



RESEARCH ARTICLE

# Synergistic and independent effects of crop residue and nitrogen fertilizer incorporation on nitrogen dynamics and GHG emissions

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## Abstract

Crop residues with a high carbon-to-nitrogen (C/N) ratio immobilise nitrogen (N) released during decomposition in soil, thereby reducing nitrogen losses through leaching, denitrification and nitrous oxide (N<sub>2</sub>O) emissions. As microbial decomposition is mainly governed by C:N ratio, residues with wider C:N ratio promote nitrogen immobilisation, limit mineral N availability and slow down nitrification-denitrification pathways, ultimately influencing both CO<sub>2</sub> and N<sub>2</sub>O fluxes. A laboratory incubation experiment was conducted over 120 days to investigate the influence of crop residues (paddy, maize, red gram, green gram and cotton) and mineral nitrogen additions on nitrogen immobilisation and greenhouse gas emissions. The experiment was laid out in a completely randomised design (CRD) with three replications and data were analysed using ANOVA to determine treatment differences. The experiment was performed under controlled conditions at room temperature with a moisture content of 55 % water-filled pore space. Residues were added at the rate of harvest biomass typically available to farmers in Telangana. Results indicated that maximum N<sub>2</sub>O emissions occurred within the first 30 days of incubation across all treatments. On average, incorporating residues into the soil increased N<sub>2</sub>O emissions by 66.78 % over control. Soil amended with residues exhibited significantly lower nitrate (NO<sub>3</sub><sup>-</sup>-N) levels, with minimum values of 21 mg N kg<sup>-1</sup> for maize and 27 mg N kg<sup>-1</sup> for paddy observed on day 30. There was an increase in soil CO<sub>2</sub> fluxes immediately following residue incorporation, with the highest CO<sub>2</sub> emissions recorded in soil treated with cotton residue and nitrogen (19.95 µg C g<sup>-1</sup> of soil). Cumulative CO<sub>2</sub> emissions were highest in soil amended with green gram residue and inorganic nitrogen, which is attributed to the lower C:N ratio (21.81) of the residue, which enhanced the decomposition process and increased CO<sub>2</sub> emissions. Cumulative N<sub>2</sub>O emissions were significantly higher when inorganic fertilizer nitrogen was applied along with residues, accounting for approximately 79.75 % more cumulative N<sub>2</sub>O emissions, compared to soil amended with residues alone.

**Keywords:** crop residues; C:N ratio; decomposition; GHG emissions; NO<sub>3</sub><sup>-</sup>-N; NH<sub>4</sub><sup>+</sup>-N; nitrogen immobilisation

## Introduction

One of the most essential components that sustain human existence is nitrogen. Globally, approximately 67.84 million tonnes of nitrogen are applied to agricultural land annually (1). However, a significant portion of the applied N is lost from the agricultural system, leading to environmental pollution, which has led to synthetic N fertilizer being characterised as "too much of a good thing" (2). Gaining a deeper understanding of how various farming methods affect the dynamics of soil mineral N is crucial for increasing its efficiency and lowering pollution. An efficient way to maintain soil organic matter concentration, boost biological activity, improve physical characteristics and increase nutrient availability is to return crop residues to the soil (3). One important

component of soil that has been significantly affected by crop residue management is organic matter. The primary form of nitrogen that is available to plants is soil inorganic nitrogen, which is obtained from fertilizer and soil organic nitrogen. Historically, relation between soil N dynamics and plant residue quality has not been an important aspect (4). Recent studies, however, have shown that the characteristics of the returned crop residues affect the amounts of inorganic nitrogen in the soil. For instance, higher quality plant residues with low lignin and cellulose concentrations, high N concentrations and low C:N and lignin:N ratios often give rise to high N mineralization rates. Low-quality residues, on the other hand, have a slower rate of N mineralization, which might have a detrimental effect on plant-available nitrogen because of its effect on nitrogen immobilization (5).

Large amounts of available N in the soil can result in the production of NO<sub>x</sub> gases, which include nitrous oxide (N<sub>2</sub>O), a greenhouse gas linked to global warming. N<sub>2</sub>O emissions from soil corresponds to the mineral N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) produced, provided other parameters (temperature, soil moisture, organic carbon, pH) are favourable for denitrification (6). Since residue quality influences decomposition (7), using N fertilizer in conjunction with low-quality (C/N ratio of > 42:1) maize (*Zea mays* L.) stover residue could regulate the balance of N mineralization and immobilization (8) and thereby reduce N losses (9). Previous researchers discovered that when residues and fertilizer N were applied together, the patterns of C mineralization and N immobilization in a 60-day incubation were affected by residue quality (10).

The application of residue and fertilizer inputs together still resulted in a lower mineral N pool because of the immobilization of fertilizer-derived N (11). Wheat (*Triticum aestivum* L.) straw and cotton (*Gossypium hirsutum* L.) residue led to a rapid immobilization of N that affected microbial activity and further mineralization. Lower N<sub>2</sub>O losses may result from the first decrease in mineral N caused by immobilization from the application of crop residue with a high C/N ratio (12). Accordingly, this soil fertility management technique could be used in locations with higher soil N losses due to the benefit of combining low-quality crop residue with N fertilizer in reducing N mineralization and N losses (9).

The hot semi-arid agro-climatic conditions of Telangana state is marked by high summer temperatures (35–45 °C), 750–900 mm rainfall during monsoon, low organic carbon, predominately red and black soils create conditions under which nitrogen transformations and greenhouse gas emissions become highly responsive to residue quality and fertilizer inputs. The major residues producing crops in Telangana are paddy (*Oryza sativa*), maize (*Zea mays*), red gram (*Cajanus cajan*), green gram (*Vigna radiata*), cotton (*Gossypium hirsutum*) residues. Due to decrease in soil fertility, the residues of these crops may be incorporated into soil along with inorganic fertilizers. Although many studies have been carried out to determine impact of residue quality on N mineralization and immobilization, there is limited information available on how diverse crop residues that are generally generated in Telangana influence CO<sub>2</sub> and N<sub>2</sub>O emissions under controlled conditions especially when these residues are combined with N fertilizer and incorporated into soil. Most of the earlier studies focus on single residue or short incubation period, which usually provide partial information of residue-N interactions. The semi-arid soils have not been sampled in terms of comprehensive comparisons of various residues that vary in C/N ratio and biochemical composition and the semi-arid soils have not been monitored in detail on the inorganic components of N and greenhouse gases.

Thus, this paper will fill this gap in the literature by assessing the effect of residues of major crops grown in Telangana, which is a continuum of C/N ratios, on GHG emissions and time changes in inorganic N decomposition. The piece of work offers geographical information that is critical in the maximization of residue and fertilizer use to reduce nitrogen losses and environmental effects. The objective of this study is to evaluate impact of crop residues with a range of C/N ratios and biochemical characteristics when incorporated into soil along with or without N fertilizer on GHG emissions and the release pattern of inorganic N fractions during the decomposition of these residues.

