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Enhancing phosphorus use efficiency and soil quality indicators in lowland paddy ecosystem through *Azolla*, rice straw, and NPKS fertilizers

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Purpose: This study investigates the influence of incorporating *Azolla*, rice straw, and NPKS fertilizers on phosphorus use efficiency (PUE) and rice productivity in lowland paddy fields. Despite *Azolla*'s well-known role as a nitrogen-fixing aquatic fern in rice production, its specific impact on PUE remains unclear. The primary objective is to explore diverse treatment combinations to identify synergies that enhance both PUE and overall rice productivity.

Methods: The study was conducted at Mkula Irrigation Scheme in the Kilombero Valley, Tanzania; the field experiment employed a randomized complete block design with 13 treatments and three replications. Treatments comprised various combinations of *Azolla*, rice straw, and chemical fertilizers, incorporating 50% and 100% rates of nitrogen (N) applied with phosphorus (P), potassium (K), and sulfur (S).

Results: The study reveals the substantial impact of *Azolla* application on total nitrogen, available phosphorus, and exchangeable potassium levels in the soil. Particularly noteworthy were treatment combinations involving *Azolla*, rice straw, and reduced rates of synthetic nitrogen, along with specific P, K, and S applications, which exhibited the highest phosphorus uptake and PUE. Specifically, combining rice straw and *Azolla* with reduced N rates, alongside 30 kg P ha^{-1} $^{+}$ 30 kg K ha^{-1} $^{+}$ 20 kg S ha^{-1}, resulted in the highest phosphorus uptake (73.57 kg/ha) and PUE (46.24%).

Conclusion: Integrated nutrient management, incorporating rice straw and *Azolla* alongside synthetic fertilizers, demonstrates synergistic effects on phosphorus uptake and efficiency while maintaining soil quality. The study underscores the potential of such integrated strategies to optimize PUE and contribute to sustainable rice production in lowland paddy fields.

KEYWORDS

biofertilizers, crop nutrient recovery efficiency, improved food systems, nutrient omission, smallholder farming systems, sustainable environment

1 Introduction

Phosphorus (P) deficiency is among the major constraints on plant growth and crop production globally. This vital nutrient plays a crucial role in numerous biochemical and physiological processes, including energy transfer, genetic constituents' relocation (DNA and RNA), and promoting vegetative growth in rice plants (Bird et al., 2001; Verzeaux et al., 2017; Azene et al., 2022; Mboyerwa et al., 2022). However, P availability is limited in many parent materials and becomes even more complicated in waterlogged conditions associated with high levels of iron, aluminum, and calcium, leading to low availability for plant uptake in both acidic and alkaline soils (Fageria et al., 2010, 2011; Rivaie et al., 2013; Lemanowicz, 2018). Additionally, several factors, such as pH, soil parent material, management practices, and climate change, influence P fractions and solubility of phosphorus (Azene et al., 2022). Phosphorus may be present in soil parent materials in relatively high amounts, yet more than 80% gets fixed as immobile stock and a fraction of P that is available to plant does not exceed 15%–20% (van de Wiel et al., 2016; Biswas Chowdhury and Zhang, 2021; Jiang et al., 2021). Previous studies demonstrated that fertilization of P into soil results in its distribution among the solid phase and clay-sized minerals. Increased clay content enhances P retention, with adsorption being reversible. Initial P fertilization sees low efficiency, but with additional P, efficiency improves. Equilibrium leads to equally rapid adsorption and desorption reactions. In highly calcareous soils, soluble P retention occurs mainly through precipitation, posing a challenge due to the availability of Ca for P precipitation. Organic matter retention of P is an inefficient process, causing P accumulation, but immobilization is reversible (Syers et al., 2008; Fixen et al., 2014). Therefore, for better sustainable soil management practices, there is a need for further research to optimize P use efficiency (PUE) and to explore the implications of these findings for global food security and environmental sustainability.

Soil organic matter serves as a significant reservoir of soil P, influencing nutrient availability, soil structure, cation exchange capacity, soil buffering capacity, and water holding capacity (Oyange et al., 2020). It was observed that *Azolla* compost and rice straw are common organic residues in rice cropping systems, but rice straw takes time to mineralize due to its wider C/N ratio (Wan et al., 2016). Co-application of straw with other crop residues with lower C:N ratio, such as milk vetch, green manure, and *Azolla*, can minimize this issue (Wan et al., 2016; Van Hung et al., 2020). *Azolla* not only acts as an eco-friendly amendment but also fixes nitrogen, enhancing nitrogen use efficiency and promoting rice growth and yield (Cabangon et al., 2015; Fosu-Mensah et al., 2015; Cissé and Vlek, 2022). However, more research is needed to explore the potential *of Azolla* in lowland rice production systems, especially its impact on PUE and enzyme activities.

Maintaining an appropriate level of plant-available P in soils is crucial. Over-application can lead to water eutrophication and disturbance in marine ecosystems while insufficient P levels reduce crop yield and limit the uptake of other essential nutrients like nitrogen (Ladha et al., 2000; Mandana et al., 2014; Zhang et al., 2017; Bhunia et al., 2021; Zhang et al., 2022b). Several strategies, such as improving soil structure, managing P sources, and using appropriate amounts of P and timing of its application can enhance PUE and solubilization (Syers et al., 2008).

Assessing soil health and quality through physical, chemical, and biological indicators is essential (Konare et al., 2010; Hengl et al., 2015). However, focusing solely on physiochemical indicators may not fully explain the effect of organic matter on nutrient availability through microbiological activities (Reddy et al., 2001; Yadav et al., 2020). Soil enzymes play an important role in the transformation of soil organic carbon (e.g., B-amylase, maltase, cellulase, β-galactosidase, and β-glucosidase), nitrogen (e.g., urease, protease, amidase, and nitrate reductase), P (phytases, acid phosphomonoestarases, and alkaline phosphomonoestarases), and S (sulfatase) cycle (Liang et al., 2014; Dotaniya and Meena, 2015; Bagheri Novair et al., 2020). Since enzymes are catalysts and mediators of various important soil functions (Abraham, 2010). Increased activity of these enzymes directly reflects proper availability of specific nutrients related to the enzymes (Gu et al., 2009). For example, soil urease catalyzes the hydrolysis of urea in the soil to CO_2 and NH_3/NH_4^+ (Saha et al., 2008; Fisher et al., 2017) (Equation 1).

$$\mathbf{CO(NH_2)_2 + 2H_2O + 2H^+ \rightarrow CO_2 + 2NH_4^+} \qquad (1)$$

This enzyme is released by many groups of microbes like bacteria, fungi, yeast, and algae, and they are responsible for the availability of N added through urea or free nitrogen fixed by microorganisms (Gu et al., 2009). Another important enzyme is phosphomonoestarases, which are responsible for hydrolysis of organic phosphorus compounds in either acidic or alkaline soils. Various types of phosphatases depend on the number of bonds hydrolyzed and optimum pH. Plant roots also release acid phosphatase at the P deficiency stage and mobilize available P in the soil. Fungi also produce acid phosphatase and most bacteria produce alkaline phosphatase. Other factors like soil organic carbon, total nitrogen, soil pH, clay content, and moisture can also influence soil enzymes (Gu et al., 2009; Kumari et al., 2017). Therefore, by utilizing enzyme activities as indicators, researchers can assess the effects of the application of organic materials on soil fertility, nutrient cycling, soil health, organic residue decomposition, and plant physiological processes in paddy fields. This information could contribute to a comprehensive understanding of the benefits and impacts of organic practices on paddy performances and help to guide sustainable agriculture practices.

Therefore, the objective of this study is to examine the effect of integrating chemical fertilizers (NPKS) with *Azolla* compost and rice straw having different C:N ratios on P uptake and PUE, as well as its implications on soil chemical fertility and enzyme activity in lowland paddy ecosystems.

2 Materials and methods

2.1 Description and location of the study area

The field layout was carried out at Mkula Irrigation Scheme (7° 47′57.084′′ S, 36°54′47.592′′ E), Kilombero district. The district is located within agroecological zone E10 in "*Eastern Plateaux and Mountain Blocks*" in Morogoro Region-Tanzania. The climate is classified as a tropical savanna climate with a bimodal rainfall distribution pattern, having dry spells separating a short rainy season from October to December and a long rainy season from

March to May (Kwesiga et al., 2020; Michael et al., 2023). The mean annual rainfall and temperature range from 1,200 to 1,400 mm and 22–23°C, respectively (Alaivasha et al., 2022; Marzouk et al., 2023). The site receives sufficient water drained from the forest reservoir on the eastern side of Udzungwa Mountain.

2.2 Experimental design and treatments

The field experiments were carried out for two consecutive rice-growing seasons from 2022 to 2023. Randomized complete block design (RCBD) was adopted with 13 treatment levels with half and full recommended levels of N (i.e., 50 kg N ha^{-1} and 100 kg N ha^{-1}) applied with recommended levels of P and or K and S coupled with *Azolla* or rice straw incorporation in three replications. Details of the treatment combination are shown in Table 1. The dimensions of the individual experimental plot were 3 m × 6 m, and the space between replicate blocks was 1.5 m and that between plots within a replicate was 0.5 m. The ridges protruded 30 cm above the soil to prevent any fertilizer runoff and lateral contamination.

