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Nitrogen Release in Soils Amended with Different Organic and Inorganic Fertilizers under Contrasting Moisture Regimes: A Laboratory Incubation Study

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Abstract: Understanding nitrogen (N) release patterns and kinetics is a key challenge for improving N use efficiency in any agroecosystem. An incubation experiment was done to study the N release pattern and kinetics of contrasting soils amended with compost (CO), poultry manure (PM), rice husk biochar (RHB), poultry manure biochar (PMB) and cowdung (CD) combined with chemical fertilizer (integrated plant nutrient system, IPNS approach) under two moisture regimes, viz. field capacity (FC) and continuous standing water (CSW) at 25 °C for 120 days. Our results revealed that NH_4^+ -N was the dominant under CSW conditions, whereas NO_3^- -N was dominant under FC conditions. Net mineral N data fitted well to the first order kinetic model. Both N release potential (N_0) and rate constant (k) were greater in acidic soil than those of charland soil. The maximum N release varied between 24.90–76.29% of input depending on soil type and moisture status. N mineralization was strongly correlated with urea N application. PM and PMB mineralized in all soil and moisture conditions whereas N immobilization was observed in the case of RHB. N mineralization was strongly correlated with urea N application. Gaseous N losses were different for the organic amendments exhibiting more gaseous N losses in PM, CD and CO based IPNS whereas the lowest gaseous N loss was observed in PMB based IPNS. Biochar based IPNS increased soil pH in all conditions. Thus, the present study suggests that N release depends on soil type, soil moisture and type of organic amendment. However, CO, PM and CD based IPNS can be recommended for both acidic and charland soils in terms of N release as short duration crops will suffer from N deficiency if biochar based IPNS is used in the field.

Keywords: biochar; nitrogen release; N release kinetics; integrated plant nutrient system; organic amendment; field capacity; continuous standing water; soil pH

1. Introduction

In Bangladesh, SOM content is dwindling day by day as a result of inadequate management, intensive manipulation, and excessive use of chemical fertilizer. Acidic and charland soils in Bangladesh are two deteriorated soils with poor organic matter and nutritional status, posing a hazard to crop productivity on approximately 36% of the country's land [1,2]. In order to improve crop yields, minimize contamination, and attain sustainable farming, fertility in these soils must be maintained or restored to a sufficient level [3,4]. An

integrated plant nutrient system (IPNS) is an approach that allows for the applying of nutrients from both organic and inorganic sources to reduce the application of synthetic fertilizers, achieve a close fit between nutrient supplies and plant nutrient demands, maintain soil health, and provide a continuous nutrient supply to plants. Organic amendment (OA) is an important nutrient supplier to the plants and a means of soil rejuvenation [5,6]. According to some researchers, organic amendments are most effective when combined with mineral fertilizers [7,8]. OA, especially cowdung (CD), poultry manure (PM) and biochar (BC), improves soil physical properties [9,10], chemical properties [11,12], and biological properties [13].

Nitrogen (N) is one of the most important yet scarce elements in all ecological systems, and it influences ecosystem functioning significantly [14,15]. The conversion of N from organic to inorganic form is known as mineralization, and the reversal of the process is known as immobilization [16]. The net mineralization or net immobilization of the resultant consequences of these two processes determines the nitrogen supply to growing crops. Many factors influence mineralization processes, including organic matter source, soil properties [17,18], crop management [19], and other soil environmental factors, particularly temperature, moisture, and soil pH [20,21]. Lehmann and Joseph [22] described biochar as a carbon-rich substance produced from the pyrolysis of organic material at low temperatures. Biochar can modulate soil N dynamics by boosting nitrification [23], lowering denitrification [24], adsorbing NH_4^+ and reducing ammonia volatilization [25,26], minimizing N_2O emission [24,27] and enhancing crop's N bioavailability [25]. However, biochar application has not always enhanced N release [28,29], but was also found to have little or no effect [28–30], or to even reduce N release [31,32]. These incoherent results may be due to the differences in biochar feedstock, the pyrolytic condition, soil properties, or various other underlying processes as reported previously. The soil moisture regime is one of the key factors affecting the N mineralization process [33]. Soil moisture affects N release through regulating oxygen consumption rates, as well as microorganism specific growth rates and enzymatic activity [34]. Field capacity conditions of soil moisture favor N mineralization, resulting in greater mineral N release compared to the saturated or continuous standing water conditions [35,36]. Guntiñas et al. [37] reported that soil moisture at 80% of the field capacity was optimum for N mineralization. In contrast, Ono [38] postulated that flooding increased soil pH as well as N mineralization. Therefore, the effect of soil moisture on N mineralization is still a controversy and needs further research to reach a conclusion.

Previous research on N mineralization dynamics was mostly focused on N release from only OAs in agricultural ecosystems, such as livestock manures, plant residues, and composts [35,36,39]. As OAs differ considerably in composition and degree of stability, and their ability to release nutrients is frequently overlooked or exaggerated, resulting in nutrient shortages or plant toxicity [40]. Nutrients in organic chemical structures are generally complex; this implies that organic amendments can serve as a slow-release fertilizer, and the amount of nutrients released is highly dependent on the rate and degree of mineralization. The mineralization process is aided by the use of chemical fertilizer as it lowers the C:N ratio of the soil [41]. IPNS is necessary for improving nutrient availability as sole application of OAs may cause nutrient shortage and sole application of chemical fertilizer enhances plant toxicity and environmental hazards [40]. It is still unclear how much of the applied N (i.e., IPNS basis) will be available to the current crop, when to apply IPNS for synchronization of N release and plant requirements, and how environmental factors influence N release in acidic and charland soils. Thus, the present laboratory incubation study was designed to (1) evaluate the N release pattern and kinetics of different IPNS treatments in two degraded N poor soils of Bangladesh under different moisture regimes; (2) measure the changes in soil total nitrogen (STN) and soil mineral N after the addition of various IPNS treatments during the incubation period; and (3) investigate the subsequent changes in soil pH as a result of IPNS treatments throughout the incubation period.

2. Materials and Methods

2.1. Soil Sampling and Preparation

24 soil samples (8–10 kg each) for both acidic soil and charland soil were collected at 0–15 cm depth from an agricultural field of 0.072 ha. Acidic soil samples were collected from Madhupur, Mymensingh (24°59.82' N, 90°03.99' E) and charland soil samples from Islampur, Jamalpur (25°80.73' N, 89°81.90' E). Madhupur belongs to the Madhupur Tract (AEZ-28) and Islampur to the Old Brahmaputra Floodplain (AEZ-9). The general soil type of the acid soil is a deep red to brown terrace soil [42] and the charland soil is a Non-Calcareous Dark Grey Floodplain Soil [42]. In the laboratory, 24 soil samples were mixed to make a composite soil sample in each type and spread on brown paper to air dry. To remove roots and other debris, the air-dry soil was crushed and passed through a 2-mm sieve. After sieving, the soil was kept in a polyethylene bag and water was added to wet the soil to 50–72% of water holding capacity, and then incubated aerobically for three weeks at 25 °C in order to reduce the effect of handling on soil microbial respiration before the start of the incubation experiment. Table 1 shows the physicochemical properties of the soils under experiment.

Table 1. Physical and chemical properties of the soils.

