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Nitrogen Dynamics in Soil Treated with Plant-growth Promoting Bacteria and Urea Fertilizer

Amaily Akter[1], Ali Tan Kee Zuan[1]*, Susilawati Kasim[1], Adibah Mohd Amin[1], Zakry Fitri Ab Aziz[2], Noor Md Rahmatullah[3], Buraq Musa Sadeq[1], Sayma Serine Chompa[1] and Md Ekhlasur Rahman[1,4]

[1]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

[2]Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus, 97008, Sarawak, Malaysia

[3]Department of Agricultural Statistics, Faculty of Agribusiness Management, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

[4]Divisional Laboratory, Soil Resource Development Institute, Krishi Khamar Sarak, Farmgate, Dhaka-1215, Bangladesh

ABSTRACT

The mineralization of urea fertilizer significantly impacts nitrogen movement in the soil. An incubation study was done on a lab scale basis to examine nitrogen dynamics in soil inoculated with plant growth-promoting bacteria (PGPB) supplemented with varying levels of nitrogen fertilizer in the form of urea (0% N, 25% N, 50% N, 75% N, and 100% N). In the present experiment, sandy clay loam soil was used and incubated for four weeks, and the concentrations of NH_4^+–N and NO_3^-–N were monitored using the destructive method (Kjeldahl) to determine the mineralization rate of urea. Results showed higher NH_4^+–N (11.880 mg/kg mineralized with UPMRB9N50 treatment) and NO_3^-–N (20.060 mg/kg mineralized with UPMRB9N50 treatment) concentrations in the bacteria-treated soil compared to the uninoculated control. Urea-N remains higher (0.0353% and 0.0253% from UPMRB9N50 treatment in the first and second weeks, respectively) in bacteria-treated soil during the first two weeks, then gradually becomes zero towards the end of the observing period. Nitrogen (N) leaching loss was lower in bacterial inoculated soil compared to the control, and the leaching loss of N was greater with the

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E-mail addresses:
amailyakter2554@gmail.com (Amaily Akter)
tkz@upm.edu.my (Ali Tan Kee Zuan)
susilawati@upm.edu.my (Susilawati Kasim)
adibahamin@upm.edu.my (Adibah Mohd Amin)
zakryfitri@upm.edu.my (Zakry Fitri Ab Aziz)
rahmatnm@sau.edu.bd (Noor Md Rahmatullah)
buraqmosa1991@gmail.com (Buraq Musa Sadeq)
serinesayma@gmail.com (Sayma Serine Chompa)
ekhlasurrahman02@gmail.com (Md Ekhlasur Rahman)
* Corresponding author

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increased N fertilizer rates. Cumulative N leaching loss is higher (29.797 mg/kg) in 100% N-treated soil than in other treatments. The findings observed that the beneficial bacteria could enhance the N mineralization to make the nutrient available for the crop while, at the same time, reducing leaching losses of fertilizer when supplied with a minimum amount of chemical fertilizer, thereby saving the input cost and protecting the environment.

Keywords: Ammonium ion, nitrate ion, nitrogen leaching, nitrogen mineralization, plant growth-promoting bacteria

INTRODUCTION

A vital component of plants, nitrogen (N) is also a major component of genetic material, amino acids, chlorophyll, and adenosine triphosphate (ATP); it enhances agricultural productivity by 30–50% globally (Leghari et al., 2016). The primary source of N fertilizer that substantially affects agricultural productivity worldwide is urea (Kira et al., 2019). It is the most frequently utilized N fertilizer due to its simplicity and high N content (46%) (Motasim et al., 2021). About 73.4% of all nitrogen fertilizer used worldwide is urea (Heffer & Prud'homme, 2016). However, the worrisome issue with using granular urea fertilizer is its significant nitrogen loss and inefficient use of nitrogen fertilizer, which varies from 10% to 50% (Almaz et al., 2017). When urea is broadcast onto the field, more than 50% of the nitrogen in urea cannot be taken up by plants if fertilization management is not done properly (Rochette et al., 2009). It turns into a risk factor for environmental deterioration, which includes loss of stratospheric ozone, acidic precipitation, the excessive richness of nutrients in a lake or other body of water, water contamination, NH_3 volatilization loss, and N_2O emissions (Puga et al., 2020). A significant gaseous loss is reported when granular urea is applied to the surface (Lichiheb et al., 2019; Motasim et al., 2021) and nitrogen loss via leaching (Puga et al., 2020).

