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Article

N_2O Emission and Mineral N Release in a Tropical Acrisol Incorporated with Mixed Cowpea and Maize Residues

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Abstract: A laboratory microcosm incubation was conducted to study the influence of mixed cowpea-maize residues on N_2O emission and N mineralization in a tropical acrisol. The soils were incorporated with different ratios of cowpea:maize mixtures on weight basis: 100:0, 75:25, 50:50, 25:75 and 0:100, and a control treatment in which there was no residue incorporation. The results show that N_2O and CO_2 emissions were higher in the sole cowpea treatment (100:0) than the sole maize treatment (0:100) and the control. However, cowpea-maize residue mixtures increased the proportion of N lost as N_2O compared to the sole treatments. This interactive effect was highest in the 75:25 treatment. The 50:50 treatment showed moderate N_2O emission compared to the 100:0, 75:25 and 25:75 treatments but with corresponding steady N mineralization and appreciable mineral N concentration. It is concluded that mixing cowpea-maize residues might increase the proportion of N lost as N_2O in a tropical acrisol. However, compared to the other residue mixture treatments, mixing cowpea-maize residues in equal proportions on weight basis might offer a path to reducing N_2O emissions while maintaining a steady N mineralization without risking good N supply in acrisols. The study therefore offers potential for mitigating greenhouse gas emissions while maintaining soil fertility in tropical acrisols.

However, further studies under both laboratory and field conditions will be required to verify and validate this claim.

Keywords: cowpea-maize residue; nitrous oxide emission; N mineralization; tropical acrisol

1. Introduction

Agro-ecological research has recently become focused not only on economically-viable soil fertility management but also on environmentally-friendly systems [1]. Emission of N_2O is of global concern because N_2O contributes 6% of global radiative forcing of atmospheric greenhouse gas emissions and its concentration in the atmosphere is estimated to be rising at a rate of 0.3% per annum [2]. N_2O has a residence time of about 120 years and a global warming potential of 298 times greater than carbon dioxide over a 100 year period [2]. It is believed that 90% of global anthropogenic N_2O emissions originate from soils [3] and 6.3 Tg of N_2O-N is emitted from agricultural systems, representing more than half of anthropogenic N_2O emissions [4]. Both nitrification and denitrification produce N_2O as intermediate product from organic and inorganic N sources in soils [5]. Emission of N_2O from soils is reported to be enhanced by the addition of nitrogenous fertilizers, nitrogen fixation by legumes and biomass burning [6]. Africa accounts for 15% of global N_2O emission from soils and agriculture accounts for 42% of total N_2O emissions from Africa [2]. In Ghana, agriculture accounts for 65% of the 3.07 Gg N_2O emissions (1994 baseline year), while biomass burning contributes 27% [7].

Cereal-legume intercropping is a common crop production system in Africa with several benefits, key among them being the improvement in household food security and soil fertility [8–12]. Among the various possible cereal-legume combinations, cowpea (*Vigna unguiculata*) and maize (*Zea mays*) intercropping is common as shown for Southern Africa [9,10], for Western Africa [8,11,12] and for Eastern Africa [13]. Western Africa (mainly Nigeria, Niger, Burkina Faso and Ghana) accounts for 70% of world cowpea production and this largely comes from mixed cropping systems [12]. Although cowpea-maize intercropping is an important practice from food security and soil fertility perspectives, statistics concerning the precise extent of this practice in Africa is currently unavailable. In cereal-legume intercropping systems, residues are either left on the surface or incorporated into the soils to replenish soil fertility, but the rapid decomposition of leguminous crop residues provides NH_4^+, NO_3^- and organic C substrates for N_2O production through nitrification and denitrification, respectively [14–16].

Millar *et al.* [17] have shown that the magnitude of N_2O emission from soils incorporated with organic N inputs varies depending on residue chemical composition and quantity of biomass added. It is practically not feasible to considerably alter the quality or chemical composition of particular species during growth, but residue quality can be manipulated by mixing high C:N and low C:N residues [18]. Most of the environmental consequences associated with inorganic N in agricultural systems occur as a result of accumulation of inorganic N (NO_3^- and NH_4^+) forms, and loss of excess N, particularly in the presence of water [19]. Therefore, regardless of whether N is applied as inorganic fertiliser or as organic N inputs, any management strategy that minimises excess N loss can promote the attainment of synchrony. Baggs *et al.* [20] reported that more rapid N release and greater N_2O

emission are measured in soils amended with materials of higher rather than low nitrogen content. Leguminous crop residues, which contain high N content, offer the potential to replenish soil N fertility if they are incorporated in soils, but this practice is likely to result in increased N_2O emission. There is therefore the need, in the interest of climate change and food security, to identify management practices that lower N_2O emission from soils amended with leguminous crops without decreasing N availability for crop uptake.

One strategy to achieve this is the addition of a high C:N ratio cereal residue, which ensures prolonged N mineralization [18–20] and ultimately lowers N_2O emission [21] compared to sole addition of low C:N legume residues [18,21,22]. The interactive effect of mixing residues of different C:N ratio on N release is attributed to net N immobilisation through enhanced microbial activity, stimulated by the presence of labile C from the high C:N residue [22,23]. Net N immobilisation following incorporation of mixed residues can promote a synchrony between inorganic N release from decomposing residues and N uptake by plants. In agricultural systems synchrony refers to the supply of N to match the rates of plant N demand or uptake [24,25]. Thus, the delayed N mineralization following addition of high C:N ratio species might better match N supply with N demand, resulting in a higher N use efficiency from the high N inputs. Previous authors have reported that the incorporation of rice residue together with groundnut residue delayed N mineralization for up to 8 weeks after incorporation [21,23]. Furthermore, maize residue has been identified as a high C:N ratio material capable of delaying N mineralization when mixed with low C:N residues [26]. Since cowpea and maize intercropping is common in Africa, it is important to examine the effect of incorporating mixtures of cowpea-maize residues on N_2O emission and N mineralization in tropical African soils. Therefore, this laboratory soil microcosm study was aimed at investigating the effect of incorporating mixtures of cowpea-maize residues at different ratios on N mineralization and N_2O emission from a tropical acrisol.

2. Materials and Methods

2.1. Soil

Soil samples obtained from arable fields at the Crop Research Institute (Kumasi) in the semi-deciduous agro-ecological zone of Ghana were used in this study. The soils were air-dried, sieved (<2 mm) and packed to a bulk density of 1.23 g cm^{-3} in 500 mL kilner jars. The sandy clay loam soil contained 59.1% sand, 13.5% silt and 27.4% clay. Other properties included pH (H_2O) of 5.8, total N (0.2%) and organic C (1.2%). The soil is classified as Orthic Ferric Acrisol according to the FAO 1998 system. The soil was pre-incubated at 45% WFPS and 25 °C for 7 days prior to addition of the residues to re-initiate microbial activity after 1 year of cold storage (−4 °C), and to minimize changes in soil water filled pore space (WFPS) at the start of the experiment. WFPS was calculated based on soil volumetric water content, bulk density and a particle size of 2.3 g cm^{-3}.

