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THE MICROBIAL RESPONSE TO THE ORGANIC AND NATURAL FERTILISERS UNDER MAIZE GROWN FOR SILAGE IN MONOCULTURE

Summary

The aim of the study was to assess the influence of organic and natural fertilisation under maize plantation grown in monoculture on the number of selected groups of microorganisms. The experiment was conducted between 2005 and 2007 in plots of the Experimental and Educational Station in Swadzim, Department of Agronomy, Poznań University of Life Sciences, Poland. Soil taken from maize for silage was the research material. Soil samples for microbiological analyses were collected at a particular phase of maize development: before sowing; at the phase of maize emergence (BBCH 11-13); at the phase of 3-4 maize leaves (BBCH 13-14), at the phase of 6-7 maize leaves (BBCH 16-17), at the phase of panicle florescence (BBCH 63-69), at the phase of wax maturity (BBCH 87-89). The number of selected groups of microorganisms in the soil samples (moulds, total bacterial count, proteolytic and ammonifying bacteria, copiotrophs, oligotrophs and actinobacteria) was measured with the pour plate method developed by Koch. Between 2005 and 2007 natural and organic fertilisation in plots with a maize monoculture grown for silage increased the intensity of proliferation in all the groups of microorganisms under study. The trend and rate of variation in the microorganisms depended on the type of organic matter applied to soil and the term of analyses related with the plants' stage of development.
***Key words**: bacteria, fungi, actinobacteria, microbiological indicators*

ODPOWIEDŹ MIKROBIOLOGICZNA NA WPROWADZENIE NAWOZÓW ORGANICZNYCH I NATURALNYCH POD UPRAWĄ KUKURYDZY NA KISZONKĘ W MONOKULTURZE

Streszczenie

Celem pracy była ocena wpływu nawożenia organicznego i naturalnego w uprawie kukurydzy, w monokulturze na liczebność wybranych grup mikroorganizmów. Doświadczenie prowadzono na poletkach Zakładu Doświadczalno-Dydaktycznego w Swadzimiu, należącym do Katedry Agronomii, Uniwersytetu Przyrodniczego w Poznaniu, w latach 2005–2007. Gleba pobrana spod kukurydzy przeznaczonej na kiszonkę była przedmiotem badań. Gleba pobrana spod kukurydzy przeznaczonej na kiszonkę, była przedmiotem badań. Próbki glebowe do analiz mikrobiologicznych pobierane były w określonej fazie rozwojowej kukurydzy: przed siewem; w fazie wschodów kukurydzy (BBCH 11 – 13); w fazie 3 – 4 liści kukurydzy (BBCH 13 – 14), w fazie 6 – 7 liści kukurydzy (BBCH 16 – 17), w fazie kwitnienia wiech (BBCH 63 – 69), w fazie dojrzałości woskowej (BBCH 87 – 89). W pobranych próbach glebowych oznaczano liczebność wybranych grup drobnoustrojów (grzyby pleśniowe, ogólna liczba bakterii, bakterie proteolityczne i amonifikacyjne, kopiotrofy, oligotrofy i promieniowce) metodą płytek lanych według Kocha. W przeprowadzonych badaniach, w latach 2005-2007 nawożenie naturalne i organiczne na stanowiskach z monokulturą kukurydzy uprawianej na kiszonkę przyczyniło się do wzrostu intensywności namnażania wszystkich badanych grup drobnoustrojów. Kierunek i tempo zmian mikroorganizmów zależały od rodzaju materii organicznej trafiającej do gleby oraz terminu analiz związanego z fazą rozwojową rośliny.
***Słowa kluczowe**: bakterie, grzyby, promieniowce, wskaźniki mikrobiologiczne*

1. Introduction

In Poland cereals still have a high share in the structure of crops. In consequence, there are often irregularities in crop rotation. In the 1950s and 1960s it was commonly thought that even long-term maize growing without crop rotation did not reduce the yield, whereas the use of chemicals and mineral fertilisers replaced rotation successfully. However, many reports indicate that reducing crop rotation even to monoculture limits the microbial biodiversity in the environment, causes the accumulation of phenolic compounds in soil, increases the amount of phytotoxins, which induce autotoxicity and result in lower yield. Apart from that, monoculture causes acidification in the upper soil layers and one-sided exhaustion of soil nutrients, it disorders the physical properties of soil, increases its density and compaction, decreases the available water capacity and makes unfavourable changes in the content of humus in soil [7]. Due to the deficit of humus compounds in Polish soils, it seems highly significant to choose an appropriate organic or natural fertiliser, which will enable adequate management of humus. It is particularly important for protection of the natural environment, maintenance of biodiversity in agro-ecosystems and improvement of soil fertility. The choice of an adequate organic or natural fertiliser may significantly increase soil fertility and intensify the humification process. The biological equilibrium in soil may be disordered not only by monoculture but also by organic fertilisers [23]. The application of natural and organic fertilisers to soil may significantly affect the growth and development

of microbial cells. According to Koper et al. [4], excessive and one-sided fertilisation, which is common agricultural practice, may disorder the balance between nutrients in an aqueous solution.

The aim of the study was to assess the influence of fertilisation and crop residues under maize plantation grown in monoculture on the number of selected groups of microorganisms.

