

# Nitrous Oxide Emissions from Soils Amended with Polyphenols and Cowpea Residues

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## Abstract

Polyphenols can influence the rate of N<sub>2</sub>O emission and N mineralization in leguminous crop residues by affecting the activities of residue decomposers or by forming protein complexes. A laboratory microcosm incubation study was conducted to assess the effect of three concentrations of ferulic, vanillic and tannic acids on N<sub>2</sub>O emissions and inorganic N dynamics in a tropical soil amended with cowpea residue. The results show that N<sub>2</sub>O emission and mineral N concentrations in the sole cowpea amended soils were significantly higher than in all the polyphenol treatments. Decrease in N<sub>2</sub>O emissions and N concentrations showed a direct relation with the polyphenol concentrations. However, at the same concentration, the polyphenols did not differ significantly in their ability to decrease N<sub>2</sub>O emissions and N concentrations even though tannic acid showed the highest numerical decrease. The tannic acid lowered N mineralisation and N<sub>2</sub>O production through protein binding while ferulic and vanillic acids decreased N<sub>2</sub>O production through N immobilisation by stimulating microbial activity. It is concluded that the addition of polyphenols to tropical soils amended with cowpea residue is likely to lower N<sub>2</sub>O emissions and inorganic N concentration, but the magnitude of reduction will depend on the type and concentration of the polyphenol compounds added.

## Introduction

Polyphenols include a wide range of plant compounds such as coumarins, flavonoids and tannins, which differ in size, solubility and reactivity (Haslam, 1989). Polyphenols have been reported to influence the rate of litter decomposition by directly inhibiting the growth or functioning of the residue decomposers (Palm & Sanchez, 1991), or through complex-forming interactions with proteins including nitrogen (Myers *et al.*, 1994; Mafongoya *et al.*, 1998). Soluble polyphenols can bind and immobilise different forms of N (Martin & Haider, 1980). The capacity of polyphenols to bind proteins

derives from the presence of multi-dentate ligands, at different points on their surfaces that react with plant residue amide groups to form polyphenol-protein complexes which resist microbial decomposition (Haslam, 1989). This capacity to bind proteins is the most important property of polyphenols that affects plant N mineralisation (Mole & Waterman, 1986) even though some polyphenols stimulate microbial N immobilisation by providing soluble carbon that enhances microbial growth (Kraus *et al.*, 2004).

During the early periods of decomposition, plant residues with high N, low lignin and

low polyphenol concentrations are reported to mineralise rapidly to supply a high concentration of mineral N while 'poor' quality residues decompose slowly and contribute little initially to the plant available N pool (Palm & Sanchez, 1991). Palm & Sanchez (1991) indicated that N-rich legumes with greater than 15% lignin and, or 4% active polyphenols content qualify as 'poor quality' residues. Thus, little plant N mineralization is expected to occur after the incorporation of plant residues with a high concentration of polyphenol (Palm & Sanchez, 1991) and a high capacity to bind plant protein (Handayanto *et al.*, 1997).

Frimpong & Baggs (2010) have reported that net N mineralisation and nitrous oxide (N<sub>2</sub>O) emissions from soils amended with high N residues of *Leucaena*, *Mucuna* and cowpea, solely or in combination with fertiliser N, are regulated by their lignin content (in agreement with Moorhead *et al.*, 1996) and polyphenol concentration (as observed also by Constantinides & Fownes, 1994). In addition, Frimpong & Baggs (2010) reported that total N<sub>2</sub>O emitted from these high N residues amended soils were negatively correlated with residue polyphenol:N ratio and lignin + polyphenol:N content. Moreover, the <sup>15</sup>N-N<sub>2</sub>O emission, which indicates the contribution of residue-<sup>15</sup>N to measured N<sub>2</sub>O emission, was significantly lower in the higher polyphenol (4.6%) *Leucaena* amended treatments than the lower polyphenol *Mucuna* (2.2%) and cowpea (1.3%) amended treatments.

Mineralization, nitrification and denitrification of leguminous crop residues combine to contribute to N<sub>2</sub>O emissions from agricultural soils (Abdalla *et al.*, 2010). The effect of polyphenols on N<sub>2</sub>O emission or N mineralization has been studied previously (e.g. De Neve *et al.*, 2004; Rahn *et al.*, 2003;

Chavez *et al.*, 2005), using the higher molecular weight tannic acid as a model polyphenol. However, no study has yet tested the effect of low molecular weight polyphenols (such as ferulic and vanillic acids) on N<sub>2</sub>O emission and mineral N concentrations in tropical soils amended with N-rich crop residues, or compared this effect with a high molecular weight polyphenol. Both ferulic and vanillic acids are natural constituents of raw legumes, peas and lentils (Lopez-Amóros *et al.*, 2006). Ferulic acid occurs naturally as a product of lignin degradation by white-rot fungi (Kirk, 1971), and vanillic acid is formed as a result of degradation of ferulic acid. Therefore, the study examined and compared the effect of ferulic, vanillic and tannic acids on N<sub>2</sub>O emission and inorganic N dynamics in a tropical soil amended with cowpea residues.

## Materials and methods

### Soils

The soils (0–15 cm) used in this study were sampled from the Savanna Agricultural Research Institute, Tamale, Ghana. The Soil has been classified as Ferric Luvisol (FAO, 1998) with a sandy loam texture (72.5% sand, 17.5% Clay and 10% clay). The soil had a pH (H<sub>2</sub>O) of 6.1, 1.2% organic carbon and 0.06% total N. The soils were air-dried, crushed and sieved through a 2-mm mesh and pre-incubated at 40% WFPS for 7 days prior to the start of the incubations to stimulate microbial activity and to minimize changes in water content at the start of the experiment.

### Plant material

Cowpea (*Vigna unguiculata*) residue was used in this study because of its high N content and low C:N ratio, lignin and polyphenol contents. The above-soil biomass

of 7 week-old cowpea seedlings was harvested just before flowering and dried at 40 °C to a constant weight to determine the dry matter content. The dry leaf residues were ground (< 1 mm) in a rotary mill and analyzed for total N, total C, lignin and polyphenol contents (Table 1). Lignin content was determined using the Ankom acid detergent fibre (ADF) method and 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.

TABLE 1  
*Biochemical characteristics of the cowpea residue used*

<i>Biochemical characteristics</i>	<i>Value</i>
Polyphenol	1.28%
Lignin	7.21%
C	39.6%
N	3.4%

#### *Experimental set-up*

The study involved two separate laboratory microcosm incubations carried out in a completely randomised design with three replicates of each treatment. Both incubations were undertaken at 27 °C for a period of 21 days in 500-ml Kilner jars with 200 g of soil. The soil was mixed with the ground cowpea

residues to supply 100 mg N kg<sup>-1</sup> soil, based on the % N content of the residue at the start (day 0) of the incubation. Three rates (0.1, 0.25 and 0.5 g kg<sup>-1</sup> soil) of each polyphenol compound (Table 2) were added to the cowpea residue amended soil, representing approximately 6%, 15% and 30%, respectively, of residue biomass incorporated. Each treatment was replicated three times for gas sampling. Three additional replicates were added per treatment for destructive soil sampling. The soil WFPS was brought up to 60% on day 0 with deionised water.

