

Do combined applications of crop residues and inorganic fertilizer lower emission of N₂O from soil?

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Abstract

Emissions of N₂O were measured following addition of ¹⁵N-labelled residues of tropical plant species [*Vigna unguiculata* (cowpea), *Mucuna pruriens* and *Leucaena leucocephala*] to a Ferric Luvisol from Ghana at a rate of 100 mg N/kg soil under controlled environment conditions. Residues were also applied in different ratio combinations with inorganic N fertilizer, at a total rate of 100 mg N/kg soil. N₂O emissions were increased after addition of residues, and further increased with combined (ratio) applications of residues and inorganic N fertilizer. However, ¹⁵N-N₂O production was low and short-lived in all treatments, suggesting that most of the measured N₂O-N was derived from the applied fertilizer or native soil mineral N pools. There was no consistent trend in magnitude of emissions with increasing proportion of inorganic fertilizer in the application. The positive interactive effect between residue- and fertilizer-N sources was most pronounced in the 25:75 *Leucaena*:fertilizer and cowpea:fertilizer treatments where 1082 and 1130 mg N₂O-N/g residue were emitted over 30 days. N₂O (log_e) emission from all residue amended treatments was positively correlated with the residue C:N ratio, and negatively correlated with residue polyphenol content, polyphenol:N ratio and (lignin + polyphenol):N ratio, indicating the role of residue chemical composition in regulating emissions even when combined with inorganic fertilizer. The positive interactive effect in our treatments suggests that it is unlikely that combined applications of residues and inorganic fertilizer can lower N₂O emissions unless the residue is of very low quality promoting strong immobilisation of soil mineral N.

Keywords: Nitrous oxide, crop residues, inorganic fertilizer, residue quality, stable isotopes, tropical soil

Introduction

Integrated soil fertility management aims to optimize the use of a range of local suitable resource inputs for sustainable crop production (Kimani *et al.*, 2003). One example is the combined application of plant or crop residues with inorganic fertilizer which is thought to better optimize N use-efficiency, either through promoting temporary immobilisation of inorganic fertilizer N or by improving soil organic matter content, and thereby offering the potential to lower losses of N (Kirchmann & Thorvaldsson, 2000; Vanlauwe *et al.*, 2001). Such an integrated approach is essential in much of sub-Saharan Africa where a decrease in soil fertility is often also coupled with low availability or affordability of inorganic fertilizers and low quantities of N-rich crop residues.

However, there is evidence that combined applications of crop residues and inorganic fertilizer may have an interactive effect on N release and on emissions of the greenhouse gas nitrous oxide (N₂O), the magnitude and direction of which is thought to depend on the chemical composition, or quality, of the organic inputs (Garcia-Ruiz & Baggs, 2007; Gentile *et al.*, 2008), and therefore also on the ratio of inorganic-to-organic N applied (Garcia-Ruiz & Baggs, 2007).

Addition of plant material or inorganic N-based fertilizers to soil typically increase N₂O emissions, with emission factors based on the quantity of N applied (Eichner, 1990; Baggs *et al.*, 2000; Millar *et al.*, 2004; Novoa & Tejeda, 2006; Snyder *et al.*, 2007). The emission factor from plant material is harder to predict because of the role of the chemical composition of this material (Novoa & Tejeda, 2006). Agricultural soils are a major source of atmospheric N₂O, contributing to global warming and the destruction of stratospheric ozone (Solomon *et al.*, 2007; Ravishankara

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et al., 2009), with the contribution of tropical soils, currently thought to be ca. 20–50%, anticipated to increase with continuing application of inorganic fertilizers. In tropical systems this also represents a crucial lowering in N-use efficiency in often N-limited cropping systems.

The chemical composition of residues added to soil has been shown to be important in regulating the magnitude of N₂O emissions, both in temperate (Baggs *et al.*, 2000; Harrison *et al.*, 2002; Baggs *et al.*, 2003) and tropical agricultural systems (Millar *et al.*, 2004; Gentile *et al.*, 2008), and after addition of temperate and tropical species under controlled environment conditions (Millar & Baggs, 2004, 2005; Huang *et al.*, 2004; Garcia-Ruiz & Baggs, 2007). We have previously demonstrated strong negative relationships between N₂O emission and residue C-to-N ratio in tropical agroforestry systems (Millar *et al.*, 2004) and with residue lignin content and (lignin + polyphenol)-to-N ratio after applying tropical agroforestry residues and olive crop weed residues to soil under controlled environment conditions (Millar & Baggs, 2004; Garcia-Ruiz & Baggs, 2007). The latter relationship was attributed to lower N release when the residue contains >15% lignin and/or >3–4% total extractable polyphenol content (Palm *et al.*, 2001; Constantinides & Fownes, 1994). The threshold for N mineralization is generally accepted to be above 1.7–1.8% N, with temporary immobilisation after addition of material with lower N contents (Melillo *et al.*, 1982; Constantinides & Fownes, 1994; Seneviratne, 2000). Combined application of inorganic fertilizer-N with residues may overcome such immobilisation, and such an integrated approach will also lower fertilizer-N requirements through N supply from the residues, thereby holding the potential to regulate N release for crop uptake, whilst minimizing detrimental effects on the environment. However, little is known of the extent to which these different N sources interact in soil, nor the nature or underlying mechanisms of any interaction (Garcia-Ruiz & Baggs, 2007; Gentile *et al.*, 2008), but are likely to be strongly influenced by the chemical composition of the residue.

Here, we report a controlled environment experiment, the objective of which was to determine the optimum combination of residue-to-fertilizer N to increase N release from the residue, but to also lower N₂O emission after addition of leguminous tropical species to a tropical Ferric Luvisol. Applied residues ranged in their chemical compositions, and were ¹⁵N-labelled to enable the influence of lignin and polyphenol contents of the residue N to be determined, to quantify the contribution of this residue ¹⁵N to measured mineral N pools and N₂O production, and to enable a better understanding of any interaction between the two N sources. The hypothesis was that the extent of interaction would depend on the chemical composition of the residue applied, with a high residue-to-fertilizer ratio only being able to lower soil N availability and N₂O emissions after addition of these high N leguminous residues if they

also have high lignin and high polyphenol contents, and conversely greater N availability and potential for N₂O loss with a low residue-to-fertilizer ratio where the residue has a high N content and lower lignin and polyphenol contents.

Materials and methods

Soil

Soil (0–15 cm depth) was sampled from an arable field at the Savanna Research Institute, Tamale, Ghana, in February 2008. This soil is a reddish brown sandy loam [72.5% silt, 10% clay, 17.5% sand, 1.1% organic C, 0.07% total N, pH (H₂O) 6.1, bulk density 1.03 g/cm³], of the Nyankpala series, classified as a Ferric Luvisol (FAO, 1998). The soil was thoroughly mixed, air-dried and sieved <2 mm prior to establishment of the experiment.

