

## SOIL PHOSPHOMONOSESTERASES ACTIVITY AND PHOSPHORUS AVAILABILITY IN DIFFERENT TILLAGE SYSTEMS AND INOCULATIONS WITH *Bacillus megaterium* var. *phosphaticum*

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### Abstract

Phosphorus (P) is a crop-limiting nutrient, and its availability is challenging due to low solubility and high fixation rate. Rhizosphere activity, which mediates P uptake, can be promoted by inoculating phosphorus-solubilizing bacteria (PSB). This study aimed to evaluate soil total and accessible phosphorus (TP and AP), acid and alkaline phosphatases activity in response to PSB inoculation in different tillage practices. The field experiment was conducted on winter wheat, cultivated under conventional (CT), no-tillage (NT) and reduced tillage (RT). Both inoculations and the tillage systems were found to be associated with changes in TP and AP. In the CT and RT systems, alkaline phosphatase showed an increase in the bacterial inoculum treatments, 2-31% higher than the control in CT and 15-41% in RT. Across tillage systems, acid phosphatase activity was highest in RT (1.59-1.86  $\mu\text{g p-nitrophenol g}^{-1} \text{h}^{-1}$ ), followed by NT (1.46-1.72  $\mu\text{g p-nitrophenol g}^{-1} \text{h}^{-1}$ ) and CT (1.16-1.28  $\mu\text{g p-nitrophenol g}^{-1} \text{h}^{-1}$ ). The soil air-water regime controlled by tillage practices influences rhizosphere activity and, together with bacterial inoculation, leads to variations in the investigated parameters.

**Key words:** acid phosphatase, alkaline phosphatase, no-tillage, phosphorus-solubilizing bacteria.

### INTRODUCTION

Phosphorus (P) is a limiting element of crop nutrition, and its management requires particular attention. Even though P is found in large organic (Po) and inorganic (Pi) amounts, it has a low mobility (Milić et al., 2019) and only a small percentage is accessible to plants as  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$  ions in soil solution. In addition, phosphorus natural sources are non-renewable and declining, making fertilization with large amounts unsustainable and polluting (Prasad and Chakraborty, 2019). P dynamics are regulated by biological and biochemical processes, which depend on soil physical properties, pH, and soil organic matter (SOM) content (Davidescu & Madjar, 2009).

Most biochemical reactions are driven by enzymes, which are sensitive to changes in the soil environment. Enzymatic activity regulates and improves soil ecology, physical-chemical characteristics and, by extension, soil fertility (Das & Varma, 2010; Huang et al., 2023).

Of the enzymes found in soil, phosphatases are vital in the processes of Po mineralization. Acid

and alkaline phosphatases originated from microorganisms or plant roots. Their activity involves releasing free phosphates from inorganic fertilizers and catalyzing the breakdown of organic compounds with P (Acosta-Martínez & Tabatabai, 2011).

To improve P mobility and availability to plants, many studies have successfully tested the effectiveness of multiplication and soil fertilization with phosphorus solubilizing bacteria (PSB). *Bacillus* sp. is one of the most common and documented genera with extensive field applications. This is due to its species characterized by high adaptability and complex metabolic functions. Among their mechanisms of actions, we mention the synthesis of phytohormones (Tiwari et al., 2019), antibiotics (Shafi et al., 2017), organic acids and enzymes that enhance crop resistance, promoting growth and productivity (Robles Montoya et al., 2020). Studying the species *Bacillus megaterium*, Ibarra-Galeana et al. (2017) found that it exhibits both alkaline and acid phosphatase activity, thus contributing to enhanced solubilization of organic phosphates.

Considering that mineral fertilization is almost unavoidable, there is a potential to promote the accessibility of P to plants by using integrated crop nutrition formulations with the application of bacterial inoculants (Bargaz et al., 2021). Mixed use of mineral fertilizers with PSB can increase uptake efficiency as well as environmental protection.

Wheat (*Triticum aestivum* L.) is the second most widespread crop in Romania. The cultivated area is 2.2 million ha, with a total production of 9.6 million tons in 2024, ranking fourth in the European Union (INSSE). Achieving high yields is a challenge and involves extreme management measures such as intensive tillage and the use of large amounts of fertilizers, which are also the main causes of environmental degradation in the agricultural sector (Gomiero, 2016; Sun et al., 2024).

