Incubation study of kinetics and mineralization rate of nitrogen in organic sources of Ultisol

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Age-old traditional farming practices are generally followed by tribal inhabitants using locally available organic sources of plant nutrients. The aim of the present study was to determine the kinetics and rate of mineralization of different local organic sources of North East region of India. An incubation study of 100 days was carried out using locally available organic sources, i.e. farmyard manure (FYM; T_1), poultry manure (T_2), pig manure (T_3) and vermicompost (T_4) at the rate of 120 kg N/ha (considering recommended dose of fertilizer of rice as 120 kg N/ha). Bulk soil sample of Typic kandihumultis at 0–15 cm was collected from the College of Post Graduate Studies in Agricultural Sciences, Umiam, Meghalaya research farm and treated with organic sources and kept in an incubator at field capacity soil moisture and 25°C temperature. Observations were taken at 10 days interval up to 100 days of incubation (DOI). A control treatment (T_0) of no organic source was used for comparison. The results showed that the average nitrogen mineralization rate (N_{min}) of T_3 was highest (64.88%), followed by T_2 (57.77%), T_4 (42.98%) and T_1 (35.24%). The highest N_{min} rate of T_3 and T_2 was noted at 60 DOI as 79.37% and 76.10% respectively. At 50–60 DOI, total nitrogen, available nitrogen and nitrogen fractions (ammonical nitrogen and nitrate nitrogen) released were the highest irrespective of the organic sources. R^2 (coefficient of determinate) of first-order kinetics of all organic sources was found to be: 0.91 (T_3) > 0.90 (T_2) > 0.89 (T_4) > 0.88 (T_1), while R^2 of second-order kinetics was: 0.66 (T_3) > 0.65 (T_2) > 0.64 (T_1 and T_4). It has been concluded that T_3 is the best organic nutrient source among the treatments considered for this study.

Keywords: Incubation study, kinetics, nitrogen mineralization, organic sources, traditional farming practice.

THE prudent management of locally accessible organic resources has been the best option for tribal communities who use traditional farming methods. However, the suitability of organic resources as a source of nutrients depends on the kinetics and rate of mineralization[1]. Kinetics is the study of the rate of chemical processes and the rate of a reaction is the change in chemical concentration over time. Due to scarcity of scientific interventions and equipment, it was difficult to determine the kinetics and rate of nitrogen (N) mineralization of local organic sources. By measuring the kinetics and rate of mineralization of various locally accessible organic sources, farmers could increase agricultural output and better feed the people. Both parameters depend upon soil moisture, temperature, type of soil and its properties, organic matter addition, microbial activity and management practices[2,3]. Rapid N mineralization occurs in the pH range 5.5–10 with optimum at 8.5 (ref. 4). The dissociation of functional groups of soil organic matter and reduction in the number of bonds between the organic constituents and clay increased with increase in soil pH, i.e. solubility and mineralizable nitrogen[5]. Farmyard manure (FYM)[6], cattle manure, pig manure, poultry manure, compost and vermicompost are good nutrient sources[7]. About 3.30 million tonnes (mt) of poultry waste per year is generated in India. Notably, poultry droppings are rich in nutrients, cellulose, hemicellulose and lignin[8]. Pig manure contains all essential plant nutrients. As North East India is one of the world's major hotspots for biodiversity, forest waste is another source of plant nutrients. Due to the abundance of locally available organic sources, an effort had been made to study them with the goal of determining the kinetics of the various sources as well as the rate at which they are mineralized.

Materials and methods

Study site

The study site is located at 91°18′–92°18′E long. and 25°40′–26°20′N lat., at an altitude of 950 m amsl. It falls under the subtropical humid climate characterized by high rainfall and cold winters. Monsoon normally sets in during the first fortnight of June and remains active till October. The area receives 2617.10 mm mean annual rainfall with a good amount of pre-monsoon showers during March–May, with high humidity (above 80%) and low sunshine hours. The maximum temperature goes up to 35°C in July–August and the minimum falls to 5°–6°C during the first week of January. The soil belongs to Typic kandihumultis[9].