Experimental set-up

A laboratory soil microcosm experiment was established in 500 mL Kilner jars to each of which 100 g of the sieved soil was added and packed to a bulk density of 1.23 g/cm³. The soil was pre-incubated at 45% water-filled pore space (WFPS) for 7 days prior to the start of the experiment to re-initiate microbial activity after dry storage, and to minimize changes in soil WFPS at the start of the experiment. WFPS was calculated based on the soil volumetric water content, bulk density and a particle size density of 2.65 g/cm³, and was achieved by applying an appropriate volume of distilled H₂O using a pipette and mixing thoroughly. Fertilizer (NH₄NO₃) and ¹⁵N-labelled residues (leaf material, chopped to 3–5 mm) of cowpea (*Vigna unguiculata*), *Mucuna* (*Mucuna pruriens*) and *Leucaena* (*Leucaena leucocephala*) were mixed in to the soil on day 0 in the following treatments of residue-to-fertilizer ratio: 100:0, 75:25, 50:50, 25:75, 0:100 at a rate of 100 mg N/kg soil per treatment. A control treatment was also included which had no residue or fertilizer addition, but this soil was disturbed in the same way as the other treatments when the target WFPS was established. Each treatment was replicated three times for gas sampling and a further three times for destructive soil sampling. Three days prior to residue and fertilizer addition on day 0, soil in all treatments and the control was wetted to 60% WFPS and maintained at this WFPS on a weight basis for the duration of the experiment. The experiment was conducted at 21 °C in the dark for 30 days.

¹⁵N-labelling of plant species

Seedlings of *L. leucocephala*, *M. pruriens*, and *V. unguiculata* were grown in vermiculite for 6 months in a greenhouse at the University of Aberdeen. Nodulation of the leguminous species was prevented by previous addition of 1% sodium hypochlorite solution to the pots, after Millar & Baggs

(2004). The seedlings were fed regularly with a 5 mM $^{14}\text{NH}_4^{15}\text{NO}_3$ solution (10 atom percent excess ^{15}N) at a rate of 200 to 400 mL per pot per day depending on plant demand. Other macro and micro nutrients were provided in a nutrient solution adapted from Yoshida *et al.* (1976). The leaves were harvested after 6 months, oven dried at 40 °C and their chemical composition determined as described below. The atom percent excess ^{15}N of the leaves was determined using a SerCon 20/20 isotope ratio mass spectrometer (SerCon Ltd, Crewe, UK) (Table 1).

Chemical composition of the plant residues

Dried leaf residues were ground (<1 mm) in a rotary mill and analyzed for total N, total C, lignin and total extractable polyphenol contents (Table 1). Lignin content was determined in an Ankom 220 fiber analyser (acid detergent fibre). Total extractable polyphenol content was measured using Folin-Ciocalteu reagent in a method adapted from Anderson & Ingram (1993). Total C and total N contents were determined using a Metler Toledo AG 2455 C/N autoanalyser.

Gas sampling and analysis

Gas samples for N_2O and CO_2 analysis were collected from the Kilner jar headspaces on day 0, 2 h prior to addition of fertilizer and residues, and on days 1, 2, 3, 5, 7, 10, 14 and 30 after addition. Kilner jars were closed for an hour during gas sampling. The gas samples were taken through a gas sampling port in the Kilner jar lids at 0, 30 and 60 min after their closure and stored in pre-evacuated 12 mL gas vials (Labco, UK). N_2O concentration was determined on a Perkin Elmer autosystem gas chromatograph fitted with an electron capture detector, and CO_2 concentration was determined using a Chrompack CP9001 gas chromatograph fitted with a methaniser and flame ionization detector. Oven and detector temperatures were 50 °C and 250 °C, respectively. The increase in N_2O concentration during the 60 min headspace closure period was used to calculate by linear interpolation a daily flux of N_2O from the soil. Total N_2O and CO_2 emissions over specified periods were calculated by linear interpolation between daily fluxes.

Samples (120 mL) for ^{15}N - N_2O determination were taken after 60 min closure of the headspace, and an equal

volume of air was replaced to the headspace. Samples were stored in helium-flushed and pre-evacuated 120 mL gas-tight glass bottles (Supelco, UK) and the ^{15}N -enrichment of the N_2O molecule determined on a SerCon 20/20 isotope ratio mass spectrometer following condensing and cryofocusing of the sample in an ANCA TGII gas module (SerCon Ltd). ^{15}N -enrichments of the gas fluxes were calculated from the atom percent excess of samples, taking into account the atom percent excess of the residues applied.

Soil mineral N and total organic carbon analyses

Soil was destructively sampled from three additional replicates per sampling date on days 0 (prior to fertilizer and residue addition), 1, 3, 7, 14 and 30. Subsamples (40 g) of the fresh soil were extracted in 1 M KCl at a soil-to-extractant ratio of 1-to-5 and filtered through Whatmann no.1 filter paper. NH_4^+ -N and NO_3^- -N concentrations in the extracts were determined colorimetrically by continuous flow analysis on an FIA star 5010 analyser fitted with a cadmium column. The ^{15}N -enrichments of NH_4^+ and NO_3^- were determined by diffusion methodology (Brooks *et al.*, 1989) and analysis on the SerCon 20/20 isotope ratio mass spectrometer. Water extractable C (cold water) was quantified in soil sampled on days 0, 1, 7 and 30. A 10-g (air-dried basis) subsample was shaken in 40 mL deionised water for 2 h, and filtered through Whatmann no. 42 filter paper. Further filtration of the extract was undertaken with a 0.45- μm micropore filter. Concentration of C in the supernatant liquid was determined using a total organic carbon analyzer (TOC-5000A, Shimadzu).

Statistical analysis

Data obtained in the study were subjected to statistical analysis using the MINITAB 15 statistical package. Tukey's HSD (Honestly Significant Difference) method was used for means' comparison.

Results

Emissions of $^{14+15}\text{N}$ - N_2O and C- CO_2

Emissions of $^{14+15}\text{N}$ - N_2O and CO_2 over the 30-day experimental period were increased ($P < 0.001$) after

Table 1 Chemical composition of the tropical residues applied in this experiment

Treatment	N (%)	C (%)	C-to-N ratio	ADL (%)	TEP (%)	Atom % ^{15}N excess
<i>Leucaena leucocephala</i>	6.1	41.7	6.8	8.3	4.6	4.50
<i>Mucuna pruriens</i>	4.3	39.8	9.2	10.1	2.2	4.28
<i>Vigna unguiculata</i>	3.4	39.6	11.7	7.2	1.3	4.08

ADL, acid detergent lignin; TEP, total extractable polyphenols.

combined addition of residues and fertilizer-N, compared to measured emission from the control (Tables 2 and 3). Between 51 and 87% of the total N₂O or CO₂ measured throughout the 30-day experiment was emitted in the first 7 days after addition. Of the 100:0 residue:fertilizer treatments, the cowpea:fertilizer treatment resulted in the highest N₂O and CO₂ emissions (37.2 mg N₂O-N/m² per 30 days; 14.5 g CO₂-C/m² per 30 days; $P < 0.05$). This emission of N₂O was increased ($P < 0.05$), but CO₂ emission lowered ($P < 0.05$), when the cowpea residue was combined with fertilizer-N, with 63.1 mg N₂O-N/m² per 30 days emitted from the 50:50 cowpea:fertilizer treatment, 87% of which was emitted in the first 7 days. Emissions of both N₂O and CO₂ were low from the fertilizer only treatment (0:100 residue:fertilizer; 18.5 mg N₂O-N/m² per 30 days; 4.7 g CO₂-C/m² per 30 days). Increasing the proportion of fertilizer-N combined with the residues had no consistent effect on emissions between the different residue species, but emissions of both N₂O and CO₂ were lower ($P < 0.05$) from the 75:25 ratio of all residues than from the 25:50 and 50:50 residue:fertilizer combinations. Total N₂O (log_e) and CO₂ (log_e) emitted by 30 days were positively correlated in the 25:75, 50:50 and 75:25 residue:fertilizer treatments ($r = 0.93$; $P < 0.05$; Figure 1). N₂O (log_e) emission over the 30-day experiment from all residue-amended treatments was positively correlated with residue C:N ratio ($r = 0.63$; $P < 0.05$), and negatively correlated with residue polyphenol content ($r = -0.59$; $P < 0.05$), polyphenol:N ratio ($r =$

-0.61 ; $P < 0.05$) and (lignin + polyphenol):N ratio ($r = -0.61$; $P < 0.05$) (Figure 1).