Due to these situations, people are beginning to learn about an additional or alternative greener approach (Ladha et al., 1997), and using soil microorganisms is one of the techniques. This eco-friendly method utilizes the beneficial microorganisms called plant growth-promoting rhizobacteria (PGPR), which promotes biological nitrogen fixation (BNF), inorganic phosphate solubilization, the synthesis of phytohormones, siderophores, and hydrolyzing enzymes to promote plant development and productivity (Ali-Tan et al., 2017). These microbes play a crucial role in the nitrogen transformation that enhances nitrification in the soil, which leads to an increase in NO_3^- production (Mandal et al., 2016). Nitrogen in the soil is relatively more stable in NO_3^- form than in NH_4^+ form (Wang et al., 2018). Loss of NH_4^+–N and NO_3^-–N in topsoil was positively correlated, according to (Shan et al., 2015). Ineffective techniques and without proper urea use management encourage nitrogen losses (Zhao et al., 2015). With the optimization of N fertilizer rates and the application of PGPB, it is possible to maximize crop productivity, minimize N losses, and improve mineralization and nutrient uptake. Only a few research have been reported

regarding the results of optimum N fertilizer levels with PGPB-treated soil, especially on the nitrogen mineralization on tropical acidic soils. Thus, this study aims to assess the N mineralization pattern that microbial inoculation affects to better understand and reduce urea–N losses from soils in tropical climates.

MATERIALS AND METHODS

Sampling and Preparation of Soils

In this study, sandy clay loam soil was used, and they were based on the USDA's soil classification (Table 1). The top 15 cm of the soil was sampled and air-dried in the drying room at the Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia. The soils were ground by laboratory pestle and mortar, followed by sieving with a 2.0 mm metallic sieve and stored in a clean container for analysis.

Table 1
Physicochemical characteristics of soils

Features of the soil	Values	References
USDA's class for soil texture	Sandy clay loam	
Sand (%)	52.36 ± 0.012	
Silt (%)	12.46 ± 0.012	(Teh & Talib, 2006)
Clay (%)	35.12 ± 0.006	
Moisture content at field capacity (%)	23.95 ± 0.006	(Richards & Fireman, 1943)
pH	4.91 ± 0.003	(Jones, 2001; Sharifuddin et al., 1990)
Total C (%)	2.31 ± 0.003	(LECO., 2018)
Total N (%)	0.199 ± 0.001	
CEC (cmol+/kg)	8.7 ± 0.115	(Chapman, 1965; Keeney & Nelson., 1982)
K (me/ 100g)	0.23 ± 0.003	
Ca (me/100g)	0.31 ± 0.012	
Mg (me/100g)	0.10 ± 0.003	
P (mg/kg)	39.71 ± 0.012	(Sharifuddin et al., 1990)

Experimental Design

The incubation study used a completely randomized design (CRD) with two factors: Nitrogen levels (0%, 25%, 750%, 75%, and 100%) and PGPB strains (*Bacillus subbtilis* and *Bacillus tequilensis*).

Characterization of the Soil

Analysis of Soil Particle Size. The particle size distribution of soil was analyzed by pipette method (Teh & Talib, 2006) with modifications. In a 1000 ml beaker, 20 g of sieved soil was taken, and H_2O_2 was added in a 50-milliliter amount and left overnight. After 24 hours,

the mixture was heated at 100°C for about one hour, and the remaining quantities of H_2O_2 were added until no frothing remained. In case of bubbling from the heated mixer, some drops of alcohol were added. It was followed by adding 0.2 N HCl in 50 milliliters and making the volume 200 milliliters by adding distilled water. After an hour, the mixture was allowed to cool and washed twice with distilled water of 200 milliliters. The mixture was then set on a mechanical agitator by adding 40 mL Calgon solution for 5 minutes, and then the mixture was passed through a 50 µm sieve for collecting sand fraction. Then, the mixture was transposed to a 1000-milliliter cylinder, and distilled water was used to get the volume at the mark. A hot water bath at 23°C was used to place the cylinders, and a plunger thoroughly mixed the solution for a minute. Then, the suspensions were left to settle for 7 hours; after that, a pipette was placed at 10 cm depth, and an aliquot was pipetted and transferred into a porcelain pot. These pots containing soil suspension were dried for 24 hours at 105°C in the oven, then transferred into a 200-milliliter desiccator and cooled before weighing. The previously collected sand fraction was also dried in the oven at 105°C, transferred to a desiccator, and allowed to cool before weighing.

The sand, silt, and clay percentages were calculated by the following Equations 1, 2, and 3:

$$\%\ Sand = weight\ of\ oven\ dried\ sand\ particles(g) \times \frac{100}{weight\ of\ the\ soil(g)} \quad [1]$$

$$\%\ Clay = \left\{\frac{weight\ of\ oven\ dried\ clay(g) \times 1000}{volume\ pipetted} - C\right\} \times \frac{100}{weight\ of\ the\ soil(g)} \quad [2]$$

$$\%\ Silt = 100 - (\%\ sand + \%\ clay) \quad [3]$$

where; C = weight of Calgon in the solution (g)
The textural classes were determined using the USDA textural triangle.

