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Effects of Eco-Friendly Product Application and Sustainable Agricultural Management Practices on Soil Properties and Phytosanitary Condition of Winter Wheat Crops

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Abstract: Despite the eco-political difficulties that accompany the application of principles of the European Green Deal policy on agriculture in the current world crisis, the need of their implementation seems to be absolutely necessary. The practices recommended within the sustainable agriculture strategy include replacing traditional fertilizers and pesticides with eco-friendly preparations and optimizing the management of biomass produced on farms. The aim of the research was to determine the effect of eco-friendly preparations application combined with straw incorporation on the chemical and microbiological soil parameters and plant sanitary status of winter wheat. The soil analyses included the determination of total organic carbon (TOC) and total nitrogen (TN) content; mineral nitrogen (MN), phosphorus (P), potassium (K), and magnesium (Mg) content, and the pH value. The number of soil bacteria (B), actinobacteria (A), fungi (F), and the total number of microorganisms (TNM) were also analyzed. The application of Effective Microorganisms resulted in an increase in TOC and TN concentration. The influence of biostimulator Asahi was diversified. The beneficial effect of straw on TOC, TN, and K content and microbial growth was also observed. Despite a number of limitations, the potential benefits of application of eco-friendly preparations provide ample reasons to continue experiments with their use.

Keywords: eco-friendly preparations; effective microorganisms; biostimulator; sustainable agriculture; soil properties; fungal plant diseases; winter wheat

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1. Introduction

The dilemmas of modern agriculture, which center on maintaining a stable balance between maximizing economic profit and acting responsibly toward environmental resources, have been growing significantly in recent years. This results from the rapid and accelerating rate of climate change and from the global geopolitical destabilization that in many countries is threatening to disturb food security. Despite the difficulties that these crises bring to realizing the goals of the European Green Deal, as well as the unsatisfactory rate of implementation of beneficial transformations in agriculture, it nevertheless seems crucial to achieve those objectives [1]. Agricultural practices to minimize the negative consequences of intensifying agricultural production that are recommended as part of a sustainable agriculture strategy include replacing traditional fertilizers and plant protection products with environmentally friendly preparations and optimizing the management of biomass produced on farms [2–7].

Of the wide range of alternative products to conventional preparations used in agriculture, biostimulants deserve special attention. According to the EU 2019 definition, these are products that stimulate plant growth, increase nutrient availability in the soil, and improve the efficacy of the use of nutrients and thus also the measurable quality of crops [8]. They also increase plant tolerance to abiotic stresses, which is especially valuable under the more frequent occurrence (including in temperate climate zones) of prolonged droughts or the need to cultivate crops on soils with high salinity [9,10]. Biostimulants are classified, depending on the nature of the active substance, as microbiological and non-microbiological. Their application to soil primarily affects the structure of plant roots, while foliar application supports plants in fighting biotic and abiotic stressors [11]. The biostimulator Asahi SL used in the research contains the phenolic compounds found naturally in plants, meaning that its use does not pose a risk of environmental contamination [12].

The preparations of Effective Microorganisms (EM) comprise a consortium of microorganisms, mainly including photosynthetic bacteria, yeast, lactic acid bacteria, and filamentous fungi, and are activated on various substrates before application [13]. The idea of using EM was presented in the 1990s in Japan by Higa [14]. The wide range of uses of these biopreparations include a number of benefits from having been introduced into agricultural practice. The producers of individual commercial preparations containing EM declare that applying them to soil or topically to plants provides many positive effects that ultimately increase yields [15]. The most important include improving physico-chemical and biological soil parameters, providing plants with nutrients, breaking down soil toxins, or reducing the occurrence of phytopathogens [16]. Despite many studies questioning the scope of benefits of EM use, it seems that reliable research to assess their efficacy is greatly justified. Optimizing the application parameters and tailoring them to specific growing conditions may contribute to the broader recognition of EM as wholesome products to support or replace conventional pesticides and fertilizers.

Introducing pro-ecological products to the cultivation systems reduce the number of chemical pollutants in the environment, resulting in the increased quality of soil and yields. Agricultural production with the use of eco-friendly preparations provides foods with health-promoting values, which is an important element in the prevention of many diseases [17,18]. Besides their applications, other activities to implement sustainable agriculture are recommended, including practices that enrich the soil with organic matter. Leaving harvest residues, including straw, in the field has a positive effect on soil structure and its biological and chemical properties [19]. Increasing the amount of organic matter is also considered to support soil carbon sequestration [20].

The present research aimed to determine the effect that the individual and combined action of an EM microbiological preparation and the Asahi SL biostimulator in various stubble management systems had on the biological and chemical soil properties and the phytosanitary condition of plants in winter wheat cultivation.

2. Materials and Methods

2.1. Study Area and Soil Sampling

The study was carried out during the three-year period of 2011–2014 on a field of winter wheat field, on a farm in Tarnówko (52°36′45.0″ N 18°24′30.0″ E) on the Inowrocław Plain (Kuyavia Lakeland, northern Poland). The soils of the experimental plots were Cambic Stagnic Phaeozem soils [21]. The soil texture was qualified as loamy: 42.1% of sand fraction (2.0–0.05 mm), 40.7% of silt fraction (0.05–0.002 mm), and 17.2% of clay fraction [22]. The mean TOC in the soil was 26.4 $g \cdot kg^{-1}$ and pH 7.2 (in 1M KCl). The grain size composition was measured by Mastersizer 2000 analyzer (Malvern Instruments, Malvern, UK). A ploughing tillage system was used, with fertilization using nitrogen at a dose of 100 kg $N \cdot ha^{-1}$ and phosphorus and potassium at a dose of 30 $kg \cdot P_2O_5 \cdot ha^{-1}$ and 60 $kg \cdot K_2O \cdot ha^{-1}$, respectively. The qualified seed material of winter wheat cv. Arktis dressed with Maxim Star 025 FS (cyproconazole + difluorobenzene) at a rate of 200 g of product per 100 kg of grain was sown at a density of 400 $grains \cdot m^{-2}$ in September each year. Over the study years, for each spring after the start of growth (BBCH 25–29), a herbicide tank mixture was used: Atlantis 12 OD at a dose of 0.45 $dm^3 \cdot ha^{-1}$ + Sekator 125 OD at a dose of 150 $mL \cdot ha^{-1}$ + Esteron 600 EC (2,4-D) at a dose of 0.45 $dm^3 \cdot ha^{-1}$. At the BBCH 32 stage, a mixture of growth regulators was used (Antywylegacz 750 SL and

Moddus 250 EC at doses of 1.0 and 0.3 $dm^3 \cdot ha^{-1}$, respectively), along with the fungicide Capalo 337.5 SL (epoxiconazole + fenpropimorf + metrafenone) at a dose of 1.5 $dm^3 \cdot ha^{-1}$ in order to reduce the occurrence of fusarium wilt, eyespot, and powdery mildew. As a prophylaxis to reduce ear infections (BBCH 49-51) the product Artea 330 EC (propiconazole + cyproconazole) was applied at a dose of 0.5 $dm^3 \cdot ha^{-1}$. The distribution of precipitation and temperature during the field study period, the grain yield of winter wheat [$t \cdot ha^{-1}$], and agrotechnical treatments were all described in detail in the work of Lamparski and Kotwica [23].

