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The Cumulative Effect of Various Tillage Systems and Stubble Management on the Biological and Chemical Properties of Soil in Winter Wheat Monoculture

Karol Kotwica [1], Barbara Breza-Boruta [2], Justyna Bauza-Kaszewska [2,*], Piotr Kanarek [2], Iwona Jaskulska [1] and Dariusz Jaskulski [1]

[1] Department of Agronomy, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, 7 Prof. S. Kaliskiego St., 85-796 Bydgoszcz, Poland; kotwica@utp.edu.pl (K.K.); jaskulska@utp.edu.pl (I.J.); darekjas@utp.edu.pl (D.J.)

[2] Department of Microbiology and Food Technology, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, 6 Bernardynska Street, 85-029 Bydgoszcz, Poland; breza@utp.edu.pl (B.B.-B.); piotrek.kanarek@gmail.com (P.K.)

* Correspondence: bauza@utp.edu.pl

Abstract: Agricultural practices, including tillage systems and postharvest residue management, strongly affect a wide range of soil properties. Depending on the degree of soil inversion, both negative and beneficial changes of its structure, chemical composition, and biological activity may occur as a result of these treatments. The three-year experiment was carried out on the soil under winter wheat (cv. Arktis) monoculture. The effect of various tillage systems and stubble management on the soil environment's biological and chemical features was investigated. The total number of microorganisms (TNM); bacteria (B); actinobacteria(A); fungi (F); soil respiratory activity (SR); pH in 1 M KCl (pH); organic carbon content (OC); content of available phosphorus (Pa); potassium (Ka); magnesium (Mg); and content of total nitrogen (TN) and mineral nitrogen forms were determined in soil samples. It was shown that manure application was the factor with the greatest influence on soil properties. The manure fertilization resulted in a higher number of microorganisms in arable soil. Conventional tillage procedures combined with manure application increased the amounts of available forms of phosphorus, potassium, magnesium, and mineral nitrogen. Introduction of the chopped straw in the field enhanced the beneficial effect of manure on soil properties.

Keywords: tillage system; stubble management; soil environment; nutrients; microorganisms

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1. Introduction

The planet's predicted 30% population growth by 2050 may be one of the main factors determining the increased demand for wheat as one of the most important cereal crops cultivated worldwide [1]. Consequently, intensification of research can be expected to develop new or optimize already used agricultural techniques, allowing for the increase of wheat yield per unit area [2]. When making decisions aimed at achieving this goal, related to modifying the applied method of tillage, fertilization, or plant protection' the impact of those changes on the soil environment and its most important biological and physicochemical parameters should be considered [3].

One of the important reasons for the degradation of arable soils is an intensive agricultural activity using traditional cultivation methods based, among other factors, on deep plowing and other treatments that strongly interfere with the soil structure [4,5]. Conventional tillage practices include soil inversion, multiple loosening, and mixing, which strongly alter the soil environment, resulting in a decrease in soil organic matter content, a reduction in soil biological activity, and a reduction in biodiversity [6]. To prevent these unfavorable phenomena in agricultural practice, the current plow tillage is often simplified by introducing non-inversive methods generally limited to the loosening the soil structure, e.g., a non-tillage system or direct sowing [7,8]. However, similarly to the excessive intensification of agricultural production, ill-conceived simplifications may sometimes lead to ecologically and economically dubious consequences, requiring additional measures to be taken to preserve or improve soil biodiversity and fertility, including diverse crop rotations, perennial crops, organic fertilizer use, growing legumes, incorporating cover crops to the soil or integrating pest and weed management [9].

Bringing these key properties of the soil environment to the optimal level for the proper growth of plants determines the effectiveness of cultivation. This requires monitoring the values of certain parameters, e.g., organic matter content, pH, electrical conductivity, C/N ratio, macro and micronutrient content, and undertaking interventions in the event of disturbances that may have a negative impact on the quantity and quality of crops or on plant health. The necessary in modern agriculture augmentation of soil with organic matter, the level of which determines, among others, the ability to retain water and nutrients, the proper structure of soil, and efficient carbon management, including the sequestration of this element from the atmosphere, is mainly achieved by using organic fertilizers [6,10,11]. In critical situations of a deficit of manure or a large share of cereals in the structure of crops, both of which negatively affect the balance of organic matter in the soil, it is reasonable to supplement its deficiencies by using organic carbon-rich crop residues such as straw and intercrop biomass [12].

The content of organic matter in soil is also related to the activity of the microorganisms inhabiting it. These are responsible for the most important biochemical processes occurring in this environment and enabling the fulfillment of the basic soil functions, from the agricultural point of view [13,14]. The number and composition of microbial populations are also influenced by several other factors, both those changing the properties of the soil environment independently of human activity and typically anthropogenic ones. Modification of the cultivation method, directed either at its intensification or its limitation, may cause significant changes in the proportions of particular groups of soil microorganisms, their activity, and mutual interactions [15,16].

The microbial activity and diversity may be supported by the enrichment of soil with specific biopreparations containing the so-called consortia of effective microorganisms (EM) composed mainly of photosynthetic bacteria, lactic acid bacteria, actinomycetes, yeast, and filamentous fungi [17]. Although the results of the research considering their effect on soil and crops can be contradictory, a number of them confirm the beneficial effect of EM on soil fertility, crop yield, and plant disease reduction [18,19].

This study aimed to analyse the effect of different tillage systems and methods of incorporating post-harvest residues into soil on the various groups of soil microorganisms and soil chemical properties including pH, organic carbon (OC), total (TN) and mineral nitrogen ($N\text{-}NO_3$ and $N\text{-}NH_4$), phosphorous (Pa), potassium (Ka), and magnesium (Mg) in extended monocultures of winter wheat.

2. Materials and Methods

2.1. Experiment Location and Layout

The three-year experiment was carried out on an individual farm in Chełmce, the Kuyavian-Pomeranian Voivodeship (or province), in Poland (52°61′ N; 18°44′ E). A static (second- and and third-year of winter wheat monoculture) three-way experiment was set up in a split-plot-split-block design in three replications involving 20 experimental plots. The qualified seed material of winter wheat cv. Arktis was sown at a density of 400 grains$\cdot$ m^{-2} in September 2011 and 2012.

