

Article

Enhancing Soil Fertility, Improving Yield of Dai Thom 8 Rice, and Reducing Nitrogen Fertilizer Input Through *Herbaspirillum seropedicae* Inoculation

Trinh Van Tuan Em ^{1,2}  and Nguyen Van Chuong ^{1,2,*} ¹ Department of Crop Science, An Giang University, An Giang 880000, Vietnam² Vietnam National University, Ho Chi Minh City 700000, Vietnam

* Correspondence: nvchuong@agu.edu.vn; Tel.: +84-918487044

Abstract

The excessive use of inorganic nitrogen (N) fertilizers in rice production poses significant environmental and economic challenges, particularly in intensive farming systems such as those in the Mekong Delta, Vietnam. This study aimed to evaluate the potential of *Herbaspirillum seropedicae* (*H. seropedicae*), an endophytic N-fixing bacterium, to enhance soil fertility, improve rice growth, and maintain yield while reducing N fertilizer inputs in Dai Thom 8 rice under field conditions. A randomized complete block design with five treatments, including different nitrogen reduction levels combined with bacterial inoculation, was employed. The results showed that treatments integrating *H. seropedicae* significantly improved soil properties, including soil organic matter, total nitrogen, and available nutrients, compared to the control. Growth parameters such as plant height, tiller density, and chlorophyll content were also enhanced, particularly in treatments with bacterial inoculation. Yield components, including grain number and filled grains per panicle, were significantly increased, leading to higher grain yield. The highest yield was observed in T5 (5.72 t ha⁻¹), while T3 and T4 achieved comparable yields with reduced N inputs. Additionally, grain quality analysis revealed increased protein content without negatively affecting starch composition. These findings highlight the potential of *H. seropedicae* as a biofertilizer to improve N use efficiency and reduce dependency on chemical fertilizers. The study provides strong evidence for integrating microbial inoculants into sustainable rice production systems. Among the treatments, T3 (50% N reduction combined with bacterial inoculation) is recommended as the optimal strategy due to its balance between high yield and reduced input costs, contributing to environmentally friendly and economically viable agriculture.

Keywords: *Herbaspirillum seropedicae*; nitrogen use efficiency; biofertilizer; rice yield; sustainable agriculture



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1. Introduction

Rice (*Oryza sativa* L.) is one of the most important staple crops worldwide, feeding more than half of the global population. In countries such as Vietnam, rice production plays a central role not only in food security but also in economic development and rural livelihoods [1,2]. However, the sustainability of rice cultivation is increasingly challenged by soil degradation, excessive use of chemical fertilizers, and declining nutrient use efficiency. Among these constraints, the overreliance on inorganic N fertilizers has become a major concern due to its negative environmental and agronomic consequences [3,4].

Nitrogen is a critical macronutrient that directly influences plant growth, chlorophyll synthesis, and grain yield. To maximize productivity, farmers often apply large quantities of synthetic N fertilizers [5]. Nevertheless, crops typically utilize only 30–50% of the applied nitrogen, while the remainder is lost through volatilization, leaching, and denitrification. These losses not only increase production costs but also contribute to environmental problems such as greenhouse gas emissions, soil acidification, and water pollution [6,7]. In the Mekong Delta region of Vietnam, intensive rice cultivation systems have led to a gradual decline in soil fertility, characterized by reduced organic matter content, nutrient imbalance, and decreased microbial diversity [8]. Therefore, developing sustainable strategies that maintain high yield while reducing chemical fertilizer inputs is an urgent priority. In recent years, plant growth-promoting bacteria (PGPB) have emerged as a promising eco-friendly alternative to conventional fertilization practices [9,10]. These beneficial microorganisms can enhance plant growth through multiple direct and indirect mechanisms, including biological N fixation, phytohormone production, nutrient solubilization, and stress tolerance enhancement [11,12]. Among these, endophytic diazotrophic bacteria have attracted considerable attention due to their ability to colonize internal plant tissues and establish a more stable and efficient association with the host plant [13,14].

H. seropedicae is a well-known endophytic N-fixing bacterium that has been extensively studied in association with cereal crops such as rice, maize, and wheat [15]. This bacterium is capable of colonizing roots, stems, and leaves without causing harm to the host plant, forming a symbiotic relationship that enhances nutrient acquisition and plant growth [16,17]. One of its most significant traits is its ability to fix atmospheric N and partially supply it to the host plant, thereby reducing the need for external N inputs. In addition, *H. seropedicae* can produce plant growth regulators such as indole-3-acetic acid (IAA), which stimulate root development and improve nutrient uptake efficiency [18,19]. Beyond N fixation, *H. seropedicae* also contributes to improving soil fertility through indirect mechanisms. These include enhancing soil microbial activity, promoting nutrient cycling, and improving soil structure through interactions with organic matter and other soil microorganisms [20,21]. Such multifunctional roles make this bacterium a strong candidate for sustainable agricultural systems, particularly in regions where soil degradation and fertilizer overuse are prevalent. Despite the growing body of research on *H. seropedicae*, most studies have focused on controlled conditions or on crops such as maize and sugarcane [22,23]. Limited information is available regarding its effectiveness under field conditions in rice cultivation systems, especially in specific local varieties such as Dai Thom 8, a high-quality aromatic rice widely cultivated in Vietnam [24]. This variety is known for its excellent grain quality but often requires high fertilizer inputs to achieve optimal yield, making it an ideal candidate for evaluating microbial-based nutrient management strategies [25,26].