## Materials and Methods

### Soil and crop residues

The surface soil (0–15 cm) used for this study was collected from B Block of college farm, College of Agriculture, Rajendranagar, PJTSAU, at an altitude of 542.6 m above mean sea level, 78°23' E longitude and 17°19' N latitude. It comes under Southern Telangana Agroclimatic Zone of Telangana and according to Troll's classification, it falls under Semi-Arid Tropics (SAT). The soil is sandy clay loam and contains 0.57% OC, available N of 177 kg ha<sup>-1</sup>. The soil was non-saline (EC<sub>1:2.5</sub>, 0.45 dS m<sup>-1</sup>) and pH<sub>12.5</sub> was 8.4. The soil was air-dried, pounded and then passed through 2 mm sieve. Crop residues of paddy (*Oryza sativa* L.), maize (*Zea mays* L.), cotton, red gram and green gram were used in this study. The residues were dried at 65 °C for 48 hr for gravimetric water content assessment and ground and sieved to <2 mm for chemical analysis. The total C in residues and soil was determined by loss on ignition method. Total N was determined by catalyst digestion and heating up to 420 °C until a clear mixture was observed (13). The mineral N in air-dried soil was extracted with 2 M KCl at a ratio of 1:10 (14). The extract was analysed for nitrate (NO<sub>3</sub><sup>-</sup>), ammonium (NH<sub>4</sub><sup>+</sup>) colorimetrically. The lignin, cellulose and fiber content of the crop residues were determined with the acid detergent fiber method.

### Incubation

#### Pre-incubation

The experiment was conducted by taking 100 g of the soil in 150 mL plastic cups and the soil was kept at 55% WFPS (water filled pore space) by adding 13 mL of distilled water and kept in dark for 10 days at room temperature (25 °C), for pre-incubation. Pre-incubation was done prior to the start of incubation experiment to initiate microbial activity in the soil

#### Incubation

After pre-incubation, residues were mixed thoroughly in the soil as per treatments (Table 1) and urea (7.76 mg/100 g) was added along with distilled water into the soil and bulk density of 1.0 Mg m<sup>-3</sup> was maintained in all treatments. After treatment imposition, the cups were incubated at room temperature (25 °C), for 120 days. The entire experiment was divided into two sets. First set was used to collect greenhouse gas emissions and second set was used to conduct mineralization study where destructive sampling was done at given intervals and the samples were used for analysis of NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, C:N ratio and available N. Treatments are given in Table 2. Biochemical properties of residues used in the study were given in Table 3. The properties of soil used for incubation are mentioned in Table 4.

**Rate of residue to be employed:** Different (based on top residue available from these kharif crops that is possible to deploy in succeeding rabi (maize) crop upon estimating them in field).

Paddy straw yield: 4.12 t/ha

Maize stover yield: 16.75 t/ha

Red gram stalks yield: 6 t/ha

**Table 1.** Amount of residue added per 100 g soil (g)

1.	Rice	0.18
2.	Maize	0.74
3.	Cotton	0.50
4.	Green gram	0.20
5.	Red gram	0.26

**Table 2.** Treatment details of laboratory study

T <sub>1</sub>	Control (Soil with no N and no residue)
T <sub>2</sub>	Soil + 80 kg N ha <sup>-1</sup> *
T <sub>3</sub>	Soil + Residue 1 (Maize straw)
T <sub>4</sub>	Soil +Residue 2 (Paddy straw)
T <sub>5</sub>	Soil +Residue 3 (Cotton stalks)
T <sub>6</sub>	Soil +Residue 4 (Green gram stalks)
T <sub>7</sub>	Soil +Residue 5 (Red gram stalks)
T <sub>8</sub>	Soil + Residue 1 (Maize straw) + 80 kg N ha <sup>-1</sup> *
T <sub>9</sub>	Soil + Residue 2 (Paddy straw) + 80 kg N ha <sup>-1</sup> *
T <sub>10</sub>	Soil + Residue 3 (Cotton stalks) + 80 kg N ha <sup>-1</sup> *
T <sub>11</sub>	Soil + Residue 4 (Green gram stalks) + 80 kg N ha <sup>-1</sup> *
T <sub>12</sub>	Soil + Residue 5 (Red gram stalks) + 80 kg N ha <sup>-1</sup> *

\*80kg ha<sup>-1</sup> is chosen because it is the amount employed for rabi maize at the time of sowing as 1/3<sup>rd</sup> of RDN.

**Table 3.** Properties of residues used in lab study 1

S. No.	Particulars	Rice	Maize	Cotton	Green Gram	Red Gram
1.	Lignin (%)	24.7	14.8	21.0	4.5	6.5
2.	Cellulose (%)	35.0	38.1	36.8	37.2	34.0
3.	Hemicellulose (%)	13.6	30.1	16.0	6.13	25.2
4.	Total C (%)	48.98	49.91	50.07	37.52	56.86
5.	Total N (%)	0.66	0.35	2.13	1.72	1.28
6.	C:N	74.21	142.60	23.51	21.81	44.42
7.	ADF (Acid Detergent Fiber) (%)	43.6	48.0	64.0	28.3	69.3
8.	Ash (%)	15.53	12.74	13.59	37.62	1.98
9.	Proteins (%)	4.12	2.20	13.31	10.75	8.00
10.	Total phenols (mg GAE (Gallic Acid Equivalent) / 100 g)	31.03	95.55	11.850	100.750	98.375

**Table 4.** Initial properties of soil used for lab studies

S. No.	Property	Value
1.	pH	7.9
2.	EC (dS m <sup>-1</sup> )	1.14
3.	Organic Carbon (%)	0.74
4.	Total C (%)	2.002
5.	Total N (%)	0.122
6.	C:N	16.4
7.	Bulk Density (Mg m <sup>-3</sup> )	1.00
8.	% Water Filled Pore Space (WFPS)	42.11
9.	Total Porosity	62
10.	NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	98
11.	NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	42

Cotton stalks yield: 11.25 t/ ha

Green gram residue yield: 4.50 t/ ha

**Frequency of sampling:** 2, 4, 6, 8, 10, 20, 30, 45, 60, 75, 90, 105, 120 days after incubation.

**Duration of lab experiment:** 120 days

All the treatments were organized in a completely randomized design (CRD). Experimental units were individual incubation cups and were put in laboratory shelves in one controlled room. Each cup was given a random number to designate its position and rearranged back to its original position after each sampling of the gases to eliminate positional bias. Cups were stored at the minimum spacing between cups of about 5–7 cm to avoid any micro-environmental interference (gradients of temperature and moisture) and to avoid unintentional cross-contamination during handling. The gas-tight lids (and perforated lids respectively) were used on each cup according to its purpose and made sure that the gaseous exchange or moisture loss of one unit did not affect another. Each treatment was mixed and sampled using separate spatulas, funnels and gloves and tools were washed with 70 % ethanol between treatments to ensure isolation. The two sets of incubation were processed separately in the experiment.

The results of the incubation set were put under the three replicates, which were sampled by gas during the period of 120 days. The vacuum grease was applied to the inside vertical rim of every chamber lid to ensure that it did not leak. The columns containing the incubation were aerated at 30 sec of time to provide an aerated environment, before removing the top cap through an airtight three-way stopcock syringe (60 mL capacity) into the head space to obtain the gas. A homogenization of the headspace air was also done before extraction with pumping of the syringe two times to prevent stratification of the concentration. The gas collected was transferred at once in pre-evacuated Labco exetainer vials.

Concentrations of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O were measured in 2–3 days with a modified gas chromatograph (Bruker 450, Bruker Corporation, USA) with three detectors: an electronic capture detector (ECD) to measure N<sub>2</sub>O, a flame ionization detector (FID) to measure CH<sub>4</sub> and a thermal conductivity detector (TCD) to measure CO<sub>2</sub>. Two stainless-steel columns packed with 80100 mesh Porapak Q (column 1: 1 m x 2.2 mm i.d.; column 2: 3 m x 2.2 mm i.d.) were used to separate nitrous oxide which was detected by the ECD. The stainless-steel column (2 m x 2.2 mm i.d.) filled with 5080 mesh Porapak Q was employed to isolate carbon dioxide and after that, Hydrogen was added to reduce CO<sub>2</sub> to CH<sub>4</sub> in a nickel catalytic converter kept at 37.5 °C; finally, CH<sub>4</sub> was measured via FID. The GC oven temperature was set to 55 °C, ECD and FID temperature were set to 330 °C and 220 °C, respectively. Aquarium air pump (model AP -208) was used to aerate plastics occasionally to stop the buildup of CO<sub>2</sub> in the interior of the incubation jars. The jars were opened and the cups briefly taken off and then the jars were resealed before flushing with room air for 1 min.

