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INFLUENCE OF NUTRIENT FACTORS AND COVER CROPS ON THE COMPOSITION OF BACTERIA, FUNGI, AND ACTINOMYCETES IN SOILS IN SOUTHEASTERN KAZAKHSTAN

G. YESSENBAYEVA*, S. KENENBAYEV, Y. DUTBAYEV, and K. ZHUMANOV

Kazakh National Agrarian Research University, Kazakhstan
*Corresponding author's email: gyessenbayeva@list.ru
Email addresses of co-authors: skenenbayev@bk.ru, yerlan.dutbayev@bk.ru, kairat.zhumanov.51@bk.ru

SUMMARY

The paper reports the results of a study conducted in 2023 in the Baltabai rural district, Yenbekshikazakh district, Almaty Region, Kazakhstan. The study centered on analyzing soil samples collected as part of field experiments. The research goal was to assess the influence of various organic farming technologies on soil microorganisms, such as bacteria, fungi, and actinomycetes. The results proved highly significant for microbiological research as they highlight the effect of various environmental conditions on the growth and development of microorganisms. The study reveals that using organic farming technology in the combination of winter peas and corn as a cover crop promotes a higher concentration of bacteria than traditional cultivation methods. Cultivating soybeans with organic agriculture resulted in the highest levels of bacteria. Cultivation of winter peas for green mass, its use of soybean cover in organic agriculture, and their variants becoming straw as organic fertilizers helped increase actinomycete concentration. It proved that Gause's agar, Ashby's medium, and meat-peptone agar provide optimal conditions for the growth of bacteria.

Meanwhile, Czapek-Dox and Hutchison's media do not support their growth at specified dilution rates. Actinomycetes fruitfully multiplied in Gause's and Hutchison's media at the 10^-3 dilution rate. Ashby's medium, meat-peptone agar, and starch ammonia agar at the dilution rate of 10^-5 showed no positive effect on the growth of fungi, while Czapek-Dox, Gause's, and Hutchison's media diluted at the rate of 10^-3 provided optimal conditions for soil fungi production. The findings of this study can find practical applications in quality control and safety assurance in medicine, food production, and agriculture.

Keywords: Cover crops, culture media, soil bacteria, fungi, actinomycetes, organic farming, Kazakhstan

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Key findings: Organic farming methods, particularly winter peas and winter peas intercropped with corn, resulted in higher bacterial concentrations than conventional farming practices. Soybean cultivation within the organic farming framework displayed the highest bacterial concentration among the tested crops. Cultivating winter peas for green mass and using soybean as a cover crop in organic farming, especially when combined with straw as organic fertilizer, led to elevated concentrations of actinomycetes.

INTRODUCTION

Environmental issues and sustainable agriculture became increasingly pressing, spurred by new challenges like climate change, depleting natural resources, and biodiversity loss, particularly in developing countries (Scavo *et al*., 2022). With these problems, organic agriculture becomes highly relevant. The global market for organic products has grown more than six-fold over the past two decades, surpassing the USD 106 billion mark in 2020. A projected growth continues at a 15%–16% rate annually. The Grand View Research expects the market to reach USD 212–230 billion by 2025. In this context, Kazakhstan ranks 29th among 186 countries by volume of organic agriculture (Karabassov *et al*., 2022).

However, intensifying agricultural processes cause serious environmental problems. Depleting soil microbial composition, decreasing beneficial soil microorganisms, and greenhouse gas release are becoming crucial issues. In turn, microorganisms and their activity, including breathing, methanogenesis, nitrification, and denitrification, have a critical part in regulating greenhouse gas flows over time (Singh and Kumar, 2021; Kussainova *et al*., 2023).

Research shows that various factors, such as soil temperature, water content, accessibility of nutrients, pH, and soil processing methods, influence microbial processes in soils, thus being critical for the sustainability of agriculture (Stark and Firestone, 1995; Lauber *et al*., 2009; Singh and Kumar, 2021). In addition, different plant species and crop rotations can also considerably affect the composition and biomass of soil microbial communities (Mitchell *et al*., 2010; Calderón *et al*., 2017).

Soil bacteria, fungi, and actinomycetes, as key players in the soil ecosystem, are fundamental to maintaining soil stability and ensuring soil fertility (Mącik *et al*., 2020). Long-term application of composted manure contributes to soil carbon stock buildup, retaining nitrogen as microbial or stabilized organic nitrogen, and the increased abundance of microorganisms involved in denitrification, lowering C_2O emissions. This process is conducive to the completion of denitrification for dinitrogen production (Lazcano *et al*., 2021; Amangaliev *et al*., 2023; Kunypiyaeva *et al*., 2023). These premises were the basis for this study, which aims to assess the influence of nutrient substrate and cover crops on the structure of soil microbial communities in the Almaty Region of Kazakhstan.

MATERIALS AND METHODS

Soil samples collected during the vegetation period of plants were part of field experiments conducted by the Baltabai 2030 LLP in 2023 at the Baltabai rural district, Yenbekshikazakh, Kazakhstan (latitude 43°30’23.256”, longitude N E 77°32’38.76”). The study assessed the effect of the factor of organic farming technology on the variable counts of bacteria, actinomycetes, and fungi. The variants of the experiment are available in Table 1.

In evaluating the effect of artificial nutrient media on the content of bacteria, actinomycetes, and fungi in soil, the following experiment variants progressed (Table 2). The number of grown colonies calculated in each Petri dish ensued by counting individual colonies. The best dilution rates were those that produced 30 to 300 colonies when sown on an agarized nutrient medium.