Soil sample collection and processing

A bulk soil sample was prepared from 20 randomly collected soil samples at 0–15 cm depth from the College of

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Post Graduate Studies in Agricultural Sciences (CPGS-AS), Umiam, Meghalaya research farm (2 ha) (Figure 1).

The samples were air-dried, ground and passed through a 2.0 mm sieve. Analysis of soil texture[10], pH and EC (1 : 2.5 ratio of soil : water suspension[11,12], soil organic carbon (SOC; dichromate wet oxidation)[13], water-soluble carbon (WSC)[14], cation exchange capacity (CEC; 1 N ammonium acetate extraction)[15], available nitrogen (alkaline permanganate method)[16], total nitrogen[17], nitrate nitrogen[18] and ammonical nitrogen[19] was carried out. WSC was extracted by shaking 5 g soil with 10 ml deionized water for 60 min, followed by centrifugation at 10,000 *g* at 4°C for 30 min. The supernatant was filtered with suction through a 47 mm diameter, 0.2 μm membrane filter previously washed with 150 ml deionized water. Table 1 presents the initial soil properties.

Incubation study

An incubation study of 100 days was carried out under control conditions (moisture at field capacity and temperature at 25°C) in a BOD incubator[20]. Fifty grams of soil was treated with organic sources, viz. FYM (T_1), poultry manure (T_2), pig manure (T_3) and vermicompost (T_4) at the equivalent dose of 120 kg N/ha and placed in 50 ml capacity centrifuge tube for 100 days. A control treatment (T_0) with no organic source was used for comparison. The treatments were replicated four times in a completely randomized design (CRD) and observations were recorded at every 10 days interval, i.e. 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 days of incubation (DOI). As soon as the samples were taken out, they were immediately extracted for analysis. Table 2 shows the initial nutrient content and ratio of organic sources.

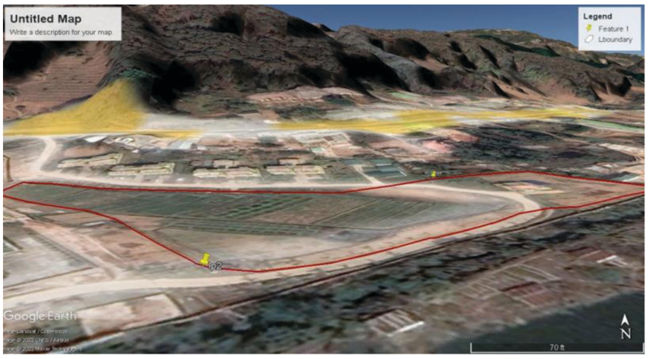

Figure 1. Sampling site with boundary area (2 ha).

Table 1. Initial physico-chemical properties of the soil

Soil properties	Value
Texture	
Sand (%)	42.6
Silt (%)	9.56
Clay (%)	39.67
Soil $pH_{1:2.5}$	4.73
Soil $EC_{1:2.5}$ (dSm/m)	0.11
Cation exchange capacity (milliequivalence/100 g)	11.01
Soil organic carbon (%)	1.31
Total nitrogen (%)	0.15
Available nitrogen (ppm)	213
Nitrate nitrogen (ppm)	17.5
Ammonical nitrogen (ppm)	28.0

EC, Electrical conductivity.

Determination of nutrient mineralization rate

N mineralization rate was determined at every 10 days interval of DOI till 100 DOI using the following equation as[21]

$$\text{N mineralization rate (\%)} = (\{(N_f - S_f) - (N_i - S_i)\} \times 100)/N_0,$$

where N_f is the total nitrogen at final sampling time, S_f the total nitrogen in control soil at final sampling time, N_i the total N at initial sampling time in amended soil, S_i the total nitrogen in control soil at initial sampling time, and N_0 is the initial organic N content.