#### *Gas sampling for N<sub>2</sub>O and CO<sub>2</sub> analysis*

Gas samples for N<sub>2</sub>O and CO<sub>2</sub> determination were taken from Kilner jar headspace 1 h after jar closure on days 0, 1, 2, 3, 5, 7, 10, 14 and 30 after incubation and stored in pre-evacuated 12-ml gas vials (Labco, UK). Determination of N<sub>2</sub>O concentration was done with a Perkin Elmer autosystem gas chromatograph fitted with an electron capture detector (ECD). CO<sub>2</sub> concentration in the gas samples was determined using a Chrompack CP9001 gas chromatograph fitted with a methaniser and flame ionisation detector (FID). Oven and determination temperatures were 50 °C and 250 °C, respectively. Linearity of gas diffusion into the headspace over the 1 h closure period had previously been determined, so that each flux could be

TABLE 2  
*The names and sources of the polyphenol compounds used*

<i>Common name</i>	<i>Chemical name</i>	<i>Formula mass</i>	<i>Source</i>
Ferulic acid	Trans-4-hydroxymethoxycinnamic acid(C <sub>10</sub> H <sub>10</sub> O <sub>4</sub> )	194.14	Aldrich, UK
Vanillic acid	4-hydroxy-3-methoxybenzoic acid(C <sub>8</sub> H <sub>8</sub> O <sub>4</sub> )	168.15	Aldrich, UK
Tannic acid	Penta-O-galloyl- D-glucose(C <sub>7</sub> H <sub>52</sub> O <sub>48</sub> )	1701.23	Aldrich, UK

calculated from a single determination at the end of the closure. Total N<sub>2</sub>O and CO<sub>2</sub> emissions over specified periods were calculated by linear interpolation between daily fluxes.

#### Soil mineral N

Destructive soil sampling of three replicates of each treatment was done at days 0, 1, 3, 7, 14 and 30. A subsample (40 g) from each of the fresh soil for each treatment was mixed with 1 M KCl (1:5 extraction ratio) and filtered through Whatman No.1 filter paper after mechanically shaking for 1 h. NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations in the extracts were determined colorimetrically by continuous flow analysis on an FIA star 5010 analyser fitted with a cadmium column. Soil pH was analysed on a 1:5 soil:H<sub>2</sub>O ratio on days 0, 1, 3, 7, 14 and 21 after incubation using a pH meter.

#### Microbial biomass carbon

Microbial biomass carbon (MBC) was determined using the chloroform fumigation-incubation technique (Anderson & Domsch, 1978). Soil samples (15 g) placed in 100-ml glass beakers were fumigated in a large dessicator lined with moist tissue paper. A separate 100-ml beaker containing approximately 50 ml of alcohol-free chloroform was also placed in the desiccator together with the soil samples. The dessicator was connected to a pump, tightly closed and evacuated until the chloroform started boiling. The pump was then disconnected and the tightly closed dessicators placed in the dark for 24 h at laboratory temperature (18 °C ± 2). A second set of non-fumigated (control) soil samples were kept in a dessicator in a dark room at the same temperature for 24 h. Carbon from the fumigated and control soils

was extracted with 75 ml of 0.5 M potassium sulphate (K<sub>2</sub>SO<sub>4</sub>) solution after shaking the suspension for 2 h, centrifugation at 3600 r.p.m. for 1 h and filtration through Whatman No. 5 filter paper. The C concentration in extract obtained from the fumigated and control samples were analysed using the total organic carbon analyser (TOC-5000A, Shimadzu, Japan). Microbial biomass carbon content (MBC) was calculated as:

$$\text{MBC } (\mu\text{g C g}^{-1} \text{ soil}) = \text{Extracted C} \times 2.64$$

where

$$\text{Extracted C} = \text{TOC}_{\text{fumigated}} - \text{TOC}_{\text{non-fumigated}}$$

$$\text{TOC}_{\text{fumigated}} = \text{Total organic carbon concentration from fumigated soil}$$

$$\text{TOC}_{\text{non-fumigated}} = \text{Total organic carbon concentration in non-fumigated soil.}$$

2.64 = Correction factor applied to compensate for the incomplete recovery of microbial constituents extracted from soil after fumigation (Vance *et al.* 1987).

#### Statistical analyses

All data were analysed using the MINITAB 15 statistical package. Data was checked for normality and homogeneity of variance. Data was log-transformed where necessary. Analysis of variance (ANOVA) was used for multiple comparisons of means and Tukey's honestly significant difference (HSD) test was applied to establish significance between means, if any.

Correlations and regressions were used to determine relationships between N<sub>2</sub>O and mineral N concentrations, and to determine relationships between total N<sub>2</sub>O and total CO<sub>2</sub> emissions, and concentrations of the polyphenols added.

## Results

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

Total N<sub>2</sub>O emitted from the sole cowpea treatment over the 7-day period was significantly higher ( $P < 0.05$ ) than emissions from all other treatments over the same period (Table 3). The cowpea and 0.1 g ferulic acid treatment was also significantly different from all the remaining treatments. Total N<sub>2</sub>O emitted from all the 0.5 g polyphenol treatments and the control did not differ significantly. For tannic acid and vanillic acid, treatments with 0.1 g and 0.25 g did not differ in their N<sub>2</sub>O emission. Over the 30-day period, total N<sub>2</sub>O emitted from the sole cowpea treatment was significantly higher ( $P < 0.05$ ) than emissions from all other treatments over the same period. Among the polyphenol treatments, total N<sub>2</sub>O emitted was generally higher ( $P < 0.05$ ) from the 0.1 and 0.25 g kg<sup>-1</sup> treatments than from the corresponding 0.5 g kg<sup>-1</sup> treatments.

Total CO<sub>2</sub> emitted over 7 days from the sole cowpea, 0.1 and 0.25 ferulic acid and

vanillic acid were not similar but significantly different from the other treatments (Table 3). Total CO<sub>2</sub> emitted from all the 0.5 g kg<sup>-1</sup> polyphenol treatments were similar. Over the 30 days, total CO<sub>2</sub> emitted from the 0.1 and 0.25 g kg<sup>-1</sup> tannic acid treatments were significantly lower than from the corresponding 0.1 and 0.25 g kg<sup>-1</sup> vanillic and ferulic acid treatments. A strongly negative relationship ( $r = -0.70$ ,  $P < 0.05$ ) was observed between the polyphenol concentration and total N<sub>2</sub>O emissions over the 30 days across all the treatments (data not shown).

