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Original Research

The Effect of Soil Tillage and Nitrogen Fertilization on Microbiological Parameters of Soil on which Spring Triticale is Grown

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Abstract

In a field experiment conducted on leached brown soil in Malice, near Zamość (southeastern Poland) in 2007-08, the effect of soil tillage and nitrogen fertilization on microbiological parameters of the soil under spring Triticale cultivation was investigated. Soil tillage – conventional and simplified with double or single cultivation – was combined with different variants of nitrogen fertilization – 60, 90, and 120 kg N·ha^{-1}. The soil was sampled three times – I - end of tillering stage (BBCH 29), II - end of heading stage (BBCH 59) and III – late milk stage (BBCH 77) – and its microbiological properties were determined.

Generally, the simplified soil tillage and nitrogen fertilization up to 90 kg N·ha^{-1} favors bacteria, *Actinomycetes,* and fungi content, as well as dehydrogenase activity. The conventional soil tillage and nitrogen fertilization at a level of 120 kg·ha^{-1} reduced the number of microflora. The highest numbers of micro-organisms in the soil were noted at the stage (BBCH 59) of the spring Triticale, but dehydrogenase activity was highest at that stage (BBCH 29). In formulating the biological index of soil fertility (BISF), the biological activity of the soil (M), organic carbon content (H) and soil absorbing capacity (T) were taken into account. Biological activity of the soil (M) was expressed as numbers of micro-organisms, or as dehydrogenase activity. The BISF, calculated based on dehydrogenase activity, is significantly dependent on the all factors investigated. However, BISF calculated as the sum of the numbers of bacteria and *Actinomycetes* in relation to fungi (B+A/F) was positively correlated with grain yield. Grain yield was highest following conventional soil tillage and nitrogen fertilization at a level of 60 kg N·ha^{-1}.

Keywords: spring Triticale, soil tillage, nitrogen fertilization, microbiological parameters of the soil

Introduction

In natural soils a favorable soil structure is formed by the interaction of plant roots, earthworms, and micro-organisms [1, 2]. Cultivation, fertilization, protection, and contamination of soil modify its physico-chemical properties and change its biological activity. A measure of biological activity comprising all compound and energy conversions may be enzymatic activity and the dynamics of the development of select groups of micro-organisms living in the soil.

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It depends on type of soil, the depth of the soil profile, vegetation cover, atmospheric conditions, cultivation regime and fertilization, and many other factors affecting soil [3]. Factors affecting the activity of micro-organisms in soil and their quality and quantity include content of organic substances, nitrogen compounds, macro- and micro-elements, water, and oxygen, as well as soil pH and temperature [4, 5]. Soil organisms mobilize many main and trace nutrients (particularly N) for the plant through their conversion processes alone, and are involved in particular in the nutrient cycles of carbon, nitrogen, phosphorus, and sulfur [6].

Depth, granulation and structure, soil reaction, nutrient content, (including harmful materials), humus content and composition, sorption, and microbial activity are important factors determining soil fertility [7-9].

A suitable indicator for the microbial activity of the soil is dehydrogenase activity [10-12]. Dehydrogenases are catalytically involved in various redox reactions and their activity reflects the activity of the entire microbial population. Dehydrogenase activity is usually correlated with the organic carbon content in the soil as well as with the total nitrogen content.

Only a few studies have found a direct relationship between biological soil activity and crop yield. Wyczółkowski et al. [13] argue that the number of bacteria in the soil depends on the plant and its stage of growth. Tys-Jezierska et al. [14] showed that the dehydrogenase activity of the soil was significantly affected by the cultivar and line of winter wheat. Furczak [15] argues that growing soybeans in an 8-year-old monoculture interferes to some degree with the biochemical activity of soil. A similar phenomenon was observed by Kucharski et al. [16]. Cultivation and nitrogen fertilization positively influence microbial activity in the soil, which in turn positively affects yield. The present study examined the influence of different methods of soil tillage and nitrogen fertilization on microbial activity in the soil and the yield of spring Triticale.

Materials and Methods

The subject of our research was the Wanad variety of spring Triticale. A two-factorial experiment was conducted in 2007-08, in Malice (a village near Zamość, Poland; 50º42' N, 23º15' E), using the split-plot method in four replications. The experiment was carried out on leached brown soil formed from light loamy silty soil. Soil properties, identified by the methods described by Mocek et al. [17] and Ostrowska et. al. [18] directly before the experiment, are given in Table 1.

Thirty-six plots were marked off with an area of 30 m^2 each. The area of the plots put into harvest was 19.5 m^2 (3 m × 6.5 m). The forecrop of the spring Triticale was potatoes, fertilized with 30 $t \cdot ha^{-1}$ of cattle manure. After harvesting potatoes we carried out 3 doses of nitrogen fertilization (1N – 60, 1.5 N – 90, 2N – 120 kg $N \cdot ha^{-1}$ – as ammonium nitrate) and 3 different methods of soil tillage. Nitrogen fertilization was used three times:

1st dose – 1/3 before sowing
2nd dose – 1/3 in the tillering stage (BBCH 20-29)
3rd dose – 1/3 in the stem elongation stage (BBCH 30/31).

Phosphorus-potassium fertilization depended on the abundance of plant-available forms and was 90 kg P_2O_5 (as mineral superphosphate) and 100 kg K_2O ha^{-1} (as potassium sulphate).

