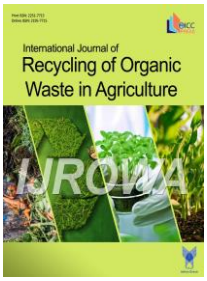

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Research Article

# Impact of Bacterial Inoculation on Enzyme Activity in Vermicompost Derived from Various Vermi-beds

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**Abstract**

**Purpose:** Vermicomposting can be enhanced by inoculating vermibeds with beneficial microbes, thereby improving its biofertilizer properties. This study examined how phosphate-solubilizing bacteria (*Pseudomonas putida* Tabriz) and nitrogen-fixing bacteria (*Azotobacter* sp.) influence enzyme activity in vermicompost produced from different organic substrates (litter, wheat straw, wood dust, and compost).

**Method:** A mixture of cow manure and organic materials (1:8 w/w) was vermicomposted in 3-kg pots with *Eisenia fetida* earthworms. After four months, bacterial inoculants were introduced, and samples were analyzed at 0, 4, 7, and 10 months for acid phosphatase, urease, and cellulase activities.

**Results:** Bacterial inoculation significantly increased enzyme activity, with peaks observed at 4–7 months. Overall, bacterial inoculation significantly increased enzymatic activity, with the highest values observed at 4 and 7 months after inoculation. Specifically, *P. putida* Tabriz enhanced cellulase and urease activities, while *Azotobacter* sp. improved phosphatase activity in the vermibeds. The highest enzyme activities were recorded in: compost (acid phosphatase, 1864 µg pNP/g.h), leaf litter (urease, 460 µg $NH_4^+$-N/2h), and wood dust (cellulase, 1568 µg GE/g.24h). Enzyme activity gradually increased during the experiment but declined toward the end of the study period.

**Conclusion:** Vermicompost effectively serves as a carrier for beneficial microbes, enhancing enzyme activity and soil health. This approach offers a sustainable way to improve agricultural productivity through biofertilization.

**Keywords:** Acid phosphatase, Bio-inoculation, Biofertilizer, Cellulase, PGPR, Urease, Vermicomposting

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## 1. Introduction

Solid waste management is a growing challenge in both developed and developing countries (Kumar et al., 2025). Vermicomposting, a specialized form of composting that involves the use of earthworms, is an effective method for converting organic waste into nutrient-rich fertilizer under controlled conditions (Devi et al., 2024; Bhartiya et al., 2024; Iraji et al., 2025). Earthworms such as *Eisenia fetida* play a vital role in this process by enhancing organic matter decomposition, soil aeration, and nutrient cycling through their feeding, burrowing, and casting activities (Singh et al., 2023).

Unlike conventional composting, vermicomposting is a non-thermophilic process, relying on the synergistic actions of earthworms and microorganisms to break down organic waste (Zhou et al., 2022). The efficiency of vermicomposting is influenced

by several factors, including the type of organic substrate, earthworm species, and environmental conditions (Devi et al., 2024). Commonly used substrates include agricultural residues, animal manure, and industrial waste, which provide the necessary carbon and nitrogen for microbial and earthworm activity. For instance, a study by Das & Deka (2021) investigated the vermicomposting of potato crop waste using *E. fetida*. The study compared two treatments: one with only potato plant biomass and another with a mixture of potato biomass and cattle manure (5:1 ratio). The results demonstrated that the addition of cattle manure significantly improved the quality and quantity of the vermicompost, as well as enhanced the earthworm population and activity.

Vermicompost, the end product of vermicomposting, has been shown to enhance the growth of a wide range of crops, including cereals, vegetables, and ornamental plants, by improving soil fertility and nutrient availability (Parewa et al., 2024). Microorganisms are an essential component of biodiversity and play a pivotal role in maintaining ecosystem structure and function. They act as primary decomposers of organic waste, facilitating nutrient cycling and soil health (Kumar et al., 2025). The cast of earthworms contain significantly higher populations of microorganisms compared to the surrounding soil, making them effective vectors for microbial dispersal (Kumar et al., 2025). This unique characteristic has led to the exploration of vermicompost as a carrier for biofertilizers, which can enhance soil fertility and plant growth (Singh et al., 2020). For instance, studies have demonstrated that vermicompost enriched with phosphate-solubilizing bacteria (PSB) can serve as an effective phosphorus biofertilizer, improving plant growth and nutrient uptake (Sharma et al., 2024; Rajasekar et al., 2012). In a study by Shariati & Alikhani (2015), the survival of *Pseudomonas fluorescens* as a PSB in vermicompost and its impact on maize growth indicators were evaluated. The results showed that vermicompost enriched with PSB significantly increased plant growth parameters and phosphorus content in maize shoots, highlighting its potential as a sustainable biofertilizer.

The integration of vermicompost with microbial fertilizers has been found to significantly improve crop yields. In some cases, beneficial bacteria are inoculated into vermicompost to enhance its fertility and nutrient content (Singh et al., 2023). Recent studies have demonstrated that the inoculation of efficient microorganisms during vermicomposting can reduce decomposition time, increase nutrient levels (such as total nitrogen and phosphorus), and enhance the content of humic substances, leading to greater stabilization of the organic fraction (Lirikum et al., 2022; Rajasekar et al., 2012). For example, a study by Mahanta et al. (2012) investigated the use of microbial inoculants, including *Azotobacter*, *Azospirillum*, *Pseudomonas*, and their consortium, to enrich vermicompost produced from various plant residues. The results indicated that locally available plant biomass, such as *Ipomoea carnea* a weed abundant in northeast India can be effectively converted into a nutrient-rich, environmentally friendly fertilizer through vermicomposting. The inclusion of plant growth-promoting bacteria like *Azotobacter chroococcum* and *Azospirillum brasilense* further enhanced the quality of the vermicompost (Mahanta et al., 2012). In another study, Pramanik et al. (2009) examined the effects of inoculating three microbial strains into two different organic waste materials. The study focused on acid phosphatase activity and microbial properties in the final stabilized product. The findings revealed that vermicomposting significantly increased acid phosphatase activity in the organic substrates, and microbial inoculation further accelerated enzyme activity, improving the overall quality of the compost. Similarly, a study by Chang et al. (2024) evaluated enzyme activity, available nitrogen, and bacterial succession during the composting of pig manure with and without microbial inoculation. The results demonstrated that microbial inoculation not only accelerated the composting process but also regulated microbial functions, leading to a more efficient decomposition process.

Boruah & Deka (2023a) investigated the efficacy of biological indicators for understanding the stabilization/maturity of the vermicomposting products of citronella bagasse and paper mill sludge. They found significantly higher dehydrogenase and urease activity in citronella bagasse vermicompost compared to raw materials, with >80% germination index indicating phytotoxin-free products. In a subsequent study, they observed low enzyme activity in the first 15 days of cereal sludge vermicomposting, followed by a sharp increase (days 15-60) and gradual decline until harvest, monitoring seven enzyme types (including cellulase, amylase, invertase, phosphatase, protease, dehydrogenase, and urease) across three earthworm species (Boruah & Deka, 2023b).

