

## Mineralization of Three Organic Manures Used as Nitrogen Source in a Soil Incubated under Laboratory Conditions

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**Abstract:** The rate and timing of manure application when used as nitrogen (N) fertilizer depend on N-releasing capacity (mineralization) of manures. A soil incubation study was undertaken to establish relative potential rates of mineralization of three organic manures to estimate the value of manure as N fertilizer. Surface soil samples of 0–15 cm were collected and amended with cattle manure (CM), sheep manure (SM), and poultry manure (PM) at a rate equivalent to 200 mg N kg<sup>-1</sup> soil. Soil without any amendment was used as a check (control). Nitrogen-release potential of organic manures was determined by measuring changes in total mineral N [ammonium-N + nitrate-N (NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N)], NH<sub>4</sub><sup>+</sup>-N, and accumulation of NO<sub>3</sub><sup>-</sup>-N periodically over 120 days. Results indicated that the control soil (without any amendment) released a maximum of 33 mg N kg<sup>-1</sup> soil at day 90, a fourfold increase (significant) over initial concentration, indicating that soil had substantial potential for mineralization. Soil with CM, SM, and PM released a maximum of 50, 40, and 52 mg N kg<sup>-1</sup> soil, respectively. Addition of organic manures (i.e., CM, SM, and PM) increased net N released by 42, 25, and 43% over the control (average). No significant differences were observed among manures. Net mineralization of organic N was observed for all manures, and the net rates varied between 0.01 and 0.74 mg N kg<sup>-1</sup> soil day<sup>-1</sup>. Net N released, as percent of organic N added, was 9, 10, and 8% for CM, SM, and PM. Four phases of mineralization were observed; initial rapid release phase in 10–20 days followed by slow phase in 30–40 days, a maximum mineralization in 55–90 days, and finally a declined phase in 120 days.

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Accumulation of  $\text{NO}_3^-$ -N was 13.2, 10.6, and 14.6  $\text{mg kg}^{-1}$  soil relative to 7.4  $\text{mg NO}_3^-$ -N  $\text{kg}^{-1}$  in the control soil, indicating that manures accumulated  $\text{NO}_3^-$ -N almost double than the control. The proportion of total mineral N to  $\text{NO}_3^-$ -N revealed that a total of 44–61% of mineral N is converted into  $\text{NO}_3^-$ -N, indicating that nitrifiers were unable to completely oxidize the available  $\text{NH}_4^+$ . The net rates of mineralization were highest during the initial 10–20 days, showing that application of manures 1–2 months before sowing generally practiced in the field may cause a substantial loss of mineralized N. The rates of mineralization and nitrification in the present study indicated that release of inorganic N from the organic pool of manures was very low; therefore, manures have a low N fertilizer effect in our conditions.

**Keywords:** Aerobic incubation, immobilization, manure nitrogen, nitrification, N transformation

## INTRODUCTION

Organic sources of nitrogen (N) such as crop residues, cattle manures, and compost, when applied to soil, play key roles in sustaining soil fertility and crop productivity (Soumare, Tack, and Verloo 2003). This practice is particularly important under low-input agriculture systems where nutrient availability is a serious constraint for agriculture and food production. Increased surface runoff and soil erosion in the hilly areas of Azad Jammu and Kashmir and other mountainous areas may erode the surface layer of the soil and expose the subsurface material. The capacity of this material to hold nutrients becomes crucial, and as a consequence, soil fertility and quality deteriorates. Use of organic manures to improve the quality and fertility status of these soils is very important. Manure can serve as a source of important plant nutrients including P and N (Gilley and Eghball 2002). The organic fraction of manure can significantly increase soil aggregation, infiltration, microbial activity, structure, and water-holding capacity and can reduce soil compaction and erosion (Gilley and Risse 2000; Haynes and Naidu 1998). Chemical properties improved by manure application include cation exchange capacity and soil buffering potential (Tisdale et al. 1993). Improvement and maintenance of a good supply of organic matter (OM) through recycling in soil is a precondition for efficient recycling of nutrients. Castillo, Benito, and Fernandez (2003) reported that when manures are managed properly, they become valuable soil amendments and their application to agriculture land receives considerable attention because of their natural value, liming effect, and environmental friendly behavior. The land application of manure can produce crops similar to those obtained using inorganic fertilizers (Eghball and Power 1999).

The N mineralization potential of manures, composts, and soils has conventionally been estimated using laboratory incubation (Abbasi, Shah, and Adams 2001; Hadas and Portnoy 1994; Sorensen 1998). According to Calderon, McCarty, and Reeves (2005), during manure decomposition the

mineralized N may take several routes: (1) the mineral N may remain in the soil and may become part of the net mineralized N pool; (2) the mineral N may be immobilized by microbes and become part of the microbial biomass pool; or (3) nitrate ( $\text{NO}_3^-$ )-N derived from manure may be denitrified and lost from the soil as either nitrous oxide ( $\text{N}_2\text{O}$ ) or dinitrogen ( $\text{N}_2$ ). Within this set of conditions, manure with high N content should result in high net N mineralization, increased soil microbial biomass N, high denitrification, or a combination of these outcomes.

The proportion of manure organic N mineralized during an aerobic incubation also varied widely, from  $-29$  (net immobilization) to  $55\%$  (Van Kessel, Reeves, and Meisinger 2000). A  $35\%$  mineralization factor is used in Maryland to estimate the first year availability of the organic N fraction of dairy manure (Van Kessel and Reeves 2002). Mineralization of organic N from cow manure in the first year of application was highly variable and ranged from  $0\%$  to  $50\%$  (Kirchmann and Lundvall 1993; Paul and Beauchamp 1994; Serna and Pomares 1991). Van Kessel, Reeves, and Meisinger (2000) demonstrated that amendment composition has a significant impact on the rate and extent of organic N mineralization. Recently, Agehara and Warncke (2005) reported that the net N released as a percent of organic N in blood meals, alfalfa pellets, and chicken manure was  $56-61$ ,  $41-52$ , and  $37-45\%$ , respectively. The variation in N availability among different types of animal manures and plant residues has been attributed to the chemical composition, such as total N content (Aulakh, Khera, and Doran 2000; Constantinides and Fownes, 1994; Fox, Myers, and Vallis 1990), C/N ratio (Aulakh, Khera, and Doran 2000; Rowell, Prescott, and Preston 2001; Trinsoutrot et al., 2000) and lignin/N ratio (Constantinides and Fownes 1994; Kumar and Goh 2003; Melillo, Aber, and Muratore 1982).