### *Daily N<sub>2</sub>O and CO<sub>2</sub> emissions*

Daily N<sub>2</sub>O emissions from all the treatments peaked on day 1 and decreased sharply thereafter until day 30 (Fig. 1). Peak N<sub>2</sub>O flux measured from the sole cowpea treatment on day 1 (3.2 mg N<sub>2</sub>O-N m<sup>-2</sup> day<sup>-1</sup>) was significantly higher ( $P < 0.05$ ) than peak fluxes from all the polyphenol treatments. The N<sub>2</sub>O emissions from the 0.1

TABLE 3  
Total N<sub>2</sub>O and CO<sub>2</sub> emissions over 7 and 30 days periods

Treatment	Total N <sub>2</sub> O(mg N <sub>2</sub> O-N m <sup>-2</sup> 7/30 d <sup>-1</sup> )		Total CO <sub>2</sub> (g CO <sub>2</sub> -C m <sup>-2</sup> 7/30 d <sup>-1</sup> )	
	7 d	30 d	7 d	30 d
Cowpea only	16.86 ± 0.35a	29.37 ± 0.71a	19.88 ± 1.13a	30.67 ± 1.17a
Cowpea + ferulic acid(0.1 g kg <sup>-1</sup> )	8.14 ± 0.28 b	23.0 ± 0.98b	24.97 ± 0.91a	31.6 ± 1.46a
Cowpea + ferulic acid(0.25 g kg <sup>-1</sup> )	8.58 ± 0.23c	21.08 ± 0.53bc	27.61 ± 0.56a	33.58 ± 0.43a
Cowpea + ferulic acid(0.5 g kg <sup>-1</sup> )	5.01 ± 0.15d	17.37 ± 0.28d	6.74 ± 0.5b	12.6 ± 1.08d
Cowpea + tannic acid(0.1 g kg <sup>-1</sup> )	8.92 ± 0.45 c	18.45 ± 0.67c	10.97 ± 1.7b	23.35 ± 1.8b
Cowpea + tannic acid(0.25 g kg <sup>-1</sup> )	7.61 ± 1.05c	17.41 ± 1.61bc	9.56 ± 0.29b	13.94 ± 1.2c
Cowpea + tannic acid(0.5 g kg <sup>-1</sup> )	5.04 ± 0.12d	12.47 ± 0.47d	5.52 ± 0.3b	12.64 ± 0.36c
Cowpea + vanillic acid(0.1 g kg <sup>-1</sup> )	8.75 ± 0.28c	21.07 ± 0.75b	23.51 ± 0.86a	30.54 ± 1.52a
Cowpea + vanillic acid(0.25 g kg <sup>-1</sup> )	8.09 ± 1.37c	20.54 ± 1.43bc	22.04 ± 0.34a	29.28 ± 1.07a
Cowpea + vanillic acid(0.5 g kg <sup>-1</sup> )	6.06 ± 0.18d	18.66 ± 0.47cd	6.59 ± 0.5b	12.8 ± 1.15c
control	6.43 ± 0.54d	16.48 ± 1.28bc	8.95 ± 0.68b	17.14 ± 1.62c

Same letters (superscripts) indicate significant difference at  $P < 0.05$ .

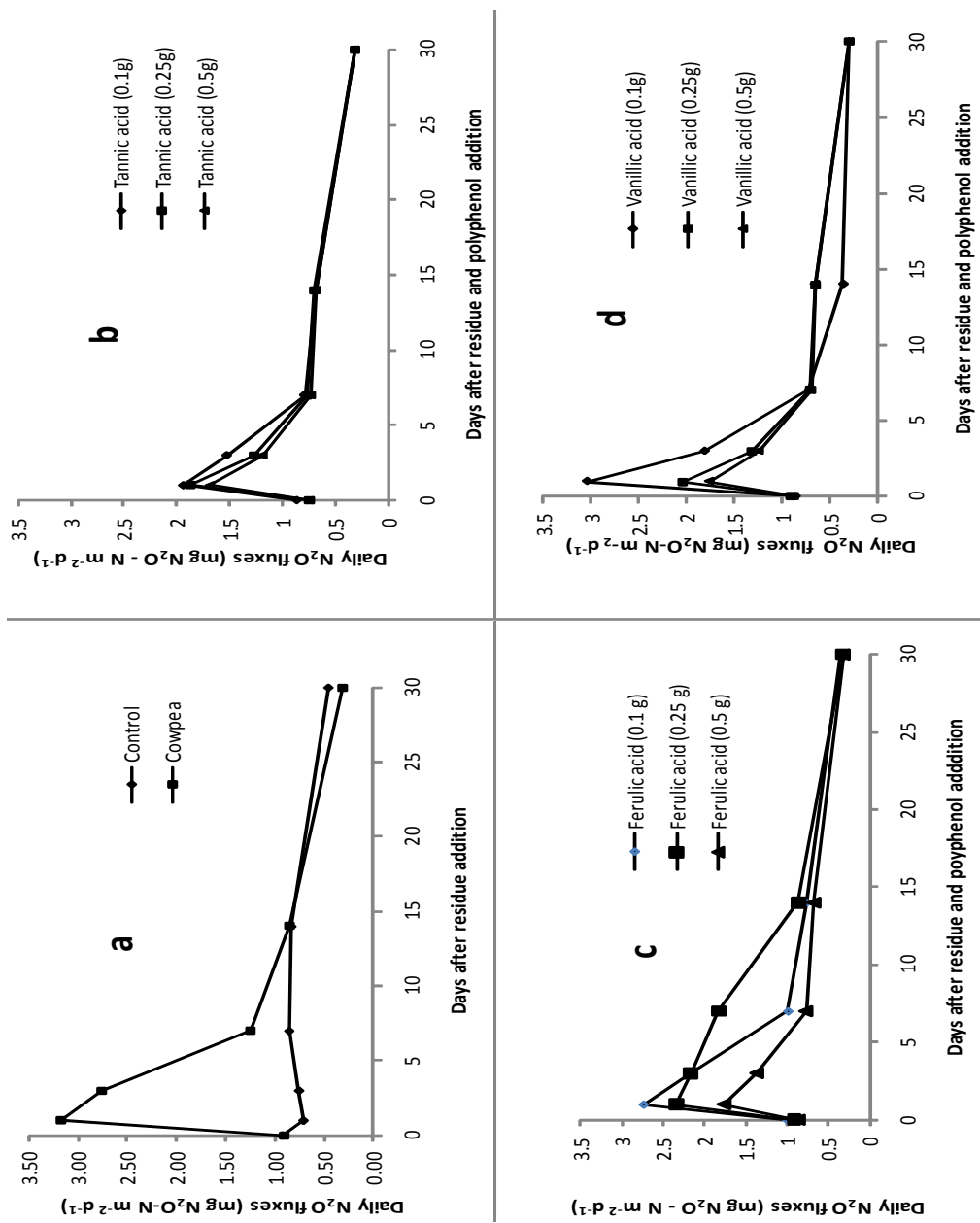


Fig. 1. Pattern of daily  $N_2O$  emissions from the amended soils

and 0.25 g kg<sup>-1</sup> soil vanillic acid and ferulic acid treatments on days 1 and 3 were higher ( $P < 0.001$ ) than emissions from their corresponding 0.5 g kg<sup>-1</sup> treatments, but N<sub>2</sub>O fluxes measured on days 14 and 30 were similar in all the treatments.