2.3 Organic amendment application and agro-techniques

2.3.1 Preparation of *Azolla* and rice straw

Azolla plant was collected from the Aquaculture Unit of the Sokoine University of Agriculture (6°51′9.5′′ S, 37°38′59.7′′ E) in Tanzania. Before establishing culture, *Azolla* was harvested and analyzed for organic C, total N, P, and C/N ratio. Subsequently, 6 kg of fresh *Azolla* was multiplied in the propagation pond (6 m × 5 m) of the same aquaculture unit at the university campus. During preparation, 7.5 kg 30 m^{-2} of cow dung and triple superphosphate [TSP, $Ca_3(PO_4)_2$] fertilizer at 75 g P 30 m^{-2} were applied in three split doses at 4-day intervals for 22 days (Watanabe and Berja, 1983; Bagheri Novair et al., 2020). Nitrogen content of *Azolla* dry matter, surface water pH, water temperature, and electrical conductivity were analyzed by taking representative samples after every 5 days until the *Azolla* had accumulated maximum nitrogen in biomass (Table 2). Thereafter, *Azolla* was harvested, drained, and transferred to the field for inoculation (Figure 1). Rice straw was collected from farmers' fields, cutting manually using a knife and incorporated into the soil for the respective straw treatments during land preparation using a hand hoe. By obtaining representative samples, the straw was dried and transported to the laboratory for chemical analysis of N through Kjeldahl and P by the wet digestion method (Okalebo et al., 2002).

2.3.2 Application of *Azolla*, rice straw, and inorganic fertilizers

Azolla in its fresh form was applied at a rate of 2 kg per 18 m^2 and inoculated during nursery preparation and incorporated in the soil after 15 days. At this stage, *Azolla* had covered the surface of the water completely (see Figure 1). Fresh *Azolla* biomass at 36.4 kg 18 m^{-2} was incorporated to the soil using a hand hoe 3 days before transplanting of rice seedlings. Another 2 kg 18 m^{-2} was inoculated 6 days after transplanting (DAT) and incorporated at a rate of 29.2 kg 18 m^{-2} into the soil at 40 DAT. Before incorporation, *Azolla* was harvested within a 1 m × 1 m wooden frame (Watanabe et al., 1991), collected, dried, and analyzed for total N through Kjeldahl and P by the wet digestion method (Okalebo et al., 2002) (see Table 3). The fertilizers containing the primary (NPK) and S secondary macronutrients were applied. Fertilizers containing P, K, and S were applied uniformly in all experimental plots while N from urea (46% N) was applied at two rates of 100 kg N ha^{-1}, being the recommended rate and half rate (50 kg N ha^{-1}). Urea fertilizer for both rates of N was applied in

TABLE 1 Summary of the experimental treatments of chemical fertilizers, *Azolla*, and rice straw application.

Treatment code	*Azolla* application	Rice straw	Nitrogen application	Other synthetic fertilizer applications
T1	–	–	–	–
T2	–	–	100 kg urea-N ha^{-1}	–
T3	–	–	100 kg urea-N ha^{-1}	30 kg P ha^{-1}
T4	–	–	100 kg urea-N ha^{-1}	30 kg P ha^{-1} $^{+}$ 30 kg K
T5	–	–	100 kg urea-N ha^{-1}	30 kg P ha^{-1} $^{+}$ 30 kg K ha^{-1} $^{+}$ 20 kg S ha^{-1}
T6	–	–	50 kg urea-N ha^{-1}	30 kg P ha^{-1} $^{+}$ 30 kg K ha^{-1} $^{+}$ 20 kg S ha^{-1}
T7	–	6.9-ton rice straw ha^{-1}	–	30 kg P ha^{-1}
T8	3.4-ton dry *Azolla* ha^{-1}	–	–	30 kg P ha^{-1}
T9	–	6.9-ton rice straw ha^{-1}	50 kg urea-N ha^{-1}	30 kg P ha^{-1} $^{+}$ 30 kg K ha^{-1} $^{+}$ 20 kg S ha^{-1}
T10	3.4-ton dry *Azolla* ha^{-1}	–	50 kg urea-N ha^{-1}	30 kg P ha^{-1} $^{+}$ 30 kg K ha^{-1} $^{+}$ 20 kg S ha^{-1}
T11	3.4-ton dry *Azolla* ha^{-1}	6.9-ton rice straw ha^{-1}	–	30 kg P ha^{-1}
T12	3.4-ton dry *Azolla* ha^{-1}	6.9-ton rice straw ha^{-1}	50 kg urea-N ha^{-1}	30 kg P ha^{-1} $^{+}$ 30 kg K ha^{-1} $^{+}$ 20 kg S ha^{-1}
T13	3.4-ton dry *Azolla* ha^{-1}	6.9-ton rice straw ha^{-1}	100 kg urea-N ha^{-1}	30 kg P ha^{-1} $^{+}$ 30 kg K ha^{-1} $^{+}$ 20 kg S ha^{-1}

TABLE 2 Characterization of *Azolla* prior to application in the experiments.

Dates of sample collection	Water pH	Electrical conductivity (ms/cm)	Water temperature (°C)	Total N in dry matter (%)
02/06/2022	6.80	0.16	26.2	2.63
07/02/2022	6.65	0.15	23.6	2.41
12/06/2022	6.66	0.16	23.5	2.69
17/06/2022	6.57	0.14	23.4	2.81
22/06/2022	6.61	0.15	23.4	2.80

two splits of 50% basal application at 7 DAT and another 50% was top-dressed at 45 DAT, which was close to the booting stage. The triple superphosphate fertilizer (30 kg P ha^{-1}), muriate of potash (30 kg K ha^{-1}), and ammonium sulfate (21.0% N and 24.0% S) at 20 kg S ha^{-1} were applied through broadcasting as basal fertilizers, except for the absolute control plots. A rice cultivar (c.v SARO-5 TXD 360) was used in this experiment, and it was obtained from the Tanzania Research Institute at Katrini-Ifakara (TARI-CATRINI). Rice seedlings (at 18 days old) were transplanted into well-puddled soils at a spacing of 20 cm × 20 cm in all treatments. Rice straw 11.3 kg 18 m^{-2} was spread evenly across the designated straw treatment plots and incorporated into the soil using a hand hoe during the farm preparation stage.

2.4 Plant tissue sampling, analysis, and calculation of phosphorus use efficiency

Fifteen randomly selected rice plants were harvested at the booting stage (75 DAT). All aboveground plant samples were taken, oven-dried at 70°C to a constant weight, and ground to pass through a 1-mm sieve and digested using H_2SO_4. P nutrient content was calorimetrically determined as described by Watanabe and Olsen (1965), Using the Micro Kjeldahl method, total N content was determined as described by Seleiman et al. (2022).

Phosphorus uptake (PU) (kg ha^{-1}) was calculated by multiplying their nutrient concentration (%) by their biological weight (kg ha^{-1}) (Assefa et al., 2021) (Equation 2).

$$\mathrm{PU}\ (\mathrm{kg\ ha^{-1}}) = \frac{\mathrm{P\ content\ in\ aboveground\ biomass\ (\%)} \times \mathrm{biological\ yield\ (kg\ ha^{-1})}}{100} \tag{2}$$

PUE (%) was calculated using the balanced method (Equation 3) described by Fixen et al. (2014) and Syers et al. (2008).

$$\mathrm{PUE} = \frac{\mathrm{Total\ P\ uptake\ in\ aboveground\ biomass\ (kg\ ha^{-1})}}{\mathrm{P\ input\ \ (kg\ ha^{-1})}}100 \tag{3}$$

The agronomic efficiency of applied P (AE) (kg kg^{-1}) was calculated according to Andriamananjara et al. (2019) (Equation 4).

$$\mathrm{AE}\ (\mathrm{kg\ kg^{-1}}) = \frac{\mathrm{Grain\ yield\ fertilized\ (kg\ ha^{-1})} - \mathrm{Grain\ yield\ control (kg\ ha^{-1})}}{P\ \ \mathrm{input (kg\ ha^{-1})}} \tag{4}$$

2.5 Soil sampling and analysis

Before planting rice, soil samples were collected for the analyses of selected physicochemical properties. The composite soil samples were taken from the experimental site from a depth of 0–20 cm using augur randomly from 10 spots by walking in a zigzag pattern. At the end of each experiment, soil sampling was done in each treatment plot. After carefully mixing the composite samples, 2 kg

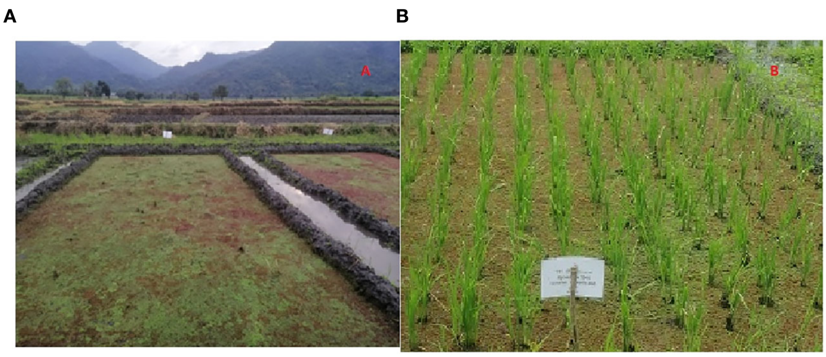


FIGURE 1
Azolla cover before rice transplanting **(A)** and at 35 DAT **(B)**.

of subsample was taken and brought to Sokoine University of Agriculture soil laboratory. The submitted sample was air-dried and grounded to pass a 2-mm mesh-sized sieve and analyzed using the standard procedure described in Table 3.