The amount of N potentially mineralized from manures is an important variable to be considered when recommending the appropriate rate to apply to meet N needs for optimal crop production (Logan 1990). Thus, the organic N mineralized from manures needs to be determined to apply these resources to provide adequate N for the crop without adverse effects. The aim of the present investigation was to evaluate the potential rates of mineralization and nitrification of three animal manures normally used in agriculture in Azad Jammu and Kashmir and to access the potential of these manures to be used as N sources (fertilizer) in our farming system.

## MATERIALS AND METHODS

### Soil Sampling/Collection

The soil used in this study was collected from an arable field located at the research farm, Faculty of Agriculture, Rawalakot, Azad Jammu and Kashmir. Detailed soil survey/classification and soil profile studies in this

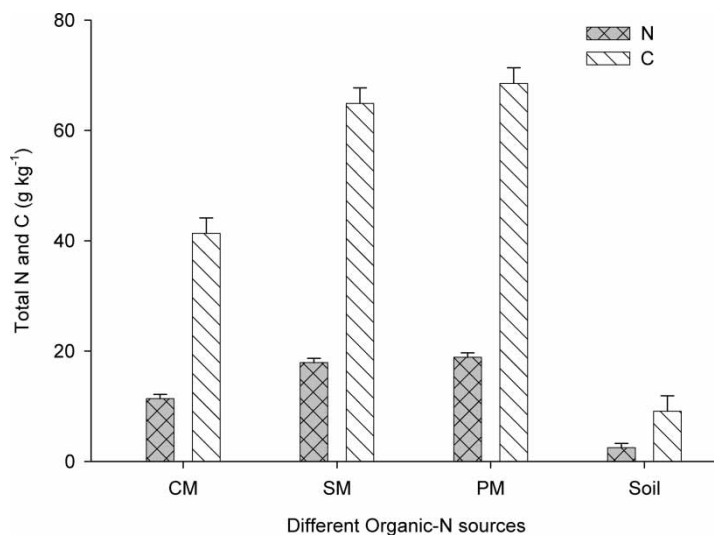
area have not been done so far. The field was barren at the time of sampling but was previously cultivated for wheat and maize production. The selected area was uniform in topography and well leveled. Soil samples were collected from 0- to 15-cm depth at random from 3–5 locations with a spade, and a composite sample was prepared accordingly. The sample was air dried and sifted (2-mm mesh) to eliminate coarse rock and plant material and thoroughly mixed to ensure uniformity. The soil sample was stored in a dry place at room temperature (20–22°C) until the incubation was initiated to minimize disturbance of the microbial population. A subsample was used to determine the physical and chemical characteristics of the soil used in the study (Table 1).

Manure Sampling/Collection

Three different organic manures [cattle manure (CM), sheep manure (SM), and poultry manure (PM)] were selected because of their availability and use in agricultural cropping systems of Azad Jammu and Kashmir. Cattle and poultry manures were collected from the farmer’s farm nearby Rawalakot, and sheep manure was collected from the sheep farm at the Faculty of Agriculture, Rawalakot. Manure samples were air dried (after taking the composite sample of each manure) and screened through a 2-mm sieve to remove coarse material and other unwanted materials. Triplicate samples of each of

*Table 1.* Some physicochemical characteristics of the soil used in the experiment

Property	Unit	Value
Bulk density	(Mg m <sup>-3</sup> )	1.31
Particle density	(Mg m <sup>-3</sup> )	2.61
Porosity	(%)	49.8
Particle size distribution		
Sand	(%)	68.0
Silt	(%)	19.0
Clay	(%)	13.0
Texture class		Sandy loam
Calcium carbonate content	(%)	0.68
Organic matter	(%)	0.85
pH (1:1 water)		7.8
Total N	(mg kg <sup>-1</sup> )	850
Total mineral N (TMN)	(mg kg <sup>-1</sup> )	8.3
Organic N	(mg kg <sup>-1</sup> )	841.7
Available phosphorus	(mg kg <sup>-1</sup> )	2.5
Available potassium	(mg kg <sup>-1</sup> )	83.0



**Figure 1.** Total N and C contents ( $\text{g kg}^{-1}$ ) of different organic manures and soil used in the experiment. Vertical bars indicate least significant difference ( $P \leq 0.05$ ). CM = cattle manure; SM = sheep manure; PM = poultry manure.

the manures were taken and analyzed for their C and N contents (Figure 1). Total N in manure samples was determined by the Kjeldahl method of Bremner and Mulvaney (1982). Organic matter content was estimated from the weight loss on ignition at  $400^{\circ}\text{C}$  (Ball 1964). Organic N concentration in the samples was estimated as the difference between total nitrogen (TN) and available N as described by Griffin, He, and Honeycutt (2005).

### Laboratory Incubation

A hundred grams of finely ground, air-dried (after sieving through 2-mm mesh) soil were weighed and transferred into 200-mL glass jars. The initial moisture content of soil was 20% (w/w). Distilled water was then added to achieve a final moisture level of 45%; the resulting water-filled pore space (WFPS) of approximately 60% was maintained throughout the incubation period. Phosphorus (P) was added to all jars at the rate of  $90 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$  soil in the form of single superphosphate (SSP). Samples of different manures were weighed and added in the glass jars at a rate equivalent to  $200 \text{ mg N kg}^{-1}$  soil. The application rates were calculated to provide approximately an equal amount (i.e.,  $200 \text{ mg N kg}^{-1}$  soil) as described by Agehara and Warncke (2005). Manures were mixed with soil to trap any volatilized ammonia ( $\text{NH}_3$ ). Soil without manure was also used as a control. Treatments

include four N sources including a control [i.e., CM, SM, PM, and control/check (without manure)] and nine incubation times. Each treatment was replicated three times to give a total of 135 experimental units at the start of the experiment.

