Asian Journal of Agriculture and Biology
https://doi.org/10.35495/ajab.2025.193

Original Article

Nano nitrogen application enhances the physiological response of rice leaves under different soil types

Nurwulan Agustiani[1], Sujinah[1*], Etty Pratiwi[1], Wiwik Hartatik[1*], Antonius Kasno[1], Chendy Tafakresnanto[1], A. Arivin Rivaie[1*], Terry Ayu Adriany[1], Selly Salma[1], Antonius Fransisco Nababan[2], Fasa Aditya Hanindipto[3], Yudhistira Nugraha[1]
[1]Research Center for Food Crops, Research Organization Agriculture and Food, National Research and Innovation Agency, KST Soekarno, Cibinong, West Java, Indonesia
[2]Directorate for Laboratory Management, Research Facilities and Science and Technology Park, National Research and Innovation Agency, KST Soekarno, Cibinong, West Java, Indonesia
[3]Indonesian Fertilizer Holding Company, Jakarta, Indonesia

**Corresponding author's email:* sujinah@brin.go.id; wiwik_hartatik@yahoo.com; aari001@brin.go.id
Received: 02 August 2025 / Revised: 28 November 2025 / Accepted: 16 December 2025 / Published Online: 21 January 2026

Abstract

Nano-nitrogen (nano-N) fertilizers have been developed to boost the efficiency and lessen the environmental footprint of conventional nitrogen (N) fertilizers in lowland rice cultivation. This study examined the impact of combining nano-N with existing N fertilization on rice leaf physiology. A greenhouse experiment tested five concentrations of nano-N (0 to 4 mL L^{-1}) alongside a one-third reduction in the standard N rates across Aeric Epiaquents, Vertic Endoaquepts, and Typic Endoaquepts. Observations were made primarily on leaf dry weight, leaf area, chlorophyll content, photosynthesis rate, dissolved sugar levels, nitrate reductase activity (NRA), and glutamine synthase activity. The results showed that nano-N application increased chlorophyll a and b levels and enhanced NRA even with a 50% reduction in top-dressed nitrogen, equivalent to 46 kg of urea. In particular, the 2 mL L^{-1} nano-N treatment resulted in 34% higher starch and 28% more total non-structural carbohydrates than farmer-practice fertilization, driven by increased chlorophyll at panicle initiation and elevated NRA during grain filling. These findings suggest that nano-N fertilization can increase chlorophyll content, enzyme activity, and carbohydrate accumulation in rice leaves, providing a promising approach to improve nitrogen use efficiency, reduce environmental risks linked to overfertilization with N, and enhance yields through improved plant physiological processes.

Keywords: Nano-nitrogen, Non-structural carbohydrate, Physiological traits of leaves, Rice plant, Soil types

How to cite this article:

Agustiani N, Sujinah, Pratiwi E, Hartatik W, Kasno A, Tafakresnanto C, Rivaie AA, Adriany TA, Salma S, Nababan AF, Hanindipto FA and Nugraha Y. Nano nitrogen application enhances the physiological response of rice leaves under different soil types. Asian J. Agric. Biol. 2026: e2025193. DOI: https://doi.org/10.35495/ajab.2025.193

Introduction

Nitrogen (N) is a fundamental macronutrient for the growth and productivity of rice, one of the world's most important staple crops. Low soil N availability cannot sustain the increasing demand for rice yield, making N fertilization essential in most rice-growing systems. Rice requires N in large quantities, and it is often the most limiting factor in determining crop productivity. The application of N fertilizer significantly increases grain yield, depending on N rate, water condition, timing, and other management factors (Zhao et al., 2022; Proud et al., 2023; Hu et al., 2024). Farmers commonly apply large amounts of N fertilizer because soil N alone is insufficient to meet crop requirements.

Plant physiological processes are strongly influenced by N availability. In rice, adequate N enhances photosynthetic performance and sugar metabolism (Cao et al., 2025). Chlorophyll a, chlorophyll b, and carotenoid concentrations increase with higher N application at booting, heading, and maturity stages, supporting light capture and enhancing photosynthetic rate (Peng et al., 2021; Qi et al., 2025). Nitrogen deficiency, by contrast, accelerates pigment degradation, leading to leaf yellowing, reduced photosynthesis, and impaired growth (Chen et al., 2022). N also improves Photosystem II (PSII) activity and photochemical efficiency, thereby promoting high yield potential (Peng et al., 2021). Nitrogen availability, especially in the nitrate (NO_3^-) form, regulates NO_3^- reductase (NR) activity, a key enzyme in N assimilation. NR catalyzes the reduction of nitrate (NO_3^-) to nitrite (NO_2^-), initiating the pathway toward ammonium (NH_4^+) assimilation. Rice generally shows higher NR activity under NO_3^- nutrition, as NO_3^- directly induces NR gene expression (Handoyo et al., 2021). Partial substitution of NH_4^+ with NO_3^- has been shown to enhance NR activity, improve N uptake, increase biomass accumulation, and strengthen overall growth. Environmental conditions such as soil aeration strongly influence the relative availability of NH_4^+ and NO_3^-, making NR particularly important in soil systems where aerobic and anaerobic phases alternate (Duan et al., 2006).

Nitrogen also regulates glutamine synthetase (GS), the central enzyme responsible for assimilating NH_4^+ into glutamine. GS activity responds dynamically to N status, tissue type, and developmental stage. Under N deficiency, GS2 activity increases in rice roots as a compensatory mechanism to maintain assimilation (Liang et al., 2021). Therefore, optimizing GS activity is therefore essential for improving nitrogen use efficiency (NUE) and reducing environmental losses. At the metabolic level, N further affects key enzymes involved in sucrose and starch synthesis, demonstrating the interconnectedness of N availability, carbohydrate metabolism, and yield formation (Hu et al., 2024).

Recent advances have focused on nano-nitrogen (nano-N) fertilizers to address inefficiencies associated with conventional N inputs. Nano-formulations are designed for controlled and sustained nutrient release, improved NUE, and reduced losses via volatilization or leaching (Hegab et al., 2018). Nano-N can influence enzyme activities, protein synthesis, NO_3^- signaling, and the carbon–nitrogen balance at both physiological and molecular levels (Pathak et al., 2008). However, despite these promising characteristics, the performance of nano-N may vary across soil environments because soil physicochemical properties strongly affect nutrient retention, transformation, and plant uptake. Leaf physiological traits are directly linked to rice yield because leaves serve as the primary organs responsible for photosynthesis, transpiration, and temporary storage of assimilates before translocation to reproductive tissues. Enhancing leaf photosynthetic potential remains a key strategy to improve C_3 crop yield, including rice (Xiong et al., 2015; Ouyang et al., 2022). Soil characteristics such as pH, texture, organic carbon (C), cation-exchange capacity (CEC), and mineralogy strongly influence N dynamics and optimal N fertilizer management. Proper nutrient management can increase the efficiency of N fertilizer use in rice fields, typically leading to a 40-50% reduction in the amount applied (Aleminew et al., 2020; Lee, 2021). Therefore, rice grown in different soil types may exhibit different responses to the same N fertilizer input, including nano-N. However, information on nano-N behavior and its physiological effects across contrasting Indonesian paddy soils remains extremely limited.

Although nano-N fertilizers have been increasingly explored for improving N use efficiency, most existing studies are confined to single soil environments and rarely consider how soil physicochemical differences shape plant responses to nano-N. Limited attention has been given to how soil texture, mineralogy, organic C content, and CEC modulate nano-N performance, despite their central role in determining N retention and assimilation pathways (Ye et al., 2024). Moreover, only few studies have tested whether nano-N can offset reduced conventional N inputs while sustaining physiological processes such as chlorophyll formation, nitrate reductase activity (NRA), and non-structural carbohydrate (NSC) accumulation (Gondwal et al., 2024; Kumar et al., 2024; Liu et al., 2024).

To address these gaps, the present study evaluates the combined effects of nano-N and reduced N fertilization across three distinct paddy soil types in three Indonesian main rice producing areas, namely Aeric Epiaquents (Kediri, East Java), Vertic Endoaquepts (Ngawi, East Java), and Typic Endoaquepts (Subang, West Java) to determine how soil-specific properties influence leaf physiological responses and N metabolism. It is expected that these three soil types represent meaningful variability for nitrogen response because they have several quite different soil properties, such as organic C and CEC. This study provides the first comparative evidence of nano-N performance across diverse Indonesian paddy soils and identifies soil-dependent optimal nano-N dosages for improving N assimilation and leaf physiological functioning. In these regards, this study aimed to assesses the physiological responses of rice leaves to combined nano-N and conventional N fertilization and evaluates the key variables contributing to enhanced photosynthetic and metabolic activity across different soil types. The outcomes are expected to support more efficient and sustainable N management strategies tailored to soil-specific conditions.