Nitrogen kinetics in soil

Kinetics helps determine the rate of nutrient transformation and associated chemical reactions. It also aids in collecting and analysing information regarding the mechanism of the reaction and defining the features of a chemical reaction. The change in the rate of mineralizable nitrogen was estimated as (d*N*/d*t*) and fitted to the kinetic equations

First-order equation[22]: $q_t = q_0 \cdot e^{-k1t}$,

Second order equation[6]: $1/qt = 1/qt - k_2 t$,

where q_0 is the initial amount of nutrients present in different organic sources (mg/kg) and q_t is the amount of nutrients present in different organic sources (mg/kg) at time *t* (days). k_1 and k_2 are the constants of first- and second-order kinetics respectively.

Statistical analysis

One-way ANOVA and Duncan's multiple range test (DMRT) were performed to compare the means and deter-

Table 2. Initial total nitrogen and organic carbon content, and C : N ratio of different organic sources

Treatment	Total N (%)	Organic carbon (%)	C : N
T_1 (FYM)	0.153	31.2	37.59
T_2 (poultry)	1.54	26.1	16.94
T_3 (pig manure)	1.72	26.8	15.58
T_4 (vermicompost)	0.76	23.00	30.26

mine the significant effect of organic sources using MS-Excel 2010 and Web Agri Stat Package (WASP) version 2.0.

Results and discussion

Nitrogen releasing pattern

Figure 2 shows the nitrogen-releasing pattern of different organic sources during the incubation period (days). The soil total nitrogen (TN) varies between 1490 (T_0 at 60 DOI) and 1980 ppm (T_3 at 80 DOI). The TN content gradually increased with organic manure treated soil, however, TN content of non-organic treated soil shows a wavy nature with incubation time, a little depression of TN content observed at 60 DOI. The TN curve attains a maximum at 70 DOI and maintains this level till 100 DOI. Among the organic treatments, T_3 recorded the highest TN (1833.03 ppm; increase of 14.69% over T_0) followed by T_2 (1779.09 ppm; increase of 2.94% over T_2), T_4 (1766.66 ppm; increase of 3.62% over T_4) and T_1 (1697.57 ppm; increase of 7.38% over T_1) during the entire incubation period. The TN variable in the soil is due to the mineralization of organic matter present in the organic sources and not from the inorganic soil. The high mineralization rate of T_3 resulted in the highest TN (1980 ppm) at 80 DOI among all organic treatments. Also T_3 recorded higher TN content (1.72% or 1720 ppm), followed by T_2 (1.54% or 1540 ppm), T_4 (0.76% or 760 ppm) and T_1 (0.153% or 153 ppm) depending upon the type of feed given to the pigs and poultry manure which is also classified as the concentrate nutrient sources[23]. FYM (T_1) prepared from the forest waste had a wide C/N ratio and low nitrogen content.

The available N (AN) also increased with organic matter application over T_0 (95.08 ppm). The highest AN was recorded with T_3 (133.81 ppm) and T_2 (129.73 ppm), which increased by about 26.47% and 24.17% over T_0 respectively. The lowest AN was recorded with T_1 (117.46 ppm; 16.25% more than the control). Abbasi and Khaliq[23] classified the poultry and pig feed as concentrated feed. Nair[24] suggested that the bioavailability of AN is controlled by the interactions effect of N, P and K present in the manure. AN attained a maximum of 60 DOI for T_2, T_3 and T_4 and 70 DOI for T_1. The availability of nitrogen from the organic sources through mineralization was maximum at 60–70 DOI.

The ammonical nitrogen (NH_4^+; AmN) varied between 28.0 and 72.3 ppm. AmN release pattern during the incubation period (days) was found to attain a peak at 10–40 DOI. The ammonical nitrogen (AmN) released gradually decreased with increasing days of incubation. Initially, the AmN released might not get fixed to the soil and the rate of fixation may increase with increasing DOI. Maximum AmN was observed with T_3 (61.26 ppm; 50.88% increase over T_0) at 20–40 DOI, followed by T_4 (54.16 ppm; 44.44% increase over T_0), T_2 (38.88 ppm; 22.60% increase over T_0) and T_1 (36.13 ppm; 16.71% increase over T_0).

