Hungarian University of Agriculture and Life Sciences

The impact of soil-isolated inoculants in ecological farming with various soil management technologies

PHD THESIS

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1. INTRODUCTION AND OBJECTIVES

Preserving soil fertility and health is essential for sustainable agriculture and global food security. Excessive use of fertilizers and chemicals, as well as improper soil management, leads to soil degradation and a reduction in fertility, which negatively affects the environment, including water resources and biodiversity (Kertész, 2019).

Two main movements have emerged to reduce chemical use and prevent soil degradation: organic farming, which uses only approved natural substances (Tirado, 2015), and regenerative agriculture, which strives to minimize tillage (Brown, 2018). These practices serve as alternatives to chemical-based solutions, promoting the maintenance of natural nutrient cycling in soil, increasing biodiversity, and applying eco-friendly techniques. Weed management is crucial in both approaches, achieved on a smaller scale by mulching and on a larger scale through the integration of cover crops and livestock (Meredith et al., 2018; Koltai, 2010).

Hungary is rich in mineral resources, which were previously used in state-managed soil amendment practices to improve soil physicochemical properties, such as structure and acidity, supporting the goals of organic farming and reducing the harmful effects of tillage (Várallyay, 2001). The use of microbial inoculants is also a permitted, globally widespread practice, despite uncertainties about their effectiveness. The application of industrial inoculants could be promising for enhancing soil biological activity and plant-soil health.

These principles not only strengthen the sustainability of agriculture but also contribute to combating climate change. Supporting innovative sustainable practices is crucial for maintaining soil fertility and health, considering the conservation of natural resources and addressing global environmental challenges.

The thesis began with the formulation of hypotheses, which were the focus of the research for verification.

1. Our hypothesis was that microbial strains isolated from the specific soil and pre-tested would be more effective than commercial inoculants not developed for this soil.

2. We assumed that soil microorganisms possess not only known properties but also, through adaptation, may exhibit new, less studied characteristics such as potassium solubilization. This dynamic adaptation allows them to optimally respond to environmental changes and plant needs, thus facilitating plant nutrient supply and stress tolerance.

3. Alginite, a commonly used domestic ameliorative mineral, could favorably influence soil biological properties, thereby affecting the efficacy of inoculants. Nevertheless, its application might be limited on soils that are neither acidified nor nutrient-depleted.

4. We assumed that since different organic matter and "soil carbon" measurement methods target different compound groups, the extent of changes shown by treatments would also differ. In this context, we examined methods measuring labile organic matter content in soil, such as POXC, DOC, and the NaOH extraction method.

5. Various soil treatments can improve soil organic matter content, but the degree of change will vary depending on the organic matter analyzed. Short-term changes are more detectable when measuring labile fractions, but these are less reliable over the long term as seasonality and immediate effects can quickly saturate. Therefore, the use of various methods is

recommended for longer-term monitoring of changes. Although our research timeframe is limited, we aim to indicate directions for longer-term study opportunities.

With these studies, we aim to expand knowledge on the effectiveness of various soil improvement methods and the effects of the combined application of inoculants and mineral soil amendments, especially from the perspective of organic farming. Our goal is to contribute to the development of sustainable agricultural practices and the long-term preservation of soil fertility. Our research particularly focuses on monitoring changes in soil organic matter content. Our results could help alter agriculture's reliance on chemical-based practices, thereby providing protection for the environment and promoting sustainable food production.

2. METHODS AND MATERIALS

2.1 Experimental designs

During the research, we progressed in stages and gradually increased the experimental scale. This process of "upscaling" encompasses both the treatments applied and the testing methods used. The research progressed through the following steps:

Isolation and testing of microorganisms were initially conducted in Petri dishes and cell trays, then in cultivation containers at the Department of Agro-Environmental Sciences (AES) and its light room at the Hungarian University of Agricultural and Life Sciences (MATE), previously known as SZIE. In the field experiment, the effects of microbial inoculants and their interactions with other soil regenertive treatments were studied over several years at the MATE (formerly SZIE) Soroksár ecological institute's demonstration farm.

2.1.1 Isolation, Selection, and Identification of PGP Strains

Isolation and selection of microorganisms were carried out on nutrient media, focusing on strains that we isolated. Molecular identification of the selected and isolated strains was performed by BIOMI Ltd. based in Gödöllő. For identification purposes, we utilized the gene segment coding for the bacteria's 16S rRNA.

2.1.2 PGP Strain Germination Plant Tests

Evaluation involved small-scale, cell-tray experiments based on the effects on plants and soil enzyme activity. The microbial PGPM strains used for soil inoculation and their designations were as follows: A5: *Enterobacter ludwigii*; Bm: *Bacillus megaterium*; Bs: *Bacillus subtilis*; D1: *Kosakonia cowanii*; Pf: *Pseudomonas fluorescens* Hx1; Th: *Trichoderma harzianum* T-22. As test plants, white mustard (*Sinapis alba* L.) and perennial ryegrass (*Lolium perenne* L.) were used.

In this experiment, the following properties were measured: wet and dry mass (g), growth length (mm) related to plant shoots, and the number of germinated seeds for mustard. Additionally, we measured the fluorescein diacetate (FDA) activity values in the soil of the mustard plants.

The effect of microbial inoculations on seed germination was calculated as follows: the average relative response ratios (Response Ratio - RR) compare the percentage change (%) between the results of the applied strains and the control.

2.1.3 Cultivation Container Test with Basil and Lettuce Inoculated with *Bacillus megaterium*

As test plants, we used head lettuce (*Lactuca sativa* var. *capitata* L.) and basil (*Ocimum basilicum* L.). The plants were grown on lightly humus-rich, calcareous sandy soil (Soroksár1)

and humus-rich, loose-textured meadow soil (Martonvásár). We examined the release of mineral nutrients with the addition of 5% (m/m) alginite. Soil sterilization was applied to simulate a degraded soil microbiome. After pre-cultivation, plants of similar developmental stages were randomly transplanted into cultivation containers. Inoculation with Bacillus megaterium occurred 1 day after transplanting for basil and 10 days for lettuce. We applied 1 ml of inoculant per plant at a concentration of $1{,}735\times10^6$ CFU/ml.

Biological parameters of the soils were measured. Using the MPN method, we determined the count of culturable aerobic spore-forming bacteria and microscopic fungi. For basil, we measured the DHA values of the soils, and for lettuce, the FDA values were assessed.

2.1.4 Pot experiment with Agricultural Soils, Bean and Corn as Test Plants

The soils have a neutral to slightly alkaline pH but vary in their compactness and organic matter content. These soils correspond to genetic soil types commonly used in cultivation. The key physicochemical properties of the soils used are presented in Table 1.

Table 1. Main Physicochemical Characteristics of Soils Used in the Cultivation Container Experiment.

	T1	**T2**	**T3**	**T4**	**T5**
Sampling Location	Soroksár 1	Soroksár 2	Tófej	Hatvan	Szegalom
WRB Classification	Arenosol	Gleysol	Chernozem	Luvisol	Gleysol
Coordinates	47°23’33” 19°08’55”	47°24’02” 19°09’18”	46.65473 16.78882	47.65475 19.61545	47.12483 21.07916
Platicity (K_A)	26	43,4	49	54	57,5
Physical Texture	Sand	Clay loam	Clay loam	Clay	Clay
$pH_{(H2O)}$	7,49	7,42	7,44	7,50	7,61
$pH_{(KCl)}$	6,94	7,13	6,58	6,74	6,45
Water-Soluble Salts (m/m %)	0,0317	0,0216	0,02677	0,0555	0,0665
Organic Matter Content (SOM %)	2,18	4,09	4,63	3,89	3,75

As test plants, we used yellow-podded bush beans (*Phaseolus vulgaris* var. 'Maxidor'), followed by sweet corn (*Zea mays* var. saccharata) planted in the same spots. The microbial inoculation was carried out simultaneously with the sowing and transplanting. The strains used were: D1 - *Kosakonia cowanii*, Pf - *Pseudomonas fluorescens*, A5 - *Enterobacter ludwigii*, Bs - *Bacillus subtilis*, Th - *Trichoderma harzianum* T-22.

Experiments were also conducted on Soroksár 1 and Szeghalom soils (selected as the two extremes based on physical texture) using Vázsonyi alginite in conjunction. Thus, inoculant treatments were applied to five soils, and two of these soils were additionally combined with alginite (5% m/m).

2.1.5 Field Experiments with Mixtures of Inoculant Strains and Ecological Treatments

The experiment was conducted on the premises of the Experimental Farm and Demonstration Farm of the Hungarian University of Agricultural and Life Sciences (MATE). The site features humus-rich sandy soil, which has high drainage capacity, poor water management, and very low water retention capabilities. The properties of this soil are presented in column Soroksár 1 of Table 1.

This experiment particularly focused on studying microbial changes in the soils, as well as various organic matter and carbon measurements in the soil.

Experimental Setup

Treatments:Vázsonyi alginite (40 t/ha); Mulch (triticale straw in a 10 cm layer, replenished as it decomposed); Cover crop: (Demeter-mix) cover crop mixture.

Microbial inoculants (combined with the above treatments): 2019-2020: Own mixture, 2021: Phy - commercial inoculant preparation consisting of *Pseudomonas putida*, *Azotobacter chroococcum, Bacillus circulans,* and *Bacillus megaterium*.

2.2 Materials

2.2.1 Applied microorganisms

During the research, we worked with numerous microorganisms and utilized them in the experiments in the combinations described above. Some of these were already available in the laboratory's culture collection, others were included in commercial products, and we also had our own strains isolated from the Soroksár experimental area. The main strains are presented with code names, origins, and presumed properties with which we worked during the research, as shown in Table 2.

Table 2: Possible and Expected Properties of the Microorganisms Used. (C – Collection, P – Product, I – Isolated). In the case of products, a code name is not necessarily given.

Code and Name	Source	PGP Properties
Bacteria		
Bm - *Bacillus megaterium*	**C, P**	PSB, KSB, N bonding, *Fusarium* Antagonism, IAA-, Siderophore and Extracellular Polysaccharide Production (EPS)
Bacillus circulans	**P**	Cellulase Production, PSB, IAA production. Biocontrol
Bs - *Bacillus subtilis*	**C**	PSB, IAA-, Siderophore production, Antibiose
A3 - *Bacillus coreaensis*	**I**	Xylanase Activity, Siderophore and Extracellular Polysaccharide Production (EPS)
Ac - *Azotobacter chroococcum*	**C, P**	PSB, N bonding, *Fusarium* Antagonism, Biodegradation
A5 - *Enterobacter ludwigii*	**I**	PSB, KSB, IAA-, EPS Production, Antifungal effects, Alkane Biodegradation
D1 - *Kosakonia cowanii*	**I**	N bonding, IAA-, Siderophore Production
C1 - *Lelliottia amnigena*	**I**	PSB, ACC-Deaminase Activity, IAA-, Siderophore Production
Pf - *Pseudomonas fluorescens*	**C**	PSB, Biocontrol, IAA-, Siderophore Production
Pseudomonas putida	**T**	PSB, Biocontrol, IAA-, Siderophore Production
Fungi		
Th - *Trichoderma harzianum*	**T**	Antifungális hatás, cellulóz bontás

2.2.2 Soil Biological Activity: Enzyme Assays and Cellulose Test

The *fluorescein diacetate (FDA) hydrolysis* method is a frequently used technique to measure soil enzymatic activity. We applied the method based on the work of Adam and Duncan (2001) and Villányi et al. (2006). This method allows for the assessment of the extent to which the microbial community is capable of degrading organic materials, providing important information about soil fertility and biological activity. It can detect the activity of multiple, non-specific extracellular enzymes, thus offering a general view of the activity of microbial communities in the soil. Indirectly, it also allows for the estimation of the total biomass of bacteria and filamentous fungi in the soil.

