THESES OF THE DOCTORAL (PhD) DISSERTATION

Nándor Prettl

Budapest

2025

HUNGARIAN UNIVERSITY OF
AGRICULTURE AND LIFE SCIENCES

EFFECTS OF MICROBIAL INOCULATION AND LIMING OF SOILS ON PLANT GROWTH DEPENDING ON NUTRIENT SUPPLY

Nándor Prettl

Budapest

2025

Name of Doctoral School: Horticultural Sciences Doctoral School

Topics: Agriculture and Horticulture

Head of doctoral school: Éva Zámboriné Németh, DSc. Professor

Hungarian University of Agriculture and Life Sciences,
Institute of Horticulture

Supervisor: Borbála Biró, DSc. Professor Emerita
Hungarian University of Agriculture and Life Sciences,
Institute of Environmental Science, Department of Agro Environmental Studies

Co-Supervisor: Katalin Juhos, PhD. Associate Professor
Hungarian University of Agriculture and Life Sciences,
Institute of Environmental Science, Department of Agro Environmental Studies

....
Head of Doctoral School Supervisor

....
Co-Supervisor

1. INTRODUCTION AND OBJECTIVES

Meeting growing food demands in a sustainable way is one of the biggest global challenges, as the world's population is expected to reach 9-10 billion by 2050. Presently, nutrient supplementation in agricultural production is mainly achieved using synthetic fertilizers, which however does not equate to cultivation methods that reintroducing organic matter and stimulate soil biological activity. Soil organic matter and soil organisms, including bacteria and fungi, protect soils from extreme weather events and improve nutrient uptake and soil structure. It is important to thoroughly evaluate the impact of agrotechnical elements on soil health and plant growth using measurements.

As part of its "From Farm to Table" strategy, the European Commission has set a target to reduce synthetic fertilizer use by 20% by 2030, and agriculture is one of the key players in the UN's 17-point Sustainable Development Goals for 2030 (Veerman et al., 2020). At the same time, the scientific and practical role of plant-protecting and nutrient-mobilizing microorganisms in agriculture is becoming increasingly important (Nkebiwe et al., 2024).

The Ministry of Agriculture introduced an incentive form of support in 2023 – the Agro-ecological Program – which aims to encourage farmers to make environmentally beneficial voluntary commitments to mitigate climate change, protect soil and water, and protect biodiversity. One of the optional elements of the program is the use of microbiological inoculants on arable land, which has significantly increased the number of areas treated with inoculants in Hungary. According to the Ministry's data, in 2023, land users used microbiological inoculants on at least 14,4% of arable land in Hungary. Despite this, many people question the practical effect and right to exist of the inoculants. The majority of the users expect effects from the inoculants that, due to the unfavourable environmental conditions, cannot be achieved even in the case of an ideal microbe-plant relationship. Instead of judging the inoculants based on subjective opinions, it would be important to conduct more professionally set up experiments, mainly in open fields, on various soil types and in different years, which would support the practical use of microbial inoculants. There is a little scientifically based research in Hungary on inoculants, and the results of international literature are rather one-sided. Most of the foreign examples do not mention negative or neutral effects on inoculants, and most of the scientific results published show positive effects on plants, and most of the publications do not come from the continental climate region.

During the research, we examined the effect of microbiological inoculations (PSP, Plant Strenghtening Products) on corn and winter wheat in soils with different nutrient supply and pH under open field and pot conditions. The experiments were mostly set up on acidic brown forest soil with poor nutrient supply and neutral meadow soil with a good nutrient supply, examining the correlation between soil biological parameters and crop growth. During our work we made the following hypotheses:

We assumed that

- in addition to weaker nutrient supply capacity and soil biological activity, the use of starter fertilizers has significant effect on corn yield;
- the differences between the physicochemical properties of the soils and the intensity of fertilization fundamentally determine the effectiveness of bacterial and mycorrhizal inoculations;
- the efficiency of bacterial inoculant and soil biological activity can be improved on acidic brown forest soil by liming the soil;
- the indigenous bacterial community of the soil influences the effect of the applied bacterial inoculant, but the effect depends on the nutrient supply of the soil;
- the beneficial effects of inoculants can be predicted by monitoring soil biological and plant growth parameters.

2. MATERIALS AND METHODS

2.1. Field experiments

2.1.1. Soil types of growing sites and their cultivation

We set our field experiments on two types of soils with significantly different defining characteristics (Table 1.). The experimental sites were located in Baranya County, north of Mecsek.

Table 1. The soil properties measured in a 0-25 cm layer at the two experimental sites prior to the experiment, before the application of the first microbial inoculant (2020).

Soil parameters	Unit of measure	Site 1 Luvisol	Site 2 Gleysol
Soil texture	-	loam	clay loam
pH	-	4.91	6.75
Soil organic carbon	m/m%	0.94	1.45
Permanganate oxidizable labile carbon	mg/kg	371	647
Potential available P	mg/kg	29	132
Total organic P	mg/kg	27	169
Total inorganic P	mg/kg	489	819
Available (exchangeable) K	mg/kg	128	201
Cation exchange capacity	meq/100g	12.79	18.78
Water holding capacity (pF 2.4)	v/v%	26.74	33.46

The soil type of Site 1 could be classified as acidic brown forest soil (Luvisol), with loam texture, low amount of organic matter and a highly acidic pH. In addition to good water and cation exchange capacity, this growing site has also good potassium supply. However, the total amount of P is low, and due to the acidic pH, the potentially available fraction is also low (only 6% of inorganic P is available). The proportion of organic P is very low (5.2% of the total P). There is also limited available zinc is in this Luvisol. In contrast, the soil type at Site 2 is classified as meadow soil (Gleysol) with a clay loam texture and has higher water holding and cation exchange capacity compared to Site 1. It has a neutral pH and more soil organic matter content than the Luvisol. Total organic and inorganic P levels are much higher at Site 2 compared to Site 1. Available P consisting 16% of the total inorganic P. The neutral pH and higher organic matter content contribute to better P uptake. The amount of available potassium and zinc levels are sufficient. The difference in biological activity between the soils is well reflected in their permanganate oxidizable labile carbon content, which is almost twice as high in the Gleysol compared to the Luvisol.

The experiments were conducted over four consecutive years, with corn (*Zea mays* L.) as the test crop in the first, second and fourth years, and winter wheat (*Triticum aestivum* L.) in the third year. During and prior to the trial, the experimental sites were managed conventionally, with the application of synthetic fertilizers containing nitrogen, phosphorus, and potassium. No animal manure was applied in the previous 20 years, but all the plant residues were left on the ground every year. Mouldboard ploughing was not used during the experiment.

2.1.2. Meteorological conditions

The experimental areas have warm-summer and humid continental climate. We placed a rain gauge midway between the two experimental sites to record precipitation after each rainfall. During the corn growing season precipitation recorded was 383 mm in 2020, 210 mm in 2021, 362 mm in 2022. Of the three corn growing seasons studied, one can be considered as dry, while the other two can be considered average in terms of precipitation. The wheat year had average precipitation in terms of growing season in 2022 (458 mm). The precipitation significantly impacted crop development and yield on both soils.

