

RESEARCH ARTICLE

IMPACT OF DIFFERENT SOIL AMENDMENTS ON CARBON AND NITROGEN MINERALIZATION IN ULTISOLS

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ABSTRACT

Quantifying the nutrient mineralization in soils amended with different materials is vital for understanding the nutrient availability for plant uptake. It helps to improve nutrient management by minimizing nutrient loss from the soil. The present research was conducted as a laboratory incubation to assess the nitrogen (N) and carbon (C) mineralization in Ultisols amended with cattle manure, spent poultry layer litter, *Gliricidia* leaves (*Gliricidia sepium*), leaf litter compost, urea, and Siam weed leaves (*Chromolaena odorata*). A Completely Randomized Design (CRD) was used with four replicates, and means were compared with Duncan's multiple range test (DMRT) after analyzing the data with the SPSS 25 statistical package. CO₂-C evolution in amended soils increased up to day 14 of incubation and became stable as incubation progressed. The highest total flux of CO₂-C (171.89 g/kg) was obtained in cattle manure-amended soil. Compost-amended soil showed significantly higher ($p=0.05$) release of mineralized nitrogen (1530.31 mg/kg) over unamended control. The pH in all the amended soils increased over time, with the highest pH in soil amended with compost. Thus, the present study indicated rapid C and N mineralization when different amendments are integrated into the soil.

Keywords: Carbon, Incubation, Mineralization, Nitrogen, Nutrient availability, Soil amendments

INTRODUCTION

Mineralization plays a crucial role in assessing soil nutrient availability, as it determines the conversion of organic matter into essential nutrients that plants can readily absorb. Organic matter accumulates from plant, animal, and microbial residues that exist in the soil or are added as organic amendments. Compost, manure, sewage, green manure, and other organic materials can be added to soil to improve its physicochemical and biological quality and are considered organic amendments (Liyanage *et al.*, 2021). Organic amendments exhibit substantial variability in their composition and stabilization levels, resulting in varying capacities for nutrient release (Sikora *et al.* 2001). Thus, identifying the mineralization patterns after applying soil

amendments is essential for optimizing their nutrient release and synchronizing it with plant nutrient demand. Synchronizing the carbon (C) and nitrogen (N) release patterns with plant requirements and nutrient transformations can improve the efficiency and effectiveness of soil-plant systems, enhancing carbon mineralization, minimizing nitrogen losses, and reducing phosphorus fixation in the soil (Bai *et al.* 2020). Hence, incubation studies on incorporating soil amendments and synchronizing nutrient availability with crop demand should be conducted before amendment practices are applied in agricultural lands.

Many incubation studies have been conducted to determine the mineralization of organic amendments (Liyanage *et al.* 2021; Khalil *et*

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al. 2005; Haer and Benbi 2003). An incubation study is essential to identify the temporal nutrient release pattern by incorporating different amendments into the soil (Bankole and Azeez 2024; Impraim *et al.* 2022). Furthermore, many researchers have used incubation experiments to model the mineralization kinetics of N and C (Liyanage *et al.* 2021; Moharana *et al.* 2015; Wyngaard *et al.* 2018; Stanford and Smith 1972). These studies have shown that N mineralization of various soils follows the first order (exponential) kinetics, where the constant (*k*) remains the same for most soils. At the same time, the potential mineralizable N varies significantly. Additionally, according to the kinetics of C mineralization, incorporating various organic amendments substantially boosts the amount of mineralized carbon, reaching its peak in the early stages of incubation.

Mineralization is a microbial-driven process influenced by several factors, mainly soil properties such as texture, mineralogy, acidity, biological activity, presence of other nutrients, temperature, moisture content, and characteristics of the amendments (Tian *et al.* 2010; Wang *et al.* 2018). The type and quality of organic amendments will affect the stabilization of microbial biomass (Ladd *et al.* 1996). Amendments with high nitrogen content and low carbon-to-nitrogen ratios, such as *Gliricidia* leaves, tend to mineralize rapidly, providing a quick release of nutrients to plants (Liyanage *et al.* 2021; Dossa *et al.* 2015; Kumar *et al.* 2016). High-quality amendments containing >2% nitrogen and C/N ratios <25 may rapidly release N when incorporated into the soil (Abbasi *et al.* 2015). Organic N goes through the mineralization process, which converts the organic N into inorganic N (NO_3^- , NO_2^- or NH_4^+) (Wang *et al.*, 2023; Chatterjee and Lal, 2022). This decomposition process drives soil microorganisms to release plant-available nutrients and CO_2 . The added amendments will supply an energy source (C) to microorganisms involved in N transformations. Thus, the mineralization process can be investigated by measuring either the consumption of substrates or the production of CO_2 . The release of CO_2 is directly