A static (second and third year of winter wheat monoculture) three-way experiment was set up in a split-plot–split-block design in three replications involving 18 experimental plots. The experimental factors included:

- Factor A levels: A1 (straw + EM single application, introduced into the soil during post-harvest cultivation in autumn at a dose of 40 dm^3 ha^{-1}), A2 (straw + EM dual application introduced into the soil during post-harvest cultivation in autumn at a dose of 20 $dm^3 \cdot ha^{-1}$ and EM applied on leaves in a dose of 20 dm^3 ha^{-1} at BBCH 20–22), A3 (straw + no EM application), A4 (straw removed + EM single application), A5 (straw removed + EM dual application), and A6 (straw + no EM application);
- Factor B levels: B1 (biostimulant Asahi SL applied once on leaves at a dose of 1.0 $dm^3 \cdot ha^{-1}$ at BBCH 20–22), B2 (biostimulant Asahi SL applied twice on leaves in two doses of 0.5 $dm^3 \cdot ha^{-1}$ at BBCH 20–22 and BBCH 27–29), and B3 (no biostimulant application).

The biopreparation EM "Naturally Active" is a suspension of microorganisms (Greenland Technologia EM sp. Z o.o.) with certificate PZH/HT-1448/2002 and the Institute of Soil Science and Plant Cultivation qualification certificate No. NE/1/2004. EM is a product that is safe for people, animals, and the environment. According to the manufacturer's information, the preparation consists of photosynthetic and lactic acid bacteria, yeast, actinobacteria, *Azotobacter* spp. bacteria, and cane molasses. It does not contain GMO (genetically modified organisms) components. It is manufactured under license from EMRO-EM Research Organization Japan. The biostimulant Asahi SL, in accordance with the annex to the decision of the Ministry of Agriculture and Rural Development No. R-357/2010d of 27.12.2010 is a stimulator of plant growth and yields supplied as a liquid for dilution in water. It contains the active substances (nitrophenol derivative compounds): 0.3% sodium para-nitrophenolate, 0.2% sodium orthonitrophenolate, and 0.1% sodium 5-nitroguayacolate. The commercial product EM "Naturally Active" and Asahi SL products were applied using a self-propelled sprayer.

The soil samples for microbiological and chemical analysis were collected for the first time in 2011 (at the beginning of the experiment and before winter wheat sowing) and for the last time in 2014 (after plant harvesting and before the beginning of the post-harvest cultivation). The content of mineral nitrogen and its forms were the only parameters determined in the autumn and spring seasons during each year of the study. On each plot, the soil samples were extracted from the tilled soil layer (0–25 cm depth). Ten individual soil samples were collected from each experimental plot for each treatment. The soil from a single plot was thoroughly mixed and homogenized by sieving to create a pooled sample. All the soil samples were analyzed in triplicate.

2.2. Chemical Properties

The chemical properties were determined according to the standard methods used in soil sciences and each sample was analyzed in triplicate. The pH was measured in a solution of 1 M KCl using the potentiometric method [24]; the contents of total organic carbon (TOC) and total nitrogen (TN) were determined using a Vario Max CN dry combustion analyzer (Elementar, Germany). The contents of available forms of phosphorus (P) [25] and potassium (K) were determined by the Egner–Riehm method [26], whereas the content of magnesium available to plants (Mg) was analyzed following the Schachtschabel method [27]. The contents of forms available to plants were determined by atomic absorption spectroscopy and atomic emission spectroscopy using a Solaar S4 spectrometer. The forms of mineral nitrogen (N-NO_3^- and N-NH_4^+) were extracted from field-moist soil using KCl and K_2SO_4, respectively, and determined by flow colorimetry using a Skalar San Plus Analyzer.

2.3. Soil Microbiological Parameters

The microbiological analyses in the collected soil samples involved determining the number of heterotrophic bacteria (B), filamentous fungi (F), and actinobacteria (A). Ten grams of each soil sample was added to 90 mL of Ringer's solution. After homogenization for 30 min, tenfold serial dilutions were created (from 10^{-1} to 10^{-6}). Then, inoculations of the prepared soil solutions were created on the appropriate culture media. The total numbers of microorganisms were determined using standard nutrient agar for bacteria and Rose–Bengal agar containing 30 $\mu g \cdot mL^{-1}$ streptomycin for filamentous fungi [28]. The actinobacteria were isolated on a modified yeast extract–glucose medium (YGA) with 100 nystatin $\mu g \cdot mL^{-1}$ [29]. The bacteria and fungi were incubated at 25 °C for five days, while the actinobacteria were incubated at 28 °C for seven days. The microbiological analyses were created in four replicates and, after incubation, the colonies were counted. The determined numbers of microorganisms were expressed as colony-forming units (CFUs) per 1 g of dry soil matter.

For the obtained values of microbiological and selected chemical parameters (pH, TOC, TN, and available forms of P, K, and Mg) the relative change index (Ic) was determined as the quotient of each specific microbiological and chemical parameters value at the beginning of the research and the value at the end for each experimental plot. The index values above 1.0 indicate a favorable impact of a given combination of levels of the analyzed factors, while values below 1.0 indicate the opposite.

2.4. Plant Diseases Occurrence

The health assessment included an analysis of the occurrence of root rot or leaf or ear diseases. The health observations were performed in the BBCH 75–77 phase. In the course of the research, the severity of occurrence of all observed diseases was determined. The severity of disease symptoms was determined on leaves: powdery mildew (*Blumeria graminis*), Septoria leaf blotch (*Zymoseptoria tritici*, *Septoria glumarum*), and brown rust (*Puccinia recondita*). The health status of flag (L1) and sub-flag (L2) leaves was assessed on 20 randomly selected plants and was expressed as the average share of leaf area showing symptoms of particular diseases. The health of ears was also determined as the share of ear area showing symptoms of ear fusarium disease (*Fusarium* spp.). This assessment was performed on 50 randomly selected ears from a plot. Moreover, in the same wheat development phase, the severity of food and root rot diseases was assessed. The severity of symptoms of take-all (*Gaeumannomyces graminis*), Fusarium root rot (*Fusarium* spp.), Eyespot (*Oculimacula acuformis*, *O. yallundae*), and sharp eyespot (*Rhizoctonia cerealis*, *R. solani*) was determined. One hundred generative tillers of each combination were assessed for health. The plant samples were dug up (with roots) and transported to the laboratory. In laboratory conditions, after removing and washing the shoots and leaf sheaths, the percentage of stalks or plants (in the case of take-all) showing symptoms of particular diseases were assessed. The severity of these diseases was determined on a 0–4° scale [30]. The severity of infestation was transformed into the disease index (DI) according to the Townsend and Heuberger's transformation [31].

2.5. Data Analyses

The results of analyses of individual parameters were statistically verified using the variance of multiple experiments, according to the model appropriate for the randomized sub-block design. The analysis of variance (two-way ANOVA) was used, where the first factor (A) was the application of the biopreparation EM at different rates and straw management and the second factor (B) was the application of the Asahi biostimulant. To assess the significance of the factors' influences and their interactions, a Tukey's post hoc test was used with a 95% confidence interval to compare the mean values of features. The differences between the examined objects were analyzed by principal component analysis (PCA) based on the mean data values of all of the studied properties. The first two principal components (PC1 and PC2) were selected for the ordination of the cases. A cluster analysis was performed to determine the groups in the dataset based on a dendrogram. Due to the diverse ranges of absolute quantities of individual soil characteristics, multidimensional analyses were performed on standardized data. The statistical analyses of the results were conducted using the StatSoft's Statistica 12 PL software package [32].

