Agroecological characteristics of the effect of a mixture of probiotic preparations with concomitant formation water on soil microorganisms

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Abstract. In the context of energy and environmental crisis, the search for new substances ensuring the formation of microbial cenosis with a rich composition of agronomically valuable groups of bacteria, optimal level of humification and increase of organic matter in the soil will allow to substantiate innovative environmentally safe types of fertilizers and plant protection under specific soil and climatic conditions. Therefore, the aim of the research was to study the peculiarities of formation and functioning of microbial cenosis of podzolic chernozem soil and the intensity of soil-biological processes upon application of a mixture of probiotic preparations and concomitant formation water in different concentrations. Different concentrations of concomitant formation water (CFW) and probiotic preparations were applied to the soil in the selected plots and the soil microbial cenoses of farmland were evaluated in the spring and autumn periods on days 15, 30, and 60 after application of the mixtures. Soil without application of any substances was considered as a control variant. The most effective impact is observed on day 30 after application, there is an activation of microbiological processes on day 15, a significant decrease is observed on day 60, although higher than the control due to the prolonged action of CFW. It was determined that the best variant of the experiment in both spring and autumn periods to improve the viability of soil microbial cenosis is the option of joint use of CFW at a concentration of 900 L ha^{-1} and probiotic Sviteco-Agrobiotic-01 diluted in a ratio of 1:10 (dose 100 L ha^{-1}). In particular, the total number of all groups of bacteria in the soil increases with the use of probiotics diluted in a ratio of 1:10 (15-31% compared to control) and is the maximum when using a mixture of CFW at a dose of 900 L ha^{-1} and 10% probiotic (by 82-102% compared to control). Based on the analysis of the coefficients of mineralization-immobilization, oligotrophy and pedotrophy, it was found that the application of CFW mixture and probiotic increases the soil nutrient content for different ecological and trophic groups of bacteria, reduces the rate of humus decomposition and creates favourable conditions for the development of soil bacteria.

Key words: soil, probiotic preparations, concomitant formation water, soil bacteria, soil-biological processes.

1. Introduction

Sown areas in Ukraine are reducing as a consequence of military operations. However, underproduction of food grains could lead to a global food crisis. There is a need to maximize the involvement of land in agricultural turnover in Ukraine in order to ensure environmental and food security, sustainable functioning of agro-ecosystems (Kulyk et al., 2020; Pysarenko et al., 2022; Shevnikov et al., 2022). This requires solving the problems of cleaning and reclamation of anthropogenically contaminated soils, in particular by oil products.

Biochemical activity of bacteria plays a leading role in the complex of natural and anthropogenic factors affecting the formation of soil fertility. It predetermines the specifics of organic matter transformation and humus synthesis (Aranda & Comino, 2014; Bogomazov et al., 2016; Yong et al., 2020).

Based on the research conducted by (Obire & Amusan, 2003; Reva, 2016) it was found that CFW contains a significant amount of mineral elements and inorganic compounds (about 60 different micro- and macroelements), in particular sulfates and chlorides, the total mineralization is in the range of 140-180 g dm^{-3}. However, the impact of CFW in different doses on the soil has not been studied sufficiently.

The previous research conducted by Markina (2019) established the possibility of using CFW as an environmentally friendly substitute for agrochemicals on the crops of cereals in order to increase their yields. As a result of warfare in Ukraine, sown areas are being reduced, which raises the issue of maximizing the yield from smaller areas of farmland, as well as the protection of crops from weeds by inexpensive and environmentally safe means. This issue is especially important nowadays, since the lack of food grains can cause a global food crisis. Therefore, it is necessary to search for new approaches of using environmentally friendly and cost-effective plant protection agents in agriculture. The use of mineralized stratum water, which is a by-product of oil production, can become one of such methods.

The possibility of using CFW in order to improve the technology of obtaining high-quality organic fertilizers was determined earlier, Pisarenko et al. (2019). The phytosanitary impact of MSW on the crops of cereals was studied as well (Pysarenko et al., 2021a).

As pointed out by reseaches (Aktar et al., 2009; Margesin & Niklinska, 2019; Möhring et al., 2020), pesticide application in the agrosphere has a significant impact on the volume, structural and functional characteristics of microbial groupings and soil biodynamic processes. This suggests that changes in the soil microbial complex will determine a certain tendencyof humification processes (Galytska et al., 2021; Taranenko et al., 2021). Under the conditions of military operations on the territory of Ukraine, sown areas are reducing, which may cause a global food crisis. Therefore, the search for new substances ensuring the formation of microbial cenosis with rich composition of agronomically valuable groups of bacteria, optimal level of humification and increase of organic matter in the soil will allow diagnosing the direction of its fertility evolution to justify the environmentally friendly, resource-saving system of using new types of fertilizers and plant protection under specific soil and climatic conditions (Kwiatkowska-Malina, 2018; Ma et al., 2020; Degaltseva et al., 2021).