Materials and Methods

Study site and design of the experimental

The research was conducted using a pot experiment in the greenhouse of the Indonesian Soil Research Institute (ISRI), Bogor (6°34'32" S, 106°45'15" E), from September 2023 to March 2024, using three soil types collected from paddy fields. Before starting the trials, the bulk samples of paddy field soils (depth 0–20 cm) were collected from three different locations to determine selected soil properties. The three soil types used were Aeric Epiaquents (Kediri, East Java - 07°50’33” S; 112°06’10” E), Vertic Endoaquepts (Ngawi, East Java - 07°25’13” S; 111°07’52” E), and Typic Endoaquepts (Subang, West Java - 06°20’26” S; 107°39’00” E) (Figure 1). This N fertilization response experiment employed a fully randomized scheme (CRD); each treatment was repeated three times. Nitrogen treatment consisted of five levels of nano-N fertilizer (0, 1, 2, 3, and 4 mL L^{-1}) with a reduction of 50% of the top-dressing dosage, following the previous studies (Wang et al., 2023; Gondwal et al., 2024; Kumar et al., 2024). The standard treatment was 100% common N (urea, 46% N) according to soil nutrient status. N was applied three times, while nano-N was sprayed on the stems and leaves according to the treatment dosage. The characteristics of the nano-N fertilizer revealed that 48% of the particles were 11-13 nm in diameter, and 52% were 80-93 nm in diameter, with a total N content of 4.9%. Zeta potential is 32.4 mV, and pH is 4.9. P and K fertilizers were applied to all treatments, followed by soil nutrient status. The arrangement of treatments and fertilizer application dosage is listed in Table 1. After air drying, soil samples from the three locations (Kediri, Ngawi, and Subang) were ground finely and filtered using a 5 mm sieve. Then the filtered soil is weighed and put into black plastic pots, each weighing 8 kg. Water was added to the soil to a depth of 2-4 cm, then it was stirred and allowed to settle for 1 hour weekly. The rice variety used, Inpari 48, was transplanted into three seedling pots 14 days after sowing (DAS).

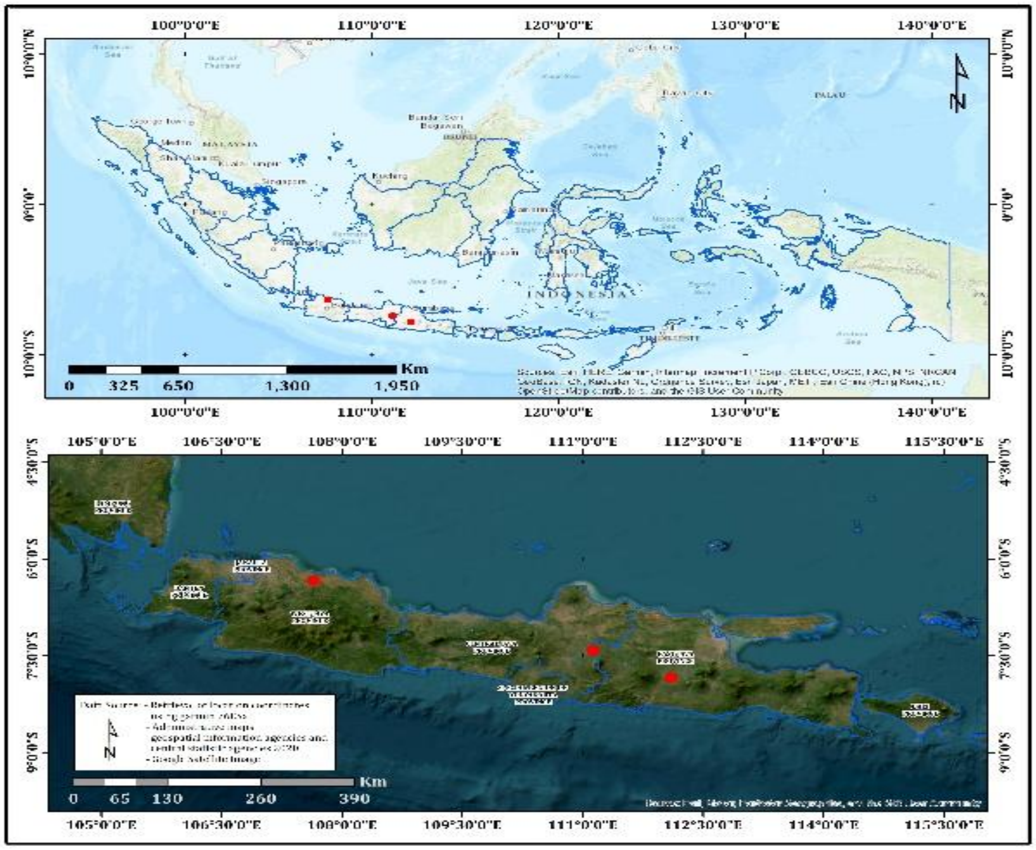

Figure-1. Origin of the soil treatment in Java Island, Indonesia.

Table-1. Treatment arrangement and fertilizer dosage.

Code	Treatment	Urea (kg ha^{-1})			Nano-N (L ha^{-1})	Total N* (kg ha^{-1})
		Base	TD 1	TD 2		
P1	Standard	100	100	100	0	138.0
P2	Basal N + 50% TD	100	50	50	0	92.0
P3	P2 + nano-N 1	100	50	50	0.5	94.5
P4	P2 + nano-N 2	100	50	50	1.0	96.9
P5	P2 + nano-N 3	100	50	50	1.5	99.4
P6	P2 + nano-N 4	100	50	50	2.0	101.8

P and K fertilizers based on soil status were applied in the form of SP-36 (50 kg ha^{-1}) and KCl (100 kg ha^{-1}).
TD: top dressing
* Calculation of total Nitrogen (N) applied in one rice planting period (Urea 46% N and Nano N 4.9% N)

Soil sampling and analysis

Each soil sample underwent air-drying, grinding, and sieving using a 2 mm diameter sieve before being dispatched to the soil laboratory of the ISRI to determine the mineralogical composition of the fine sand fractions and soil chemical properties. Crop residues, gravel, and rocks from soil samples were discarded at a depth of 0-20 cm. The samples were air-dried, crushed with a mortar, and then sieved with a 2 mm diameter sieve, including visible crop residue. Next, the soil samples were sent to a soil laboratory for analysis of the total sand fraction's mineral composition (50-500 μm) using a polarization microscope. The minerals were identified through the line-counting method. Data from sand mineral analysis is grouped according to the similarity of mineral types to determine the source and nature of the parent material, as well as the amount of easily weathered mineral reserves as a source of nutrients in the soil. Types and percentages of easily weathered minerals follow the method proposed by Buurman (1990).

In addition, some other soil samples were analyzed for N total and NH_4^+ (Blakemore et al., 1987) and NO_3^- (Keeney and Nelson, 1982). Soil pH was analyzed using water extraction with a ratio of 1:5 (soil/water). Soil suspensions were blended and kept at a controlled temperature of 20±2°C overnight to ensure accurate measurements. pH values were then measured using a pH meter according to the described method. Samples were heated in a Leco furnace to determine the percentage of carbon, indicating soil organic matter content. A high-quality oxygen stream was introduced to convert organic matter to carbon dioxide (CO_2) during this process. The infrared detector measured the amount of CO_2, which was used to calculate the total organic C content. Exchangeable cations and CEC were measured by leaching with ammonium acetate at pH 7. The amounts of potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) in the leachates were analyzed using atomic absorption spectrometry (AAS) (Blakemore et al., 1987).

Physiological characteristics of leave

The collected physiological data includes leaf dry weight, leaf area, chlorophyll content, photosynthesis rate, dissolved sugar content (sugar and starch), NRA, and GS. For this study, a LI-3000C Portable Leaf Area Meter was used to measure leaf area, and then oven-dried the biomass at 70°C until it reached a constant dry weight.

The chlorophyll content

The chlorophyll content, which includes chlorophyll a, b, carotenoids, and total chlorophyll, was extracted using 95% ethanol, according to Lichtenthaler (1987). About 0.05 grams of fresh leaves were cut into small pieces, added to the liquid N, and then ground. The sample was placed into a tube, immersed in 5 mL of ethanol, and then vortexed and centrifuged at 10,000 rpm for 4 minutes. The spectrophotometer was used to measure the absorption of the supernatant at 665, 649, and 480 nm. The analysis results were calculated using the following equation:

$$\mathbf{Chlorophyll\ a = 13.36 * A_{665} - 5.19 * A_{649}}$$

$$\mathbf{Chlorophyll\ b = 27.43 * A_{649} - 8.12 * A_{665}}$$

$$\mathbf{Chlorophyll\ total = 5.24 * A_{665} + 22.24 * A_{649}}$$

$$\mathbf{Carotenoid = \frac{1000 * A_{480} - 2.13 * Chl\ a - 97.64 * Chl\ b}{209}}$$

Sugar analysis

Sugar extraction was performed by heating 0.05 g of ground rice grain with 80% ethanol in a water bath (80–85°C) followed by centrifugation. The extraction was repeated three times, and all supernatants were combined and evaporated to approximately 2 mL. The concentrate was transferred to a 25 mL volumetric flask and brought to volume with distilled water to obtain the sugar extract. A glucose standard curve (0-20 ppm) was prepared from a 20-ppm stock solution. Anthrone reagent was prepared by dissolving anthrone in concentrated sulfuric acid (H_2SO_4). For color development, 2.5 mL of each standard or sample extract was mixed with 5 mL anthrone reagent in an ice bath, heated in a water bath for 7.5 min, then cooled. Absorbance was measured at 630 nm using a UV-VIS spectrophotometer following the Anthrone-Sulfuric Acid method of Yoshida et al. (1976).