3. Results

3.1. Soil Total Organic Carbon and Total Nitrogen Content

Introducing the EM biopreparation to the winter wheat cultivation system resulted in a statistically significant increase in the average amounts of TOC in the studied soils. This was true of samples taken from plots where straw was left, where the TOC content ranged from 27.20 g·kg^{-1} (without EM) to 29.90 g·kg^{-1} (1 × EM) and of those whose surfaces were cleared of residues after harvest, where TOC ranged from 26.23 g·kg^{-1} (without EM) to 29.40 g·kg^{-1} (1 × EM). The statistically lowest average TOC was also observed in the experimental variants for which the biostimulator had not been applied. Although the TOC concentration was higher in straw-enriched soils, the differences were not statistically significant. However, the straws' capacity to increase the TOC content in the soil throughout the experiment is evidenced by the fact that Ic exceeded 1 only in the variants in which crop residues had been ploughed down (Table 1).

Table 1. Total organic carbon (TOC) and total nitrogen (TN) content and index of relative change (Ic) depending on the application of the biopreparation EM and straw management and the application of the biostimulant Asahi in winter wheat cultivation.

Biopreparation and Straw Management (Factor A)	Biostimulant (Factor B)						Mean
	1		2		3		
	Content	Ic	Content	Ic	Content	Ic	
Total Organic Carbon—TOC (g C kg^{-1} soil)							
1	29.6	1.03	31.2	1.03	28.9	1.02	29.90
2	30.1	1.02	29.6	1.03	29.3	1.03	29.67
3	26.5	1.01	27.2	1.02	27.9	1.01	27.20
4	27.8	0.98	30.2	0.97	30.1	0.98	29.40
5	28.2	0.97	29.2	0.98	27.5	0.99	28.30
6	27.8	0.95	26.4	0.94	24.5	0.98	26.23
Mean	28.4		29.0		28.0		28.45
$LSD_{0.05}$ for Factor A = 1.448; Factor B = 0.822; Interaction A/B = 2.508; B/A = 2.014							
Total Nitrogen—TN (g N kg^{-1} soil)							
1	2.99	1.04	2.92	1.06	2.66	1.04	2.86
2	2.78	1.05	2.82	1.04	2.52	1.04	2.71
3	2.66	1.02	2.64	1.04	2.48	1.03	2.59
4	2.56	0.92	2.62	0.94	2.78	0.94	2.65
5	2.59	0.94	2.54	0.92	2.66	0.93	2.60
6	2.44	0.91	2.52	0.89	2.46	0.91	2.47
Mean	2.67		2.68		2.59		2.65
$LSD_{0.05}$ for Factor A = n.s.; Factor B = n.s.; Interaction A/B = n.s.; B/A = n.s.							

Factor A levels: A1 (straw + EM single application), A2 (straw + EM dual application), A3 (straw + no EM application), A4 (straw removed + EM single application), A5 (straw removed + EM dual application), A6 (straw + no EM application); factor B levels: B1 (biostimulant single application), B2 (biostimulant double application), B3 (no biostimulant application); n.s. not significant, Ic index of relative change, LSD least significant difference, the significance of differences by Tukey's test at $p \leq 0.05$.

The average amount of TN in the studied soils ranged from 2.47 g·kg^{-1} (A6) to 2.86 g·kg^{-1} (A1). In general, the trends for the influence of EM biopreparation and Asahi biostimulator on TN content in soil were similar to those for TOC. The lowest average values were recorded in variants in which the tested eco-friendly products were not applied at all, but the differences were not statistically significant. The values of the relative change coefficient confirmed the positive effect that incorporating straw into the soil had on increasing total nitrogen content (Table 1).

3.2. Mineral Nitrogen Content

The minimum mineral nitrogen content (MN) in the examined soils in autumn was 21.9 mg·kg^{-1} (A1), while the maximum was 26.2 mg·kg^{-1} (A6). In the spring, these values were, respectively, 18.7 mg·kg^{-1} (A6) and 25.3 mg·kg^{-1} (A1). The statistically significant differences related to the application of EM were observed only in the autumn, when the combination without the addition of EM and with straw removed contained on average more mineral forms of nitrogen than when EM was not used and straw was left in the field. Using the biostimulator did not statistically significantly affect the concentration of these combinations in the studied soils. Analyzing the impact that straw management practices in cultivating winter wheat had on the average N-NO_3 and N-NH_4 in the soil, it was found that, in spring, these values were higher in soils with straw added than in variants in which it was removed. Conversely, the inverse trend was observed in autumn (Table 2).

Table 2. Content of mineral forms nitrogen (N-NO_3 and N-NH_4) depending on the application of the biopreparation EM and straw management and the application of the biostimulant Asahi in winter wheat cultivation.

Biopreparation and Straw Management (Factor A) *	Biostimulant (Factor B)			
	1	2	3	Mean
N-NO_3 and N-NH_4 (mg·kg^{-1})—autumn				
1	23.2	20.6	21.8	21.9
2	24.0	21.4	24.5	23.3
3	21.8	20.6	24.9	22.4
4	23.1	25.5	25.6	24.7
5	23.2	27.5	26.7	25.8
6	24.2	27.7	26.5	26.1
Mean	23.3	23.9	25.0	24.0
$LSD_{0.05}$ for Factor A = 3.46; Factor B = n.s.; Interaction A/B = n.s.; B/A = n.s.				
N-NO_3 and N-NH_4 (mg·kg^{-1})—spring				
1	26.3	24.0	25.6	25.3
2	25.6	23.2	25.2	24.7
3	26.1	20.6	24.7	23.8
4	21.9	19.7	20.7	20.8
5	21.8	17.8	21.1	20.2
6	20.9	16.6	18.5	18.7
Mean	23.8	20.3	22.6	22.2
$LSD_{0.05}$ for Factor A = 6.09; Factor B = n.s.; Interaction A/B = n.s.; B/A = n.s.				

* See: Table 1.

3.3. Soil P, K, and Mg Content, and Soil pH

The maximum available phosphorus content in the soils was 188.1 mg·kg^{-1} and the minimum was 134.0 mg·kg^{-1}. The use of EM and biostimulators did not have a clear effect on these values. However, it was observed that the coefficient of relative change, despite values being close, was always lowest in the variant without the use of EM and highest when the Asahi biostimulator was not applied (Table 3). The potassium content in the soil ranged from 90.3 mg·kg^{-1} to 234.0 mg·kg^{-1} and was not dependent on the bacterial preparation having been applied. The relative change coefficient was slightly higher in the soil samples from plots where Asahi was not applied than in the variants where it was. In contrast to phosphorus, leaving crop residues in the field significantly changed the potassium content in the soil. In the soil samples from the plots where the shredded straw was not removed, high values of the relative change index of this element content were recorded that exceeded 1, proving the increase in its quantity over the entire study period (Table 3). Introducing EM and a biostimulator to the cultivation system did not affect the concentrations of available Mg forms in the soil. They were within the range of 20.3–43.9 mg·kg^{-1}. The relative change index values were higher when using systems that incorporated straw into the soil, but they did not exceed a value of 1. The pH value and its relative change coefficient were slightly lower in soils from plots on which chopped straw was left. However, no differences were found to result from using the tested eco-friendly preparations were found (Table 3).