As noted by other scientists (Pisarenko et al., 2019; Fitzner et al., 2021; He et al., 2017; Semiz & Suarez, 2019), one of the eco-friendly methods to improve the soil quality, including enhancing microbial vitality, is to use natural minerals and concentrations, in particular concomitant formation water, which is a by-product of oil production. Studies of Pysarenko et al. (2021a) found that the best dose of CFW used to improve agrobiocenosis productivity is a dose of 1200 L ha^{-1}. Also, a number of scientists (Li et al., 2014; Vandenberghe et al., 2017; Pisarenko et al., 2019) point out the positive effects of probiotic preparations, in particular on the main bacteria of the genus *Bacillus,* on improving soil microbiota activity and phytosanitary effects on agrocenoses. The issues of integrated use of a mixture of CFW and probiotics, as well as the identification of optimal doses of their joint application to justify the ecologically safe system of new types of fertilizers and plant protection are relevant and poorly investigated today.

The aim of the research was to study the peculiarities of formation and functioning of microbial cenosis of podzolic chernozem soil and intensity of soil-biological processes under the conditions of application of CFW mixture of different concentrations and probiotic preparations.

2. Materials and methods

The field experiments were carried out on the experimental farm of Poltava State Agrarian University during the period of 2016–2021. Different mixtures of concomitant formation water (CFW) and probiotic preparations were applied to the soil in some plots and the viability of soil microbial cenoses of farmland soil was evaluated. Soil without application of any substances was considered as a control variant. Concomitant formation water of Reshetnykivskyi gas and oil field located in Poltava region, Ukraine which refers to highly mineralized (salt concentration 40.21 g L^{-1}) and brines with mineralization 157.98 g L^{-1} according to the mineralization criteria were used for the research. The ionic composition is of the chlorcalcium type.

Bacteria of the genus *Bacillus* (*B. subtilis* and *B. subtilis* var. *amyloliquefaciens*), in particular the probiotic preparation Sviteco-Agrobiotic-01 (R&D enterprise Eco-Kraina Ltd, Switzerland), were used as basic probiotic cultures in the experiment.

2.1 Field research

Previous studies (Nakano & Zuber, 1998; Pysarenko et al., 2021b; Tsentylo, 2019;) found that the best dose of probiotic Sviteco-Agrobiotic-01 to improve soil quality and phytosanitary status of crops is manifested in 10% dilution and dose of 100 L ha^{-1}. The following mixtures were applied in the experimental plots: I – control; II – probiotic Sviteco-Agrobiotic-01 in a dilution 1:10 (100 L ha^{-1}); III – CFW (600 L ha^{-1}) and probiotic Sviteco-Agrobiotic-01 in a dilution 1:10

(100 L ha^{-1}); IV – CFW (900 L ha^{-1}) and probiotic Sviteco-Agrobiotic-01 in a dilution 1:10 (100 L ha^{-1}); V – CFW (1200 L ha^{-1}) and probiotic Sviteco-Agrobiotic-01 in a dilution 1:10 (100 L ha^{-1}); VI – CFW (2400 L ha^{-1}) and probiotic Sviteco-Agrobiotic-01 in a dilution 1:10 (100 L ha^{-1}).

The impact of joint application of CFW and probiotic preparations compared to the control variant was evaluated in the spring and autumn periods on days 15, 30, and 60 after application.

2.2 Determination of ecological and trophic groups of soil bacteria

Ten grammes of soil for each experiment variant was taken for microbiological analyses and examined in three replicates. The samples were transferred into sterile mortars and bacteria were dispersed by the Zvyagintsev (1991) method. A tenfold dilution of the original soil suspension was used for seeding on selective media.

The ecological and trophic groups of soil bacteria were determined by sowing specific dilutions of the soil suspensions on appropriate nutrient media (Ovreås et al., 1998). The number of bacteria was measured by sowing the soil suspension on standard nutrient media: ammonifying bacteria on meat-and-peptone agar (MPA), streptomycetes and bacteria using mineral nitrogen (amylolytic bacteria) on starch -ammonia agar (SAA), pedotrophic bacteria on soil extract agar (SEA), microfungi on Czapek's agar medium with lactic acid, oligotrophic bacteria on starvation agar (SA) (Titan Biotech LTD, India). After sowing the nutrient media, they were incubated at 28°C for 5-14 days (depending on the growth rate of bacteria of certain groups) (Titova & Kozlov, 2012; Liuta & Kononov, 2018). The number of bacteria was expressed in colony-forming units (CFU) per 1 g of absolutely dry soil. For this purpose, the moisture content of the soil sample taken for the experiment was determined by the thermostatic weight method, and the obtained number of colonies was calculated taking into account the coefficient of moisture and dilution of the soil suspension. The experiments were carried out in three replications. The direction of microbiological processes in the soil was determined by the mineralization-immobilization, oligotrophy, and pedotrophy coefficients (Iutynska, 2017; Romero-Olivares et al., 2017). The statistical analysis was performed by the analysis of variance in Excel and Statistica, version 6.0.

