agriculture

MDPI

Article

Evaluating the Efficacy of Selected Plant Growth-Promoting Microorganisms in Optimizing Plant Growth and Soil Health in Diverse Soil Types

Sándor Attila Pabar [1,2], Zsolt Kotroczó [2,*], Tünde Takács [1,*] and Borbála Biró [2]

[1] Department of Soil Biology, Institute for Soil Sciences, Centre for Agricultural Research, HUN-REN, Herman Ottó Str. 15, H-1022 Budapest, Hungary; pabar.sandor@atk.hun-ren.hu
[2] Department of Agro-Environmental Studies, Hungarian University of Agriculture and Life Sciences, Villányi Str. 29–43, H-1118 Budapest, Hungary; biro.borbala@gmail.com
* Correspondence: kotroczo.zsolt@gmail.com (Z.K.); takacs.tunde@atk.hun-ren.hu (T.T.)

Abstract: This study explores the efficacy of bio-efficient solutions, specifically plant growth-promoting microorganisms (PGPMs), in sustainable soil management. This research was conducted in 2020. It evaluates the impact of various single microbial inoculants, including *Enterobacter ludwigii*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Kosakonia cowanii*, and *Trichoderma harzianum*, on plant growth soil enzyme activity and organism abundance. Perennial ryegrass and mustard were used as test plants, in controlled environmental conditions. The results show generally positive effects of microbial inoculants on plant biomass (*E. ludwigii* increased ryegrass biomass by 9.75%, and *P. fluorescens* increased mustard biomass by up to 38.81% compared to the control) and on soil microbial activities. Our study further investigated the combined application of all these strains in five different soil types and textures. The results highlight the significance of soil physicochemical properties in determining inoculant efficacy; we found that clayey soils with higher colloid content support more robust microbial activity. Additionally, using natural clay minerals like alginite for enhancing soil conditions showed promising interactions with microbial inoculants, although application requires further optimization. These findings suggest that integrating microbial inoculants in sustainable agricultural practices could enhance plant growth, improve soil health, and reduce the need of chemical fertilizers. Future research should aim to refine the combinations and application methods of these bio-efficient solutions for broader agricultural applicability.

Keywords: plant growth-promoting microorganisms (PGPMs); microbial inoculants; sustainable soil management; alginite; soil enzyme activity

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

Academic Editor: Andong Cai

Received: 17 July 2024
Revised: 6 September 2024
Accepted: 10 September 2024
Published: 12 September 2024

1. Introduction

Bio-efficient solutions, such as biocontrol agents, biopesticides, and biofertilizers, can play an important role in sustainable soil management by providing nutrient supply and biological plant protection in a single step [1,2]. Such soil treatments include the application of microbial inoculations [3–5]. The literature suggests that, depending on the environment, microbial biomass per hectare ranges from 3 to 15 tons; thus, its significance cannot be overlooked [6–8].

Plant growth-promoting microorganisms (PGPMs) can assist plant growth in various ways and synergistically strengthen each other's effects [9]. The development of plant growth-promoting (PGP) effects can occur through several mechanisms: (1) beneficial microorganisms can directly increase plant biomass by producing auxin, gibberellin, and cytokinins, while abscisic acid and reduction of ethylene plays a key role in stress response and regulation [10,11]; (2) assist nutrient uptake through the symbiotic relationship with mycorrhizal (AMF) fungi [12]; (3) indirectly enhance nutrient availability through biological nitrogen fixation and phosphorus mobilization [13]; (4) reduce yield losses through

biocontrol functions such as hyperparasitism and iron uptake [14,15], or strengthen crop resistance to biotic stresses [16]. Despite the recognized benefits of PGPMs, there is limited research on their effectiveness across different soil types, particularly when combined with natural amendments, such as alginite. Furthermore, the synergistic effects of combining multiple species under varying environmental conditions have not been fully explored.

Several previous studies have established the beneficial functions of plant growth-promoting rhizobacteria (PGPR) and their ability to synergize with each other [17,18]. In this study, the following PGPM species were used both as single inoculants and in combination as a microbial consortium: *Enterobacter ludwigii*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Kosakonia cowanii*, and *Trichoderma harzianum*.

Enterobacter ludwigii is a phosphorus-solubilizing and potassium-solubilizing bacterium (PSB, KSB) with biocontrol properties, capable of nitrogen bonding, indole-3-acetic acid (IAA) and siderophore production, and enhancing plant biomass [19–22]. *Bacillus subtilis* is also recognized for its biocontrol capabilities, phosphorus-solubilizing (PSB) activity, and cellulose-degrading properties [23–25]. *Pseudomonas fluorescens* is commonly used in microbial inoculants due to its ability to control plant diseases, produce siderophores, function as a phosphate-solubilizing bacterium (PSB), and produce the plant hormone indole-3-acetic acid (IAA) [14,24]. *Kosakonia cowanii* is capable of biological nitrogen fixation, and its related species is known for its phosphate solubilization (PSB) and indole-3-acetic acid (IAA)- and siderophore-producing capabilities, suggesting that the species of *K. cowanii* likely possesses these traits as well [26–30]. Furthermore, both *Enterobacter ludwigii* and *Kosakonia cowanii* species have been observed to produce extracellular polysaccharides (EPSs) [31,32], which can improve soil structure [33]. *Trichoderma harzianum* is known for its biocontrol and cellulose-degrading properties [34,35].

In addition to the species listed above, there are many more PGP microorganisms, and these beneficial traits are often characteristic of their respective genera [12,36,37].

There are still unknown factors that need further investigation to enhance their effectiveness [38]. Inoculants can behave differently and may not necessarily function well in different soils influenced by several key factors, including soil characteristics, microbial community interactions, and management practices. Soil properties include pH, moisture, texture, and the presence of indigenous microorganisms [23,39–41]. For example, research indicates that microbial inoculants, such as *Bacillus* species, demonstrate varying success in sandy versus clay soils. For instance, a study found that a specific inoculant (P1) improved wheat shoot weight under drought conditions in sandy soil but not in clay soil, highlighting the importance of soil texture in inoculant performance [42].

To address this and improve the viability of introduced strains, natural clay minerals can be helpful in certain soil types [43]. These minerals can enhance the primary physical and chemical conditions of soils, creating more favorable conditions for both plants and microbes [44]. Their mineral content can also support plant growth and nutrient uptake [45,46]. One such clay mineral is alginite, which is high in carbonate and can alter soil pH, as well as increase the Ca content of soil [47]. It contains nearly 60 mineral elements, including micro- and macronutrients [45]. For these reasons, it can improve soil functions and boosts plant production in certain soil types. Its application is environmentally friendly and poses lower health risks compared to chemical methods [48]. Through selecting and matching the appropriate species of inoculants, it is possible to implement biotic and abiotic stress-tolerant inoculations adapted to specific soil and environmental conditions [49,50].

Since microbial products typically contain various species and their strains, rapid testing options for these PGP properties are important. Most modern solutions are based on methods like PCR [51]. The need for quick methods for assessing plant germination rates and biomass production has increased, bridging the gap between laboratory tests and field applications [52]. These tests preempt the usual and accepted scaling-up impact assessments from laboratory to field conditions for microbial inoculants. We sought a method that is rapid, cost-effective, and feasible with real soil, facilitating the transition and providing suitable results for selecting the most effective strains. Consequently, we

tested the effectiveness of various bacterial species and a microscopic fungus on the growth of different plant species.

In this study, we tested the (PGP) effects of PGPMs on perennial ryegrass (*Lolium perenne* L.) and mustard (*Sinapis alba* L.) at a small scale, using cell trays. We investigated the ability of the PGPM strains to increase biomass in different test plants, and their impact on soil enzyme activity. Subsequently, from the tested strains, we created an inoculant combination from all the previously tested strains, since it was reported to be more effective than the single inoculations [17,18], and scaled up the experiment. We tested this combination in pots with bean seedlings in five different soils to assess how the physical and chemical properties of soils influence the effectiveness of our selected microbial inoculant.

Our goal was to test the PGP effects of these strains, evaluate their combination on various soil types mixed with the soil amendment alginite, and examine whether there were differences between the different environments, as this is crucial for the efficient application of environmentally friendly solutions such as inoculants and soil amendments.

Our hypotheses were that PGP strains would increase plant biomass production, both individually and in combination, except for *Trichoderma harzianum*, which is primarily a biocontrol species but was included in the combined inoculant; therefore, we also tested this strain individually. We also hypothesized that this biomass increase would correlate with higher soil enzyme activity. Furthermore, we proposed that the effectiveness of the inoculant could be influenced by soil type, prompting us to test it on diverse soils. Since the **S1** and **S4** strains were isolated from Soroksár 1 soil, we hypothesized that these strains would be more effective on this soil, as they were already adapted to this environment. Additionally, we expected that alginite would influence the effectiveness of the inoculation, depending on soil plasticity, which is why it was tested on the two soil types with the most distinct plasticity.

2. Materials and Methods

2.1. Used Microorganisms

For the experiments, the following PGP strains were used for soil inoculation. Their abbreviations are described in Table 1. Some of the used PGPM strains were the results of our isolation work (**S1**—*Enterobacter ludwigii*—**A5**; **S4**—*Kosakonia cowanii*—**D1**). These were isolated from Soroksár 1 soil. The strains were selected using traditional Pikovskaya's agar. The **S4** strain was isolated from a bean plant root nodule that was grown in this soil. **S2** and **S3** were from our strain collection (**S2**—*Bacillus subtilis*—B 01035; **S3**—*Pseudomonas fluorescens*—Hx1), and the fungus was a commercial product (**S5**—*Trichorderma harzianum*—T-22 (Koppert B.V., Rotterdam, The Netherlands). These strains were selected based on their PGP effects, with plans to use them in future field experiments.

Table 1. Potential and expected properties of used microorganism species. **S1–5** are identifiers in this article.