Table 1. Variants of the experiment.

Variant	Description
1	winter peas for green mass
2	winter peas for sideral fertilizer (in the budding phase)
3	winter peas for grain (with straw removed from the field)
4	winter peas for grain (with straw as organic fertilizer)
5	winter peas under corn cover (organic farming technology)
6	winter peas under soybean cover (organic farming technology)
7	corn cultivated under traditional technology
8	soybeans cultivated under traditional technology

(Source: compiled by the authors)

Table 2. Variants of the experiment in different media.

<table>
<tr><th>Medium</th><th>Composition</th><th>Description</th></tr>
<tr><td>Gause's agar</td><td>Composition (g): Starch – 20.0, KNO3 – 1.0, K2HPO4 – 0.5, MgSO4 – 0.5, NaCI – 0.5, FeSO4 – 0.01, distilled water – 1 L, agar – 12. Goal: To estimate the population size of actinomycetes.</td><td rowspan="3">Gause's agar, Ashby's medium, and meat-peptone agar are designed for cultivating microorganisms in laboratory conditions. It is important to note that exact proportions and concentrations may vary slightly depending on the specific protocol or technique used in the laboratory (Nguyen et al., 2020; Coyne et al., 2023; Gorsuch and Buckman, 2023).</td></tr>
<tr><td>Ashby's medium</td><td>Composition (g): Mannitol – 20.0, K2HPO4 – 0.2, MgSO4*7H2O – 0.2, NaCl – 0.2, K2SO4 – 0.1, CaCO3 – 5.0, distilled water – 1 L, agar – 12. Goal: To estimate the population size of anaerobic nitrogen fixers (Akhmetov et al., 2022).</td></tr>
<tr><td>Meat-peptone agar (MPA) medium</td><td>Composition (g): Meat broth – 1.0 L, dry fermentation peptone – 10.0, NaCl – 5.0, agar – 10.0. Goal: To estimate the amount of microorganisms that utilize the organic form of nitrogen.</td></tr>
<tr><td>Starch ammonia agar (SAA)</td><td>Composition (g): Starch – 20.0, (NH4)2SO4 – 1.0, MgSO4*7H2O – 1.0, NaCl – 1.0, CaCO3 – 3.0, distilled water – 1 L, agar – 12. Goal: To detect actinomycetes and bacteria that use the inorganic form of nitrogen.</td><td rowspan="2">The Czapek-Dox and Hutchison's media are two different culture media used in microbiology to isolate and cultivate various microorganisms. Both are designed for growing microorganisms in laboratory conditions (Romero-Jiménez et al., 2022; Pinchi-Davila et al., 2023). Substances, such as sucrose, sodium nitrate, and others, provide essential nutrients for microbial growth and reproduction. Agar serves as a gelling agent, making the medium solid to allow bacterial growth in the form of colonies (Romero-Jiménez et al., 2022; Pinchi-Davila et al., 2023).</td></tr>
<tr><td>Czapek-Dox medium</td><td>Composition (g): Sucrose – 20.0, NaNO3 – 2.0, K2HPO4 – 1.0, MgSO4 – 0.5, KCI – 0.5, FeSO4 – 0.01, distilled water – 1 L, agar – 12. Goal: To estimate the population size of microscopic fungi.</td></tr>
</table>

The calculation of CFU per 1 ml of the biopreparation used the following formula:

$$M = \frac{a \times 10^n}{V} \quad (1)$$

Where:
M – Cell count per 1 ml;
A – Average number of colonies when sown from the given dilution;
V – Volume of the suspension taken for sowing in ml – 0.1 ml;

10 – Dilution rate;
n – Serial number of the dilution (Akhmetov *et al*., 2022).

The statistical data processing employed the Integrated Development Environment (IDE) for the R programming language. Given that the study involved three or more samples, an assessment of the significance of the variables used the nonparametric one-factor Kruskal-Wallis analysis of variance, with the p-value determining statistical significance. The research applied a critical significance level of <0.05 (Dutbayev *et al*., 2022a, b).

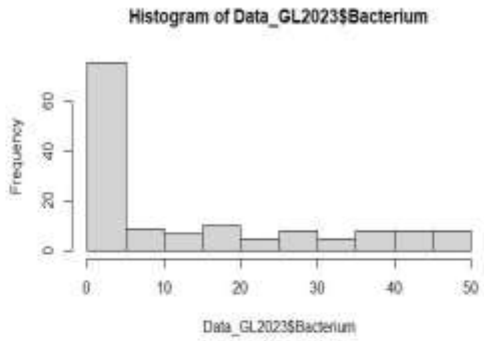
Boxplot generation helped visualize the impact of cultural practices and culture media factors on the soil's number of bacteria, actinomycetes, and fungi. These visual representations help illustrate the distribution and variability of the data, providing insights into the observed effects (Kenenbayev *et al*., 2023). The general population of the variables of bacteria, actinomycetes, and fungi counts in soil incurred analyses. The variables emerged to be non-normal, skewed to the left. Therefore, we applied the nonparametric analog of Kruskal-Wallis one-factor analysis of variance.

