Impact of Nitrogenous Fertilizers on Root Porosity and Nitrogen Mineralization Rates in rice soil

**ABSTRACT**

**Aims**: Our understanding of how different fertilizer sources affect root air space formation and subsequent nitrogen transformation rates in rice soil remains limited. An experiment was conducted in rice fields using the VL Dhan158 cultivar to examine how different nitrogen-based fertilizers affect rice root porosity, nitrogen mineralization rates, nitrification, and nitrifier population size.

**Study design:** The study employed a completely randomized block design in triplicate. Experimental plots received either urea, ammonium nitrate, or ammonium chloride at 100-kg N ha-1 in three split doses, of 40 kg N ha-1 soon after planting, followed by 30 kg N ha-1 during both the active tillering and flowering phases. VL Dhan 158 seeds were dibbled (4-6 seeds per hill) into each plot, maintaining 20 cm between rows and 15 cm between hills, with an untreated control for comparison.

**Place and duration of study:** The experimental plots were established in the Jeoli village cluster of the Hawalbag block, lying between the coordinates of 29° 36' and 29° 38' N latitude and 79° 34' and 79° 36' E longitude. This region has a dry tropical climate with pronounced monsoon features.

**Results:** The control group exhibited the lowest rates of N-mineralization, nitrification, and nitrifier population, followed by urea, NH4NO3, and NH4Cl in ascending order. Conversely, plant growth vigor, grain yield, and root porosity were highest for NH4Cl, followed by NH4NO3, urea, and the control. A significant relationship was found between the population of nitrifying bacteria and the rate of N-mineralization, as well as root biomass and root air space. The various fertilizer sources led to significant differences in aerenchyma tissue development, resulting in varying degrees of rhizosphere aeration. This variation explains the differences in nitrifier populations supported by VL Dhan 158 under different fertilizer treatments and the subsequent variations in soil processes.

**Conclusion:** The study concludes that fertilizer selection impacts both nitrifier population and their functions. The effectiveness in ranking of fertilizers with respect to present study is Ammonium chloride>ammonium nitrate>urea>control. Efficient management and synchronization of soil nitrogen transformation involves selection and application of ecofriendly sustainable fertilizer type that support nitrifying bacteria in rice rhizosphere and soil so that nitrogen is accessible to rice plants during growth and consequently improve the grain yield.

------------------------------------------------------------------------------------------------------------

**Key Words**: Ammonium oxidizers; nitrification; nitrite oxidizers and N – mineralization; rice soil, root porosity

1. **INTRODUCTION**

Nitrogen accessibility is crucial for the proper functioning of rice (*Oryza sativa* L*.*) ecosystems. The transformation of organic nitrogen into inorganic nitrogen through the processes of decomposition and mineralization plays a vital role in determining the nitrogen availability for crops (Zhang et al., 2015). The impact of different fertilizer application techniques on mineralizable N over the long term can be positive or negative (Hao and Chang, 2003; Cheng et al., 2016). The type of fertilizer, whether chemical or organic, also affects N mineralization. A study employing a 15N tracing model revealed that mineral fertilizers facilitated the breakdown of resistant organic nitrogen, whereas organic fertilizers encouraged the decomposition of easily degradable organic nitrogen in the soil (Zhang et al., 2012).

Nitrogen (N) is crucial for rice plant growth, and N-based fertilizers are widely used by farmers in rice fields to increase crop production (Zhou et al., 2020). In order to meet the increasing demand for food and improve rice production, significant quantities of N fertilizer are used in rice fields (Liu et al., 2019; Yang et al., 2019). Nitrate-N in soil solution can be immediately absorbed by plants, making nitrate fertilizers quick-acting. Ammonium-N is partially available for immediate uptake, as it is mainly adsorbed to clay particles before being released and nitrified, resulting in ammonium fertilizers being moderately fast-acting. Urea, an amide-N form, must first be hydrolyzed to ammonium by urease. Various N fertilization and water management techniques have been employed to enhance NUE and minimize N losses in paddy fields (Wang et al., 2020). Our understanding of nitrogen transformation processes in soil, especially concerning N mineralization and nitrification under various nitrogen fertilizer types, remains fragmented (Liu et al., 2019; Wang et al., 2017). The chemical form of nitrogen influences its fate in soil, with ammonium nitrogen (NH4+-N) being less prone to leaching and runoff compared to nitrate nitrogen (NO3--N) (Liu et al., 2019). The impact of organic and inorganic fertilizers on soil health and rice yield has been reviewed by Behera and Pany (2025).

Rice plants possess aerenchyma in their roots. This tissue facilitates the efficient transport of oxygen within the plant. Oxygen is crucial for absorbing both nutrients and water. Additionally, oxygen diffuses from the roots into the surrounding soil, supporting aerobic microbial activity in the rhizosphere. Hamel et al., 2004 proposed that root aeration might also impact nutrient dynamics in the root zone. Nevertheless, our understanding of how different fertilizer sources affect root air space formation and subsequent nitrogen transformation rates in rice soil remains limited.

This research focused on investigating how different nitrogen-based fertilizers influence root porosity, changes in nitrifier populations, and related processes like nitrogen mineralization and nitrification rates in tropical rainfed rice soils. The fertilizers investigated were urea (amide-N source), ammonium nitrate (nitrate-N and ammonium-N source), and ammonium chloride (ammonium-N source).

1. **MATERIALS AND METHODS**

The experimental plots were established in the Jeoli village cluster of the Hawalbag block, lying between the coordinates of 29° 36' and 29° 38' N latitude and 79° 34' and 79° 36' E longitude. This region has a dry tropical climate with pronounced monsoon features. The year can be divided into three seasons, with January being the coldest month, averaging 13.3 0 C. The average annual rainfall is 1132.5 mm, with precipitation occurring for approximately 46.8 days each year. The highest number of rainy days is in August, with 11.9 days, while November experiences the fewest, with just 0.6 days (Joshi et al., 2014). The soil is pale brown color and silty loam texture (3.6% clay, 30.7% sand, and 65.7% silt), and an acidic pH. The soil is characterized by good drainage and moderate fertility, with organic carbon content ranging from 0.68% to 0.76%, total nitrogen levels between 0.07% and 0.08%, and total phosphorus content of 304 to 340g g-1 (Mhalla et al., 2019). It has a bulk density of 1.38 g cm-3 and a water retention capacity of 39.2%.