Strain Composition	Properties, Abilities
S1—*Enterobacter ludwigii*	PSB, KSB, N bonding, Fusarium fungal antagonism, IAA, siderophore, and extracellular polysaccharide production [19–22]
S2—*Bacillus subtilis*	Cellulose decomposition, PSB, biocontrol [23–25]
S3—*Pseudomonas fluorescens*	PSB, biocontrol effect, IAA, and siderophore production [14,23]
S4—*Kosakonia cowanii*	Nitrogen bonding, PSB, KSB, and EPS production [26–30]
S5—*Trichorderma harzianum*	Biocontrol effect, cellulose decomposition [31,32]

The microbial strains were cultivated for 48 h in an orbital shaker at 28 °C and 125 rpm. Microbial suspensions were centrifuged after cultivation and then resuspended in a physiological saline solution to eliminate the effect of nutrient broth on the microorganisms used in the experiment. The cell concentration was adjusted to 10^7 cells/mL using OD600

measurement for uniform and standard application with a spectrophotometer at 600 nm (Libra S22 UV/VIS spectrophotometer (Biochrom Ltd., Cambridge, England)) [53].

Trichoderma harzianum, a microscopic fungus, was only available in powder form and did not require prior cultivation. After preparing the solution with appropriate concentration, it was applied in the same manner as the bacteria.

2.2. Experimental Design

There were two parts of this experiment. First was the small-scale experimental design for testing the effectivity of the PGPM strains individually; then, the mixed inoculation of the used PGPM strains in a larger pot experiment was the second part. The experiment was conducted under controlled conditions in a light room, where light, temperature, and humidity could be precisely regulated.

2.2.1. Experiment for the PGP Strain Test

The experiment was conducted using cell trays typically used for germinating plants. Each cell in the trays was individually inoculated with different strains. For inoculation, 0.5 mL of the prepared suspension was applied to each cell. The control cells were treated with a sterilized inoculant mixture of the previously described strains.

A randomized arrangement was employed across the cell trays. A total of six treatments were applied, including the control, allowing for the investigation of 12 independent strains.

White mustard (*Sinapis alba* L.) and perennial ryegrass (*Lolium perenne* L.) were used as test plants. The seeds were distributed into the tray cells as follows: mustard—4 seeds/cell; ryegrass—0.6 g of grass seed/cell. The experiment was concluded 2 weeks after sowing, and for mustard, the number of germinated seeds was counted relative to the initial 4 seeds per cell. The dry biomass was measured for both mustard and perennial ryegrass. The impact of microbial inoculations on the seed germination was calculated as follows: the mean relative response ratio (RR), which represents the percentage change (%) based on a comparison of results between the used strains against the control (RR = (Control − Strain)/Strain × 100).

Calcareous sandy (arenosol) soil from the Research and Experimental Farm of Hungarian University of Agriculture and Life Sciences, Horticultural Faculty, Ecological Farming Division in Soroksár, Hungary, was used for the experiment, amended with compost in a ratio of 90:10 by mass percentage. The most important chemical properties of the soil used for the experiment were as follows: pH (H_2O) of 7.79, soil organic matter (SOM) content of 3.29 *w*/*w*%, calcium (Ca) concentration of 2979 mg/kg, ammonium lactate soluble phosphorus pentoxide (AL-P_2O_5) concentration of 424 mg/kg, and potassium oxide (AL-K_2O) concentration of 460 mg/kg.

2.2.2. Pot Experiment with Mixed Microbial Inoculations and Alginite-Amended Soils

We used pencil pod yellow wax beans (*Phaseolus vulgaris* var. Maxidor) as test plants. The plants were sown in pots (4 seeds per pot), and then thinned to the same number of plants per pot (1 plant per pot) after germination. Microbial inoculation was carried out simultaneously with sowing. The inoculant was a mixture of all the previously tested strains in equal ratios, with the potential and expected properties of each microorganism species described in Table 1. Each pot received 1 mL of inoculant, and as a control, autoclaved bacterial suspension was applied. The autoclave was operated at 121 °C for 20 min under a pressure of 1 bar (2 bar absolute pressure). Soil samples were taken at the test plants' 60% flowering during the simultaneous harvesting of the experiment. We measured the dry biomass of the plants, which were air-dried to weight equality. Each treatment was replicated four times.

Under controlled conditions, the plants were cultivated in a growth chamber with a 16/8 h day/night cycle. The temperature was maintained at 22 ± 2 °C, and the relative

humidity was 55 ± 5%. Each pot was filled with 850 g of dry soil, adjusted to a 60% water holding capacity, and watered accordingly after gravimetric control.

In the second phase of this experiment, our primary focus was on observing differences between soils and the effects of the inoculum on the examined parameters. However, we also examined the effects of alginite and its interactions with the soils and inoculum mixture. We evaluated the impact of alginite solely on two soils with the most extreme textures: Soroksár 1, a limey sandy soil (arenosol), and Szeghalom, a heavy, clayey soil (gleysol). Alginite was added to these soils at a 5% *w*/*w* ratio. In these instances, we investigated the interactions among alginite, soil types, and the inoculant.

The plants were grown in five different soils with varying physicochemical characteristics (Table 2). The soils have neutral to slightly alkaline pH levels and differ in their texture and organic matter content. These soils correspond to genetic soil types commonly used in cultivation based on WRB classification [54]. The key physicochemical properties of the soils used are presented in Table 2.

Table 2. The main physicochemical characteristics of the used soils in the pot experiment.

Measured Parameters	Soroksár 1	Soroksár 2	Hatvan	Tófej	Szeghalom
Soil type	Arenosol	Gleysol	Chernozem	Luvisol	Gleysol
Texture	Sand	Clay loam	Clay loam	Clay	Clay
Soil plasticity (K_A)	26	43.4	49	54	57.5
$pH_{(H2O)}$	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 *w*/*w*%	0.0317	0.0216	0.0268	0.0555	0.0665
Humus content (H%):	2.18	4.09	4.63	3.89	3.75

2.3. Determination of the Soils' Physico-Chemical Parameters

The soil samples were air-dried until weight equality, the plant materials were removed, and the samples were homogenized. The $pH_{(KCl)}$ and soil available Ca, P, and K content were measured using an ammonium lactate solution [55], assessed by ion-flame chromatograph (FP910 flame photometer (PG Instruments Ltd., Leicestershire, England)) and a spectrophotometer (Libra S22 UV/VIS spectrophotometer (Biochrom Ltd., Cambridge, England)).

The total organic carbon (TOC) content was determined after sulfochromic oxidation followed by titration of the excess $K_2Cr_2O_7$ with $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ [56]. SOM (*w*/*w*%) were obtained by multiplying the TOC values by 1.724. The pH of the soils was measured in 1:2.5 air-dried soil–distilled water and 1:2.5 air-dried soil–1 M KCl solution suspension after 24 h equilibration [57]. Water-soluble salts (*w*/*w*%) were also measured using this standard [57]. The soil plasticity (K_A) was measured as described in the following method book: [58].

The plant biomass was measured, which was oven-dried at 70 °C to constant weight.

2.4. Examination of the Soils' Biological Parameters

For the microbial tests, the soil samples were stored at 4 °C after sampling until use.

2.4.1. Examination of Microbial Abundance in the Soils

The most probable number (MPN) method was used to quantify the culturable mesophilic aerobic bacterial, spore-forming bacterial, and microscopic fungal populations within the soil samples [59]. This was conducted using a microplate technique as described by Reichart [60]. Ten-fold serial dilutions of the soil were prepared, ranging from 10^{-1} to 10^{-8}. For bacterial quantification, nutrient broth (composed of 3 g L^{-1} meat extract, 5 g L^{-1} peptone, 5 g L^{-1} glucose, and 0.5 g L^{-1} NaCl, with a pH of 7.0 ± 0.2) was used. Spore-forming bacteria were measured on nutrient medium, after soil samples treated at 80 °C for 20 min and Sabouraud broth (containing 5 g L^{-1} casein, 5 g L^{-1} meat extract, and 20 g L^{-1} glucose, with a pH of 5.7 ± 0.2) was used for fungi. The plates were incubated at

28 °C for 5 days. After visual evaluation, the MPN values were calculated using Cochran's MPN table [61].

2.4.2. Methodology for Determining Soil Enzyme Activities

Soil enzymatic activity was assessed using the dehydrogenase activity (DHA) and fluorescein-diacetate (FDA) hydrolysis method measurements. A Libra S22 UV/VIS spectrophotometer (Biochrom Ltd., Cambridge, England) was used.

DHA was assessed using a modified method based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) [62,63]. Fresh soil samples (1 g) were vortexed with 1 mL of TTC solution and incubated at 30 °C. The control contained only 1 mL of TRIS buffer. After 24 h of incubation, the resulting triphenyl formazan (TPF) was extracted by adding 5 mL of methanol to each tube. The samples were incubated in the dark at room temperature for 2 h. The soil suspensions (6 mL) were centrifuged, and the optical density of the clear supernatant was measured at 546 nm against a methanol blank.

The fluorescein diacetate (FDA) activity test was conducted based on the method described by Villányi et al. [64]. Briefly, 7.5 mL of 60 mM potassium phosphate buffer (pH 7.6) and 100 µL of 4.8 mM fluorescein diacetate were added to 1 g of fresh soil. Samples were incubated at 30 °C and 150 rpm for 1 h. The samples were measured out as 700 µL to Eppendorfs, and the reaction was stopped with 700 µL of 50% acetone, followed by centrifugation at 2000 rpm for 2 min at room temperature. The amount of hydrolyzed FDA was measured at 490 nm using a calibration curve prepared with a fluorescein standard solution (0–100 mg L^{-1}).

2.5. Measurement of Organic Matter Decomposition

We examined the decomposition of organic matter by placing 1 g of cellulose in the soil, which was measured back at the end of the experiment [65].

2.6. Data Analysis

The results were processed using R (4.1.3.) and Microsoft Excel (The version of the software is Microsoft® Excel® LTSC MSO (16.0.14332.20761) 64-bit.). Normality and homogeneity of variances were assessed using the Kolmogorov–Smirnov and Levene tests, respectively, justifying the use of parametric methods. ANOVA was used to evaluate main effects and interactions, given its suitability for multi-factorial designs. Significant differences were further explored using Tukey's post hoc test, which controls for Type I error in multiple comparisons.