Table 3. Contents of available forms of phosphorus, potassium, magnesium, and pH of the soil depending on the application of the biopreparation EM and straw management and the application of the biostimulant Asahi in winter wheat cultivation.

Biopreparation and Straw Management (Factor A) *	Biostimulant (Factor B)						Mean
	1		2		3		
	Content	Ic	Content	Ic	Content	Ic	
Phosphorus—P (mg kg^{-1} soil)							
1	155.3	0.95	178.7	0.96	171.3	0.98	168.4
2	170.0	0.96	144.4	0.97	187.5	0.98	167.3
3	161.6	0.91	152.5	0.91	134.0	0.96	149.4
4	188.1	0.98	185.7	0.96	137.5	0.99	170.4
5	154.1	0.96	165.0	0.98	153.1	0.99	157.4
6	166.5	0.91	154.2	0.95	160.8	0.98	160.5
Mean	165.9		163.4		157.4		162.2
Potassium—K (mg kg^{-1} soil)							
1	176.2	1.04	135.9	1.04	114.8	1.09	142.3
2	166.8	1.02	131.4	1.03	156.8	1.07	151.7
3	198.4	1.02	188.2	1.04	199.4	1.06	195.3
4	234.0	0.88	134.6	0.81	202.3	0.91	190.3
5	162.8	0.86	144.5	0.92	160.9	0.96	156.1
6	103.9	0.78	90.3	0.78	158.4	0.89	117.5
Mean	173.7		137.5		165.4		158.9
Magnesium—Mg (mg kg^{-1} soil)							
1	32.9	0.91	33.1	0.91	22.2	0.88	29.4
2	23.9	0.84	26.7	0.84	27.1	0.90	25.9
3	30.7	0.86	27.1	0.86	27.2	0.90	28.3
4	43.9	0.86	25.8	0.86	20.3	0.84	30.0
5	27.3	0.81	27.6	0.81	32.4	0.89	29.1
6	27.1	0.81	27.8	0.83	28.3	0.82	27.7
Mean	31.0		28.0		26.3		28.4
pH							
1	7.0	0.96	7.1	0.96	7.2	0.97	7.1
2	6.9	0.96	7.0	0.94	7.2	0.97	7.0
3	6.9	0.95	7.0	0.92	7.4	0.95	7.1
4	7.1	0.99	7.0	0.98	7.4	0.99	7.2
5	7.2	0.97	7.1	0.97	7.2	0.97	7.2
6	7.4	0.97	7.4	0.96	7.3	0.96	7.4
Mean	7.1		7.1		7.3		7.2

* See: Table 1.

3.4. Soil Microorganisms Number

Applying the EM biopreparation had a statistically significant effect on the number of heterotrophic bacteria in the analyzed soils. Regardless of the method of straw management, the mean numbers of these microorganisms were statistically highest after a double dose of EM, whereas they were significantly lowest in the variant in which they were not applied. The highest concentration of bacteria in the soil (72.5×10^6 cfu·g^{-1}) was obtained by combining double doses of EM and the Asahi biostimulator with the incorporation of straw (A2B2). The average indices of relative change in number of soil heterotrophic bacteria did not exceed 1 in the two variants without the addition of straw but reached 2.37 in the plots where straw was incorporated into the soil, thus evidencing that enriching the soil with organic matter greatly stimulated the growth of these microorganisms (Table 4).

Table 4. The number of the soil microorganisms depending on the application of the biopreparation EM, straw management, and the application of the biostimulant Asahi in winter wheat cultivation.

Biopreparation and Straw Management (Factor A) *	Biostimulant (Factor B)						Mean
	1		2		3		
	Content	Ic	Content	Ic	Content	Ic	
Heterotrophic bacteria (10^6 cfu g^{-1})							
1	48.5	1.60	49.8	1.61	52.8	1.66	50.3
2	71.7	2.37	72.5	2.34	59.3	1.86	67.8
3	26.0	1.47	27.0	1.37	43.3	1.36	32.1
4	35.7	1.19	37.1	1.06	38.3	1.10	37.0
5	39.7	1.32	48.0	1.24	43.3	1.37	43.7
6	32.7	1.09	30.0	0.86	27.0	0.77	29.9
Mean	42.4		44.1		44.0		43.5
$LSD_{0.05}$ for Factor A = 3.78; Factor B = n.s.; Interaction A/B = 5.69; B/A = 7.36							
Actinobacteria (10^5 cfu g^{-1})							
1	40.1	1.15	40.7	1.12	40.9	1.09	40.6
2	41.3	1.18	42.0	1.15	47.0	0.95	43.4
3	37.7	1.08	38.3	1.05	35.7	1.25	37.2
4	39.9	1.06	40.6	1.06	34.9	1.07	38.5
5	37.3	1.00	38.0	0.99	39.0	1.11	38.1
6	44.7	1.09	45.7	1.02	33.3	1.03	41.2
Mean	40.2		40.9		38.5		39.8
$LSD_{0.05}$ for Factor A = n.s.; Factor B = 2.24.; Interaction A/B = 12.82; B/A = 5.67							
Fungi filamentous (10^4 cfu g^{-1})							
1	13.3	1.21	13.7	1.30	12.7	1.21	13.2
2	11.0	1.37	11.3	1.21	11.7	1.28	11.3
3	16.3	1.48	16.7	1.59	14.7	1.40	15.9
4	8.3	1.06	8.6	1.04	13.8	1.06	10.2
5	9.7	1.04	10.0	1.13	14.7	1.06	11.4
6	6.3	1.05	6.7	1.12	13.3	1.03	8.8
Mean	10.8		11.2		13.5		11.8
$LSD_{0.05}$ for Factor A = 3.46; Factor B = n.s.; Interaction A/B = 4.21.; B/A = 4.62							
Total number of microorganisms (10^6 cfu g^{-1})							
1	52.6	1.56	54.0	1.56	57.0	1.60	54.5
2	75.9	2.24	76.8	2.21	64.4	1.76	72.5
3	29.3	1.30	31.0	1.31	47.0	1.35	35.8
4	39.8	1.06	41.2	1.09	42.0	1.10	41.0
5	43.5	1.14	51.9	1.13	47.3	1.18	47.6
6	37.2	1.01	34.6	1.02	30.5	1.05	34.1
Mean	46.5		48.3		48.0		47.6
$LSD_{0.05}$ for Factor A = 4.65; Factor B = n.s.; Interaction A/B = 6.45; B/A = 7.29							

* See: Table 1.

The maximum average number of actinobacteria was 43.4 × 10^5 cfu·g^{-1} and, as with bacteria, was observed for the cultivation variant that combined double doses of EM preparation and the incorporation of straw. However, the influence of these factors on the growth of actinobacteria was not statistically significant, in contrast to the action of the biostimulator. The average concentration of these microorganisms in the soil after a double dose of Asahi (40.9 × 10^5 cfu·g^{-1}) was statistically significantly higher than in the samples from the plots where the biostimulator was not used (38.5 × 10^5 cfu·g^{-1}). The relative change index values exceeding 1 (indicating an increase in the number of actinobacteria over the study period) were determined for each of the tested experimental variants (Table 4).