N₂O fluxes were temporarily increased ($P < 0.01$) compared to the control after addition of residues, with peak fluxes measured from all residue treatments on day 1 (Figure 2). Maximum fluxes of 24 and 20 mg N₂O-N/m²/day ($P < 0.05$) were measured from the 50:50 cowpea:fertilizer and 50:50 *Mucuna*:fertilizer treatments, respectively, on day 1. In contrast, addition of fertilizer on its own (0:100 residue:fertilizer treatment) only raised emissions compared to the unamended control on days 1 and 7. The trend was for an exponential decrease in CO₂ fluxes from all treatments with time after addition of residues and fertilizer (Figure 3). The highest CO₂ flux of 1.6 g CO₂-C/m²/day was measured in both the 50:50 and 75:25 cowpea:fertilizer treatments on day 0. Both N₂O and CO₂ fluxes from the residue only treatments (100:0 residue:fertilizer) decreased the least rapidly after day 1, and after day 10 were the only treatments for which CO₂ fluxes remained higher ($P < 0.05$) than from the control. N₂O and CO₂ fluxes from the 50:50 and 100:0 cowpea:fertilizer treatments were positively correlated ($r = 0.54$ and 0.63 for the 50:50 and 100:0 treatments, respectively; $P < 0.05$).

¹⁵N-N₂O emissions

Combining residues with fertilizer addition increased ($P < 0.001$) the total ¹⁵N-N₂O emitted over the first 7 days

Table 2 Total emissions of N₂O (mg N/m² and mg N per g residue) and ¹⁵N-N₂O (μg ¹⁵N/m²) after addition of residues and fertilizer to soil

Treatment	¹⁴⁺¹⁵ N-N ₂ O emission (mg N/m ²)		¹⁵ N-N ₂ O emission (μg ¹⁵ N/m ²)	¹⁴⁺¹⁵ N-N ₂ O emission (mg N per g residue)	
	7 days	30 days	7 days	7 days	30 days
<i>Leucaena leucocephala</i>					
25:75 residue:fertilizer	20.3 (± 1.6) ^b	26.4 (± 2.1) ^b	74 (± 2) ⁱ	753 (± 63) ^b	1082 (± 66) ^a
50:50 residue:fertilizer	21.9 (± 1.5) ^b	29.0 (± 2.2) ^c	127 (± 1) ^h	416 (± 29) ^c	606 (± 37) ^b
75:25 residue:fertilizer	9.7 (± 0.4) ^d	15.8 (± 0.8) ^d	230 (± 2) ^g	78 (± 1.2) ^f	189 (± 0.7) ^d
100:0 residue:fertilizer	11.4 (± 1.1) ^d	16.3 (± 1.5) ^d	27 (± 1) ^j	78 (± 10) ^f	147 (± 10) ^e
<i>Mucuna pruriens</i>					
25:75 residue:fertilizer	23.0 (± 2.7) ^b	29.7 (± 3.0) ^b	1823 (± 13) ^c	490 (± 66) ^a	691 (± 63) ^b
50:50 residue:fertilizer	31.3 (± 1.2) ^b	37.3 (± 1.6) ^b	1012 (± 2) ^e	357 (± 12) ^d	448 (± 12) ^c
75:25 residue:fertilizer	16.0 (± 2.7) ^c	22.4 (± 3.1) ^c	1549 (± 76) ^d	131 (± 28) ^e	215 (± 29) ^d
100:0 residue:fertilizer	13.8 (± 1.0) ^d	18.9 (± 1.5) ^d	109 (± 3) ^h	78 (± 6.4) ^f	130 (± 6.7) ^e
<i>Vigna unguiculata</i> (cowpea)					
25:75 residue:fertilizer	32.2 (± 3.6) ^b	45.8 (± 4.3) ^b	1456 (± 21) ^d	931 (± 91) ^a	1130 (± 97) ^a
50:50 residue:fertilizer	54.7 (± 1.0) ^a	63.1 (± 11) ^a	6618 (± 72) ^a	675 (± 131) ^b	799 (± 136) ^b
75:25 residue:fertilizer	23.6 (± 3.7) ^b	29.9 (± 4.1) ^b	3301 (± 16) ^b	168 (± 31) ^c	232 (± 31) ^d
100:0 residue:fertilizer	30.9 (± 2.2) ^b	37.2 (± 2.9) ^c	884 (± 17) ^f	176 (± 13) ^c	224 (± 15) ^d
0:100 residue:fertilizer	4.9 (± 0.3) ^e	18.5 (± 1.1) ^d			
Control	5.0 (± 0.3) ^e	17.2 (± 1.1) ^d			

Different superscript letters within a column indicate significant differences between treatments at $P < 0.05$.

Treatment	CO ₂ emission (g C/m ²)		CO ₂ emission (g C per g residue)	
	7 days	30 days	7 days	30 days
<i>Leucaena leucocephala</i>				
25:75 residue:fertilizer	4.1 (±0.2) ^c	6.7 (±0.6) ^c	102 (±16) ^a	120 (±7.6) ^a
50:50 residue:fertilizer	4.0 (±1.2) ^c	6.2 (±0.3) ^c	49 (±8.5) ^c	47 (±10) ^d
75:25 residue:fertilizer	2.7 (±0.1) ^f	4.8 (±0.2) ^e	11 (±6.6) ^e	8 (±7.7) ^f
100:0 residue:fertilizer	4.3 (±0.3) ^c	10.6 (±0.6) ^b	28 (±3.2) ^d	77 (±1.9) ^c
<i>Mucuna pruriens</i>				
25:75 residue:fertilizer	3.8 (±0.2) ^c	7.0 (±0.4) ^c	60 (±1.4) ^b	72 (±2.5) ^c
50:50 residue:fertilizer	3.7 (±0.1) ^e	6.4 (±0.1) ^c	49 (±3.3) ^c	76 (±3.6) ^c
75:25 residue:fertilizer	3.0 (±0.1) ^d	5.9 (±0.3) ^d	46 (±3.5) ^c	66 (±4.3) ^c
100:0 residue:fertilizer	5.2 (±0.4) ^b	11.4 (±0.7) ^b	46 (±0.8) ^c	91 (±0.1) ^b
<i>Vigna unguiculata</i> (cowpea)				
25:75 residue:fertilizer	4.3 (±0.5) ^c	6.9 (±0.8) ^c	47 (±8.5) ^c	74 (±8.8) ^c
50:50 residue:fertilizer	5.7 (±0.3) ^b	9.9 (±0.4) ^b	22 (±6.3) ^c	29 (±7.7) ^c
75:25 residue:fertilizer	6.0 (±0.2) ^b	9.8 (±0.3) ^b	8 (±3.5) ^e	15 (±3.6) ^f
100:0 residue:fertilizer	7.3 (±0.4) ^a	14.5 (±0.7) ^a	21 (±0.9) ^d	49 (±0.3) ^d
0:100 residue:fertilizer	2.1 (±0.2) ^f	4.7 (±0.3) ^c		
Control	2.1 (±0.5) ^f	4.3 (±0.7) ^c		

