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Rice straw incorporation and Azolla application improves agronomic nitrogen-use-efficiency and rice grain yields in paddy fields

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In paddy soils, excessive application of N fertilizer often results in substantial N losses due to low N utilization efficiency. However, this condition can be mitigated by enhancing soil organic carbon content. Two-year field experiment was carried out at Mkula Irrigation Scheme in Kilombero Valley, Tanzania with the aim of investigating the impact of Azolla, rice straw incorporation and reduced levels of nitrogen input from NPKS-containing fertilizers on N use efficiency, soil chemical properties and rice grain yield. Assuming that this technology will introduce a novel perspective to the research, shedding light on alternative and potentially more sustainable methods for nitrogen management in paddy soils, it will be particularly relevant in sub-Saharan Africa, where the annual cost of chemical fertilizers is expected to continue rising. The treatments involved absolute control, half dose N (50 kg N ha^{-1}), full dose N (100 kg N ha^{-1}), and combination of these N doses with PKS, dry Azolla (3.4 *t* ha^{-1}) and rice straw (6.9 t ha^{-1}) through omission approach. The soil of the experimental area was sandy clay loam in texture, very strongly acid (pH 4.8), normal electrical conductivity (0.06 dS m^{-1}), low amounts of recorded organic carbon (1.35%), total nitrogen (0.33%), 0.68 mg kg^{-1} available P, exchangeable potassium (0.15 cmol$_{(+)}$ kg^{-1}), calcium (0.19 mg kg^{-1}) and sodium percentage (3.75%), with very low cation exchange capacity (1.6 cmol$_{(+)}$ kg^{-1}). The results showed that combination of Azolla, rice straw +100 kg N ha^{-1} $^{+}$ 30 kg P ha^{-1} $^{+}$ 30 kg K ha^{-1} $^{+}$ 20 kg S ha^{-1} resulted in higher rice grain yield, nitrogen uptake and agronomic efficiency of N. Azolla, being an effective biofertilizer, significantly contributes to nitrogen fixation and soil enrichment. Interestingly, this study demonstrates that co-application of Azolla, rice straw, and 50% reduced N is effective for achieving high rice yields, minimizing over-dependence on chemical N fertilizer, sustainable agricultural development, and environmental conservation.

KEYWORDS

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

1 Introduction

Nitrogen (N) plays a significant role in crop plants' photosynthesis, protein synthesis, amino acids, chlorophyll, nucleic acids, ATP, and phytohormone production. Nitrogen use efficiency (NUE) the relationship between the dry matter production or economic yield of a crop and the quantity of N applied) is highly dependent on applied N fertilizer and soil (1). The NUE indices refer to key metrics used to assess the effectiveness with which plants utilize nitrogen in their growth and development (2, 3). These indices help measure the efficiency of nitrogen uptake, assimilation, and utilization by plants, which is crucial for optimizing fertilizer application and reducing environmental impacts such as nitrogen leaching and greenhouse gas emissions (4–6). According to Congreves et al. (7), the common NUE indices include nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE), and nitrogen recovery efficiency (NRE). NUpE evaluates the ability of plants to acquire nitrogen from the soil, NUtE assesses the conversion of absorbed nitrogen into biomass, and NRE quantifies the proportion of applied nitrogen that is taken up by plants. These soil-based indices provide valuable insights into nutrient dynamics and plant-soil interactions, contributing to sustainable nitrogen management in agriculture (7). The capacity of crops to absorb nitrogen (N) relies on several interconnected factors, including soil fertility, crop variety, soil moisture, temperature, seasonal timing, N uptake patterns, pest and disease prevalence, farmer knowledge, and socioeconomic conditions on the farm (6). The primary objective of this study is to explore fertilizer-based indices, specifically focusing on guiding efficient nitrogen management practices. According to Kimani et al. (8) nitrogen use efficiency for cereal crop production globally is approximately 30–50%. Similarly Liu et al. (9) reported average apparent recovery efficiency (AREN) and agronomic use efficiency of N were 39.0% and 12.7 kg kg^{-1}, respectively. Therefore, improving NUE can enhance plant performance and increase crop yields (10) especially in rice producing areas. However, the demand for rice (*Oryza sativa* L.) cultivation is set to soar along with a growing global population, causing nitrogen fertilizer consumption to increase by about 2-fold by 2050 (11).

Excessive application of N fertilizer in paddy soils with characteristically low N utilization efficiency leads to a large amount of N losses (12). Previous studies estimated that 10–40% of chemical N fertilizer applied in paddy is lost either through Ammonia volatilization (9, 13–16), or through conditions associated with potential environmental and ecological disturbances, such as soil acidification (17, 18), eutrophication (19), reduced soil biodiversity (20), nutrient imbalance (21, 22) and enhanced salt accumulation (9). Thus, rice cropping systems require sustainable agronomic and soil management practices to improve N use efficiency and minimize negative environmental impacts on paddy fields (1, 10, 23).

Several options have been practiced to minimize the effects of N-loss and improve nitrogen use efficiency in lowland rice production systems. These include application of urea super-granules, urease inhibitors, and slow-release urea (16, 24–26). However, these technologies result in high economic costs and become unaffordable by smallholder farmers (12, 27).

While approaches such as incorporation of organic materials with wide C/N ratios, such as rice straw can increase microbial activity and lead to increased N immobilization (28–30), their combined application with narrow C/N ratio materials such as Azolla might be a good option to mitigate methane emissions (31), improve soil organic carbon and other nutrients supply as well as minimizing N losses (32–34). Despite the widespread recognition of these materials' potential benefits in agriculture (35, 36). Yet in Tanzania, this is the first study for Azolla and rice straw to assess N use efficiency. Hence, this study aims to address this gap by investigating the effects of azolla, rice straw, and NPK fertilizers on Tanzanian soils rather than solely focusing on traditional chemical fertilizers. This is because, Azolla application will allow resource-poor farmers to substitute chemical fertilizer N with biologically fixed N- the latter being not immediately subject to ammonia volatilization.

2 Materials and methods

2.1 Description and location of the study area

This experiment 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 *Eastern Plateaux and Mountain Blocks* in Morogoro Region-Tanzania. The climate is classified as tropical savanna climate with bimodal rainfall distribution pattern; having dry spells separating short rainy season from October to December and long rainy season from March to May (37, 38). The mean annual rainfall and temperature range from 1200-1400 mm and 22-23°C, respectively (39, 40). 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 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.