Bulk Density and Moisture Content Determination of Soils

The soil core method (Okalebo et al., 2002) was used during bulk density determination. A metallic core/ring (known as weigh-w_1 and volume-v) was inserted into the soil after removing the surface layer in the field. The ring was excavated around the soil, and cut excess soil beneath the ring, followed by the removal of excess soil at the ends of the ring with a knife. Immediately, the soil was dried for 24 hours at 105°C in the oven and weighed (w_2).

$$Bulk\ density, Db\ (^{g}/_{cm3}) = \frac{[w2\ (g) - w1\ (g)]}{v\ (cm3)} \quad [4]$$

For moisture content determination, in a porcelain pot, 10 g of soil that had been air-dried was weighed and dried in an oven for 24 hours at 105°C, and then the pot was cooled after transferring from the oven to the desiccator.

The following Equation 5 was used to determine the soil's moisture content:

$$Soil\ moisture\ content\ (\%) = \frac{[initial\ soil\ weight\ (g) - ovendried\ soil\ weight\ (g)]}{oven\ dried\ soil\ weight\ (g)} \times 100 \quad [5]$$

Determination of Water Content at Field Capacity

The water at field capacity was estimated using a pressure plate method (Richards & Fireman, 1943). A core sampler was used at the desired depth, and the pressure was maintained at 33 kPa. From the top, pound into the earth a core ring with a known weight of approximately 7.6 cm in diameter and 4.0 cm deep. Carefully remove the core ring from the ground; clean off any excess soil up to the level of the core ring's two ends. Take the core ring and use the plastic caps to seal the ends before bringing it to the lab for analysis. On the porous plates, place the retaining rings for 33 kPa pressure. Five pieces of nearly identical sizes should be created from the undisturbed core samples. One sample piece should be placed in a holding ring on a coarse wire mesh, and one piece should be placed on a porous plate for water saturation (0 bar pressure). Keep the water below the ring edge while soaking ceramic plates for 24 hours. Insert the plate containing the samples into the 33 kPa pressure chamber and attach the outlet tube. Apply pressure by closing the chamber. Equilibrium is attained when no more water outflows and opens the chamber by releasing the pressure slowly. Take away the samples from the chamber and take the weight. The samples should be oven-dried for 24 hours at 105°C, then weighed again.

Soil pH Determination

The pH of the soil was determined (Jones, 2001; Sharifuddin et al., 1990) 'by adding water into soil at a ratio of 1:2.5 (soil to water)', 50 milliliters of deionized distilled water and 20 g of soil were added to a 100 milliliters plastic vial, and the mixture was agitated for 30 minutes and allowed to be settled, and then pH value was measured using Metrohm827 pH meter, Metrohm AG, Switzerland.

Cation Exchange Capacity Determination of Soil

The leaching method determined the soil's cation exchange capacity (Chapman, 1965). A hundred ml of 1 M NH_4OAC was introduced to 10 g of soil sample in a leaching tube (pH 7). After discarding the leachate that had been collected, two rounds of 95% alcohol washing were performed on the soil to remove any remaining NH_4OAC. Then 100 ml of

0.1 N K_2SO_4 was added, the leachate was collected, and reading was taken following the distillation (Keeney & Nelson., 1982) method.

Total N, remaining N (%) and C Content of Soil

The initial total C, N content in soil and remaining N (%) after the end of the study periods in the soils were estimated by LECO's new TruMac CNS Macro Analyser (LECO., 2018), LECO FP-2000 (LECO Corp. Michigan, USA). By putting around 0.10 g of soil in a C-free combustion boat and burning it at 1350°C in an O_2 environment, the total (%) of C, N, and S was determined. The boat was inserted into the TruMac CNS analysis machine using an auto-sampler stand.

Preparation of Inoculants for Soil Application

The microorganisms UPMB10 and UPMRB9 were inoculated into a 250 ml conical flask containing 100 ml of tryptic soy broth (TSB). The flask was incubated after inoculation in an orbital shaking incubator (Model OSI-503 LD; Firstek Scientific, Japan) for 48 hours at 28°C with 150 rpm of shaking. A UV-Visible spectrophotometer set the optical density (OD_{600}) of the two strains to 1.

Treatments of the Soil Sample

There were fifteen treatments used for this incubation study, below mentioned as follows:

B0N0 = Control
B0N25 = Uninoculated control with 25% of nitrogen fertilizer applied @ 180 kg/ha
B0N50 = Uninoculated control with 50% of nitrogen fertilizer applied @ 180 kg/ha
B0N75 = Uninoculated control with 75% of nitrogen fertilizer applied @ 180 kg/ha
B0N100 = Uninoculated control with 100% of nitrogen fertilizer applied @ 180 kg/ha
UPMB10N0 = *Bacillus subtilis* (10^8 CFU/mL) inoculated without nitrogen fertilizer
UPMB10N25 = *Bacillus subtilis* (10^8 CFU/mL) inoculated with 25% nitrogen fertilizer applied @180 kg/ha
UPMB10N50 = *Bacillus subtilis* (10^8 CFU/mL) inoculated with 50% nitrogen fertilizer applied @180 kg/ha
UPMB10N75 = *Bacillus subtilis* (10^8 CFU/mL) inoculated with 75% nitrogen fertilizer applied @180 kg/ha
UPMB10N100 = *Bacillus subtilis* (10^8 CFU/mL) inoculated with 100% nitrogen fertilizer applied @180 kg/ha
UPMRB9N0 = *Bacillus tequilensis* (10^8 CFU/mL) inoculated without nitrogen fertilizer
UPMRB9N25 = *Bacillus tequilensis* (10^8 CFU/mL) inoculated with 25% nitrogen fertilizer applied @180 kg/ha

