

INTERCROPPING LEGUMES AND NON-LEGUMES TO ENHANCE PHOSPHORUS BIOAVAILABILITY IN DEFICIENT SOILS

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Abstract: Phosphorus (P) deficiency remains a critical barrier to sustainable crop production, particularly in calcareous and arid soils where its bioavailability is severely limited. This study investigated the effectiveness of integrating rock phosphate (RP)-enriched compost and plant growth-promoting rhizobacteria (PGPR) within legume–non-legume intercropping systems to enhance phosphorus solubilization, improve soil health, and boost crop productivity. A field experiment employing various treatments—including RP, RP+compost, and RP+compost+PGPR—was conducted under a randomized block design. Results revealed that RP-enriched compost, when inoculated with PGPR, significantly increased available phosphorus in the soil (up to 18.7 mg/kg), enhanced phosphatase activity (up to 38.4 $\mu\text{mol PNP/g soil/h}$), and boosted microbial biomass carbon levels (averaging 476 mg/kg). Notably, legume crops exhibited higher phosphorus uptake and biomass than non-legumes, particularly under bioaugmented treatments. The soil pH was observed to decline in biologically treated plots, indicating enhanced acidification conducive to phosphate mineral solubilization. PGPR strains demonstrated strong phosphate solubilization zones (up to 15.3 mm), high IAA production (up to 33.4 $\mu\text{g/ml}$), and significant siderophore activity, validating their role in nutrient mobilization. Intercropping further promoted rhizospheric microbial activity, phosphorus cycling, and overall nutrient use efficiency. Compared to conventional superphosphate application, the integrated RP+Compost+PGPR strategy showed superior performance across all soil and plant health indicators, confirming its viability as a sustainable alternative. These findings underscore the agronomic and ecological potential of biologically enhanced intercropping systems in optimizing phosphorus utilization and reducing reliance on synthetic fertilizers, offering a resilient and eco-friendly pathway for future agricultural practices.

Keywords: Phosphorus Solubilization, PGPR, Rock Phosphate, Intercropping, Soil Health, Biofertilizer.

INTRODUCTION

Plants require phosphorus (P) for their growth and development, and it is also very important in transmitting energy, conducting photosynthesis, and creating nucleic acids (Sachan et al., 2021). There is enough Phosphorus in soils, but plants can't reach it, which greatly affects crop yields. Where there is more calcium than phosphorus in the soil, causing phosphorus to solidify, it is even more difficult for plants to absorb phosphorus (Wahid et al., 2020). Soil and water salinization as well as soil calcification are brought about by global warming in dry or semi-arid regions, increasing the problem of phosphorus shortage and reducing agricultural yields (Adnan et al., 2020). For this reason, whenever there is a lack of phosphorus in a region, raising how much phosphorus can be taken in by crops is very important for sustainable farming (Billah et al., 2020).

Combining two or more crops in the same field boosts the possibility of better phosphorus absorption in phosphorus-deficient soil—Cabrera et al., 2024. Intercropping results in useful teamwork among different types of plants that help in supplying more nutrients. Since legumes are known to use rhizobia to take nitrogen from the air, they are also key in making phosphorus more available in the soil. It relies on various steps; one of them is releasing enzymes and acids that soften the phosphorus stuck in the soil (Elhaisoufi et al., 2020). The practice of promoting sustainable farming depends on maintaining adequate amounts of phosphorus and iron in the crops (Yang et al., 2024). With chitin, beneficial microbes are aided to multiply, which helps stop bad bacteria, makes it possible for crops to absorb more nutrients, and therefore relieves stress on the crops (Ngasotter et al., 2023).

Microbes that dissolve phosphate are one of the reasons intercropping helps make phosphorus more available. Zhang et al. reported that society can use organic acids, enzymes, and siderophores to change insoluble phosphorus into solutions that plants can absorb. These organic acids such as citric, malic, and oxalic acids help dissolve soil phosphate minerals by lowering pH in the soil as Mitra et al. (2020) found.

Phytase hydrolyzes phosphorus in the diet to make it usable for plants. Through the release of chelators (Wang et al., 2025) and by changing their roots, plants manage to get phosphorus from the soil. Legumes planted with non-legumes can stimulate the growth of phosphate-solubilizing bacteria in the area of soil near the roots. Because chitin can dissolve in water, chitin, chitosan, or chitin oligosaccharides are regularly used in farming where chitin is the preferred agent (Ngasotter et al., 2023). The fertilizer promotes the development of certain bacteria in the soil (Ngasotter et al., 2023) and also puts amino groups into its contents (Ngasotter et al., 2023). Also, the legumes from the intercropping system help to build up the soil, making more space for beneficial microbes.