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 fertilizers application
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}

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 multiplicated in the propagation pond (6 m × 5 m) of the same aquaculture unit at the university campus.

During preparation, 7.5 kg of cow dung and 75 g P were applied as triple superphosphate (TSP, $Ca_3(PO_4)_2$) fertilizer in three split doses at 4-day interval for 22 days (41, 42). Nitrogen content of Azolla, 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. Rice straw was collected from farmers' fields, cutting manually using knife and incorporated into the soil in to respective straw treatments during land preparation using hand hoe. Representative samples of rice straw were dried and transported to the laboratory for chemical analysis of N and P (43).

2.3.2 Application of Azolla, rice straw and inorganic fertilizers

1 kg fresh Azolla was applied during nursery preparation and incorporated in the soil after 15 days. At this stage Azolla had covered the surface of water completely (See Figure 1). 36.4 kg of

TABLE 2 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		59:01:00	46:01:00	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.

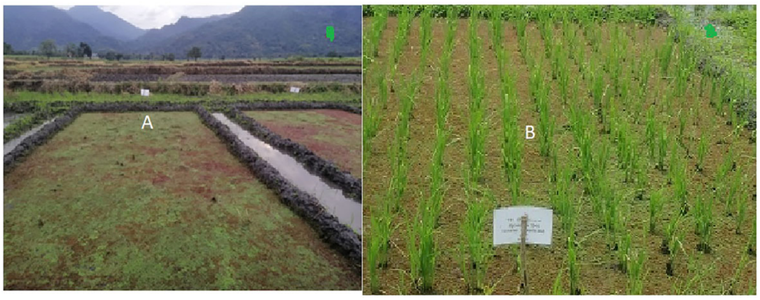

FIGURE 1
Azolla cover before rice transplanting **(A)** and at 35 DAT **(B)**.

fresh Azolla biomass was incorporated to the soil using a hand hoe three days before transplanting of rice seedlings to allow Azolla to decompose partially and minimize competition between rice seedling and Azolla. Another 2 kg of fresh Azolla was inoculated six days after transplanting (DAT) and at a rate of 29.2 kg and incorporated into the soil at 40 DAT. By this time rice seedlings have taken roots and start to grow actively. Before incorporation, Azolla was harvested within a 1 m × 1 m wooden frame (44), dried and analyzed for total N through Kjeldahl and P by the wet digestion method (43) (See Table 3). The fertilizers containing NPKS 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 the recommended rate (50 kg N ha^{-1}). Urea fertilizer for both rates of N was applied in two splits of 50% basal application at seven 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 sulphate (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 Soil sampling and analysis

Soil samples were collected for the analysis of the selected physicochemical properties before transplanting of the rice seedlings. The composite soil samples were taken from experimental site from a depth of 0–20 cm using soil auger randomly from 10 spots following a zigzag pattern. Soil samples were also taken from each experimental plot at the end of experiment. 2 kg of soil sample was taken and sent to the laboratory for analysis. The samples were air-dried, grounded, and sieved through a 2 mm wire-mesh and analyzed through standard procedures described in Table 3.

2.5 Sample analysis and calculation of nitrogen use efficiency

Rice was harvested at maturity (116 DAT), with rice ears and straw carefully separated and air-dried for two weeks. Grain yields were adjusted and reported on a basis of 14% moisture content (52). All above-ground plant samples were taken at the booting stage (75 DAT) oven-dried at 70°C to a constant weight then analyzed for total

TABLE 3 Procedure of 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 hours	Rochette and Bertrand (45)
Soil texture	Bouyoucos hydrometer method, followed by dispersion of soil particles	Beretta et al. (46)
Soil pH and electrical conductivity	Soil: water suspension (1:2.5) using glass electrode pH meter	Okalebo et al. (43)
Organic carbon	Wet oxidation by the Black and Walkley method	Nelson and Sommers (47)
Total nitrogen	Micro-Kjeldahl wet digestion-distillation method	Bremner (48)
C: N ratio		
Available P	Bray 1 method following color development using molybdenum blue method	Bray and Kurtz (49)
Cation exchange capacity (CEC)	Neutral ammonium acetate saturation method (NH4-Ac, pH 7.0) followed by Kjeldahl distillation.	Mattigod and Zachara (50)
Exchangeable bases (K^+, Mg^{2+}, Ca^{2+} and Na^+)	1N NH4-Ac (pH 7.0) method Mg and Ca were read by UV-VIS Spectrophotometer and K and Na Flame Photometer	Okalebo et al. (43)
Extractable micronutrients (Fe, Cu, Zn, and Mn)	DTPA extraction and determined by atomic absorption spectroscopy (AAS)s	Okalebo et al. (43) and Lindsay and Norvell (51)

N by the Kjeldahl method. Shoot length was measured at 30 and 60 DAT. Number of tillers and effective tillers was counted at 90 DAT.

The apparent recovery efficiency for N (ARE_N), the percentage of N applied recovered in above-ground biomass was calculated using Equation 1.

$$ARE_N(\%) = \frac{N_f - N_u}{N_u} \times 100 \tag{1}$$

Where N_f is the N accumulation by above-ground biomass in the fertilized pots (kg), N_u is the N accumulation by above-ground biomass in the unfertilized pots (kg), and Ni is the quantity of N applied (kg) for each treatment, as shown in Table 1.

The soil N-dependent rate (SNDR; the ratio of TNU without fertilization to TNU with fertilization), was calculated using Equation 2.

$$SNDR(\%) = \frac{N_u}{N_f} \times 100 \tag{2}$$

The agronomic efficiency of nitrogen (AEN), an expression of unit weight increases in grain yield per N applied) was calculated using Equation 3.

$$AEN(kg\ kg^{-1}) = \frac{G_f - G_u}{N_i} \tag{3}$$

Where G_f and G_u represent the grain yield (kg) of the fertilized pots and unfertilized pots, respectively, for each replicate.

The physiological nitrogen efficiency (PEN), the unit weight increases in grain yield per unit weight increase in N uptake from N fertilizer), was calculated using Equation 4.

$$PEN(kg\ kg^{-1}) = \frac{Y_f - Y_u}{N_f - N_u} \tag{4}$$

Where Y_f and Y_u represent the total biomass (kg) of fertilized pots and total biomass of unfertilized pots (kg), respectively, for each treatment.