RESULTS

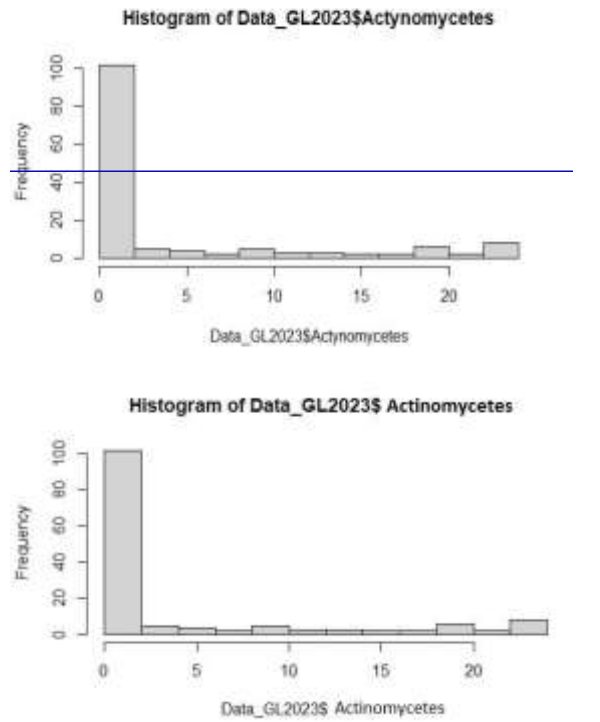
The results of the nonparametric equivalent of the Kruskal-Wallis one-factor analysis of variance appear in Figure 1. As shown in columns 1 and 2 of Figure 2, data present the cultivation of winter peas for grain with straw removed from the field and their cultivation with corn as a cover crop (in the framework of organic agriculture technology), respectively. The average number of soil bacteria in these two variants ranges from 4.4 to 6 million CFU/ml. Columns 3 and 4 (winter peas cultivated for grain with straw as an organic fertilizer and for sideral fertilization) show higher concentrations of bacteria: 24.1 and 26.7 million CFU/ml, respectively. In the soil sample representing the traditional technology of corn cultivation, bacteria concentration amounts to 39.8 million CFU/ml. The variant of winter pea cultivation for green mass demonstrates a much higher concentration of bacteria – 54.0 million CFU/ml. Finally, the greatest concentration of bacteria is evident in the variants of soybean cultivation under the traditional technology and winter peas with soybean as a cover crop (using organic farming technology) – 92.9 and 95.8 million CFU/ml, respectively (Figures 2, 7).

The variants with winter peas, including the cultivation of winter peas with soybean as a cover crop (organic farming technology), corn cultivated under traditional technology, and winter peas for grain (with straw removed from the field), demonstrated similar concentrations of actinomycetes, ranging around 7.3*10^4 to 11.2*10^4 CFU/ml. Under traditional soybean cultivation, the average actinomycete concentration in soil rises to 10.7*10^4 CFU/ml. Cultivation of winter peas for green mass also showed a high n actinomycete concentration at 17.8*10^4 CFU/ml. The variant of winter peas for grain (with straw as organic fertilizer) demonstrated an even superior actinomycete concentration, reaching up to 24.6*10^4 CFU/ml. All the discovered differences between variants by concentration of actinomycetes are statistically significant, as confirmed by p-value < 0.001 (Figures 3, 7).

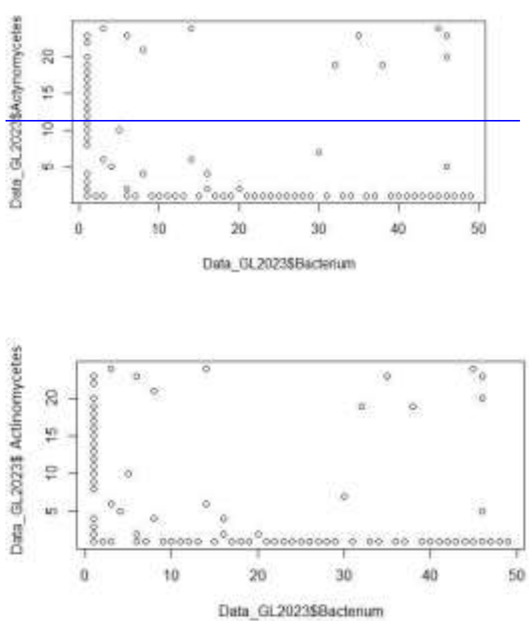
The variants of corn cultivated per traditional technology, winter peas for green mass, winter peas under corn cover (organic farming technology), and soybean under traditional technology have a low concentration of fungi (1–1.2*10^4 CFU/ml), which may indicate that the other variants have some shared features with customary corn cultivation. The variant of winter peas grown for sideral fertilizer showed some increase in the fungi concentration (1.5*10^4 CFU/ml). Using straw as an organic fertilizer considerably increases fungi content (2*10^4 CFU/ml), suggesting a positive impact of organic components on the soil's biological activity. In the case of straw removed from the field, the fungi concentration rose to 2.7*10^4 CFU/ml, possibly because of the loss of organic material, hence, worse conditions for fungi. Organic agricultural technology in soybean cultivation came with a primary increase in fungi concentration (3.7*10^4 CFU/ml), which



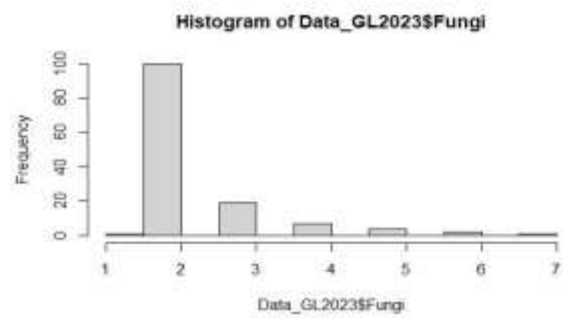
A – general population of data on bacteria counts