Figure 2 shows that among the organic sources, T_2, T_3 and T_4 enhance nitrate released, with the peak of nitrate released during 50–60 DOI. Soils without organic treatment (control) shows little fluctuation in nitrate release during incubation; the incubation period (days) was independent of nitrate released from such soils. This might be due to the chemical properties of nitrate and its ability to high leach from the soil. The nitrate nitrogen (NO_3^-) ranged from 17.50 (T_0) to 39.6 ppm (T_3) during the incubation period and was maximum during 30–70 DOI. The average NO_3^- contribution during the incubation period was 33.90% (T_3) > 31.33% (T_4) > 26.16% (T_2) and 21.49% (T_1) with an increase of 44.27%, 39.70%, 27.79% and 12.09% over T_0 respectively.

Rate of nitrogen mineralization

The N_{min} rate (%) was estimated at every 10 days interval up to 100 DOI (Figure 3). Among the organic treatments, T_3 had the highest nitrogen mineralization rate (64.88%) followed by T_2 (57.77%), T_4 (42.98%) and T_1 (35.24%). The N_{min} rate of T_3 and T_2 increased rapidly raised from 0 to 60 DOI, after which it decreased gradually. T_4 and T_1 followed a similar trend of N mineralization rate. However, after 60 DOI till the end of incubation period, the N mineralization rate remained the same. The N_{min} rate of T_3 increased by about 10.95% over T_2, 33.75% over T_4 and 45.68% over T_1. The nitrogen concentration and C : N ratio of manure significantly affected the N mineralization rate of manure. The mineralization rate decreases with decreasing amount of manure (as a reactant) concentration, i.e. (–d(manure))/(d*t*)[25]. Joffre and Agren[26], and Singh *et al.*[27] also reported a similar findings.

Kinetics of nitrogen mineralization

Table 3 and Figure 4 present the kinetics of nitrogen mineralization. Analysis of the kinetics of N release from organic sources helps reveal whether the release of N is independent of its initial concentration (zero order), or is concentration-dependent, where a constant proportion of N is mineralized (first order), or the release of a nutrient is dependent on the square of its concentration or the concentration of some other nutrients (second order). The first-order kinetic constants of N from different organic treatments were T_1 (0.1066 μg/g/day), T_2 (0.1058 μg/g/day), T_3 (0.1059 μg/g/day) and T_4 (0.1054 μg/g/day), while the second-order kinetic constants were T_1 (–0.0007 μg/g/day), T_2 (–0.0006 μg/g/day), T_3 (–0.0006 μg/g/day) and T_4 (–0.0007 μg/g/day). The coefficient of determination (R^2) using the first-order kinetic equation varies between 0.88 (T_1) and 0.91 (T_3) significant at $P \leq 0.01$) but the R^2 of the second-order kinetic equation varied from 0.64 (T_1 and T_4) to 0.66 (T_3) (Table 3). The nitrogen content in organic treatments was well mineralizationed (N_{min} rate) and