Methods of soil tillage were as follows:

1. (CST) – conventional soil tillage: medium ploughing in autumn (20 cm), harrowing (5 cm), and cultivation (10 cm). In spring: PK fertilization, harrowing (5 cm), sowing, and harrowing (5 cm).
2. (DST) – simplified soil tillage, with double cultivation in autumn (10 cm). In spring: cultivation (10 cm), PK fertilization, harrowing (5 cm), sowing, and harrowing (5 cm).
3. (SST) simplified soil tillage, with simple cultivation (10 cm) – without tillage in autumn. In spring: cultivation (10 cm), PK fertilization, harrowing (5 cm), sowing, and harrowing (5 cm).

Spring Triticale was sown in the first 10 days of April, at a density of 550 grains m^2. Grains before sowing dressed of Vitavax 200 FS (a.i. carboxin + tiuram) in quantities of 300 ml 100 kg^{-1}. To eliminate mono- and dicotyledonous weeds a mixture of herbicides was used: Granstar 75 WG (75% of tribenuron methyl) (205 $g \cdot ha^{-1}$) and the Puma Super 069 EW (fenoxaprop-P-ethyl) (1 $L \cdot ha^{-1}$) at the tillering stage (BBCH 28). At the stem elongation stage (BBCH 30-32) the growth regulator Terpal C 420 SL (2-chloroethulphosphonic + mepiqat chloride) was applied at a level of 2 $L \cdot ha^{-1}$. The occurrence of foot and root rot diseases was reduced at the stem elongation phase (BBCH 30-32) with Alert 375 SC (flusilazole + carbendazim) at a level of 1.0 $L \cdot ha^{-1}$, while in BBCH 58-59 Tilt CB 37.5 (propiconazole + carbendazim) at 1 $L \cdot ha^{-1}$ was used to prevent leaf and head diseases. Pesticides were applied with a 12-m-wide tractor-mounted sprayer that delivered 200 $L \cdot ha^{-1}$ spray solution through 80-02 flat fan nozzles (model PILMET – P-412, Poland) at a spray pressure of 200 kPa.

Total precipitation in both tests (IV-VIII) were higher than the average sums of long-term period (1971-2005: 341.2 mm): 26.0 mm in 2007 and a 128.2 mm in 2008. In 2007 the highest precipitation was recorded in July, while in 2008 generally in all months (with the exception of June) rainfall exceeded the average long-term period sums. Total air temperatures in growing seasons that were higher than the sum of the years (1971-2005: 2304ºC). And so, in the 2007 season it is 365ºC, and in 2008 – 317ºC. Generally, each month that year the air temperature exceeded the average temperature in the long-term period. It was noted, however, that the distribution of precipitation and temperature significantly affects the differentiation of phases of development of spring Triticale. However, a weather indicator changed significantly the population of micro-organisms and the average yield of plants, as described later in the work. Table 2 shows the precipitation and temperature. Sielianinow's hydrothermal factor was calculated according to the formula:

Table 1. Physical and chemical properties before the experiment (Malice 2007).

Specifications	Unit	Depth of soil (cm)		
		0-25	25-70	70-150
		Layer		
		A/A_3	B(B)	C
1. Granulometric composition				
1.0.1 mm	%	58	64	64
0.1-0.02 mm		29	24	20
<-0.02 mm		13	12	16
2. Soil color		grey-brown	brown	yellow
3. Structure		fine- crumb	prismatical	prismatical
4. Solid phase density	$Mg \cdot m^{-3}$	2.63	2.65	2.67
5. Soil bulk density	$Mg \cdot m^{-3}$	1.38	1.43	1.59
6. Soil moisture	%	23.6	22.1	20.3
7. Porosity	%	37.6	37.6	32.3
9. Soil compaction	kPa	334	808	-
10. Hydrolitic acidity	$cmol(H) \cdot kg^{-1}$	19.0	9.7	10.5
11. Sum of exchangeable bases	$cmol(+) \cdot kg^{-1}$	26.0	10.6	120.0
12. Soil absorbing capacity	$cmol(+) \cdot kg^{-1}$	45.0	20.3	130.5
13. Saturation with cations	%	57.8	52.1	91.9
14. pH in H_2O		6.8	7.1	7.0
15. pH in 1M $CaCl_2$		5.6	6.1	6.0
16. C-organic	$g \cdot kg^{-1}$	10.2	9.0	2.5
17. $CaCO_3$	%	<1	<1	<1
18. N-total	$g \cdot kg^{-1}$	0.9	0.7	0.3
19. P-available	$mg \cdot kg^{-1}$	65.5	35.4	35.1
20. K-available	$mg \cdot kg^{-1}$	96.2	76.0	83.1
21. Mg-available	$mg \cdot kg^{-1}$	37.1	22.6	67.2
22. S-SO_4^{2-}-available	$mg \cdot kg^{-1}$	14.2	15.8	17.2

$$k = \frac{p \times 10}{\sum t} \quad (1)$$

...where:

p – the sum of monthly precipitation (mm),

$\sum t$ – the sum of the mean daily air temperatures of the month (ºC).

On the basis of the model were thus hydrothermal factors for the growing season of spring Triticale (IV-VIII). Individual seasons for growing spring Triticale are defined as: 2007 – dry (1.0), 2008 – quite humid (1.4). The long-term period was defined as quite humid (1.5).

