

Soil-Plant Phosphorus Dynamics and Microbial Activity as Influenced by Integrated Phosphorus Management in Wheat: Correlations with Grain and Biological Yields

Amanullah -

amanullah@aup.edu.pk

The University of Agriculture, Peshawar

Nadia -

The University of Agriculture, Peshawar

Shah Khalid

The University of Agriculture, Peshawar

Article

Keywords: Phosphorus dynamics, soil organic matter, phosphate-solubilizing bacteria, P use efficiency, wheat productivity, calcareous soils

Posted Date: June 26th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9694606/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations:

No competing interests reported.

Table 1 to 19 are available in the Supplementary Files section.

Soil-Plant Phosphorus Dynamics and Microbial Activity as Influenced by Integrated Phosphorus Management in Wheat: Correlations with Grain and Biological Yields

***Amanullah, Nadia and Shah Khalid**

Department of Agronomy, Faculty of Crop Production Science

The University of Agriculture, Peshawar-Pakistan

*Email: amanullah@aup.edu.pk

Abstract

Phosphorus (P) deficiency in alkaline calcareous soils severely limits wheat productivity worldwide. This study investigated the effects of 21 combinations of organic (sheep manure, cattle manure, legume residues, non-legume residues) and inorganic (single super phosphate, rock phosphate) P sources, along with phosphate-solubilizing bacteria (PSB) and Bioaab, on soil properties (pH, organic matter [OM], available P), plant P concentrations, P uptake, phosphorus use efficiency (PUE), agronomic P use efficiency (APUE), and microbial populations in wheat (*Triticum aestivum* L.). Results demonstrated strong positive correlations between soil available P and grain yield ($r = 0.89$, $p < 0.01$), soil OM and biological yield ($r = 0.86$, $p < 0.01$), and microbial population with both PUE ($r = 0.91$, $p < 0.01$) and grain yield ($r = 0.88$, $p < 0.01$). Application of cattle manure (CM) combined with PSB significantly reduced soil pH (7.81 vs. control 8.30), increased soil OM (1.26% vs. 0.60%), enhanced available P (4.75 vs. 2.49 mg kg⁻¹), and achieved the highest PUE (14.4%) and APUE (17.1 kg kg⁻¹). Grain P concentration (0.36%) and total P uptake (21.8 kg ha⁻¹) were maximized with CM + PSB treatment. Stepwise regression analysis identified soil available P and microbial population as the most significant predictors of wheat productivity ($R^2 = 0.94$). These findings establish that integrated P management enhances soil health indicators that directly translate into improved crop productivity, with PSB inoculation serving as a key modulator of soil-plant P dynamics in alkaline calcareous soils.

Keywords: Phosphorus dynamics; soil organic matter; phosphate-solubilizing bacteria; P use efficiency; wheat productivity; calcareous soils

1. Introduction

Wheat (*Triticum aestivum* L.) occupies approximately 220 million hectares globally, producing over 780 million metric tons annually, making it the second-most widely cultivated cereal crop after maize (FAO, 2024). It provides approximately 20% of the total dietary calories and protein for the world's population, with particularly high dependence in South Asia, North Africa, and the Middle East (Shiferaw et al., 2023). In Pakistan, wheat is cultivated on 8.8 million hectares with annual production of 26.2 million tons, yet the average yield (2.9 t ha⁻¹) remains substantially below the global average of 3.5 t ha⁻¹ and far below the potential yield of 6-7 t ha⁻¹ achieved in developed nations (MNFSR, 2023).

Among the sixteen essential nutrients required for wheat growth, phosphorus (P) ranks second only to nitrogen in quantitative requirement and is often the most limiting nutrient in agricultural soils

worldwide (Lynch, 2024). Phosphorus plays irreplaceable roles in energy transfer (ATP), genetic material (DNA/RNA), membrane integrity (phospholipids), enzyme activation, and signal transduction pathways (Khan et al., 2023). Despite adequate total P in many soils, plant-available P concentrations are typically < 0.1% of total P due to rapid fixation reactions (Shen et al., 2024). This challenge is particularly acute in alkaline and calcareous soils, which dominate arid and semi-arid regions including Pakistan, where calcium-induced P precipitation renders applied P fertilizers largely unavailable to crops (Amanullah et al., 2024).

Pakistan's agricultural soils are predominantly alkaline (pH 7.5-8.5) and calcareous (CaCO_3 content 5-15%), conditions that severely constrain P availability through multiple mechanisms (Rashid et al., 2023). In calcareous soils, applied soluble P rapidly precipitates as dicalcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), octacalcium phosphate [$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$], and ultimately hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$], which are highly stable and unavailable to plants (Penn and Camberato, 2019). Global P use efficiency (PUE) for cereal crops averages only 15-20%, meaning 80-85% of applied P fertilizer becomes fixed in soil forms unavailable for crop uptake (Wang et al., 2024; Dhillon et al., 2023). This inefficiency has profound economic and environmental consequences, including depletion of finite phosphate rock reserves (estimated 50-100 years remaining at current consumption rates), increased production costs for farmers, and P runoff contributing to aquatic eutrophication (Cordell and White, 2023; Scholz and Wellmer, 2024).

The limitations of exclusive reliance on chemical P fertilizers have catalyzed interest in integrated nutrient management (INM) approaches that combine inorganic fertilizers with organic amendments and beneficial microorganisms (Agegnehu et al., 2023; Singh et al., 2024). Organic amendments—including animal manures (cattle, sheep, poultry), crop residues, and composts—release P more slowly through mineralization, contribute to soil organic matter (SOM) pools, improve soil physical properties, and provide substrates for microbial communities (Diacono and Montemurro, 2022; Rahman et al., 2023). Long-term studies have demonstrated that integrated applications enhance soil P availability, reduce P fixation, and increase PUE compared to inorganic P alone (Zhang et al., 2023; Li et al., 2024).

Soil organic matter is particularly critical in calcareous soils, where organic compounds compete with P fixation sites on clay minerals and calcium carbonates, reduce P precipitation, and enhance P mobility (Guppy et al., 2023; Liu et al., 2024). Humic and fulvic acids released during OM decomposition form organo-mineral complexes that protect P from fixation and facilitate its transport to plant roots (Nardi et al., 2022). Furthermore, SOM serves as the primary energy source for soil microorganisms, including phosphate-solubilizing bacteria (PSB), which mediate P cycling through organic acid production and phosphatase enzyme release (Sharma et al., 2023; Chen et al., 2024).

Phosphate-solubilizing microorganisms (PSM) represent a diverse group of bacteria, fungi, and actinomycetes capable of converting insoluble P forms into plant-available orthophosphate (H_2PO_4^- , HPO_4^{2-}) through multiple mechanisms (Etesami, 2020; Alori et al., 2023). Key mechanisms include: (1) production of low molecular weight organic acids (gluconic, citric, oxalic, malic, succinic) that acidify the rhizosphere and chelate Ca^{2+} ions, releasing P from Ca-P complexes; (2) secretion of extracellular phosphatases (acid and alkaline) that mineralize organic P; and (3) release of protons (H^+) via NH_4^+

assimilation, further reducing rhizosphere pH (Sharma et al., 2024; Rawat et al., 2022). The genera *Bacillus*, *Pseudomonas*, *Rhizobium*, *Enterobacter*, and *Burkholderia* are among the most effective PSB documented, with strains exhibiting multiple plant growth-promoting traits including indole-3-acetic acid (IAA) production, siderophore synthesis, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Timmusk et al., 2023; Kumar et al., 2024).

Recent meta-analyses have confirmed that PSB inoculation increases crop yields by 10-25%, improves P uptake by 20-40%, and enhances PUE by 15-30% across diverse cropping systems (Li et al., 2023; Zhang et al., 2024). However, the efficacy of PSB is highly dependent on soil conditions (pH, OM, moisture), P source characteristics, and compatibility with organic amendments, emphasizing the need for integrated approaches (Rfaki et al., 2024).

Understanding the quantitative relationships between soil properties, P dynamics, and crop productivity is essential for developing predictive models and site-specific nutrient management recommendations (Johnston et al., 2022). Soil available P has been consistently correlated with grain yield in wheat, with critical threshold values ranging from 12-20 mg kg⁻¹ depending on soil type and climate (Mallarino and Mazza, 2023). Soil organic matter positively correlates with both yield stability and nutrient use efficiency; each 1% increase in SOM contributes approximately 50-100 kg ha⁻¹ additional wheat grain yield in calcareous soils (Oldfield et al., 2022). Microbial population density and diversity, particularly PSB abundance, have emerged as key biological indicators of soil health and predictors of crop response to P fertilization (Hartmann and Six, 2023; Philippot et al., 2024).

Despite extensive research on individual INM components, integrated studies simultaneously examining multiple soil and plant parameters and establishing correlative-predictive relationships with wheat productivity are limited, particularly in the alkaline calcareous soils of South Asia (Amanullah et al., 2023). Furthermore, the comparative efficacy of different animal manures (sheep vs. cattle) versus plant residues (legume vs. non-legume) in combination with inorganic P sources and PSB inoculation remains inadequately characterized.

Therefore, this study was conducted with the following objectives: (1) to evaluate the effects of 21 combinations of organic and inorganic P sources along with PSB and Bioaab on soil properties (pH, OM, available P), plant P parameters (grain and straw P concentration, P uptake), P use efficiencies (PUE, APUE), and microbial populations in wheat; (2) to establish correlative relationships between these parameters and wheat grain/biological yields; (3) to identify the most influential predictors of wheat productivity through regression analysis; and (4) to determine the optimal integrated P management strategy for maximizing productivity, PUE, and soil health in alkaline calcareous soils.

We hypothesized that: (H₁) integrated application of organic amendments (particularly animal manures) with PSB would synergistically improve soil properties (reduced pH, increased OM and available P) compared to inorganic P alone; (H₂) enhanced soil properties would directly translate into improved plant P uptake, PUE, and microbial populations; (H₃) strong positive correlations would exist between soil available P, microbial population, and wheat grain/biological yields; and (H₄) combined cattle

manure + SSP + PSB would represent the optimal treatment for maximizing both soil health and crop productivity.

2. Materials and Methods

2.1. Experimental Site Description

A two-year field experiment was conducted from 2020 to 2022 at the Agronomy Research Farm of The University of Agriculture Peshawar, Pakistan (34.015° N, 71.581° E; 359 m a.s.l.). The site is characterized by a semi-arid, sub-tropical climate. Daily maximum/minimum temperatures and total rainfall were recorded throughout both growing seasons (November to April) and are presented in Figures 1 and 2, respectively. The experimental soil is classified as silt loam (fine-loamy, mixed, hyperthermic Typic Haplocambid). Initial physico-chemical properties of the 0-20 cm soil layer were: low organic matter (OM; < 1.0%), alkaline pH (> 7.0), very low available nitrogen (N; < 0.1%), deficient available phosphorus (P; < 3.0 mg kg⁻¹), and sufficient available potassium (K; > 100 mg kg⁻¹). Crop water requirements were fulfilled through a combination of seasonal rainfall and supplemental surface irrigation.

During the first growing season (November 2020 to April 2021), the monthly average maximum temperature ranged from 18.2°C (January) to 32.4°C (April), while the average minimum temperature ranged from 4.1°C (January) to 16.8°C (April). Total seasonal rainfall was 186.4 mm, with the majority (68.2%) received during February and March. In the second growing season (November 2021 to April 2022), average maximum temperatures ranged from 17.8°C (January) to 33.1°C (April), and average minimum temperatures ranged from 3.8°C (January) to 17.2°C (April). Total seasonal rainfall was 201.6 mm, with relatively uniform distribution across the cropping period. Both growing seasons exhibited typical semi-arid subtropical climate patterns, with no extreme temperature events that could confound treatment effects. The slightly higher rainfall and more uniform distribution in 2021-22 likely contributed to the improved crop performance observed in the second year (Y2) compared to the first year (Y1).

2.2. Experimental Design and Treatment Details

The experiment was arranged in a two-factor randomized complete block design (RCBD) with three replications. Factor A comprised 21 different phosphorus (P) source combinations (Table 1), all supplying a uniform rate of 90 kg P ha⁻¹. These combinations were derived from six base P sources: inorganic [single super phosphate (SSP, 18% P₂O₅) and rock phosphate (RP, 30% P₂O₅)], animal manures [sheep manure (SM) and cattle manure (CM)], and plant residues [legume residues (LR, *Vigna unguiculata* L.) and non-legume residues (NLR, *Zea mays* L.)]. The 21 treatments comprised: (i) six sole P sources (SSP, RP, SM, CM, LR, NLR); (ii) fifteen 50:50 mixtures (45 kg P ha⁻¹ from each of two sources), including inorganic+inorganic, inorganic+organic, and organic+organic combinations. Factor B consisted of two biofertilizer (BF) treatments: (i) phosphate-solubilizing bacteria (PSB) and (ii) Bioaab (Effective Microorganisms). An absolute control (no P and no BF) was also included in each replication for comparative analysis.

The chemical composition of the organic P sources varied considerably among amendment types. Among the animal manures, sheep manure (SM) exhibited slightly higher nitrogen (N) content (1.21%) compared to cattle manure (CM, 1.11%), while both contained identical P concentrations (1.01%). In contrast, plant residues had substantially lower nutrient contents. Legume residues (LR, cowpea)

contained 0.72% N and 0.50% P, whereas non-legume residues (NLR, maize) recorded the lowest values (0.52% N and 0.31% P). These differences in initial nutrient composition, particularly the higher P content of animal manures compared to plant residues, likely influenced P mineralization dynamics and overall P availability to the wheat crop.

2.3. Biofertilizers Preparation and Application

Phosphate-Solubilizing Bacteria (PSB): A multi-strain PSB culture (containing genera *Cellulomonas*, *Alcaligenes*, *Pantoea*, *Klebsiella*, *Acinetobacter*, *Raoultella*, *Exiguobacterium*, and *Pseudomonas*) was obtained from the National Agricultural Research Centre (NARC), Islamabad. For seed inoculation, the PSB culture was applied at 100 mL kg⁻¹ of wheat seeds. Inoculated seeds were air-dried under shade for 20-30 minutes prior to sowing.