Gas sampling was done at the interval of 2, 4, 6, 8, 10, 20, 30, 45, 60, 75, 90, 105 and 120 days of incubations. Cumulative CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emissions time-dependent cumulative CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emissions have been determined by linear interpolating the natural log-transformed fluxes of the sample dates.

## Statistical analysis

All data was statistically analysed by one-way analysis of variance using the OPSTAT software package. Critical difference (CD at  $p < 0.05$ ) between means was used to identify differences in inorganic N,  $N_2O$ ,  $CO_2$  and  $CH_4$ .

## Results and Discussion

### Mineral N status ( $NH_4^+$ and $NO_3^-$ )

The effect of different crop residues on changes in mineral N status ( $NH_4^+$  and  $NO_3^-$ ) was studied for 120 days, where the sampling was done on 2,4,6,8,10,20,30,45,60,75,90,105 and 120 DAI (Fig. 1a, b). The  $NH_4^+$  and  $NO_3^-$  content in soil under incubation was significantly influenced by different crop residues and nitrogen fertilizer throughout the incubation period (Table 5). In control ( $T_1$ ) and soil + N ( $T_2$ ), the  $NH_4^+$  - N increased significantly over time and reached

peak on day 10, later decreased up to 120<sup>th</sup> day. In soil amended with paddy ( $T_4$ ) and maize ( $T_3$ ) residue as well as soil amended with paddy residue along with first split dose of nitrogen ( $T_8$ ) and maize residue along with first split dose of nitrogen ( $T_9$ ), there was approximately 10 % decrease in the release of  $NH_4^+$  - N up to 45<sup>th</sup> day, compared to start of the experiment and later it increased up to 120<sup>th</sup> day. In the soil amended with red gram residue ( $T_7$ ) and red gram residue along with first split dose of nitrogen ( $T_{12}$ ) the  $NH_4^+$  - N was decreased from day 2 to day 20, later there was an increase upto 120<sup>th</sup> day. In soil amended with green gram residue ( $T_6$ ) and soil amended with cotton residue ( $T_5$ ) there was increase in the release of  $NH_4^+$  - N up to 60<sup>th</sup> day and later it has reduced up to 120<sup>th</sup> day. In soil amended with cotton residue along with first split dose of nitrogen ( $T_{10}$ ) and green gram residue along with first split dose of nitrogen ( $T_{11}$ ), the  $NH_4^+$  - N increased up to 90<sup>th</sup> day and later there was a decrease up to 120<sup>th</sup> day.

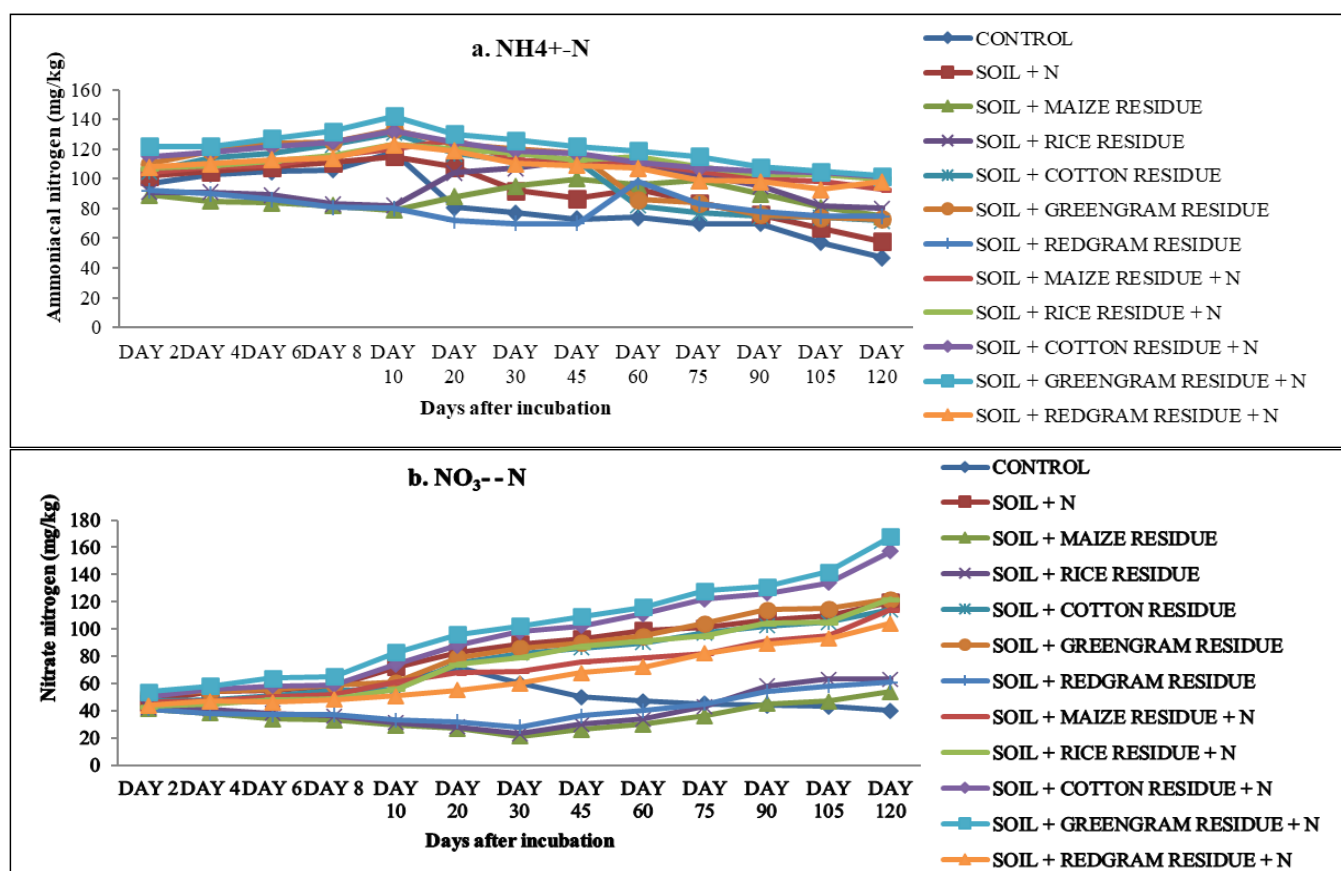


Fig. 1. Effect of different crop residues and N application on inorganic nitrogen fractions during incubation. a:  $NH_4^+$ -N; b:  $NO_3^-$ -N.