The internal utilization efficiency (IUEN), the amount of produced grain yield by unit weight plant nutrient accumulation in the total biomass) was calculated using Equation 5.

$$IUEN(kg\ kg^{-1}) = \frac{Yf - Yu}{Ni} \tag{5}$$

The partial factor productivity (PFPN, unit of grain yield per N applied) was calculated from Equation 6.

$$PFPN(kg\ kg^{-1}) = \frac{Gf}{Ni} \tag{6}$$

All parameters were calculated according to other researchers (10, 52).

2.6 Statistical data analysis

The data was subjected to analysis of variance (ANOVA) to examine the effect of Azolla, rice straw and synthetic fertilizers on rice performance and nitrogen use efficiency parameters. reliability of treatments for the non-significant effects observed in ANOVA was detected by in-depth analysis for normality of residuals confirmed through the Shapiro-Wilk test and the homogeneity of variances was confirmed through Bartlett's test. The significant treatment means were compared by Turke's test at 5% level of probability.

3 Results

3.1 The effect of Azolla, rice straw and NPKS fertilizer combinations on rice growth and yield

Results on the effect of treatments on rice plant height, number of tillers and grain yield are presented in Table 4. The fertilized treatments significantly ($p< 0.05$) influenced plant height compared to the control group (Figure 2). The control plots exhibited the lowest plant height (36.4 cm to 52.8 cm). No significant difference was observed between the control and the treatment involving rice straw + 30 kg P ha^{-1}. The significantly ($p< 0.001$) higher plant height (59.0 cm to 80 cm) was recorded in the combination of Azolla with full dose N and other treatments, including rice straw and NPS compared with other treatment combinations. Furthermore, the results revealed significant ($p< 0.05$) variations in the total number and effective tillers among different treatments (Figure 2). The co-application of NPKS (100 kg N ha^{-1}) resulted in significantly ($p< 0.05$) higher number and productive tillers among other treatment combinations. Treatment combination also resulted in the significantly ($p< 0.05$) higher variation in total biomass and rice grain yield (Figure 3).

3.2 Effect of rice straw, Azolla and 50% reduced N on N uptake and agronomic N use efficiency

The parameters related to N uptake and its use efficiency are presented in Table 5. All treatments demonstrated significant ($p< 0.05$) improvements in nitrogen uptake compared to the control. Comparable results of agronomic use efficiency of N were observed in treatment combinations with 50 and 100 kg N ha^{-1}. The apparent recovery efficiency of nitrogen ranged from 29.8% to 163.3% for the two seasons, with the second season lagging a bit behind in the amount of N recovered. The inclusion of Azolla in other treatment combinations with 50% N resulted in significant ($p< 0.05$) higher apparent recovery efficiency of nitrogen compared to other treatment combinations.

Whereas comparable results of the calculated internal utilization efficiency of nitrogen were observed for both full dose and 50% reduced N co-application with other treatments (~76 kg kg^{-1}), the 50% N reduced-tailored combination significantly ($p< 0.05$) outperformed others with 102.22 kg kg^{-1} recorded. Physiological nitrogen efficiency ranged 10.66 kg kg^{-1} to 68.82 kg kg^{-1} and 53.72 kg kg^{-1} to 123.28 kg kg^{-1} for first and second season respectively. Highest value 68.82 kg kg^{-1} was recorded under application of reduced N along with NPKS in the first season and sole rice straw (23.28 kg $kg^{-1)}$ in the second season. The highest

TABLE 4 The effect of applying Azolla, rice straw and 50% reduced N on rice growth attributes and yield of rice plants.

Treatments	2022						2023					
	Plant height 30DAT	Plant height (60 DAT)	Total number of tillers	Number of effective tillers	Grain yield (kg ha^{-1})	Total biomass (kg ha^{-1})	Plant height 30DAT	Plant height (60 DAT)	Total number of tillers	Number of effective tillers	Grain yield (kg ha^{-1})	Total biomass (kg ha^{-1})
T1	36.40 a	53.13 a	7.47 a	6.87 a	833 a	1889 a	35.67 a	52.80 a	8.83 a	7.66 a	796 a	1648 a
T2	43.60 b	65.33 abc	13.27 bc	11.00 abc	1407 b	2926 b	43.47 b	68.60 bcd	12.77 ab	12.77 abc	2278 bc	4000 b
T3	45.33 bc	68.27 abc	17.00 cdef	18.67 fg	1833 c	3926 cd	44.40 bc	69.67 bcd	16.23 abcd	16.23 bcd	4352 ef	7185 def
T4	50.40 de	71.80 bc	15.67 bcde	14.67 cdef	2537 de	5185 e	47.40 cd	71.33 bcd	18.01 bcd	17.94 cd	4352 ef	8241 fg
T5	52.27 e	63.80 abc	22.67 g	20.67 g	2778 e	5704 e	50.80 de	69.67 bcd	22.82 d	21.35 d	4685 fg	7722 efg
T6	47.47 cd	64.67 abc	14.60 bcd	13.00 bcde	2204 cd	4519 d	47.07 bcd	68.60 bcd	13.13 ab	13.13 abc	2407 c	5222 bc
T7	35.73 a	58.07 abc	10.87 ab	8.67 ab	944 a	2296 a	35.33 a	60.40 ab	10.13 a	9.73 ab	1333 ab	2704 a
T8	46.87 bc	55.80 ab	19.13 defg	17.73 defg	2019 c	4222 cd	46.47 bc	60.17 ab	19.80 bcd	19.47 cd	3963 def	7315 def
T9	51.33 e	59.40 abc	19.07 defg	17.87 defg	1907 c	3852 c	50.87 de	64.53 abc	15.49 abcd	15.42 bcd	3407 de	6370 cd
T10	53.53 e	72.80 bc	18.67 defg	18.33 efg	2796 e	5704 e	53.00 e	74.93 cd	21.33 cd	19.67 cd	3815 def	6759 de
T11	47.60 cd	59.00 abc	14.07 bcd	12.40 abcd	1852 c	3685 c	47.27 bcd	62.13 abc	13.73 abc	13.00 abc	3185 cd	6241 cd
T12	52.20 e	65.73 abc	20.33 efg	19.53 fg	3352 f	6778 f	51.73 e	67.53 bcd	23.00 d	22.33 d	4704 fg	8074 fg
T13	59.00 f	76.37 c	22.33 fg	20.00 fg	4556 g	9537 g	57.73 f	80.05 d	22.81 d	22.74 d	5333 g	8778 g
***P* value**	<.001	0.003	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
c.v (%)	2.4	9.6	10.9	12.1	6	4.5	2.8	7	15.4	15.3	9.5	6.8
s.e.d	0.946	5.035	1.468	1.518	109.8	171.3	1.064	3.801	2.107	2.038	267.4	343.3
Seasons												
2022	47.83 a	64.17 a	16.55 a	15.34 a	2232 a	4632 a						
2023	47.02 a	66.95 b	16.77 a	16.26 a	3432 b	6174 b						
LSD (0.05)	0.978	1.21	1.271	1.83	102	315.7						
P-value	0.07	0.01	0.525	0.162	<.001	0.002						