For the soil-micro-biological investigations in 2007 and 2008 in three terms (I – end of tillering phase (BBCH 29), II – end of heading phase (BBCH 59), and III – late milk phase (BBCH 77)) soil samples in the scope of the roots of spring Triticale were taken from the arable layer (0-20 cm) of each plot (dates for phenological growth phases are given in Table 3). After mixing within each treatment, the soil material was dried and screened through sieves of 2 mm mesh size.

In the soil samples were determinate using the plate method on respective agar media (in five replications). The mean number of colonies was calculated as converted to 1 g^{-1} dry substance (DS) of soil.

- The total bacterial counts (B) in the soil extract (35% it soil extract with 0.05% K_2HPO_4; 0.1% glucose; 1.5% agar, pH = 7). The dilution of the soil extract amounted to 10^{-5} or 10^{-6} [19].

Table 2. Sums of rainfall (mm) and mean temperature (ºC) in the 2007-08 growing seasons and over a long-term period. Research Station in Zamość.

Sums of rainfall (mm)							
Year	IV	V	VI	VII	VIII	Sums IV-VIII	S*
2007	21.7	41.1	54.0	118.9	31.6	267.3	1.0
2008	71.5	74.8	48.9	104.6	69.7	369.5	1.4
Long-term period 1971-2005	44.1	65.5	78.9	98.4	54.3	341.2	1.5
Mean temperature (ºC)							
2007	10.0	17.6	19.8	21.1	18.6	2,669	-
2008	10.7	15.5	19.4	20.2	19.7	2,621	-
Long-term period 1971-2005	7.9	14.1	16.8	18.4	17.8	2,304	-

* Sielianinow's hydrothermal factor

Table 3. Dates of phenological growth phases (BBCH) of spring Triticale in the experiment.

Years	BBCH00	BBCH29	BBCH59	BBCH77	BBCH99
2007	8.04	24.05	11.06	4.08	19.08
2008	15.04	11.06	27.06	9.09	22.09

- *Actinomycetes* (A) in the soil extract (with 0.2% $(NH_4)_2SO_4$, 0.1% K_2HPO_4, 0.1% $MgSO_4$ ($7H_2O$, 0.1% NaCl, 0.3% $CaCO_3$, 1% glucose, 2% agar). The dilution of the soil extract amounted to 10^{-5} or 10^{-6} [19].
- Fungi (F) in the soil extract after Martin [20] (0.5% Pepton, 1% glucose, mineral salts, antibiotics: 0.2% Chlorotetracyclin and 0.003% Streptomycin; 0.0033% Bengalrosa, pH= 5.5). The dilution of the soil extract amounted to 10^{-3} or 10^{-4}.
- Determination of activity of dehydrogenases used the Thalmann method [21]. In 100 ml flask are affixed 5 g sieved soil and add 5 ml of 1% TTC (2, 3, 5-triphenyltertrazolinum chloride), then dissolved in Tris-HCl pH 7.4. These samples were incubated 24 hours at 30ºC. After incubation to flask add 20 ml of methanol and shake 5 minutes. The filtrate mark of spectophotometry was at a wavelength of 485 nm and it was expressed in [$cm^3 \cdot H_2 \cdot kg^{-1} \cdot 24\ h^{-1}$]. All data in this work refer to dry substance (DS) of soil.

On the basis of the chemical soil properties and soil-micro-biological analyses the so-called "Biological Factor of the Soil Fertility – BISF" after Myśków et al. [22] in the following way computes:

$$BISF = \sqrt{M^2 + H^2 + T^2 \times 100} \qquad (2)$$

...where:

M – Biological activity of the soil (*M*) was expressed as the number of bacteria and *Actinomycetes*, the number of bacteria and *Actinomycetes* in relation to that of fungi [$10^{-3} \cdot g^{-1}$ DS of soil], or as dehydrogenase activity [TPF $mg \cdot kg^{-1} \cdot d^{-1}$] (TPF – Triphenyl Formazon).

H – Content of organic carbon in the soil [$g \cdot kg^{-1}$ DS soil],

T – Soil absorbing capacity [cmol(+) kg^{-1} DS soil].

There the values for *M*, *H*, and *T* in different and not comparable units are present (see above), became the values in accordance with Myśków et al. [22] weights and standardizes (0 1). These values were then used for the calculation of the biological factor of soil fertility (BISF).

The analysis of variance carried out using the ANOVA was based on Tukey's HSD test at a significance level of $p < 0.05$ and $p < 0.01$ using the Statistica PL (Polish Edition, Statistica for Windows 1997 by StatSoft, Poland, Krakow sp. z o.o.). The correlation coefficients also were determined [23].

