological and biochemical activity, and the indices' value were determined as described previously (Malynovska et al., 2020).

Results and discussion

Quantity of ammonifying microbes in the rhizosphere of soya, as a legume crop, differed from the rhizosphere of other investigated crops: the number of ammonifying microbes in the soybean rhizosphere was higher than in the soil of wheat rhizosphere by 60.1 % in the conditions of intensive crop farming and by 8.24 % - in the conditions of extensive crop farming (Table 1). It can be explained that amino acids and amines prevail in legumes' root secretions (Ivanov, 1973; Samtsevich, 1965; Parinkina, 1993). We consider the number of ammonifying microbes as an indicator of the intensity of root secretions and their enrichment of protein substances because proteins of root secretions and dead fragments of roots and root hairs are the substrate for the growth of ammonifying microbes. We observed many ammonifying microbes in the rhizosphere of wheat and flax, particularly in extensive crop farming conditions. It can be explained by the fact that in the conditions of extensive crop farming, plants pass through the phases of ontogenesis faster compared to the plants cultivated in the conditions of extensive crop farming. When research wheat and flax have already passed through the vegetation process's main phases, dead root hairs in their rhizosphere began rotting. The increase in the number of cellulolytic bacteria in 2.14 and 1.47 times in these experiment variants could prove this hypothesis (Table 1).

Table 1. The number of rhizosphere microorganisms in the dark-gray podzolic soil in the conditions of intensive (1) and extensive (2) crop farming, mln. CFU/dehydrated soil

Crop / Variant		Ammonifiers	Im. min. N	Oligonitrophils	Azotobacter, %	Denitrifiers	Pedotrophs	Cel.-des.	Pol.-synt.	Aut.	Str.	Micromicetes	Min. ph. mob.	Total number
Corn	1	271.1	102.6	61.0	30.7	57.7	108.8	57.3	2.99	10.1	28.7	0.16	20.5	772.3
Corn	2	298.6	72.5	59.3	100.0	3.90	60.5	47.0	2.61	10.6	15.7	0.19	16.8	704.9
Wheat	1	330.2	61.9	105.4	19.3	181.3	55.2	36.3	5.18	12.7	10.8	0.18	18.6	856.0
Wheat	2	434.5	180.8	96.4	82.7	150.2	85.6	77.8	5.91	13.3	24.1	0.21	24.6	1176.8
Linen	1	334.2	158.7	67.3	48.0	118.5	104.1	58.0	2.66	5.66	42.5	0.13	12.4	965.9
Linen	2	402.0	142.3	42.7	100.0	15.3	232.9	85.1	1.08	8.61	12.4	0.07	12.6	1054.9
Soybeans	1	528.5	127.0	49.5	25.3	172.1	64.3	30.0	4.10	5.98	39.9	0.13	8.19	1055.1
Soybeans	2	470.3	40.1	31.4	83.3	10.8	41.5	26.1	1.89	5.29	6.81	0.16	8.70	435.7
Row spacing	1	136.8	174.0	120.2	24.7	149.8	165.0	125.2	9.27	33.8	26.7	0.41	20.3	994.3
Row spacing	2	146.3	86.8	65.0	100.0	21.6	153.7	36.9	3.24	43.4	15.1	0.11	14,3	692.4
LSD_{05}		8.12	6.58	2.01	4.87	4.56	4.86	5.36	0.04	0.28	1.05	0.03	0.20	

Note: CFU - colony-forming unit, Im. min. N – immobilizers of mineral nitrogen, Azt., % - Azotobacter, % fouling of soil lumps, Cel.-des. - cellulose-destructive, Pol.-synt. - polysaccharide-synthesizing, Aut. – autochthonous, Str. – streptomycetes, Min. ph. mob. - mineral phosphate mobilizers.