However, the timing of bacterial inoculation whether at the beginning or end of the vermicomposting process and its impact on enzyme activity and nutrient dynamics require further investigation. To address this gap, the present study aimed to evaluate the effects of different vermibeds and bacterial inoculation on enzyme activity in vermicompost. The vermibeds were prepared using a base of cow manure mixed with various organic materials, including: (1) leaf litter from the University of Tabriz campus, (2) municipal garbage compost from Tabriz city,

(3) wheat straw, and (4) wood dust. Bacterial inoculation was performed in the fourth month of the vermicomposting process, using a consortium of beneficial bacteria, including the phosphate-solubilizing bacterium *Pseudomonas putida* Tabriz and the nitrogen-fixing bacterium *Azotobacter* sp. The study focused on key enzyme activities, such as acid phosphatase, urease, and cellulase, to assess the impact of bacterial enrichment on the biochemical properties of the vermicompost.

## 2. Material and method

### 2.1. Preparation of the vermi-beds

In this study, four different organic substrates were evaluated as vermibeds. Further details regarding the materials used in this study are outlined in Table 1. The primary substrate, cow manure, was mixed with other organic materials in separate treatments, including (1) leaf litter collected from the University of Tabriz campus, (2) municipal garbage compost from Tabriz city, (3) wheat straw, and (4) wood dust.

Vermicompost was produced using the earthworm species *Eisenia fetida*. To prepare the vermibeds, the cow manure was first sieved through a 4 mm mesh to ensure a homogeneous texture. The sieved manure was then mixed with each of the four organic substrates at a ratio of 1:8 (substrate to manure). Water was sprayed onto the mixtures to achieve an approximate moisture content of 50%.

The prepared vermibeds were transferred into 3-kg pots. Before introducing the earthworms, the pots were rinsed to reduce initial salinity and allowed to stabilize for two weeks to adjust the electrical conductivity. After this stabilization period, 10 adult *E. fetida* worms were added to each pot to initiate the vermicomposting process.

**Table 1.** Comparative analysis of organic materials used for vermicomposting in this study

| | OC (%) | N (%) | C:N ratio | P (%) | K (%) | $pH_{(1:2)}$ | $EC_{(1:2)}$ (dS/m) |
|---|---|---|---|---|---|---|---|
| Cow manure | 21.7 | 1.21 | 18 | 0.96 | 2.25 | 8.2 | 6.8 |
| Compost | 13.9 | 0.73 | 19 | 0.65 | 0.70 | 7.3 | 10 |
| Leaf litter | 38.8 | 0.55 | 70.6 | 0.20 | 0.50 | 6.9 | 3.9 |
| Wood dust | 54.0 | 0.30 | 180 | 0.10 | 0.20 | 7.1 | 0.5 |
| Wheat straw | 45.9 | 0.60 | 76.6 | 0.15 | 0.50 | 6.5 | 1.2 |

**Table 2.** Auxin production and phosphate solubilization by bacterial strains used in the study

| Bacterial isolates | Auxin production (mg/L) | | Phosphate-solubilizing (mg/L) | |
|---|---|---|---|---|
| | +T | -T | TCP | RP |
| *Azotobacter* sp. | 2.1 | 1.4 | 59.5 | 5.7 |
| *P. putida* Tabriz | 3.1 | 3.5 | 345.9 | 25.3 |

Note: +T: With 100 mg/L tryptophan, -T: Without tryptophan, TCP: Tricalcium phosphate, RP: Rock phosphate.

### 2.2. Bacterial inoculation

In this study, two types of beneficial microorganisms were utilized: phosphate-solubilizing bacteria (*Pseudomonas putida* Tabriz) and nitrogen-fixing bacteria (*Azotobacter* sp.). These bacterial strains were obtained from the Soil Biology Laboratory of the Soil Science Department at the University of Tabriz, and their key characteristics are summarized in Table 2. A fresh culture of each bacterial strain was prepared by streaking onto nutrient agar (NA) plates. A single colony from each strain was then transferred into nutrient broth (NB) medium and incubated overnight to achieve optimal growth. Bacterial inoculation was performed after the completion of the vermicomposting process, specifically after harvesting the earthworms at the end of the four-month period. A 50 ml inoculum of the bacterial suspension ($10^9$ CFU/ml) was uniformly added to each vermicompost bed, ensuring that no earthworms were present at the time of inoculation. To maintain consistent moisture levels, the volume of the inoculum was accounted for by adjusting the total moisture content in the vermibeds. In the control treatment, where no bacterial inoculation was applied, 50 ml of sterile nutrient broth (NB) was added to the pots instead of the bacterial inoculum.

### 2.3. Measurement of enzyme activities

Enzyme activity was measured for acid phosphatase, urease, and cellulase at different time intervals: at the beginning of the experiment (0 months), at the end of the vermicomposting process and bacterial inoculation (4 months), and after 3 and 6 months of bacterial inoculation (7 and 10 months, respectively). In this study, enzyme assay substrates—pNPP (p-Nitrophenyl phosphate), urea, and CMC (carboxymethyl cellulose) were purchased from

Sigma-Aldrich (Merck). The specific product details are as follows: Pnpp (CAS No. 4264-83-9), Urea (CAS No. 57-13-6), CMC (Catalog No. C4888). At each sampling time, vermicompost samples were collected and passed through a 2 mm sieve to ensure uniformity. Acid phosphatase activity was determined using the method described by Tabatabai (1994).

This method quantifies the enzyme activity based on the release of p-nitrophenol (pNP) from the hydrolysis of sodium p-nitrophenyl phosphate (pNPP), which is measured spectrophotometrically at 410 nm. Further details on this procedure can be found in Moradi et al. (2024).

One gram of vermibed was weighed into test tubes, 4 ml of modified universal buffer (MUB) reagent (acidic or basic) and 1 ml of pNPP substrate (in acidic or basic buffer) were added to it. After mixing the sample, to provide the optimum temperature for enzyme activity, the tube was placed in a water bath at a temperature of 37◦C and maintained under these conditions for 1 h.

Then, the reaction was stopped by adding stopping solutions (1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH) and before reading the absorbance, the samples were filtered using filter paper. This substrate is hydrolyzed to phosphorus and pNP due to phosphatase activity. To measure the amount of pNP released, its absorbance was read at a wavelength of 410 nm with a spectrophotometer.

Urease activity (UA) was assessed by measuring the amount of ammonium released from urea through the hydrolyzing activity of urease, following the method outlined by Ebrahimi et al. (2021).

One gram of vermi-bed was weighed into test tubes, 2 ml of urea solution (10%) and 4 ml of phosphate buffer (pH 7) were added to it.