2.3 Determination of soil and biological processes parameters

The next stage was to study the parameters of microbiological coefficients of intensity of soil-biological processes in the soils at different doses of CFW application. The following parameters were calculated: 1) index of mineralization-immobilization (IMI) is the ratio of amylolytic bacteria that utilize ammonia (mineral) nitrogen to ammonifying bacteria that assimilate organic nitrogen (soil proteins). IMI > 1 indicates the increased rate of humus decomposition or unfavourable conditions for microbial development (Mary & Recous, 1994); 2) index of pedotrophy (IP) is the ratio of pedotrophic bacteria involved in the transformation of the water-soluble fraction of soil nutrients to ammonifying bacteria that assimilate organic nitrogen. IP > 1 indicates humus recovery and approach to virgin lands (>6) (Bongiorno et al., 2020; Taranenko et al., 2019); 3) index of oligotrophy (IO) is the ratio of oligotrophic bacteria which complete the mineralization of soil organic compounds to ammonifying bacteria which assimilate organic nitrogen. IO > 1 indicates unfavourable degradation processes in the soil (Primpas & Karydis, 2011).

3. Results and discussion

The research on the main ecological and trophic groups of bacteria showed that the soil was more enriched with bacteria in the spring period compared to the autumn one, which is explained by the active recovery of microbiota in autumn (Table 1).

Table 1. The number of the main groups of bacteria in the soil, number of cells in 1 gramme of absolutely dry soil (spring and autumn sample, average for 2016–2021, 10^6 CFU g soil^{-1}).

Treat-ment	**Total number of bacteria, ×10^6 cfu g^{-1}**	**Pedotrophic bacteria (SEA), ×10^6 cfu g^{-1}**	**Oligotrophic microorganism (SA), ×10^6 cfu g^{-1}**	**Ammonifiers (MPA), ×10^6 cfu g^{-1}**	**Amylolytic bacteria (SAA), ×10^6 cfu g^{-1}**	**Actinomycetes, ×10^6 cfu g^{-1}**	**Microfungi, ×10^3 cfu g^{-1}**
1. Spring sample							
Day 15							
I	4.5 ±0.05	7.0 ±0.17	10.0 ±0.04	9.2 ±0.40	9.4 ±0.09	0.301 ±0.01	22.5 ±1.00
II	5.0 ±0.03	10.3 ±0.45	9.5 ±0.20	9.5 ±0.15	9.1 ±0.03	0.320 ±0.01	24.8 ±0.31
III	6.0 ±0.12	12.5±0.15	9.5 ±0.30	10.3 ±0.12	9.0 ±0.03	0.402 ±0.02	35.2 ±0.15
IV	8.6 ±0.03	26.8± 0.75	9.6 ±0.14	14.4 ±0.10	10.1 ±0.17	0.500 ±0.02	43.3 ±1.1
V	4.5 ±0.02	8.9 ±0.38	8.5 ±0.25	9.1 ±0.38	8.2 ±0.03	0.422 ±0.02	20.5 ±0.8
VI	3.1 ±0.11	8.5±0.15	8.3±0.02	8.4 ±0.35	8.6 ±0.33	0.212 ±0.00	15.5 ±02
Day 30							
I	5.9 ±0.15	7.2 ±0.24	14.0 ±0.35	13.8 ±0.40	14.2 ±0.09	0.412 ±0.02	36. 4 ±1.01
II	6.8 ±0.23	19.7 ±0.87	13.7 ±0.26	14.3 ±0.55	13.8 ±0.03	0.450 ±0.01	40.8 ±1.09
III	8.0 ±0.12	27.5±0.65	13.1 ±0.30	16.1 ±0.32	14.5 ±0.03	0.445 ±0.01	45.2 ±1.15
IV	11.9 ±0.13	36.1± 0.75	12.5 ±0.44	19.2 ±0.10	14.0 ±0.17	0.610 ±0.02	53.3 ±1.30
V	6.5 ±0.12	10.2 ±0.18	9.4 ±0.15	8.2 ±0.18	7.1 ±0.03	0.602 ±0.01	29.4 ±1.04
VI	3.7 ±0.07	7.7±0.12	10.1±0.02	6.8 ±0.24	6.7 ±0.30	0.040 ±0.00	20.1 ±0.15
Day 60							
I	4.8 ±0.20	5.4 ±0.20	8.1 ±0.03	7.2 ±0.05	7.9 ±0.09	0.101 ±0.00	20.0 ±1.00
II	4.7±0.15	9.2 ±0.20	7.3 ±0.04	7.5 ±0.05	7.3 ±0.08	0.524 ±0.00	22.2 ±0.65
III	6.7 ±0.18	13.5 ±0.45	8.2 ±0.05	9.4 ±0.11	7.0 ±0.35	0.578 ±0.00	24.9 ±0.95
IV	7.0 ±0.15	19.4 ±0.20	8.4 ±0.16	11.2 ±0.40	7.1 ±0.03	0.412 ±0.01	27.1 ±0.65
V	5.5 ±0.20	6.9 ±0.17	6.5 ±0.02	6.8 ±0.06	5.9±0.15	0.504 ±0.02	18.5 ±0.50
VI	2.5 ±0.11	5.8 ±0.05	6.4±0.03	6.5 ±0.00	6.2 ±0.00	0.111 ±0.00	14.7 ±0.45
2. Autumn sample							
Day 15							
I	4,7 ±0.15	5.5 ±0.27	7.7 ±0.35	6.9 ±0.33	7.6 ±0.09	0.019 ±0.001	15.5 ±0.14
II	6.0 ±0.13	8.3 ±0.11	7.0 ±0.26	7.3 ±0.15	7.5 ±0.03	0.019 ±0.001	15.6 ±0.21
III	6.4 ±0.12	12.3±0.65	7.1 ±0.10	9.0 ±0.12	7.3 ±0.03	0.018 ±0.000	17.6 ±0.64
IV	8.6 ±0.13	16.9± 0.75	7.2 ±0.14	10.1 ±0.10	7.7 ±0.04	0.053 ±0.001	23.0 ±0.31
V	4.5 ±0.10	7.9 ±0.31	6.4 ±0.05	6.5 ±0.28	7.1 ±0.03	0.125 ±0.000	24.4 ±0.11
VI	3.1 ±0.14	5.0±0.12	6.3±0.02	6.2 ±0.15	6.9 ±0.13	0.015 ±0.001	10.3 ±0.04