Characteristics	Acidic Soil	Charland Soil
Mechanical fractions (USDA system)		
Sand (0.2–0.02) mm %	34.24	58.91
Silt (0.02–0.002 mm) %	35.95	31.95
Clay (<0.002 mm) %	29.81	9.14
Textural class	Clay loam	Sandy loam
Bulk density (g cm ⁻³)	1.18	1.29
Organic matter (%)	1.84	0.88
Water holding capacity (%)	24.10	17.28
pH	5.45	6.56
Total N (g kg ⁻¹ soil)	0.90	0.50
Available P (mg kg ⁻¹)	7.37	6.95
Exchangeable K (mg kg ⁻¹)	70.20	62.40
Available S (mg kg ⁻¹)	13.26	19.50

2.2. Collection of Organic Materials

Five types of organic materials, viz. compost (CO), poultry manure (PM), rice husk biochar (RHB), poultry manure biochar (PMB) and cowdung (CD) were used in this study. Compost was collected from Mazim Agro Industries Ltd., poultry manure from a poultry farm of Bangladesh Agricultural University (BAU), rice husk (mainly unfilled grains) from the warehouse of the BAU farm, and cowdung was collected from the dairy farm of BAU, Mymensingh. Rice husk biochar and poultry manure biochar were prepared at the Soil Science Field Laboratory, BAU. Biochars were produced by an anaerobic pyrolysis process at 400 °C for 2–2.5 h. The manures were air dried to 15% moisture content, pulverized and sieved with a 2-mm mesh. Table 2 shows the chemical compositions of the manures.

Table 2. Chemical properties of different organic amendments.

Manure	C (%)	N (%)	P (%)	K (%)	S (%)	C:N	C:P	C:K	C:S	pH	CEC (meq/100 g)
CO	15.27	0.61	0.08	0.11	0.04	25.0	190.9	138.8	381.8	7.3	10.07
PM	28.56	1.85	0.08	0.10	0.08	15.4	357.0	285.6	357.0	8.3	12.29
RHB	38.62	1.57	0.11	0.13	0.11	24.6	351.1	297.1	351.1	7.5	19.54
PMB	47.76	2.74	0.14	0.14	0.13	17.4	341.1	341.1	367.4	8.5	35.68
CD	25.02	1.40	0.09	0.10	0.09	17.9	278.0	250.2	278.0	7.6	14.11

i.e., CO = compost; PM = poultry manure; RHB = rice husk biochar; PMB = poultry manure biochar; and CD = cowdung.

2.3. Incubation Experiment

The experiment was laid out in a factorial design with three replications. The experiment comprised three main factors, i.e., soil type (acidic soil and charland soil), moisture level (field capacity and 1 cm of standing water) and treatments. Treatments included compost + chemical fertilizers, poultry manure + chemical fertilizers, rice husk biochar + chemical fertilizers, poultry manure biochar + chemical fertilizers, and cowdung + chemical fertilizers. Organic amendments were applied at 6 t ha⁻¹ (4 g kg⁻¹ soil). All of the treatments were applied at a rate of 120 mg N kg⁻¹ soil on an N-equivalent basis. N was applied from both organic and chemical fertilizer (i.e., urea) in all the treatments. Table 3 is the summary table of the N sources from various treatments. P, K, and S were also added in the form of triple superphosphate (TSP), muriate of potash (MoP) and gypsum fertilizer (dry), respectively, at rates of 10.67, 50.67, and 4 mg P, K, and S kg⁻¹ soil, respectively, to each plastic container. Altogether, there were twenty treatments [2 × 2 × 5 (two moisture levels for each soil)] with three replications of each. Unamended soil was incubated as control, to calculate net N mineralization by subtracting amount of mineral N in amended soils with amount if mineral N in control. A total of 250 g soil was weighed into a succession of plastic containers on an oven dry weight basis. During the entire incubation period, 648 plastic containers were used (i.e., 540 for the treatments and 108 for control (unamended soil)). The organic additions were fully incorporated into the soil (except control). To stabilize microbial activity, the soil moisture content was adjusted 1 week before to actual incubation to maintain field capacity (22–25% moisture) and 1 cm standing water conditions. The soils in the plastic containers were incubated at room temperature (25 °C) for up to 120 days. A polythene covering was placed over each container to avoid evaporation and quick loss of water. Every other day, the containers were weighed, and the weight loss was supplemented with distilled water. Incubation period involved nine sampling dates, i.e., 0, 3, 7, 15, 21, 30, 60, 90, and 120 days (after amendments application). For N quantification, destructive sampling was used to take a series of samples at each sampling date. The results were adjusted for moisture content and expressed on a dry weight basis determined in the oven.

Table 3. Summary of the nitrogen source from different treatments used in the experiment.

Treatment Combination	N from Organic Amendment (mg N kg ⁻¹ Soil)	N from Chemical Fertilizer i.e., Urea (mg N kg ⁻¹ Soil)
Control	0	0
CO + CF	51.2	68.8
PM + CF	74.0	46.0
RHB + CF	62.8	57.2
PMB + CF	98.8	21.2
CD + CF	56.0	64.0

i.e., CO = compost; CF = chemical fertilizer; PM = poultry manure; RHB = rice husk biochar; PMB = poultry manure biochar; and CD = cowdung.

2.4. Soil Extraction and Analysis

Total nitrogen (TN), mineral nitrogen (NH₄⁺-N and NO₃⁻-N), and soil pH were measured in soil samples from all treatments incubated at various intervals. Extraction of soil samples immediately after incorporation of each amendment was used to determine the initial concentration of TN and mineral N at day zero.

2.4.1. Mineral N

Mineral N was determined by colorimetric method [43]. For this purpose, 50 g soil sample from each plastic container was extracted by shaking with 125 mL 2 M KCl (soil:KCl solution = 1:2.5) for 2 h, after that filtration was done using Whatman No. 41 filter paper. To determine NH₄⁺-N, 25 mL of the aliquot was steam distilled with 0.7 g of MgO for 4 min and the ammonia was trapped in a 20 mL boric acid indicator solution similar to

total N determination. Then 0.3 g more MgO and 0.4 g Devarda's alloy were added to the same aliquot derived after first distillation for NO_3^- -N determination. Before starting the second distillation, the conical flask containing 20 mL boric acid indicator solution was replaced with a fresh boric acid indicator. After completion of distillation, the colour of the boric acid indicator solution changed from pink to green and the indicator solutions were then titrated with 0.1 N H_2SO_4 separately to determine the concentration of NH_4^+ -N and NO_3^- -N [43].