2.1.3. Experimental setup, treatments and fertilization levels

In 2020, 2021 and 2023, we examined the effect of starter fertilization (Starter) on corn. From 2020 to 2023, we examined the effect of bacterial inoculation (Bact1, Bact2) for four years. In 2023, we investigated the effect of liming with $CaCO_3$ and its combination with bacterial inoculation (Bact2). Furthermore, in 2021-2023, we examined the effect of arbuscular mycorrhizal inoculation (Myc) in two soil types. The experimental plots were placed in the same field and soil types throughout the four years of the study. After 2020, the experimental plots were moved within the field to an area where no experimental setup had been conducted in previous years, but in 2021 and 2022, the experimental plots with the same treatment were placed in the exact same location. We considered it particularly important during the experiments planning, that the area that had previously received microbial inoculant treatment, should not be used as a control plot in the next years. We set up three repetitions of the treatments in randomised arrangement, with each plot measuring 4.5 metres wide and 100 metres long.

As a test crop, we sowed FAO 330, an early maturing hybrid corn variety DKC3972, at a density of 72.000 seed/ha. For wheat we sowed the early maturing Gaudio at a density of 4 million seed/ha.

The fertilization levels used in the experiments were as follows: In 2020, high fertilization level, 120 kg N, 17.5 kg P, 47 kg/ha K per hectare. In 2021 high fertilization level, 120 kg N, 19 kg P, 55 kg/ha K per hectare. In 2022 (wheat test crop) high fertilization level, 142.5 kg N, 35 kg P, 17.5 kg/ha K per hectare. In 2023 low fertilization level, 46 kg N, per hectare, and medium fertilization level 87 kg N per hectare, furthermore high fertilization level, 120 kg N, 9 kg P, 17.5 kg/ha K per hectare. Markings related to the fertilization levels in the thesis: low fertilization level: 46N; medium fertilization level: 87N; high fertilization level: 120N, 143N.

2.2. Pot experiments

Our pot experiments conducted in two years, in 2022 and 2023. In 2022 we set up a liming experiment with two soil types and corn as a test plant. The soils belonged to the same soil type, Luvisol, but differed significantly in their soil test parameters, especially in their phosphorus content: „Luvisol P100" soil (pH=4,88; humus=1,43%; available P_2O_5=99 mg/kg); „Luvisol P175" soil (pH=4,95; humus=1,73%; available P_2O_5=173 mg/kg). Two treatments were set up for each soil type: Control and treated with CaO lime. The CaO dose was 0.1 g/100 g soil, which corresponds to 2.66 t/ha. Thus, a total of 16 culture pots were set up, with 4 replicates per treatment and 3 kg of soil per pot.

In 2023, we conducted a sterile treatment model experiment under *in vitro* laboratory conditions to study the effect of live and inactivated microbial inoculants using two types of soils also used in field experiments. Both soil types were treated with three types of soil treatment: cooled, so kept at 4°C; kept at 22°C and treated with molasses; and received sterile treatment (microwave treatment - 2450 MHz, 800W - then stored in a thin layer for one week under UV light). These soil treatments were combined with the inoculum and its autoclaved version: Control, Bact2, and AutoclavedBact2. A total of 72 pots were set up, each containing 450 g of soil, with 4 replicates per treatment.

2.3. Applied microbial inoculants and chemical products

Bact1 is a nutrient solution suspension containing the strains *Pseudomonas putida*, *Azotobacter chrococcum*, *Bacillus circulans*, *Bacillus megaterium* in 10^9 pieces/cm^3 CFU, with recommended dose of 15l/ha. Bact2 is also a nutrient solution suspension containing the strains *Bacillus simplex, Pseudomonas frederiksbergensis, Agreia pratensis, Paenibacillus peoriae, Exiguobacterium acetylicum, Azospirillum largimobile, Azospirillum brasilense* in minimum 10^9 pieces/cm^3 CFU/ml, and is able to slightly compensate for the yield reducing effect of acidic soils due to their low pH, with recommended dose of 1,5l/ha. The Myc product contains spores, myceliums and dried root fragments of the genus *Funneliformis*, *Rhizophagus* and *Claroideoglomus*. It contains five types of mycorrhiza from the three fungal colonies, so according to its manufacturer, it is also effective on different soil types, at a dose of 10 kg/ha. Composition of the starter fertilizer used in our experiments: N: 15%, P_2O_5: 20%, i.e. P: 9%, K_2O: 9%, i.e. K: 8.3%, MgO: 3%, SO_3: 10%, zinc: 1% and boron: 0.05%, dose: 150 kg/ha. The lime used is an ultrafine preparation containing 87% $CaCO_3$ from a natural source according to the manufacturer's description, at a dose of 2 t/ha. Molasses is a by-product of sugar production, which can be used for soil improvement and feeding microbes in the soil in agriculture, at a dose of 20 ml, 1.2 g/l solution 4 times.

2.4. Measurement methods

2.4.1. Soil sampling and analysis

During soil sampling we took 10-10 homogenous soil samples per plot from the 0-20 cm soil layer, with10 samples representing the entire length of the plots. Soil enzyme activity measurements were performed using refrigerated soil samples. Additionally, samples were dried in an oven set at 105 °C and we measured the weight loss of the dried soil (m/m%).

Measurement methods used in both pot and field experiments:

The method of measuring dehydrogenase enzyme (DHA) activity is used to estimate total microbial activity in soil. Dehydrogenase (DHA) enzyme activity was measured according to Veres et al. (2013).

The method currently used to measure microbial activity in soils is the measurement of labile carbon (POXC – permanganate oxidizable carbon). This method measures the carbon content available to plants and microbes, and its value changes more rapidly than the total organic carbon content. We used for our measurements the method developed by Weil et al. (2003).

Measurement methods used in field experiments:

Acid phosphatase enzyme activity (PHO) was measured according to the method of Sinsabaugh et al. (1999), which enzyme we use to detect the presence of microorganisms with phosphatase activity.

We measured the amount of glucosidase enzyme (GLU) using the same method (Sinsabaugh et al., 1999). Glucosidases are involved in carbon metabolism and play an important role in the breakdown of low molecular weight carbohydrates, during which they produce sugars.

To determine the number of spore-forming bacteria in soil samples, we used the most probable number (MPN) method (Cochran, 1950), which uses statistical methods to estimate the number of bacteria present.

The extent to which plant roots were colonised by arbuscular mycorrhizal fungi was determined using a modified version of the method developed by

Phillips and Hayman (1970). We placed the root fragments at the intersection of the grid lines and then examined them under the microscope at the intersection to determine whether AMF colonization was visible or not at each intersection.

2.4.2. Plant analysis and yield measurement

We measured the above ground dry shoot biomass and yield of the plants during the four years. When measuring biomass, we weighed eight corn plants per plot, excluding their yield. In the case of wheat, we tied 10 x 30 plants into bundles and weighed them, also excluding their yield. We measured the yield every year with harvesting machine, from which we unloaded the yield onto a trailer using weighing pads beneath. After that, we measured the moisture content of the crop using Wile 200 handheld moisture meter, thereby correcting the yield weight to the same moisture content.

In 2023, when the corn plants were flowering, we measured their plant height, root neck diameter, chlorophyll content, and root capacity at the same time on the same plants. The electrical capacity between the electrode inserted into the soil and the electrode placed on the plant is the root capacity. In the case of corn plants, there is a significant positive correlation between the measured root capacity values and the root surface area and root mass of corn. We randomly selected 15 plants per plot for the measurement. In 2022 and 2023 we measured the plant height and the biomass of the plants in the pot experiments.

2.4.3. Statistical evaluation

The significance of differences between our measured parameters was tested using ANOVA and IBM SPSS Statistics 27-29. The effects of mycorrhiza inoculantion, soil type, and their interaction on plant parameters were analysed using multivariate analysis of variance (MANOVA). Following significant ANOVA and MANOVA, we used Tukey post hoc test if the homogeneity of variance was satisfied, and the Games Howell method if it was not satisfied. In all cases, we considered the results to be significant at $p<0.05$ level.