Table 5. Effect of different crop residues and N application on cumulative GHG emissions during incubation

Treatment	Cumulative $CO_2$ Emissions ( $\mu g C g^{-1}$ of soil)	Cumulative $CH_4$ Emissions ( $\mu g C g^{-1}$ of soil)	Cumulative $N_2O$ Emissions ( $\mu g N g^{-1}$ of soil)
Control ( $T_1$ )	42.64	0.011	0.09
Soil + N ( $T_2$ )	72.33	0.012	0.30
Soil + Maize Residue ( $T_3$ )	84.52	0.013	0.15
Soil + Rice Residue ( $T_4$ )	100.61	0.013	0.09
Soil + Cotton Residue ( $T_5$ )	82.42	0.012	0.07
Soil + Greengram Residue ( $T_6$ )	81.95	0.012	0.10
Soil + Redgram Residue ( $T_7$ )	125.82	0.012	0.06
Soil + Maize Residue + N ( $T_8$ )	134.90	0.012	0.54
Soil + Rice Residue + N ( $T_9$ )	147.83	0.011	0.38
Soil + Cotton Residue + N ( $T_{10}$ )	134.31	0.011	0.65
Soil + Greengram Residue + N ( $T_{11}$ )	148.38	0.012	0.42
Soil + Redgram Residue + N ( $T_{12}$ )	156.20	0.012	0.36
C.D. ( $p=0.05$ )	6.294	NS	0.02
SE(m) $\pm$	2.144	0.001	0.01
SE(d)	3.032	0.001	0.01

The data indicated that the dynamics of ammoniacal nitrogen were primarily governed by the C:N ratio of the incorporated crop residues. In soil amended with residues having high C:N ratio viz., maize and paddy, there was an apparent decline in the  $\text{NH}_4^+$ -N up to 45<sup>th</sup> day which is a result of temporary N immobilization due to high C:N ratio of residues. The crop residues of maize and paddy immobilized soil  $\text{NH}_4^+$ -N, with maximum reduction in  $\text{NH}_4^+$ -N at 45 days of incubation. Approximately 10% of the  $\text{NH}_4^+$ -N was immobilized from day 2 to day 45. Among residues having high C:N ratio, maize residue has greater effect on reducing the soil N mineralization compared to paddy. In crop residues having higher C:N ratio, there was a decrease in mineral N pool due to immobilization of N by soil microbes, as the C:N ratio was high (15). In contrast, residues having lower C:N ratio viz., cotton, green gram had increased the  $\text{NH}_4^+$ -N immediately after addition to soil. In soil amended with cotton and green gram residue without inorganic N addition ( $T_5$  and  $T_6$  respectively), there was an increase of  $\text{NH}_4^+$ -N upto 30<sup>th</sup> day and later there was a slow decrease towards the end of the incubation. In soil amended with cotton and greengram residue with inorganic N ( $T_8$  and  $T_{11}$  respectively) addition at the start of the experiment, the  $\text{NH}_4^+$ -N showed an increase upto 90<sup>th</sup> day and then after, there was a slight decrease upto 120<sup>th</sup> day. Among all the treatments, the soil amended with cotton and green gram residue along with first split of inorganic N addition at the initiation of the experiment showed highest  $\text{NH}_4^+$ -N at different sampling dates.

The residues having lower C:N ratio, when added to soil along with inorganic N fertilizer, led to rapid ammonification and subsequent increase in  $\text{NH}_4^+$ -N. In soil amended with inorganic N fertilizer, the  $\text{NH}_4^+$ -N was increased upto 10<sup>th</sup> day and thereafter the concentration tended to decline with time. In previous reports, there is a strong negative correlation between the N mineralised from crop residues and their C:N ratio (16). The results were in accordance with the findings of previous researchers, who reported that in soil amended with urea, there was an increase in the  $\text{NH}_4^+$ -N during the initial period i.e., upto 6<sup>th</sup> day and later it was decreased, after working on the impact of addition of residues (sugar beet leaves) to soil on N mineralization, have concluded that soil amended with residues showed significantly higher mineral N compared to unamended soil (17, 18). Combining inorganic N fertilizer with organic N sources slows down the mineralization process to decompose crop residues. Therefore, the  $\text{NH}_4^+$ -N remains stable in soil for longer periods in the mineral pool and there is a chance for delaying of nitrification process, consequently N losses will be minimized.

In soil amended with inorganic N ( $T_2$ ), the  $\text{NO}_3^-$ -N increased significantly over time upto 120<sup>th</sup> day. In soil amended with paddy ( $T_4$ ), red gram ( $T_7$ ) and maize ( $T_3$ ) residue as well as soil amended with paddy residue along with first split dose of nitrogen ( $T_8$ ) and maize residue along with first split dose of nitrogen ( $T_9$ ), there was decrease in the release of  $\text{NO}_3^-$ -N upto 30<sup>th</sup> day and later it has been increased upto 120<sup>th</sup> day. In the soil amended with red gram residue along with first split dose of nitrogen ( $T_{12}$ ) the  $\text{NO}_3^-$ -N was decreased from day 2 to day 10, later there was an increase upto 120<sup>th</sup> day. In soil amended with green gram residue ( $T_6$ ) and soil amended with cotton residue ( $T_5$ ) there was increase in the release of  $\text{NO}_3^-$ -N upto 120<sup>th</sup> day. In soil amended with cotton residue along with first split dose of nitrogen ( $T_{10}$ ) and green gram residue along with first split dose of nitrogen ( $T_{11}$ ), the  $\text{NO}_3^-$ -N increased throughout the

incubation period. Among all the treatments, the soil amended with residues and first split of inorganic nitrogen recorded significantly higher  $\text{NO}_3^-$ -N compared to soil amended only with residues. The results were in consistent with the findings of previous researchers, who have reported that accumulation of  $\text{NO}_3^-$ -N under poultry manure (PM), white clover (*Trifolium repens*) residues (WCR) and PM + WCR was 16, 20 and 38 %, respectively, that had been significantly increased to 55, 63 and 69 % when urea nitrogen (UN) was combined with these amendments (19). The increasing nitrification in the residue amended treatments was due to the increasing microbial biomass C and N that may have affected microbial activity, thereby improving N transformation processes, including nitrification. Various losses of mineral N can be reduced by combining inorganic N fertilizer with organic N sources.

The temporal variation of  $\text{NH}_4^+$ -N in the 120 days incubation was very much an expression of the biochemical quality of the residue controlling the soil microbial processes and the residue C:N ratio proved to be the critical factor in the immobilization-mineralization activities. Maize and paddy are the high C:N residues that caused strong microbial N immobilization in the initial incubation due to high requires of N by decomposers to produce enzymes and biomass in order to break down lignin-rich and cellulose-rich substrates, which in turn drained the mineral N pool of soil and had a strong effect on the observed data on the decrease in both  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ . With continued decomposition processes and an accelerated microbial turnover, the immobilized N was returned to the mineral pool during microbial lysis and oxidative depolymerization, which explains the gradual increase in  $\text{NH}_4^+$ -N and the following increase in  $\text{NO}_3^-$ -N after the nitrifiers can resume their sources of nutrition. Conversely, low C:N residues like cotton and green gram had more labile carbohydrates and soluble N compounds, so less microbial N was required to break them down; this favored rapid mineralization, immediate rises in  $\text{NH}_4^+$ -N and rapid nitrification by the presence of ammonium to stimulate autotrophic nitrifiers. Combination of residues and inorganic N eased the N limitation in microbes, decreasing the strength of immobilization and increasing the initial ammonification rate, but preserving adequate  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$  levels in the residues + N treatment; this was why the  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$  levels were always higher in the residue + N treatment. The steady increase in  $\text{NO}_3^-$ -N with most of the treatments post-mid-incubation indicates a change in microbial N uptake with oxidative N conversions, promoted by the enhancement of aeration and reduced competitive microbial demand. The urea-only treatments exhibited a typical behavior of rapid initial  $\text{NH}_4^+$  accumulation due to the hydrolysis process, which is then reduced to form  $\text{NO}_3^-$  due to active nitrification. Taken together, the dynamics between the mineral N and minerals were regulated by (i) the demand by microbes in C and N through residue quality, (ii) the ratio between the immobilization during the initial decomposition and the remineralization during microbial turnover, (iii) the demand in  $\text{NH}_4^+$  availability and microbial competition in nitrification and (iv) the integrative effect of residue combination with inorganic N, which enhanced microbial activity, coordinated N release and reduced N bottlenecks in the course of decomposition.