2. Materials and methods

The experiment was conducted between 2005 and 2007 in plots of the Experimental and Educational Station in Swadzim, Department of Agronomy, Poznań University of Life Sciences, Poland. Maize grown for silage was the research material. There were two experiments conducted in fields in the village of Swadzim, where maize had been sown in monoculture for six years. The experiments were conducted on lessivé soil formed from light loamy sands, classified as IVb, very good rye complex.

The weather conditions were monitored during the experiments. The average temperatures ranged between 13.3 and 14.8ºC and were 1-2.5°C higher than the average temperatures noted between 1957 and 2008. 2006 was the hottest year – high temperatures in individual months ideally met the thermal requirements of maize. There were also considerable differences in the amount of rainfall in consecutive years of the experiment. The second year of the research (2006) was the least favourable, because there was considerable deficit at the time when maize was in high demand for rainfall. 2007 was the best year, because the annual rainfall was lower only by 21.5 mm than the average amount in the long-term period.

The type of fertilisation was the experimental factor: 1) the control variant – NPK mineral fertilisation; 2) a full dose of manure 30 $t \cdot ha^{-1}$; 3) half a dose of manure 15 $t \cdot ha^{-1}$; 4) 40 $m^3 \cdot ha^{-1}$ slurry; 5) 5 $t \cdot ha^{-1}$ rye straw + 40 $m^3 \cdot ha^{-1}$ slurry; 6) 5 $t \cdot ha^{-1}$ rye straw + mineral nitrogen; 7) winter intercrop – winter rye and winter vetch (1:1). Each year the same experimental plots were fertilised naturally and organically and an aftercrop was sown in autumn before the soil was ploughed for winter. In spring, immediately before pre-sowing cultivation the soil was additionally treated with a mineral fertiliser to supplement the amount of nutrients provided with the organic fertilisers. The dosage of supplementary mineral fertilisation was based on equivalents for individual natural and organic fertilisers. The dosage was balanced, where nutrients from mineral fertilisers were used up to the following amounts: N: 130 kg $N \cdot ha^{-1}$, P: 80 kg $P_2O_5 \cdot ha^{-1}$, K: 140 kg $K_2O \cdot ha^{-1}$. Additionally, immediately before the pre-sowing cultivation the intercrop of winter rye and vetch was ploughed. Maize seeds of the PR39G12 cultivar were sown in four rows spaced at 0.7 m from each other. In all the years there were similar numbers of plants sown, i.e. 8.0-9.5 plants per metre. The experimental plot area was 42 m^2 (width: 2.8 m, length: 15 m).

Soil samples for microbiological analyses were collected at a particular phase of maize development: before sowing; at the phase of maize emergence (BBCH 11-13); at the phase of 3-4 maize leaves (BBCH 13-14), at the phase of 6-7 maize leaves (BBCH 16-17), at the phase of panicle florescence (BBCH 63-69), at the phase of wax maturity (BBCH 87-89).

The count of selected groups of microorganisms in the soil samples (moulds, total bacterial count, proteolytic and ammonifying bacteria, copiotrophs, oligotrophs and actinobacteria) was measured with the serial dilution method developed by Koch. The count of individual groups of microorganisms was measured three times on specially prepared selective mediums, which corresponded to individual groups under analysis:

– the total bacterial number – on a selective medium made from a soil extract, incubated at 25°C for 7 days;
– moulds – on a selective medium developed by Martin [8], incubated at 24°C for 7 days;
– actinobacteria – on a selective medium developed by Pochon after 5 days of culturing at 25°C [18];
– copiotrophs – on a selective nutrient broth (NB) medium for copiotrophs, incubated at 25°C for 7 days [16];
– oligotrophs – on a selective DNB medium for oligotrophic microorganisms, incubated at 25°C for 14 days [16];
– proteolytic bacteria – on a selective medium for proteolytic microorganisms developed by Rodina [18], incubated at 22°C for 48 hours;
– ammonifying bacteria – on a selective medium for ammonifying microorganisms developed by Rodina [18], incubated at 28°C for 6 days.

The research results were subject to univariate analysis of variance for orthogonal factorial experiments in a randomised block design in Statistica 9.0 [2]. Apart from that, Pearson's correlation was calculated. The significance of the coefficients of correlation between the parameters under study was estimated at a confidence level $\alpha_{0.05}$ (*significant difference). The least significant difference (LSD) was calculated by means of an F-test at a confidence level $\alpha_{0.05}$.

3. Results and discussion

Between 2005 and 2007 natural and organic fertilisation in plots with a maize monoculture grown for silage increased the intensity of proliferation in all the groups of microorganisms under study. The trend and rate of variation in the microorganisms depended on the type of organic matter applied to soil and the term of analyses related with the plants' stage of development.

The total bacteria count was diversified during the growth of maize plants and it depended on the type of fertilisation (Fig. 1). The time before plants' emergence was the best for the development of total bacterial count in soil. During that period the microorganisms tended to proliferate when each natural fertiliser was applied. Manure, especially at a dose of 30 $t \cdot ha^{-1}$, proved to be the best fertiliser for these microorganisms during the whole research period, except the phase of plants' emergence. Slurry was also effective during the first three phases of development. The observations and analyses showed that the highest total bacteria count was measured before emergence when half a dose of manure was applied (85.9 cfu 10^6 g^{-1} d.m. of soil). The lowest total bacterial count was measured at the phase of panicle florescence when slurry was applied (27.0 cfu 10^6 g^{-1} d.m. of soil). During the entire period of plants' growth differences in the bacterial count between the methods of fertilisation were the smallest at the ripening period.

