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Changes in soil microbial community structure after fertilisation with organic fertilisers and biological additives

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Abstract

Various forms of organic fertilisers are often used in agriculture. It is useful to have information on how microbial communities in arable land are affected by fertilisation with different types of fertilisers. One of the indicators of the stability and fertility of the soil ecosystem is the population structure and abundance of soil microorganisms. The aim of the study was to determine how fertilisation and biological additives affected the most active soil microorganisms of the studied functional groups in 2021 and 2022. It was found that organotrophic ($113 \pm 3 \times 10^3$ CFU g⁻¹) and diazotrophic ($161 \pm 5 \times 10^3$ CFU g⁻¹) bacteria were most abundant in the spring 2021. However, in 2022, the amount of organotrophs decreased, and their abundance was approximately the same throughout the growing season, probably due to a decrease in the amount of free organic matter in the soil. In 2022, the most abundant organotrophs ($91 \pm 2 \times 10^3$ CFU g⁻¹) and diazotrophs ($100 \pm 1 \times 10^3$ CFU g⁻¹) were also in the spring. Organic fertilisers and their different rates had a significant or very significant effect on the abundance of organotrophic, mineral nitrogen-assimilating, and non-symbiotic diazotrophic bacteria, except for diazotrophs in treatments with the biological additive *Trichoderma* spp. In 2021, nitrifiers ($162 \pm 5 \times 10^3$ CFU g⁻¹) and fungi ($2.29 \pm 0.13 \times 10^3$ CFU g⁻¹) were most abundant in the autumn. In 2022, nitrifiers were most abundant in the spring ($128 \pm 2 \times 10^3$ CFU g⁻¹) but less abundant than in the autumn 2021. During the experimental period, the abundance of fungi and yeasts was highest in the autumn, and this trend was not affected by fertilisation. In the treatments with the biological additive *Trichoderma* spp., organic fertilisers significantly or very significantly affected the abundance of fungi and yeasts. However, no significant differences were found in the treatments with the biological additive *Azotobacter* spp.

Keywords: fungi, bacteria, abundance, *Trichoderma* spp., *Azotobacter* spp.

Introduction

Soil is rich in microorganisms – one gram of soil contains hundreds of fungi and bacteria species (Schlatter et al., 2018). Soil microorganisms are very important and directly affect the biochemical activity, biological productivity, and stability of soil (Sahu et al., 2017; Kaur, Kaur, 2021; Pu et al., 2022). They are actively involved in the carbon cycle and other processes by regulating the circulation of the elements in the soil. They make them available to plants and create the conditions for the optimal growth (Barabasz et al., 2002; Jacoby et al., 2017; Iminov et al., 2020). Climatic conditions, plant diversity, soil depth and properties, including the application of fertilisers to the soil, affect and in some cases stimulate certain groups of microorganisms, which are often involved in phosphorus solubilisation, nitrogen mineralisation, methane decomposition, and the

degradation of complex organic compounds (Tedersoo et al., 2014; Ko et al., 2017; Schlatter et al., 2018).

Land use and anthropogenic activities drive agroecosystem changes (Wang et al., 2019; Katai et al., 2020; Labouyrie et al., 2022). Studies by other authors showed that the rational application of organic fertilisers is of great importance for improving of soil organic matter and is positively related to soil carbon accumulation, conservation of arable land, and increase in plant productivity and recultivation of livestock manure (Watts et al., 2010; Piaszczyk et al., 2017; Guo et al., 2019; Dinca et al., 2022). However, the increasing input of nitrogen and the long-term use of mineral fertilisers usually reduce the soil microbial biomass, change the composition of microbial community, and cause severe soil acidification and degradation, resulting in significant

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changes in microbiocenosis (Geisseler, Scow, 2014; Xun et al., 2016).

To determine the short-term effect of a fertiliser on the soil bacterial community, Ye et al. (2020) conducted a short-term field experiment using pig and chicken manure and composted pig manure. The authors found that the application of organic fertilisers increased the population of soil microorganisms such as aerobes and actinomycetes. Chicken and composted pig manure significantly increased the richness of soil bacteria (Lee, 2010; Ye et al., 2020). Gautam et al. (2020) conducted a 16-year long-term experiment aimed to determine the influence of long-term manure and mineral fertiliser application on biological indicators using mineral fertilisers and beef manure. The results of their study showed that higher amounts of organic manure significantly increased the enzyme activity.

Doyeni et al. (2023) conducted a study to evaluate the influence of animal waste-based digestate on the soil microbial activity in the Lithuanian climate conditions. It was found that the long-term application of animal manure affected the amount of microbial biomass, which is one of the most important components of the biological conditions of the soil. Another valuable study was conducted by Jurys and Feizienė (2021). The influence of *Trichoderma reesei* on the changes in soil organic carbon, microbial biodiversity, and other parameters in loamy *Cambisol* in Lithuania was evaluated. The results of the study showed that under drought conditions, the biological preparation *T. reesei* in a mixture with other components increased the microbial biodiversity.

A significant effect on the bacterial taxonomic composition was also found. Pu et al. (2022) conducted an experiment to use basic organic and microbial organic fertilisers to restore soil microorganisms after fumigation. The results of the study showed that organic fertilisers can promote microbial diversity and increase the relative abundance of beneficial species. Li et al. (2020) analysed the composition and diversity of soil microorganisms in rice paddy fields after fertilisation. It was found that the application of chicken and pig manure increased the abundance of bacteria involved in the nitrogen cycle and played a positive role in regulating the nitrogen balance in paddy soils. Ren et al. (2018) conducted an extensive study and quantified the effects of manure application on soil microbial biomass carbon and nitrogen in croplands at a national scale. It was determined that a sufficient amount of nitrogen and carbon obtained by supplementing with organic fertilisers promotes the abundance of soil microorganisms. Wu et al. (2021) used an organic fertiliser composted from pig manure and studied the soil response to the application of organic fertilisers. The results of the study showed that organic fertiliser increased the number of mineral nitrogen-assimilating bacteria but decreased the number of nitrite-oxidising bacteria. Li et al. (2020) concluded that the addition of chicken and fresh pig manure increased the abundance of pathogenic bacteria.