Previous research demonstrated that planting of legume (in monoculture and as a part of legume grass mixtures) leads to the intensification of the rhizosphere's denitrification process (Malinovska, 2011). In our research, this phenomenon was observed only in soybean planting in intensive crop farming conditions: there was observed the highest among all crops quantity and physiological and biochemical activity of denitrifying microbes (Table 1, 2). A high content of nitrogen compounds, synthesized due to symbiotic and associative nitrogen fixation, is a prerequisite for activation of the denitrification process in the soya rhizosphere because nitrogen compounds are the substrate necessary. The quantity of alkali hydrolyzed, nitrate, and ammonium nitrogen in the soybean rhizosphere soil were higher by 47.8, 73.5, and 54.4 %, respectively, than in the soil of the corn rhizosphere (Table 3).

Table 2. Probability of formation of rhizosphere microbial colonies (λ, h $^{-1.}$ 10^{-2}) in the dark-gray podzolic soil in the conditions of intensive (1) and extensive (2) crop farming

Crop / Variant		Ammo-nifiers	Im. min. *	Oligo-nitro-phils	Nitri-fiers	Deni-trifiers	Pedo-trophs	Auto-chto-nous	Cellu-lose-destru-stive	Micro-micetes	Mineral phosphate mobilizers
Corn	1	2.69	1.15	3.17	0.117	0.010	2.75	4.60	3.02	1.12	2.24
Corn	2	1.34	2.32	2.98	0.118	0.106	3.08	4.58	2.15	1.53	5.01
Wheat	1	1.57	2.83	1.26	0.020	0.194	1.93	2.22	1.36	2.41	1.54
Wheat	2	5.50	1.32	2.16	0.260	0.116	2.80	2.30	4.23	4.48	3.93
Linen	1	6.34	1.51	1.26	0.082	0.187	2.53	2.97	3.23	5.00	1.61
Linen	2	3.04	1.60	3.68	0.046	0.153	1.92	3.19	2.19	5.75	1.76
Soybeans	1	2.67	1.60	2.89	0.412	0.205	2.11	2.21	3.26	7.35	1.76
Soybeans	2	3.38	1.54	3.58	0.153	0.138	3.42	4.03	3.03	4.04	1.83
Row spacing	1	2.68	0.89	5.32	0.045	0.821	1.64	4.90	2.74	2.81	3.15
Row spacing	2	3.44	0.55	4.64	0.101	0.058	1.78	6.55	3.60	2.50	4.68

Note: Im. min. N – Immobilizers of mineral nitrogen.

Table 3. Content of macroelements in the dark-gray podzolic soil in the conditions of intensive (1) and extensive (2) crop farming

Crop / Variant		Content, mg/kg					Degree of mobility, P_2O_5, mg/100 g
		N alkali hydrolyzed	N-NO_3	N-NH_4	P_2O_5	K_2O	
Maize	1	64.4	6.80	12.5	445.0	152.0	0.38
Maize	2	56.0	2.41	10.0	240.0	76.0	0.19
Wheat	1	64.4	22.4	13.3	335.0	303.0	0.29
Wheat	2	61.6	4.50	11.8	225.0	200.1	0.18
Flax	1	72.8	1.92	11.8	285.0	139.0	0.21
Flax	2	61.6	4.43	10.8	210.1	70.5	0.17
Soybeans	1	95.2	11.8	19.3	225.0	78.5	0.18
Soybeans	2	70.0	13.5	12.5	160.3	68.5	0.15
Row spacing	1	72.8	10.7	16.8	410.0	130.0	0.42
Row spacing	2	64.4	2.31	14.3	240.0	76.0	0.20
LSD_{05}		3.00	1.01	1.05	7.14	2.00	0.02

There was observed an active denitrification process in the wheat rhizosphere in both conditions of extensive and intensive crop farming. The number and physiological and biochemical activity of denitrifying microbes in intensive crop farming conditions were higher than one in the conditions of extensive crop farming (Table 1, 2). In intensive crop farming conditions, many denitrifying microbes were found in the soil rhizosphere of almost all crops and the soil of row spacing. It indicated the existence of unproductive nitrogen losses due to the application of unsustainable doses of fertilizers and perhaps the limiting factors for crop growth. The number of denitrifying microbes was low only in the maize rhizosphere – 2.6 times lower than in the soil of row spacing. Their physiological and biochemical activity was 82.0 times less than in the soil of row spacing. In the model experiments conducted in intensive crop farming conditions, the denitrification process's low activity was also observed in the rhizosphere of maize at the early stages of ontogeny (Malynovska, 2011). Perhaps the root exudates of this crop have inhibitory properties concerning the denitrifying microbes.