After mixing the sample, to provide the optimum temperature for enzyme activity, the tube was placed in a water bath at a temperature of 37 °C and kept in this condition for 2 h.

After incubation, 20 mL of KCl 2M was added to the mixture and shaken well.

Then before reading the absorbance, the samples were filtered using filter paper.

In this research, indophenol blue method was used to quantify the ammonia produced.

Ammonia in the supernatant reacts with phenol and hypochlorite in the presence of a catalyst (e.g., sodium nitroprusside) and its absorbance was read at a wavelength of 630 nm with a spectrophotometer.

Cellulas activity was measured on colorimetric determination using Spectrophotometer at 690 nm according to the method of Aliasgharzad (2010). At first 10 g of vermi-bed was added to the test tubes and homogenized in a 1:10 (w/v) ratio with acetate buffer (pH 5.0). The mixture at 6000 rpm for 10–15 minutes was centrifuged to separate the supernatant (enzyme extract). Then 0.5 ml of enzyme extract was added to 0.5 ml of CMC solution (1%) in a test tube. It was mixed well and incubated at 50 °C for 30 minutes.

After incubation, 1 ml of DNS reagent (3,5-Dinitrosalicylic acid) was added to the reaction mixture. Cellulase activity was expressed by this unit (µg GE/g.24h) which represents the amount of glucose equivalents released by cellulase activity in 1 gram of vermibed material over 24 hours.

### 2.4. Data analysis

The experiment followed a completely randomized factorial design with three replications. Data were analyzed using analysis of variance (ANOVA) in SPSS to assess the effects of substrate type (four levels; including litter, straw, wood and compost), bacterial inoculation (three levels; containing *Pseudomonas*, *Azotobacter* and control), and time (0, 4, 7, and 10 months). Mean comparisons were performed using Duncan's test ($p < 0.01$).

## 3. Result and discussion

The findings of this study highlight the significant potential of integrating bacterial inoculation into vermicomposting to enhance the biological and enzymatic properties of vermicompost, thereby enhancing its efficacy as a biofertilizer. The results demonstrate organic substrate type, bacterial inoculants, and incubation time collectively influence enzyme activities, which are critical indicators of vermicompost quality and its potential to enhance soil health and plant growth.

### 3.1. Substrate influence on enzyme activities

Variance analysis revealed that the main effects and interactions of all three factors substrate type, bacterial inoculation, and incubation time significantly influenced the activity of phosphatase, urease, and cellulase in vermicompost at $p < 0.01$. Vermicompost produced from all three substrates garbage compost, leaf litter, and wood dust exhibited comparatively higher acid phosphatase activity than that from the straw bed, which recorded the lowest average value (Figure 1a). This finding can likely be attributed to the richer organic matter content and higher nutrient availability of the three materials, which promote microbial proliferation and enzyme synthesis (Ebrahimi et al., 2021).

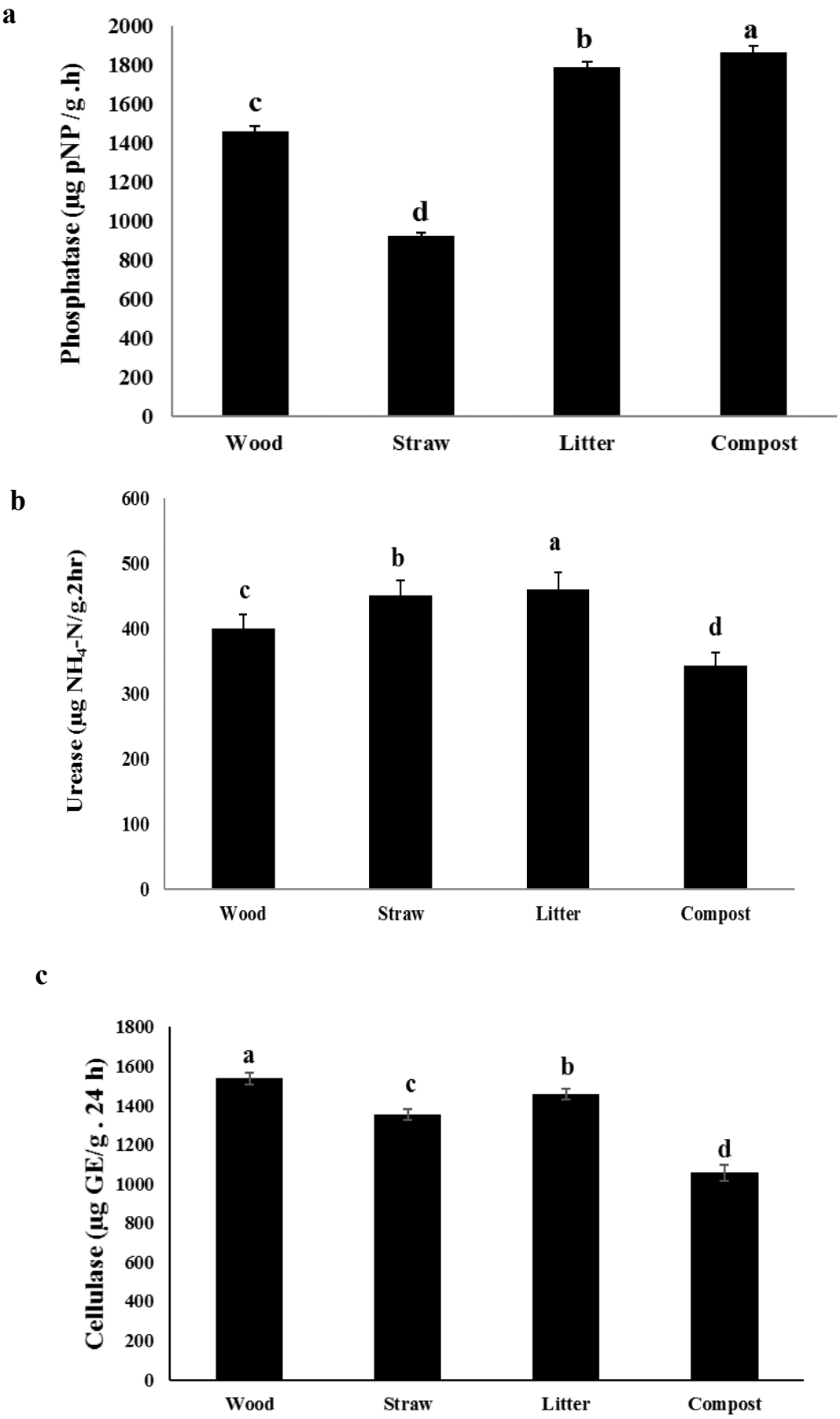


**Figure 1.** Effect of different vermi-beds or substrate on a) acid phosphatase activity, b) urease activity and c) cellulase activity

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The variation in enzyme activities across different vermibeds underscores the importance of substrate selection in vermicomposting. The highest acid phosphatase activity (1864 µg pNP/g·h) in the compost-based vermibed can be attributed to the high organic matter content and nutrient availability in compost, which likely provided an optimal environment for microbial activity and enzyme production. Phosphatase activity serves as an indicator of phosphorus (P) mineralization by microorganisms. Acid phosphatase plays a crucial role in phosphorus mineralization, making it a key enzyme for enhancing phosphorus availability in soils (Ebrahimi et al., 2021).