It has been stated that the soil microbial community is sensitive to fertilisation, so understanding

the response of soil microbiota to organic fertiliser application is of great importance.

This study investigated how the application of various fertilisers affects the most active soil microorganisms. For the experiment, two types of organic fertilisers were selected – litter and granulated poultry and cattle manure. The aim of the study was to determine how fertilisation and biological additives affected the most active soil microorganisms of different functional groups. It was hypothesised that different fertilisers and their combinations with biological additives, differences in nutrients amount, and the intensity of nutrient release would have distinct effects on different functional groups of bacteria and fungi.

Material and methods

Experimental design and treatments. The experiment on the abundance of the most active soil microorganisms was carried out in 2021 and 2022 as a part of the experiment started in 2018. The soil of the experimental site is basic illimerised soil, or sandy loam *Haplic Luvisol* according to FAO (WRB, 2022). The study area was situated in Vokė Branch (54.609344, 25.125962), Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. There were no changes in the study area during the experiment.

The abbreviations of fertilisers used in the field experiment are given in Table 1.

The field experiment was carried out according to the field experiment scheme adapted to all crops in the rotation presented in Table 2.

Soil sampling and microbial counts. In 2021 and 2022, the soil samples were taken in four replicates of the respective experimental fields in spring, summer, and autumn from a depth of 10–20 cm arable layer. Each soil sample was dried and passed through a 1.0 mm sieve to remove rocks and pieces of plants and before further analysis stored at 4°C no longer than 2–3 weeks. To analyse cultivable microorganisms, the plate-count technique using different selective media was used (Davet, Rouxel, 2000). Serial dilutions were prepared, and 0.1 mL of 10^{-2} and 10^{-3} dilutions were spread on the specific nutrient agar in Petri plates in five replicates. For fungi and yeasts, Sabouraud agar with chloramphenicol (200 ppm) (Liofilchem, Italy), for organotrophic bacteria, meat peptone nutrient agar (Liofilchem), for mineral nitrogen (N_{min})-assimilating bacteria, starch-ammonium agar, and for non-symbiotic diazotrophic bacteria, Ashby's mannitol agar were used. Petri dishes with the inoculated samples were cultivated at 25°C in the dark for 7–12 days (Sivojiene et al., 2021). Quantity of microorganisms was determined as colony-forming units (CFU) per 1 g of dry soil (Carter, Gregorich, 2007). The studies conducted using this method allow observation of the most active organotrophs, nitrifiers, diazotrophs, the populations of soil fungi and yeast-like fungi, and changes of their abundance in the soil (Gans et al., 2005; Furtak, Gajda, 2018).

Table 1. Description of the fertilisers used in the experiment

Abbreviation	Fertiliser type	Explanation
Control ($N_0P_0K_0$)	–	without fertilisers
MF	mineral	mineral fertilisers, $N_{90/60}$
PLM ₁₇₀	organic	poultry litter manure, N_{170}
GPM ₈₅	organic	granulated poultry manure, N_{85}
GPM ₁₇₀	organic	granulated poultry manure, N_{170}
GPM ₁₇₀ + A	organic	granulated poultry manure, N_{170} + biological additive No. 1: <i>Azotobacter</i> spp., a mixture of two cultures (<i>A. chroococcum</i> and <i>A. vinelandii</i>) together with the residues of the culture medium, microelements (manganese, iron, copper, molybdenum, zinc, and cobalt) and vitamins (B1, B3, and B6), no more than 0.02%;
GPM ₁₇₀ + T	organic	granulated poultry manure, N_{170} + biological additive No. 2: <i>Trichoderma</i> spp., a non-commercial biological additive enriched with phytohormones – a mixture of three cultures (<i>T. harzianum</i> , <i>T. tomentosum</i> , and <i>T. viride</i>)
CLM ₁₇₀	organic	cattle litter manure, N_{170}
GCM ₈₅	organic	granulated cattle manure, N_{85}
GCM ₁₇₀	organic	granulated cattle manure, N_{170}
GCM ₁₇₀ + A	organic	granulated cattle manure, N_{170} + biological additive No. 1: <i>Azotobacter</i> spp., a mixture of two cultures (<i>A. chroococcum</i> and <i>A. vinelandii</i>) together with the residues of the culture medium, microelements (manganese, iron, copper, molybdenum, zinc, and cobalt), and vitamins (B1, B3, and B6), no more than 0.02%;
GCM ₁₇₀ + T	organic	granulated cattle manure (N_{170}) + biological additive No. 2: <i>Trichoderma</i> spp., a non-commercial biological additive enriched with phytohormones – a mixture of three cultures (<i>T. harzianum</i> , <i>T. tomentosum</i> , and <i>T. viride</i>)
GPM ₈₅ + MF	organic + mineral	granulated poultry manure, N_{85} + mineral fertilisers, $N_{90/60}$
GCM ₈₅ + MF	organic + mineral	granulated cattle manure, N_{85} + mineral fertilisers, $N_{90/60}$

