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Enhancing Soil Fertility Through Azolla Incorporation: Impacts on Nitrogen Cycling and Cation Exchange Capacity

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Abstract: The incorporation of Azolla into soil was investigated in this study for its potential to enhance soil fertility by influencing key parameters, including organic carbon (Organic-C) content, total nitrogen (Total-N), and cation exchange capacity (CEC). This study was conducted in a controlled greenhouse environment using a Completely Randomized Design (CRD) with eight treatments and three replications. The primary objective was to evaluate the effects of Azolla on soil quality, particularly in improving organic matter content and nitrogen (N) retention, both of which are essential for sustainable agricultural management. The findings indicate that Azolla incorporation led to a 29% increase in soil Organic-C and a 21% increase in Total-N compared to control treatments ($p < 0.05$). Additionally, CEC was enhanced by 33.4%, demonstrating improved nutrient retention capacity. A strong positive correlation was observed between Organic-C content, soil pH, and CEC, suggesting that Azolla contributes to optimizing soil nutrient dynamics. These results highlight the capacity of Azolla to function as a biofertilizer, improving soil fertility and nitrogen cycling while reducing dependence on synthetic fertilizers. The potential of Azolla to serve as an eco-friendly amendment aligns with sustainable agricultural practices aimed at enhancing soil health and long-term productivity. The findings contribute to the growing body of research on biofertilizers, offering valuable insights for soil management strategies that prioritize environmental sustainability and resource efficiency.

Keywords: Azolla biofertilizer; Soil fertility enhancement; Nitrogen fixation; Cation exchange capacity; Sustainable agriculture

1. Introduction

Nitrogen is a crucial element for plant growth and optimizing crop yields. With the global population projected to reach 9.7 billion by 2050, the demand for nitrogen fertilizers is expected to rise significantly (United Nations, 2023a). However, the extensive use of chemical fertilizers has led to various environmental concerns, including soil degradation, increased salinity, and nutrient runoff that contaminates water bodies (Tyagi et al., 2022). This has raised awareness about the need for sustainable alternatives. Biofertilizers, such as Azolla, offer an environmentally friendly solution by enhancing nitrogen use efficiency, promoting soil health, and improving microbial diversity (Fitriatin et al., 2021a; Mahanty et al., 2017). These benefits help reduce the reliance on synthetic fertilizers and contribute to long-term soil fertility (Yang et al., 2022).

Since the Industrial Revolution, the use of chemical fertilizers has become widespread, but their overuse has caused soil acidity and nutrient imbalances and reduced organic matter, ultimately affecting crop yields (Guo & Wang, 2021). Additionally, the rising cost of chemical fertilizers is a concern for the future (United Nations, 2023b). As a result, the adoption of biofertilizers, which facilitate natural nitrogen fixation, has been proposed as a more sustainable approach (Fitriatin et al., 2021a). These alternatives not only reduce synthetic fertilizer dependence but also enhance soil health, support biodiversity, and improve crop resilience to abiotic stresses, which is particularly important in the face of climate change (Fasusi et al., 2021; Zafar et al., 2024).

Azolla, a floating aquatic fern, is recognized for its nitrogen-fixing capabilities through a symbiotic relationship with Anabaena azollae, converting atmospheric nitrogen (N_2) into ammonia (NH_3), which is bioavailable for plant uptake. This process enriches the soil as Azolla decomposes, releasing fixed nitrogen and contributing to sustainable nitrogen cycling (Thapa & Poudel, 2021). Research has shown that Azolla can contribute up to 40–60 kg of nitrogen ha^{-1} per growing season, reducing the need for synthetic fertilizers while promoting soil health (Akhtar et al., 2021).

In addition to nitrogen fixation, Azolla incorporation improves soil properties by increasing organic matter content, enhancing microbial activity, and improving CEC. The decomposition of Azolla biomass further enriches soil Organic-C, fostering beneficial microbial communities that enhance nutrient cycling and overall soil health (Korsa et al., 2024). Moreover, Azolla-derived humic substances increase CEC, improving nutrient retention and the availability of essential cations such as potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and ammonium (NH_4^+) (Aytenew & Bore, 2020).

Azolla can be incorporated into the soil, either before or after transplanting crops, enhancing soil fertility and reducing dependence on synthetic fertilizers. Recent studies have shown that Azolla not only improves nitrogen availability but also enhances soil microbial diversity, contributing to long-term soil health (Akhtar et al., 2021). Azolla also sequesters atmospheric carbon (C), adding to its environmental benefits as a sustainable farming practice (Bharali et al., 2021).