This enzyme is particularly relevant for evaluating the composting process, as microorganisms play a major role in its synthesis. Acid phosphatase activity in vermicompost is directly correlated with microbial population size; higher microbial activity leads to higher enzyme activity. Our study has shown that both microbial and phosphatase activities peak around the 7$^{th}$ month of composting. Treatments inoculated with bacteria exhibited significantly higher phosphatase activity compared to non-inoculated treatments, highlighting the indirect role of earthworms in enhancing phosphatase activity by stimulating microbial flora (Kumar et al., 2021). The presence of organic compounds has been shown to enhance phosphatase activity, promoting the conversion of organic phosphorus during composting (Marinari et al., 2000). However, during the final stages of composting, phosphatase activity declines due to a reduction in bacterial community viability (Pramanik et al., 2009). Additionally, changes in microbial abundance resulting from inoculation can influence enzyme activity, thereby affecting phosphorus transformation pathways (Kumar et al., 2021).

The results indicated that the leaf litter substrate had a greater impact on urease activity, with the highest average activity observed in this treatment, showing a significant difference compared to other substrates (Figure 1b). The leaf litter vermibed exhibited the highest urease activity (460 µg $NH_4^+$-N/2h), likely due to the high nitrogen content and rapid decomposition rate of leaf litter, which facilitated nitrogen mineralization. Urease is essential for converting urea into ammonia, a form of nitrogen readily available for plant uptake (Moradi et al., 2024; Ebrahimi et al., 2021). The elevated urease activity in leaf litter suggests a more active microbial community, likely driven by the labile carbon and optimal C:N ratio of this substrate, fostering efficient enzyme synthesis (Boruah & Deka, 2023a). Furthermore, the faster breakdown of leaf litter may have increased the availability of organic nitrogen compounds, further stimulating urease-producing microorganisms.

The results indicated that the highest cellulase activity was observed in the wood dust substrate, followed by a decrease in activity in the leaf litter, straw, and garbage compost substrates, respectively. Significant differences were noted between all substrates (Figure 1c). The wood dust vermibed, on the other hand, showed the highest cellulase activity (1568 µg GE/g·24h), which can be linked to the lignocellulosic nature of wood dust, providing a rich substrate for cellulolytic microorganisms. The highest organic carbon content and C:N ratio were observed in this vermibed compared to the other three beds used in the study (Table 1).

Cellulase is critical for breaking down cellulose into simpler sugars, which are essential for microbial metabolism and organic matter decomposition (Ansari et al., 2024).

Cellulase activity is closely linked to the availability of carbon-rich materials. We found higher cellulase activity in substrates with greater carbon content, such as wood dust, while garbage compost beds exhibited lower activity due to their lower organic carbon content.

As composting progresses to maturity, cellulase activity declines, likely due to a reduction in microbial population and the depletion of available organic substrates (Lakshmi et al., 2014). Similar findings were reported by Shanthi (2018). Cellulase activity, dependent on microorganisms, increased with microbial inoculation and decreased as organic matter and microbial activity declined. It is synthesized in the presence of rapidly decomposing carbon materials; as microbial populations grow, decomposition and cellulase activity rise. Over time, reduced carbon availability lowers microbial activity, decreasing cellulase activity (Kumar et al., 2018). Kumar et al. (2018) also noted that cellulase activity was lower in leaf litter and crop residue composts compared to wheat and rice straw composts, likely due to faster decomposition depleting suitable carbon sources and the presence of easily available nutrients in the former.

### 3.2. Role of bacterial inoculation

Inoculation with *Pseudomonas putida* Tabriz significantly increased urease activity in the litter-based vermibed and cellulase activity in the wood dust-based vermibed. Conversely, inoculation with *Azotobacter* sp. enhanced phosphatase activity in the compost and litter-based vermibeds.

Vermicompost inoculated with *Pseudomonas putida* showed no significant difference in phosphatase activity compared to the non-inoculated treatment. In contrast, the *Azotobacter* strain was more effective in enhancing acid phosphatase activity than *Pseudomonas putida* (Figure 2a).