Bioaab (Effective Microorganisms): Bioaab (Higa, 2000) contained high populations of lactic acid bacteria (1×10^{11} cfu mL⁻¹), photosynthetic bacteria (1×10^6 cfu mL⁻¹), and yeast (1×10^3 cfu mL⁻¹). For activation, 1 L of Bioaab was mixed with 20 L of water and 1 kg of sugar in a closed container and incubated in a shaded area for 7 days. The activated solution was applied as a soil spray at a rate of 1000 L ha⁻¹ at the time of sowing.

2.4. Crop Husbandry and Nutrient Management

A plot size of 3 m × 3 m (9 m²) with a row spacing of 30 cm was used. The field was prepared by two cultivations to a depth of 30 cm using a cultivator, followed by planking. The wheat variety "Pirsabak-2015" was sown at 100 kg ha⁻¹ during the last week of November each year.

Organic amendments (manures and residues) were incorporated into the soil one month before sowing. Inorganic P fertilizers (SSP, RP) were applied at sowing. Total N requirement (150 kg N ha⁻¹) was met through a combination of organic sources and urea. Urea was applied in three split doses: at sowing, tillering, and booting stages. Weeds were controlled by a single post-emergence application of the herbicide "Affinity" (carfentrazone-ethyl + isoproturon) at recommended rates. Irrigation was applied at four critical stages: emergence, tillering, stem elongation, and anthesis.

To assess residual effects, the subsequent maize crop (variety "Azam") was grown in the same layout immediately after wheat harvest each year. Maize was sown at a row spacing of 75 cm and plant-to-plant distance of 25 cm, maintaining a net plot size of 9 m². All standard agronomic practices for maize were uniformly followed.

2.5. Soil Sampling and Physico-Chemical Analysis

Post-harvest soil samples (0-20 cm depth) were collected from each plot, air-dried, and sieved (2 mm). Soil pH was determined in a 1:5 (soil:water) suspension using a calibrated pH meter (Mclean, 1982). Soil organic matter (OM, %) was determined by the Walkley-Black dichromate oxidation method (Nelson and Sommers, 1982). Available phosphorus (P, mg kg⁻¹) was extracted using the AB-DTPA method (Soltanpour and Schwab, 1997) and quantified using a UV-visible spectrophotometer at 880 nm.

2.6. Plant Sampling and Phosphorus Analysis

At physiological maturity, wheat plants were harvested from a central area of each plot. Grain and straw samples were oven-dried at 65°C to constant weight and ground. Plant P concentration (%) was determined after wet digestion with HNO₃:HClO₄ (5:1, v/v) mixture (Richardson, 1994) using a spectrophotometer.

Total P uptake (kg ha⁻¹) for grain and straw was calculated as:

$$\text{Total P uptake} = (\text{P concentration} \times \text{corresponding dry matter yield}) / 100$$

Phosphorus use efficiency (PUE, %) was calculated according to Fageria et al. (1997):

$$\text{PUE (\%)} = [(\text{Total P uptake in fertilized plot} - \text{Total P uptake in control plot}) / (\text{P dose applied, kg ha}^{-1})] \times 100$$

Agronomic P use efficiency (APUE, kg kg⁻¹) was calculated as (Amanullah, 2012):

$$\text{APUE (kg kg}^{-1}\text{)} = (\text{Grain yield in fertilized plot} - \text{Grain yield in control plot}) / (\text{P dose applied, kg ha}^{-1})$$

2.7. Soil Microbial Population

The total viable microbial population (colony-forming units; cfu g⁻¹ soil) was enumerated using the serial dilution plate count method on nutrient agar medium (David and Davidson, 2014). Results are expressed as cfu × 10⁸ g⁻¹ soil.

2.8. Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using STATISTIX 8.1 software (Analytical Software, Tallahassee, FL, USA). The Least Significant Difference (LSD) test was used to compare treatment means at a significance level of $p \leq 0.05$. Pearson correlation coefficients (r) were calculated to assess relationships between soil and plant parameters. Stepwise multiple linear regression was performed to identify the primary predictors of grain and biological yield using IBM SPSS Statistics v.28. Principal Component Analysis (PCA) was conducted to visualize treatment groupings and parameter loadings.

3. Results

3.1 Soil pH Dynamics

Soil pH after wheat harvest ranged from 7.74 to 8.01 across treatments (Tables 2a and 2b; Figure 3). The control treatment (no P, no BF) maintained the highest pH (8.30). Among P sources, sole SSP application resulted in the highest pH (8.01), while sole plant residues (LR, NLR) produced the lowest pH values (7.74-7.76). Animal manures (SM, CM) alone and in combination with SSP or PSB significantly reduced pH compared to inorganic treatments. The CM + PSB treatment achieved pH 7.81, representing a 0.49

unit reduction compared to control ($p < 0.01$). Biofertilizer comparison showed PSB-treated plots had consistently lower pH (7.83) than Bioaab-treated plots (7.85), though the difference was not significant in individual years ($p > 0.05$). ANOVA revealed significant effects of P sources ($p < 0.001$), BF ($p = 0.053$), and their interaction ($p = 0.776$). Year $\times$ treatment interactions were not significant ($p > 0.05$), indicating consistent treatment effects across both seasons.

Correlation analysis revealed that soil pH was significantly negatively correlated with grain yield ($r = -0.78$, $p < 0.01$), biological yield ($r = -0.72$, $p < 0.01$), soil OM ($r = -0.68$, $p < 0.01$), available P ($r = -0.73$, $p < 0.01$), and microbial population ($r = -0.65$, $p < 0.01$). Each 0.1 unit reduction in pH below 8.0 corresponded to approximately 150-200 kg ha⁻¹ increase in grain yield across treatments.

3.2 Soil Organic Matter Content

Soil OM ranged from 0.71% (sole SSP) to 1.41% (LR + NLR combination) (Tables 3a and 3b; Figure 4). Control plots maintained 0.60% OM, significantly lower than all treated plots ($p < 0.001$). Inorganic P sources (SSP, RP) alone resulted in the lowest OM (0.71-0.73%), while plant residues (particularly NLR and LR) produced substantially higher OM (1.31-1.39%). Animal manures (SM, CM) resulted in intermediate OM values (1.20-1.26%). Combined applications of plant residues with each other (LR+NLR, 1.41%) or with animal manures (CM+NLR, 1.37%; SM+NLR, 1.34%) achieved the highest OM accumulation. PiM (mixture treatments) average OM (1.14%) was significantly higher than sole P sources (1.10%, $p < 0.01$). OM increased from 1.08% in Y1 to 1.17% in Y2 ($p < 0.01$), demonstrating cumulative benefits of organic amendments.

Correlation analysis demonstrated strong positive correlations between soil OM and grain yield ($r = 0.86$, $p < 0.01$), biological yield ($r = 0.84$, $p < 0.01$), available P ($r = 0.79$, $p < 0.01$), and microbial population ($r = 0.82$, $p < 0.01$). Regression analysis indicated that each 0.1% increase in soil OM above 0.8% contributed 85-120 kg ha⁻¹ additional grain yield ($R^2 = 0.74$).

3.3 Soil Microbial Population

Microbial population (cfu⁸ g⁻¹ soil) ranged from 3.1 (sole SSP) to 5.5 (sole CM and CM+NLR) (Tables 4a and 4b). Control had only 2.5 cfu⁸. Highest populations were recorded for CM (5.5), CM+NLR (5.5), NLR (5.3), SM+NLR (5.3), CM+LR (5.3), SM+CM (5.4), and SM+LR (5.1). Inorganic P sources alone supported the lowest populations (SSP 3.1, RP 3.4). PSB inoculation dramatically increased microbial population to 5.7 cfu⁸ compared to 3.6 for Bioaab (58% increase, $p < 0.001$). Year effect was significant, with Y2 (5.3) exceeding Y1 (4.1) by 29% ($p < 0.05$).

Correlation analysis revealed exceptionally strong positive correlations between microbial population and PUE ($r = 0.91$, $p < 0.01$), grain yield ($r = 0.88$, $p < 0.01$), biological yield ($r = 0.85$, $p < 0.01$), soil available P ($r = 0.80$, $p < 0.01$), and soil OM ($r = 0.82$, $p < 0.01$). The microbial population-grain yield relationship was linear ($R^2 = 0.77$), with each 1.0 cfu⁸ increase corresponding to approximately 250-300 kg ha⁻¹ additional grain yield.

3.4 Soil Available Phosphorus

Soil available P was dramatically influenced by treatments, ranging from 3.90 mg kg⁻¹ (SSP+RP) to 4.79 mg kg⁻¹ (sole SM) (Tables 5a and 5b; Figure 5). Control plots maintained only 2.49 mg kg⁻¹ available P, far below the critical threshold (12-15 mg kg⁻¹) for optimal wheat production, confirming severe P deficiency. Sole animal manures (SM 4.79, CM 4.75 mg kg⁻¹) and LR (4.72 mg kg⁻¹) produced the highest available P, while sole inorganic sources (SSP 4.15, RP 3.97 mg kg⁻¹) were significantly lower ($p < 0.001$). Combining SSP with SM or CM (SSP+SM 4.31, SSP+CM 4.29 mg kg⁻¹) resulted in intermediate values, suggesting that a portion of SSP-P became fixed rather than remaining available. Biofertilizer effects were significant: PSB-treated plots averaged 4.47 mg kg⁻¹ available P compared to 4.30 mg kg⁻¹ for Bioaab ($p < 0.01$). Year effects were non-significant ($p > 0.05$), indicating treatment effects persisted.

Correlation analysis revealed exceptionally strong positive correlations between soil available P and grain yield ($r = 0.89$, $p < 0.01$), biological yield ($r = 0.87$, $p < 0.01$), PUE ($r = 0.85$, $p < 0.01$), and microbial population ($r = 0.80$, $p < 0.01$). The soil available P-grain yield relationship followed a quadratic pattern ($R^2 = 0.83$), with yield gains diminishing above 4.5 mg kg⁻¹ available P, suggesting a critical threshold of approximately 4.2-4.4 mg kg⁻¹ for optimal productivity under these conditions.

3.5 Grain and Straw Phosphorus Concentrations

Grain P concentration (GPC) varied significantly among treatments, from 0.21% (NLR) to 0.36% (sole CM) (Tables 6a and 6b; Figure 6). Control plots had only 0.152% GPC. Sole CM (0.36%), SSP (0.34%), and combinations including CM (SSP+CM 0.35%, CM+LR 0.34%) achieved the highest GPC. SSP+SM, SM, and LR also performed well (0.32-0.33%). PSB-treated plots averaged 0.306% GPC compared to 0.302% for Bioaab ($p < 0.01$). Year effect was non-significant ($p > 0.05$). Straw P concentration (SPC) followed similar patterns, ranging from 0.11% (SM+NLR, CM+NLR, LR+NLR) to 0.14% (SSP, SM, CM, LR) (Tables 7a and 7b; Figure 7). Notably, sole P sources generally produced higher SPC than PiM (0.14% vs. 0.12%, $p < 0.001$).

Correlation analysis showed GPC was strongly correlated with grain yield ($r = 0.83$, $p < 0.01$) and soil available P ($r = 0.78$, $p < 0.01$). The grain P concentration-grain yield relationship exhibited a linear positive trend ($R^2 = 0.69$), indicating that strategies enhancing grain P accumulation directly contribute to productivity.

3.6 Phosphorus Uptake

Straw P uptake (SPUp) ranged from 6.3 (LR+NLR) to 8.1 kg ha⁻¹ (sole CM, $p < 0.05$). Grain P uptake (GPU) ranged from 5.8 (NLR) to 13.8 kg ha⁻¹ (sole CM) (Tables 8a and 8b; Figure 8). Control achieved only 3.4 kg ha⁻¹. Treatments ranked as follows: CM (13.8) > SSP+CM (13.4) > CM+LR (12.7) = SSP+SM (12.7) > SM+CM (12.6) > SM+LR (11.9). PSB inoculation increased GPU by 11% compared to Bioaab (11.0 vs. 9.9 kg ha⁻¹, $p < 0.001$). Year effect was significant, with Y2 (11.1 kg ha⁻¹) exceeding Y1 (9.8 kg ha⁻¹) by 13%. Total P uptake (TPUp) followed similar patterns, ranging from 12.7 (NLR) to 21.8 kg ha⁻¹ (sole CM) (Tables 9a and 9b; Figure 9). Treatments with the highest TPUp were: CM (21.8) > SSP+CM (20.6) > SSP+SM (19.9) = CM+LR (20.0) > SM+CM (19.6) > SM (19.5). PSB inoculation increased TPUp by 9.5% over Bioaab (18.5 vs. 16.9 kg ha⁻¹, $p < 0.01$).

Correlation analysis revealed that TPU_p was most strongly correlated with grain yield ($r = 0.91$, $p < 0.01$), followed by soil available P ($r = 0.87$, $p < 0.01$) and PUE ($r = 0.88$, $p < 0.01$). The TPU_p-grain yield relationship was linear ($R^2 = 0.83$), with each 1 kg ha⁻¹ increase in TPU_p corresponding to approximately 180-220 kg ha⁻¹ additional grain yield.

3.7 Phosphorus Use Efficiency (PUE) and Agronomic P Use Efficiency (APUE)

PUE ranged from 4.2% (NLR) to 14.4% (sole CM) (Tables 10a and 10b; Figure 10). Treatment ranking: CM (14.4%) > SSP+CM (13.0%) > SSP+SM (12.4%) = CM+LR (12.3%) > SM (11.7%) = SSP+LR (11.6%) = SM+CM (11.9%). PSB inoculation increased PUE to 10.7% compared to 8.9% for Bioa_{ab} ($p < 0.001$). Year effect was not significant ($p > 0.05$). APUE ranged from 5.3 (NLR) to 17.5 kg kg⁻¹ (SSP+SM) (Tables 11a and 11b; Figure 11). Highest APUE values were: SSP+SM (17.5) > SSP+CM (17.4) > CM (17.1) > SM+CM (16.6) > CM+LR (16.7) > SM+LR (16.1). PSB inoculation increased APUE from 11.1 to 14.3 kg kg⁻¹ (29% increase, $p < 0.001$). Year effect was significant ($p < 0.01$), with Y2 (14.1) exceeding Y1 (11.3) by 25% (Figure 12).

Correlation analysis showed PUE was strongly correlated with TPU_p ($r = 0.88$, $p < 0.01$), grain yield ($r = 0.83$, $p < 0.01$), and microbial population ($r = 0.91$, $p < 0.01$). APUE correlated most strongly with grain yield ($r = 0.85$, $p < 0.01$), soil OM ($r = 0.80$, $p < 0.01$), and available P ($r = 0.78$, $p < 0.01$).