Starch analysis

Following sugar extraction, the residual pellet was dried at 80°C and hydrolyzed sequentially with distilled water and perchloric acid ($HClO_4$) (9.2 N followed by 4.6 N). Supernatants from both extraction steps were combined and diluted to 50 mL to obtain the starch extract. A 0-20 ppm glucose calibration series was prepared in 0.46 N $HClO_4$. Anthrone reagent was prepared in concentrated H_2SO_4. For colorimetric analysis, 2.5 mL of each standard or starch extract was mixed with 5 mL of anthrone reagent in an ice bath, heated for 7.5 min, cooled, and measured at 630 nm using a UV-VIS spectrophotometer (Yoshida et al., 1976).

The calculation of sugar and starch contents was made as per the following equation:

$$\mathbf{Sugar\ (\%) = ppm\ curve\ x\ \frac{ml\ extract}{1000\ mL}\ x\ \frac{100\ g}{mg\ sample}\ x\ fk\ x\ fp\ x\ 0.9}$$

$$\mathbf{Starch\ (\%) = ppm\ x\ \frac{ml\ extract}{1000\ mL}\ x\ \frac{100\ g}{mg\ sample}\ x\ fp\ x\ fk}$$

Nitrate reductase activity (NRA)

NR analysis was conducted based on Moro et al. (2017) and Singh et al. (2019). Leaf samples in their mature stage were collected and placed in a test tube with a solution of 2% 1-propanol, 100 mM KH_2PO_4 (pH 7.5), and 30 mM KNO_3. The samples were then incubated under vacuum for 5 minutes, followed by incubation in a shaking water bath at 25°C for 30 minutes in the dark. Next, 1 mL of the sample extract was transferred to a new tube, where 1 mL of sulphanilamide (1% w/v in 1.5 M HCl) and 1 mL of N-(1-naphthyl)-ethylenediamine dihydrochloride (0.02% w/v in 0.2 M HCl) were added. The mixture was incubated at room temperature for 30 minutes. The samples were analyzed using a spectrophotometer at a wavelength of 540 nm.

Glutamine synthase (GS)

Leaf tissue was homogenized in extraction buffer (Tris-HCl, sucrose, EDTA, β-mercaptoethanol, KCl, and $MgCl_2$) and centrifuged at 10,000 g. GS activity was assayed by incubating the supernatant with a reaction mixture containing $MgSO_4$, L-glutamate, ATP, and hydroxylamine at 40 °C for 30 min. The reaction was terminated with ferric chloride-TCA-HCl reagent, and the resulting color was measured at 540 nm. All steps were performed under chilled conditions (Rajesh et al., 2017).

Statistical analysis

The data collected were then analyzed using ANOVA, followed by the Duncan Multiple Range Test (DMRT) at a 5% significance level to identify differences between treatments. A stepwise analytical approach was used to determine the relationship between variables and improve the interpretation of the results.

Results

Soil characteristic

Kediri rice fields are developed from young volcanic material, characterized by the presence of opaque volcanic glass (Table 2). The main characteristics of the Kediri rice fields' soil are a silty clay soil texture, a slightly acidic soil reaction (pH 6.2), and exchangeable bases (Ca, Mg, and K) that vary in concentration from low to high. Kediri soil is classified as Aeric Epiaquents (Ae-ent) (Soil Survey Staff, 2022). The Kediri rice fields are classified as young soil and have not undergone weathering, which means they lack quartz minerals. Rock fragments are very numerous (40%), and minerals are easily weathered, with more than 20% being easily weathered, so mineral reserves are classified as very high. The clay minerals of Kediri rice fields are dominated by vermiculite (type 2:1), a little halloysite (type 1:1), and a little illite (type 2:1). These clay minerals do not expand or contract. By paying attention to soil texture, mineral reserves, and easily weathered minerals, it is evident that the Kediri rice fields are considered young soils with very high mineral reserves.

The soil of the Ngawi rice fields is derived from volcanic material, characterized by the presence of opaque volcanic glass and numerous labradorite (feldspar) minerals with a clay-like texture (Table 2). The main characteristics of Ngawi rice fields' soil are clay texture, slightly acidic soil reaction (pH 6.2), and high exchangeable bases (Ca, Mg, and K) (Table 3). Ngawi soil is classified into Vertic Endoaquepts (Vep-ept) (Soil Survey Staff, 2022). The Ngawi rice fields have not been further weathered and are characterized by the presence of 5% quartz minerals. There are relatively many rock fragments (24%) and easily weathered minerals >10%, so mineral reserves are classified as very high. The clay minerals in the Ngawi rice fields are dominated by chlorite (type 2:1:1), which is composed of layers covered with layers of octahedra, and substitution generally occurs for Al by Si in the tetrahedral, resulting in a negative charge. Additionally, there is a small amount of halloysite clay mineral (a 1:1 type). These clay minerals do not expand or contract. By paying attention to the soil texture, mineral reserves, and easily weathered minerals, Ngawi's rice fields, in terms of land resources, are classified as very fertile.

Table-2. Mineral composition of the sand fraction under three different soil types in the research area.

Location	Opaque	Cloudy quartz	Clear Quartz	Iron Concretion	Organic SiO_2	Zeolite	Weathering Minerals	Rock Fragments	Volkan Glass	Andisin	Labradorite	Bitownit	Green Hornblende	Augite	Hypersthene	Epidote	Tourmaline
Kediri (Ae-ent)	10			4			2	40	1		3	7	3	7	20	1	2
Ngawi (Vep-ept)	2	4	1	nd	nd	1	3	24	3	1	42	1	5	7	2	2	3
Subang (Tep-ept)	8	53	4	1	7	2	6	11	2			1	1	4	2	nd	

Subang's rice fields are classified as Typic Endoaquepts (Tep-ept) (Soil Survey Staff, 2022). Soil materials come from alluvial deposits, which are characterized by the presence of opaque and cloudy quartz (Table 2). The role of these minerals is very important; apart from being a source of nutrients, they also play a role in determining soil load. It is developed from alluvial deposits with a silty clay loam texture. The main characteristics of the Subang rice fields' soil are silty clay loam soil texture, acidic soil reaction (pH 4.5), and low to moderate exchangeable bases (Ca, Mg, and K) (Table 3). Subang's rice fields have been further weathered and are characterized by sand minerals dominated by quartz (53%). Approximately 11% of rock fragments remain, and easily weathered minerals account for more than 5%, indicating that mineral reserves are considered sufficient. The Subang rice fields are characterized by a mixture of clay minerals, predominantly kaolinite (type 1:1) and vermiculite (type 2:1). These clay minerals do not expand or contract. Considering the soil texture, mineral reserves, and the proportion of easily weathered minerals, Subang’s rice fields can be classified as fertile in terms of land resources.

Table-3. Chemical soil properties under three different soil types in the research area.

Soil properties	Unit	Kediri (Ae-ent)	Ngawi (Vep-ept)	Subang (Tep-ept)
Texture				
Sand	%	27	6	2
Silt	%	63	30	63
Clay	%	10	64	35
pH (H_2O)		6.2	6.2	4.5
C-organic	%	0.69	3.29	1.83
N total	%	0.10	0.26	0.15
C/N ratio		7	13	12
P Olsen / Bray I	mg kg^{-1}	118 *	253 *	2.5 **
P_2O_5 HCl	mg 100 g^{-1}	101	162	63
K_2O	mg 100 g^{-1}	8	44	9
Cation exchange capacity (CEC)	$cmol_{(+)}kg^{-1}$	9.28	47.72	13.31
Ca	$cmol_{(+)}kg^{-1}$	5.38	25.82	3.87
Mg	$cmol_{(+)}kg^{-1}$	1.83	12.92	1.07
K	$cmol_{(+)}kg^{-1}$	0.16	0.86	0.16
Na	$cmol_{(+)}kg^{-1}$	0.31	0.79	0.60
Base saturation	%	83	85	43
Fe	mg kg^{-1}	66	179	374
Mn	mg kg^{-1}	30	256	67
Zn	mg kg^{-1}	4	6	2
Cu	mg kg^{-1}	2	2	4

*: P Olsen; **: P Bray I.