Different superscript letters within a column indicate significant differences between treatments at $P < 0.05$.

compared to emissions from the residue only (100:0 residue:fertilizer) treatments (Table 2). The highest ($P < 0.001$) emission of 6.6 mg ¹⁵N-N₂O/m²/7 days was emitted from the 50:50 cowpea:fertilizer treatment. Emissions from all *Mucuna* and cowpea treatments were greater ($P < 0.05$) than from the corresponding *Leucaena* treatments. Much of this higher emission was due to the fluxes on day 1, with 5.1 and 3.3 mg ¹⁵N-N₂O/m²/day measured from the 50:50 *Mucuna*:fertilizer and cowpea:fertilizer treatments, respectively (Figure 4). Emission of ¹⁵N-N₂O (log_e) over the first 7 days from all treatments was negatively correlated with residue polyphenol content ($r = -0.78$; $P < 0.05$), N content ($r = -0.77$; $P < 0.05$), polyphenol:N ($r = -0.77$; $P < 0.05$), and (lignin + polyphenol):N ($r = -0.57$; $P < 0.05$), and positively correlated with residue C:N ratio ($r = 0.73$; $P < 0.05$) and lignin:N ratio ($r = 0.70$; $P < 0.05$).

Concentrations of soil mineral N

Available soil NH₄⁺ concentrations increased after residue and fertilizer addition, with the highest ($P < 0.05$) concentrations in the 100:0 cowpea:fertilizer and *Leucaena*:fertilizer treatments (Figure 5). ¹⁵N-NH₄⁺ concentrations in these treatments on day 14 (7.4 and 8.8 mg ¹⁵N-NH₄⁺/kg soil, cowpea and *Leucaena*, respectively) accounted for 30% and 26% of the ¹⁴⁺¹⁵N-NH₄⁺ concentrations. Fertilizer addition maintained concentrations between 41 and 53 mg NH₄⁺/kg soil during the experiment, and by the end of the experiment (day 30) concentrations remained high in the cowpea and *Leucaena* 100:0

Table 3 Total emissions of CO₂ (g C/m² and g C per g residue) after addition of residues and fertilizer to soil

residue:fertilizer treatments and in the *Mucuna* 25:75 and 50:50 treatments. NH₄⁺ concentrations in the 50:50 *Mucuna*:fertilizer, 50:50 cowpea:fertilizer and 100:0 *Leucaena*:fertilizer treatments were strongly negatively correlated with CO₂ fluxes ($r = -0.93$ for each of these treatments; $P < 0.05$).

The trend was for increasing NO₃⁻ concentrations after addition of residues, both solely and in combination with fertilizer (Figure 6). The highest ($P < 0.005$) concentration of 117 mg NO₃⁻-N/kg soil was measured in the 50:50 *Leucaena*:fertilizer treatment on day 30, with the residue-derived ¹⁵N only contributing to 16% of this ¹⁴⁺¹⁵N-NO₃⁻ pool. On this day concentrations in the 75:25 *Mucuna*:fertilizer and cowpea:fertilizer treatments were greater ($P < 0.05$) than in the other *Mucuna* and cowpea treatments. Also on this day 50% (9 mg ¹⁵N-NO₃⁻/kg soil) of the ¹⁴⁺¹⁵N-NO₃⁻ pool in the 100:0 cowpea:fertilizer treatment was derived from the ¹⁵N-residue. NO₃⁻ concentrations in the *Mucuna* and cowpea treatments were negatively correlated with CO₂ fluxes ($r = -0.5$ to -0.64 , varying with residue:fertilizer ratio; $P < 0.05$), and the NO₃⁻ concentrations in the 100:0 *Mucuna*:fertilizer treatment were negatively correlated with N₂O fluxes ($r = -0.67$; $P < 0.05$).

Water extractable C

Water extractable C decreased in all treatments with time after addition of residues and fertilizer. Concentrations in treatments to which residue had been applied were greater ($P < 0.05$) than in the fertilizer only (0:100) treatment and in

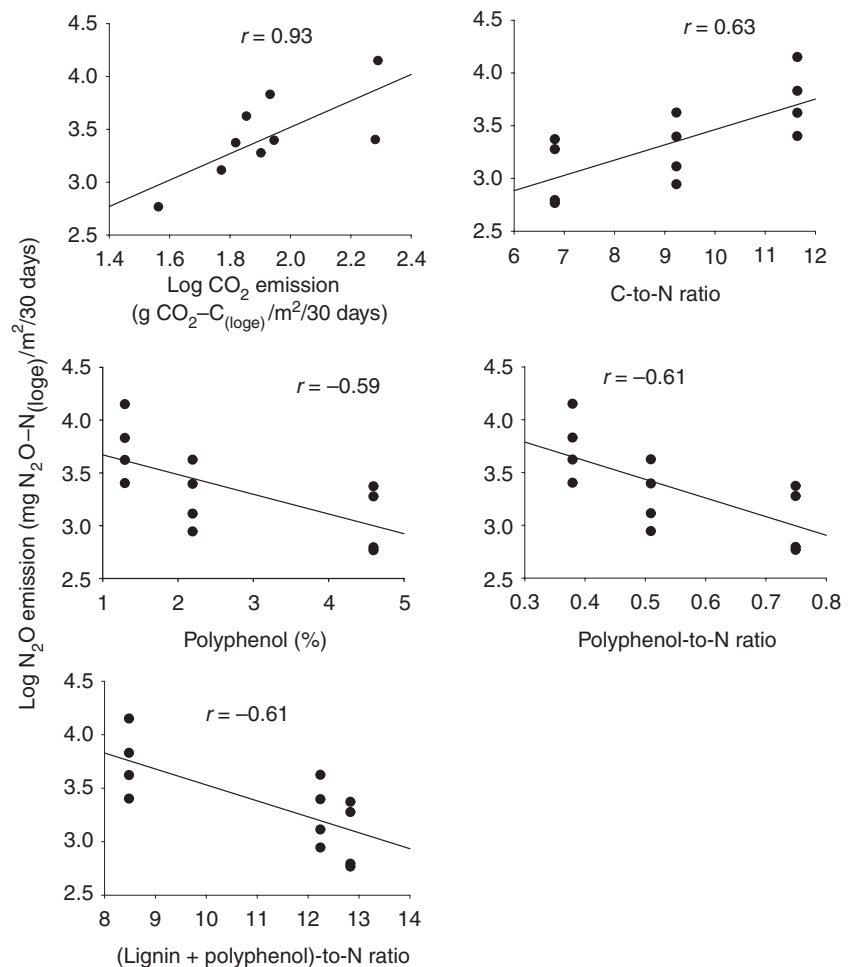


Figure 1 Correlations between N₂O emissions (log_e) and CO₂ emissions (log_e), residue C-to-N ratio, polyphenol content, polyphenol-to-N ratio and (lignin + polyphenol)-to-N ratio.

the control (Figure 7). The highest concentration of 357 mg C/kg soil was in the 100:0 cowpea:fertilizer treatment on day 0, but had fallen to 149 mg C/kg soil by day 1. Concentrations in the 100:0 *Leucaena*:fertilizer and 100:0 *Mucuna*:fertilizer treatments on day 7 were greater ($P < 0.05$) than in the other ratio treatments for these residues. C concentrations were negatively correlated with ¹⁵N-NO₃⁻ concentrations in all the residue treatments ($r = -0.51$ to -0.88 ; $P < 0.05$), and with ¹⁵N-NH₄⁺ concentrations in the *Leucaena* and *Mucuna* treatments ($r = -0.61$ to -0.99 ; $P < 0.05$), and positively correlated with CO₂ emissions from all residue treatments ($r = 0.71$ to 0.97 ; $P < 0.05$).