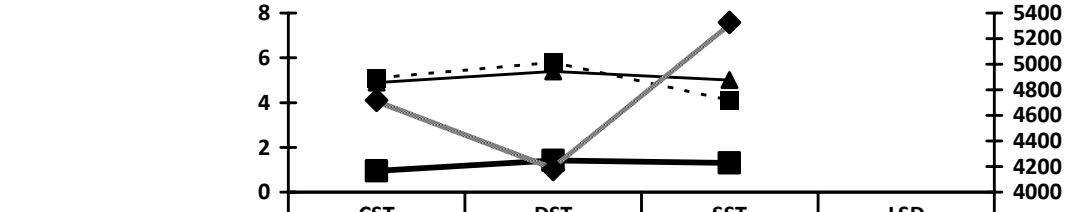
Results and Discussion

Tillage systems affect the physical and chemical environment in which soil organisms live, thereby affecting these organisms [24]. Tillage practices change soil water content, temperature, aeration, and the degree of mixing of crop residues within the soil matrix. These changes in the physical environment and the food supply of the organisms affect different groups of organisms in different ways. Soil organisms perform important functions in soil, including structure improvement, nutrient cycling, and organic matter decomposition. Although there is a wide range of responses among different species, most groups of organisms have greater abundance or biomass in no-till systems than in conventional tillage systems. Larger organisms in general appear to be more sensitive to tillage operations than smaller organisms, due to the physical disruption of the soil, burial of crop residue, and the change in soil water and tem-

perature resulting from residue incorporation. Variations in responses found in different studies reflect different magnitudes of tillage disruption and residue burial, timing of the tillage operations, timing of measurements, and different soil, crop, and climate combinations [24].

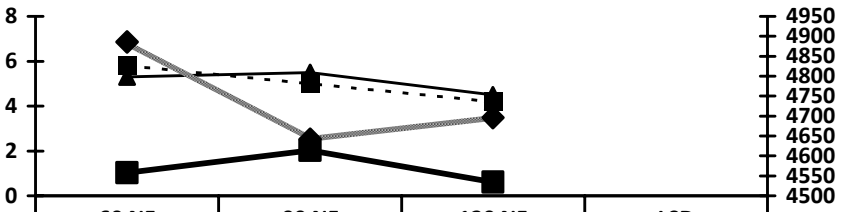
The different methods of soil tillage and nitrogen fertilization influenced the number of micro-organisms in the soil on which spring Triticale was grown. Simplified soil tillage with single cultivation (SST) significantly increased the number of bacteria – by about 610 CFU×10^{-3}·g^{-1} DS (11%) compared to conventional soil tillage (CST) and by about 1.150 CFU×10^{-3}·g^{-1} DS (22%) compared to simplified soil tillage with double cultivation (DST) (Fig. 1). The bacterial number did not depend directly on nitrogen fertilization (Fig. 2). Niewiadomska et al. [3] observed that lawn fertilization with nitrogen had no significant effect on bacteria cell numbers. The significantly highest number of bacteria in the soil [5.635 CFU×10^{-3}·g^{-1} DS] was noted in the case of the combination of simplified soil tillage with single cultivation (SST) with N fertilizer in the amount of 120 kg N·ha^{-1}, whereas only 67% of this number [3.797 CFU×10^{-3}·g^{-1} DS] was noted in the case of the simplified soil tillage with double cultivation (DST) with N fertilization at 90 kg N·ha^{-1}. The highest number of bacteria was observed in soil samples at the end of the heading stage of the spring Triticale (BBCH 59) [5.914 CFU×10^{-3}·g^{-1} DS], with only 75% of this number [3.856 CFU×10^{-3}·g^{-1} DS] found in the late milk phase (BBCH 77) and 24% [4.454 CFU×10^{-3}·g^{-1} DS] at the end of the tillering phase (BBCH 29) (Fig. 3). A significantly higher number of bacteria, by about 7%, was noted in 2007 over 2008 (Fig. 4). The diverse dynamics of microbiological alterations during the growing season were confirmed by Niewiadomska et al. [3] and Furczak and Turska [25]. The authors noted the highest microbial cell number and highest microbial activity in the spring.

Simplified soil tillage with double cultivation (CST) significantly increased the number of *Actinomycetes*, by about 7 CFU×10^{-3}·g^{-1} DS (12%) in relation to conventional soil tillage (CST) and by about 17 CFU×10^{-3}·g^{-1} DS (29%) in relation to simplified soil tillage with single cultivation (SST) (Fig. 1). The number of *Actinomycetes* depended directly on nitrogen fertilization. The highest number of *Actinomycetes* was found in the soil fertilized with 60 kg N·ha^{-1}, with substantially less (14 and 28%) noted at 90 and 120 kg N·ha^{-1} (Fig. 2). Niewiadomska et al. [3] determined that lawn fertilization with nitrogen had no effect on cell numbers of *Actinomycetes*. The authors, however, wrote that in fertile soils the number of *Actinomycetes* is usually lower in relation to bacteria (the ratio of bacteria to



	CST	DST	SST	LSD
Dehydrogenases	0,95	1,41	1,31	'0,1
Fungi	4,9	5,4	5	n.s.
Actinomycetes	5,1	5,8	4,1	'0,6
Bacteria	4718	4178	5328	'514

Fig. 1. Number of microorganisms [CFU×10^{-3}·g^{-1} DS] and dehydrogenase activity of soil (TPF) [cm^3·H$_2$·kg^{-1}·24 h^{-1}] depending on soil tillage: CST – conventional soil tillage, DST – simplified soil tillage with double cultivation, SST – simplified soil tillage with single cultivation.



	60 NF	90 NF	120 NF	LSD
Dehydrogenases	1,03	2,03	0,61	'0,1
Fungi	5,3	5,5	4,5	n.s.
Actinomycetes	5,8	5	4,2	'0,6
Bacteria	4886	4642	4696	n.s.