## **N<sub>2</sub>O emissions**

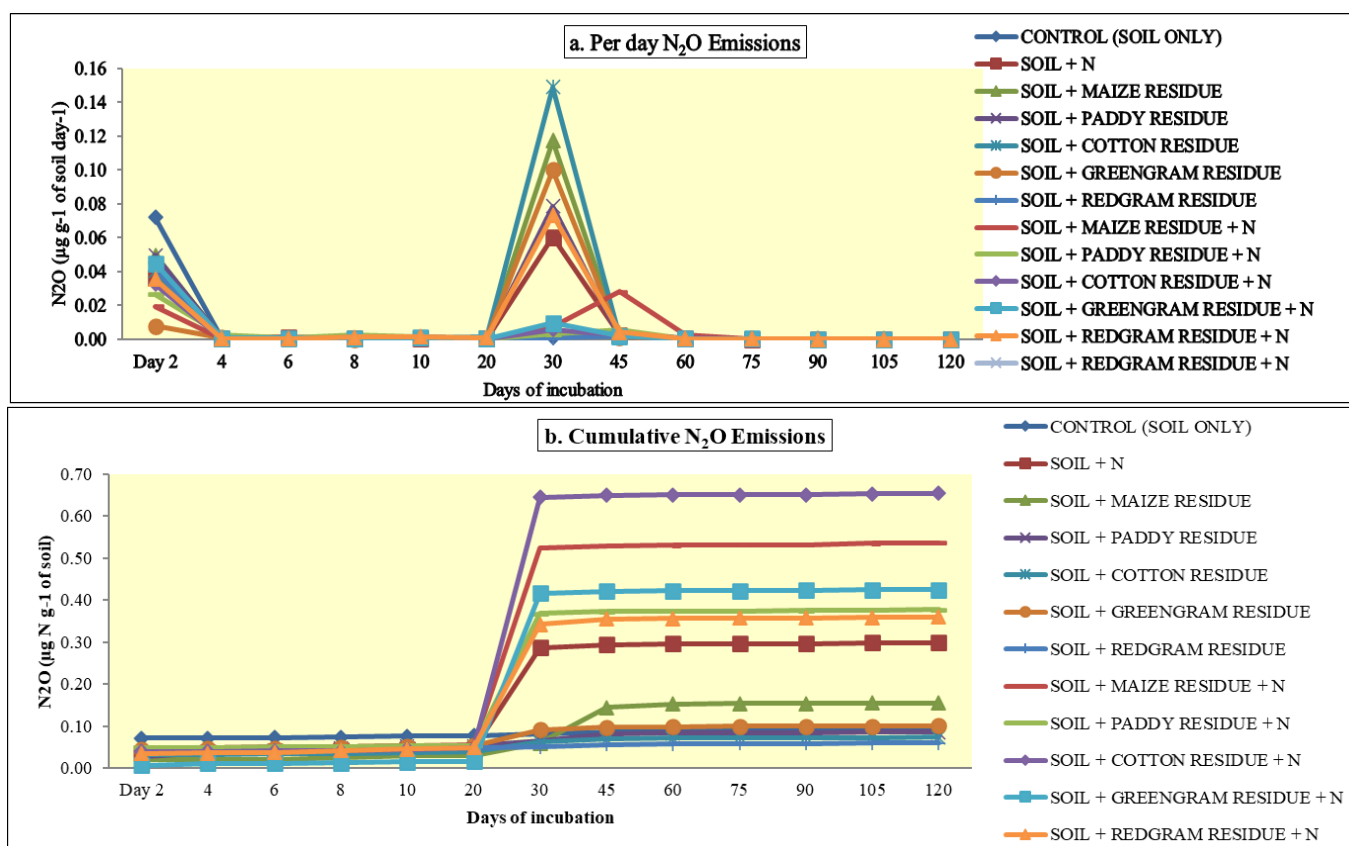
Results revealed that maximum  $\text{N}_2\text{O}$  emissions occurred during 30 days after incubation, among all the treatments. Impact of amendment of soil with different residues on  $\text{N}_2\text{O}$  emissions is

depicted in Fig. 2a, b. The soil amended with cotton residue ( $T_5$ ) recorded significantly higher  $N_2O$  emissions ( $p < 0.05$ ) on 30<sup>th</sup> day. Data indicated that, immediately after the addition of residue and fertilizer, there was emission of  $N_2O$  from soil. On day 2 there were higher emissions, later it decreased and became almost zero from day 4 to day 20. From day 20 there was exponential increase in  $N_2O$  emissions and it peaked on 30<sup>th</sup> day. On 30<sup>th</sup> day, highest  $N_2O$  emissions were observed in soil amended with cotton residue ( $T_5$ ), this is in correspondence with the  $NH_4^+ - N$  concentration in soil amended with cotton residue ( $T_5$ ), which increased upto 30 days and later there was a decrease. The next higher cumulative  $N_2O$  emissions were recorded in soil amended with maize residue ( $T_3$ ) and the control ( $T_1$ ) treatment recorded lowest emissions among all the treatments. However, in soil amended with maize residue along with inorganic nitrogen ( $T_8$ ), the peak was observed on 45<sup>th</sup> day. The same was reflected in the ammoniacal nitrogen status, where there was an increase upto 45<sup>th</sup> day and later there was a decrease. This might be due to higher C:N ratio of maize. After 30<sup>th</sup> day, there was decline in  $N_2O$  emissions throughout the incubation period. In soil amended with maize residue along with inorganic nitrogen ( $T_8$ ), after 45<sup>th</sup> day, the  $N_2O$  emissions decreased towards the end of the incubation period.

The emissions reflect mineral N in the soil.  $N_2O$  emissions are the net result of differences in biochemical composition of the residues. The residues with lower C:N ratio add maximum amount of N to the soil during initial days of incubation. This leads to apparent increase in the  $N_2O$  emissions during early stages of incubation. Rise in  $N_2O$  emissions after N fertilization was also noticed previously (20). Increase in  $N_2O$  emissions immediately after amendment of soil with residues and N fertilizer might be due to addition of easily oxidisable organic matter to soil, under aerobic conditions which led to N oxidation and subsequent emission of

$N_2O$  from soil. Previous researchers noticed an increase in  $N_2O$  emissions after application of manure to soil (21). After incorporation of readily degradable crop residues to soil, there was increase in the  $N_2O$  emissions (22).