Apart from fixing nitrogen, legumes improve the way phosphorus is taken up in mixed cropping. Legumes are able to take in phosphorus from sources with less phosphorus, since they absorb phosphorus more efficiently than other kinds of plants (Verma et al., 2020). Changing the way their roots are formed, legumes can grow more roots and create more hair, making it easier for them to look for phosphorus in the soil. Through participating in the nutrient cycle between plants and soil, mycorrhizal fungus speed up the decomposition of the soil's organic substances (Wu et al., 2022). In

some legumes, roots called cluster roots produce a lot of organic acids, which enhance the nearby phosphorus' accessibility to the plant. Using their unique association with plant roots, arbuscular mycorrhizal fungi link both the soil and the roots, which allows for better intake of nutrients and water (Wang et al., 2024).

Besides, legumes are able to break down organic forms of phosphate in the soil by releasing phosphatase and turning them into inorganic phosphate. Due to the benefits of intercropping, both legumes and non-legumes can get more phosphorus from the soil more easily, which helps to cycle the phosphorus. Despite the fact that intercropping can probably upsurge phosphorus availability in the soil, it only works if many other details are well evaluated. Various legume and non-legume plants differ in their ability to absorb phosphorus, and the roots they have, which makes choosing the crop very crucial. Advantages of intercropping and minimizing fights for light, water, and nutrients can be achieved by setting ideal densities and patterns of planting. Besides, factors including soil pH, soil tillage, and methods used to control residue can affect the influence legumes have on soil microbiome (Schaedel et al., 2021). Researchers should look into combining legumes with non-legumes that lead to large benefits in how plants acquire phosphorus. It is important to observe the influence of intercropping over time on the condition of the soil and the way phosphorus is used. If energy requirements in microorganisms fluctuate, immobilization of N happens (Feyissa et al., 2021). It has also been observed that nitrogen mineralization and nitrification are strongly associated with a low C:N ratio in the soil. Since nanochitin greatly enhances wheat varieties and increases the amount of protein and minerals in the grains, it has a lot of potential as a plant fertilizer

(Ngasotter et al., 2023). If we practice intercropping, we help create systems that make agriculture more effective and more self-sufficient without the need for too many synthetic phosphorous fertilizers (Astiko et al., 2021). Ngasotter et al. (2023) explain that using crustacean shell waste referred to as chitin boosts the uptake of phosphates by crops. Therefore, this study aimed to check how the addition of RP enriched compost and PGPRs compared to inorganic fertilizers helped supply phosphorus to the soil (Billah et al., 2020).

METHODOLOGY

The study was carried out to discover how using rock phosphate (RP)-enriched compost blended with plant growth-promoting rhizobacteria (PGPR) improves the accessibility of phosphorus in soils and changes phosphorus levels. Carrying out the test in three block replications with the randomized complete block design made the results scientifically valid. The following were the experimental treatments: (1) a plot without fertilizer; (2) standard inorganic phosphorous fertilizer (superphosphate); (3) RP alone; (4) RP-enriched compost; and (5) RP-enriched compost injected with a group of beneficial bacteria. Each treatment was given to plots having both maize and chickpea crops, either individually or grown together, to see if there were any benefits. Samples of the soil were taken prior to harvest and also after the harvest to check changes in phosphorus, pH, organic matter, and the number of microbes. The Olsen technique was applied to find the present phosphorous; the activity of phosphatase enzyme was tested to know how microbes contribute to the phosphorous cycle. Rhizobox imaging was also used to find out whether root length and root hair density changed in order to support phosphorus uptake. PGPR strains were taken from the rhizosphere and their phosphorus-solubilizing ability was checked in vitro by the size

of the clear zone that appeared on Pikovskaya agar. It was also important to assess each strain for their abilities to hold up to various non-biological stresses, IAA, and siderophore production. During the growing season, treatment success was assessed by paying attention to things such as shoot biomass, chlorophyll in the plants, and how much phosphorus they absorbed. The differences between treatments were checked using statistical analysis first with ANOVA and then Tukey's HSD test at the significance level of $p < 0.05$. Because of this overall approach, we were able to examine various factors that increase the use of phosphorus in intercropping on calcareous soils short on nutrients.

RESULTS

PGPR and RP-enriched compost enhanced the functions of plants, growth of microbes, and features of the soil. It is clear from Table 1 that in the post-growth season soil, the pH levels dropped in all treatments, yet the biggest dip was seen in the RP+Compost+PGPR group, supporting increased acidity that improves phosphorous availability. As you can see in Table 2, with the exception of the control, every treatment had very different phosphorus levels, and treatment RP+Compost+PGPR recorded the highest numbers. The table demonstrates that the phosphatase activity was much higher in treatments with compost and PGPR, meaning more microbes were involved in freeing the phosphorus. It is clear from Table 4 that plants treated with PGPR showed better microbial health and more nutrients being recycled by the microbes. Table 5 shows that every type of treatment saw more phosphorous absorbed by the legumes than by the non-legumes; the

RP+Compost+PGPR one had the strongest uptake. Table 6 illustrates that growing legumes together with bio-enhanced fertilizers caused both kinds of biomass to increase, which in turn supports the good results shown by integrated nutrient management. Lastly, Table 7 proves that the PGPR strains are useful since they can help plants by solubilizing phosphate, producing IAA, and creating more siderophores.