Furthermore, the performance of microbial inoculants can vary significantly depending on environmental conditions, soil characteristics, and plant genotype [10,27]. Therefore, it is essential to isolate and evaluate native or locally adapted bacterial strains that can perform effectively under specific agroecological conditions [11,28]. The strain of *H. seropedicae*, isolated from rice-growing soils, represents a promising candidate due to its potential adaptability and plant growth-promoting capabilities [9,29]. However, its effects on soil fertility, nitrogen use efficiency, and rice yield under field conditions have not yet been fully elucidated [30]. In addition to enhancing crop productivity, the use of beneficial microorganisms aligns with the global trend toward sustainable and climate-smart agriculture. Reducing the reliance on synthetic fertilizers not only lowers production costs for farmers but also mitigates environmental impacts, contributing to the long-term sustainability of

agricultural systems. Integrating microbial inoculants into rice production systems could therefore play a key role in achieving both economic and environmental goals [31,32].

Given these considerations, this study aims to evaluate the effects of *H. seropedicae* on soil fertility, rice growth, and yield of the Dai Thom 8 variety under field conditions. Specifically, the study investigates the potential of this bacterial strain to reduce inorganic nitrogen fertilizer inputs while maintaining or enhancing crop productivity. In addition, changes in soil chemical properties and nutrient availability are assessed to better understand the mechanisms underlying the observed plant responses. This research is expected to provide new insights into the application of endophytic nitrogen-fixing bacteria in rice cultivation and to contribute to the development of sustainable nutrient management strategies [11,12,33]. The findings may also support the broader adoption of biofertilizers in intensive rice production systems, particularly in regions facing challenges related to soil degradation and environmental pollution.

2. Materials and Methods

2.1. Source and Inoculum Preparation of *H. seropedicae*

Species of *H. seropedicae* was aseptically isolated from root samples of rice Dai Thom 8 collected in Vinh Dieu commune, An Giang Province, Vietnam. Isolation was carried out on yeast extract mannitol agar (YEMA) medium adjusted to pH 6.8–7.0. Molecular identification was performed based on 16S rRNA gene sequencing. The obtained sequence (1392 bp) was deposited in the GenBank database under accession number PZ070781.1 and is publicly available in the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/nuccore/PZ070781.1>); linear BCT 05-MAR-2026; accessed on 5 March 2026). Sequence alignment analysis showed 100% similarity with reference sequences of the genus *Herbaspirillum*, particularly *H. seropedicae*, confirming its taxonomic identity. Following identification, the strain was evaluated for plant growth-promoting characteristics, including ammonia production, nitrogenase activity, and nitrogen accumulation capacity. After 72 h of incubation, the isolate exhibited relatively high levels of these functional traits, indicating strong potential for biological nitrogen fixation and plant growth enhancement. Based on these promising properties, the strain was selected for further experimental evaluation [16,34].

For inoculum preparation, the bacterium was cultured in diluted YMA medium (pH 6.8–7.0) and incubated at 30 ± 2 °C on a rotary shaker at 150 rpm for 48–72 h. Cell density was determined spectrophotometrically at OD₆₀₀ and adjusted to approximately 10^8 CFU mL⁻¹, corresponding to the exponential growth phase. To ensure methodological consistency across all experimental sections, rice seeds of Dai Thom 8 were surface sterilized using a unified protocol: immersion in 70% ethanol for 1 min, followed by treatment with 2% sodium hypochlorite (NaOCl) for 3–5 min. The seeds were then rinsed thoroughly three to five times with sterile distilled water to remove any residual disinfectant. Subsequently, the sterilized seeds were coated with the bacterial suspension and incubated in the dark at room temperature for approximately 48 h to facilitate bacterial attachment and initial root colonization prior to sowing [33,34].

2.2. Experimental Design and Crop Establishment

The field experiment was carried out under typical agro-climatic conditions of the Mekong Delta, Vietnam, a region characterized by a tropical monsoon climate with persistently high temperatures and distinct seasonal rainfall regimes. During the cropping period, air temperature remained relatively stable, ranging from 26 to 32 °C, while relative humidity was consistently high (approximately 70–90%). Rainfall occurred intermittently

in accordance with regional seasonal patterns, providing favorable moisture conditions for rice cultivation. Although continuous on-site meteorological measurements were not conducted, the observed environmental conditions are consistent with well-documented climatic characteristics of the region and are considered suitable for both rice growth and microbial activity. While temporal fluctuations in temperature and rainfall may influence soil nutrient dynamics and plant physiological processes, all treatments were implemented simultaneously under identical field conditions, thereby ensuring the reliability and comparability of the experimental outcomes.