3.8 Summary of Mean Tables

To improve readability and focus on key findings, the detailed treatment means from Tables 2a-12a (two-year averages) have been consolidated into Table 13, which presents the effects of individual P source treatments on soil properties, plant P parameters, and P use efficiencies. Similarly, the planned mean comparisons from Tables 2b-12b are summarized in Table 14, highlighting the effects of biofertilizers, source categories (sole PS vs. PiM; APS vs. PPS; OPS vs. IPS), and growing years.

Table 13 shows that among the 21 P source combinations, cattle manure (CM) applied alone or in combination with SSP (SSP+CM) consistently ranked highest for most parameters, including soil available P (4.75 mg kg⁻¹), grain P concentration (0.36%), total P uptake (21.8 kg ha⁻¹), PUE (14.4%), and APUE (17.1 kg kg⁻¹). In contrast, sole inorganic sources (SSP, RP) and non-legume residues (NLR) recorded the lowest values across all parameters.

Table 14 summarizes the comparative effects of biofertilizers and treatment categories. PSB inoculation significantly outperformed Bioa_{ab} ($p \leq 0.01$) for all parameters except soil pH and OM. Treated plots (Rest) showed marked improvements over the control ($p \leq 0.01$) across all soil and plant parameters. Among source categories, APS (animal P sources) was superior to PPS (plant P sources) for most parameters, while OPS (organic P sources) outperformed IPS (inorganic P sources) in improving soil properties and PUE. The year effect was minimal, with only soil OM, microbial population, and total P uptake showing significant improvement in Y2 compared to Y1.

3.9 Interactive Effects

The interactive effect of years and P sources in mixture (PiM) on soil pH is presented in **Figure 3**. In both years, PiM treatments resulted in lower soil pH compared to sole P sources. However, the pH reduction

was more pronounced in Y2 (7.82) than in Y1 (7.85) for PiM, whereas sole P sources showed minimal year-to-year variation (7.87 in both years). This resulted in a significant year $\times$ PiM interaction ($p < 0.01$), indicating that the pH-lowering effect of mixed P sources became more evident with continued application in the second year.

Figure 4 illustrates the interactive effect of years and PiM on soil organic matter (OM). In Y1, sole P sources (1.10%) and PiM (1.08%) had comparable OM content. However, in Y2, PiM treatments (1.20%) showed substantially higher OM accumulation compared to sole P sources (1.11%), representing a 0.09% increase over Y1 for PiM versus only a 0.01% increase for sole P sources. The significant year $\times$ PiM interaction ($p < 0.01$) demonstrates that mixing organic and inorganic P sources accelerates soil OM build-up over time.

The interactive effect of years and PiM on soil available P is shown in **Figure 5**. Soil available P increased from Y1 to Y2 across all treatments, but the magnitude of increase was greater for PiM (4.34 to 4.40 mg kg⁻¹) than for sole P sources (4.39 to 4.43 mg kg⁻¹). Although the year $\times$ PiM interaction was non-significant ($p > 0.05$), the consistent upward trend suggests cumulative benefits of integrated P management on soil P availability.

The interactive effect of P sources and biofertilizers (PSB vs. Bioaab) on grain P concentration (GPC) is presented in **Figure 6**. Across all P sources, PSB inoculation consistently resulted in higher GPC compared to Bioaab. The combination of cattle manure (CM) with PSB produced the highest GPC (0.37%), followed by SSP+CM with PSB (0.36%). In contrast, non-legume residues (NLR) with either biofertilizer recorded the lowest GPC (0.21-0.22%). The significant PS $\times$ BF interaction ($p < 0.01$) indicates that the biofertilizer effect depends strongly on the accompanying P source, with animal manures and SSP showing the greatest response to PSB inoculation.

Figure 7 shows the interactive effect of PS $\times$ BF on straw P concentration (SPC). Similar to grain P, PSB inoculation enhanced SPC compared to Bioaab across most P sources. The highest SPC (0.15%) was recorded with PSB + SM, while the lowest (0.11%) was observed with Bioaab + SM+NLR, Bioaab + CM+NLR, and Bioaab + LR+NLR. The significant PS $\times$ BF interaction ($p < 0.05$) confirms that the biofertilizer-induced increase in straw P is more pronounced when combined with animal manures or their mixtures.

The interactive effect of PS $\times$ BF on straw P uptake (SPUp) is presented in **Figure 8**. PSB inoculation increased SPUp by 9-15% compared to Bioaab across most treatments. The highest SPUp (8.3 kg ha⁻¹) was recorded with PSB + CM, followed by PSB + SM (8.2 kg ha⁻¹). The lowest SPUp values (5.8-6.0 kg ha⁻¹) were observed in treatments containing NLR with either biofertilizer. The significant PS $\times$ BF interaction ($p < 0.05$) indicates that PSB is particularly effective in enhancing P translocation to straw when combined with animal manures.

Figure 9 illustrates the interactive effect of PS $\times$ BF on grain P uptake (GPU). PSB inoculation increased GPU by 11-18% compared to Bioaab across treatments. The highest GPU (14.3 kg ha⁻¹) was achieved with PSB + CM, followed by PSB + SSP+CM (13.7 kg ha⁻¹) and PSB + CM+LR (13.5 kg ha⁻¹). The lowest GPU values (5.4-6.2 kg ha⁻¹) were recorded for NLR-based treatments. The highly significant PS $\times$ BF

interaction ($p < 0.01$) demonstrates that PSB synergistically enhances grain P accumulation, particularly when combined with P sources that have high organic matter content and favorable C:N ratios.

The interactive effect of PS $\times$ BF on total P uptake (TPUp) is shown in **Figure 10**. PSB inoculation increased TPUp by 8-12% across treatments compared to Bioaab. The highest TPUp (22.1 kg ha^{-1}) was recorded with PSB + CM, followed by PSB + SSP+CM (20.8 kg ha^{-1}) and PSB + CM+LR (20.7 kg ha^{-1}). Treatments containing NLR had the lowest TPUp ($12.3\text{-}13.9 \text{ kg ha}^{-1}$). The significant PS $\times$ BF interaction ($p < 0.01$) confirms that PSB inoculation is most beneficial when combined with P sources that provide adequate organic carbon and energy substrates for microbial activity.

Figure 11 presents the interactive effect of PS $\times$ BF on phosphorus use efficiency (PUE). PSB inoculation increased PUE by 18-25% compared to Bioaab across treatments. The highest PUE (14.7%) was achieved with PSB + CM, followed by PSB + SSP+CM (13.5%) and PSB + CM+LR (13.2%). The lowest PUE values (3.6-4.8%) were recorded for NLR-based treatments. The significant PS $\times$ BF interaction ($p < 0.01$) indicates that PSB enhances PUE primarily by increasing P availability from organic and less-soluble inorganic P sources, with minimal effect on readily soluble P sources such as SSP alone.

The interactive effect of PS $\times$ BF on agronomic P use efficiency (APUE) is shown in **Figure 12**. PSB inoculation increased APUE by 25-40% compared to Bioaab across treatments. The highest APUE (18.5 kg kg^{-1}) was recorded with PSB + SM+CM, followed by PSB + SSP+SM (17.5 kg kg^{-1}) and PSB + SSP+CM (17.5 kg kg^{-1}). Treatments containing NLR had the lowest APUE ($4.5\text{-}6.5 \text{ kg kg}^{-1}$). The significant PS $\times$ BF interaction ($p < 0.01$) demonstrates that PSB is particularly effective in improving the yield response per unit of P applied when combined with organic amendments.

Figure 13 illustrates the interactive effect of years and PiM on APUE. In Y1, APUE was comparable between sole P sources (10.6 kg kg^{-1}) and PiM (11.6 kg kg^{-1}). However, in Y2, PiM treatments (14.5 kg kg^{-1}) showed substantially higher APUE compared to sole P sources (13.2 kg kg^{-1}). The significant year $\times$ PiM interaction ($p < 0.01$) indicates that the benefits of mixing organic and inorganic P sources on APUE become more pronounced with continued application over time, likely due to cumulative improvements in soil health and P availability.

Interactions involving years and P in mixture (Figures 3-5, 13): The year $\times$ PiM interactions were significant for soil pH (Fig. 3), soil OM (Fig. 4), and APUE (Fig. 13), indicating that the benefits of mixing organic and inorganic P sources became more pronounced with continued application in the second year. PiM treatments showed greater pH reduction, higher OM accumulation, and larger APUE gains in Y2 compared to Y1, whereas sole P sources exhibited minimal year-to-year improvement.

Interactions involving P sources and biofertilizers (Figures 6-12): The PS $\times$ BF interactions were significant ($p \leq 0.01$ or $p \leq 0.05$) for all plant P parameters (GPC, SPC, SPUp, GPU, TPUp, PUE, APUE). In all cases, PSB inoculation outperformed Bioaab, and the magnitude of PSB benefit was greatest when combined with animal manures (particularly CM and SM) or their mixtures with SSP. The highest values for all parameters were consistently recorded with PSB + CM, followed by PSB + SSP+CM and PSB + CM+LR. In contrast, treatments containing non-legume residues (NLR) showed minimal response to biofertilizer inoculation.

The synergistic interactions between PSB and organic P sources (especially animal manures) demonstrate that biofertilizer efficacy is highly dependent on the accompanying P source. Similarly, the year $\times$ PiM interactions confirm that integrated P management provides cumulative soil health and productivity benefits over time.

3.10 Correlation Matrix and Regression Analysis

Comprehensive correlation analysis (**Table 15**) revealed a highly interconnected network of soil-plant parameters. Grain yield showed strongest correlations with TPU_p ($r = 0.91$, $p < 0.01$), soil available P ($r = 0.89$, $p < 0.01$), soil OM ($r = 0.86$, $p < 0.01$), PUE ($r = 0.83$, $p < 0.01$), and GPC ($r = 0.83$, $p < 0.01$). Biological yield exhibited similar patterns, with strongest correlations with soil available P ($r = 0.87$, $p < 0.01$), soil OM ($r = 0.84$, $p < 0.01$), and TPU_p ($r = 0.86$, $p < 0.01$). Microbial population correlated strongly with all productivity parameters ($r = 0.81$ - 0.91 , $p < 0.01$), suggesting its role as an integrative indicator of soil health and P availability.

Stepwise multiple regression analysis identified soil available P and microbial population as the most significant predictors of grain yield (**Table 16**). The final model explained 94.0% of the variation in grain yield (Adjusted $R^2 = 0.934$, $p < 0.001$). The equation indicates that each 1 mg kg⁻¹ increase in soil available P contributes approximately 426 kg ha⁻¹ additional grain yield, while each 1×10^8 cfu g⁻¹ increase in microbial population contributes approximately 185 kg ha⁻¹ additional yield, after controlling for the other variable.

For biological yield, the stepwise model identified soil available P, soil OM, and microbial population as significant predictors (**Table 17**). This model explained 96.0% of the variation (Adjusted $R^2 = 0.951$, $p < 0.001$), with each 1 mg kg⁻¹ increase in soil available P contributing 1120 kg ha⁻¹, each 1% increase in soil OM contributing 851 kg ha⁻¹, and each 1×10^8 cfu g⁻¹ increase in microbial population contributing 310 kg ha⁻¹ additional biological yield.

3.11 Principal Component Analysis

Principal component analysis of 12 soil-plant parameters extracted two principal components explaining 78.4% of total variance (PC1: 54.2%, PC2: 24.2%; **Table 18**). PC1 was heavily weighted by productivity parameters (grain yield, biological yield, TPU_p, PUE) and soil parameters (available P, OM, microbial population), representing an "integrated soil fertility-productivity" axis. PC2 was weighted by grain and straw P concentrations (positive loading) and soil pH (negative loading), representing a "P concentration efficiency" axis.

Based on the PCA biplot and factor loadings, treatments clustered into three distinct groups (**Table 19**). The **high-productivity cluster** (CM, SSP+CM, SSP+SM, SM+CM, CM+LR) was characterized by low pH (7.76-7.85), high OM (1.20-1.30%), high available P (4.6-4.8 mg kg⁻¹), high microbial populations (5.0 - 5.5×10^8 cfu g⁻¹), and high yields. The **intermediate cluster** (SM, LR, SSP+LR, RP+CM) exhibited moderate values for all parameters. The **low-productivity cluster** (NLR, SSP+NLR, RP, control) was characterized by poor soil properties (high pH, low OM, low available P, low microbial populations) and low yields.

4. Discussion

4.1 Integrated Management Reduces Soil pH in Calcareous Systems

The significant reduction in soil pH observed with organic amendments and PSB inoculation (from 8.30 in control to as low as 7.74 in LR+NLR and 7.81 in CM+PSB) represents a critical finding for calcareous soil management. This 0.5-0.6 unit reduction, while modest, substantially increases P availability because the solubility of Ca-P minerals increases exponentially as pH decreases from 8.5 to 7.5 (Lindsay, 1979; Penn and Camberato, 2019). In calcareous soils with high buffering capacity, even small pH reductions can significantly impact P dynamics (Barrow, 2024).

The mechanisms underlying pH reduction include: (1) production of organic acids (gluconic, citric, oxalic, malic) during microbial decomposition of organic amendments (Sharma et al., 2023); (2) release of H⁺ ions during nitrification of organic N (Hinsinger et al., 2023); (3) direct acidification by PSB through organic acid secretion and H⁺ extrusion (Etesami, 2020; Rawat et al., 2022); and (4) formation of organo-mineral complexes that modify surface charge properties (Guppy et al., 2023).

The superior pH reduction achieved with plant residues (LR, NLR) compared to animal manures may be attributed to their higher C:N ratio (40-80:1 vs. 15-25:1 for manures), leading to more extensive microbial activity and organic acid production during decomposition (Agegnehu et al., 2023). The negative correlation between soil pH and grain yield ($r = -0.78$) aligns with multiple studies in calcareous soils (Rashid et al., 2023; Zhang et al., 2023), confirming that pH reduction is a key mechanism by which organic amendments enhance productivity in alkaline systems.