UPMRB9N50 = *Bacillus tequilensis* (10^8 CFU/mL) inoculated with 50% nitrogen fertilizer applied @180 kg/ha
UPMRB9N75 = *Bacillus tequilensis* (10^8 CFU/mL) inoculated with 75% nitrogen fertilizer applied @180 kg/ha
UPMRB9N100 = *Bacillus tequilensis* (10^8 CFU/mL) inoculated with 100% nitrogen fertilizer applied @180 kg/ha

Determination of N Mineralization of Soil

Finely ground and air-dried samples were analyzed for NH_4^+–N, NO_3^-–N, and urea–N concentration after extracting the soil with 2M potassium chloride-phenyl mercuric acetate (KCl-PMA) solution. The soil surface in the 100 cm^3 plastic pots received the 15 treatments listed below. Fifty grams of sieved, air-dried soil was left open to preserve the aerobic environment. Adding water to retain the initial weight allowed the moisture content to be kept at a field-capacity level throughout the observing period. Weekly analyses of the N mineralization were conducted using destructive methods (Junejo et al., 2011; Keeney & Nelson., 1982). This procedure involved extracting 20 g of soil with 40 mL of a potassium chloride-phenyl mercuric acetate (KCl-PMA) solution, distilling it with a micro-Kjeldahl steam distillation unit, titrating it against a solution of 0.01 N HCl, and then determining the amount of mineral N (Keeney & Nelson, 1982) and urea-N (using a colorimetric method) (Douglas & Bremner, 1970).

Urea-N Determination of Soil Samples

Twenty grams of soil were extracted with the help of 40 mL of KCl-PMA solution to determine the amount of urea-N. Di-acetyl monoxime and Thio-semi-carbazide were used to color the solution, and the intensity of color was evaluated using a calibrated spectrophotometer at 528 nm wavelength (Douglas & Bremner, 1970).

Leaching Loss of N Determination in Soil

The 100 g of sieved, air-dried soil used in the leaching investigation was placed in leachate tubes (10 cm in diameter and 60 cm in height). The bottom of the leachate tubes was closed with ash flock and filter paper to allow only liquid to be leached. In the soil sample, twenty-eight soil columns with 8 cm depth were made by adding the dried, ground, and 2.00 mm sieved soil samples. Fifteen levels of treatments were added to soil samples. A blank treatment was used for the study. The soil columns were moistened by adding distilled water overnight, and the field-capable moisture level remained. After two days of treatment, a hundred mL of distilled water was added, and the leachate was kept until 10 volumes of pore (Zadeh, 2010). From the leachate, 10 mL was taken for analysis using a distillation plant, and steam was collected in a boric acid solution. The trapped solution

was titrated against 0.01N HCL solution. The leached solutions were analyzed for NH_4^+–N and NO_3^-–N concentration (Keeney & Nelson., 1982). The incubation room's temperature was kept at 25 ± 0.5°C throughout the investigation. The experimental units were set up in a completely randomized design with three replicates, and the experiment was carried out as a complete factorial design.

Analysis of Statistics

The data was statistically examined using ANOVA analysis by Statistical analysis software (SAS) 9.4 (SAS Institute Inc., Cary, NC, USA, 2013) at a 5% level of confidence; the treatment means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS

Effects of Treatments on NH_4^+–N and NO_3^-–N Concentrations in Soil

An increase in NH_4^+–N and NO_3^-–N concentrations were observed in B0N100, UPMRB9N50, and UPMB10N50 treated soils compared to other treatments (Tables 2 and 3). The concentrations of NH_4^+–N declined, whereas concentrations of NO_3^-–N were higher with the increased incubation time, and concentrations of both were higher with

Table 2
NH_4^+-N concentration (mg/kg) in soil treated with different levels of N with 2 strains of PGPB throughout the observing period of four weeks

Treatments	NH_4^+–N concentrations (mg/kg)			
	1st week	2nd week	3rd week	4th week
B0N0	2.127n	1.257n	0.0827m	0.085i
B0N25	3.017k	2.177k	1.443j	0.383g
B0N50	6.643g	4.803g	3.157g	0.843f
B0N75	8.123d	5.873d	3.863d	1.033d
B0N100	10.507c	7.593c	4.987c	1.333c
UPMB10N0	2.187m	1.583m	1.040l	0.283h
UPMB10N25	6.973f	5.027f	3.313f	0.883ef
UPMB10N50	11.243b	8.117b	5.353b	1.417b
UPMB10N75	3.113i	2.250i	1.483i	0.393g
UPMB10N100	3.053j	2.203j	1.453j	0.383g
UPMRB9N0	2.227l	1.607l	1.057k	0.283h
UPMRB9N25	7.303e	5.267e	3.467e	0.923e
UPMRB9N50	11.880a	8.583a	5.653a	1.500a
UPMRB9N75	3.227h	2.333h	1.527h	0.407g
UPMRB9N100	3.113i	2.253i	1.483i	0.393g