In our previous experiments with winter and spring wheat, soybean, and other crops, we found that soil, in which organic fertilizers and meliorates have not been applied for 28 years (control variants), contained a high number of *Azotobacter* (88-99 % of soil lump overgrowing) (Malynovska & Dombrovska, 2011). It allowed us to conclude that *Azotobacter* could be used as an indicator of soil health rather than an indicator of sufficient soil fertility and phosphorus compounds' availability. The maximum number of *Azotobacter* was observed in soils of control variants with the lowest level of contamination by pollutants. The number of *Azotobacter* in the soil in the conditions of extensive crop farming was much higher than one in the conditions of intensive crop farming: in case of corn planting - in 3.26 times; wheat - in 4.28 times; flax - in 2.08 times; soybean – in 3.29 times; row-spacing - in 4.05 times (Table 1). However, the number of *Azotobacter* in the plant rhizosphere depended on crop species and varied in extensive and intensive crop farming conditions. The lowest quantity of *Azotobacter* was observed in the wheat rhizosphere and row spacing in intensive crop farming and the rhizosphere of soybean and wheat in extensive crop farming conditions. Therefore the quantity of *Azotobacter* depended on the content of pollutants in the soil, the dose of nitrogen fertilizers, composition of crops root secretions, and ontogeny phase.

Polysaccharide synthesizing microorganisms play an essential role in natural biogeocenoses. There are several reasons for that; one of them is the protective properties of polysaccharides against different pollutants and stressors (Malynovska, 2007; Dudman, 1977). Protective functions of polysaccharides are not specific for their producers' cells; they can protect other organisms and even those that are not in trophic relations with producers of polysaccharides. Our data indicated that the quantity and biochemical and physiological activity of polysaccharide synthesizing microorganisms depended on crop species and crop farming type (Table 1, 2). In particular, the number of polysaccharides synthesizing microorganisms in most crops' rhizosphere was higher in intensive crop farming conditions. An exception to this pattern was the wheat, in the rhizosphere, of which the number of polysaccharides synthesizing microorganisms increased in extensive crop farming conditions. The number of polysaccharides synthesizing microorganisms in the wheat rhizosphere was higher in 2.26, 5.47, and 3.13 times than in the rhizosphere of maize, flax, and soybeans, respectively (in the conditions of extensive crop farming). The number of polysaccharides synthesizing microorganisms in the soil of row spacing varied substantially depending on crop farming conditions: higher 2.86 times in intensive crop farming than the extensive one. We can assume that the soil in intensive crop farming conditions is more contaminated with various pollutants. So polysaccharides synthesizing microorganisms just in it are getting a competitive advantage in its development.

It is known that the proportion of autochthonous microorganisms is one of the most critical characteristics of microbial communities. According to the data, the lowest quantity and level of physiological and biochemical activity of autochthonous microorganisms were observed in the root zone of soya (Table 1, 2). The activity of humus mineralization processes in the soybean rhizosphere was lower than that one in the wheat root zone by 147.3 % (in intensive crop farming) and 21.1 % (in extensive crop farming) (Table 4). It confirms the results of previous long-term observations that activity of humus mineralization processes in the rhizosphere of legumes (monoculture) and legume-grass mixtures is lower in comparison with cereals (Malynovska, 2011; Malynovska et al., 2009). In flax planting, there was also the lowest number of autochthonous microorganisms and activity of humus mineralization processes (Table 1, 4).