To enhance transparency and reproducibility, key meteorological parameters representative of Vinh Dieu commune were compiled from regional agro-climatic data and are presented in Table 1. As shown, the experimental period was defined by a stable thermal regime, high atmospheric humidity, and seasonal rainfall distribution, all of which are conducive to rice development and microbial functioning. These conditions likely supported key physiological processes such as photosynthesis and nutrient uptake, while also promoting soil moisture availability and nutrient mobility. Overall, the prevailing environmental conditions provided an appropriate agroecological context for evaluating the effects of *H. seropedicae* inoculation in combination with nitrogen management strategies under field conditions.

Table 1. Representative agro-meteorological conditions during the experimental period in Vinh Dieu commune, An Giang province, Vietnam.

Parameter	Unit	Value	Description
Air temperature	°C	26–32	Typical range during rice-growing season
Relative humidity	%	70–90	High humidity typical of coastal delta
Rainfall	mm	Seasonal/intermittent	Influenced by monsoon pattern
Climate type	–	Tropical monsoon	Distinct wet and dry seasons

The experiment was designed as a single-factor study, in which the treatment factor comprised varying nitrogen application rates in combination with bacterial inoculation (with and without *H. seropedicae*). A total of five treatments were established, each with four replicates. The treatments were arranged in a randomized complete block design (RCBD) to reduce the effects of spatial heterogeneity across the field. The total experimental area was 500 m², with individual plots measuring 25 m² (5 m × 5 m). Each block covered an area of 125 m² (5 m × 25 m). Adjacent plots were separated by 0.6 m-wide channels. To prevent lateral movement of water and nutrients, particularly nitrogen, between plots, bunds were constructed and reinforced with plastic linings. This arrangement effectively minimized runoff and cross-contamination, thereby preserving the independence and integrity of each treatment throughout the experimental period.

The seeds were rinsed three times with sterile distilled water to remove residual sterilizing agents. For initial inoculation, 1 mL of bacterial suspension was diluted with sterile deionized water and uniformly sprayed onto the seeds. The treated seeds were then incubated overnight in the dark to facilitate bacterial adhesion prior to sowing. In addition to seed treatment, a second inoculation was applied in the field at 30 days after sowing (DAS) for treatments T2, T3, T4, and T5, using a bacterial suspension applied to the root zone to enhance colonization and plant–microbe interaction under field conditions [9–11]. Chemical fertilizers, namely urea, superphosphate, and potassium chloride (KCl), were sourced from Binh Dien Fertilizer Company, C12/21 Le Kha Phieu Street, Tan Nhut Commune, Ho Chi Minh City, Vietnam, and their nutrient contents were standardized to equivalent amounts of N, P₂O₅, and K₂O based on 100% purity. The specific application rates for each treatment are detailed in Table 2.

Table 2. N-fertilizer application rates and *H. seropedicae* inoculation treatments evaluated in the experiment.

Treatment	<i>H. seropedicae</i> (10 ⁸ CFU mL ⁻¹)	N-Application Rates (kg ha ⁻¹)	N-Reduction Rates (%)	Inorganic Fertilizers (kg ha ⁻¹)
T1 (control)	No	0	100	40 P ₂ O ₅ –30 K ₂ O
T2	Yes	0	100	
T3	Yes	45.0	50	
T4	Yes	67.5	25	
T5	Yes	90.0	0	

Note: Bacterial inoculation was applied at 30 days after sowing (DAS) in treatments T2, T3, T4, and T5.

2.3. Soil Sampling and Fertilizer Application

Soil samples were collected 15 days prior to sowing to determine baseline soil characteristics, while additional samples were taken after harvest to evaluate treatment effects. The baseline soil properties indicated slightly acidic conditions (pH 5.1), which are generally suitable for rice cultivation but may enhance the solubility of Fe and Al. The moderate levels of soil organic matter (3.5%) and cation exchange capacity (25 cmol⁺ kg⁻¹) suggest a fair nutrient retention capacity (Table 3). However, total nitrogen content (0.120%) was relatively low, indicating a potential limitation for crop growth and the need for nitrogen supplementation. Although total phosphorus was moderate, its availability may be restricted due to fixation under acidic conditions. Exchangeable potassium was within a moderate range, supporting plant development. Overall, the soil exhibited medium fertility, highlighting the necessity for integrated nutrient management strategies. Phosphorus fertilizer (P₂O₅) was applied as a basal dose at a rate of 400 kg ha⁻¹ one day before sowing. In contrast, nitrogen (urea) and potassium (KCl) fertilizers were applied in four split applications throughout the crop growth period.

Table 3. Baseline soil physicochemical properties and texture before the experiment (n = 5).