In rice fields, Azolla can be cultivated using monoculture or intercropping systems. Monoculture planting is done before rice planting, while intercropping is carried out by growing rice and Azolla together. Subsequently, Azolla can be incorporated into the soil or harvested for use elsewhere (Marzouk et al., 2023). Azolla seedlings are planted or scattered immediately after rice seedlings are transplanted into the field. Incorporating Azolla into the soil, either before or after rice transplantation, has proven to be more effective in improving soil fertility and reducing dependence on chemical fertilizers (Akhtar et al., 2021). Besides enhancing soil fertility, numerous studies have reported that using Azolla can increase crop yields (Seleiman et al., 2022). Azolla also plays a role in restoring macro and micronutrients in the soil, such as Ca^{2+}, Mg^{2+}, P, Fe, S, and K^+, compared to monoculture rice systems (Thapa & Poudel, 2021). Additionally, Azolla can lower the pH of standing water and reduce temperature, thereby minimizing ammonia volatilization (Mahapatra et al., 2022). Research has also shown that the integration of Azolla into rice systems can contribute to a more efficient use of water, offering an additional benefit in water-scarce regions (Kour et al., 2024a).

Although Azolla has many benefits in the fields of agriculture and the environment, its global utilization is currently hindered due to the lack of economic, social, and scientific research analysis on Azolla, leading many parties to still doubt its benefits (Kulasooriya, 1991). This study aims to determine the effect of Azolla incorporation and intercropping on soil fertility and quality. Additionally, it also aims to understand the relationship between each parameter and nitrogen uptake (N-uptake).

2. Methodology

2.1 Time and Place

The research was conducted at the Experimental Field of Faculty of Agriculture (KPFP), Udayana University, Denpasar, Bali, from February to July 2024. Soil samples were collected from paddy fields, air-dried, and sieved before experimental use.

2.2 Materials and Equipment

The materials used in this study include rice seedlings, Azolla, NPK fertilizer, pesticides, and chemicals for analyzing soil and compost fertilizer's chemical, biological, and physical properties. The equipment used includes agricultural tools, plant growth measurement tools, and instruments for analyzing the chemical, biological, and physical properties of soil.

A total of 28 kg of soil for the study was collected from KPFP. The soil was then air-dried and sieved through a 5 mm sieve before being mixed and used. Rice seeds were sown in seedling trays (three seeds per hole). After five weeks, the seedlings were transplanted into pots. Each pot contained 7 kg of soil (5 kg dry soil weight). The water depth in the pots was maintained above 5 cm by adding water as needed.

2.3 Research Design

This study commenced with a comprehensive literature review aimed at identifying key research gaps and refining the experimental framework. Insights from the review guided the subsequent preliminary survey, which involved examining Azolla specimens and the specific paddy field soil intended for use in the main experiment (Figure 1). Data collected during this phase informed the preparation of research instruments, including the design of experimental protocols and sampling procedures. Following these preparatory stages, the main experiment was conducted by cultivating paddy in the surveyed soil, with Azolla incorporated according to the experimental design. Upon reaching maturity, the paddy plants were harvested, and a thorough assessment of nutrient uptake was carried out by analyzing relevant plant tissues. Concurrently, soil fertility parameters were evaluated to determine any alterations attributable to the presence of Azolla or varying management practices. All data analyses were conducted using appropriate statistical methods to discern significant differences among treatments. The findings from these analyses were then synthesized to draw conclusions about the effectiveness of Azolla as a soil amendment and to propose recommendations for future research and practical applications in sustainable paddy cultivation.