Although conventional tillage (CT) maximizes yields, this is often associated with negative effects on soil quality caused by repeated mechanical operations. Conversely, conservative practices create a balance between soil chemical, physical and biological attributes by reducing (minimum tillage) or avoiding soil disturbance (no-tillage) storing a significant quantity of organic matter (SOM) (Szczepanek et al., 2023; Singh et al., 2023). SOM accumulation leads to an abundant and diversified microbial community with a positive impact on soil nutrient cycling and plant uptake (Saini, 2024).

This study aimed to evaluate the enzymatic and chemical response to mineral and biological fertilization of autumn wheat under different management soil systems. We hypothesized that

bacterial inoculum together with mineral fertilizer could increase accessible phosphorus in the soil. Another hypothesis was that combined fertilization might increase the intensity of phosphomonoesterases activity, and this could be higher with mulch soil management practices.

## MATERIALS AND METHODS

### *Experimental Site and Treatments*

The research was conducted at the Ezăreni Student Research and Practice Station of the “Ion Ionescu de la Brad” Iasi University of Life Sciences (47°7'24.6"N and 27°30'59.76"E). For the field experiment, the growing season of winter wheat from October 2023 to June 2024 was considered. This crop is part of a multiyear rotation along with maize, sunflower and peas. Based on long-term meteorological reports, the experimental field is defined by a temperate continental climate. From sowing to grain harvest, the mean temperature was 9.5°C and the sum of precipitation was about 354.4 mm (Figure 1). The environmental conditions from the time of sowing and during crop growth were favorable. Thus, before the winter season, the crop received the required sum of active temperatures of 500°C. From the end of winter until harvest, both temperatures and rainfall were at optimal levels, so that plant development and ripening proceeded at a normal physiological rate. The recorded climatic data were also favorable for the functioning of *Bacillus megaterium*, although this bacterium is known for its resistance to extreme environmental influences (Biedendieck et al., 2021).

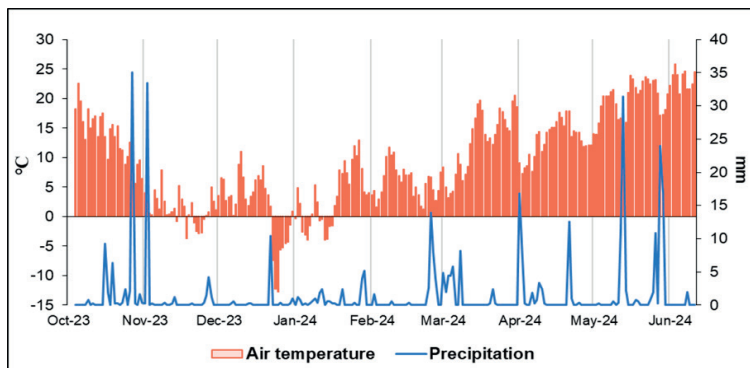


Figure 1. Air temperatures and precipitation from October 2023 to June 2024 (www.fieldclimate.com)

The soil type in the research area is Cambic Chernozem, according to the Romanian Taxonomic Soil System (SRTS, 2012). For a general description of the soil in the

experimental field before establishment, the basic basic soil properties are summarized Table 1.

Table 1. Soil chemical and physical properties

| Soil parameter                    | Tillage system                 |        |        |
|-----------------------------------|--------------------------------|--------|--------|
|                                   | CT                             | NT     | RT     |
| pH                                | 6.2                            | 6.2    | 6.3    |
| Organic C (%)                     | 2.06                           | 1.94   | 2.43   |
| Total N (%)                       | 0.178                          | 0.169  | 0.207  |
| Total P (%)                       | 0.0425                         | 0.0478 | 0.0463 |
| Available P (ppm)                 | 25                             | 52     | 51     |
| Texture                           | Clay-loam (Mihiu et al., 2022) |        |        |
| Bulk density (g/cm <sup>3</sup> ) | 1.14                           | 1.36   | 1.33   |

The field experiment was bifactorial and considered the tillage systems and fertilizer treatments described in table 2 and table 3. The local Glosa wheat variety was sown on October

17, 2023 at a row spacing of 17 cm. NP fertilizer was applied at crop sowing over the entire experimental area and the bacterial strain was inoculated manually.