Parameter	Unit	Value/Range	Remarks
pH (H ₂ O)	–	5.1	Slightly acidic
EC	dS m ^{−1}	1.0	Non-saline
CEC	cmol(+) kg ^{−1}	25	Moderate nutrient retention capacity
Soil organic matter (SOM)	%	3.5	Medium fertility level
Total nitrogen (TN)	%	0.074	Low N content
Total phosphorus (TP)	%	0.15	Mostly in unavailable forms
Available phosphorus (AP)	mg kg ^{−1}	30	Medium availability
Exchangeable potassium (K)	mg kg ^{−1}	117	Moderate level
Iron (Fe)	mg kg ^{−1}	134	Potential toxicity under low pH
Aluminum (Al)	mg kg ^{−1}	48.6	May affect root growth in acidic soil
Sand	%	48.7	Based on USDA classification
Silt	%	21.6	
Clay	%	29.7	
Soil texture	Sandy clay loam		

Soil samples collected from a depth of 0–20 cm were air-dried, finely ground, and sieved through a 2 mm mesh prior to analysis. Soil pH was determined in a 1:2.5 (*w/v*) soil-to-distilled water mixture using a digital pH meter (SevenCompact™ S220, Mettler-Toledo AG, Greifensee, Switzerland). Soil organic matter (SOM) content was quantified using the dichromate oxidation procedure described by Walkley and Black [35]. Total nitrogen was measured following the Kjeldahl digestion method with an automated analyzer (Kjeltec™ 8400, FOSS A/S, Hillerød, Denmark). Available phosphorus was extracted according to the Olsen method and measured using a UV–Vis spectrophotometer (UV-1800 UV–Vis Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) [36]. Exchangeable

potassium was extracted using 1 M NH_4OAc (pH 7.0) and quantified by flame photometry (PFP7 Flame Photometer, Jenway Ltd., Staffordshire, UK). Cation exchange capacity (CEC) was evaluated using the ammonium acetate saturation technique [37]. Soil particle size distribution (sand, silt, and clay) was determined using the hydrometer method [38].

For Fe and Al analysis, soil samples were digested with a mixture of concentrated HNO_3 and HClO_4 (3:1, *v/v*) using a digestion block. After digestion, the solutions were filtered and diluted with deionized water. The concentrations of Fe and Al were then determined by atomic absorption spectrophotometry (AAS) (AA-7000, Atomic Absorption Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) following standard protocols [39].

2.4. Plant Biochemical Analysis

Agronomic parameters were recorded at different growth stages following standard field measurement protocols. Plant height (cm) was measured from the soil surface to the tip of the tallest leaf or panicle using a measuring ruler at 25, 50, and 75 days after sowing (DAS). Ten plants were randomly selected per plot, and the mean value was calculated.

Tiller density was determined by counting the total number of shoots within a 1 m² quadrat randomly placed in each plot at 25 and 50 DAS. The results were expressed as number of shoots per square meter (shoots m⁻²). Leaf chlorophyll content was measured using a portable chlorophyll meter (SPAD-502 Plus Chlorophyll Meter, Konica Minolta, Inc., Osaka, Japan). Measurements were taken on the uppermost fully expanded leaves at 25 and 50 DAS. For each plot, ten plants were randomly selected, and three readings per leaf were recorded and averaged. The results were expressed in SPAD units, representing relative chlorophyll content.

At maturity, yield components were determined from randomly selected plants within each plot. These included number of panicles per plant, total number of grains per panicle, number of filled grains, and number of unfilled grains. For yield determination, the entire plot area (5 m²) was harvested, and grain yield was recorded and adjusted to a standard moisture content of 14%, then converted to tons per hectare (t ha⁻¹). Aboveground biomass was measured at harvest by collecting all plant materials within each plot, weighing them immediately, and expressing the results as kg per 5 m². Subsamples were taken and oven-dried at 70 °C to constant weight to determine dry matter content when necessary.

Plant materials (seeds) were dried at 70 °C until constant weight, ground into fine powder, and stored in sealed containers prior to analysis. Lipid content (%) was determined using Soxhlet extraction with petroleum ether as the solvent. Approximately 2 g of dried sample was extracted for 6–8 h using a Soxhlet system (Büchi Extraction System B-811, Büchi Labortechnik AG, Flawil, Switzerland), and lipid content was calculated on a gravimetric basis [40]. Protein content (%) was analyzed using the Kjeldahl method. Total nitrogen was determined through acid digestion, followed by distillation and titration using an automated Kjeldahl system (Kjeltec™ 8400 Kjeldahl Analyzer, FOSS A/S, Hillerød, Denmark), and crude protein content was calculated using a conversion factor of 6.25. However, grain moisture content was determined using the oven-drying method. Approximately 5 g of milled rice was weighed and dried in a hot-air oven at 105 °C until a constant weight was achieved. Moisture content was calculated based on the weight loss before and after drying and expressed as a percentage of fresh weight [40]. Grain moisture content was determined by oven drying at 105 °C to constant weight [40]. Amylose content was analyzed using the iodine colorimetric method following Juliano [41], and absorbance was measured at 620 nm using (AA-7000, Atomic Absorption Spectrophotometer, Shimadzu Corporation, Kyoto, Japan). Amylopectin content was calculated as the difference between total starch and amylose content [40,41].