Note. Using Duncan's multiple range test (DMRT) at the 0.05 confidence level, different letters within a column indicate significant variations between means

Table 3
NO_3^-–N concentration (mg/kg) in soil treated with different levels of N with 2 strains of PGPB throughout the observing period of four weeks

Treatments	NO_3^-–N concentrations (mg/kg)			
	1st week	2nd week	3rd week	4th week
B0N0	1.4867n	1.4933n	1.5033n	1.4767d
B0N25	3.3933k	4.2533k	5.1033k	4.9267c
B0N50	7.4567g	9.3533g	11.2167g	10.8533b
B0N75	9.1233d	11.4333d	13.7167d	13.2567b
B0N100	11.8033c	14.7933c	17.7467c	17.1567a
UPMB10N0	1.5533m	1.5633m	1.6167m	1.5400d
UPMB10N25	7.8333f	9.8167f	11.7833f	11.3933b
UPMB10N50	12.6267b	15.8267b	18.9867b	18.3567a
UPMB10N75	3.4933i	4.3833i	5.2533i	5.0833c
UPMB10N100	3.4333j	4.3033j	5.1633j	4.9857c
UPMRB9N0	1.5867l	1.6000l	1.6533l	1.5757d
UPMRB9N25	8.2067e	10.2933e	12.3433e	11.9367b
UPMRB9N50	13.3333a	16.1333a	20.0600a	17.3933a
UPMRB9N75	3.6333h	4.5533h	5.4633h	5.2767c
UPMRB9N100	3.4933i	4.3833i	5.2533i	5.0767c

Note. Using Duncan's multiple range test (DMRT) at the 0.05 confidence level, different letters within a column indicate significant variations between means

more fertilizer-N applied. Among the treatments, NH_4^+–N and NO_3^-–N concentrations were greater in bacteria-treated soil than in the uninoculated control. The concentrations of NH_4^+–N were greater in the first week and then gradually declined afterward, which is true in all treatments. As opposed to that, the concentrations of NO_3^-–N were lower in the first week, then increased significantly and peaked in the third week. Similar results were found in both soils, with and without bacterial inoculations. In bacteria-treated soil, the amount of NH_4^+–N concentrations and level of urea mineralization (%) in the soils were greater during the initial incubation and then became zero incubation in the fourth week (Table 2). Soil applied with 50% of fertilizer–N and inoculated with UPMRB9 showed 15.15%, 10.95%, 7.21%, and 1.91% of mineralized urea into NH_4^+–N in the week of the first, second, third, and fourth of incubation respectively which, when compared to all treatments, were the highest. Similar patterns were observed with the inoculation of UPMB10. This study revealed that the application of 50% fertilizer–N along with bacterial inoculations mineralized the highest N amount compared to other treatments, and interestingly, the mineralization rate became lower with the increase in N rate (Tables 2 and 3). The highest NO_3^-–N mineralization rate was recorded in soil treated with UPMRB9 supplied with 50% fertilizer-N. The highest mineralization rates of NH_4^+–N and NO_3^-–N occur in the first and third weeks, respectively (Tables 2 and 3).

Effect of Treatments on Remaining Urea-N in the Soil (%)

With time, less urea–N was left in the soil. The soil treated with bacteria had a larger amount of urea–N remaining compared to the uninoculated treatments. The quantities of remaining urea–N in both bacteria and without bacteria-treated soils were high in the first week of incubation and afterward became lower towards the end of the incubation (Table 4). During the first week of incubation, treatments with UPMB10 and UPMRB9 with 50% N recorded 0.0333% and 0.0353% of N remained in the soil, whereas the control with 100% N recorded 0.0307% of urea-N remained, all three are significantly higher than other treatments. A similar pattern was observed throughout the incubation period.