After adding different amendments, all the jars were weighed, and their weight was recorded. The jars were covered with parafilms with three or four small holes on the top to allow O<sub>2</sub> exchange within glass jars. Soil samples were incubated under controlled conditions in the dark at 20°C. Soil moisture was adjusted every week by weighing the glass jars and adding the required amount of distilled water when the loss was greater than 0.05 g. During this process, care was taken not to disturb the soil either through stirring or shaking.

### Soil Extraction and Analysis

Samples of all four treatments incubated for different timings were analyzed for total mineral nitrogen (TMN) and ammonium-N (NH<sub>4</sub><sup>+</sup>-N). Initial concentration of TMN and NH<sub>4</sub><sup>+</sup>-N at day 0 were determined in samples extracted with 200 mL of 1 M potassium chloride (KCl) added directly to the flask immediately after incorporation of each N source. Thereafter, triplicate samples from different treatments were removed randomly from the incubator at different incubation periods (i.e., 10, 20, 30, 40, 55, 70, 90, and 120 days). The samples were extracted by shaking for 1 h with 200 mL of 1 M KCl followed by filtration through Whatman's No. 40 filter paper. The mineral N contents of the extract were determined using the steam distillation and titration method of Keeney and Nelson (1982). Aliquots (40 mL) of the extracts were added via pipette into a distillation flask, and steam distillation was carried out after adding magnesium oxide (MgO) and Deverda's alloy in the case of TMN and by adding only MgO in the case of NH<sub>4</sub><sup>+</sup>-N. The distillate was then collected in 5 mL of boric acid containing bromocresol green/methyl red mixed indicator and titrated against 0.05 M hydrochloric acid (HCl). Nitrate-N was calculated by subtracting NH<sub>4</sub><sup>+</sup>-N from total mineral N. Any NO<sub>2</sub> present would have been included in the NO<sub>3</sub><sup>-</sup> fraction.

### Calculations

Manure organic N mineralized (N<sub>org</sub>) and cumulative net nitrification (N<sub>cum</sub>) at each sampling time (*t*) were calculated according to Griffin, He, and Honeycutt (2005).

$$N_{org}(\text{mg kg}^{-1} \text{ soil}) = \{[Ni]_t - \{[Ni]_{t=0}\}_{\text{manure}} - [Ni]_t\} - \{[Ni]_{t=0}\}_{\text{unamended}}$$

where  $N_i$  is the sum of  $\text{NO}_3$  and  $\text{NH}_4$  at time  $t$ ;

$$N_{\text{cum}}(\text{mg kg}^{-1} \text{ soil}) = \{[\text{NO}_3]_t - \{[\text{NO}_3]_{t=0}\}_{\text{manure}} \\ - \{[\text{NO}_3]_t - \{[\text{NO}_3]_{t=0}\}_{\text{unamended}}\}$$

accounting for changes in soil  $\text{NO}_3$  concentration between  $t = 0$  and  $t$  and correcting for the N nitrified in the unamended soil.

The percentage of organic N released from an applied N source at time  $t$  was calculated as

$$(\% \text{Nrel})_{\text{N source}} = \left[ \frac{(\text{Nrel})_{\text{N source}}}{N_o(\text{N source})} \right] \times 100$$

where  $N_o$  is the organic N (total N – inorganic N) content (i.e., N applied through organic sources).

### Statistical Analysis

All data were statistically analyzed by multifactorial analysis of variance (ANOVA) using the software package Statgraphics (1992). Least significant differences (LSD) are given to indicate significant variations between the values of either manures or time intervals. Confidence values (P) are given in the text for the significance among treatments, manures, time interval, and their interactions. A probability level of  $\leq 0.05$  was considered significant.

## RESULTS

### Mineralization of Organic Manures

The changes in total mineral nitrogen (TMN) in soil with and without added organic manures during different incubation periods were used to estimate the mineralization of manures/soil. Release of N from organic pool ( $N_{\text{org}}$ ) of manures /mineralization was separately calculated following the methods described earlier. Tables 2–4 depict the ANOVA, showing the significance level of manures (M), timings (T), and their interactions ( $M \times T$ ). Statistical analysis showed a significant difference among manures ( $P \leq 0.01$ ), timings ( $P \leq 0.01$ ), and their interactions ( $M \times T$ ) ( $P \leq 0.01$ ). The overall manure effect was determined by taking the average value across the timings (Table 2), indicating that mineral N released from the soil without amendments was  $16.7 \text{ mg kg}^{-1}$ , whereas CM, SM, and PM were able to release 23.7, 20.8, and  $23.9 \text{ mg kg}^{-1}$  of mineral N from the organic pool. All the three manures had significantly higher concentration of mineral N than the control soil. The percentage increases were 42, 25, and 43% for CM, SM, and PM, respectively. Among manures, no significant difference was

**Table 2.** ANOVA statistical analysis of the mineralization pattern of different manures applied to a soil incubated over 120 days

Main effects	Sum of square	d.f.	Mean square	F. ratio	Significance level
Manure (M)	678.42	3	226.14	4.90	0.004
Timings (T)	11770.36	8	1471.29	31.89	0.001
M × T	2249.50	24	93.72	2.03	0.01
Residual	3321.45	72	46.13		
Total	18019.72	107			

observed. However, CM and PM released 14 and 15% more mineral N than the SM. The effect of different incubation timings on mineralization was determined by taking the average values of three manures at each incubation time (Table 2). Release of mineral N (mineralization) from the organic pool increased over time, and the significant difference was noticed in the later stages of incubation (i.e., after 40 days of incubation). The maximum mineralization occurred at day 90 where 43 mg kg<sup>-1</sup> (average) of mineral N was recorded.