The analysis of the results showed a diverse but statistically significant influence of EM on the number of filamentous fungi, the average number of which ranged from 8.8 to 15.9 × 10^4 cfu·g^{-1}. After applying a double dose of the bacterial preparation on plots on which straw was left (A3), the number of these microorganisms was higher than all other variants (A1, A2). Conversely, after the removal of post-harvest residues, the reverse tendency was observed and the lowest concentration of fungi was observed after the application of EM twice. The cultivation variants without EM differed statistically from each other depending on the straw management method—the number of fungi was statistically significantly lower in the soil cultivated without straw than in the straw-enriched soil. The coefficient of relative change was slightly higher for plots with shredded straw, but for the other experimental combinations its value also exceeded 1 (Table 4).

The total number of microorganisms, ranging from 34.1 to 72.5 × 10^6 cfu·g^{-1}, showed a statistically significant dependence on the number of EM treatments and the use of crop residues. The abundances and relative change index values were both higher in the soils where chopped straw was left and, in the case of the EM variants, these differences were statistically significant. Regardless of the straw management model used, a double dose of EM resulted in a statistically higher total number of microorganisms than did a single application of the preparation. Meanwhile, the concentration of microorganisms in soil samples with no addition of EM was statistically the lowest. The use of the Asahi biostimulator had no statistically significant effect on the average number of the studied soil microorganisms, neither in one dose nor in two. The coefficient of relative change ranged from 1.01 to 2.24, evidencing an increase in the total number of microorganisms during the research (Table 4).

3.5. Occurrence of Wheat Diseases

In assessing the effect that applying pro-ecological treatments and incorporating straw into the soil had on the occurrence of root-rot diseases in winter wheat, these plants were shown to be infected by take-all (Ta), fusarium root rot (Ff), eyespot (E), and sharp eyespot (S). The lowest rate of infection, from 1.1 to 13.3%, was for eyespot, while the pathogens responsible for take-all infected the most plants (74.4–94.4%) (Figure 1). The take-all diseases infected wheat slightly more on plots with the straw removed, as confirmed by the DI index values for the analyzed wheat root rot diseases (Table 5).

The rate of infection with winter wheat leaf and ear diseases was found not to have exceeded 11.0%, which was the rate for leaf septoriosis (Sl). The wheat was least infected by powdery mildew (Pm): 1.2–3.8%. Most leaf diseases occurred on the tested plants at similar rates, whether they were grown in soil with straw residue or not. The type and dose of preparations did not significantly differentiate the severity of the identified wheat leaf and ear diseases (Figure 2).

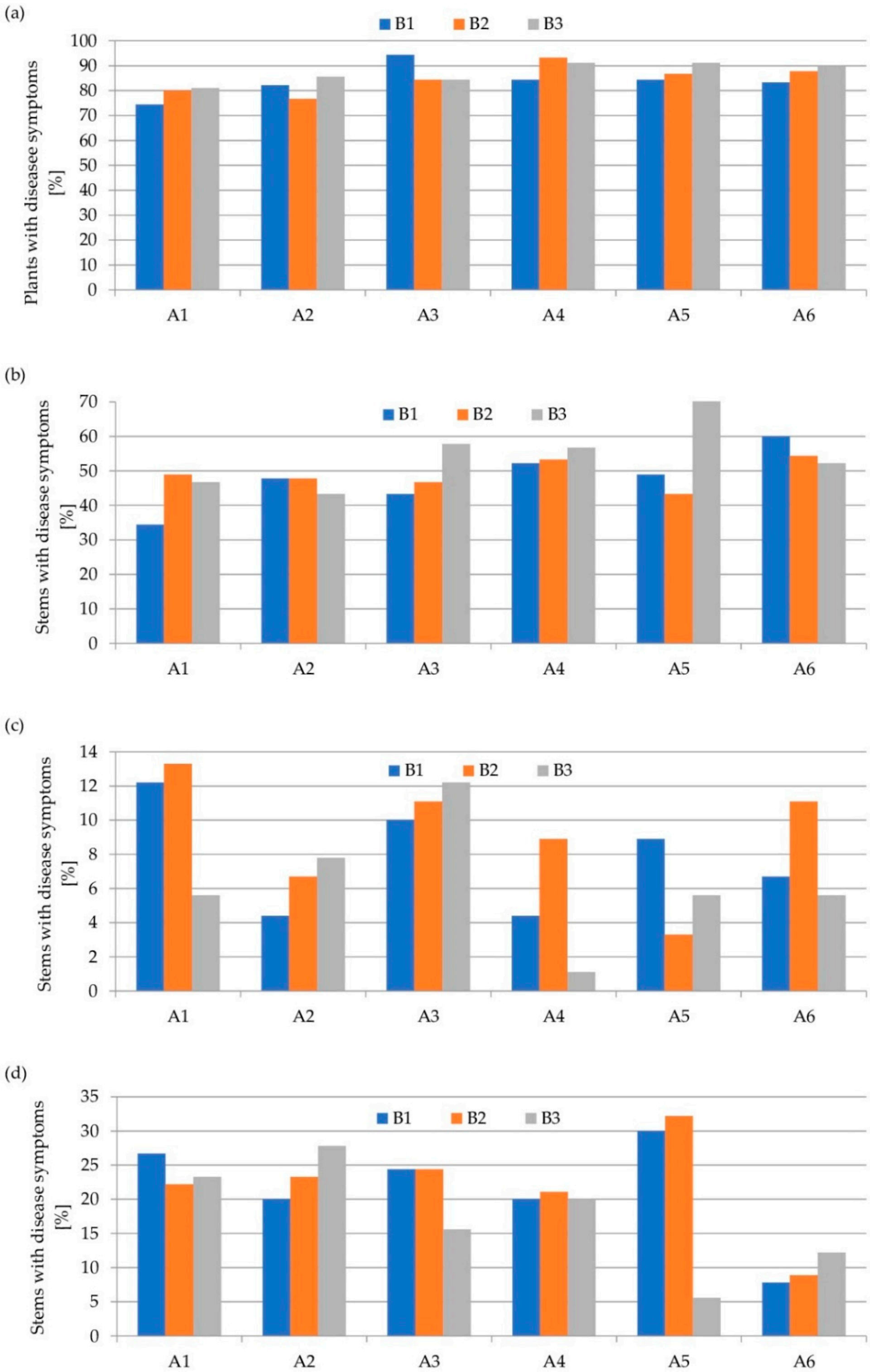

Figure 1. Foot and root rot disease symptoms occurrence on winter wheat depending on the application of the biopreparation EM and straw management (A factor) and the application of the biostimulant Asahi (B factor) (**a**)—take-all; (**b**)—Fusarium foot rot; (**c**)—eyespot; and (**d**)—sharp eyespot.

Table 5. Foot and root rot disease symptoms occurrence on winter wheat depending on the application of the biopreparation EM and straw management and the application of the biostimulant Asahi (Disease index, %).