Note: Ae-ent = Aeric Epiaquents; Vep-ept = Vertic Endoaquepts; Tep-ept = Typic Endoaquepts.

The difference between the three types of soil lies in their organic C content, which is very low in the other two types but high in Vep-ept soil. In the present study, although the soil pH in Ae-ent and Vep-ept was the same, the organic C in Vep-ept was much greater than in Ae-ent. However, the total N in this soil was also greater than in the other two types of soil. Furthermore, CEC, which is the ability of soil to store and release nutrients, was high in Vep-ept, while the others were low (Fig. 2).

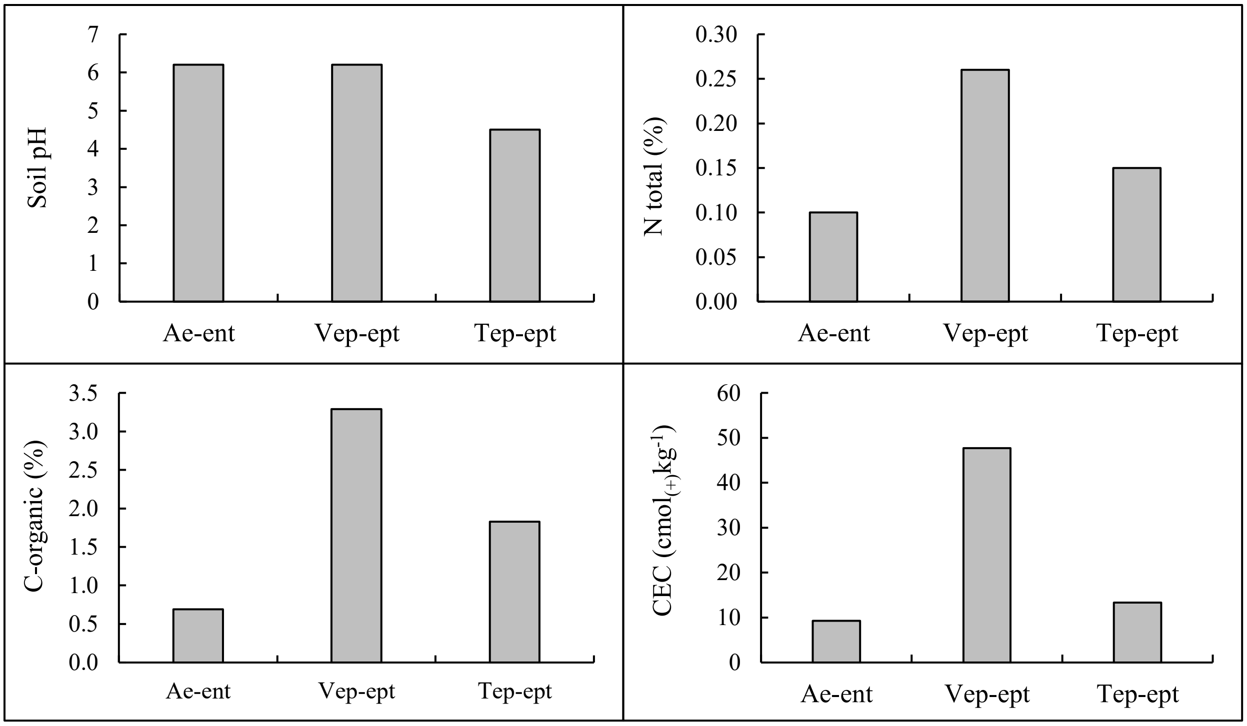

Figure-2. The key parameters of soil characteristics of Indonesia three main rice producing areas.

Physiological characteristics of leaves under different soil types

Soil types exhibit varying abilities to support physiological activities in rice leaves. The highest leaf dry weight and leaf area were observed in the Vep-ept soil type. At the same time, the lowest were found in the Tep-ept soil types (Fig. 3). The dry weight of leaves on Vep-ept soil was 43% higher than on Ae-ent (Fig. 3A). Additionally, the leaf area on the Vep-ept was 3.6 and 2.4 times greater than on the Ae-ent during the heading and grain-filling phases, respectively (Fig. 3B). The number of leaves, leaf dry weight, leaf area, and chlorophyll a, b, and carotenoids content in the Vep-ept soil type were higher than in the aeric, especially during the panicle initiation phase (Table 4). Meanwhile, the number of leaves, leaf area, chlorophyll a, b, and carotenoids in the typical soil were slightly higher than in the Ae-ent. Interestingly, the Tep-ept soil showed the highest photosynthetic rate during the grain-filling phase, surpassing that of the Vep-ept soils.

https://doi.org/10.35495/ajab.2025.193

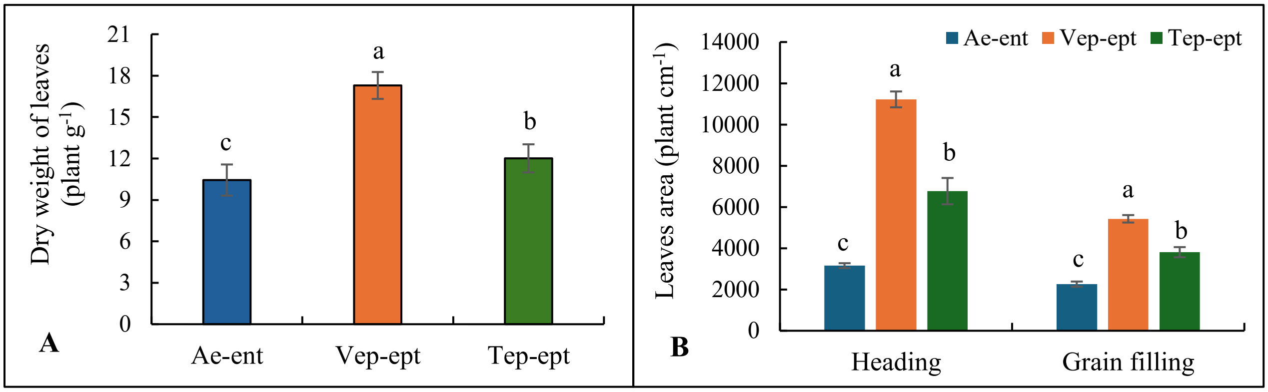

Figure-3. Dry weight of leaves (A) and leaf area (B) of Inpari 48 in different soil types.
Lowercase letters with different symbols show significant differences between treatments at $p \leq 0.05$ (DMRT). Values represent the means ± standard error.

Table-4. Chlorophyll a, b, and carotenoid (mg g^{-1}) of the Inpari 48 variety in various soil types and fertilization treatments.

No	Treatment	Vegetative			Panicle initiation		
		chl a	**chl b**	**car**	**chl a**	**chl b**	**car**
Type of soil							
S1	Ae-ent	1.34b ± 0.05	1.06b ± 0.02	0.623a ± 0.02	1.40c ± 0.07	1.14b ± 0.03	0.540c ± 0.02
S2	Vep-ept	1.34b ± 0.04	1.07b ± 0.03	0.622a ± 0.02	2.18a ± 0.09	1.28a ± 0.03	0.767a ± 0.03
S3	Tep-ept	1.51a ± 0.05	1.20a ± 0.02	0.427b ± 0.02	1.75b ±0.10	1.12b ± 0.03	0.676b ± 0.03
Fertilization							
P1	Standard	1.47a ± 0.06	1.12a ± 0.04	0.516a ± 0.05	1.74ab ± 0.13	1.16ab ± 0.04	0.660a ± 0.05
P2	Basal N + 50% TD	1.20b ± 0.07	1.02a ± 0.03	0.580a ± 0.05	1.57b ± 0.14	1.14ab ± 0.07	0.709a ± 0.05
P3	P2 + nano-N 1	1.54a ± 0.06	1.13a ± 0.03	0.558a ± 0.05	1.55b ± 0.12	1.08b ± 0.04	0.626a ± 0.04
P4	P2 + nano-N 2	1.44ab ± 0.08	1.15a ± 0.04	0.556a ± 0.04	2.05a ± 0.21	1.26ab ± 0.09	0.674a ± 0.06
P5	P2 + nano-N 3	1.40ab ± 0.07	1.11a ± 0.06	0.548a ± 0.06	2.02a ± 0.16	1.28a ± 0.04	0.672a ± 0.05
P6	P2 + nano-N 4	1.33ab ± 0.07	1.12a ± 0.04	0.589a ± 0.06	1.73ab ± 0.10	1.18ab ± 0.03	0.625a ± 0.03
Mean		1.40	1.10	0.557	1.78	1.18	0.661
CV (%)		13.59	10.76	24.95	15.91	11.58	14.66

Lowercase letters that differ significantly represent distinct treatments at $p \leq 0.05$ (DMRT). Values are means ± standard error. chl = chlorophyll, car = carotenoid.