The method for measuring *dehydrogenase enzyme activity (DHA)* is one of the most commonly used indicators of microbial activity in soil. Dehydrogenases are enzymes located

within microbial cells that participate in the oxidation of organic materials, playing a direct role in electron transfer processes. These enzymes are active only in viable cells, hence their activity reflects the microbial viability and health of the soil effectively (Veres et al., 2013).

In the pot experiment, we measured soil biological activity by examining *cellulose degradation*. We placed 1 gram of cotton in each cultivation container at an equal depth. The cotton was neither sterilized nor pre-treated in any way. Throughout the experiment, we measured the cotton to assess its decomposition (Unger, 1960).

2.2.3 Determination of the Most Probable Number (MPN) of Microorganisms: Aerobic Bacteria, Spore-forming Bacteria, and Microscopic Fungi

The Most Probable Number (MPN) of viable microorganisms in the soils was determined using the Hoskins table (Cochran 1950). This method allowed us to estimate the count of all culturable microorganisms, microscopic fungi, and inoculated spore-formers. By utilizing this quantitative technique, we were able to assess the density and distribution of these microbial populations within the experimental soil samples.

2.2.4 Examined Plant Parameters

During the experiments, various plant biological parameters were measured. In the cell tray and cultivation container experiments, we measured shoot length, and wet and dry mass of both shoots and roots.

In the field experiment, crop yield values were recorded. The *normalization of yield values* was done by dividing the measured values by the average values for that year. The purpose of this process is to ensure comparability among crop yields across different years, taking into account that different crops were cultivated each year. As a result, the Year factor was excluded from the analysis of this variable.

2.2.5 Measurements of Soil Carbon Forms

A *teljes szerves szén (TOC)* tartalmát Kálium-dikromátos oxidáció után határoztuk meg, (Tyurin, 1951). Az *Oldott Szerves Szén (Dissolved Organic Carbon - DOC)* mennyiséget Az oldatok DOC koncentrációját folyékony (liquid) üzemmódban az Elementar Vario TOC cube eszközzel mértük. Vizsgáltuk a talajok *Labilis-C, vagy másnéven POXC (Permanganate Oxidizable Carbon)* tartalmát is Weil et al. (2003) alapján. A *glomalint (Easily extractable glomalin-related soil proteins - EE-GRSP)*. A humuszminőséget a *Hargitai-féle két oldószeres eljárással* vizsgáltuk (Hargitai, 1955). A Hargitai módszer mellett használtuk az *E4/E6* módszert. A *mikrobiális biomassza szén (Microbial Biomass Carbon, MBC)* a talaj mikrobiális közösségében tárolt szerves szén mennyiségét

Total Organic Carbon (TOC) content was determined following potassium dichromate oxidation (Tyurin, 1951). The quantity of *Dissolved Organic Carbon (DOC)* was measured in liquid mode using an Elementar Vario TOC cube device. We also examined the soil's labile carbon, also known as *POXC (Permanganate Oxidizable Carbon)*, following the methodology of Weil et al. (2003). *Easily extractable glomalin-related soil proteins (EE-GRSP)* and soil humus quality were assessed using *Hargitai's dual-solvent method* (Hargitai, 1955). Alongside the Hargitai method, the *E4/E6 ratio* technique was used. *Microbial Biomass Carbon (MBC)* represents the amount of organic carbon stored within the soil microbial community.

2.2.6 Applied Statistical Methods

For data analysis, I used IBM SPSS 25, R 4.3.1, and the Excel software suite. One-way and multi-way ANOVA, along with Tukey’s post-hoc test, were applied to determine differences between factors. Additionally, correlation analyses were conducted to establish

relationships among various examined parameters. Normality for ANOVA was checked using the Kolmogorov-Smirnov test, and homogeneity of variances was verified with the Levene test. If conditions were not met, one-way Welch ANOVA was employed with Games-Howell's post-hoc test, allowing non-parametric examination of the factors.

3. RESULTS AND DISCUSSION

3.1 Verification of the Effects of Isolates

For selection, according to literature, Aleksandrow medium was used (Etesami et al., 2017). After selection on solid Aleksandrow medium, the species were cultivated in liquid Aleksandrow broth and their potassium solubilizing capacity was measured using a flame photometer across different pH ranges (pH=5.0, 7.1, and 8.0). Notably, their potassium solubilizing capability was most pronounced at a neutral pH of 7.1 (Figure 1).

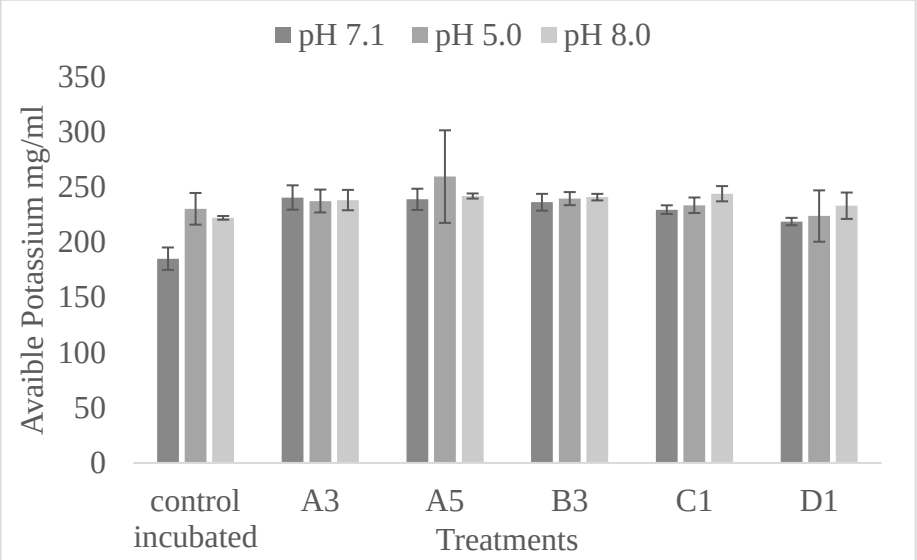

Figure 1. Examination of the available potassium solubilizing capacity of isolated strains using a flame photometer.

3.2 Identification of Isolated Microorganisms (Genetic Analysis)

Based on preliminary tests, we selected several microbial isolates for genetic identification. The results are presented in the table below (Table 3).

Table 3. Species identified based on the genetic analysis of the isolates and their corresponding probability percentages.

	Identified species	Similarity %	Base pair (bp)
A3	*Bacillus coreaensis*	98,52 %	1481
A5	*Enterobacter ludwigii*	99,72 %	1438
B3	*Enterobacter tabaci*	100,00 %	1079
C1	*Lelliottia amnigena*	99,44 %	1438
D1	*Kosakonia cowanii*	99,51 %	1441

Among the isolated microorganisms, we selected strains A5 and D1 for further investigation, taking into account our preliminary laboratory observations. The selection and strain screening were facilitated by their properties, such as phosphate solubilizing bacteria (PSB), nitrogen-fixing, and extracellular polysaccharide (EPS) production capabilities.

3.3 Investigation of the Effects of Isolated and Subsequently Applied Microorganisms Using Germination Tests with White Mustard (*Sinapis alba*) and Perennial Ryegrass (*Lolium perenne*)

3.3.1 Germination Percentage and Seedling Growth

During the testing and selection experiments of PGPM strains, we observed higher growth parameters due to the effects of microbial strains compared to the control.

For the mustard test plant, although not statistically significant, we obtained higher values for plant properties in comparison to the control for every tested strain. The highest value (+30% for germination and biomass) was observed with strain A5: *Enterobacter ludwigii* (Figure 2). There was a strong correlation between the germination percentage and the resulting biomass of mustard. Based on this, the strains did not necessarily have a substantive effect on biomass, but significantly stimulated germination, which was reflected as an increase in biomass.

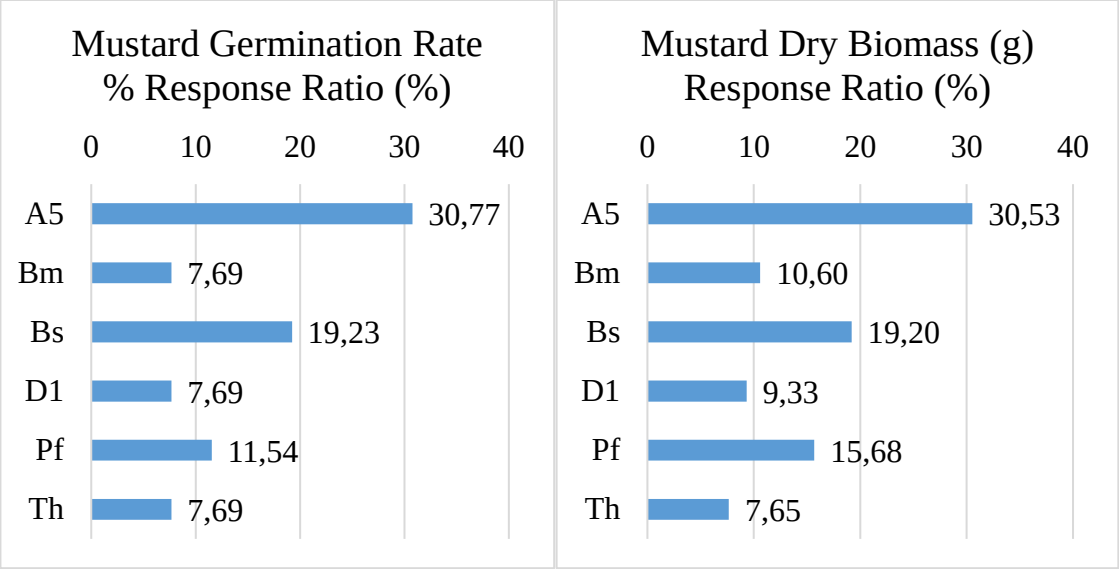

Figure 2. Percentage of germination and percentage change in dry mass of mustard plants in response to treatments compared to the control, shown as "response ratio" (RR) values due to inoculations: A5 - *Enterobacter ludwigii*, Bm - *Bacillus megaterium*, Bs – *Bacillus subtilis*, D1 - *Kosakonia cowanii*, Pf – *Pseudomonas fluorescens*, Th - *Trichoderma harzianum*.

We can reach a similar conclusion for the perennial ryegrass test plant. There is no significant difference in the results, but all treatments, except for D1: *Kosakonia cowanii* and Bs: *Bacillus subtilis*, showed greater biomass compared to the control. This may indicate an interaction between the bacterial strains and the target plant, suggesting that their positive PGP effects are not universal across all plant species. The greatest differences were observed with A5: *Enterobacter ludwigii* (RR = +9.75%) and Bm: *Bacillus megaterium* (RR = +11.24%), which showed the most significant growth compared to the control (Figure 3).