3. RESULTS AND DISCUSSION

3.1. The effect of P-starter fertilizer on two soils with different nutrient supply and soil biological activity

In terms of crop yields, based on data from several years (Figure 1), we did not measure any yield effect of Starter fertilization on corn at high fertilizer rates (120N) or medium fertilizer rates (87N). Overall, during the three years studied, precipitation had a significant effect on corn yield, while Starter treatment and the interaction between treatment and precipitation did not show any effect on yield (Table 2).

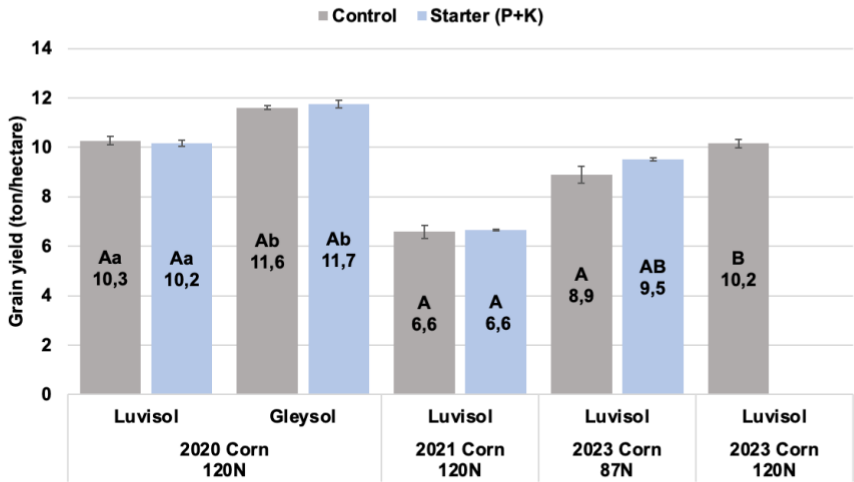

Figure 1. The effect of Starter fertilizer on grain yield (86% dry matter) in field experiment on Luvisol and on Gleysol soil with corn (*Zea mays* FAO 330) test plant. Mean values and standard errors of measurement. ***The capital letters in the columns (A) means a significant difference between the treatment per soil type, the lowercase letters (a) means a significant difference among soil types per treatments ($p<0.05$)***

Table 2. The effect of Starter treatment and precipitation interactions on yield (ANOVA) in 2020, 2021 and 2023 years on Luvisol soil. The asterisk indicates the significant level: * = $p<0.05$; *** = $p<0.001$.

Predictors	Response variable	F-value	Sig level	Effect size (h^2)
Starter treatment	Yield	0.002	ns	0.995
Precipitation	Yield	130.152	***	0.879
Starter treatment * Precipitation	Yield	0.085	ns	0.005

The biomass results are similar to the crop results, no significant difference was found in any of the years due to the treatments (although some biomass increase was measurable).

In terms of plant height, we observed the visible height increasing and phosphorus deficiency reducing effect of Starter fertilizer on the lower quality Luvisol soil every year. The same was not observed on Gleysol soil, which may have been due to higher nutrient supply and favorable pH. This initial height difference in 2021 was significant due to the Starter treatment and the plants showed less signs of anthocyanin discoloration this year as well. However, this difference in height and color on Luvisol soil was equalized every year during flowering, including in 2021.

We measured the PHO enzyme activity results of soil samples three times during the growing season in 2021. At the initial stage of plant development, during flowering, and at maturity. We not detected the effect of Starter fertilization at any time or at any soil sampling depth.

In our three-year experiment, the amount of precipitation during the growing season had the greatest effect on yield, which is very common in Hungary. In our 2023 experiments, the amount of fertilizer applied had a significant effect on yield, but the phosphorus-containing Starter fertilizer did not increase corn yield in any of the years studied.

Similar results were found by Roller et al. (2022), who examined the reaction of several corn hybrids and found that anthocyanin discoloration of corn due to P deficiency is a highly hybrid-dependent trait. Our experiment did not confirm the findings of Geist et al. (2023), that starter phosphorus fertilizer is recommended when P uptake is low. Several other studies, such as Quinn et al. (2020), have also obtained similar results to our experiment. Quinn et al. (2020) found in their meta-analysis that the yield-increasing effect of starter fertilizer occurs at a rate of 5.2% and generally decreases with increasing yield levels.

In our experiments, neither lower nor higher yield levels influenced the yield-increasing effect of starter fertilizer, because higher basic fertilizer application and the nutrient supply of the growing sites were together sufficient for the plants to develop properly under the given precipitation conditions. However, the initial growth of plants treated with Starter was more vigorous, especially on poorer quality, acidic Luvisol soil, indicating that the initial nutrient deficiency was compensated, but these differences in height were made even by the end of the growing season in all cases, meaning that the nutrients taken up later during plant growth compensated for the initial nutrient deficiencies. According to Margalef et al. (2021), P fertilization reduces soil phosphatase activity. We did not demonstrate this in our trial, which may be due to the fact that the basic fertilizer applied also to the control plots contained P fertilizer, and the additional phosphorus in the starter treatment was not sufficient to produce a significant difference. Our hypothesis that the use of starter fertilizers has a significant effect on corn yield was not confirmed.

3.2. The effect of bacterial inoculation depending on soil type and fertilization

Bacterial inoculation (Bact1 and Bact2) had no effect on corn or wheat yield in the years of high fertilization (2020-2022) (Figure 2). In 2020, we set up an experiment on two types of soil with Bact1 inoculation and we could not detect a significant effect of the inoculation on either soil, however, the yield of the Gleysol soil significantly exceeded the yield of the Luvisol soil. In 2023, the year of low fertilization, the Bact2 inoculation significantly increased corn yield, so in addition to the low nutrient supply, the applied bacterial strains contributed to the nutrient uptake of the plants on the Luvisol soil with a weaker nutrient supply. Furthermore, the basic fertilizer dose also had a significant yield-increasing effect this year, which was contributed by the appropriate amount of precipitation that year. Based on the results of the Bact1 treatment in 2020 and 2021 and the Control results of 120N in 2023, the Bact1 treatment had no effect on yield, while it was significantly affected by the precipitation of the years (Table 3.). The interaction of the Bact1 treatment and precipitation did not affect yield.

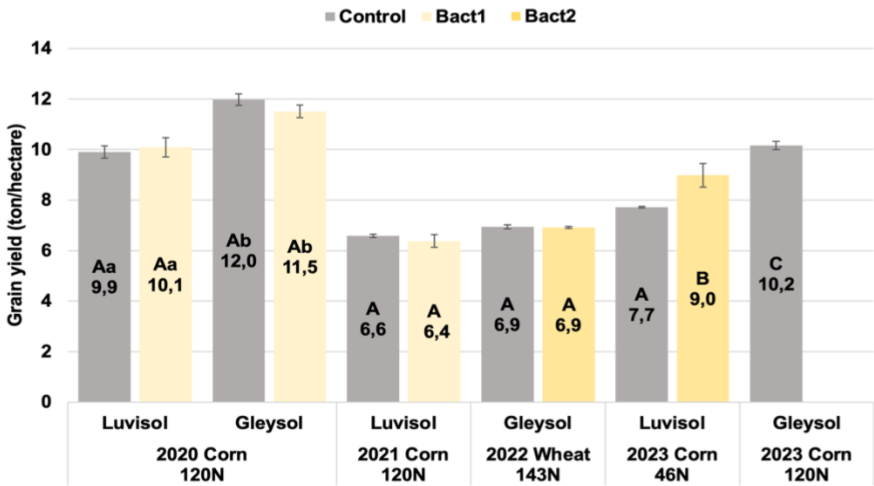

Figure 2. The effect of Bact1 and Bact2 inoculants on grain yield (86% dry matter) in field experiment on Luvisol and on Gleysol soil with corn (*Zea mays* FAO 330) test plant. Mean values and standard errors of measurement. ***The capital letters in the columns (A) means a significant difference between the treatment per soil type, the lowercase letters (a) means a significant difference among soil types per treatments ($p<0.05$)***

Table 3. The effect of Bact1 treatment and precipitation interactions on yield (ANOVA) in 2020, 2021 and 2023 (Control treatment) years on Luvisol soil. The asterisk indicates the significant level: * = p<0.05; *** = p<0.001.