Day 30							
I	4.2 ±0.15	6.1 ±0.17	10.8 ±0.15	10.0 ±0.07	10.6 ±0.01	0.100 ±0.005	20 4 ±0.55
II	5.0 ±0.13	13.2 ±0.45	10.2 ±0.20	10.3 ±0.45	10.4 ±0.08	0.095 ±0.001	21.8 ±0.17
III	7.2 ±0.12	20.6±0.65	10.9 ±0.04	11.1 ±0.32	10.8 ±0.10	0.117 ±0.003	20.2 ±0.78
IV	8.5 ±0.03	27.7± 0.35	11.0 ±0.07	14.2 ±0.10	10.5 ±0.11	0.162 ±0.004	25.5 ±0.23
V	7.5 ±0.12	11.6 ±0.18	7.1 ±0.12	7.2 ±0.18	6.1 ±0.03	0.090 ±0.001	17.4 ±0.15
VI	2.1 ±0.07	9.4±0.22	6.5±0.05	5.8 ±0.11	5.5 ±0.07	0.099 ±0.002	18.1 ±0.33
Day 60							
I	2.2 ±0.04	4.6 ±0.07	9.2 ±0.13	4.5 ±0.15	5.8 ±0.14	0.056 ±0.002	17.7 ±0.45
II	2.9±0.01	8.1 ±0.15	9.3 ±0.04	5.5 ±0.14	3.5 ±0.15	0.060 ±0.001	20.2 ±0.15
III	4.7 ±0.11	10.5 ±0.84	7.4 ±0.05	7.7 ±0.31	3.4 ±0.10	0.054 ±0.002	21.4 ±0.95
IV	3.0 ±0.07	14.5 ±0.22	8.3 ±0.36	9.5 ±0.44	4.7 ±0.03	0.069 ±0.000	25.5±1.01
V	1.5 ±0.04	11.7 ±0.47	8.0 ±0.02	7.8 ±0.10	3.5±0.07	0.048 ±0.002	17.5 ±0.50
VI	0.8 ±0.01	5.7 ±0.14	7.1±0.12	6.1 ±0.11	3.2 ±0.04	0.035 ±0.001	16.0 ±0.42

Note: Significance was tested by applying the *Student t-test*. Values of less than 0.05 ($p < 0.05$) were considered significant. Data are provided as mean ± standard deviation.