2.6 Analysis of soil enzyme activities

Three types of soil enzymes (urease, acid phosphatase, and alkaline phosphatase) were selected as indicators of microbial capacity to drive nutrient cycling (N and P, respectively). Soil urease activity was assessed by the method described by Tabatabai (1994). Five grams of oven-dry soil was placed in a 25-mL volumetric flask followed by the addition of 5 mL of 2 mg/mL urea solution and incubated at 37°C for 5 h using a Memmert 0214 incubator. After 5 h, 50 mL of 2 M potassium mercuric acetate solution (KCI-PMA) was added to stop the enzymatic reaction and the solution was shaken for 1 h. The solution was then filtered through filter paper Whatman no. 42. Then, 1 mL of aliquot was placed in a 50-mL volumetric flask followed by the addition of 10 mL of KCI-PMA solution and 30 mL of coloring reagents (25 mL of 2.5% diacetyl monoxime + 10 mL of 0.25% Thiosemicarbazide). The solution was placed in a water bath for 30 min to allow chemical reactions to proceed and the formation of a complex-colored compound. After cooling solution was diluted to 50 mL, a concentration of urea was determined by an AR-2000 spectrophotometer at a wavelength of 512 nm. Acid and alkaline phosphatases were analyzed spectrophotometrically by the method described by Kandeler et al. (1999). Oven-dry soil (1 g) was placed in a glass bottle followed by the addition of 0.2 mL of toluene to arrest microbial activities. Then, 4 mL of Modified Universal Buffer (MUB) of pH 6.5 was added. For acidic phosphatase and for alkaline phosphatase (pH 11), MUB was prepared by mixing 12.1 g of Tris (hydroxymethyl) aminomethane (THAM), 11.6 g of maleic acid, 14 g of citric acid, and 6.3 g of boric acid in 488 mL of 1 N sodium hydroxide and the solution was diluted to 1 L with distilled water. Then, 1 mL of 0.05 M P-nitrophenyl phosphate solution was mixed and incubated for 1 h. After incubation, 1 mL of 0.5 M calcium chloride and 4 mL of 0.5 M sodium hydroxide were added and mixed properly and then filtered through Whatman No. 42. A concentration of p-nitrophenol solution was determined by an AR-2000 spectrophotometer at a wavelength of 440 nm (Adams, 1992; Kandeler et al., 1999).

2.7 Statistical analysis

Data were analyzed using GenStat software version 15th edition. The experiment unit was arranged in an RCBD with three replications. Grain yield, grain P uptake, and available soil P were subjected to one-way ANOVA to examine the effects of treatments, and the mean was compared by Tukey test ($p< 0.05$). Before ANOVA, the normality of the data and homogeneity of variance were checked using the Shapiro–Wilk and Bartlett tests, respectively. Spearman rank correlation analysis among rice yield attributes, PUE, and soil properties was performed by GenStat software. The coefficient values and their associated significance was tested at 5% confidence level and reported under different p-values: $^*p< 0.05$, $^{**}p< 0.01$, and $^{***}p< 0.001$.

3 Results

3.1 Characteristics of soil and inputs used in the experiments

The soil in the experimental site has sandy clay loam texture topsoil (61.68% sand, 27.04% silt, and 11.28% clay). The topsoil (0–

TABLE 3 Laboratory analysis of soil samples.

Parameter	Method of analysis	References
Soil bulk density and moisture characteristics	Dying undisturbed core soil samples at 105°C for 24 h	(Rochette and Bertrand, 2007)
Soil texture	Bouyoucos hydrometer method, followed by dispersion of soil particles	(Beretta et al., 2014)
Soil pH and electrical conductivity	Soil:water suspension (1:2.5) using a glass electrode pH meter	(Okalebo et al., 2002)
Organic carbon	Wet oxidation by the Black and Walkley method	(Walkley and Black, 1934; Nelson and Sommers, 1996)
Total nitrogen	Micro-Kjeldahl wet digestion-distillation method	(Bremner, 1996)
C:N ratio		
Available P	Bray 1 method following color development using molybdenum blue method	(Bray and Kurtz, 2009)
Cation exchange capacity (CEC)	Neutral ammonium acetate saturation method (NH4-Ac, pH 7.0) followed by Kjeldahl distillation	(Mattigod and Zachara, 1996).
Exchangeable bases (K^+, Mg^{2+}, Ca^{2+}, and Na^+)	1N NH4-Ac (pH 7.0) method; Mg and Ca were read by a UV–VIS Spectrophotometer and a K and Na Flame Photometer	(Okalebo et al., 2002)
Extractable micronutrients (Fe, Cu, Zn, and Mn)	DTPA extraction and determined by atomic absorption spectroscopy (AAS)	(Okalebo et al., 2002; Lindsay and Norvell, 1978)

20 cm) has a pH $_{(H_2O)}$ of 4.8, E.C 0.06 dS/m, and contains 1.35% organic C, 0.33% total N, 0.68 mg kg^{-1} available P, 58.70 ppm available K, 0.19 mg kg^{-1} exchangeable Ca, 3.75% exchangeable sodium percentage, and 1.6 cmol kg^{-1} cation exchange capacity. Details of organic amendments are shown in Table 4.

3.2 Effects of *Azolla* rice straw and NPKS fertilizer combinations on soil chemical properties

Data in Table 5 show that except soil pH, all other tested soil chemical parameters were significantly affected by the different treatment combinations of synthetic fertilizers (NPKS), *Azolla*, and rice straw incorporation. Soil pH at the end of the first and second season ranged from 4.87 to 5.3 and 4.2 to 4.7, respectively. There is no significant difference in pH among treatments (p = 0.84). However, significant effects of pH were noted between the seasons ($p < 0.05$). The experiment results indicate a marked increase in the mean of total N compared to the pretreatment levels. The total N of soil ranged from medium (0.18%) to high (0.46%) for the first season and medium (0.13) to very high (0.65) in the second season. There is a significant ($p < 0.05$) increase in total N between the first and second season. The treatments involving the application of organic amendments (*Azolla* and rice straw incorporation) significantly impact the change in soil TN reserve over sole synthetic fertilizers. The concentration of SOC increased significantly ($p < 0.05$) between seasons compared to the pretreatment levels. At the end of the first season, the highest concentration of SOC (2.9%) was recorded under the treatment of sole rice straw incorporation, which was statistically ($p < 0.05$) higher than other treatments. At the end of the second experiment, the highest value of SOC (5.79%) was recorded under the treatment involving *Azolla* and 50% reduced N along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}, which was statistically similar to other organic amended treatments and superior over sole synthetic treatments. The C:N ratio of soil at a depth of 0–20 cm varied between 4.37 to 11.33 and 4.6 to 29.3 for the first and second seasons, respectively. The highest C:N ratio was recorded under sole rice straw treatments in both seasons. There is a significant difference ($p < 0.05$) in C:N ratio between different treatments and between seasons. The exchangeable K$^+$ concentrations in the soil at a depth of 0–20 cm ranged from 28.19 to 122.12 ppm in the first season and from 29.58 to 98.43 ppm in the second season. Treatments incorporating *Azolla* (T8, T10, T11, T12, and T13) consistently demonstrated increasing soil K$^+$ levels. The results indicate that the total P content in the soil significantly ($p < 0.05$) varied with treatment and seasons. In the first and second seasons, the total P content ranged from 0.31 to 2.39 mg kg^{-1} and 0.15 to 2.95 mg kg^{-1}, respectively. The highest P content was recorded under organic matter application (*Azolla* and rice straw) that was statistically higher over sole synthetic fertilizer treatments.

3.3 Effect of *Azolla*, rice straw, and NPKS fertilizers on enzyme activities

The data showed that the co-application of *Azolla*, rice straw, and synthetic fertilizers resulted in significantly higher enzymatic activities in both seasons compared to control and sole synthetic fertilizer treatments (Table 4). The rate of urea hydrolysis varied from 85.4 to 160.2 μg urea-N hydrolyzed g^{-1} dw soil 5 h^{-1} in the first season and 51.1 to 188.5 μg urea-N hydrolyzed g^{-1} dw soil 5 h^{-1} in the second season. The treatment involving *Azolla* combined with 50% reduced N, along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}, and co-treatment of *Azolla*, rice straw + 100 kg N ha^{-1}, 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} exhibited the highest urease activity for the first and second seasons, respectively. These two treatments significantly ($p < 0.001$) outperformed sole synthetic fertilizer combinations. No significant difference in urease activity was observed between different synthetic fertilizer applications. The treatments *Azolla* + 30 kg P ha^{-1} and *Azolla* + 50% reduced N, along

TABLE 4 Characteristics of *Azolla* and rice straw used in the experiment.