Different lower case letters over each column indicate significant ($P< 0.05$) differences of the mean within input treatments (T1-T13) as presented in Table 1. DAT represent days after transplanting.

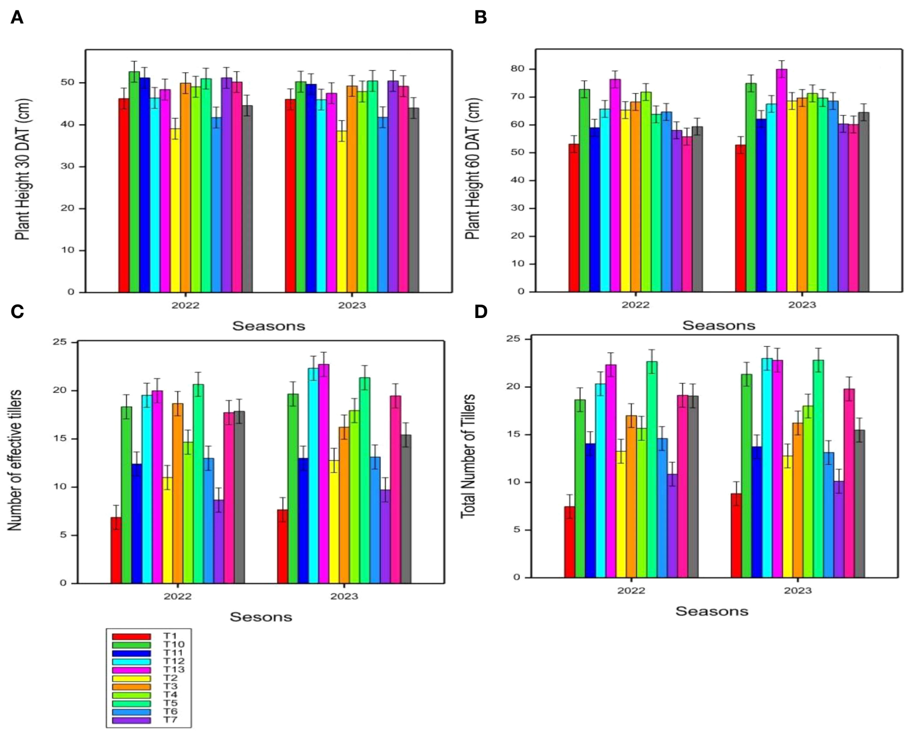

FIGURE 2
Effects of applying rice straw, Azolla and NPKS fertilizer combinations (T1-T13) on plant height, total number of tillers and effective tillers, small bars are standard errors at (P< 0.05). **(A)** Plant height 30 DAT (cm). **(B)** Plant height 60 DAT (cm). **(C)** Number of effective tillers. **(D)** Total number of tillers.

value of partial factor productivity of nitrogen was recorded under sole application of Azolla with 50% N reduced-based combination 55.93 kg kg^{-1} to 76.30 kg kg^{-1} for first and second season respectively, that significantly ($p< 0.001$) outperforming other treatments. The agronomic efficiency of applied nitrogen exhibited significant ($p< 0.001$) variation among treatments, with the highest values being 39.26 kg grain per kilogram of applied nitrogen in the first season and 60.37 kg grain per kilogram in the second season. These remarkable results were observed under 50% N reduced-based combinations. Soil nitrogen dependent rate ranged from 7.56% to 100% during the first season and 6.31% to 100% during the second season, with the highest dependence recorded in absolute control and the lowest dependence recorded in full N dose-based combination with other treatments.

3.3 Estimation N fixation and total nitrogen enrichment by *Azolla* in both seasons

Characteristics of Azolla used in the experiments are shown in Table 2. In first season, fresh Azolla incorporated was 1,956 kg and 1,503.4 kg ha^{-1}, with nitrogen contents of 2.17% and 1.92% for the first and second incorporations, respectively. The estimated nitrogen fixed was 42.44 kg and 29.01 kg per hectare based on nitrogen fixation efficiency resulted in 71.4 kg N fixation ha^{-1} in the first season. In the second season, Azolla biomass stood at 1,784 and 1,640 kg fresh Azolla ha^{-1}, with nitrogen contents of 1.8% and 2.2% respectively. The estimated nitrogen fixed was 32.6 kg and 36.08 kg N ha^{-1} for the first and second incorporations, resulting in a total estimated nitrogen of 68.68 kg ha^{-1}.