Note: Ae-ent = Aeric Epiaquents; Vep-ept = Vertic Endoaquepts; Tep-ept = Typic Endoaquepts.

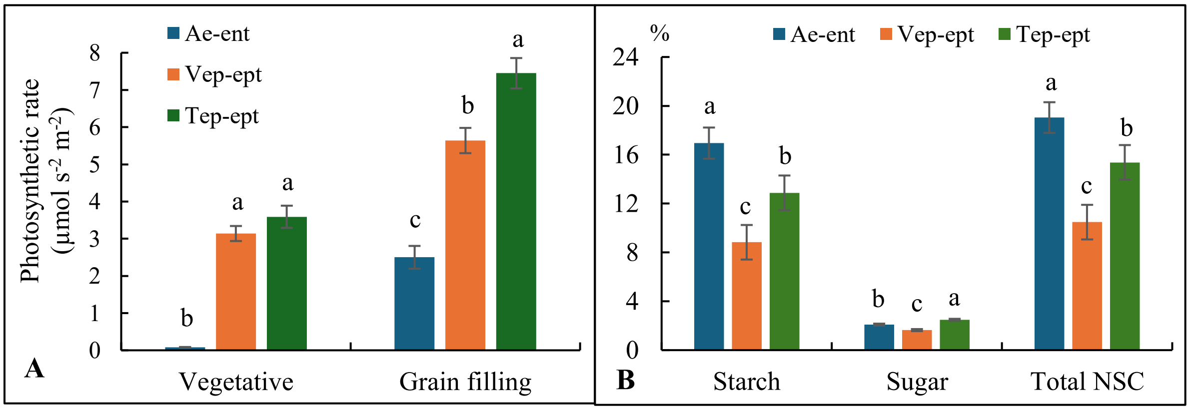

Figure-4. Photosynthetic rate (A) and starch, sugar, and total NSC (B) of Inpari 48 in different soils.
Lowercase letters show notable differences between treatments at $p \leq 0.05$ (DMRT). Values are a means ± standard error.

Table-5. Nitrate reductase activity and glutamine synthetase of the Inpari 48 variety in different soil types and fertilization.

No	Treatment	**Nitrate reductase activity (μmol NO_2^- g^{-1} h^{-1})**		**Glutamine synthetase (μmol g^{-1} min^{-1})**
		Vegetative	Grain filling	
Type of soil				
S1	Ae-ent	1.652a ± 0.24	0.664c ± 0.05	0.788b ± 0.02
S2	Vep-ept	2.395a ± 0.23	1.153b ± 0.07	0.556c ± 0.02
S3	Tep-ept	2.234a ± 0.24	2.530a ± 0.07	0.927a ± 0.03
Fertilization				
P1	Standard	2.000a ± 0.33	1.754b ± 0.36	0.789a ± 0.09
P2	Basal N + 50% TD	2.658a ± 0.37	1.202d ± 0.28	0.767a ± 0.10
P3	P2 + nano-N 1	2.054a ± 0.28	1.113d ± 0.19	0.736a ± 0.05
P4	P2 + nano-N 2	2.008a ± 0.32	1.953a ± 0.50	0.781a ± 0.09
P5	P2 + nano-N 3	1.931a ± 0.29	1.306c ± 0.13	0.733a ± 0.06
P6	P2 + nano-N 4	1.911a ± 0.21	1.364c ± 0.29	0.738a ± 0.05
Mean		2.094	1.449	0.757
CV (%)		45.06	17.92	22.90

Lowercase letters in different treatments indicate a significant difference at $p \leq 0.05$ (DMRT)

Values are a means ± standard error.

Note: Ae-ent = Aeric Epiaquents; Vep-ept = Vertic Endoaquepts; Tep-ept = Typic Endoaquepts.

At the start of growth (vegetative phase), all three soil types had similar levels of NRA. However, during the grain-filling phase, the Tep-ept soil type exhibited higher NRA and GS contents compared to the others (Table 4). The pattern of these results was similar to the trend seen in the photosynthesis rate results in Figure 3. NRA and GS are important indicators of nitrogen availability in plants. During the grain filling phase, NRA in Tep-ept soil was 2.8 and 1.2 times higher, and GS content was 18% and 67% higher than in Ae-ent and Vep-ept soils, respectively (Table 5).

Leaves' physiological character under different nano-N fertilizer levels

Fertilizer treatments did not significantly affect leaf dry weight or leaf area during heading or grain filling (Fig. 5A-B). However, nano-N clearly influenced pigment content. All nano-N treatments increased chlorophyll a and b during the vegetative and panicle-initiation stages, with nano-N 2 and nano-N 3 producing the highest chlorophyll b during panicle initiation (Table 4). Carotenoids did not differ among treatments. Photosynthetic rate did not respond to fertilization during heading or grain filling (Fig. 6A). Despite this, carbohydrate accumulation did. Nano-N 1 and especially nano-N 2 increased grain starch (13% and 34%) and total NSC (12% and 28%) relative to the standard treatment (Fig. 6B), showing that nano-N 2 consistently enhanced carbon storage. Furthermore, NRA did not differ among treatments during the vegetative phase, but clear differences appeared during grain filling (Table 6). Nano-N 2 increased NRA by 11% compared with the standard treatment, while nano-N 1, 3, and 4 reduced it. GS remained unchanged across treatments, indicating that replacing 50% of the top dressing with nano-N did not reduce GS activity.

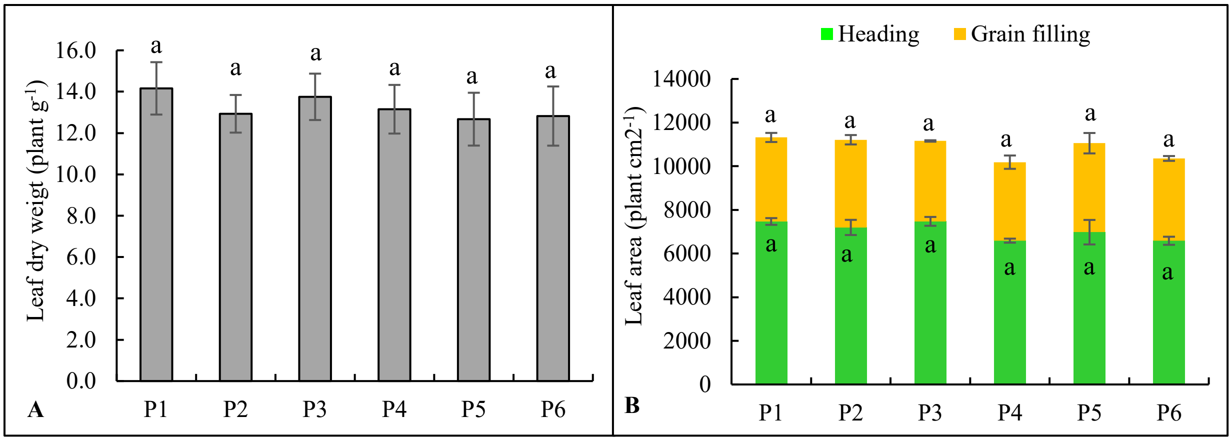

Figure-5. Dry weight of leaves (A) and leaf area (B) of Inpari 48 in different fertilization treatments.
Lowercase letters in different fonts highlight notable differences between treatments at $p \leq 0.05$ (DMRT). Values are expressed as a mean ± standard deviation error.
Note: P1 = Standard; P2 = Basal N + 50% TD; P3 = P2 + nano-N 1; P4 = P2 + nano-N 2; P5 = P2 + nano-N 3; P6 = P2 + nano-N 4.

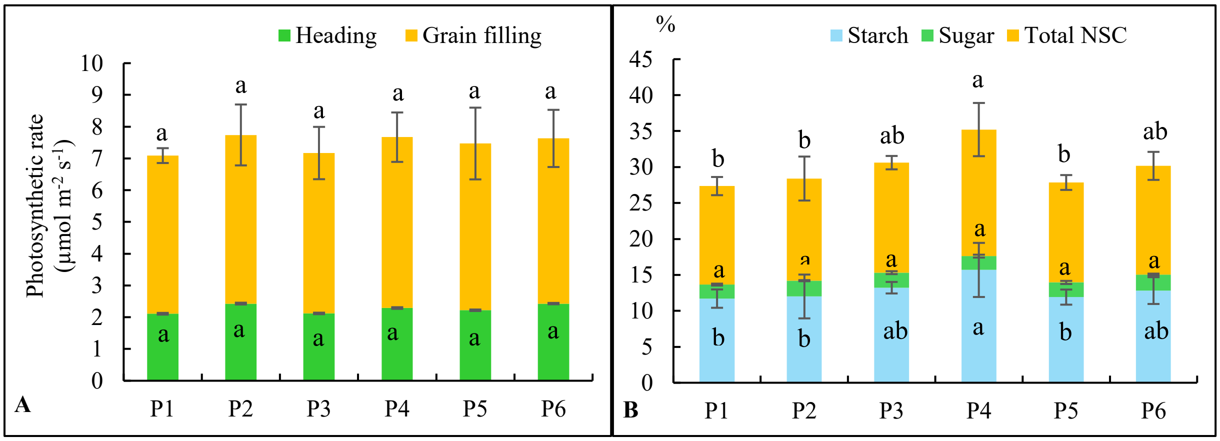

Figure-6. Photosynthetic rate (A) and starch, sugar, and total NSC (B) of Inpari 48 under different fertilization treatments.
Lowercase letters with different styles highlight notable differences between treatments at $p \leq 0.05$ (DMRT). Values are a means ± standard error.
Note: P1 = Standard; P2 = Basal N + 50% TD; P3 = P2 + nano-N 1; P4 = P2 + nano-N 2; P5 = P2 + nano-N 3; P6 = P2 + nano-N 4.