On day 0, no significant difference was found between the CO<sub>2</sub> fluxes measured from any of polyphenol treatments and the sole cowpea treatment (Fig. 2). Daily CO<sub>2</sub> fluxes peaked in almost all the treatments on day 1 and decreased thereafter till day 30, with the 0.1 and 0.25 g kg<sup>-1</sup> vanillic acid and ferulic acid treatments showing significantly higher ( $P < 0.05$ ) peaks (4.7–5.9) than the sole cowpea treatment (2.3) and the control. In contrast, daily CO<sub>2</sub> fluxes from all the tannic acid treatments were less than 2 g CO<sub>2</sub>-C m<sup>-2</sup> day<sup>-1</sup> throughout the 30 days and were significantly lower.

#### *Mineral N concentration*

Available NH<sub>4</sub><sup>+</sup> concentrations measured over the 30 days in all the polyphenol treatments were lower ( $P < 0.05$ ) than in the sole cowpea treatment (Fig. 3). The highest ( $P < 0.05$ ) NH<sub>4</sub><sup>+</sup> concentration of 25.6 mg kg<sup>-1</sup> soil was measured in the sole cowpea treatment on day 1 after residue incorporation. The available NH<sub>4</sub><sup>+</sup> concentrations in all the polyphenol treatments decreased from day 0 to day 30 but, by day 21, NH<sub>4</sub><sup>+</sup> concentrations in all the polyphenol treatments were < 2 mg N kg<sup>-1</sup> soil. The NH<sub>4</sub><sup>+</sup> concentrations in the tannic acid treatments were generally lower.

The highest ( $P < 0.05$ ) NO<sub>3</sub><sup>-</sup> concentration of 42.6 mg kg<sup>-1</sup> soil was measured on day 14 after residue addition in the sole cowpea treatment (Fig. 4). Unlike the polyphenol treatments, the sole cowpea treatment showed steep increase in NO<sub>3</sub><sup>-</sup> concentration from day 0 to a peak on day 14. The NO<sub>3</sub><sup>-</sup>

concentration in the sole cowpea was significantly higher ( $P < 0.05$ ) than the polyphenol treatments. While the NO<sub>3</sub><sup>-</sup> concentrations increased gradually from day 1 to day 30 for the ferulic acid treatments and from day 3 to day 30 for vanillic acid treatments, intra- and inter-treatment differences were not significant. However, both were higher than the tannic acid treatments which were similar throughout the 30 day period.

#### *Relationships between mineral N concentrations and N<sub>2</sub>O and CO<sub>2</sub> emissions*

Over the 30 days, positive and significant correlations were found between log N<sub>2</sub>O emissions and mineral N concentrations (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and available N) for ferulic and vanillic acid treatments (Table 4). However, NO<sub>3</sub><sup>-</sup> concentration was not significantly correlated with tannic acid treatment. CO<sub>2</sub> emission was positively and significantly correlated with mineral N concentrations only in the vanillic acid treatments.

#### *Microbial biomass concentration*

The MBC in all the amended soils peaked on day 1 and decreased thereafter till day 30 (Fig. 5). On day 1, the MBC of the 0.1 and 0.25 g ferulic and vanillic acid treatments were significantly higher ( $P < 0.05$ ) than in all other treatments. Among the polyphenol treatments MBC was higher ( $P < 0.05$ ) in the 0.1 and 0.25 g kg<sup>-1</sup> vanillic, tannic and ferulic acid treatments than their corresponding 0.5 g kg<sup>-1</sup> treatments. The trend was a rapidly decreasing MBC in the polyphenol treatments with time such that by day 14, all the MBC in all the polyphenol treatments were not different but lower than the MBC of the sole cowpea treatment. By day 21, MBC had declined by up to 78% in