associated with the mineralization of organic materials in the soil. In contrast, materials with higher C: N ratios, like leaf litter compost, may mineralize more slowly, offering a more prolonged nutrient release (Abbasi *et al.* 2015). However, other controlling factors such as the lignin to nitrogen ratio other than C and N ratio, effectively support nitrogen release patterns (Melillo *et al.* 1984). Further, Fox *et al.* (1990) stated that the total lignin and polyphenol to N ratio would indicate initial mineralization. Several other factors affect the mineralization, such as cellulose, hemicellulose and lignin contents of the amendments (Van *et al.* 2000; Hao and Benke 2008; Xie *et al.* 2022).

Many researchers have recognized the positive impact of using plant residues, composts, and manures as organic amendments to enhance soil fertility. Using an incubation study, Kalita *et al.* (2023) studied organic amendments such as farmyard manure, poultry manure, pig manure, and vermicompost. They showed that pig and poultry manure had a maximum nutrient release within 50-60 days of incubation. Liyanage *et al.* (2021) showed that *Gliricidia* leaves decomposed rapidly, with the highest mineral C content in the mineralization process. Incorporating 1 t of *Gliricidia* leaf manure adds 21 kg N, 2.5 kg P, 18 kg K, and other trace elements (Nagavallemma 2000). Furthermore, this study mentioned that *Gliricidia* lopping has consistently supplied N from 7 - 121 mg kg^{-1} soil in 150 days of incubation. Moreover, Siam weed has many soil nutrients, including high N content (Vineela *et al.* 2008). These residues contribute nitrogen (3.79%) to the soil and contain a total % N content of 0.6% (Murthy 2013). Stark *et al.* (2007) stated that green manure crops add labile organic matter to the soil after incorporation. In addition, manure contains a lot of N and P, and is recycled again to the soil and provides 30% of the N requirement for plants. However, aerobically decomposed manure releases less N to the soil. On the other hand, compost in a decomposed state continuously supplies nutrients (Agustina *et al.*, 2019). Poultry litter, a mixture of poultry excreta, bedding material, feathers, and ration, provides plants with 30-50% readily

available N (Bittenbender *et al.* 1998). Prolonged field application of organic amendments may affect soil's organic N composition and mineralization rate (He *et al.* 2014; Nicholson *et al.* 1997). Meanwhile, several studies compare the mineralization of organic amendments and urea (inorganic fertilizer), highlighting how these treatments affect soil nitrogen dynamics compared to organic amendments (Tambone and Adani 2017; Singh *et al.* 2016; Stark *et al.* 2007). In urea, the hydrolysis converts it to ammonia and carbon dioxide in relatively fast reactions using urease enzyme by soil microorganisms (Piotrowska-Długosz, 2020). Stark *et al.* (2007) incubated soil amended with a ground legume (lupin 4.3 t/ha) as an organic form and urea (100 kg/ha) as an inorganic form.

Evaluating soil properties under consistent conditions allows for individually examining soil moisture levels, temperature, microbial interactions, and soil type using incubation studies (Stark *et al.*, 2007). Soil pH is crucial in carbon (C) and nitrogen (N) mineralization among various soil properties. Ultisols, known for their high acidity and low organic matter content, often exhibit low fertility. Numerous studies have utilized acidic soils in incubation experiments (O'Connor, 2023; Uddin *et al.*, 2021; Yang *et al.*, 2021). Atoloye *et al.* (2024) investigated compost and urea mineralization and microbial activity in Ultisols. Uddin *et al.* (2021) studied the nitrogen release patterns and kinetics in acidic soils treated with compost, poultry manure, rice husk biochar, poultry manure biochar, and cow dung combined with urea fertilizer.

In Sri Lanka, common organic amendments incorporated into agricultural lands include compost, Gliricidia, Siam weed, cattle manure, and poultry litter. Researchers amended soils with different soil amendments in varying quantities and forms to investigate the impact of microbial communities on C and N mineralization using incubation studies of various soil types in Sri Lanka (Jayarathna *et al.* 2024; Liyanage *et al.* 2021; Amarasinghe and Gunawardana, 2020; Gunarathne *et al.* 2020). However, organic forms of soil amendments and urea in acidic soils have yet

to be extensively studied in Sri Lanka. Therefore, this study focuses on acidic soils (Ultisols), examining the effects of the widely used soil amendments and urea. It aims to investigate the C and N mineralization dynamics during incubation to compare their effectiveness in releasing inorganic N and stimulating microbial activity.