Furczak and Turska [3] and Niewiadomska et al. [11] also proved that the term of analysis and type of fertiliser influenced the total bacteria count in soil. They found the most bacteria before maize was sown in plots fertilised with slurry and during the plants' emergence in the plots fertilised with straw and mineral nitrogen. The high fertilising value of slurry was also confirmed by Vargova et al. [20] and Kucharski and Wyszkowska [6].

Similarly to other groups of microorganisms under analysis, the count of moulds depended on the phase of plants' development and the type of fertilisation applied (Fig. 2).

The synthesis for the 2005-2007 period revealed increased activity of moulds at the phase of 3-4 maize leaves and at the phase of wax maturity. At the phase of 3-4 maize leaves the count of moulds in all fertilisation variants was significantly higher than in the control variant. The experiments proved that before the emergence of plants there was a statistically significant increase in the activity of moulds in the variants fertilised with manure at a dose of 30 t·ha^{-1}(16.1 cfu 10^5 g^{-1} d.m. of soil), at the phase of maize emergence – after ploughing the winter aftercrop (16.5 cfu 10^6 g^{-1} d.m. of soil) and at later phases – there was a strong stimulating effect of straw with mineral nitrogen (Fig. 2). Kucharski and Wyszkowska [6] proved very intense development of moulds when brown soil was fertilised with slurry. However, manure did not have significant influence on the count of these microorganisms. Intense development of the total count of moulds was also observed by Wielgosz and Szember [22] in legume plantation. Nowak et al. [15] found that manure stimulated the growth of moulds most, whereas the effect of straw was much weaker.

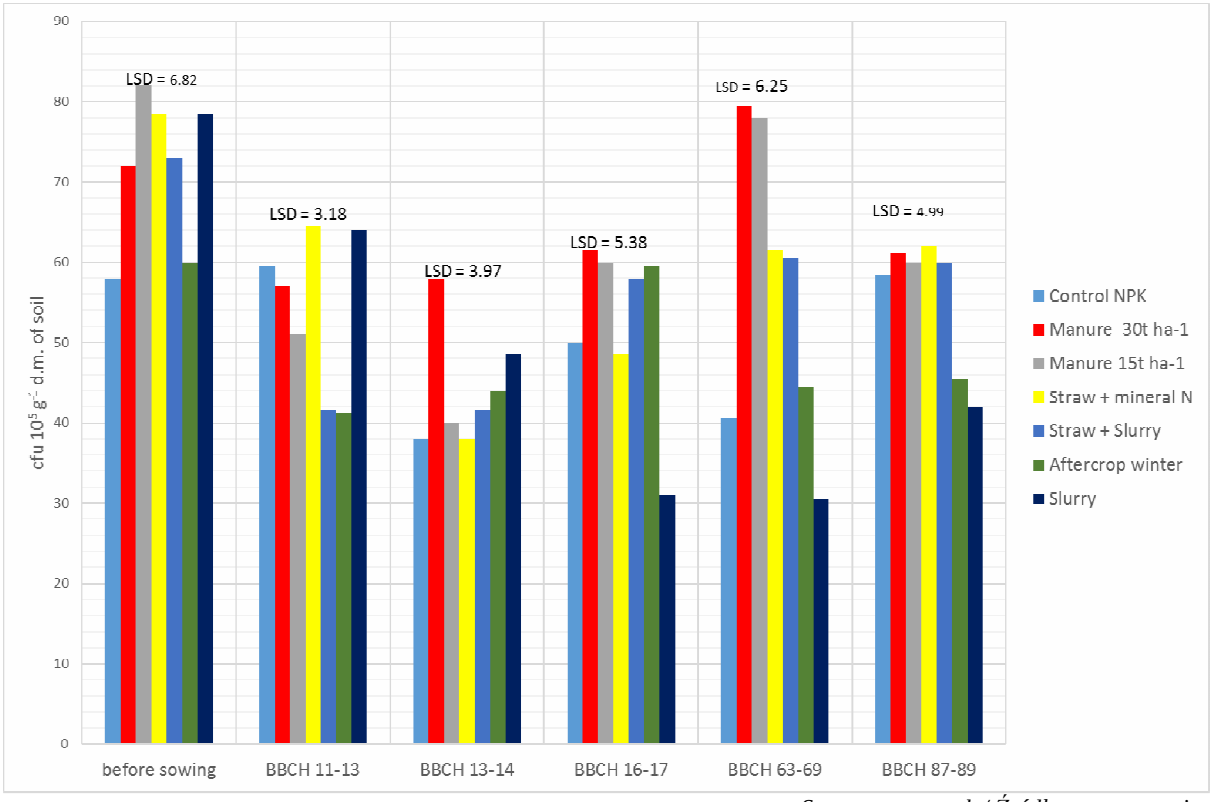

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Fig. 1. The dependence between the total bacteria count and the type of fertilisation (cfu 10^5 g^{-1} d.m. of soil)
Rys. 1. Ogólna liczebność bakterii w zależności od rodzaju zastosowanego nawożenia (jtk 10^5 g^{-1} s.m. gleby)