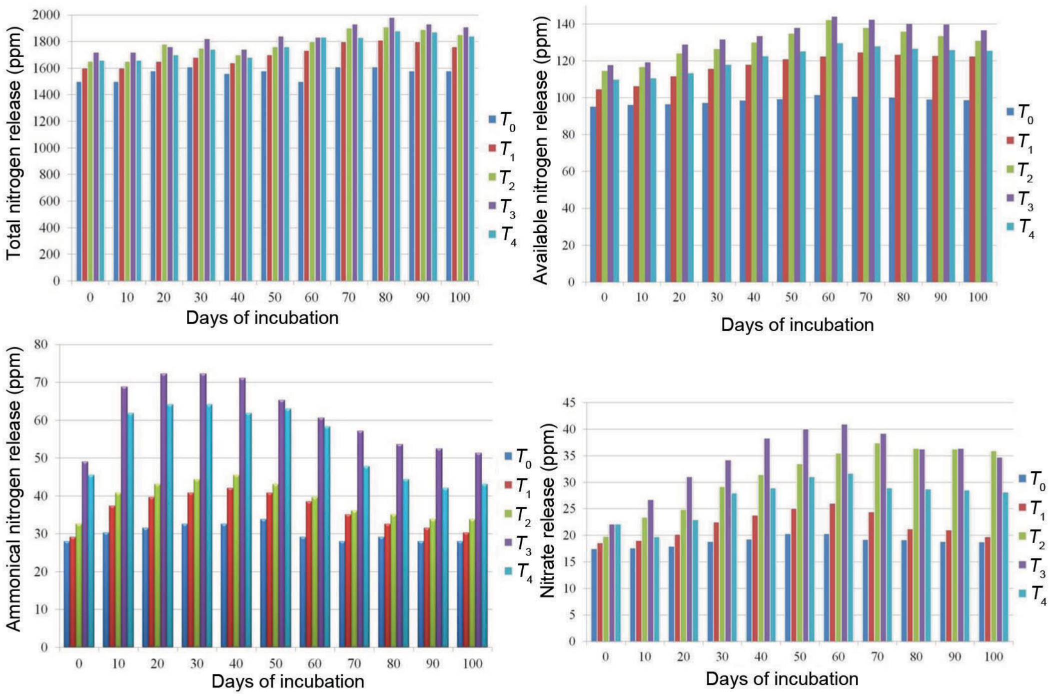

Figure 2. Effect of local organic sources on total, available, ammonical and nitrate nitrogen release (ppm) during the incubation period (days).

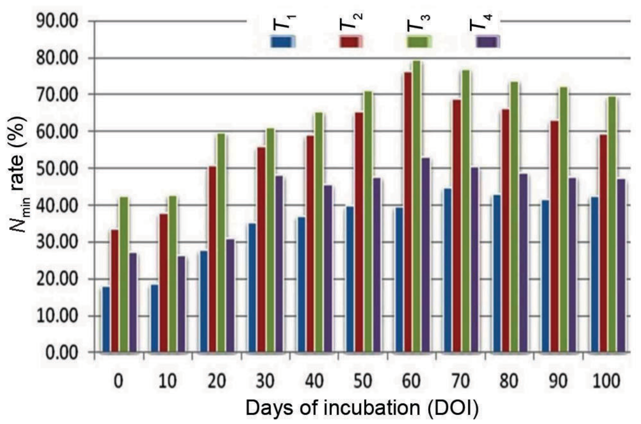

Figure 3. Nitrogen mineralization (N_{min}) rate of different organic sources during the incubation period.

released *N*. The high R^2 of the first-order kinetic equation shows changes in the particle structure which directly affect the organic matter degradation process. The N releases (mineralization, N_{min}) from different organic sources conform to the first-order kinetics, while second-order kinetics shows the immobilization of N. The results are in agreement with those of Preusch *et al.*[21] and Dey *et al.*[6]. The potentially mineralizable nitrogen (N_0) is the amount of N present that can be mineralized. The initial nitrogen (N_0) for the first-order kinetics was 2.20 ppm (T_3) > 2.17 ppm (T_2) > 2.14 ppm (T_4) > 2.11 ppm (T_1). T_3 exhibited the highest kinetic rate (0.41 mg/kg/day) followed by T_2 (0.34 mg/kg/day), T_4 (0.25 mg/kg/day) and T_1 (0.2 mg/kg/day). Ge *et al.*[28] found that manure particles were initially large with a relatively smooth external surface which facilitated the mineralization process in the internal structure of pig manure during the mesophilic–thermophilic phase (after seven days of incubation). Baharuddin *et al.*[29] noticed the particles decreased in size, with cracks developing on the surface (cracks deepened with increasing days of incubation, more fragments attached to a surface), and increased the internal pores (elongated shape between 28 and 35 days) and microbial activities. The kinetics rapidly decreased from 10 to 100 DOI, and this might be the reason for the highest kinetics of nitrogen mineralization of T_3 among the organic treatments. A similar result was reported by Nair[24]. Other studies found that the particles of pig manure decreased in size and increased in surface roughness and pores during mineralization (decomposition)[29–31]. The rate of ion exchange increased with decreasing particle size of the exchanger[32], i.e. ion exchange controlled by diffusion, mass-transfer phenomena limited reaction rate. Diffusion occurred under the influence of the electric field caused by ionic charge[33–35]. The decreasing kinetics of pig manure (T_3) is also influenced by the cellular structure[36] and crystal structure of pig manure particles[37,38]. The amorphous structure and polysaccharide nature of hemicellulose is highly biodegradable compared to lignin[39,40] and increasing the specific surface area for mineralization[41,42]. The growth of microorganisms (i.e. *Nitrosomonas* and *Nitrobacter*) has been described by the Michaelis–Menten kinetics, which explains the rate at which the organisms grow and the mechanism of enzyme-catalysed reactions[43]. It assumes the rapid reversible formation of a complex between