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

Results of the experiment indicated that all tested soil chemical parameters were significantly affected by different treatment combinations (Table 6). Results of the first and second experiments showed that soil pH ranged from 4.87 to 5.3 and 4.2 to 4.7 at the end of first and second season respectively. While there was no significant difference in pH among treatments ($p = 0.84$), pH varied significantly between seasons ($p< 0.05$). The mean total N increased compared to pretreatment levels. In the first season, total N ranged from medium (0.18%) to high (0.46%). In the second season, it ranged from medium (0.13%) to very high (0.65%). There was a significant ($p< 0.05$) increase in total N between the two seasons. Organic amendments (Azolla and rice straw) impacted soil TN reserve more than sole synthetic fertilizers. SOC concentration increased significantly ($p< 0.05$) between seasons. At the end of the first season, the highest SOC concentration (2.9%) was under sole rice straw incorporation, statistically higher than other treatments. In the second experiment, the highest SOC value (5.79%) recorded under the Azolla treatment with 50% reduced N, along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}. This was comparable to other

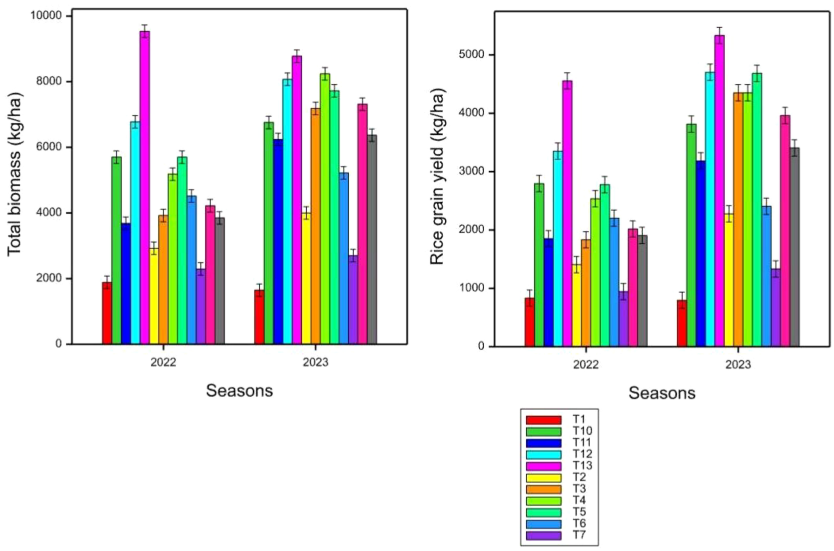

FIGURE 3
Effects of applying rice straw, Azolla and NPKS fertilizer combinations (T1-T13) on agronomic N use efficiency components and indices, small bars are standard errors at (P< 0.05).

organic amended treatments and superior to sole synthetic treatments. Soil C:N ratio at a depth of 0-20 cm varied between treatments, in the first season it ranges from 4.37 to 11.33 and 4.6 to 29.3 for the second season. The highest C:N ratio was observed under sole rice straw treatments in both seasons.

4 Discussion

4.1 The effect of Azolla, rice straw and 50% reduce N on rice growth and yield attributes

Plant height serves as an indicator of overall vegetative growth in rice crops (53, 54). Results of the study indicated that application of Azolla along with rice straw incorporation with 50% reduced N statistically yields comparable results in terms of plant height compared to the full recommended dose of NPKS (Figure 4). This increase in height could be attributed to several factors, including improved soil structure, enhanced nutrient cycling, and increased microbial activity due to the balanced application of synthetic fertilizers and organic matter (8, 52, 55). Also, could be attributed to the high nitrogen supply capacity of Azolla to rice crops (36, 56). These favorable conditions promote the rice crop's vegetative growth, leading to a larger leaf area, higher photo assimilates, greater dry matter accumulation, and increased cell division (57, 58). According to research by Feyisa et al. (59), Azolla can fix nitrogen in the range of 53-1000 kg ha-1. When Azolla is grown either as a monocrop or intercropped with rice, it can contribute 40-170 kg N ha^{-1}, which is gradually released 40-60% after 20 days and 55-90% after 40 days once incorporated into paddy soils. The reduced plant height observed in the control, as well as in treatments involving rice straw + 30 kg P ha^{-1} and rice straw combined with 50% reduced N (along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}), may be attributed to the limited supply capacity of these treatments. Additionally, the high carbon-to-nitrogen (C-to-N) ratio of rice straw could have contributed to soil nitrogen assimilation by microorganisms (58, 60). The reduced plant height observed in the sole application of nitrogen (N) may be attributed to the absence of other essential nutrients like phosphorus (P) fertilizer. Phosphorus plays a crucial role in enhancing root anatomy and promoting higher panicle formation (14, 53). The authors emphasized a significant finding related to the correlation between nitrogen application rates and rice plant height. As nitrogen doses increase, rice plant heights also tend to rise, thereby extending the overall crop cycle. To achieve an optimal balance between crop growth and maturity timing, it is essential to implement a precise and well-calibrated nitrogen application strategy (61–63). The number of tillers and productive tillers per hill significantly impacts rice yield (64). These tillers provide the necessary stalks for optimal production (65). In control plots and sole rice straw application, the low number of tillers and productive tillers could be attributed to the limited soil supply capacity when no additional inputs are provided. Research by (66) highlights that the actual tillering ability of rice is closely tied to N input and spacing. Our study corroborates this finding, showing that N supply significantly affects the number of tillers and effective tillers. Interestingly, balanced fertilizer application plays a crucial role in tiller formation. When fertilizers are judiciously applied between early and late emerging tillers, it leads to improved crop yield and a greater number of productive tillers (67). The application of Azolla + 30 kg P ha^{-1} yielded comparable tillers and effective tillers to the full recommended level of NPKS and when Azolla and rice straw coupled with full or 50% reduced N + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} outperformed sole synthetic fertilizers applications. This can be attributed to Azolla's high supply capacity of nitrogen to rice crops (68). When **Azolla** and **rice straw** are combined, they complement

TABLE 5 Effect of Azolla, rice straw and 50% reduced N along with PKS fertilizers combinations on Agronomic Nitrogen Use Efficiency.