Cumulative  $N_2O$  emissions were significantly influenced by amendment of soil with different residues (Table 5). Fig. 2b shows cumulative emissions of  $N_2O$  emissions within 120 days incubation period. Apparently, all the residues enhanced  $N_2O$  emissions. On average, incorporation of residues into soil increased  $N_2O$  emissions by 66.78 %, compared to control. The cumulative  $N_2O$  emissions were significantly higher when inorganic fertilizer nitrogen was applied along with residues. It accounted for approximately 79.75 % more cumulative  $N_2O$  emissions compared to soil amended with residues without fertilizer. Among all the treatments, soil + cotton residue + inorganic N ( $T_{10}$ ) recorded significantly highest ( $p < 0.05$ ) cumulative  $N_2O$  emissions ( $0.65 \mu\text{g N g}^{-1}$  of soil), followed by soil + maize residue + inorganic N ( $T_8$ ) i.e.,  $0.54 \mu\text{g N g}^{-1}$  of soil. Our results were in line with findings of (23), who reported that addition of lucerne (which has lower C:N ratio) to soil, led to rise in  $N_2O$  emissions during incubation period. The cotton residue with C:N ratio of 23:1 has produced higher  $N_2O$  emissions, which is because it gets easily decomposed and produced more dissolved organic carbon and nitrogen, hence resulting in more  $N_2O$  emissions. This is supported by the findings of former researchers, who reported that residues with lower C:N ratio produced more dissolved organic carbon and higher  $N_2O$  emissions (24). The maize residue, despite having higher C:N ratio, produced higher cumulative emissions, but as many researchers reported, C:N ratio itself is not a reliable predictor for  $N_2O$  emissions from residues (25). Incorporation of crop residues > 100, increased  $N_2O$  emissions was reported earlier (26).



**Fig. 2.** Effect of different crop residues and N application on  $N_2O$  emissions during incubation. a: Per day  $N_2O$  emissions; b: Cumulative  $N_2O$  emissions.

When maize residue is added to soil, there is increased availability of organic substrates to microbes, which led to creation of anaerobic microsites in soil, where rates of  $O_2$  consumption exceed diffusion, this might have increased the denitrifiers population and increased  $N_2O$  emissions. Similar results were obtained previously, in which residues with higher C:N ratio and moderate lignin content, increased the denitrifiers population in soil, which further enhanced  $N_2O$  emissions (27). The cumulative  $N_2O$  emissions from soil + N ( $T_2$ ) treatment was higher than all residue amended treatments without fertilizer application, but lower than residue + inorganic N applied treatments by 36.40 %. Cumulative  $N_2O$  emission was considerably higher in N fertilizer treatment and residues with N-fertilizer treatments (28). Among the residue amended treatments with or without inorganic N, lowest cumulative  $N_2O$  emissions were observed in soil amended with red gram residue ( $T_7$ ) i.e.,  $0.06 \mu\text{g N g}^{-1}$  of soil. This is due to higher C:N ratio, which caused immobilization, which was evidenced in our study. The temporal patterns of  $N_2O$  emissions observed in the study can be mechanistically explained by the interplay between biochemical residue, microbial demand for nitrogen and shifts between nitrification and denitrification pathways. As observed from the data, the sharp emission peak on 2<sup>nd</sup> day might be due to immediate availability of easily oxidisable carbon and mineral N released from freshly added residues and N fertilizer, which promotes heterotrophic respiration and rapid ammonification increasing oxygen consumption in soil microsites and creating short-lived anaerobic niches where denitrifiers produce  $N_2O$ . The close to zero emission levels between days 4 and 20 is an indicator of a temporary condition of intense microbial N immobilization in the treatments given higher C:N ratios residues (maize, paddy, redgram), where the microbes take up mineral N to form biomass, thus temporarily inhibiting both nitrification and denitrification. The rapid increase during day 20 to day 30 (or day 45 in maize + N) of the emission to the peak of the emission is indicative of a transition to net mineralization as the microbial biomass starts to turnover to release the previously immobilized N; concurrently, the degradation of residues grows the dissolved organic carbon (DOC) to augment microbial respiration,  $O_2$  depletion and the development of denitrifying hotspots. Cotton residue, characterised by a relatively lower C: N ratio and more labile carbon, degraded quickly and emitted a lot of DOC and mineral N at the beginning of the incubation, which is why it had the highest  $N_2O$  peak on day 30 and the leading to increasing  $NH_4^+$ - N up to this point. Incorporation of maize residue, however, had a comparatively high cumulative emissions as it has moderate lignin and hemicellulose content, which degraded gradually and kept releasing DOC and preserving anaerobic microsites during longer periods, thereby delaying the peak until day 45 when used with inorganic N. The introduction of inorganic nitrogen also increased emissions by providing a large pool of  $NH_4^+$  to undergo nitrification and  $NO_3^-$  to undergo denitrification, which decreased the strength of early immobilization and changed the system towards more N-transforming microbial pathways that can produce  $N_2O$ . The cumulative emissions monotonic increase across residues with cotton + N and maize + N recording the highest values indicate that under the conditions of a simultaneous presence of energy sources (DOC) and electron acceptors ( $NO_3^-$ ), the soil conditions are along with the coupled nitrification-denitrification process that optimizes the emission of  $N_2O$ . Low emission in red gram residue indicates its high C:N ratio and slower decomposition which increased the

duration of immobilization and maintained pools of mineral N in a suppressed state that limited the activity of both the nitrifiers and denitrifiers during the incubation. The measured dynamics of  $N_2O$  are therefore due to treatment specific interactions between treatment residue C:N ratio, DOC release patterns, microbial immobilization mineralization cycles and availability of oxygen in soil microsites-mechanisms that simply provide an explanation of the timing and magnitude of  $N_2O$  peaks in this experiment and not merely a current correlation to existing literature.