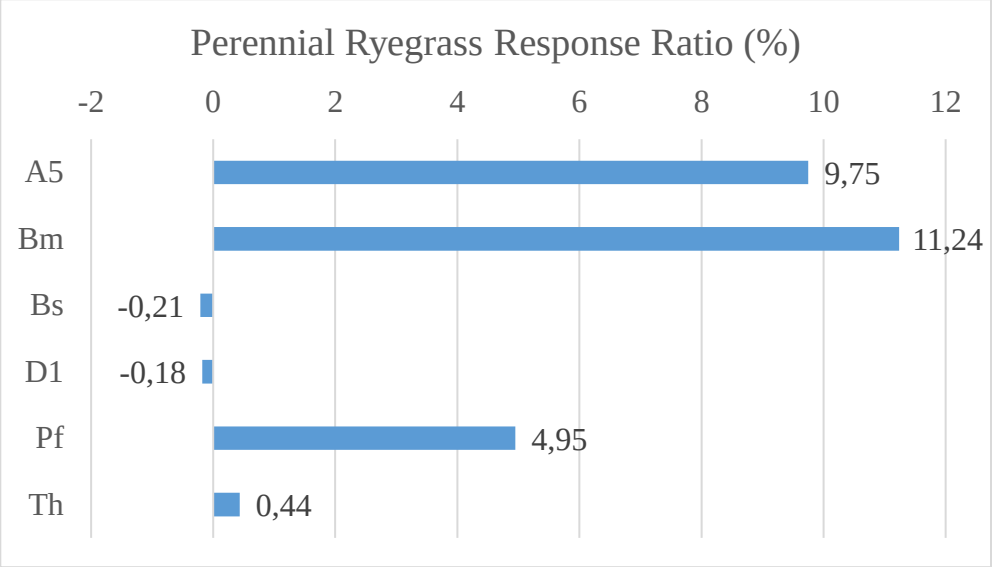

Figure 3. Percentage change in dry mass of perennial ryegrass in response to treatments compared to the control, shown as "response ratio" (RR) values due to inoculations: A5 - *Enterobacter ludwigii*, Bm - *Bacillus megaterium*, Bs – *Bacillus subtilis*, D1 - *Kosakonia cowanii*, Pf – *Pseudomonas fluorescens*, Th - *Trichoderma harzianum*.

3.3.2 The Effect of Applied Microbial Strains on Soil Enzyme Activity

We conducted a correlation analysis between FDA enzyme activity and measured plant properties. We found that FDA generally showed a positive correlation with all measured plant parameters, except for the length of mustard shoots, which requires further investigation. However, the results were not statistically significant due to the limited amount of soil available, which meant that only informative average samples were generated for each treatment for FDA measurement (Table 4).

Table 4. Correlation of FDA with Measured Parameters of Mustard Plants

	r	*P-value*
Germintaion rate	0,3087	0,5005
Wet Biomass (g)	0,3622	0,4247
Dry Biomass (g)	0,3657	0,4199
Length (mm)	-0,5497	0,2012

3.4 The Effect of Soil Sterilization and Alginite Application on the Success of Microbial Inoculation

3.4.1 The Effects of Treatments on Soil Biological Activity

We examined the *FDA enzyme activity* in the soils of both plants. There was a significant difference in FDA activity between the plants ($F(1, 90) = 48.003$, $p < 0.001$), where the lettuce soil exhibited higher soil biological activity.

Other parameters were examined separately for each plant. A significant difference between the soils was only observed with lettuce ($F(1, 43) = 17.804$, $p < 0.001$), where the Martonvásár soil showed higher values.

Sterilization significantly lowered FDA enzyme activities in the soils for both plants, including basil and lettuce (Basil ($F(1, 43) = 7.403$, $p < 0.01$); Lettuce ($F(1, 43) = 30.147$, $p < 0.001$)) (Figure 4). No significant differences were observed in other parameters.

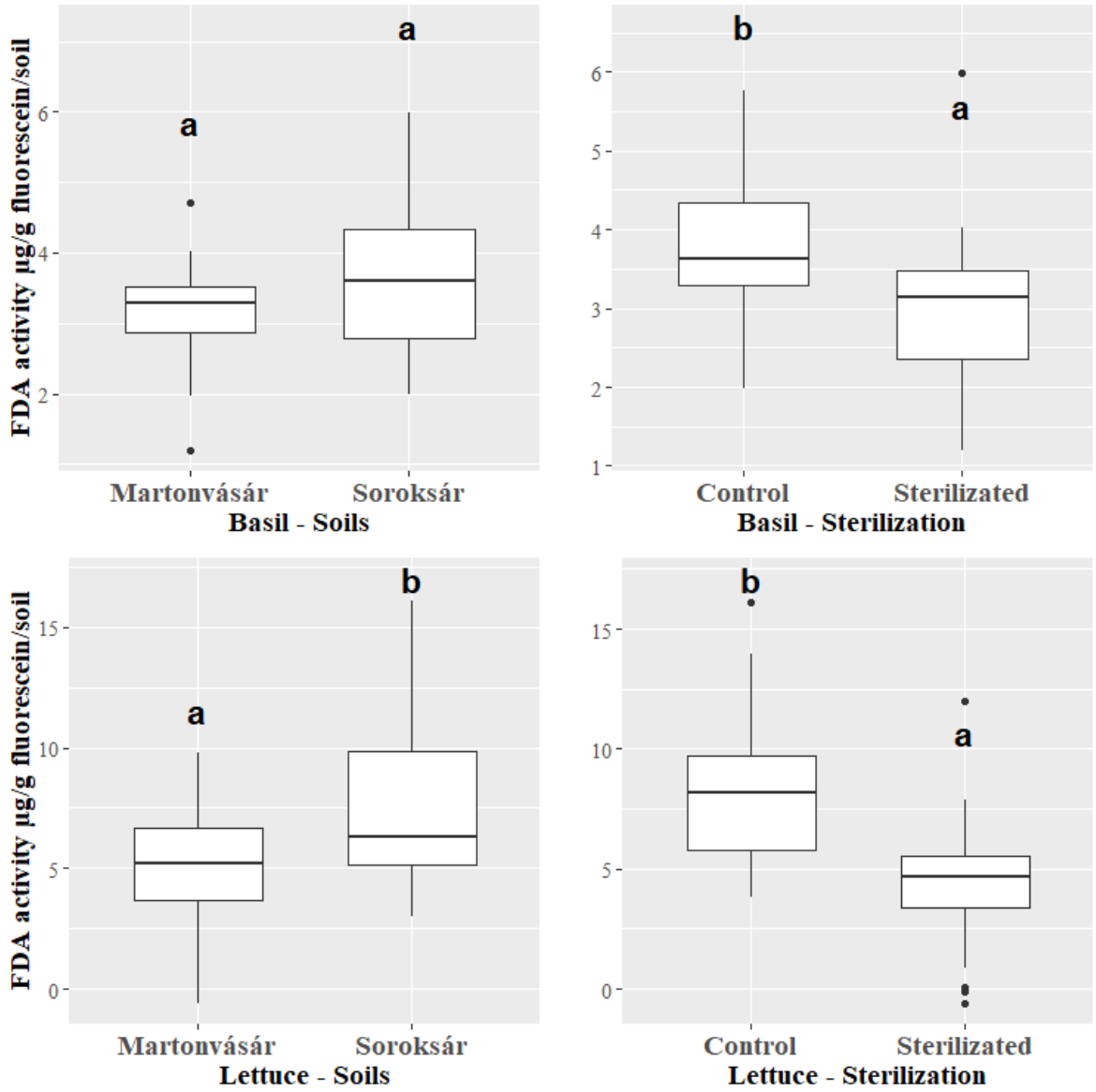

Figure 4. Soil FDA activity in response to different plants, soils, and the effect of soil sterilization.

DHA was exclusively analyzed in the soil of basil plants, where significant results were observed for every factor studied. Among the soils, the Martonvásár soil showed higher DHA values ($F(1, 43) = 90.563$, $p < 0.001$). After soil sterilization, the control samples displayed higher DHA values ($F(1, 43) = 24.267$, $p < 0.001$). The incorporation of alginite also resulted in higher values ($F(1, 43) = 4.896$, $p < 0.05$). To our knowledge, the effect of alginite on soil enzyme activity has not been studied in this system before. The application of the inoculant also led to higher enzyme activity values ($F(1, 43) = 12.821$, $p < 0.001$) (Figure 5).

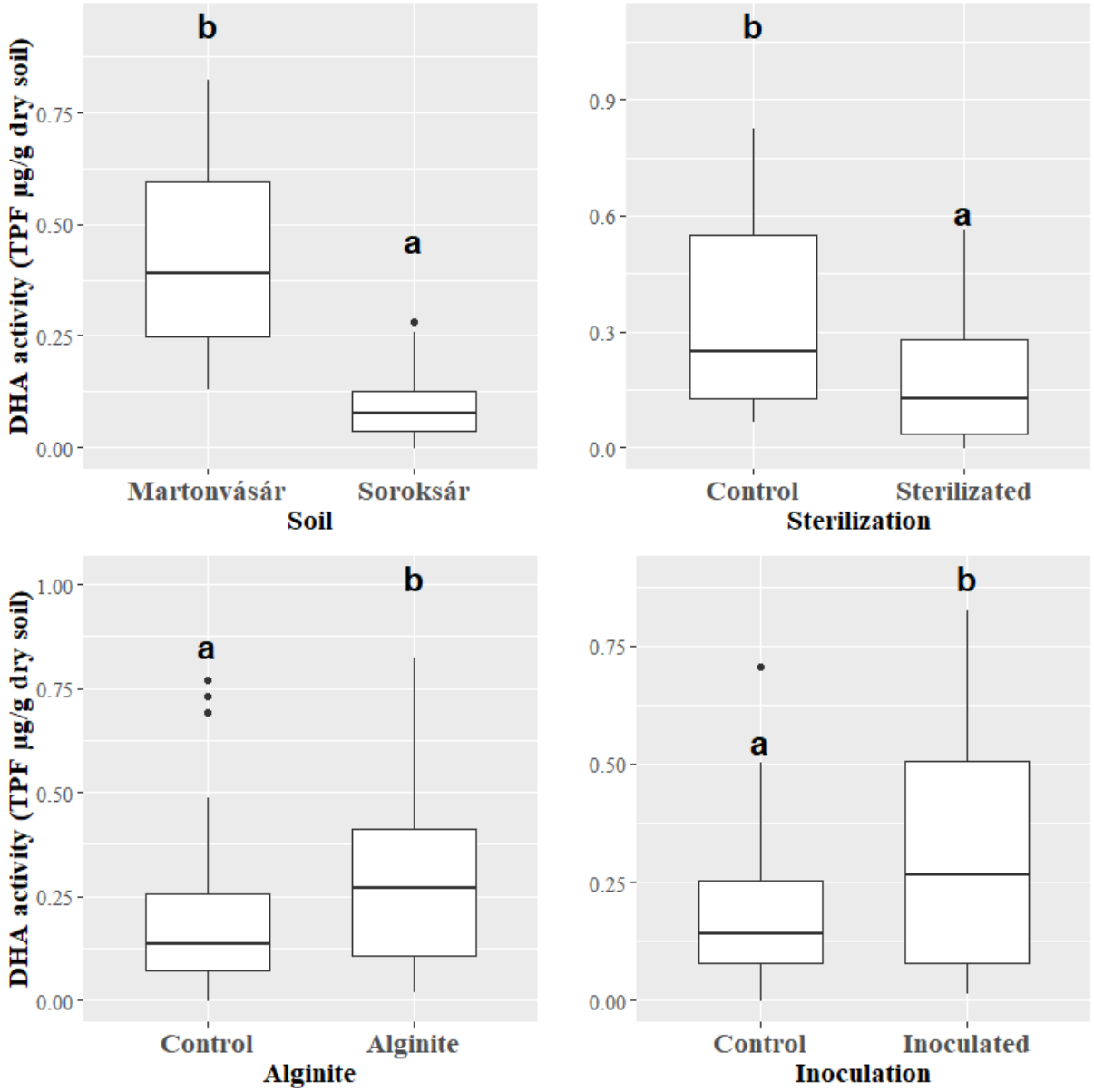

Figure 5. Changes in soil DHA activity in basil plants due to different soils, alginite treatment and the effect of soil sterilization.