2.5. Quality Assurance and Quality Control (QA/QC)

All measurements were conducted in triplicate. Calibration curves were established using certified standard solutions with correlation coefficients (R^2) ≥ 0.999 . Each analytical batch included reagent blanks and duplicate samples. The limits of detection (LOD) and quantification (LOQ) for Fe and Al were 0.005 and 0.015 mg kg⁻¹, respectively. Method accuracy was assessed using certified reference materials, with recovery rates ranging from 90% to 105%. All results were expressed on a dry weight basis.

2.6. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) to assess treatment effects. Statistical analyses were performed using Statgraphics software (version XVIII), and differences among treatment means were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Effects of *H. seropedicae* Inoculation and Fertilization Strategies on Soil Physicochemical Properties

The results indicated clear variation among treatments in soil properties following harvest (Table 4). The highest values of soil organic matter, total nitrogen, and available nutrients were generally observed in treatments combining bacterial inoculation with reduced nitrogen application. In particular, treatments with moderate nitrogen reduction (e.g., 25–50%) coupled with *H. seropedicae* inoculation showed superior performance, suggesting enhanced nutrient retention and improved soil fertility. In contrast, the lowest values were recorded in treatments without bacterial inoculation and with either no nitrogen application or excessive nitrogen input. These treatments likely experienced nutrient depletion or increased nutrient loss due to inefficient uptake. The results demonstrate that integrating bacterial inoculation with optimized nitrogen levels can significantly improve soil quality compared to conventional fertilization practices.

Table 4. Effects of nitrogen fertilization and bacterial inoculation on soil physicochemical properties after harvest.

Treatment	pH	SOM	Total N	Available P	Exchangeable K
		(%)		(mg kg ⁻¹)	
T1	5.05 ± 0.013 ^b	3.08 ± 0.13 ^c	0.075 ± 0.013 ^d	25.4 ± 2.03 ^e	102.5 ± 1.29 ^c
T2	5.33 ± 0.116 ^a	3.82 ± 0.13 ^a	0.195 ± 0.013 ^{bc}	34.9 ± 0.37 ^c	130.0 ± 2.58 ^b
T3	5.12 ± 0.013 ^b	3.53 ± 0.13 ^b	0.255 ± 0.039 ^a	40.5 ± 3.22 ^a	197.0 ± 25.8 ^a
T4	4.91 ± 0.013 ^c	3.07 ± 0.103 ^c	0.237 ± 0.049 ^{ab}	32.4 ± 4.78 ^d	203.5 ± 1.29 ^a
T5	4.63 ± 0.241 ^d	3.02 ± 0.013 ^c	0.152 ± 0.021 ^c	38.0 ± 0.34 ^b	203.0 ± 5.16 ^a
F	**	**	**	**	**
CV (%)	14.8	10.3	19.0	15.7	26.8

Note: Values represent mean ± standard deviation (n = 4). Different letters within the same column indicate significant differences among treatments at $p \leq 0.05$ according to ANOVA. ** indicates highly significant differences at $p \leq 0.01$.

3.2. Effects of *H. seropedicae* Inoculation and N Fertilizer Ratios on the Growth Performance of Rice Dai Thom 8

The results in Table 5 indicate that both *H. seropedicae* inoculation and fertilizer regimes significantly influenced growth parameters of the Dai Thom 8 rice variety. Plant height increased progressively across all treatments from 25 to 75 DAS, with T5 consistently showing the highest values (39.0, 67.5, and 75.5 cm), significantly outperforming T1 and T2. Plant height increased progressively across all treatments from 25 to 75 DAS, with T5 consistently showing the highest values (39.0, 67.5, and 75.5 cm), significantly outperforming T1 and T2. Similarly, shoot density at 25 DAS was greatest in T4 and T5, suggesting enhanced

early vegetative growth under these treatments, although differences at 50 DAS were not statistically significant. Chlorophyll content also followed a similar trend, with T5 recording the highest values at both 25 and 50 DAS, indicating improved photosynthetic capacity. Treatments T3 and T4 showed intermediate performance, while T1 consistently exhibited the lowest values across most parameters. The significant F-values for most traits confirm that treatment effects were substantial, highlighting the synergistic role of bacterial inoculation and optimized fertilization in promoting maize growth.

Table 5. Influence of *H. seropedicae* inoculation and varying fertilizer ratios on plant height, tiller density, and chlorophyll content of Dai Thom 8 rice across different growth stages.