Contribution of variables to leaf physiology

Stepwise regression was employed to identify key predictors of non-structural carbohydrate (NSC) accumulation in rice leaves. The results in Table 6 showed that the model demonstrated an exceptional fit, with an R-squared and adjusted R-squared value of 1.0000, indicating that the selected variables collectively explained the entire variability in NSC accumulation. Significant positive predictors included chlorophyll a during both the heading and panicle initiation phases, as well as carotenoids at both stages. Additionally, the photosynthetic rate at heading showed a positive influence, with sugar and starch contents exhibiting the strongest influence ($p < 0.01$). Conversely, chlorophyll b during both growth stages and biomass dry weight were negatively associated with NSC levels. Although variables like glutamine synthetase and leaf area were part of the model, they did not show statistical significance ($p > 0.05$), indicating a minor direct impact on NSC accumulation. The low root mean square error (0.0030) and lack of residual error also support the model's robustness. These results highlight the key role of photosynthetic pigment dynamics, photosynthetic rate, and carbohydrate metabolites in controlling NSC accumulation during crucial developmental stages in rice.

Table-6. Stepwise regression for predicting non-structural carbohydrate buildup in leaves.

Variable	Estimate	Standard error	T value	Probability
Intercept	0.1264	0.0499	2.53	0.0524
Leaf area 1	-0.0000	0.0000	-2.13	0.0869
Chlorophyll a-1	0.0813	0.0204	3.98	0.0105*
Chlorophyll b - 1	-0.1304	0.0434	-3.01	0.0299*
Carotenoid-1	0.1388	0.0443	3.13	0.0259*
Chlorophyll a – 2	0.0368	0.0134	2.74	0.0407*
Chlorophyll b – 2	-0.1186	0.0418	-2.84	0.0363*
Carotenoid – 2	0.0487	0.0201	2.42	0.0601
Photosynthesis rate – 1	0.008	0.0030	2.68	0.0438*
Biomass dry weight	-0.0057	0.0020	-2.88	0.0345*
Glutamine synthetase	0.0323	0.0201	1.61	0.1692
Starch	0.9980	0.0007	1399.04	0.0000**
Sugar	0.9775	0.0088	111.20	0.0000**

Source	df	Sum of squares	Mean square	F value	Pr (>F)
Model	12	378.0564	31.5047	3495817.36	0.0000
Error	5	0.0000	0.0000		
Total	17	378.0564			

Model summary

Root MSE	NSC Mean	CV (%)	R - Square	Adj R-Sq
0.0030	14.96	31.52	1.0000	1.0000

Note: 1 = heading phase, 2 = panicle initiation phase, * = significant $p < 0.05$, ** = significant $p < 0.01$

Discussion

How soil characteristics, the N cycle, and plant physiology interact is crucial to agricultural productivity and ecosystem health. Soil properties have a significant impact on many aspects of plant growth and metabolism (Sahrawat, 2008; Hayatsu et al., 2021). Soil's ability to support the biological N cycle depends on factors like soil temperature, pH, physical and chemical properties, and water content (van Groenigen et al., 2015; Hirsch and Mauchline, 2015). The differences in the three soil types used in this study matched the varying responses to the N treatment. Vep-ept and Tep-ept soils supported better plant growth than Ae-ent soils, as indicated by the plants' chlorophyll content, biomass, leaf area, photosynthesis rate, and NRA. Both soil types had higher levels of organic C and CEC than Ae-ent. Healthy soil helps prevent the loss of inorganic elements and enhances nutrient absorption, leading to better photosynthesis and more chlorophyll content (Iqbal et al., 2020). Furthermore, Vep-ept performed best is most probably because their markedly higher organic C content and very large CEC provide a stronger nutrient-retentive and biologically active rooting environment, resulting in greater N availability, and improved base-cation supply than the other soils. These properties reinforced by the clay-rich, weatherable mineralogy of Vep-ept, enhance nutrient uptake and sustain leaf chlorophyll formation, photosynthetic activity, and carbohydrate (starch/NSC) accumulation. This explains the superior leaf area, biomass, and pigment concentrations observed in this soil. Recent studies also show that soils with high organic C and CEC respond more efficiently to reduced N doses combined with nano-N foliar application, because soil nutrient suffering complements the rapid physiological effects of nano-

N on chlorophyll, photosynthesis, and N metabolism (Li et al., 2018; Wei et al., 2021; Kumar et al., 2024).

It is noteworthy that Tep-ept soils, although lower in baseline fertility, may have supported the highest photosynthesis and NRA during grain filling. This phenomenon is most likely because they enabled sustained post-anthesis nitrate uptake, a process known to maintain leaf N status, induce NR activity, and preserve photosynthetic capacity even under modest soil N availability (Iqbal et al., 2020; Wei et al., 2021). Favourable soil physical conditions in Tep-ept (silty clay loam texture) due to better root aeration and more stable redox dynamics can further enhance late-season root NO_3^- acquisition and NR induction, processes that often decline in more reduced or compacted soils (Li et al., 2018; Chunmei et al., 2020). In addition, strong sink demand from actively filling grains can signal leaves to maintain C and N assimilation, upregulating both photosynthesis and NR activity regardless of initial soil fertility, a mechanism widely reported in cereals during grain filling (Wang et al., 2023; Kumar et al., 2024).

The present study showed that reducing basal N fertilizer by 50% (equivalent to 46 kg ha^{-1}) maintained chlorophyll levels comparable to the standard treatment. Nano-N application (2 mL L^{-1}) increased ANR, starch, and NSC, with NSC strongly associated with leaf area at heading, chlorophyll a and b, carotenoids, photosynthesis rate (vegetative phase), GS activity, and sugar–starch content. Nitrogen is essential for chlorophyll formation and, together with water, CO_2, and light availability, regulates photosynthesis. Insufficient N lowers chlorophyll, photosynthesis rate, and sugar production (Farhan et al., 2024), whereas adequate N enhances photosynthesis, though excessive N can suppress it. In rice, photosynthesis peaks before full heading (Zhang et al., 2020).

Nitrogen cycling is controlled by climate and soil properties. Plants absorb N mainly as NH_4^+ or NO_3^-, with NO_3^- commonly used in aerated soils. Nitrate must be reduced to NH_3 to form amino acids. This process involves NR converting NO_3^- to NO_2^- in the cytosol, followed by its reduction to NH_3 in the chloroplast (Rosenblueth et al., 2018). NR regulates N flow into organic compounds and influences growth (Fu et al., 2022). Its activity increases with N supply up to an optimal level but may decline under excess N, and typically peaks at heading (Quan et al., 2005). Amino acids, especially glutamic acid and its derivatives (glutamine and asparagine), are transported through the xylem to support metabolism and organ development (Rivai et al., 2021; Akhtar et al., 2024). Although high N inputs support growth and yield, their use efficiency is often low. Zhang et al. (2020) emphasized applying the right N rate for optimal rice yield and quality, reporting partial factor productivity of 40–70 kg grain kg^{-1} N, agronomic efficiency of 10–30 kg kg^{-1} N, and N use efficiency of 30–50%.

Studies reported that nano-N enhances plant performance through several tightly linked mechanisms that extend beyond the effects of conventional fertilizers. Its nanoscale properties improve foliar and root uptake while enabling controlled and sustained N release, reducing losses and maintaining nitrate availability during critical reproductive stages, conditions that prolong NR and GS-dependent assimilation rather than producing the short-lived spikes typical of bulk urea (Wang et al., 2023; Gondwal et al., 2024; Kumar et al., 2024; Liu et al., 2024). This sustained N assimilation increases amino-acid production and modulates nitrate-glutamine signaling that drives the up-regulation of sucrose and starch metabolic enzymes (SUS, AGPase, starch synthases) and sugar-transport pathways, strengthening sink capacity and enhancing starch and NSC accumulation during grain filling (Liu et al., 2024). Nano-N also preserves chloroplast structure and PSII efficiency, improving photosynthetic C supply, while simultaneously enhancing root biomass, tiller formation, and rhizosphere N dynamics together contributing to higher NUE, NRA, and assimilate allocation (Dey et al., 2025). Empirical evidence shows that nano-N can raise total N uptake by 15-65% when combined with conventional fertilizers (Abhiram, 2023) and can mitigate N pollution through reduced losses and more efficient assimilation (Wang et al., 2023). However, these agronomic benefits must be balanced with environmental and food-safety considerations, as excessive N, regardless of form, can enhance the uptake and grain translocation of toxic metals such as Cd (Rahimi et al., 2017). Therefore, integrating nano-enabled fertilizers into sustainable nutrient strategies requires optimizing application rates, minimizing N losses, and managing co-occurring risks to ensure both productivity and ecological safety in rice-based systems.