Table 4. Parameters of mineralization processes and level of phytotoxicity of dark-gray podzolic soil in the conditions of intensive (1) and extensive (2) crop farming

Variant		Pedotrophy Index	Oligotrophy index	Index of nitrogen mineralization	Rate of humus mineralization, %	The mass of 100 plants of winter wheat, g		
						stem	roots	the total mass
Maize	1	0.40	0.23	0.38	9.28	5.12	4.88	10.0
Maize	2	0.21	0.20	0.24	17.5	5.05	4.41	9.46
Wheat	1	0.15	0.32	0.42	23.0	6.15	4.20	10.4
Wheat	2	0.26	0.22	0.48	15.5	6.69	4.80	11.5
Flax	1	0.31	0.20	0.85	5.48	5.09	5.00	10.1
Flax	2	0.58	0.11	0.35	3.69	5.60	4.32	9.92
Soybeans	1	0.12	0.09	0.24	9.30	7.83	6.52	14.4
Soybeans	2	0.09	0.07	0.09	12.8	6.12	6.23	12.4
Row spacing	1	1.21	0.88	1.27	20.4	5.12	3.92	9.04
Row spacing	2	1.05	0.44	0.59	28.2	4.63	3.39	8.02
LSD_{05}						0.07	0.05	0.05

The maximum quantity of autochthonous microorganisms and their physiological and biochemical activity were observed in the soil of row spacing. The number of autochthonous microorganisms in the soil of row spacing was higher than one in the soybean rhizosphere soil 5.64 times (in the conditions of intensive crop farming) and 8.20 times (in the conditions of extensive crop farming) (Table 1 and 2). The physiological and biochemical activity of autochthonous soil microorganisms in the row spacing was higher than one in the soybean rhizosphere soil at 2.22 and 1.63 times, respectively (Table 2). Thus decomposition of humus compounds in the soil of row spacing, with no input of root secretions containing easily utilizable substances, was intensified approximately on the same level (in 2.19–2.20 times) in both conditions (extensive and intensive crop farming) in comparison with that one in the soybean rhizosphere soil (Table 4). The maximum rate of humus decomposition was observed in the soil of row spacing in the conditions of extensive crop farming: it was higher by 38.2 % compared to that in the soil of row-spacing in the conditions of intensive crop farming. The soil of row spacing was also characterized by increased organic matter loss rate, which was higher than one in the soybean rhizosphere in 5.04–8.75 times. Thus the extensive use of soil led to negative consequences: the rate of mineralization of organic matter and humus was accelerated; the activity of nitrogen mineralization decreased 2.15 times, and the phytotoxicity increased by 12.7 %.

The minimum phytotoxicity level was observed in the soil of soybean rhizosphere, and the maximum one - in the soil of wheat rhizosphere and the soil of row spacing (Table 4). The wheat rhizosphere soil's phytotoxicity was higher than one of the soybean rhizosphere soil by 7.83 % (in the conditions of extensive crop farming) and by 38.5 % (in the conditions of intensive crop farming). It confirms previous long-term research findings (Malinovska, 2011; Malinovska et al., 2009).

Thus crop plants, by their root secretions, regulate the quantity and physiological and biochemical activity of microorganisms of specific functional and taxonomic groups responsible for synthesizing and mineralization processes in the rhizosphere.

Conclusions

1. Quantity and physiologo-biochemical activity microorganisms of the rhizosphere in the dark gray soil depended on crop species and varied substantially in extensive and intensive crop farming.
2. The number of *Azotobacter* in the soil where organic and mineral fertilizers have not been applied since 1987 was significantly higher in comparison with the intensively fertilized soil. This number was higher by 3.26 (4/28, 2.08, 3.29, and 4.05) times in corn (wheat, flax, soybeans, and with row spacing). Therefore this indicator is not sufficient to properly diagnose soil fertility.
3. Quantity of polysaccharide synthesizing microorganisms in most cultures' rhizosphere was higher in the conditions of intensive crop farming. In the conditions of extensive crop farming, the highest number of polysaccharide synthesizing microorganisms were found in the rhizosphere of winter wheat (it was 2.26, 5.47, and 3.13 times higher than one in the rhizosphere of maize, flax, and soybeans, respectively).
4. We determined that the rate of humus mineralization in the rhizosphere of legumes was lower than that in the rhizosphere of cereal crops. In most cases, the rate of soil organic matter loss and humus mineralization was higher in extensive crop farming; and vice versa, the rate of nitrogen mineralization was higher in intensive crop farming.
5. We observed the minimum phytotoxicity level in the soil of soybean rhizosphere and the maximum one - in the soil of wheat rhizosphere and the soil of row spacing. It confirms the findings of previous long-term research.

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