B – population of data on actinomycete counts



C – correlation between the number of bacteria and the number of actinomycetes



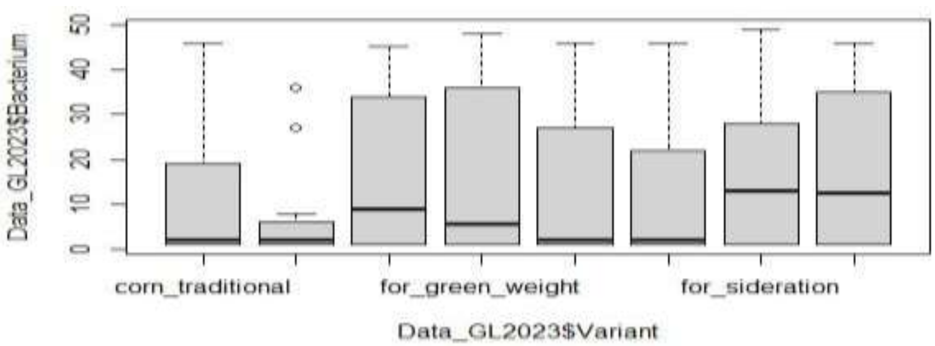
D – population of data on fungi counts

Figure 1. Statistical data on the content of microorganisms in the soil (Baltabai Rural District, 2023).

may indicate more favorable conditions for fungi in this variant. Thus, data analysis highlighted the effect of different cultivation methods on the fungi content in soil, emphasizing the importance of applying organic methods to stimulate soil biological activity (Table 3, Figure 7).

In the medium of starch ammonia agar (SAA), bacteria count reached a moderately high level, and with the medium dilution rate at 10^-5, it amounted to 11.42*10^6 CFU/ml. In Gause's agar (dilution rate 10^-3, 29.96*10^6 CFU/ml), Ashby's medium (dilution rate 10^-5, 63.32*10^6 CFU/ml), and meat-peptone agar (MPA) (dilution rate 10^-5, 72.73*10^6 CFU/ml), the concentration of bacteria was high, indicating favorable conditions for their development. Importantly, bacteria showed no growth in the Czapek-Dox medium (dilution rate 10^-3) and Hutchison's medium (dilution rate 10^-3). Thus, the presented data provided insight into the varying activity of microorganisms in different media and their sensitivity to dilution rate, which is a critical factor when conducting microbiological studies (Figures 4, 7).

Actinomycetes visibly grew in the Gause's agar diluted to 10^-3, with their concentration reaching 10.36*10^4 CFU/ml. In Hutchison's medium at a dilution rate of 10^-3, it showed a concentration of 17.58*10^4 CFU/ml. The concentrations of microorganisms observed in these media are moderate, which may indicate favorable conditions for their multiplication. Thus, the presented data suggest that microorganisms can distinctly grow in different media and their dilutions. This finding is vital for understanding the biological activity of microorganisms and their adaptation to the environment (Figures 5, 7).



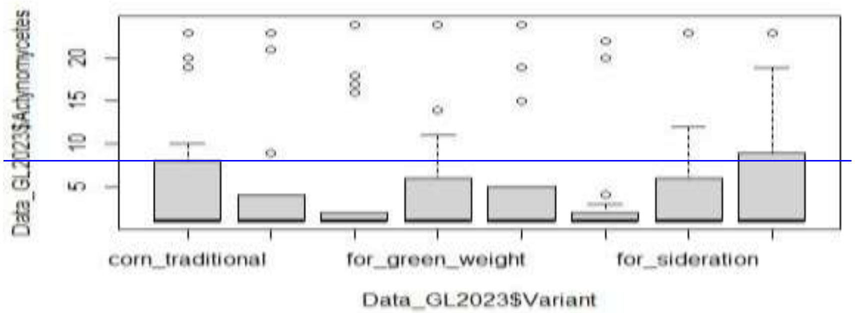
1 2 3 4 5 6 7 8

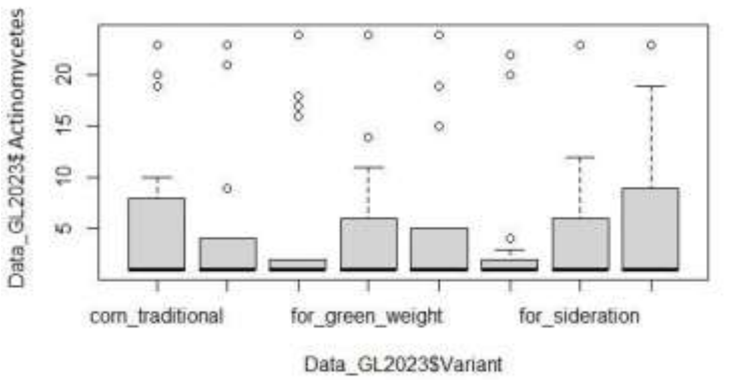
A – bacteria count variable

Note: 1 – corn (traditional technology); 2 – winter peas for grain (with straw removed from the field); 3 – winter peas for green mass; 4 – 5 – winter peas under corn cover (organic farming technology) and winter peas for grain (with straw as organic fertilizer); 6 – winter peas under soybean cover (organic farming technology); 7 – winter peas for sideral fertilizer (in the budding phase); 8 – soybeans cultivated under traditional technology.