Table 3. Kinetics of nitrogen mineralization of different organic sources

Parameters	Kinetic equation	Treatment T_1	T_2	T_3	T_4
Nitrogen cumumlative (ppm)	First-order kinetic	1172.46	1312.5	1354.09	1225.08
	Second-order kinetic	1172.46	1312.5	1354.09	1225.08
N_0 (ppm)	First-order kinetic	2.11	2.17	2.2	2.14
	Second-order kinetic	0.0068	0.0063	0.0067	0.0059
K (µg/g/day) (kinetic constant)	First-order kinetic	0.1066	0.1058	0.1059	0.1054
	Second-order kinetic	–0.0007	–0.0006	–0.0006	–0.0007
R^2 (coefficient of determination)	First-order kinetic	0.88	0.9	0.91	0.89
	Second-order kinetic	0.64	0.65	0.66	0.64

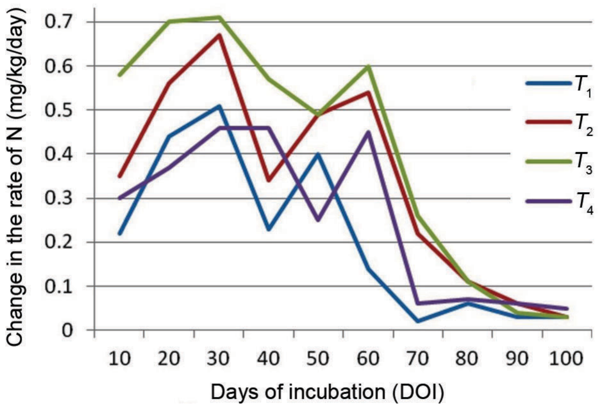

Figure 4. Change in the rate of nitrogen for different organic sources during the incubation time period.

an enzyme and its substrate, and also that the rate of product formation is proportional to the concentration of the complex. Hence, the initial rate of reaction is more and declines later. Beek and Frissel[44] reported that NH_4^+ oxidation, mineralization of proteins, sugar, cellulose, lignin and living biomass follow the first-order kinetics. The sharp decline in the rate of nitrogen after 70 DOI might be due to the immobilization, NH_3 volatilization by diffusion and NH_4^+ clay fixation[43,45]. Soil organic matter aggregates the soils[46].

Conclusion

The present study reveals that different organic treatments (sources) affect the rate of nitrogen release, which could be well accounted for by the first-order kinetics ($R^2 = 0.91$) compared to second-order kinetics. FYM from forest waste has the lowest nutrient supplying capacity (117.46 ppm AN), however, the nutrient supplying capacity of FYM of forest waste is gradually increased till 70 DOI. Pig (T_3) and poultry (T_2) manure are ideal options for nutrient sources with maximum nutrients released at 50–60 DOI. The combination of pig/poultry manure with FYM might be the best solution for enhancing crop production. However, further research on the kinetics and mineralization rate is necessary for nutrient and land management practices.

Conflict of interest: The authors declare no conflict of interest.

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