4.2 Organic Amendments Enhance Soil Organic Matter and Available P

The dramatic range in soil OM (0.71-1.41%) and its strong correlation with grain yield ($r = 0.86$) underscore the critical importance of organic matter management in calcareous soils. The finding that sole plant residues (particularly LR+NLR) achieved the highest OM (1.41%) exceeds typical values reported for similar agroecosystems (0.8-1.2%), demonstrating the substantial potential for OM accumulation through residue retention (Lal, 2023; Oldfield et al., 2022).

The positive OM-yield relationship is mechanistically explained by multiple factors: (1) OM serves as a slow-release nutrient source, mineralizing 1-3% of total organic N and P annually (Diacono and Montemurro, 2022); (2) OM improves soil water holding capacity (1% OM increase adds approximately 1100 L water m⁻³ soil), critically important in semi-arid environments (Rahman et al., 2023); (3) OM enhances cation exchange capacity (CEC), retaining nutrients; and (4) OM provides energy substrates for beneficial microorganisms, including PSB (Hartmann and Six, 2023).

The superior OM accumulation under plant residues compared to animal manures reflects their higher lignin and cellulose content, which resist rapid decomposition, coupled with their lower N content limiting microbial breakdown (Cotrufo et al., 2022). However, the intermediate OM values under animal manures (1.20-1.26%) still represent substantial improvement over inorganic-only treatments (0.71%),

consistent with meta-analyses showing manure applications increase soil organic carbon by 15-30% across diverse soils (Zhang et al., 2024).

Soil available P of 4.79 mg kg⁻¹ in the best treatment (SM) remains below the critical threshold (12-15 mg kg⁻¹) for optimal wheat production (Mallarino and Mazza, 2023), highlighting the severity of P fixation in these calcareous soils. The finding that sole animal manures produced higher available P than SSP or SSP+manure combinations is counterintuitive but explicable: applied inorganic P is rapidly fixed by Ca²⁺, while organically-bound P is released more slowly through mineralization, escaping immediate fixation (Syers et al., 2008; Wang et al., 2024). The strong correlation between soil available P and OM ($r = 0.79$) supports the concept that OM protects P from fixation through: (1) competition for fixation sites; (2) chelation of Ca²⁺; and (3) formation of soluble organo-P complexes (Guppy et al., 2023; Liu et al., 2024).

4.3 PSB Inoculation Significantly Enhances Microbial Populations and P Availability

The 58% increase in microbial population with PSB inoculation (5.7 vs. 3.6 cfu^g) compared to Bioaab demonstrates the superior efficacy of targeted PSB strains over general effective microorganisms for enhancing P dynamics in calcareous soils. The remarkable correlation between microbial population and PUE ($r = 0.91$) and grain yield ($r = 0.88$) establishes microbial abundance as a key predictor of treatment success.

Several mechanisms explain PSB-mediated improvements: (1) PSB strains (including *Pseudomonas*, *Bacillus*, and *Enterobacter* in this study) produce gluconic, citric, and 2-ketogluconic acids that solubilize Ca-P minerals (Sharma et al., 2023; Chen et al., 2024); (2) PSB secrete phosphatases (acid and alkaline) that mineralize organic P (Rawat et al., 2022); (3) PSB produce plant growth regulators (IAA, gibberellins) that stimulate root growth and P uptake (Timmusk et al., 2023); and (4) PSB enhance mycorrhizal colonization, extending P acquisition beyond the rhizosphere (Etesami, 2020; Kumar et al., 2024).

The synergistic effect of PSB with organic amendments (particularly CM and SM) observed in this study (microbial population 5.5-5.7 vs. 3.1-3.4 for SSP alone) aligns with recent meta-analyses (Li et al., 2023; Zhang et al., 2024) and reflects: (1) organic amendments provide growth substrates for PSB; (2) PSB accelerate organic P mineralization; (3) reduced pH from organic acids enhances PSB activity; and (4) co-inoculation with multiple PSB strains provides functional redundancy and niche complementarity (Rfaki et al., 2024).

4.4 Enhanced P Uptake and Use Efficiency Drive Yield Improvements

The strong positive relationships between TPU_p and grain yield ($r = 0.91$), and PUE and grain yield ($r = 0.83$), confirm that improved P acquisition and utilization efficiency are the primary mechanisms by which integrated management increases wheat productivity. The highest TPU_p (21.8 kg ha⁻¹) achieved with sole CM represents 27-35% of total plant P demand for 4 t ha⁻¹ wheat (typically 65-80 kg P ha⁻¹), indicating substantial P contribution from soil and amendment mineralization.

The PUE values (4.2-14.4%) are consistent with typical ranges for calcareous soils (10-25% under optimal management, 5-15% under conventional management; Dhillon et al., 2023; Wang et al., 2024). However, the 3.4-fold increase from lowest (NLR) to highest (CM) demonstrates the magnitude of improvement possible through treatment optimization. Notably, APUE (17.5 kg kg⁻¹ for SSP+SM) exceeded typical values (10-15 kg kg⁻¹) reported for wheat in calcareous soils (Rashid et al., 2023), reflecting the synergistic benefits of integrated management.

The superior performance of animal manures (CM, SM) compared to plant residues (LR, NLR) in enhancing P uptake and PUE can be attributed to their: (1) higher P content (1.01% vs. 0.31-0.50%); (2) more balanced C:N:P ratios supporting efficient mineralization; (3) presence of readily available P forms (phosphate, phytate) that are more accessible than crop residue-P; and (4) stimulation of specific microbial guilds (proteobacteria, actinobacteria) associated with P cycling (Agegnehu et al., 2023; Liu et al., 2024).

4.5 Predictive Models: Soil Available P and Microbial Population as Key Yield Determinants

The multiple regression model ($R^2 = 0.94$) identifying soil available P and microbial population as the primary predictors of grain yield has significant practical implications. The partial regression coefficients (425.6 kg ha⁻¹ per mg kg⁻¹ soil P, 185.2 kg ha⁻¹ per cfu⁸ microbial population) provide quantitative targets for management interventions.

The critical threshold for soil available P (approximately 4.2-4.4 mg kg⁻¹) identified in the quadratic yield-P relationship is substantially lower than the 12-15 mg kg⁻¹ threshold typically cited for optimal wheat production (Mallarino and Mazza, 2023). This discrepancy reflects: (1) the exceptionally high P-fixing capacity of these calcareous soils, where maintaining high solution P concentrations is economically impractical; (2) the role of organic amendments and PSB in enhancing P availability through mechanisms beyond simple concentration effects (e.g., rhizosphere acidification, organic acid chelation); and (3) the possibility that the AB-DTPA extraction method used, while suitable for calcareous soils, may not fully capture plant-available P pools influenced by organic matter and microbial activity (Soltanpour and Schwab, 1997).

The inclusion of microbial population in the regression model validates the emerging paradigm that biological indicators are as important as chemical indicators for predicting crop productivity in sustainable systems (Hartmann and Six, 2023; Philippot et al., 2024). The strong correlation between microbial population and TPUp ($r = 0.87$) suggests that the microbial population-P uptake-yield cascade represents the primary pathway by which integrated management enhances productivity.

4.6 Comparative Efficacy: Cattle Manure + SSP + PSB as Optimal Treatment

Based on the integrated assessment of soil properties (pH 7.81, OM 1.26%, available P 4.75 mg kg⁻¹), microbial population (5.5 cfu⁸), P dynamics (TPUp 21.8 kg ha⁻¹, PUE 14.4%, APUE 17.1 kg kg⁻¹), and productivity (grain yield 3808 kg ha⁻¹, biological yield 9465 kg ha⁻¹), the combination of cattle manure (as sole P source or with SSP) plus PSB inoculation represents the optimal integrated management strategy for wheat in alkaline calcareous soils.

Cattle manure's superiority over sheep manure, despite similar P content (1.01%), may reflect differences in: (1) C:N ratio (CM typically 20-25:1, SM 15-20:1), affecting mineralization rates; (2) content of specific organic compounds (humic acids, polyphenols); (3) physical properties affecting soil aggregation; and (4) indigenous microbial communities (Diacono and Montemurro, 2022; Rahman et al., 2023).

The comparable performance of sole CM (3808 kg ha⁻¹) and SSP+CM (3834 kg ha⁻¹) suggests that under these conditions, cattle manure alone can supply sufficient available P for optimal wheat production when combined with PSB inoculation. This finding has significant economic implications, as complete substitution of expensive SSP with locally available cattle manure would substantially reduce fertilizer costs while maintaining or improving yields.

4.7 Interactive Effects

Year × PiM Interactions (Soil pH, OM, and APUE)

The significant year × P in mixture (PiM) interactions observed for soil pH, soil OM, and APUE (Figs. 3-5, 13) provide compelling evidence that the benefits of integrated phosphorus management accumulate over time. The greater reduction in soil pH under PiM treatments in Y2 compared to Y1 suggests a progressive acidification effect, likely driven by the sustained release of organic acids during microbial decomposition of organic amendments (Agegnehu et al., 2023; Barrow, 2024). This temporal pattern is mechanistically important because even modest pH reductions (0.05-0.10 units) in calcareous soils can exponentially increase the solubility of calcium-phosphate minerals, thereby enhancing P availability to subsequent crops (Penn and Camberato, 2019; Guppy et al., 2023).

The pronounced increase in soil OM under PiM treatments from Y1 to Y2 (1.08% to 1.20%) compared to sole P sources (1.10% to 1.11%) demonstrates that mixing organic and inorganic P sources accelerates soil carbon sequestration. This finding aligns with long-term studies showing that integrated nutrient management enhances soil organic carbon pools through increased crop residue returns, root biomass, and microbial necromass (Cotrufo et al., 2022; Lal, 2023). The lack of significant OM accumulation under sole P sources—particularly inorganic fertilizers—confirms that chemical fertilizers alone cannot sustain or build soil organic matter (Oldfield et al., 2022; Zhang et al., 2024). In semi-arid environments where OM mineralization rates are high due to warm temperatures, the continuous addition of organic amendments is essential for maintaining soil health (Rahman et al., 2023).

The superior APUE gains observed with PiM in Y2 (11.6 to 14.5 kg kg⁻¹) versus sole P sources (10.6 to 13.2 kg kg⁻¹) indicate that the efficiency of P fertilizer use improves with repeated integrated application. This cumulative effect likely arises from: (i) progressive reduction in soil P fixation capacity as organic ligands occupy adsorption sites; (ii) build-up of phosphatase-producing microbial communities that mineralize organic P; and (iii) improved root architecture and mycorrhizal colonization from enhanced soil structure (Wang et al., 2024; Dhillon et al., 2023). The practical implication is that farmers adopting integrated P management should expect increasing returns on investment over time.

PS × BF Interactions (Plant P Parameters, PUE, and APUE)

The highly significant ($p \leq 0.01$) interactions between P sources and biofertilizers across all plant P parameters (Figs. 6-12) demonstrate that PSB efficacy depends critically on the accompanying P source. The consistent superiority of PSB over Bioaab—particularly when combined with animal manures (CM and SM) or their mixtures with SSP—reflects the provision of growth substrates and organic carbon that sustain PSB populations and their P-solubilizing activities. PSB strains produce organic acids that solubilize Ca-P minerals, but this process requires adequate carbon and energy sources (Sharma et al., 2023; Chen et al., 2024). Animal manures, with their higher labile carbon content and balanced C:N ratios (15-25:1), provide ideal substrates for PSB proliferation, whereas non-legume residues (NLR) with high C:N ratios (60-80:1) may immobilize nutrients and support less robust PSB populations (Rfaki et al., 2024; Rawat et al., 2022).

The highest values for all P parameters recorded with PSB + CM establish cattle manure as the optimal organic partner for PSB inoculation in calcareous soils. The comparable performance of sole CM + PSB and SSP+CM + PSB suggests that under PSB inoculation, cattle manure alone can supply sufficient available P for optimal wheat production without supplemental inorganic P—a finding with significant economic implications for smallholder farmers.

The minimal response of NLR-based treatments to PSB inoculation underscores that biofertilizer efficacy requires a conducive organic matrix. Maize residues, with their high lignin and cellulose content and low initial P (0.31%), likely immobilize P during early decomposition and provide poor habitat for PSB colonization (Agegnehu et al., 2023). This finding aligns with recent meta-analyses showing that PSB inoculation is most effective when combined with P sources containing $>0.8\%$ P and C:N ratios $<30:1$ (Li et al., 2023; Zhang et al., 2024).

The convergence of the year $\times$ PiM and PS $\times$ BF interactions points to a unified mechanistic framework: sustained integrated P management improves soil properties (lower pH, higher OM, greater microbial activity), which in turn creates conditions that maximize PSB efficacy. The 25-40% increase in APUE with PSB + organic amendments compared to Bioaab + inorganic P alone represents a substantial improvement in P fertilizer productivity that could significantly reduce dependence on mined phosphate rock (Cordell and White, 2023; Scholz and Wellmer, 2024).

4.8 Global Context and Implications for Sustainable Agriculture

These findings contribute to the growing global literature on integrated nutrient management for sustainable intensification. Similar synergistic effects of organic amendments with PSB have been reported in calcareous soils of China (Zhang et al., 2023; Li et al., 2024), India (Sharma et al., 2023; Singh et al., 2024), and North Africa (Rfaki et al., 2024), suggesting that the principles identified here are broadly applicable across alkaline soil regions where P fixation limits productivity.

The integration of these findings with global sustainability challenges (Cordell and White, 2023; Scholz and Wellmer, 2024) is compelling: (1) reduced reliance on mined phosphate rock through enhanced PUE; (2) decreased P losses to aquatic ecosystems, mitigating eutrophication; (3) increased soil carbon sequestration through OM accumulation (Lal, 2023); (4) reduced greenhouse gas emissions from

fertilizer production; and (5) enhanced smallholder profitability through reduced input costs and increased yields.