2.2. Plant Residues

Maize and cowpea residues were chosen because of their varying N content and C:N ratios and the widespread use of these species as intercrop or multi-crop species in African farming systems. The

cowpea and maize were grown in vermiculite in the greenhouse at the University of Aberdeen. The cowpea and maize plants were both harvested after 7 weeks (just before tasseling of maize and when adequate biomass could be obtained for the cowpea treatment). Subsamples of the maize and cowpea leaves were dried at 45 °C for the determination of dry matter (%), and then milled (<1 mm) for further laboratory analyses. Total C and N contents were determined on a 0.5 g dry subsample through dry combustion using a Metler-Toledo AG 2455 C/N auto analyser (Sercon Ltd., Cheshire, UK). Lignin content was determined in an Ankom 220 fibre analyser (Acid detergent fibre) and the total extractable polyphenol was measured using Folin-ciolcateau reagent in a method adapted from [27] (Table 1).

Table 1. The biochemical characteristics of cowpea and maize residues.

Chemical characteristics	Cowpea	Maize
Total extractable polyphenol (%)	1.3	1.14
Acid detergent lignin (%)	7.2	7.4
Total nitrogen (%)	3.4	0.92
Organic carbon	39.6	42.1
C:N ratio	11.7	45.6

2.3. Experimental Setup

2.3.1. Incubation

A laboratory microcosm incubation was set up with 200 g of soil in 500 mL Kilner jars using a completely randomized design. Ground (<2 mm) cowpea and maize residues from the above-ground biomass were incorporated into each soil solely or in combination at different cowpea:maize ratios of 100: 0, 75:25, 50:50 and 25:75 and 0:100 on dry matter basis at a rate of 4 t ha^{-1}. A control treatment without residue incorporation was included. Each treatment was replicated 4 times for gas sampling and 3 times for destructive soil sampling. The incubation was carried out in the dark at 25 ºC for 30 days. Kilner jars were kept open during the storage in between sampling times. The soil was brought to 60% WFPS at the start of incubation (Day 0). Soil WFPS was maintained constant throughout the experimental period by mass balance through the addition of deionized water. Parton *et al.* [28] suggested that at 60% WFPS, neither the diffusion of substrates nor the diffusion of oxygen is limited, indicating that WFPS of 60% probably offers the optimum conditions whereby both nitrification and denitrification contribute to N_2O production [29].

2.3.2. Gas Sampling and Analysis

Gas samples were collected on days 0, 1, 2, 3, 5, 7, 10, 14 and 30 using gas-tight syringes and stored in pre-evacuated 12 mL vials for N_2O and CO_2 analysis. Gas samples were analysed for N_2O concentration on a Pye-Unicam gas chromatograph fitted with an electron capture detector and a HAYESEP Q column. CO_2 concentration in the gas samples was determined using a Chrompack CP9001 gas chromatograph fitted with a methaniser and a flame ionisation detector (FID) [16]. Detector and oven temperatures were 250 °C and 50 °C respectively. Gas samples were collected after Kilner jars were closed for 1 h. Preliminary trials established that the flux was linear and over a 1-h period under controlled conditions. In between sampling the lids of the Kilner jars were removed to

maintain the treatments under aerobic conditions. Total N_2O emissions were calculated by linear interpolation of the daily fluxes.

2.3.3. Soil Sampling and Analysis

The soils were destructively sampled on days 0, 1, 3, 7, 14, and 30. A Subsample (40 g) from each treatment was mixed with 1 M KCl (1:5 extraction ratio) and filtered through Whatmann No. 42 filter paper, after shaking the suspension on a mechanical shaker for 1 h. The extracts were analyzed for NH_4^+ and NO_3^- concentrations colorimetrically by continuous flow analysis on a FIA star 5010 autoanalyser fitted with a cadmium column [16].

2.3.4. Water Extractable Carbon

Water extractable carbon (WEC) was quantified in soil sampled on days 0, 1, 7, 14 and 30 using a method adapted from [30]. To extract the WEC, a 10 g (air-dried basis) subsample was shaken in 40 mL deionized water for 2 h, and filtered through Whatmann No.42 filter paper. Further filtration of the extract was undertaken with a 0.45 µL micropore filter. Concentration of WEC in the supernatant liquid was determined using a total organic carbon analyser (TOC-5000A, Shimadzu).

2.3.5. Calculations

Net N_2O emission (mg N_2O-N m^{-2} d^{-1}) from residue amended treatments (mixed or single) was calculated using the "difference method" as shown below:

$$\text{Net } N_2O = [\text{Tr}(N_2O) - \text{Co}(N_2O)] \quad (1)$$

Where

$\text{Tr}(N_2O)$ is the total N_2O production from the residue amended soil,

$\text{Co}(N_2O)$ is the total N_2O production from the control.

Where maize and cowpea residues were applied as a mixture, residue N was calculated as weighted average N of the two species.

Net N mineralized or immobilized was calculated as:

$$\text{Net N} = \text{Tr(N)} - \text{Co(N)} \quad (2)$$

Where:

Tr(N) is the sum of NO_3^- and NH_4^+ in the residue amended soil;

Co(N) is the sum of NO_3^- and NH_4^+ in the control.

When Net N is positive, it indicates mineralized N and negative indicates immobilized N.

Cumulative N_2O emission was calculated as:

$$\text{Cum } N_2O_x + [(\text{Day}_y + \text{Day}_x)/2] \times [|\text{Day}_y| - |\text{Day}_x|] \quad (3)$$

Where:

$\text{Cum } N_2O_x$ = Cumulative N_2O calculated for preceding day.

(N/B: *Cum* N_2O_x for day 0 represents N_2O flux for day 0, but *Cum* N_2O_x for day 1 is the *Cum* N_2O calculated for day 1 using the formula).

Day_y = Current day of sampling
Day_x = previous day of sampling
$Day_y - Day_x$ = Previous day − Current day

Emission factors were calculated as:

$$[Tr(N_2O) - Co(N_2O)]/N_R \text{ added (mg N 200 g soil}^{-1}) \times 100 \quad (4)$$

mg N added was calculated by weighted average and percentage total N content of residues.

2.4. Statistical Analysis

All data were analyzed using the Minitab 15 statistical package. All data were tested for normality and homogeneity and log-transformed where necessary. ANOVA was carried out using the measured values of N_2O, mineral N (NH_4^+ and NO_3^-) and WEC. Significant difference was tested using pooled standard error of the difference (SED) and Tukey's HSD at 0.05 significance level. Pearson correlation analyses were carried out to establish relationships if any, between crop residue C:N ratio and total N_2O and CO_2 emissions, and between WEC and mineral N concentrations, and CO_2 and N_2O fluxes.