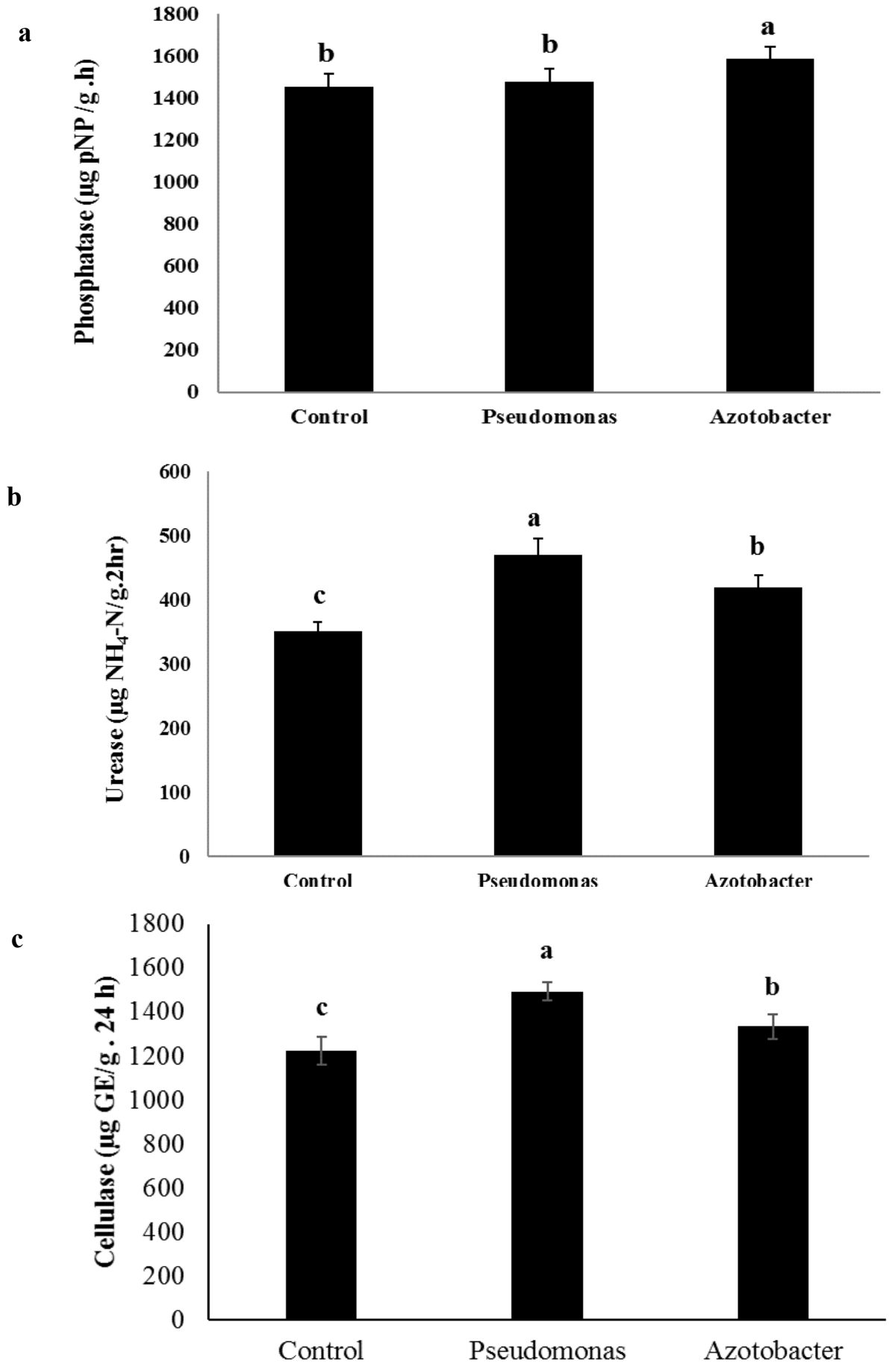


**Figure 2.** The effect of bacterial inoculation on a) acid phosphatase activity, b) urease activity and c) cellulase activity

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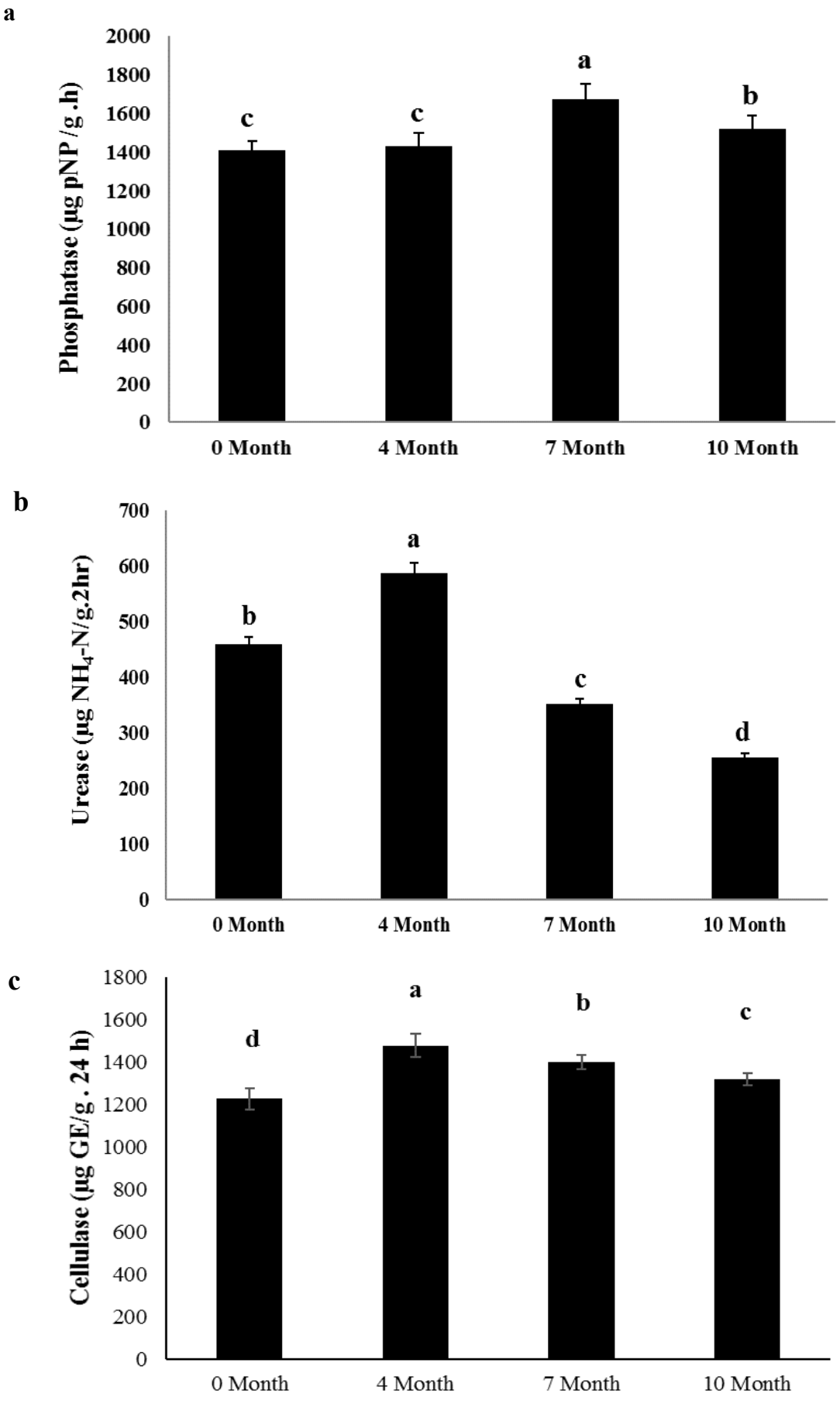


**Figure 3.** The effect of incubation time on a) acid phosphatase activity, b) urease activity and c) cellulase activity

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Inoculation withn*Pseudomonas* in wood dust and leaf litter beds increased phosphatase activity compared to the non-inoculated treatment; however, this increase was significant only in the wood dust treatment. The lowest enzyme activity was observed in straw and garbage compost inoculated with *Pseudomonas*. Furthermore, *Azotobacter* inoculation significantly increased phosphatase activity in all substrates except for the compost substrate, which showed no significant difference compared to the non-inoculated treatment (Figure 4a). The inoculation of beneficial microorganisms, particularly *Pseudomonas putida* Tabriz and *Azotobacter* sp., significantly enhanced enzyme activities in the vermicompost. *P. putida* Tabriz, a phosphate-solubilizing bacterium, was particularly effective in increasing cellulase and urease activities.