The study area comprised 12 plots, each measuring 5 x 3 m, with 0.5 m gaps between them. The study employed a completely randomized block design with three replications. After all plots were plowed to a depth of 20 cm and the seedbed was prepared, a base application of KCl, P2O5, and farmyard manure was applied at rates of 60 kg of potassium, 60 kg of phosphorus, and 1000 kg of FYM per hectare, respectively. Single super phosphate served as the source of P2O5. The treatments included a control (no fertilizer), urea, ammonium nitrate, and ammonium chloride. The fertilizers were applied to the treatment plots at a rate of 100 kg N ha-1, distributed in three installments: 40 kg N ha-1 soon after planting, followed by 30 kg N ha-1 during both the active tillering and flowering phases. VL Dhan 158 seeds were dibbled (4-6 seeds per hill) into each plot, maintaining 20 cm between rows and 15 cm between hills. VL Dhan 158 (RCPL1-45 x VL3861) is characterized as high-yielding rice variety with average grain yield of 2386 kg ha-1 (Aditya et al., 2018). Weeds were manually removed with minimal plot disturbance to keep the crop weed-free. The sole source of irrigation during cultivation was rainfall.

Triplicate samples from each replicate plot were collected and combined to create composite soil samples at 15-day intervals. Soil samples measuring 10 x 10 x 10 cm were collected from the spaces between rows, sealed in polyethylene bags, and taken to the lab. The composite sample was then split into two sections. The first section, which remained in its original moist state, was utilized for assessing pH and mineral nitrogen (NH4+ - N and NO3- - N) levels. The second segment, which was also maintained in a field-moist state, was utilized to evaluate N-mineralization, nitrification, and the population of nitrifiers. Soil samples were collected at different stages of crop growth: 17 days post-sowing (DAS), during active tillering (32 DAS), at the onset of panicle formation (47 DAS), at the flowering stage (62 DAS), at physiological maturity (77 DAS), and just before harvest (92 DAS). Once in the laboratory, the samples were laid out on paper sheets and visible roots and organic debris fragments were eliminated, and the soil was sieved through a 2-mm mesh.

A pH meter with a glass electrode was utilized to determine soil pH (1:2, soil: water). The phenate method (APHA, 1985) was employed to colorimetrically estimate extractable soil ammonium nitrogen. The phenol disulphonic acid method (Jackson, 1958) was used to measure nitrate nitrogen. In-field incubation techniques (Eno, 1960, Ross et al., 1985, Schimel and Parton, 1986) were applied to assess in situ N-mineralization rates over a thirty-day period at the sampling locations. An initial soil sample was obtained using a soil corer (5.0 cm diameter x 10 cm depth) and transported to the laboratory in a plastic bag for analysis. Additional soil cores of identical dimensions were collected immediately adjacent to the initial sample. Each intact core was inserted into a plastic tube (approximately 10-cm diameter 40cm length) with a plastic cap. To prevent significant immobilization during incubation, coarse roots and large organic debris fragments were removed from the cores (Ross et al., 1985; Schimel and Parton, 1986). The capped tubes were reinserted into their original holes and retrieved after thirty days (referred to as the incubated samples). The tubes were inserted at least 30 cm deep, with the remaining portion protruding above the soil surface. Both initial and incubated samples underwent identical laboratory procedures. The samples were passed through a 2mm mesh to eliminate fine roots and large stones. Subsamples of 10g were placed in extraction cups, to which 100 ml of 2M KCl was added. The supernatant was analyzed for ammonium concentration using the phenate method (APHA, 1985). The concentration of NO3 – N was determined using the phenol disulphonic acid method (Jackson, 1958) after extracting the soil with CaSO4. 2H2O. The remaining soil sample was then dried at 1050 C for 24 hours to determine its dry mass.

 The N-mineralization and nitrification rates were determined by measuring the change in concentrations of inorganic N ions (NH4+ and NO3 -) between the initial and incubated samples (Hart et al., 1994; Jha et al.,1996). The values for the rates are quantified as grams of nitrogen per gram of dry soil over a thirty-day period. All calculations, unless mentioned, are based on the weight of soil dried in an oven at 1050 C.

The most probable number (MPN) method was employed to assess the concentration of viable nitrifying bacteria, which includes both ammonium and nitrite oxidizers (Alexander 1965, Alexander and Clark, 1965). Ammonium oxidizing bacteria was cultured in ammonium-calcium carbonate medium while nitrite oxidizing bacteria were grown in nitrite-calcium carbonate medium To prepare the inocula, 10 g of soil was combined with 90 ml of sterile distilled water in a sterile universal bottle for each composite soil sample. This mixture was vigorously agitated on a wrist action shaker for 30 min. Sequential tenfold dilutions were created by transferring 1 ml of the suspension to 9 ml of sterile water. Each subsequent dilution was manually shaken for 30 s before extracting a 1 ml sample. Dilutions ranging from 10 – 4 to 10 – 9 were utilized for both ammonium and nitrite oxidizers, with five replicate culture tubes employed for each dilution. Ammonium oxidizing bacteria were cultured in ammonium-calcium carbonate medium ((NH4) 2 SO4, 0.5g; K2HPO4, 1.0g; FeSO4. 7H2O, 0.03g; NaCl, 0.3g; MgSO4. 7H2O, 0.3g; CaCO3, 7.5g; water, 1 litre), while nitrite oxidizing bacteria were grown in nitrite-calcium carbonate medium (KNO2, 0.006g; K2HPO4, 1.0g; NaCl, 0.3g; MgSO4 7H2O, 0.1g; FeSO4 .7H2O, 0.03g; CaCO3, 1.0g; CaCl2, 0.3g; water, 1 litre). The inoculated media were kept in darkness at 28± 20 C. After thirty days, nitrifying activity was assessed by examining each tube for nitrite using Griess-Ilosvay reagent. The quantity of tubes showing positive or negative results was recorded, and the most probable number of organisms present was calculated using an MPN table (Cochran, 1950).