Table 2. Soil tillage systems

| Tillage system                  | Description                                            |
|---------------------------------|--------------------------------------------------------|
| Conventional (CT)               | plowing at 25-30 cm; seedbed preparation at 8-10 cm    |
| No-tillage (NT) with mulch      | direct seeding in the last 10 years                    |
| Reduced tillage (RT) with mulch | loosening with scarifier at 40 cm, after 9 years of NT |

In the CT system, fertilizers were mixed into the soil when preparing the seedbed for sowing. In NT and RT, the biofertilizer was applied to the soil surface and NP fertilizer was inoculated at the same time as the seed. For the second

fertilization, Ecofertil was mixed with Corona N for T2, T3 and T4 and sprayed on plants and soil. Ecofertil is a commercial product based on *Bacillus megaterium* var. *phosphaticum*, produced by Antibiotice S.A. (Iasi).

Table 3. Fertilizer treatments

| Fertilizer treatment | Initial fertilization                                         |                                                                                             | Second fertilization                       |   |
|----------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------------------|--------------------------------------------|---|
|                      | OCTOBER 17, 2023 (before sowing)                              |                                                                                             | APRIL 01, 2024                             |   |
|                      | N <sub>10</sub> P <sub>24</sub><br>(200 kg ha <sup>-1</sup> ) | PSB<br>(Ecofertil - 1× 10 <sup>11</sup> CFU)<br>(15 l ha <sup>-1</sup> year <sup>-1</sup> ) | Corona N (21%)<br>(3 kg ha <sup>-1</sup> ) |   |
| T0 (control)         | +                                                             | -                                                                                           | -                                          | - |
| T1                   | +                                                             | 100 %                                                                                       | 100 %                                      | - |
| T2                   | +                                                             | 75 %                                                                                        | 75 %                                       | + |
| T3                   | +                                                             | 100 %                                                                                       | 100 %                                      | + |
| T4                   | +                                                             | 125 %                                                                                       | 125 %                                      | + |

Note: PSB - Phosphorus Solubilizing Bacteria; CFU - Colony Forming Unit; “+” means that the listed fertilizer has been applied; “-” means that the listed fertilizer has not been applied; % of PSB means the fraction of the manufacturer's recommended rate.

Soil Sampling and Analysis Methods

Soil sampling was conducted considering the analysis to be performed: acid and alkaline phosphatases, total and available phosphorus. For total and available phosphorus (TP and AP), the soil was collected with a hand auger to a depth of 15 cm from 20 points in paper bags, thoroughly homogenized and transported to the

laboratory. Samples for TP and AP were collected from each treatment at two different times: the first was between the two PSB inoculations, when the plants were at stem elongation, and the second at harvest. For the analysis of the two phosphatases, soil samples were collected from the 0-25 cm soil layer at harvest from five points on each treatment plot.

The soil was dried at room temperature and plant residues were removed from the samples. After that soil was sieved at a 2 mm diameter and stored in a dry place until analysis. For all analyses, samples were processed in triplicate. TP and AP were assessed by the molybdenum blue colorimetric method (Rusu et al., 2025) and the results were expressed as % for TP and ppm for AP. Acid and alkaline phosphatase activities were measured by incubating 1 g of soil at 37°C for 1 h. Spectrophotometric quantification was performed at an optical density of 420 nm. Results were calculated from the calibration curve equations and expressed as  $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$  (Tabatabai & Bremner, 1969; Eivazi & Tabatabai, 1977, Aruna Kumari et al., 2018).

### Statistical Analysis

SPSS Statistics 20 software was used for one-way analysis of variance (ANOVA) and comparing the means of fertilizer treatments both within and between tillage systems. This was followed by Tukey post-hoc test to determine differences between group means. Spearman rank correlation (2-tailed) was used to

check the significance and direction of correlation of the studied indicators. This was performed for each tillage system individually.

## RESULTS AND DISCUSSIONS

### Acid and Alkaline Phosphatases

Acid and alkaline phosphatases are indicators of enzymatic activity and overall soil fertility, being involved in the hydrolysis of substrate phosphomonoesters and the release of available P (Banerjee et al., 2012; Futa et al., 2021). The use of fertilizers containing PSB contributes to increased phosphomonoesterases activity and provides accessible P through solubilization of mineral fertilizers (Chesti et al., 2013) or by decomposition of organic matter (Tahir et al., 2018).