Results were interpreted at a 95% significance level ($p < 0.05$). Non-significant results indicate that no statistically meaningful differences were detected, though this does not rule out the presence of smaller effects not detected in this study.

When a specific factor showed significant effects, but other factors did not, there may have been no interactions.

3. Results and Discussion

Microbial communities play a crucial role in plant–soil systems, contributing to numerous functions such as nutrient cycling, disease suppression, and plant growth promotion [5,13,22]. The synergistic effects of combining different microbial strains can enhance these benefits, offering potential for improved agricultural productivity and sustainability [2,18,35]. However, to fully harness these benefits, rigorous testing and refinement across various scales are essential to optimize microbial consortia for specific applications [17,52].

In the PGPM strain test and selection experiment, we observed higher plant growth parameters in the PGPM-treated samples compared to the control. In the case of mustard as the test plant, we observed higher, though not statistically significant, values compared to the control for both germination rate ($F(5,62) = 0.791$, $p = 0.56$) and dry biomass ($F(5,62) = 0.592$, $p = 0.706$). The highest germination rate was associated with **S3**, *Pseudomonas fluorescens* (RR = +38.81%), while the highest dry biomass was recorded with **S1**, *Enterobacter*

ludwigii (RR = +30.77%) (Figure 1). The literature is lacking on seed germination on mustard seeds treated with PGPMs, but other plants species react positively [66]; for example other plant species (*Brassica napus* L., *Amaranthus retroflexus* L., *Linum usitatissimum* L., *Panicum miliaceum* L., and *Rumex patientia* L.) had increased biomass after *P. fluorescens* treatments [67].

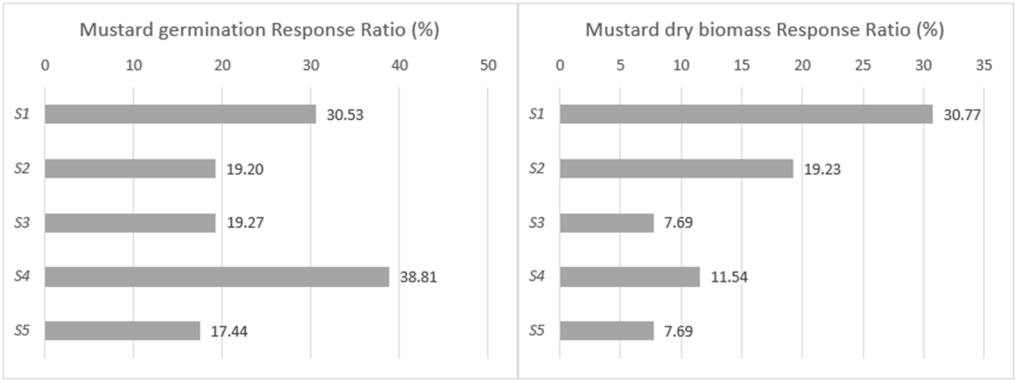

Figure 1. Mean relative response ratios (RRs) representing the percentage change (%) based on the comparison of results between the used strains (**S1**—*Enterobacter ludwigii*, **S2**—*Bacillus subtilis*, **S3**—*Pseudomonas fluorescens*, **S4**—*Kosakonia cowanii*, **S5**—*Trichorderma harzianum*) against the control (RR = (Control − Strain)/Strain × 100). (**Left**): mustard seed germination. (**Right**): mustard dry biomass. Further information in text.

Similar conclusions can be drawn regarding the dry biomass of perennial ryegrass ($F(5,66) = 0.376$, $p = 0.864$). There were no significant differences in the results obtained, and the overall biomass growth differences among strains were less pronounced. **S1**, *Enterobacter ludwigii* (RR = +9.75%), showed the highest growth compared to the control (Figure 2). To our knowledge, no study has addressed *Enterobacter ludwigii* inoculation on *Lolium perenne* so far. However, Zaballa et al. reported a positive effect of this bacterium on barley biomass, and it was isolated from ryegrass [68].

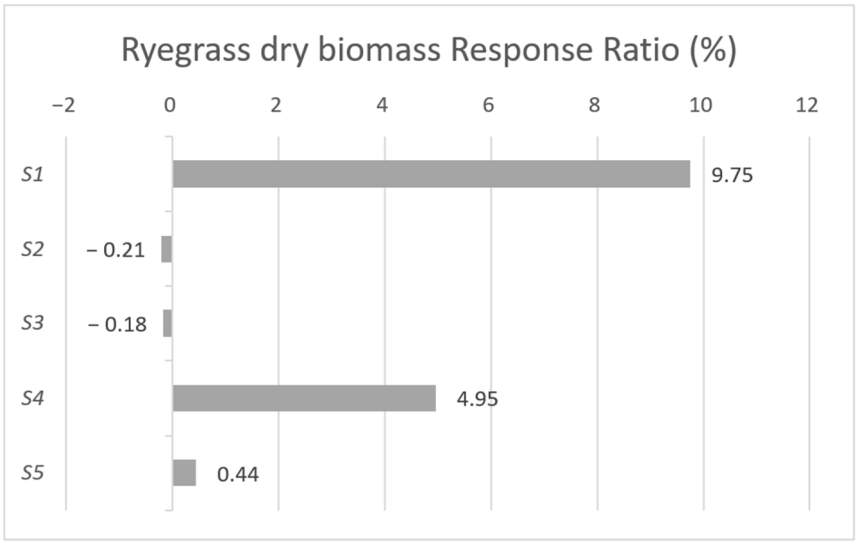

Figure 2. Mean relative response ratios (RRs) representing the percentage change (%) based on the comparison of results between the used strains (**S1**—*Enterobacter ludwigii*; **S2**—*Bacillus subtilis*; **S3**—*Pseudomonas fluorescens*; **S4**—*Kosakonia cowanii*; **S5**—*Trichorderma harzianum*) against the control (RR = (Control − Strain)/Strain × 100). Ryegrass dry biomass. Further information in text.

Trichoderma harzianum (**S5**), a microscopic fungus known for its biocontrol properties [34,35], achieved the lowest RR in the tests. Therefore, its role primarily focuses on

plant protection rather than direct crop yield enhancement, which aligns with the expected outcome (Figure 2).

The increase in germination rate and plant biomass is promising, but the lack of significant results indicates that further testing and validation are needed.

Soil enzyme activity and its response ratio are presented in Figure 3, showing the results of soil FDA (fluorescein diacetate hydrolysis) activity for mustard and perennial ryegrass. In both plant species, higher FDA activity values were consistently observed compared to the control, indicating increased biological activity due to the treatments. The **S3** bacterial treatment was an exception to this trend. Pereira et al. found similar results for this species, with maize test plants under water deficit conditions [69].

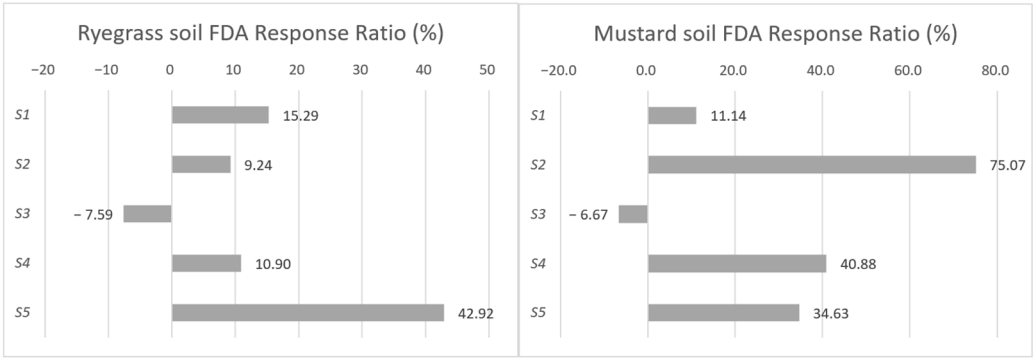

Figure 3. Mean relative response ratios (RRs) representing the percentage change (%) based on the comparison of results between the used strains (**S1**—*Enterobacter ludwigii*; **S2**—*Bacillus subtilis*; **S3**—*Pseudomonas fluorescens*; **S4**—*Kosakonia cowanii*; **S5**—*Trichorderma harzianum*) against the control (RR = (Control − Strain)/Strain × 100). (**Left**): FDA enzyme activity in inoculated ryegrass soil. (**Right**): FDA enzyme activity in inoculated mustard soil. Further information in text.

Additional data for Figures 1–3 are available in the Supplementary Material.

We also conducted correlation–regression analysis between FDA enzyme activity and measured plant characteristics. Generally, FDA showed positive correlations with all measured plant parameters. However, these results were not statistically significant, as we used only informative average samples due to limited soil quantity for FDA measurements per treatment (Table 3). Manjutha et al. found a positive correlation between wheat yield and both FDA and DHA [70].

Table 3. Correlation of FDA (fluorescein diacetate hydrolysis) enzyme activity with the examined plant parameters.

	FDA	***p*-Value**
Mustard germination%	0.29418	0.5715
Mustard dry biomass (g)	0.4300	0.4033
Perennial ryegrass dry biomass (g)	0.1331	0.8015

In the second part of the experiment, where we tested the inoculum consortium on different soils with alginite, there were no significant differences in plant dry biomass weight across the inoculant or alginite treatments. The results in the literature regarding the effects of microbial combinations on plant biomass are diverse, indicating that more research is needed to optimize inoculums [2,39]. Alginite mostly positively affects plant biomass, but this effect is documented primarily in soils with lower pH and/or low nutrient content [46,49]. However, dry biomass was significantly different across different soils ($F(4,34) = 9.794$, $p < 0.001$) (Figure 4). These results indicate that the primary factor influencing plant biomass was the type of soil used. Neither the microbial consortia nor the alginite had a significant effect under these conditions. Despite the previously

demonstrated positive effects on plant biomass, the combination of the strains did not further enhance the PGP effect.