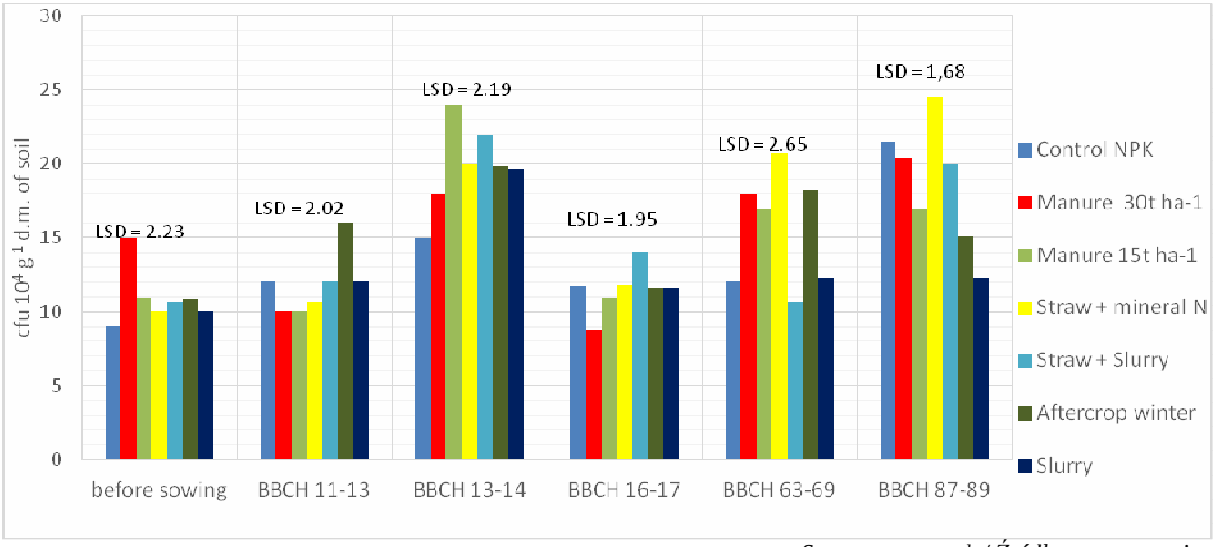

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Fig. 2. The dynamics of variation in the count of moulds according to the type of fertilisation applied (cfu 10^4 g^{-1} d.m. of soil)
Rys. 2. Dynamika zmian liczebności grzybów pleśniowych w zależności od rodzaju zastosowanego nawożenia (jtk 10^4 g^{-1} s.m. gleby)

The investigations on actinobacteria revealed that, similarly to other groups of microorganisms, their count varied during the growth period, depending on the type of fertilisation applied. The average results during the three years of the research showed that the highest count of this group of microorganisms (Fig. 3) was observed before the emergence of maize. At that time in all combinations except the one with the winter intercrop the count of actinobacteria was greater than in the control variant. There were statistically significant differences between the variant with slurry as well as the one with straw, mineral nitrogen and slurry, as compared with the control variant. Nowak et al. [15] also observed that the straw fertiliser stimulated the count of this group of microorganisms. Barabasz et al. [1] confirmed that compounds containing nitrogen increased the count of actinobacteria in soil. Niewiadomska et al. [11] indicated that substances with hardly accessible forms of protein favoured the development of actinobacteria. Kucharski and Wyszkowska [6] observed that manure stimulated the development of this group of microorganisms more than slurry.

The data recorded during the three years of the research showed that fertilisation stimulated the growth of copiotrophs. The most intense growth and the greatest diversification between the types of fertilisation was noted at the initial period of vegetation, immediately before the emergence of plants. The influence of slurry on the count of copiotrophs was limited, but straw with slurry and the winter intercrop caused intense proliferation of the microorganisms. In comparison with the control variant their counts were more than 250% greater and amounted to 92.3 cfu·10^5·1g d.w.$^{-1}$ of soil and 95.3 cfu·10^5·1g d.w.$^{-1}$ of soil, respectively (Fig. 4). Further assessment of the results revealed that the count of copiotrophs decreased significantly at the phase of maize emergence, but the effect of fertilisation was still statistically significant. It was the strongest after the application of slurry (62.3 cfu·10^5·1g d.w.$^{-1}$ of soil) and the winter aftercrop (59.3 cfu·10^5·1g d.w.$^{-1}$ of soil). In the combinations with a full dose of manure there was a noticeable, statistically significant increase in the count of copiotrophs during the whole period of vegetation, as compared with the control variant (Fig. 5).

The analysis of the research findings confirmed that the ploughing of natural and organic fertilisers resulted in strong dynamics of the proliferation of copiotrophs at the earliest phases of vegetation. The research by Kucharski and Wyszkowska [6] revealed that the count of copiotrophs increased both in the plots fertilised with slurry and those fertilised with manure. However, the double dose of slurry did not increase the count of copiotrophs more than in the variant fertilised with manure. According to Pengthamkeerati et al. [17], the positive effect of organic fertilisation on the biomass of microorganisms results not only from the fact that they are supplied with nourishment but also from the change in the physical properties of soil. Both excessive soil density over 1.8 Mg·m^{-3} and excessive soil looseness below 1.2 Mg·m^{-3} cause unfavourable conditions for the biological life in soil.