Parameters	SI units	Rice straw		*Azolla* biofertilizers			
		2022	2023	2022		2023	
				1st incorporation	2nd incorporation at 40 DAT	1st incorporation	2nd incorporation at 40 DAT
Organic C	%	40.3	38.24	31.3	34.87	34.3	35.8
Total N	%	0.68	0.82	2.17	1.92	1.8	2.2
C:N ratio		59:01	46:01	15:01	17:01	19:01	16:01
Total P	mg kg^{-1}	0.23	0.31	0.83	0.87	0.89	0.63
Azolla biomass dry basis	kg ha^{-1}	nd	nd	1,956	1,503.40	1,784	1,640
Azolla wet basis	t ha^{-1}	nd	nd	20.2	16.22	19.38	17.38
Estimate N fixed	kg ha^{-1}	nd	nd	42.4	29.02	32.11	36.08

DAT, days after transplanting; nd, not determined.

with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}, consistently resulted in statistically similar urea hydrolysis as per full recommended synthetic fertilizer application. The highest activities of acid phosphatase in the first season, 99.98 µg nitro phenol hydrolyzed g^{-1} DW soil 1 h^{-1}, were recorded in rice straw + 50 kg N ha^{-1} $^{+}$ 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}. In the second season, the highest activity of acid phosphatase, 136.38 µg nitro phenol hydrolyzed g^{-1} dw soil 1 h^{-1}, was recorded under *Azolla* + rice straw with 100 kg N ha^{-1} $^{+}$ 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}. Except for the sole application of 100 Kg N h^{-1}, all other treatments significantly ($p< 0.001$) differed from the control. Interestingly, treatments involving co-application of P with *Azolla* and rice straw significantly ($p< 0.001$) outperformed synthetic fertilizer combination treatments. Despite the population being normally distributed as per the Shapiro–Wilk test for Normality (4.597), there is no significant difference ($p< 0.001$) observed in alkaline phosphatase between fertilized treatments and even control.

3.4 Effect of *Azolla*, rice straw, and NPKS fertilizer treatments on rice grain yield, P uptake, and P use efficiency

The data of rice grain yield, P uptake, PUE, and AE of two cropping seasons are listed in Table 5. Results of this study indicated that the total biomass and rice grain yield increased significantly ($p< 0.001$) under co-application of *Azolla*, rice straw, and synthetic fertilizer treatments (Figure 2). The highest effect of P applied fertilizer on rice grain yield was observed under co-application of *Azolla* and rice straw along with 100 kg N ha^{-1} $^{+}$ 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}. No significant difference was observed between rice straw application and 30 kg P ha^{-1} with control. However, the application of rice straw with 50% reduced N along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} significantly increased rice grain yield over control. Interestingly, the application of *Azolla* with 50% reduced N along with + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} significantly ($p< 0.001$) maintained a higher grain yield as per full application of 100 kg N ha^{-1} $^{+}$ 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} through synthetic fertilizers. The total P uptake, PUE, and AE of P were affected by co-application of organic and synthetic fertilizers, suggesting the residual effect of treatments on P uptake and PUE (Table 5). The total phosphorus concentration in the above rice plant biomass ranges from 2.6 to 73.57 kg ha^{-1} and from 2.37 to 81.45 kg ha^{-1} for the first and second seasons, respectively. The study found that co-application of *Azolla* and rice straw + 100 kg N ha^{-1} along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} consistently resulted in higher P uptake and was significantly ($p< 0.001$) higher compared to other treatments. The sole application of 100 kg N ha^{-1} significantly ($p< 0.001$) increased aboveground P concentration over control. The application of *Azolla* + 30 kg P ha^{-1} and *Azolla* + 50% reduced N along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} resulted in similar P uptake ($p< 0.001$) over the balanced recommended level of synthetic fertilizers. No significant difference was observed between control and sole rice straw + 30 kg P ha^{-1}. According to the study, the application of rice straw along with 30 kg P ha^{-1} resulted in lower PUE and was significantly lower in the average PUE over other treatments. On the other hand, the application of *Azolla*, rice straw, and 100 kg N ha^{-1} along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} consistently resulted in higher PUE that significantly outperformed other treatments. The treatment involving *Azolla* + 50% reduced N along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} resulted in similar PUE with a balanced application of 100 kg N ha^{-1} $^{+}$ 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}. The mean values of AE generally increased with increased with increasing N rates in years 1 and 2. Co-application of *Azolla*, rice straw, and 100 kg N ha^{-1} along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} consistently increased AE by 91.4% and 16.6% compared to full application of 100 kg N ha^{-1} along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} through synthetic fertilizers for first and second seasons, respectively. The application of rice straw with 30 kg P ha^{-1} consistently resulted in low AE of P that was statistically ($p< 0.001$) lower compared to other treatments.

3.5 Association between rice yield, phosphorus use efficiency, and soil chemical and enzyme activities

The results for Spearman rank correlation analysis among rice yield attributes, PUE, and soil properties are shown in Figure 3. There was significant positive correlation between rice grain yield vis-à-vis agronomic efficiency of P (AEP), nitrogen uptake (NU), PU, and PUE (r = 0.99***, 0.877***, 0.895*** and 0.895***, respectively). Soil total N (TN) has a very strong positive correlation with soil organic carbon, acid phosphatase, exchangeable K^{+} and soil urease (URE) (r = 0.616***, 0.704***, 0.620***, and 0.704***, respectively). It also moderately correlated with NU (r = 0.251*). URE has a strong positive correlation with acid phosphatase (Ac.PH) and organic carbon (r = 1.000*** and 0.448**, respectively). PUE has strong positive correlations with PU and NU (r = 0.875***, and 0.895***, respectively), and moderately correlated with acid and alkaline phosphatase (r = 0.354* and 0.273*, respectively).

4 Discussion

4.1 Effects of treatments on soil chemical properties

The present study demonstrated that short-term application of *Azolla* and rice straw impact change in most chemical fertility (Figure 2). The stability of pH within certain ranges suggests that the treatments were effective in maintaining a suitable soil environment for rice cultivation. However, the observed seasonal disparity in pH emphasizes the dynamic nature of soil processes, influenced by both applied treatments and external factors associated with seasonal changes. Soil chemical fertility depends on several factors such as climate, topography, nature of the soil, and type of amendments (Körschens et al., 2013; Tian et al., 2015a). Similar

TABLE 5 The effect of applying *Azolla*, rice straw, and NPKS fertilizer combinations on soil chemical properties.

Treatments	pH	Organic carbon (%)	Total N (%)	C: N ratio	TP (mg/kg)	Exchangeable K (ppm)	pH	Organic carbon (%)	Total N (%)	C: N ratio	Total P (mg/kg)	Exchangeable K (ppm)
T1	5.1 a	1.7 ab	0.18 a	10.29 cd	0.495 ab	34.43 a	4.7 a	0.94 a	0.13 a	7.37 a	0.330 a	36.24 ab
T2	4.8 a	1.6 ab	0.25 abc	6.34 abc	0.31 a	31.55 a	4.5 a	0.85 a	0.19 a	4.68 a	0.152 a	32.01 a
T3	5.3 a	1.9 ab	0.34 c	5.62 ab	1.09 bc	31.09 a	4.2 a	1.638 a	0.13 a	11.43 ab	1.387 b	34.09 ab
T4	4.8 a	1.4 a	0.32 bc	4.78 ab	1.33 cde	44.96 ab	4.2 a	1.06 a	0.18 a	5.81 a	1.651 bc	47.72 abcd
T5	5.0 a	1.7 ab	0.21 ab	8.04 abcd	1.25 cd	29.30 a	4.5 a	1.10 a	0.18 a	9.56 a	1.80 bcd	29.58 a
T6	4.9 a	1.9 ab	0.30 bc	6.50 abc	1.15 cd	41.62 a	4.2 a	1.21 a	0.21 a	5.96 a	1.701 bc	40.42 abc
T7	5.1 a	2.9 c	0.26 abc	11.33 d	2.16 fg	28.19 a	4.4 a	5.62 b	0.19 a	29.31 b	2.51 ef	31.91 a
T8	5.1 a	2.3 bc	0.35 cd	6.64 abc	2.11 fg	122.12 c	4.4 a	5.41 b	0.65 c	8.33 a	2.22 de	82.09 bcde
T9	4.9 a	1.9 ab	0.29 abc	6.42 abc	2.39 g	104.75 c	4.4 a	4.88 b	0.37 ab	20.09 ab	1.93 cd	79.31 abcde
T10	5.0 a	2.0 ab	0.46 d	4.37 a	1.70 cdef	114.51 c	4.3 a	5.79 b	0.63 bc	9.21 a	2.59 efg	98.43 e
T11	5.0 a	2.1 ab	0.34 cd	8.49 bcd	1.95 efg	91.40 bc	4.5 a	5.35 b	0.56 bc	9.59 a	2.44 ef	94.31 de
T12	4.9 a	2.3 abc	0.36 cd	6.50 abc	1.78 defg	99.79 c	4.6 a	5.62 b	0.62 bc	9.09 a	2.87 fg	71.71 abcde
T13	5.0 a	2.0 ab	0.35 cd	7.33 abcd	1.78 defg	105.51 c	4.4 a	5.18 b	0.61 bc	8.59 a	2.95 g	86.20 cde
p-value	0.84	<0.001	0.001	<0.001	<0.001	<0.001	0.087	<0.00	<0.001	0.003	<0.001	<0.001
Cv	6.3	14.2	12.6	19.2	14.4	23.8	4.0	10.9	24.3	56.5	0.1217	28.3
s.e.d.	0.2606	0.23	0.03	1.118	0.17	13.16	0.144	0.30	0.0717	4.932	7.9	13.58
Seasons												
2022	5.037 b	2.124 a	0.3127 a	7.13 a	1.503	74.0 a						
2023	4.437 a	3.438 b	0.3612 a	10.69 b	1.889	58.8 a						
***p*-value**	0.036	0.013	0.089	0.020	0.002	0.196						
Lcd	0.5034	0.6529	0.0665	2.217	0.0792	34.27						

Different lowercase letters over each column indicate significant ($p< 0.05$) differences of the mean within input treatments (T1–T13) as presented in Table 1.