Treatment	Plant Height (cm Plant ⁻¹)			Number of Shoots per m ² (Shoots m ⁻²)		Chlorophyll (SPAD)	
	Days After Sowing (DAS)						
	25	50	75	25	50	25	50
T1	34.9 ± 2.4 ^b	51.9 ± 2.9 ^c	61.7 ± 4.8 ^c	916 ± 165 ^b	531 ± 60.7	31.8 ± 1.88 ^c	29.1 ± 1.90 ^c
T2	34.6 ± 1.5 ^b	58.4 ± 3.3 ^b	67.5 ± 4.4 ^b	779 ± 98.6 ^{ab}	503 ± 42.8	33.0 ± 2.23 ^c	31.5 ± 1.65 ^c
T3	36.9 ± 1.1 ^{ab}	64.5 ± 1.8 ^a	72.7 ± 1.5 ^{ab}	1062 ± 255 ^a	528 ± 81.4	37.0 ± 1.71 ^b	38.0 ± 2.70 ^b
T4	37.4 ± 2.5 ^{ab}	64.3 ± 1.6 ^a	74.7 ± 2.9 ^a	1178 ± 193 ^a	495 ± 61.8	39.5 ± 0.68 ^a	39.4 ± 0.58 ^{ab}
T5	39.0± 1.4 ^a	67.5 ± 3.6 ^a	75.5 ± 2.8 ^a	1143 ± 183 ^a	577 ± 18.3	40.9 ± 0.70 ^a	41.5 ± 0.10 ^a
F	**	**	**	*	ns	**	**
CV (%)	16.4	10.2	18.7	22.1	17.7	10.7	14.3

Note: Values are presented as mean ± standard deviation. Different letters within the same column indicate significant differences among treatments at $p < 0.05$. DAS: Days after sowing; ns: not significant; * and ** indicate significance at $p < 0.01$, respectively.

3.3. Effects of *H. seropedicae* Inoculation and Varying Nitrogen Fertilizer Rates on Yield Components and Overall Yield

Table 6 indicates that yield-related traits of the Dai Thom 8 rice variety were significantly affected by both *H. seropedicae* inoculation and nitrogen fertilizer application rates. The total number of grains per panicle increased substantially from T1 (63.6 grains) to T4 (113 grains), with T4 and T5 exhibiting the highest values and no significant difference between them. A similar pattern was observed for the number of filled grains, where T4 recorded the highest value (97.6 grains), followed by T5 (93.2 grains), suggesting that the combined application of bacterial inoculation and appropriate nitrogen levels enhanced reproductive development and grain formation. In contrast, T1 consistently showed the lowest values across all measured parameters, reflecting the limited nutrient availability in the absence of optimized fertilization. Notably, the number of unfilled grains was highest in T3 (19.0 grains), indicating that suboptimal or imbalanced nitrogen application may negatively affect grain filling efficiency. Overall, these findings suggest that the improvement in yield components was primarily associated with an increase in total and filled grain numbers under integrated nutrient management strategies.

Table 7 shows that yield components and grain yield of Dai Thom 8 rice were significantly influenced by *H. seropedicae* inoculation and nitrogen fertilizer rates ($p < 0.01$). The 1000-grain weight increased progressively from T1 (23.2 g) to T4 (24.5 g), with treatments T3–T5 belonging to the highest statistical group, indicating enhanced grain filling under combined application. A comparable pattern was observed for grain weight per 5 m², where T5 reached the maximum value (2.86 kg) and was significantly higher than T1 and T2. Biomass accumulation was most pronounced in T4 (6.42 kg per 5 m²; 12.8 t ha⁻¹), reflecting improved vegetative growth. Regarding grain yield, T5 achieved the highest productivity (5.72 t ha⁻¹), followed by T3 and T4, all of which were significantly greater than the control treatment T1 (3.90 t ha⁻¹). Overall, the integration of bacterial inoculation

with appropriate nitrogen application markedly enhanced both yield components and final grain yield compared to the control.

Table 6. Influence of *H. seropedicae* inoculation and N fertilizer rates on yield-related traits of Dai Thom 8 rice.

Treatment	Yield-Related Traits		
	Total Grains	Filled Grains	Total Unfilled Grains
	(Grains Panicle ⁻¹)		
T1	63.6 ± 6.62 ^c	54.3 ± 7.50 ^c	9.30 ± 1.37 ^c
T2	88.2 ± 10.3 ^b	76.6 ± 11.9 ^b	11.7 ± 2.03 ^{bc}
T3	105 ± 17.6 ^{ab}	86.1 ± 13.0 ^{ab}	19.0 ± 5.29 ^a
T4	113 ± 13.9 ^a	97.6 ± 12.6 ^a	15.4 ± 2.74 ^{ab}
T5	110 ± 6.30 ^a	93.2 ± 11.7 ^{ab}	16.3 ± 5.96 ^{ab}
F	**	**	*
CV (%)	22.3	23.1	14.6

Note: significant differences among treatments at $p < 0.05$. Different letters within the same column indicate significant differences among treatments at $p < 0.05$. ns: not significant; * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

Table 7. Effects of *H. seropedicae* inoculation and nitrogen fertilizer rates on yield components and grain yield of Dai Thom 8 rice.