MATERIALS AND METHODS

Soil characteristics

Soil (0 - 15 cm depth) was collected from a bare land at the Faculty of Agriculture, University of Ruhuna, Kamburupitiya (6° 3' 38.86" N and 80° 33' 50.53" E). According to the USDA soil classification, the soil belongs to the order Ultisols and Hapludults (Ju *et al.* 2006). The collected soil samples were air-dried and sieved through a 4 mm mesh. A sample was used to measure the physico-chemical properties of the experimental soil (Table 1).

Table 1: Some physicochemical properties of soil selected for the incubation study

Soil Properties	Amount in soil
Bulk density	1.5 g/cm ³
Particle density	2.5 g/cm ³
Sand %	65%
Silt %	20%
Clay %	15%
Soil Texture	Sandy loam
pH _w (1:2.5 w/v)	4.4
EC _w (1:5 w/v)	0.07 dS/m
Total organic matter	1.97%
Total nitrogen content	0.053%
Available NO ₃ ⁻ -N	2.74 ppm
Available Ammonium NH ₄ ⁺ -N	25.08 ppm

Characteristics of soil amendments

All organic materials (cattle manure, spent poultry layer litter, Gliricidia, leaf litter compost, and Siam weed) were collected from the farm at the Faculty of Agriculture, University of Ruhuna, Sri Lanka. Urea was purchased from a fertilizer shop. The carbon percentage of all amendments was measured using the Walkley-Black method, and the organic matter percentage was calculated by applying the conversion factor of 1.72. Total nitrogen was determined using the Kjeldahl method (Velp UDK 139, Italy) (Mapa *et al.* 1999). Subsequently, the C/N ratio was

calculated. The total C%, total N%, and the C ratio are presented in Table 2.

Table 2: Characteristics of the amendments used for the incubation study

Type of amendment	Total C %	Total N %	C: N ratio
Cattle manure	15.44	2.98	5.18
Spent poultry litter	24.98	3.53	7.07
Gliricidia leaves	18.30	2.80	6.54
Dry leaf litter compost	41.50	1.49	27.85
Urea	20.00*	46.00*	0.43
Siam weed leaves	18.32	1.65	11.10

*By calculation

Treatments and laboratory incubation study

The incubation study was conducted as a batch experiment with seven treatments: T1 - dry cattle manure, T2 - spent poultry layer litter, T3 - Gliricidia, T4 - leaf litter compost, T5 - urea, T6 - Siam weed, T7 - soil without any treatment. To create experimental units, air-dried and sieved (4 mm) 100 g of soil was added to clean 500 ml glass bottles. Daily monitoring and weighing of the bottles containing soil samples carefully regulated soil moisture content. If moisture levels dropped below the desired threshold, water was added to maintain 60% of the soil's field capacity, ensuring optimal soil moisture conditions throughout the experiment.

The bottles were placed in a dark room at ambient temperature and pre-incubated for two weeks. At the end of the pre-incubation period, each of the seven amendments was separately applied to the glass bottles and thoroughly mixed. Organic amendments were applied at field application rates equivalent to 10 t/ha, and urea was applied at 100 kg/ha after adjusting for the 100 grams of soil used in each bottle.

The tops of the bottles were covered with polythene film with small punctures for aeration and placed inside larger jars for sealing. The experiment was arranged in a Completely Randomized Design with batches for destructive sampling. Each treatment was replicated three times within each batch.

To measure microbial activity via CO₂-C

evolution, another set of bottles was prepared and incubated in the dark at ambient temperature. During incubation, a vial of 1M NaOH and a distilled water tube were maintained inside each bottle. On the relevant day of incubation, NaOH was titrated with 0.1M HCl (McGill and Figueiredo, 1993). Samples were destructively collected from the respective batches for C and N analysis.

Soil sampling and Analysis

Soil samples were collected from the bottles on days 1, 3, 5, 7, 14, 21, 28, 35, and 42 of the incubation periods. The pH, NH₄⁺-N, NO₃⁻-N, and CO₂-C evolution were determined using the methods and instruments described in Table 3.

