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Humic Acid and Nitrogen Fertilizer Effects on Nitrogen Availability and Bacterial Population in Sipramin-Affected Paddy Soil

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Abstract

The accumulation of sodium (Na) due to the continuous use of sipramin reduces the physical, chemical, and biological quality of soil, thereby affecting nitrogen (N) availability and microbial activity. This study aimed to evaluate the combined effect of humic acid and different nitrogen fertilizer sources on nitrogen availability (NH_4^+ and NO_3^-) and bacterial population dynamics in sipramin-affected paddy soil. The experiment was conducted under greenhouse conditions using a completely randomized factorial design with two factors: humic acid doses (0, 20, 40, and 60 kg ha^{-1}) and nitrogen fertilizer sources (NPK Phonska, urea, KNO_3, and MAP) at an equivalent rate of 92 kg N ha^{-1}. Observations included NH_4^+, NO_3^-, and total bacterial populations, and data were analyzed using analysis of variance (ANOVA). The results showed that the interaction between humic acid and nitrogen fertilizer did not significantly affect nitrogen availability. However, both factors individually showed significant effects on NO_3^- availability, indicating their role in influencing nitrogen dynamics. Bacterial populations increased from 7 to 21 Day After Treatment (DAT) and decreased at 42 DAT, reflecting temporal microbial dynamics during incubation. Although changes in bacterial populations coincided with variations in nitrogen forms, this relationship could not be directly confirmed due to the limitation of total culturable bacteria analysis. These findings highlight the importance of humic acid and nitrogen fertilizer management in regulating nitrogen dynamics under high-Na soil conditions, while further studies on specific microbial groups are needed to better understand the underlying mechanisms.

1. Introduction

Sipramin is a liquid organic by-product of food seasoning production derived from sugarcane molasses (Bangun and Suryanto, 2020). It contains several nutrients, including nitrogen (N), phosphorus (P), potassium (K), and sodium (Na), as well as relatively low levels of heavy metals. Continuous application of sipramin can lead to sodium accumulation in the soil. This accumulation negatively affects the physical, chemical, and biological properties of the soil. High sodium concentrations cause soil dispersion, leading to surface crust formation and compaction, which reduces soil aeration and limits root as well as microbial activity (Gyhan and Rauf, 2023). In addition, Na^+ can compete with essential cations (K^+, Ca^{2+}, and Mg^{2+}) on soil colloids, thereby reducing cation exchange capacity and nutrient availability (Jumarni et al., 2021). These conditions not only degrade soil physical quality but also influence microbial activity and biogeochemical processes, including nitrogen transformation.

Nitrogen transformation in soil is strongly influenced by microbial activity, particularly through mineralization, nitrification, and denitrification processes. However, high sodium levels disrupt microbial community balance and

suppress the activity of microorganisms involved in the nitrogen cycle. Consequently, nitrogen availability becomes unstable and tends to decrease, even when nitrogen sources are present in the soil. On the other hand, nitrogen is an essential nutrient that plays a critical role in plant growth but is highly susceptible to loss through volatilization, leaching, and denitrification (Rahayu et al., 2021). This indicates that nitrogen management in sipramin-affected soils requires an approach to increase nutrient inputs, as well as improve the soil conditions and microbial activity.

The application of humic acid as a soil amendment has been widely reported to improve soil chemical and biological properties due to the presence of active functional groups, such as carboxyl and phenolic groups, which play a role in nutrient binding (Maimunawaro et al., 2020). In addition, enhancing nitrogen retention in the form of ammonium (NH_4^+), humic acid is also known to modulate soil microbial activity, including microorganisms involved in the nitrification process (Ampong et al., 2022). Furthermore, humic acid can stimulate microbial processes associated with nutrient cycling (Lestari et al., 2023). Thus, humic acid has the potential to play a dual role as both a nutrient binder and a regulator of microbial dynamics in soil.

However, most previous studies have focused on the individual effects or interactions of humic acid and nitrogen fertilizers without considering the high-sodium (Na) soils, such as sipramin-affected soils. Moreover, studies examining the interaction between humic acid and different nitrogen fertilizer sources in influencing microbial populations and nitrogen transformation under such conditions remain limited. Therefore, this study offers a more comprehensive approach by investigating the relationships among sodium accumulation, microbial activity, and nitrogen transformation through the combined application of humic acid and various nitrogen fertilizer sources. This study aims to analyze the effects of humic acid combined with different nitrogen fertilizer sources on nitrogen availability and bacterial populations in sipramin-affected paddy soil.