The experimental factors included:

A—tillage system (five variants):
A1—post-harvest: Grubber with a roll; pre-sowing: grubber + seeder-cultivator unit
A2—post-harvest: grubber with a roll; sow ploughing + seeder-cultivator unit
A3—single ploughing + seeder-cultivator unit

A4—post-harvest: manure + grubber with a roll; pre-sowing: sow ploughing + seeder-cultivator unit
A5—direct sowing

Variant A1 was a non-tillage system, and together with A5 variant (direct sowing) was characterized by a limited interference in the soil structure. Variants A2, A3, and A4, involving ploughing procedure and soil inversion, were considered more intense systems.

The sowing depth was 4 cm with the row spacing of 14.3 cm. A seeder-cultivator unit was used for sowing with an active cyclotiller section and seeder (Horsch Pronto 4DC equipped with disk coulters). In variant A5 (direct sowing), the cyclotiller section was inactive.

Individual tillage systems variants were labeled 1, 2, 3, 4 and 5 in the tables presenting final research results.

Cattle manure was applied at a dose of 30 t·ha^{-1} in an A4 experimental variant. Seven days before direct sowing, glyphosate was applied in the form of a preparation of Roundup Max 680SG (2 kg·ha^{-1});

B—method of managing the post-harvest residues:
B1—leaving shredded straw
B2—leaving shredded straw + EM
B3—removing straw + EM
B4—removing straw

(EM—preparation containing effective microorganisms "EM-A" at a dose of 40 dm^3·ha^{-1});

Individual tillage systems variants were labeled 1, 2, 3, and 4 in the tables presenting research results.

An experimental plot with a classic tillage method was considered as the control, with the following combination of A2B4 levels: coarse stubble cultivation + sowing plowing (A2) made after removing the straw without the use of EM (B4). The chemical properties of light loam soil sampled in 2011 were as follows: pH in 1 M KCl (pH) 7.6; organic carbon content (OC) 2.31%, content of available phosphorus (Pa), potassium (Ka) and magnesium (Mg): 16.1; 21.8 and 4.80 mg 100 g^{-1}, respectively.

Habitat conditions during the performance of the research, including the distribution of rainfall and temperature, as well as agrotechnical conditions and wheat yielding, were described in the previous paper [20].

2.2. Soil Samples

Ten individual soil samples were collected from each experimental plot for all the treatments. The soil from a single plot was thoroughly mixed and homogenized by sieving to create a pooled sample. All soil samples were analysed in triplicate.

Soil samples for microbiological analysis were collected for the first time in 2011, at the beginning of the experimentand before winter wheat sowing, and the last time in 2013, after plant harvesting and before the beginning of post-harvest cultivation. On each plot, soil samples were taken from the tilled soil layer (0–25 cm depth).

Soil samples for chemical analysis; including the measurement of soil pH, the content of organic carbon (OC), total nitrogen (TN), and available P (Pa), K (Ka), and Mg (Mg) forms in individual experimental plots; were collected from a depth of 0–25 cm on dates analogous to microbiological tests.

The content of mineral nitrogen and its forms was determined in the autumn and spring seasons in the following years of research based on average samples, from three replications from each experimental plot.

2.3. Soil Microbiological Parameters

The plate count method was used to estimate the number of aerobic heterotrophic bacteria, filamentous fungi, and actinomycetes. Ten grams of each soil sample were added to 90 mL of Ringer's solution. After homogenization for 30 min, ten-fold serial dilutions were prepared (10^{-1} to 10^{-6}) and inoculations of the prepared soil solutions were inoculated on the proper culture media. To determine the total number of bacteria (B), a yeast extract-peptone-soil extract medium (YPS) was used [21]. Actinomycetes (A) were isolated on yeast extract glucose agar (YGA) with 100 µg mL^{-1} nystatin [22] and filamentous fungi (F) were isolated on Rose-Bengal agar containing 30 µg mL^{-1} streptomycin [21]. Incubation of the fungi and bacteria was performed at 25 °C for five days, while incubation of the actinomycetes was carried out at 25 °C for ten days. All analyses were performed in four replications. After the incubation period, the colonies that had grown on the Petri dishes were counted. The number of colony-forming units (cfus) was determined per 1 g of soil dry matter (cfu g^{-1} d.m. of soil).

Based on the obtained values of the total number of microorganisms, the relative change index (W_k) was determined. The W_k index was calculated as the quotient of the value of the mean microorganisms number from the individual experimental plots and also in a control plot. The values of the index above 1.0 indicate the favorable impact of a given combination of levels of the analyzed factors, and the values lower than 1.0 indicated the contrary.

In order to characterize the biological properties of the soil, the soil phytosanitary index was calculated as the quotient of the sum of the total number of bacteria (B) and actinomycetes (A) to the number of fungi (F) [23]. Higher values of the soil phytosanitary index indicate a more favorable phytosanitary condition of the soil, as well as greater fertility.

2.4. Soil Respiration Measurement

Soil biological activity was measured based on the analysis of soil respiration intensity performed in each year of the study, at four dates each year, using the SRC-1 Soil Respiration Chamber with the PP Systems EGM-4 analyzer. The intensity of respiration was determined by changes in the concentration of carbon dioxide, which was measured in 5-min cycles, in each experimental plot.

2.5. Soil Chemical Properties

In air-dried disturbed soil samples sieved through a 2-mm mesh, selected physicochemical properties were determined, i.e., pH in 1 M KCl by potentiometric method [24], organic carbon (OC), and total nitrogen (TN) concentrations using a Vario Max CN analyzer (Elementar, Germany). The contents of available forms of phosphorus (Pa) [25] and potassium (Ka) were determined by the Egner–Riehm method [26], while the content of magnesium available to plants (Mg) was analyzed following the Schachtschabel method [27]. The content of forms available to plants was determined by atomic absorption spectroscopy and atomic emission spectroscopy using a Solaar S4 spectrometer. The forms of the mineral nitrogen, i.e., ammonium ($N\text{-}NH_4$) and nitrate ($N\text{-}NO_3$), were determined by flow colorimetry following soil extraction in 1% K_2SO_4 using the Skalar San Plus Analyzer.

Based on the obtained values of selected chemical parameters (soil pH, content of OC, TN, and available P, K, and Mg forms) at two dates, before the start of the research and after its completion, the relative change index (W_z) was determined. The W_z index was calculated as the quotient of the value of a specific chemical parameter at the beginning and the end of research in a given experimental plot. The values of the index above 1.0 indicate a favorable impact of a given combination of levels of the analyzed factors, and the values lower than 1.0 indicated the contrary.