Weather conditions in the research years were typical for the zone of unstable moisture in the central forest-steppe of Ukraine. The climate of the research area is temperate continental, characterized by hot summers and relatively cold winters. Weather conditions during the research years (2016–2021) were typical for the zone of unstable moisture in the central Forest Steppe of Ukraine. The average annual air temperature was 7.2°C, and the average amount of precipitation was 470 mm. The average temperature of the coldest period (January) is minus 6.9°C, the hottest (July) is +24.5°C. The absolute maximum temperature is +40°C. The average monthly relative humidity was 87% of the coldest month and 63% of the hottest month.

The soil of the experimental field was typical deep low-humus medium-loam soil *Haplic Luvisol* (according to WRB, 2014): organic matter – 3.17%, nitrogen (N) – 81 mg kg^{-1} dry soil, phosphorus (P) – 139 mg kg^{-1} dry soil, potassium (K) – 118 mg kg^{-1} dry soil, acidity (pH) – 6.8.

It was found that the influence of CFW and probiotic on soil microbiological cenosis depends on both the dose and the period of aftereffect. The most active influence is manifested on day 30 after application, the activation of microbiological processes occurs on day 15, a significant decrease in these processes is observed on day 60, although it is higher than the control, which is connected with a long aftereffect of CFW (Fig. 1).

The differences between the treatments on the total number of bacteria on the sampling season and experimental day are confirmed by the statistical analysis. MANOVA showed significant differences ($p < 0.05$) on the treatments from the experimental day, total number of bacteria on the sampling season and experimental day at significance levels.

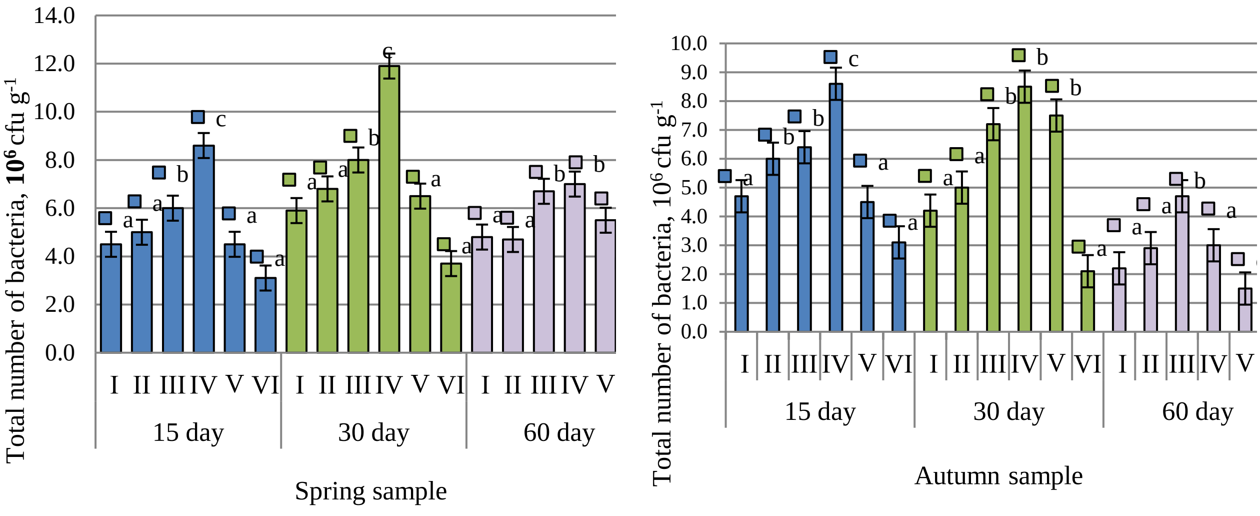

Figure 1. Dependence of the total number of bacteria in the soil on the experimental day and treatment. Different letters indicate significant ($p < 0.05$) differences between the means; I – control; II – probiotic Sviteco-Agrobiotic-01 in a dilution 1:10 (100 l ha^{-1}); III – CFW (600 l ha^{-1}) and probiotic Sviteco-Agrobiotic-01 in a dilution 1:10 (100 l ha^{-1}); IV – CFW (900 l ha^{-1}) and probiotic Sviteco-Agrobiotic-01 in a dilution 1:10 (100 l ha^{-1}); V – CFW (1200 l ha^{-1}) and probiotic Sviteco-Agrobiotic-01 in a dilution 1:10 (100 l ha^{-1}); VI – CFW (2400 l ha^{-1}) and probiotic Sviteco-Agrobiotic-01 in a dilution 1:10 (100 l ha^{-1})

The variant with CFW concentration of 900 L ha^{-1} and probiotic Sviteco-Agrobiotic-01 in a dilution 1:10 (dose 100 L ha^{-1}) was determined to be the best experiment variant for increasing viability of soil microbial cenosis both in spring and in autumn. In particular, the total number of all groups of bacteria in the soil increases upon application of probiotic dilution 1:10 (by 15-31% compared to control) and is maximum with the integrated use of CFW concentration at a dose of 900 L ha^{-1} and probiotic dilution 1:10 (82-102% compared to control).