Treatments	2022							2023						
	N-uptake (kg N ha^{-1})	ARE$_N$ (%)	IUNE	SNDR (%)	AE$_N$ (kg kg^{-1})	PE$_N$ (kg kg^{-1})	PFP$_N$ (kg kg^{-1})	N-uptake (kg N ha^{-1})	ARE$_N$ (%)	IUNE	SNDR (%)	AE$_N$ (kg kg^{-1})	PE$_N$ (kg kg^{-1})	PFP$_N$ (kg kg^{-1})
T1	13.42 a	0.00 a	0.00 a	100.00 f	0.00 a	0.00 a	0.00 a	8.62 a	0.00 a	0.00 a	100.00 e	0.00 a	0.00 a	0.00 a
T2	43.93 bc	30.52 b	10.37 b	30.38 cd	5.74 b	34.03 abc	14.07 b	38.51 ab	29.89 ab	23.52 b	23.71 c	14.81 b	83.34 b	22.78 b
T3	57.81 c	44.39 bc	20.37 c	23.51 bcd	10.00 b	46.69 abc	18.33 c	88.57 cdef	79.95 cd	55.37 c	9.84 abc	35.56 cd	71.74 ab	43.52 c
T4	77.52 d	64.10 cd	32.96 d	17.28 abc	17.04 c	51.73 bc	25.37 d	111.58 efg	102.95 cde	65.93 cd	7.81 ab	35.56 cd	65.06 ab	43.52 c
T5	86.93 d	73.52 d	38.15 d	15.49 ab	19.44 c	51.93 bc	27.78 d	122.51 fg	113.89 de	60.74 cd	7.19 ab	38.89 cd	53.72 ab	46.85 c
T6	52.74 bc	78.64 d	52.59 e	25.62 bcd	27.41 e	68.82 c	44.07 g	39.78 ab	62.31 bc	71.48 d	21.77 c	32.22 c	116.05 b	48.15 c
T7	21.32 a	0.00 a	0.00 a	64.07 e	0.00 a	10.66 ab	0.00 a	19.16 a	0.00 a	0.00 a	48.12 d	0.00 a	123.28 b	0.00 a
T8	49.61 bc	0.00 a	0.00 a	27.37 bcd	0.00 a	22.20 abc	0.00 a	84.26 cde	0.00 a	0.00 a	10.20 abc	0.00 a	75.74 b	0.00 a
T9	44.32 bc	61.81 cd	39.26 d	30.33 cd	21.48 cd	63.62 c	38.15 f	70.58 bcd	123.92 e	94.44 e	12.45 abc	52.22 ef	76.99 ab	68.15 d
T10	76.39 d	125.94 e	76.30 f	17.53 abc	39.26 f	60.85 c	55.93 h	99.05 cdef	180.85 f	102.22 e	8.71 abc	60.37 f	56.65 ab	76.30 d
T11	39.32 b	0.00 a	0.00 a	34.04 d	0.00 a	19.88 abc	0.00 a	65.55 bc	0.00 a	0.00 a	13.19 abc	0.00 a	81.45 b	0.00 a
T12	87.50 d	74.08 d	48.89 e	15.32 ab	25.19 de	66.07 c	33.52 e	108.13 defg	99.51 cde	64.26 cd	8.13 ab	39.07 cd	67.47 ab	47.04 c
T13	176.72 e	163.31 f	76.48 f	7.56 a	37.22 f	46.94 abc	45.56 g	136.97 g	128.35 e	71.30 d	6.31 a	45.37de	55.68 ab	53.33 c
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
CV (%)	9.3	13.3	8.3	14.6	9.7	40.1	5.5	16.4	19.2	10.9	23.8	13.9	37	10.7
s.e.d	4.828	5.98	2.055	3.738	1.239	13.68	1.054	10.26	11.14	4.163	4.143	3.095	21.57	3.011
Seasons														
2022	63.7 a	55.1 a	30.41 a	31.42 b	15.60 a	41.8 a	23.29 a							
2023	76.4 a	70.9 b	46.87 b	21.34 a	27.24 b	71.3 b	34.59 b							
LSD (0.05)	12.73	13.37	3.165	9.093	1.796	10.63	2.032							
P-value	0.05	0.037	0.002	0.041	0.001	0.007	0.002							

Means in a column followed by different letter(s) differ significantly at (p< 0.05). ARE$_N$ is apparent N recovery efficiency (kg grain N increase kg^{-1} N applied); AE$_N$ is agronomic N use efficiency (kg grain increase kg−1 N applied). T1 – T13 represent treatment combinations as presented in Table 1.

TABLE 6 The effect of applying Azolla, rice straw and NPKS fertilizers 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 bcd	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 def	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 efg	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 bcd	1.78 efg	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	<.001	0.001	<.001	<.001	<.001	0.087	<.00	<.001	0.003	<.001	<.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	0.26	0.23	0.03	1.118	0.17	13.16	0.144	0.30	0.077	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 lower-case letters over each column indicate significant ($P< 0.05$) differences of the mean within input treatments (T1-T13) as presented in Table 1.

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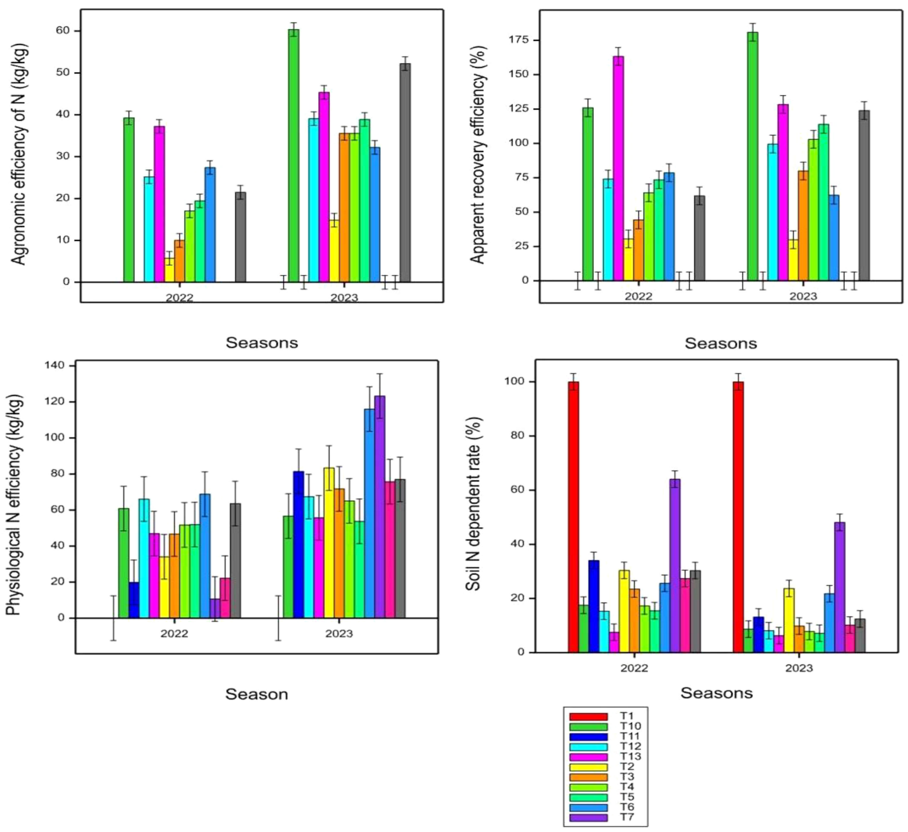