2.6. Data Analysis

The final results are the mean of three replications from each experimental plot in each sampling time. Soil characteristic data were normally distributed and the results were statistically analyzed using the variance of multiple experiments, according to the model appropriate for the randomized subblock design. Analysis of variance (two-way ANOVA) was used, where the first factor was the tillage systems (A) and the second was the method of managing post-harvest residues (B). The significance of differences between the plot means was determined by Tukey's test, at $p \leq 0.05$. The studied parameters were evaluated using principal component analysis (PCA). The results of this analysis are presented as graphics that display traits in the arrangement of the first two principal components (PC1 and PC2), which synthetically represent mutually correlated variables. The similarity of the impact of tillage methods and stubble management systems on the chemical and biological properties of the soil was assessed by cluster analysis using Ward's method and presented in a dendrogram. Due to the diverse ranges of absolute quantities of individual soil characteristics, multidimensional analyses were performed on standardized data. The statistical analysis of the results was performed using the Statistica.PL 12 [28] software package.

3. Results

Among the factors related to the method of winter wheat cultivation, the use of manure (A4) had a significant impact on the number of bacteria and actinomycetes in the soil. The mean number of bacteria in soil samples from these plots was 109.3×10^6 cfu g^{-1}, and was significantly higher than in the remaining cultivation variants, where this value ranged from 41.4×10^6 cfu g^{-1} (A3) to 58.5×10^6 cfu g^{-1} (A5) (Table 1). The numbers of actinomycetes and fungi reached 10^5 cfu g^{-1} and 10^4 cfu g^{-1}, respectively, and the differences between individual experimental plots did not exceed one log. Leaving chopped straw in the field, especially when using direct sowing, also stimulated the growth of all the studied groups of soil microorganisms (A5). In the latter cultivation plot, a significantly higher number of bacteria was also found after applying the EM preparation to the stubble (Table 1).

The analysis of the total number of microorganisms confirmed the results obtained for individual groups of microorganisms. Values exceeding 10^8 cfu g^{-1} were found after the two-year application of manure and the introduction of intercrop biomass (A4) to the soil, and they were significantly higher than those observed in the other experimental plots (Table 2). The percentage difference among treatments including conventional tillage combined with manure application (A4) and simplified treatment reduced to a single ploughing (A3), was 61.2%. The introduction of shredded straw to the soil, particularly if previously treated with the EM (B2) vaccine, resulted in a significant increase in the total number of microorganisms in the cultivation plots using manure (A4) and direct sowing (A5) (Table 2).

Table 1. Total number of bacteria, actinomycetes, and fungi in the soil, depending on the tillage method (factor A) and stubble management (factor B) in the short-term monoculture of winter wheat.

Levels of Soil Management (A)	Levels of Organic Input (B)				
	1	2	3	4	Mean
	bacteria (10^6 cfu g^{-1})				
1	51.3 ^{bc}	53.3 ^{c}	49.3 ^{bc}	48.7 ^{b}	50.7 ^{c}
2	46.7 ^{c}	48.7 ^{cd}	43.3 ^{bc}	41.7 ^{b}	45.1 ^{cd}
3	44.0 ^{c}	39.7 ^{d}	40.3 ^{c}	41.7 ^{b}	41.4 ^{d}
4	115.0 ^{a}	118.3 ^{a}	100.0 ^{a}	104.0 ^{a}	109.3 ^{a}
5	61.3 ^{b}	72.7 ^{b}	53.3 ^{b}	46.7 ^{b}	58.5 ^{b}
Mean	63.7 $_{AB}$	66.5 $_{A}$	57.2 $_{B}$	56.6 $_{B}$	
LSD for: Factor A = 6.1; Factor B = 8.5; Interaction A/B = 11.6; B/A = 12.7					
	Actinomycetes (10^5 cfu g^{-1})				
1	28.7 ^{b}	36.0 ^{b}	24.0 ^{b}	22.7 ^{b}	27.8 ^{bc}
2	26.0 ^{b}	27.3 ^{c}	29.0 ^{b}	24.3 ^{b}	26.7 ^{c}
3	26.7 ^{b}	36.0 ^{b}	22.0 ^{b}	21.7 ^{b}	26.6 ^{c}
4	47.3 ^{a}	44.7 ^{a}	46.7 ^{a}	44.0 ^{a}	45.7 ^{a}
5	32.3 ^{b}	32.3 ^{bc}	28.3 ^{b}	21.0 ^{b}	31.1 ^{b}
Mean	32.2 $_{AB}$	35.3 $_{A}$	30.0 $_{BC}$	26.7 $_{C}$	
LSD for: Factor A = 4.2; Factor B = 4.1; Interaction A/B = 7.6; B/A = 7.4					

Table 1. *Cont.*

Levels of Soil Management (A)	Levels of Organic Input (B)				
	1	2	3	4	Mean
	Fungi (10^4 cfu g^{-1})				
1	26.0 ^{b}	28.7 ^{b}	26.7 ^{b}	25.3 ^{b}	26.7 ^{c}
2	19.3 ^{c}	24.0 ^{c}	18.0 ^{c}	15.3 ^{d}	19.2 ^{d}
3	20.0 ^{c}	23.3 ^{c}	18.0 ^{c}	14.7 ^{d}	19.0 ^{d}
4	33.3 ^{a}	31.3 ^{b}	31.7 ^{a}	32.7 ^{a}	32.3 ^{a}
5	34.3 ^{a}	36.0 ^{a}	28.7 ^{ab}	20.0 ^{c}	29.7 ^{b}
Mean	26.6 $_{AB}$	28.7 $_{A}$	24.6 $_{BC}$	21.6 $_{C}$	
LSD for: Factor A = 1.9, Factor B = 3.3; Interaction A/B = 4.3; B/A = 4.9					

Factor A levels: 1—post-harvest: grubber with a roll; pre-sowing: grubber + seeder-cultivator unit, 2—post-harvest: grubber with a roll; sow ploughing + seeder-cultivator unit, 3—single ploughing + seeder-cultivator unit, 4—post-harvest: manure + grubber with a roll; pre-sowing: sow ploughing + seeder-cultivator unit, 5—direct sowing; Factor B levels: 1—leaving the shredded straw, 2—leaving the shredded straw + EM, 3—removing the straw + EM in the stubble, 4—moving the straw. $^{a–d}$—letters in columns indicate significant differences at $p < 0.05$. $^{A–C}$—letters in row indicate significant differences at $p < 0.05$.

Table 2. Total number of microorganisms in the soil, depending on the tillage method (factor A) and stubble management (factor B) in the short-term monoculture of winter wheat [10^6 cfu g^{-1}].