Table 2. Description of the field experiment crop rotation fertilisation scheme

Fertilisation	Winter rye (2018–2019)	Spring barley (2020)	Potatoes (2021)	Spring wheat (2022)	Winter rye (2023–2024)
Control ($N_0P_0K_0$)	–	–	–	–	–
MF*	$N_{90}P_{60}K_{90}$	$N_{60}P_{60}K_{60}$	$N_{90}P_{60}K_{90}$	$N_{60}P_{60}K_{60}$	$N_{90}P_{60}K_{90}$
PLM ₁₇₀ **	N_{170}	–	N_{170}	–	N_{170}
GPM ₈₅ ***	N_{85}	–	N_{85}	–	N_{85}
GPM ₁₇₀ **	N_{170}	–	N_{170}	–	N_{170}
GPM ₁₇₀ + A**	N_{170} + A	A	N_{170} + A	A	N_{170} + A
GPM ₁₇₀ + T**	N_{170} + T	T	N_{170} + T	T	N_{170} + T
CLM ₁₇₀ **	N_{170}	–	N_{170}	–	N_{170}
GCM ₈₅ ***	N_{85}	–	N_{85}	–	N_{85}
GCM ₁₇₀ **	N_{170}	–	N_{170}	–	N_{170}
GCM ₁₇₀ + A**	N_{170} + A	A	N_{170} + A	A	N_{170} + A
GCM ₁₇₀ + T**	N_{170} + T	T	N_{170} + T	T	N_{170} + T
GPM ₈₅ *** + MF*	N_{85} + $N_{90}P_{60}K_{90}$	$N_{60}P_{60}K_{60}$	N_{85} + $N_{90}P_{60}K_{90}$	$N_{60}P_{60}K_{60}$	N_{85} + $N_{90}P_{60}K_{90}$
GCM ₈₅ *** + MF*	N_{85} + $N_{90}P_{60}K_{90}$	$N_{60}P_{60}K_{60}$	N_{170} + $N_{90}P_{60}K_{90}$	$N_{60}P_{60}K_{60}$	N_{85} + $N_{90}P_{60}K_{90}$

Note. * – N_{90} kg ha⁻¹ for winter rye and potatoes and N_{60} kg ha⁻¹ for spring barley and spring wheat; the rate of organic fertiliser was calculated based on ** – 170 kg ha⁻¹ and *** – 85 kg ha⁻¹ of N active substance.

The meteorological conditions are an important indicator influencing the abundance of soil microorganisms, which are physiologically active in wet soils and dormant in dry ones (Dwevedi et al., 2017). The

meteorological conditions are described using the data of the Lithuanian Hydrometeorological Service, recorded in Trakų Vokė, Vilnius City Station, and presented in Figure 1.

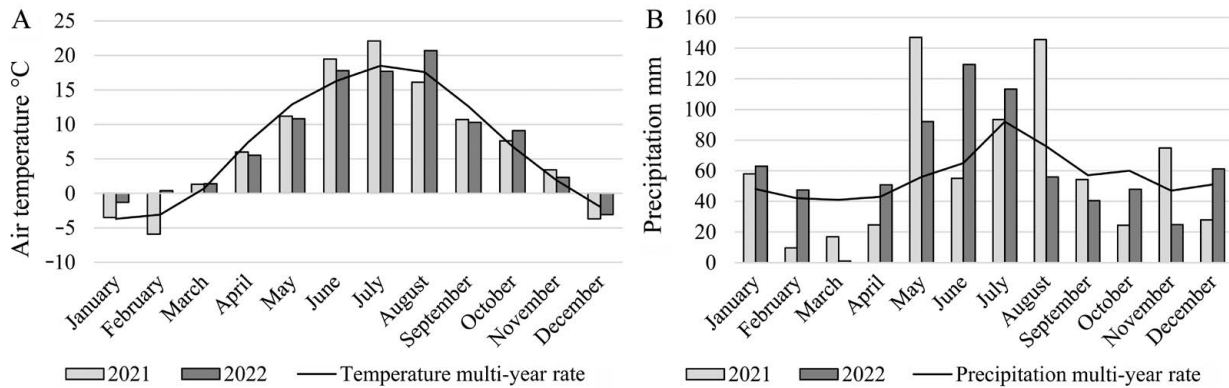


Figure 1. Average temperature (A) and average precipitation (B) in 2021 and 2022

The spring of 2021 started relatively warm, with temperatures 0.6°C above the multi-year rate (MYR) in March, but April and May were cooler. The summer of 2021 was warm, with the temperature 3.2°C above the MYR in June, 3.6°C above the MYR in July, and August was cooler. The summer of 2021 was warmest since the beginning of the meteorological observations. 2021 became the third hottest year in a row. All months of the growing season were particularly rainy. Twice as much precipitation fell in August and four times more above the MYR in May. March of 2022 was very dry and slightly warmer than normal -0.6°C above the MYR. There was very little precipitation during the month. April and May were relatively cold (1.5°C and 1.8°C below the MYR) and relatively wet. June was warm and significantly wetter than normal but contrasting in terms of temperature and precipitation. July was cooler and wetter than usual. August was warm (2.8°C above the MYR) and dry (52% of the MYR). September was cooler and drier than usual. The average air temperature in September was 10.7°C (3.8°C above the MYR). The average precipitation during this period was 1.5 mm (7% of the MYR).

Statistical analysis. Data of the abundance of soil microorganisms are presented as the mean \pm standard error (SE). The analysis was performed using the SPSS Statistics, version 26 (IBM Corp., USA). To assess the difference between the 2021 and 2022 data, the Duncan's multiple range test was used ($p < 0.05$).

Results and discussion

Abundance of different functional groups of soil microorganisms. To evaluate the influence of fertilisation on the most active soil microorganisms, a quantitative analysis of different cultivable functional groups of soil microorganisms was performed. The abundance of cultivable fungal, organotrophic, N_{\min} -assimilating, and non-symbiotic diazotrophic bacteria in 2021 and 2022 was analysed (Figures 2–9).