Variant	Bacterium CFU/ml
winter peas for grain (with straw removed from the field)	$4.4*10^6$
winter peas under corn cover (organic farming technology)	$6.0*10^6$
winter peas for grain (with straw as organic fertilizer)	$24.1*10^6$
winter peas for sideral fertilizer (in the budding phase)	$26.7*10^6$
corn cultivated under traditional technology	$39.8*10^6$
winter peas for green mass	$54.0*10^6$
soybeans cultivated under traditional technology	$92.9*10^6$
winter peas under soybean cover (organic farming technology)	$95.8*10^6$
p-value	<0.001

Figure 2. Influence of different organic farming technologies on the number of actinomycetes in soil (Baltabai Rural District, 2023).





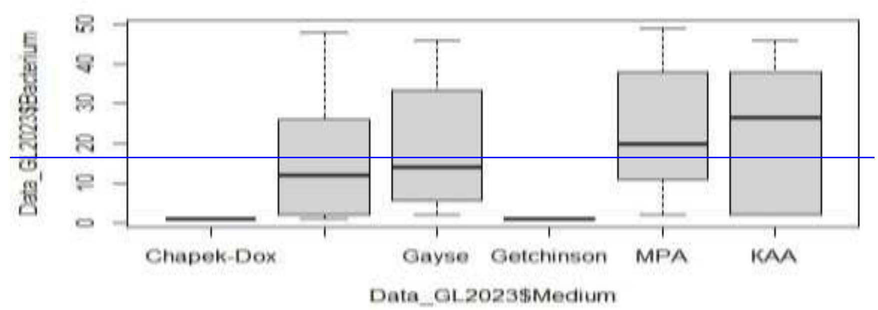
1 2 3 4 5 6 7 8
B – actinomycetes count variable
Note: 1 – corn cultivated under traditional technology; 2 – winter peas for grain (with straw removed from the field); 3 – winter peas for green mass; 4 and 5 – winter peas under corn cover (organic farming technology) and winter peas for grain (with straw as organic fertilizer); 6 – winter peas under soybean cover (organic farming technology); 7 – winter peas for sideral fertilizer (in the budding phase); 8 – soybeans cultivated under traditional technology.

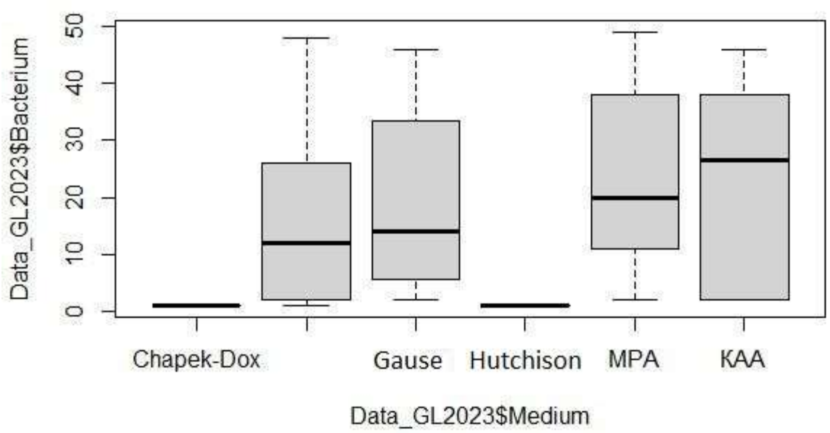
Variant	Actinomycetes, CFU/ml
winter peas under corn cover (organic farming technology)	$7.3*10^4$
winter peas under soybean cover (organic farming technology)	$9.2*10^4$
corn cultivated under traditional technology	$10.7*10^4$
winter peas for grain (with straw removed from the field)	$11.2*10^4$
soybeans cultivated under traditional technology	$16.3*10^4$
winter peas for sideral fertilizer (in the budding phase)	$16.8*10^4$
winter peas for green mass	$17.8*10^4$
winter peas for grain (with straw as organic fertilizer)	$24.6*10^4$
p-value	<0.001

Figure 3. Influence of the factor of different organic farming technologies on the number of actinomycetes in soil (Baltabai Rural District, 2023).

Table 3. Influence of different organic farming technologies on the amount of fungi in soil (Baltabai Rural District, 2023, CFU/ml, $*10^6$).

Variant	Fungi, CFU/ml
corn cultivated under traditional technology	$1*10^4$
winter peas for green mass	$1.2*10^4$
winter peas under corn cover (organic farming technology)	$1.2*10^4$
soybeans cultivated under traditional technology	$1.2*10^4$
winter peas for sideral fertilizer (in the budding phase)	$1.5*10^4$
winter peas for grain (with straw as organic fertilizer)	$2*10^4$
winter peas for grain (with straw removed from the field)	$2.7*10^4$
winter peas under soybean cover (organic farming technology)	$3.7*10^4$
p-value	<0.001