Discussion

Residue addition and the influence of residue chemical composition

As hypothesized, and in accordance with several other studies (e.g. Baggs *et al.*, 2000; Velthof *et al.*, 2002; Millar *et al.*, 2004; Toma & Hatano, 2007), N₂O fluxes were raised after

addition of residues (100:0 residue:fertilizer) to soil, with greater magnitude than fluxes measured from the unamended control soil. This increase in emission was short-lived, and the positive correlation between N₂O and CO₂ fluxes in the cowpea (100:0 cowpea:fertilizer) treatment, suggests that the immediate increase in N₂O flux after addition of this residue resulted from a direct stimulation of microbial activity (Azam *et al.*, 2002). Addition of residues to soil would also have resulted in an increase in sub-oxic microsites within the soil structure (Tiedje *et al.*, 1984), which along with the provision of residue-C and -N would have created ideal conditions for the stimulation of N₂O-genic processes such as denitrification or nitrate ammonification. Alternatively, release of residue-N may have increased ammonia oxidation in more oxic microsites. Any stimulation in microbial activity in the 100:0 residue:fertilizer treatments was longer-lived than in the fertilizer treatments as exemplified by the more gradual decrease in CO₂ fluxes after residue addition. The negative correlation between CO₂ fluxes and NO₃⁻ concentrations in the *Mucuna* and cowpea treatments, and between N₂O fluxes and NO₃⁻ concentrations in the 100:0 *Mucuna*:fertilizer treatment may be indicative of NO₃⁻ reduction during

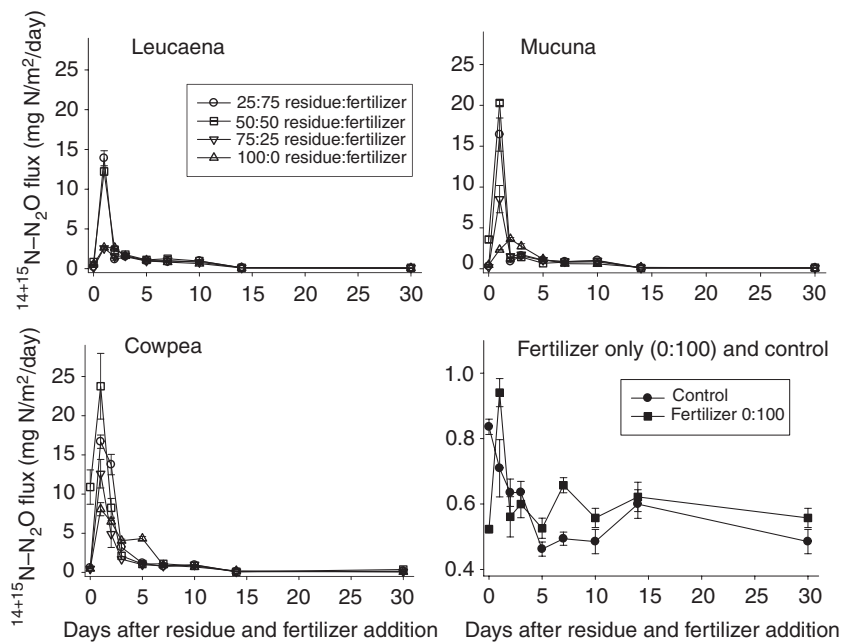


Figure 2 Daily fluxes of N_2O following addition of residues and residue:fertilizer combinations to soil. Error bars represent \pm one standard error of the mean.

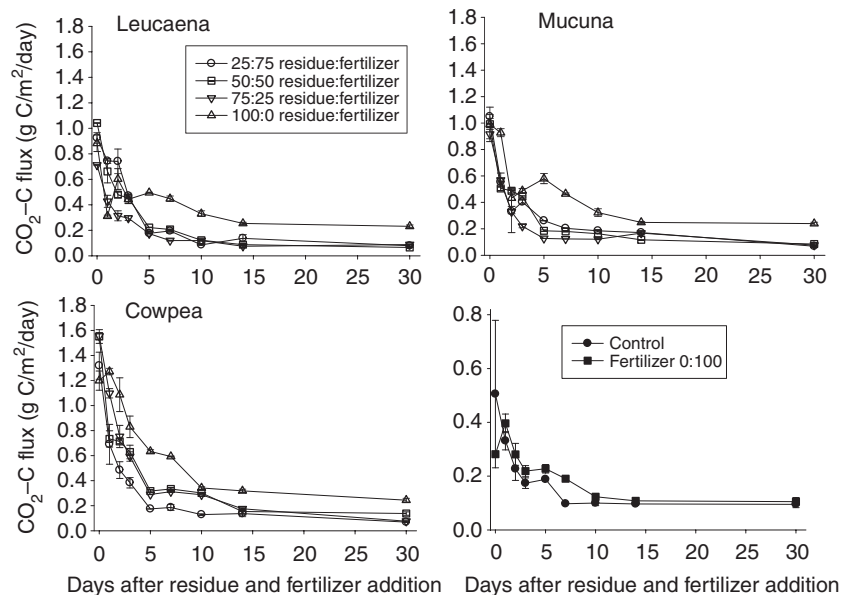


Figure 3 Daily fluxes of CO_2 following addition of residues and residue:fertilizer combinations to soil. Error bars represent \pm one standard error of the mean.

denitrification. This is supported by the negative correlations between total organic C and $^{15}\text{N}-\text{NO}_3^-$ concentrations in all the residue treatments. Water extractable C concentrations were highest in the 100:0 cowpea:fertilizer treatment which was attributed to solubilization of more C in the low lignin, low polyphenol cowpea residue compared to the relatively higher lignin *Mucuna* and higher polyphenol *Leucaena* residues. This may account for the higher $^{15}\text{N}-\text{N}_2\text{O}$ (7 days) and $^{14+15}\text{N}-\text{N}_2\text{O}$ emission from this cowpea treatment than from the *Leucaena* and *Mucuna* 100:0 treatments.