2.4.2. Total N

Total N was determined using Kjeldahl method [44]. The soil derived after extraction of mineral N was then oven dried. 1 g of oven-dried ground soil sample, 1.1 g catalyst mixture ($\text{K}_2\text{SO}_4:\text{CuSO}_4\cdot 5\text{H}_2\text{O}:\text{Se} = 100:10:1$), and 5 mL H_2SO_4 were added to a digestion flask. After swirling the flasks, they were set aside for around 10 min. The digest was then heated to 380 °C until it was clear and colourless. After cooling, the contents were transferred to a 100 mL volumetric flask and filled with distilled water to the desired volume. In the same way, a reagent blank was made. The total N content of this digest was determined. For distillation, 40% NaOH was added to the digest after it was finished digesting. The ammonia was trapped in a 4% H_3BO_3 solution with five drops of bromocresol green ($\text{C}_{21}\text{H}_{14}\text{Br}_4\text{S}$) and methyl red ($\text{C}_{10}\text{H}_{10}\text{N}_3\text{O}_3$) mixed indicator solution. Finally, standard 0.01 N H_2SO_4 was used to titrate the distillate until the colour changed from green to pink [44]. The amount of N was calculated using the following formula:

$$\text{TN (g kg}^{-1}\text{)} = \frac{(\text{T} - \text{B}) \times \text{N} \times 0.0144 \times 100 \times 10000}{\text{S} \times 1000}$$

where, T = Sample titration value (mL) of standard H_2SO_4 ; B = Blank titration value (mL) of standard H_2SO_4 ; N = concentration of H_2SO_4 ; S = Sample weight in gram; 4 = Dilution factor.

2.4.3. Soil pH

20 g oven dry soil sample was taken to measure soil pH with the help of a glass electrode pH meter using a soil:water suspension of 1:2.5, as described by Peech [45].

2.5. Calculating Net Mineral N Data, Maximum N Release, N Release Per Unit Urea and Gaseous N Loss

The net mineralization data was calculated by subtracting the mineral N of unamended soil from mineral N data of amended soil at a particular sampling date [46].

Net mineral N = Mineral N content in amended soil – Mineral N content in unamended soil

Maximum N release is the highest amount of net mineral N content of a particular treatment during the entire sampling period [46].

N release per unit urea was calculated by following equation:

$$\text{N release per unit urea} = \frac{\text{Mean mineral N conc. in a treatment (mg/kg)}}{\text{Urea N applied in a particular treatment (mg)}}$$

If, the value of N release per unit urea > 1, it indicates mineralization of organic amendment and if the value of N release per unit urea < 1, it indicates immobilization of urea N fertilizer

Gaseous N loss was calculated by following equation [41]:

$$\text{Gaseous N loss} = \text{Loss of total N} - \text{Mineral N conc. after 120 days of incubation}$$

(note: all the units are expresses in mg N kg⁻¹)

where, loss of total N is the difference between initial and final total N concentration.

2.6. Fitting of N Release Data to the First Order Kinetic Model

Different kinetic models were used to fit the net N release values. The first-order kinetic model provided the best fit. An exponential equation was used to compute the N release potential (N_0) and rate constant (k) for various sources of organic materials [47].

The equation for N kinetics was

$$N_t = N_0 (1 - e^{-kt})$$

where, N_t is the net N release at time t , N_0 is the N release potential, and k is the rate constant.

2.7. Statistical Analysis

The data were subjected to three-way analysis of variance (ANOVA) technique using Statistix 10 software package by Factorial Design. Normality of residuals and homogeneity of variances were checked before performing ANOVA. N release kinetics were determined using Sigma-Plot 14.0 software. Post-hoc tests were performed to separate differences among treatments using the Tukey-Kramer multiple comparison. All statistical analyses were considered significant at $p \leq 0.05$, unless otherwise mentioned. Correlations between total mineral N (TMN) and STN were calculated using Microsoft EXCEL package (Microsoft Corporation, Pullman, WA, USA).

3. Results

3.1. NH_4^+ -N Release

The release of NH_4^+ -N from the IPNS treatments was significantly influenced by the soil types ($p < 0.01$), soil moisture regimes ($p < 0.001$), and treatment combination ($p < 0.01$). The NH_4^+ -N was significantly higher in acidic soil than in charland soil ($p < 0.01$), exhibiting a mean value of 24.20 and 19.65 mg N kg⁻¹, respectively, in acidic soil and charland soil, irrespective of soil moisture status and treatment combination (Table 4). With regard to soil moisture status, the mean NH_4^+ -N was significantly higher in soils kept under continuous standing water (CSW) compared to the soils kept under field capacity (FC), being 35.47 and 8.38 mg N kg⁻¹ soil, respectively, in CSW and FC, irrespective of all other factors (Table 4). Likewise, the highest mean NH_4^+ -N release was observed in compost + chemical fertilizer (CO + CF), which was identical to cowdung + chemical fertilizer (CD + CF) and poultry manure + chemical fertilizer (PM + CF), whereas the lowest value was observed in poultry manure biochar + chemical fertilizer (PMB + CF) (Table 4). The interactions between soil type and moisture status had a significant effect on NH_4^+ -N release, recording the highest value in acidic soil with CSW, and the lowest value in either soil type with FC. Treatment combinations and soil moisture status interactions had also significant effect on NH_4^+ -N. CO + CF, CD + CF and PM + CF with CSW had the highest NH_4^+ -N release than any other combination. In contrast, soil type and treatment combination interactions were non-significant. Similarly, three-way interactions were non-significant.

Table 4. Mean NH_4^+ -N, NO_3^- -N, Mineral N concentration and pH of different soil type, moisture status and treatments (mean \pm SE); $n = 3$.

	Net NH_4^+ Concentration (mg N kg ⁻¹)	Net NO_3^- Concentration (mg N kg ⁻¹)	Net Mineral N Concentration (mg N kg ⁻¹)	Soil pH
Soil type				
Acidic soil	24.2 \pm 5.38 a	26.6 \pm 8.37 a	50.8 \pm 4.46 a	5.6 \pm 0.11 b
Charland soil	19.7 \pm 4.22 b	23.7 \pm 7.61 b	36.9 \pm 2.83 b	6.5 \pm 0.06 a
level of significance	**	***	***	***

Table 4. Cont.

	Net NH ₄ ⁺ Concentration (mg N kg ^{−1})	Net NO ₃ [−] Concentration (mg N kg ^{−1})	Net Mineral N Concentration (mg N kg ^{−1})	Soil pH
Moisture Status				
FC	8.4 ± 0.67 b	48.1 ± 3.30 a	50.8 ± 4.46 a	5.8 ± 0.17 b
CSW	35.5 ± 2.58 a	2.2 ± 0.17 b	36.9 ± 2.83 b	6.3 ± 0.13 a
level of significance	***	***	***	***
Treatments				
CO + CF	26.6 ± 9.47 a	31.6 ± 16.69 a	53.6 ± 7.85 a	5.9 ± 0.29 b
PM + CF	23.1 ± 9.08 ab	25.3 ± 13.27 b	45.9 ± 4.52 ab	6.0 ± 0.32 ab
RHB + CF	18.9 ± 6.97 b	21.4 ± 11.44 c	38.2 ± 5.17 b	6.2 ± 0.25 a
PMB + CF	16.0 ± 5.46 c	18.1 ± 9.59 d	31.2 ± 4.37 c	6.2 ± 0.26 a
CD + CF	25.0 ± 9.12 a	29.4 ± 15.62 a	50.3 ± 7.11 a	6.0 ± 0.31 ab
level of significance	**	***	**	*
Statistical analysis for interaction				
Soil type × Soil Moisture	**	**	***	***
Soil type × Treatment	ns	***	**	*
Soil Moisture × Treatment	**	**	**	*
Soil type × Soil Moisture × Treatment	ns	ns	ns	ns

i.e., CO = compost, CF = chemical fertilizer, PM = poultry manure, RHB = rice husk biochar, PMB = poultry manure biochar, CD = cowdung, FC = field capacity, CSW = continuous standing water. ns indicates non-significant, * indicates significant at 5% level of significance, ** indicates significant at 1% level of significance, *** indicates significant at 0.1% level of significance.