3. Results

3.1. N_2O and CO_2 Emissions

The daily N_2O emissions from all the treatments were similar on first sampling date (day 0) (Figure 1). On the second sampling date, the N_2O emission was highest (5.26 mg N m^{-2} d^{-1}) in the 75:25 cowpea:maize treatment and was significantly different ($P < 0.05$) from the other treatments. The N_2O emissions from the 100:0 and 25:75 treatments were similar but significantly different ($P < 0.05$) from the remaining treatments while the 50:50 treatment was not significantly different from the 0:100 and the control. On the third sampling date, the N_2O emissions in the 0:100 and the control were significantly lower ($P < 0.05$) than all other treatments. N_2O emissions from the 0:100 treatment and the control were similar and lowest on all sampling dates. However, N_2O emissions from all the treatments returned to background value (represented by the control) by the sixth sampling date (day 7).

Cumulative N_2O emitted from the 75:25 cowpea:maize treatment was substantially greater than emissions from all the other residue mixture treatments (Figure 2). Cumulative N_2O emission from the 100:0 was higher than cumulative emissions from the remaining treatments. However, cumulative emissions from the 0:100 and control were similar. The cumulative N_2O emissions from the 50:50 and 25:75 treatments do not appear to differ substantially but are higher than those of the sole maize treatment and the control. Overall, the pattern of cumulative N_2O emissions (Figure 2) appears consistent with the total N input from the residue mixtures, which decreased from 136 kg N ha^{-1} for the 100:0 treatment to 36.8 kg N ha^{-1} for the 0:100 treatment (Table 2). The emission factor of the sole cowpea treatment was greater than the sole maize treatment. However, the cowpea:maize mixtures substantially increased the emission factors compared to the sole treatments, with the highest observed in the 75:25 treatment and the lowest in the 50:50 treatment (Table 2).

Figure 1. N_2O emission from soil after addition of cowpea-maize residue mixtures (0 indicates first sampling date and start of incubation; error bars represent standard error of means).

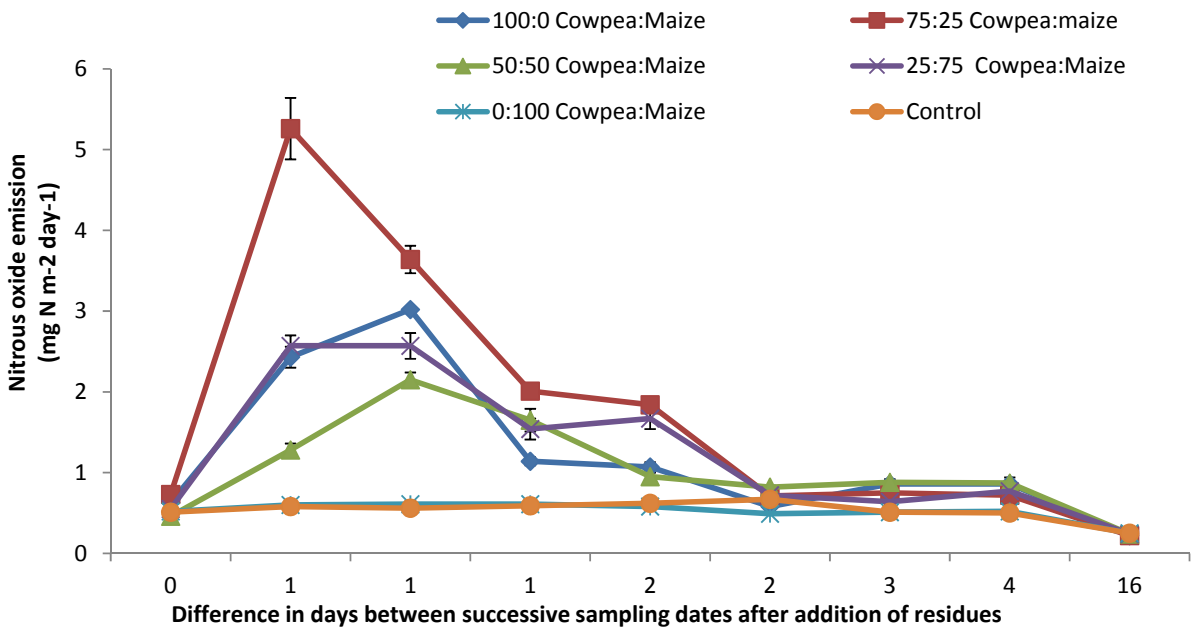


Figure 2. Cumulative N_2O emissions from the soils (0 indicates first sampling date and start of incubation).