Levels of Soil Management (A)	Levels of Organic Input (B)								Mean
	1		2		3		4		
	Count	W_k	Count	W_k	Count	W_k	Count	W_k	
1	54.5 ^{bc}	1.23	57.2 ^{c}	1.29	52.0 ^{bc}	1.18	51.2 ^{b}	1.16	53.7 ^{c}
2	49.5 ^{c}	1.12	51.6 ^{cd}	1.17	46.4 ^{bc}	1.05	44.3 ^{b}	1.00	47.9 ^{cd}
3	46.9 ^{c}	1.06	43.5 ^{d}	0.98	42.7 ^{c}	0.97	44.0 ^{b}	0.99	44.3 ^{d}
4	120.1 ^{a}	2.71	123.1 ^{a}	2.78	105.0 ^{a}	2.37	108.8 ^{a}	2.46	114.2 ^{a}
5	64.9 ^{b}	1.47	76.3 ^{b}	1.72	56.5 ^{b}	1.28	49.0 ^{b}	1.11	61.6 ^{b}
Mean	67.2 $_{AB}$		70.3 $_{A}$		60.5 $_{B}$		59.4 $_{B}$		
LSD for: Factor A = 6.1; Factor B = 8.4; Interaction A/B = 11.7; B/A = 12.7									

$^{a–d}$—letters in columns indicate significant differences at $p < 0.05$. $^{A, B}$—letters in row indicate significant differences at $p < 0.05$.

The highest values of the phytosanitary index were observed in the soil treated with a single plough procedure (A3) and conventional tillage (A2). On the contrary, in the soil samples from traditional tillage systems combined with manure application (A4), the number of fungi in relation to the total number of bacteria and actinomycetes was higher, which resulted in a lower value of the phytosanitary index (Table 3).

Table 3. Phytosanitary index of the soil depending on the tillage method (factor A) and stubble management (factor B) in the short-term monoculture of winter wheat.

Levels of Soil Management (A)	Levels of Organic Input (B)				
	1	2	3	4	Mean
1	176.9 ^{c}	151.1 ^{b}	168.4 ^{b}	177.4 ^{d}	168.5 ^{b}
2	240.3 ^{a}	175.1 ^{a}	236.7 ^{a}	270.9 ^{b}	230.7 ^{a}
3	218.7 ^{b}	181.2 ^{a}	248.9 ^{a}	312.6 ^{a}	240.3 ^{a}
4	135.2 ^{d}	140.9 ^{b}	145.9 ^{c}	148.3 ^{e}	142.6 ^{c}
5	135.9 ^{d}	120.4 ^{c}	152.8 ^{c}	217.5 ^{c}	156.7 ^{b}
Mean	181.4 $_{BC}$	153.7 $_{C}$	190.5 $_{AB}$	225.3 $_{A}$	
LSD for: Factor A = 12.75. Factor B = 35.61; Interaction A/B = 32.131; B/A = 45.368					

$^{a–d}$—letters in columns indicate significant differences at $p < 0.05$. $^{A–C}$—letters in row indicate significant differences at $p < 0.05$.

Trends in changes in soil respiration intensity were similar to those reported for soil microorganisms. Manure introduced into the soil significantly intensified respiration to the maximum value of 0.444 μL CO_2 m^{-2} h^{-1} (A4, B2). On the other hand, leaving the shredded straw on the stubble significantly increased respiration in all experimental plots except A4 when the soil was fertilized with manure (Table 4). The percentage difference among the significantly highest and lowest respiration rates observed in experimental variants A4 (conventional tillage system combined with manure) and A3 (single plough) was 45.5%.

Table 4. Soil respiration intensity (μL CO_2 m^{-2} h^{-1}), depending on the method of tillage (factor A) and stubble management (factor B) in winter wheat monoculture.

Levels of Soil Management (A)	Levels of Organic Input (B)				
	1	2	3	4	Mean
1	0.364 ^{ab}	0.395 ^{ab}	0.204 ^{b}	0.179 ^{b}	0.285 ^{b}
2	0.332 ^{b}	0.374 ^{bc}	0.196 ^{b}	0.164 ^{b}	0.266 ^{b}
3	0.278 ^{c}	0.324 ^{c}	0.127 ^{c}	0.124 ^{b}	0.213 ^{c}
4	0.406 ^{a}	0.444 ^{a}	0.351 ^{a}	0.361 ^{a}	0.391 ^{a}
5	0.357 ^{ab}	0.396 ^{ab}	0.175 ^{bc}	0.160 ^{b}	0.272 ^{b}
Mean	0.347 $_{A}$	0.387 $_{A}$	0.211 $_{B}$	0.198 $_{B}$	
LSD for: Factor A = 0.045, Factor B = 0.081; Interaction A/B = 0.053; B/A = 0.079					

$^{a–c}$—letters in columns indicate significant differences at $p < 0.05$. $^{A, B}$—letters in row indicate significant differences at $p < 0.05$.

The pH of the soil in the monoculture winter wheat cultivation was close to neutral and ranged from 6.9 to 7.5. Fertilization of soil with straw resulted in a pH decrease, while relatively high values of this index were found with traditional cultivation (A2) and after fertilization with manure, especially with the simultaneous removal of straw and application of the EM vaccine directly to the stubble. The values of the relative change index mostly indicate a slight decrease in pH during the experiment (Table 5).

Table 5. Chemical properties of soil depending on the method of tillage (factor A) and stubble management (factor B) in winter wheat monoculture.

Level of Soil Management (A)	Level of Organic Input (B)								Mean
	1		2		3		4		
	Content	W_z	Content	W_z	Content	W_z	Content	W_z	
pH									
1	7.0	0.91	7.5	0.97	7.2	0.98	7.3	0.96	7.3
2	7.4	0.99	7.3	1.00	7.5	1.03	7.4	1.00	7.4
3	7.1	0.93	7.2	0.96	7.4	0.99	7.5	0.96	7.3
4	7.5	0.97	7.3	0.99	7.5	0.99	7.5	0.99	7.5
5	6.9	0.88	7.2	0.95	7.1	0.96	7.3	0.94	7.1
Mean	7.2		7.3		7.3		7.4		
Organic Carbon—OC (g C kg^{-1} d.m. soil)									
1	20.0	1.01	16.3	1.03	21.7	1.00	29.9	1.01	21.9
2	35.1	1.02	27.8	1.02	40.4	0.99	25.9	0.98	32.3
3	38.9	1.02	36.9	1.03	30.8	0.99	36.5	0.98	35.8
4	29.5	1.06	27.6	1.05	32.4	1.02	23.8	1.02	23.3
5	40.4	1.03	39.4	1.03	22.1	1.01	34.5	1.01	34.1
Mean	32.9		29.6		29.5		30.1		