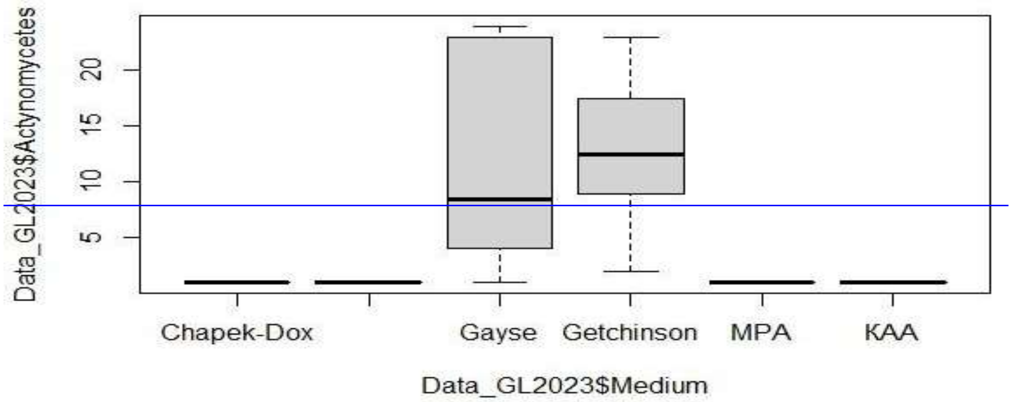
1 2 3 4 5 6
A – bacteria count variable

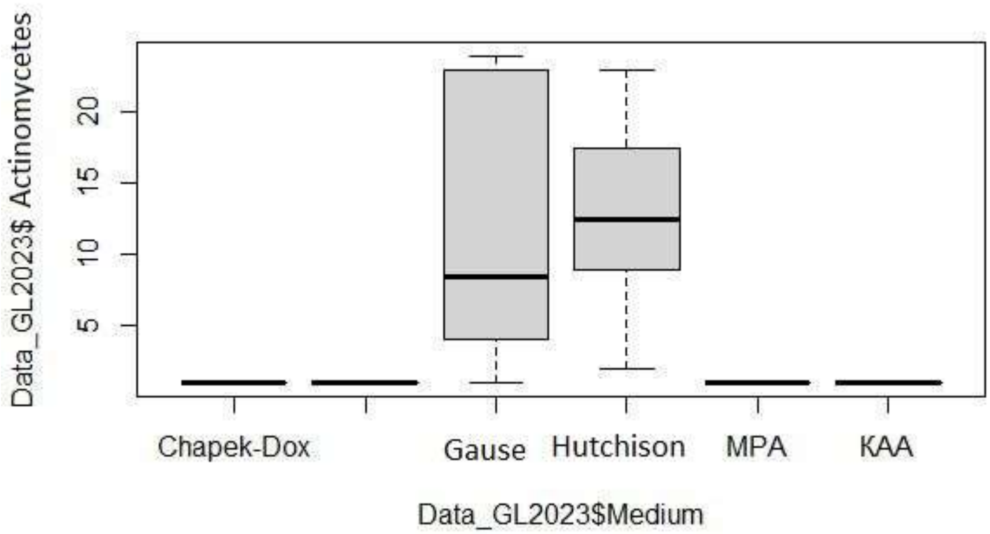
Medium	Bacterium CFU/ml
SAA medium, dilution rate 10^{-5}	$11.42*10^6$
Gause's agar, dilution rate 10^{-3}	$29.96*10^6$
Ashby's medium, dilution rate 10^{-5}	$63.32*10^6$
MPA medium, dilution rate 10^{-5}	$72.73*10^6$
Czapek-Dox medium, dilution rate 10^{-3}	0.0
Hutchison's medium, dilution rate 10^{-3}	0.0
p-value	<0.001

Note: 1 – Czapek-Dox; 2 – Ashby's; 3 – Gause's; 4 – Hutchison's; 5 – MPA; 6 – SAA

Figure 4. Influence of artificial nutrient media on the number of microorganisms in the soil (Baltabai Rural District, 2023, CFU/ml, $*10^6$).

In Ashby's, MPA, and SAA media diluted to 10^-5, the fungi concentration amounts to 0.0 CFU/ml. It may indicate a negative influence of these media on fungal growth at this concentration. In the Czapek-Dox medium at a 10^-3 dilution rate, the fungi concentration reached 1.33*10^4 CFU/ml. Thus, the Czapek-Dox medium could be more suitable for fungal growth at this dilution rate than the previous media. The fungi concentration in Gause's agar and Hutchison's medium at a 10^-3 dilution rate amounted to 1.25*10^4 CFU/ml and 2.89*10^4 CFU/ml, respectively. These media also provided favorable conditions for fungal growth at the specified dilution rate. P-value is below 0.001 suggesting that the described differences in the media influence on microorganism concentrations are statistically significant (Figures 6, 7).