2. Research Methods

2.1. Research Location

The research was conducted from February 2025 to August 2025 at the Greenhouse of the Faculty of Agriculture, UPN ““Veteran” Jawa Timur. The soil used as a planting medium in this study was taken from paddy fields in Winongo Village, Manguharjo District, Madiun City, East Java. This research was conducted in the greenhouse of the Faculty of Agriculture, UPN “Veteran” Jawa Timur. At the same time, laboratory analysis was carried out in the Land Resources Laboratory and Plant Health Laboratory of the Faculty of Agriculture, “Veteran” Jawa TimurJava.

2.2. Research Implementation

The study was designed using a completely randomized factorial design with two factors. The first factor was the dose of humic acid, which consisted of four treatment levels, namely H0 (0 kg ha^{-1}), H1 (20 kg ha^{-1}), H2 (40 kg ha^{-1}), and H3 (60 kg ha^{-1}). The second factor was the source of nitrogen fertilizer, which consisted of five treatment levels, namely N0 (without N fertilizer), N1 (NPK Phonska), N2 (Urea), N3 (KNO_3), and N4 (MAP). Nitrogen fertilizer was applied at a dose of 92 kg N ha^{-1}. This application rate was selected based on agronomic recommendations from research of Jauhari et al. (2021), following the Paddy Soil Test Kit (PUTS) approach for site-specific nutrient management. Although the soil conditions differ, this rate was used as a baseline to evaluate its effectiveness in sipramin-affected soil with high sodium (Na) content. There was a total of 20 treatment combinations, each repeated three times, resulting in 60 randomly arranged experimental units.

This study was conducted in several stages, including soil collection, preparation of planting media, preparation of soil conditioners and fertilizers, seedling cultivation, application of soil conditioners and fertilizers, planting, maintenance, growth observation, soil sampling, harvesting, and laboratory analysis. Soil was collected at a depth of 0–20 cm using a composite sampling method taken from several points. The collected soil was air-dried, gently crushed, and passed through a 2 mm sieve to ensure uniformity and homogeneity. The processed soil was then used for baseline analysis, as presented in Table 1. For the experiment, the prepared soil was weighed at 3 kg (oven-dry basis) and placed into pots. Soil samples for laboratory analysis were collected at specific observation times. Samples were taken from each pot at several points and separated for analysis of nitrogen utilization and microbial populations. Soil samples were stored in a refrigerator to maintain their existing condition before analysis.

Humic acid and nitrogen fertilizer were applied at the beginning, prior to transplanting, and then incubated for one week to ensure uniform distribution in the soil. Humic acid was first dissolved in water and applied to the soil, followed by nitrogen fertilizer, which was also applied in solution form. All applications were carried out under field capacity conditions to ensure uniform distribution in the soil. Seedlings were grown for two weeks and transplanted seven day after treatment (DAT). The used rice seedlings were the Ciherang variety, as they were suitable for the variety commonly used in the Madiun paddy fields. Plant maintenance included watering (in flooded conditions), weeding, and controlling pests and diseases. Plant growth observations were conducted every two weeks to monitor the parameters of plant length and the

number of tillers. Soil samples were taken at 7 DAT, 21 DAT, and 42 DAT, then analyzed for bacterial population using the total plate count (TPC) method. The NH_4^+ and NO_3^- content was analyzed using the titrimetric distillation method (Eviati et al., 2023).

Table 1. Basic soil characteristics

Parameter	Unit	Result	Criteria
pH	-	6.39	Slightly sour
Organic-C	%	2.90	Moderate
CEC	cmol (+) kg^{-1}	39.79	Height
Exchangeable Na	cmol (+) kg^{-1}	1.10	Very high
NH_4^+	ppm	239.55	-
NO_3^-	ppm	31.94	-
Sand	%	4.06	-
Silt	%	50.79	-
Clay	%	45.15	-
Soil Texture	-		Silty clay loam

2.3. Data Analysis

The research data were analyzed for diversity (ANOVA) with a significance level of 5% to determine whether there was a significant effect of the treatment. If a significant difference was found between treatments, a further Honest Significant Difference (HSD) test was conducted at a 5% error level to determine the most effective treatment.