Table 5. *Cont.*

Level of Soil Management (A)	Level of Organic Input (B)								Mean
	1		2		3		4		
	Content	W_z	Content	W_z	Content	W_z	Content	W_z	
	Total Nitrogen—TN (g N kg^{-1} d.m. soil)								
1	1.59	1.05	1.35	1.04	1.53	1.03	2.12	1.01	1.65
2	2.26	0.94	2.04	1.03	2.60	0.97	2.07	1.04	2.24
3	2.56	1.04	2.46	1.04	2.25	0.93	2.39	0.98	2.42
4	1.54	0.86	1.38	0.93	2.29	0.89	1.74	1.10	1.73
5	2.67	0.99	2.71	1.10	1.86	1.22	2.37	1.04	2.40
Mean	2.12		1.99		2.11		2.14		
	Phosphorus—Pa (mg P kg^{-1} d.m. soil)								
1	172.7	0.99	173.5	1.00	82.8	1.01	79.8	0.97	127.2
2	217.1	0.98	218.4	0.98	99.4	0.97	99.4	0.95	158.6
3	176.1	0.95	182.3	0.98	173.5	0.96	173.9	0.95	176.5
4	248.5	1.41	239.8	1.36	233.3	1.22	239.8	1.25	240.3
5	194.0	1.01	196.2	1.02	202.7	0.99	198.4	0.97	197.8
	201.7		202.0		158.3		158.3		
	Potassium—Ka (mg K kg^{-1} d.m. soil)								
1	220.8	1.18	223.4	1.16	224.8	1.06	225.8	1.07	223.7
2	236.6	1.14	214.9	1.13	218.3	1.02	220.6	1.06	222.6
3	218.9	1.16	218.5	1.17	184.3	1.04	188.3	1.05	202.5
4	307.8	1.49	306.2	1.49	260.8	1.33	261.3	1.33	284.0
5	233.8	1.17	232.5	1.17	212.1	1.04	211.4	1.06	222.5
Mean	243.6		239.1		220.1		221.5		
	Magnesium—Mg (mg Mg kg^{-1} d.m. soil)								
1	28.3	0.84	28.1	0.83	55.3	0.91	55.3	0.92	41.7
2	27.5	0.86	27.1	0.85	40.7	0.89	41.3	0.89	34.2
3	37.9	0.90	37.9	0.90	26.4	0.87	37.1	0.83	34.8
4	57.0	1.03	58.1	1.05	42.3	1.03	38.4	1.01	48.9
5	39.4	0.88	31.2	0.86	21.6	0.87	34.6	0.87	31.7
Mean	38.0		36.5		37.3		41.3		

The OC content in the studied soils ranged from 16.3 to 40.4 g OC kg^{-1} d.m. soil. The relative change index with values > 1 suggests that the accumulation of OC in the soil was favored by the introduction of organic biomass, i.e., manure, straw, and intercrop. Values < 1, demonstrating a decrease in OC, were observed only in the experimental plots of stubble management combined with straw removal (Table 5). The mean total nitrogen content in all cultivation plots ranged from 1.35 to 2.71 g N kg^{-1} d.m. soil. When thickening was applied in stubble cultivation and pre-sowing (A1), the values of the index of the relative change in nitrogen content exceeded 1, regardless of the method of stubble management (Table 5).

The content of available phosphorus in the soils tested was particularly high after using manure (B4) and reached 248.5 mg P kg^{-1}. Fertilization with manure also caused the highest increases in the amount of this element in the soil, as evidenced by the values of the relative change index reaching 1.41 (Table 5). The higher content of this component in the soil was favored by the annual application of the effective microorganisms (EM) vaccine, independent of straw usage, during post-harvest cultivation. As for using only a grubber or coarse and traditional plow tillage (A1, A2), a significant increase in the content of available phosphorus was found in the plots fertilized annually with shredded straw.

The content of assimilable potassium was also the highest in the soil of the experimental plots fertilized with manure and those in which shredded straw was left. Its content on these plots exceeded 300 mg K kg^{-1} (Table 5). Annual fertilization with manure also increased the content of available magnesium forms in the soil during the winter wheat monoculture to the value of 57–58 mg kg^{-1}. Only in this cultivation plot (B4) did the values of the relative change index in Mg content exceed 1 (Table 5).

Limiting tillage, especially its lack and the use of direct sowing (A5), reduced the mean content of mineral nitrogen in the soil. This effect was found both in spring and autumn, when the average content of mineral forms of nitrogen in the soil was 23.6 and 33.7 mg N kg^{-1}, respectively. Significantly higher values exceeding 50 mg N kg^{-1} were reported after conventional tillage systems combined with manure introduction into the soil (A4) (Table 6).

Table 6. Content of N-NH$_4$, N-NO$_3$ and TMT total mineral nitrogen (mg kg^{-1}) in the soil depending on the methods of tillage (factor A) and stubble management (factor B) under winter wheat monoculture in autumn and spring.