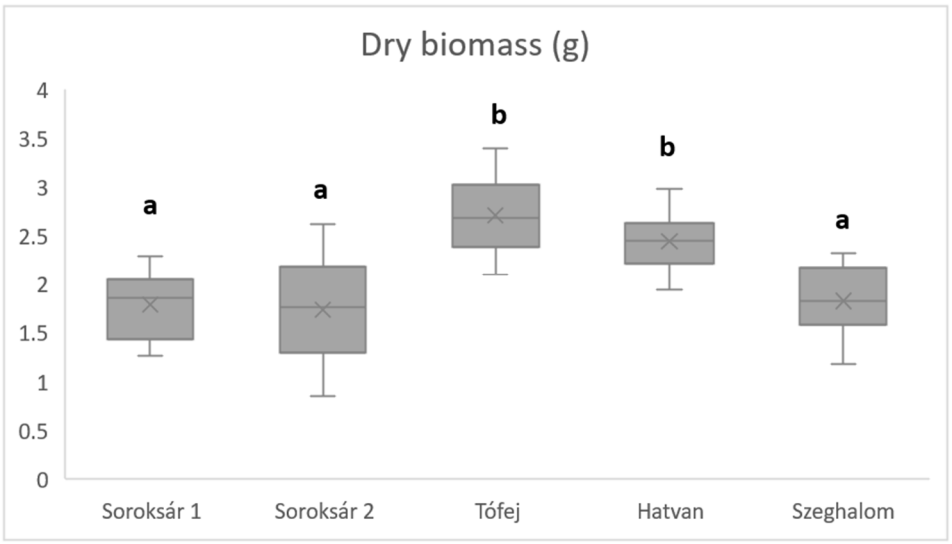

Figure 4. Bean plant dry biomass (g) production across different soils (without alginite treatment). Data include the inoculation, but the results are not separated by inoculation treatments because no statistically significant differences were observed between them. Different letters mean statistically significant differences. Further information in text.

Among the soils, there was a significant difference in DHA enzyme activities ($F(4,34) = 14.75$, $p < 0.001$). The effect of the inoculant on DHA was also significant ($F(1,34) = 14.18$, $p < 0.001$), with higher values attributed to the inoculant. There was an interaction observed, where the inoculant-treated soil had higher DHA in Tófej ($F(4,34) = 3.194$, $p < 0.05$) (Figure 5). There was no significant change found that could be attributed to alginite. The type of soil continued to have the greatest influence on soil DHA, but the inoculum also showed positive effects, indicating its overall beneficial impact on the soil–plant system.

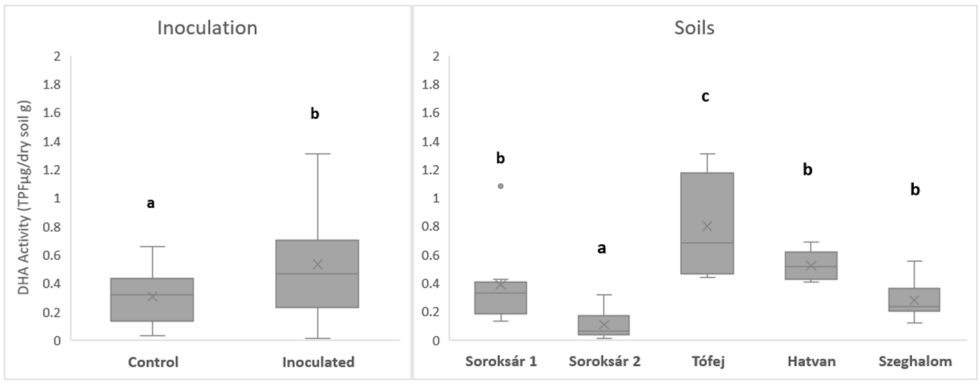

Figure 5. Variation in DHA enzyme activity values in relation to the inoculant treatments and the five different soil types and textures. (**Left**): effect of inoculation. (**Right**): effect of soils. Different letters mean statistically significant differences. Further information in text.

Telesiński et al. [71] found that dehydrogenases (DHAs) are highly sensitive to contamination with coal tar creosote, with significant variations observed in different soil types. Abou-Zeid [72] reported significant differences in DHAs due to inoculations with PGPR bacteria. Bandyopadhyay and Maiti [73] emphasized dehydrogenase activity as a crucial indicator of soil functionality and disturbance, noting lower DHA functionality in coal mine soil due to damaged microflora and reduced organic matter content. Collectively,

these studies indicate that DHA enzyme activities can vary significantly among soils due to pollution levels, soil types, and other influencing factors.

The main factors did not show significant effects on the MPN-based aerobic bacteria abundance. In the comparison involving alginite, the main factors did not show significance, but the interaction revealed that in Soroksár 1 soil, where inoculant was applied, the MPN of the aerobic bacteria significantly decreased ($F(1,24) = 12.518$, $p < 0.01$) (Figure 6). This result shows that alginite, which is high in clay and calcium carbonate, can alter the soil bacteria abundance in arenosol-type soil. To our knowledge, no other study has investigated the interaction between soil type and the effects of inoculation on bacterial abundance in the soil.

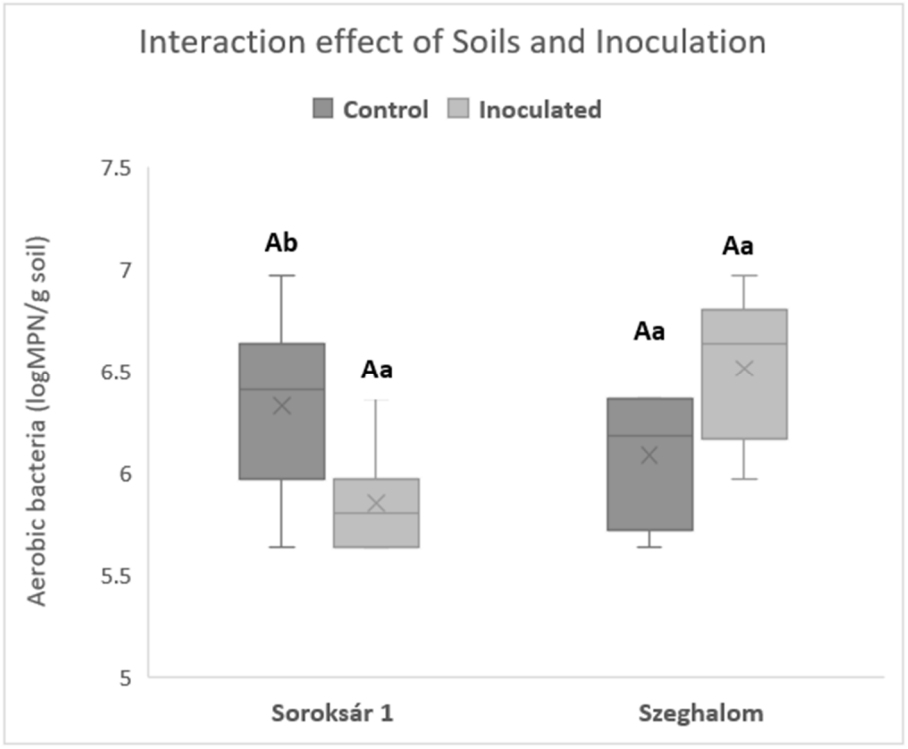

Figure 6. The number of aerobic bacteria in response to the inoculant and in different two soils. Measured on nutrient medium with the MPN method. The results are presented on a logarithmic scale. Large capital letters indicate statistical differences between soils; small capital letters mean statistical differences due to the inoculation on one soil. Same letters mean no statistical differences. Further information in text.

Soil type and inoculant treatment both showed significant effects [$F(4,34) = 14.81$, $p < 0.001$ and $F(4,34) = 14.96$, $p < 0.001$, respectively] on changes in microscopic fungal logarithmic values (Figure 7). Kutateladze et al. also found that soil type has a major effect on the abundance of microscopic fungi [74]. Furthermore, Gazdag et al. found that the soil type and texture had a stronger effect on *Alphaproteobaceria* community composition than farming systems [75]. The effects of PGPM inoculation on microscopic fungi are rarely studied. Most research focuses on evaluating antifungal effects in Petri dishes and through plant infection [19], or on synergistic effects with arbuscular mycorrhizal fungi (AMF) and PGPR bacteria [76]. Interaction was significant between soil type and inoculants on bean plants. In Tófej and Szeghalom soils, the inoculation resulted in microscopical fungi abundance ($F(4,30) = 8.319$, $p < 0.001$). This indicates that fungal components, likely primarily **S5**—*T. harzianum*, successfully survived inoculation and persisted in the soil until the time of measurement. Therefore, it can be concluded that the fungal component of the inoculant had the most effective impact on the most clayey soil types (soil plasticity (K_A))

of the following soils: Tófej, 54% and Szeghalom, 57.5%). However, this contradicts the literature, which indicates no correlation between fungal biomass and soil clay content [77].

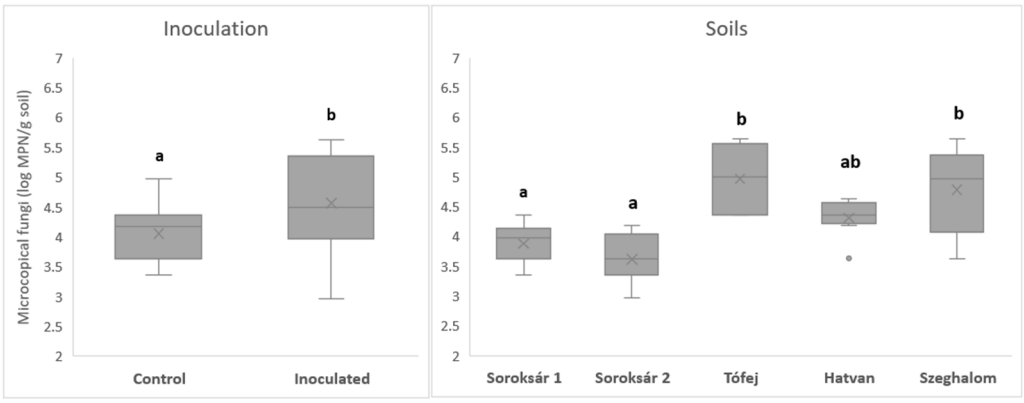

Figure 7. The number of microscopical fungi in response to the inoculant and in five different soils. Measured on sabouraud dextrose broth with MPN. The results are presented on a logarithmic scale. (**Left**): effect of inoculation. (**Right**): effect of soils. Different letters mean statistically significant differences. Further information in text.