Biopreparation and Straw Management (Factor A)	Biostimulant (Factor B)			
	B1	**B2**	**B3**	**Mean**
Take-all (Ta)				
A1	21.4	22.8	23.3	22.5
A2	26.9	23.9	25.3	25.4
A3	33.6	24.7	26.1	28.1
A4	25.8	32.5	29.2	29.2
A5	25.8	27.8	30.6	28.1
A6	25.3	29.2	29.4	28.0
Mean	26.5	26.8	27.3	
Fusarium foot rot (Ff)				
A1	10.3	16.1	16.7	14.4
A2	14.7	15.0	13.6	14.4
A3	15.0	16.4	21.1	17.5
A4	17.5	20.3	19.7	19.2
A5	15.3	15.3	24.7	18.4
A6	19.4	17.5	17.8	18.2
Mean	15.4	16.8	18.9	
Eyespot (E)				
A1	4.2	5.0	1.4	3.5
A2	2.2	1.7	2.8	2.2
A3	3.1	4.7	3.9	3.9
A4	2.5	3.9	0.3	2.2
A5	2.5	0.8	1.4	1.6
A6	2.8	4.2	1.9	3.0
Mean	2.9	3.4	2.0	
Sharp eyespot (S)				
A1	9.7	9.4	10.8	10.0
A2	6.9	8.6	11.1	8.9
A3	12.5	10.6	6.7	9.9
A4	7.8	13.1	9.7	10.2
A5	9.4	16.9	1.4	9.2
A6	3.6	4.2	4.7	4.2
Mean	8.3	10.5	7.4	

3.6. Relationship between the Studied Properties—PCA

A multivariate principal component analysis (PCA) was used to determine the nature and strength of the relationships between all the parameters tested. These were: soil pH; content of total organic carbon (TOC); content of available phosphorus (P), potassium (K) and magnesium (Mg); content of total nitrogen (TN), mineral nitrogen forms N-NO3, and N-NH4 (MN); total number of microorganisms (TNM); number of heterotrophic bacteria (B), actinobacteria (A), and filamentous fungi (F); disease index of take-all (Ta,) fusarium root rot (Ff), eyespot (E), and sharp eyespot (S); the applied dose of EM preparation and Asahi biostimulator; and the addition of straw PCA (Figure 3). The two principal components *PC1* (40.8%) and *PC2* (16.8%) were distinguished in the data, accounting for a total of 57.6% of the variance. The PCA showed that the first principal component (*PC1*) was strongly positively correlated with TNM, B, TOC, and TN. On the other hand, *PC1* was significantly negatively associated with the disease index Ff, Ta, and soil pH. The second component (*PC2*) was significantly positively correlated with the number of fungi (F) and K content and strongly negatively correlated only with the number of Actinobacteria (A) (Figure 3a). However, the PCA analysis based on projections of cases (Figure 3b) revealed plots with straw and the use of EM and Asahi preparations had a strong and positive correlation with *PC1*. On the other hand, plots without straw and without the addition of EM biopreparation (A6B1 and A6B2) had the most negative correlations with *PC2*.

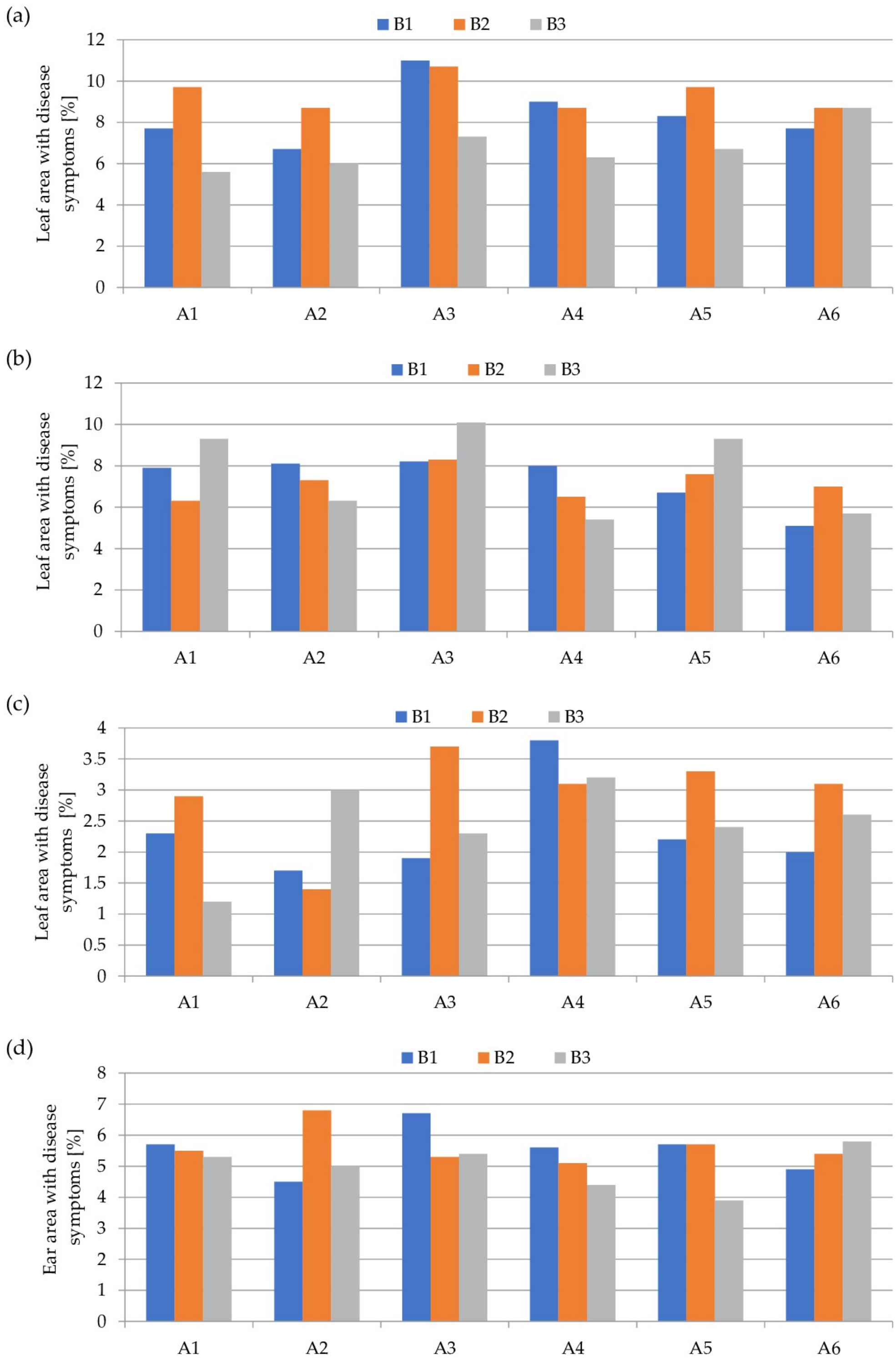

Figure 2. Leaf and ear disease occurrence on winter wheat depending on the application of the biopreparation EM and straw management (A factor) and the application of the biostimulant Asahi (B factor) (leaf or ear area with disease symptoms (%)) (**a**)—Septoria leaf blotch; (**b**)—brown rust; (**c**)—powdery mildew; and (**d**)—Fusarium head blight.