Predictors	Response variable	F-value	Sig level	Effect size (h^2)
Bact1 treatment	Yield	0.095	ns	0.009
Precipitation	Yield	260.34	***	0.959
Bact1 treatment * Precipitation	Yield	0.351	ns	0.031

The biomass of plants was measured during the year of low fertilization in 2023, but the yield increase caused by Bact2 did not cause proportional biomass increase. The amount of increased fertilizer applied was causing a noticeable, but not significant increase in biomass results.

In the years of high fertilization in 2021-2022 (120N, 143N), we measured PHO enzyme activity at several times, but we could not detect significant differences in the effect of bacterial inoculations. In the year of low fertilization level in 2023 (46N), similar to yield and plant height, the PHO activity of soil samples in the Luvisol soil was significantly increased in comparison with Control.

In 2023, under low fertilization level (46N), we examined the effect of the inoculation on four plant parameters, which appears to have a significant effect on the plant height, similar to the yield. In the case of the root neck diameter, we measured a higher average value, but this was not significantly different. No effect of inoculation was shown regarding the root capacity and chlorophyll content results.

In our experiments conducted over four consecutive years, we did not observe any yield-enhancing effect of bacterial inoculation in wheat crops grown on Luvisol and Gleysol soils with high fertilizer application rates (143N), similar to the results reported by Mayer et al. (2010). In corn crops, we also found no inoculation effect at high fertilizer levels (120N), which is consistent with the findings of Santos et al. (2023). According to Santos et al. (2023), soil cultivation methods play a much more important role in corn yield than inoculation. Contrary to the results obtained at high fertilizer rates, at low fertilizer rates (46N) we observed a significant increase in corn yield due to inoculation on lower-quality Luvisol soil, which can be attributed to the low nutrient content of the soil.

Plant height is an important plant growth parameter, which was able to predict the effect of inoculation in our low fertilizer experiment. Furthermore, the inoculation effect was also indicated by an increase in PHO enzyme activity in our experiment, which is in line with the findings of Campdelacreu Rocabruna et al. (2024), who found that the use of beneficial microbes can increase PHO activity and that total microbial activity is positively correlated with PHO activity.

Our hypothesis that soil type and fertilization determine the effectiveness of bacterial inoculation has been partially confirmed.

3.3. The effect of soil biological status on the effectiveness of bacterial inoculants in a model pot experiment

The results show that no statistically significant effect was found on the biomass of plants in any soil type or soil treatment. The root capacity measurement also gave similar result, but on the lower quality Luvisol the root capacity of plants treated with molasses and AutoclavedBact2 significantly exceeded Control plants, and Bact2 inoculation took the value between the two treatments.

In case of sterile treatment on both soil types, a significant decrease in DHA enzyme activity of up to 80% detected compared to the two other soil treatments. On these soils, DHA enzyme activity was increased by Bact2 inoculation and in Luvisol soil with weaker nutrient supply and biological activity resulted in a significant difference (Figure 3.).

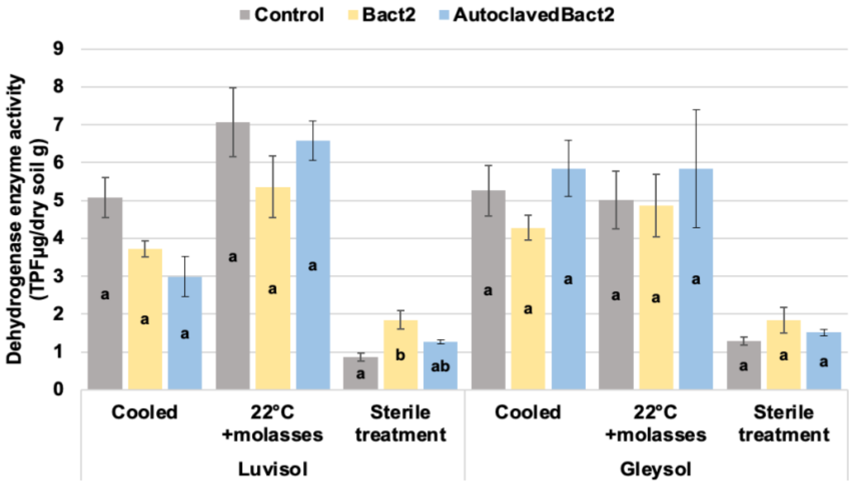

Figure 3. Bact2 and AutoclavedBact2 inoculation effect on the dehydrogenase enzyme activity (DHA) in pot experiment on Luvisol and on Gleysol soil with corn (*Zea mays* FAO 330) test plant. Mean values and standard errors of measurement. ***The letters in the columns (a) means a significant difference between the treatment per soil treatments, (p<0.05)***

We measured the labile carbon content of soil samples, but the effect of sterile treatment was not detected on any soil type. In soil samples incubated at 22°C and treated with molasses, a significant increase in DHA, i.e. general microbiological activity, was observed in Luvisol soil, which may have been caused by the easily and quickly absorbable amount of sugar (Nugroho et al., 2023c). The inoculation also showed its positive effect on sterile treated soil because the reduced number of the original bacterial mass was no longer competing for nutrients, so the free living space due to sterile treatment could be more easily occupied by the inoculated strains. This result suggests that the number, composition and physiological status of the original microbes of the soil is very decisive for the effectiveness of the inoculation, which is in line with Zhang et al. (2011). Our hypothesis that the indigenous bacteria community of soil affects the effect of the bacterium added, but the effect depends on the nutritional supply of soil has been demonstrated.

3.4. Effect of liming on plant growth and the bacterial inoculations efficiency in 2023

3.4.1. Effect of liming on P mobilization in pot experiment

No significant differences were found between the results of the two soil types with different nutrient supply. Within soil types, the labile carbon content of soil samples increased significantly as a result of liming on both nutrient-supplying Luvisol soils. The measurement of labile carbon clearly illustrates the difference in biological activity under pot conditions. Considering plant height and biomass production, we measured higher average values as a result of liming, but none of the results were significant, only a trend emerged. As a result of liming, the KCl pH value of the soil samples increased from 4.91 to 7.43 over 11 weeks by the end of the experiment. Based on Abdi's (2024) findings, the long-term beneficial effect of liming can be explained by an increase in pH, available P, cation exchange capacity, microbial activity, organic carbon, total nitrogen, and a decrease in nutrient leaching and exchangeable aluminium. Our hypothesis that soil biological activity can be improved in acidic brown forest soils by liming the soil pH has been confirmed.

3.4.2. Effect of liming on bacterial inoculations in field experiment

We measured the pH-increasing effect of liming on soil samples of flowering control and limed parcels. Due to liming, pH (H_2O) increased from 6.00 to 6.70, while pH (KCl) increased from 4.68 to 6.24. Based on the yield results, liming alone caused higher average yields, but a significant increase in yield could be caused by the Bact2 inoculation alone, and the combination of two treatments, but the combination of these two treatments did not exceed the single effect of the Bact2, and the synergistic effect of the treatments did not occur.