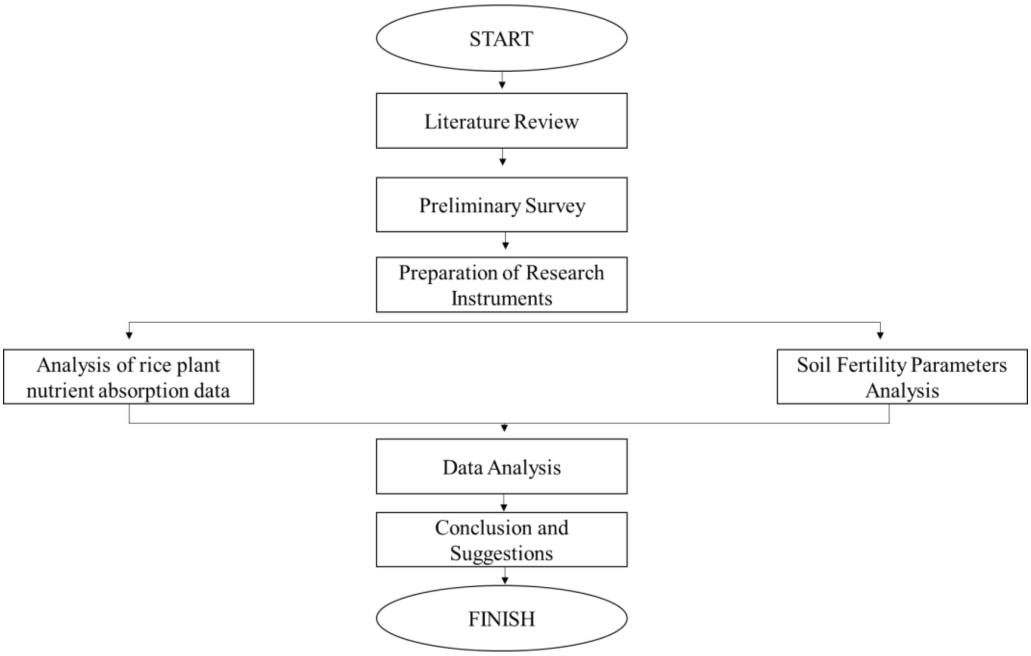

Figure 1. Research flow chart

The study was a pot experiment conducted at the KPFP greenhouse. CRD consisted of eight treatments replicated three times. The randomization process was performed using a computer-generated sequence to ensure an unbiased allocation of treatments across experimental units. Each treatment was assigned randomly to the pots to eliminate environmental variability within the greenhouse setting. CRD was chosen for this study because the research environment was considered sufficiently homogeneous. Additionally, this method simplifies randomization and analysis, making it efficient for this experiment. The treatments applied in this study are shown in Table 1.

Table 1. Addition of chemical fertilizers and Azolla in each treatment

No.	Treatment Code	Chemical Fertilizer (g pot^{-1})	Azolla Incorporation (g pot^{-1})	Intercropping with Azolla
1	K0	-	-	No
2	K1A	0.35 SP-36, and 0.35 KCl	240	Yes
3	K2A	0.35 SP-36, and 0.35 KCl	280	Yes
4	K3A	0.35 SP-36, and 0.35 KCl	320	Yes
5	K1At	0.35 SP-36, and 0.35 KCl	240	No
6	K2At	0.35 SP-36, and 0.35 KCl	280	No
7	K3At	0.35 SP-36, and 0.35 KCl	320	No
8	Ka	0.52 Urea, 0.35 SP-36, and 0.35 KCl	0	No

The application of chemical fertilizers follows the fertilization recommendations from the Agricultural Office of Buleleng Regency (2020), with adjustments made to convert the prescribed rates into pot-based units. The total number of experimental units is 24, derived from a factorial combination of eight treatments with three replications each.

2.4 Soil Sampling and Analyses

Soil samples were taken before and after treatments to assess soil fertility properties. Approximately 1 kg of soil was collected, air-dried, and sieved. The chemical properties of the soil samples were analyzed based on several parameters, as shown in Table 2.

Table 2. Soil parameters analyzed

No.	Soil Parameters	Methods
1	pH (H_2O)	H_2O 1:2.5
2	Electrical conductivity (EC) (mmhos cm^{-1})	H_2O 1:2.5
3	Organic-C (%)	Walkley and Black
4	Total-N (%)	Kjeldahl
5	C/N	Ratio of carbon to nitrogen
6	CEC (me $100g^{-1}$)	NH_4OAc 1N pH 7
7	BS (%)	NH_4OAc 1N pH 7
8	Total-P (ppm)	Bray-1
9	Total-K (me $100g^{-1}$)	Bray-1

The pH (H_2O) and EC (DHL) of the soil were determined using the H_2O 1:2.5 method, where the soil was mixed with distilled water in a 1:2.5 ratio. The pH was measured using a pH meter, while the EC was measured using a conductivity meter (Hendershot et al., 1993). Organic-C content was analyzed using the Walkley and Black method, a widely used wet oxidation technique where potassium dichromate ($K_2Cr_2O_7$) in sulfuric acid (H_2SO_4) oxidizes organic matter, and the residual dichromate was titrated with ferrous sulfate to determine carbon content (Walkley & Black, 1934). Total-N content was measured using the Kjeldahl method, which involves digesting soil samples with concentrated sulfuric acid and a catalyst to convert organic nitrogen into ammonium, followed by distillation and titration to determine nitrogen concentration (Bremner, 1965). The C/N ratio, representing the balance between carbon and nitrogen in the soil, was calculated by dividing the Organic-C content by the Total-N content (Weil & Brady, 2016). CEC (KTK) was analyzed using the 1N ammonium acetate (NH_4OAc) pH 7 method, where ammonium acetate was used to extract exchangeable cations, and the soil's capacity to retain and exchange cations was determined (Sumner & Miller, 1996). Similarly, base saturation (BS) was measured using the 1N ammonium acetate pH 7 method, determining the percentage of exchangeable bases (Ca, Mg, K, and Na) relative to total CEC (Chapman, 1965). Total phosphorus (Total-P) was determined using the Bray-1 method, which extracts phosphorus (P) with ammonium fluoride (NH_4F) and hydrochloric acid (HCl) to estimate plant-available phosphorus (Bray & Kurtz, 1945). Likewise, total potassium (Total-K) was analyzed using the Bray-1 method, which extracts potassium from the soil and quantifies it using atomic absorption spectrophotometry (AAS) or flame photometry (Thomas, 1982). These methods provide essential insights into soil fertility and nutrient availability for plant growth.