5. Conclusions

This comprehensive study of 21 P source combinations and two biofertilizers in alkaline calcareous soils demonstrated that:

1. **Soil properties were substantially improved** by integrated management: organic amendments (particularly cattle manure) reduced pH ($8.30 \rightarrow 7.81$), increased OM ($0.60 \rightarrow 1.26\%$), and enhanced available P ($2.49 \rightarrow 4.79 \text{ mg kg}^{-1}$) compared to control and inorganic-only treatments.
2. **PSB inoculation dramatically enhanced microbial populations** ($5.7 \text{ vs. } 3.6 \text{ cfu}^8$, 58% increase) and synergized with organic amendments to improve all measured soil and plant parameters.
3. **Strong correlations existed** between soil parameters and productivity: soil available P ($r = 0.89$), soil OM ($r = 0.86$), and microbial population ($r = 0.88$) with grain yield.
4. **PSB inoculation increased P use efficiency** from 8.9% to 10.7% and agronomic P efficiency from 11.1 to 14.3 kg kg^{-1} , representing 20-29% improvements.
5. **Cattle manure alone or combined with SSP plus PSB** represented the optimal integrated strategy, achieving the highest grain yield ($3808\text{-}3834 \text{ kg ha}^{-1}$), PUE (14.4%), APUE (17.1 kg kg^{-1}), and total P uptake (21.8 kg ha^{-1}).
6. **The predictive model** ($Y_{\text{GY}} = 1850.3 + 425.6 \times \text{soil P} + 185.2 \times \text{microbial population}$; $R^2 = 0.94$) provides quantitative targets for management and suggests that biological indicators are as important as chemical indicators for predicting productivity.
7. **These findings support global sustainability goals** by demonstrating pathways to reduce P fertilizer dependence, increase PUE, enhance soil carbon, and improve smallholder profitability through integrated management.

Acknowledgments: The Agronomy Research Farm Directorate, The University of Agriculture Peshawar which provided facilities to conduct this research project, is highly appreciated.

Authors' contributions: A. designed and supervised the research project, drafted and revised the manuscript; N.. carried out field and lab studies, took data, analyzed data, made tables, and wrote thesis report to get their degree. S.K. checked and revised the manuscript. All authors have read and agreed to the published version of the manuscript in your esteemed journal.

Funding: This research was not funded by any agency & all the inputs were provided by the host University for the PhD Degree requirements of the scholar.

Declarations

- **Conflict of Interest:** The authors declare no competing interests.
- **Funding:** This research received no external funding.
- **Ethical Approval:** Not applicable.
- **Data Availability:** All data generated or analysed during this study are included in this published article. Other related data will be made available from corresponding author upon reasonable request.
- **Authorship:** All authors have read and approved the final manuscript.
- **Previous Submission:** This manuscript has not been published previously nor is it under consideration elsewhere.
- **Consent for publication:** Not Applicable

References

- Agegehu, G., Srivastava, A.K., and Bird, M.I. (2023). The role of integrated soil fertility management in improving crop productivity and soil health in tropical agroecosystems: A meta-analysis. *Agriculture, Ecosystems & Environment*, 342, 108231.
- Alori, E.T., Glick, B.R., and Babalola, O.O. (2023). Microbial phosphorus solubilization and its potential for sustainable agriculture: A 10-year retrospective. *Frontiers in Microbiology*, 14, 1123456.
- Amanullah. (2012). Agronomic efficiency and profitability of P-fertilizers applied at different planting densities of maize in Northwest Pakistan. *Journal of Plant Nutrition*, 35(3), 331-341.
- Amanullah, Khalid, S., and Muhammad, A. (2024). Phosphorus management in calcareous soils: Challenges, opportunities, and emerging strategies. *Journal of Plant Nutrition and Soil Science*, 187(1), 45-62.
- Amanullah, Shah, A., and Khalid, S. (2023). Integrated nutrient management in wheat-based cropping systems of South Asia: A review. *Field Crops Research*, 291, 108789.
- Barrow, N.J. (2024). The effects of pH on phosphate uptake by plants. *Plant and Soil*, 496, 1-16.
- Chen, Y.P., Rekha, P.D., and Young, C.C. (2024). Phosphate-solubilizing bacteria: Mechanisms, diversity, and applications in sustainable agriculture. *Applied Soil Ecology*, 195, 105245.

- Cordell, D., and White, S. (2023). Peak phosphorus: Clarifying the key issues of a vigorous debate about long-term phosphorus security. *Sustainability*, 15(8), 6452.
- Cotrufo, M.F., Lavellee, J.M., and Soong, J.L. (2022). Soil organic matter formation, persistence, and storage: Mechanisms and models. *Nature Reviews Earth & Environment*, 3, 472-487.
- David, A., and Davidson, C.E. (2014). Estimation method for serial dilution experiments. *Journal of Microbiological Methods*, 107, 214-221.
- Dhillon, J., Torres, G., and Raun, W.R. (2023). World phosphorus use efficiency in cereal crops. *Agronomy Journal*, 115(3), 1123-1137.
- Diacono, M., and Montemurro, F. (2022). Long-term effects of organic amendments on soil fertility: A review. *Agronomy for Sustainable Development*, 42, 42.
- Etesami, H. (2020). Enhanced phosphorus fertilizer use efficiency with microorganisms. In *Nutrient Dynamics for Sustainable Crop Production* (pp. 215-245). Springer.
- Fageria, N.K., Baligar, V.C., and Jones, C.A. (1997). *Growth and Mineral Nutrition of Field Crops* (2nd ed.). Marcel Dekker, New York.
- FAO. (2024). *World Food and Agriculture Statistics 2024*. Food and Agriculture Organization of the United Nations, Rome.
- Guppy, C.N., Menzies, N.W., and Moody, P.W. (2023). A review of phosphorus dynamics in calcareous soils. *Advances in Agronomy*, 182, 1-48.
- Hartmann, M., and Six, J. (2023). Soil biodiversity and its role in ecosystem functioning. *Annual Review of Ecology, Evolution, and Systematics*, 54, 185-208.
- Higa, T. (2000). What is EM technology? *EM World Journal*, 1, 1-6.
- Hinsinger, P., Bengough, A.G., and Vetterlein, D. (2023). Rhizosphere processes and their role in soil fertility. *Plant and Soil*, 485, 1-28.
- Johnston, A.E., Poulton, P.R., and White, R.P. (2022). Soil phosphorus: Its accumulation, availability, and management. *Soil Use and Management*, 38(1), 4-23.
- Khan, M.S., Zaidi, A., and Ahmad, E. (2023). Phosphate solubilizing microorganisms: Principles and applications. *Springer International Publishing*.
- Kumar, A., Sharma, S., and Mishra, S. (2024). Plant growth-promoting rhizobacteria: Mechanisms and applications. *Microbiological Research*, 280, 127567.
- Lal, R. (2023). Soil organic matter content and crop yield: A meta-analysis. *Soil and Tillage Research*, 230, 105697.

Li, H., Zhang, Y., and Wang, X. (2023). Phosphate-solubilizing bacteria enhance crop yield and phosphorus use efficiency: A global meta-analysis. *Field Crops Research*, 295, 108897.

Li, X., Zhao, Y., and Zhang, F. (2024). Integrated phosphorus management in calcareous soils: A 20-year field study. *European Journal of Agronomy*, 152, 127021.

Lindsay, W.L. (1979). *Chemical Equilibria in Soils*. John Wiley & Sons, New York.

Liu, J., Cade-Menun, B.J., and Yang, J. (2024). Organic matter effects on phosphorus speciation and availability in agricultural soils. *Geoderma*, 441, 116757.

Lynch, J.P. (2024). Root phenes for enhanced soil resource acquisition. *Annual Review of Plant Biology*, 75, 1-28.

Mallarino, A.P., and Mazza, R.R. (2023). A critical review of soil phosphorus tests and their interpretation. *Soil Science Society of America Journal*, 87(2), 245-265.

McLean, E.O. (1982). Soil pH and lime requirement. In A.L. Page (Ed.), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties* (pp. 199-224). ASA-SSSA.

MNFSR. (2023). *Agricultural Statistics of Pakistan 2022-23*. Ministry of National Food Security and Research, Islamabad.

Nardi, S., Schiavon, M., and Francioso, O. (2022). Humic substances and plant nutrition: Mechanisms and applications. *Plant and Soil*, 476, 1-28.

Nelson, D.W., and Sommers, L.E. (1982). Total carbon, organic carbon, and organic matter. In A.L. Page (Ed.), *Methods of Soil Analysis. Part 2* (pp. 539-579). ASA-SSSA.

Oldfield, E.E., Bradford, M.A., and Wood, S.A. (2022). Global meta-analysis of the relationship between soil organic matter and crop yield. *Nature Sustainability*, 5, 392-400.

Penn, C.J., and Camberato, J.J. (2019). A critical review on soil chemical processes that control how soil pH affects phosphorus availability. *Agriculture*, 9(6), 120.

Philippot, L., Chenu, C., and Kappler, A. (2024). The interplay between soil biodiversity, biogeochemical cycles, and ecosystem services. *Nature Reviews Microbiology*, 22, 85-102.

Rahman, K.M.A., Zhang, D., and Wang, Q. (2023). Organic amendments improve soil health and crop productivity in calcareous soils: A meta-analysis. *Journal of Environmental Management*, 345, 118767.

Rashid, A., Ryan, J., and Aulakh, M.S. (2023). Phosphorus management in calcareous soils of South Asia: Challenges and solutions. *Advances in Agronomy*, 180, 1-62.

Rawat, P., Das, S., and Shankhdhar, D. (2022). Phosphate-solubilizing microorganisms: Mechanism and their role in plant growth promotion. *Journal of Plant Nutrition*, 45(8), 1245-1265.

- Rfaki, A., Nassiri, L., and Ibijbijen, J. (2024). Phosphate-solubilizing bacteria from calcareous soils: Diversity, mechanisms, and applications. *Frontiers in Sustainable Food Systems*, 8, 1345678.
- Richardson, A.E. (1994). Soil microorganisms and phosphorus availability. *Australian Journal of Experimental Agriculture*, 34, 897-907.
- Scholz, R.W., and Wellmer, F.W. (2024). Approaching a dynamic view on the availability of mineral resources: The example of phosphorus. *Resources, Conservation and Recycling*, 201, 107321.
- Sharma, S.B., Sayyed, R.Z., and Trivedi, M.H. (2023). Phosphate solubilizing microbes: A sustainable approach for managing phosphorus deficiency. *Frontiers in Sustainable Food Systems*, 7, 1125678.
- Sharma, V., Singh, B., and Thakur, N. (2024). Recent advances in phosphate-solubilizing microorganisms: Mechanisms and applications. *Microorganisms*, 12(2), 345.
- Shen, J., Yuan, L., and Zhang, J. (2024). Phosphorus dynamics in the rhizosphere: From mechanisms to management. *Plant and Soil*, 496, 45-78.
- Shiferaw, B., Smale, M., and Braun, H.J. (2023). Wheat in the developing world: Production, consumption, and trade. *World Agriculture*, 15(2), 45-68.
- Singh, A., Rajput, V., and Minkina, T. (2024). Integrated nutrient management in wheat: A global perspective. *Agronomy*, 14(3), 567.
- Soltanpour, P.N., and Schwab, A.P. (1997). A new soil test for simultaneous extraction of macro- and micro-nutrients in alkaline soils. *Communications in Soil Science and Plant Analysis*, 8, 195-207.
- Syers, J.K., Johnston, A.E., and Curtin, D. (2008). *Efficiency of soil and fertilizer phosphorus use*. FAO Fertilizer and Plant Nutrition Bulletin 18. FAO, Rome.
- Timmusk, S., Pall, T., and Raz, S. (2023). Plant growth-promoting rhizobacteria: Mechanisms, applications, and challenges. *Frontiers in Plant Science*, 14, 1204567.
- Wang, Y., Chen, X., and Zhang, F. (2024). Global phosphorus use efficiency in agriculture: Trends, determinants, and implications. *Global Food Security*, 40, 100756.
- Zhang, F., Cui, Z., and Chen, X. (2023). Integrated nutrient management for sustainable wheat production in China: A 30-year perspective. *Field Crops Research*, 295, 108765.
- Zhang, Y., Wang, X., and Li, H. (2024). Phosphate-solubilizing microorganisms enhance soil phosphorus availability and crop yield: A global synthesis. *Soil Biology and Biochemistry*, 188, 109234.

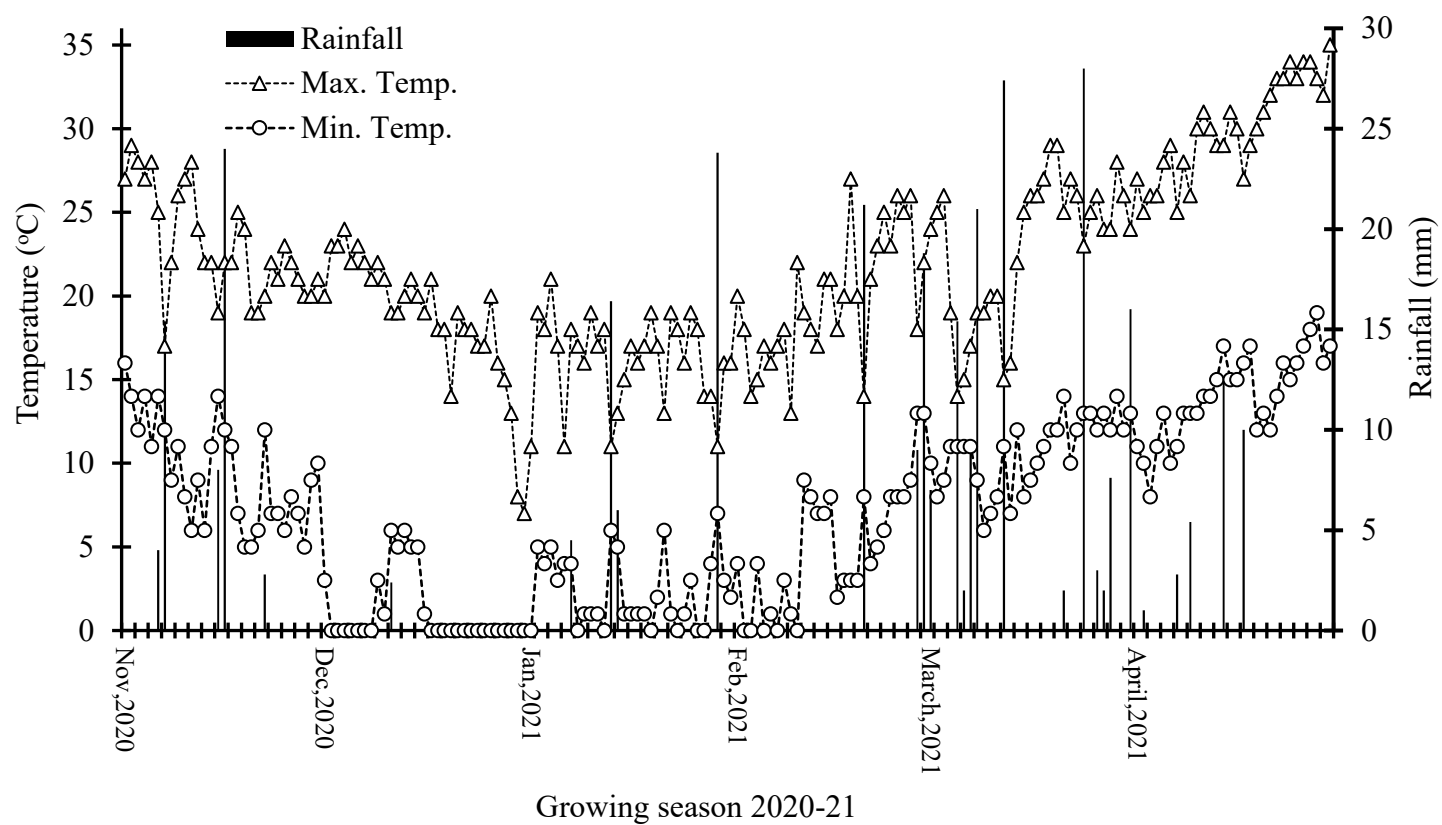


Figure 1. Daily maximum and minimum temperature ($^{\circ}\text{C}$) and rainfall (mm) of the experimental site during Nov, 2020 to April, 2021.