In terms of plant height, when we examined the three treatments, we found a significant difference due to liming and combined treatment, and compared to bacterial inoculation and liming, the combined treatment did not cause any further differences in plant height. For the other plant growth parameters, the treatments did not cause any significant changes.

In our opinion, a higher dose of liming on our low-nutrient Luvisol soil with low fertilization (46N) could have increased the yield of corn plants similar to the results of Bossolani et al. (2022). Presumably, by increasing the pH, you can reduce the amount of P bound by aluminium in the soil (Bossolani et al., 2022), thus increasing the amount of P that can be absorbed, which would be very important in Transdanubian brown forest soils. Our hypothesis that the effectiveness of bacterial inoculation can be improved on acidic brown forest soil by pH liming has not been confirmed.

3.5. The effect of mycorrhizal inoculation depending on soil type and fertilization

In the field experiment, in terms of yield results (Figure 4), at high fertilization (120N), we did not measure the effect of Myc inoculation in either corn or wheat on either soil type. However, in 2023, at low fertilization levels (46N), Myc inoculation significantly increased corn yield by 12.5% on the lower quality Luvisol soil. Inoculation had no significant effect on yield on the Gleysol soil, and even at this nutrient supply level, a significant yield advantage of the Control treatment over the Luvisol Control treatment was observed on the Gleysol soil. In 2023, looking at the Control plots, it is clear that the level of fertilization had a significant effect on yield. The high fertilizer dose (120N) increased the yield by an average of 31.4% and 21.9% in the uninoculated Control plots on Luvisol and Gleysol in 2023. This effect was supported by the fact that 2023 had sufficient rainfall, which contributed to the uptake of conventional amounts of fertilizer (Pepó, 2018).

Examining the 2021 results and the 2022 control results together, it appears that the soil type, precipitation and interaction of the two had a significant impact on the yield (Table 4).

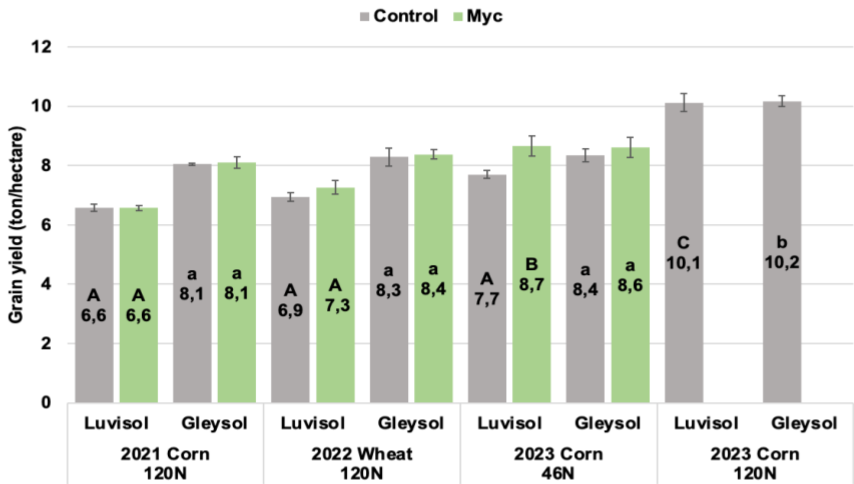

Figure 4. The effect of mycorrhizal inoculation (Myc) on grain yield (86% dry matter) in field experiment on Luvisol and on Gleysol soil with corn (*Zea mays* FAO 330) and wheat (*Triticum aestivum*) test plants. Mean values and standard errors of measurement. *The capital letters in the columns (A) means a significant difference between the treatment per Luvisol, the lowercase letters (a) means a significant difference between the treatment per Gleysol (p<0.05)*

Table 4. The effect of soil type and precipitation interactions on yield (ANOVA) in 2021 and 2023 years in case of Control treatments on Luvisol soil. The asterisk indicates the significant level: * = p<0.05; *** = p<0.001.

Predictors	Response variable	F-value	Sig level	Effect size (h^2)
Soil type	Yield	36.027	***	0.818
Precipitation	Yield	510.363	***	0.985
Soil type * Precipitation	Yield	34.454	***	0.812

Biomass results approximately follow yield results, but we did not detect significant biomass differences in case of Myc inoculation.

The effect of Myc treatment on PHO enzyme activity was only detected once in the high fertilization years (120N, 143N) of 2021 and 2022, in the flowering time of 2021 in July, on the low quality Luvisol. The results of Gleysol soil PHO enzyme activity in several cases significantly exceeded the PHO results measured on Luvisol soil and in several cases a significant difference between sampling depths was found. In the year of low fertilization (46N), in 2023, we measured a significant increase in PHO activity in response to Myc treatment compared to the uninoculated control with the same fertilizer application on lower quality Luvisol. However, the difference was not significant on Gleysol soil.

Plant parameters measured in 2023 at low fertilizer levels (46N), plant height, root capacity, root neck diameter, and chlorophyll content showed significant increases in most cases as a result of Myc treatment. Furthermore, soil type also had a significant effect on plant parameters in this year, except for plant height. Examining the results of the post-hoc test, root capacity was the most sensitive parameter to Myc inoculation on Luvisol soil, while plant height was the most sensitive parameter on Gleysol soil

We found a moderately strong linear regression relationship between plant height and the other measured plant parameters. The regression analysis resulted in the strongest correlation (R^2) when plant height was compared to the other parameters. On Luvisol soil, we found a strong linear relationship between plant height and root capacity (R^2=0.5795) and between plant height and root collar diameter (R^2=0.6091). On Gleysol soil, we also found a strong linear relationship between plant height and root capacity (R^2=0.5889) and between plant height and chlorophyll values (R^2=0.6963).

Myc inoculation generally had a significant effect on root colonization in the three years studied. The only exception was the root colonization of 2021 corn on Luvisol soil, but on average colonization was higher in inoculated plots, but the difference was not significant. In corn, we measured significantly lower root colonization values in 2023 at low fertilization levels (46N) than in 2021 at high fertilization levels during a drier growing season.

In our three-year experiment, precipitation (Table 4) had the greatest effect on yield (Figure 4), which also greatly influenced the yield-increasing effect of fertilization. Similar to bacterial inoculation, the effect of Myc treatment on grain yield and biomass was only observed in 2023 with reduced N-P-K fertilizer application (Prettl et al., 2024), in line with the findings of Bakonyi and Csitári (2023). In drier and average years (2021-2022), the grain yield and biomass of Gleysol soil significantly outperformed that of Luvisol soil. This could be due to the higher nutrient and humus content of Gleysol soil and its better water management properties, which had a more significant effect in drier years (Juhos et al., 2015). In 2023, when rainfall distribution was better, grain yield and biomass were very similar on both soil types. However, in 2023, with low fertilizer application rates, the effect of Myc treatment on yield was more pronounced on Luvisol soil (Figure 4). In our opinion, this is because fertilizer reduction has a stronger negative effect on Luvisol, which is fundamentally limited in nutrients and has a lower humus content, so the nutrients provided by mycorrhiza had a greater effect on yield. Qin et al. (2019) conclude that the effect of mycorrhiza is more significant at low organic matter content and neutral pH. Our hypothesis that soil type and fertilizer intensity determine the effectiveness of mycorrhizal inoculation has been confirmed

Despite better nutrient supply, Myc treatments on Gleysol soil significantly increased the height, root neck diameter and chlorophyll concentrations of corn plants, while on Luvisol soil only plant height and chlorophyll concentrations changed. Whether Myc treatments had a significant impact on yields, all plant development parameters we measured could have shown an increase in potential nutrient uptake capacity on both soils. Similar conclusions were concluded, for example, Colla et al. (2015). Similar to the yield, the significant increase in the PHO enzyme activity of soil samples was shown only on Luvisol soil, due to the lack of nutrients on Luvisol soil (in consideration of the fact that we did not applied P fertilizer in the year of low fertilization) (Margalef et al., 2021). Our hypothesis that soil biological and plant growth parameters may predict the effects of the inoculation of the mycorrhiza have been partially confirmed.