In the case of comparisons involving alginite, all factors were deemed significant (Figure 8) (soil—($F(1,24) = 62.959$, $p < 0.001$); alginite—($F(1,24) = 17.619$, $p < 0.001$); inoculant—($F(1,24) = 8.195$, $p < 0.01$), along with a relevant interaction. In treatments without alginite, the effect of the inoculant on the microscopical fungi abundance was significant ($F(1,24) = 8.515$, $p < 0.001$). There were no relevant significant effects in the case of alginite comparisons.

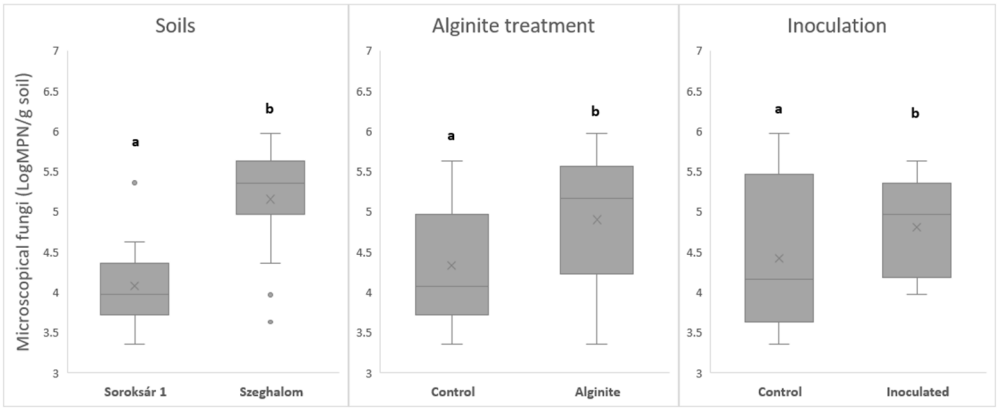

Figure 8. Changes in microscopical fungi numbers across two different soils in response to alginite and inoculant treatments in common bean plants. Measured on sabouraud dextrose broth with MPN method. The results are presented on a logarithmic scale. (**Left**): difference between soils. (**Mid**): difference due to alginite treatment. (**Right**): difference due to inoculation. Different letters mean statistically significant differences. Further information in text.

Both parameters were significant for spore-forming bacteria [soil ($F(4,34) = 3.595$, $p < 0.05$), inoculum ($F(1,34) = 5.929$, $p < 0.05$)] (Figure 9), but no interaction was found. This indicates that inoculation can boost the abundance of spore-forming bacteria in the investigated soils overall.

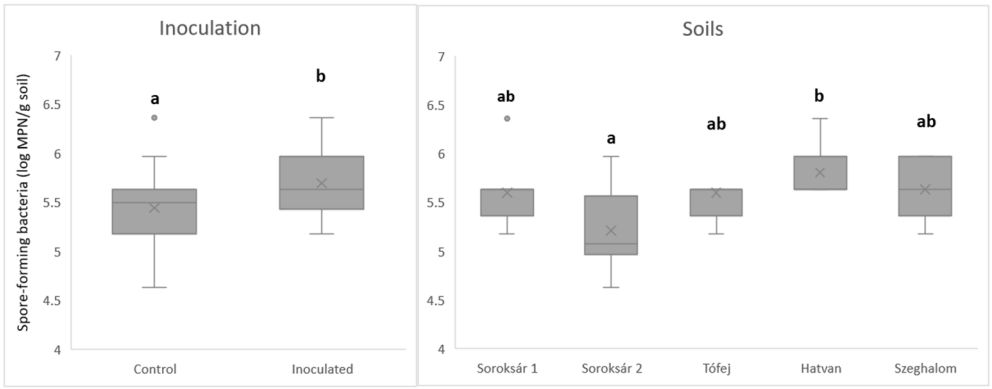

Figure 9. The number of spore-forming bacteria in response to the inoculant and in different five soils. Measured on nutrient medium with the MPN method after soil samples treated at 80 °C for 20 min. The results are presented on a logarithmic scale. (**Left**): effect of inoculation. (**Right**): effect of soils. Different letters mean statistically significant differences. Further information in text.

The inoculation effect on the composition and abundance of native soil bacterial communities is controversial, with no clear trends observed [78,79].

There were no significant effects in the case of alginite comparisons.

We examined the decomposition of organic matter by placing 1 g of cellulose in the soil, which was measured back at the end of the experiment. It was significant only between the different soil types ($F(4,34) = 9.794$, $p < 0.001$). The difference between the soils probably stems from the physical nature of the soils. The Soroksár 1 soil is sandy soil, and the Szeghalom soil is a heavily bound, clayey soil, so it is difficult for oxygen to pass through. Soil moisture and temperature could impair the decomposition ability of Soroksár 1 soil, since it is sand and therefore dries out quickly, but the soil moisture was kept at the same level in the experimental conditions (Figure 10). The effect of soil texture on the rate of decomposition is well supported by the literature [80,81].

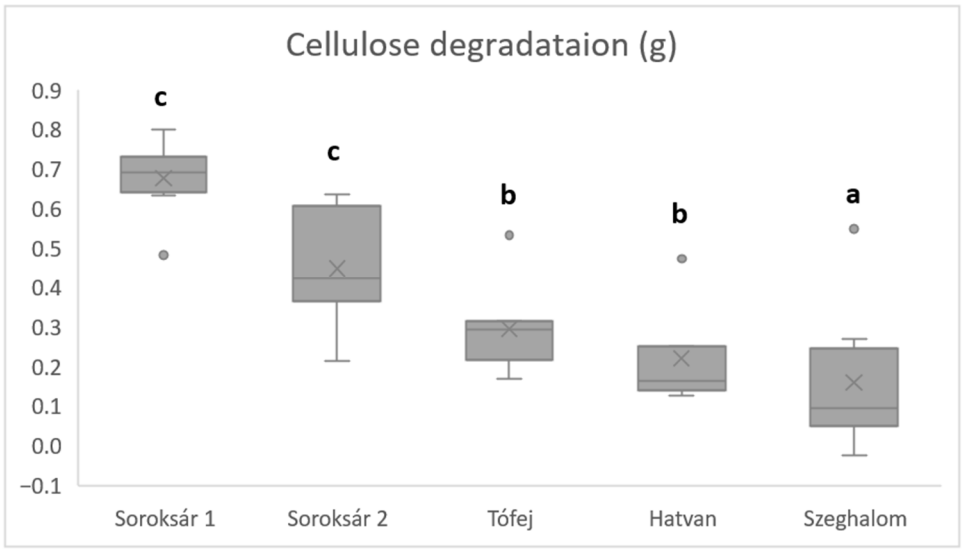

Figure 10. The rate of cellulose degradation in five different soils (using a 1 g starting cellulose/pot) during the experiment. Control and inoculated pots evaluated together, without alginite-treated pots. Different letters mean statistically significant differences. Further information in text.

In the alginite-treated soils, cellulose degradation differed significantly between soil types differences [$F(1,24) = 101.892$, $p < 0.001$], whereas in the absence of alginite, there were no significant differences. In addition, cellulose loss was significantly reduced in the pots treated with both alginite and inoculum [$F(1,24) = 10.353$, $p < 0.01$], likely due to

the negative synergistic effects of alginite and inoculum on organic matter decomposition (Figure 11).

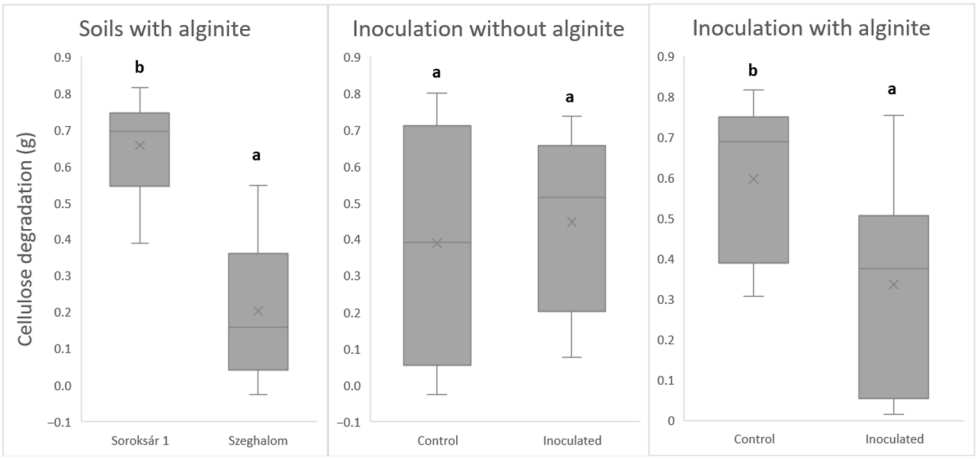

Figure 11. The rate of cellulose degradation in alginite and inoculant treated soils (using a 1 g starting cellulose/pot) during the experiment. (**Left**): alginite treatment. (**Mid**): the effects of microbial inoculation. (**Right**): alginite interaction with inoculation. Different letters mean statistically significant differences. Further information in text.

4. Conclusions

This study highlights the significant potential of plant growth-promoting microorganisms (PGPMs) in enhancing plant growth and soil enzyme activity across various soil types. Specifically, strains such as *Enterobacter ludwigii*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Kosakonia cowanii*, and *Trichoderma harzianum* demonstrated generally positive effects on the growth of mustard and perennial ryegrass in controlled environments. While the effectiveness of individual strains varied, their combined application significantly improved microbial activity and soil enzyme functions across different soils.