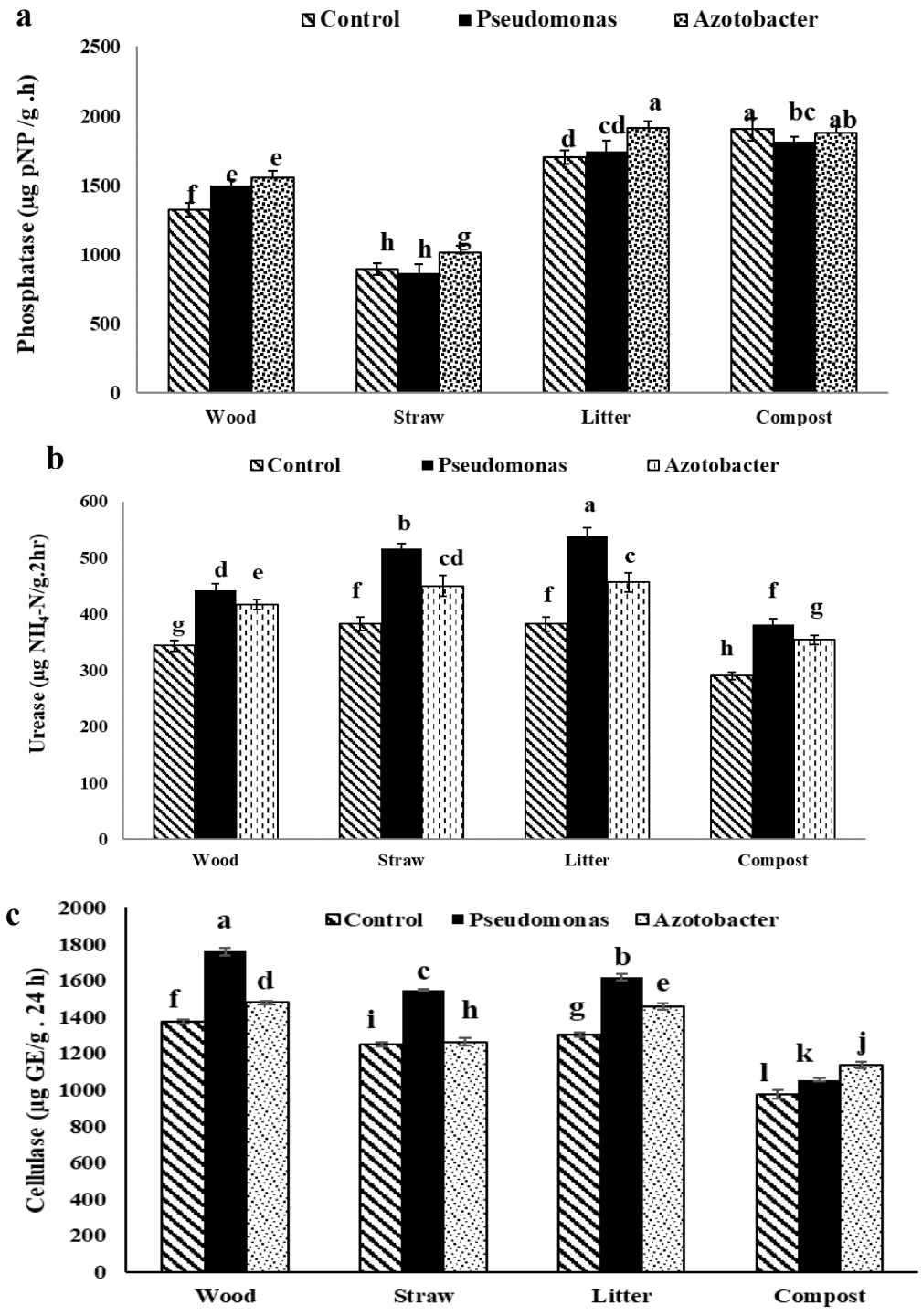


**Figure 4.** Interaction effects of substrate type and bacterial inoculation on a) acid phosphatase activity, b) urease activity and c) cellulase activity

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This aligns with previous studies showing that phosphate-solubilizing bacteria not only enhance phosphorus availability but also promote the production of hydrolytic enzymes involved in organic matter decomposition (Yadav et al., 2022). Similarly, *Azotobacter* sp., a nitrogen-fixing bacterium, significantly improved phosphatase activity, likely due to its role in nitrogen cycling and its ability to stimulate microbial communities involved in phosphorus mineralization (Kumar et al., 2021). These findings suggest that bacterial inoculants can serve as bioenhancers, accelerating the decomposition process and improving the nutrient content of vermicompost. Our study found that *Azotobacter* strains were more effective than *Pseudomonas putida* in boosting acid phosphatase activity. Maximum phosphatase activity was observed with the inoculation of *A. chroococcum* and *E. fetida*, likely due to increased earthworm biomass stimulating microbial metabolism. Microbes in earthworm guts consumed nitrogenous compounds from mucus, enhancing their activity and contributing enzymes to digestive processes. This synergy between earthworms and microbes increased microbial activity and phosphatase accumulation, highlighting their role in P-cycle substrate metabolism during composting (Lakshmi et al., 2014). The highest urease activity was recorded in the treatment inoculated with *Pseudomonas*, which demonstrated a significant difference compared to the treatment inoculated with *Azotobacter* (Figure 2b). In our study, bacterial inoculation significantly increased urease activity compared to non-inoculated treatments, with the highest activity observed in *Pseudomonas*-inoculated substrates, showing a notable difference over *Azotobacter*.

The litter bed exhibited the highest urease activity, likely due to its proper C/N ratio and reduced $NH_4^+$ production, as $NH_4^+$ inhibits urease activity and synthesis (Kumar, 2018). Earthworm inoculation further enhanced urease activity, possibly due to increased earthworm and microbial biomass, as well as the presence of N-fixing bacteria that convert $NH_4^+$ to $NO_3^-$ (Kumar, 2018; Lakshmi et al., 2014). However, in our study, *Pseudomonas putida* outperformed *Azotobacter* in boosting urease activity, likely due to its ability to accelerate the decomposition of nitrogen-containing organic materials. This aligns with findings by Singh et al. (2020), who reported lower urease activity in *Azotobacter*-inoculated substrates and correlated urease activity with initial total organic carbon content.

Bacterial inoculation significantly increased cellulase activity compared to the non-inoculated treatment. Furthermore, a significant difference was observed between the treatment inoculated with *Pseudomonas* and the treatment inoculated with *Azotobacter* (Figure 2c).

### 3.3. Temporal dynamics of enzyme activities

The comparison of incubation times revealed that phosphatase activity initially increased but declined after 7 months (Figure 3a). The results indicated that after vermicomposting, the highest acid phosphatase activity was observed in the wood dust and straw beds during the seventh month, with no significant differences between them at other incubation times. On the other hand, phosphatase activity in leaf litter and garbage compost increased until the seventh month but declined thereafter (Figure 5a). Similarly, in both inoculated and non-inoculated treatments, phosphatase activity increased until the seventh month, after which it decreased (Figure 6a).