The variant of experiment with application of CFW at a dose of 2400 L ha^{-1} and probiotics (dilution 1:10, 100 L ha^{-1}) showed a decrease in the total number of bacteria in the soil compared to the control, which is connected with the inhibition of microbial cenosis at this concentration of CFW.

The same dependence can be found for other groups of soil microflora (Table 1). The number of pedotrophic bacteria increases upon probiotic application by 50-66% on day 15 of application, by 116-173% on day 30, by 70-76% on day 60, respectively. The best result was recorded when the CFW mixture was applied at a dose of 900 L ha^{-1} and probiotic (by 207-257% on day 15 of application, by 354-401% on day 30, by 215-257% on day 60). When the dose of CFW is increased to 1200 L ha^{-1} there is an increase of pedotrophic bacteria compared to the control, but a decrease compared to the CFW concentration of 900 L ha^{-1} and their value decreases sharply at a concentration of 2400 L ha^{-1}. The joint application of CFW at a dose of

900 L ha^{-1} and probiotic in a dilution 1:10 on day 30 after application had the highest activity for pedotrophic bacteria (36.1 million in the spring sample and 27.7 million in the autumn sample, respectively).

It was found that the content of oligotrophic bacteria reduced by the integrated use of CFW at a dose of 600 L ha^{-1} and probiotics by 7-10%, but at CFW concentration of 900 L ha^{-1} it slightly increased and was at a level of control. Thus, no significant aftereffect of CFW and probiotic was observed for this group of bacteria.

Ammonifiers and amylolytic bacteria play an important part in the biological cycle of nutrients, especially nitrogen. It was established that when probiotics were used, the number of ammonifying bacteria increased by 2-5% in the spring period and by 3-22% in the autumn period compared with the control, while the joint use of CFW at a dose of 900 L ha^{-1} and probiotics increased the number of bacteria of this group by 39-55% in the spring period and by 42-111% in the autumn period compared with the control. The number of ammonifiers is significantly reduced when the dose of CFW is increased to 1200 and 2400 L ha^{-1} compared to the control. The number of amylolytic bacteria in the spring period is reduced by 3-8% compared with control when probiotics are used in a dilution of 1:10. The joint use of CFW with concentrations of 600 and 900 L ha^{-1} and probiotics in a dilution of 1:10 reduced the number of bacteria by 2-11% compared to the control variant. This is explained by the fact that the use of probiotics and CFW with a concentration of up to 900 L ha^{-1} enhances the development of bacteria which assimilate organic nitrogen, and the number of bacteria using ammonia (mineral) nitrogen was decreased slightly, but not significantly compared to the control. The CFW concentration of 1200 and 2400 L ha^{-1} in combination with probiotic greatly suppresses the development of ammonifiers and amylolytic bacteria.

The number of actinomycetes increased with the application of probiotic in a dilution of 1:10, and the highest number of bacteria of this group was recorded upon the joint use of probiotic and CFW at a dose of 900 L ha^{-1} (increased 1.5-4 times in the spring period and 1.6–2.7 times in the autumn period compared to the control).

Analysis of the total number of microfungi showed that the number of this ecological and trophic group was significantly higher in the variant of joint application of CFW at a dose of up to 900 L ha^{-1} and probiotic compared to control (37-58% higher compared to control in the spring period and 25-48% higher in the autumn period). It was found that microfungi grew most strongly on day 15 due to the effect of probiotic, but there is also a significant aftereffect on day 60, which is connected with the effect of CFW as food for these bacteria.The parameters of microbiological coefficients of intensity of soil-biological processes in soils such as mineralization-immobilization of nitrogen (IMI = SAA/MPA); pedotrophy (IP = SEA/MPA), oligotrophy (IO = SA/MPA) at different doses of CFW and probiotics application were studied (Table 3).

Table 3. Microbiological coefficients of intensity of soil-biological processes in soil (spring and autumn sample, average for 2016–2021).