As far as the next group of microorganisms is concerned, i.e. oligotrophs, the average results of the three-year research period revealed that they were the most numerous at the beginning of the growth season, before the emergence of maize plants. The growth of oligotrophs was most stimulated by half a dose of manure (209.2 cfu·10^5·1g d.w.$^{-1}$ of soil), a full dose of manure (163.5 cfu·10^5·1g d.w.$^{-1}$ of soil) and the winter intercrop (164.3 cfu·10^5·1g d.w.$^{-1}$ of soil). Niewiadomska et al. [13] observed the highest count of oligotrophs in spring, but later the count and activity of these microorganisms decreased. Kucharski et al. [5] and Kucharski and Wyszkowska [6] proved that the count of oligotrophs dropped when slurry was entered into soil, whereas manure inhibited the growth of these microorganisms to the greatest extent.

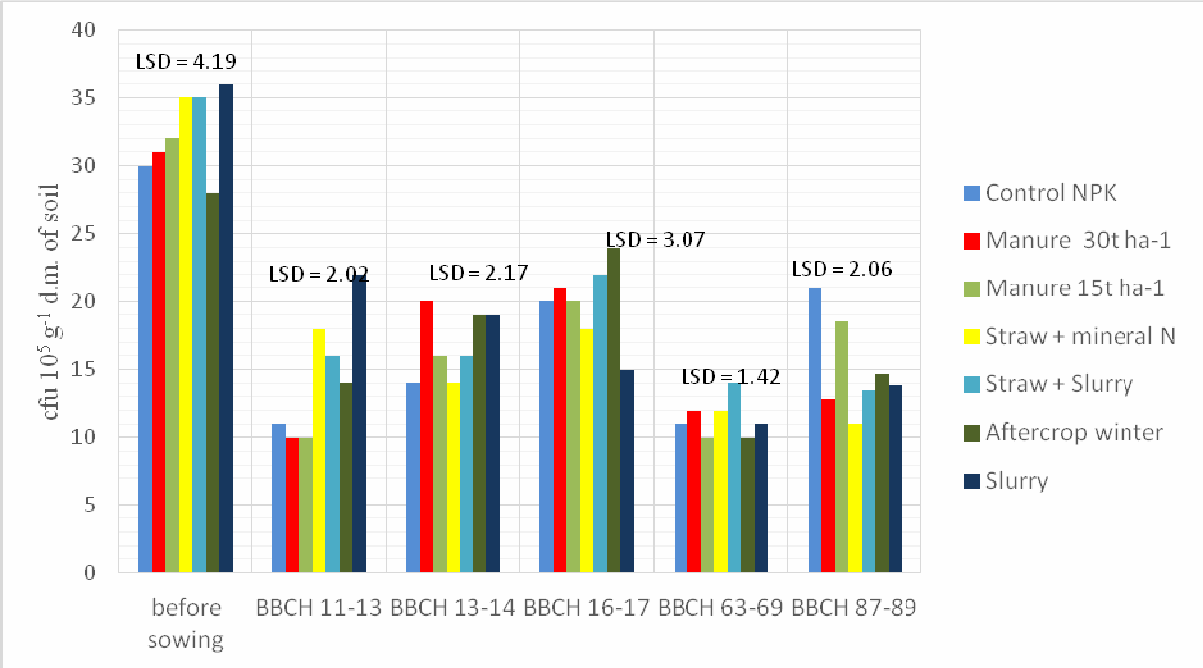

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Fig. 3. The dynamics of variation in the count of actinobacteria according to the type of fertilisation applied (cfu 10^5 g^{-1} d.m. of soil)

Rys. 3. Dynamika zmian liczebności promieniowców w zależności od rodzaju zastosowanego nawożenia (jtk 10^5 g^{-1} s.m. gleby)