There is a significant difference in the counts of *aerobic bacteria* (log CFU/g) between the plants ($F(1, 90) = 5.053$, $p < 0.05$), with lettuce having higher aerobic counts. However, sterilization proved to be significant for both plants. For basil, the impact of sterilization was marked ($F(1, 43) = 32.453$, $p < 0.001$), and for lettuce, it was even more significant ($F(1, 43) = 51.191$, $p < 0.001$). For both plants, sterilization increased the count of aerobic microorganisms in the soil by the fourth week post-sterilization. The treatment was significant only for lettuce ($F(1, 43) = 4.464$, $p < 0.05$). However, the inoculant treatment showed significant results only for basil ($F(1, 43) = 15.518$, $p < 0.001$) (Figure 6).

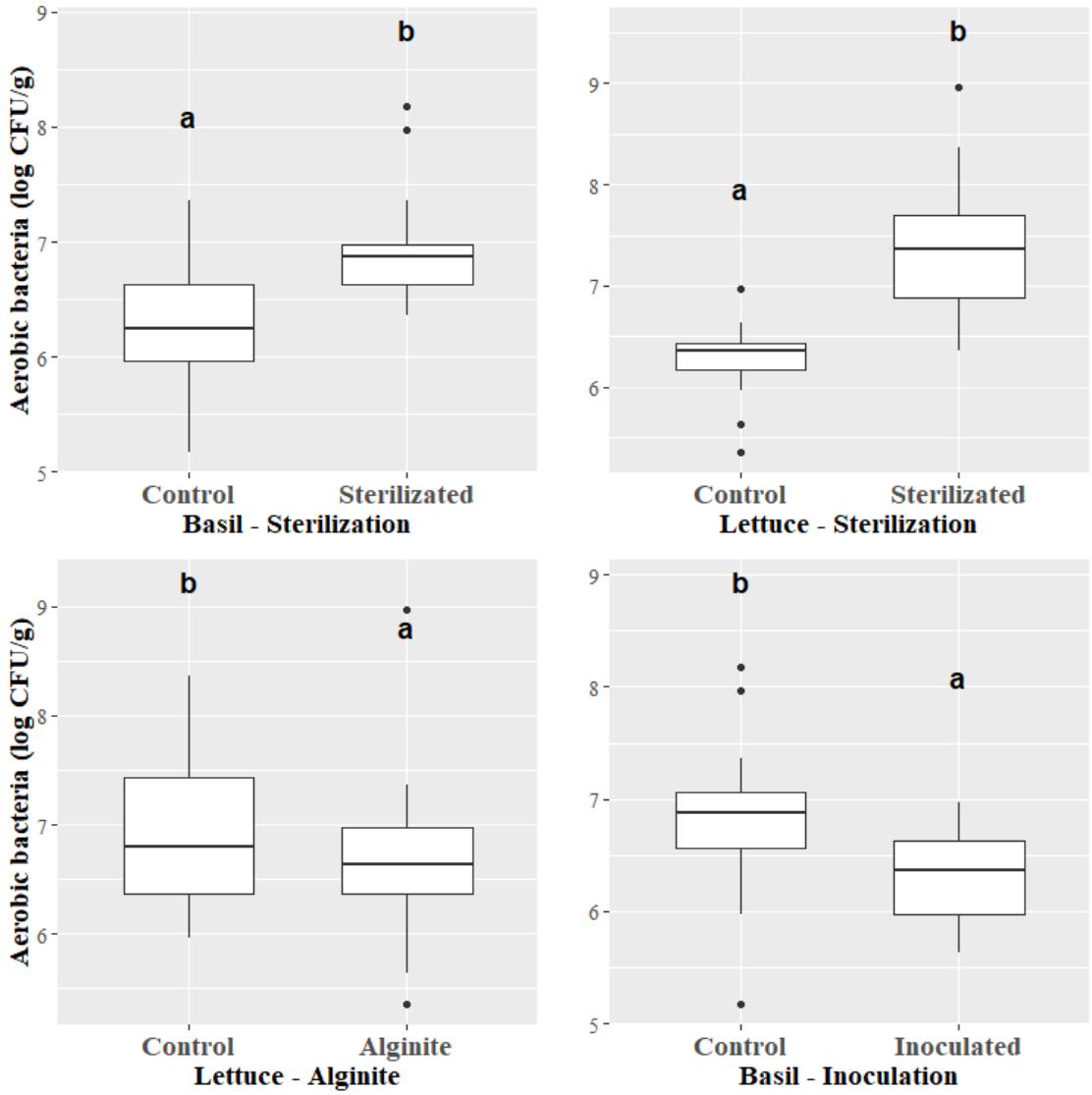

Figure 6. Counts of Aerobic bacteria (log CFU/g) in response to soil sterilization, alginite treatment, and inoculant application across different plants soils

Sterilization significantly influenced the viable cell counts of microorganisms and soil enzyme activity. During the sterilization process, the naturally present soil microorganisms were destroyed, but the vacant ecological niches were quickly colonized by airborne microorganisms and those introduced during plant transplantation. Four weeks post-transplantation, microbial presence was detectable even in sterilized soil, and microbial counts were higher than in non-sterilized conditions. However, enzyme activities did not keep pace. This could be due to the destruction of extracellular enzymes, and their replenishment may require more time.

Alginite treatment did not have a significant impact on either soil DHA enzyme activity or microbial abundance.

For basil, the Soroksár soil showed significantly higher counts of culturable microscopic fungi ($F(1, 43) = 6.250$, $p < 0.05$), as did the soil for lettuce ($F(1, 43) = 14.010$, $p < 0.001$). Soil sterilization resulted in significant increases in fungal counts for both plants: for basil ($F(1, 43) = 40.674$, $p < 0.001$), and for lettuce ($F(1, 43) = 11.418$, $p < 0.01$). In both cases, sterilization increased the counts of microscopic fungi in the soil by the fourth week post-sterilization. While alginite treatment did not show significant effects, the inoculant had significant results for basil ($F(1, 43) = 6.250$, $p < 0.05$) (Figure 7).

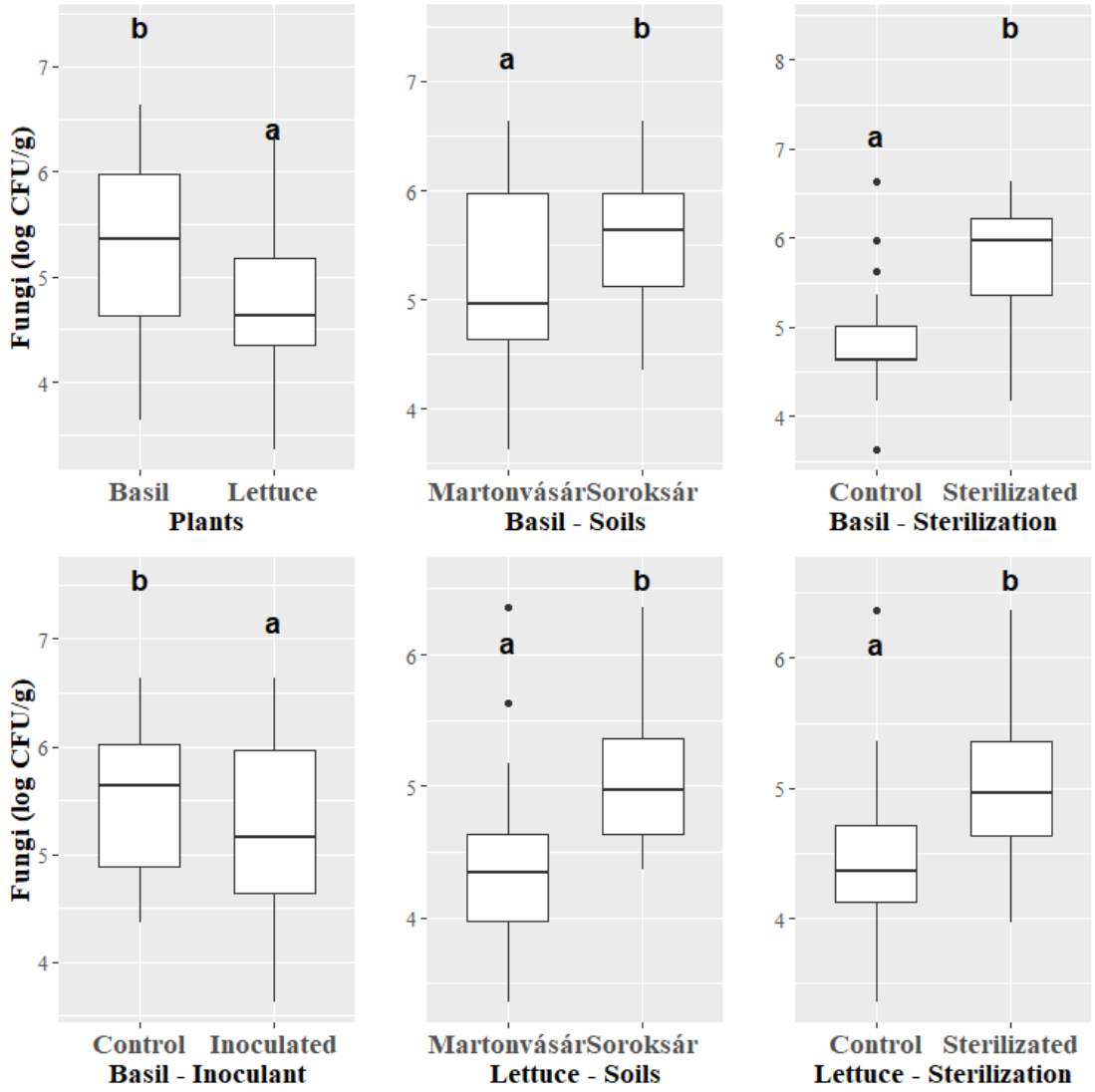

Figure 7. Log (CFU/g) values of microscopic fungi in soils affected by different plants, soil types, soil sterilization, and inoculant treatment.

The observation that inoculation with *B. megaterium* reduced the counts of culturable aerobic bacteria and microscopic fungi is contrary to the literature (Zhao et al., 2021), where inoculation typically increased the abundance of the inoculated microorganisms and altered the composition of the microbial community. It is also possible that *B. megaterium* occupied a portion of the ecological niche, which could explain the observed decrease in abundance values, while the overall microbial biomass in the soil remained constant. This is because *B. megaterium* has a larger cell size compared to other *Bacillus* species (hence its name). This could account for the increase in DHA enzyme activity following inoculation. However, to confirm this hypothesis, more direct microbial biomass assessments would be required.

3.4.2 Changes in Plant Biomass Values in Response to the Factors Examined

We assessed the changes in dry biomass mass for each plant. For basil, the Martonvásár soil showed significantly higher values ($F(1, 43) = 15.161$, $p < 0.01$). Sterilization resulted in significant differences for both plants. In the case of basil ($F(1, 43) = 19.793$, $p < 0.001$) and lettuce ($F(1, 43) = 11.604$, $p < 0.01$), the dry biomass mass of plants increased as a result of soil sterilization. This increase could be attributed to the additional nutrients released. The alginite treatment and the inoculant had no significant effect on the dry biomass mass of the plants, and no interaction effects were found.