The results of the study showed that the organotrophic bacteria (except the sample with MF) were most abundant ($113 \pm 3 \times 10^3 \text{ CFU g}^{-1}$) in the

spring 2021. An increase in organotrophs was expected because the manure was added in the autumn 2020. The highest abundance of organotrophs in the spring was found in the soil fertilised with GCM_{170} (Figure 2). Their abundance was also high in other soils fertilised with granulated cattle manure and PLM_{170} . Compared to 2020, the highest number of organotrophs ($120 \pm 3 \times 10^3 \text{ CFU g}^{-1}$) was found in the summer (Sivojiene et al., 2021). It is likely that there was no active manure processing in the spring 2020, because the environmental conditions were unfavourable. The results of the quantitative analysis of organotrophic bacteria in 2022 showed that organotrophs were most abundant ($91 \pm 2 \times 10^3 \text{ CFU g}^{-1}$) in the spring (Figure 3). The experimental data of summer and autumn were very similar and showed a slight decrease in the abundance of organotrophic bacteria. It is likely that in 2022, the amount of organic matter in the soil was similar in the spring–autumn period. Organic fertilisers were added to the soil in the autumn 2020, and most of the organic matter was decomposed by organotrophs in 2021. The results of Ren et al. (2018) study also showed that the application of organic fertilisers provided a sufficient amount of carbon and nitrogen for microbial growth and reproduction.

During the experiment, it was analysed whether different organic fertilisers and their rates based on the N content affect the abundance of organotrophic bacteria. It was found that the use of organic fertilisers had a significant or very significant effect on the abundance of organotrophic bacteria in the PLM_{170} , GPM_{170} , $\text{GPM}_{170} + \text{A}$, $\text{GPM}_{170} + \text{T}$, CLM_{170} , GCM_{170} , $\text{GCM}_{170} + \text{A}$, and $\text{GCM}_{170} + \text{T}$ treatments during the 2021 and 2022 experimental years. However, in the MF treatments and where the rate of the organic fertilisers was twice as low as the N content, no significant differences and no effect of fertilisers on the abundance of organotrophic bacteria were found.

The highest abundance of N_{\min} -assimilating bacteria ($162 \pm 5 \times 10^3 \text{ CFU g}^{-1}$) was in the autumn 2021, and it increased in most samples compared to the control (Figure 4). Nitrifiers were highly abundant in MF, granulated poultry manure, and CLM_{170} fertilised soils. The lowest abundance of N_{\min} -assimilating bacteria

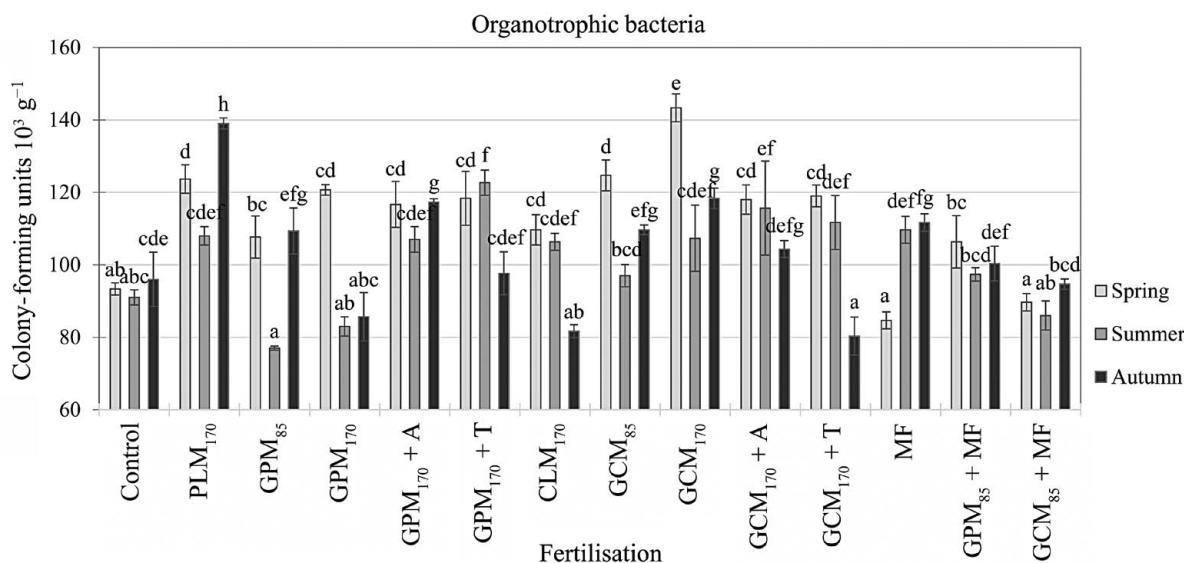


Figure 2. The abundance of organotrophic bacteria in 2021 ($p < 0.05$)