Measurement of $^{15}\text{N}-\text{N}_2\text{O}$ fluxes and ^{15}N -enrichment of soil mineral N pools enabled us to determine the fate of applied ^{15}N -labelled residues, and to quantify the contribution of this residue- ^{15}N to N_2O emissions. Over the 30-day experiment 9.9% ($442 \mu\text{g } ^{15}\text{N}-\text{N}_2\text{O}/\text{kg soil}$), 1.1% ($54 \mu\text{g } ^{15}\text{N}-\text{N}_2\text{O}/\text{kg soil}$) and 0.3% ($13 \mu\text{g } ^{15}\text{N}-\text{N}_2\text{O}/\text{kg soil}$) of the ^{15}N applied in the cowpea, *Mucuna* and *Leucaena* (all 100:0 residue:fertilizer) treatments, respectively, was emitted as $^{15}\text{N}-\text{N}_2\text{O}$. Only up to 2.9% of N_2O emission was enriched in ^{15}N , meaning that a significant proportion of the measured

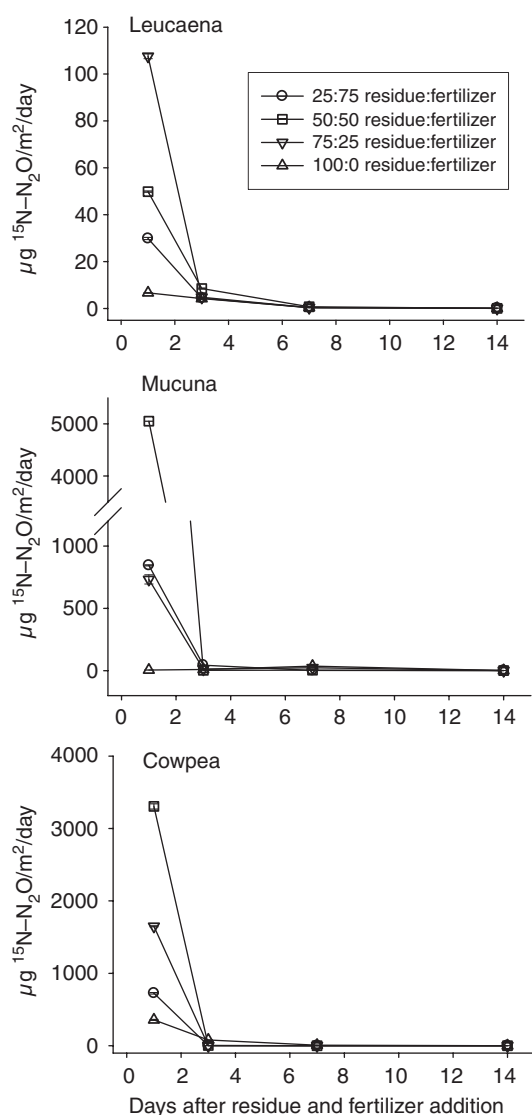


Figure 4 Daily fluxes of ¹⁵N-N₂O following addition of residues and residue:fertilizer combinations to soil. Error bars represent ± one standard error of the mean.

¹⁴+¹⁵N-N₂O emission was derived from unlabelled residue N, native soil mineral N, or a priming of N release from soil organic matter. Recovery of applied ¹⁵N was greater as mineral N than as N₂O in the *Mucuna* and *Leucaena* treatments, with 2.4% and 1.2% of applied ¹⁵N recovered as ¹⁵N-NO₃⁻ on day 14 in these treatments, and 1.4% and 8.5% recovered as ¹⁵N-NH₄⁺. Conversely, in the cowpea treatment proportionally more applied ¹⁵N was recovered as ¹⁵N-N₂O (9.9%), with 6.3 and 8.3% of ¹⁵N applied recovered as ¹⁵N-NH₄⁺ and ¹⁵N-NO₃⁻, respectively, at day 14.

Total N₂O emission at 30 days was positively correlated with residue C-to-N ratio in the 100:0 residue:fertilizer treatments ($r = 0.97$; $P < 0.05$). Although this is in agreement with data of Millar & Baggs (2004), it is against

the generally accepted negative relationship in which N₂O emissions increase with greater residue-N content (Kaiser *et al.*, 1998; Baggs *et al.*, 2000; Akiyama & Tsuruta, 2003; Millar *et al.*, 2004; Toma & Hatano, 2007). Our result may reflect that C was limiting in this system for denitrification of available NO₃⁻, or that release of N from the residues was slow. N₂O emission from the 100:0 residue:fertilizer treatments was negatively correlated with residue polyphenol ($r = -0.87$; $P < 0.05$) and lignin ($r = -0.6$; $P < 0.05$) contents, and polyphenol:N ($r = -0.91$; $P < 0.05$) and (lignin + polyphenol):N ($r = -0.99$; $P < 0.05$) ratios. The lower (lignin + polyphenol):N ratio of the cowpea coupled with its higher C:N ratio compared to the other residues may account for emission from the 100:0 cowpea:fertilizer treatment (374 mg N₂O-N/m²/30 days, 2.5 g N₂O-N/m²/kg biomass/30 days) being greater than that emitted from the 100:0 *Mucuna* and *Leucaena* treatments. Lignin and polyphenol contents are known to delay N release from the residue, either by forming recalcitrant N compounds, or binding to soil microbial enzymes (Baldwin *et al.*, 1983; Mole & Waterman, 1986), thereby lowering the substrate availability for N₂O-genic processes. Millar & Baggs (2004) also showed a strong relationship between N₂O emission and the (lignin + polyphenol):N ratio of agroforestry residues when combining their data with that of Baggs *et al.* (2001). However, here our residue lignin of all three species, and polyphenol contents of *Mucuna* and cowpea were below the thresholds generally accepted to lower N release; >15% lignin and/or >3–4% total extractable polyphenol content (Constantinides & Fownes, 1994; Palm & Rowland, 1997).

Combined application of residue and fertilizer

Application of residues and fertilizer increased the total N₂O emitted compared to the sum of sole applications of residue and fertilizer (100:0 and 0:100 residue:fertilizer treatments), by up to 114% (25:75 *Mucuna*:fertilizer treatment) indicating a positive interactive effect on emissions. This, coupled with the lack of difference in N₂O emission between the 0:100 residue:fertilizer treatment and unamended control, suggests that the combined addition in part alleviated any limitation on N₂O production in the sole treatments. Previously this has been attributed to the greater C availability from residues driving dissimilatory reduction of NO₃⁻ (denitrification or nitrate ammonification) (de Catanzaro & Beauchamp, 1985; Sarkodie-Addo *et al.*, 2003). However, here water extractable C was not significantly correlated with daily N₂O fluxes, suggesting that whilst microbial activity was increased (exemplified by increased CO₂ fluxes, and strong positive correlation between water extractable C and CO₂), residue C was not solely responsible for stimulating N₂O production. It is possible that ammonia oxidation was contributing to N₂O production in our soil, as has previously been demonstrated for the 60% WFPS at which the soil was maintained here