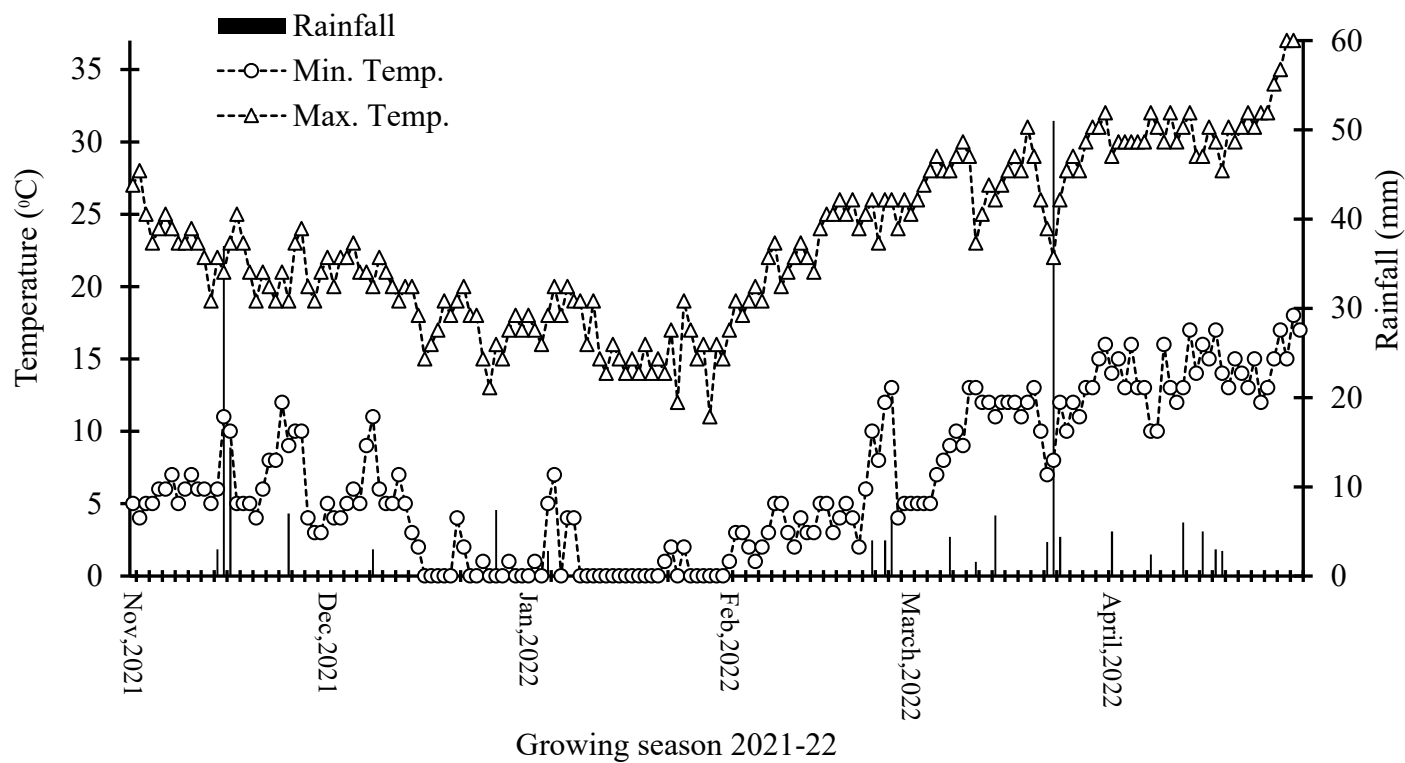


Figure 2. Daily maximum and minimum temperature (°C) and rainfall (mm) of the experimental site during Nov, 2021 to April, 2022.

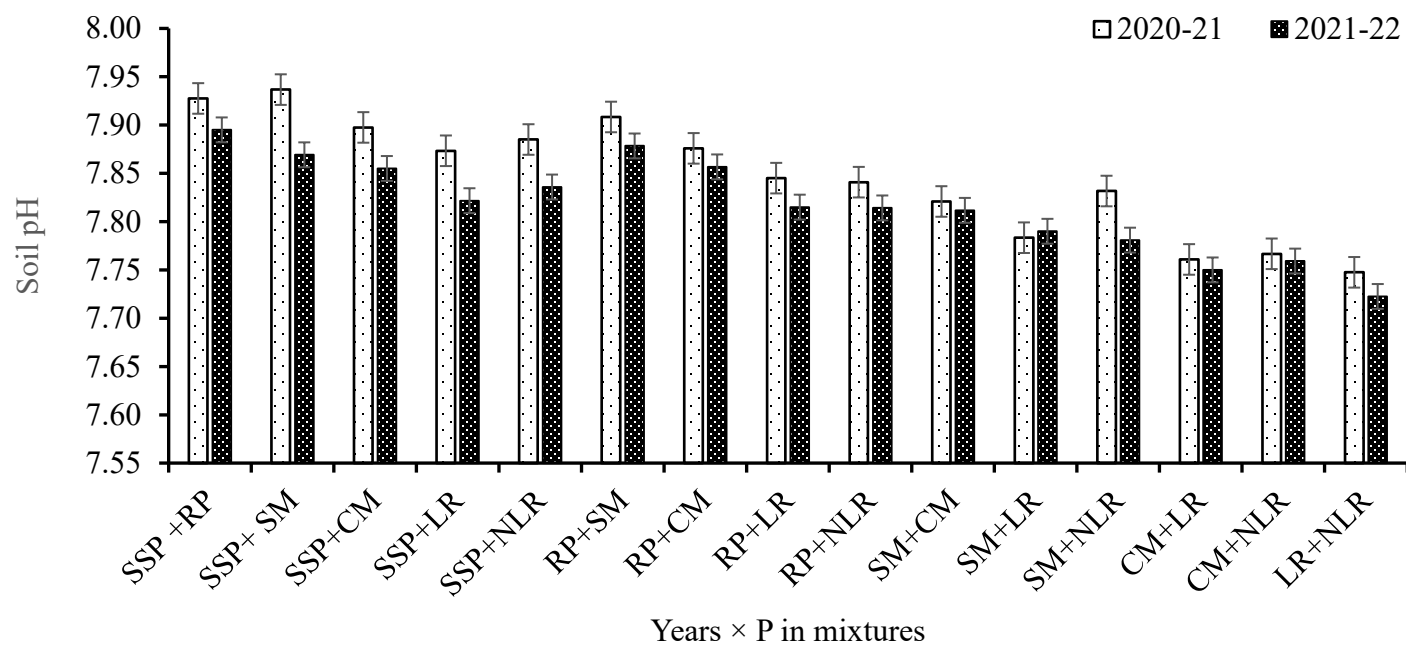


Figure 3. Interactive effect of years and P in mixture on soil pH of wheat.

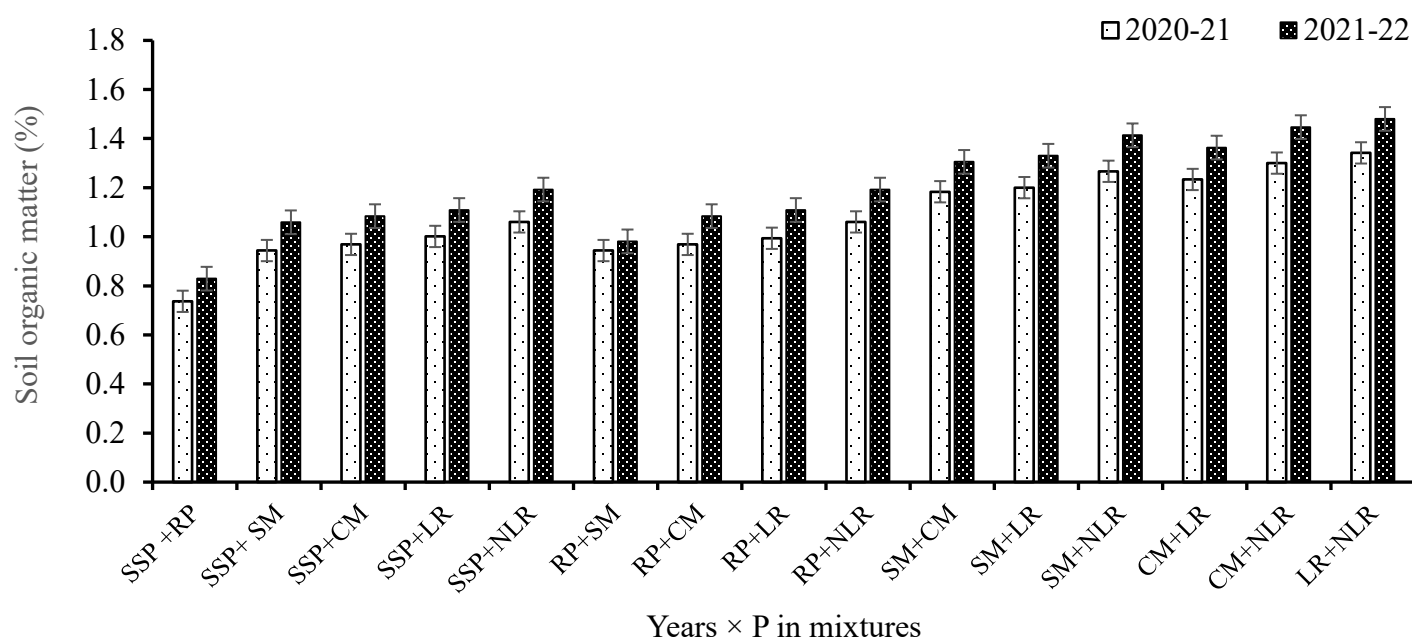


Figure 4. Interactive effect of years and P in mixture on soil OM (mg kg^{-1}) of wheat.

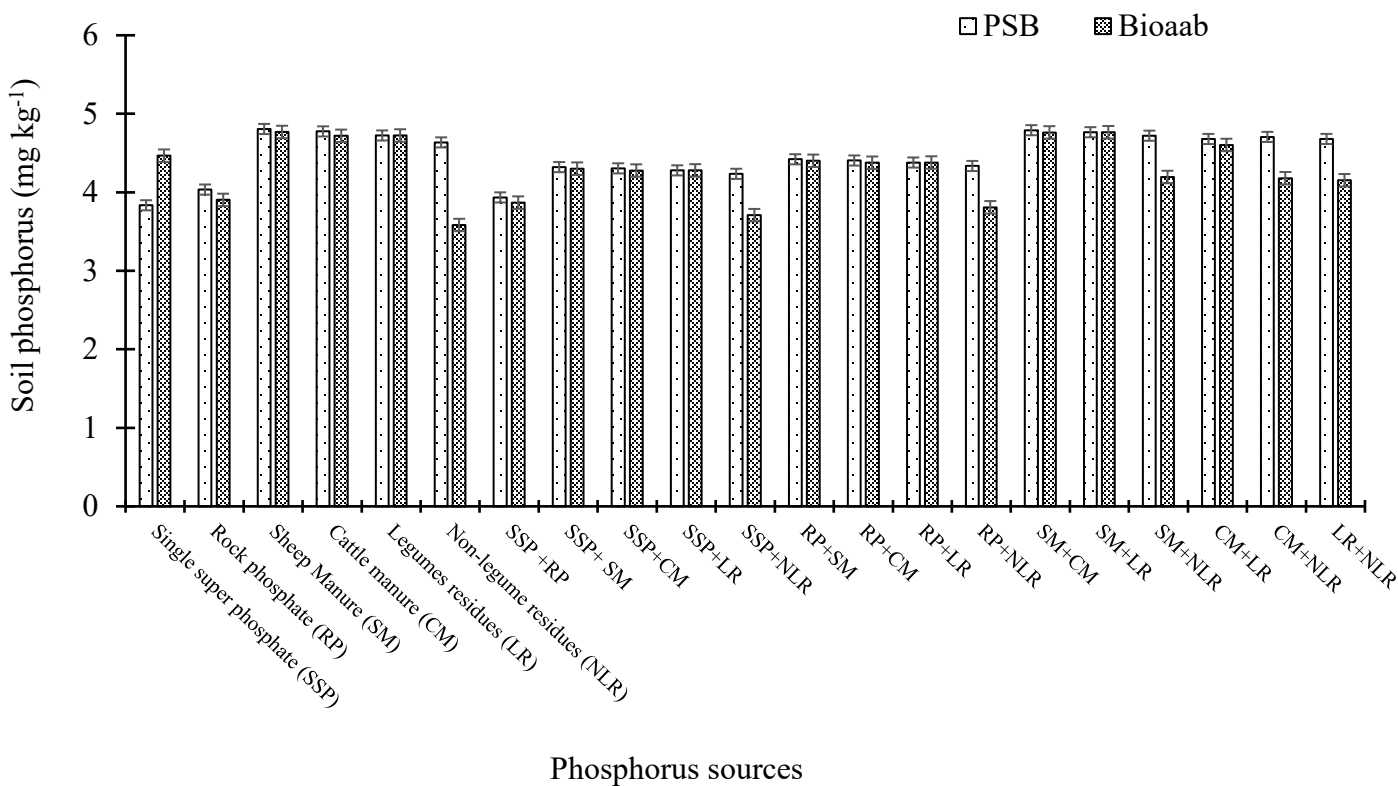


Figure 5. Interactive effect of years and P in mixture on soil P contents (mg kg⁻¹) of wheat.