Table 4
Urea-N remaining (%) in the soil treated with different levels of N with bacteria throughout the observing period of four weeks

Treatments	Urea-N remaining (%)			
	1st week	2nd week	3rd week	4th week
B0N0	0.0000j	0.0000j	0.0000h	0.0000f
B0N25	0.0093i	0.0073i	0.0050g	0.0023e
B0N50	0.0203h	0.0137h	0.0083f	0.0033d
B0N75	0.0243d	0.0167de	0.0103e	0.0037d
B0N100	0.0307c	0.0223c	0.0147c	0.0050bc
UPMB10N0	0.000j	0.0000j	0.0000h	0.0000f
UPMB10N25	0.0213fg	0.0153g	0.0103e	0.0033d
UPMB10N50	0.0333b	0.0243b	0.0167b	0.0063b
UPMB10N75	0.0227def	0.0170de	0.0110de	0.0050c
UPMB10N100	0.0217efg	0.0157fg	0.0103e	0.0040d
UPMRB9N0	0.000j	0.0000j	0.0000h	0.0000f
UPMRB9N25	0.0217efg	0.0163ef	0.0110de	0.0040d
UPMRB9N50	0.0353a	0.0253a	0.0177a	0.0093a
UPMRB9N75	0.0237de	0.0173d	0.0117d	0.0057bc
UPMRB9N100	0.0233ef	0.0170de	0.0110de	0.0053c

Note. Using Duncan's multiple range test (DMRT) at the 0.05 confidence level, different letters within a column indicate significant variations between means

N Leaching (NH_4^+–N and NO_3^-–N) from Soil

The NH_4^+–N and NO_3^-–N leaching loss were greater in the treatment with just fertilizer–N alone than in inoculated soil. The amount of leached N is higher as the amount of N applied is higher (Table 5 and Figure 1). Leachate was greater in the second and third pores and significantly reduced in bacteria and without bacteria-treated soil. In the fertilizer-treated soil, NH_4^+–N leaching loss was low in the first two pores, which is insignificant. In the third to fifth pore, noticeably greater quantities of NH_4^+–N were lost by leaching

Table 5
Leaching loss of NH_4^+-N (mg/kg) in soil treated with different levels of N with bacteria in 10 pore volumes

Treatments	Leaching loss of NH_4^+-N (mg/kg) in soil									
	1st pore	2nd pore	3rd pore	4th pore	5th pore	6th pore	7th pore	8th pore	9th pore	10th pore
B0N0	0.195l	0.382m	0.416g	0.366l	0.344m	0.322j	0.304j	0.304j	0.304j	0.304j
B0N25	0.391i	0.458j	2.326defg	1.424i	0.522j	0.321kl	0.301k	0.301k	0.301k	0.301k
B0N50	0.861f	1.008g	5.118bcd	3.134f	1.15g	0.690g	0.662g	0.662g	0.662g	0.662g
B0N75	1.053d	1.232d	6.247ab	3.832c	1.406d	0.844d	0.809d	0.809d	0.809d	0.809d
B0N100	1.363a	1.594a	8.097a	4.958a	1.819a	1.092a	1.046a	1.046a	1.046a	1.046a
UPMB10N0	0.192lm	0.372n	0.412g	0.354m	0.337n	0.314k	0.297l	0.297l	0.297l	0.297l
UPMB10N25	0.372j	0.435k	2.21efg	1.353j	0.496k	0.297m	0.285n	0.285n	0.285n	0.285n
UPMB10N50	0.801g	0.937h	4.76bbcde	2.915g	1.063h	0.642h	0.615h	0.615h	0.615h	0.615h
UPMB10N75	1.05d	1.195e	6.071ab	3.717d	1.364e	0.819e	0.785e	0.785e	0.785e	0.785e
UPMB10N100	1.347b	1.575b	8.003a	4.898b	1.797b	1.079b	1.033b	1.033b	1.033b	1.033b
UPMRB9N0	0.188m	0.367o	0.408g	0.352m	0.333n	0.308l	0.291m	0.291m	0.291m	0.291m
UPMRB9N25	0.352k	0.412l	1.531fg	1.281k	0.47l	0.282n	0.270o	0.270o	0.270o	0.270o
UPMRB9N50	0.749h	0.877i	3.105cdefg	2.726h	1.001i	0.600i	0.575i	0.575i	0.575i	0.575i
UPMRB9N75	0.977e	1.146f	4.017bcdef	3.564e	1.308f	0.785f	0.752f	0.752f	0.752f	0.752f
UPMRB9N100	1.322c	1.546c	5.372abc	4.958b	1.764c	1.053c	1.015c	1.015c	1.015c	1.015c

Note. Using Duncan's multiple range test (DMRT) at the 0.05 confidence level, different letters within a column indicate significant variations between means

through fertilizer-treated soil compared to bacteria-treated soil, with the same levels of fertilizer–N application (Table 5). The greatest total NH_4^+–N concentrations of NH_4^+–N were observed in leachate from B0N100 treated soil (23.109 mg/kg) compared to treatments of UPMB10N100 and UPMRB9N100 at (22.834 mg/kg) and (22.023 mg/kg) respectively (Table 6). The NO_3^-–N leachate was greater in the second pore volumes and drastically reduced afterward. In the first three pore volumes, noticeably more NO_3^-–N was leached through fertilizer-treated soil, and the cumulative loss was greater with the increase in fertilizer-N application rates (Figure 1).