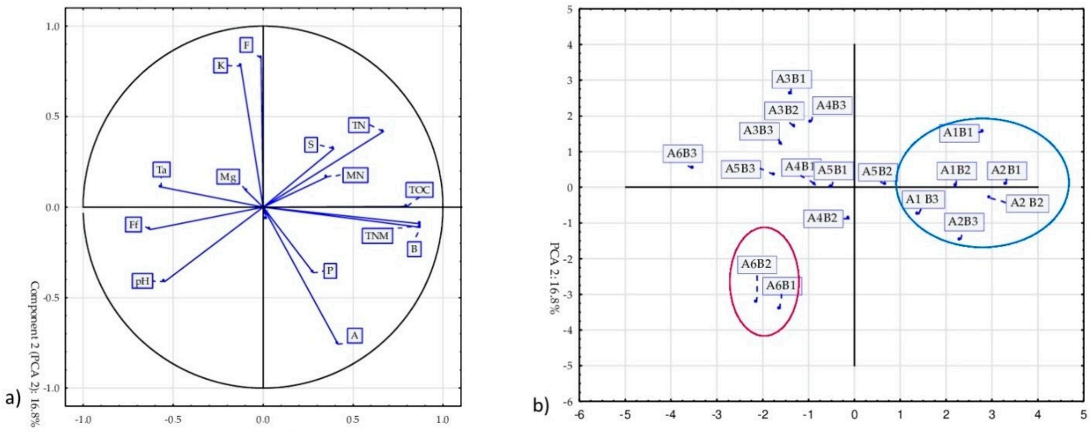

Figure 3. Principal component analysis (PCA) derived from the studied properties. (**a**) Plot of configuration of variables in the system of the PC1 and PC2 of principal components for the measured parameters: content of TOC; content of available P, K, and Mg; content of TN and MN; pH, TNM, B, A, and F; disease index of Ta, Ff, E, and S. (**b**) Principal component analysis of the variables—projection of the cases on the factor-plane for all experimental objects (explanation of abbreviations in Table 1).

Using statistical cluster analysis (Ward's method) based on Euclidean distances, five clusters were distinguished on the basis of the differentiation of variables; these are presented as a dendrogram (Figure 4). Cluster 1 was of plots that differed in terms of the use of eco-friendly products and straw management; of these, one (A6B3) had a lower TOC content, number of actinobacteria and total number of microorganisms than all the others. All five plots in cluster 2 had the highest potassium content, while two (A3B1, A3B2) had the lowest concentration of heterotrophic bacteria and the highest concentration of filamentous fungi. The EM biopreparation was applied at all four sites found in cluster 3. They had a high content of bioavailable forms of phosphorus and two of them (A1B2, A4B2) had the highest TOC content recorded. The three plots forming cluster 4 (A2B1, A2B2, A2B3) were fertilized with straw and received a double EM dose; they had a high content of TOC and the highest number of heterotrophic bacteria and total number of microorganisms. Lastly, cluster 5 comprised two plots (A6B1, A6B2) that had been cleared of straw and to which EM had not been applied. They had the lowest potassium (K) content and the lowest number of filamentous fungi.

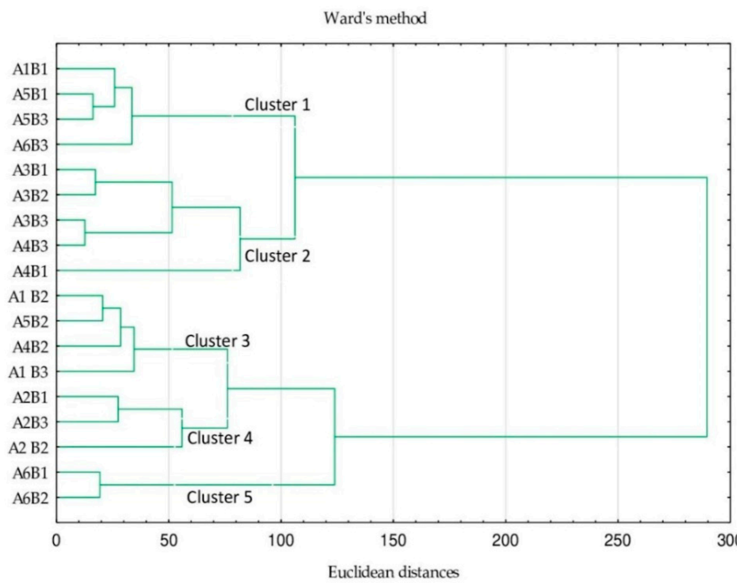

Figure 4. Cluster analysis—dendrogram of studied soil and plants properties (explanation of abbreviations in Table 1).

4. Discussion

The excessive and uncontrolled use of fertilizers and pesticides in conventional agriculture degrades arable soil quality via the environmental accumulation of the products' toxic residues. Another negative effect of such practices is that they reduce the soil's natural resistance to stressors. The environmental losses are accompanied by a reduction in the quantity and quality of yields and, thereby, measurable economic losses. To counteract these changes in keeping with the goals of sustainable agriculture, biological preparations that are environmentally neutral or have a positive effect on its functioning are being introduced into cultivation systems. According to the assumptions, replacing traditional fertilizers and plant protection products with biostimulators and microbiological preparations is expected to improve soil fertility, preserve soil biodiversity, and be beneficial to the health and quality parameters of crops [33,34].

Despite the studies on the effects of EM preparations on soil characteristics and crop yields being numerous, their results are very diverse. Many authors claim that applying EM in various forms was effective in increasing the yields of high-quality crops. The improved productivity was ascribed to, inter alia, improved plant symbiosis with atmospheric nitrogen-fixing bacteria, or the prolonged optimal photosynthetic efficiency of leaves. Conversely, other studies have questioned the validity of using bacterial EM preparations and that the beneficial effects on soil and plants are minimal or non-existent [6,35–39]. In the authors' own research, applying an EM biopreparation led to the highest organic carbon content and total N content in the studied soils, amounting to 29.9 $g \cdot kg^{-1}$ and 2.86 $g \cdot kg^{-1}$, respectively (Table 1). In the case of other chemical parameters (P, K, Mg, pH) no clear trends were observed (Table 2). The results of Szymanek et al. [40] showed that using EM lowered the content of bioavailable forms of phosphorus, potassium and magnesium in soil, though, compared to control soils, the differences were not statistically significant. Hu and Qi [36] observed statistically significant increases in content of available P and K forms in compost-enriched soil with the addition of EM. For phosphorus, these were 50.69 $mg \cdot kg^{-1}$ in soil fertilized with EM and 4.07 $mg \cdot kg^{-1}$ in soil from a control plot, and for potassium, 207.21 $mg \cdot kg^{-1}$ and 80.86 $mg \cdot kg^{-1}$, respectively. In their research, adding a microbiological preparation also led to a drop in pH.

One of the key elements ensuring the proper functioning of a soil ecosystem is the group of microorganisms inhabiting it. The soil microbiomes are very diverse and imbalances that cultivation practices bring can have serious consequences. The environmentally safe composition of EM biopreparations means that they can be applied to soil without threatening the natural microbiological processes going on within it and, in a stressful situation, they should play a regulating and supporting role in the activity of native soil microorganisms. Our research results indicate an increase in the number of bacteria and total number of microorganisms in soils with the addition of EM, especially when applied twice. The values of these parameters in the variant combining their operation with leaving straw on the plots were statistically significantly higher than the others, amounting to 67.8×10^6 $cfu \cdot g^{-1}$ (B) and 72.5×10^6 $cfu \cdot g^{-1}$ (TNM) (Table 4). We observed similar dependencies in our previous experiments, which could indicate the stable tendency [41]. Other researchers have formed the opposite opinion, however, suggesting that the possibility for concentrations of microorganisms in microbiologically rich environments to be modified by the introduction of EM are limited. They assert that any fluctuations in the number of soil microorganisms after the application of doses of biopreparations recommended by producers will be short-lived and will not lead to permanent changes [13]. Shin et al. [33] state that the efficacy of microbiological biopreparations largely depends on the type of soil and is greater for soils with low microbiological activity.