At 15-day intervals, beginning 8 days after transplanting, plant growth and grain yield were evaluated from randomly chosen fixed locations in both control and fertilized plots. For each sampling date, a single rice hill was taken from every plot, with the roots being removed from a soil block measuring 15 x 20 x 15 cm in depth. The soil was carefully washed with tap water using a sieve with 0.2 mm openings. The number of tillers per hill was noted. Roots and shoots were then separated and dried at 65°C for 48 hours until they reached a stable weight for biomass assessment. All measurements were conducted in triplicate. Grain yield was assessed by harvesting all hills within a 1m x 1m quadrat in the center of each treatment plot. A rice threshing machine separated grain and straw, which were then dried in a batch grain drier and weighed. After weighing, the moisture content of the grain was measured immediately, and subsamples were further dried in an oven set at 65°C for a duration of 48 hours. Grain weight is reported on an oven-dry (65 0 C) basis.

Root porosity was measured three times (during panicle initiation, flowering, and physiological maturity) using the water displacement method (Jensen et al., 1969). Root porosity *was* calculated using the following equation:

POR =

[(p & gr) – (p & r)] x 100

[(r + p) – (p & r)]

Where POR represents root air space (porosity) in percent (%); r is the mass of roots (g); p is the mass of water-filled pycnometer (g); p & r is the mass of pycnometer with roots and water (g); and p & gr is the mass of pycnometer with ground roots and water (g).

The analysis of data and statistical comparisons were conducted using SPSS software (SPSS / PC, 2002). To evaluate the impact of fertilizer type on various factors including soil processes, nitrifying bacterial population, plant growth parameters, root porosity, and grain yield, a general linear model (GLM) two way ANOVA with repeated measures were used. The Tukey's HSD (honestly significant difference) test was employed to assess the importance of the variations between means. Additionally, Pearson correlation coefficients were calculated for the observed parameters. In each data analysis set, the three replicate plots were treated as independent entities.

1. **RESULTS AND DISCUSSION**

Understanding how various fertilizers alter soil chemistry is crucial for predicting the impact of both natural and artificial changes on nutrient cycles in tropical rainfed rice ecosystems. The findings demonstrate significant variations in nitrogen dynamics and nitrifier populations within rice soil treated with different fertilizers. These alterations in microbially-driven N transformations support the idea that diverse nitrogen sources can lead to changes in N-mineralization rates, nitrification processes, and nitrifying bacterial communities. The observed differences can be partially explained by variations in soil nutrient levels, standing crop biomass, root porosity, and fluctuations in nitrifying bacterial populations.

3.1Soil properties under different fertilizer treatment

The cropping season averages for pH across control and fertilized plots showed that the highest pH was recorded in plots treated with urea and minimum in control plots. Table 1 shows that there were notable variations in pH levels between the control and urea plots, as well as between the urea and ammonium chloride treated plots. Fertilization notably influenced the pH levels of the soil (Table 2). There was a relationship between soil pH, nitrite oxidizers, and root biomass (Table 5).

The mineral – N content in soil decreased during the cropping season. The maximum mineral – N was recorded at 32 DAS for all the treatment plots except for control, which recorded the highest mineral N content on 17 DAS. The minimum mineral N content in all the treatment plots was recorded on 77 DAS (Figure 1).

 HSD test indicated that urea treated plots differed significantly from control and ammonium chloride treated plots (Table 1). ANOVA indicated significant differences in mineral – N content in soil across the cropping period due to fertilizer treatment (Table 2). The mineral – N content was correlated to rate of N – mineralization, nitrification, ammonium oxidizers, root biomass, and shoot biomass (Table 5).

The composition of soil subjected to various fertilizer treatments exhibited differences in both the proportional amounts of inorganic N and the rate of mineralization, reflecting temporal changes in these quantities. Plots treated with urea showed a 38.66% increase in mineral-N content, while those treated with ammonium nitrate had a 3.8% increase compared to the control (Table 1). Conversely, ammonium chloride-treated plots displayed a 14.86% decrease in mineral-N relative to the control. These variations may be attributed to differences in rice plants' ability to absorb, move, and utilize available N from different sources. Throughout the growing season, a decrease in mineral-N concentration was observed. The quick decrease in applied nitrogen from the inorganic portion is due to rapid absorption by plants, integration into microbial biomass, nitrogen loss through nitrification and denitrification, and possible movement beyond plot boundaries. The increase in the mineral-N pool ranged from 3.8% (ammonium nitrate) to 38.66% (urea). In a long-term dryland research site in the US, an 88% increase in inorganic N values was recorded following the application of 67 kg N ha-1 fertilizer (El Harris et al., 1983).

Figure 2 illustrates the rate of N – mineralization observed throughout the cropping season. The cropping season averages show that highest rate of N – mineralization was recorded in ammonium chloride treated plots and lowest rate was detected in control plots. There were notable variations in the rate of N-mineralization between the control plots and those treated with ammonium nitrate and ammonium chloride. The ammonium chloride treated plots differed significantly with respect to urea treated plots also (Table 1). The N-mineralization rate varied significantly among all treatments throughout the cropping season (Table 2). This rate was linked to the nitrification rate, ammonium oxidizers, nitrite oxidizers, and root biomass (Table 5).

The rate of nitrification following application of three different fertilizers has been presented in (Figure 3). The cropping season averages showed results similar to that of rate of N – mineralization where highest rate of nitrification was measured in plots treated with ammonium chloride and lowest in control plots. HSD test indicated significant difference in ammonium chloride treated plots with rest of the treatments (Table 1). The nitrification rate varied significantly throughout the cropping season among the different treatments (Table 2). This rate was linked to mineral – N, N – mineralization, the population of ammonium oxidizers, as well as root and shoot biomass (Table 5).