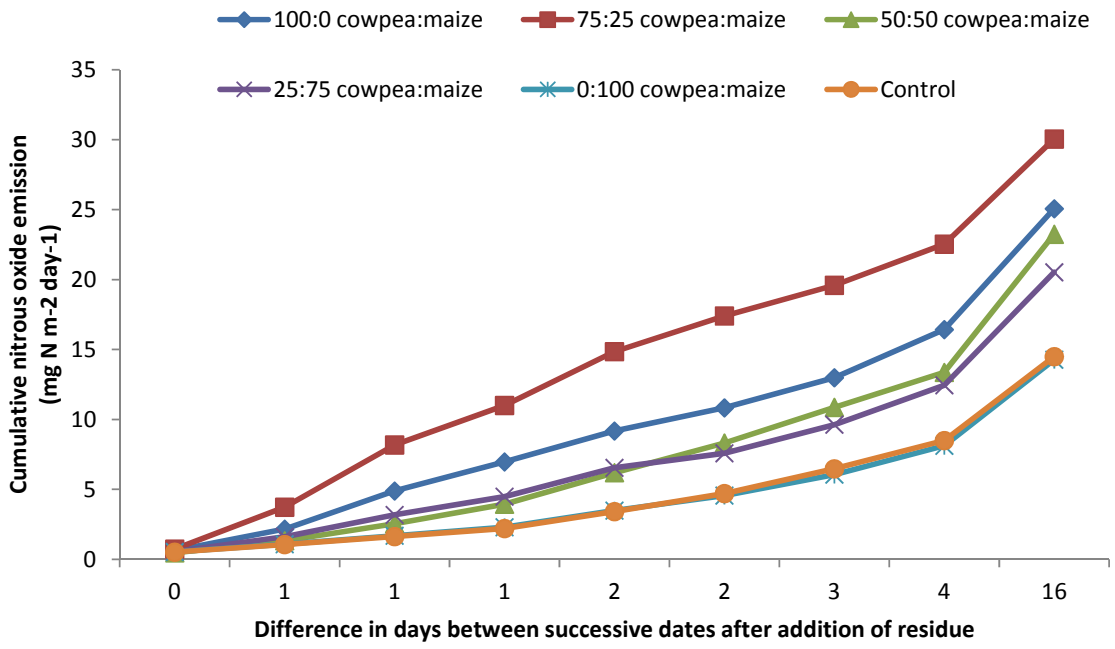
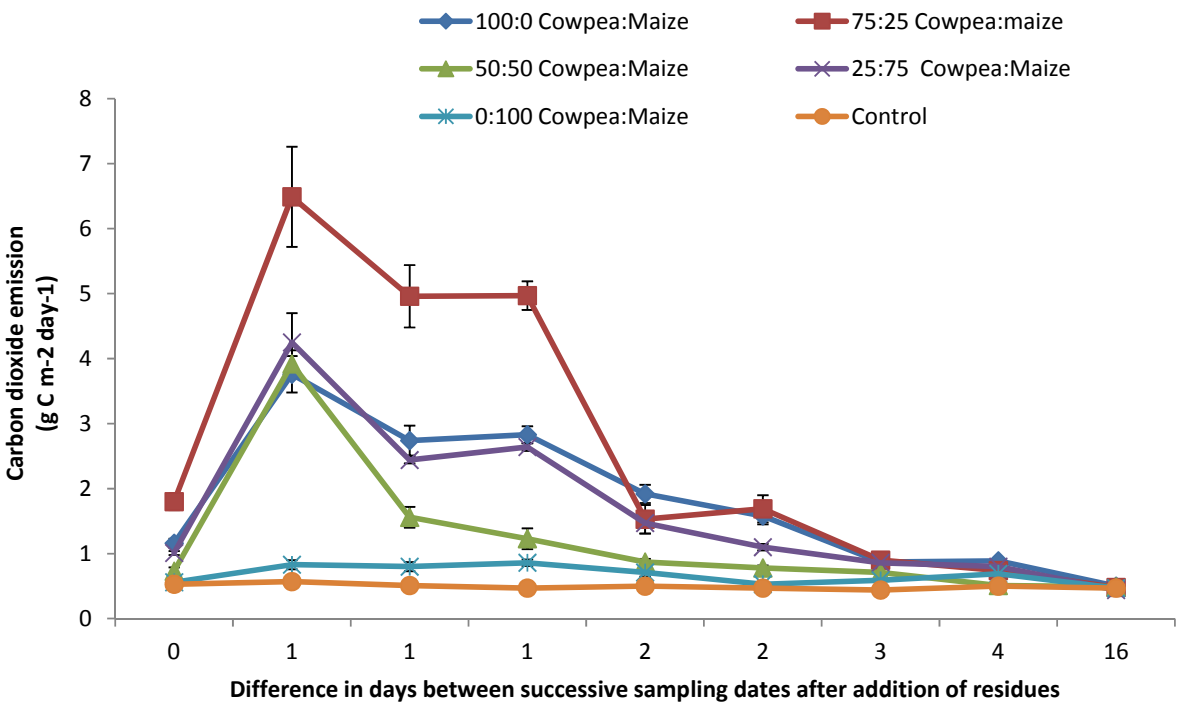


Table 2. Emission factors of the soils with different cowpea-maize residue mixtures.

Treatment	N input from residues (kg N ha^{-1})	Emission factor (%)
100:0 cowpea:maize	136.0	5.4
75:25 cowpea:maize	111.2	12.1
50:50 cowpea:maize	86.4	9.1
25:75 cowpea:maize	61.6	11.1
0:100 cowpea:maize	36.8	0.16

The CO_2 emissions from the treatments were not different on the first sampling date (day 0) (Figure 3). On the second sampling date (day 1), the 75:25 cowpea:maize treatment had the highest CO_2 emission which was significantly different ($P < 0.05$) from the other treatments. The 100:0, 50:50 and the 25:75 were similar but differed ($P < 0.05$) from the 0:100 treatment and the control. On the third sampling date (day 2), the CO_2 emission from the 75:25 treatment was different ($P < 0.05$) from the other treatments, while the 100:0 and the 25:75 were similar but different ($P < 0.05$) from the 0:100 and the control. On the fourth sampling date (day 3), the CO_2 emission from the 75:25 was still highest ($P < 0.05$) than all other treatments. The 100:0 and 25:75 were also similar but different ($P < 0.05$) from the remaining treatments. Daily CO_2 fluxes peaked on day 1 in the 100:0, 75:25, 50:50 and 25:75 treatments. CO_2 emissions from the 50:50 treatment returned to background value on day 10, but emissions from the 100:0, 75:25 and 25:75 treatments returned to background levels on day 30. Throughout the 30 days CO_2 fluxes measured from both the 0:100 cowpea:maize treatment and the control were low and not substantially different from each other.

Figure 3. Effect of cowpea-maize residue amendment on CO_2 emissions (0 indicates first sampling date and start of incubation; error bars represent standard error of means).



3.2. Water Extractable Carbon

Water extractable carbon (WEC) concentrations measured in all the treatments decreased from first to final sampling dates (Figure 4). The highest ($P < 0.05$) WEC concentration of 254.53 mg C kg^{-1} soil was measured in the 0:100 cowpea:maize treatment on first sampling date (day 0). Apart from the 0:100 treatment, the other treatments did not differ substantially. WEC concentrations were not significantly correlated with NH_4^+ and NO_3^- concentrations, but were positively correlated with N_2O emission (Table 3). The highest correlations were observed for the 25:75 and the 50:50 treatments. However, the correlation was not significant for the 0:100 treatment. CO_2 and N_2O emissions were also positively correlated. The strongest correlation was found in the 75:25 treatment, followed by the 25:75 treatment.

Figure 4. Effect of cowpea-maize residue amendment on water extractable carbon (0 indicates first sampling date and start of incubation; error bars represent standard error of means).

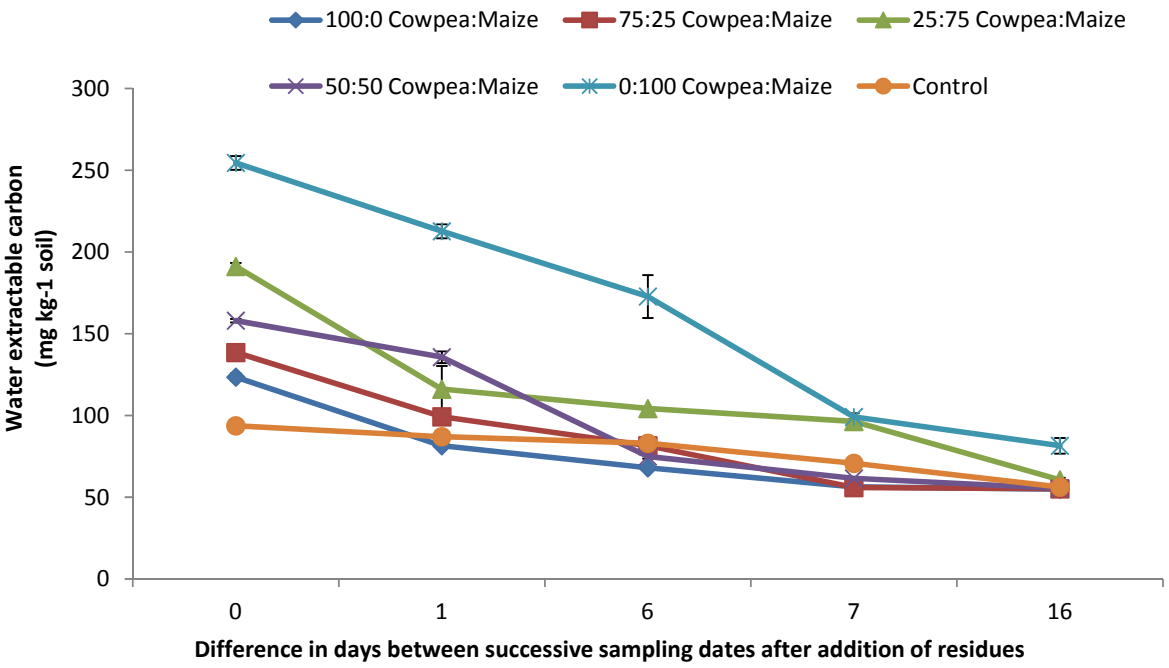


Table 3. Correlation between water extractable carbon (WEC) and daily N_2O and CO_2 and daily N_2O emissions.