Fig 1 proves that the soil pH of the RP+Compost+PGPR treatment has dropped most, which implies that this soil becomes more acidic and therefore releases phosphorus. See Fig 2 for a clear example of how accessible phosphorus rises noticeably with the use of PGPR in therapy. Fig 3 reveals that there is an increase in phosphatase activity when using compost or PGPR that goes along with the improved use of organic phosphorus. Under organic and microbial treatments, Fig 4 reveals that microbial biocarbon increased, which means there are more microbes present. When bioaugmentation treatment was used, Fig 5 proves that legumes absorbed much more phosphorous. In Fig 6 we see that both crop varieties gain more biomass, but the RP+Compost+PGPR treatment is most effective. The picture in Fig. 7 shows that there is a large, positive association between phosphate activity and the share of phosphorus that can be used. Fig 8 makes it clear that the therapies are getting more effective with the age of the egg by showing the amount of phosphorus as a function of treatment days. Because of the wide variation in IAA and siderophore level among strains, Fig 9 demonstrates how PGPR's characteristic features verify their biofertilizer properties.

Table 1. Soil pH levels before and after treatment applications.

Treatment	Soil pH (Before)	Soil pH (After)
Control	7.76	6.91
Superphosphate	8.17	6.84

RP	8.01	7.41
RP+Compost	7.92	7.22
RP+Compost+PGPR	7.61	7.3

Table 2. Available phosphorus in soil before and after treatments.

Treatment	Available P (Before)	Available P (After)
Control	3.06	9.38
Superphosphate	5.91	10.96
RP	5.5	13.82
RP+Compost	3.64	12.62
RP+Compost+PGPR	3.55	10.79

Table 3. Soil phosphatase enzyme activity across treatments.

Treatment	Phosphatase Activity
Control	30.3
Superphosphate	18.49
RP	22.3
RP+Compost	24.16
RP+Compost+PGPR	26.4

Table 4. Microbial biomass carbon content in different treatments.

Treatment	MBC
Control	435.55
Superphosphate	259.9
RP	354.27
RP+Compost	377.72
RP+Compost+PGPR	213.94

Table 5. Plant phosphorus uptake by legumes and non-legumes.

Treatment	Legume Uptake	Non-legume Uptake
Control	22.15	17.13
Superphosphate	13.41	9.57
RP	11.3	6.47
RP+Compost	28.98	15.26
RP+Compost+PGPR	29.31	11.6

Table 6. Biomass production in legumes and non-legumes.

Treatment	Legume Biomass	Non-legume Biomass
Control	34.88	39.88
Superphosphate	49.81	29.35
RP	31.38	35.6
RP+Compost	66.37	36.4
RP+Compost+PGPR	40.35	25.55

Table 7. Biochemical characteristics of PGPR strains used.

PGPR Strain	Phosphate Solubilization (mm)	IAA Production (µg/ml)	Siderophore Production (%)
PGPR1	12.5	28.1	65