Our findings indicate that the initial physical and chemical properties of soils play a critical role in determining the efficacy of microbial inoculants. Clayey soils with higher colloid content, for example, provide a more favorable environment for microbial activity, as evidenced by higher MPN values and enzyme activity. Although the use of natural clay minerals like alginite showed potential in improving soil conditions, further research is needed to optimize its interaction with microbial inoculants.

Additionally, our results reveal that alginite, high in clay and calcium carbonate, could alter the soil microbial composition, particularly in arenosol-type soils, and impact the efficacy of inoculation treatments. For instance, while alginite treatment decreased aerobic bacteria abundance in Soroksár 1 soil, it increased the abundance of microscopic fungi, suggesting its complex role in modifying soil microbial dynamics.

This study also observed that inoculation significantly boosted DHA enzyme activity, reflecting enhanced soil biological activity. However, the combination of strains did not further enhance the plant growth-promoting effects significantly, highlighting the need for further testing and validation.

Future research should focus on optimizing microbial strain combinations and understanding the specific interactions between these inoculants and various soil types. Tailored microbial inoculant formulations could be developed to maximize their benefits in diverse agricultural settings, thereby contributing to more sustainable and environmentally friendly soil management practices.

In summary, while the positive effects of these microbial inoculants are promising, further research and refinement are necessary to fully harness their potential in enhancing plant growth and soil health in different environmental conditions.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture14091586/s1, auxiliary file—Figures 1–3.

Author Contributions: This work was carried out in collaboration with all authors. Conceptualization, S.A.P., T.T. and B.B.; methodology, Z.K.; data curation, S.A.P.; writing—original draft preparation, S.A.P. and T.T.; writing—review and editing, S.A.P., Z.K., T.T. and B.B.; supervision, B.B.; project administration, Z.K. All authors have read and agreed to the published version of the manuscript.

Funding: The research was funded by the Sustainable Development and Technologies National Programme of the Hungarian Academy of Sciences (FFT NP FTA) and Hungarian Research Network (grant no. SA-26/2021). Zsolt Kotroczó was supported by the MATE Research Excellence Program 2024.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are contained within the article (and Supplementary Materials).

Acknowledgments: The authors gratefully acknowledge the Department of Agro-Environmental Studies, MATE, and are indebted to the Institute for Soil Sciences, Centre for Agricultural Research, HUN-REN.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Tripathi, A.N.; Meena, B.R.; Pandey, K.K.; Singh, J. Microbial Bioagents in Agriculture: Current Status and Prospects. In *New Frontiers in Stress Management for Durable Agriculture*; Rakshit, A., Singh, H.B., Singh, A.K., Singh, U.S., Fraceto, L., Eds.; Springer: Singapore, 2020; pp. 331–368. [CrossRef]
2. Woo, S.L.; Pepe, O. Microbial Consortia: Promising Probiotics as Plant Biostimulants for Sustainable Agriculture. *Front. Plant Sci.* **2018**, *9*, 1801. [CrossRef] [PubMed]
3. Dudas, A.; Kotroczó, Z.; Videki, E.; Wass-Matics, H.; Kocsis, T.; Szalai, Z.; Végvári, G.; Biró, B. Fruit Quality of Tomato Affected by Single and Combined Bioeffectors in Organically System. *Pak. J. Agric. Sci.* **2017**, *54*, 847–856. [CrossRef]
4. Dudás, A.; Szalai, Z.; Vidéki, E.; Wass-Matics, H.; Kocsis, T.; Végvári, G.; Kotroczó, Z.; Biró, B. Sporeforming *Bacillus* Bioeffectors for Healthier Fruit Quality of Tomato in Pots and Field. *Appl. Ecol. Environ. Res.* **2017**, *15*, 1399–1418. [CrossRef]
5. Wei, X.; Xie, B.; Wan, C.; Song, R.; Zhong, W.; Xin, S.; Song, K. Enhancing Soil Health and Plant Growth through Microbial Fertilizers: Mechanisms, Benefits, and Sustainable Agricultural Practices. *Agronomy* **2024**, *14*, 609. [CrossRef]
6. Béni, Á.; Lajtha, K.; Kozma, J.; Fekete, I. Application of a Stir Bar Sorptive Extraction Sample Preparation Method with HPLC for Soil Fungal Biomass Determination in Soils from a Detrital Manipulation Study. *J. Microbiol. Methods* **2017**, *136*, 1–5. [CrossRef]
7. Fekete, I.; Lajtha, K.; Kotroczó, Z.; Várbíró, G.; Varga, C.; Tóth, J.A.; Demeter, I.; Veperdi, G.; Berki, I. Long-Term Effects of Climate Change on Carbon Storage and Tree Species Composition in a Dry Deciduous Forest. *Glob. Chang. Biol.* **2017**, *23*, 3154–3168. [CrossRef]
8. Liddle, K.; McGonigle, T.; Koiter, A. Microbe Biomass in Relation to Organic Carbon and Clay in Soil. *Soil Syst.* **2020**, *4*, 41. [CrossRef]
9. Etesami, H.; Maheshwari, D.K. Use of Plant Growth Promoting Rhizobacteria (PGPRs) with Multiple Plant Growth Promoting Traits in Stress Agriculture: Action Mechanisms and Future Prospects. *Ecotoxicol. Environ. Saf.* **2018**, *156*, 225–246. [CrossRef]
10. Etesami, H.; Glick, B.R. Bacterial Indole-3-Acetic Acid: A Key Regulator for Plant Growth, Plant-Microbe Interactions, and Agricultural Adaptive Resilience. *Microbiol. Res.* **2024**, *281*, 127602. [CrossRef]
11. Lopes, M.J.d.S.; Dias-Filho, M.B.; Gurgel, E.S.C. Successful Plant Growth-Promoting Microbes: Inoculation Methods and Abiotic Factors. *Front. Sustain. Food Syst.* **2021**, *5*, 606454. [CrossRef]
12. Khan, W.; Zhu, Y.; Khan, A.; Zhao, L.; Yang, Y.-M.; Wang, N.; Hao, M.; Ma, Y.; Nepal, J.; Ullah, F.; et al. Above-and below-Ground Feedback Loop of Maize Is Jointly Enhanced by Plant Growth-Promoting Rhizobacteria and Arbuscular Mycorrhizal Fungi in Drier Soil. *Sci. Total Environ.* **2024**, *917*, 170417. [CrossRef]
13. Nagrale, D.T.; Chaurasia, A.; Kumar, S.; Gawande, S.P.; Hiremani, N.S.; Shankar, R.; Gokte-Narkhedkar, N.; Renu; Prasad, Y.G. PGPR: The Treasure of Multifarious Beneficial Microorganisms for Nutrient Mobilization, Pest Biocontrol and Plant Growth Promotion in Field Crops. *World J. Microbiol. Biotechnol.* **2023**, *39*, 100. [CrossRef]
14. David, B.V.; Chandrasehar, G.; Selvam, P.N. Pseudomonas fluorescens: A Plant-Growth-Promoting Rhizobacterium (PGPR) With Potential Role in Biocontrol of Pests of Crops. In *Crop Improvement Through Microbial Biotechnology*; Prasad, R., Gill, S.S., Tuteja, N., Eds.; Elsevier: Amsterdam, The Netherlands, 2018; pp. 221–243. [CrossRef]
15. Orhan, E.; Esitken, A.; Ercisli, S.; Turan, M.; Sahin, F. Effects of Plant Growth Promoting Rhizobacteria (PGPR) on Yield, Growth and Nutrient Contents in Organically Growing Raspberry. *Sci. Hortic.* **2006**, *111*, 38–43. [CrossRef]
16. Shah, A.; Nazari, M.; Antar, M.; Msimbira, L.A.; Naamala, J.; Lyu, D.; Rabileh, M.; Zajonc, J.; Smith, D.L. PGPR in Agriculture: A Sustainable Approach to Increasing Climate Change Resilience. *Front. Sustain. Food Syst.* **2021**, *5*, 667546. [CrossRef]