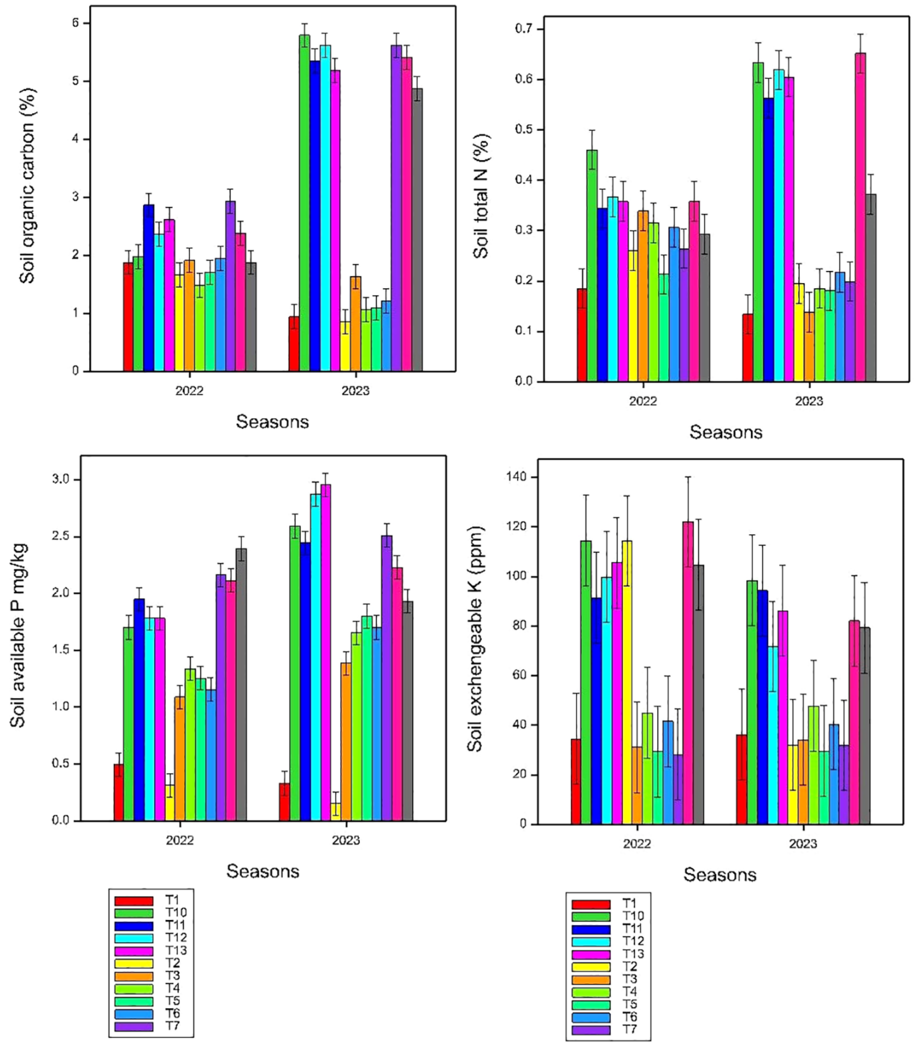


FIGURE 2
Effects of co-application of Azolla, rice straw, and NPKS fertilizer combination on some selected soil properties, small bars are standard errors at $p < 0.05$.

results were reported by Zhou et al. (2015) in which application of chemical fertilizers alone or partially substituted with organic fertilizers increased the soil pH by 0.71 to 0.96 units over the control. This might be due to the severe microbial nitrification process that led to soil acidification, which is largely attributed to ammonium fertilizers applied in the soil (Tian et al., 2015b). Soil total nitrogen is the major determinant and indicator of soil fertility and quality in an agricultural ecosystem and is closely related to soil productivity (Li et al., 2022). The significant increase in total nitrogen (TN) levels observed in the soil, particularly in the *Azolla*-fertilized treatments, might be attributed to *Azolla* N fixing capacity. *Azolla* is known for its ability to fix atmospheric nitrogen with the help of nitrogen-fixing cyanobacteria present in its symbiotic relationship (Akhtar et al., 2020). This process results in an increase in available nitrogen in the soil (Dey and Datta, 2008). The combination of *Azolla* and rice straw incorporation might have synergistic effects on nitrogen availability. *Azolla* provides nitrogen through biological nitrogen fixation, while rice straw helps in accumulation of TN stocks. Together, they create a more comprehensive impact on soil TN levels. Rice straw incorporation significantly ($p < 0.05$) affected the soil organic carbon. This might be due to the wider C/N (59.1) ratio of straw that takes longer to decompose and thus improve soil aggregation and soil water retention and reduce bulk density of the soil, promoting crop growth and TN stocks (Ekawati and Purwanto, 2014; Li et al., 2022). The observed increase in SOC concentrations is statistically significant ($p < 0.05$), emphasizing the reliability of the results. This suggests that the changes in SOC are not due to random variability but are attributed to the applied treatments. This observed outcome highlights the importance of nutrient management strategies that integrate organic inputs such as *Azolla* and rice straw and the synergy between organic amendments and adjusted synthetic fertilizers contributes to the improvement of SOC, indicating a more sustainable and holistic approach to soil health. Soil organic carbon and its stoichiometric characteristics are important indicators for the quality and quantity of soil organic matter (Tong et al., 2023). The carbon-to-nitrogen (C:N) ratio in the soil is a crucial indicator of nutrient availability and microbial activity (Landon, 1991). A higher C:N ratio generally indicates slower

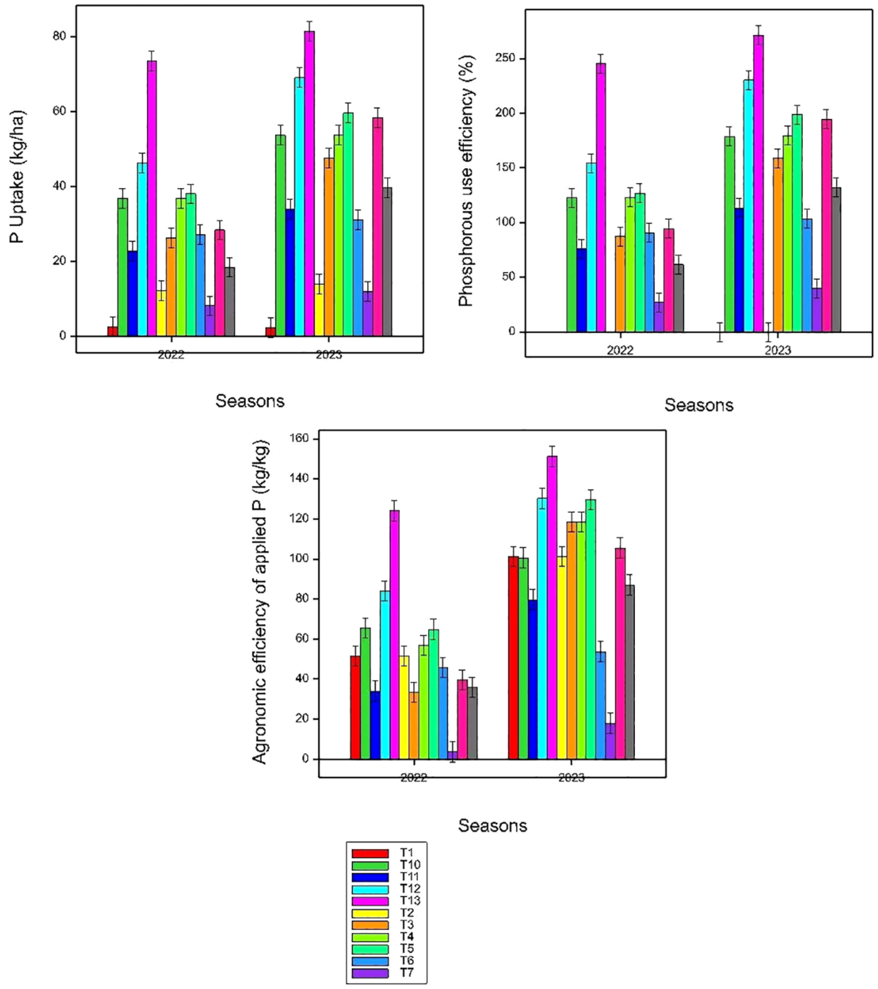


FIGURE 3
Effects of co-application of Azolla, rice straw, and NPKS fertilizer combination on soil urease, acid, and alkaline phosphatases; small bars are standard errors at p< 0.05.