3.2. NO₃[−]-N Releases

NO₃[−]-N release was significantly influenced by soil type ($p < 0.001$), soil moisture status ($p < 0.001$) and treatment combination ($p < 0.001$). Irrespective of moisture status and treatment, significantly higher NO₃[−]-N was observed in acidic soil than in charland soil (ca. 26.58 and 23.70 mg N kg^{−1} soil in acidic soil and charland soil, respectively) (Table 4). The NO₃[−]-N was significantly higher in FC than in CSW, displaying a mean value of 48.13 and 2.16 mg N kg^{−1} soil, respectively, in FC and CSW, irrespective of soil type and treatment combinations (Table 4). Likewise, CO + CF had the highest NO₃[−]-N, which was similar to CD + CF, whereas PMB + CF showed the lowest value (Table 4). Treatment combinations and soil moisture status interactions had a significant effect on NO₃[−]-N production. CO + CF produced the highest NO₃[−]-N while PMB + CF produced the lowest amount of NO₃[−]-N under FC conditions. Similar trend was also observed in case of CSW condition. Similarly, soil type and treatment combination interactions were also significant. OF + CF had the highest and PMB + CF had the lowest NO₃[−]-N concentration in both soils. In contrast, three-way interactions were non-significant.

3.3. Mineral N (NH₄⁺-N + NO₃[−]-N) Releases

The availability of N (NH₄⁺-N + NO₃[−]-N) was significantly influenced by soil types ($p < 0.001$, Table 4). The mean mineral N was higher in acidic soil than in charland soil, being 50.78 and 36.93 mg N kg^{−1} soil, respectively, irrespective of all other factors. Likewise, soil moisture status also influenced the mean mineral N ($p < 0.001$, Table 4). N mineralization was higher in soils kept under FC moisture status than in soils with CSW of 1 cm (ca. 50.78 and 36.93 mg N kg^{−1} soil in FC and CSW, respectively). Mineral N content was significantly influenced by treatment combinations ($p < 0.001$, Table 4). The highest mean mineral N was observed in CO + CF, which was similar to CD + CF, and the lowest mineral N was found in PMB + CF. The trend of mineral N showed that the mineral N content reached its peak at day 7, and remained more or less constant in the case of acidic soil incubated at FC, and decreased thereafter with time in charland soil with FC and CSW conditions in both the soil types (Figure 1).

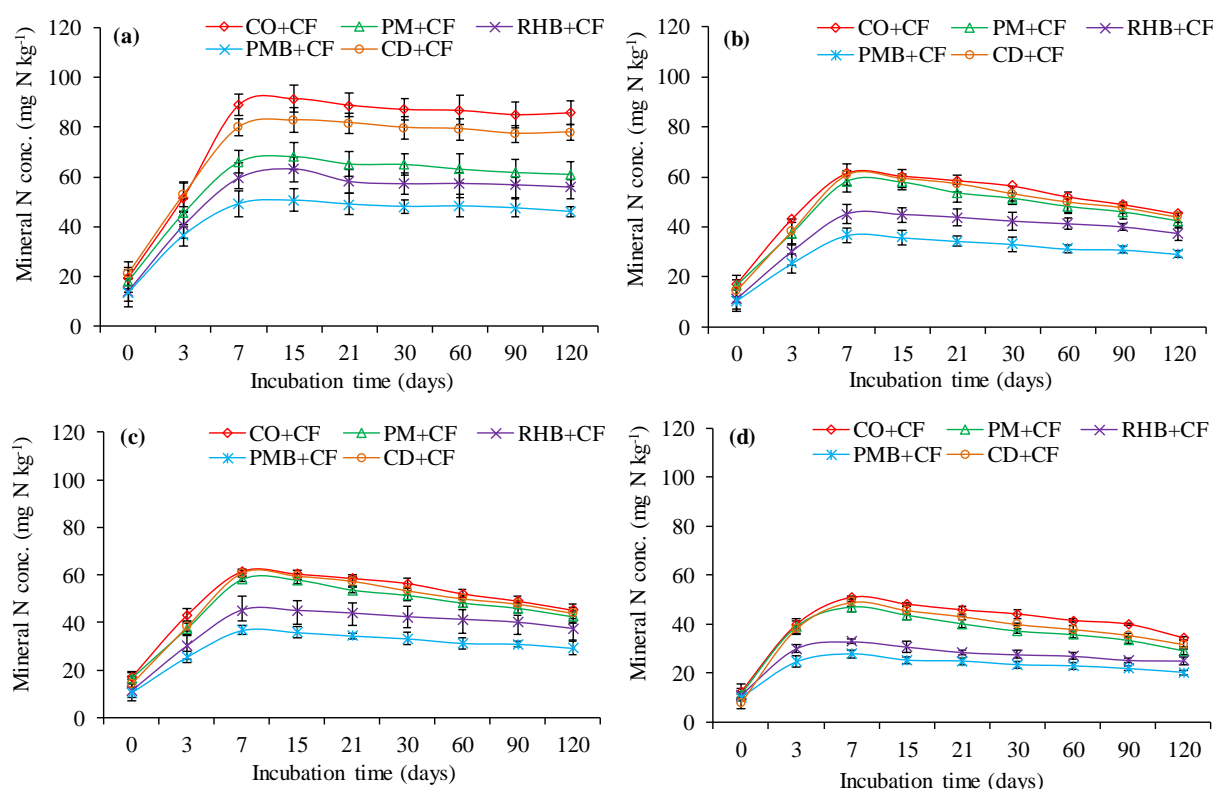


Figure 1. Trend of net mineral N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) mineralization from different treatments in (a) acidic soil with FC; (b) acidic soil with CSW; (c) charland soil with FC; and (d) charland soil with CSW. Net mineralization is calculated as the difference in amount between amended soil and control. Error bars represent standard errors of the means ($n = 3$).

The interaction effects of the soil type and soil moisture status were significant. The highest mineral N was observed in either soil type having FC moisture status, and the lowest value was observed in either soil type with CSW. Considering the soil type and treatment combination interactions, mean mineral N was significantly higher in acidic soil treated with CO + CF, which was statistically similar to CD + CF and PM + CF in either soil, whereas the lowest value was observed in either soil with PMB + CF. Likewise, treatment combination and soil moisture status interactions had a significant effect on mean mineral N, exhibiting the highest mineral N content in CO + CF with FC which was identical to CD + CF along with FC moisture status and lowest value in PMB + CF with FC and CSW, respectively. Unlike interactions among soil types, treatment combinations and soil moisture status were found to be non-significant.