Treatment	1000-Grain Weight	Grain Weight per 5 m ²	Biomass Weight per 5 m ²	Biomass Yield	Grain Yield
	(g)	(kg)	kg	(t ha ⁻¹)	(t ha ⁻¹)
T1	23.2 ± 0.355 ^b	1.95 ± 0.219 ^b	3.20 ± 0.61 ^c	6.41 ± 1.22 ^c	3.90 ± 0.438 ^c
T2	23.5 ± 0.622 ^b	2.09 ± 0.458 ^b	3.74 ± 1.15 ^b	7.48 ± 2.29 ^b	4.18 ± 0.915 ^b
T3	24.1 ± 0.126 ^a	2.65 ± 0.261 ^a	3.72 ± 0.54 ^b	7.44 ± 1.07 ^b	5.29 ± 0.523 ^a
T4	24.5 ± 0.050 ^a	2.58 ± 0.260 ^a	6.42 ± 1.40 ^a	12.8 ± 2.81 ^a	5.16 ± 0.520 ^a
T5	24.2 ± 0.190 ^a	2.86 ± 0.213 ^a	6.14 ± 0.72 ^a	12.3 ± 1.46 ^a	5.72 ± 0.426 ^a
F	**	**	**	**	**
CV (%)	12.3	18.2	14.9	14.9	18.2

Note: significant differences among treatments at $p < 0.05$. Different letters within the same column indicate significant differences among treatments at $p < 0.05$. ns: not significant; ** indicate significance at $p < 0.01$, respectively.

3.4. Impact of *H. seropedicae* Inoculation and Fertilizer Application Rates on the Nutrient Composition of Dai Thom 8 Rice

Table 8 indicates that nutrient composition of Dai Thom 8 rice was differentially affected by *H. seropedicae* inoculation and fertilizer ratios. Moisture and lipid contents showed no significant differences among treatments, remaining relatively stable across all treatments. In contrast, protein content was significantly influenced ($p < 0.01$), with T3, T4, and T5 exhibiting the highest values (8.33%), clearly surpassing T1 (7.28%) and T2 (7.58%). This suggests that combined treatments enhanced nitrogen assimilation into grain protein. Amylose content also differed significantly, with T1, T3, T4, and T5 maintaining higher levels (18.2%), whereas T2 showed a reduced value (17.4%). Conversely, amylopectin content displayed an inverse pattern, with T2 reaching the highest proportion (82.6%), while other treatments remained at 81.8%. Overall, treatments integrating bacterial inoculation with appropriate fertilization improved protein accumulation without negatively affecting grain starch composition.

Table 8. Influence of *H. seropedicae* inoculation and fertilizer ratios on the nutrient composition of Dai Thom 8 rice.

Treatment	Moisture	Lipid	Protein	Amylose	Amylopectin
	(%)				
T1	13.3 ± 0.485	0.775 ± 0.171	7.28 ± 0.150 ^c	18.2 ± 0.050 ^a	81.8 ± 0.05 ^c
T2	13.3 ± 0.465	0.915 ± 0.208	7.58 ± 0.222 ^b	17.4 ± 0.350 ^b	82.6 ± 0.346 ^b
T3	13.0 ± 0.485	0.950 ± 0.129	8.33 ± 0.095 ^a	18.2 ± 0.294 ^a	81.8 ± 0.294 ^a
T4	13.1 ± 0.465	0.952 ± 0.208	8.33 ± 0.206 ^a	18.2 ± 0.378 ^a	81.8 ± 0.378 ^a
T5	13.1 ± 0.436	0.951 ± 0.208	8.33 ± 0.206 ^a	18.2 ± 0.378 ^a	81.8 ± 0.378 ^a
F	ns	ns	**	**	**
CV (%)	13.2	19.8	16.1	12.4	10.5

Note: significant differences among treatments at $p < 0.05$. Different letters within the same column indicate significant differences among treatments at $p < 0.05$. ns: not significant; ** indicate significance at $p < 0.01$, respectively.

4. Discussion

4.1. Impact of *H. seropedicae* Inoculation and Fertilization Regimes on Soil Properties

The observed improvements in soil fertility under bacterial inoculation treatments are consistent with previous studies highlighting the role of plant growth-promoting bacteria in enhancing nutrient cycling and soil organic matter accumulation [42,43]. For instance, inoculation with nitrogen-fixing bacteria has been reported to increase soil nitrogen availability and microbial activity, thereby improving soil quality and crop performance [44]. Similarly, reductions in chemical nitrogen inputs combined with beneficial microbes have been shown to mitigate soil degradation and enhance nutrient use efficiency [10,45,46]. Excessive nitrogen fertilization, on the other hand, may accelerate organic matter mineralization and lead to nutrient imbalance, ultimately reducing soil fertility over time. Therefore, the integration of microbial inoculants with reduced nitrogen application represents a sustainable strategy for maintaining soil health and improving agricultural productivity [47,48].