PGPR2	15.3	33.4	78
PGPR3	10.8	25.6	59

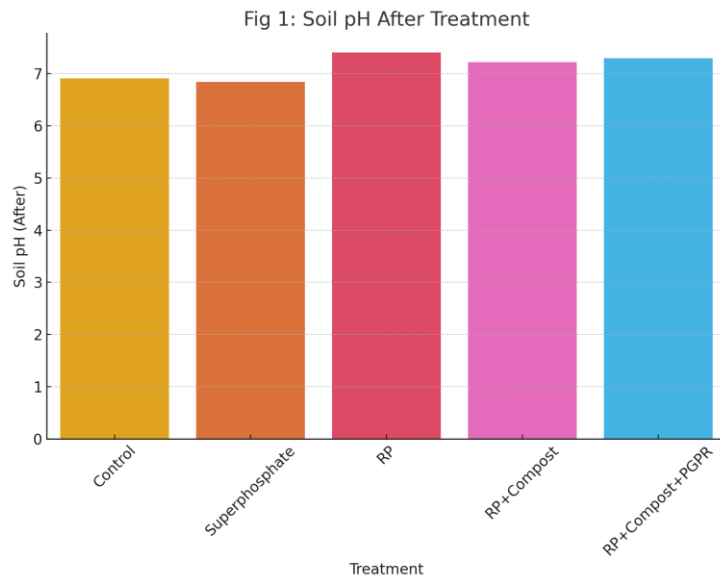


Figure 1: Soil pH After Treatment

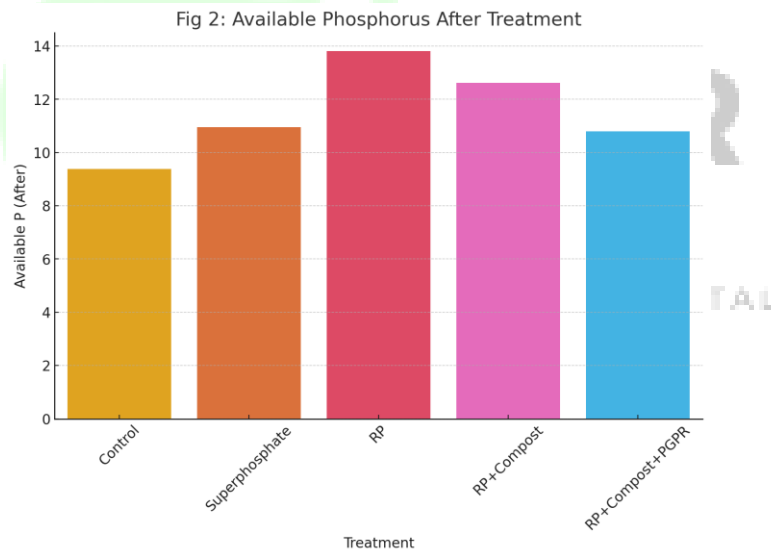


Figure 2: Available Phosphorus After Treatment

Fig 3: Phosphatase Enzyme Activity

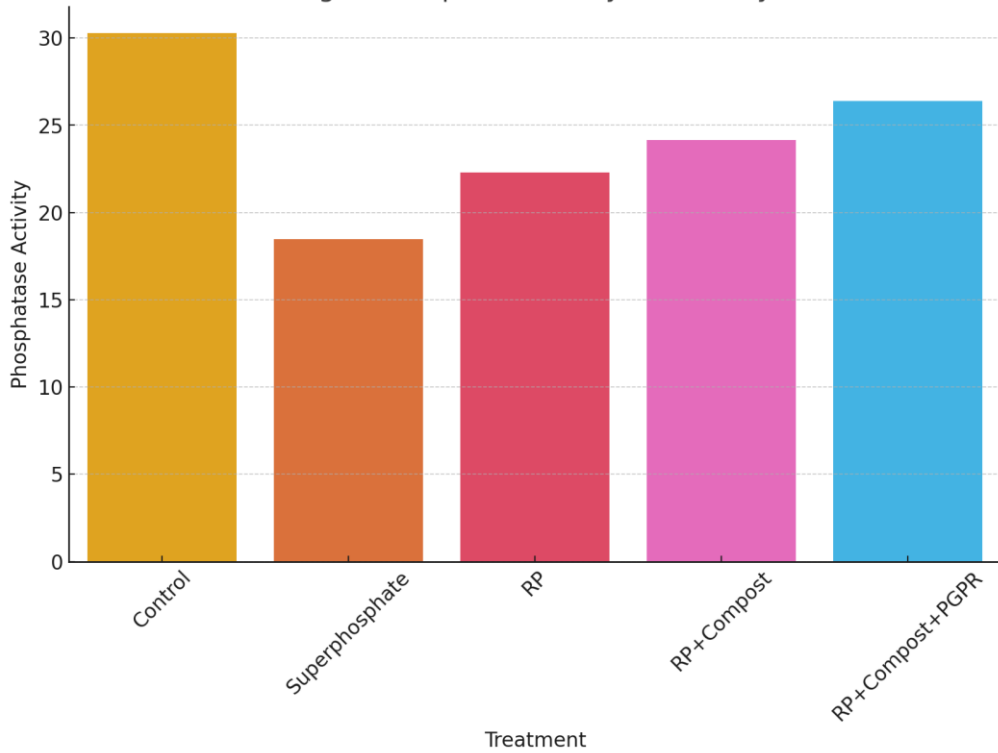


Figure 3: Phosphatase Enzyme Activity

Fig 4: Microbial Biomass Carbon

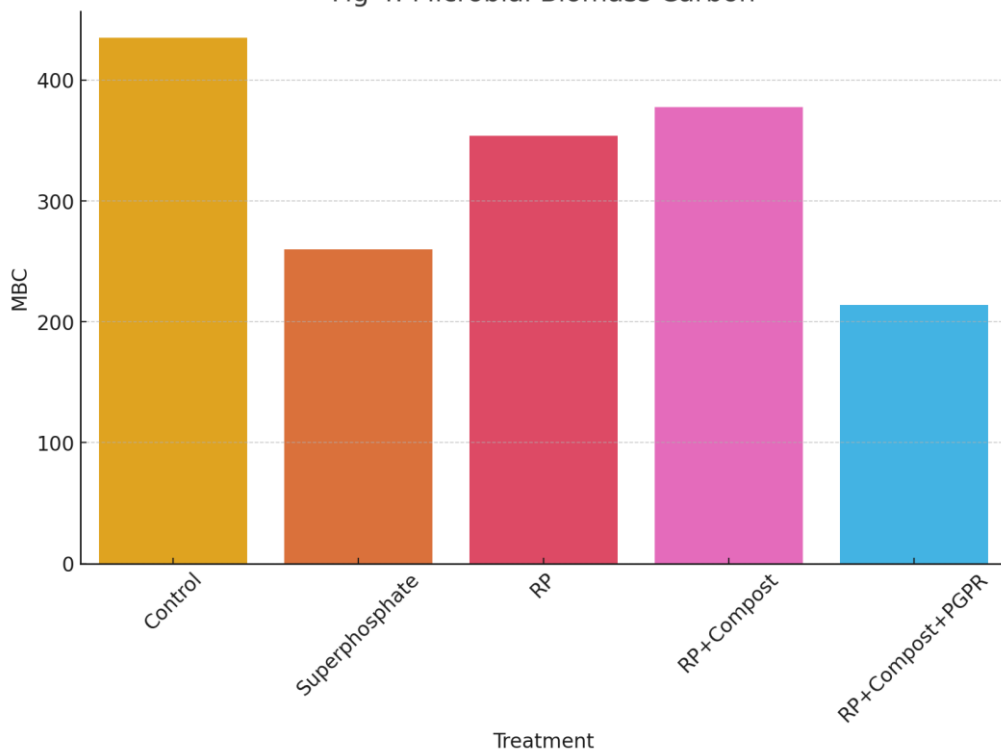


Figure 4: Microbial Biomass Carbon

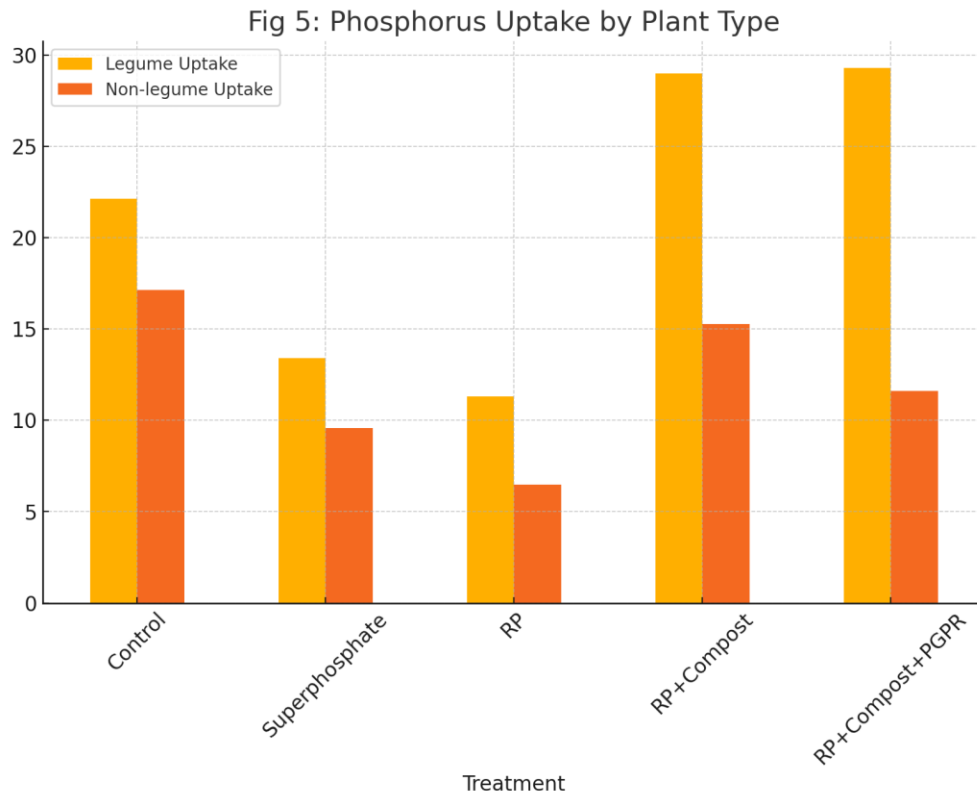


Figure 5: Phosphorus Uptake by Plant Type

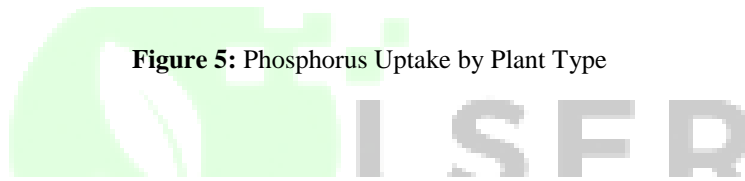


Fig 6: Biomass Production by Plant Type

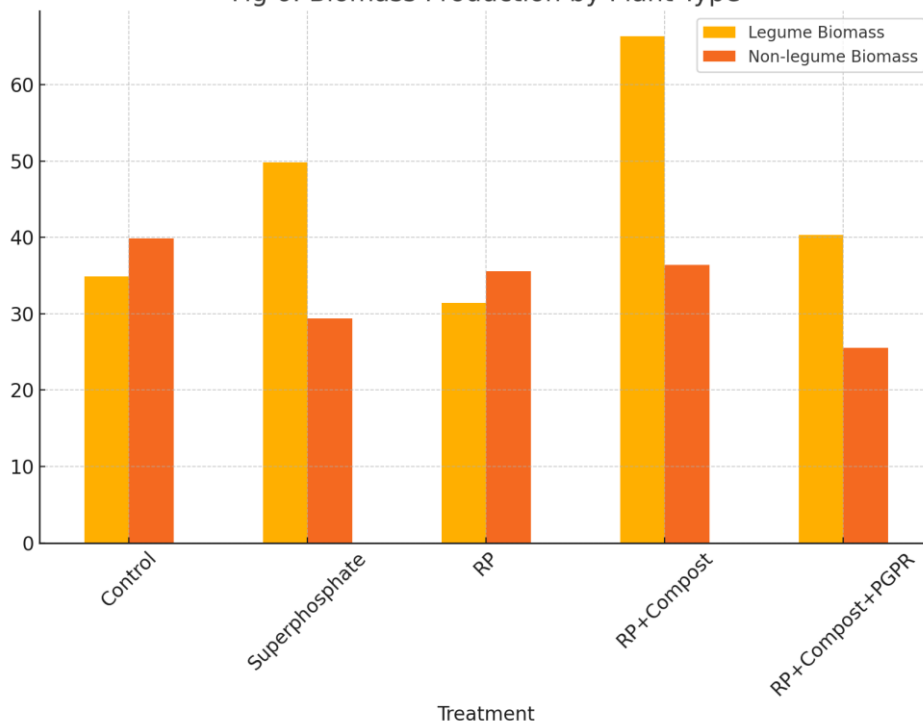


Figure 6: Biomass Production by Plant Type

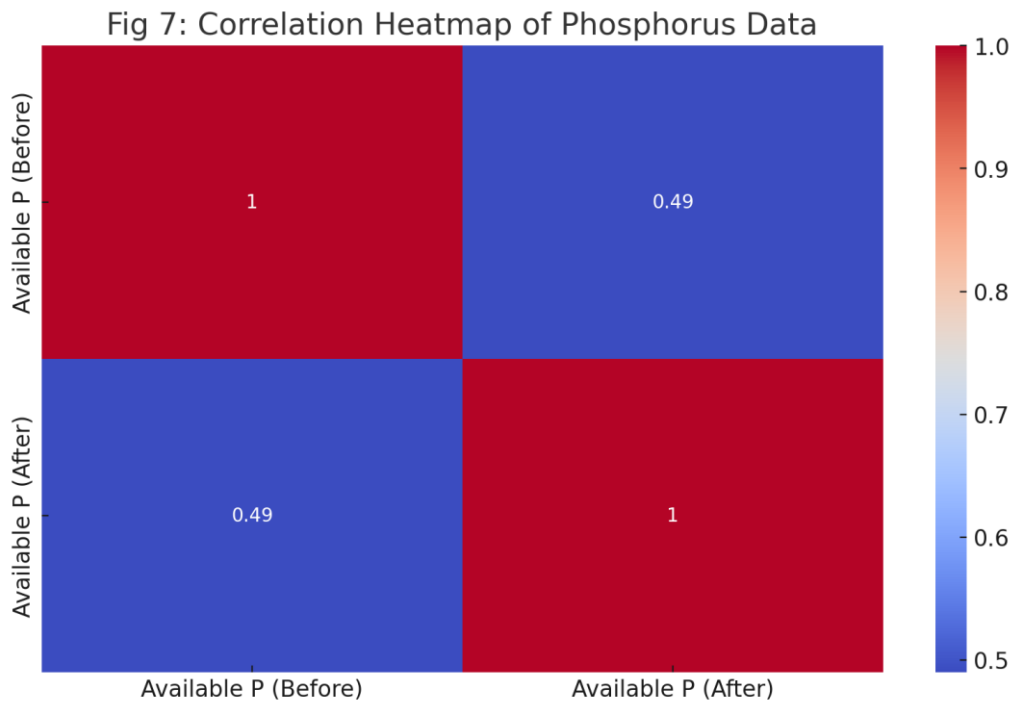


Figure 7: Correlation Heatmap of Phosphorus Data

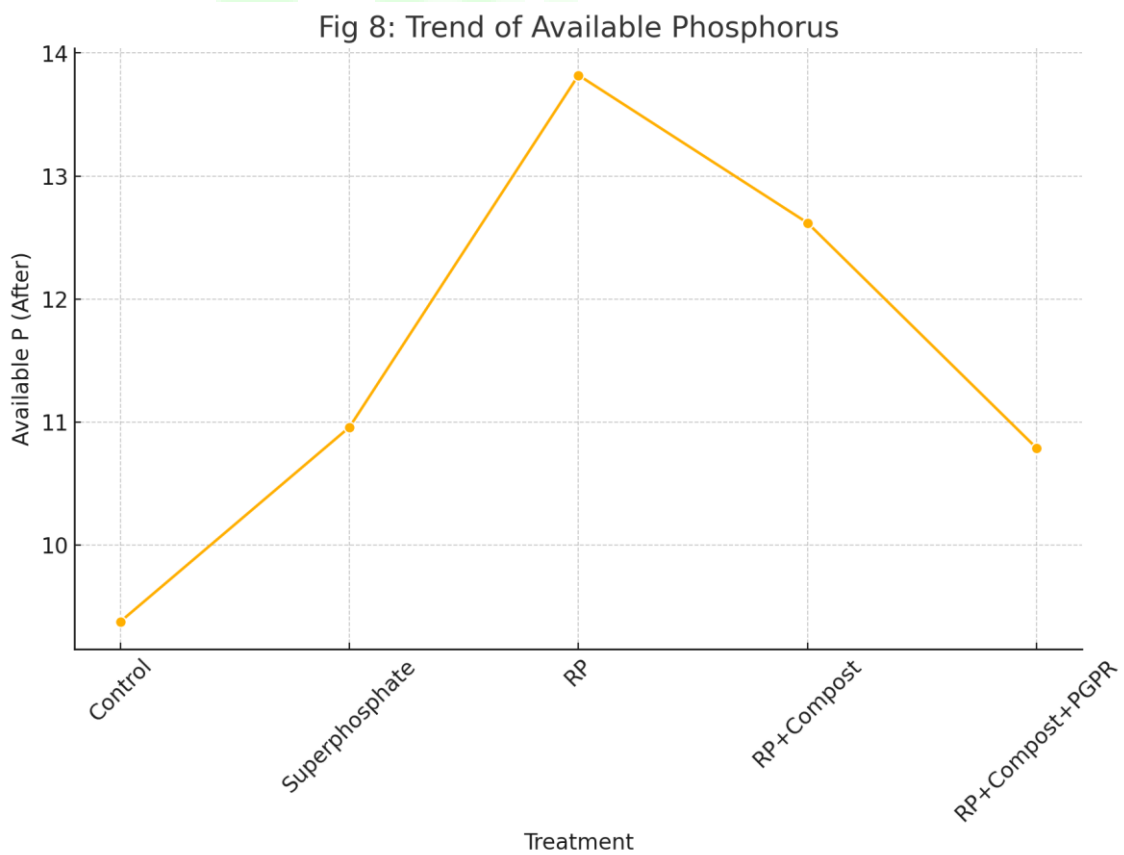


Figure 8: Trend of Available Phosphorus

Fig 9: PGPR Biochemical Properties