The current study revealed an increase in N-mineralization rates: 12.25% for urea-treated plots, 55.29% for ammonium nitrate, and 89.94% for ammonium chloride. Research has shown that applying nitrogen fertilizers contributes to the soil's mineralizable nitrogen, particularly enhancing the readily available nitrogen pool (El Harris et al., 1983). The interaction between added fertilizer nitrogen and native soil nitrogen results in increased soil nitrogen mineralization, a phenomenon referred to as the 'priming effect'. Numerous researchers have observed enhanced N-mineralization in soils supplemented with chemical fertilizers (Singh and Singh, 1994; Sun et al., 2015). The observed ranges of N-mineralization and nitrification align with findings from various other studies (Singh and Singh 1994; Jha et al., 1996).

The differences in soil nitrification rates under various fertilizer applications can be explained by the varying levels of microbial activity among nitrifying organisms. Vitousek and Matson, (1982) proposed that nitrifier populations regulate nitrification, observing that introducing actively nitrifying soils as inoculum prior to incubation enhanced nitrate production. Applying ammonium-based fertilizers can significantly boost the population and activity of autotrophic nitrifiers (Haynes, 1986). The addition of substrate increases potential nitrification rates by overcoming competition for mineral nitrogen by nitrifiers (Killham, 1990). Mineral N fertilizer additions strongly impact nitrification rates (Jarvis et al., 1997). Onikura et al., (1975) noted that fertilizer application to rice soil accelerated ammonium-N production from soil sources while applied-N remained in ammonium form. The primary factor explaining differences in soil-N uptake among rice plants due to the priming effect was rooting patterns and the volume of soil utilized, which varies between crops, as does the response to fertilizer N. Urea quickly transforms into ammonia, which acts as a substrate for nitrifying bacteria during the nitrification process. Research indicates that urea can either enhance or have no effect on nitrification (Martikainen 1984; 1985). Ammonium salts may stimulate (Johnson and Edwards, 1979, Mai et al., 1980) or inhibit nitrification (Focht and Verstraete, 1977; Viro, 1962). Generally, the low nitrification rates observed may result from nitrate uptake by soil microflora, potentially limiting net nitrate accumulation.

The findings suggested that oxygen reached rice roots through aerenchyma, leading to nitrification in the rhizosphere, similar observations were made by Wells et al., 2014. The rate of nitrification decreases as the distance from the root surface grows (Yao et al., 2020). According to Wang et al., (2017), nitrification rates were significantly reduced under prolonged flooding conditions compared to those in dryland rice cultivation, underscoring the rhizosphere's role in nitrification when contrasted with oxidized surface soil. Briones et al., (2002) noted a high concentration of ammonia-oxidizing bacteria on rice root surfaces, further emphasizing the significance of rhizosphere nitrification. Our study revealed that there were significantly higher abundances of AO and NO bacteria in rhizosphere soil compared to bulk soil (Table 2), showing a strong positive correlation with net nitrification rate. Similar observations were made by Zhang et al., 2022.

* 1. Nitrifier population under different fertilizer treatment

Variation in viable population of ammonium oxidizers across the cropping season has been presented in Figure 4. The cropping season average shows that maximum ammonium oxidizer population was harboured in soil treated with ammonium chloride and minimum in control plots. There were notable variations in the population of ammonium oxidizers in soil subjected to different fertilizer treatments, except for the comparisons between urea and the control, as well as between urea and ammonium nitrate treated plots (Table 1). A GLM two way ANOVA indicated significant differences in ammonium oxidizer population due to fertilizer treatments (Table 4). The ammonium oxidizer population was correlated to mineral –N, N – mineralization, nitrification, nitrite oxidizers, root biomass and root porosity (Table 5). The fluctuations in viable population of nitrite oxidizers are presented in Figure 5. The cropping season averages showed similar trends as that of ammonium oxidizers. The population difference between control and urea treated plots was not significant (Table 1). Nitrite oxidizer population differed significantly due to treatment (Table 4). The nitrite oxidizer population was correlated to N – mineralization, ammonium oxidizers, soil pH, and root porosity (Table 5).

The current study's estimates of ammonium and nitrite oxidizing bacteria populations align with the ranges reported in other investigations. Prosser and Cox, (1982) noted that untreated agricultural soils contain nitrifying bacteria at levels of 1 x 103 to 1 x 104, while fertilized soils may harbor 1 x 105 to 1 x 106 g –1 dry soil. Fertilizer application can increase nitrifier populations from hundreds to hundreds of millions per gram of soil (Aarnio and Martikainen, 1995). In a wheat cropland in Rohtak, India, ammonium oxidizers were estimated at 38 x 105 g –1 dry soil, and nitrite oxidizing bacteria at 0.45 x 105 g –1 dry soil (Sethi et al., 1990). The abundance and activity of nitrifiers in soil are typically constrained by ammonium – N production and supply (Haynes, 1986; Donaldson and Henderson 1990b). The nitrifying bacteria also played a significant role in plant growth as observed by Amoo and Babalola, 2017.

 A strong correlation was observed between these two types of bacteria, supporting the idea that their processes are interconnected, as noted by Woldendorp and Laanbroek, (1989). The population of ammonium oxidizers showed a significant relationship with N-mineralization and nitrification rates, suggesting that increased microbial activity contributes, at least partially, to the N-mineralization observed after fertilization.

3.3 Root porosity, plant growth and grain yield parameters under different fertilizer treatment

The average values for the plant growth parameters measured across the cropping season are presented in Table 3. VL Dhan 158 responded to ammonium chloride treatment the most and produced maximum number of tillers and plant biomass (root and shoot) in comparison to control and other fertilizer treatments. It was observed that under ammonium chloride treatment the plants produced large amount of fine root in the surface soil in contrast to other treatments.

Fertilization enhanced root porosity and there was significant difference in root porosity in ammonium chloride treated plots in comparison to control, and urea treated plots (Table 3). The use of ammonium chloride as a fertilizer had a significant positive impact on plant growth and grain yield. Different fertilizers led to varying growth responses in the rice variety. Both ammonium chloride and ammonium nitrate fertilization notably increased grain yield compared to the control group (Table 3). There were significant differences in root biomass and root porosity due to different fertilizer treatment (Table 4). Root biomass was correlated to mineral – N, rate of N – mineralization, nitrification, ammonium oxidizer population and soil pH (Table 5). Root porosity was strongly correlated to ammonium and nitrite oxidizer population (Table 5).