Levels of Soil Management (A)	Levels of Organic Input (B)									
	1	2	3	4	Mean	1	2	3	4	Mean
	Autumn					Spring				
	N-NH$_4$ [mg kg^{-1}]									
1	15.4	12.7	17.6	18.8	16.1	12.9	16.9	15.0	11.1	13.9
2	18.6	11.1	19.1	19.3	17.0	19.1	19.2	19.3	10.2	16.9
3	19.6	18.0	23.2	24.1	21.2	21.4	25.5	18.2	17.0	20.5
4	24.6	17.9	25.5	26.8	23.7	24.1	25.4	22.6	16.5	22.1
5	11.3	10.7	17.0	11.8	12.7	11.9	11.7	12.3	10.8	11.7
Mean	17.9	14.1	20.5	20.2		17.9	19.7	17.5	13.1	
	N-NO$_3$ [mg kg^{-1}]									
1	24.8	16.7	26.4	31.5	24.9	28.2	29.2	26.7	24.3	27.1
2	26.0	19.9	30.2	31.0	26.8	28.2	30.0	26.9	26.2	27.8
3	24.3	14.2	29.8	41.3	27.4	28.7	31.2	28.2	20.7	27.2
4	30.5	32.5	30.5	47.1	35.1	32.2	29.4	28.2	26.1	28.9
5	17.3	19.2	24.4	23.2	21.0	8.0	16.7	14.7	8.1	11.9
Mean	24.6	20.5	28.3	34.8		25.1	27.3	24.9	21.1	
	TMN									
1	40.2 ^{ab}	29.4 ^{b}	44.0 ^{a}	50.2 ^{bc}	40.9 ^{bc}	41.1 ^{c}	46.2 ^{b}	41.7 ^{b}	35.4 ^{ab}	41.1 ^{c}
2	44.5 ^{a}	31.0 ^{b}	49.3 ^{a}	50.3 ^{bc}	43.8 ^{b}	47.3 ^{bc}	49.2 ^{ab}	46.2 ^{ab}	29.6 ^{b}	43.1 ^{c}
3	43.9 ^{ab}	32.2 ^{b}	53.0 ^{a}	65.4 ^{ab}	48.6 ^{b}	50.0 ^{ab}	56.7 ^{a}	46.4 ^{ab}	37.7 ^{ab}	47.7 ^{b}
4	55.1 ^{a}	50.4 ^{a}	55.9 ^{a}	73.9 ^{a}	58.8 ^{a}	56.3 ^{a}	54.8 ^{a}	50.8 ^{a}	42.6 ^{a}	51.1 ^{a}
5	28.6 ^{b}	29.9 ^{b}	41.4 ^{a}	35.0 ^{c}	33.7 ^{c}	19.9 ^{d}	28.4 ^{c}	27.0 ^{c}	18.9 ^{c}	23.6 ^{d}
Mean	42.5 $_{B}$	34.6 $_{BC}$	48.7 $_{AB}$	55.0 $_{A}$		42.9 $_{B}$	47.0 $_{A}$	42.4 $_{B}$	32.8 $_{C}$	
	LSD for: Factor A = 9.2; Factor B = 8.5; Interaction A/B = 15.5; B/A = 14.7					LSD for: Factor A = 2.8; Factor B = 3.6 Interaction A/B = 8.1 B/A = 8.2;				

$^{a–d}$—letters in columns indicate significant differences at $p < 0.05$. $^{A–C}$—letters in row indicate significant differences at $p < 0.05$.

The analysis of individual forms of mineral nitrogen in the soil, N-NH4^{+} and N-NO$_{3,}$ confirmed their low content in samples from experimental plots where only direct sowing (A5) was used, mainly the nitrate form in spring. The average content of mineral nitrogen in the soils where straw was introduced as an element of stubble management was lower in autumn, particularly with the ammonium form. In spring, these differences were less significant. The positive effect of fertilization with manure on the content of the nitrate form of mineral nitrogen was also found, mainly in the autumn period (Table 6).

The content of all forms of mineral nitrogen in the soil in autumn ranged from 28.6 to 73.9 mg N kg^{-1} d.m. soil. Its amount was significantly higher in the soils from experimental plots where manure (A4) was applied and shredded straw (B1, B2) was left than in the other cultivation variants. In spring, significantly lower values were found in plots with direct sowing (A5). They ranged from 18.9 to 28.4 mg N kg^{-1} d.m. soil. The addition of organic matter in the form of manure increased the average content of mineral nitrogen in the soil in the spring to the significantly highest value of 51.1 mg N kg^{-1} (Table 6). The percentage difference among treatments including conventional tillage combined with (A4) and without manure application (A2) was 15.7%.

The PCA method was used to explain the differentiation of the soil in terms of selected parameters (TNM, B, A, F, SR, pH, OC, Pa, Ka, Mg, TN, N-NO_3 N-NH_4) based on two main components. The first two major components, PC1 and PC2, account for 67.84% of the total variance (Figure 1). The first component (PC1) showed 48.65% of the total variance and was negatively related to TNM (−0.964), B (−0.962), A (−0.926), F (−0.702), SR (−0.719), Pa (−0.616), and Ka (−0.932). PC2 discriminated 19.19% of the total variance and was negatively associated with pH (−0.719), N-NO_3 (−0.821), N-NH_4 (−0.606). Most of the parameters studied were grouped on the side of PC1 that can, therefore, be identified with the anthropogenic impact on the soil.

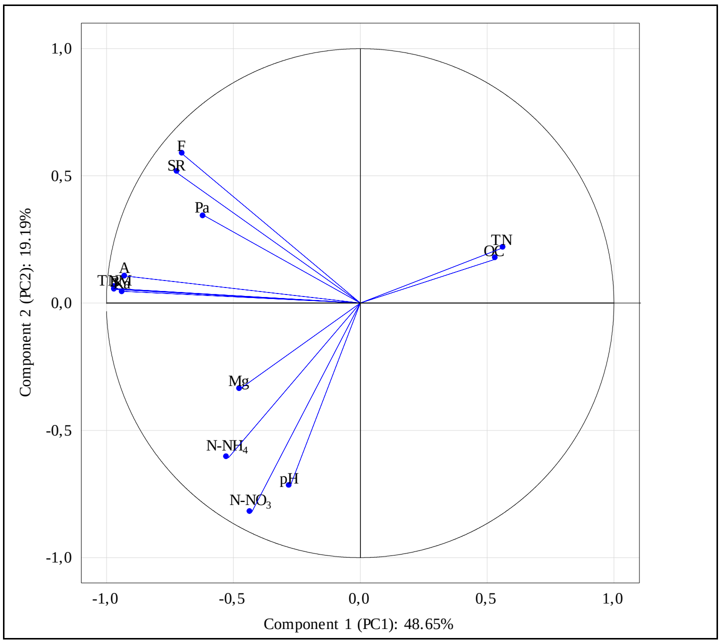

Figure 1. PCA analysis (principal component analysis PC1, PC2) of soil parameters: total number of microorganisms (TNM), bacteria (B), actinobacteria(A) fungi (F); soil respiratory activity (SR); pH in 1 M KCl (pH); organic carbon content (OC); content of available phosphorus (Pa), potassium (Ka), magnesium (Mg); content of total nitrogen (TN), mineral nitrogen forms: N-NO_3 and N-NH_4.

Due to the use of PCA, it was possible to investigate the significance of mutual correlations between the values of individual parameters. The number of microorganisms (TNM) was significantly correlated with the content of available Mg (r = 0.482), Pa (r = 0.630), Ka (r = 0.916), and N-NH_4 (r = 0.453). On the other hand, soil pH was correlated with N-NO_3 (r = 0.642) and N-NH_4 (r = 0.532) (Figure 1).