2.5 Azolla Analyses

The Azolla used in this study was *Azolla pinnata*, sourced from a local farmer in Bali. Prior to application, its chemical composition was analyzed to ensure consistency and accuracy in the experimental treatments. The chemical properties of the Azolla were analyzed based on several parameters, as shown in Table 3.

Table 3. Azolla parameters analyzed

No.	Azolla Parameters	Methods
1	Organic-C (%)	Walkley and Black
2	Total-N (%)	Kjeldahl
3	C/N	The ratio of carbon to nitrogen

To determine the chemical composition of Azolla, samples were collected, oven-dried at 70°C for 48 hours, and ground to a fine powder. The carbon analysis of Azolla was conducted using the Loss on Ignition (LOI) method. The dried sample was placed in a porcelain crucible and then heated in a furnace at a temperature of 450–600℃ for four hours. The carbon percentage was determined by calculating the difference between the dry sample weight and the post-combustion weight after ignition in the furnace (Ball, 1964). The nitrogen analysis of Azolla was conducted using the wet digestion method (Lindner & Harley, 1942). The digestion process involves the use of sulfuric acid and hydrogen peroxide (H_2O_2), followed by distillation. The nitrogen concentration was then determined through titration with sulfuric acid. The C/N ratio was calculated based on the balance between organic-C and nitrogen values (Weil & Brady, 2016). Then the results of the Azolla chemical analysis were compared to the Indonesian National Standard (SNI) for solid organic fertilizers (SNI 7763:2024) to evaluate its suitability as an organic soil fertilizer.

2.6 Statistical Analyses

All collected data were analyzed using descriptive and inferential statistical methods to assess the impact of Azolla incorporation on soil fertility parameters. The statistical analysis was conducted using SPSS 26.0 and MS Excel. One-way Analysis of Variance (ANOVA) was performed to determine significant differences between the different Azolla incorporation treatments and the control group. The Duncan's Multiple Range Test (DMRT) at a 5% significance level ($p < 0.05$) was applied for post hoc comparisons, identifying which treatments significantly differed from each other. A Pearson correlation test was conducted to examine the relationships between key soil parameters, such as pH, Organic-C, Total-N, Total-P, Total-K, CEC, and BS. A correlation matrix was created to visualize interactions between soil fertility indicators.

3. Results

3.1 Initial Soil Analysis

Table 4 shows the initial soil parameters analyzed before the experiment. The soil used in this study was paddy soil collected from rice fields in Pemecutan Klod, Denpasar City. Paddy soil was selected to maintain uniformity in its chemical, biological, and physical properties. Initial soil analysis indicated a neutral pH, moderate levels of Organic-C and Total-N, high CEC, and very high BS, Total-P, and Total-K. According to the soil fertility classification by Pusat Penelitian Tanah (1995), the soil used in this study was classified as highly fertile.

Table 4. Initial soil parameters analyzed before the experiment

No.	Soil Parameters	Value	Status
1	pH (H_2O)	7.27	Neutral
2	EC (mmhos cm^{-1})	0.61	-
3	Organic-C (%)	2.89	Moderate
4	Total-N (%)	0.23	Moderate
5	C/N	12.38	-
6	CEC (me $100g^{-1}$)	32.50	High
7	BS (%)	92.52	Very high
8	Total-P (ppm)	2808.89	Very high
9	Total-K (me $100g^{-1}$)	70.10	Very high

3.2 Initial Azolla Properties

Table 5 shows the Azolla chemical properties. Laboratory analysis of Azolla nutrient content indicated that its Organic-C and Total-N were 44.89% and 3.48%, respectively, with a C/N ratio of 12.91. These values are consistent with findings from previous studies. According to the study by Weil & Brady (2016), *Azolla pinnata* contains 3.63% nitrogen, 0.88% phosphorus, and 3.03% potassium. When compared with SNI for solid organic fertilizers (SNI 7763:2024), the nutrient composition of Azolla meets the required criteria. This indicates that Azolla has strong potential as a viable organic fertilizer source for sustainable agricultural development.