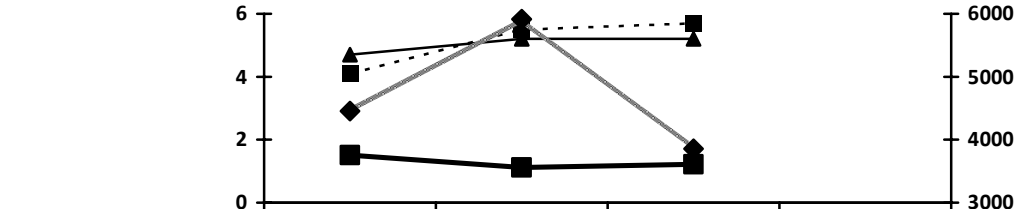
Fig. 2. Number of microorganisms [CFU×10^{-3}·g^{-1} DS] and dehydrogenase activity of soil (TPF) [cm^3·H$_2$·kg^{-1}·24 h^{-1}] depending on nitrogen fertilization (60, 90, 120 kg N·ha^{-1}).

Actinomycetes is 60:40). This phenomenon was not observed in the presented study. The significantly highest number of *Actinomycetes* [77 CFU×10^{-3}·g^{-1} DS] was noted in the combination of simplified soil tillage with double cultivation (DST) with 60 kg N·ha^{-1}, whereas only 30% of this number [29 CFU×10^{-3}·g^{-1} DS] was found in the combination of simplified soil tillage with single cultivation (SST) with N fertilization at 120 kg N·ha^{-1}. The highest numbers of *Actinomycetes* were observed in soil samples from the end of the heading stage (BBCH 59) and in the late milk stage (BBCH 77) [52 CFU×10^{-3}·g^{-1} DS] of the spring Triticale, with only 90% of this number [47 CFU×10^{-3}·g^{-1} DS] noted at the end of the tillering phase (BBCH 29) (Fig. 3). A significantly higher number of *Actinomycetes*, about 67%, was noted in 2007 over 2008 (Fig. 4).

Kaczmarek et al. [26] stated that *Actinomycetes* (which are counted among bacteria) are, after bacteria proper, the second most populous group of micro-organisms carrying out important transformations of complex carbon and nitrogen compounds in soil. *Actinomycetes* play a decisive role in numerous biochemical processes in the soil. Due to their capacity for distribution and transformation of various organic substances, they are an important factor in humus creation. One of the main factors affecting their numbers is the moisture content of the soil.

In addition to bacteria proper and *Actinomycetes*, fungi play a key role in nutrient and energy circulation in soil. There are many indicators that they are powerful agents of geochemical change. Due to their small size, the ratio of surface area to volume is very large, which allows the rapid exchange of substances between the cell and its environment. Equally important is their rapid reproduction. From the point of view of agriculture, due to their physiological capacity to accumulate water, produce organic acids, and release many nutrients from soil minerals, they play an important role in soil processes and plant nutrition. As saprophytes they carry out heavily overlapping processes of mineralization of organic matter, thus increasing the fertility of soils [26].

In the present study, no direct influence was observed of soil tillage, nitrogen fertilization, and phenological phases of spring Triticale on the number of fungi (Figs. 1 and 2). It was only noted that the highest level of nitrogen fertilization (120 kg·ha^{-1}) reduced the number of fungi by 17% in relation to 60 and 90 kg N·ha^{-1}. Significant differences were found in the interrelation between the phenological phases of spring Triticale and the year of the study. The highest number of fungi was noted in the soil samples taken in the late milk stage (BBCH 77) in 2008 [84 CFU×10^{-3}·g^{-1} DS], whereas only 36% of this number [30 CFU×10^{-3}·g^{-1} DS]



	BBCH29	BBCH59	BBCH77	LSD
Dehydrogenases	1,51	1,12	1,22	n.s.
Fungi	4,7	5,2	5,2	'0,5
Actinomycetes	4,1	5,5	5,7	n.s.
Bacteria	4454	5914	3856	'514

Fig. 3. Number of microorganisms [CFU×10^{-3}·g^{-1} DS] and dehydrogenase activity of soil (TPF) [cm^3·H_2·kg^{-1}·24 h^{-1}] depending on phenological growth phases of spring Triticale.

	2007	2008	LSD
Dehydrogenases	1,23	1,22	n.s.
Fungi	3,6	6,6	'0,4
Actinomycetes	4	6	'0,5
Bacteria	5158	4325	'419

Fig. 4. Number of microorganisms [CFU×10^{-3}·g^{-1} DS] and dehydrogenase activity of soil (TPF) [cm^3·H_2·kg^{-1}·24 h^{-1}] depending on the year of study.

was observed at this stage in 2007 (Fig. 3). Moreover, a significantly higher number of fungi, about 45%, was noted in 2008 than in 2007 (Fig. 4). Niewiadomska et al. [3] also observed that lawn fertilization with nitrogen had no significant effect on fungi cell numbers. Significant factors were sampling date and atmospheric conditions.

The correlation analysis showed no significant correlation between numbers of bacteria, *Actinomycetes,* and fungi.