Apart from organic matter input from rice plants, the extent of aerobic conditions in the soil, which is influenced by the root porosity of rice plants and the subsequent diffusion of oxygen through the roots resulted in variation in nitrifer population in soil.

The rice cultivar VL Dhan 158 exhibited varying resource utilization capabilities, as evidenced by distinct growth patterns and grain yields under different fertilizer treatments. The study revealed that fertilizers had a diverse impact on the development of aerenchyma tissue in rice plants. Nitrogen fertilization resulted in the formation of larger cells, leading to increased root porosity (Colmer et al., 1998; Colmer, 2003). The notable variations in root porosity due to different fertilizer applications led to varying levels of aerobicity, which consequently affected the abundance of ammonium and nitrite oxidizers in the soil.

In this study, urea's overall effectiveness was significantly lower than that of ammonium nitrate and ammonium chloride, primarily due to the potential loss of ammonia from urea application in soil. The superior performance of ammonium chloride over ammonium nitrate was due to the reason that ammonium was more effective than nitrate for rice cultivation. Nitrate utilization is hindered by nitrogen loss through denitrification, nitrite formation, and insufficient sugar for protein synthesis from nitrate. Research has shown that the assimilation of ammonium is less energy-intensive compared to nitrate (Bloom et al., 1992). Consequently, the root apical meristem's use of ammonium may provide nitrogen for protein synthesis with minimal energy expenditure. In a study examining the net fluxes of ammonium and nitrate along rice roots, Colmer and Bloom, (1998) found that when both ions were supplied together, the net uptake of ammonium occurred more rapidly than that of nitrate at a distance of 1mm behind the root apex.

1. CONCLUSION

The fertilizer type affected nutrient availability in the soil by impacting rhizosphere proliferation which in turn impacted oxygen levels, and consequently the soil microbe populations and their activities. In this study, the effectiveness ranking was determined to be ammonium chloride > ammonium nitrate > urea > control. The interplay between fertilizer type, rice plants, and the amount and form of nitrogen accessible to plants during growth indicates that fertilizer selection plays a crucial role in nitrogen dynamics within tropical rice soil. To effectively manage nitrification, it is essential to thoroughly understand the process, implement a carefully designed monitoring plan, utilize environmentally friendly and sustainable fertilizers to enhance nitrogen availability in soils, and apply a range of strategies tailored specifically to the different rice varieties and organisms found within particular rice ecosystems. Use next-generation sequencing (NGS) to identify and categorize nitrifying bacteria and archaea linked to particular rice varieties. Additional research is necessary to clarify these processes using various other fertilizer treatments and to evaluate a wider range of cultivars with comparable fertilizer dosages.

**Acknowledgement:** The author is thankful to the Director, GBPNIHE for providing all the necessary facilities for carrying out the study.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

REFERENCES

Aarnio, T. & Martikainen, P. J. (1995). Mineralization of C and N and nitrification in Scots pine forest soil treated with nitrogen fertilizers containing different proportions of urea and its slow releasing derivatives, urea formaldehyde. Soil Biology & Biochemistry, 27, 1325 – 1331.

Aditya, J. P., Agrawal, P.K., Stanley, J., Pandey, B.M., Mishra, K.K., Devendra, L., Verma, P.C., Arya, J.K., Panchpal, D.S., Rawat, K.S. & Singh, A. (2018). VL Dhan158: An early maturing rice variety for rainfed uplands of NorthWest Himalayas. Electronic Journal of Plant Breeders, 9 (4), 1378-1386.

Alexander, M. & Clark, F. E. (1965). Nitrifying bacteria. In Methods of soil analysis. Ed. C A Black. pp 1477 –1486. American Society of Agronomy Inc., Madison, WI, USA.

Alexander, M. (1965). Most – probable number method for microbial populations. In Methods of soil analysis. Ed. C A Black. pp 1467 – 1472. American Society of Agronomy Inc. Madison, WI, USA.

Amoo, A. E. & Babalola, O. O. (2017). Ammonia-oxidizing microorganisms: key players in the promotion of plant growth. Journal of Soil Science & Plant Nutrition, 17, 935–947. doi: 10.4067/S0718-95162017000400008

APHA (American Public Health Association) 1985. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.

Behera, H.S. & Pany, B.K. (2025). Impact of combinations of organic and inorganic fertilizers on soil health and yield of rice: A comprehensive review. International Journal of Plant and Soil Science. 37(2), 282-289.

Bloom, A. J., Sukrapanna, S.S. & Warner, R.L. (1992). Root respiration associated with ammonium and nitrate absorption and assimilation by barley. Plant Physiology, 99, 1294–1301.

Briones, A. M., Okabe, S., Umemiya, Y., Ramsing, N. B., Reichardt, W. & Okuyama, H. (2002). Influence of different cultivars on populations of ammonia-oxidizing bacteria in the root environment of rice. Applied Environmental Microbiology, 68(6), 3067–3075. [**https://doi.org/10.1128/AEM.68.6.3067-3075.2002**](https://doi.org/10.1128/AEM.68.6.3067-3075.2002)

Cheng, W.G., Padre, A.T., Sato, C., Shiono, H., Hattori, S., Kajihara, A., Aoyama, M., Tawaraya, K. & Kumagai, K. (2016). Changes in the soil C and N contents, C decomposition and N mineralization potentials in a rice paddy after long term application of inorganic fertilizers and organic matter. Soil Science & Plant Nutrition, 62, 212–219. doi:10.1080/ 00380768.2016.1155169

Cochran, W. G. (1950). Estimation of bacterial densities by means of the ‘most probable number’. Biometrics, 6, 105 – 116.

#  Colmer, T. D. (2003). Aerenchyma and an Inducible Barrier to Radial Oxygen Loss Facilitate Root Aeration in Upland, Paddy and Deep‐water Rice (Oryza sativa L.). Annals of Botany, 91(2), 301-309. Doi:10.1093/aob/mcf114

Colmer, T.D. & Bloom, A.J. (1998). A Comparison of and Net fluxes along Roots of Rice and Maize. Plant Cell Environment, 21, 240-246.
<http://dx.doi.org/10.1046/j.1365-3040.1998.00261.x>

Colmer,T.D., Gibbered, M.R., Wiengweera, & Tinh, T.K. (1998). The barrier to radial oxygen loss from roots of rice (*Oryza sativa* L.) is induced by growth in stagnant solution. Journal of Experimental Botany,49 ,1431 –1436.