Under our experimental field test conditions, the intensity of activity of both enzymes varied with fertilizer treatments and tillage.

The acid phosphatase activity ranged from 1.16  $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$  in T3 from CT soil to 1.86  $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$  in RT control (T0) (Figure 2).

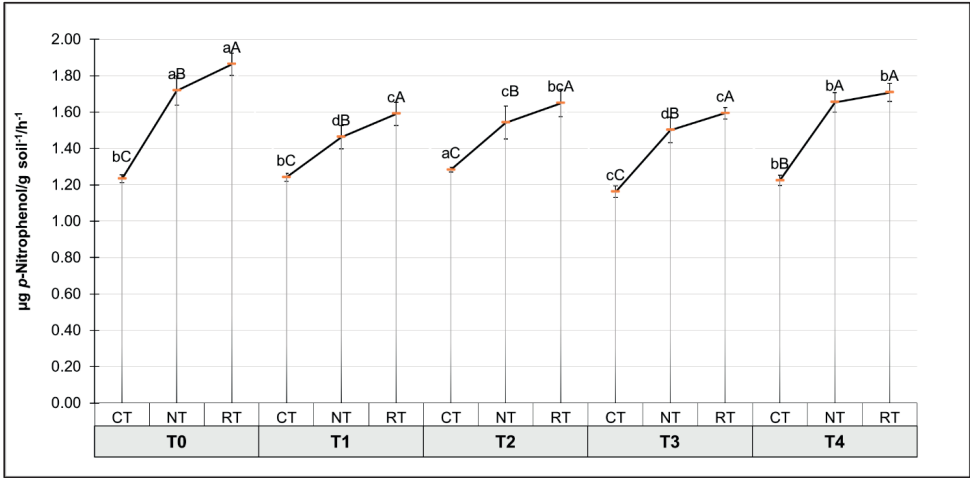


Figure 2. Acid phosphatase activity

Note: Distinct lower case letters express that the differences are significant between fertilization treatments within the same tillage system; distinct upper case letters express that differences are significant for the same fertilization treatment within different tillage systems

Of the tillage practices, in CT within fertilizer treatments, inoculation of 75% PSB + N (T2) was significantly higher (1.28  $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$ ) than all other treatments. At the opposite extreme, T3 was found to produce the lowest acid phosphatase activity. Between T0, T1 and T4, there were no significant differences and the

means were 1.23, 1.24 and 1.22  $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$ , respectively.

Results were similar in NT and RT soils, with T0 significantly above bacterial fertilizations (1.72  $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$  in NT and 1.86  $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$  in RT).

Between tillage systems, acid phosphatase activity was significantly greater in RT, followed by NT and then CT, a tendency that lasted regardless of fertilization treatment. Alkaline phosphatase originates mainly from bacterial synthesis. Inoculation at the recommended rate of *Bacillus megaterium* var. *phosphaticum* without N supply in the CT

system promotes alkaline phosphatase synthesis ( $0.28 \mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$ ) significantly higher than control and the other bacterial treatments (Figure 3). No significant changes in alkaline phosphatase activity were observed between T0, T3 and T4, whereas the mean activity of T2 was significantly reduced.