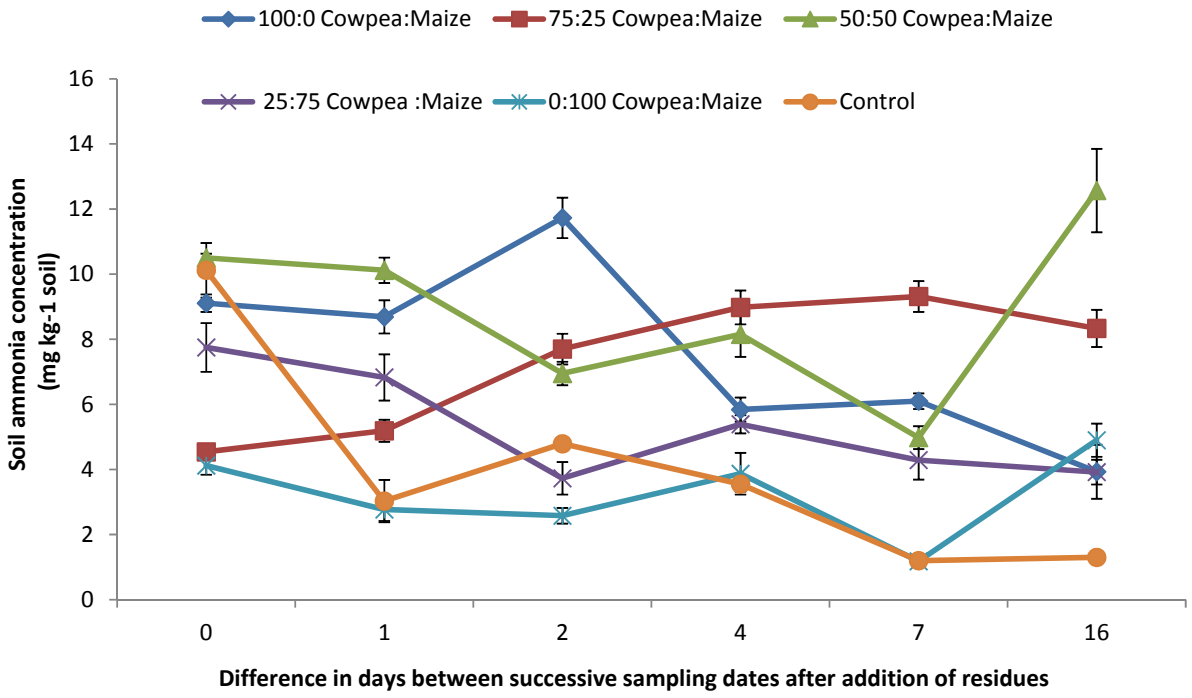
Treatment	WEC and N_2O	CO_2 and N_2O
100:0 Cowpea:maize	$r = 0.76$ *	$r = 0.64$, $P < 0.05$
75:25 cowpea:maize	$r = 0.71$ *	$r = 0.83$ *
50:50 cowpea:maize	$r = 0.94$ **	$r = 0.20$
25:75 cowpea:maize	$r = 0.97$ **	$r = 0.78$ *
0:100 cowpea:maize	$r = 0.56$	$r = 0.68$

* significant at 0.05; ** significant at 0.01.

3.3. Soil Available N (NH_4^+-N and NO_3^--N) and Net N Mineralized

On the first sampling date (day 0), the 75:25 and 0:100 treatments had similar NH_4^+ concentrations and were the lowest ($P < 0.05$) than all other treatments (Figure 5). The NH_4^+ concentrations of the control and the 50:50 treatments were similar and greater than all other treatments. On the second sampling date, the NH_4^+ concentration of the 50:50 treatment was highest and different ($P < 0.05$) from the other treatments except the 100:0 treatment. The NH_4^+ concentrations of all the treatments were greater than those of the 0:100 treatment and the control. The NH_4^+ concentration of the 100:0 treatment was highest ($P < 0.05$) on the third sampling date but declined on subsequent sampling dates. NH_4^+ concentration in the 75:25 treatment, however, increased gradually between successive sampling dates from day 0 to day 30. NH_4^+ concentration of the 50:50 treatment also decreased gradually from day zero to day 14 (5th sampling date) but recorded the highest ($P < 0.05$) NH_4^+ concentration of 12.57 NH_4^+ kg^{-1} soil on day 30. NH_4^+ concentrations of the remaining treatments decreased gradually in successive sampling dates relative to their initial NH_4^+ concentrations.

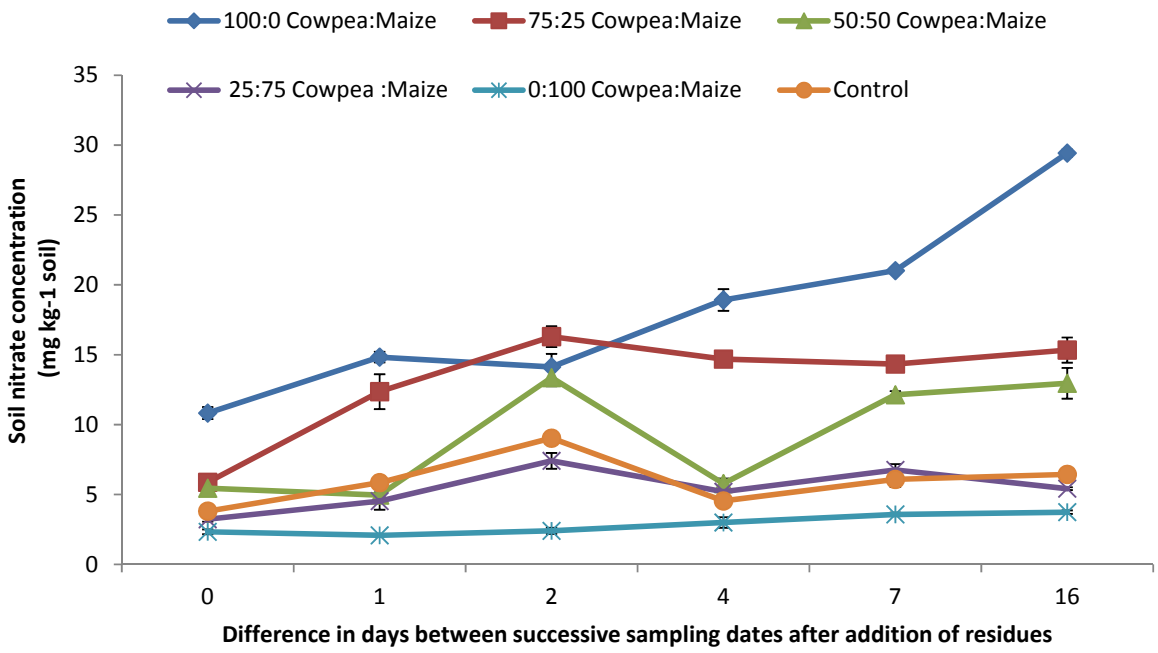
Figure 5. Soil NH_4^+ concentration after addition of cowpea-maize residue mixtures (0 indicates the day on which incubation started; error bars represent standard error of means).