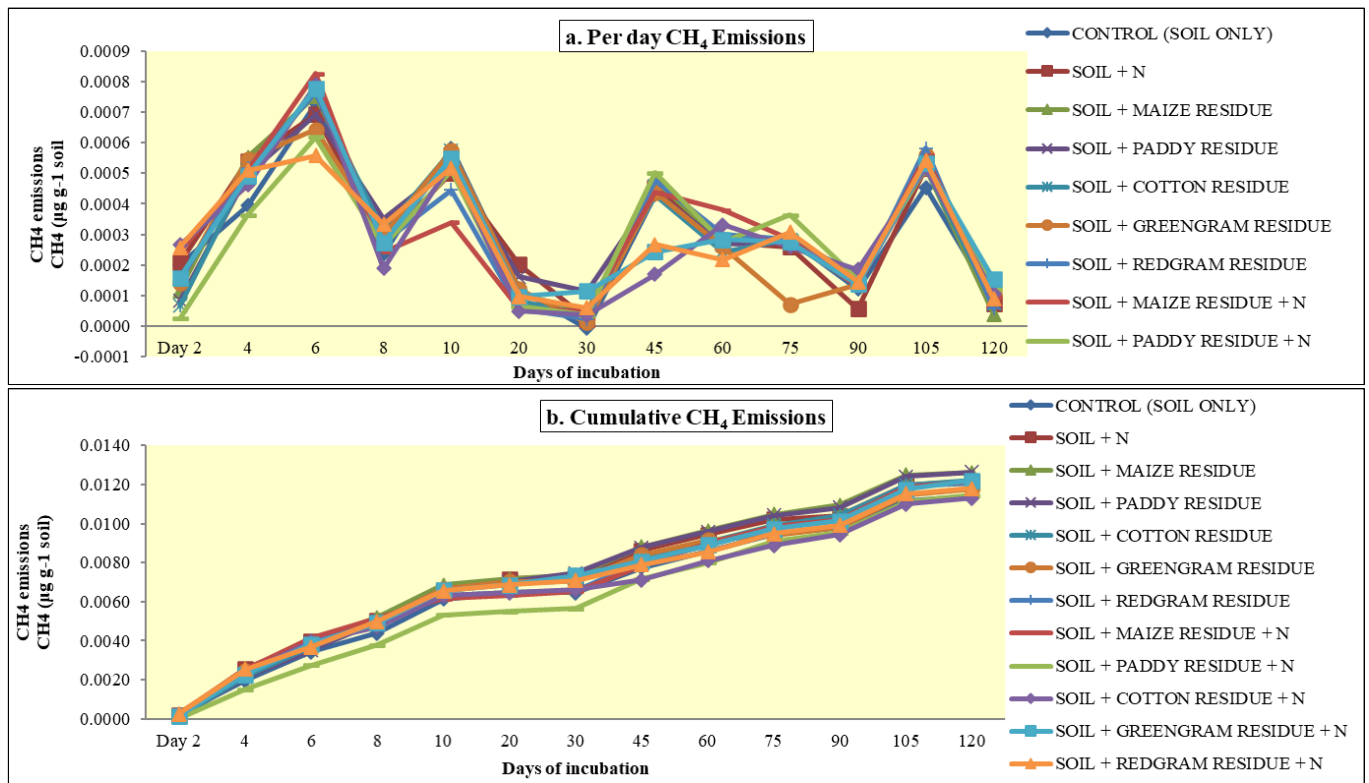
### CH<sub>4</sub> emissions

The  $CH_4$  emissions increased from day 2 and peaked on day 6 in all the treatments (Fig. 3a, b). Later there was a decrease in  $CH_4$  emissions upto day 8. After that, it has increased upto day 10. From day 10, there was a decrease in  $CH_4$  emissions upto day 20 and further decreased and reached almost zero on day 30. From day 30,  $CH_4$  emissions increased upto day 45. After day 45, there was decrease in  $CH_4$  emissions upto day 60 in all treatments except in soil + green gram residue ( $T_6$ ), where the  $CH_4$  emissions reduced to 75. In remaining treatments, there was increase in  $CH_4$  emissions upto day 75. Later it declined upto 90 days, after which it reached peak on day 105. After day 105, there was a sudden decrease in  $CH_4$  emissions upto day 120.

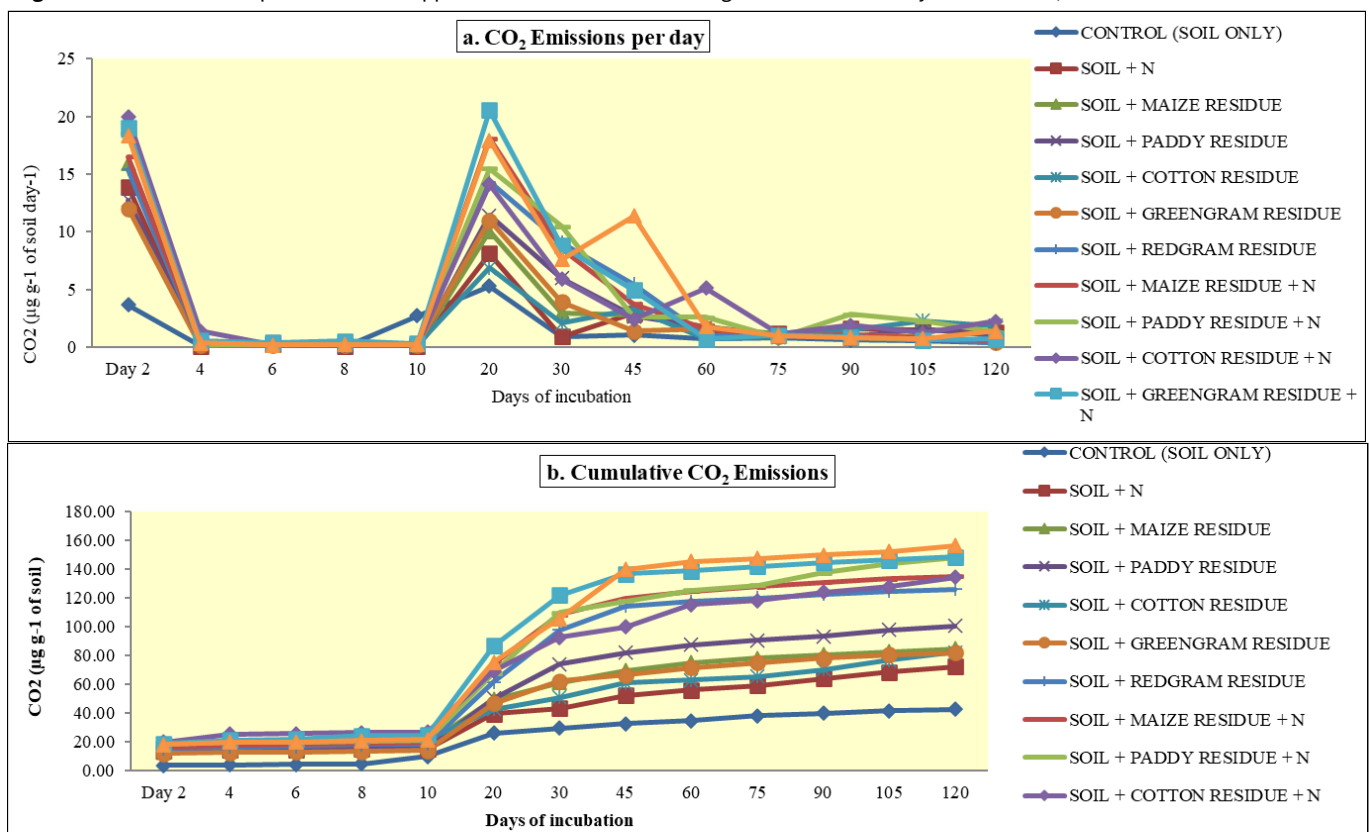
Throughout the incubation period, highest  $CH_4$  emissions were recorded on day 6. On day 6, soil amended with maize residue along with inorganic N ( $T_8$ ). The lowest  $CH_4$  emissions were recorded in soil + red gram residue + inorganic nitrogen ( $T_{12}$ ). The lowest  $CH_4$  emissions were observed on day 30. On day 30, the soil + green gram residue + inorganic nitrogen ( $T_{11}$ ) recorded lowest emissions. However, there is no significant difference in the cumulative  $CH_4$  emissions among the treatments. Similar results were obtained by former researchers, who assessed the *in situ* GHG emissions after the application of different residues which had broad variation in C:N ratios (5-521) (17). They have concluded that  $CH_4$  emissions changed over time but cumulative  $CH_4$  emissions did not vary significantly ( $p < 0.05$ ) among different residues. The temporal variations in  $CH_4$  emissions are due to C mineralization, which caused variations in the carbon content. However, the cumulative methane emissions (Table 5) did not vary significantly with incorporation of different residues, because aerobic conditions were maintained throughout the study, under which there might be limited accumulation of methanotrophs. Previous researchers compared different moisture regimes (Field capacity and submergence) on cumulative  $CH_4$  emissions (27). They have concluded that cumulative  $CH_4$  emissions were significantly lower in field capacity than in submergence.

### CO<sub>2</sub> emissions

The temporal dynamics of soil  $CO_2$  fluxes for 120 days incubation period is presented in Fig. 4a. The data indicated that, there was increase in the soil  $CO_2$  fluxes immediately after incorporation of residue, with the maximum values of  $CO_2$  emissions recorded in soil + cotton residue + N ( $19.95 \mu\text{g C g}^{-1}$  of soil). After first 1-2 days, soil  $CO_2$  emissions declined upto 10 days and later the  $CO_2$  emissions increased exponentially and reached peak on 20<sup>th</sup> day, after which  $CO_2$  emissions decreased until the end of the experiment. However, in some of the treatments like soil + red gram residue + N and soil + cotton residue + N there were some short-lived spikes observed during 45<sup>th</sup> day and 60<sup>th</sup> respectively. The soil cumulative  $CO_2$  emissions were significantly lower in control (soil only) treatment ( $p < 0.05$ ), compared to residue amended treatments. The



**Fig. 3.** Effect of different crop residues and N application on CH<sub>4</sub> emissions during incubation. a: Per day CH<sub>4</sub> emissions; b: Cumulative CH<sub>4</sub> emissions.