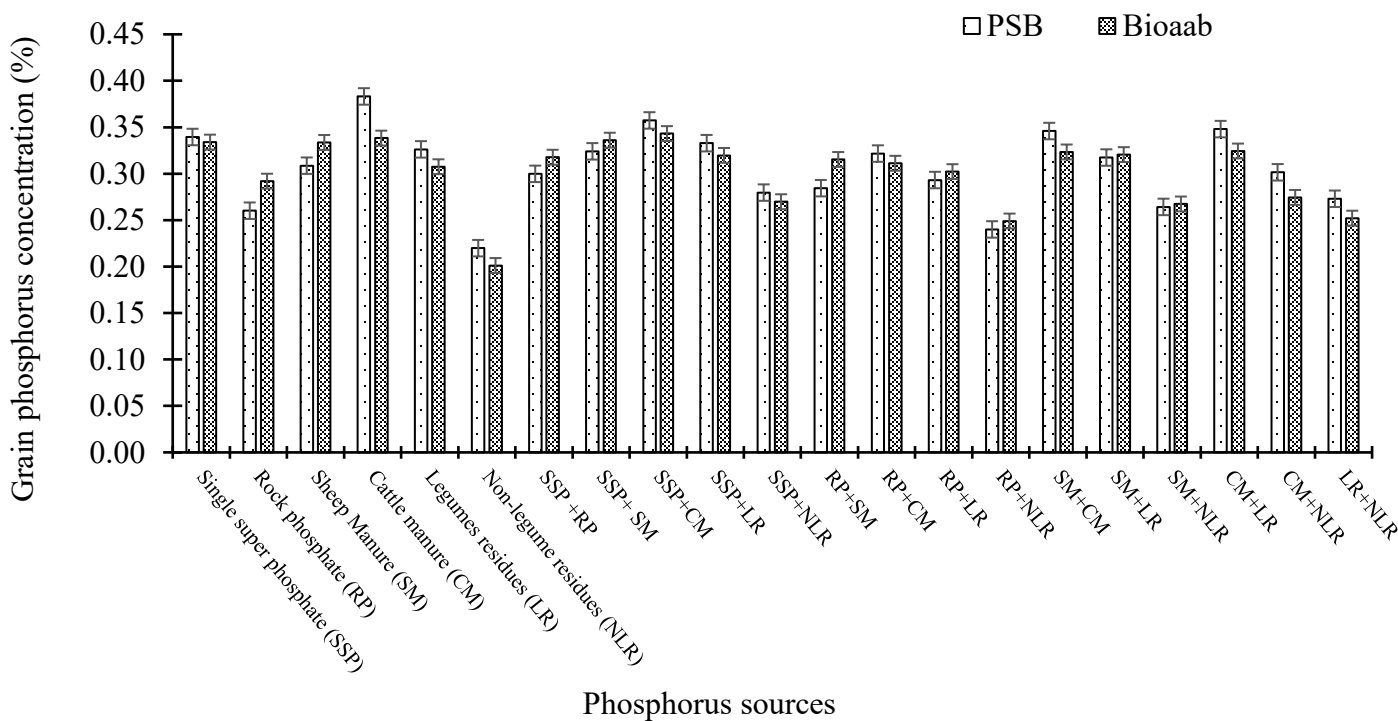


Figure 6. Interactive effect of P sources and BF on grain P concentration of wheat.

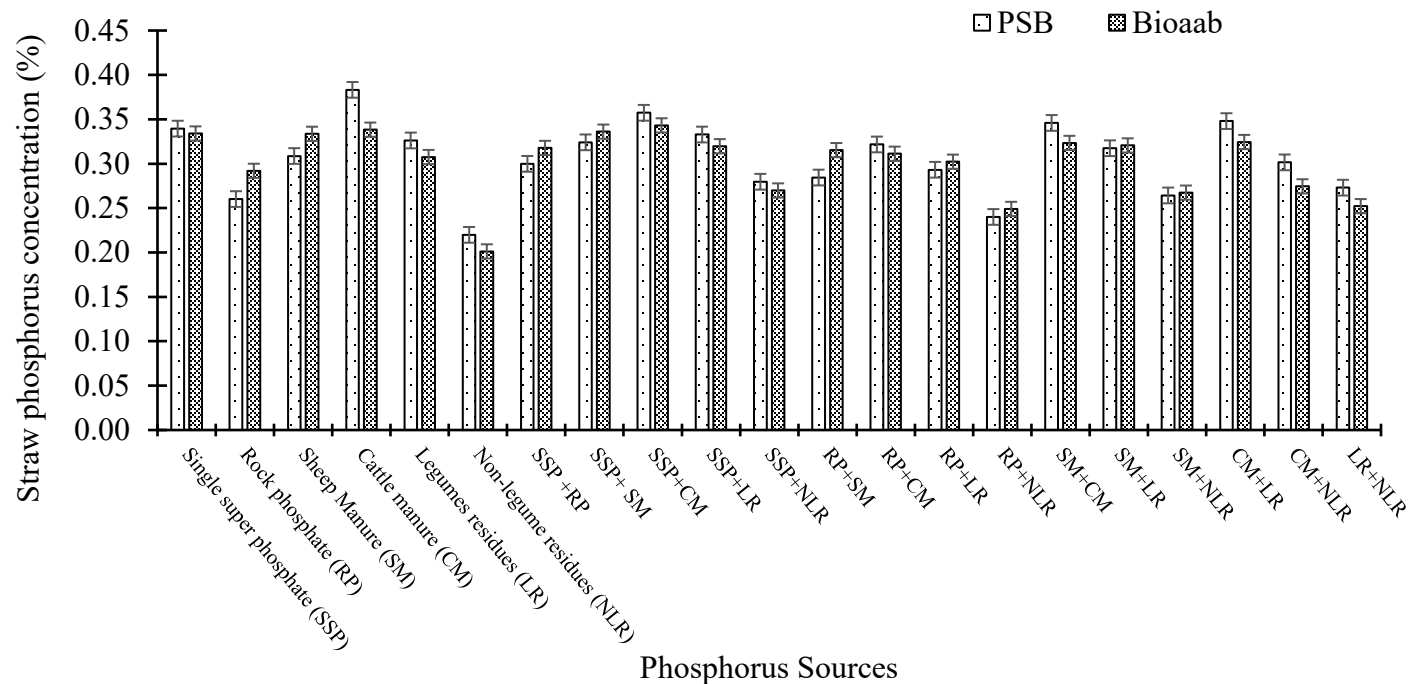


Figure 7. Interactive effect of PS $\times$ BF on straw P concentration (%) of wheat.

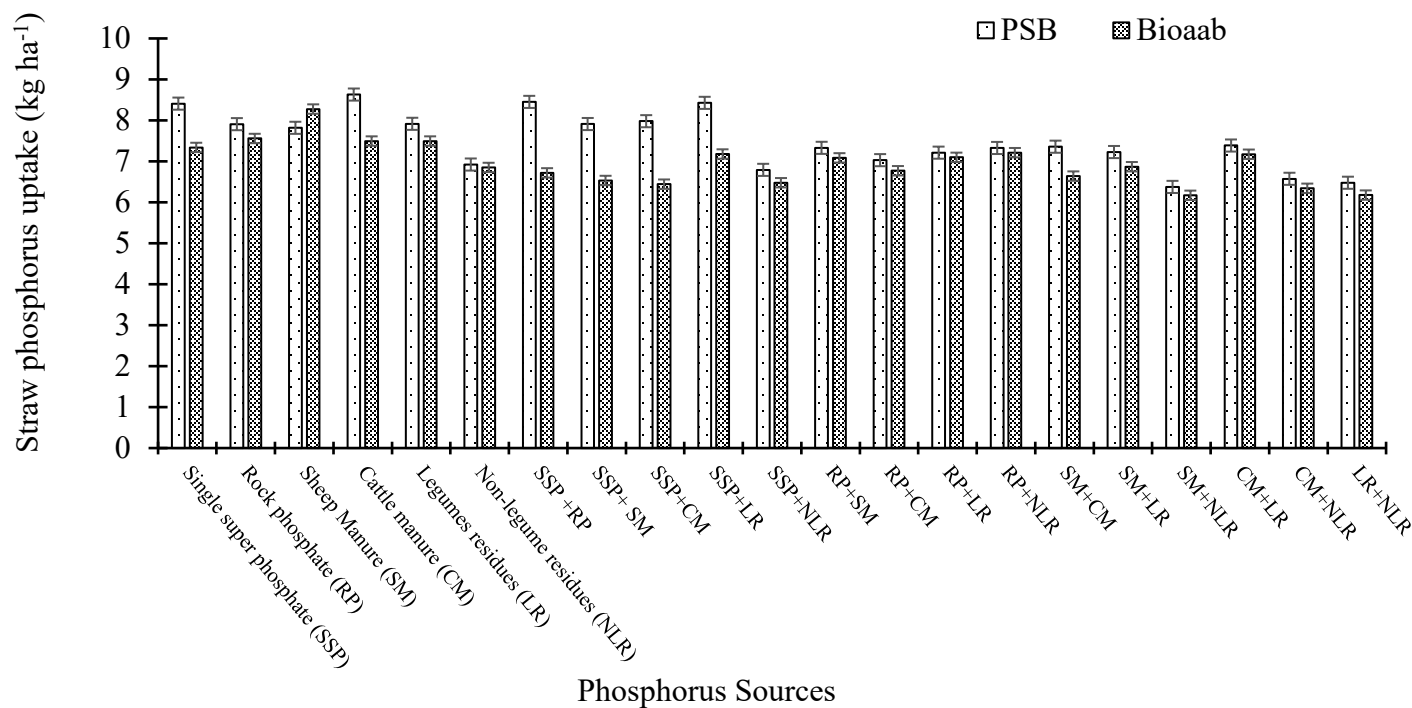


Figure 8. Interactive effect of PS × BF on straw P uptake (kg ha⁻¹) of wheat.

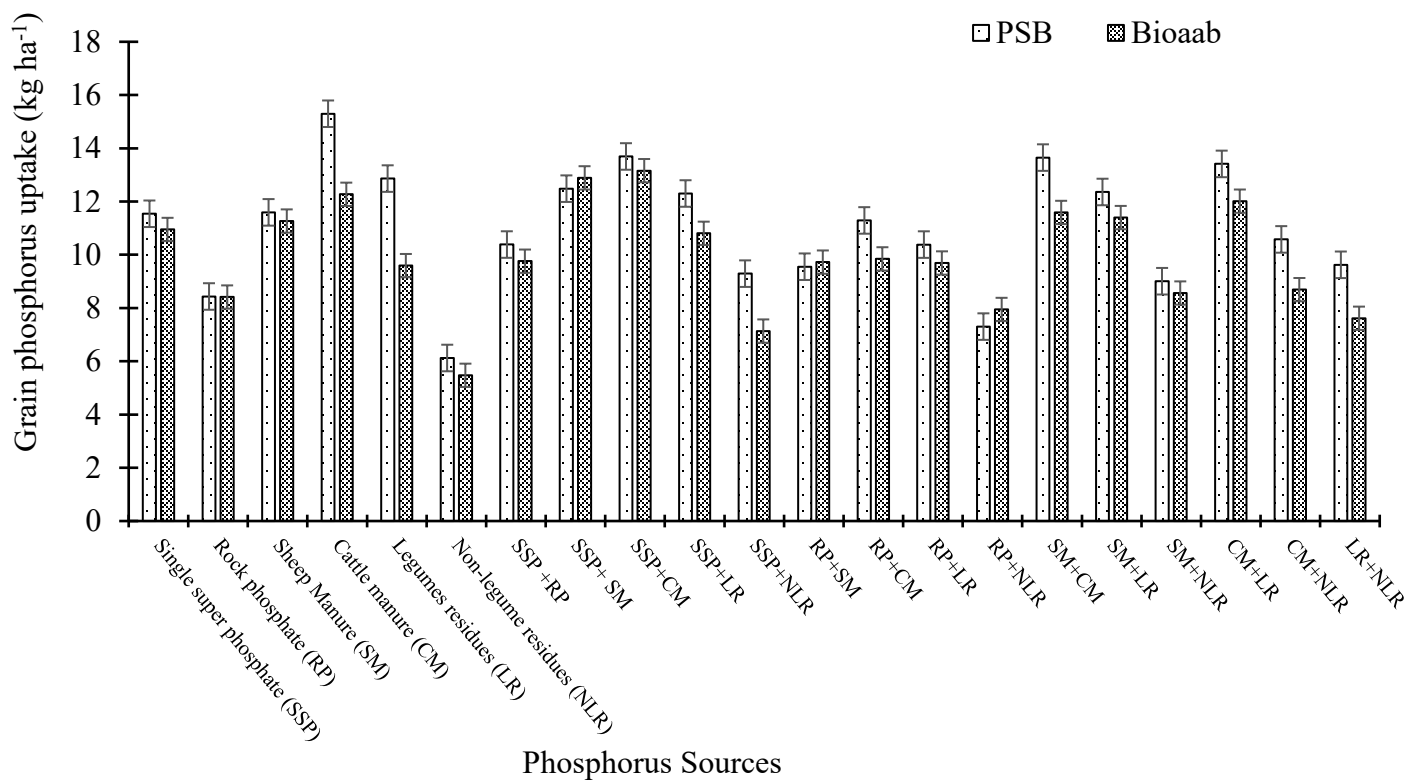


Figure 9. Interactive effect of PS $\times$ BF on grain P uptake (kg ha^{-1}) of wheat.