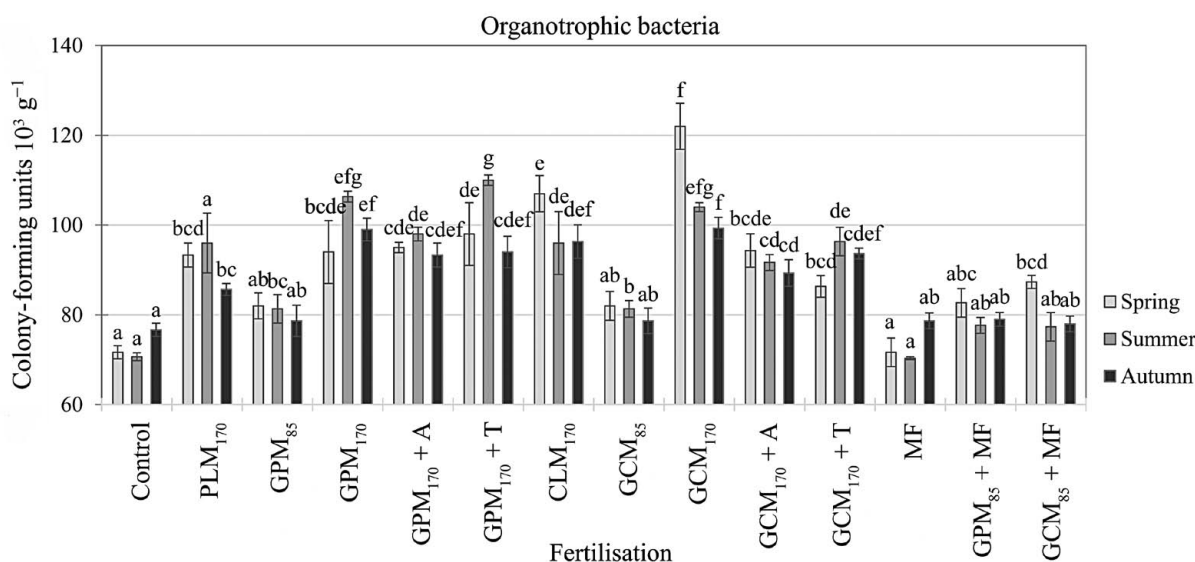


Figure 3. The abundance of organotrophic bacteria in 2022 ($p < 0.05$)

was in the spring 2021, which is understandable because organotrophs dominated in the spring. As in 2020, an increase in the abundance of N_{\min} -assimilating bacteria ($181 \pm 6 \times 10^3$ CFU g^{-1}) was found in the autumn 2021 (Sivojiene et al., 2021).

According to the studies of other authors (Piaulokaitė-Motuzienė, Končius, 2006; Bakšienė et al., 2014), the amount of organotrophic bacteria increases at the beginning of the growing season after fertilisation with organic fertilisers. As in the present study, the amount of N_{\min} -assimilating bacteria increased at the end of the growing season. The results of the similar study of Ye et al. (2020) showed that the relative abundance of some nitrifying bacteria decreased by 40% after application of organic fertilisers. In the present study, fertilisation with organic fertilisers had a significant effect on the soil functional bacteria involved in the soil carbon and nitrogen cycle.

Microorganisms of many functional groups are involved in the decomposition of organic matter. First, bacteria decompose organic matter. After evaluating the abundance of soil microorganisms during the transformation of organic matter, the performance of organotrophs was initially observed. Later, their activity weakened. Then the number of N_{\min} -assimilating bacteria which use nitrate nitrogen to form their cellular structure increased (Piaulokaitė-Motuzienė, Končius, 2006).

The results of 2022 showed that the highest abundance of nitrifiers ($128 \pm 2 \times 10^3$ CFU g^{-1}) was in the spring, while their abundance continuously decreased in the summer and autumn (Figure 5). This is the opposite of the trend in 2021, as the abundance of N_{\min} -assimilating bacteria increased continuously from the spring to autumn in 2021. The amount of N_{\min} in the soil decreased in 2022, which resulted in a decrease of nitrifiers as well as organotrophs.

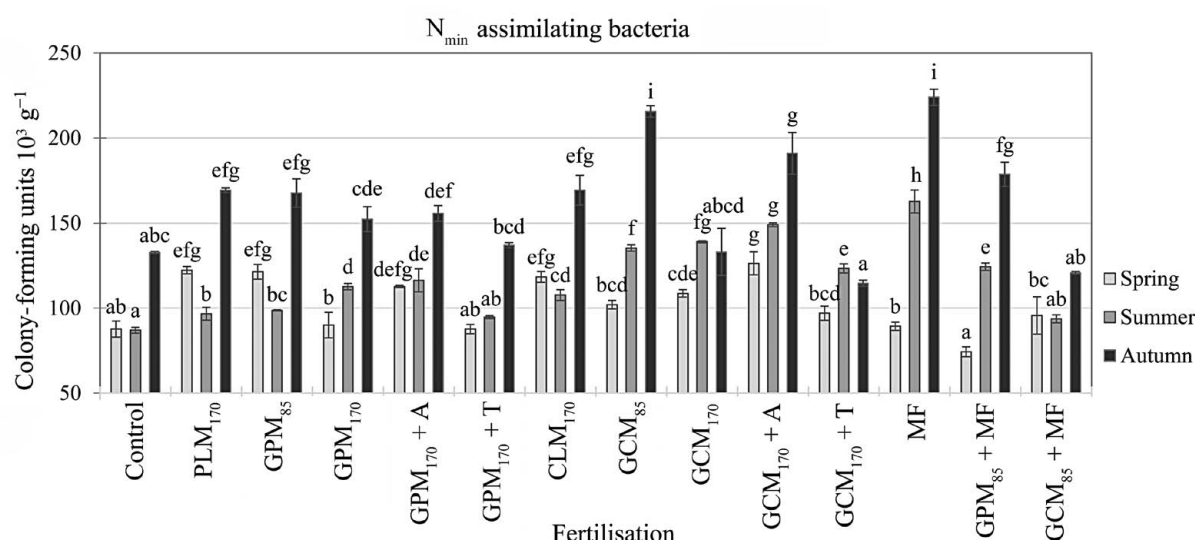


Figure 4. The abundance of mineral nitrogen (N_{\min})-assimilating bacteria in 2021 ($p < 0.05$)