On the day of incubation (day 0), apart from the 100:0 cowpea:maize treatment, all the treatments showed similar NO_3^- concentrations (Figure 6). On the second sampling date, the NO_3^- concentrations in the 100:0 and 75:25 treatments were significantly higher ($P < 0.05$) but the other treatments did not differ substantially. On the third sampling date, NO_3^- concentrations in the 100:0, 75:25 and the 50:50 did not differ substantially but were higher than the remaining treatments. The control and the 25:75 did not differ substantially but were also higher than the 0:100 treatment. On the fourth sampling date,

the NO_3^- concentrations in the 100:0 and 75:25 were significantly higher ($P < 0.05$) than all other treatments. On subsequent sampling dates, however, the NO_3^- concentration in the 100:0 was significantly higher than the other treatments while the 75:25 and the 50:50 treatments were higher than the remaining treatments. The NO_3^- concentration in the 0:100 treatment did not vary substantially throughout the study period. However, the 100:0 cowpea:maize treatment showed gradual increase successive sampling dates but the increase was steepest from the third to the final sampling dates.

Figure 6. Soil NO_3^- concentration after addition of cowpea-maize residue mixtures (0 indicates the day on which incubation started; error bars represent standard error of means)



On day 0, N mineralization occurred only in the 100:0 and 50:50 cowpea:maize treatments (Figure 7), but between days 1 and 14, net mineralization occurred in all the treatments except in the 0:100 cowpea:maize which showed net N immobilization up to day 14. At the end of the experimental period, the 100:0 treatment recorded the highest ($P < 0.05$) net N mineralization. Net N mineralization for the 100:0 and 75:25 treatments did not appear to differ substantially on the third, fourth and fifth sampling dates (days 3, 7 and 14) as is also the case for the 75:25 and 50:50 treatments on days 1, 3 and 30. However, the difference between the 50:50 and 25:75 appears substantial.

Net N mineralized on day 30 is consistent with the total available N (Table 4). The total available N and net N mineralized for the 100:0 cowpea:maize treatment were significantly different from all other treatments. Net N mineralized in the 75:25 and the 50:50 treatments were not significantly different but both differed from the remaining treatments. However, the 25:75, 0:100 treatments and control did not differ significantly in their total available N and net N mineralized.

Figure 7. Effect of cowpea-maize residue amendment on N mineralized or immobilized (error bars represent standard error of means).

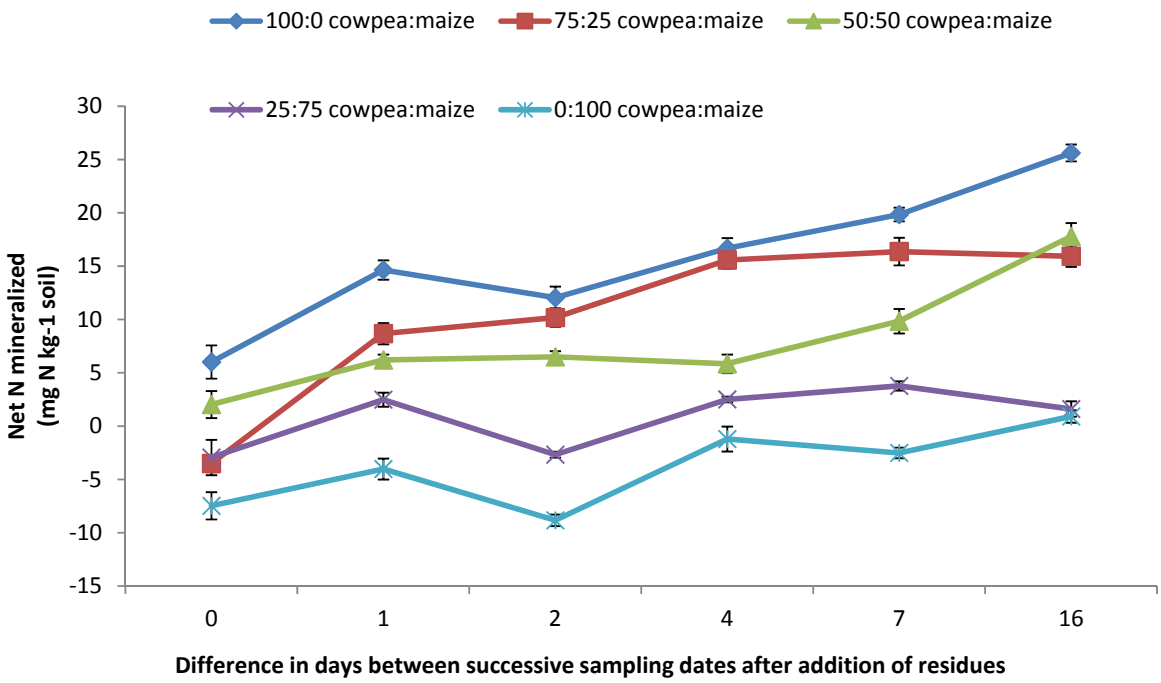


Table 4. Differences in total available N and net N mineralized on day 30 after addition of residue mixtures.

Treatment	$NH_4^+ + NO_3^-$ (mg kg^{-1} soil)	Net N mineralized
100:0 cowpea:maize	34.5 ± 1.0a	26.9 ± 1.2a
75:25 cowpea:maize	25.6 ± 0.6b	16.0 ± 0.8b
50:50 cowpea:maize	24.1 ± 1.8b	16.6 ± 1.8b
25:75 cowpea:maize	9.32 ± 0.32c	1.8 ± 0.7c
0:100 cowpea:maize	7.98 ± 1.2c	0.47 ± 0.7c
Control	7.5 ± 1.2c	-

Note: same letters indicate no significant difference.