**Fig. 4.** Effect of different crop residues and N application on CO<sub>2</sub> emissions during incubation. a: CO<sub>2</sub> emissions per day; b: Cumulative CO<sub>2</sub> emissions.

cumulative CO<sub>2</sub> emissions from control treatment was 42.64  $\mu\text{g C g}^{-1}$  of soil, which was approximately 64.38 % lower than residue amended treatments. The soil + N treatment (T<sub>2</sub>) also have recorded lower cumulative CO<sub>2</sub> emissions (72.33  $\mu\text{g C g}^{-1}$  of soil) compared to residue amended treatments which accounted to an average of 39.57 % lower emissions in comparison with residue amended treatments.

Among the residue amended treatments, with or without inorganic N, soil + redgram residue + inorganic N (T<sub>12</sub>) treatment recorded significantly higher cumulative emissions ( $p < 0.05$ ) (156.20  $\mu\text{g C g}^{-1}$  of soil), followed by soil + green gram residue + inorganic N (T<sub>11</sub>) i.e., 148.38  $\mu\text{g C g}^{-1}$  of soil. This treatment (T<sub>11</sub>) is on par to soil + paddy residue + inorganic N (T<sub>9</sub>) where the cumulative CO<sub>2</sub> emissions recorded was 147.83  $\mu\text{g C g}^{-1}$  of soil. The cumulative emissions in soil + maize residue + N (T<sub>8</sub>) and soil + cotton residue + N

(T<sub>10</sub>) were almost same (134.90 and 134.31  $\mu\text{g C g}^{-1}$  of soil respectively). Among soil + residue amended treatments without inorganic N, the soil + red gram residue (T<sub>7</sub>) recorded significantly higher cumulative CO<sub>2</sub> emissions ( $p < 0.05$ ) (125.82  $\mu\text{g C g}^{-1}$  of soil), followed by soil + paddy residue (T<sub>4</sub>) i.e., (100.61  $\mu\text{g C g}^{-1}$  of soil). Soil amended with maize, green gram and cotton recorded similar cumulative emissions (84.52, 81.95 and 82.42  $\mu\text{g C g}^{-1}$  of soil respectively).

The rate of decomposition and the resultant emission of CO<sub>2</sub> or N<sub>2</sub>O mainly depends on the quality of the residues (lignin content, C:N ratio, cellulose content, hemicellulose content etc.). Addition of residues to soil enhanced cumulative CO<sub>2</sub> emissions. The highest emissions in red gram amended soil is due to lower lignin content (6.5 %) in the residue used, which is easily biodegradable and there was higher microbial activity which increased CO<sub>2</sub> emissions. Similar results were noticed by (28), who found that, despite having high C:N ratio (57–140), sugarcane, maize and sorghum residues produced high CO<sub>2</sub> emissions due to lower lignin content (5.5–8.3 %) compared to cotton residue, which had lower C:N ratio (29 %).

The higher cumulative CO<sub>2</sub> emissions in soil amended with green gram residue along with inorganic N is due to lower C:N ratio (21.81), which enhanced the decomposition process and increased CO<sub>2</sub> emissions (Table 5; Fig. 4b). Our results were in line with the findings of previous researchers, who observed that the addition of mung bean residues which have higher N content and lower C:N ratio facilitated higher decomposition and increased cumulative CO<sub>2</sub> emissions when they are mixed with rice or wheat residues and added to soil (29). The cumulative emissions from soil amended with paddy residue along with inorganic N (T<sub>9</sub>) is like soil amended with green gram residue along with inorganic N (T<sub>11</sub>). This is due to comparatively lower lignin content (18.70 %) and addition of inorganic N, apparently lowered the C:N ratio, which eased the decomposition and increased cumulative CO<sub>2</sub> emissions (30).

The dynamics of decomposition of residues and microbial activity were well illustrated by the temporal pattern of CO<sub>2</sub> fluxes to soil during the 120 days incubation period. The CO<sub>2</sub> emissions were high right after the incorporation of residues, which signifies that the equivalent of the readily decomposable substances was mineralised swiftly. The fluxes started to decrease in the initial 10 days, but another peak was observed at approximately the 20<sup>th</sup> day indicating that the microbes were becoming active again as the decomposition went on. Intermittent spikes in red gram and cotton residue treatments also indicate periodic peaks of microbial respiration in relation to the availability of the substrate and its quality. Addition of the residue significantly increased cumulative CO<sub>2</sub> emissions over the control soil indicating the high potential of organic inputs in microbial respiration. The non-residential treatments (control and soil + N) had significantly reduced cumulative emissions and this result confirmed that the supply of organic carbon is the dominant source of soil CO<sub>2</sub> efflux.

In the treatments that were amended with residues, red gram residue along with inorganic N had the highest cumulative CO<sub>2</sub> emissions. This is explained by the low lignin content of the residue (6.5 %), which enhances the rate of decomposition and the metabolism of the microorganisms. The same has been observed in previous research where low lignin residues with relatively high C:N ratios also resulted in high CO<sub>2</sub> emission, as they are easily biodegraded. Inorganic N residue that contained green gram also

gave high emissions, presumably because it had low C:N ratio, thus decomposition was rapid. The emissions with paddy residue and inorganic N were similar and this indicates that moderate lignin content and N addition is responsible in supporting the breakdown by the microbes.

Residues of lower quality or with a higher percentage of lignin, e.g., maize or cotton, demonstrated lower cumulative CO<sub>2</sub> emissions. This helps in realizing that the quality of residue, especially that of lignin, cellulose, soluble components and C:N ratio all has a role to play in determining decomposition rates and not just one. Another point that has been highlighted in past literature is that soluble carbon compounds and residue maturity have strong impacts on possible CO<sub>2</sub> emission. One of the patterns was also the fact that the inclusion of inorganic N led to higher CO<sub>2</sub> emissions than the unfertilized residue-amended soils. This indicates that N supplements reduce N deficit caused by microbes and improve mineralization of residues. Such constructive relationships between the residues and N fertilizer on soil CO<sub>2</sub> fluxes have been reported to exist in the studies conducted with cereals and sugarcane residues.

## Conclusion

The current study was taken up for 120 days in a sandy clay loam soil with low organic carbon and under semi-arid tropical climatic conditions. Residue amended soil showed consistently lower mineral N due to an immobilization of soil and fertilizer-derived N. Maximum emissions of N<sub>2</sub>O from residue amended soil, with and without N-fertilizer treatment, occurred in the first 30 days of incubation. The cumulative N<sub>2</sub>O emission was significantly altered by residue addition. The highest being observed in cotton along with inorganic N and lowest emissions were observed in red gram residue. The CO<sub>2</sub> emissions were markedly increased immediately after addition of residue to soil. Significantly higher cumulative CO<sub>2</sub> emissions were observed in red gram residue. We conclude that crop residue of wide C/N ratio such as maize, paddy and red gram can be used to immobilize nitrate N during the fallow period and this reduces not only the risk of nitrate leaching but also N<sub>2</sub>O emissions. Similarly, residues with lower C/N ratio like green gram, cotton can improve the N release into soil immediately after their incorporation.

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## Authors' contributions

CR carried out the investigation and drafted the manuscript. GJ participated in the study design and conceptualization. KSR contributed to data curation and validation. GP participated in the study design, supervision and data curation. ST was involved in data validation and writing. RNR and AAL reviewed the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors declare that there is no conflict of interest.

**Ethical issues:** None

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