decomposition of organic matter relative to nitrogen release. The consistently highest C:N ratio recorded under sole rice straw treatments in both seasons suggests that the decomposition of rice straw, which is rich in carbon, outpaced nitrogen release. This leads to a higher C:N ratio, indicating a relative abundance of carbon compared to nitrogen. This is in accordance with some results of Zhang et al. (2015). However, results demonstrated that the incorporation of synthetic fertilizers, *Azolla*, and rice straw each contributes differently to the carbon and nitrogen content of the soil, influencing the overall C:N ratio. The significant differences in C:N ratio between seasons suggest that continuous application of treatment combinations could enhance organic matter reserve and decomposition that subsequently impacts change in nutrient release. This might be a holistic approach to nutrient management and sustainable agricultural practices. Results of the experiments indicated that application of *Azolla* and rice straw enhances P content in soil. The rise in soil P content can be attributed to *Azolla*'s high P absorption capacity, directly promoting *Azolla* biomass growth. The elevated PUE in these treatment combinations provides clear evidence that upon the decomposition of *Azolla* plants, the organic nitrogen and phosphorus undergo rapid mineralization, releasing them as biofertilizers available for the thriving rice plants. The study by Chatterjee et al. (2021) demonstrated that long-term organic fertilization, including the application of *Azolla*, consistently yielded the highest P content in soil, surpassing the levels observed in treatments solely reliant on synthetic fertilizers. These findings emphasize the effectiveness of organic matter (*Azolla* and rice straw) in enhancing soil phosphorus levels, suggesting its potential as a sustainable alternative to synthetic fertilizers.

4.2 Enzyme activity

Soil microbial communities produce enzymes in response to soil changes that modify organic substrates and other soil factors (Lagos et al., 2015). Hence, it is possible to use enzymatic activity as an indicator of soil changes (Saha et al., 2008; Liang et al., 2014). This study showed clear response of enzyme activities by co-application of *Azolla*, rice straw, and synthetic fertilizers on URE activity over sole

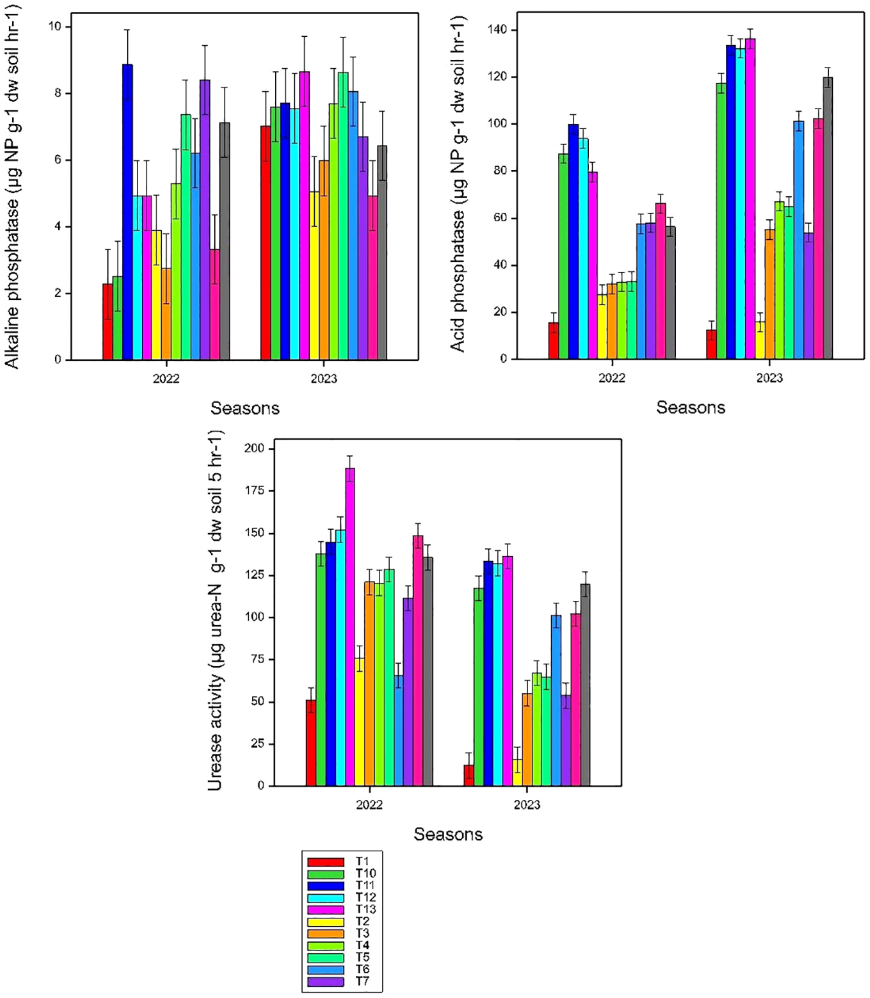


FIGURE 4
Effects of applying rice straw, Azolla, and NPKS fertilizer combinations (T1–T13) on total biomass (kg ha^{-1}) and rice grain yield (kg ha^{-1}); small bars are standard errors at p< 0.05.

synthetic fertilizers (Figure 4). The higher urease activity in treatment involve *Azolla* combined with 50% reduced N, along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}, and co-treatment of *Azolla*, rice straw + 100 kg N ha^{-1}, 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} could be attributed to improvement of soil organic carbon content that directly enhances microbial community (Luo et al., 2016; Sharma et al., 2021; Singh et al., 2023). Generally, an increase in urease activity indicates an improvement in the ability of microorganisms in the soil to convert urea into ammonium and make it available to plants. This is crucial for NU by crops (Apoorva et al., 2018). Similar urease activities in rice straw treatment and 50% reduced level of N along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} enhanced soil microbial population and increased soil N availability. A previous study by Sharma et al. (2021) demonstrated that the co-application of rice straw with N fertilizer significantly increases soil enzyme activity through enhanced substrate availability. Another study (Jat et al., 2019) found that if cereal residues are incorporated in soil, N immobilization might require higher levels of fertilizer N in the short term as compared with no residue. The return of crop residues may, however, lead to a net buildup of readily mineralized soil organic nitrogen, which may reduce crop fertilizer requirements in the future. Phosphomonoestarases are large groups of enzymes that increase the rate of organic phosphate hydrolysis (Sırt Çıplak and Akoğlu, 2020). The higher activities of acid phosphomonoestarases than alkaline phosphomonoestarases might be due to the pH nature of the soil, since the optimal activity of these enzymes is pH dependent (Nannipieri et al., 2012). Previous studies demonstrated that in adaptation to different environmental conditions, acidic soils tend to have higher levels of acid phosphomonoestarases, while alkaline soils tend to have higher levels of alkaline phosphomonoestarases; for this reason, neither enzyme is positively correlated with the other (Nannipieri et al., 2012; Dotaniya et al., 2018). Higher levels of acid phosphomonoestarases might also be influenced by wet soil conditions as observed by Grierson and Adams (2000) in moist winter and spring; acid phosphatase activity was between 30 and 40 mmol p-Nitrophenol g^{-1} h^{-1} that declined to less than 10 mmol p-Nitrophenol g^{-1} h^{-1} during the dry summer. The highest activities of acid phosphatase under rice straw + 50 kg N ha^{-1} + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} in the first season and

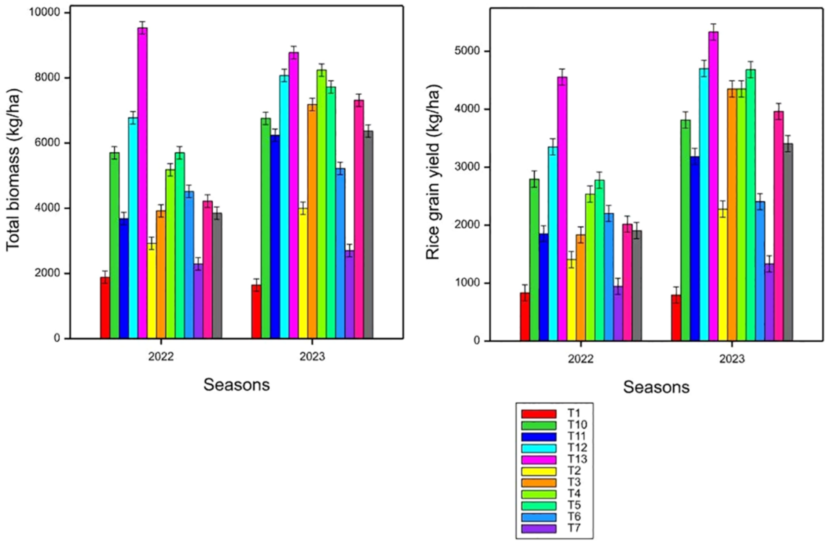


FIGURE 5
Effects of applying rice straw, Azolla, and NPKS fertilizer combinations (T1–T13) on P uptake, agronomic efficiency of P, and P use efficiency; small bars are standard errors at $p< 0.05$.