The temporal analysis of enzyme activities revealed a peak in activity at 4 and 7 months after inoculation, followed by a gradual decline toward the end of the study period. This pattern can be explained by the dynamic nature of microbial communities and substrate availability during vermicomposting. Initially, the addition of bacterial inoculants and the presence of readily decomposable organic matter likely stimulated microbial activity, leading to increased enzyme production. However, as the organic matter became more stabilized and nutrient availability decreased, microbial activity and enzyme production declined. This trend is consistent with previous studies reporting a similar temporal pattern in enzyme activities during vermicomposting (Pramanik et al., 2009; Boruah & Deka, 2003b). However, the highest enzyme activities were observed 4 to 7 months after bacterial inoculation, suggesting that the inherent enzymatic potential of these bacterial strains played a key role in the observed increases. Urease plays a key role in the conversion of urea to ammonia and $CO_2$, making it a critical indicator of nitrogen metabolism during composting (Boruah & Deka, 2023a). Like other enzymes, urease synthesis depends on microorganisms. In our study, urease activity peaked at the end of the 4$^{th}$ month, likely due to the presence of rapidly decomposable nitrogen sources such as amino acids and proteins. High initial urease activity was driven by the availability of easily degradable substrates, which stimulated microbial activity. However, activity declined in later stages, possibly due to the depletion of carbon sources, leading to reduced microbial biomass (Lakshmi et al., 2014). Consistent with observations by Kumar (2018) and Boruah & Deka (2023a), urease activity in our study exhibited a progressive decline throughout the composting process.

This trend likely reflects diminishing substrate availability, as urease a key enzyme in nitrogen (N) mineralization hydrolyzes urea into plant-available ammonium ($NH_4^+$).

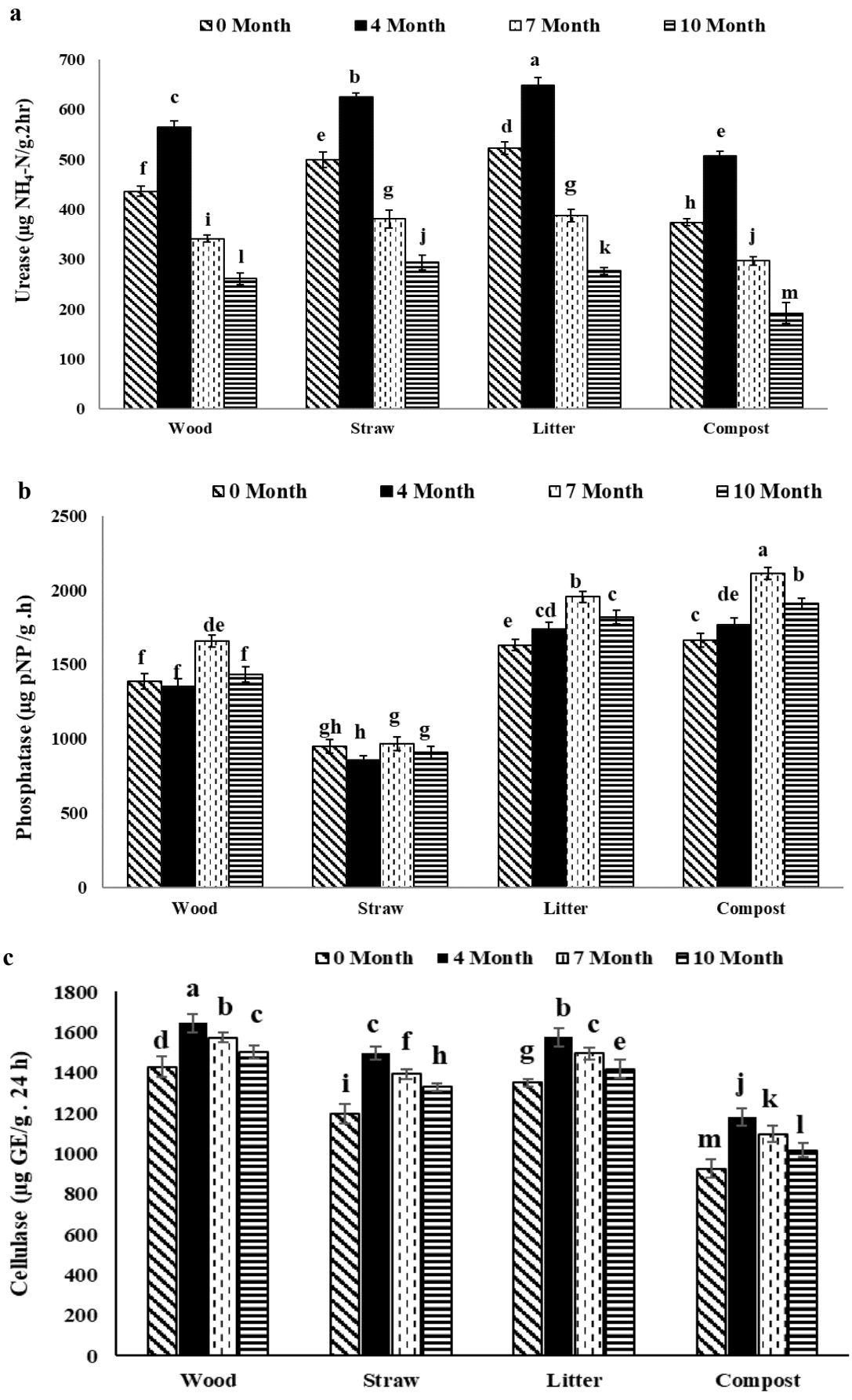


**Figure 5.** Interaction effect of substrate type and incubation time on a) acid phosphatase activity, b) urease activity and c) cellulase activity