3.5 Testing of Inoculant Mixtures on Five Different Soils Using Bush Bean and Sweet Corn as Test Plants in a Pot Experiment

3.5.1 Soil Biological Results: Comparing Different Soils and Alginite Treatment

Significant differences in *DHA* were observed between the soils for both bush beans ($F(4,34) = 14.75$, $p < 0.001$) and sweet corn ($F(4,34) = 11.347$, $p < 0.001$). The effect of the inoculant was also significant for beans ($F(1,34) = 14.18$, $p < 0.001$) (Figure 8). In the case of beans, there was an interaction effect between the soils and the inoculant. On the Tófej soil, the inoculant treatment resulted in significantly higher dehydrogenase enzyme activity ($F(4,34) = 3.194$, $p < 0.05$).

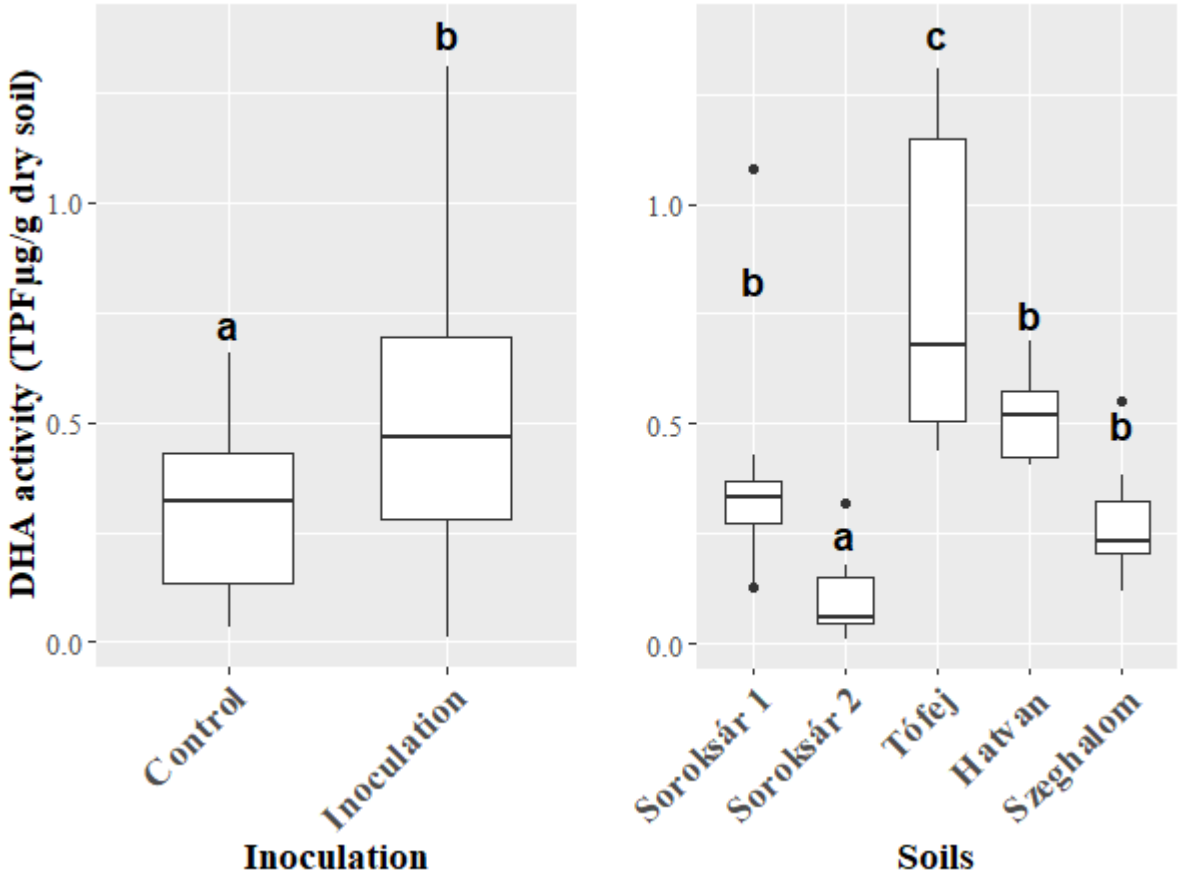

Figure 8. DHA Values in Response to Inoculant Treatment and Different Soils for Bush Bean (Only the results for beans are presented in the figure, as the same pattern was observed for both beans and corn regarding soil effects).

The significant effect of alginite on the corn test plant was confirmed, resulting in higher DHA values in the Soroksár 1 soil ($F(1,24) = 32.506$, $p < 0.001$) and an alginite-soil interaction. Simultaneously, the treatment led to lower DHA activity compared to the control ($F(1,24) = 7.577$, $p < 0.05$) (Figure 9).

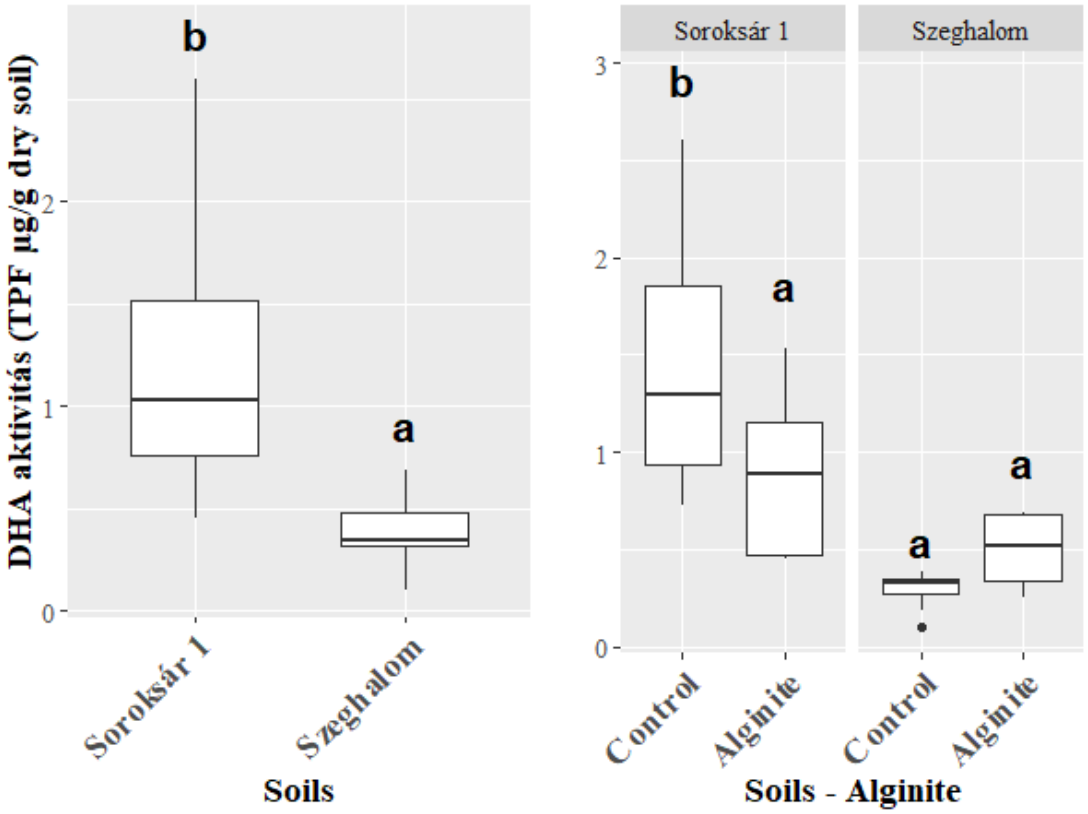

Figure 9. Changes in DHA values in sweet corn plant soils with inoculant and various soils treated with alginite.

The significant effect of alginite was confirmed in the sweet corn test, where it resulted in higher DHA values in the Soroksár 1 soil ($F(1,24) = 32.506$, $p < 0.001$) and an alginite-soil interaction. Additionally, the treatment simultaneously caused lower DHA activity compared to the control ($F(1,24) = 7.577$, $p < 0.05$)

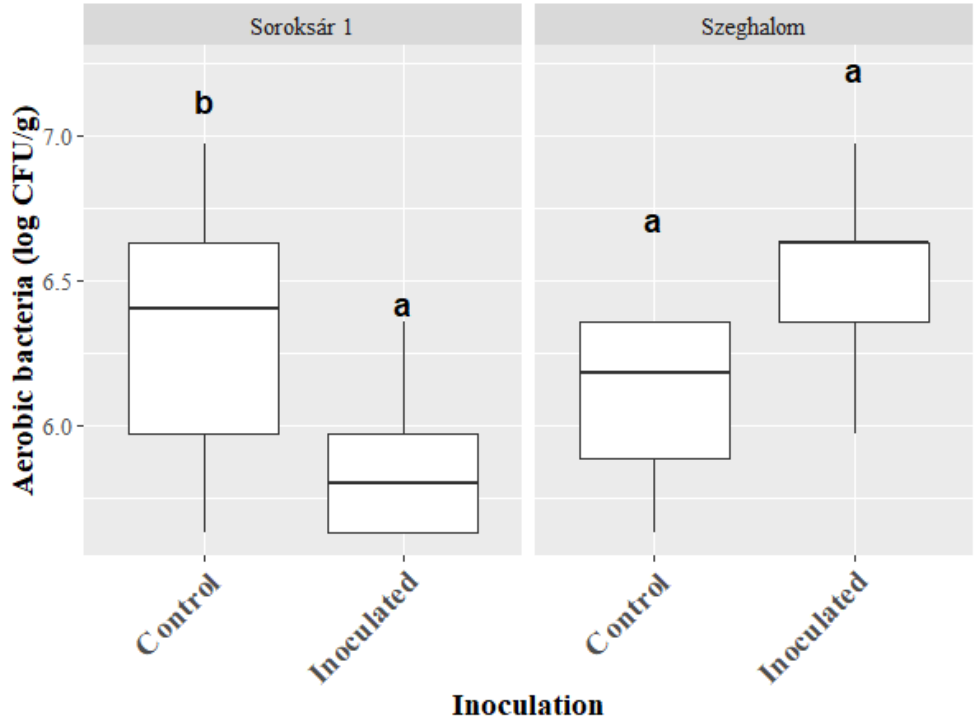

Figure 10. Changes in the abundance of aerobic bacteria (log CFU/g) in different soils due to inoculant treatment in the bush bean experiment.

An interaction effect was observed on the *abundance of aerobic bacteria*, where inoculation on the Soroksár1 soil with the inoculant in the bean experiment resulted in a significantly lower aerobic bacteria count ($F(1,24) = 12.518$, $p < 0.01$) (Figure 10). This indicates that the effect of inoculation was soil-dependent when evaluating alginite (and its controls) with respect to inoculant effects. To our knowledge, no study has investigated the impact of soil type and inoculation on bacterial counts in the soil alongside alginite treatments.

The *quantity of culturable fungi (log CFU/g)* was significantly affected by both the soil types ($F(4,34) = 14.81$, $p < 0.001$) and the inoculant ($F(4,34) = 14.96$, $p < 0.001$) in the bush bean experiment (Figure 11). However, neither factor was significant for corn.