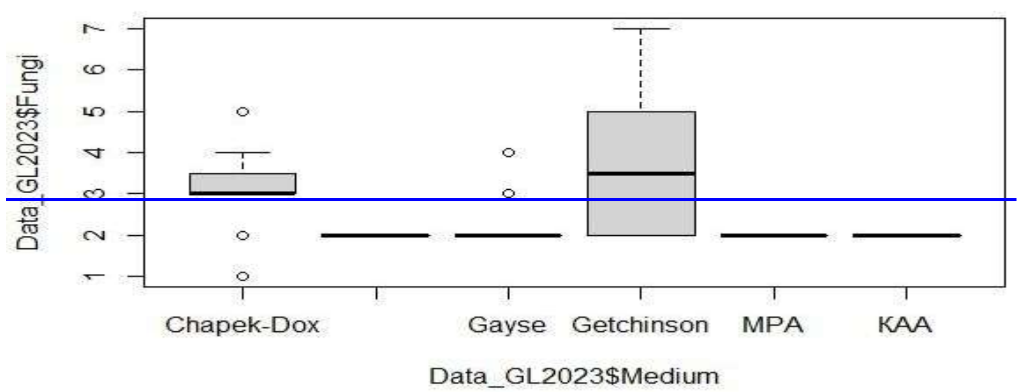


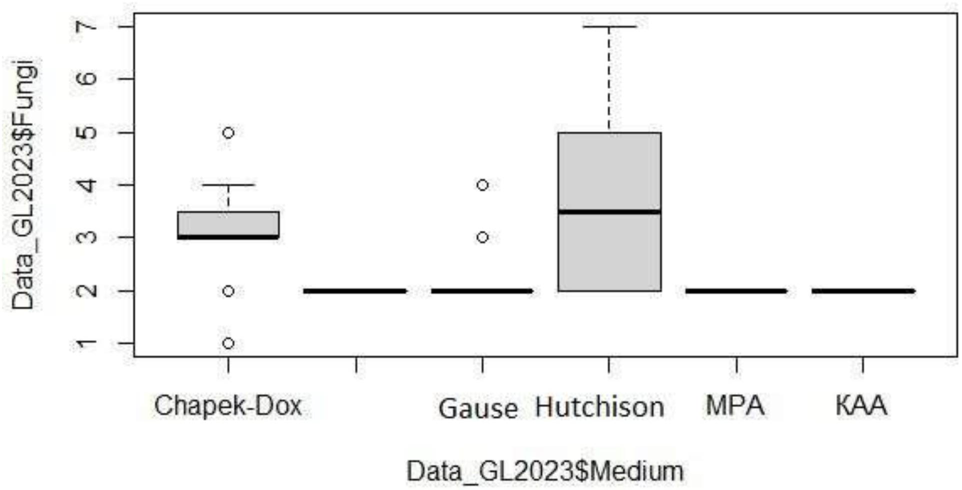
1 2 3 4 5 6
B – actinomycete count variable

Medium	Actinomycetes, CFU/ml,
Czapek-Dox medium, dilution rate 10^{-3}	0.0
Ashby's medium, dilution rate 10^{-5}	0.0
Gause's agar, dilution rate 10^{-3}	$10.36*10^4$
Hutchison's medium, dilution rate 10^{-3}	$17.58*10^4$
MPA medium, dilution rate 10^{-5}	0.0
SAA medium, dilution rate 10^{-5}	0.0
p-value	<0.001

Note: 1 – Czapek-Dox; 2 Ashby's; 3 – Gause's; 4 – Hutchison's; 5 – MPA; 6 – SAA

Figure 5. Influence of artificial nutrient media on the number of actinomycetes in soil (Baltabai rural district, 2023, CFU/ml, $*10^6$).





1 2 3 4 5 6
C – fungi count variable

Medium	Fungi CFU/ml,
Ashby's medium, dilution rate 10^{-5}	0.0
MPA medium, dilution rate 10^{-5}	0.0
SAA medium, dilution rate 10^{-5}	0.0
Czapek-Dox medium, dilution rate 10^{-3}	$1.33*10^{4}$
Gause's agar, dilution rate 10^{-3}	$1.25*10^{4}$
Hutchison's medium, dilution rate 10^{-3}	$2.89*10^{4}$
p-value	<0.001

Note: 1 – Czapek-Dox; 2 Ashby's; 3 – Gause's; 4 – Hutchison's; 5 – MPA; 6 – SAA

Figure 6. Influence of artificial nutrient media on the number of fungi in soil (Baltabai Rural District, 2023, CFU/ml, $*10^{6}$).