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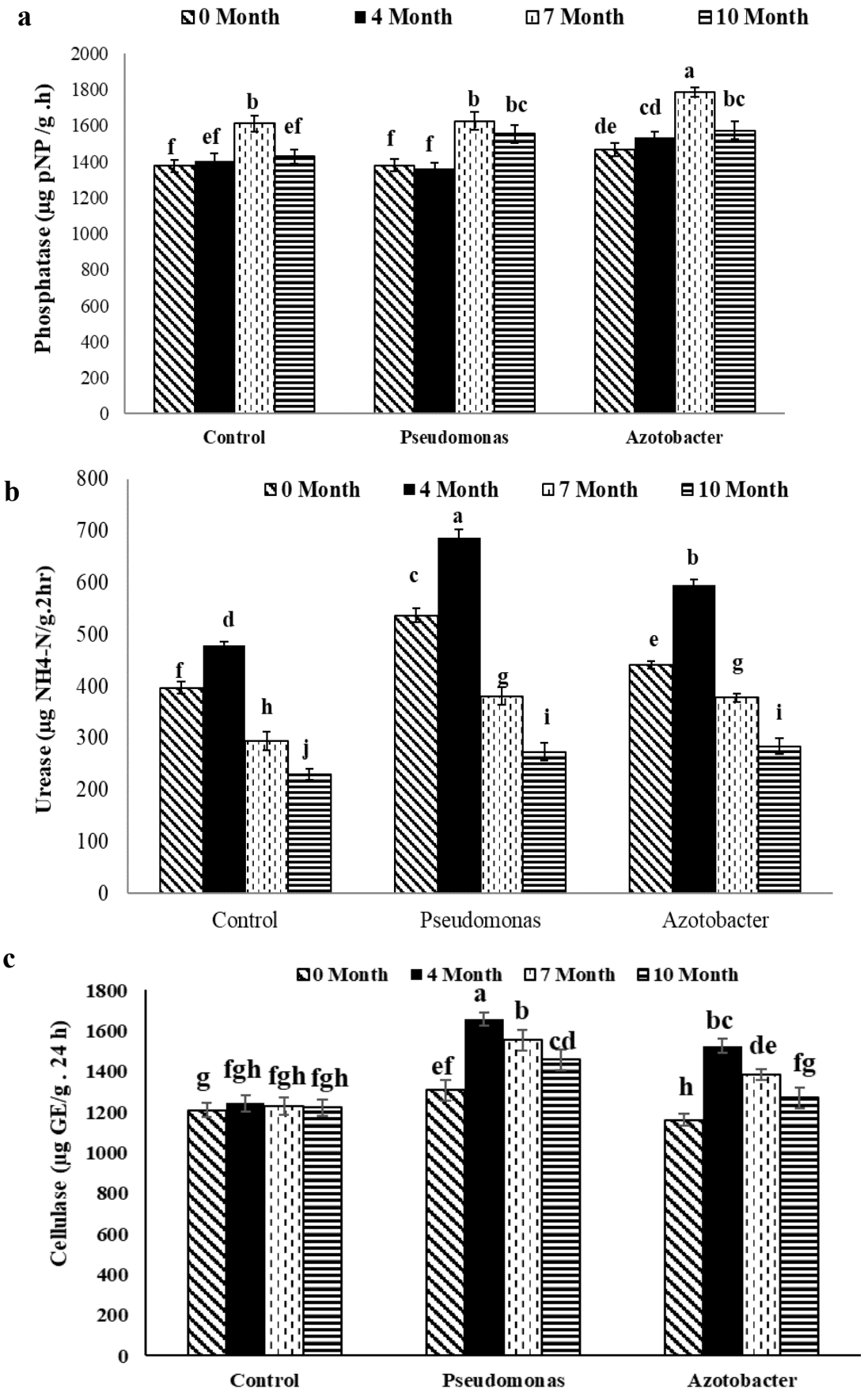


**Figure 6.** Interaction effects of bacterial inoculation and incubation time on a) acid phosphatase activity, b) urease activity and c) cellulase activity

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The decline suggests that readily degradable organic N compounds (e.g., proteins, urea) were preferentially metabolized in earlier stages, leaving recalcitrant N forms in later phases (Boruah & Deka, 2023a).

In the analysis of incubation time and the interaction between treatment and time, urease activity exhibited an increasing trend up to the fourth month, after which it declined, reaching its lowest level by the tenth month (Figures 3b, 4b, and 5b). Regarding the interaction between substrate and bacterial inoculation, the highest urease activity was observed in treatments with bacterial inoculation compared to those without inoculation. Specifically, the treatment inoculated with *Pseudomonas* showed the highest activity and was significantly different from the treatment inoculated with *Azotobacter* (Figure 6b). The investigation of the interaction between substrate type, bacterial inoculation, and incubation time revealed that the highest urease activity was observed in the leaf litter substrate inoculated with *Pseudomonas putida* during the fourth month (data not shown).

In the investigation of the effect of incubation time and the interaction between treatments and time on cellulase activity, it was observed that all experimental substrates and treatments inoculated with *Pseudomonas* and *Azotobacter* exhibited high cellulase activity up to the fourth month. After this period, the activity declined, with the lowest value recorded at the beginning of the experiment (Figures 3c, 4c, and 5c).

The results showed that cellulase activity was higher in substrates inoculated with *Pseudomonas*, with a significant difference compared to those inoculated with *Azotobacter* (Figure 6c). In this experiment, the highest cellulase activity was observed in the wood dust substrate inoculated with *Pseudomonas putida* at four months (data not shown). Cellulase activity, dependent on microorganisms, increased with microbial inoculation and decreased as organic matter and microbial activity declined. It is synthesized in the presence of rapidly decomposing carbon materials; as microbial populations grow, decomposition and cellulase activity rise.

### 3.4. Implications for sustainable agriculture

The integration of bacterial inoculation with vermicomposting offers a promising strategy for producing high-quality biofertilizers with enhanced enzymatic and nutrient properties.

By optimizing the combination of organic substrates and microbial inoculants, this approach can significantly improve the efficiency of organic waste recycling and contribute to sustainable soil management.

The use of vermicompost enriched with beneficial microorganisms not only enhances soil fertility but also promotes plant growth by improving nutrient availability and stimulating root development (Yadav et al., 2022). Furthermore, this method aligns with the principles of circular economy and environmental sustainability by reducing reliance on chemical fertilizers and minimizing organic waste accumulation. While this study provides valuable insights into the effects of bacterial inoculation and substrate type on enzyme activities, further research is needed to explore the long-term impacts of enriched vermicompost on soil health and crop productivity. Additionally, investigating the interactions between different microbial inoculants and their effects on enzyme dynamics could provide a deeper understanding of the mechanisms underlying vermicompost enrichment. The key finding of this study is that it demonstrates that integrating bacterial inoculation with vermicomposting can significantly enhance the enzymatic and nutrient properties of vermicompost, making it a valuable biofertilizer for sustainable agriculture. This study provides novel evidence of a synergistic effect from integrating bacterial inoculation with specific vermi-bed substrates, significantly enhancing a comprehensive suite of enzymatic activities (e.g., cellulase, urease, phosphatase) and nutrient profiles an interaction that has been insufficiently explored in prior research. Studies focusing on the application of enriched vermicompost in field conditions and its effects on diverse cropping systems would also be beneficial for validating its practical utility.

## 4. Conclusion

In conclusion, this study demonstrates that the integration of bacterial inoculation into vermicomposting can significantly enhance the enzymatic and nutritional properties of vermicompost, thereby making it a valuable biofertilizer for sustainable agriculture. The findings underscore the importance of substrate selection, microbial inoculation, and incubation time in optimizing vermicompost quality. By leveraging the synergistic effects of earthworms and beneficial microorganisms, this approach offers a sustainable solution for organic waste management and soil fertility enhancement, contributing to the broader goals of environmental sustainability and food security. However, further experiments at both pot and field scales are necessary to assess the effects of these products on plant growth.

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**Authors Contribution**

Mitra Ebrahimi: Conceptualization, Writing – Original Draft. Farshad Shakuri: Formal analysis, Data Curation, Visualization. Mohammad Reza Sarikhani: Supervision, Project administration, Resources, Validation, Conceptualization, Methodology. Zahra Delfruz: Investigation, Methodology. Nasser Aliasgharzad: Conceptualization, Methodology, Supervision. Nosratollah Najafi: Conceptualization, Methodology, Software.

**Availability of data and materials**

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

**Conflict of interests**

The authors of the paper declare that they have no conflict of interest.

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