3.4. Nitrogen Release Kinetics

The net N mineralization data for all the treatments in both soil type and moisture status were fitted well to the first order kinetic model ($N_t = N_0 (1 - e^{-kt})$). The adjusted R^2 values ranged from 0.565 to 0.870 when mineral N data were fitted to the first order kinetic model (Table 5). The N mineralization potential and rate constant for different treatment combinations were calculated for both the soil type and moisture status (Table 4). In both soils, mineralization potential for treatments was higher under FC compared to CSW. In both the soils and moisture status, among the treatments, CO + CF showed the highest mineralization potential, followed by CD + CF, PM + CF, RHB + CF, and PMB + CF (Table 1). The N release potential (N_0) of the treatment combination was significantly influenced by soil type ($p < 0.001$), soil moisture status ($p < 0.001$), and treatment combination ($p < 0.001$). The N_0 was significantly higher in acidic soil than in charland soil, being 57.42 and 48.50 mg N kg^{-1} soil in acidic soil and charland soil, respectively, irrespective of moisture status and treatment combination. FC moisture status had higher N_0 than in CSW moisture status (ca. 64.09 and 41.63 in FC and CSW, respectively) irrespective of soil

type and treatment combination. The highest N_0 was observed in CO + CF and CD + CF than in any other treatment combination (Table 4).

Table 5. Parameter values, regression equation and coefficients of determination for the N release kinetics of soils amended with different organic sources along with chemical fertilizers under two moisture status during 120 days of incubation.

Soil Type	Moisture Status	Treatment Combination	N_0 (mg N kg ⁻¹ Soil)	k (mg N kg ⁻¹ Soil Day ⁻¹)	Regression Equation	R ² adj *	p-Value
Acidic soil	FC	CO + CF	88.20	0.885	$y = 1.175x - 15.32$	0.870	$p < 0.0001$
		PM + CF	64.57	0.648	$y = 1.302x - 19.26$	0.775	$p < 0.0001$
		RHB + CF	58.73	0.589	$y = 1.247x - 14.30$	0.843	$p < 0.0001$
		PMB + CF	48.63	0.488	$y = 1.332x - 15.83$	0.801	$p < 0.0001$
		CD + CF	80.31	0.806	$y = 1.282x - 22.13$	0.830	$p < 0.0001$
	CSW	CO + CF	54.68	0.54	$y = 1.195x - 11.51$	0.594	$p < 0.0001$
		PM + CF	50.90	0.503	$y = 1.164x - 9.27$	0.565	$p < 0.0001$
		RHB + CF	42.12	0.416	$y = 1.240x - 10.22$	0.764	$p < 0.0001$
		PMB + CF	32.99	0.326	$y = 1.281x - 9.48$	0.641	$p < 0.0001$
		CD + CF	53.02	0.524	$y = 1.104x - 6.45$	0.667	$p < 0.0001$
Charland soil	FC	CO + CF	75.66	0.577	$y = 1.249x - 18.41$	0.855	$p < 0.0001$
		PM + CF	61.10	0.466	$y = 1.480x - 28.71$	0.597	$p < 0.0001$
		RHB + CF	50.51	0.385	$y = 1.422x - 20.93$	0.709	$p < 0.0001$
		PMB + CF	43.99	0.335	$y = 1.384x - 16.60$	0.743	$p < 0.0001$
		CD + CF	69.23	0.528	$y = 1.367x - 24.96$	0.670	$p < 0.0001$
	CSW	CO + CF	45.92	0.487	$y = 1.253x - 11.87$	0.750	$p < 0.0001$
		PM + CF	39.20	0.416	$y = 1.142x - 6.34$	0.608	$p < 0.0001$
		RHB + CF	29.61	0.314	$y = 1.326x - 9.79$	0.687	$p < 0.0001$
		PMB + CF	26.75	0.284	$y = 1.203x - 5.57$	0.833	$p < 0.0001$
		CD + CF	43.07	0.457	$y = 1.122x - 5.57$	0.856	$p < 0.0001$

R² adj * value: 0.0 to 0.2—very weak fit, 0.2 to 0.4—weak fit, 0.4 to 0.7—moderate fit, 0.7 to 0.9—strong fit, 0.9 to 1.0—very strong fit. The regression equations in the table represent the relationship between the calculated value (x) and the predicted value by model (y).

Mineralization rate constant (k) was significantly higher in acidic soil than in charland soil ($p < 0.001$). Likewise, moisture status significantly influenced the mineralization rate ($p < 0.001$). The highest mineralization rate constant was observed in soil kept under CSW rather than in FC. Similarly, mineralization rate constant was significantly influenced by different treatments ($p < 0.01$). CO + CF had the highest mineralization rate constant, which was statistically similar to CD + CF and PM + CF, whereas biochar based IPNS had the lowest mineralization rate constant (Table 5). The interaction effects between soil type and soil moisture status were significant. Other interactions were non-significant. The calculated net mineral N concentration and the modeled mineral N concentration had a linear relationship. The linear regression equations in Table 5 can be used as a tool for calculating the first order kinetic model value of different treatments under different conditions.

The N release was significantly higher in acidic soil than in charland soil. The N release ranged from about 30–76% of N input in acidic soil, whereas the value was about 25–64% of N input in the case of charland soil (Table 6). For both soil types, N release was significantly higher when incubated at FC than in CSW. A significant amount of N was released due to the addition of CO + CF in both the soil type and moisture status, which was followed by CD + CF, PM + CF, RHB + CF, and PMB + CF (Table 6). N release per unit urea application is the indication of the sources of mineral N. The data of N release per unit urea application revealed that mineral N came from both organic and inorganic sources in most of the cases in acidic soil compared to the charland soil. The results revealed that FC conditions were more favourable for mineralization of organic sources, whereas immobilization took place in most of the cases under CSW conditions (Table 6). PM and PMB mineralized in both soil types and moisture regimes. Immobilization of urea N took

place in CO + CF, RHB + CF and CD + CF in all the cases except in acidic soil kept under FC conditions.

Table 6. Maximum net mineral N release, % N release, and N release per unit urea of different soil type, moisture status and treatments.

Soil Type	Moisture Regime	Treatment	Maximum Net Mineral N (mg N kg ⁻¹)	N Release (% of Input)	N Release (Per Unit Urea)
Acidic soil	FC	CO + CF	91.55	76.29	1.33
		PM + CF	68.32	56.93	1.49
		RHB + CF	63.38	52.82	1.11
		PMB + CF	50.68	42.23	2.39
		CD + CF	82.73	68.94	1.29
	CSW	CO + CF	61.60	51.33	0.90
		PM + CF	58.14	48.45	1.26
		RHB + CF	45.13	37.61	0.79
		PMB + CF	36.66	30.55	1.73
		CD + CF	60.76	50.64	0.95
Charland soil	FC	CO + CF	61.60	51.33	0.90
		PM + CF	58.14	48.45	1.26
		RHB + CF	45.13	37.61	0.79
		PMB + CF	36.66	30.55	1.73
		CD + CF	60.76	50.64	0.95
	CSW	CO + CF	50.76	42.30	0.74
		PM + CF	46.98	39.15	1.02
		RHB + CF	32.77	27.31	0.57
		PMB + CF	27.87	23.23	1.31
		CD + CF	48.83	40.69	0.76

3.5. Nitrogen Balance

The initial total N concentration was 1020 and 620 mg N kg⁻¹ in acidic and charland soil, respectively. The final total N concentration ranged from 928.4 to 983.3 mg N kg⁻¹ in acidic soil and 558.4 to 592.1 mg N kg⁻¹ in charland soil, respectively (Table 7). A decreasing trend of changes in soil total N during the 120 days of incubation was observed (Figure S1). Nitrogen balance of acidic soil and charland soil amended with organic and inorganic fertilizer under FC and CSW conditions after 120 days of incubation period are shown in Table 7. Data revealed that total N loss ranged from 27.9 to 91.6 mg kg⁻¹ soil. Total N loss was 31.7% higher in acidic soil than in charland soil. Similarly, total N loss was 31.7% higher in FC conditions compared to the CSW conditions. The highest total N loss was observed in CO + CF and the lowest in PMB + CF. Unaccounted N loss/Gaseous N loss (volatilization & denitrification) was 40.4% higher in charland soil than in acidic soil. Likewise, gaseous N loss was significantly higher under CSW conditions compared to the FC conditions. Considering treatment combination, gaseous N loss was significantly higher in PM + CF, CD + CF and CO + CF whereas the lowest gaseous N loss was observed in PMB + CF.