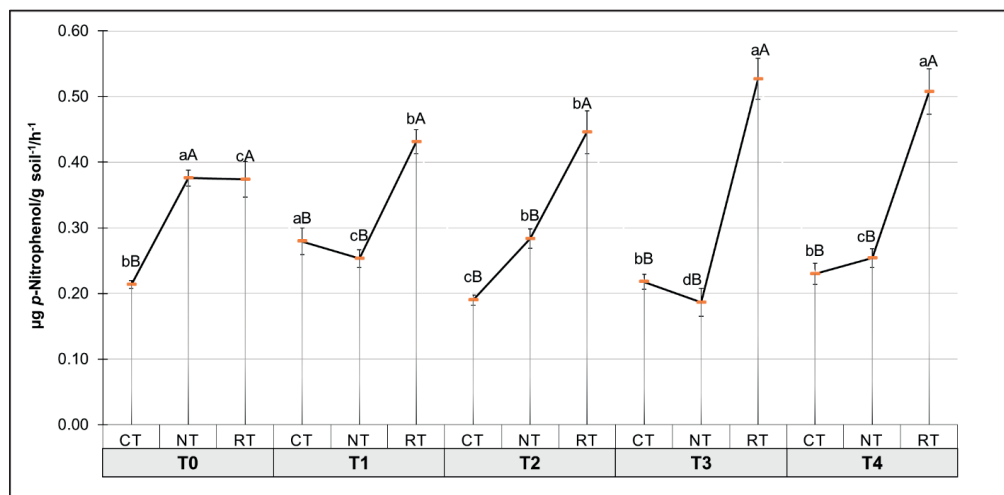


Figure 3. Alkaline phosphatase activity

Note: Distinct lower case letters express that the differences are significant between fertilization treatments within the same tillage system; distinct upper case letters express that differences are significant for the same fertilization treatment within different tillage systems

An opposite trend was found in the NT system, where the mean value of the control ( $0.38 \mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$ ) was significantly higher than that of bacterial treatments. Of the bacterial inoculations, T2 ranked first, close but with a significant decrease, followed by T1 and T4. The lowest activity was measured in T3 soil samples ( $0.19 \mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$ ).

Alkaline phosphatase activity increased with microbial inoculation under RT with all treatments significantly above control.

The general trend of alkaline phosphatase activity measurements in all three soil management practices was the same as for acid phosphatase (RT > NT > CT). However, differences between CT and NT were significant only in the control, and for treatments T1 and T3, mean values in the CT system slightly exceeded those in NT.

### Total and Available Phosphorus

Phosphorus (P) is a nutrient with multiple constitutive and functional implications in

plants. The amount and availability of soil P depends on soil type, SOM, biological activity, pH, climatic conditions and land management in terms of cultivated crops, soil tillage and fertilization.

In our study, analysis of soil TP content showed mean values between 0.0191 and 0.0542% and significant differences ( $p < 0.05$ ) across both fertilization treatments and tillage practices (Figure 4).

In CT system, soil TP levels remained on the same tendency at both times of the determinations. The control outperformed the *Bacillus* sp. treatments, with means of 0.0410 - 0.0450% and T2 (75% PSB + N) reported significant lowest values (0.0343 - 0.0307%). Of the bacterial treatments, T1 and T4 were similar in total P content, significantly higher than T2 and T3. There were no significant differences between control, T1 and T4 in the samples collected at crop harvest.

Instead, in the NT system, between the two bacterial inoculations, a significantly higher

level of TP was observed in T3 (0.0433%), and between the other treatments the variation in means was slight and insignificant. At harvest,

in NT the total P level fluctuated quite widely, and the ascending order of averages was T1 > T0 > T4 > T3 > T2.

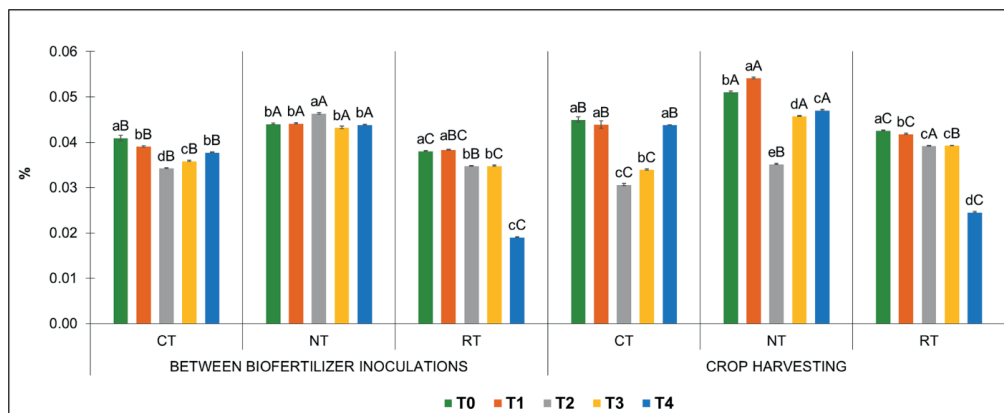


Figure 4. Soil total phosphorus content

Note: Distinct lower case letters express that the differences are significant between fertilization treatments within the same tillage system; distinct upper case letters express that differences are significant for the same fertilization treatment within different tillage systems

In the RT system, the same behavior of the fertilizer treatments was maintained at both soil sampling times. The highest means were recorded in T0 and T1 (100% PSB), followed by T2 and T3 and finally T4, with significantly lower values. As a general pattern among bacterial treatments, 100 % inoculation without the addition of N fertilizer resulted in higher total soil phosphorus content.