Table 5 shows the individual effects of different treatments on mineralization. During 120 days of incubation, the concentration of inorganic N in different manures/soil increased 2–6 fold to the concentration recorded at the start of the experiment at day 0. The extent of mineralization over time was such that a slow release of mineral N was noticed up to day 40; thereafter, a significant increase in mineral N was found in all the treatments. The maximum concentration was recorded at day 90, and significant reduction was found at day 120. The variation among different manures/soils was inconsistent. A significant difference among manures/soils was found at days 10, 20, 55, 90, and 120, whereas nonsignificant difference was observed at days 0, 30, 40, and 70.

The mineral N released from the organic pool of the manures (N<sub>org</sub>) is shown in Figure 2. The pattern of changes in TMN over time is inconsistent

**Table 3.** Manure effects

Parameter	Total mineral N over 120 days, mg kg <sup>-1</sup> soil (average)
Control soil	16.70
Cattle manure	23.72
Sheep manure	20.84
Poultry manure	23.89
LSD (P ≤ 0.05)	3.68



**Table 4.** Timing effects, days after incubation

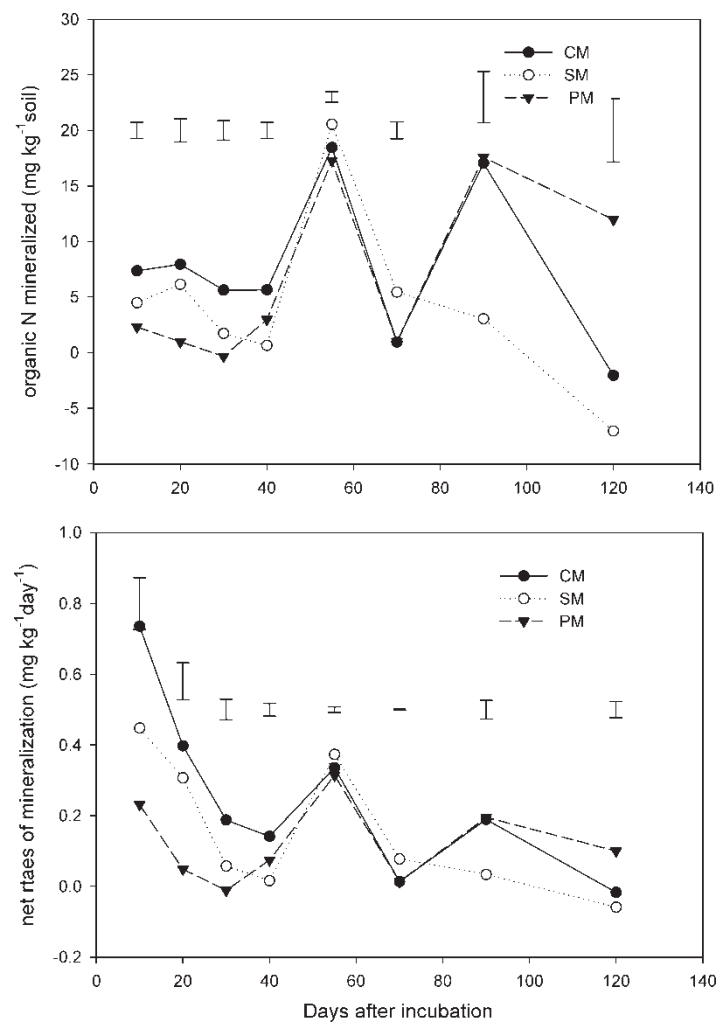
Parameter	Total mineral N, mg kg <sup>-1</sup> soil (average)
0	8.74
10	11.53
20	14.70
30	13.41
40	18.65
55	34.10
70	22.08
90	42.55
120	25.85
LSD (P ≤ 0.05)	5.53

(i.e., it increased/decreased with time). Cattle and sheep manure were able to release a substantial amount of mineral N in the initial stage whereas PM mineralized more in the later stages of incubation. However, the highest level of mineral N (i.e., 18.5, 20.5, and 17.3 mg kg<sup>-1</sup> for CM, SM, and PM) was released at day 55. On the basis of this maximum release of mineral N, net N released as percent of added N for CM, SM, and PM was 9, 10, and 8.5%, respectively. The net rates of mineralization calculated from the mineral N released from manures ranged from 0.01 to 0.74, 0.02 to 0.45, and 0.01 to 0.45 mg kg<sup>-1</sup> day<sup>-1</sup> for CM, SM, and PM, respectively (Figure 2). All the three manures also showed negative values at different timings. Taking averages (10–120 days), the daily rates of mineralization were 0.25, 0.16, and 0.12 mg kg<sup>-1</sup> day<sup>-1</sup> for CM, SM, and PM, respectively.

**Table 5.** Total mineral N (NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N) mg kg<sup>-1</sup> soil with and without added organic manures incubated under controlled laboratory conditions for 120 days

Treatments	Days after incubation									LSD
	0	10	20	30	40	55	70	90	120	
Control	8.3	7.6	10.5	11.2	15.9	19.6	19.8	32.7	24.7	10.3
CM	8.6	15.2	18.7	17.1	21.8	38.3	21.0	50.0	22.9	15.7
SM	8.6	12.3	16.9	13.2	16.8	40.4	25.5	36.0	17.9	12.4
PM	9.5	11.1	12.7	12.1	20.1	38.1	22.0	51.5	37.9	6.2
LSD	NS	4.7	5.4	NS	NS	11.9	NS	13.7	9.6	
(P ≤ 0.05)										

*Note.* CM, SM, and PM indicate cattle manure, sheep manure and poultry manure, respectively, while NS denotes nonsignificant at P ≤ 0.05. LSD indicates least significant difference.