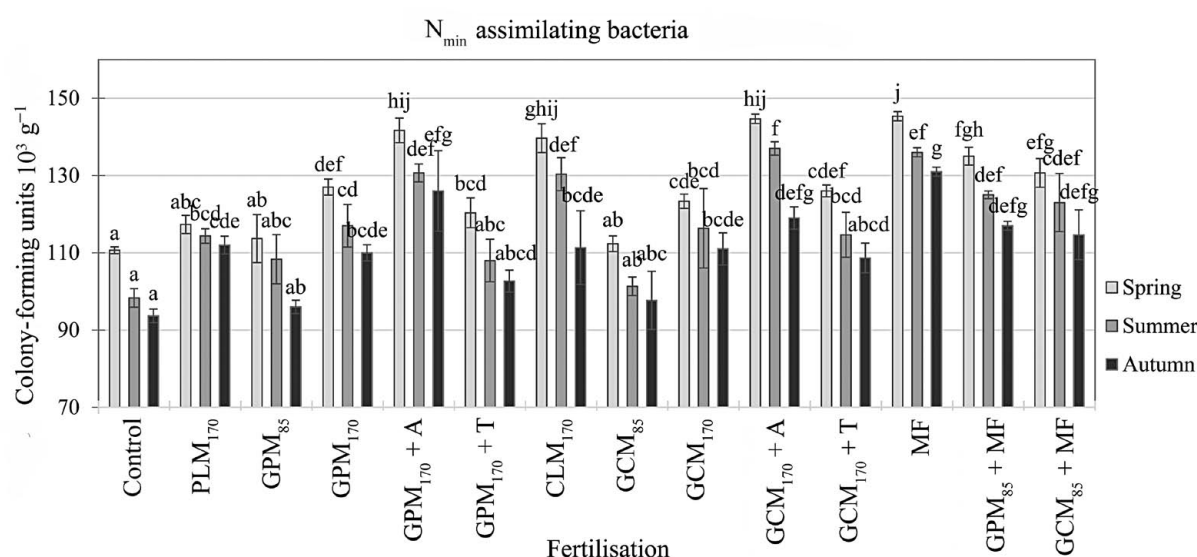


Figure 5. The abundance of mineral nitrogen (N_{\min})-assimilating bacteria in 2022 ($p < 0.05$)

The analysis of different fertilisation treatments revealed that in almost all cases the abundance of N_{\min} -assimilating bacteria was related to the use of fertilisers, and a significant or very significant effect on the abundance of nitrifiers was found. Significant differences and the effect of fertilisers on the abundance of nitrifiers were not determined at the $GPM_{170} + T$ and $GCM_{85} + MF$ treatments in 2021 and at the GPM_{85} and GCM_{85} ones in 2022. Gautam et al. (2020) also found that the application of organic fertilisers stimulated some groups of microorganisms and influenced changes in the composition of bacteria in the soil depending on the fertiliser and its rate.

Diazotrophs were most abundant ($161 \pm 6 \times 10^3$ CFU g^{-1}) in the spring 2021, and their abundance decreased significantly in the summer and slightly increased in the autumn (Figure 6). The highest abundance of diazotrophs was in the soil fertilised with MF compared to the control. A high abundance of diazotrophs was also found

in the soils fertilised with PLM_{170} , granulated poultry and cattle manure. The lowest abundance of diazotrophs was found in the soils treated with the biological additive *Trichoderma* spp. In 2022, diazotrophs were also most abundant ($100 \pm 1 \times 10^3$ CFU g^{-1}) in the spring. The abundance of diazotrophs decreased slightly in the summer, while in the autumn it was very similar.

Evaluating the effect of fertilisation on the abundance of non-symbiotic diazotrophic bacteria, significant differences were determined in the PLM_{170} , GPM_{85} , GPM_{170} , $GPM_{170} + A$, CLM_{170} , GCM_{170} , and $GCM_{170} + A$ treatments. However, no significant differences were found in the biological additive *Trichoderma* spp. and $GPM_{85} + MF$, $GCM_{85} + MF$, and GCM_{85} treatments (Figure 7).

The results of Wu et al. (2021) study showed that increasing the amount of organic fertilisers promotes the growth of N_{\min} -assimilating bacteria. It was also found that the determinant factor of bacterial composition was

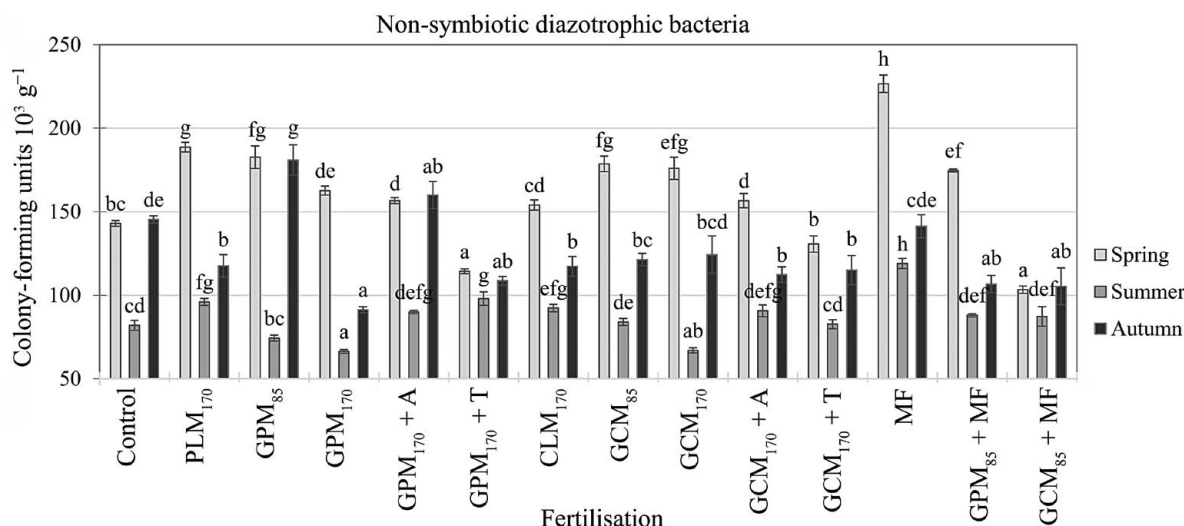


Figure 6. The abundance of non-symbiotic diazotrophic bacteria in 2021 ($p < 0.05$)