Microbio-logical coefficients	**Treatment**					
	control	**probiotic 1:10**	**CFW 600+probiotic 1:10**	**CFW 900+probiotic 1:10**	**CFW 1200+probiotic 1:10**	**CFW 2400+probiotic 1:10**
1. Spring sample						
Day 15						
IMI	1.04	0.99	0.78	0.70	0.90	1.02
IP	0.74	1.21	1.54	1.86	0.98	1.01
IO	1.04	0.93	0.75	0.67	0.93	0.99
Day 30						
IMI	1.03	0.97	0.90	0.73	0.87	0.99
IP	0.52	1.38	1.71	1.88	1.24	1.13
IO	1.01	0.96	0.81	0.65	1.15	1.49
Day 60						
IMI	1.10	0.97	0.74	0.63	0.87	0.95
IP	0.75	1.23	1.44	1.73	1.01	0.89
IO	1.13	0.97	0.87	0.75	0.96	0.98
2. Autumn sample						
Day 15						
IMI	1.10	1.03	0.81	0.76	1.09	1.11
IP	0.80	1.14	1.37	1.67	1.22	0.81
IO	1.12	0.96	0.79	0.71	0.98	1.02
Day 30						
IMI	1.06	1.01	0.97	0.74	0.85	0.95
IP	0.61	1.28	1.86	1.95	1.61	1.62
IO	1.08	0.99	0.98	0.77	0.99	1.12
Day 60						
IMI	1.29	0.64	0.44	0.49	0.45	0.52
IP	1.02	1.47	1.36	1.53	1.50	0.93
IO	2.04	1.69	0.96	0.87	1.03	1.16

It was found that IMI > 1 in the control samples in both spring and autumn periods, which indicates the predominance of organic matter destruction over synthesis. The minimum IMI index was observed at the CFW dose of 900 L ha^{-1} upon the integrated application of CFW and probiotic in a dilution 1:10 (100 L ha^{-1}) that proves the slowdown of humus decomposition rate and creation of favourable conditions for the development of soil bacteria. In spring, the reduction of IMI index was 30-42%, and in autumn - 30-62% compared to the control, and the best effect compared to the control was observed on day 60, which is connected with the positive aftereffect of CFW and probiotic application on soil microcenoses. The application of CFW at a dose of 600 L ha^{-1} and probiotic had a positive impact on soil bacteria (reduction of IMI index was 12-32% in spring and 8-65% in autumn compared to control). When the CFW dose was increased up to 1200 and 2400 L ha^{-1}, there was a little IMI increase compared to the CFW dose of 900 L ha^{-1}, although these indices were generally less than 1 and smaller compared to controls. Thus, the addition of probiotic in a dilution of 1:10 (100 L ha^{-1} dose) and the joint application of CFW at a dose of 600-2400 L ha^{-1} improved the conditions for the development of soil bacteria (Fig. 3).

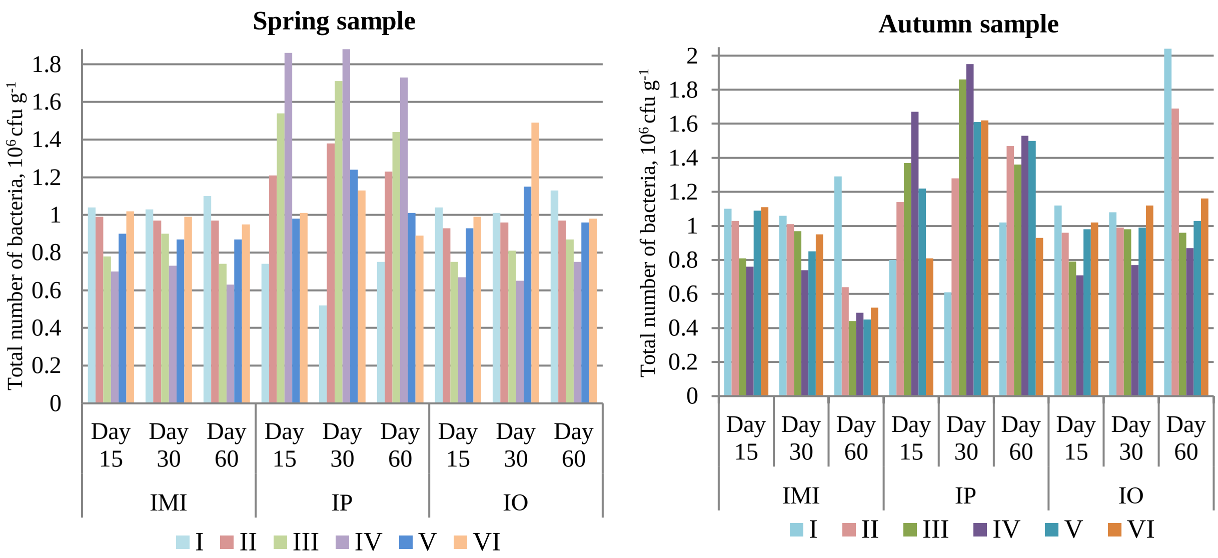

Figure 3. Dependence of microbiological coefficients of intensity of soil-biological processes on the experimental day and treatment. Explanation under Figure 1.