4. Discussion

4.1. N_2O and CO_2 Emissions

The N_2O emission from the sole cowpea (100:0 cowpea:maize) treatment was significantly higher ($P < 0.05$) than that of the sole maize (0:100 cowpea:maize) treatment, suggesting that in the sole residue amended treatments residue N availability was the main driving factor for N_2O production. This observation agrees with several authors, e.g., [17,23,31,32], who have also recorded enhanced N_2O emission from soils amended with crop residues and have attributed it to residue N availability. Previous studies [16,33] have found that in addition to their percentage residue N content and C:N ratios, N_2O emissions from soils amended with crop residue have also been influenced by their

lignin and polyphenol contents, and lignin:N ratio, polyphenol:N ratio or (lignin + polyphenol): N ratio. [16,31] have also recommended that, for the purposes of controlling N release and subsequent N_2O emission, high N residues with high polyphenol content may be more useful. Lignin and polyphenol contents are known to delay N release from the residues, either by forming recalcitratnt N compounds or by binding to soil microbial enzymes, thereby lowering the substrate availability for N_2O production [34].

In this study the lignin and extractable polyphenol contents of the cowpea and maize residues used were lower than the 15% lignin and 3%–4% polyphenol threshold levels, respectively proposed in the Organic Resources Database [35] and by other authors [36,37] to retard residue N mineralization. Therefore, in this experiment, only the initial N contents and the C:N ratios of the residues were expected to affect N_2O emission and mineral N concentrations. Incorporation of sole cowpea resulted in net N mineralization but sole addition of maize residues led to a net N immobilization and low N_2O emission throughout the 30 days. This observation confirmed the initial hypothesis that incorporation of low C:N ratio cowpea residues will promote rapid net mineralization while the addition of high C:N ratio maize residue will result in initial N immobilization [38,39]. Immediate net mineralization is known to occur often after incorporation of residues with C:N ratios below approximately 20–25 [24,40]. The initial N concentration (3.4%) in the cowpea residue used in this study was higher than the threshold of 1.8%–2.5% N suggested by [35], but that of the maize residue (0.9%) was less than this threshold.

N_2O emissions measured from the mixed cowpea and maize treatments, except for the 75:25 cowpea:maize treatment, were lower than from the sole cowpea treatment, indicating an effect of the maize residue on N_2O emission and perhaps the lower N input from the residues. The N_2O and CO_2 emissions (Figures 1 and 3 respectively) suggest that disproportionate mixing of cowpea:maize residues on weight basis might lead to relatively higher N_2O and CO_2 emissions shortly after the incorporation. This is also reflected in the relatively higher emission factors of the cowpea:maize mixture treatments compared to the sole cowpea or maize treatments (Table 2). While the N_2O emission pattern might reflect a decreasing N input from the residues, the substantially higher N_2O and CO_2 emissions from the 75:25 treatment is interesting and requires further investigation to explain the interactive effects. The same can be said of the 50:50 treatment which has N input lower than the 75:25 treatment but higher than that of the 25:75 treatment, yet has an emission factor lower than both treatments. Thus, the pattern of N_2O emission from the treatments does not reflect just a reduction in N input from the mixtures but an interactive effect from the imbalance between the N and C sources [40,41]. This observation, therefore, seems to suggest that incorporating sole cowpea residue in a tropical acrisol might increase N_2O emission over the control and sole maize treatments, but mixing cowpea and maize residues might potentially lead to relatively higher N_2O and CO_2 emissions in relation to the magnitude of N input from the residues.

Greater C availability from residues is reported to drive dissimilatory reduction of NO_3^- [41,42]. In this study, WEC significantly correlated with N_2O emissions, indicative that whilst microbial activity was increased, exemplified by increased CO_2 emissions, denitrification also potentially increased. This is further confirmed by the strongly positive correlation found between CO_2 and N_2O fluxes measured from the different treatments (Table 3).

4.2. Mineral N (NO_3^- and NH_4^+) Concentrations and N_2O Emission

NO_3^- concentration was highest in the 100:0 cowpea:maize treatment than all other treatments. This can be attributed to the higher residue N input and the absence of high C:N maize residue. The addition of high C:N maize residue probably modified the total residue-N input and C:N ratio in the cowpea:maize mixtures depending on the proportions of residues in the mixture. However, the concentration of NO_3^- in the 50:50 treatment appeared to differ substantially from the other cowpea:maize treatments except the 75:25 treatment on days 3, 14 and 30. The pattern of NH_4^+ concentration seems to reflect the pattern of NO_3^- concentration and thus suggest the occurrence of nitrification. Thus, reducing the quantity of cowpea residue and increasing the proportion of low N maize residues could have lowered the total N substrate available for mineralization. Again, the apparent lower inorganic N concentration in the cowpea:maize mixtures could have further been exacerbated by the large supply of available C from the high C:N maize residues coupled with an insufficient N supply. This is particularly reflected by the patterns of immobilization and low mineralization in the 25:75 treatment. Unlike the other treatments which showed steep changes in net N mineralization, the 50:50 treatment showed a steady, slow increase in net N mineralization and had no immobilization. The results agree with Gartner and Cardon [43] who reported that decomposition patterns in leaf mixtures are not always easily predictable. They further argued that the characteristics of decomposition in such litter mixes might deviate from responses predicted from decomposition of single species alone.

N_2O emission in the 100:0 treatment was highest on day 2 but declined sharply from day 3 (Figure 1) but NH_4^+ concentration peaked on day 3 and decreased steeply thereafter (Figure 5). NO_3^- concentration increased significantly after day 3 and continued to increase till day 30 (Figure 6). The same pattern can be observed in the 75:25 treatment. These patterns seem to suggest rapid nitrification of the organic N from the cowpea residue. Denitrification requires a C source as electron donor. The high N_2O emission from the 75:25 treatment might suggest accelerated denitrification due to the pulse of C substrate from the 25% maize residue, even though with decreased N input from the cowpea residue. This might also explain the rapid mineralization found in the 75:25 (Figure 7) and why even though the NH_4^+ concentration in the 75:25 was higher than the 100:0 from day 7 to day 30, this did not correspond with higher NO_3^-. Thus, the relatively lower N_2O emission in the 100:0 treatment (which had higher N input) with corresponding high mineral N concentrations and net N mineralization might be attributed to inadequate C source. Notwithstanding, it is noteworthy that, in this study, mixing maize with cowpea increases the proportion of added N that is lost as N_2O compared to adding cowpea alone (Table 2).