3. Results and Discussion

3.1. Nitrogen Availability

Nitrogen is an essential nutrient that is fundamental to all aspects of plant growth and development, including protein synthesis and photosynthesis, and stress resistance (Ashari et al., 2024). Nitrogen can be absorbed by plants in the form of ammonium (NH_4^+) and nitrate (NO_3^-) ions. Nitrate (NO_3^-) is an anion that is highly mobile in the soil solution, making it is easily absorbed by plant roots but also prone to leaching. In contrast, ammonium (NH_4^+) is a cation that can be retained by soil colloids, making it less susceptible to leaching (Febriyansyah et al., 2024). The results of the analysis of variance showed that the interaction between humic acid and nitrogen fertilizer had no significant effect on nitrogen availability, both NH_4^+ and NO_3^- ($p > 0.05$).

Treatment H1 (20 kg ha^{-1} humic acid) produced the highest NH_4^+ concentration of 328.48 ppm at 42 DAT, as shown in Table 2, although the differences among treatments were not statistically significant ($p > 0.05$). Although H3 represents the highest dose of humic acid, the NH_4^+ concentration was lower than that of H1. This may indicate that increasing humic acid beyond an optimal level does not necessarily enhance nitrogen retention and may influence soil chemical or microbial processes. This result may be associated with the presence of functional groups in humic acid, such as carboxyl and phenolic groups, which have been reported to enhance ammonium retention in soil (Radite and Simanjuntak, 2020). Humic acid is known to contain active functional groups (–COOH and –OH) that interact with soil nutrients and contribute to nutrient retention (Maimunawaro et al., 2020). However, this mechanism was not directly measured in this study and is inferred based on previous findings.

Based on the results of ANOVA at 42 DAT, the single factor of humic acid and nitrogen fertilizer had a significant effect on Nitrate (NO_3^-) content ($p < 0.05$). Treatment H1 (20 kg ha^{-1} humic acid) also produced the highest NO_3^- content of 53.73 ppm, which was significantly different from treatments H2 and H3 (Table 2). Although H3 represents the highest dose of humic acid, the NO_3^- concentration was lower than H1. This suggests that the effect of humic acid on nitrate availability might not be linear, and the intermediate dose might be more effective under the given soil conditions. This pattern was associated with the influence of humic acid on nitrification processes. Previous studies had reported that humic acid affects the activity of nitrifying microorganisms, which might alter nitrate formation in soil (Ampong et al., 2022). In addition, humic substances have been suggested to regulate nitrogen transformation by influencing the conversion of ammonium to nitrate (Nuraini and Zahro, 2020).

Treatment N4 (MAP) showed the highest NH_4^+ concentration of 350.44 ppm at 42 DAT, although it was not statistically significant (Table 2). This result may be related to the form of nitrogen contained in MAP fertilizer, which supplies nitrogen predominantly in the ammonium form. The N0 treatment (without N fertilizer) produced the highest NO_3^- concentration of 63.49 ppm, which was significantly different from treatments N2, N3, and N4 (Table 2). This could be associated with lower plant uptake or differences in microbial processes compared to fertilized treatments. Nitrate was highly mobile in the soil and could be lost through leaching or denitrification processes, especially under anaerobic conditions (Permatasari et al., 2019).

Table 2. NH_4^+ and NO_3^- content

Treatment	0 DAT (ppm)		42 DAT (ppm)	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Humic Acid				
H0= 0 kg ha^{-1}	239.55	31.94	300.71	47.47^{ab}
H1= 20 kg ha^{-1}	239.55	31.94	328.48	53.73^{b}
H2= 40 kg ha^{-1}	239.55	31.94	304.72	27.88^{a}
H3= 60 kg ha^{-1}	239.55	31.94	322.61	28.96^{a}
Tukey's HSD 5%	**ns**	**ns**	**ns**	**22.16**
Nitrogen Fertilizer				
N0=No N Fertilizer	239.55	31.94	307.54	63.49^{b}
N1=NPK Phonska	239.55	31.94	301.39	42.18^{ab}
N2= Urea	239.55	31.94	304.90	34.00^{a}
N3= KNO_3	239.55	31.94	306.37	2099^{a}
N4= MAP	239.55	31.94	350.44	36.90^{a}
Tukey's HSD 5%	**ns**	**ns**	**ns**	**26.41**