**Figure 2.** Organic N mineralized ( $N_{org}$ ) and net mineralization rates of soil with and without organic manures applied at the rate of  $200 \text{ mg organic N kg}^{-1} \text{ soil}$  during 120 days of aerobic incubation under controlled laboratory conditions. Vertical bars indicate standard error of means (SEM;  $n = 3$ ).

**Nitrification of Organic Manures**

Tables 6–8 indicate the ANOVA for nitrification of four soils with and without added organic manures at nine timings. Statistical analysis showed a significant difference among manures ( $P \leq 0.05$ ), timings ( $P \leq 0.01$ ), and their interactions ( $M \times T$ ) ( $P \leq 0.05$ ). The overall effect of manure on nitrification revealed that all three manures accumulated significantly more  $\text{NO}_3^-$  –

**Table 6.** ANOVA statistical analysis of the nitrification pattern of different manures applied to the soil and incubated over 120 days

Main effects	Sum of square	d.f.	Mean square	F. ratio	Significance level
Manure (M)	443.51	3	147.84	3.28	0.02
Timings (T)	9642.87	8	1205.36	26.71	0.001
M × T	2041.99	24	85.08	1.88	0.02
Residual	3249.32	72	45.13		
Total	15377.70	107			

N than control soil. The level of increase in  $\text{NO}_3^-$ -N concentration was 78, 44, and 98% for CM, SP, and PM, respectively, over the control. Among manures, CM and PM had significantly higher concentration of  $\text{NO}_3^-$ -N than SM, and the difference between CM and PM was nonsignificant. The effect of timings on nitrification was significant and was determined by taking the average values of four treatment manures at each incubation time. Nitrification of mineral N was slow initially up to day 40, and thereafter accumulation of  $\text{NO}_3^-$ -N significantly increased over time up to day 90. The maximum concentration of  $\text{NO}_3^-$ -N was  $30.4 \text{ mg kg}^{-1}$ , recorded at day 90.

The individual effects of different treatments on nitrification of organic manures revealed that the initial concentration of  $\text{NO}_3^-$ -N was generally negligible (Table 9). A detectable amount of  $\text{NO}_3^-$ -N was found at day 55 and consistently increased over time up to day 90. In the control soil without organic manures, concentration was in the range of  $0.19\text{--}21.4 \text{ mg kg}^{-1}$ . Nitrate-N in the CM was ranging from  $1.72\text{ to }35.64 \text{ mg kg}^{-1}$ , while the corresponding values for SM and PM were  $0.78\text{--}29.6$  and  $1.6\text{--}40.4 \text{ mg kg}^{-1}$  for SM and PM, respectively. Generally, soil amended with organic manures accumulated significantly more  $\text{NO}_3^-$  than soil without organic manures. The accumulation of  $\text{NO}_3^-$ -N showed a significant response to time. A significant increase in  $\text{NO}_3^-$ -N was noticed in the later stages on incubation (i.e., after day 55). However, concentration of  $\text{NO}_3^-$ -N in all the four treatment

**Table 7.** Manure effects

Parameter	$\text{NO}_3^-$ -N over 120 days, $\text{mg kg}^{-1}$ soil (average)
Control soil	7.39
Cattle manure	13.20
Sheep manure	10.63
Poultry manure	14.60
LSD ( $P \leq 0.05$ )	2.64

Table 8. Timing effects days after incubation

Parameter	NO <sub>3</sub> <sup>-</sup> -N, mg kg <sup>-1</sup> soil (average)
0	1.06
10	1.78
20	7.0
30	4.96
40	7.78
55	22.27
90	13.96
70	30.38
120	13.90
LSD ( <i>P</i> ≤ 0.05)	3.47

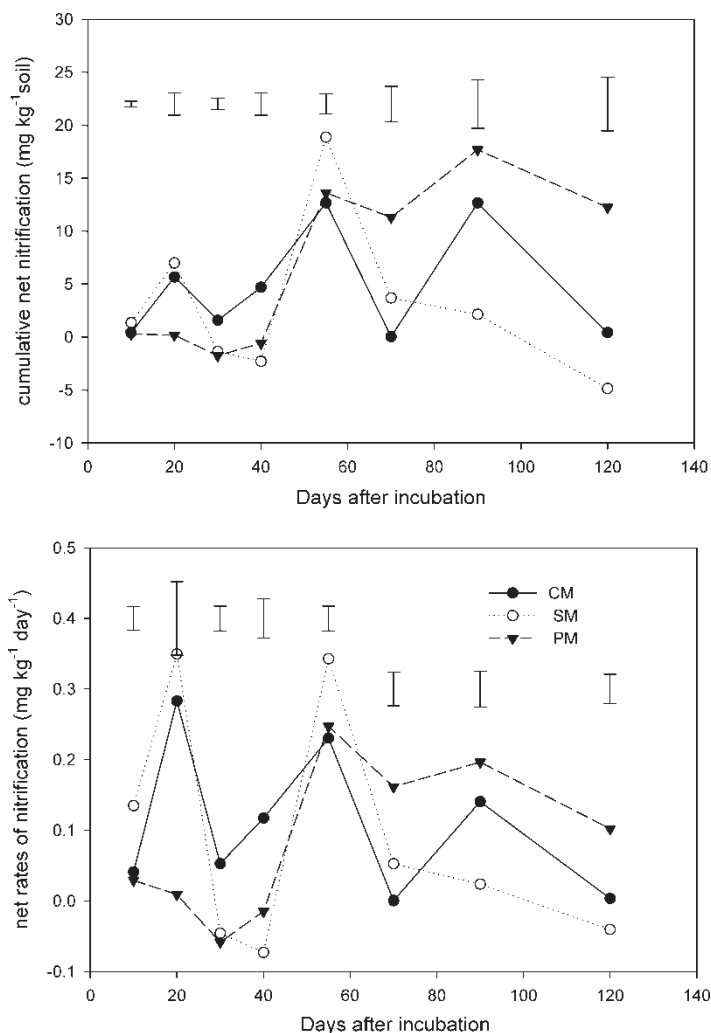
decreased at the end (i.e., day 120). The extent of reduction in CM and SM was higher than the reduction level of PM.