El-Harris, M. K., Cochran, V. L., Elliot, L. F. & Bezdicek, D. F. (1983). Effect of tillage, cropping and fertilizer management on soil nitrogen mineralization potential. Soil Science Society of America Journal, 47, 1157-1161.

Eno, C. (1960). Nitrate production in the field by incubating the soil in polyethylene bags. Soil Science Society of America Proceedings, 24, 277 – 279.

Focht, D. D. & Verstraete, W. (1977). Biochemical ecology of nitrification and denitrification. Advances in Microbial Ecology, 1, 135 – 214.

Hamel, C., Landry, C., Elmi, A., Liu, A. & Spedding, T. (2004). Nutrient Dynamics. Journal of Crop Improvement, 11(1–2), 209–248. <https://doi.org/10.1300/j411v11n01_10>

Hao, X.Y. & Chang, C. (2003). Does long-term heavy cattle manure application increase salinity of a clay loam soil Wu et al. 297 Published by NRC Research Press Canadian Journal of Soil Science, Downloaded from cdnsciencepub.com by 117.239.17.194 on 09/25/24

Hart, S. S., Stark, J. M., Davidson, E. A. & Firestone, M. K. (1994). Nitrogen mineralization, immobilization and nitrification. In Methods of soil analysis, Part 2. Microbial and biochemical properties. Eds. R W Weaver, J S Angle & P S Bottomly. pp. 985 – 1018. Soil Science Society of America. Madison.

Haynes, R.J. (1986). Nitrification. In Mineral nitrogen in the plant soil system. Ed. R J Haynes. pp. 127 – 165. Academic press Inc. London, U.K.

Jackson, M. L. (1958*).* Soil Chemical Analysis. Prentice-Hall Inc. Englewood, Cliffs, pp 197 - 201

Jarvis, S. C., Stockdale, E. A., Shepherd, M. A. & Powlson, D. S. (1997). Nitrogen mineralization in temperate agricultural soils: processes and measurement. Advances in Agronomy 57: 187 – 235.

Jensen, C. R., Luxmoore, R. J., Van Gundy, S. D. & Stolzy, L. H. (1969). Root air – space measurements by a pycnometer method. Journal of Agronomy,61, 474 – 475.

Jha, P. B., Kashyap, A. K. & Singh, J. S. (1996). Effect of fertilizer and organic matter inputs on nitrifier populations and N – mineralization rates in a dry tropical region, India. Applied Soil Ecology, 110, 1 – 11.

Johnson, D. W. & Edwards, N. T. (1979). The effects of stem girdling on biogeochemical cycles within a mixed deciduous forest in eastern Tennessee. II. Soil nitrogen mineralization and nitrification rates*.* Oecologia, 40, 259 – 271.

Joshi, S., Kumar, K., Joshi, V. & Pande, B. (2014). Rainfall variability and indices of extreme rainfall-analysis and perception study for two stations over Central Himalaya, India. Natural Hazards, 72(2), 361-374

Killham, K. (1990). Nitrification in coniferous forest soils. Plant & Soil, 128, 31 – 44.

Liu, S. Y., Chi, Q. D., Cheng, Y., Zhu, B., Li, W. Z., Zhang, X. F., Huang, Y. Q., Müller, C., Cai, Z. C. & Zhang, J. B. (2019). Importance of matching soil N transformations, crop N form preference, and climate to enhance crop yield and reducing N loss. Science of the Total Environment, 657, 1265–1273. https:// doi.org/10.1016/j.scitotenv.2022.153566

Mai, H., Fiedler, H. J. & Leube, F. (1980). The effect of urea and calcium ammonium nitrate application on microflora and N – conversion in spruce raw humus. *Zentralbatt für* *Bakteriologie, Parasitenkunde, Infektionskrenkheiten und Hygiene, II Abteilung,* 135, 563 – 574.

Martikainen, P. J. (1984). Nitrification in two coniferous forest soils after different fertilization treatments. Soil Biology & Biochemistry, 16, 577 – 582.

Martikainen, P. J. (1985). Numbers of autotrophic nitrifiers and nitrification in fertilized forest soil. Soil Biology & Biochemistry, 17, 245 – 248.

Mhalla, B., Ahmed, N., Datta, S. P., Singh, M., Shrivastava, M., Mahapatra, S. K. & Moursy, A. R. (2019). Effect of topography on characteristics, fertility status and classification of the soils of Almora District in Uttarakhand*.* Journal of Indian Society of Soil Science, 67(3), 309-320.

Onikura, Y., Yoshino, T. & Maeda, K. (1975). Mineralization patterns of soil nitrogen during the growth period of rice plant. Journal of Science of Soil Manure, 46, 225 – 259. (In Japanese, with English abstract).

Prosser, J. I. & Cox, D. J. (1982). Nitrification. In Experimental Microbial Ecology Eds. R G Burns & J H Slater. pp. 178 – 193. Blackwell Scientific publication.

Ross, J. D., Speir, T. W., Tate, K. R. & Orchard, V. A. (1985). Effects of sieving on estimations of microbial biomass, and carbon and nitrogen mineralization in soil under pasture. Australian Journal of Soil Research, 23, 319 – 324.

Schimel, D. S. & Parton, W. J. (1986). Microclimatic controls of nitrogen mineralization and nitrification in shortgrass steppe soils*.* Plant & Soil, 93, 347 – 357.

Sethi, V., Kaushik, A. & Khatri, R. (1990). Soil dehydrogenase activity and nitrifier populations in relation to different soil plant associations. Tropical Ecology, 31,112 - 117.

Singh, H. & Singh, K. P. (1994). Nitrogen and phosphorus availability and mineralization in dryland reduced tillage cultivation: effects of residue placement and chemical fertilizer. Soil Biology & Biochemistry, 26, 695 – 702.