A significant interaction was observed in the bush bean experiment; the Tófej and Szeghalom soils treated with inoculant exhibited a higher number of culturable fungi ($F(4,30) = 8.319$, $p < 0.001$). This suggests that the fungal components—most likely *T. harzianum*—successfully survived the application and were able to persist in the soil until the time of measurement. Additionally, the inoculant might have promoted the abundance of microscopic fungi in these soils. Therefore, we can conclude that the inoculant had the most pronounced effect on the microscopic fungi in the most compact soils (Tófej KA 54, Szeghalom KA 57.5).

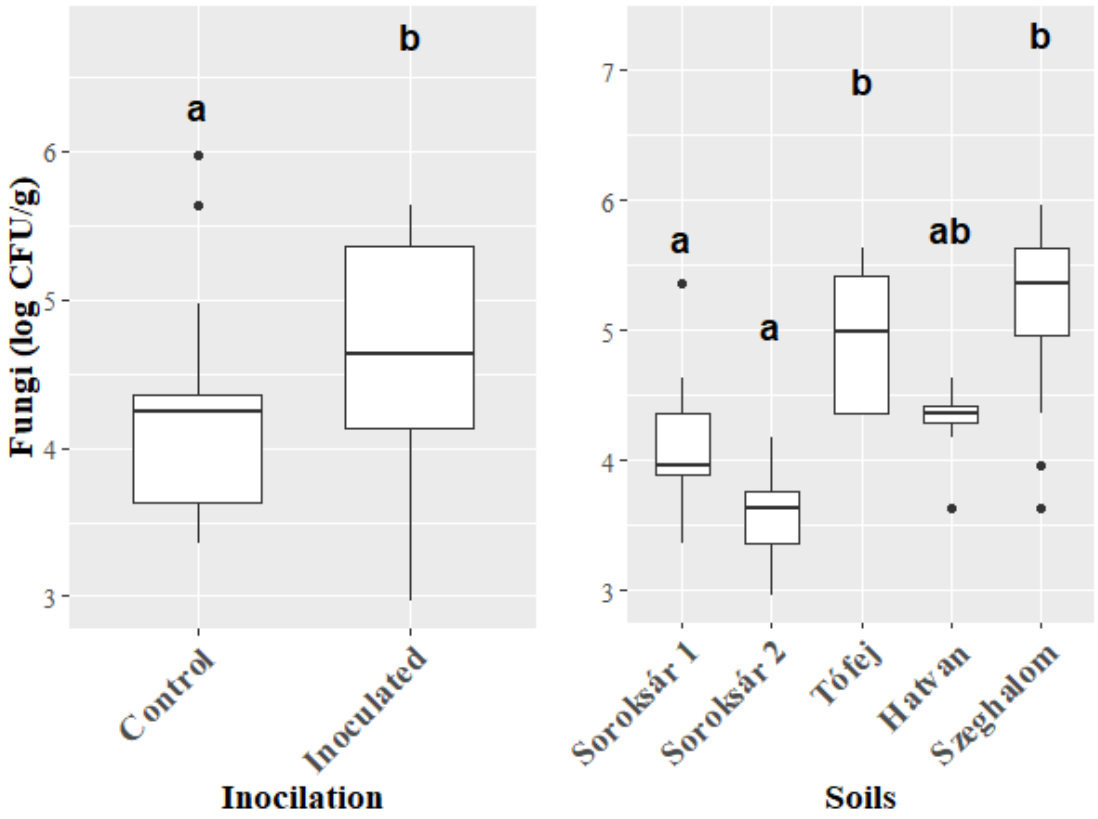

Figure 11. Changes in the number of cultivable fungi (log CFU/g) in different soils under the influence of inoculant treatment with bush bean test plant.

In comparisons involving alginite, all factors showed significant differences in the number of culturable fungi in the bush bean experiment (Figure 12). The results were as follows: Soil - ($F(1,24) = 62.959$, $p < 0.001$); Alginite - ($F(1,24) = 17.619$, $p < 0.001$); Inoculant - ($F(1,24) = 8.195$, $p < 0.01$). Additionally, a relevant interaction was observed between the factors, where in alginite-free treatments, the effect of the inoculant was significant ($F(1,24) = 8.515$, $p < 0.001$).

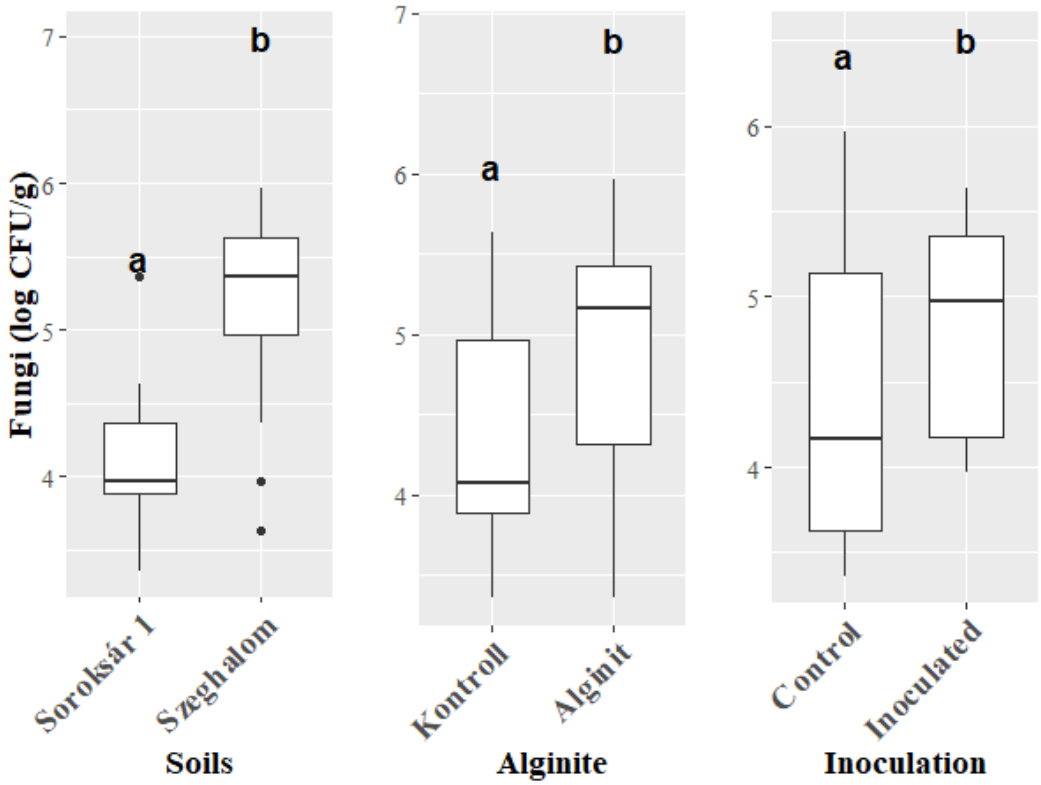

Figure 12. Changes in fungal (log CFU/g) values in different soils due to alginite and inoculant treatment with bush bean test plant

The *spore-forming bacteria* (log CFU/g) counts for the bush bean plants showed significant differences for both parameters: Soil ($F(4,34) = 3.595$, $p < 0.05$) and Inoculant ($F(1,34) = 5.929$, $p < 0.05$) (Figure 13). However, there was no significant interaction between the soil types and the inoculant concerning the abundance of spore-forming bacteria.

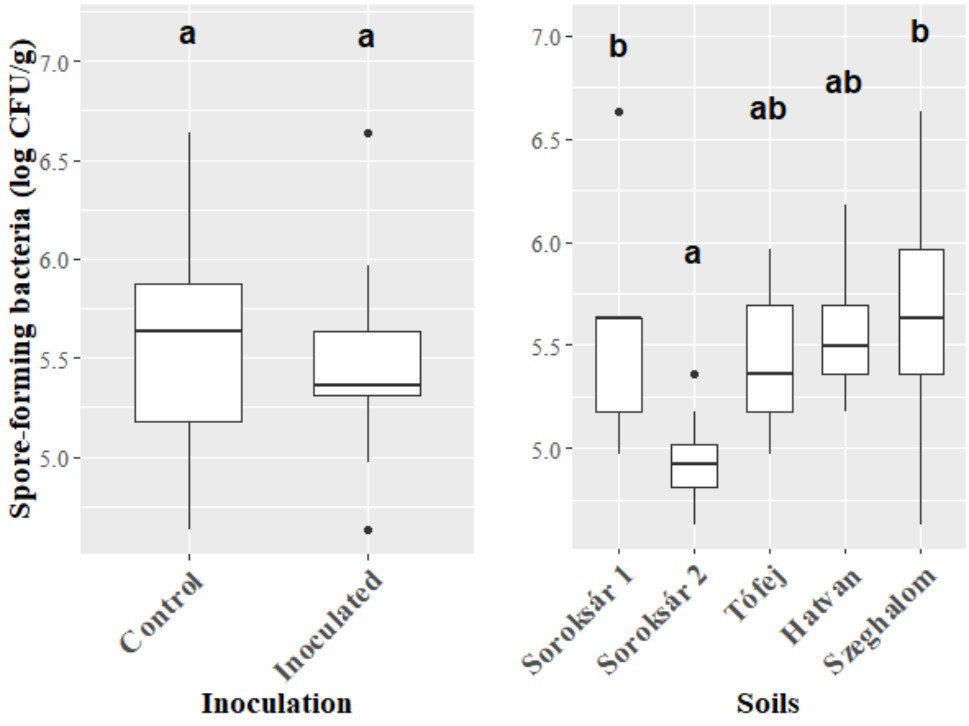

Figure 13. The number of spore-forming bacteria (log CFU/g) in different soils in response to inoculant treatment with sweet corn test plant.

In summary of the microbial results: The most pronounced differences were found between the soil types. The inoculant was only significant for the bush bean plants in terms of DHA, fungi, and spore-forming bacteria. The impact of inoculation on the composition and abundance of native soil bacterial communities is quite controversial, with no clear trends observed (Mallon et al., 2015; Mawarda et al., 2022).

The DHA and microbial parameters generally show a positive correlation, where DHA indicates the success of the inoculant in enhancing soil microbial parameters. Interaction effects were observed between the soil and microbial inoculant in the Tófej and Szeghalom soils. This suggests that the initial physical properties of the soil fundamentally influence its "receptiveness" to microorganisms (Kincses et al., 2008). The clayey, highly colloidal Tófej, Hatvan, and Szeghalom soils provided more habitat for microorganisms, leading to generally higher MPN values.

We also examined organic matter decomposition using the *Unger test* (1960). Cellulose decomposition showed significant differences only related to soil type ($F(4,34) = 9.794$, $p < 0.001$). The differences between soils are likely due to their physical properties. The Soroksár 1 soil is sandy, while the Szeghalom soil is heavy, clayey, and compact, making it less permeable to oxygen. Soil moisture might have reduced the decomposition capability in the Soroksár 1 soil since it's sandy and dries out quickly, but under experimental conditions, soil moisture was continuously controlled with regular irrigation.

In the case of alginite treatments, cellulose degradation continued to show significant differences between soils ($F(1,24) = 101.892$, $p < 0.001$). An interaction was observed where significantly more cellulose was degraded in alginite-treated pots without inoculant ($F(1,24) = 10.353$, $p < 0.01$) (Figure 14).