17. Liu, X.; Mei, S.; Salles, J.F. Do Inoculated Microbial Consortia Perform Better than Single Strains in Living Soil? A Meta-Analysis. *Appl. Soil Ecol.* **2023**, *190*, 105011. [CrossRef]
18. Ribeiro, V.P.; Gomes, E.A.; de Sousa, S.M.; de Paula Lana, U.G.; Coelho, A.M.; Marriel, I.E.; de Oliveira-Paiva, C.A. Co-Inoculation with Tropical Strains of *Azospirillum* and *Bacillus* Is More Efficient than Single Inoculation for Improving Plant Growth and Nutrient Uptake in Maize. *Arch. Microbiol.* **2022**, *204*, 143. [CrossRef] [PubMed]
19. Shoebitz, M.; Ribaudo, C.M.; Pardo, M.A.; Cantore, M.L.; Ciampi, L.; Curá, J.A. Plant Growth Promoting Properties of a Strain of *Enterobacter ludwigii* Isolated from *Lolium perenne* Rhizosphere. *Soil Biol. Biochem.* **2009**, *41*, 1768–1774. [CrossRef]
20. Dolkar, D.; Dolkar, P.; Angmo, S.; Chaurasia, O.P.; Stobdan, T. Stress Tolerance and Plant Growth Promotion Potential of *Enterobacter ludwigii* PS1 Isolated from Seabuckthorn Rhizosphere. *Biocatal. Agric. Biotechnol.* **2018**, *14*, 438–443. [CrossRef]
21. Singh, R.; Mishra, S.; Jha, P.; Raghuvanshi, S.; Jha, P. Effect of Inoculation of Zinc-Resistant Bacterium *Enterobacter ludwigii* CDP-14 on Growth, Biochemical Parameters and Zinc Uptake in Wheat (*Triticum aestivum* L.) Plant. *Ecol. Eng.* **2018**, *116*, 163–173. [CrossRef]
22. Do, T.T.T.; Dang, T.N.T.; Minh, T.H. Characterization of Phosphate and Potassium Solubilization, and Antifungal Activity of Bacteria Isolated from Rhizosphere of *Allium Ascalonicum* (L.) Grown in Ninh Hai District, Ninh Thuan Province, Vietnam. *World J. Adv. Res. Rev.* **2023**, *17*, 018–027. [CrossRef]
23. Xiao-ying, G.; Chun-e, H.; Tao, L.; Zhu, O. Effect of *Bacillus subtilis* and *Pseudomonas fluorescens* on Growth of Greenhouse Tomato and Rhizosphere Microbial Community. *J. Northeast. Agric. Univ.* **2015**, *22*, 32–42. [CrossRef]
24. Ahmad, M.; Adil, Z.; Hussain, A.; Mumtaz, M.; Nafees, M.; Ahmad, I.; Jamil, M. Potential of Phosphate Solubilizing *Bacillus* Strains for Improving Growth and Nutrient Uptake in Mungbean and Maize Crops. *Pak. J. Agric. Sci.* **2019**, *56*, 283–289. [CrossRef]
25. Kim, Y.-K.; Lee, S.-C.; Cho, Y.-Y.; Oh, H.-J.; Ko, Y.H. Isolation of Cellulolytic *Bacillus subtilis* Strains from Agricultural Environments. *ISRN Microbiol.* **2012**, *2012*, 650563. [CrossRef] [PubMed]
26. Menéndez, E.; Ramirez-Bahena, M.H.; Peix, A.; Tejedor, C.; Mulas, R.; González-Andrés, F.; Martínez-Molina, E.; Velázquez, E. Analysis of Cultivable Endophytic Bacteria in Roots of Maize in a Soil from León Province in Mainland Spain. In *Biological Nitrogen Fixation and Beneficial Plant-Microbe Interaction*; González-Andrés, F., James, E., Eds.; Springer International Publishing: Cham, Switzerland, 2016; pp. 45–53. [CrossRef]
27. Brady, C.; Cleenwerck, I.; Venter, S.; Coutinho, T.; De Vos, P. Taxonomic evaluation of the genus *Enterobacter* based on multilocus sequence analysis (MLSA): Proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrinus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov. and *Pluralibacter pyrinus* comb. nov., respectively, *E. cowanii*, *E. radicincitans*, *E. oryzae* and *E. orachidis* into *Kosakonia* gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia radicincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. *Syst. Appl. Microbiol.* **2013**, *36*, 309–319. [CrossRef] [PubMed]
28. Zhu, B.; Zhou, Q.; Lin, L.; Hu, C.; Shen, P.; Yang, L.; An, Q.; Xie, G.; Li, Y. *Enterobacter sacchari* sp. nov., a Nitrogen-Fixing Bacterium Associated with Sugar Cane (*Saccharum officinarum* L.). *Int. J. Syst. Evol. Microbiol.* **2013**, *63 Pt 7*, 2577–2582. [CrossRef] [PubMed]
29. Lin, L.; Li, Z.; Hu, C.; Zhang, X.; Chang, S.; Yang, L.; Li, Y.; An, Q. Plant Growth-Promoting Nitrogen-Fixing *Enterobacteria* Are in Association with Sugarcane Plants Growing in Guangxi, China. *Microbes Environ.* **2012**, *27*, 391–398. [CrossRef]
30. Sherpa, M.T.; Sharma, L.; Bag, N.; Das, S. Isolation, Characterization, and Evaluation of Native Rhizobacterial Consortia Developed From the Rhizosphere of Rice Grown in Organic State Sikkim, India, and Their Effect on Plant Growth. *Front. Microbiol.* **2021**, *12*, 713660. [CrossRef]
31. Pau-Roblot, C.; Lequart-Pillon, M.; Apanga, L.; Pilard, S.; Courtois, J.; Pawlicki-Jullian, N. Structural Features and Bioremediation Activity of an Exopolysaccharide Produced by a Strain of *Enterobacter Ludwigii* Isolated in the Chernobyl Exclusion Zone. *Carbohydr. Polym.* **2013**, *93*, 154–162. [CrossRef]
32. Gao, H.; Lu, C.; Wang, H.; Wang, L.; Yang, Y.; Jiang, T.; Li, S.; Xu, D.; Wu, L. Production Exopolysaccharide from *Kosakonia Cowanii* LT-1 through Solid-State Fermentation and Its Application as a Plant Growth Promoter. *Int. J. Biol. Macromol.* **2020**, *150*, 955–964. [CrossRef]
33. Redmile-Gordon, M.; Gregory, A.S.; White, R.P.; Watts, C.W. Soil Organic Carbon, Extracellular Polymeric Substances (EPS), and Soil Structural Stability as Affected by Previous and Current Land-Use. *Geoderma* **2020**, *363*, 114143. [CrossRef]
34. Abdel-Fattah, G.M.; Shabana, Y.M.; Ismail, A.E.; Rashad, Y.M. *Trichoderma harzianum*: A Biocontrol Agent against *Bipolaris oryzae*. *Mycopathologia* **2007**, *164*, 81–89. [CrossRef]
35. Haddadin, M.S.Y.; Haddadin, J.; Arabiyat, O.I.; Hattar, B. Biological Conversion of Olive Pomace into Compost by Using *Trichoderma harzianum* and Phanerochaete chrysosporium. *Bioresour. Technol.* **2009**, *100*, 4773–4782. [CrossRef] [PubMed]
36. Khoso, M.A.; Wagan, S.; Alam, I.; Hussain, A.; Ali, Q.; Saha, S.; Poudel, T.R.; Manghwar, H.; Liu, F. Impact of Plant Growth-Promoting Rhizobacteria (PGPR) on Plant Nutrition and Root Characteristics: Current Perspective. *Plant Stress* **2024**, *11*, 100341. [CrossRef]
37. Borah, P.; Gogoi, N.; Asad, S.A.; Rabha, A.J.; Farooq, M. An Insight into Plant Growth-Promoting Rhizobacteria-Mediated Mitigation of Stresses in Plant. *J. Plant Growth Regul.* **2023**, *42*, 3229–3256. [CrossRef]