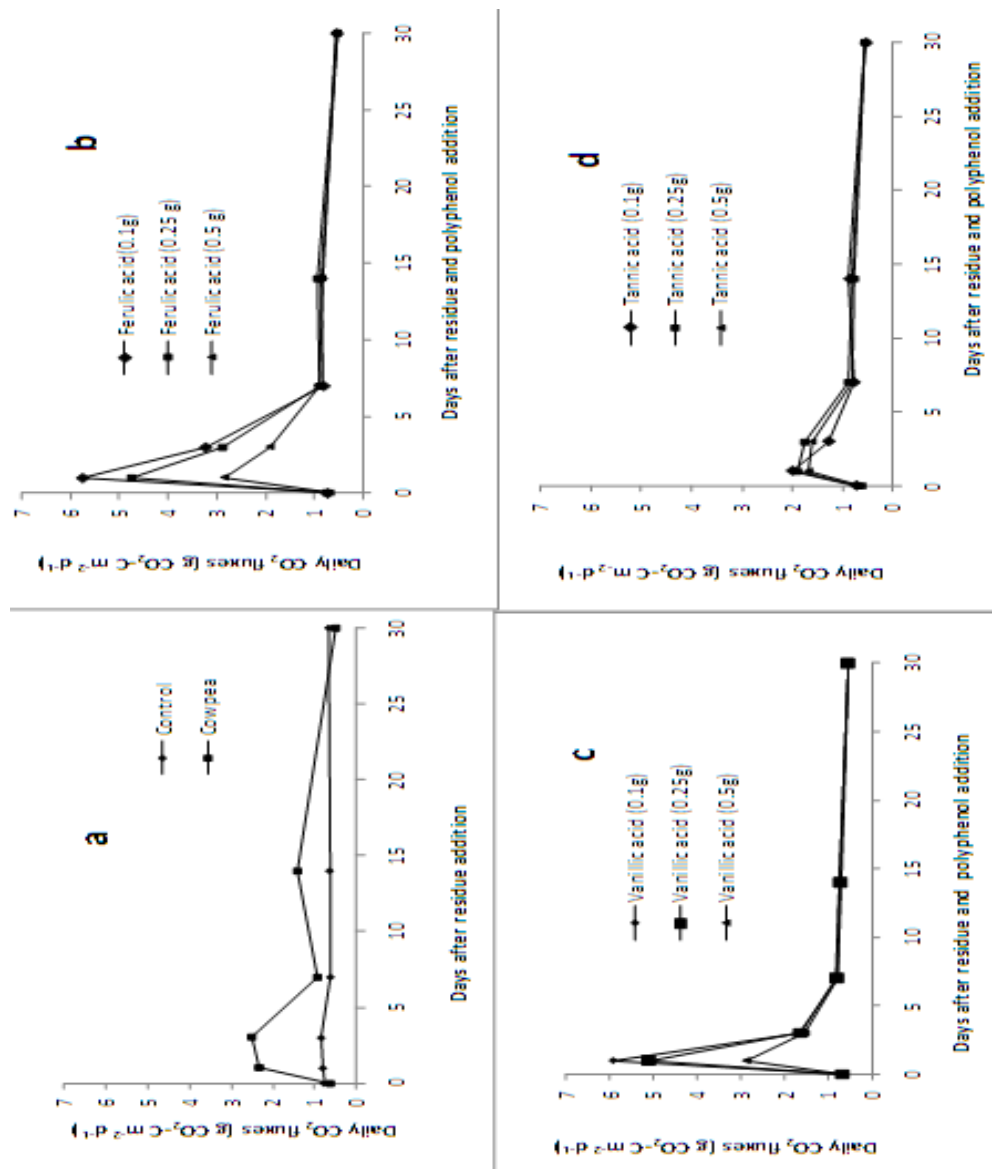


Fig. 2 Pattern of daily CO<sub>2</sub> emissions from the amended soils



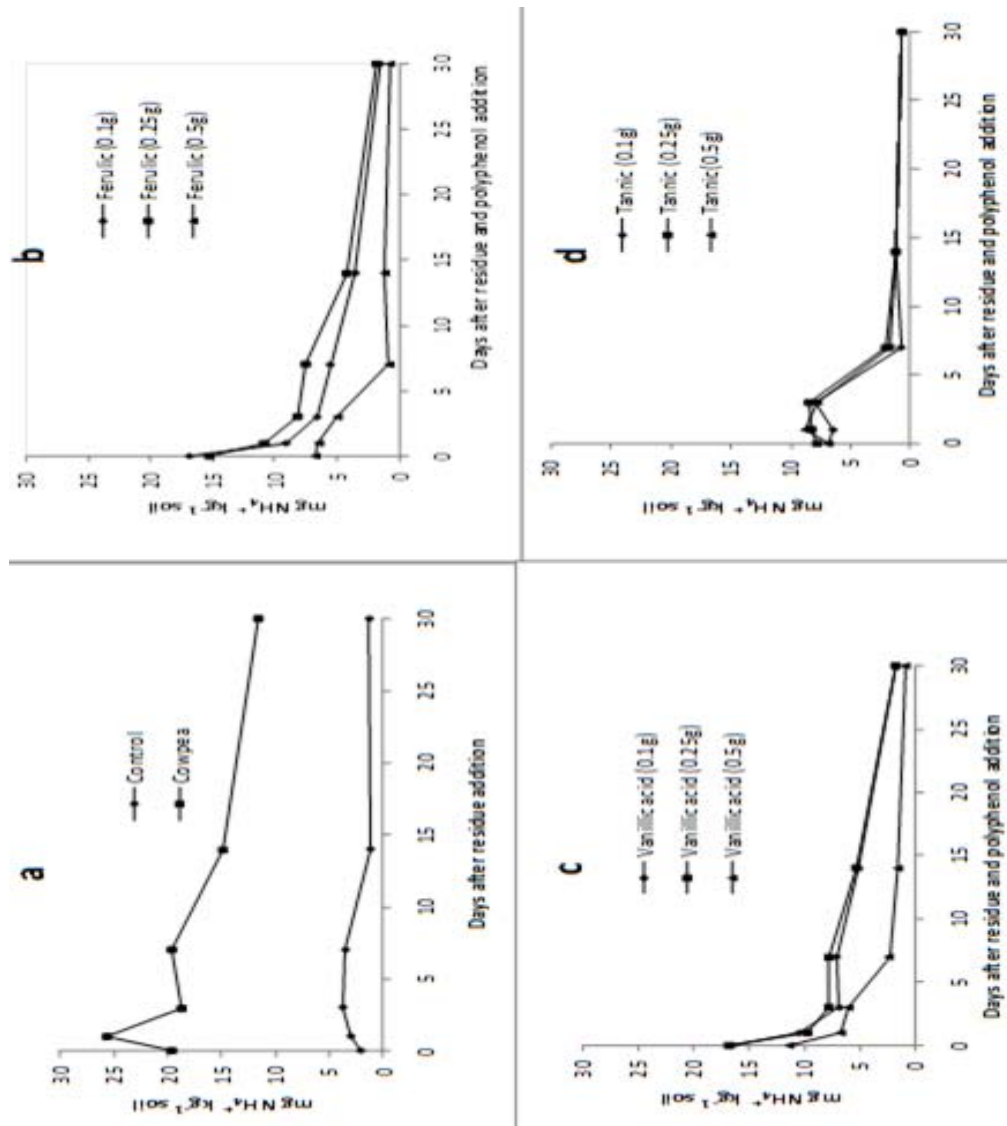


Fig. 3.  $\text{NH}_4^+$  concentration in the amended soils

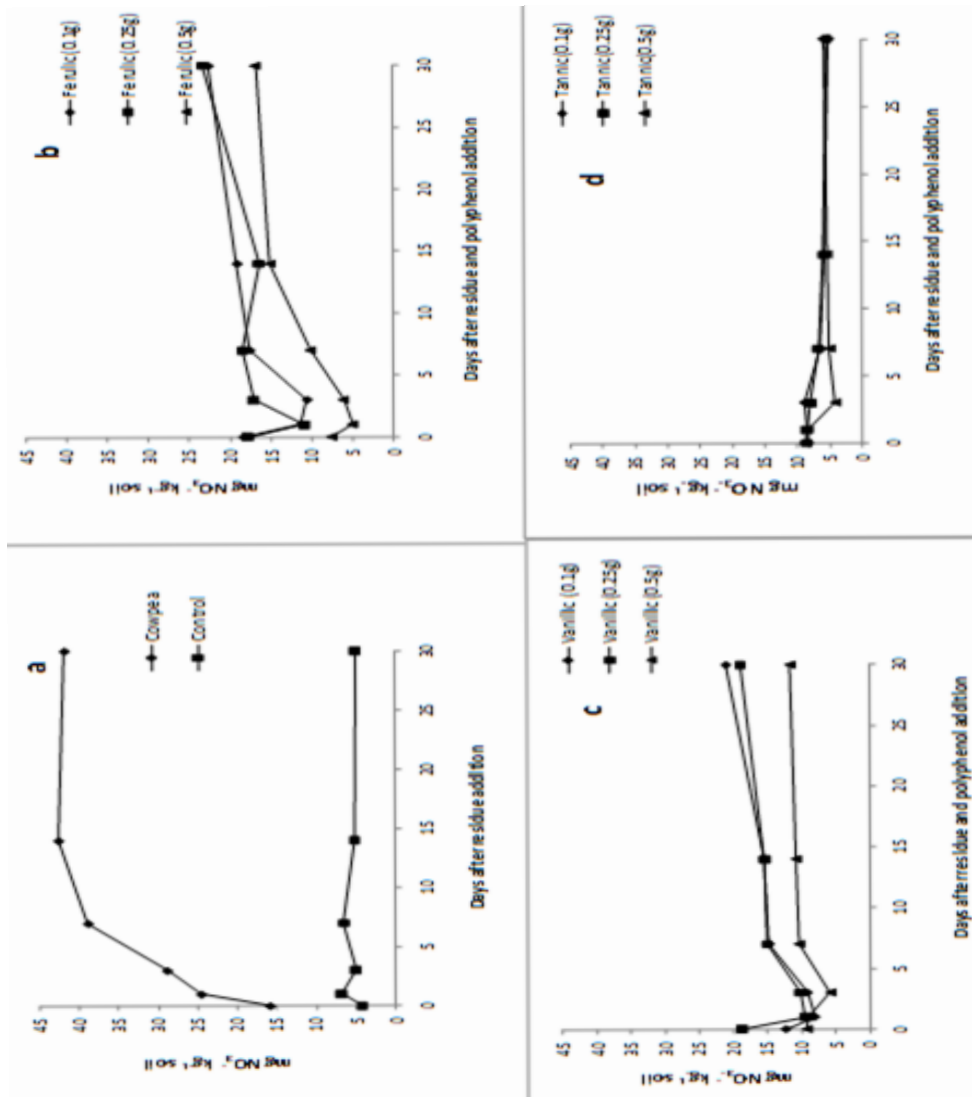


Fig. 4.  $\text{NO}_3^-$  concentration in the amended soils

TABLE 4  
Relationships between mineral N concentrations and N<sub>2</sub>O and CO<sub>2</sub> emissions

Polyphenol	Mineral N concentrations (mg kg <sup>-1</sup> soil)		
	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	Available N (NH <sub>4</sub> <sup>+</sup> + NO <sub>3</sub> <sup>-</sup> )
<i>Ferulic acid</i>			
log N <sub>2</sub> O	0.6 *	0.45 *	0.75 **
CO <sub>2</sub>	0.45	0.39	0.6
<i>Vanillic acid</i>			
log N <sub>2</sub> O	0.75 **	0.54 *	0.74 **
CO <sub>2</sub>	0.57 *	0.52 *	0.68 **
<i>Tannic acid</i>			
log N <sub>2</sub> O	0.61 *	0.13 ns	0.58 *
CO <sub>2</sub>	0.19 ns	0.17 ns	0.08 ns

\* represents significance at 0.05; \*\* represents significance at 0.01

the 0.1 and 0.25 g kg<sup>-1</sup> ferulic and vanillic acid treatments.

Pooling the results from all the treatments together, a strong positive relationship was found between MBC and both N<sub>2</sub>O ( $r = 0.80$ ;  $P < 0.001$ ) and CO<sub>2</sub> ( $r = 0.86$ ,  $P < 0.001$ ) measured on day 1 (Fig. 6).

### Discussion

#### *Influence of polyphenol type on N<sub>2</sub>O emission and N mineralization*

The N<sub>2</sub>O emissions were lower ( $P < 0.05$ ) in the polyphenol treatments compared with the sole cowpea treatment (Table 3). This could be attributed to a lowered inorganic N availability for N<sub>2</sub>O production via nitrification and denitrification because by day 