Azolla + rice straw with 100 kg N ha^{-1} + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} in the second season might be due to enhancing a favorable environment for microbial activity and nutrient cycling, specifically benefiting acid phosphatase activity in the soil. The study by Nannipieri et al. (2012) demonstrated that acid phosphatase activities are correlated with the content of organic matter and decrease with soil depth. Another study (Mohamed et al., 2021) reported that incorporating rice straw stimulates soil microorganisms and accordingly enhances soil dehydrogenase, catalase, and acid phosphatase activities.

4.3 Effect of *Azolla*, rice straw, and NPKS fertilizer treatments on grain yield, P uptake, and P use efficiency

The observed outcomes in rice grain yield based on different treatments suggest that the interaction between organic amendments (rice straw and *Azolla*) and synthetic fertilizers, along with variations in nutrient management, plays a crucial role in influencing crop productivity (Figure 5). The application of *Azolla* and rice straw along with 100 kg N ha^{-1}, 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} has a significant effect on the total biomass and grain yield. This effect may be attributed to the physicochemical changes in the rhizosphere soil, including (1) improved soil water holding capacity (Andriamananjara et al., 2019); (2) decreased soil bulk density, thereby improving root nutrient acquisition (Awodun, 2008); (3) enhance availability of N, P, and other essential nutrients (Division et al., 1995; Ali et al., 2014; Chawngthu et al., 2020; Liu et al., 2021); (4) decreased P sorption sites after interaction of organic anion released during *Azolla* and rice straw decomposition in the soil through complexation or precipitation of soluble Al or competitive sorption sites; and (5) promotion of soil microbial biomass and activity (Andriamananjara et al., 2019; Dhar et al., 2022; Khmelevtsova et al., 2022). Additionally, *Azolla*, being a nitrogen-fixing aquatic fern, may have contributed nitrogen to the soil, compensating for the reduced synthetic nitrogen input, fostering optimal conditions for rice growth. The maintenance of a higher grain yield in sole *Azolla* along with 30 kg P ha^{-1} compared to full synthetic fertilizer application suggests the efficiency of *Azolla* in nutrient provision. Because the application of *Azolla*, rice straw, and 50% reduced N along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} did not significantly affect total biomass and rice grain yield over the full recommended level of NPKS through synthetic fertilizers, our results suggest that this combination may contribute to yield and long-term environmental and soil quality management.

The study reveals interesting findings regarding phosphorus (P) uptake in plants under various treatment conditions (Table 6); higher P uptake under co-application of *Azolla* and rice straw along with 100 kg N ha^{-1}, 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} might be due to the synergistic effect of *Azolla*, rice straw, and synthetic fertilizers, which likely created an optimal environment for phosphorus availability and uptake by rice plants. Compared to the control using the full dose of chemical N fertilizer alone (100 kg N ha^{-1}) seems to have a positive effect on phosphorus concentration possibly due to stimulating plant growth and biomass production, which can increase the demand and uptake of P by plants (Tang et al., 2021; Zhang et al., 2022a). However, the effect of N input on P uptake may depend on the type, amount, and timing of N input, the plant species and functional traits, the soil properties and P status, and the interactions with other factors (Zhang et al., 2022b; Chen et al., 2023). Therefore, the response of P uptake to N input may vary across different ecosystems and experimental conditions. The addition of rice straw alone with 30 kg P ha^{-1} may not provide a

TABLE 6 Effects of *Azolla*, synthetic fertilizers, and rice straw on P use efficiency and enzyme activities.

Treatments		First season 2022								Second season 2023					
	P-rates (kg P ha^{-1})	Grain yield kg/ha	P uptake (kg/ha)	PUE (%)	Urease	Agronomic efficiency (kg^{-1})	Acid phosphatase	Alkaline phosphatase	Grain yield kg ha^{-1}	P uptake (kg/ha)	PUE (%)	Agronomic efficiency	Urease	Acid phosphatase	Alkaline phosphatase
T1	**0**	833 a	2.61 a	0	85.4 ab	0	15.72 a	2.287 a	796 a	2.37 a	0.0 a	0	51.1 a	12.37 a	7.02 ab
T2	**0**	1,407 b	12.18 bc	0	64.7 a	0	27.51 ab	3.905 a	2278 bc	14.01 ab	0.0 a	0	75.8 abc	15.83 a	5.06 a
T3	**30**	1,833 c	26.22 de	87.39 bc	98.0 abc	33.33 b	32.02 ab	2.749 a	4352 ef	47.59 cde	158.6 bcd	118.52de	121.1 cd	55.13 b	5.98 ab
T4	**30**	2,537 de	36.85 fg	122.83 de	67.6 a	56.79 cd	32.94 b	5.292 a	4352 ef	53.82 def	198.9 de	118.52def	120.6 cd	67.15 b	7.70 ab
T5	**30**	2,778 e	38.05 gh	126.82 ef	75.8 a	64.81 d	33.06 b	7.372 a	4685 fg	59.67 ef	179.4 cde	129.63ef	128.8 d	64.96 b	8.64 b
T6	**30**	2,204 cd	27.19 e	90.64 c	91.7abc	45.68 bc	57.67 c	6.216 a	2407 c	31.04 bc	103.5 b	53.70 b	65.6 ab	101.36 c	8.06 ab
T7	**30**	944 a	7.27 ab	24.24 a	125.4 cde	3.70 a	58.14 c	8.412 a	1333 ab	11.99 a	40.0 a	17.90 a	111.7 bcd	53.86 b	6.71 ab
T8	**30**	2,019 c	28.36 ef	94.55 cd	120.8bcd	39.51 b	66.23 cd	3.327 a	3963 def	58.41 ef	194.7 de	105.56 cde	148.6 de	102.40 c	4.94 a
T9	**30**	1,907 c	18.44 cd	61.48 b	116.0bcd	35.80 b	56.40 c	7.141 a	3407 de	39.61 cd	132.0 bc	87.04 bcd	135.8 d	119.86 cd	6.43 ab
T10	**30**	2,796 e	36.82 fg	122.73 de	93.7abc	65.43 d	87.49 ef	2.518 a	3815 def	53.67 def	178.9 cde	100.62 cde	137.9 d	117.43 cd	7.60 ab
T11	**30**	1,852 c	22.79 de	75.96bc	147.0 de	33.95 b	99.98 f	8.875 a	3185 cd	33.99 c	113.3 b	79.63 bc	144.9 de	133.50 d	7.71 ab
T12	**30**	3,352 f	46.24 h	154.12 f	160.2 e	83.95 e	93.97ef	4.945 a	4704 fg	69.11 fg	230.4 ef	130.25 ef	152.2 de	132.22 d	7.55 ab
T13	**30**	4,556 g	73.57 i	245.24 g	140.0 de	124.07 f	79.63 de	4.945 a	5333 g	81.45 g	271.5 f	151.23 f	188.5 e	136.38 d	8.66 b
***p*-value**		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.008
Cv		6.0	10.0	10.3	11.4	9.1	9.9	43.4	9.5	13.3	13.8	11.7	13.8	9.5	16.6
s.e.d		109.8	2.364	7.78	9.92	3.971	4.597	1.855	267.4	4.666	15.58	9.48	13.69	6.65	0.962
Seasons															
2022		2232 a	29.03 a	93.0 a	121.7 b	53.1 a	56.98 a	5.23 a							
2023		3432 b	42.82 a	138.5 a	85.6 a	99.6 b	85.57 b	7.09 b							

(Continued)

TABLE 6 Continued

Treatments		First season 2022							Second season 2023						
	P-rates (kg P ha^{-1})	Grain yield kg/ha	P uptake (kg/ha)	PUE (%)	Urease	Agronomic efficiency (kg^{-1})	Acid phosphatase	Alkaline phosphatase	Grain yield kg ha^{-1}	P uptake (kg/ha)	PUE (%)	Agronomic efficiency	Urease	Acid phosphatase	Alkaline phosphatase
Seasons															
***p*-value**		<0.001	0.006	0.005	0.003	0.001	0.004	0.047							
Lcd		102.0	4.543	14.06	8.34	6.54	8.165	1.794							

The data are expressed as mean (n = 13). Different letters within columns indicate significant differences (p< 0.05) (urease activity was expressed as µg urea-N hydrolyzed g^{-1} dw soil 5 h^{-1}; acid phosphatase and alkaline phosphatase were expressed as µg nitro phenol hydrolyzed g^{-1} dw soil 1 h^{-1}).

substantial boost in PU compared to the control, possibly due to the high carbon-to-phosphorus ratio of rice straw that may lead to microbial immobilization of phosphorus, reducing its availability for plant uptake (Mi et al., 2016). However, rice straw decomposition may be accelerated by the presence of organic amendments with low C:N ratio (Gummert et al., 2020; Van Hung et al., 2020). These results provide valuable insight into effective and environmentally conscious nutrient management practices, which can have positive implications for crop productivity, soil health, and agricultural sustainability. PUE was expressed using a balanced method to show how much P is being taken out of the system compared with the amount applied and the residual (Syers et al., 2008). Inorganic P was applied as triple super phosphate (TSP) at a constant rate (30 kg P ha^{-1}). However, it was observed that different treatments had a significant effect ($p \leq 0.05$) on PUE, indicating that phosphorus input does not solely determine PUE, but various other factors also affect it. Diverging responses of sole rice straw incorporation along with 30 kg P ha^{-1} with respect to PUE were observed. This suggests that, under the specific conditions of the study, the use of rice straw with 30 kg P ha^{-1} did not efficiently contribute to phosphorus utilization by the plants, possibly due to the high C/N ratio of straw that creates a nitrogen deficiency in the soil (Zheng et al., 2015). This was also confirmed by researchers (Nekir, 2019; Liu et al., 2020; Chen et al., 2021) in which nitrogen is required for proper P uptake and utilization, and its deficiency can limit the efficiency of P uptake. The higher PUE under the co-application of *Azolla*, rice straw, and 100 kg N ha^{-1} along with P, K, and S might be due to the synergistic effect of this treatment combination, particularly with added nitrogen and balanced nutrients, which appears to enhance PUE, possibly due to improved nutrient availability and microbial activity. There is substantial evidence that application of a high rate of N results in soil acidification, which decreases exchangeable Ca and Mg concentrations while mobilizing Fe and Al with significant effects on repressing soil microbial population and soil phosphatase activity (Kimani et al., 2020; Chen et al., 2021; Zhang et al., 2022b).