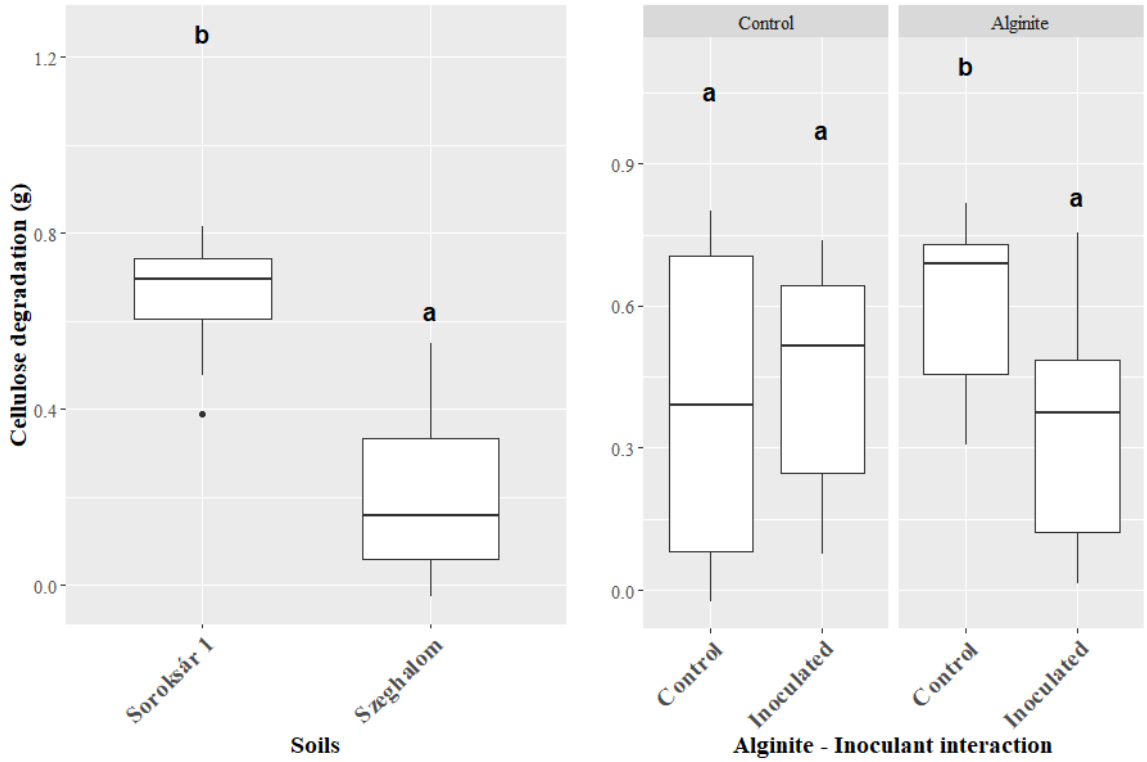

Figure 14. The extent of cellulose decomposition in case of bean plants soils, across different soils, under the influence of microbial inoculation and alginite treatment.

3.5.2 Dry Biomass (g)

The *dry biomass* of the plants showed significant differences across the different soils for both test plants; however, no significant effect was observed for the inoculant. For bush bean plants, the soil factor was highly significant ($F(4,34) = 9.794$, $p < 0.001$), and for corn plants, it was also significant ($F(4,34) = 3.121$, $p < 0.05$). However, no interaction was found between the soils and either the inoculant or the alginite treatments.

3.6 Field Experiment

The measurements conducted between 2019 and 2021 were evaluated for the 0-20 cm soil depth. However, for certain parameters, we also examined the differences between 0-10 cm and 10-20 cm depths, and these will be discussed in the relevant sections where justified.

A multi-factor ANOVA was used to evaluate the results. The main factors considered were: soil rejuvenation methods (treatments), use of inoculants, time (changes observed across the years), and, in certain cases, the soil sampling depth. Additionally, we performed correlation analyses to explore relationships between various soil parameters.

We monitored the effects of alginite, mulch, cover crop treatments, and microbial inoculants on soil and crop yield over the years. Our primary focus was on assessing their impact on various biological parameters, soil carbon, and crop yield. Additionally, we examined various soil chemical and physical properties, primarily for verification purposes.

3.6.1 Soil Biological Results in the Field Experiment: Impact of Soil Management Technologies and Their Combination with Inoculants

For *aerobic bacteria* (log CFU/g), neither the treatments nor the inoculant had a significant effect, but a significant decline was observed across the years ($F(2,65) = 158.409$, $p < 0.001$).

Spore-forming bacteria showed similar results to the abundance of aerobic bacteria. The treatment and inoculant did not result in significant differences in the number of culturable spore-forming bacteria, while the time factor ($F(2,65) = 25.017$, $p < 0.001$) did, showing a declining trend.

The MPN (Most Probable Number) values of *microscopic fungi* also showed a significant decrease over the years ($F(2,65) = 111.844$, $p < 0.001$). The treatment and inoculant did not show significant differences.

These declining trends could have been caused by the decrease in precipitation during the 2019-2021 period, despite the fact that the area was irrigated.

In the case of *DHA*, significant differences were observed over the years ($F(2,65) = 52.323$, $p < 0.001$), but no clear trend was established. At the end of the experiment, DHA values were significantly higher compared to the initial values.

The trend-like changes in soil biological parameters over the years might seem contradictory. Despite the declining number of culturable microorganisms, DHA did not follow this trend. One possible explanation is that while the MPN-detectable number of microorganisms decreased, soil biodiversity increased. As the soil food web became more complex, more organisms that feed on microorganisms may have appeared, reducing their numbers but increasing overall soil biological activity. However, confirming this would require results from microbial biomass measurements. Indirectly, examining soil biological activity through DHA enzyme activity is also suitable for this purpose (Sinsabaugh et al., 2016).

The decrease in microorganisms might seem contradictory in a minimum-till system, but it could indicate a transformation in the soil food web. The number of microorganisms detectable with the applied methods decreased. Several sources confirm that under minimum-till practices, the abundance of soil microorganisms and biological activity generally increase (Khangura et al., 2023; Govednik et al., 2023). However, in this experiment, there was no conventional tillage treatment for comparison, so no definitive conclusions can be drawn.

3.6.2 Normalized Yield Results Under the Influence of Soil Management Technologies

Significant differences were observed between the treatments ($F(3,67) = 13.627$, $p < 0.001$). According to the post hoc test, the yield results from mulched plots were significantly higher than those from other treatments ($p < 0.001$). The primary reason for this could be the weed-suppressing properties of the mulch, especially since the area underwent minimal tillage, only at the beginning and end of the season, leading to significant weed problems. Additionally, the mulch may have influenced the results by regulating moisture, temperature, and pH, as well as providing extra available nutrients from the straw mulch.

3.6.3 Measurement of Soil Carbon (C%) Content Under the Influence of Soil Management Practices

The total organic carbon (TOC) content (%) of the soil showed a significant increase over the three-year period. Specifically, the average TOC value increased from 1.906% in 2019 to 2.055% in 2020, representing a +7.8% relative increase in the first year. This significant increase continued from 2020 to 2021, further rising to 2.116%, which represents an additional +3% relative increase in the second year.

Although the effects of time and treatment factors were not statistically significant, the inoculant treatment resulted in a significantly higher TOC ($F(1,65) = 4.072$, $p < 0.05$).

3.6.4 Analysis of Soil Organic Matter and Quality Indicators Under the Influence of Soil Management Technologies and Their Combinations with Inoculants

The analysis of *NaF-extractable organic matter* showed significant changes due to treatments ($F(3,65) = 4.382$, $p = 0.007$) and inoculants ($F(1,65) = 4.557$, $p = 0.037$). Cover crops produced significantly higher results compared to the alginite and mulch treatments, and the inoculant-treated plots showed significantly higher values compared to the control. This may be related to the fact that both the cover crop and the inoculant can produce nitrogen-rich organic matter through nitrogen fixation. In contrast, mulch and alginite primarily enriched the soil with carbon-dominant compounds.

NaOH-extractable organic matter represents fresh organic matter in the early stages of humification. The results indicated significant differences across all three factors. In terms of treatment ($F(3,65) = 4.009$, $p = 0.011$), the mulch treatment showed the highest values, which were significantly different from the alginite treatment. For inoculants ($F(1,65) = 9.235$, $p = 0.003$), the inoculated plots had significantly higher values compared to the control. The most significant change, however, was observed over the years ($F(2,65) = 35.502$, $p < 0.001$). Post hoc analysis revealed significant differences between 2021-2019 ($p < 0.001$) and 2021-2020 ($p < 0.001$), indicating a 35.89% increase in NaOH-extractable organic matter between 2019 and 2021. The difference between 2020 and 2019 was less pronounced but still significant ($p < 0.05$) (Figure 15). This suggests that NaOH-extractable organic matter is an excellent indicator of fresh organic matter growth in the soil.

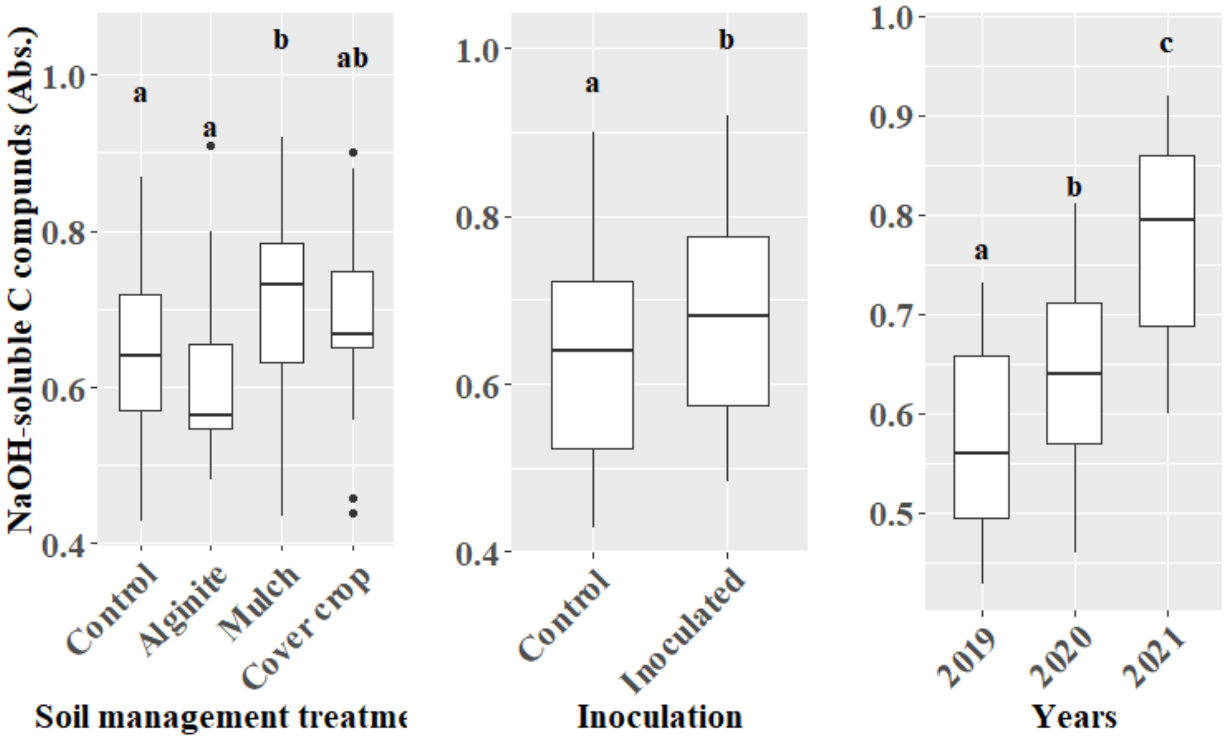

Figure 15. Amount of NaOH-Extractable organic matter under the influence of Soil management treatments, Inoculants, and Time.