Table 7. Nitrogen balance (mg N kg^{-1}) of different soil type, moisture status and treatments after 120 days of incubation.

Soil Type	Moisture Regime	Treatment	Initial Total N (mg N kg^{-1})	Final Total N (mg N kg^{-1})	Loss of Total N (mg N kg^{-1})	Mineral N Conc. after 120 Days (mg N kg^{-1})	Unaccounted N/Gaseous N Loss (mg N kg^{-1})
Acidic soil	FC	CO + CF	1020.0	928.4	91.6	85.9	5.7
		PM + CF	1020.0	951.7	68.3	61.2	7.1
		RHB + CF	1020.0	956.6	63.4	56.1	7.3
		PMB + CF	1020.0	969.3	50.7	46.1	4.6
		CD + CF	1020.0	937.3	82.7	78.0	4.7
	CSW	CO + CF	1020.0	958.4	61.6	45.2	16.4
		PM + CF	1020.0	961.9	58.1	42.3	15.8
		RHB + CF	1020.0	974.9	45.1	37.4	7.7
		PMB + CF	1020.0	983.3	36.7	29.2	7.5
		CD + CF	1020.0	959.2	60.8	43.8	17.0
Charland soil	FC	CO + CF	620.0	558.4	61.6	45.2	16.4
		PM + CF	620.0	561.9	58.1	42.3	15.8
		RHB + CF	620.0	574.9	45.1	37.4	7.7
		PMB + CF	620.0	583.3	36.7	29.2	7.5
		CD + CF	620.0	559.2	60.8	43.8	17.0
	CSW	CO + CF	620.0	569.2	50.8	34.3	16.5
		PM + CF	620.0	573.0	47.0	29.1	17.9
		RHB + CF	620.0	587.2	32.8	25.0	7.8
		PMB + CF	620.0	592.1	27.9	20.2	7.7
		CD + CF	620.0	571.2	48.8	31.6	17.2

3.6. Changes in Soil pH

The trend of changes in soil pH showed that soil moisture status had a significant influence on soil pH (Figure S2). In both the soils incubated at FC, all the treatments significantly decreased soil pH after 120 days of incubation compared to the initial soil pH except RHB + CF and PMB + CF (Figure 2). In contrast, soil pH significantly increased in both the soils incubated at CSW (Figure 2). Charland soil had a significantly higher pH than acidic soil (ca. 6.47 and 5.60 in charland soil and acidic soil, respectively) ($p < 0.001$). Likewise, soil moisture and treatment combinations also significantly influenced soil pH ($p < 0.001$). Soil pH was significantly higher in CSW than in FC (ca. 6.23 and 5.84 in CSW and FC, respectively). Irrespective of soil type and soil moisture status, RHB + CF and PMB + CF had higher soil pH than any other treatment combinations, being 6.19, 6.18, 6.02, 5.98, 5.97 and 5.87 in RHB + CF, PMB + CF, CD + CF, PM + CF, control and CO + CF, respectively. Interactions between soil type and soil moisture were significant. Charland soil incubated at CSW had higher soil pH than any other combination. Similarly, interactions between soil type and treatment combination were also significant. The charland soil treated with either of the treatments had significantly higher soil pH than treated acidic soil. Likewise, soil moisture status and treatment combination had a significant influence on soil pH. RHB + CF and PMB + CF treated soils incubated at CSW had a higher soil pH than any other combination, which were similar to PM + CF and CD + CF treated soils incubated at CSW. The three-way interactions were non-significant.

Nitrogen mineralization was very strongly correlated with urea N application in both soil types and moisture regimes (Figure 3). The R^2 value ranged from 0.61 to 0.75 in different combinations. The highest correlation was observed in acidic soil kept under FC conditions and the lowest in charland soil kept under CSW conditions.

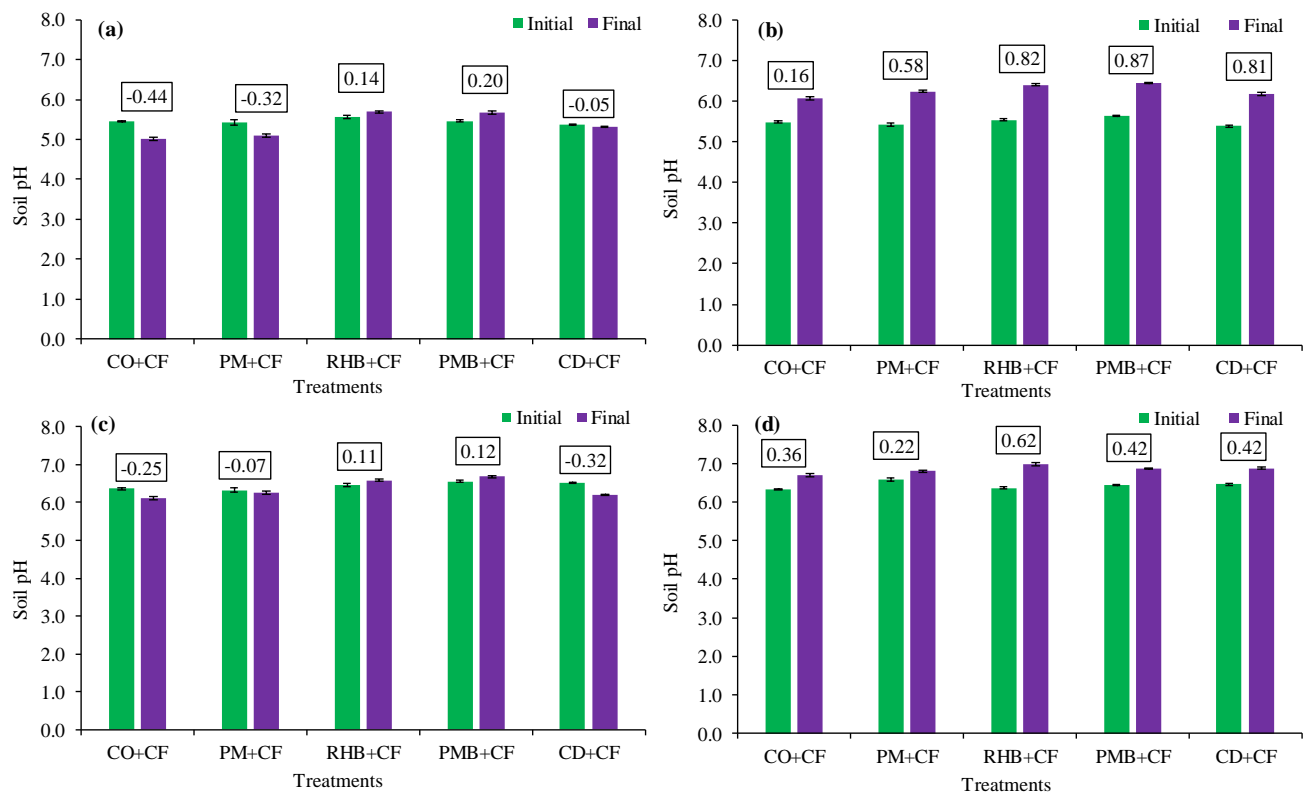


Figure 2. Changes in soil pH after 120 days of incubation of different treatments in (a) acidic soil with FC; (b) acidic soil with CSW; (c) charland soil with FC; and (d) charland soil with CSW. Values in the box represents delta pH, Δ pH. Δ pH = Final pH—Initial pH.