Comparing the tillage systems with each other, for the same fertilization treatment, between the two inoculations with *Bacillus megaterium* var. *phosphaticum*, NT ranked first in terms of TP level, with significantly higher averages, followed by CT and RT. Also, at crop harvest the mean values were highest in NT, except for T3.

Even if soil TP levels are constant and substantial, the accessibility of this element to crops requires careful assessment to ensure optimal plant nutrition. Figure 5 shows soil AP levels at the two sampling times, highlighting the differences between fertilization treatments as well as between tillage systems.

Comparatively, the mean values in CT, NT and RT, reveal a decrease in AP from the time of the first soil sampling until crop harvest. There were also significant differences between fertilizer treatments in all three systems.

In the first analyses, in CT and RT systems the highest averages were found in T4 (38.48 and 38.32 ppm) and under NT in T3 (50.23 ppm). In both CT and RT, the control (T0) ranked second with an AP level of 31.49 and 44.34 ppm, and the lowest values were found in T2 (22.80-34.08 ppm). In RT system all PSB variants outperformed the control. At wheat harvest, the evaluation of the soil accessible phosphorus content in the CT system resulted the maximum value in the control (27.20 ppm), significantly exceeding the other treatments. In contrast, in NT, biological inoculations had the highest values (T3 > T4 > T1 > T2). Finally, in the RT system, the highest mean was obtained in T4 (37.31 ppm), followed by the control (31.34 ppm), T3 (25.92 ppm), T2 (21.755 ppm) and T1 (20.10 ppm).

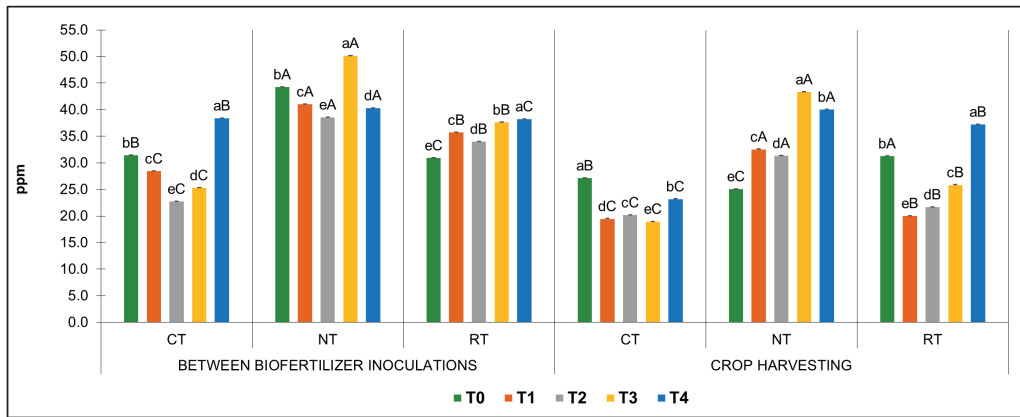


Figure 5. Soil available phosphorus content

Note: Distinct lower case letters express that the differences are significant between fertilization treatments within the same tillage system; distinct upper case letters express that differences are significant for the same fertilization treatment within different tillage systems

The lower accessibility of P could be observed in the conventional system compared to NT and RT.

Spearman correlation revealed the significance of the relationships between the investigated properties. (Table 4).

Table 4. The correlation between the indicators considered in the study for each tillage system

| Tillage system | Parameter            | Acid phosphatase | Alkaline phosphatase | TP     | AP |
|----------------|----------------------|------------------|----------------------|--------|----|
| CT             | Acid phosphatase     | 1                |                      |        |    |
|                | Alkaline phosphatase | 0.156            | 1                    |        |    |
|                | TP                   | -0.042           | 0.227                | 1      |    |
|                | AP                   | 0.570*           | 0.402                | 0.511  | 1  |
| NT             | Acid phosphatase     | 1                |                      |        |    |
|                | Alkaline phosphatase | 0.560**          | 1                    |        |    |
|                | TP                   | 0.046            | -0.157               | 1      |    |
|                | AP                   | -0.868**         | -0.218               | -0.189 | 1  |
| RT             | Acid phosphatase     | 1                |                      |        |    |
|                | Alkaline phosphatase | 0.469**          | 1                    |        |    |
|                | TP                   | 0.234            | 0.316                | 1      |    |
|                | AP                   | 0.396            | 0.634*               | -0.322 | 1  |

Note: \* means that correlation is significant at the 0.05 level (2-tailed); \*\* means that correlation is significant at the 0.01 level (2-tailed).