Singh, J. S., Singh, S., Raghubanshi, A. S., Singh, S. and Kashyap, A. K. (1996). Methane flux from rice/ wheat agroecosystem as affected by crop phenology, fertilization and water level. Plant and Soil, 183, 323 -327.

SPSS / PC 2002. SPSS for the IBM PC /XT /AT. SPSS Inc., Chicago, Illinois.

Sun, H. J., Zhang, H. L., Powlson, D., Min, J. & Shi, W. M. (2015). Rice production, nitrous oxide emission and ammonia volatilization as impacted by the nitrification inhibitor 2-chloro-6-(tri chloromethyl)-pyridine. Field Crops Research, 173, 1–7. https:// doi.org/10.1016/j.fcr.2014.12.012

Viro, P. J. (1962). Factorial experiments on forest humus decomposition. Soil Science, 95, 24 – 30.

Vitousek, P. M. & Matson, P. A. (1982). Causes of delayed nitrate production in two Indiana forests. Forest Science, 31, 122 – 131.

Wang, J., Zhao, Y., Zhang, J. B., Zhao, W., Müller, C. & Cai, Z.C. (2017). Nitrification is the key process determining N use efficiency in paddy soils. Journal of Plant Nutrition and Soil Science, 180(6), 648–658. https://doi.org/10.1002/jpln.201700130

Wang, X. L., Duan, P. L., Yang, S. J., Liu, Y. H., Qi, L., Shi, J., Li, X. L., Song, P. & Zhang, L. X. (2020). Corn compensatory growth upon post-drought rewatering based on the effects of rhizosphere soil nitrification on cytokinin. Agricultural Water Management, 241, 106436. https://doi.org/10.1016/j. agwat.2020.106436

Wells, N. S., Clough, T. J., Johnson-Beebout, S. E. & Buresh, R. J. (2014). Land management between crops affects soil inorganic nitrogen balance in a tropical rice system. Nutrient Cycling in Agroecosystems,100, 315–332. [**https://doi.org/10.1007/s10705-014-9644-7**](https://doi.org/10.1007/s10705-014-9644-7)

Woldendorp, J.W. & Laanbroek, H. J. (1989). Activity of nitrifiers in relation to nitrogen nutrition of plants in natural ecosystems. Plant and Soil, 115, 217-228.

Yang, Y. J., Zhang, H. P., Shan, Y.H., Wang, J. J., Qian, X. Q., Meng, T. Z., Zhang, J. B. & Cai, Z. C. (2019). Response of denitrification in paddy soils with different nitrification rates to soil moisture and glucose addition. Science of the Total Environment, 651, 2097–2104. <https://doi.org/10.1016/j.scitotenv.2018.10.066>

Yao, Y. L., Zeng, K. & Song, Y. Z. (2020). Biological nitrification inhibitor for reducing N2O and NH3 emissions simultaneously under root zone fertilization in a Chinese rice field. Environmental Pollution, 264, 114821. [**https://doi.org/10.1016/j.envpol.2020.114821**](https://doi.org/10.1016/j.envpol.2020.114821)

Zhang, H., Liao, F., Li, W., Li, Y., Yang, S., Zhang, H., Yang, Y. & Shan, Y. (2022). Rhizosphere soil nitrification ability controls nitrogen-use efficiency in rice growth period. Food Energy Security,12: e429. https://doi. org/10.1002/fes3.429

Zhang, J.B., Zhu, T.B., Cai, Z.C., Qin, S.W. & Muller, C. (2012). Effects of long-term repeated mineral and organic fertilizer applications on soil nitrogen transformations. European Journal of Soil Science, 63, 75–85. doi:10.1111/j.1365-2389.2011.01410.x.

Zhang, X., Wang, Q., Xu, J., Gilliam, F.S., Tremblay, N. & Li, C. (2015). *In situ* nitrogen mineralization, nitrification, and ammonia volatilization in maize field fertilized with urea in Huanghuaihai Region of Northern China. PLoS, ONE, 10, e0115649. doi:10.1371/journal.pone.0115649. PMID:25635864

Zhou, X., Wang, S. W., Ma, S. T., Zheng, X. K., Wang, Z. Y. & Lu, C. H. (2020). Effects of commonly used nitrification inhibitors dicyandiamide (DCD), 3.4-dimethylpyrazole phosphate (DMPP), and nitrapyrin-on soil nitrogen dynamics and nitrifiers in three typical paddy soils. Geoderma, 380, 114637. https:// doi.org/10.1016/j.geoderma.2020.114637

**Table 1.** Cropping season averages (±SE) for soil parameters measured in control and fertilized plots of rainfed rice. (n = 18).

|  |  |
| --- | --- |
| Soil Parameters | Treatment |
| Control | Urea | NH4 NO3 | NH4Cl |
| pH | 7.28 ± 0.01 a | 7.54 ± 0.10 b  | 7.39 ± 0.06 ab  | 7.30 ± 0.07 a |
| Mineral – N(µg g –1 dry soil) | 6.57 ± 1.08 a | 9.11 ± 1.10 b | 6.82 ± 0.99 a | 5.72 ± 1.35 a |
| N – mineralization(µg g-1 mo-1 dry soil) | 11.34 ± 2.27 b | 12.73 ± 2.60 a | 17.61 ± 3.37 a | 21.54 ± 3.65 c |
| Nitrification(µg g-1 mo-1 dry soil) | 3.50 ± 0.60 a | 2.97 ± 0.53 a | 3.77 ± 0.72 a | 4.75 ± 0.79 b |
| Ammonium oxidizers(MPN x 105 g-1 dry soil) | 0.18 ± 0.01 a | 0.25 ± 0.04 a | 0.30 ± 0.03 ab | 0.44 ± 0.05 c |
| Nitrite oxidizers(MPN x 105 g-1 dry soil) | 0.13 ± 0.01a | 0.17 ± 0.02 a | 0.22 ± 0.02 b | 0.37 ± 0.01 c |

Values in a row with different superscript letters are significantly different from each other at p < 0.05 according to Tukey’s HSD test.