Table 5. Azolla chemical properties

No.	Parameters	Value	Status (SNI 7763:2024)
1	Organic-C (%)	44.89	Minimum 15
2	Total-N (%)	3.48	Minimum 2
3	C/N	12.91	Maximum 25

3.3 Soil Chemical Properties at Harvest

Table 6 presents the soil properties at harvest across different treatments. The results indicate that soil pH remained within a neutral range (7.36–7.46) with no significant differences among treatments ($p > 0.05$). EC ranged from 0.79 to 1.05 mmhos cm^{-1}, with K3AT showing significantly higher EC values compared to other treatments ($p < 0.05$). Organic-C content varied significantly across treatments ($p < 0.05$). The highest Organic-C content was observed in K3AT (3.75%) and K3A (3.73%), which were significantly higher than K0 (2.90%) and KA (3.28%). These results indicate that Azolla incorporation increased organic matter accumulation in the soil, particularly at higher application rates. Total-N content also exhibited significant differences ($p < 0.05$), with the highest values in K3A (0.28%) and K3AT (0.28%), significantly differing from K0, K1AT, and KA (all 0.24%). This suggests that higher Azolla incorporation improved nitrogen retention in the soil. CEC showed significant variation across treatments ($p < 0.05$). The highest CEC values were recorded in K3A (42.28 me $100g^{-1}$) and K3AT (41.25 me $100g^{-1}$), significantly higher than K0 (31.71 me $100g^{-1}$) and KA (31.54 me $100g^{-1}$). This indicates that Azolla incorporation improved soil nutrient-holding capacity, enhancing soil fertility. BS remained consistently high across treatments (92.54% to 94.99%) with no statistically significant differences ($p > 0.05$).

Table 6. Soil properties during harvest

Treatment Code	pH H_2O	EC (mmhos cm^{-1})	Organic-C (%)	Total-N (%)	Total-P (ppm)	Total-K (mg $100g^{-1}$)	CEC (me $100g^{-1}$)	BS (%)
K0	7.36	0.80	2.90 c	0.24 c	2896.54	70.52	31.71 f	93.09
K1A	7.43	0.90	3.68 a	0.25 bc	2985.97	70.34	39.49 c	93.31
K2A	7.41	0.85	3.70 a	0.26 b	2839.96	70.29	36.72 e	94.99
K3A	7.46	0.94	3.73 a	0.28 a	2844.90	70.95	42.28 a	94.00
K1AT	7.42	0.85	3.64 a	0.24 c	2857.34	70.95	38.99 cd	92.54
K2AT	7.44	0.79	3.69 a	0.25 bc	2930.27	71.56	38.68 d	93.45
K3AT	7.42	1.05	3.75 a	0.28 a	3037.68	71.73	41.25 b	93.83
KA	7.39	0.85	3.28 b	0.24 c	2879.01	68.92	31.54 f	93.00

Note: Different superscript letters in each column indicate significant differences between treatments based on DMRT ($p < 0.05$).

3.4 Correlation between soil and paddy crop parameters

Table 7. Correlation matrix between soil parameters

Parameters	Soil pH H_2O	Soil EC	Soil Organic-C	Soil Total-N	Soil Total-P	Soil Total-K	Soil CEC	Soil BS	Paddy Organic-C	Paddy Total-N	N-Uptake
Soil pH H_2O											
Soil EC	0.43										
Soil Organic-C	0.89	0.49									
Soil Total-N	0.62	0.78	0.58								
Soil Total-P	0.08	0.54	0.19	0.23							
Soil Total-K	0.49	0.35	0.48	0.47	0.41						
Soil CEC	0.90	0.63	0.87	0.70	0.29	0.71					
Soil BS	0.27	0.27	0.42	0.68	-0.10	0.13	0.28				
Paddy Organic-C	0.73	0.15	0.74	0.06	0.02	0.38	0.72	-0.17			
Paddy Total-N	0.75	0.79	0.74	0.83	0.57	0.56	0.86	0.43	0.39		
N-Uptake	0.86	0.66	0.89	0.71	0.45	0.67	0.96	0.37	0.66	0.93	

Confidence interval	Relationship
0.00-0.199	Very Low
0.20-0.399	Low
0.40-0.599	Medium
0.60-0.799	Strong
0.80-1.000	Very Strong