The dendrogram was created based on the cluster analysis and Euclidean distances between the traits analyzed and on the method of their agglomeration according to Ward (Figure 2). As shown in the dendrogram, four clusters could be distinguished that corroborate with the studied chemical and biological properties of the soil. The highest similarity was found between the plots A1B1, A1B2, A3B1, and A3B2 (cluster 1). These plots are characterized by lower values of microbiological and most chemical parameters. Cluster 2 brings together the plots A2B1, A2B2, A5B3, and A5B4, in which the lowest content of mineral nitrogen in the soil was found. These two clusters are followed by A1B3, A1B4, A2B3, A2B4 (cluster 3). Cluster 4 covers all experimental plots with a manure-based cultivation system with stubble cultivation and sowing (A4B1, A4B2, A4B3, A4B4). In these four plots, the highest number of bacteria, actinomycetes, and fungi, the intensity of respiration, and the content of assimilable forms of nutrients were reported. The cluster that is covering them has the lowest similarity with the others.

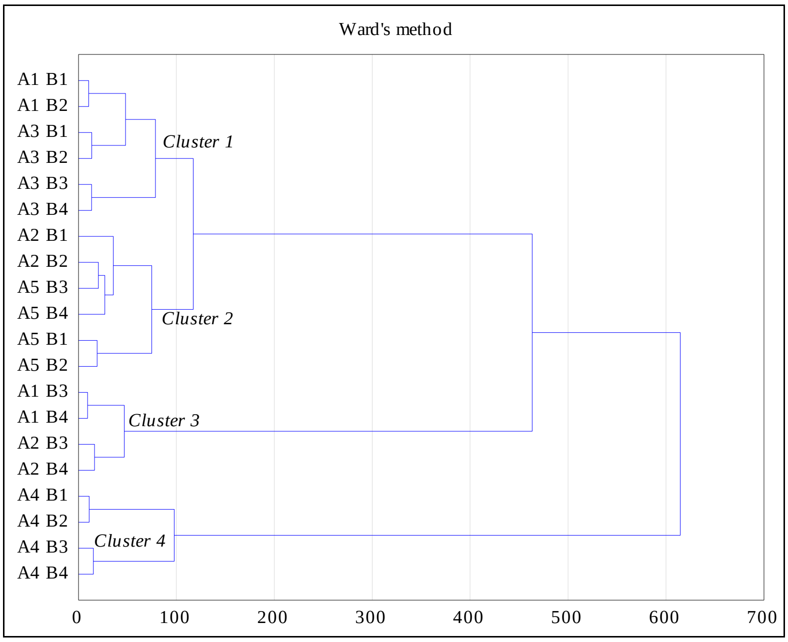

Figure 2. Dendrogram of tillage methods: A1—post-harvest: grubber with a roll; pre-sowing: grubber + seeder-cultivator unit, A2—post-harvest: grubber with a roll; sow ploughing + seeder-cultivator unit, A3—single ploughing + seeder-cultivator unit, A4—post-harvest: manure + grubber with a roll; pre-sowing: sow ploughing + seeder-cultivator unit, A5—direct sowing, and stubble managements: B1—leaving the shredded straw, B2—leaving the shredded straw + EM, B3—removing the straw + EM in the stubble, B4—moving the straw.

4. Discussion

Simplification of the soil cultivation system is done to obtain beneficial effects, i.e., the improvement of its structure, protection against erosion and excessive evaporation, or the increasing of the OC content [29–32]. Agrotechnical treatments based on minimal soil disturbance (zero or no plowing cultivation) also have a confirmed positive impact on the level of CO_2 emissions in the atmosphere, which is of particular importance in the context of the growing ecological threats that humanity is currently struggling with [33]. While the expectations related to the possibility of carbon sequestration in soil and the relationship between the efficiency of this process and the cultivation method seem overestimated and are increasingly being questioned, the issue of optimizing carbon management during simplified tillage remains essential [6].

Organic carbon (OC) is key to the functioning of the soil ecosystem, influencing water content, nutrient availability, and the biological activity of microorganisms [34,35]. Modifications to agricultural practices regulate the quantity and quality of OC, which affects the nutrient delivery and, ultimately, the yield level of crops [36]. In the study by Ozpinar and Cay [37], the organic carbon content in the soil subjected to simplified tillage was 11.5 g OC kg^{-1}, while the total nitrogen concentration reached 0.11 g N kg^{-1}. These values were higher than in the conventional system (8.8 g OC kg^{-1} and 0.008 g N kg^{-1}, respectively). The probable cause of this phenomenon could be the slow oxidation of organic matter and the decomposition of crop residues in the soil subjected to simplified cultivation [37]. In the present study, the highest contents of organic carbon (40.4 g OC kg^{-1}) and total nitrogen (2.71 g N kg^{-1}) were found in the soil of the experimental plots where the cultivation system with direct sowing of winter wheat was used (A5). The lowest values of these parameters (16.3 g OC kg^{-1} and 1.35 g N kg^{-1}, respectively) were reported with no-plowing cultivation, during which the field was grubbed both pre-sowing and post-harvest (A1) (Table 5). The PCA analysis also showed a correlation between the content of OC and N in the studied soils (Figure 1). In the study by Krauss et al. [38], the 15-year-long simplified cultivation increased the OC content by 25% compared to the conventional system. On the other hand, Koch and Stockfisch [39] reported that a single plowing operation performed after several years of conservation tillage was enough to confirm the reduction of organic matter content in the soil within a few weeks after its implementation.

The reduced mineralization rate of organic matter in the topsoil, accompanying the simplified methods of cultivation, results from the incorporation of organic carbon into soil macroaggregates that limit the access of microorganisms performing the oxidation processes [9,40]. However, the association between soil organic carbon and microbial activity is much more complex. The results of several studies have shown that microbial residues play an important role in the formation of soil organic matter (SOM), and the carbon content from dead microbes may account for up to 59% of the total soil carbon in agricultural systems [41–43]. According to Jaskulska et al. [7], replacing plowing with loosening treatments for eight years of simplified tillage resulted in increased activity of soil microorganisms and in the number of bacteria and fungi by 17.3% and 45.1%, respectively, compared to conventional tillage. In the present study, the least number microorganisms were isolated from the soil after the use of single-grain plowing (A3), while the highest total number of microorganisms, exceeding the value of 10^8 cfu g^{-1}, was reported in the system based on two-year manure application (A4) and when no cultivation system was used with direct sowing (A5) (Table 2). The high number of microorganisms in these cases was undoubtedly associated with the additional augmentation of the soil with organic matter in the form of manure that had a significant impact not only on the microbiological parameters of the soil. According to the dendrogram, all plots with the manure-based farming systems (A4B1, A4B2, A4B3, A4B4) are included in a single cluster (4) characterized by the lowest similarity with the other clusters (Figure 2).