Dehydrogenase activity in the soil is regarded as an indicator of the intensity of the respiratory metabolism of soil microbes, mainly bacteria proper and *Actinomycetes* [26]. Dehydrogenases, together with oxidases, reductases, and oxigenases, comprise the oxidoreductase class of enzymes, which are the largest group of enzymes [27]. Since many specific dehydrogenases are involved in biological substrate oxidation, the sum of their activity is often regarded as an indicator of biological redox processes, as well as a measure of the intensity of microbiological material conversions in the soil [28, 29]. In the present study, the highest dehydrogenase activity was noted in the soil where simplified soil tillage with double cultivation (DST) was used [1.41 TPF mg·kg^{-1}·day]; it was 8% less in the case of simplified soil tillage with single cultivation (SST), and 23% less in the case of conventional soil tillage (CST) (Fig. 1). In regard to nitrogen fertilization, the highest dehydrogenase activity was noted in the soil fertilized with 90 kg N·ha^{-1}; it was lower for fertilization with 60 kg N·ha^{-1} (50%) and 120 kg N·ha^{-1} (70%) (Fig. 2). The significantly highest dehydrogenase activity [2.80 TPF mg·kg^{-1}·day] was observed with the combination of simplified soil tillage with double cultivation (DST) and 90 kg N·ha^{-1}, compared to only 18% of this value [0.5 TPF mg·kg^{-1}·day] in the case of conventional soil tillage (CST) with nitrogen fertilization at 120 kg N·ha^{-1}. In regard to nitrogen fertilization, Acosta-Martinez and Tabatai [30] believe that organic fertilizers are more favourable to the overall biological activity of the soil than mineral fertilizers, which, while improving the physical-chemical properties of the soil, can have a negative effect on the activity of the enzyme. Kucharski [16] reports that in the case of mineral fertilization excessive amounts of nitrogen (240 kg·ha^{-1}) lead to a substantial reduction in dehydrogenase activity. Similarly, Natywa et al. [31] found that the greatest respiration activity in soil was in the spring, in the absence of nitrogen fertilization. Mysków et al. [22] also observed that the use of large amounts of nitrogen reduced dehydrogenase activity. The growth phase of spring Triticale and the year of the study were found to have no significant direct effect on dehydrogenase activity. It was only noted that the highest dehydrogenase activity in the soil was at the end of the tillering stage (BBCH 29) [1.51 TPF mg·kg^{-1}·day], while at other stages of growth it was on average 33% less (Fig. 3).

Siwik-Ziomek and Koper [32] argue that changes in the activity of enzymes in the growing season are probably due to changes in the content of substrates in the soil, as well as to differences in temperature and humidity. The authors observed an increase in activity in the spring. The high enzyme activity recorded in autumn, after the plants were harvested, may be linked to the long period of decomposition of organic matter in the soil, as crop residues constitute an ideal energy source for soil micro-organisms. Wielgosz and Szember [33] make a similar claim, noting the decline in dehydrogenase activity in the summer. The cause of this could be inhibition of the mineralization of organic matter, since at this time high temperatures were coupled with low moisture content in the soil due to inadequate rainfall. As Natywa et al. [31] observe, dehydrogenase activity is dependent on the physiological activity of micro-organisms. The activity of these enzymes is often used in the study of soil as an indicator of changes in microbial activity that occur under the influence of pesticides, heavy metals, industrial waste, and wastewater, and also in studies on the effect of fertilization, crop rotation, and the periodic flooding of soil on its status [34, 35].

In summary, it should be noted that simplified soil tillage with single cultivation (SST) increases the number of bacteria in the soil, while simplified soil tillage with double cultivation (DST) increases the number of *Actinomycetes* and fungi. The number of bacteria and *Actinomycetes* is higher following nitrogen fertilization at the level of 60 kg N·ha^{-1}, while the number of fungi was higher at 60 and 90 kg N·ha^{-1}. Nitrogen at the level of 120 kg·ha^{-1} reduced the presence of microflora and reduced dehydrogenase activity. Vetanovetz and Peterson [36] found that mineral nitrogen fertilization led to an increase in the populations of bacteria, *Actinomycetes,* and fungi. According to Niewiadomska et al. [40], the action of enzymes varies at different times of the year. In the spring the enzymes are relatively active, in summer activity declines, and in autumn it increases again. The authors also show that factors affecting the activity of micro-organisms in the soil and their qualitative and quantitative status include content of organic matter, nitrogen compounds, macro-and micro-nutrients, water, oxygen, and the pH and temperature of the soil.

In the present study, the method of soil tillage and nitrogen fertilization did not affect the pH value of the soil, organic carbon content, or soil absorbing capacity (Table 4). A change in pH was observed only between years of the study: in 2007 the pH of the soil was 6.1, while in 2008 it decreased to 5.4. The reverse was true for organic carbon content; in 2007 it was 8.9 g·kg^{-1}, while in 2008 it was about 4% higher. The significantly highest organic carbon content [9.9 g·kg^{-1}] was observed in the case of conventional soil tillage (CST) in 2007, while it was only 86% of this value in the case of the same tillage in 2008 (Table 4).