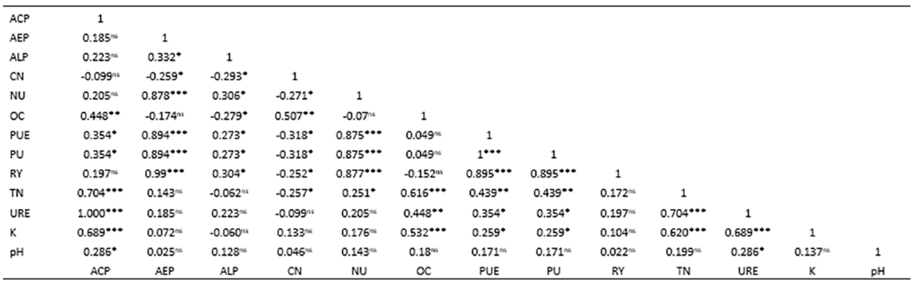
The consistency increase in mean values of agronomic efficiency (AE) with higher nitrogen rates in both years suggests that, in the context of this study, increasing the nitrogen input positively influenced agronomic efficiency, likely leading to improved crop productivity. A similar observation was made by Biswas Chowdhury and Zhang (2021) and Chen et al. (2021). This indicates a more effective use of applied nutrients for crop yield. The high AE in the co-application of *Azolla* and rice straw along with 100 kg N ha^{-1}, 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} treatments was attributed to soil C status and total nutrients added that increase microbial activity and carbon availability, and caused indirect modifications of the soil. By adding organic matter and nutrients to paddy soils, organic amendment can enhance soil biological activity and sustain soil quality (Andriamananjara et al., 2019). The combination of *Azolla*, rice straw, and half the amount of N, along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}, may have improved the agronomic efficiency of P in this study. This may have achieved a better balance of nitrogen and phosphorus in the soil and promoted the soil nutrient cycling and microbial activity. Therefore, based on our research findings, we strongly recommend adopting alternative fertilization strategies to mitigate the negative impacts associated with overapplication of synthetic chemical fertilizers. Specifically, we propose the application of rice straw and *Azolla* with reduced nitrogen as a viable solution. This approach can be considered a form of slow-release fertilization, offering several benefits. Notably, it enhances phosphorus (P) recovery efficiency and contributes to better soil health management for the future.

4.4 Association between rice yield, phosphorus use efficiency, and soil quality indicators

In this study, the positive correlation coefficients indicate that as one variable increases, the other tends to increase as well. A coefficient value close to 1 suggests a strong linear relationship between the variables, implying that changes in one variable are highly predictive of changes in the other. The Spearman rank correlation analysis conducted on rice yield attributes, PUE, and soil properties revealed significant associations among them (Figure 6). Rice grain yield exhibits a remarkably strong positive correlation with agronomic efficiency of P (AEP), NU, PU, and PUE (r = 0.99***, 0.877***, 0.895***, and 0.895***, respectively). Nitrogen and phosphorus play an important role in supporting rice growth and yield (Fomba et al., 2020; Rajï, 2020; Rupngam et al., 2023). Proper utilization of N and P enhances root development, shoot growth, photosynthesis, effective tiller formation, and rice grain yield (Kalayu, 2019; Margalef et al., 2021; Aimen et al., 2022; Tariq et al., 2022), emphasizing the critical role of nutrient use efficiency in achieving higher yields. Soil total nitrogen (TN) demonstrates strong positive correlations with soil organic carbon, P uptake, PUE, acid phosphatase, exchangeable K^+, and URE highlighting the intricate relationships between soil fertility indicators. These correlations suggest that a balanced availability of nitrogen might play a role in enhancing the efficiency of phosphorus utilization by rice plants (Marklein and Houlton, 2011; Arenberg and Arai, 2021). Optimal nitrogen levels might support metabolic processes that contribute to more efficient PU and utilization, leading to higher PUE (Hogan et al., 2010). Moreover, TN moderately correlates with NU, indicating its influence on soil N availability. However, the moderate correlation between TN and N uptake might be due to increasing N losses associated with excessive N application. Moreover, excess nitrogen prolongs the vegetative growth period, delays maturity, and attracts insect pests, leading to disease epidemics (Anas et al., 2020). The strong positive correlations exist between URE, acid phosphatase, and organic carbon, underscoring the interconnected nature of soil enzymatic activities and organic matter application. Soil organic matter inputs have been found to alter soil enzyme activity and allocation patterns (Weintraub et al., 2013). However, the correlation between soil enzymes and organic matter is intricate and dependent on the enzyme type, as well as the quality and quantity of organic matter (Margalef et al., 2021). Interestingly, PUE demonstrates strong positive associations with PU and NU, and moderate correlations with acid and alkaline phosphatase, illustrating the interplay between nutrient utilization efficiency and enzymatic processes in soil.

5 Conclusion and recommendation

The results suggest that the short-term application of *Azolla* and rice straw can induce significant soil chemical fertility and enzyme activity. In addition, the results indicated that the synergistic effect of *Azolla* and rice straw contributes to higher soil total nitrogen levels, maintaining pH stability for rice cultivation, and enhances soil total N, organic carbon, and total P highlight dynamic soil processes influenced by treatments and external factors. Total nitrogen increase, particularly in *Azolla*-treated soils, suggests its nitrogen-fixing capacity. Rice straw incorporation influences soil organic carbon, impacting soil health sustainably. The study further indicates that the co-application of *Azolla* and rice straw with 50% reduced N and balanced 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} improves rice yield, PU, and PUE, demonstrating a sustainable practice for enhancing crop performance and soil health. The study underlines that short-term application of *Azolla* and rice straw with



	ACP	AEP	ALP	CN	NU	OC	PUE	PU	RY	TN	URE	K	pH
ACP	1												
AEP	0.185^{ns}	1											
ALP	0.223^{ns}	0.332*	1										
CN	-0.099^{ns}	-0.259*	-0.293*	1									
NU	0.205^{ns}	0.878***	0.306*	-0.271*	1								
OC	0.448**	-0.174^{ns}	-0.279*	0.507**	-0.07^{ns}	1							
PUE	0.354*	0.894***	0.273*	-0.318*	0.875***	0.049^{ns}	1						
PU	0.354*	0.894***	0.273*	-0.318*	0.875***	0.049^{ns}	1***	1					
RY	0.197^{ns}	0.99***	0.304*	-0.252*	0.877***	-0.152^{ns}	0.895***	0.895***	1				
TN	0.704***	0.143^{ns}	-0.062^{ns}	-0.257*	0.251*	0.616***	0.439**	0.439**	0.172^{ns}	1			
URE	1.000***	0.185^{ns}	0.223^{ns}	-0.099^{ns}	0.205^{ns}	0.448**	0.354*	0.354*	0.197^{ns}	0.704***	1		
K	0.689***	0.072^{ns}	-0.060^{ns}	0.133^{ns}	0.176^{ns}	0.532***	0.259*	0.259*	0.104^{ns}	0.620***	0.689***	1	
pH	0.286*	0.025^{ns}	0.128^{ns}	0.046^{ns}	0.143^{ns}	0.18^{ns}	0.171^{ns}	0.171^{ns}	0.022^{ns}	0.199^{ns}	0.286*	0.137^{ns}	1

FIGURE 6
Correlation matrix displaying correlations among rice yield, phosphorus use efficiency, enzyme activity, and other soil properties. The values indicate coefficients and their associated significance *p< 0.05, **p< 0.01, ***p< 0.001 at 5% confidence level (ACP, acid phosphatase; AEP, agronomic efficiency of P; ALP, alkaline phosphatase; NU, nitrogen uptake; PUE, P use efficiency; PU, P uptake; RY, rice grain yield; TN, total N; URE, urease). NS, not significance.

reduced N and balanced PKS enhances phosphorus content in soils and underscores *Azolla*'s high absorption capacity and its role in enhancing soil fertility and PUE. These findings emphasize the potential of organic amendments for holistic nutrient management and sustainable agriculture practices.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

SM: Conceptualization, Methodology, Resources, Writing – original draft. HT: Methodology, Supervision, Writing – original draft. NA: Supervision, Writing – original draft. HC: Project administration, Resources, Writing – original draft. JS: Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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