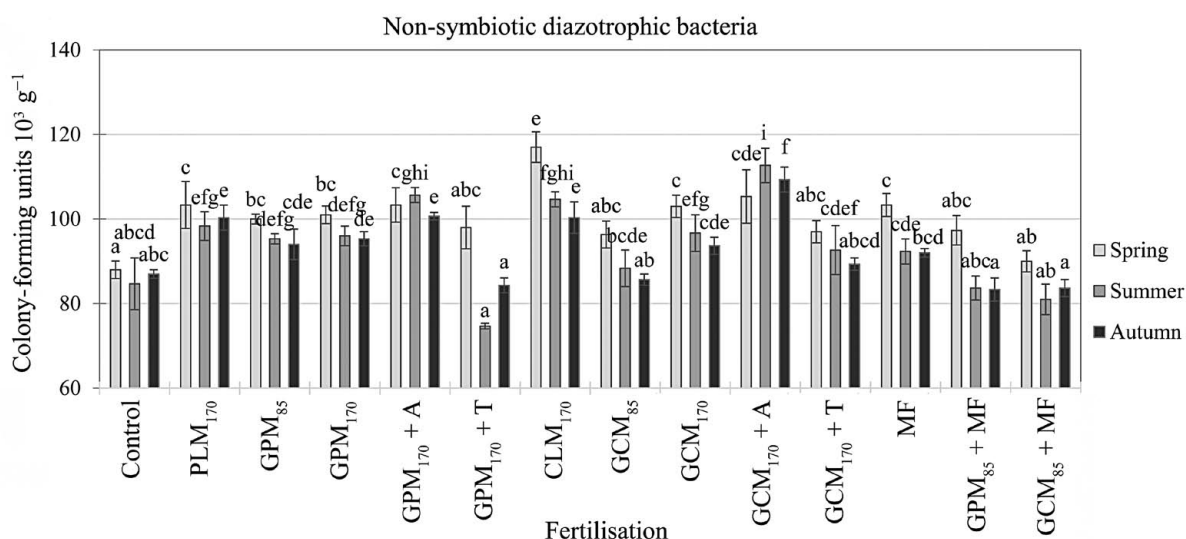


Figure 7. The abundance of non-symbiotic diazotrophic bacteria in 2022 ($p < 0.05$)

closely related to the fertilisation rate. According to the Wu et al. (2021) study results, it can be assumed that a significant decrease of diazotrophs in the summer 2021 was related to the fertilisation rates. It is also likely that it could have been caused by climatic conditions.

In the autumn 2021, the amounts of fungi and yeasts were exceptionally high ($2.54 \pm 0.11 \times 10^3$ CFU g^{-1}) (Figure 8). The lowest abundance of fungi and yeasts was observed in the spring, and with a continuous growth in the autumn, it was almost twice as high as in the spring. The lower abundance of fungi was observed in the soil samples with the biological additive *Azotobacter* spp., MF, and GCM₈₅ compared to the control. The highest abundance of fungi and yeasts was found in the soils treated with the biological additive *Trichoderma* spp. Evaluating the abundance of fungi and yeasts showed that in 2021, the highest abundance of fungi and yeasts was in the autumn. In 2022, the trend remained the same – fungi and yeasts were most abundant ($2.01 \pm$

0.10×10^3 CFU g^{-1}) in the autumn (Figure 9). However, compared to the results of autumn 2021, the abundance of fungi and yeasts was lower in 2022. That was likely due to a lower amount of precipitation than in October–November 2021.

Organic fertilisers significantly or very significantly affected the abundance of fungi and yeasts in the PLM₁₇₀, GPM₈₅, GPM₁₇₀, GPM₁₇₀ + T, GCM₁₇₀, and GCM₁₇₀ + T treatments. In other treatments, no significant effect of fertilisation on the abundance of fungi and yeasts was found. The treatment with the biological additive *Trichoderma* spp. had a higher abundance of fungi and yeasts compared to the treatments without the additive. This may have been influenced by the additives GPM₁₇₀ + T and GCM₁₇₀ + T sprayed on the fields every year.

Evaluating the total abundance of cultivable microorganisms, the organotrophic and non-symbiotic diazotrophic bacteria were most abundant in the spring 2021, and nitrifiers, fungi, and yeasts in the autumn

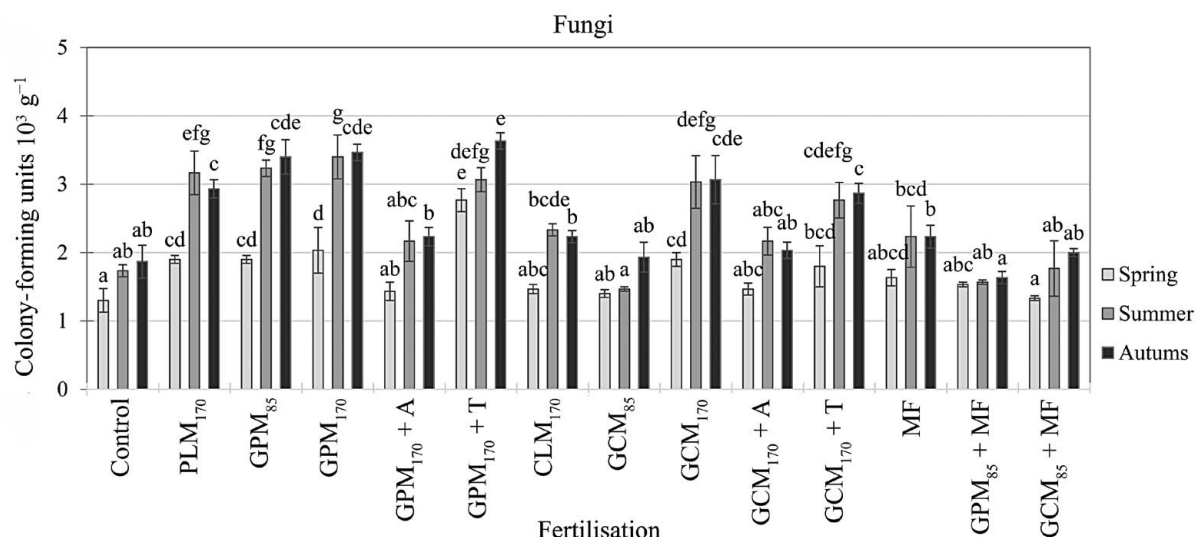


Figure 8. The abundance of fungi and yeast-like fungi in 2021 ($p < 0.05$)