3.6. The importance of soil biological activity in bacterial and mycorrhizal inoculation effectiveness, the relationship between soil biological activity and soil types

In addition to phosphatase (PHO) enzyme activity, permanganate oxidizable labile carbon content (POXC), dehydrogenase (DHA) and β-glucosidase (GLU) enzyme activities in soil were measured in field experiments. The effect of the treatments could not be demonstrated by any of the parameter. However, there were significant differences between the results of different sampling times and sampling depths. In the case of corn flowering, the DHA and GLU enzyme activity results were higher in the upper 10 cm layer, while in the case of wheat we experienced a rather reverse trend. In the case of POXC, we measured a significant difference between the two depths during wheat flowering, in favor of the 10-20 cm depth.

When examining the three soil parameters in the year 2023, which was characterized by low fertilization (46N), we measured significant GLU enzyme activity in the upper 10 cm soil layer during the corn flowering as a result of Bact2 inoculation. Similar to yield, plant height and PHO enzyme activity, the GLU enzyme activity values indicated the effect of the inoculation. In the case of DHA enzyme activity and POXC, we found no difference in the effect of inoculation in 2023 either.

In 2022, with high fertilizer application (143N) in a year when wheat was the main crop, the GLU enzyme showed a significant linear relationship with grain yield (R^2=0.412), while the DHA enzyme (R^2=0.6003) PHO enzyme (R^2=0.791) and labile carbon (R^2=0.772) showed a strong linear relationship with grain yield when the data from the two growing sites were examined together.

Evaluation of the years of high fertilizer level (120N) in 2021 and low fertilizer level (46N) in 2023 as main crop years for corn, we found linear relationships between grain yield, soil enzymes and labile carbon parameters. The GLU (R^2=0.3876) and PHO (R^2=0.3663) enzymes showed a weak linear relationship with yield, while a strong and significant linear correlation (R^2=0.6581) was found between labile carbon and grain yield.

Based on the combined analysis of soil enzymes and POXC results measured during the experiments, we concluded that the sampling time, soil type, and their interaction significantly influenced the changes in the measured parameters.

Based on the correlation analysis of the data (Table 5), the DHA enzyme correlates with the PHO enzyme, and the PHO enzyme correlates with the POXC content and soil moisture. The GLU enzyme correlates with POXC content and soil moisture, and POXC and soil moisture correlate with each other (Table 5). Most of our findings are consistent with those reported by Nugroho et al. (2024) in their soil cultivation experiment.

Table 5. Correlation matrix among soil enzymes, labile carbon content (POXC) and soil water content, in 2021, 2022 and 2023 years, under different fertilization levels. The asterisk indicates the significant level: * = p<0.05; *** = p<0.001.

	DHA	**PHO**	**GLU**	**POXC**	**Soil water content**
DHA	1	0,563**	0,067	-0,31	0,097
PHO	0,563**	1	0,105	0,194**	0,224**
GLU	0,067	0,105	1	0,227**	0,480**
POXC	-0,31	0,194**	0,227**	1	0,504**
Soil water content	0,097	0,224**	0,480**	0,504**	1

The change in GLU activity at the beginning of flowering in 2023 may have been due to the fact that the applied inoculant increased the rate of soil organic matter decomposition, which was reflected in the increase in GLU activity.

Considering the correlation analyses between grain yield and enzyme activities and the amount of POXC, it can be stated that soil biological parameters measured at flowering may be related to crop yields. This is probably due to the fact that these parameters actually develop as a result of the soil organic matter and moisture content and also indicate the reactions of the plants. Gangwar et al. (2022) reached similar results in their study by examining DHA and PHO activities in fields under arable cultivation with different soil types.

4. CONCLUSIONS AND RECOMMENDATIONS

In our field trials conducted over four years, the yield was the most significantly influenced by the amount of the precipitation during the growing season and the water and nutrient management properties of the soil type. In comparison, the effect of the applied starter and basic fertilizer (PK) was much less, but the nitrogen dose had a significant effect on both soils. A decrease in corn yield can be observed below a nitrogen dose of approximately 87 kg/ha. Based on multi-year results, the use of starter fertilizer in corn parallel with sowing, did not result in a significant yield increase at the growing sites examined. Our hypothesis that the use of starter fertilizers has a significant effect on the yield of corn has not been demonstrated.

With high fertilizer application rates (120N, 143N), the nutrients applied and present in the soil were probably sufficient for the plants to develop properly in both soil types under the given precipitation conditions. Under these conditions, bacterial and mycorrhizal inoculation had no significant effect on crop yields, which may be due to two reasons: 1) the plants did not need their presumed nutrient-mobilizing effect, or 2) the conditions for the microbes in the inoculation materials were not ideal. However, at lower nitrogen fertilization levels (46N) on Luvisol soil with poorer nutrient supply capacity, the plants needed the nutrient mobilizing capacity of the externally introduced bacteria and fungi, and their effect was reflected in the yield. However, due to the reduced nitrogen doses, crop yields decreased significantly, which could not be compensated by microbial inoculations. Our hypothesis that soil type and fertilization determine the effectiveness of bacterial and mycorrhizal inoculations was partially confirmed.

The effect of bacterial inoculation on plant height at flowering and PHO activity, as well as the effect of mycorrhiza treatment on any flowering plant development parameter, cold also be demonstrated. The inoculations probably supported the plants until flowering by the microbiome of the root zone, but the effect of the subsequent water shortage could no longer be compensated for. Root colonization has increased every year on both soils as a result of the mycorrhiza inoculation, regardless of fertilizer use, so greater root colonization does not always mean a yield increasing effect. Our hypothesis that soil biology and plant growth parameters can predict the effect of inoculations has been partially confirmed.

In a field experiment, under low fertilization (46N) liming on its own increased corn yields on acidic Luvisol soil, presumably by raising the pH and reducing the amount of aluminium-bound P in the soil, thus increasing the volume of available P. The bacterial strains we applied had beneficial effects on crop yield even without liming, so there is no evidence that the acidic conditions limited the effectiveness of the inoculants. The results of the liming experiment suggest that liming may have contributed to an increase in biological activity (POXC) by raising the pH. Our hypothesis that the efficiency of bacterial inoculant and soil biological activity can be improved on acidic brown forest soil by liming partially confirmed.

In a sterile treatment model experiment, the DHA enzyme, as a measure of total microbial activity, showed the effect of inoculation only in sterile-treated soil. Due to sterile treatment, the number of original indigenous bacteria decreased, so they competed less with the introduced strains. In addition, during sterile treatment, nutrients that help the introduced bacteria could also be released, helping them occupy the empty living space and increase their activity. The result suggests that the number and composition of the original microbes of the soil is very decisive for the effectiveness of the inoculation. Our hypothesis, that the indigenous bacterial community of the soil influences the effect of the applied bacterial inoculant, but the effect depends on the nutrient supply of the soil confirmed.