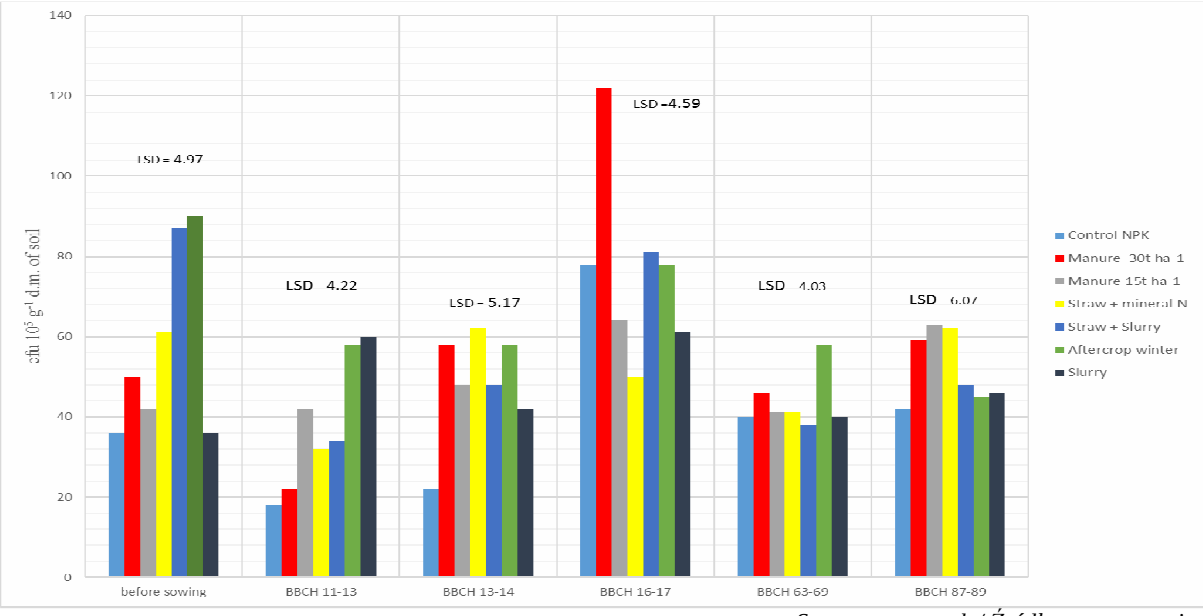

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Fig. 4. The dynamics of variation in the count of copiotrophs according to the type of fertilisation applied (cfu 10^5 g^{-1} d.m. of soil)

Rys. 4. Dynamika zmian liczebności kopiotrofów w zależności od rodzaju zastosowanego nawożenia ((jtk 10^5 g^{-1} s.m. gleby)

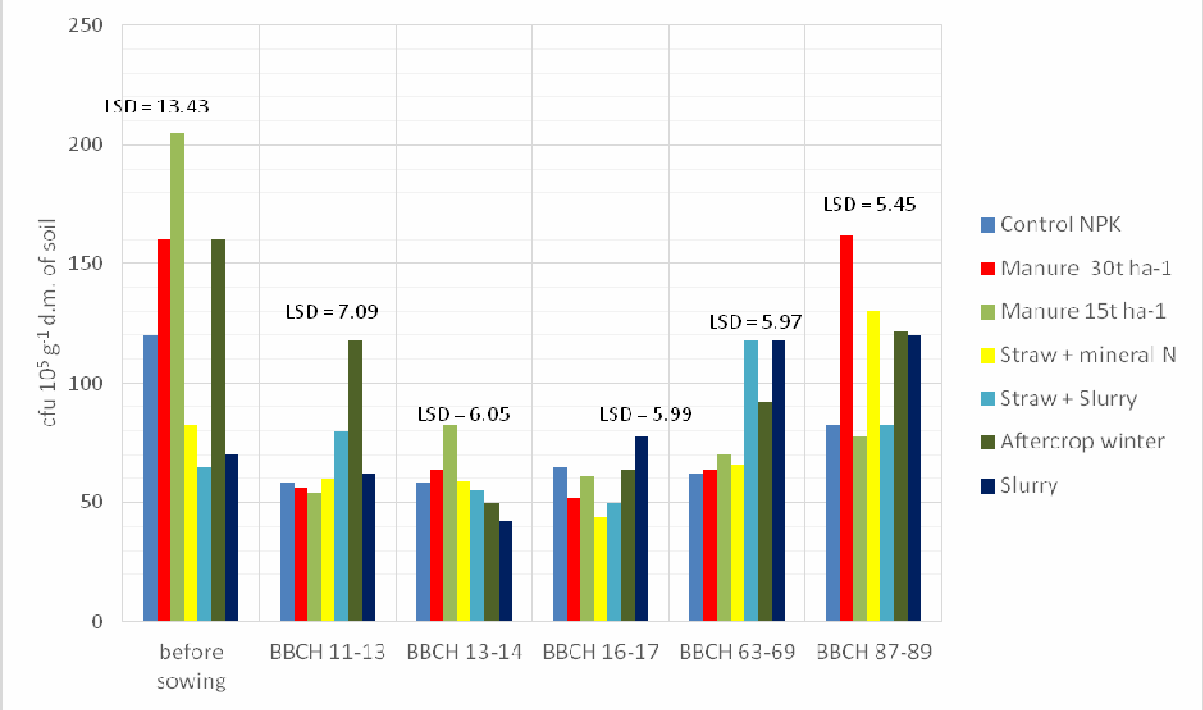

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Fig. 5. The dynamics of variation in the count of oligotrophs according to the type of fertilisation applied (cfu 10^5 g^{-1} d.m. of soil)

Rys. 5. Dynamika zmian liczebności oligotrofów w zależności od rodzaju zastosowanego nawożenia (jtk 10^5 g^{-1} s.m. gleby)

The synthesis for the three-year research period revealed that fertilisation had statistically significant influence on the count of proteolytic bacteria (Fig. 6). The average count of these bacteria in early spring was much higher than at the phase of maize emergence and at the phase of 3-4 maize leaves, when the count decreased considerably. The evaluation of the influence of individual fertilisers on the count of proteolytic bacteria before the emergence of maize plants showed that manure applied at a dose of 30 $t \cdot ha^{-1}$ stimulated the growth of this group of microorganisms. It resulted in a statistically significant increase by 79.5%, as compared with the control variant. At the phase of panicle florescence the increase was even greater as it amounted to 122.9% (Fig. 6). The winter aftercrop, slurry and straw with slurry also resulted in intense stimulation of the development of proteolytic bacteria. Niewiadomska et al. [12] observed similar dynamics in the count of these microorganisms when natural and organic fertilisers were applied. This fluctuation in the development of proteolytic bacteria was caused by the inflow of organic substances

with high content of compounds with protein and amino acids, which supplied direct nourishment to bacteria. These observations were also confirmed by Szwed and Furczak [19], who studied the proteolytic activity and proved that it was minimal in spring, but it reached the maximum in the late summer and early autumn. Nowak et al. [15] noted the highest activity of proteolytic bacteria in early spring. They observed the greatest diversification in the count of these microorganisms in early summer in consequence of organic fertilisation. Like in our study, these authors observed increased proliferation of proteolytic bacteria at the end of the growth season.