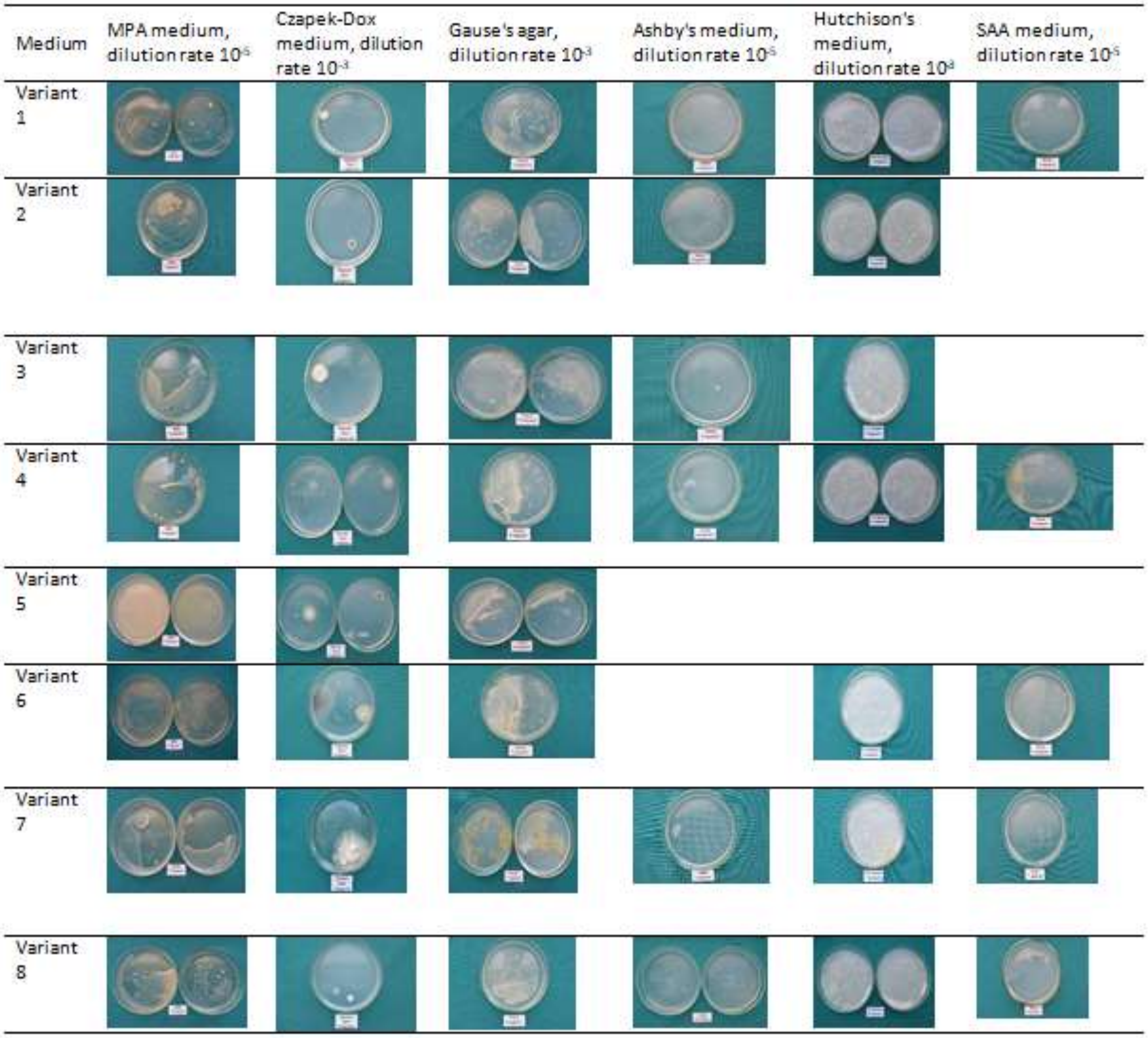


Figure 7. Influence of artificial nutrient media on the number of bacteria, actinomycetes, and fungi in soil (Baltabai Rural District, 2023, CFU/ml, *10^6).

DISCUSSION

In a study by Crystal-Ornelas *et al*. (2021), organic agriculture practices have appeared to raise organic and microbial biomass carbon concentrations. Among these methods, organic fertilizers and soil conservation practices have positively affected soil health. According to Canadian researchers, pea monoculture alone caused a sturdy reduction in bacterial and fungal richness and diversity compared with wheat monoculture and pea-wheat rotation (Woo *et al*., 2022). The results of Ning *et al*. (2021) indicated that corn straw buried deep in the soil can increase the number of bacteria, actinomycetes, and fungi and enhance the activity of urease, invertase, dehydrogenase, polyphenol oxidase, and phospholipid fatty acids, resulting in higher corn grain yields. Our research demonstrated that winter pea variants and winter peas with corn as a cover crop under organic farming technology exhibited higher bacteria concentrations than variants of conventional cultivation.

Sohn *et al*. (2021) determined that the structure of the microbial community of the rhizosphere and nodules of soybean roots depends on the variety and growth stage of soybeans. Actinomycetes were the most abundant in the rhizosphere at all growth stages, followed by Alphaproteobacteria and Acidobacteria, and the Bacteroidota phylum showed supreme changes. However, Alphaproteobacteria dominated root nodules. This study’s findings confirmed that soybean cultivation using organic agriculture results in the maximum concentration of bacteria.

In conclusion, researchers found that a better understanding of the bacterial environment will make it possible to develop new culture media and new growing conditions more adapted to specific bacteria that are difficult to isolate. Agar was the dominant gelling agent used in solid culture media. Using agar has some limitations because some bacteria sensitive to oxygen cannot grow on agar media. For this reason, the proposal and testing of alternative gelling agents emerged (Bonnet *et al*., 2020).

The study found high concentrations of bacteria in Gause's agar, Ashby's medium, and MPA. The Czapek-Dox medium and Hutchison's medium do not support bacterial growth at respective dilution rates. Thus, the research findings may have practical significance in quality control and safety assurance in various fields, including medicine, the food industry, and agriculture.

CONCLUSIONS

Variants with winter peas and winter peas with corn as a cover crop under organic farming technology demonstrated higher bacteria concentrations than variants of conventional cultivation. Soybean cultivation using organic agriculture has linkages with the maximum concentration of bacteria. Cultivation of winter peas for green mass and with soybean covering with organic agricultural practices and variants with straw as organic fertilizer resulted in a high concentration of actinomycetes. High concentrations of bacteria were evident in Gause's agar, Ashby's medium, and MPA, showing they offer favorable conditions for the growth and development of these microorganisms. The Czapek-Dox medium and Hutchison's medium do not support bacterial growth at the respective dilution rates. Actinomycetes exhibited growth in Gause's agar and Hutchison's medium at a dilution of 10^-3, suggesting favorable conditions for their reproduction. Ashby's medium, MPA, and SAA at the dilution rate of 10^-5 revealed a negative impact on the growth of fungi. The Czapek-Dox medium, Gause's agar, and Hutchison's medium diluted at the rate of 10^-3 offer better conditions for the growth of soil fungi. The obtained findings have valuable implications for microbiological research because they emphasize the effects of different environmental conditions on the growth and development of microorganisms.

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