Based on our experiments, we make the following recommendations:

- Further experiments are needed to evaluate the effectiveness of starter fertilizers, on poorer Luvisol soils with lower nutrient content than the test site, using several corn hybrids, possibly in combination with liming. This would reveal the P supply levels below which starter fertilizers are recommended.
- The use of fertilizers helps nutrient uptake processes, thus so there is less need to mobilize nutrients in the soil. However, at reduced fertilizer application rates, the microbes applied help nutrient uptake but do not replace fertilizers economically. Therefore, their intermediate use could be a solution, whereby the plant is initially supported with fertilizer and later with microbes. Various agrotechnical elements intended to replace the application of bacteria and fungi, which improve soil life, such as foliar fertilization, reduced tillage, and the use of cover crops, could also be a solution.
- In a controlled environment, we demonstrated the need for scale-up testing methods in the practical application of microbial inoculants in a model pot experiment, compared to field experiments with high fertilization levels. In field experiments, the effect of microbial inoculants can be positively influenced by the weaker nutrient uptake capacity of soils. However, it would be important to conduct individual experimental studies of the microbes present in the inoculants, combined with a preliminary knowledge of the number, composition and activity of indigenous bacteria in the soil.
- Our research only partially answered the question of what nutrient supply and soil conditions are required for microbial inoculants to have a significant effect in conventional crop production. Further experiments would be necessary under field conditions with different nutrient doses and combinations of inoculants, possibly looking for greater differences in nutrient supply within soil types.

5. NEW SCIENTIFIC RESULTS

1. The use of starter fertilizer on acidic brown soil (Luvisol) with low phosphorus and organic matter content causes visible initial biomass growth and a reduction in nutrient deficiency symptoms in corn. However, based on data from several years with different precipitation levels at the study sites, it does not result in yield increases in conventional farming, and its effect on yield at the end of the growing season cannot be detected on these soils.

2. From the acidic brown forest soil (Luvisol) area studied, we can conclude that corn yield is mainly determined by weather conditions, followed by the amount of nitrogen applied, and that the influence of the microbial inoculants and liming used by us is significantly smaller.

3. The bacterial and fungal inoculants we used only showed their yield-increasing effect when fertilizer use was reduced. However, inoculants can only partially compensate for the yield-increasing effect of the fertilizer.

4. The lack of yield-increasing effect of starter fertilizers applied to corn also predicts the ineffectiveness of microbial inoculants at high fertilizer application rates. However, the initial growth stimulation of starter fertilizers may indicate the expected yield-increasing effect of microbial inoculation at low N fertilizer levels compared to the control.

5. We have shown that soil enzymes measured at flowering (β-glucosidase, dehydrogenase, acid phosphatase) and permanganate-oxidable labile carbon content measured at flowering correlate well with wheat and corn yields, as they indicate increased plant nutrient demand and nutrient uptake at flowering. However, they not necessarily indicate the effect of microbial inoculants on soil biological activity.

6. LIST OF PUBLICATIONS RELATED TO THE DOCTORAL THESIS

Journal articles:

1. **Prettl, N.**, Biró, B., Nugroho, P. A., Kotroczó, Z., Kabalan, S., Kovács, F., Papdi, E., & Juhos, K. (2024). Limited effect of mycorrhizal inoculation depending on soil type and fertilization level in a central European field trial. *Plant Growth Regulation*, *104*(3), 1669–1681. https://doi.org/10.1007/s10725-024-01251-w, **Scopus: Q1, Impact factor: 3,6 (2023)**
2. **Prettl, N.**, Biró, B., Nugroho, P. A., & Juhos, K. (2022). Labile carbon as an indicator of soil biological activity in the application of microbial inoculants and soil conditioner containing Ca. *Journal of Central European Green Innovation*, *10*(Suppl 3), 13–25. https://doi.org/10.33038/jcegi.3559

International conference (full paper):

3. **Prettl, N.**, Kotroczó, Zs., Pabar, S.A., Juhos, K., Biró, B. (2021) Corn response to starter fertilizers in two different soils, with a main focus on soil biology. XVI. Carpathian Basin Conference for Environmental Sciences (Budapest (online), Hungary, 2021. March 30. -April 1.) Abstract book: ISBN: 978-963-8221-82-7., 193-199.
4. **Prettl, N.**, Kotroczó, Zs., Priyo, A.N., Pabar, S.A., Biró, B., Juhos, K. (2022) The effect of bio- and phosphorus starter fertilizers, on corn growth in two different soils. XVII. Carpathian Basin Conference for Environmental Sciences, (Kolozsvar, Romania, 2022. April 6-9.) Abstract book: ISSN: 1842-9815., 160-166.

International conference (oral/poster):

5. **Prettl, N.**, Biró, B., Nugroho, P.A., Kotroczó, Zs., Kabalan, S., Kovács, F., Juhos, K., (2024) Corn and wheat response to bacterial and fungal inoculation in a 3-years field trial. XIX. Carpathian Basin Conference for Environmental Sciences (April 03-05, 2024, Nuclear research Institute, Debrecen), Abstract book: 46-47.
6. **Prettl, N.**, Juhos, K., Nugroho, P.A., Kotroczó, Zs., Kabalan, S., Kovács, F., Biró, B. (2024) Effect of mycorrhiza inoculation depending on fertilization level and soil type at Central European field trial. Centennial Celebration and Congress of the International Union of Soil Sciences (May 19-21, 2024, Florence, Italy) Abstract book: 1276.

7. **Prettl, N.**, Biró, B., Nugroho, P.A., Kotroczó, Zs., Kabalan, S., Juhos, K., (2023) The effect of mycorrhizal fungi inoculant application in conventional agriculture practice on soil biological parameters and yield. XVIII. Carpathian Basin Conference for Environmental Sciences (May 17-19, 2023, University of Szeged, Szeged), Abstract book: 51-52.

8. **Prettl, N.**; Kotroczó, Z.; Pabar, S.A.; Nugroho, P.A; Juhos, K. and Biró, B. (2022) Soil characteristics and soil biological activity is a key in P-supply of corn at cold early spring in Central-European soils. XXII. World Congress of Soil Science (Glasgow, Scotland, 31 July – 5 August 2022.)

9. **Prettl, N.**, Kotroczó, Zs., Pabar, S.A., Juhos, K., Biró, B. (2021) Corn response to starter fertilizers in two different soils, with a main focus on soil biology. XVI. Carpathian Basin Conference for Environmental Sciences (Budapest (online), Hungary, 2021. March 30. -April 1.) Abstract book: ISBN: 978-963-8221-82-7., 100.