30 inorganic N (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) concentrations in the polyphenol treatments were significantly lower ( $P < 0.05$ ) than in the sole cowpea treatment. In the low molecular weight ferulic and vanillic acid treatments, NO<sub>3</sub><sup>-</sup> concentration, correlated negatively with the polyphenol concentrations ( $r = -0.45$  and  $0.54$ ;  $P < 0.05$ , respectively), indicative that an increasing polyphenol

concentration was associated with a decreasing NO<sub>3</sub><sup>-</sup> concentration. This is in agreement with previous observations that polyphenols form recalcitrant protein-polyphenol complexes (Mutabaruka *et al.*, 2007; Zibliske & Bradford, 2007) and, or inhibit microbial enzymes activity (Mole & Waterman, 1986). Handayanto (1997) noted that all these processes inhibit N mineralisation and, subsequently, limit NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> supply for the nitrification and denitrification processes that are mainly responsible for soil N<sub>2</sub>O production (Millar & Baggs, 2004). In agreement with findings from this study, Gamba *et al.* (2005) found a negative correlation between polyphenol content and soil NO<sub>3</sub><sup>-</sup> concentrations after the addition of 0, 80 and 160 kg ha<sup>-1</sup> of high polyphenol olive oil waste. Similarly, Millar & Baggs (2004) reported a negative correlation between N<sub>2</sub>O fluxes and both NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in a Kenyan oxisol amended with high polyphenol residues from some tropical agroforestry species (*Calliandra calothyrsus*, *Sesbania sesban*, *Macroptillum atropurpureum* and *Crotalaria grahamiana*).