The analysis indicated a significant and moderate positive correlation in NT and RT systems between acid and alkaline phosphatase activities ( $r_s = 0.560$ ,  $p = 0.026$  in NT and  $r_s = 0.469$ ,  $p = 0.00002$  in RT). AP was found to have a positive and moderate correlation with acid phosphatase under CT system ( $r_s = 0.570$ ,  $p = 0.026$ ), but negative and very strong in NT ( $r_s = -0.868$ ,  $p = 0.00003$ ). In contrast, in RT, AP was positively and strongly correlated ( $r_s = 0.634$ ,  $p = 0.011$ ) with alkaline phosphatase.

Acid phosphatase is of microbial and roots origin (Eichler et al., 2004) and its activity is modulated by air and water regime, soil management, SOM content, soil chemistry, plant species and their physiological state

(Ciereszko et al., 2011; Adetunji et al., 2017). The higher values of acid phosphatase compared to alkaline phosphatase can be attributed to the low acid pH of the soil.

The external addition of *Bacillus megaterium* var. *phosphaticum*, together with mineral fertilization, showed an increase in acid phosphatase activity, both as a function of inoculated dose and the type of soil management. The increase in acid phosphatase activity induced by inoculation of wheat with P solubilizing bacteria consortium (*Bacillus siamensis*, *Rahnella aceris*, *Bacillus cereus*) was also reported by Ibyasser et al. (2024).

In soil inoculated with PSB, we found that both lower (75 %) and higher (125 %) doses, together with N addition, increased the synthesis of this

enzyme. Microbial enrichment promoted enzymatic activity to catalyze reactions to convert P from inaccessible forms into plant-available P from organic compounds or mineral fertilizers. Mineral fertilization, in particular with N, leads to enhanced acid phosphatase synthesis due to its acid pH-related activity (Lemanowicz, 2011; Kalembasa & Symanowicz, 2012). However, in our study, in the NT and RT systems, in treatments with the same *Bacillus* rate (100%) but different N supply (T1 and T3), this nutrient had no obvious effect on acid phosphatase activity. In CT, even a significant inhibition of this enzyme was observed with the addition of N. This is in opposition to the results obtained by Widdig et al. (2019), who found that N fertilization leads to increased phosphatase activity. We can assume that the differences between biofertilizer treatments mean (T1, T2, T3 and T4) come from a combined effect of PSB inoculum dose and nitrogen. When inoculating soil with acid-lactic acid bacteria, *Pseudomonas* spp., *Penicillium*, *Actinomyces* spp., Długosz and Piotrowska-Długosz (2023) identified an inhibition of phosphomonoesterase activity, as we reported in NT and RT systems, but an increase in available P content. Thus, acid phosphatase activity decreased from 2.20 in the control to 2.16 mM *p*NP kg<sup>-1</sup> h<sup>-1</sup> in the inoculated treatment, alkaline phosphatase from 2.17 to 1.68 mM *p*NP kg<sup>-1</sup> h<sup>-1</sup>, and AP increased in the biological inoculum treatment to 79.4 from 66.6 mg kg<sup>-1</sup> in the control (Długosz and Piotrowska-Długosz, 2023). Also, Sednev (2017) found no influence of *Bacillus megaterium* var. *phosphaticum* inoculation with N on phosphatase activity or P content and a low and insignificant positive correlation between soil P level and phosphatase activity. Biological inoculum consisting of several *Bacillus* species and active organic matter in combination with NPK<sub>20:10:10</sub> application was shown to stimulate alkaline phosphatase. In addition, it was suggested that mineral fertilizers can be replaced by biofertilizers to increase enzyme activity and thus more efficiently utilize soil P pools by the wheat crop (Ali et al., 2024).