Note: Numbers followed by the same letter in the same column indicate insignificantly different results at the 5% HSD level; ns is not significant

Based on the t-test results comparing nitrogen availability between 0 and 42 DAT (Table 3), NH_4^+ content showed a significant difference ($p < 0.05$). This indicates that nitrogen transformation processes occurred during the incubation period, likely influenced by microbial activity and soil conditions. These changes may be associated with processes such as mineralization, immobilization, and ammonification, which are regulated by soil microbial activity (Grzyb et al., 2021). In contrast, NO_3^- showed no significant difference ($p = 0.18 > 0.05$). This suggests that nitrification processes were relatively stable or limited under the experimental conditions. It might also be influenced by microbial immobilization or environmental conditions that were not optimal for nitrifying bacteria. Overall, these results suggest that nitrogen transformation during the incubation period was more pronounced in the NH_4^+ form than in NO_3^-, indicating that both treatment effects and inherent soil processes have exerted a greater influence on ammonium dynamics.

Table 3. Results of the t-test of nitrogen availability between 0 and 42 DAT

	NH_4^+ (ppm)	NO_3^- (ppm)
t-Test p<0.05	**0.00***	0.18

Note: * = significant results in the t-test $p < 0.05$

The results of the analysis of available nitrogen levels show that NH_4^+ levels yield higher results than NO_3^- levels in all treatments involving humic acid and nitrogen fertilizer. This condition occurs due to environmental factors and the properties of these nitrogen compounds. Waterlogged soil conditions turn the soil anaerobic, thereby inhibiting the mineralization process, especially the activity of nitrifying bacteria (Hartono et al., 2021). This causes the available nitrogen in the soil to be dominated by ammonium compounds. The higher NH_4^+content was also due to the slower movement of this compound compared to NO_3^-. Ammonium (NH_4^+), a form of nitrogen, that was bound to soil colloids, making it more stable and less prone to leaching, while NO_3^- was a form of nitrogen that moves freely in soil solution, making its availability susceptible to leaching along with water flow (Febriyansyah et al., 2024).

3.2. Bacterial Population

Bacteria are the most abundant microbes in the soil, with an estimated 60.000 different species and billions of bacterial cells (Islamiah et al., 2023). Bacteria play a vital role in supplying nutrients through the mineralization process in the soil, while fungi contribute to humification (Azzahra et al., 2025). Based on this, the study analyzed the bacterial population in the soil after treatment to determine its effect on nutrient provision. Soil bacterial populations were analyzed to evaluate the effect of humic acid and nitrogen fertilizer treatments on microbial dynamics. The results showed that bacterial populations varied across treatments and observation times (Table 4).

The results of the observation indicate that the soil bacterial population exhibited distinct dynamics at each observation time. At 7 DAT, bacterial populations ranged from 2.0×10^7 to 8.1×10^7 CFU ml^{-1}, with the highest value observed in treatment H0N0. This suggests that, at the early stage of incubation, microbial populations were likely still influenced by the initial soil conditions rather than treatment effects. The relatively wide range of values indicates variability in microbial response across treatments, although no consistent pattern among humic acid or nitrogen fertilizer levels was observed at this stage. This pattern may be related to the characteristics of humic

acid, which is a relatively stable organic fraction that decomposes slowly and may not provide an immediate energy source for microorganisms (Perdana et al., 2024). As a result, the effect of humic acid on bacterial growth may not be clearly observed during the early incubation period.