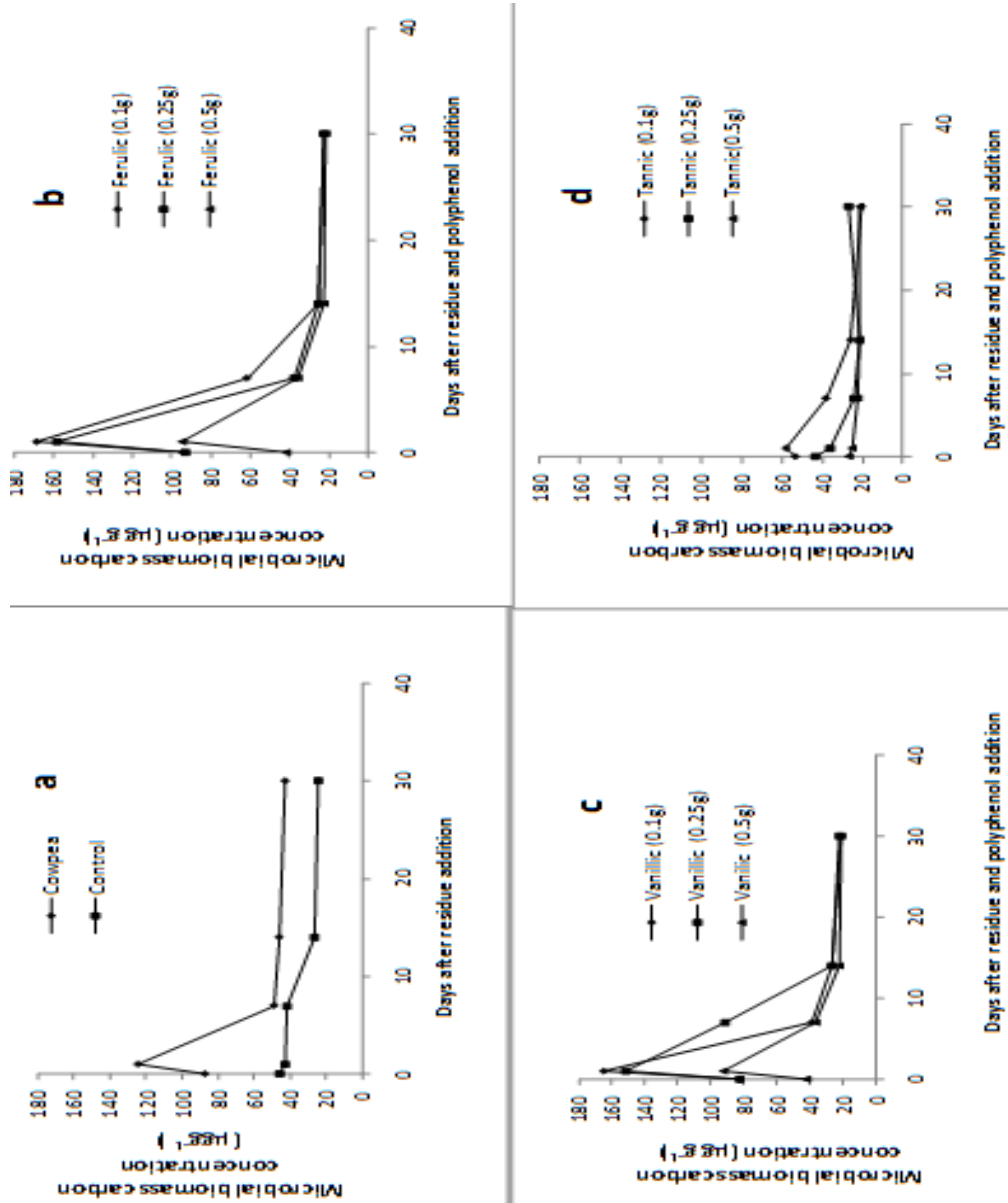


Fig. 5. Microbial biomass carbon in the amended soils

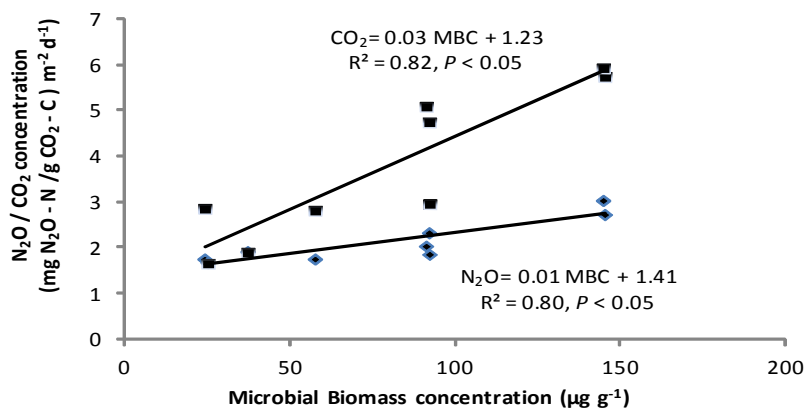


Fig. 6. Relationship between MBC and N<sub>2</sub>O and CO<sub>2</sub>

Over the 30 days, available NH<sub>4</sub><sup>+</sup> concentrations in all the polyphenol treatments were lower ( $P < 0.05$ ) than in the sole cowpea treatment. The declining NH<sub>4</sub><sup>+</sup> concentration in the sole cowpea treatment was associated with increasing NO<sub>3</sub><sup>-</sup> concentration, indicative of net N nitrification, but the decreasing NH<sub>4</sub><sup>+</sup> concentration in the polyphenol treatments did not translate into increases in NO<sub>3</sub><sup>-</sup> concentration. This observation further confirms the potential of the added polyphenol compounds to lower N mineralisation and lower inorganic N availability for N<sub>2</sub>O production (Schimel *et al.*, 1996; Kawamoto *et al.*, 1996).

Previous authors have attributed the effect of polyphenol compounds on N availability to different reactions. For instance, Joshua *et al.* (1998) and Cowan (1999) have all reported that polyphenols decreased N mineralisation of added residue by inhibiting microbial activity. In contrast, Indejit & Mallik (1997) concluded that polyphenol compounds released from *K. angustifolia* residues rather served as microbial C sources, leading to enhanced microbial activity and

net N immobilisation. This view was supported by Kraus *et al.* (2004), who further argued that polyphenols comprise a substantial pool of carbon which can be used by heterotrophic soil microorganisms leading to increased microbial activity and temporary immobilisation of N in microbial biomass.

Apparently, results from the ferulic and vanillic acid treatments were consistent with the previous reports by Indejit & Malik (1997) and Kraus *et al.* (2004), who attributed lower N<sub>2</sub>O emissions from polyphenol amended soils to net N immobilisation in that MBC measured in the 0.1 and 0.25 g ferulic and vanillic acid treatments on day 1 were significantly higher ( $P < 0.05$ ) than in the tannic acid treatments. This suggests that at lower rates of application (0.1 and 0.25 g kg<sup>-1</sup> soil) the low molecular weight vanillic and ferulic acids provided soluble C sources directly to the denitrifiers. At a higher application rate of 0.5 g kg<sup>-1</sup> soil, the effect of the low molecular weight polyphenols on MBC was not evident. The reason for this observation was not clear but it could be partly attributed to a lowering of the soil pH (data not shown),

which might have lowered microbial activity. Increased soluble C supply would have increased oxygen consumption through the stimulation of microbial activity, thereby, creating sub-oxic conditions for denitrification (Tiedje *et al.*, 1984). However, in this study the increased CO<sub>2</sub> and N<sub>2</sub>O fluxes from the 0.1 and 0.25 g kg<sup>-1</sup> ferulic and vanillic acid treatments were short-lived, indicating that the soluble C supply was depleted quickly following the rapid increase in microbial growth.

Tannic acid is a member of the gallotanins family of compounds having a number of gallic moieties connected to glucose molecule by ester linkages (Siebert, 1999), but ferulic acid and vanillic acid do not have the galloyl moiety in their structure (Andjelkovic *et al.*, 2005). Therefore, in accordance with previous studies, it is likely that the low molecular weight ferulic and vanillic acids decreased N<sub>2</sub>O production through N immobilisation (Fierer *et al.*, 2001; Hättenschwiler & Vitousek, 2000; Kraus *et al.*, 2004), while the more complex tannic acid decreased N availability and N<sub>2</sub>O production through protein binding (Chavez *et al.*, 2005; De Neve *et al.*, 2004).

Fierer *et al.* (2001) and Hättenschwiler & Vitousek (2000) reported that low molecular weight polyphenol can be easily degraded by microbes, thereby, contributing to increases in N immobilisation through the supply of soluble C, whilst more complex polyphenols such as condensed tannins slow decomposition and N mineralisation by forming complexes with proteins, including cellular enzymes in the soil. Thus, the low NH<sub>4</sub><sup>+</sup> concentration measured in the ferulic acid and vanillic acid amended treatments, might be an indication of N immobilisation through temporary increase in microbial

activity following degradation of the polyphenol (Sugai & Schimel, 1993), and that the low NO<sub>3</sub><sup>-</sup> concentration in these treatments could be due to decrease in nitrification following NH<sub>4</sub><sup>+</sup> substrate depletion through immobilisation. In contrast, Castells (2004) concluded that low NO<sub>3</sub><sup>-</sup> concentration in polyphenol amended soils was due to the inhibition of the nitrifying bacteria.