The results for *permanganate-oxidizable carbon (POXC)* showed similar but even more pronounced effects related to changes over the years, with an overall increase of 68.97% between 2019 and 2021. The Year factor was significant ($F(2, 24) = 41.943$, $p < 0.001$). The post hoc test revealed significant differences between all years ($p < 0.001$). However, there were no significant differences in the treatment and inoculant factors.

For *dissolved organic carbon (DOC)*, the results showed that the time factor had a significant effect ($F(2, 64) = 147.66$, $p < 0.001$). DOC values increased significantly after the first year but then returned to initial levels. This is likely due to the high sensitivity of DOC to seasonal effects. The treatments also had a significant impact on DOC ($F(3, 64) = 5.906$, $p < 0.01$). Comparisons between treatment groups showed significant differences ($p < 0.01$) between the mulch-alginite and mulch-control pairs. The inoculant treatment also resulted in significantly higher DOC values compared to the control ($F(1, 64) = 5.104$, $p < 0.05$).

This suggests that, unlike other methods, DOC may be too sensitive for examining long-term carbon level increases between years but is sufficiently sensitive for assessing differences between treatments in short-term experiments.

The data for *glomalin (Easily Extractable Glomalin-Related Soil Proteins - EE-GRSP)* indicated significant differences for the treatment factor ($F(3, 65) = 11.264$, $p < 0.001$). The mulched plots showed significantly higher values compared to the Control ($p < 0.001$) and Alginite ($p < 0.001$) treatments. Additionally, the cover crop treatment had significantly higher values compared to the alginite-treated plots ($p < 0.05$). The inoculant factor was significantly higher than the control ($p > 0.01$). There was no interaction between the factors.

Glomalin showed a non-significant increase between 2019 and 2020, but there were significant differences between 2020 and 2021 ($p < 0.01$). However, the decrease in glomalin content was unexpected and contradicts the literature, which suggests that glomalin's transformation and degradation time is measured in years (Holátko et al., 2021).

The *microbial biomass-C (MBC)* values were measured in the last year (2021) from the top 10 cm of soil to examine the effects of treatments and inoculants. The inoculated plots showed higher but not significant values. Significant differences were observed between the treatments ($F(3, 16) = 5.529$, $p = 0.00846$). The cover crop plots showed significantly higher values compared to both the Alginite-treated ($p < 0.05$) and Mulched ($p < 0.001$) plots. This could be due to the greater living plant biomass in the area, as the cover crop plots had much more living plant matter, which could introduce more carbon into the soil to feed microorganisms. In contrast, the mulched plots had dead plant cover, which prevented the growth of unwanted plants (weeds), thereby limiting the carbon that could be introduced to microorganisms via root exudates.

3.6.5 Correlation Analysis Results of Measured Parameters from the Field Experiment

We conducted a correlation analysis on key parameters related to yield, soil carbon, and nitrogen to gain deeper insights into their relationships and to assess how well different carbon measurement methods correlate with each other. This analysis was performed using data from the control and the three main soil regeneration methods, as nitrogen data was also available for these treatments.

The strongest correlation was observed between stable carbon forms in the soil (NaF-extractable organic matter) and the total nitrogen content of the soil ($r = 0.80$, $p < 0.001$). Total Organic Carbon (TOC) also positively correlated with nitrogen content ($r = 0.59$, $p < 0.001$). Measurements of easily degradable carbon forms, such as NaOH-extractable organic matter and POXC, were strongly correlated ($r = 0.68$, $p < 0.001$). A strong correlation was also observed between TOC and NaOH-extractable organic matter ($r = 0.62$, $p < 0.001$).

Additionally, a strong correlation was found between Dissolved Organic Carbon (DOC) and glomalin, which is associated with fungi ($r = 0.62$, $p < 0.001$). Crop yield showed a moderate correlation with glomalin levels ($r = 0.49$, $p < 0.01$). However, according to the literature, glomalin typically shows a stronger correlation with TOC and nitrogen content (Barna et al., 2020).

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Effectiveness of Microbial Inoculations

The use of alginite is most effective in nutrient-poor, acidic soils where it helps stabilize pH levels and increase the levels of minerals such as calcium and phosphorus. In our research, minimal results were observed in the calcareous Soroksár soil, and alginite did not show significant interaction with the applied inoculants, likely because the typical issues addressed by alginite were absent in this soil type. Alginite application is recommended primarily for soils where nitrogen fertilization's acidifying effect is prevalent (Ragályi et al., 2019).

4.2 Impact of Alginite as a Soil Amendment on Soil Properties

Az alginit használata főként savanyodásra hajlamos, tápanyagban szegény talajokon hatékony, ahol segít pH értékek stabilizálásában és ásványi anyagok, mint kalcium és foszfor, szintjének növelésében. Kutatásunk során azonban a meszes Soroksári talajon minimális eredményeket észleltünk, és az alginit nem mutatott számottevő interakciót az alkalmazott oltóanyagokkal, mivel ezen a talajtípuson a jellemző problémák hiányoztak. Az alginit alkalmazását elsősorban azon talajokon ajánljuk, ahol a nitrogénműtrágyázás savanyító hatása érvényesül (Ragályi et al., 2019).

4.3 Impact of Soil Management Technologies in Organic Farming on Soil Properties

In our field experiment, we assessed the effects of regenerative soil management, evaluating changes in biological properties and organic matter forms. The most positive changes were observed in plots treated with cover crops and mulch, where nitrogen fixation by plants and carbon from mulch significantly increased soil organic carbon content.

However, minimal tillage led to weed problems, which were mitigated by using mulch. Under these conditions, we recommend using mulch and cover crops, especially when the goal is to increase biomass and soil carbon content.

The examination of microbial activity revealed an increase in DHA activity despite a decrease in microbial numbers, indicating a transformation of the soil food web. To better understand these changes, we recommend direct measurement of microbial biomass and more detailed study of soil fauna. However, these changes may also have been influenced by decreasing precipitation during the experiment, despite irrigation.

4.4 Interaction of Soil Management Methods (and Alginite) with Inoculants

Interactions between treatments were insignificant, but in some cases, the interaction between soil and inoculants influenced the number of aerobic bacteria and microscopic fungi in the Tófej and Szeghalom soils. Additionally, the combination of alginite and inoculants altered the rate of cellulose decomposition. This suggests that interactions are mainly related to soil-specific characteristics. Since inoculants rarely showed significant effects, it is difficult to draw clear conclusions about their effectiveness under different conditions.

4.5 Suitable Soil Organic Matter Measurement Methods for Assessing the Impact of Regenerative Practices Over Different Time Frames

In minimum-till systems, it is critical to select appropriate organic matter measurement methods for different time frames to accurately track the effects of treatments.

For *short-term studies*, dissolved organic carbon (DOC) measurements are recommended, as they quickly reflect changes.

For *medium-term studies*, NaOH-extractable organic matter, permanganate-oxidizable carbon (POXC), and Hargitai's humus quality assessment are ideal, as they differentiate changes in fresh and quality organic matter. It is essential to evaluate NaF and NaOH-extractable organic matter separately, not just based on the Q or K index. This allows for more precise tracking of whether the values of higher-quality, NaF-extractable organic matter remain stable while the quantity of fresher, NaOH-extractable organic matter increases.

For *long-term changes*, total organic carbon (TOC) and sodium fluoride (NaF) extractable organic matter measurements are recommended. Glomalin (EE-GRSP) is also suitable for long-term measurements (Holátko et al., 2021), although our results did not support this.

5. NEW SCIENTIFIC RESULTS

1. The A5: *Enterobacter ludwigii* strain, isolated from the specific soil used in our experiments, demonstrated a positive impact on the growth of mustard and perennial ryegrass during the initial stages of strain selection. This positive effect was also associated with higher activity of the non-specific FDA enzyme in the soil. This finding highlights the importance of utilizing strains from the specific soil in question and suggests the potential for improving the success of their application.

2. We tested the isolated strains for their potassium-solubilizing capabilities (KSB), a property that has been less studied to date. Three of our isolated strains (D1 - *Kosakonia cowanii*, A3 - *Bacillus coreaensis*, C1 - *Lelliottia amnigena*) were found to possess KSB capabilities, marking the first identification of this trait in these strains. This previously underexplored property may render these strains suitable for practical applications.

3. Our research on alginite demonstrated that the interaction between soil types and plant species is crucial in determining soil biological effects. The influence of alginite on soil biological properties (DHA, microbial abundance, organic matter decomposition) varied significantly depending on the context, either increasing or decreasing the effects. This result complements earlier studies where alginite was used to mitigate soil acidification and low nutrient content. It can be concluded that the application of alginite is most effective in a limited and site-specific manner based on known soil characteristics.

4. By comparing international and national carbon analysis methods, we identified and substantiated sensitivity differences with precise comparative measurements. The strong positive correlation ($r = 0.68$, $p < 0.001$) between POXC and NaOH-extractable organic matter supports our hypothesis that these methods provide information on fresher, more labile, and currently accessible organic matter. These results contribute to the comparability of national and international methods.

5. The choice of organic matter analysis methods for monitoring soil management practices should be based on the duration since the shift in management practices. While POXC and NaOH-extractable organic matter analysis are most effective for short-term studies, TOC and NaF-extractable organic matter analysis have proven more suitable for long-term studies.

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7. LIST OF PUBLICATIONS RELATED TO THE DOCTORAL THESES

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1. **Pabar, S. A.,** Kotroczó, Z., Takács, T., Biró, B., 2024. Evaluating the Efficacy of Selected **Plant Growth-Promoting Microorganisms** in Optimizing Plant Growth and Soil Health in Diverse Soil Types. Agriculture 14, 1586. https://doi.org/10.3390/agriculture14091586

2. Kotroczó, Zs., Juhos, K., Biró, B., Kocsis, T., **Pabar, S. A.,** Varga, Cs., & Fekete, I. (2020). *Effect of detritus manipulation on* different ***organic matter decompositions*** *in temperate deciduous forest soils.* Forests, *11(6), 675-689.* https://doi.org/10.3390/f11060675

3. Gorliczay, E., Boczonádi, I., Kiss, N. É., Tóth, F. A., **Pabar, S. A**., Biró, B., Kovács, L. R., & Tamás, J. (2021). ***Microbiological effectivity*** *evaluation of new poultry farming organic waste recycling.* Agriculture-Basel, 11(7), Paper 683. https://doi.org/10.3390/agriculture11070683

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4. **Pabar, S. A**., Mónok, D., Kotroczó, Zs., & Biró, B. (2020). *Soil microbial parameters and synergies between bean growth and* ***microbial inoculums*** *as a dependence of five soils with different characteristics*. Hungarian Agricultural Engineering, 37, 27-33. http://doi.org/10.17676/HAE.2020.37.27

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9. **Pabar S A**, Begum S R, Kotroczó Zs, Kardos L, Mónok D, Pribeli A, Biró B. *A saláta (Lactuca sativa var. capitata L.) növekedésére és* ***a talajállapotra ható bioeffektív kezelések.*** In: Füleky Gy (szerk.) XIV. Kárpát-Medencei Környezettudományi Konferencia kiadványa. Gödöllő, Magyarország, 018.04.05-2018.04.07. pp. 236-241.

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