According to Natywa et al. [31], evaluation of soil fertility on the basis only of chemical and physical properties is incomplete and insufficient. Biological parameters, i.e. respiratory and enzymatic activity, are more sensitive and better describe the state of the soil environment than physico-chemical properties. Table 5 shows the Biological Index of Soil Fertility (*BISF*). The different values depended on the variable used in calculating the biological activity of the soil (*M*). When the variable in the calculation was the sum of numbers of bacteria and *Actinomycetes* (B+A), then BISF was on average 46.1; when it was the sum of the num-

bers of bacteria and *Actinomycetes* divided by the number of fungi (B+A/F), then *BISF* was on average 10.0; when BISF was calculated with M as dehydrogenase activity, then it was on average 16.3. The factors investigated were found to substantially differentiate *BISF* where the value of M was dehydrogenase activity (D). In this case the highest *BISF* was noted in the case of simplified soil tillage (DST and SST) [18.3] and significantly decreased to 14.2 (22%) under conventional soil tillage (CST). Also, the highest BISF index dependent on dehydrogenase activity was noted after applying 90 kg N·ha^{-1} and the lowest after applying 120 kg N·ha^{-1}. Significant differences were also observed in the interaction between the factors analyzed. Only the year of the study did not affect *BISF* (Table 5). In the case of the other parameters used to express biological soil activity (*M*), there were no direct significant differences in *BISF* dependent on the study factors. Only when the value of *M* was the sum of the number of bacteria and *Actinomycetes* divided by that of fungi was there a significant difference in BISF in the interaction between soil tillage and the year of the study (Table 5). Myśków et al. [22] observed that the highest values for the indicator BISF resulted when biological activity (*M*) was calculated as the cell numbers of bacteria and *Actinomycetes*. The lowest values resulted when *M* was the sum of numbers of bacteria and *Actinomycetes* divided by numbers of fungi. The type and quantity of organic fertilization also had a substantial effect on *BISF*. According to Niewiadomska et al. [3], regular microbiological changes in soil, apart from the quantitative microbial community composition, are also shown by the interrelations between particular groups of micro-organisms, which can be expressed as the ratios of bacteria to *Actinomycetes*, total cell number of bacteria to fungi, and oligotrophic to copiotrophic bacteria. In fertile soils, bacteria predominate numerically over *Actinomycetes* (ratio = 60:40). Higher *Actinomycetes* content may be found in poor soils, since soil quality is one of the factors having a selective effect on them. In the opinion of some researchers cited by Niewiadomska et al. [3, 37], another microbiological index of soil fertility, based on the ratio of fungi to the total bacterial count, more accurately expresses the biological properties of soil than the count of each of these groups separately. Moreover, it indicates compensatory dependencies in the development of communities of bacteria and fungi. Dominance of fungi over bacteria shows a greater ability of fungi than that of bacteria to survive under deteriorating environmental conditions.

Regarding the yield of spring Triticale, the highest grain yields were obtained following conventional soil tillage (CST) and nitrogen fertilization at a level of 60 kg·ha^{-1}. Following reduced soil tillage (CST and SST), the yields were on average about 10% lower. Also, the higher levels of nitrogen reduced average yield to about 34% of that obtained with 60 kg·ha^{-1} (Table 4). Klikocka [38] and Klikocka and Wesołowski [39] obtained similar results in previous studies. The statistical analysis showed that yield was positively correlated with the number of *Actinomycetes* in the soil (r = 0.522) and the index of soil fertility (*BISF*), where *M* = (B + A/F) (r = 0.483) (Table 6). No significant

Table 4. The influence of soil tillage (ST) and nitrogen fertilization (NF) on spring Triticale yield, pH value, organic carbon content (H), and cation exchange capacity of soil (T) (averages from 2007 and 2008 of soil samples taken at the end of the tillering phase BBCH 29).

N fertilization (NF)	Soil tillage (ST)			Mean	P≤0.05
	CST	DST	SST		
Grain yield					
60	58.8	58.8	49.6	55.7	3.0
90	51.0	46.1	38.2	45.1	
120	45.7	40.4	34.2	40.1	
P ≤ 0.05 (ST*NF)		n.s.			
2007	47.0	49.7	43.5	46.8	3.5
2008 (Y)	56.6	47.1	37.8	47.1	
P ≤ 0.05 (ST*Y)		6.0			
Mean	51.8	48.4	45.4	47.0	3.0
pH_{KCl}					
60	5.8	6.0	5.7	5.8	n.s.
90	5.8	5.5	5.9	5.7	
120	5.8	5.7	5.9	5.8	
P ≤ 0.05 (ST*NF)		n.s.			
2007	6.0	6.1	6.3	6.1	0.3
2008 (Y)	5.5	5.4	5.2	5.4	
P ≤ 0.05 (ST*Y)		n.s.			
Mean	5.8	5.7	5.7	5.8	n.s.
$C_{org.}$ (H)					
60	9.8	9.3	8.8	9.3	n.s.
90	8.8	9.3	8.8	8.9	
120	9.1	9.3	9.1	9.2	
P ≤ 0.05 (ST*NF)		n.s.			
2007	8.5	9.2	9.0	8.9	0.3
2008 (Y)	9.9	9.4	8.7	9.3	
P ≤ 0.05 (ST*Y)		0.6			
Mean	9.2	9.3	8.9	9.1	n.s.
T					
60	4.09	4.01	4.14	4.08	n.s.
90	3.72	4.24	3.78	3.91	
120	3.54	4.00	3.43	3.66	
P ≤ 0.05 (ST*NF)		n.s.			
2007	3.74	4.40	3.48	3.87	n.s.
2008 (Y)	3.83	3.76	4.08	3.89	
P ≤ 0.05 (ST*Y)		n.s.			
Mean	3.79	4.08	4.00	3.88	n.s.