Table 3: Methods and instruments used to analyze the soil samples

Parameter	Instrument and method used
pH	pH meter; 1:2.5 (w/v soil: water), (HANNA multi-parameter analyzer)
NH ₄ ⁺ -N	Salicylate method by UV visible spectrophotometer (UV 160, Shimadzu, Japan) (Amarasinghe and Gunawardhana, 2020)
NO ₃ ⁻ -N	Salicylate method by UV visible spectrophotometer (UV 160, Shimadzu, Japan) (Amarasinghe and Gunawardhana, 2020)
Microbial activity	Titration method (CO ₂ -C evolution method) (Amarasinghe and Gunawardhana, 2020)

Statistical Analysis

The SPSS 25 statistical package (IBM Corp. 2017) was utilized to determine significant differences among treatments, and means were compared using Duncan's multiple range test (DMRT) at P≤0.05.

RESULTS AND DISCUSSION

Microbial activity (CO₂ evolution) in soil treated with different amendments.

The total CO₂-C evolution during the 42-day incubation period showed significant variations (p=0.05) among the treatments compared with the control. CO₂ evolution increased in all amended soils as incubation progressed, regardless of the type of amendment. However, the daily CO₂-C flux from different amendments decreased

gradually until day 14 of incubation and remained stable, except in T2 (Figure 1). Rapid CO_2 emission was observed in Gliricidia, and similar results were seen in an incubation study for acidic tea soils (Liyanage *et al.* 2021). In the early stages, decomposition rates were high due to readily decomposable components such as sugars, amino acids, and proteins (Pitta *et al.* 2012). The decomposition rate of poultry litter was lower than other treatments due to the accumulation of recalcitrant components such as lignin, tannins, and cellulose (Pitta *et al.* 2012). The breakdown of these complex compounds may occur over a longer period, sustaining microbial activity and increasing CO_2 production.

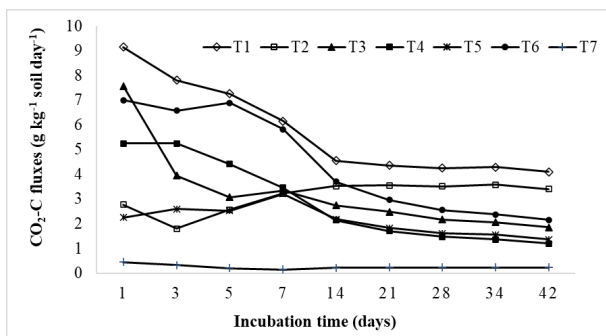


Figure 1: Evolution of daily CO_2 -C fluxes during the 42-day incubation time from soils incorporated with six amendments

T1: cattle manure amended soil, T2: spent poultry layer litter amended soil, T3: Gliricidia amended soil, T4: leaf litter compost amended soil, T5: Urea amended soil, T6: Siam weed amended soil T7: soil without any treatment

Moreover, the total CO_2 -C evolution was highest (171.89 g/kg) in cattle manure-amended soil, possibly due to the higher levels of microorganisms that enhance microbial activity (Figure 2). The control soil had deficient microbial activity because of the low availability of energy sources. The amount of CO_2 -C in Gliricidia leaves amended soil is lower than in Siam weed leaves amended soil. The study by Kausadikar *et al.* (2020) stated that the amount of CO_2 released by Gliricidia leaves is less than other plant residues. The incorporated amendments regulated the cumulative CO_2 -C evolution as cattle manure

> compost > Siam weed > Gliricidia < poultry litter > urea > control soil (Figure 2). Cattle manure contains a substantial amount of organic carbon in the form of easily degradable organic matter. The high availability of this carbon can lead to rapid microbial activity, resulting in higher carbon mineralization rates. Nitrogen in cattle manure may be more readily assimilable by microbes, promoting microbial growth and activity and further driving carbon mineralization. According to Hartz *et al.* (2000), mineralization of manure C averaged higher C content in 24 weeks, while plant residue compost C mineralization averaged only a lesser amount. However, urea-amended soil showed less CO_2 evolution. Urea does not provide carbon as an energy source for soil microbes compared to compost or manure, which provide both nitrogen and carbon, supporting a higher level of microbial activity. The soil's pH is initially increased, resulting in high pH soils in the latter part of incubation, ranging from the lowest value of 4.85 to the highest value of 8.41. When ammonia is released into the soil, the pH increases due to the formation of ammonium hydroxide. Previous studies have documented the rise in soil pH due to the application of animal manure (Whalen *et al.*, 2000; Wyngaard *et al.*, 2016).