Table 7 shows the correlation matrix between soil parameters. The correlation analysis revealed significant interdependencies among soil properties, highlighting their role in nutrient dynamics and uptake. Soil pH exhibited a strong positive correlation with soil Organic-C (r = 0.89), suggesting that higher organic matter content contributes to pH stability through buffering effects. Similarly, soil EC showed a notable correlation with Total-N (r = 0.78), indicating that soil salinity influences nitrogen availability. Soil CEC was strongly associated with soil Organic-C (r = 0.87) and Total-N (r = 0.70), reinforcing the role of organic matter in nutrient retention and soil fertility. Meanwhile, soil Total-P and Total-K showed relatively lower correlations with other soil properties (r = 0.41 and r = 0.29, respectively), suggesting that their availability might be governed by different nutrient cycling mechanisms. The relationship between soil nutrient content and plant uptake was also evident, particularly in nitrogen dynamics. Soil Organic-C had a strong correlation with paddy Organic-C (r = 0.42) and Total-N in paddy crops (r = 0.68), indicating that organic matter enhances nitrogen cycling and availability. Soil Total-N was significantly correlated with both paddy Total-N (r = 0.83) and N-uptake (r = 0.71), further emphasizing its crucial role in plant nutrition. Notably, N-uptake was highly correlated with both soil Organic-C (r = 0.89) and soil CEC (r = 0.96), suggesting that the presence of organic matter and improved soil exchange capacity enhance nutrient absorption efficiency in crops.

3.5 Rice Paddy Organic-C, Total-N, and N-Uptake

Table 8 shows the rice plant's Organic-C, Total-N, and N-uptake. The study investigated the effects of different treatments on rice plant characteristics, particularly focusing on organic-C content, total-N content, and N-uptake. The results indicated variations in these parameters across treatments, with significant differences observed in N-uptake. The organic-C content in rice plants varied across treatments, ranging from 43.10% in K0 to 45.56% in K1AT. Although some differences were observed, the overall variations remained relatively small, suggesting that organic-C content was relatively stable regardless of treatment application. The Total-N content showed a more pronounced variation, where the highest percentage was observed in K3AT (1.32%), followed by K3A (1.27%) and K1A (1.26%). In contrast, the lowest total-N content was recorded in K0 (0.99%), indicating that nitrogen availability was lower in this treatment compared to the others. The most notable differences were observed in N-uptake, which displayed statistically significant variations among treatments ($p < 0.05$). The lowest N-uptake values were recorded in K0 (0.10 g pot^{-1}) and KA (0.17 g pot^{-1}), which were significantly lower than all other treatments. Conversely, the highest N-uptake was observed in K3AT (1.87 g pot^{-1}), followed closely by K1A (1.72 g pot^{-1}) and K3A (1.70 g pot^{-1}). These findings suggest that the treatments applied in K3AT, K1A, and K3A were more effective in facilitating nitrogen absorption compared to K0 and KA. Statistical analysis using DMRT ($p < 0.05$) confirmed these differences, as indicated by different superscript letters in the dataset. The results indicate that the treatments significantly improved N-uptake. While the Organic-C content remained relatively stable, the increased Total-N and N-uptake values in certain treatments suggest that these methods enhanced nitrogen availability and utilization by rice plants. The notably higher N-uptake in K3AT, K1A, and K3A suggests that these treatments play a crucial role in optimizing nitrogen absorption efficiency. In contrast, K0 and KA exhibited significantly lower uptake levels, indicating insufficient nitrogen absorption under these conditions.

Table 8. Rice plant Organic-C, Total-N, and N-uptake

Treatment	Rice Plant Organic-C (%)	Rice Plant Total-N (%)	N-Uptake (g pot^{-1})
K0	43.10	0.99	0.10 b
K1A	45.04	1.26	1.72 a
K2A	44.11	1.12	1.12 a
K3A	44.66	1.27	1.70 a
K1AT	45.56	1.05	1.10 a
K2AT	44.64	1.15	1.37 a
K3AT	44.15	1.32	1.87 a
KA	43.60	1.00	0.17 b

Note: Different superscript letters in each column indicate significant differences between treatments based on DMRT ($p < 0.05$).

4. Discussion

4.1 Soil Fertility Improvements

The findings of this study highlight the significant impact of Azolla incorporation on soil fertility, particularly in improving Organic-C content, total-N levels, and CEC. The results indicate that Azolla application significantly enhances soil organic matter content, which is consistent with previous studies suggesting that Azolla plays a crucial role in nitrogen fixation and organic matter accumulation in agricultural soils (Fasusi et al., 2021; Prabakaran et al., 2022). The highest Organic-C content was observed in K3A (3.73%) and K3AT (3.75%), which were significantly higher than the control treatment (K0, 2.90%) ($p < 0.05$). This suggests that Azolla decomposition contributes to an increase in soil organic matter, improving soil structure and microbial activity (Gerke, 2022; Korsa et al., 2024). Furthermore, Total-N levels increased significantly in treatments incorporating Azolla, with the highest values recorded in K3A and K3AT (0.28%), demonstrating the effectiveness of Azolla in enhancing nitrogen retention. These findings align with previous research that emphasizes the ability of Azolla to supply bioavailable nitrogen through its symbiotic association with Anabaena azollae (Adhikari et al., 2020; Prabakaran et al., 2022).