According to Higa and Parr [14], the creator of EM technology, adding EM to soil gradually increases its suppressiveness and limits its content of plant pathogens and, as a result, allows lower quantities of conventional plant protection product to be used. Boligłowa and Gleń [42] examined the effect that applying an EM biopreparation had on the health of the leaves, ears, and stem base of winter wheat and they confirmed its efficacy in preventing diseases caused by *Septoria nodorum* (septoria disease of wheat leaf and ear) and *Drechslera tritici-repentis* (tan spot of the leaf), but found that it had no effect on the infestation of plants by *Gaeumannomyces* and *Pseudocercosporella herpotrichoides*. In the research by Roberti et al. [43], preparations containing EM reduced the incidence of *Rhizoctonia solani* in beans, but their efficacy was not confirmed in an in vitro experiment. Shin et al. [33] did not observe any inhibition of the development of soil-borne diseases of carrot and cucumber (*Rh. solani*, *Pythium ultimum*). Our own results did not show that the EM application affects the incidence of the analyzed wheat diseases.

The commercial EM biopreparation and its specific consortium of microorganisms is not the only example of attempts to exploit the properties of microorganisms to reduce the use of conventional fertilizers and pesticides. Other beneficial microorganisms can, alongside a number of natural chemical compounds, comprise the active ingredients in another group of eco-friendly products used in agriculture—biostimulants. The reported positive effects of biostimulants relate to both the soil environment and crops. However, as with EM, some in the scientific community are critical of the reliability and quality of the results of research investigating this issue. It seems that applying these preparations is justified under stressful growing conditions (high salinity, presence of heavy metals) to reduce their negative influence on plant growth. On the other hand, their applicability may be narrowed by the multiplicity and diversity of the factors that impact their efficacy. According to Li [9], their efficacy may be influenced by environmental conditions, but also by the method of application, e.g., biostimulants increase plant yield more effectively when introduced into the soil than when applied to leaves. Additionally, groups of crops differ from one another in their susceptibility to the effects. Analyzing the results of our own experiments, the Asahi biostimulator was found to have less effect on the tested chemical and microbiological properties of the soil than did EM. In most of our analyses, it was not possible to clearly define the direction of changes in the concentration of individual elements or chemical compounds, nor in the number of microorganisms or the severity of symptoms of wheat diseases. The statistically significant differences were observed only for: the content of organic C in soil, which was lowest on average in the experimental variant without the biostimulator, and for the number of actinobacteria, which in soil after a double dose of Asahi (40.9×10^5 cfu·g^{-1}) was statistically significantly higher than in the soil where it was not applied (38.5×10^5 cfu·g^{-1}) (Table 4). The research that used this same product has shown its positive effect on a number of important characteristics of various crops species. Michałek et al. [12] showed an improvement in the photosynthetic efficiency of beans treated with Asahi SL solution. The use of this biostimulant in the studies by Gugała et al. [44] led to an increase in the 1000-seed mass and yield of winter rape. In turn, Przybysz et al. [45] observed that using Asahi SL stimulated the development of *Arabidopsis thaliana* L. cultivated in soil containing Cd^{2+} ions, which confirms the above-mentioned hypothesis regarding the potential of biostimulants to reduce the negative effects of growing plants under abiotic stress.

While the effectiveness of using biopreparations and biostimulants sometimes raises justified doubts, the effects of the years-long practice of incorporating straw into the soil have been sufficiently investigated. Adding straw increases the amount of organic matter and organic carbon in soil, which may help minimize environmental degradation resulting from agricultural intensification [46,47]. Furthermore, this practice also contributes to increasing soil's CO_2 sequestration potential, which is of particular importance in the face of rapid global climate change. According to Wang et al. [48] and Zhao et al. [49], adding straw to the soil may lead to an increase in the content of organic carbon combinations of up to several 20%. In the authors' own research, no statistically significant differences in the C_{org} content were observed to be caused by enrichment with straw. However, the value of the Ic coefficient, which exceeded 1 only in the experimental variants in which crop residues were incorporated, indicated a quantitative increase in these parameters after the completion of the research. A similar trend was observed for the N total and bioavailable forms of K, while the content of P and K in the soil did not depend on the method of straw management. In turn, Wang et al. [50] observed that, after five years of using straw alongside mineral fertilization, the amount of phosphorus in the soil increased by 10.8%, while mineral fertilization alone led to a decrease in its content. An increase in nitrogen, potassium, and phosphorus content in straw-enriched soils was also noted by Akhtar et al. [51]. Removing residues from the surface of the experimental plots slightly increased the pH of the studied soils (7.2–7.4), as also confirmed by the results of other studies [49,50].

Enriching the soil environment with an additional load of organic matter is associated with changes in the number and activity of its native microorganisms. In our own research, the straw combined with the EM biopreparation had a statistically significant effect on the growth of heterotrophic bacteria. Their maximum number was 67.8×10^6 cfu·g^{-1} (A2), while in the variant without straw it was 43.7×10^6 cfu·g^{-1} (A5) (Table 4). The Ic values above 2 confirm the clear stimulation of these microorganisms' growth over the course of the study. The relationships relating to the influence that straw has on the total number of soil microorganisms were similar, although the concentrations of actinobacteria and fungi were less affected by this factor. The analysis of the relationship between the examined parameters by PCA showed a positive correlation between the soil organic carbon content and the total number of microorganisms and heterotrophic bacteria (Figure 3). The results we obtained are consistent with many others that confirm the soil microbiome being activated by increases in soil TOC resources after the application of straw [48,52].

An additional source of organic carbon Introduced into the soil in the form of crop residues can be used by all microorganisms in the soil, whether beneficial or detrimental, including phytopathogens [53]. In the authors' own study, however, the infection of wheat by take-all diseases was slightly greater in the plots with straw removed. Additionally, Rodgers-Gray and Shaw [54] observed a lower intensity of wheat diseases caused by *Mycosphaerella graminicola*, *Erysiphe graminis*, *Puccinia recondita*, and *Fusarium* root rot in plots in which straw had been incorporated. Meanwhile, in the studies of Jenkyn et al. [55], including straw reduced the occurrence of leaf spot caused by *P. herpotrichoides* but did not significantly affect other pathogens causing winter wheat diseases.

5. Conclusions

Adapting cultivation systems to sustainable agriculture standards is one of the most important challenges facing the modern agricultural economy. The preparations we tested that are based on the activity of microorganisms and natural phenolic compounds are treated as potential substitutes for traditional fertilizers and pesticides, the abuse of which is considered to be a cause of environmental degradation. Despite having been confirmed by many studies, the efficacy of these products fluctuates significantly, contributing to doubts as to the legitimacy of their practical application. It seems that the problems underlying these disconfirming opinions result from the variability of factors related to the application of biopreparations and biostimulants, in which variability can partially be reduced. To develop optimal methods of use for eco-friendly preparations, it is suggested to adapt them to the location-specific climatic and soil conditions, the cultivation system used, and the requirements of the plants they are intended to benefit. The formulas for individual preparations also require refinement, which, as some studies have shown, may differ by product batch. The procedures for preparing ready-to-use working solutions (e.g., EM biopreparations) should also be more carefully standardized. Despite a number of limitations, the potential benefits of introducing them into cultivation systems provide ample reason to continue experiments with their use. The results of such, by expanding the information base upon which the principles of application of eco-friendly products will be clarified, may support their wider-scale introduction into agricultural practice, while also ensuring their efficacy and environmental benefits.

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