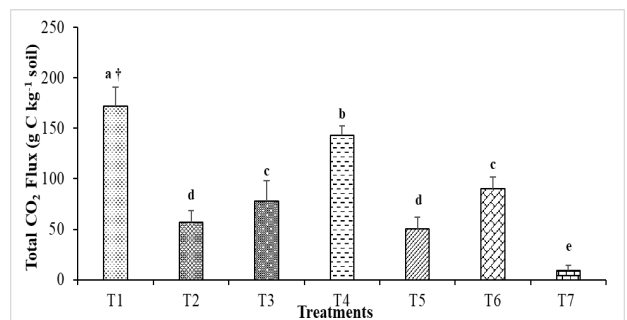


Figure 2: Total CO_2 evolution during 42-day incubation time in different amendments

T1: cattle manure amended soil, T2: spent poultry layer litter amended soil, T3: Gliricidia amended soil, T4: leaf litter compost amended soil, T5: Urea amended soil, T6: Siam weed amended soil T7: soil without any treatment. Error bars indicate $\pm$ standard deviations. †Means with the same letters are not significantly different at $p > 0.05$ by Duncan's Multiple Range Test.

Mean values designated by the same letter are not significantly different at $p < 0.05$ as determined by DMRT

C:N ratio, thus showing less NO_3^- -N content at the beginning of the incubation. However, a significant portion of the easily decomposable carbon might have already been broken down during composting. Then, nitrogen in leaf litter compost may become more available over time as the organic matter stabilizes and decomposes, resulting in higher nitrogen mineralization rates at the end of the incubation period (42 days). When compared to the control on day 1, all treatments showed significantly higher ($p=0.05$) NO_3^- -N values except the T4 treatment. On day 3, T4, T5, and T6 treatments showed significantly higher NO_3^- -N than the control treatment. However, on day 5, only the T4 treatment showed a significantly higher NO_3^- -N value than the control. On days 7, 14, and 21, all treatments showed significantly higher ($p=0.05$) values than the control. However, on day 28 of incubation, T1 and T2 showed significantly less NO_3^- -N than the control, while other treatments showed significantly higher values. The rapid release of NH_4^+ -N was found in urea up to day 5 (Table 4) due to hydrolysis, and it was reported in other research studies (Khalil *et al.* 2005; MacLean and MacRae 1987). Liyanage *et al.* (2014) stated that urea hydrolysis has increased pH within four days when hydrolysis is completed in urea-amended soils.

According to the results, the total mineralized N values varied as compost > poultry litter > Gliricidia > Siam weed > urea > cattle manure > control (Figures 3 & 4). Thus, it was clear that compost-amended soil had the highest plant-available N value ($1530.31 \text{ mg Kg}^{-1}$) compared to other treatments, while the control soil had the lowest cumulative N ($452.41 \text{ mg Kg}^{-1}$). However, the cumulative N mineralization in poultry litter-amended soil was slightly lower than in compost-amended soil. The research from a 90-day aerobic incubation study found that prolonged application of poultry litter can increase soil N mineralization in sandy loam soil (Watts *et al.* 2010). The research conducted by Wyngaard *et al.* (2018) described that cumulative N mineralization is higher in poultry litter-amended soil than inorganic N

fertilizer-amended soil. According to Bittenbender *et al.* (1998), poultry litter, a mixture of poultry manure, bedding material, feathers, and feed remnants, provides plants with 30-50% readily available nitrogen (N). The Siam weed amended soil (T6) showed higher cumulative N during the incubation than T2, T5, and T7. Siam weed has many soil nutrients, including high N content (Vineela *et al.* 2008). According to Murthy (2013), Siam weed residues contribute 3.79% nitrogen to the soil.

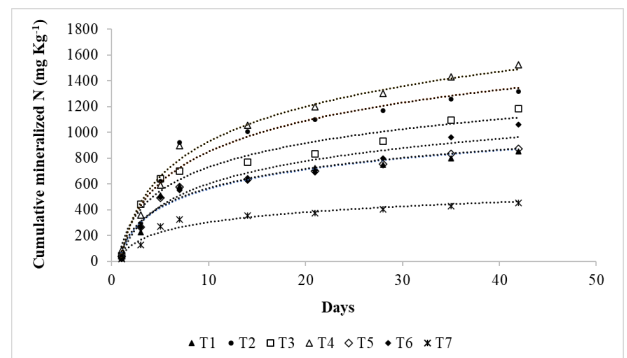


Figure 3: Cumulative amount of N mineralized by different soil amendments during incubation.