38. Bashan, Y.; Kamnev, A.; de-Bashan, L. Tricalcium Phosphate Is Inappropriate as a Universal Selection Factor for Isolating and Testing Phosphate-Solubilizing Bacteria That Enhance Plant Growth: A Proposal for an Alternative Procedure. *Biol. Fertil. Soils* **2013**, *49*, 465–469. [CrossRef]
39. Bradáčová, K.; Sittinger, M.; Tietz, K.; Neuhäuser, B.; Kandeler, E.; Berger, N.; Ludewig, U.; Neumann, G. Maize Inoculation with Microbial Consortia: Contrasting Effects on Rhizosphere Activities, Nutrient Acquisition and Early Growth in Different Soils. *Microorganisms* **2019**, *7*, 329. [CrossRef]
40. Kincses, S.; Filep, T.; Kátai, J. Effect of organic, artificial, and bacterial fertilizers on the nutrient content of soils extractable with 0.01 M $CaCl_2$ solution. *Talajvédelem* **2008**, 423–430. (In Hungarian)
41. Ayala-Zepeda, M.; Rojas-Padilla, J.; Díaz-Rodríguez, A.M.; Chávez-Luzanía, R.A.; Parra Cota, F.I.; Villalobos, S.d.l.S. Chapter 12—Performance Evaluation of Bacterial Inoculants in the Field. In *New Insights, Trends, and Challenges in the Development and Applications of Microbial Inoculants in Agriculture*; Villalobos, S.d.l.S., Ed.; Developments in Applied Microbiology and Biotechnology; Academic Press: Cambridge, MA, USA, 2024; pp. 117–127. [CrossRef]
42. Li, J.; Wang, J.; Liu, H.; Macdonald, C.A.; Singh, B.K. Microbial Inoculants with Higher Capacity to Colonize Soils Improved Wheat Drought Tolerance. *Microb. Biotechnol.* **2023**, *16*, 2131–2144. [CrossRef]
43. Iqbal, R.; Valipour, M.; Ali, B.; Zulfiqar, U.; Aziz, U.; Zaheer, M.S.; Sarfraz, A.; Javed, M.A.; Afridi, M.S.; Ercisli, S.; et al. Maximizing Wheat Yield through Soil Quality Enhancement: A Combined Approach with *Azospirillum Brasilense* and Bentonite. *Plant Stress* **2024**, *11*, 100321. [CrossRef]
44. Ntanos, E.; Kekelis, P.; Assimakopoulou, A.; Gasparatos, D.; Denaxa, N.-K.; Tsafouros, A.; Roussos, P.A. Amelioration Effects against Salinity Stress in Strawberry by Bentonite–Zeolite Mixture, Glycine Betaine, and *Bacillus Amyloliquefaciens* in Terms of Plant Growth, Nutrient Content, Soil Properties, Yield, and Fruit Quality Characteristics. *Appl. Sci.* **2021**, *11*, 8796. [CrossRef]
45. Solti, G. *Soil-Improving, and Ecological Nutrient-Management*; Mezőgazda Press: Budapest, Hungary, 2000. (In Hungarian)
46. Kátai, J.; Magdolna, T.; Sándor, Z.; Zsuposné, Á. Effect of Bentonite and Zeolite on Some Characteristics of Acidic Sandy Soil and on the Biomass of a Test Plant. *Agrokémia Talajt.* **2010**, *59*, 165–174. [CrossRef]
47. Ragályi, P.; Kádár, I.; Csathó, P.; Murányi, A.; Radimszky, L.; Gajdó, A. *Effect of Gérce Alginit on the Fertility of an Acidic Sandy Soil During Five Years*; Magyar Tudományos Akadémia Agrártudományi Kutatóközpont, Alginit Kft: Martonvásár, Hungary; Budapest, Hungary, 2019. (In Hungarian)
48. Kardos, L.; Juhasz, A.; Palko, G.; Olah, J.; Barkács, K.; Záray, G. Enzyme Activity Analyses of Anaerobic Fermented Sewage Sludges. *Appl. Ecol. Environ. Res.* **2011**, *9*, 333–339. [CrossRef]
49. Kovács, R.; Imre, C.; Puspán, I.; Rizó, B.; Imri, Á.; Pék, N.; Kárpáti, É.; Árvay, G.; Romsics, C.; Kutasi, J. Selection and creation of a strain collection of stress-tolerant bacteria adapted to degraded soils with adverse pH and salt conditions. *Talajvédelem* **2017**, 85–96. (In Hungarian)
50. O'Callaghan, M.; Ballard, R.A.; Wright, D. Soil Microbial Inoculants for Sustainable Agriculture: Limitations and Opportunities. *Soil Use Manag.* **2022**, *38*, 1340–1369. [CrossRef]
51. Iosa, I.; Agrimonti, C.; Marmiroli, N. Real-Time PCR (qtPCR) to Discover the Fate of Plant Growth-Promoting Rhizobacteria (PGPR) in Agricultural Soils. *Microorganisms* **2024**, *12*, 1002. [CrossRef]
52. Gupta, G.; Jha, P. Screening of Potential PGPR Candidates as Future Biofertilizers-A Strategic Approach from Lab to Field. *Res. J. Biotechnol.* **2015**, *10*, 48–62.
53. Zhang, X.; Wang, Y.; Guo, J.; Yu, Y.; Li, Y.; Liu, C. Comparing Two Functions for Optical Density and Cell Numbers in Bacterial Exponential Growth Phase. *J. Pure Appl. Microbiol.* **2015**, *9*, 299–305.
54. Food and Agriculture Organization of the United Nations. *World Reference Base for Soil Resources 2014, International Soil Classification System for Naming Soils and Creating Legends for Soil Maps; Update 2015*; World Soil Resources Reports No. 106; FAO: Rome, Italy, 2015.
55. Egnér, H.; Riehm, H.; Domingo, W. Untersuchungen Über Die Chemische Bodenanalyse Als Grundlage Für Die Beurteilung Des Nährstoffzustandes Der Böden II. *K. Lantbrukshögskolans Ann. Chem. Extraktionsmethoden Zur Phosphor-Und Kaliumbestimmung* **1960**, *26*, 199–215.
56. Tyurin, I.V. Analytic Procedure for a Comperative Study of Soil Humus. *Tr. PochvInst Dokuchayeva* **1951**, *38*, 5–21.
57. *MSZ-08-0206/2-1978*; Evaluation of Some Chemical Properties of the Soil. Laboratory Tests. (pH Value), Phenolphtalein Alkalinity Expressed in Soda, Total Water-Soluble Salt Content, Hydrolytic (y1 Value) and Exchangeable Acidity (y2 Value). Hungarian Standard Association: Budapest, Hungary, 1978. (In Hungarian)
58. Buzás, I. A talaj Arany-féle kötöttségi számának (KA) meghatározása kézi keveréssel. In *Talaj—és Agrokémiai Vizsgálati Módszerkönyv 1*; Buzás, I., Ed.; Inda: Budapest, Hungary, 1993; p. 48.
59. Olsen, P.E.; Sanda, E.S.; Keyser, H.H. *The Enumeration and Identification of Rhizobial Bacteria in Legume Inoculant Quality Control Procedures*; NifTAL Center: Paia, HI, USA, 1996; p. 96.
60. Reichart, O. Some Remarks on the Bias of the MPN Method. *Int. J. Food Microbiol.* **1991**, *13*, 131–141. [CrossRef]
61. Cochran, W.G. Estimation of Bacterial Densities by Means of the "Most Probable Number". *Biometrics* **1950**, *6*, 105–116. [CrossRef] [PubMed]
62. Veres, Z.; Kotroczó, Z.; Magyaros, K.; Tóth, J.A.; Tóthmérész, B. Dehydrogenase Activity in a Litter Manipulation Experiment in Temperate Forest Soil. *Acta Silv. Lignaria Hung.* **2013**, *9*, 25–33. [CrossRef]

63. Thalmann, A. Dehydrogenase activity. In *1995 Methods in Applied Soil Microbiology and Biochemistry*; Alef, K., Nannipieri, P., Eds.; Academic Press Ltd.: New York, NY, USA, 1968; pp. 228–230.
64. Villányi, I.; Füzy, A.; Angerer, I.; Biró, B. Total Catabolic Enzyme Activity of Microbial Communities. Fluorescein Diacetate Analysis (FDA). In *Understanding and Modelling Plant-Soil Interactions in the Rhizosphere Environment. Handbook of Methods Used in Rhizosphere Research*; Jones, D.L., Ed.; Swiss Federal Research Institute WSL: Birmensdorf, Switzerland, 2006; pp. 441–442.
65. Unger, H. The Cellulose Test as a Method for Determining the Cellulose-Decomposing Activity of Soil in Field Experiments. *Z. Pflanzenernahr. Dung. Bodenkd.* **1960**, *91*, 44–52. [CrossRef]
66. Kaymak, H.C. Potential of PGPR in Agricultural Innovations. In *Plant Growth and Health Promoting Bacteria*; Maheshwari, D.K., Ed.; Microbiology Monographs; Springer: Berlin/Heidelberg, Germany, 2010; Volume 18, pp. 45–79. [CrossRef]
67. Minuț, M.; Diaconu, M.; Roșca, M.; Cozma, P.; Bulgariu, L.; Gavrilescu, M. Screening of *Azotobacter*, *Bacillus* and *Pseudomonas* Species as Plant Growth-Promoting Bacteria. *Processes* **2023**, *11*, 80. [CrossRef]
68. Zaballa, J.I.; Golluscio, R.; Ribaudo, C.M. Effect of the Phosphorus-Solubilizing Bacterium *Enterobacter Ludwigii* on Barley Growth Promotion. *Am. Sci. Res. J. Eng. Technol. Sci.* **2020**, *63*, 144–157.
69. Pereira, S.I.A.; Abreu, D.; Moreira, H.; Vega, A.; Castro, P.M.L. Plant Growth-Promoting Rhizobacteria (PGPR) Improve the Growth and Nutrient Use Efficiency in Maize (*Zea mays* L.) under Water Deficit Conditions. *Heliyon* **2020**, *6*, e05106. [CrossRef]
70. Manjunath, M.; Prasanna, R.; Sharma, P.; Nain, L.; Singh, R. Developing PGPR Consortia Using Novel Genera *Providencia* and *Alcaligenes* along with Cyanobacteria for Wheat. *Arch. Agron. Soil Sci.* **2011**, *57*, 873–887. [CrossRef]
71. Telesiński, A.; Krzyśko-Łupicka, T.; Cybulska, K.; Pawłowska, B.; Biczak, R.; Śnieg, M.; Wróbel, J. Comparison of Oxidoreductive Enzyme Activities in Three Coal Tar Creosote-Contaminated Soils. *Soil Res.* **2019**, *57*, 814–824. [CrossRef]
72. Abou-Zeid, M. Improvement of Soybean Growth by Co-Inoculation with Rhizobium and Plant Growth-Promoting Rhizobacteria. *Egypt. J. Appl. Sci.* **2011**, *26*, 445–449.
73. Bandyopadhyay, S.; Maiti, S.K. Different Soil Factors Influencing Dehydrogenase Activity in Mine Degraded Lands—State-of-Art Review. *Water Air Soil Pollut.* **2021**, *232*, 360. [CrossRef]
74. Kutateladze, L.Y.; Zakariashvili, N.G.; Jobava, M.D.; Burduli, T.A.; Sadunishvili, T.A. Microscopic Fungi Spread in Different Types of Soils in Western Georgia. *Ann. Agrar. Sci.* **2016**, *14*, 227–232. [CrossRef]
75. Gazdag, O.; Takács, T.; Ködöböcz, L.; Krett, G.; Szili-Kovács, T. Alphaproteobacteria Communities Depend More on Soil Types than Land Managements. *Acta Agric. Scand. Sect. B—Soil Plant Sci.* **2019**, *69*, 147–154. [CrossRef]
76. Javed, S.; Javaid, A.; Hanif, U.; Bahadur, S.; Sultana, S.; Shuaib, M.; Ali, S. Effect of Necrotrophic Fungus and PGPR on the Comparative Histochemistry of Vigna Radiata by Using Multiple Microscopic Techniques. *Microsc. Res. Tech.* **2021**, *84*, 2737–2748. [CrossRef] [PubMed]
77. Frey, S.D.; Elliott, E.T.; Paustian, K. Bacterial and Fungal Abundance and Biomass in Conventional and No-Tillage Agroecosystems along Two Climatic Gradients. *Soil Biol. Biochem.* **1999**, *31*, 573–585. [CrossRef]
78. Mallon, C.A.; Poly, F.; Le Roux, X.; Marring, I.; van Elsas, J.D.; Salles, J.F. Resource Pulses Can Alleviate the Biodiversity–Invasion Relationship in Soil Microbial Communities. *Ecology* **2015**, *96*, 915–926. [CrossRef]
79. Mawarda, P.C.; Mallon, C.A.; Le Roux, X.; van Elsas, J.D.; Salles, J.F. Interactions between Bacterial Inoculants and Native Soil Bacterial Community: The Case of Spore-Forming *Bacillus* spp. *FEMS Microbiol. Ecol.* **2022**, *98*, fiac127. [CrossRef]
80. Chmolowska, D.; Hamda, N.; Laskowski, R. Cellulose Decomposed Faster in Fallow Soil than in Meadow Soil Due to a Shorter Lag Time. *J. Soils Sediments* **2017**, *17*, 299–305. [CrossRef]
81. Li, D.; Li, Z.; Zhao, B.; Zhang, J. Relationship between the Chemical Structure of Straw and Composition of Main Microbial Groups during the Decomposition of Wheat and Maize Straws as Affected by Soil Texture. *Biol. Fertil. Soils* **2020**, *56*, 11–24. [CrossRef]