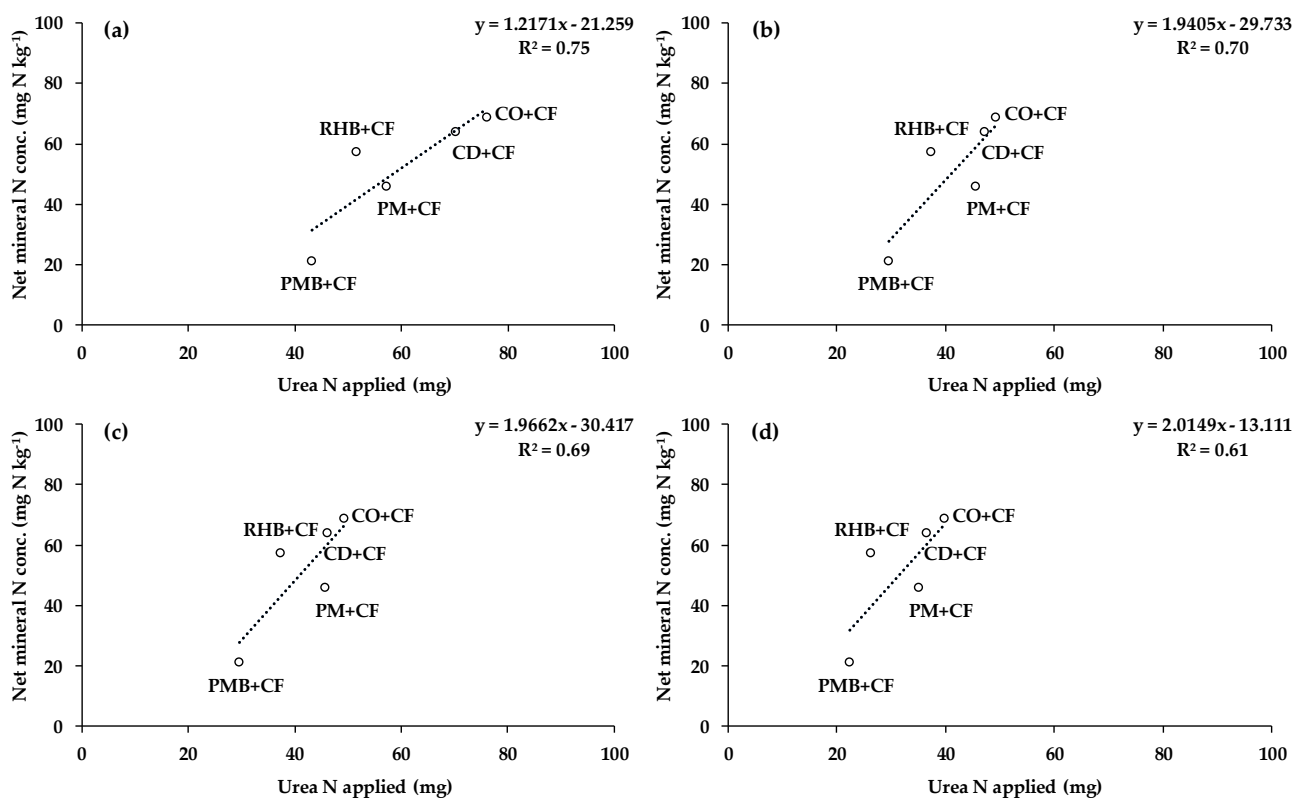


Figure 3. Correlation between net mineral N conc. and urea N application in (a) acidic soil with FC; (b) acidic soil with CSW; (c) charland soil with FC; and (d) charland soil with CSW.

4. Discussion

4.1. Influence of Soil Type on N Dynamics and Changes in Soil pH

In acid soil, NH_4^+ -N was substantially greater than in charland soil. This is attributed to the higher NH_4^+ production in acidic soil and increased gaseous N loss (Table 7) in charland soil as a result of the higher soil pH and low clay content [48]. Higher organic matter as well as clay content in the acidic soil adsorb more positive NH_4^+ ions, resulting in less gaseous N loss. The higher pH of charland soil also favors urease activity and ammonia volatilization [49]. In acidic soil, NO_3^- -N levels were much greater than in charland soil. This might be due to the rapid nitrification of the available NH_4^+ -N in acidic soil. Although it is stated that soil bacteria work effectively under neutral conditions, our results suggest that soil pH doesn't affect the function of soil bacteria in the acidic soil. Nitrifying bacteria typically function in soil with a pH ranging from 5.5 to around 10.0, with an optimum around 8.5 [50]. Furthermore, nitrification has even been observed in soils with pH levels as low as 3.8 [51]. N release through mineralization of organic amendments was only observed in the case of acidic soil, which resulted in more mineral N concentration in acidic soil. Mineralization potential (N_0) and rate constant was substantially higher in acidic soil than in charland soil due to its high organic matter content, clay content, TN content and low pH, which help to adsorb more NH_4^+ and reduce gaseous losses. Canali et al. [52] found that the potential for N release in clay textured soil was substantially higher (9%) than in silty-clay textured soil. Low N release can lead to a lot of STN retention [53]. As N release is a microbiological process, STN retention is influenced by the organic matter content of the soil. More STN was retained in charland soil because of its low organic matter content than in acidic soil which indicated lower microbial activity and mineralization. With increasing clay content, several researchers observed lower mineralization and an increased tendency to immobilize [17,54]. Hadas et al. [55] found no significant differences in net mineralization in sandy and clay soils incubated with dairy and poultry manure, but Canali et al. [52] found higher N mineralization (9%) in clay textured soil than clay loam textured soil. At the end of 120 days of incubation, the change in soil pH in different treatments ranged from -0.44 to 0.87 units in acidic soil, whereas the range was -0.32 to 0.62 units in charland soil. The higher absolute change in the magnitude of acidic soil than in charland soil may be due to the low initial pH of acidic soil than in charland soil [56]. High organic matter content of acidic soil results in increased buffering capacity and cation exchange capacity (CEC) of soil which adsorbs more H^+ and acidic cations that causes increased soil pH [56].