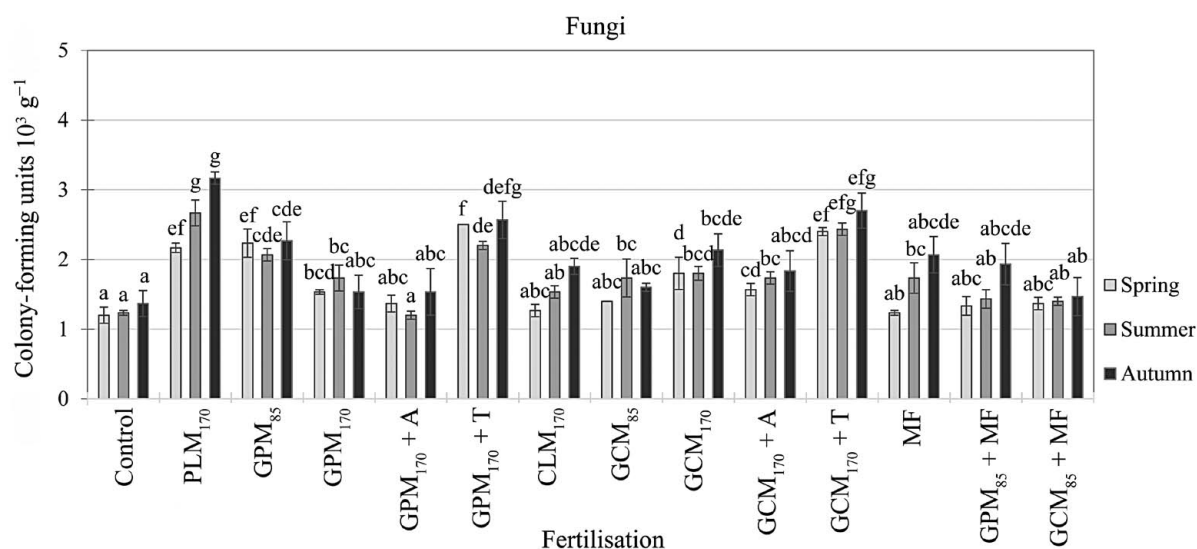


Figure 9. The abundance of fungi and yeast-like fungi in 2022 ($p < 0.05$)

2021. It is likely that the application of organic fertilisers initially increased the number of organotrophic bacteria in the spring and N_{min} -assimilating bacteria in the autumn, because fertilising with manure increases the abundance of bacteria and stimulate some microbial communities (Chen et al., 2016; Francioli et al., 2016; Wu et al., 2021). In 2022, the abundance of organotrophs was similar throughout the growing season. It is possible that these bacteria lacked a freely available organic matter, so there was no significant increase compared to the spring 2021. Unlike in 2021, the N_{min} -assimilating bacteria were most abundant in the spring 2022. This could have been influenced by the organic matter decomposed by organotrophs in 2021, and as a result, the amount of available N increased in the spring 2022. The highest abundance of non-symbiotic diazotrophic bacteria was in the spring 2022, and that of fungi and yeasts was in the autumn 2022.

Comparing the results of the analysis of bacterial abundance in 2020 (a similar study was conducted in 2020, the results were published in Sivojiene et al., 2021), 2021, and 2022, it was found that nitrifiers were more abundant than other groups of bacteria. In 2020–2022, nitrifiers were most abundant in the MF fertilised soil. Fungi and yeasts were more productive in the soils treated with the biological additive *Trichoderma* spp.

Conclusions

1. After analysing the results of the abundance of microorganisms during a two-year experiment, the fertilisation was observed to affect the abundance of the majority of active bacteria and fungi in the soil. After the fertilisation with organic fertilisers in the autumn of 2020, the amount of organotrophic and diazotrophic bacteria increased in the spring, while that of nitrifying bacteria in the autumn 2021.

2. The results of 2022 showed that the abundance of organotrophs was similar throughout the growing season, as it is likely that organotrophs did not have freely available organic matter because more organic fertilisers were not added to the soil. The highest abundance of nitrifying bacteria was found in the spring 2022, which decreased in the summer–autumn period.

3. Throughout the study period, the abundance of fungi and yeasts was highest in the autumn, and fertilisation did not correct this trend, although significant differences were found in the PLM_{170} , GPM_{85} , $GPM_{170} + T$, GGM_{170} , and $GCM_{170} + T$ treatments. Therefore, the likely abundance of fungi and yeasts is more related to seasonality and meteorological conditions.

4. After evaluating the effect of different organic fertilisers and their rates on the most active soil bacteria and fungi, it was determined that all used organic fertilisers (whose rates were calculated based on 170 kg ha⁻¹ of N active substance) had a significant or very significant effect on the abundance of organotrophic and mineral nitrogen (N_{min})-assimilating bacteria.

5. The abundance of non-symbiotic diazotrophic bacteria was also significantly or very strongly influenced by most of the organic fertilisers used, but the treatments with the biological additive *Trichoderma* spp. were the most effective. No significant differences were found in the $GPM_{170} + T$ and $GCM_{170} + T$ treatments as well as in the treatments where the rates of organic fertilisers were calculated based on 85 kg ha⁻¹ of the N active substance.

6. It was found that organic fertilisers had a significant or very significant effect on the abundance of fungi and yeasts in the treatments with the biological additive *Trichoderma* spp. as well as in the PLM_{170} , GPM_{85} , GPM_{170} , and GCM_{170} ones. However, no significant differences were found in the treatments with the biological additive *Azotobacter* spp.

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