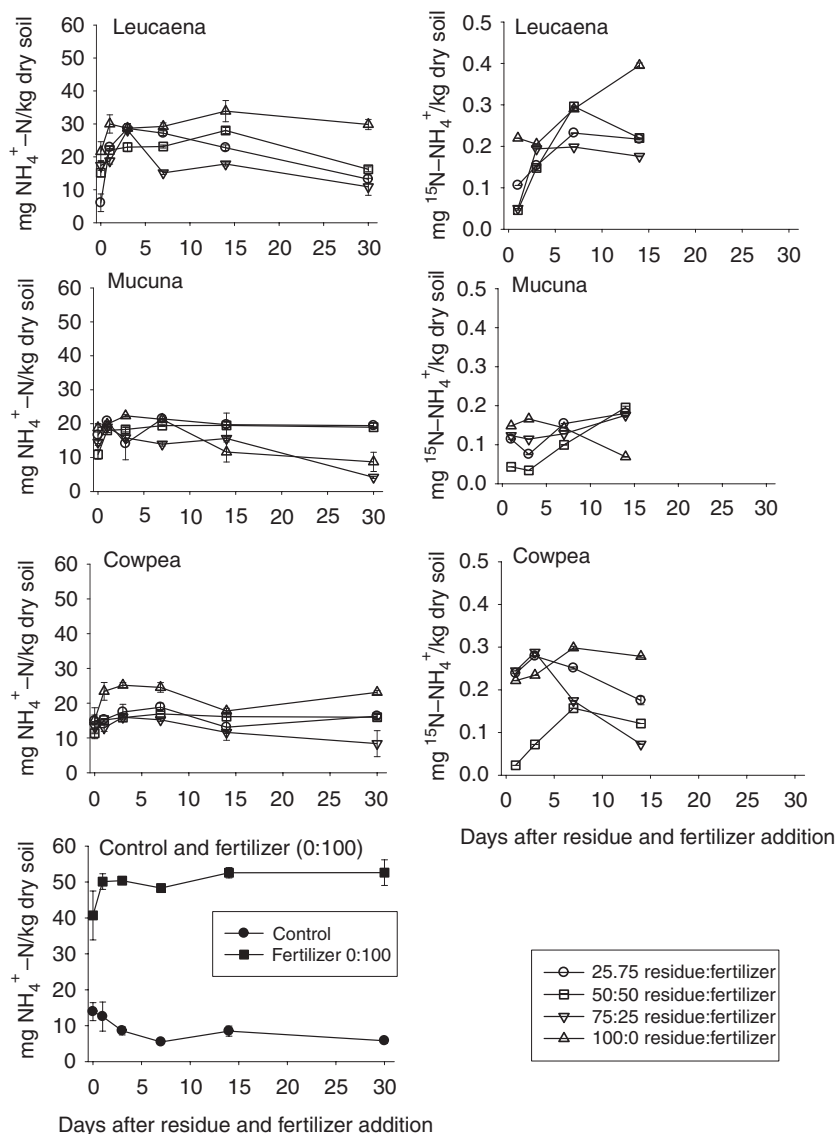


Figure 5 Concentrations of available soil NH_4^+ and $^{15}\text{N-NH}_4^+$ following addition of residues and residue:fertilizer combinations to soil. Error bars represent \pm one standard error of the mean.

(Bateman & Baggs, 2005), which as an autotrophic process would account for the lack of relationship between water extractable C or CO_2 and N_2O production. The increase in NO_3^- concentrations over time were also indicative of nitrification. The nature of such interactive effects on the microbial source of N_2O requires further investigation, for example by adopting an isotopomer approach for source partitioning the N_2O (Baggs, 2008) and a ^{15}N pool dilution approach for quantifying gross nitrification rates (Davidson *et al.*, 1991).

Increasing the proportion of fertilizer-N applied with *Leucaena* and *Mucuna* residues increased emission of N_2O (30 days) when expressed on a per residue biomass basis, with greater emissions from the 25:75 residue:fertilizer than from the 50:50 or 75:25 treatments, but this trend was not reflected in the $^{15}\text{N-N}_2\text{O}$ emission over the first 7 days. To

our knowledge this is the first study investigating interactions between residue and fertilizer-N addition on N_2O production where the residues were ^{15}N -labelled, and demonstrates that increasing the proportion of fertilizer-N applied with residues does not consistently increase or decrease the contribution of residue- ^{15}N to N_2O production. $^{15}\text{N-N}_2\text{O}$ production only accounted for up to 2.9% of the total $^{14+15}\text{N-N}_2\text{O}$ emitted after residue addition, suggesting that the N substrates for ammonia oxidation or nitrate reduction were predominantly from native soil N pools or the applied fertilizer-N.

The interactive effect between residue- and fertilizer-N sources was most pronounced in the *Leucaena*:fertilizer treatments where total $^{14+15}\text{N-N}_2\text{O}$ emission (mg N/m²/g residue/30 days) from the 25:75 *Leucaena*:fertilizer treatment was 13 times greater than the emission from the 100:0 *Leucaena*:fertilizer treatment. The contribution of

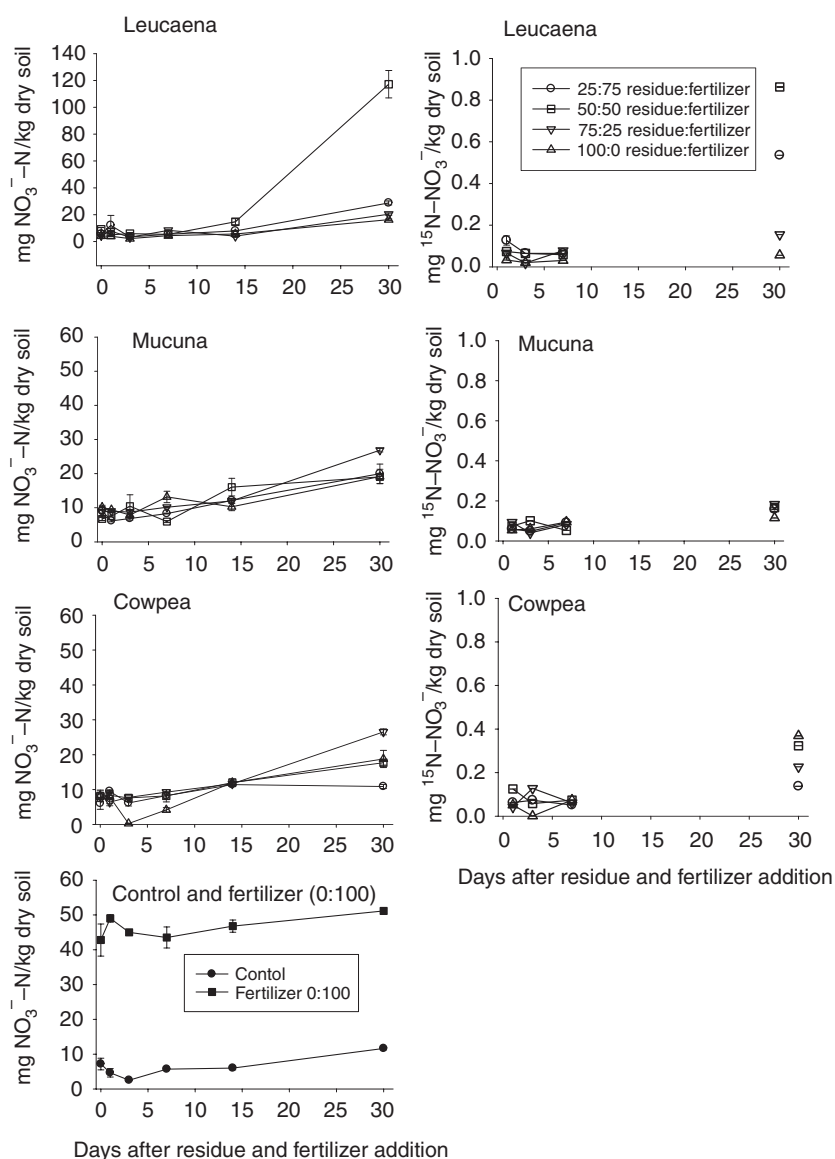


Figure 6 Concentrations of available soil NO_3^- and $^{15}\text{N-NO}_3^-$ following addition of residues and residue:fertilizer combinations to soil. Error bars represent \pm one standard error of the mean.

residue-derived ^{15}N to total N_2O emitted was also increased with joint residue and fertilizer application, and resulted in up to 10-fold greater recovery of applied ^{15}N as $^{15}\text{N-N}_2\text{O}$. This was most marked in the 50:50 cowpea:fertilizer treatment where $^{15}\text{N-N}_2\text{O}$ accounted for 88% of the total recovered ^{15}N (N_2O , NH_4^+ and NO_3^- pools). Total recovery of applied ^{15}N increased with the proportion of fertilizer-N in the combined application of residues and fertilizer. This suggests that application of fertilizer increased mineralization of ^{15}N from the residues, and further increased its utilization in N_2O -genic processes.