CST – conventional soil tillage, DST – simplified soil tillage with double cultivation, SST – simplified soil tillage with single cultivation. NF – nitrogen fertilization at 60, 90, 120 kg·ha^{-1}. Grain yield [t·ha^{-1}]; $C_{org.}$ (H) [g·kg^{-1}], soil absorbing capacity (T) [cmol(+)·kg^{-1}].

Table 5. Biological Index of Soil fertility (BISF) (according to Myśków et al. [25]). (averages from 2007 and 2008 of soil samples taken at the end of the tillering phase BBCH 29).

N fertilization (NF)	Soil tillage (ST)			Mean	P≤0.05
	CST	DST	SST		
M = B + A					
60	41.1	43.0	52.7	45.6	n.s.
90	48.9	38.0	52.9	46.6	
120	44.1	46.2	47.7	46.0	
P ≤£ 0.05 (ST*NF)		n.s.			
2007	44.9	45.5	38.5	43.0	n.s.
2008 (Y)	44.6	39.2	63.6	49.1	
P ≤ 0.05 (ST*Y)		16.3			
Mean	44.7	42.4	49.3	46.1	n.s.
M = B + A/F					
60	10.9	10.3	9.8	10.3	n.s.
90	9.7	10.2	9.7	9.9	
120	9.8	10.2	9.8	10.0	
P ≤ 0.05 (ST*NF)		n.s.			
2007	9.5	10.3	9.9	9.9	n.s.
2008 (Y)	10.7	10.2	10.0	10.2	
P ≤ 0.05 (ST*Y)		n.s.			
Mean	10.1	10.3	9.8	10.0	n.s.
M = D					
60	13.2	13.6	17.2	14.6	0.4
90	18.5	29.8	19.5	22.6	
120	11.0	11.5	12.6	11.7	
P ≤ 0.05 (ST*NF)		0.7			
2007	13.6	18.0	16.6	16.1	n.s.
2008 (Y)	14.8	18.6	16.2	16.5	
P ≤ 0.05 (ST*Y)		0.84			
Mean	14.2	18.3	18.3	16.3	0.4

A – *Actinomycetes*, B – bacteria, F – fungi, D – dehydrogenase activity, M – biological activity of soil

correlations were found between the genera of the micro-organisms. The most significant correlations were found between soil absorbing capacity (*T*) and number of bacteria (r = 0.530), the sum of the numbers of bacteria and *Actinomycetes* (r = 0.532), and BISF where *M* = (B + A) (r = 0.537) and where *M* = (B+A/F) (r = 0.488). A positive correlation was also observed between organic carbon (*H*) and *BISF* calculated with *M* = (B + A/F) (r = 0.910). The pH value of the soil does not correlate with the characteristics analyzed (Table 6).

Conclusions

1. Generally, simplified soil tillage and nitrogen fertilization up to 90 kg N·ha^{-1} of spring Triticale favours bacteria, *Actinomycetes* and fungi content, as well as dehydrogenase activity. Conventional soil tillage and nitrogen fertilization at a level of 120 kg·ha^{-1} reduces microflora. The highest numbers of soil micro-organisms were noted at the end of the heading stage (BBCH 59) of spring Triticale, while dehydrogenase activity was highest during the spring, when the spring Triticale was at the end of the tillering stage (BBCH 29).
2. In formulating the biological index of soil fertility (*BISF*), the biological activity of the soil (*M*), the organic carbon content in the soil (*H*), and soil absorbing capacity (*T*) were taken into account. (*M*) was expressed as the number of bacteria and *Actinomycetes*, the number of bacteria and *Actinomycetes* in relation to that of fungi, or as dehydrogenase activity.
3. *BISF* calculated based on dehydrogenase activity, organic carbon content, and soil absorbing capacity was influenced by the investigated factors. In the other cases no significant influence was observed. A positive correlation was observed between grain yield and *BISF* calculated as the sum of the cell number of bacteria and *Actinomycetes* divided by the cell number of fungi (*M* = B+A/F). No connection was found between the spring Triticale yield and the cell number of micro-organisms or dehydrogenase activity depending on soil tillage and nitrogen fertilization.

Table 6. The correlation coefficient between the parameters analyzed (data from 2007 and 2008 of soil samples taken at the end of the tillering phase BBCH 29).

Variables (n=18)	Yield of grain	pH	$C_{org.}$	T
Bacteria (B)	-0.394	-0.375	-0.408	0.530*
Actinomycetes (A)	0.522*	-0.282	0.193	0.235
Fungi (F)	-0.144	-0.285	0.062	-0.282
B+A	-0.383	-0.378	-0.403	0.532*
B+A/F	0.131	0.193	-0.196	0.297
Dehydrogenase activity	-0.024	-0.143	-0.180	0.201
BISF-B+A	-0.380	-0.384	-0.393	0.537*
BISF-B+A/F	0.483*	-0.142	0.910**	0.488*
BISF-D	-0.004	-0.179	-0.080	0.222

(significant difference at $P_{0.05} \geq 0.468$*, at $P_{0.01} \geq 0.590$**)

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