Table 6
The cumulative leaching loss of N (NH_4^+-N and NO_3^-–N) from soil

Treatments	NH_4^+–N (mg/kg)	NO_3^-–N (mg/kg)
B0N0	3.244 ± 0.006 f	0.622 ± 0.005m
B0N25	6.639 ± e	1.919 ± 0.005j
B0N50	14.608 ± c	4.229 ± 0.003g
B0N75	17.852 ± b	5.169 ± 0.007d
B0N100	23.109 ±0.006 a	6.688 ± 0.009a
UPMB10N0	3.172 ± 0.006f	0.574 ± 0.005n
UPMB10N25	6.305 ± 0.006e	1.823 ± 0.005k
UPMB10N50	13.580 ± 0.013cd	3.930 ± 0.005h
UPMB10N75	17.358 ± 0.007b	5.016 ± 0.005e
UPMB10N100	22.834 ± 0.003a	6.610 ± 0.005b
UPMRB9N0	3.122 ± 0.006f	0.538 ± 0.004o
UPMRB9N25	5.411 ± 0.568ef	1.726 ± 0.004l
UPMRB9N50	11.360 ± 1.354d	3.897 ± 0.003i
UPMRB9N75	14.807 ± 1.813c	4.809 ± 0.006f
UPMRB9N100	20.023 ± 2.500b	6.490 ± 0.007c

Note. Using Duncan's multiple range test (DMRT) at the 0.05 confidence level, different letters within a column indicate significant variations between means

Total N (%) Remaining in Soils

The total N remaining in the soil was greater in the inoculated treatment than in fertilizer—the amount of N still in the soil correlated with the amount of urea applied. The greatest quantities of remaining N were revealed in soil treated through UPMRB9 with 100% N (0.202%), significantly higher than other treatments. The smallest quantities were found through 25% N with and without bacterial inoculations (Figure 2).

DISCUSSION

Applying urea to soils causes it to go through hydrolysis, which produces NH_4^+ and HCO_3^- (Mariano et al., 2019). The urease enzyme catalyzes the entire reaction. According

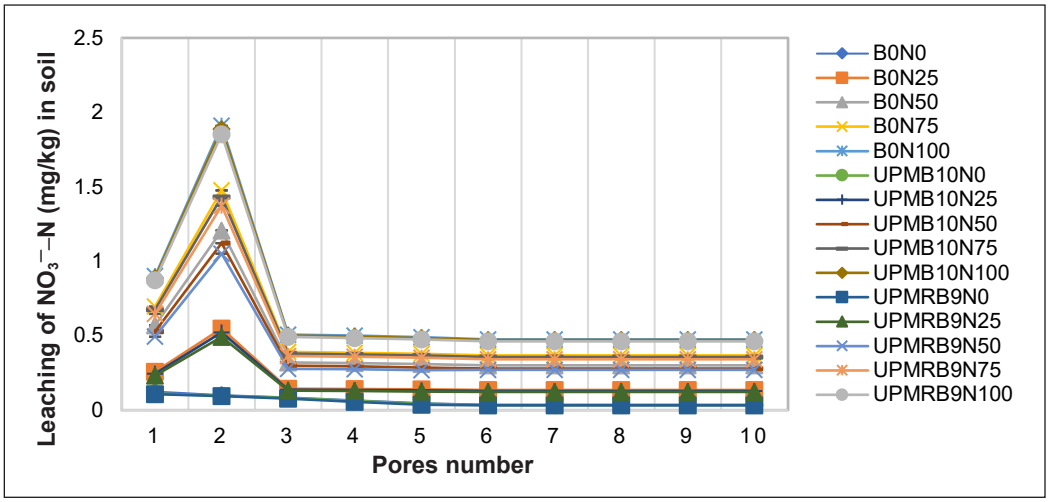

Figure 1. Leaching loss of NO_3^--N (mg/kg) in soil treated with different N levels with bacteria in 10 pore volumes. Using Duncan's multiple range test (DMRT) at the 0.05 confidence level, the standard errors are displayed as vertical bars on the graphs