A characteristic feature of the investigated enzymes is the higher activity in the RT system compared to CT and NT, and these differences are related to the variation in soil properties. Soil

disturbance in RT after a long period of no-tillage resulted in a balanced moisture and aeration regime and, as a result, stimulated microbial activity, with positive effects on enzymatic activities across treatments. Also, in the NT system, acid phosphatase increased significantly compared to CT, but alkaline phosphatase was in all cases insignificant, although higher in T2 and T3, confirming our hypothesis. Soil phosphatase activity was found to be sensitive to soil management (Gałązka et al., 2017). Soil microbial community has a key role in available P supply (Rodríguez and Fraga, 1999), which means that reduced disturbance induces increased enzymatic activity. Due to the deposition of plant residues in the surface layers corresponding to the soil sampling depth, acid phosphatase and alkaline phosphatase are abundant in soils cultivated in NT and RT (Mohammadi, 2011), a large part of soil P being organic (Nannipieri et al., 2011). Minimal soil disturbance associated with mulching creates a suitable environment for the interaction between soil physical-chemical parameters and the functioning and proliferation of microorganisms, ultimately facilitating biochemical P conversion mediated by soil enzyme systems (Calistru et al., 2024). The higher enzymatic activity in NT and RT systems can also be explained by the optimal temperature and humidity provided by the mulch layer, which prevents sudden temperature changes and rapid evaporation of water, with a positive effect on microbial activity (Mathew et al., 2012). The two enzymes studied were found to have a positive feedback interaction, as indicated by Spearman's correlation, consistent with the research of Samuel et al. (2021).

Several bacterial strains selected from the rhizosphere have revealed the potential to enhance P solubilization and efficient uptake, contributing to an environmentally friendly approach to plant nutrition (Selvi et al., 2017; Emami et al., 2020). Many studies report the viability of inoculating PSB in converting P from soil-fixed P pools or from crop fertilization, with growth and yield promoting effects. The higher level of accessible P in the treatments with 125% + N in CT systems and 100% PSB + N in NT systems suggests that the metabolic mechanisms of the microorganisms were effective in solubilizing a fraction of the

soil P reservoir and aligns with our hypothesis. Chen et al. (2021) found that PSB (*Enterobacter hormaechei*, *Pseudomonas grimonii*, *Pantoea roadsii*) can contribute to the increase in total and accessible P content as well as acid phosphatase activity intensity. However, in our study, we generally recorded a decrease in soil TP in bacterial inoculum treatments. Inoculation with PSB has been reported to significantly increase soil P availability and phosphatase activity in wheat crop and a positive correlation between them (Yahya et al., 2021). In our study, there was a positive effect of PA on acid phosphatase in the CT system and on alkaline phosphatase in the RT system, but an inhibitory effect on acid phosphatase in the NT. Increased secretion of phosphatases by soil microbiota and plant roots is related to a lack of available soil P (Kai et al., 2002; Dick et al., 2011).

When comparing tillage systems in terms of soil P content, the NT was found to have the highest level, followed by CT and then RT. This finding is in contradiction with that of Futa et al. (2021), who reported higher available P content in wheat crop at deep mineral fertilizers application. One explanation for this phenomenon observed in our study may be that, in minimum tillage systems, nutrients accumulate in the surface soil layers (Mühlbachová et al., 2024). Accessible P decreased from the first measurements stage until the end of the growing season, which means that a part of it was taken up by the root system and metabolized in plants, and some was converted into inaccessible forms.

## CONCLUSIONS

The success of soil colonization by inoculated bacteria depends on the interaction of several factors. Thus, native microorganisms, soil properties, crop species and climatic conditions all have the potential to interfere with this process, preventing the bacteria from reaching their growth and development potential (Chen et al., 2021). Inoculation of *Bacillus megaterium* var. *phosphaticum* promoted phosphomonoesterase activity in NT and RT soil management systems. Application of recommended or higher rates (100% or 125%), along with N, also increased available soil P in all tillage systems. Minimal soil disturbance provides a favorable environment for microbial

activity, stimulation of enzyme activity and P accumulation, with positive effects on plant nutrition. The mechanisms of action of BMPs are diverse and the direct or indirect influences they may have on soil P require further investigation and other research directions.

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