7. REFERENCES

1) Abdi, B. T. (2024). Studies on the effects of liming acidic soil on improving soil physicochemical properties and yield of crops: A review. *Middle East Research Journal of Agriculture and Food Science*, *4*(03), 95–103. https://doi.org/10.36348/merjafs.2024.v04i03.001
2) Bakonyi, I., & Csitári, G. (2023). Arbuscular mycorrhizal fungi inoculation of winter wheat in an intensive crop pro-ducing farm. *Journal of Agriculture and Horticulture Research*, *6*, 288–294.
3) Bossolani, J. W., Crusciol, C. A. C., Momesso, L., Portugal, J. R., Moretti, L. G., Garcia, A., De Cássia Da Fonseca, M., Rodrigues, V. A., Calonego, J. C., & Dos Reis, A. R. (2022). Surface liming triggers improvements in subsoil fertility and root distribution to boost maize crop physiology, yield and revenue. *Plant and Soil*, *477*(1–2), 319–341. https://doi.org/10.1007/s11104-022-05432-2
4) Campdelacreu Rocabruna, P., Domene, X., Preece, C., & Peñuelas, J. (2024). Relationship among soil biophysicochemical properties, agricultural practices and climate factors influencing soil phosphatase activity in agricultural land. *Agriculture*, *14*(2), 288. https://doi.org/10.3390/agriculture14020288
5) Cochran, W. G. (1950). Estimation of cacterial densities by means of the „most probable number". *Biometrics*, *6*(2), 105. https://doi.org/10.2307/3001491
6) Colla, G., Rouphael, Y., Bonini, P., & Cardarelli, M. (2015). Coating seeds with endophytic fungi enhances growth, nutrient uptake, yield and grain quality of winter wheat. *Int. J. Plant Prod*, *9*(2), 171–190.
7) Gangwar, R., Makádi, M., Bresilla, B., Zain, M., Weldmichael, T., Demeter, I., Táncsics, A., Cserháti, M., & Szegi, T. (2022). Effects of land uses and soil types on microbial activity and community structure. *International Agrophysics*, *36*(4), 323–336. https://doi.org/10.31545/intagr/155096
8) Geist, L., Wolfer, R., Thiem, R., Thielicke, M., Eichler-Löbermann, B., Eulenstein, F., & Müller, M. E. H. (2023). Alternative starter fertilization strategies in maize (*Zea mays* L.) cultivation: Agronomic potential of microgranular fertilizer and plant growth-promoting microorganisms and their impact on the soil native microbial community. *Agronomy*, *13*(12). https://doi.org/10.3390/agronomy13122900
9) Juhos, K., Czigány, S., Madarász, B., & Ladányi, M. (2019). Interpretation of soil quality indicators for land suitability assessment – A multivariate approach for Central European arable soils. *Ecological Indicators*, *99*,

261–272. https://doi.org/10.1016/j.ecolind.2018.11.063

10) Juhos, K., Szabó, S., & Ladányi, M. (2015). Influence of soil properties on crop yield: A multivariate statistical approach. *International Agrophysics*, *29*(4), 433–440. https://doi.org/10.1515/intag-2015-0049

11) Margalef, O., Sardans, J., Maspons, J., Molowny-Horas, R., Fernández-Martínez, M., Janssens, I. A., Richter, A., Ciais, P., Obersteiner, M., & Peñuelas, J. (2021). The effect of global change on soil phosphatase activity. *Global Change Biology*, *27*(22), 5989–6003. https://doi.org/10.1111/gcb.15832

12) Mayer, J., Scheid, S., Widmer, F., Fließbach, A., & Oberholzer, H.-R. (2010). How effective are 'Effective microorganisms® (EM)'? Results from a field study in temperate climate. *Applied Soil Ecology*, *46*(2), 230–239. https://doi.org/10.1016/j.apsoil.2010.08.007

13) Nkebiwe, P. M., Stevens Lekfeldt, J. D., Symanczik, S., Thonar, C., Mäder, P., Bar-Tal, A., Halpern, M., Biró, B., Bradáčová, K., Caniullan, P. C., Choudhary, K. K., Cozzolino, V., Di Stasio, E., Dobczinski, S., Geistlinger, J., Lüthi, A., Gómez-Muñoz, B., Kandeler, E., Kolberg, F., … De Neergaard, A. (2024). Effectiveness of bio-effectors on maize, wheat and tomato performance and phosphorus acquisition from greenhouse to field scales in Europe and Israel: A meta-analysis. *Frontiers in Plant Science*, *15*, 1333249. https://doi.org/10.3389/fpls.2024.1333249

14) Nugroho, P. A., Kotroczó, Z., Prettl, N., Kovács, F., Madarász, B., & Juhos, K. (2024). *Soil Biological Parameters after a 17-Year Tillage Study: Vertical and Temporal Variability Effect*. https://doi.org/10.2139/ssrn.5071357

15) Nugroho, P. A., Prettl, N., Kotroczó, Z., & Juhos, K. (2023c). The Effect of Molasses Application on Soil Biological Indicators and Maize Growth of Different Tillage Soil: A Pot Experiment. *Journal of Environmental Geography*, *16*(1–4), 119–124. https://doi.org/10.14232/jengeo-2023-44670

16) Pepó, P. (2018). Long-term experiments on chernozem soil in the University of Debrecen. *Acta Agraria Debreceniensis*, 357–369. https://doi.org/10.34101/actaagrar/150/1732

17) Phillips, J. M., & Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British mycological Society*, *55*(1), 158–161.

18) Prettl, N., Biró, B., Nugroho, P. A., Kotroczó, Z., Kabalan, S., Kovács, F., Papdi, E., & Juhos, K. (2024). Limited effect of mycorrhizal inoculation depending on soil type and fertilization level in a central European field

trial. *Plant Growth Regulation*, *104*(3), 1669–1681. https://doi.org/10.1007/s10725-024-01251-w

19) Qin, M., Zhang, Q., Pan, J., Jiang, S., Liu, Y., Bahadur, A., Peng, Z., Yang, Y., & Feng, H. (2019). Effect of arbuscular mycorrhizal fungi on soil enzyme activity is coupled with increased plant biomass. *European Journal of Soil Science*. https://doi.org/10.1111/ejss.12815

20) Quinn, D. J., Lee, C. D., & Poffenbarger, H. J. (2020). Corn yield response to sub-surface banded starter fertilizer in the U.S.: A meta-analysis. *Field Crops Research*, *254*. https://doi.org/10.1016/j.fcr.2020.107834

21) Roller, S., Weiß, T. M., Li, D., Liu, W., Schipprack, W., Melchinger, A. E., Hahn, V., Leiser, W. L., & Würschum, T. (2022). Can we abandon phosphorus starter fertilizer in maize? Results from a diverse panel of elite and doubled haploid landrace lines of maize (Zea mays L.). *Frontiers in Plant Science*, *13*, 1005931. https://doi.org/10.3389/fpls.2022.1005931

22) Santos, M. H., Aguiar, W. E., Vendruscolo, E. C. G., & Missio, R. F. (2023). Does soil cultivation practices and inoculation with *Azospirillum brasilense* affect the performance of maize genotypes? *Ciência e Agrotecnologia*, *47*. https://doi.org/10.1590/1413-7054202347005523

23) Sinsabaugh, R. L., Klug, M. J., Collins, H. P., Yeager, P. E., & Petersen, S. O. (1999). Characterizing soil microbial communities. In *Standard Soil Methods for Long-Term Ecological Research* (o. 318–348).

24) Veerman, C., Pinto Correia, T., Bastioli, C., Biro, B., Bouma, J., & Cienciela, E. (2020). Caring for soil is caring for life. *EU Soil Health and Food Mission Board: Brussels, Belgium*.

25) Veres, Z., Kotroczó, Z., Magyaros, K., Tóth, J. A., & Tóthmérész, B. (2013). Dehydrogenase activity in a litter manipulation experiment in temperate forest soil. *Acta Silvatica et Lignaria Hungarica*, *9*(1), 25–33. https://doi.org/10.2478/aslh-2013-0002

26) Weil, R. R., Islam, K. R., Stine, M. A., Gruver, J. B., & Samson-Liebig, S. E. (2003). Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. *American Journal of Alternative Agriculture*, *18*(1), 3–17. https://doi.org/10.1079/AJAA2003003

27) Zhang, G.-Y., Zhang, L.-P., Wei, M.-F., Liu, Z., Fan, Q.-L., Shen, Q.-R., & Xu, G.-H. (2011). Effect of arbuscular mycorrhizal fungi, organic fertilizer and soil sterilization on maize growth. *Acta Ecologica Sinica*, *31*(4), 192–196. https://doi.org/10.1016/j.chnaes.2011.04.005