A particularly notable effect of Azolla incorporation was the significant increase in CEC, with the highest values recorded in K3A (42.28 me 100g^{-1}) and K3AT (41.25 me 100g^{-1}), compared to the control treatment (K0, 31.71 me 100g^{-1}), representing a 33.4% improvement. This increase in CEC can be attributed to several interrelated biochemical and physicochemical mechanisms. The decomposition of Azolla releases humic substances, carboxyl, and phenolic groups, which increase the number of negatively charged sites in the soil matrix. This enhances the adsorption of essential cations such as Ca^{2+}, Mg^{2+}, K^{+}, and NH_4^{+}, improving soil nutrient-holding capacity

(Marzouk et al., 2024). The accumulation of organic residues following Azolla incorporation also creates stable soil aggregates, increasing the surface area for cation exchange (Seleiman et al., 2022). Azolla's association with nitrogen-fixing cyanobacteria promotes microbial biomass accumulation and enzymatic activity, leading to higher decomposition rates and the release of mineralized nutrients (Simarmata et al., 2021). These microbial activities increase the availability of exchangeable cations, thereby enhancing soil CEC (Akhtar et al., 2021). Azolla incorporation contributes to soil aggregation and enhanced porosity, which improves ion mobility and cation retention. This structural enhancement mitigates nutrient leaching, particularly in sandy soils, where CEC is typically lower due to reduced organic matter content. Azolla residues buffer soil pH within an optimal range (7.36–7.46), preventing excessive acidification or alkalization, both of which can lead to cation loss or immobilization (Fasusi et al., 2021). The ability of Azolla to maintain pH stability supports optimal cation exchange dynamics, further explaining the observed increase in CEC.

The observed increase in CEC following Azolla incorporation is consistent with the study by Korsa et al. (2024), who reported CEC improvements of 28–35% in Azolla-treated paddy soils, primarily due to increased humic acid formation. Similarly, Seleiman et al. (2022) found that Azolla incorporation improved cation retention by 30%, particularly enhancing the availability of Ca^{2+}, Mg^{2+}, and K^{+}. Additionally, Akhtar et al. (2021) demonstrated that Azolla decomposition leads to the release of organic acids that enhance CEC, but their study did not quantify the specific contribution of Azolla to CEC improvement. This research builds upon their findings by providing a quantified correlation between Azolla incorporation rates and CEC enhancement, demonstrating that higher incorporation levels (K3A and K3AT) yield the most significant improvements.

4.2 Correlation between Soil Parameters

A correlation matrix analysis was conducted to determine the interrelationships between key soil fertility parameters. The results indicated a strong positive correlation between soil pH, Organic-C ($r = 0.89$), and CEC ($r = 0.90$), highlighting that an increase in organic matter enhances soil nutrient retention (Abbas et al., 2020; Akhtar et al., 2021). EC was also positively correlated with Total-N ($r = 0.78$), suggesting that EC could serve as an indicator of nitrogen availability in soil fertility assessments (Barrow & Hartemink, 2023, Gu & Yang, 2022; Othaman et al., 2020). Interestingly, Total-P displayed weak correlations with pH ($r = 0.08$) and Total-N ($r = 0.23$), indicating that phosphorus availability may be regulated by microbial activity or soil mineral composition rather than by pH or nitrogen levels alone (Jiang et al., 2021; Qaswar et al., 2022; Tian et al., 2021). These findings suggest that phosphorus management should be integrated with other soil fertility strategies to optimize nutrient availability (Kour et al., 2024b).

4.3 Impact on N-Uptake

Azolla incorporation significantly improved N-uptake in rice plants, with the highest values observed in K3AT (1.87 g pot^{-1}), K1A (1.72 g pot^{-1}), and K3A (1.70 g pot^{-1}), which were significantly higher than the control treatments (K0, 0.10 g pot^{-1}; KA, 0.17 g pot^{-1}) ($p < 0.05$). These results suggest that Azolla application enhances nitrogen absorption efficiency in rice plants, thereby supporting higher growth rates and productivity (Naher et al., 2021; Tyagi et al., 2022). The Total-N content in rice plants was also significantly higher in Azolla-treated groups, with K3AT recording the highest value (1.32%), followed by K3A (1.27%). The enhanced N-uptake can be attributed to the slow release of bioavailable nitrogen from Azolla decomposition, which aligns with findings from previous studies on biofertilizers (Erfani et al., 2020; Yadav et al., 2021). The statistical validation using DMRT ($p < 0.05$) confirmed that the differences in N-uptake were statistically significant, further proving the effectiveness of Azolla in nitrogen management (Fitriatin et al., 2021b; Shukla et al., 2024).