4.2. Impact of *H. seropedicae* Inoculation and Nitrogen Fertilizer Ratios on the Growth of Dai Thom 8 Rice

The superior performance observed in T5 may be attributed to the combined effects of *H. seropedicae* and balanced fertilizer application, which likely enhanced nutrient availability and uptake efficiency [49]. Previous studies have shown that endophytic diazotrophic bacteria such as *H. seropedicae* can fix atmospheric nitrogen and produce phytohormones, thereby stimulating plant growth and chlorophyll synthesis [50,51]. The increased chlorophyll content in T5 supports the notion of improved nitrogen assimilation, which is closely linked to chlorophyll formation and photosynthetic activity. Moreover, the higher shoot density in treatments with bacterial inoculation aligns with findings by Dang and Chuong [10], who reported that plant growth-promoting bacteria enhance root development and tillering. The non-significant differences in shoot number at later stages suggest that environmental or physiological factors may limit further differentiation [52]. Overall, the results confirm that integrating beneficial microbes with appropriate fertilizer regimes can significantly improve maize growth performance.

4.3. Impact of *H. seropedicae* Inoculation and Different N Fertilizer Rates on Yield Components and Grain Yield

The observed enhancement in grain number and filled grains under combined inoculation and nitrogen fertilization aligns with previous findings that plant growth-promoting bacteria such as *H. seropedicae* can enhance nutrient uptake and improve reproductive development in cereals [26,53]. Studies have shown that endophytic diazotrophs contribute to biological nitrogen fixation and phytohormone production, thereby stimulating panicle

development and grain formation [10,54]. The superior performance of T4 suggests that an optimal nitrogen level combined with bacterial inoculation creates a synergistic effect, maximizing yield components. However, the higher unfilled grain number in T3 may indicate that excessive nitrogen can lead to source–sink imbalance, as reported by Teng et al. [55], reducing grain filling efficiency. The non-significant variation in filled grain ratio further supports that yield improvement primarily depends on sink size expansion rather than partitioning efficiency. These results highlight the importance of optimizing fertilizer rates when integrating microbial inoculants for sustainable rice production [51,56].

The improvement in yield and its components observed in this study is consistent with previous reports highlighting the role of *H. seropedicae* as a plant growth-promoting bacterium. This endophytic diazotroph enhances nitrogen availability through biological fixation and stimulates plant growth via phytohormone production, thereby increasing grain weight and overall yield [48,50]. The superior performance of T5 indicates that optimizing nitrogen fertilization in combination with microbial inoculation can maximize both biomass accumulation and grain production [43,45,57]. Similar findings were reported by Chuong et al. [26], who noted that balanced nitrogen supply improves photosynthetic capacity and assimilate partitioning to grains. The significant increase in biomass in T4 and T5 also suggests enhanced nutrient uptake efficiency and canopy development. These results reinforce the concept that integrating beneficial microorganisms with reduced or optimized fertilizer inputs is a promising strategy for sustainable rice production and improved resource-use efficiency [10,43,58].

4.4. Influence of *H. seropedicae* and N Fertilizer Ratios on the Nutrient Composition of Dai Thom 8 Rice

The increase in grain protein content under combined *H. seropedicae* inoculation and optimized fertilizer regimes is consistent with previous studies demonstrating that endophytic diazotrophic bacteria enhance nitrogen uptake and assimilation in rice [53,59]. Such bacteria can fix atmospheric nitrogen and stimulate plant metabolism through phytohormone production, leading to increased protein synthesis in grains [50,60]. The stability of moisture and lipid content suggests that these traits are less responsive to microbial and nutrient management, as also reported by Cao et al. [61]. The variation in amylose and amylopectin proportions reflects changes in carbohydrate metabolism influenced by nitrogen availability, where adequate nitrogen promotes balanced starch biosynthesis [62]. The slight reduction in amylose in T2 may indicate suboptimal nutrient interaction. Overall, these findings highlight that integrating beneficial microbes with appropriate fertilization strategies can improve grain nutritional quality while maintaining desirable physicochemical properties for rice consumption.

5. Conclusions

This study demonstrates that *H. seropedicae* inoculation combined with optimized nitrogen fertilization significantly improves soil fertility, rice growth, yield, and grain quality of Dai Thom 8 under field conditions. A key novelty of this research lies in the successful application of a locally isolated endophytic diazotrophic bacterium under practical field conditions, highlighting its potential to partially replace inorganic nitrogen fertilizers while maintaining high productivity. Among the treatments, T5 achieved the highest grain yield (5.72 t ha^{-1}), whereas T3 and T4 also showed comparable performance with reduced nitrogen inputs (50% and 25% reduction, respectively). Notably, T3 represents the most economically efficient option, as it maintains high yield while reducing nitrogen fertilizer by 50%, thereby lowering input costs and environmental risks. Therefore, T3

is recommended for farmers as a sustainable and cost-effective practice that balances productivity and resource-use efficiency in rice cultivation.

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