Emission of $^{15}\text{N-N}_2\text{O}$, indicative of the contribution of residue- ^{15}N to N_2O emission, was lower in all the *Leucaena* residue treatments than the *Mucuna* and cowpea treatments. The *Leucaena* residues had a higher polyphenol content (4.6%) than the other residues, which was at the threshold thought to

influence N release (Constantinides & Fownes, 1994; Palm & Rowland, 1997), and to lower N_2O production (Millar & Baggs, 2004). However, there was no discernable influence on soil NH_4^+ or NO_3^- concentrations, and recoveries of applied ^{15}N as $^{15}\text{N-NO}_3^-$ in the 25:75 and 50:50 *Leucaena*:fertilizer treatments (46 and 37% of ^{15}N applied) were higher than in the other residue treatments. Residue polyphenol and N contents, and (lignin + polyphenol):N ratio still exerted an effect on N_2O (\log_e) emission in the combined residue and fertilizer treatments (Figure 1), but were less strong than where the residues were applied alone ($r = -0.87$ for polyphenol content; $r = -0.90$ for N content; $r = -0.99$ for (lignin + polyphenol):N; $P < 0.05$), reflecting the contribution of applied fertilizer N as a substrate for N_2O producing processes, with the higher mineral N contents of soil resulting from immediate availability of fertilizer-N possibly

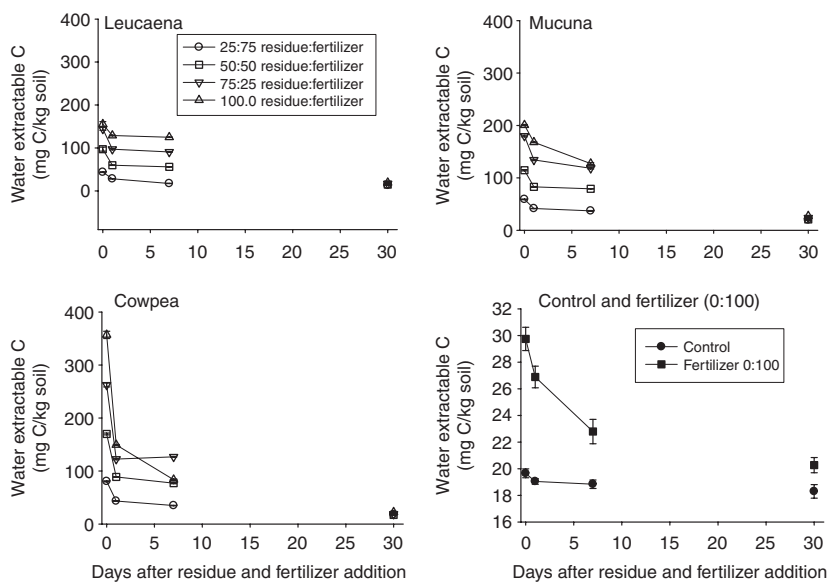


Figure 7 Concentrations of water extractable C following addition of residues and residue:fertilizer combinations to soil. Error bars represent \pm one standard error of the mean.

dampening the influence of residue composition over this 30 days experiment, and a lower quantity of residues applied.

Does the combined application of residues and fertilizer-N hold the potential for regulation of N_2O production?

Our results demonstrate that whilst there is the potential for N_2O emission to be controlled through varying ratios of residue:fertilizer input, the magnitude and direction of interactions between these N sources varies between different species as a result of their differing qualities. This is in spite of the range in qualities examined here being quite narrow. The potential for regulation may be greater where residues have a high C:N ratio, higher lignin and higher polyphenol contents, as Millar & Baggs (2004) showed that strength of relationship between N_2O emission and these residue characteristics depended on the range of chemical composition being considered. Gentile *et al.* (2008) and Palm *et al.* (2001) have recommended that for the purpose of controlling N release, high quality residues be applied alone, but low and medium quality residues be applied in combination with N fertilizers. Such relationships as we observed here may also be expected to change over time, for instance after the first flush of fertilizer-N has been utilized.

For all residues the lowest N_2O emissions over 30 days were when they were applied alone (100:0 residue:fertilizer), and also resulted in greater NH_4^+ availability. Combined application with fertilizer did not significantly lower NH_4^+ concentrations compared to the control, indicative that strong immobilisation of soil N did not occur, but raised N_2O emissions. Whilst these results confirm the positive interactive effect on N_2O emission following a 50:50 residue:fertilizer application by Sarkodie-Addo *et al.* (2003), it is in contrast to Sakala *et al.* (2000), Vanlauwe *et al.* (2002)

and Gentile *et al.* (2008) who report negative interactive effects on soil mineral N, with combined addition of maize and fertilizer-N immobilising the fertilizer-N but stimulating release of the maize-N. The lack of immobilisation of N here may reflect the low C:N ratios of the residues, and the application of residues on an equivalent N basis with a total residue- and fertilizer-N input of 100 mg N/kg soil in each of the treatments.

With combined applications the contribution of residue-derived ^{15}N to measured $^{14+15}N-N_2O$ fluxes, and the recovery of applied ^{15}N as $^{15}N-N_2O$, increased. For all residues the 75:25 residue:fertilizer treatment resulted in greater NO_3^- availability and least increase in N_2O emission compared to the 100:0 treatments, with N_2O emissions being lower than where proportionally more fertilizer-N was applied, indicating that something was limiting for optimum reduction of NO_3^- to N_2O . Although lower in magnitude, the contribution of residue- ^{15}N to N_2O emissions in the 75:25 residue:fertilizer treatments (2–14%) tended to be greater than in the 25:75 treatments (0.4–8%), whereas recovery of applied ^{15}N as $^{15}N-N_2O$ was greater in the latter. Under our controlled experimental conditions this 75:25 residue:fertilizer ratio would appear to offer the best compromise between release of N for crop uptake, appearing to enhance release of residue-N, and management of N_2O emission. Further studies are required to determine if such relationships hold true under field conditions and in response to varying environmental conditions, particularly in response to rainfall events.

Conclusion

N_2O production was increased after addition of ^{15}N -labelled residues to tropical soil under controlled environmental

conditions, and emissions were further increased when the residues were applied in combination with inorganic N fertilizer. However, there was no consistent trend in magnitude of N₂O production with increasing proportion of inorganic fertilizer in the combined applications, and contribution of residue ¹⁵N to measured N₂O emissions was low and short-lived. Correlations confirmed that the magnitude and direction of interaction between N sources was influenced by the chemical composition of the residues, and so varied with residue species. We found no direct evidence that residue C was driving N₂O production, which may either indicate a contribution of ammonia oxidation here, or that soil organic matter C was not limiting for NO₃⁻ dissimilation. Based on our applications of *Leucaena*, *Mucuna* and cowpea residues, the 75:25 residue:fertilizer ratio at 100 mg N/kg soil is recommended from this study as offering the best compromise between release of N for crop uptake and management of N₂O emission. However, the influence of residue quality is likely to change over time after addition under real field conditions, and with the range of chemical compositions being compared. In order to aid predictions of N₂O emission following addition of different residue species to soil, the biogeochemical and physical effects of residue addition now need to be separated from interactive effects of combined N sources.

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