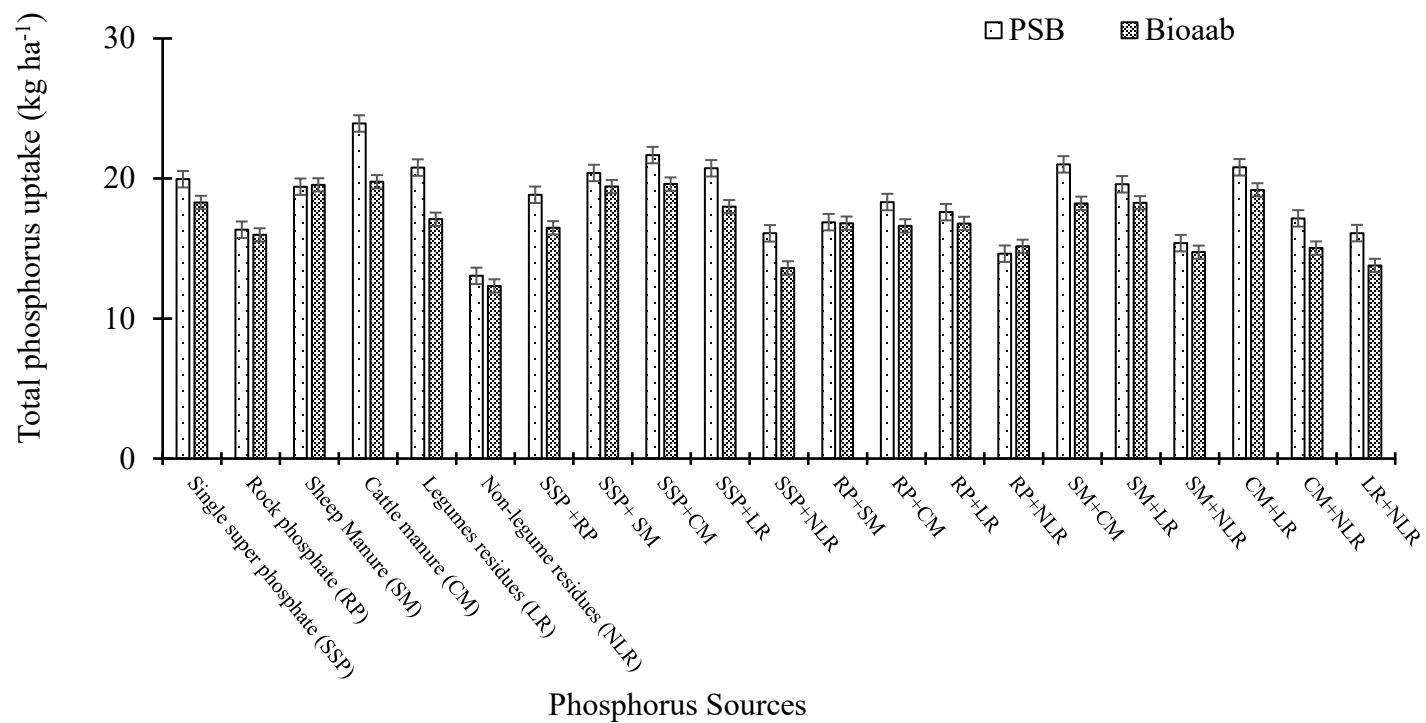


Figure 10. Interactive effect of PS $\times$ BF on total P uptake (kg ha^{-1}) of wheat.

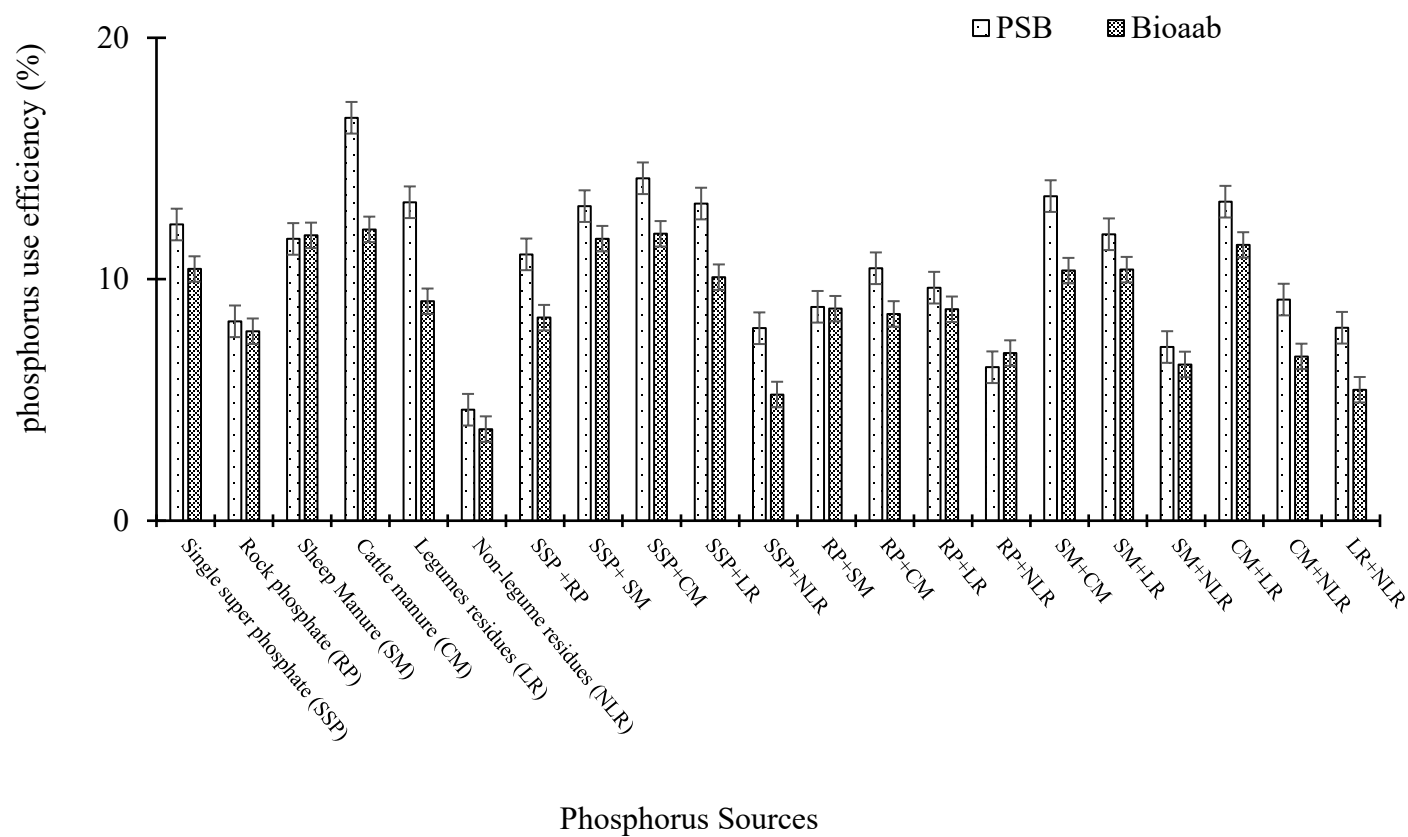


Figure 11. Interactive effect of PS $\times$ BF on P use efficiency (%) of wheat.

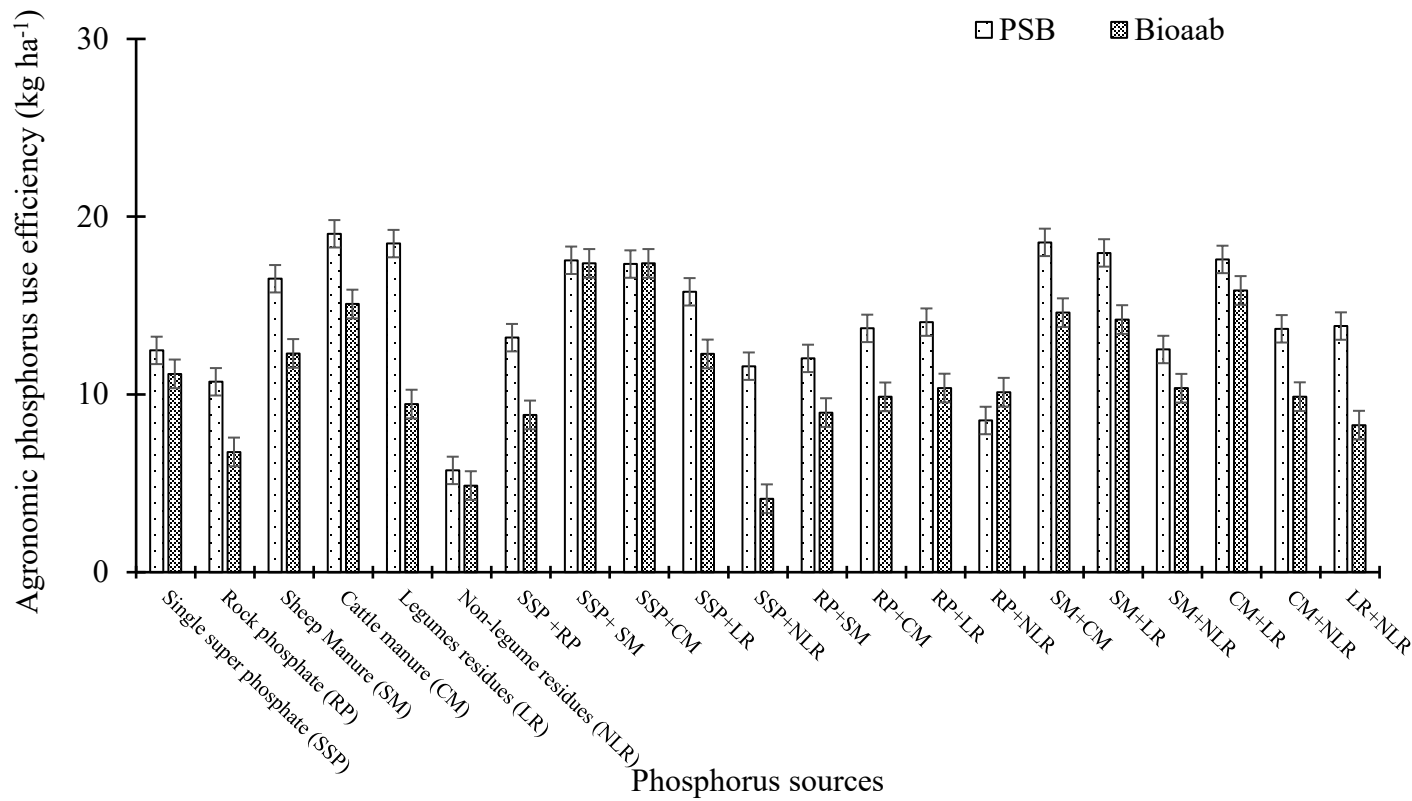


Figure 12. Interactive effect of PS × BF on agronomic P use efficiency (kg kg⁻¹) of wheat.

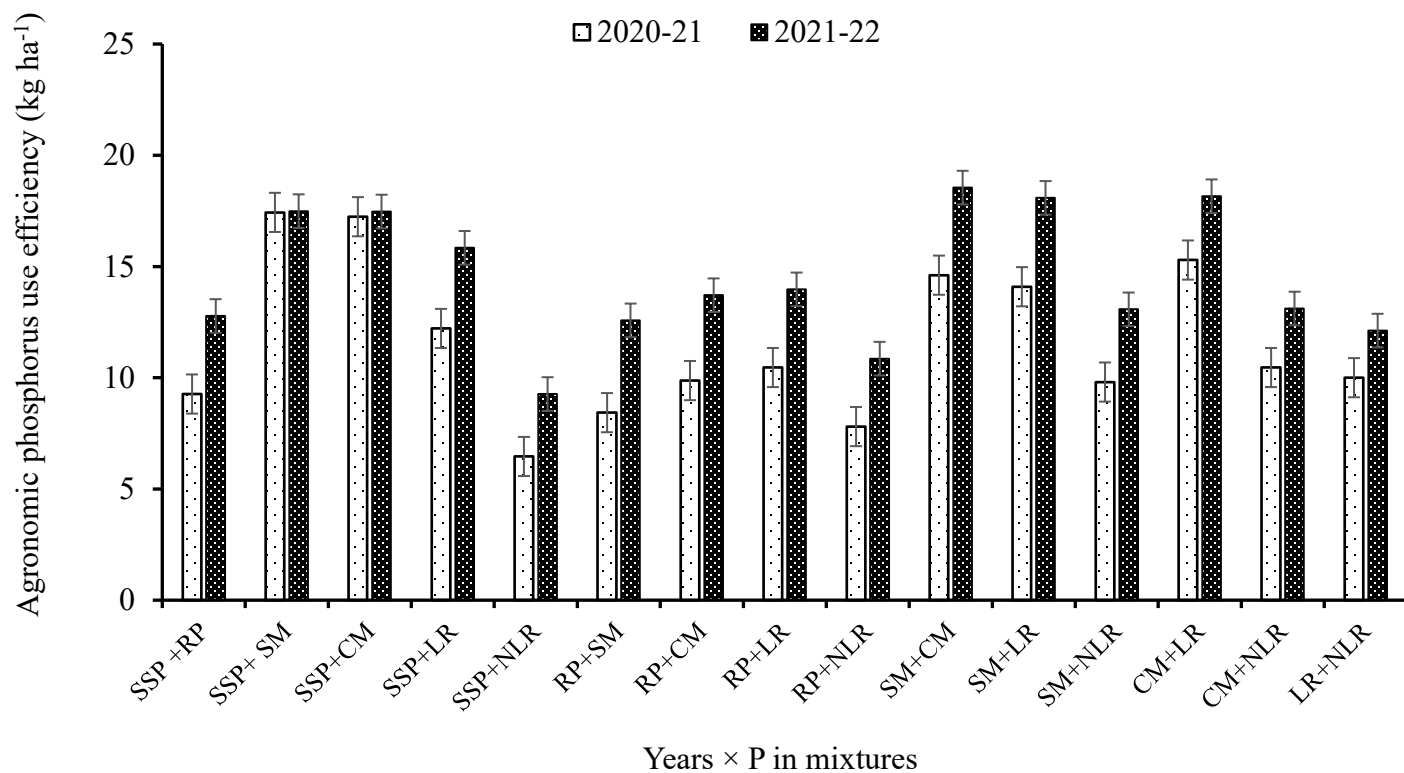


Figure 13. Interactive effect of years and P in mixture on agronomic P use efficiency (kg kg⁻¹) of wheat.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TablesFile.docx](#)