Table 4. Bacterial population after humic acid and nitrogen fertilizer treatment

Treatment	Bacterial Population (CFU ml^{-1})		
	7 DAT	21 DAT	42 DAT
H0N0	8.1 x 10^7	7.5 x 10^7	2.1 x 10^7
H0N1	7.9 x 10^7	6.6 x 10^7	5.8 x 10^7
H0N2	5.2 x 10^7	9.3 x 10^7	3.3 x 10^7
H0N3	3.1 x 10^7	5.2 x 10^7	2.7 x 10^7
H0N4	7.7 x 10^7	7.0 x 10^7	6.2 x 10^7
H1N0	2.0 x 10^7	8.9 x 10^7	5.1 x 10^7
H1N1	5.3 x 10^7	6.1 x 10^7	6.3 x 10^7
H1N2	3.1 x 10^7	7.5 x 10^7	3.8 x 10^7
H1N3	3.3 x 10^7	9.9 x 10^7	3.1 x 10^7
H1N4	5.1 x 10^7	5.6 x 10^7	3.7 x 10^7
H2N0	5.2 x 10^7	5.3 x 10^7	4.9 x 10^7
H2N1	7.1 x 10^7	4.1 x 10^7	5.8 x 10^7
H2N2	5.1 x 10^7	4.8 x 10^7	7.9 x 10^7
H2N3	4.3 x 10^7	6.9 x 10^7	6.2 x 10^7
H2N4	5.4 x 10^7	4.5 x 10^7	8.1 x 10^7
H3N0	5.2 x 10^7	1.8 x 10^7	2.1 x 10^7
H3N1	4.5 x 10^7	4.8 x 10^7	2.7 x 10^7
H3N2	3.3 x 10^7	4.3 x 10^7	2.2 x 10^7
H3N3	5.2 x 10^7	3.6 x 10^7	3.2 x 10^7
H3N4	4.4 x 10^7	2.3 x 10^7	1.7 x 10^7

Description: H0 (Humic acid 0 kg ha^{-1}), H1 (Humic acid 20 kg ha^{-1}), H2 (Humic acid 40 kg ha^{-1}), H3 (Humic acid 60 kg ha^{-1}), N0 (No N Ferilizer), N1 (NPK Phonska), N2 (Urea), N3 (KNO_3), N4 (MAP)

At 21 DAT, bacterial populations increased in several treatments, with values ranging from 1.8×10^7 to 9.9×10^7 CFU ml^{-1}. The highest population was recorded in treatment H1N3. Compared to 7 DAT, the increase in maximum population and the shift in treatment response indicate a temporal change in microbial dynamics during the incubation period. This pattern suggests that microbial populations may have responded to changes in nutrient availability over time. Nitrogen fertilizer likely provided a more readily available nutrient source, supporting microbial growth and metabolic activity (Afrianti et al., 2024). In contrast, humic acid may have influenced microbial activity indirectly. Humic acid is generally considered a relatively stable and recalcitrant organic fraction that is not readily decomposed and may not serve as an immediate carbon source for microorganisms; however, its application may improve soil conditions and gradually support microbial activity over time (Yang et al., 2021).

At 42 DAT, bacterial populations declined across most treatments, ranging from 1.7×10^7 to 8.1×10^7 CFU ml^{-1}. Compared to 21 DAT, this decrease indicates a shift in microbial dynamics during the later stage of incubation. The reduction in bacterial population suggests that the conditions supporting microbial growth during the earlier stages were no longer optimal at this time. This pattern may be related to a decrease in the availability of readily accessible nutrients in the soil due to their use by microbial activity and uptake by plants. In addition, increased competition among microorganisms for limited resources may also contribute to the observed decrease in population. According to Octaprama et al. (2020), there are several factors that influence the growth and development of bacteria in the soil, including the availability of nutrients and organic matter, as well as environmental factors such as soil pH and soil moisture.

These temporal changes in bacterial populations may be associated with variations in NH_4^+ and NO_3^- observed in this study; however, this relationship should be interpreted with caution. The results indicate that increases in bacterial populations were not always followed by corresponding increases in nitrogen availability, suggesting that the relationship between microbial abundance and nitrogen dynamics was not consistent across treatments. This may be because the total bacterial populations measured do not specifically represent functional microbial groups involved in nitrogen transformation processes, such as nitrifying or denitrifying bacteria. Therefore, the observed patterns cannot be directly used to explain specific nitrogen transformation mechanisms.

4. Conclusion

The interaction of humic acid and nitrogen fertilizer did not significantly affect nitrogen availability in sipramin-affected paddy soil. However, both humic acid and nitrogen fertilizer individually showed significant effects on

NO_3^- availability, indicating their role in influencing nitrogen dynamics. Bacterial populations increased from 7 to 21 DAT and decreased at 42 DAT, reflecting temporal microbial dynamics during incubation. Although changes in bacterial populations coincided with variations in nitrogen forms, this relationship could not be directly confirmed, as the analysis was limited to total culturable bacteria. Therefore, further research focusing on specific microbial groups involved in nitrogen transformation is recommended to better understand the underlying mechanisms.

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