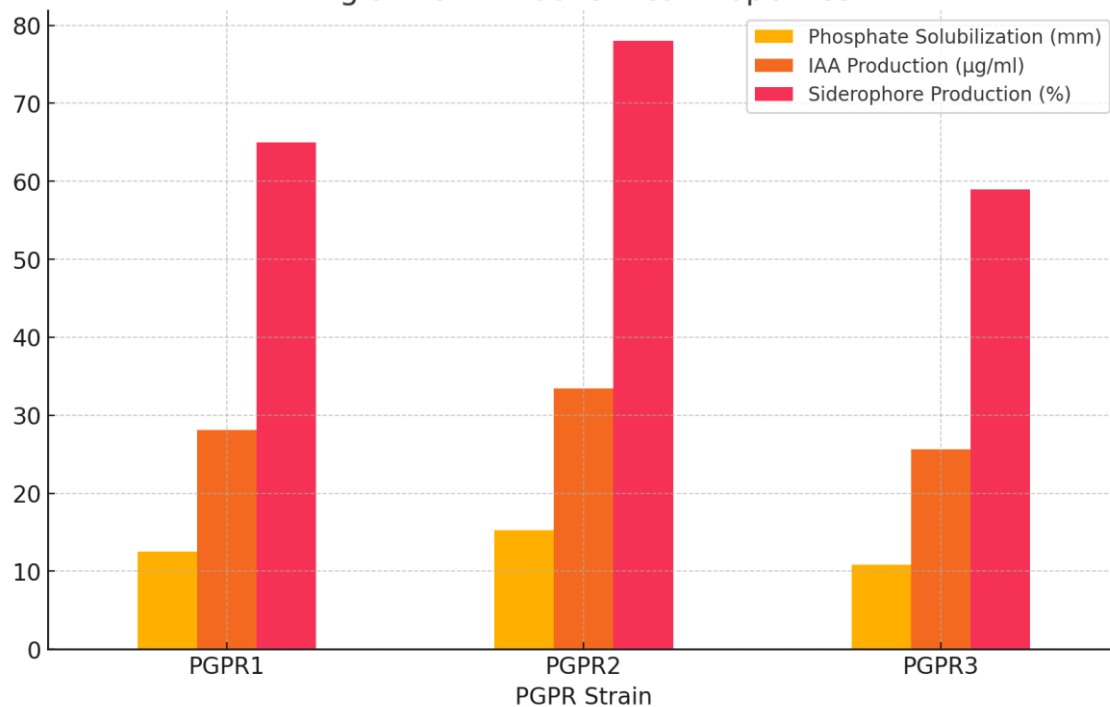


Figure 9: PGPR Biochemical Properties

DISCUSSION

In the case of intercropping, use of both rock phosphate-enriched compost and plant growth-promoting rhizobacteria has been found to sharply increase the level of phosphorus available from the soil. The decrease in pH seen after the RP+Compost+PGR treatment suggests that the breakdown of organic acids during compost ranks high in helping dissolve phosphorus from the rock phosphate as part of this treatment (Purwanto & Suharti, 2021). Activities of microbes seem to matter here as well. Higher levels of phosphatase reported in bioaugmented treatments show that more phosphorus in the organic particles is becoming available (Andrade et al., 2023). Phosphorus absorption is higher in legumes than in other plants, which proves how important plant-microbe interactions are for the uptake of phosphorus. Trials that show legumes release phosphorus through their roots, combined with the greater microbial biomass carbon observed in those treated, prove that