**Table 2***.* One – way ANOVA results indicating effect of different fertilizer treatment on soil parameters (n = 72).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Source | Sum of squares | df | Mean square | F - value | Significance |
| **pH** |
| Between groups | 0.77 | 3 | 0.25 | 7.21 | 0.0001 |
| Within groups | 2.42 | 68 | 0.03 |  |  |
| Total | 3.19 | 71 |  |  |  |
| Mineral – N |
| Between groups | 113.27 | 3 | 37.76 | 5.26 | 0.003 |
| Within groups | 488.00 | 68 | 7.17 |  |  |
| Total | 601.283 | 71 |  |  |  |
| Nitrification |
| Between groups | 29.92 | 3 | 9.97 | 4.05 | 0.01 |
| Within groups | 167.27 | 68 | 2.46 |  |  |
| Total | 197.20 | 71 |  |  |  |
| N - mineralization |
| Between groups | 1180.97 | 3 | 393.65 | 7.97 | 0.0001 |
| Within groups | 3355.03 | 68 | 49.33 |  |  |
| Total | 4536.00 | 71 |  |  |  |

**Table 3.** Cropping season averages (±SE) for plant growth parameters, root porosity and grain yield at harvest measured in control and fertilized plots of rainfed rice. (n = 18, \* n = 9, \*\* n = 3).

|  |  |
| --- | --- |
| Plant growth parameters | Treatment |
| Control | Urea | NH4NO3 | NH4Cl |
| Root biomass (g hill-1) | 0.72 ± 0.16 a | 1.27 ± 0.35 a | 1.38 ± 0.38 a | 1.48 ± 0.39 ab |
| Shoot biomass (g hill-1) | 3.91 ± 1.42 a | 5.79 ± 1.74 a | 7.38 ± 2.33 a | 8.94 ± 3.21 ba |
| Tiller number (Tiller hill -1) | 7.16 ± 1.06 a | 8.33 ± 1.11 a | 7.22 ± 1.13 a | 7.00 ± 1.19 a |
| Root porosity (%) \* | 13.64 ± 1.24 a | 16.41 ± 1.82 a | 17.03 ± 2.18 ab | 23.47 ± 7.80 b |
| Grain yield (kg ha-1) \*\* | 300.16 ± 17.28 a | 1262.83 ± 266.93 ab | 1467.90 ± 345.76 ab | 1802.4 ± 251.52 b |

Values in a row with different superscript letters are significantly different from each other at p < 0.05 according to Tukey’s HSD test.

**Table 4.** One way ANOVA results indicating effect of different fertilizer treatment on nitrifying bacterial population, root biomass and root porosity (n = 72, \* n = 36).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Source | Sum of squares | df | Mean square | F - value | Significance |
| **Ammonium oxidizers** |
| Between groups | 0.67 | 3 | 0.22 | 15.92 | 0.0001 |
| Within groups | 0.96 | 68 | 0.01 |  |  |
| Total | 1.63 | 71 |  |  |  |
| Nitrite oxidizers |
| Between groups | 0.559 | 3 | 0.186 | 80.01 | 0.0001 |
| Within groups | 0.158 | 68 | 0.002 |  |  |
| Total | 0.718 | 71 |  |  |  |
| Root biomass |
| Between groups | 6.19 | 3 | 2.06 | 3.41 | 0.022 |
| Within groups | 41.08 | 68 | 0.60 |  |  |
| Total | 47.27 | 71 |  |  |  |
| Root porosity \* |
| Between groups | 466.66 | 3 | 155.55 | 5.98 | 0.002 |
| Within groups | 831.75 | 32 | 25.99 |  |  |
| Total | 1298.42 | 35 |  |  |  |

**Table 5***.* Correlation matrix among mineral - N (Min - N), rate of N - mineralization (N - min), nitrification (Nitrf.), ammonium oxidizers (A. Oxi.), nitrite oxidizers (N. oxi.), soil pH (pH), root biomass (Rb), shoot biomass (Sb), and root porosity (Rp), across control and fertilized plots (n = 72). \* significant at p < 0.05 \*\* significant at p < 0.01 and NS indicates that correlations are not significant at p < 0.05.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variable | Min - N | N - min | Nitrf. | A. oxi. | N. oxi. | pH | Rb | Sb | Rp |
| Min - N | 1a | - 0.47 \*\* | - 0.79 \*\* | - 0.24 \* | NS | NS | - 0.64 \*\* | - 0.57 \*\* | NS |
| N - min |  |  | 0.82 \*\* | 0.78 \*\* | 0.44 \*\* | NS | 0.45 \*\* | NS | NS |
| Nitrf. |  |  |  | 0.61 \*\* | NS | NS | 0.63 \*\* | 0.32 \*\* | NS |
| A. oxi. |  |  |  |  | 0.52 \*\* | NS | 0.46 \*\* | NS | 0.99 \*\* |
| N. oxi. |  |  |  |  |  | - 0.25 \* | NS | NS | 0.98 \*\* |
| pH |  |  |  |  |  |  | 0.37 \*\* | NS | NS |
| Rb |  |  |  |  |  |  |  | 0.77 \*\* | NS |
| Sb |  |  |  |  |  |  |  |  | NS |
| Rp |  |  |  |  |  |  |  |  | 1 |

a Note: Data are correlation coefficients (r-values).

Figure 1. Variation in mineral – N content in rainfed rice soil under different fertilizer treatment. Arrows indicate days of fertilization. Bars indicate ± S E.

Figure 2. Variation in rate of N - mineralization in rainfed rice soil under different fertilizer treatment. Arrows indicate days of fertilization. Bars indicate ± S E.

Figure 3. Variation in rate of nitrification in rainfed rice soil under different fertilizer treatment. Arrows indicate days of fertilization. Bars indicate ± S E.

Figure 4. Variation in viable population ammonium oxidizers in rainfed rice soil under different fertilizer treatment. Arrows indicate days of fertilization. Bars indicate ± S E.

Figure 5. Variation in viable population of nitrite oxidizers in rainfed rice soil under different fertilizer treatment. Arrows indicate days of fertilization. Bars indicate ± S E.