The joint application of CFW at a dose of 900 L ha^{-1} and probiotic provided the best results. There was also found a significant aftereffect of their application and creation of favourable conditions for soil bacteria on day 60.

The growth of pedotrophy coefficient indicates an increase of the intensity of soil organic matter decomposition. In the control sample, IP < 1 in both spring and autumn periods in most cases, that shows a low level of humus recovery. Application of probiotic in a dilution 1:10 (dose 100 L ha^{-1}) resulted in the index exceeding 1 in all variants, and in the spring period the index was 63-65% higher than in the control, and 42-109% higher in the autumn period. When probiotic and CFW were used in combination, the best effect was provided by the CFW dose of 900 L ha^{-1} (IP increased by 130-261% in the spring period and by 50-219% in the autumn period) while the highest value of humus recovery was on day 30 in the spring and autumn periods.

A decrease of pedotrophy coefficient was observed at higher CFW doses than 1200 L ha^{-1}, which indicates less favourable conditions for soil organic matter decomposition, although in most cases IP was more than 1 and higher compared to control (at CFW dose of 1200 L ha^{-1} by 32- 38% in spring and by 47-119% in autumn compared to control, at CFW dose of 2400 L ha^{-1} it was higher by 18-36% in spring and by 1-121% in autumn). So, the maximum values of pedotrophy coefficient in the experiment were recorded upon joint application of CFW at a dose of 900 L ha^{-1} and probiotic diluted 1:10 (dose 100 L ha^{-1}) on day 30 after application, with significant aftereffect on day 60, which corresponds to the increased intensity of soil organic matter decomposition in order to satisfy the nutrient requirements of plants.

The highest values of oligotrophy coefficient (IO) were observed in the control variant, while this index was more than 1 in all variants of the experiment, indicating unfavourable degradation processes in the soil. The index was better in all variants of probiotic and CFW application at the dose up to 900 L ha^{-1} on days 15, 30 and 60 compared to the control (IO < 1). The joint application of CFW 900 L ha^{-1} and probiotic in a dilution of 1:10 at a dose of 100 L ha^{-1} produced the best effect (IO decreased by 33-35% in the spring period and by 28-57% in the autumn period compared to the control) that indicates an increase in the content of nutrients available to bacteria and high provision of nutrients.

The increase of the CFW dose of more than 1200 L ha^{-1} revealed a slight growth of the oligotrophy coefficient, but at this CFW concentration in most cases the oligotrophy coefficient was less than 1 and significantly better than the control, i.e., there was a good provision of the soil microbiota with easily digestible organic matter. Thus, it can be pointed out that the joint application of CFW and probiotic increases the nutrient content available to the bacteria, but the best option was the CFW dose of 900 L ha^{-1} and probiotic dose of 100 L ha^{-1} in a dilution of 1:10. Moreover, although the best result was recorded on day 30, the improvement of the soil by nutritional elements was also seen on day 15 due to the effective probiotics activity, and on day 60 due to the effect of CFW (an increase of nutrients for different ecological and trophic groups of bacteria).

Thus, when using CFW at a concentration of 900 L ha^{-1} and probiotics at 100 L ha^{-1}, favourable conditions are created for the vital activity of a number of soil bacteria. The microbiological indication of the studied soil showed that the introduction of CFW and probiotics contributed to the creation of a certain level of biological activity in the upper layer of the soil, which caused specific conditions for the transformation of organic matter and the productivity of agrobiocenosis. The growth and development of microscopic fungi and cellulose-destroying bacteria that participate in the decomposition of crop residues is stimulated. A significant increase in the activity of oligonitrophilic bacteria, which use low concentrations of monomers and complete the mineralization of organic residues, was also noted.

4. Conclusions

1. It was found that the application of concomitant formation water (CFW) and probiotic mixture increases the content of nutrients in the soil for different ecological and trophic groups of bacteria , reduces the rate of humus degradation and creates favourable conditions for the development of soil bacteria .
2. As a result of studying the main ecological and trophic groups of bacteria in a unit volume of soil, it was found that the combination of CFW at a dose of 900 L ha^{-1} and probiotic in a dilution of 1:10 (100 L ha^{-1}) provided the best result for the functioning of microbial cenosis of podzolic chernozem soil. Also, a significant influence of their aftereffect and the creation of favourable conditions for soil bacteria on day 60 was found.
3. The doses of CFW over 1200 L ha^{-1} show the deterioration of microbial coefficients of intensity of soil-biological processes in the soil, indicating less favourable conditions for decomposition of organic matter, although better compared to the control.
4. Thus, the mixture of CFW at a dose of 900 L ha^{-1} and probiotic in a dilution of 1:10 (100 L ha^{-1}) can be used as an environmentally friendly fertilizer in organic farming, which will improve soil and biological characteristics.

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