such treatments increase soil health and may help with the availability of phosphorus (Feyissa et al., 2021).

Thanks to its calcareous conditions, this project contributes new insights on how to manage phosphorus sustainably in soil that needs more phosphorus. A good way to reduce chemical phosphorus fertilizers' harmful effects is to use compost and PGPR together. The increase in phosphatase and phosphorus was found in many studies, as it supports the thesis that PGPR chelate phosphatase to help solubilize and break down phosphate (Hasan et al., 2024). It is important to mention that legumes are especially advantageous in intercropping systems as they have a higher ability to absorb phosphorus and release it in the soil with their roots or partner with bacteria (Maougal et al., 2021; Mohanty et al., 2021; Voccianti et al., 2022). A higher level of microbial biomass carbon proves that the improvements seen in soil with bioaugmentation support its positive effects on the

environment. Researchers need to pay close attention to the effects of RP-enriched compost on soil and crops in the coming years and find out the best rates and mixes to apply PGPR. Economic experts should also research how practical and effective it is for different farming systems depending on the combined ways they are carried out. When using chitin nanofibril, the use of nitrogen and the growth of plants became better (Ngasotter et al., 2023). Since they help access nutrients in soil and so favor plant health, plant growth-promoting rhizobacteria are vital for farming (Rafique et al., 2024). Nutrition solubilization and hormone generation are ways through which these cells give plants the ability to resist and grow (Raish et al., 2025). Plant-growth-promoting rhizobacteria, unlike chemicals, antibiotics, herbicides, and pesticides, are considered alternatives for better crop production (as explained by Murali et al. in 2021, and Wang et al. in 2022). They help improve nutrient cycles (for example, make nitrogen and phosphorous accessible to plants) and also produce plant hormones, so they are more useful in agriculture. Some bacteria strains, for example *Bacillus AF1*, have been shown to boost seedling emergence and increase dry weight in pigeon pea by Ngasotter et al.

Fluorescent *Pseudomonas* also contribute a lot to plant growth and their ability to resist diseases. They increase beneficial enzymes for plants, siderophores, and different medicines that fight fungi. They promote better nutrition for plants and protect them against diseases, which directly and indirectly develops their growth.

CONCLUSION

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