FIGURE 4
Effects of co-application of Azolla, rice straw and NPKS fertilizer combination on some selected soil properties, small bars are standard errors at (P< 0.05).

each other due to their distinct carbon-to-nitrogen (C-to-N) ratios. **Azolla**, with its lower C-to-N ratio, provides readily available nitrogen (approximately 3–5% N) when it decomposes. Meanwhile, **rice straw**, with a higher C-to-N ratio, contributes organic matter and improves soil structure (69). By using Azolla alone or in combination with reduced synthetic fertilizers, plant growth parameters can be enhanced, leading to minimized nutrient loss and improved nutrient use efficiency (70–72). The consistently high total biomass and grain yield of 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}, and Azolla, rice straw with 50% reduced N + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} or Azolla + 50% reduced N + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} treatments could be attributed to the specific combination of factors and inputs used in these treatments that favor healthy soils with good structure, high organic matter content, and a balanced pH level provide a conducive environment for root development and nutrient uptake. The observed higher total biomass and grain yield in various treatments involving Azolla, rice straw, and specific nutrient combinations can be attributed to a combination of factors. These treatments promote healthy soil conditions, including good structure, high organic matter content, and a balanced pH level, which in turn create favorable conditions for root development and efficient nutrient uptake. Specifically, the following treatments demonstrated positive effects: 1) Co-treatment of Azolla, rice straw, and specific nutrients (100 kg N ha^{-1}, 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}); 2) 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 3) Azolla alone with 50% reduced N, along with 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1}. These treatments synergistically enhance soil health and contribute to improved crop productivity. The study draws insights from previous research (35, 52, 73, 74). According to (75), the co-application of reduced chemical fertilizers and organic fertilizers effectively improves soil fertility, microbial community structure, and crop yield and limits the use of chemical fertilizers. The reduced biomass and grain yield observed in treatments involving rice straw combined with 30 kg P ha^{-1} and rice straw with 50% reduced N + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} may be attributed to the relatively high carbon-to-nitrogen ratio of the straw. This high ratio slows down decomposition upon incorporation into the soil. Additionally, a significant portion of the available nitrogen is consumed by microorganisms, resulting in nitrogen becoming temporarily tied up, making it less accessible to rice crops (58, 76–80).

4.2 Effect of fertilizer treatments on N uptake and agronomic N use efficiency

The key components of nitrogen use efficiency include agronomic efficiency of applied nitrogen (AE_N), apparent recovery efficiency of nitrogen, and physiological nitrogen efficiency.

10.3389/fsoil.2024.1378065

Additionally, partial factor productivity of nitrogen serves as a crucial index for understanding long-term productivity trends and optimizing nitrogen fertilizer use efficiency (10, 52). The apparent recovery efficiency of N is a crucial metric that quantifies how effectively plants recover and utilize nitrogen from applied fertilizers (7). It provides insights into the efficiency of N uptake by crops, which is essential for optimizing agricultural practices and sustainable nutrient management. Generally higher ARE_N values indicate more efficient nitrogen utilization by the crop. Results of the experiments indicated that the co-application of Azolla, rice straw with full and 50% reduced N + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} resulted in higher percentage of N recovery efficiency. The observed phenomenon could be attributed to several factors. Firstly, the enhanced soil structure, increased microbial activity, and improved nutrient availability collectively contribute to better nutrient uptake by plants (9, 23, 58, 81). Additionally, the presence of Azolla cover plays a role in reducing ammonia volatilization, while simultaneously minimizing nitrogen losses through surface runoff and leaching (13, 24, 82). These combined effects create a favorable environment for efficient nutrient utilization by crops. The higher apparent recovery efficiency of nitrogen observed in balanced fertilizer applications (73.52% in the first season and 113.89% in the second season) compared to sole nitrogen treatments (30.52% in the first season and 29% in the second season) can be attributed to the fact that balanced fertilizers provide a combination of essential nutrients (such as nitrogen, phosphorus, and potassium) in optimal proportions. This balanced nutrient supply promotes better nutrient uptake by plants, ultimately leading to improved recovery efficiency (52, 53, 83). Agronomic efficiency of nitrogen (AEN) can be defined as the yield increase per unit of nitrogen (N) applied, providing a more direct measure of the production impact of applied N fertilizers, nitrogen loss, and economic return (84). Higher agronomic efficiency of applied N was observed in co-application of Azolla and 50% reducing N + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} with values of 39.26 $kgkg^{-1}$ and 60.37 $kgkg^{-1}$ for the first and second seasons, respectively. This outcome is likely attributed to the accumulation of soil nutrient reserves and the rapid decomposition of Azolla green manure influenced by lower C: N ratios, which supply ample nutrients, particularly N (60, 85, 86). Additionally, this practice helps minimize ammonia volatilization (12, 13). For example, (87) reported that co-application of organic and chemical fertilizers is a better approach for enhancing soil fertility and crop yields compared to using either organic or chemical fertilizers alone. In the present study, the use of sole synthetic fertilizers aligns with the benchmark data of agronomic efficiency of N in lowland rice production systems, which typically falls within the range of 15–30 kg of nitrogen per kilogram of grain produced (52, 88). The reduced efficiency of sole synthetic fertilizers can be attributed to factors such as high ammonium volatilization, surface runoff, and leaching (12, 89, 90). The positive impact on nitrogen uses efficiency (NUE) resulting from the application of Azolla and rice straw, both in full and with 50% reduced nitrogen, aligns consistently with various NUE components, including internal utilization efficiency of nitrogen (IUNE), partial factor productivity of nitrogen (PFPN), and apparent recovery efficiency of nitrogen (PEN). This favorable influence of Azolla + rice straw and synthetic fertilizers on NUE is in line with findings reported by several authors (35, 73, 91, 92). The underlying premise is that this combination enhances N retention, reduces N losses, and ultimately improves crop N uptake. According to (93), Azolla biofertilizer holds significant promise as an approach to enhance nitrogen use efficiency (NUE) in paddy rice fields. Its remarkable potential for biological nitrogen fixation (BNF) makes it a valuable tool for sustainable agriculture. Soil nitrogen dependent rate (SNDR) signifies how much crop performance and nitrogen utilization rely on the availability of nitrogen in the soil. Results of the study indicated that the control plot and rice straw + 30 kg P ha^{-1} have higher percentage of SNDR indicating that these treatments are entirely dependent on reserved soil nitrogen for rice crop growth. The higher SNDR in rice straw treatment might be due to a higher C/N ratio that slows decomposition and release (94). On the other hand, lower SNDR in co-application Azolla, rice straw + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} suggested that these amendments consistently enhance N supply and contribute to rice crop performance. Here we demonstrate that SNDR is lowered under rice straw and 50% reduced N + 30 kg P ha^{-1}, 30 kg K ha^{-1}, and 20 kg S ha^{-1} that might be due to enhances soil hydrolysable N of straw. According to (95), proper rice straw management, especially incorporating it in winter with joint N application, improves soil fertility and mitigates greenhouse gas emissions in double rice cropping systems.