The activity of soil microorganisms is, however, closely related not only to the content of organic carbon, but also to other biological and chemical soil parameters. According to PCA analysis, the total number of microorganisms was significantly correlated with the content of available Mg, Pa, Ka, and $N\text{-}NH_4$ (Figure 1). Soil bacteria, including actinomycetes and fungi, affect the rate of decomposition of organic matter and the circulation of nutrients. The effect of microorganisms on the transformation and circulation of carbon, nitrogen, phosphorus, potassium, sulfur and iron is the mineralization of organic compounds or the transformation of forms that are not available to plants into available compounds [44–46].

Due to the high sensitivity to changes in applied soil cultivation systems, soil microbial activity, and community composition are considered useful indicators of soil quality and health [47]. In the present study the phytosanitary index value, considered as an indicator of soil sanitary condition, was the lowest in the soil enriched with manure, suggesting beneficial effect of organic fertilization on fungal community (Table 3). Rousk and Bååth [48] reported a positive correlation between N addition and fungal growth, while bacterial growth was inhibited.

The most intensive respiration activity was also reported in the experimental plots where manure and plow cultivation were used. Moreover, higher amounts of available forms of phosphorus, potassium, magnesium, and mineral nitrogen were found than in most other cultivation methods (Tables 5 and 6). A similar effect of fertilization with manure on the content of nutrients in the soil has been confirmed in several other studies. Han et al. [49] found that it significantly increased the content of organic carbon, nitrogen, available phosphorus, exchangeable potassium, and magnesium in the soil. The increased content of nitrogen, phosphorus, and potassium as a result of manure fertilization was also observed by Kaur et al. [50]. As shown in the dendrogram, all experimental plots with a manure application formed one cluster (4) that had the lowest similarity with the others. In this variant (A4), the highest number of soil microorganisms, as well as the respiration intensity and the content of nutrients, was revealed (Figure 2). Due to the high number of indigenous microorganisms, manure application may result in a temporary increase of soil microbial population. The survival of manure-borne bacteria, however, depends on various factors including soil, physical, and chemical properties. Competition between manure and soil bacteria also plays a crucial role in the survival and persistence of manure bacteria in the soil environment [51–53].

Another type of organic matter that can affect the most important parameters of arable soils is straw. The presence or absence of a relationship between leaving the shredded straw in the field and the number of soil microorganisms, as well as the content of assimilable forms of the most important elements in the soil, was reported in the present study as part of the evaluation of various methods of stubble management. Leaving the straw increased the number of microorganisms and the respiration activity, and decreased the soil pH (Tables 2, 4 and 5). While this practice increased the organic carbon content in the present study, which is confirmed by the values of the relative change index, its impact on the amount of total nitrogen was less significant. An over two-fold higher content of available forms of phosphorus in the soil, exceeding 200 mg P kg^{-1}, was found when, together with leaving the straw, pre-sowing (A1) or sowing plowing (A2) was also performed. According to Akhtar et al. [54], the application of wheat straw increases the content of organic carbon, nitrogen, and phosphorus in the soil, with a clear positive correlation between the dose of straw and the value of these parameters. On the other hand, in the study by Thomsen and Christensen [55], 12, 21, and 30% more carbon (C) were found in the soil enriched for 18 years with straw at the doses of 4, 8, and 12 t ha^{-1} year^{-1}, respectively, than in the soil without the addition of this material. Bearing in mind the deficiencies of organic matter common in arable soils, the agrotechnical use of straw from crops can be its most valuable source, especially when considering the huge scale of straw production by global agriculture [6,56].

To improve the properties of arable soils, various types of adjuvants are introduced, apart from traditional organic materials. They contain biological agents or chemicals that stimulate selected soil functions. It was reported that effective microorganisms (EM) and biopreparations positively affect the yield and quality of plants; however, scientists' opinions on their effectiveness are divergent [19,57–59]. In the present study, a nonsignificant increase in the number of microorganisms in the soil to which EM was introduced was found compared to the stubble without the application of this preparation. This phenomenon was reported in most of the experimental plots, regardless of the straw management method, with statistically significant differences occurring only with the no-till direct sowing system (Table 2). As for the changes in the content of the tested chemical components in the soil, the differences were minor or inconclusive (Tables 5 and 6). Szymanek et al. [60] suggest that the concentration of phosphorus, potassium, and magnesium in the soil treated with effective microorganisms was reduced by as much as 26%, although these changes were not statistically significant. On the other hand, research by Hu and Qi [19] suggests that the addition of EM may positively affect the nutrient availability from organic materials introduced into the soil.

5. Conclusions

The diversification of agricultural practice in the short term (two years) cultivation of winter wheat monoculture has modified the basic biological and chemical properties of soil in various ways. The differences observed among various tillage procedures applied in the study suggest, however, that the addition of organic matter is the more important factor affecting the values of the soil's biological and chemical parameters than the type of cultivation system used. The results of the present study indicate a beneficial effect of the non-cultivating system with direct sowing on the retention of organic carbon, especially if leaving chopped straw from the forecrop. On the other hand, tillage without plowing caused a decrease in the content of mineral nitrogen in the soil. Possible negative outcomes resulting from the simplified cultivation system should be compensated by introducing optimizing treatments. Manure fertilization is one of the most rational methods recommended in the event of potential soil nutrient deficiencies. In the present study, the traditional cultivation system using this fertilizer was found to be one of the most effective in stimulating the microbiological activity in the soil and increasing the content of assimilable forms of basic nutrients. As indicated by the multidimensional analysis, the number of microorganisms and the content of assimilable forms of phosphorus, potassium, and magnesium were positively correlated with each other. Despite the fact that the OC content in the soil in the cultivation system with manure application was not high, the indicators enabling a comprehensive assessment of its long-term accumulation suggest that this experimental plot resulted in the highest relative increase in the OC content throughout the entire experiment. As confirmed by previously published results, the use of this cultivation system was also optimal in terms of winter wheat yield [20]. The beneficial effect of manure addition was enhanced if the chopped straw was left in the field, which confirms the importance of introducing additional sources of organic matter into the soil as an intervention measure regulating the levels of key soil parameters.

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