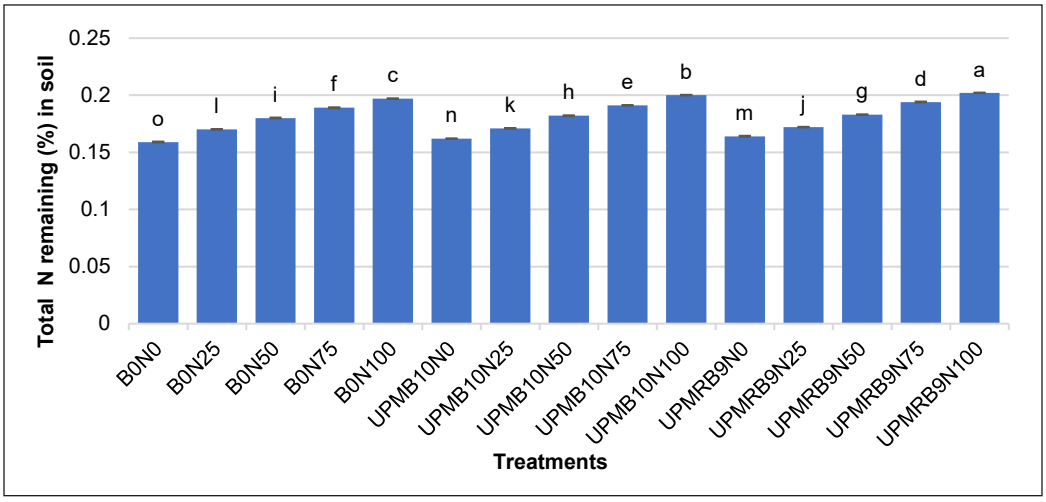

Figure 2. Total remaining N (%) in soil applied with different levels of N fertilizer with bacteria at the end of 30 days of observing period. Using Duncan's multiple range test (DMRT) at the 0.05 confidence level, different letters within a column indicate significant variations between means. The standard errors are displayed as vertical bars on the bar chart

to reports, urea hydrolysis was said to be initiated by soil moisture (Abera et al., 2012). Depending on the soil characteristics, the NH_4^+ can either be absorbed by soil colloids or converted further to NO_3^- and NH_3. Within a few days, there is quick hydrolysis, and during the first four days following treatment, 80% of the urea that has been applied can be hydrolyzed (Bundy, 2001; Cardenas et al., 2013). Urea mineralization in PGPB-treated soil was faster compared to without bacterial inoculation. The soil treated with PGPB has

evenly distributed urea throughout it since it is liquid (PGPB applied as broth where urea gets H_2O to transform and soil urease enzyme able to release available form of NH_4^+). As a result, the soil colloids absorb more NH_4^+, which may prevent NH_4^+ to NH_3 conversion (Rochette et al., 2013). Increased NH_4^+–N conversion to increase N volatilization loss is promoted by surface application of urea in soil (Rochette et al., 2009). The process of urea mineralization had an impact on how much urea-N was still present in the soils. If NH_4^+–N mineralization is increased and the nitrification process is quicker, the amount of applied urea-N that remains in the soil decreases (Junejo et al., 2011). Compared to soil treated with N-fertilizer alone, the soil treated with bacteria had a faster rate of N and higher NH_4^+-N mineralization, hence less urea-N left in the soils. Unlike uninoculated treatments, bacteria-treated soil had more NH_4^+–N and NO_3^-–N concentrations.

According to the findings of our investigation, soil treated with uninoculated treatments lost considerably more NH_4^+–N through leaching than soil treated with PGPB ($p \leq 0.05$). More NH_4^+–N concentration was present in the first few pore volumes because more urea hydrolysis in the first few days after treatment was applied before declining (Cardenas et al., 2013). Faster urea breakdown encourages greater N leaching (Gioacchini et al., 2002; Zuki et al., 2020). In contrast to the bacteria-treated soil, where urea distribution was more uniform, the urea in the uninoculated soil is more localized. The leaching loss was greater in the soil treated with fertilizer because the urea was more concentrated across a smaller soil area (Omar et al., 2015). In contrast to bacteria-treated soil, the amount of NH_4^+–N leached from uninoculated soil was greater due to the lack of bacterial inoculation, leading to a lesser and slower nitrification process (Table 6). The nitrification process can convert a lot of NH_4^+–N into NO_3^-–N. In contrast to relatively heavy textured (clay loam) soil, (Gioacchini et al., 2002) observed that increased N leaching occurred in light textured (sandy loam) soil.

The soil treated with fertilizer alone leached more NO_3^-–N compared to soil treated with PGPB, and with an increase in the rate of urea applications, the NO_3^-–N leaching also rose. In the second pore volume of the incubation research, NO_3^-–N leaching was found to be at its maximum because of the more concentrated urea in the uninoculated applied soil; increased NO_3^-–N leaching was induced (Ma et al., 2019) than PGPB treated soil, which lower NO_3^-–N resulted from the more urea even distribution throughout the soil. It was associated with increased NH_3 volatilization and soil pH (Motasim et al., 2021). Unlike bacteria-treated soil, uninoculated soil lost more of the total N (NH_4^+–N and NO_3^-–N) through leaching (Table 6). The remaining total N was greater in the inoculated treatments; possibly, the soil can absorb more NH_4^+–N. The findings of this investigation indicate that the bacteria-treated soil could retain more NH_4^+–N and NO_3^-–N concentrations, as a result, reduces loss of leaching due to more mineralization of N in the PGPB-treated soil and increased soil particle adsorption of NH_4^+–N.

CONCLUSION

According to our research findings, the beneficial bacteria UPMRB9, along with 50% of N fertilizer from the recommended rate, prove to be a better fertilization combination, mainly due to the increased NH_4^+–N and NO_3^-–N concentrations and reduced loss of leaching, compared to the single urea fertilizer treatment. The findings provide the opportunity and benefits of a better NUE, lower N losses, and reduced input cost while keeping the environment safe. These preliminary findings should be validated with a series of glasshouse and field trials for further confirmation.

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