T1: cattle manure amended soil, T2: spent poultry layer litter amended soil, T3: Gliricidia amended soil, T4: leaf litter compost amended soil, T5: Urea amended soil, T6: Siam weed amended soil T7: soil without any treatment

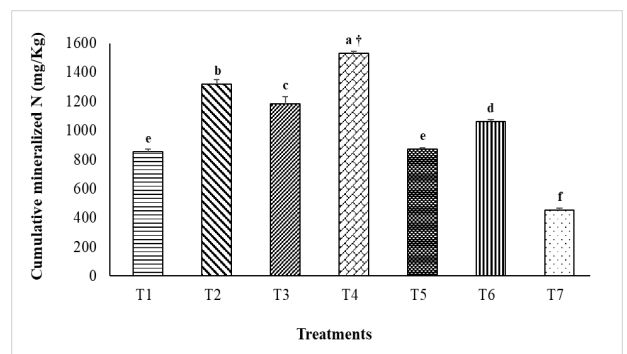


Figure 4: Cumulative mineralized N in soils amended with different amendments.

T1: cattle manure amended soil, T2: spent poultry layer litter amended soil, T3: Gliricidia amended soil, T4: leaf litter compost amended soil, T5: Urea amended soil, T6: Siam weed amended soil T7: soil without any treatment. Error bars indicate $\pm$ standard deviations. †Means with the same letters are not significantly different at $p > 0.05$ by Duncan's Multiple Range Test.

Net Nitrogen Mineralization

Net N mineralization is a balance between the rates of mineralization (release of inorganic nitrogen as $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) and immobilization (uptake of inorganic nitrogen by microorganisms), indicating the net amount of inorganic nitrogen available in the soil for plant use at the relevant incubation day 't'. It varied significantly ($p < 0.05$) among the different treatments during incubation. The mineralization of soil amendments depends upon different soil factors such as pH, temperature, oxygen replacement, type of amendment, level of other nutrients, microbial quantity and diversity and water availability. Stanford and Smith (1972) stated that soil pH had little influence on mineralization. However, according to their study, in very acidic soils, the pH increased significantly due to a gradual increase of mineralization after the initial incubation period. The present study with acidic pH (Ultisols) obtained similar results. On the other hand, Khalil *et al.* (2005) observed that nitrification increased as the pH level increased over the incubation period. Further, the net mineralized N strongly depends upon the N level of the amendments. Total soil N and organic C quantities are basic indicators of soil N mineralization (Bengtsson *et al.* 2003; Selles *et al.* 1999). It was indicated that amendments which have a low C/N ratio improve the mineralization of N (Khalil *et al.* 2005). The net N mineralization of compost is low (Hadas and Portnoy 1994; Benitez *et al.* 1998). The mineralization rate is influenced by various compost characteristics, including the type of raw material, composting method, maturity, and carbon-to-nitrogen (C/N) ratio of the compost. Factors such as the compost application rate, soil characteristics, and climatic conditions also play significant roles (Hadas *et al.* 1996; Whalen *et al.* 2001). However, in the present incubation study, the net N mineralization of soil amendments during the 42-day incubation period varied significantly among treatments, irrespective of the C/N ratio. The reason may be ammonia volatilization or immobilization during varied microbial activities.

Further, a higher quantity of N remains in

organic form in compost-treated soils, and this organic N mineralizes to a plant-available form (Moharana *et al.* 2015). Adding chemical nitrogen (N) to organic N can enhance the mineralization process due to increased microbial activity, leading to higher levels of mineral N. This approach aligns with the principles of an integrated nutrient management system. Thus, mixing chemical fertilizers may also affect the net N mineralization of organic amendments as it lessens the C/N ratio of the soil, which improves the mineralization of soil organic matter.

CONCLUSIONS

The present incubation study indicates the differences in C and N mineralization depending on the heterogeneity of amendments. The soil was amended with different amendments in the incubation study, and continuous C mineralization decline was observed until day 14 and became constant throughout the incubation period. The highest evolved $\text{CO}_2\text{-C}$ flux was observed in amended soils of cattle manure. In the case of cumulative N mineralization, soil amended with leaf litter compost reported the highest release of nitrogen. Nitrogen release from all treatments was higher up to 21 days compared to the control soil. Urea-amended soil showed less microbial activity than the other amendments, evolving a lesser amount of $\text{CO}_2\text{-C}$ during incubation. Further, efforts should be made to link the findings of the incubation study to field-level implementation.

AUTHOR CONTRIBUTION

SRA and SDW designed and supervised the study. PKPSD and SRA performed the experiments and analyzed the data. SRA drafted the manuscript and critically revised it.

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