The count of ammonifying bacteria also changed significantly as a result of fertilisation (Fig. 7). Before the emergence of plants, despite the significance of differences, the plots were characterised by the lowest variation in the count of these bacteria. There were bigger differences between individual experimental combinations at the phase of emergence in the variant with slurry (74.9 cfu·10^5·1g d.w.$^{-1}$ of soil). In comparison with the control variant the count of these bacteria increased by 93%. At this phase each type of organic substance applied to soil resulted in a greater count of ammonifying bacteria than in the control variant. The most intense proliferation of ammonifying bacteria was observed at the phase of 6-7 maize leaves and at the phase of panicle florescence. During the entire growth period the development of these bacteria was most stimulated by slurry, which also exhibited positive influence after ploughing the winter aftercrop and manure. Niewiadomska et al. [12] observed similar results – the smallest count of ammonifying bacteria was found in early spring after treatment with manure, whereas the highest count of these bacteria was measured in summer at the phase of maize florescence after treatment with the same fertiliser. The research findings also show that slurry strongly stimulated the development of ammonifying bacteria until the phase of panicle florescence. By contrast, Kucharski and Wyszkowska [6] did not note any positive influence of this fertiliser on the group of microorganisms in question. However, they noticed intense proliferation of ammonifying bacteria after treatment with manure. These authors stressed the negative influence of mineral fertilisation on the count of ammonifying bacteria.

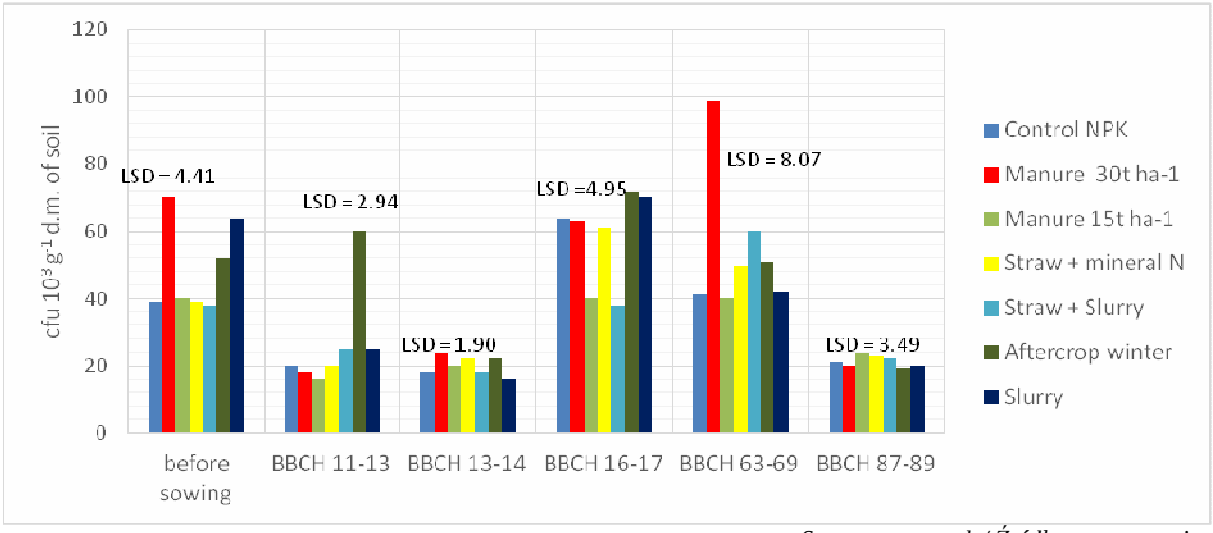

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Fig. 6. The dynamics of variation in the count of proteolytic bacteria according to the type of fertilisation applied (cfu 10^3 g^{-1} d.m. of soil)

Rys. 6. Dynamika zmian liczebności bakterii proteolitycznych w zależności od rodzaju zastosowanego nawożenia (jtk 10^3 g^{-1} s.m. gleby)