4.4 Sustainable Agricultural Implications

This study has significant implications for sustainable agricultural practices, particularly in addressing soil fertility management and nitrogen use efficiency. The findings demonstrate that the incorporation of Azolla enhances nitrogen retention and organic matter content, thereby reducing the dependence on synthetic fertilizers. This shift toward organic alternatives is crucial in mitigating the adverse environmental impacts associated with excessive fertilizer use, such as nutrient leaching, soil degradation, and greenhouse gas emissions. The observed increase in CEC further underscores the role of Azolla in improving soil nutrient retention, which is essential for maintaining soil health and productivity in the long term. Given that declining soil fertility remains a persistent challenge in modern agriculture, the adoption of Azolla-based fertilization strategies could provide a cost-effective and ecologically sound solution for farmers seeking to enhance soil resilience while minimizing external chemical inputs (Atieno et al., 2020).

From a policy perspective, integrating Azolla into national and regional agricultural frameworks aligns with global sustainability initiatives aimed at promoting climate-smart and resource-efficient farming systems. The transition toward organic and bio-based fertilizers is increasingly being recognized as a critical component of sustainable intensification, particularly in developing countries where fertilizer access and affordability pose significant challenges. Governments and agricultural policymakers could facilitate the broader adoption of Azolla through targeted interventions such as financial incentives for biofertilizer adoption, farmer capacity-building programs, and research investments that optimize Azolla application in diverse agroecosystems (Abbas et al., 2020). Furthermore, policy frameworks designed to reduce the environmental footprint of conventional fertilizers could benefit from the formal inclusion of Azolla-based fertilization strategies, particularly in policies aimed at improving nitrogen use efficiency and mitigating soil degradation. Encouraging the use of Azolla through institutional support mechanisms, extension services, and subsidy programs could accelerate its integration into mainstream farming practices, thereby contributing to a more resilient and sustainable agricultural sector.

The results of this study reinforce the potential of Azolla as a viable alternative to conventional fertilizers, particularly in enhancing soil fertility, improving nitrogen retention, and mitigating the negative impacts of chemical inputs. The ability of Azolla to enhance soil organic matter and nutrient-holding capacity makes it a valuable tool in regenerative agricultural practices, which emphasize soil restoration and sustainability. Beyond its agronomic benefits, the use of Azolla aligns with broader environmental goals, including carbon sequestration and water conservation, making it an attractive option for both smallholder farmers and large-scale agricultural enterprises. Considering the rising costs and fluctuating availability of synthetic fertilizers, Azolla presents an economically viable option that can reduce input costs while maintaining soil health and crop productivity.

Future research should explore the long-term agronomic and environmental impacts of Azolla integration, particularly in relation to soil microbial dynamics and nutrient cycling. Further studies assessing the economic feasibility of large-scale Azolla adoption, especially in the context of smallholder farming systems, are critical for informing evidence-based policy recommendations (Kulasooriya, 1991; Yadav et al., 2023). Additionally, examining the synergies between Azolla and other organic amendments, such as compost and biochar, could provide valuable insights into optimizing its benefits in different soil and climatic conditions. Given the global push toward more sustainable and resilient agricultural systems, advancing research and policy support for Azolla-based fertilization strategies could play a crucial role in shaping the future of sustainable agriculture.

5. Conclusions

This study demonstrates that Azolla incorporation significantly enhances soil fertility and N-uptake in rice cultivation. The findings indicate that Azolla application increased soil Organic-C by up to 3.75%, compared to 2.90% in control treatments, reflecting a 29% improvement. Additionally, Total-N levels in soil were enhanced, with the highest recorded value at 0.28%, compared to 0.24% in the control treatments. CEC also showed a notable increase, reaching 42.28 me $100g^{-1}$ in Azolla-treated soils—representing a 33.4% improvement over the control treatments (31.71 me $100g^{-1}$). Furthermore, Azolla incorporation significantly improved N-uptake in rice plants, with the highest N-uptake recorded at 1.87 g pot^{-1}, compared to 0.10 g pot^{-1} in the control treatments. These results emphasize the potential of Azolla as an effective biofertilizer to improve soil nutrient retention and enhance plant nitrogen absorption. Overall, the study underscores the role of Azolla in promoting sustainable agriculture by reducing reliance on chemical fertilizers while maintaining soil health. Future research should explore its long-term effects and cost-benefit implications to support broader adoption in agricultural systems.

Author Contributions

Conceptualization, I.M.A. and P.O.B.; methodology, P.O.B.; software, P.O.B.; validation, I.M.A., P.O.B. and N.G.K.R.; formal analysis, P.O.B.; investigation, P.O.B.; resources, P.O.B.; data curation, P.O.B.; writing—original draft preparation, P.O.B.; writing—review and editing, P.O.B.; visualization, P.O.B; supervision, I.M.A.; project administration, P.O.B.; funding acquisition, I.M.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Conflicts of Interest

The authors declare no conflict of interest.

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