4.2. Influence of Soil Moisture on N Dynamics and Changes in Soil pH

Our results revealed that NH_4^+ -N was found to be the dominating form of N under CSW conditions compared to FC conditions, as submergence increased NH_4^+ -N concentration [57], which is in line with Haque et al. [35] and Calderon et al. [58]. Under CSW conditions, there is insufficient O_2 to complete the nitrification process and N mineralization ends at the ammonification stage, resulting in higher NH_4^+ -N content under CSW [59]. Aeration (oxygen), low soil moisture condition, and greater quantity of ammonium ions are the most critical environmental requirements for the nitrification process in soil [60]. In the soil system, soil moisture and aeration, or soil oxygen levels, are inversely related. Because water fills most pore spaces, increased soil moisture reduces oxygen content in the soil, and it also hinders atmospheric air diffusion into the soil. As a result, NO_3^- -N concentration was higher under FC conditions. Mineral N concentration was higher in FC conditions than in CSW conditions. This is attributed to the mineralization of organic amendments under FC conditions and immobilization of urea N under CSW conditions. FC condition is more favourable for mineralization of organic amendments and N release [35]. Ammonia volatilization and denitrification are the significant N loss pathways under CSW condition [61]. 40.4% more gaseous loss under CSW condition is another reason for higher mineral N concentration under FC conditions. Mineralization potential and rate constant was also higher in FC conditions due to more mineral N production and less

gaseous N loss. As N mineralization happened more quickly under FC than under CSW, more total N retained under CSW moisture conditions, as previously observed by Islam et al. [46,62], Haque et al. [35] and Rahman et al. [36]. Our findings showed that when soil was incubated at FC conditions, the pH dropped sharply with time compared to the initial condition. However, when soil was incubated at CSW conditions, the opposite scenario was found, as previously reported by Zarate-Valdez et al. [63]. Under CSW conditions, consumption of protons during the reduction of oxidants and mineralization process as well as denitrification increased soil pH, however, under FC conditions, fast nitrification reduced soil pH by releasing many protons [64].

4.3. Influence of Treatments and C:N Ratio on N Dynamics and Changes in Soil pH

In both soil types and moisture regimes, CO + CF and CD + CF exhibited the greatest release of NH_4^+ -N because the combinations included a higher quantity of urea N. Urea application increases urease activity in soil and possesses a positive linear relationship with the N application rate [65]. The fast hydrolysis of urea fertilizer occurs owing to a favourable urease activity environment, which also promoted the breakdown of CO and CD, resulting in increased NH_4^+ -N in all instances. The two biochar treatments PMB and RHB responded contrasting to each other. The mineralization of PMB was observed in all the cases whereas mineralization of RHB was observed only in acidic soil kept under FC conditions. The results suggest that PMB contained more labile N pool which resulted in a higher N mineralization, whereas RHB contained more recalcitrant components resulting in net immobilization [66,67]. Although biochar had a higher N content, the lowest mineral NH_4^+ -N was reported in the biochar treatment combinations because biochar is more stable and less susceptible to mineralization [68]. N-enriched biochar like PMB, on the other hand, may acts as a slow-release nitrogen fertilizer [69]. The buildup of NO_3^- -N was considerably greater in CO + CF compared to CD + CF and PM + CF. This occurred as a result of the greater amount of NH_4^+ production which underwent rapid nitrification.

The amount of nitrogen released by organic additions is determined by the chemical compositions of the organic components (e.g., N content, C:N ratio, and contents of cellulose and hemicelluloses, lignin, and polyphenols) [70,71], application rate of manures [35] and on the properties of soil microorganisms [72]. Organic amendments with high nitrogen content and low C:N ratios provide more mineral N due to mineralization [73]. In OAs with lower N levels and greater C:N ratios, however, N can be immobilized [72]. The organic materials used in our study have low C:N ratio and high N content which led to mineralization of organic N in case of PM and PMB as well as all treatments in acidic soil kept under FC conditions. Several authors reported the N release by 64–91% from farmyard manure, dhaincha and legume residues [36]; 64–83% from cowdung, poultry manure, cowdung slurry and poultry manure slurry [35] which supports our results of N release that ranged from 25 to 76% of input depending on the soil type, moisture status and organic amendments. After 120 days, STN was still higher than the initial unamended soil. The lower C:N ratio of the soil amendments boosts up the mineralization process, resulting in decrease in STN concentration after 120 days compared to the initial STN concentration [74].

The principal causes of soil pH change during the incubation period are hydrolysis of urea, N mineralization from soil and organic sources and subsequent NH_4^+ nitrification [75]. Liming properties of organic manures are widely known [76]. Except for biochar, all treatments significantly enhanced soil pH in the CSW conditions compared to the control, but decreased soil pH in the FC conditions. The higher pH of the CO, PM and CD compared to the initial pH of both soils is the primary reason of increased soil pH under CSW conditions. Organic amendments enhance soil organic matter content which results in increased buffering capacity and CEC of soil adding to the increase of soil pH under CSW conditions [77]. Besides, dissociation of alkaline products which are present in the composition of the animals' diet, the metabolic activities of organic compounds, and protein degradation, which liberates ammonia into the environment [78], as well as the use of

calcium oxide during poultry house disinfection [79] may be the reasons of increased soil pH. However, mineralization of CO, PM, and CD under FC conditions releases proton which decreases the soil pH. Biochar raised soil pH in any soil and moisture conditions, as previously documented by various authors in long-term incubation trials, confirming our findings [80,81]. The high pH of RHB and PMB, which is primarily owing to the biochar being dominated by alkaline carbonates, alkali earth metals, and organic anions, may be causing the pH increase in the soil [82]. The high surface area and porous nature of biochar resulting in higher CEC of biochar-amended soils could be another explanation for an increase in soil pH. However, the addition of biochar to the soil raised the pH as a result of proton consumption processes [83].

5. Conclusions

The results of the study showed that N release was higher in acidic soil and FC moisture status. The CO, CD, and PM based IPNS treatments had higher potential for N supply in acidic soils under FC moisture status. Mineral N release reached its peak at seven days of application and remained more or less constant at FC but decreased thereafter due to loss of N in different pathways, particularly volatilization and denitrification, indicating that chemical fertilizer should be applied at least seven days prior to plants' N requirement. The maximum mineral N released from IPNS treatments varied between 24.90–76.29% of input depending on the soil type and moisture status. PM and PMB mineralized in all soil and moisture conditions but immobilization of urea N was observed in CO + CF, RHB + CF and CD + CF in all the cases except for acidic soil with FC conditions. Gaseous N loss was higher in charland soil than in acidic soil and PMB based IPNS had the lowest gaseous N loss. N mineralization was strongly correlated with urea N application. The results revealed that CO, CD, and PM based IPNS approaches can be suggested for both acid and charland soils in terms of N release to the crops in a single season. Besides, PMB with recommended chemical fertilizer (not IPNS basis) can be a good option for N supply to the crops. Biochars uplift the pH towards the neutral condition in both soils and moisture regimes, which will help to increase the availability of nutrients, especially P.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11112163/s1>, Figure S1. Change in soil total N in (a) acidic soil with FC; (b) acidic soil with CSW; (c) charland soil with FC; and (d) charland soil with CSW after addition of different IPNS treatments during the 120 days of incubation; Figure S2. Trend of changes in soil pH in (a) acidic soil with FC; (b) acidic soil with CSW; (c) charland soil with FC; and (d) charland soil with CSW after addition of organic amendments during the 120 days of incubation period.

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