However, the peak N_2O emission in the 50:50 treatment was lower than the 100:0, 75:25 and 25:75 treatments (Figure 1) and the same can be said of CO_2 emission. However, the initial NH_4^+ concentration (days 0 and 1) were relatively higher and the changes were slight except between days 14 and 30. With NO_3^- concentration, the 50:50 treatment did not appear to differ substantially from the 75:25 on days 0, 3 and 30. This reflects the stable or slow increase in net N mineralization and emission of N_2O albeit with a further low N input (compared to the 100:0 and 75:25 treatments) but with comparatively higher C substrate. The cumulative N_2O emission and N mineralization observed thus confirm the assertion that low N_2O emission will results from insufficient supply of N substrate in

the presence of a large supply of C substrate which stimulate microbial immobilization [44]. Nevertheless, there was no immobilization in the 50:50 treatment compared to the other mixtures. Thus, these findings suggest that incorporating cowpea:maize residue mixtures in equal proportions (50:50 on weight basis) might moderate the proportion of added N that is lost as N_2O compared to disproportionate mixing of cowpea:maize (*i.e.*, 75:25 and 25:75) and stabilize N mineralization in soils to match N supply to demand by plants. However, further studies will be required to confirm this under both laboratory and field conditions.

Thus, the finding in this study disagrees with previous report that showed low N_2O emission after mixing a higher C:N ratio rice straw residues with lower C:N ratio groundnut residues than from sole incorporation of groundnut residues [23]. The interactive effect of mixing residues on N release is due to the movement of soluble constituents between the residues incorporated [45]. This study was conducted under controlled moisture conditions and so the movement of soluble component as affected by rainfall could not be verified. In another study, it was reported that where residues were incorporated together, the rapid decomposition of the high quality residues in the mixture at the initial stages resulted in high N availability, which stimulated the decomposition of the low quality residues, leading to a more rapid decomposition of carbon substrates [46]. However, the interactive effect may either be positive or negative depending on the component species [47]. [46] showed that mixing residues can change the abundance and composition of soil fauna leading to alterations in residue decomposition and N mineralisation, but the abundance and composition of soil fauna following the addition of cowpea or maize residues was not examined in this study.

4.3. Implications for Residue Management in Acrisols

In this study, addition of sole cowpea residue with relatively higher N input increased the proportion of N lost as N_2O. The emission of N_2O seems to correspond with the magnitude of N input from the residues but incorporation of cowpea-maize residue mixtures (representing decreased N input) rather substantially increased the proportion of N lost as N_2O compared to the sole treatments (as shown by the emission factors). This indicates an interactive effect that requires further investigation. However, incorporating cowpea:maize residue mixture on a 50:50 weight basis resulted in lower N_2O and CO_2 emission and appreciable and steady N mineralization without immobilization. A ratio of 75:25 cowpea:maize mixture had higher N input than the 50:50 treatment but this led to a higher than proportionate N_2O emission. A 25:75 cowpea:maize mixture resulted in a higher initial N_2O emission but a cumulative emission profile that is slightly lower than but not substantially different from the 50:50 treatment, but with N immobilization. While incorporation of cowpea-maize residue mixture increases the proportion of added N lost as N_2O, the results for the 50:50 treatment show a scope for manipulating N release from N-rich cowpea residue by the addition of a proportionate amount of a high C:N maize residue. The NO_3^- concentrations measured in this treatment and the pattern of N mineralization suggest further that NO_3^- leaching and N loss as N_2O through denitrification can be minimized in this treatment. Thus, incorporation of cowpea residues mixed with maize residues has the potential to promote synchrony between residue N release for crop uptake and increased nitrogen use efficiency (NUE), but this requires further investigation on the field where NUE can be verified in the presence of a crop.

The results of this study indicate that increase in both NO_3^- availability and organic C concentration were likely to have been responsible for the difference between N_2O fluxes on day 1 from the 100:0 and the 75:25 cowpea:maize treatments. Holding WFPS constant, it appears that this observation is in contrast with [48] who stated that NO_3^- concentration and low oxygen concentration other than soluble C availability were believed to be the most important condition for denitrification at the bacterial cell level. In this study source partitioning was not done, therefore the contributions of different processes to N_2O fluxes could not be determined. However, the microbial source of N_2O requires further investigation for example by adopting ^{15}N pool dilution approach for quantifying gross nitrification [29] or by the isotopomer strategy which identifies the microbial source of N based on its position on the liner N_2O structure [49].

In situ field methods of measuring N mineralization and N_2O emissions are laborious, time consuming and are subject to confounding effects of changing edaphic and climatic factors, but they reflect more realistic temperature and moisture conditions unlike laboratory incubations, which are conducted under temperature and moisture conditions different than those occurring in the field [50]. Furthermore, in the field, soil fauna including earthworms, nematodes and arthropods may interact with microbes to alter residue decomposition and N release through communition of plant material and N mineralization [51]. In this study, the experimental conditions were supposed to be optimal for microbial decomposition and N mineralization (*i.e.*, high but not excessive soil water content, 60% WFPS, and constantly high temperature, 25 °C) [52]. Therefore, data generated under this aerobic incubation conditions may not be easily transferable or comparable to field conditions [53]. Moreover, the absence of plant N uptake in the laboratory incubation experiment implies that the NO_3^- accumulation found in this study may have been over-estimated. Again, in the field soluble carbon concentration can be further increased by root exudation, which could have increased N_2O production through denitrification. However, the results obtained from this study are useful for comparing N mineralization and N_2O emission from acrisols amended with mixed residues of differing C:N ratios as the incubation method minimizes the confounding effects of changing soil temperature and moisture conditions.

5. Conclusions

The aim of a soil fertility management strategy in the interest of climate change is to lower N_2O emission without sacrificing stable N supply. This study shows that incorporating sole cowpea residue increases N_2O emissions over the control or sole maize residue treatment. However, mixing maize and cowpea residues has the potential to increase the proportion of added N lost as N_2O compared to sole cowpea or maize incorporation in a tropical acrisol. While there was lower N input from the 75:25 cowpea:maize mixture treatment than the sole cowpea treatment, daily and cumulative N_2O emissions were substantially higher in the 75:25 compared to all the other treatments. The behavior of the 75:25 treatment, in terms of N_2O and CO_2 emissions, is interesting and requires further investigation. However, when reductions in N_2O emission and soil fertility are coupled, then the incorporation of a 50:50 cowpea:maize residue mixture might become interesting. Initial daily N_2O emission was lower in the 50:50 treatment as compared to the 100:0, 75:25 and 25:75 treatments. The 50:50 treatment also had lower emission factor than the 75:25 and 25:75 treatments and had cumulative N_2O emission not

substantially different from that of the 25:75. The 50:50 treatment also showed stable N mineralization compared to the other mixtures. This shows that amount of organic N initially added in the mixes and the ratios of mixing the different residues were important factors governing N availability for N_2O emissions. Therefore, proportionate mixing (on weight basis) of cowpea and maize residues has the potential to minimize N_2O emission and maintain stable N supply compared to disproportionate mixing. However, further studies should be done to examine the effect of varying the total weight of the 50:50 cowpea:maize mixture. Further investigation at field scale is also required to establish the effects of changing edaphic and climatic factors on the effect of mixing these residues on mineral N availability and N_2O emissions. Future field experiments should also investigate the effects of plant uptake of N and root exudation on inorganic N concentration and N_2O emissions following incorporation of mixed cowpea and maize residues in the soil.

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