The cumulative net nitrification (*N*<sub>cum</sub>) is shown in Figure 3. The pattern of changes in net NO<sub>3</sub><sup>-</sup>-N accumulation across different timings and manures was similar to that observed for mineralization. Accumulation of NO<sub>3</sub><sup>-</sup>-N up to day 40 was almost negligible, and negative values were recorded for SM and PM. The maximum concentrations of NO<sub>3</sub><sup>-</sup>-N was 17.1, 18.9, and 17.7 mg kg<sup>-1</sup> for CM, SM, and PM, respectively. Cattle manure and SM accumulated the maximum amount at day 55, whereas PM showed maximum concentration at day 90.

Table 9. Total mineral N (NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N) mg kg<sup>-1</sup> soil with and without added organic manures incubated under controlled laboratory conditions for 120 days

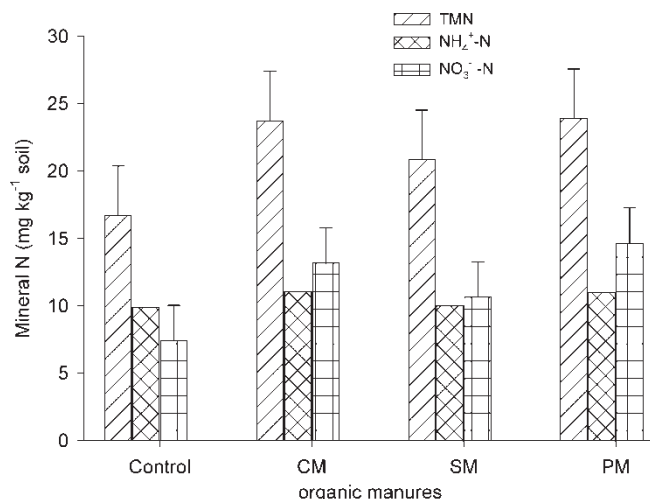
Treatments	Days after incubation									LSD ( <i>P</i> ≤ 0.05)
	0	10	20	30	40	55	70	90	120	
Control	0.2	0.4	2.9	4.5	6.6	10.1	9.3	21.4	11.1	9.4
CM	1.7	2.3	10.1	7.6	12.8	24.3	10.9	35.6	13.0	11.7
SM	0.8	2.3	10.5	3.7	4.3	29.6	13.6	24.1	6.8	8.7
PM	1.6	2.0	4.5	4.1	7.4	25.1	22.0	40.4	24.7	6.4
LSD										
( <i>P</i> ≤ 0.05)	3.8	2.7	4.9	NS	7.2	14.0	9.2	12.2	7.3	

Note. CM, SM, and PM indicate cattle manure, sheep manure, and poultry manure, respectively, while NS denotes nonsignificant at *P* ≤ 0.05. LSD indicates least significant difference.



**Figure 3.** Cumulative net nitrification ( $N_{\text{cum}}$ ) and net nitrification rates of soil with and without organic manures applied at the rate of 200 mg organic N kg<sup>-1</sup> soil during 120 days of aerobic incubation under controlled laboratory conditions. Vertical bars indicate standard error of means (SEM;  $n = 3$ ).

Net rates of nitrification were in the ranges of 0.04–0.51, –0.04–0.35, and –0.06–0.25 mg kg<sup>-1</sup> day<sup>-1</sup> for CM, SM, and PM, respectively (Figure 3). Both CM and SM showed maximum rates of nitrification during the initial stage (days 10 and 20), whereas PM exhibited maximum accumulation in the later stages of incubation. On average (over 120 days), CM had substantially higher rates of nitrification than SM and PM. When averaging over time, maximum rates were recorded at days 10, 20, and 55.



**Figure 4.** Changes in the concentration of different fractions of mineral N in soil incubated at 20°C during 120 days following the application of organic manures at a rate equivalent to 200 mg N kg<sup>-1</sup> soil. Vertical bars indicate least significant difference ( $P \leq 0.05$ ) of each mineral N constituent of different treatments.

#### Overall Changes in Different Fractions of Mineral N

The overall changes in total inorganic N,  $\text{NH}_4^+\text{-N}$ , and  $\text{NO}_3^-\text{-N}$  (mean of 9 samplings over 120 days) in soil amended with organic manures and control without amendment is presented in Figure 4. The total inorganic N in control soil mineralized during incubation was 16.7 mg kg<sup>-1</sup>, which increased significantly ( $P \leq 0.05$ ) in soils where organic manures were added. Cattle and poultry manure showed the maximum mineralization potential, releasing 23.7 and 23.9 mg mineral N kg<sup>-1</sup> soil, respectively, while SM released 20.8 mg mineral N kg<sup>-1</sup> soil. However, the difference among organic manures was nonsignificant. The percentage increases of mineralization of soils with organic manures to the soil without organic manure were 42, 25, and 43% for CM, SM, and PM, respectively. The concentration of  $\text{NH}_4^+\text{-N}$  was almost similar to all treatments including control. Concentration ranges from 9.3 in the control soil to 10.5 mg kg<sup>-1</sup> in the soil with CM. There was no significant difference among the treatments. The pattern of changes in the concentration of  $\text{NO}_3^-\text{-N}$  was similar to that observed for mineralization. In the soil without organic manure, 7.4 mg kg<sup>-1</sup> soil of  $\text{NO}_3^-\text{-N}$  were accumulated during incubation. Concentration of  $\text{NO}_3^-\text{-N}$  in soil with added manure was significantly higher than the concentration found in control soil. Among manure-added soils, soil with CM accumulated 13.2 mg  $\text{NO}_3^-\text{-N}$  kg<sup>-1</sup> soil, whereas SM and PM accumulated 10.6 and 14.6 mg  $\text{NO}_3^-\text{-N}$  kg<sup>-1</sup> soil, respectively. Soil with added SM manure had

significantly lower  $\text{NO}_3^-$ -N concentration whereas the difference between CM and PM was nonsignificant. The percentage increases over control were 78, 44, and 98% for CM, SM, and PM, respectively.