However, the results of the current study had several limitations, as follows: (i) because the potting media were disturbed and kept under continuous flooding, the experiment did not reflect the natural alternation between flooded and non-flooded conditions that shape redox processes, clay mineral behavior, N transformations, and root-soil interactions in rice fields. The controlled setup also lacked field-scale variability in soil structure, water flow, microbial activity, and climate, all of which influence nano-N behavior and plant uptake. Thus, the physiological responses observed here may not fully represent field realities, underscoring the need for multi-location field trials across diverse soils and moisture regimes to confirm the agronomic benefits and environmental safety of nano-N, and (ii) as N nano spraying was through the base of the leaves, it is most probable that the effect of differences in soil properties between Vertic Endoaquepts and Typic Endoaquepts on the leaves' physiological response was not significant.

Conclusions

The present study revealed that leaf physiological responses were strongly influenced by soil type, with Vertic Endoaquepts and Typic Endoaquepts showing superior physiological activity compared with Aeric Epiaquents. This is most likely due to their higher organic C and CEC. Importantly, nano-N application enabled a 50% reduction in top-dressing N fertilizer (equivalent to 46 kg urea ha^{-1}) while still increasing chlorophyll a (17.8%), chlorophyll b (10.3%), and nitrate reductase activity (11.3%). The Nano-N_2 treatment (2 mL L^{-1}) produced the highest starch (34%) and NSC accumulation, supported by elevated chlorophyll during panicle initiation and greater NR activity during grain filling. These findings indicate that 2 mL L^{-1} is the optimum nano-N dose for enhancing rice leaf physiology while reducing chemical N inputs.

The results of this study suggest that farmers could adopt nano-N at 2 mL L^{-1} as part of an integrated nutrient strategy to lower N fertilizer use by half without compromising crop physiological performance, thereby reducing input costs and minimizing N losses to the environment. Meanwhile, for policymakers, the results provide evidence to support guidelines or incentive programs promoting nano-enabled fertilizers as tools for improving nitrogen-use efficiency, reducing pollution, and advancing climate-smart rice production.

Further field-scale validation across diverse soils will help refine recommendations for broader adoption. In addition, for advanced understanding of nano-N fertilization and soil-plant interactions, it is recommended to conduct long-term field trials to optimize fertilization strategies and to evaluate the impact of nano-N application on rice yield, soil health, and environmental sustainability across paddy systems.

Acknowledgement

This study was supported financially by the Indonesian Fertilizer Holding Company (Research Division). We also thank the Indonesian Soil Research Institute (ISRI), Ministry of Agriculture, for helping with the greenhouse and laboratory facilities.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: The Indonesian Fertilizer Holding Company (Research Division) financially supported this research.

Contribution of Authors

Agustiani N, Sujinah & Pratiwi E: Concept and design research, conducting research/data collection, data analysis and interpretation, wrote the first and revised the final draft.
Hartatik W, Kasno A & Tafakresnanto C: Concept and design research, conducting research/data collection, data analysis and interpretation, and revised the final draft.
Rivaie AA: Conducted research/data collection, data analysis and interpretation, and revised the final draft.
Adriany TA & Nababan AF: Concept and design research, conducting research/data collection.
Salma S: Conducted research/data collection, data analysis and interpretation, and revised the final draft.
Hanindipto FA: Concept and design research, approval of the draft.
Nugraha Y: Concept and design research, data analysis and interpretation, review, and approval of the draft.

All authors read and approved the final draft of the manuscript.

References

Abhiram G, 2023. Contributions of nano-nitrogen fertilizers to sustainable development goals: A comprehensive review. Nitrogen. 4: 397-415. DOI:10.3390/nitrogen4040028.

Akhtar K, Ain N ul, Prasad PVV, Naz M, Aslam MM, Djalovic I, Riaz M, Ahmad S, Varshney RK, He B and Wen R, 2024. Physiological, molecular, and environmental insights into plant nitrogen uptake and metabolism under abiotic stresses. Plant Genome. e20461: 1–22. DOI:10.1002/tpg2.20461.

Aleminew A, Alemayehu G, Adgo E and Tadesse T, 2020. Influence of nitrogen on the growth and use efficiency of rainfed lowland rice in northwest Ethiopia. J Plant Nutr. 43(15): 1–16. DOI:10.1080/01904167.2020.1771574.

Blakemore LC, Searlej PL and Daly BK, 1987. Methods for chemical analysis of soils. New Zealand Soil Bureau Scientific Report, New Zealand.

Buurman P, 1990. Chemical, physical, and mineralogical characteristics for the soil database. Tech. Report No.7, Version 2.1. LREPP Part 2, Soil Data Base Management. CSAR. Bogor.

Cao Y, Wu N, Shi L, Xu X and Wang X, 2025. Effects of nitrogen fertilizer replacement with different sourced-black soldier fly frass on rice growth, physiological characteristics, and soil properties. Appl Soil Ecol. 208: 1-11. DOI: 10.1016/j.apsoil.2025.105975.

Chen LH, Cheng ZX, Xu M, Yang ZJ and Yang LT, 2022. Effects of nitrogen deficiency on the metabolism of organic acids and amino acids in *Oryza sativa*. Plants. 11(2576): 1-15. DOI:10.3390/plants11192576.

Chunmei X, Liping C, Song C, Guang C, Danying W and Xiufu Z, 2020. Rhizosphere aeration improves nitrogen transformation in soil, and nitrogen absorption and accumulation in rice plants. Rice Sci. 27(2): 162-174. DOI: 10.1016/j.rsci.2020.01.007.

Dey A, Jangir N, Verma D, Shekhawat RS, Yadav P and Sadhukhan A, 2025. Foliar application of nano Urea enhances vegetative growth of *Arabidopsis thaliana* over equimolar bulk urea through higher induction of biosynthesis genes but suppression of nitrogen uptake and senescence genes. Plant Growth Regul. 105: 833–859. DOI:10.1007/s10725-025-01314-6.

Duan Y-H, Zhang Y-L, Qi-Rong S and Song-Wei W, 2006. Nitrate effect on rice growth and nitrogen absorption and assimilation at different growth stages. Pedosphem. 16(6): 707–717. DOI:10.1016/S1002-0160(06)60106-9.

Farhan M, Sathish M, Kiran R, Mushtaq A, Baazeem A, Hasnain A, Hakim F, Naqvi SAH, Mubeen M, Iftikhar Y, Abbas A, Hassan MZ and Moustafa M, 2024. Plant nitrogen metabolism: balancing resilience to nutritional stress and abiotic challenges. Phyton. 93(3): 581–609. DOI:10.32604/phyton.2024.046857.

Fu YF, Yang XY, Zhang ZW and Yuan S, 2022. Synergistic effects of nitrogen metabolites on auxin-regulating plant growth and development. Front Plant Sci. 13:1–7. DOI:10.3389/fpls.2022.1098787.

Gondwal P, Rana MK, Dhankar A, Sahil, and Mohan J, 2024. Role of nano-urea in optimizing nitrogen use efficiency, yield and quality of rice: A review. Int J Res Agron. 7(10): 182-187. DOI:10.33545/2618060X.2024.v7.i10Sc.1724.

Handoyo T, Widuri LW and Restanto DP, 2021. Different nitrate and ammonium levels in the media affect changes in nitrogen assimilation enzymes in rice. Biovalentia. 7(1): 25–31. DOI:10.24233/biov.7.1.2021.204.

Hayatsu M, Katsuyama C and Tago K, 2021. Overview of recent research on nitrifying microorganisms in soil. Soil Sci Plant Nutr. 67(6): 619–632. DOI:10.1080/00380768.2021.1981119.

Hegab RH, Abou Batta WF and El-Shazly MM, 2018. Effect of mineral, nano, and bio nitrogen fertilization on nitrogen content and productivity of *Salvia officinalis* L. Plant. J Soil Sci Agric Eng. 9(9): 393–401. DOI:10.21608/jssae.2018.36159.

Hirsch PR and Mauchline TH, 2015. The importance of the microbial N cycle in soil for crop plant nutrition. pp. 45-71. In S Sariaslani and G Gadd (eds), Advances in Applied Microbiology, vol. 93. Academic Press Inc., London.