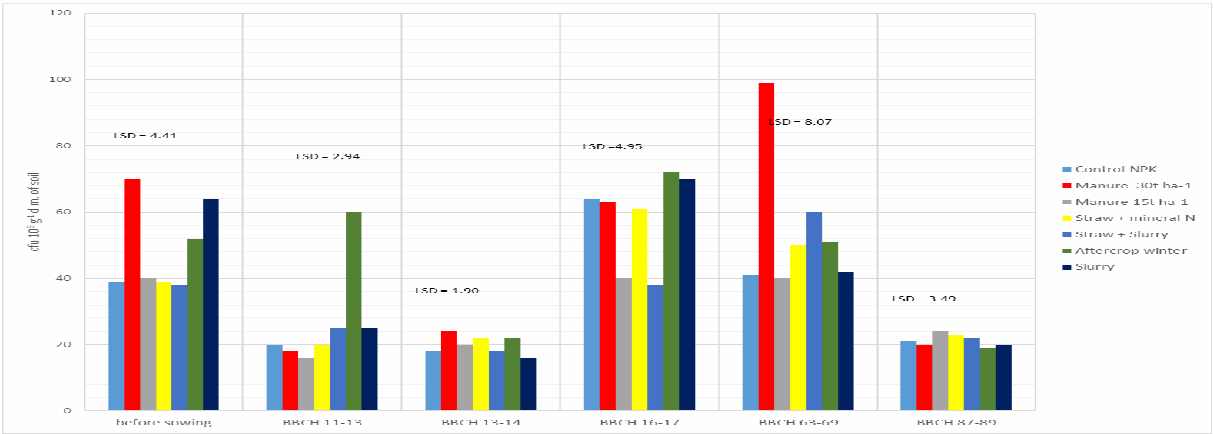

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Fig. 7. The dynamics of variation in the count of ammonifying bacteria according to the type of fertilisation applied (cfu 10^4 g^{-1} d.m. of soil)

Rys. 7. Dynamika zmian liczebności bakterii amonifikacyjnych w zależności od rodzaju zastosowanego nawożenia (jtk 10^4 g^{-1} s.m. gleby)

Tab. 1. Indicators of the microbial activity of soil.
Tab. 1. Wskaźniki aktywności mikrobiologicznej gleby

Fertilization variants	Indicators of the microbial activity		
	B: A **A**	**(B+A) x F^{-1}** **B**	**O : C** **C**
Control object - NPK mineral fertilization	2,5	3,1	1,4
Full dose of manure 30 t·ha^{-1}	4,4	3,7	2,7
Half a dose of manure 15 t·ha^{-1}	3,5	4,8	1,51
Slurry 40 m^3·ha^{-1}	5,29	3,5	2,09
Rye straw 5 t·ha^{-1}+ 40 m^3·ha^{-1} slurry	4,2	3,15	1,9
Rye straw 5 t·ha^{-1}+ N mineral	3	3,3	2,7
Intercrop winter - winter rye with winter vetch (1:1)	2,8	4,5	2,5

Abrrevation: B – bacteria, A – Actinobacteria, F – fungi, O – oligotrophs, C – copiotrophs

Source: own work / Źródło: opracowanie własne

Due to the higher volume of agricultural production the control of the state of soil which is based only on its biological parameters is increasingly often taken into consideration. Apart from the count of microorganisms, the interrelations between individual groups show the regularities of microbial changes in soil. In order to evaluate the quality of soil under maize grown in monoculture after treatment with natural and mineral fertilisers the following microbiological parameters were measured at the last term of analyses: **A** – the quantitative ratio between bacteria and actinobacteria, **B** – the ratio between the total bacterial count, actinobacteria and fungi, **C** – the quantitative ratio between oligotrophs and copiotrophs (Table 1). In fertile soils there are more bacteria than actinobacteria (**A** = 60:40). In our experiment the ratio was greater than 1.5 in all the fertilisation variants. This means that the treatment with natural and organic fertilisers stimulated the growth of microorganisms in soil under maize grown for silage in monoculture. The highest value of the ratio was noted in the variant where rye straw and slurry were applied, whereas the lowest value was measured after the winter intercrop (Table 1). The next microbiological indicator of soil fertility (**B**), i.e. the ratio between the total bacterial count, actinobacteria and fungi also confirmed the positive influence of fertilisation on the soil under maize grown for silage in monoculture. According to Myśków [10] and Myśków et al. [9], this indicator shows the biological properties of soil more precisely than the count of each group of microorganisms individually. It indicates the compensational dependence in the development of bacterial complexes and fungi, which was also observed by other authors [21]. The predominance of fungi over bacteria would indicate that the former microorganisms are more capable of existing under deteriorating conditions of the soil environment due to its acidification. This trend was not observed in our experiment (Table 1). Another important indicator of the microbial activity of soil is the ratio between oligotrophs and copiotrophs (**C**). It is an indicator of biological equilibrium, which shows the right trend in the microbial transformation of organic matter in soil. Our experiment showed that the soil under maize grown for silage in monoculture did not exhibit fatigue due to the predominance of oligotrophs over copiotrophs in all the fertilisation variants. It is significant for maintaining the level of organic matter in soil because oligotrophs process the energy substrate economically (Table 1).

4. Conclusions

The trend and rate of variation in the microorganisms depended on the type of organic matter applied to soil and the term of analyses related with the plants' stage of development.

1. Manure, especially at a dose of 30 t·ha^{-1}, proved to be the best fertiliser for total number bacteria during the whole research period.
2. The count of moulds and actinobacteria depended on the phase of plants' development and the type of fertilisation applied.
3. The influence of slurry on the count of copiotrophs was limited, but straw with slurry and the winter intercrop caused intense proliferation of the microorganisms.
4. On the basis of biological activity indicators our experiment showed that the soil under maize grown for silage in monoculture did not exhibit fatigue.

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