## DISCUSSION

The mineralization potential of a soil or any organic substrate is usually assessed by measuring the amount of  $\text{NH}_4^+ + \text{NO}_3^-$  released during a specified period under uniform conditions (Abbasi shah, and Adams 2003). The rate of release of inorganic N during incubation (N mineralization rate) reflects the potential availability of  $\text{NH}_4^+$ -N to microorganisms and plants. The  $\text{NH}_4^+$  released is further converted to  $\text{NO}_3^-$ , a readily available form of N for plant utilization. Almost all ecosystems (i.e., arable, grassland, forest) showed a wide range of mineralization potential, and this wide range reflects clearly the critical influence mineralization may assert on N cycling in these ecosystems. Organic manures and other organic substrates are important sources of plant nutrients, especially N, and the supply of N from manures/organic substrates makes an important contribution to the N demand of growing crops. In low input and organic systems, such substrates are crucial in supplying plant nutrients in the absence of inorganic fertilizer supplies (Rees, Yan, and Ferguson 1993), and even in conventional farming systems there is substantial release of N from manures, offsetting the need to provide mineral fertilizers (Goulding et al. 2001). Unfortunately, the release of nutrients from added organic substrates has proved very difficult to predict, and there is evidence to suggest that farmers often take inadequate account of the nutrient supply provided by various organic amendments (Domburg, Edwards, and Sinclair 2000). Previous studies have attempted to predict the nutrient value of manures by using their mineral N concentrations at the time of application. This is a valuable approach and can help farmers to prepare simple nutrient budgets (Chambers et al. 1999). However, such approaches do not take account of the processes of mineralization and immobilization that follow from manure application to the field and ignore any potential changes in the rates of mineralization of background organic matter.

In the present investigation, control soil without any amendments released a maximum of 32.7 mg mineral N  $\text{kg}^{-1}$  soil at day 90, whereas the initial concentration at the start of the experiment was 8.3 mg mineral N  $\text{kg}^{-1}$ , showing a fourfold increase in mineral N. This increase in mineral N was due to the mineralization of organic materials present in the soil. It showed that the soil under study has substantial potential for mineralization. The mineral N released from the soil with added organic manures was substantially higher than the control soil. It was quite expected because organic manures have higher concentration of total N than the control soil, and the variation in mineralization was due to more labile organic N compounds

and high levels of microbial biomass and activity in manures. It has been shown in a number of studies that the amount of N that is mineralized and immobilized by manures can vary as function of the availability of organic C (Chantigny, Rochette, and Angers 2001; Hadas, Kautsky, and Portnoy 1996, Van Kessel, Reeves, and Meisinger 2000).

Net mineralization of organic N was observed for all manures as measured by an increase in total mineral N after taking into account the initial mineral N of manures and the mineral N in control soil. The net rates varied, ranging between 0.01 and 0.74 mg N kg<sup>-1</sup> soil day<sup>-1</sup>. This wide variation in mineralization—pattern of manures is probably because of mineralization—immobilization turnover during the incubation. Both CM and SM showed negative values at day 120, indicating that no further mineralization occurred in these manures whereas soil without amendment (control) and PM showed a substantial release of mineral N until the end. Griffin, He, and Honeycutt (2005) reported that in some cases manure soil combinations resulted in no net mineralization through the 176 days incubation. Wichern et al. (2004) reported net mineralization of 15–39 mg kg<sup>-1</sup> soil during 0–9 days of incubation but also found negative values in the next 10–18 days. These negative values were probably because of net immobilization as reported by Van Kessel, Reeves, and Meisinger (2000). The net rates of mineralization were highest during the initial 10–20 days of incubation, indicating that application of manure 1–2 months before sowing as generally practiced in the field may cause a substantial loss of N mineralized. Therefore, application of manures with sowing is recommended so that the initial mineralized N may be utilized by the crops.

The incubation period in our study was 120 days at 20°C and is similar to a growing season of Kharif (summer) in our conditions. Organic N is mineralized at the rate of 0.18 mg N kg<sup>-1</sup> soil day<sup>-1</sup> (the mean mineralization rate of three manures); therefore 22 mg organic N kg<sup>-1</sup> soil would be mineralized during this period. Because all manures in this incubation were applied at a rate of 200 mg organic N kg<sup>-1</sup> soil, this is equivalent to a mineralization rate of 11% per growing season. This mineralization co-efficient is similar to those reported by Chadwick et al. (2000) and Serna and Pomares (1991). However, Wichern et al. (2004) reported that only 0.93% of added organic N from manures was mineralized during 18 days of incubation. Recently, Griffin, He, and Honeycutt (2005) estimated mineralization rates of different manures and reported an average of 10% per growing season. When we considered the N-release capacity of manures [i.e., manure organic N mineralized (N<sub>org</sub>)], the mean net mineralizations over 120 days were 7.62, 4.37, and 6.71 mg kg<sup>-1</sup>, showing that only 3.8, 2.2, and 3.4% of the added organic N from CM, SM, and PM was being mineralized. However, the maximum net N released from these manures was 18.5, 20.5, and 17.3 mg kg<sup>-1</sup> soil by day 55. On the basis of these values, the net N released as percent of added N from manures was 9.25, 10.25, and 8.65%, respectively. The values reported here are very low as compared to the net



N mineralization of N from organic sources reported by other workers (Agehara and Warncke, 2005; Ciavatta et al. 1997; Eneji et al. 2002; Li and Mahler 1995; Chae and Tabatabai 1986). The manures used in the study had a C:N ratio of 4:1, and it is well established that organic materials with a C:N ratio of less than 20:1 usually release inorganic N rapidly through mineralization (Whitehead 1995). Relatively low mineralization capacity of the manures is attributed to some other factors including the conditions or changes of soil developed during incubation. Soil in the jars was not disturbed throughout the incubation either by stirring or shaking, with the aim to create conditions close to the field. In most of the incubation studies, the initial moisture level is adjusted by adding water during incubation and then stirring the soil (Abbasi, Shah, and Adams 2001; Griffin, He, Honeycutt 2005). This serves to redistribute C and N and presumably would lead to conditions more favorable for microbial transformation, including mineralization, which may overestimate the rates of mineralization. In addition, particle size plays an important role in N mineralization as it affects the surface area of the N source and contact with microorganisms. Agehara and Warncke (2005) reported that organic N sources (manures or plant residues) with finer particle size may contribute to more rapid N release than the larger or coarser particles. In their study, they used a 1-mm sieve, whereas a 2-mm sieve was used in the present investigation.