4.3 Contribution of Azolla in nitrogen enrichment

The results show that Azolla is an effective biofertilizer for rice production, as it can fix significant amounts of nitrogen and enrich the soil. The results are consistent with previous studies that have demonstrated the benefits of Azolla for rice cultivation (12, 74, 96). In a study conducted by (56), it was found that *Azolla*, the fast-growing water fern, exhibits a remarkable ability to fix nitrogen and significantly contributes to rice growth and yield. Another study (74) highlighted that Azolla plays a crucial role in reducing reliance on synthetic nitrogen fertilizers, minimizing nitrogen loss, and optimizing nitrogen use efficiency. This approach not only promotes environmental conservation but also contributes to the long-term viability of agricultural practices.

4.4 Effects of treatment combinations on soil chemical properties

The present study demonstrated that application of Azolla and rice straw impact change in most chemical fertility (Figure 5). 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

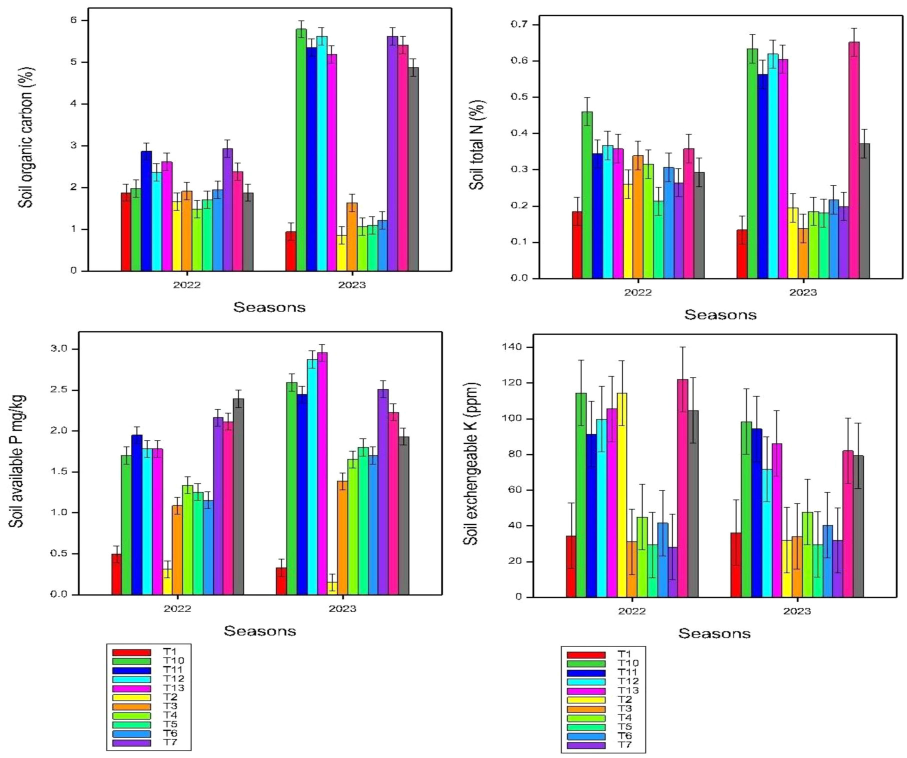

FIGURE 5
Effects of co-application of Azolla, rice straw and NPKS fertilizer combination on some selected soil properties, small bars are standard errors at ($P < 0.05$).

amendments (97, 98). Similar results were reported by (99) that 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 severe microbial nitrification process that led to soil acidification, which is largely attributed to ammonium fertilizers applied in the soil (98). 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 (100). 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 (101). This process results in an increase in available nitrogen in the soil (102). 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 help 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, soil water retention and reduce bulk density of the soil, promoting crop growth and TN stocks (100, 103). 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 along with their stoichiometric characteristics, are important indicators for the quality and quantity of soil organic matter (104). The carbon-to-nitrogen (C:N) ratio in the soil is a crucial indicator of nutrient availability and microbial activity (105). A higher C:N ratio generally indicates slower 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 (106). 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 of C:N ratio between seasons, suggest that treatment continuous application of treatment combinations could enhance organic matter reserve and decomposition that subsequently impact 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 Phosphorus Use Efficiency (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. (107) 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.

5 Conclusion and recommendation

Our study demonstrated that the co-application of Azolla, rice straw and synthetic fertilizers resulted in the best performance of rice plants in terms of plant height, tiller count, effective tiller formation and yield components. This indicates that the balanced application of synthetic and organic fertilizers can improve soil quality, nutrient availability and microbial activity, which in turn enhance rice growth and productivity. The study demonstrated that Application of Azolla 50% N + 30 kg P ha^{-1} or combined with rice straw show comparable rice grain yield to full recommended dose of NPKS, suggesting that Azolla is an effective biofertilizer that can fix significant amounts of nitrogen and enrich the soil and when applied with rice straw and reduced N provide favorable soil environment for rice growth and production. Therefore, the application of Azolla, rice straw with reduced N can improve soil quality and significantly enhance N use efficiency and sustainable rice production, especially for the local farmers.

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, Writing – original draft. HT: Conceptualization, Supervision, Writing – original draft. NA: Supervision, 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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