Hu X, Liu Y, Zhong X, Hu R, Li M, Peng B, Pan J, Liang K, Fu Y and Huang N, 2024. Optimized nitrogen management improves the grain yield of rice by regulating panicle architecture in South China. Heliyon. 10(e34607):1-11. DOI: 10.1016/j.heliyon. 2024.e34607.

Iqbal A, He L, Ali I, Ullah S, Khan Ahmad, Khan Aziz, Akhtar K, Wei S, Zhao Q, Zhang J and

Jiang L, 2020. Manure combined with chemical fertilizer increases rice productivity by improving soil health, post-anthesis biomass yield, and nitrogen metabolism. PLoS One. 15(10): 1-24. DOI: 10.1371/journal.pone.0238934.

Keeney DR and Nelson DW, 1982. Nitrogen-inorganic forms, pp. 643-698. In A Page, D Baker, R Ellis, D Keeney, R Miller and J Rhoades (eds), Methods of soil analysis: Chemical and microbiological properties. Second edition. Soil Science Society of America, Inc., Wisconsin.

Kumar A, Sheoran P, Devi S, Kumar N, Malik K, Rani M, Kumar A, Dhansu P, Kaushik S, Bhardwaj AK, Mann A and Yadav RK, 2024. Strategic switching from conventional urea to nano-urea for sustaining the rice-wheat cropping system. Plants (Basel). 17;13(24):3523. DOI:10.3390/plants13243523.

Lee S, 2021. Recent advances in nitrogen use efficiency in rice. Agronomy. 11(753): 1–7. DOI:10.3390/agronomy11040753.

Li G, Hu Q, Shi Y, Cui K, Nie L, Huang J and Peng S, 2018. Low nitrogen application enhances starch-metabolizing enzyme activity and improves accumulation and translocation of non-structural carbohydrates in rice stems. Front Plant Sci. 9:1128. DOI:10.3389/fpls.2018.01128.

Liang T, Yuan Z, Fu L, Zhu M, Luo X, Xu W, Yuan H, Zhu R, Hu Z and Wu X, 2021. Integrative transcriptomic and proteomic analysis reveals an alternative molecular network of glutamine synthetase 2 corresponding to nitrogen deficiency in rice (*Oryza sativa* L.). Int J Mol Sci. 22(7674): 1–7. DOI:10.3390/ijms22147674.

Lichtenthaler HK, 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 148: 350–382. DOI:10.1016/0076-6879(87)48036-1.

Liu X, Nadeem M and Rui Y, 2024. Effects of nano fertilizers on the mechanism of photosynthetic efficiency in plants: A review. Phyton-Int J Exp Bot. 93(12): 3197-3216. DOI:10.32604/phyton.2024.059281.

Moro E, Crusciol CAC, Nascente AS, Cantarella H, Broetto F and Moro AL, 2017. Nitrate reductase, micronutrients, and upland rice development as influenced by soil pH and nitrogen sources. Commun Soil Sci Plant Anal. 48(22): 2642–2651. DOI:10.1080/00103624.2017.1416137.

Ouyang X, Zhong X, Chang S, Qian Q, Zhang Y and Zhu X, 2022. Partially functional NARROW LEAF1 balances leaf photosynthesis and plant architecture for greater rice yield. Plant Physiol. 189(2): 772–789. DOI:10.1093/plphys/kiac135.

Pathak RR, Ahmad A, Lochab S and Raghuram N, 2008. Molecular physiology of plant nitrogen use efficiency and biotechnological options for its enhancement. Curr Sci. 94(11): 1394–403. DOI: http://ipu.ac.in/usbt/pathaketal2008a.pdf.

Peng J, Feng Y, Wang X, Li J, Xu G, Phonenasay S, Luo Q, Han Z and Lu W, 2021. Effects of nitrogen application rate on the photosynthetic pigment, leaf fluorescence characteristics, and yield of indica hybrid rice and their interrelations. Sci Rep. 11(7485): 1-10. DOI:10.1038/s41598-021-86858-z.

Proud C, Fukai S, Dunn B, Dunn T and Mitchell J, 2023. Effect of nitrogen management on grain yield of rice grown in a high-yielding environment under flooded and non-flooded conditions. Crop Environ. 2: 37–45. DOI: 10.1016/j.crope.2023.02.004.

Qi Z, Xu C, Tang R, Zhang Q, Sun W, Guan C, Wang Y, Zhang M, Ding J, Zhang Y and Ling F, 2025. Response of photosynthesis and chlorophyll fluorescence to nitrogen changes in rice with different nitrogen use efficiencies. Plants. 14(1465): 1–18. DOI:10.3390/plants14101465.

Quan D, Shim JH, Kim JD, Park HS, Cha GS and Nam H, 2005. Electrochemical determination of nitrate with nitrate reductase-immobilized electrodes under ambient air. Anal Chem. 77(14): 4467–4473. DOI:10.1021/ac050198b.

Rahimi G, Kolahchi Z and Charkhabi A, 2017. Uptake and translocation of some heavy metals by rice crops (*Oryza sativa*) in paddy soils. Agriculture (Pol'nohospodarstvo). 63(4): 163–175. DOI:10.1515/agri-2017-0016.

Rajesh K, Thatikunta R and Naik DS, 2017. Enzymatic study of glutamine synthetase activity for validating nitrogen use efficiency in rice genotypes. Int J Pure Appl Biosci. 5(4): 1416–1423. DOI:10.18782/2320-7051.5213.

Rivai RR, Miyamoto T, Awano T, Takada R, Tobimatsu Y, Umezawa T and Kobayashi M, 2021. Nitrogen deficiency results in changes to cell wall composition of sorghum seedlings. Sci Rep. 11(23309): 1-15. DOI:10.1038/s41598-021-02570-y.

Rosenblueth M, Ormeño-Orrillo E, López-López A, Rogel MA, Reyes-Hernández BJ, Martínez-Romero JC, Reddy PM and Martínez-Romero E, 2018. Nitrogen fixation in cereals. Front Microbiol. 9(1794): 1–13. DOI:10.3389/fmicb.2018.01794.

Sahrawat KL, 2008. Factors affecting nitrification in soils. Commun Soil Sci Plant Anal. 39(9): 1436–1446. DOI:10.1080/00103620802004235.

Singh P, Singh I and Shah K, 2019. Reduced activity of nitrate reductase under heavy metal cadmium stress in rice: an in silico answer. Front Plant Sci. 9(1948): 1–11. DOI:10.3389/fpls.2018.01948.

Soil Survey Staff, 2022. Keys to soil taxonomy. 13th Edition. USDA Natural Resources Conservation Service, Washington DC.

van Groenigen JW, Huygens D, Boeckx P, Kuyper TW, Lubbers IM, Rütting T and Groffman PM, 2015. The soil N cycle: New insights and key challenges. Soil. 1(1): 235–256. DOI:10.5194/soil-1-235-2015.

Wang Q, Gao L, Li Y, Shakoor N, Sun Y, Jiang Y, Zhu G, Wang F, Shen Y, Rui Y and Zhang P, 2023. Nano-agriculture and nitrogen cycling: opportunities and challenges for sustainable farming. J Clean Prod. 421: 1–11. DOI: 10.1016/j.jclepro.2023.138489.

Wei Z, Duan F, Sun X, Song X and Zhou W, 2021. Leaf photosynthetic and anatomical insights into mechanisms of acclimation in rice in response to long-term fluctuating light. Plant Cell Environ. 44: 747–761. DOI:10.1111/pce.13954.

Xiong D, Yu T, Liu X, Li Y, Peng S and Huang J, 2015. Heterogeneity of photosynthesis within leaves is associated with alteration of leaf structural features and leaf N content per leaf area in rice. Funct Plant Biol. 42(7): 687–696. DOI:10.1071/FP15057.

Ye C, Zheng G, Tao Y, Xu Y, Chu G, Xu C, Chen S, Liu Y, Zhang X and Wang D, 2024. Effect of soil texture on soil nutrient status and rice nutrient absorption in paddy soils. Agronomy. 14, 1339. DOI:10.3390/ agronomy14061339.

Yoshida S, Forno DA, Cock JH and Gomez KA, 1976. Laboratory manual for physiological studies of rice. Third Edition. The International Rise Research Institute, Manila.

Zhang J, Tong T, Potcho PM, Huang S, Ma L and Tang X, 2020. Nitrogen effects on yield, quality, and physiological characteristics of giant rice. Agronomy. 10(1816): 1–6. DOI:10.3390/agronomy10111816.

Zhao C, Liu G, Chen Y, Jiang Y, Dai Q, Huo Z, Shi Y, Zhao L, Liao P, Wang W, Xu K, Dai Q and Huo Z, 2022. Excessive nitrogen application leads to lower rice yield and grain quality by inhibiting the grain filling of inferior grains. Agriculture. 12(962): 1–7. DOI:10.3390/agriculture12070962.