Four distinct phases of mineralization were observed during the study: an initial rapid release phase (10–20 days), followed by a slow phase (30–40 days), a maximum mineralization phase (55–90 days), and then finally a declined phase (120 days). At the start of the experiment, soil was sieved after air drying, and then water was added in the jar to develop a moisture level of about 40%. The initial rapid-release pattern of mineralization was primarily because of the drying and wetting of soil. The decline phase was most probably because of immobilization. The C/N ratio of 4:1 indicated relatively more C than N resulted in immobilization of added manures. When applied to soils, manure increases the energy or food supplies available to the soil microbial population. This energy supply stimulates soil microbial activity, which consumes more available N than the mineralization processes release. Thus, high microbial activity during initial mineralization can cause a reduction of available N. During 55–90 days, most of the immobilized N and N present in different organic fractions were converted into inorganic N, which again decreased into the background level at day 120, especially in CM and SM. For a large number of organic and decomposing litter materials, several authors reported three phases of mineralization: an accumulation phase followed by a phase of N release and finally by the loss of N phases (Bosatta and Berendse 1984; Eneji et al. 2002; Kachaka, Vanlauwe, and Mercky 1993). A similar trend in mineralization of grassland soil was observed in our previous study (i.e., an initial increase phase a decline phase, and N-release phase). However, the  $^{15}\text{N}$  experiment in the study clearly indicated that immobilized N is converted into mineral

N at day 28 and more than 70% of the grass mineral N remained available as inorganic N (Abbasi, Shah, and Adams 2001).

The production of nitrate by the process of nitrification is highly dependent on other N-transforming processes in the soil, especially the accumulation of the substrate of nitrification (i.e.,  $\text{NH}_4^+$ ). The pattern of nitrification of added manure was almost similar to mineralization. Accumulation of  $\text{NO}_3^-$ -N was 13.2, 10.6, and 14.6  $\text{mg kg}^{-1}$  soil relative to 7.4  $\text{mg NO}_3^-$ -N  $\text{kg}^{-1}$  in the control, indicating that both CM and PM accumulated  $\text{NO}_3^-$ -N almost double the control soil without any amendment. Similarly, accumulation of  $\text{NO}_3^-$ -N in soil with SM was 1.5 times more than the control. Nitrification of added manures ( $\text{N}_{\text{cum}}$ ) was 4.8, 3.0, and 6.6  $\text{mg kg}^{-1}$  (average) for CM, SM, and PM, respectively, whereas the maximum accumulation of 15.0  $\text{mg kg}^{-1}$  (average of three manures) was recorded on day 55. The mineralization of added manures was very low. Because some of mineralized N remained as  $\text{NH}_4^+$  in the mineral N pool and was not 100% converted into  $\text{NO}_3^-$ -N, high values for nitrification were not expected. The proportion of total mineral N to  $\text{NO}_3^-$ -N indicated that a total of 44, 55, 51, and 61% of mineral N from control, CM, SM, and PM, respectively, is converted into  $\text{NO}_3^-$ -N, indicating that nitrifiers were unable to completely oxidize the available  $\text{NH}_4^+$  substrate even under favorable conditions of moisture, temperature, pH, and absence of plants for competition with microbes. The study indicated that soil had low potential for nitrification and a substantial amount of  $\text{NH}_4^+$ -N remained in the mineral pool after 120 days. The potential of a soil to retain the applied  $\text{NH}_4^+$  in the mineral pool is an important characteristic of a soil where leaching and denitrification are important pathways of N losses. The nitrification potential of the soil needs more investigation.

The net rates of mineralization and nitrification were generally calculated as the difference between mineral N content measured from the amended soil and that measured from the control soil (Abbasi, Shah, and Adams 2001; Eneji et al. 2002). In the present study, net rates of mineralization and nitrification were calculated according to Griffin, He, and Honeycutt (2005) by subtracting the initial mineral N in manure amended and control soil together with mineral N (control) at specific time  $t$ . In the experiments where  $^{15}\text{N}$  is not used for examining the actual route/pathways of N transformation, such calculations will provide almost the real rates of transformation of N either from soil or manures.

## CONCLUSIONS

Of the three manures investigated, organic N mineralization was highest in CM and PM, whereas cumulative net nitrification ( $\text{N}_{\text{cum}}$ ) was highest in PM. At day 120, both CM and SM showed negative values for mineralization whereas PM released a substantial level of mineral N. However, the rates of

mineralization and nitrification in the present study indicated that release of inorganic N from the organic pool of manures was very low. The net rates of mineralization were highest during the initial 10–20 days of incubation, indicating that the application of manures 1–2 months before sowing (generally practiced in the field) may cause a substantial loss of mineralized N. Therefore, application of manures with sowing is recommended so that the initial mineralized N may be utilized by the crops. The conversion of only 9–10% of added N from manures is not sufficient to fulfill the N requirement of any crop. Therefore, manures have a low N fertilizer effect in our conditions, and application of manure (alone) without mineral fertilizers will not be able to sustain crop productivity. However, the dynamics of the system are complex because the presence of high concentrations of soluble C in the manure led to a depression in the accumulation of mineral N. Furthermore, total N and C contents of manures are also important in N-release capacity of manures.

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