The rapid decrease in inorganic N concentration in the polyphenol treatments was associated with temporary increase in MBC between days 0 and 1, in the ferulic and vanillic acid treatments. This was in good agreement with Sugai & Schimel (1993), who found that 90% of the polyphenols (hydroxybenzoic acid and salicylic acid) released into a mineral soil were metabolised within 4 h, indicative of rapid microbial degradation of low molecular weight polyphenol compounds.

#### *Influence of polyphenol concentrations*

Total N<sub>2</sub>O emitted from the 0.1 and 0.25 g kg<sup>-1</sup> of each polyphenol treatment was significantly higher ( $P < 0.05$ ) than from their corresponding 0.5 g kg<sup>-1</sup> treatments, but there was no significant difference between the total N<sub>2</sub>O emitted from the 0.1 g kg<sup>-1</sup> and 0.25 g kg<sup>-1</sup> ferulic and vanillic acid treatments. In comparison with the sole cowpea treatment, the vanillic acid and ferulic acid (0.1 and 0.25 g kg<sup>-1</sup>) treatments decreased N<sub>2</sub>O emitted over the 30 days period by up to 41% whilst their corresponding 0.5 g kg<sup>-1</sup> treatments decreased N<sub>2</sub>O emission by up to 58%. This indicates that the effect of polyphenols to lower inorganic N concentration depended not only on their chemical structure (Hagerman, 2002) but also on the concentration of the polyphenol substance

incorporated (Kraus *et al.*, 2004). These results suggest that at a higher concentration of 0.5 g kg<sup>-1</sup>, the polyphenols, particularly the tannic acid, possibly provided more protein binding sites, thereby, lowering N substrate availability for N<sub>2</sub>O production more than when it was incorporated at 0.1 or 0.25 g kg<sup>-1</sup>. Talbot & Finzi, (2008) reported that the addition of humic and tannic acids at 200–4000 mg kg<sup>-1</sup> decreased protein turnover in soils, but Jan *et al.* (2009) found no significant effect of polyphenol on protein turnover in grassland soils after incorporation of 5 mg kg<sup>-1</sup> of humic and tannic acid. The highest concentration in this experiment, (0.5 g kg<sup>-1</sup>) is within the concentration range applied by Talbot & Finzi (2008).

A negative relationship ( $R^2 = -0.50$ ,  $P < 0.05$ ) was observed between the total N<sub>2</sub>O emitted over the 30 days and the concentrations of the polyphenol compounds applied. This observation was consistent with previous observations by Baggs *et al.* (2001) and Millar & Baggs (2004) that, under controlled environment, incorporation of high polyphenol residue with high protein binding capacity may result in temporary immobilisation of N. The novelty of the results from the current study, however, is that the effect of different polyphenol compounds on N<sub>2</sub>O appeared to be due to different mechanisms, which are dependent on their molecular weight and the complexity of their chemical structure. CO<sub>2</sub> fluxes from the 0.1 and 0.25 g kg<sup>-1</sup> vanillic and ferulic acid treatments were higher than from the 0.5 g kg<sup>-1</sup> treatments during days 0 and 1, but the increased CO<sub>2</sub> fluxes were short-lived such that up to 87% of the total CO<sub>2</sub> emitted over the 21 days was lost by day 7. Furthermore, MBC in the vanillic acid and

ferulic acid (0.1 and 0.25 g kg<sup>-1</sup>) treatments were significantly higher ( $P < 0.05$ ) than in the corresponding 0.5 g kg<sup>-1</sup> treatment on day 1, and pooling results from all the treatments together, a strong positive relationship was found between MBC and both N<sub>2</sub>O ( $R^2 = 0.80$ ;  $P < 0.001$ ) and CO<sub>2</sub> ( $R^2 = 0.82$   $P < 0.001$ ) measured on day 1. This observation supports reports by Garcia-Montiel *et al.* (2001) that higher CO<sub>2</sub> emissions indicate greater microbial activity, which is likely to favour the creation of anaerobic conditions favourable for N<sub>2</sub>O production.

### Conclusion

The study demonstrated that application of polyphenol compounds decreased soil mineral N concentration and N<sub>2</sub>O emissions, but the decreases in N<sub>2</sub>O emissions were as a result of different mechanisms dependent on the type of polyphenol compound added to the soil. The study showed that the higher molecular weight tannic acid lowered N mineralization and N<sub>2</sub>O production through protein binding while the lower molecular weight ferulic and vanillic acids decreased N<sub>2</sub>O production through N immobilisation by stimulating microbial activity. However, the extent to which the polyphenol compounds decreased mineral N concentration and N<sub>2</sub>O emission were variable and was dependent not only on the type but also on the concentration of polyphenol added to the soil. Thus, incorporation of the polyphenols at rates of 0.5 g kg<sup>-1</sup> soil resulted in a greater decline in mineral N concentration and N<sub>2</sub>O emissions than when they were applied at lower rates. It could then be concluded that the addition of polyphenols resulted in lower inorganic N concentration and N<sub>2</sub>O emission from soils

amended with cowpea residue, but the magnitude of N<sub>2</sub>O emission reduction was dependent on the type and concentration of the polyphenol added.

#### References

- Abdalla M., Jones M., Ambus P. and Williams M.** (2010). Emissions of nitrous oxide from Irish arable soils: effects of tillage and reduced N input. *Nutrient Cycling in Agroecosystems* **86**: 53-65.
- Anderson J. P. E. and Domsch K. H.** (1978). A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biology and Biochemistry* **10**: 215-221.
- Anderson J. M. and Ingram J. S. I.** (eds) (1993). *Tropical Soil Biology and Fertility: a Handbook of Methods*, 2nd edn. CAB International, Wallingford, UK.
- Andjelkovic M., Van Camp J., De Meulenaer B., Depaemelase G., Socacici C., Verloo M. and Verle R.** (2005). Iron chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chemistry* **98**(1): 23-31.
- Baggs E. M., Millar N., Ndufa J. K. and Cadisch G.** (2001). Effect of residue quality on N<sub>2</sub>O emissions from tropical soils. In *Sustainable Management of Soil Organic Matter*. (R. M. Rees, B. C. Ball, C. D. Campbell and C. A. Watson, eds), pp.120-125. CAB International, Edinburgh.
- Castells E., Pañuelas J. and Walentine D. W.** (2004). Are phenolic compounds released from the Mediterranean shrub *Cistus albidus* responsible for changes in N cycling in siliceous and calcareous soils? *New Phytologist* **162**: 187-195.
- Chavez B., De Neve S., Del Carmen M., Cabrera L., Boeckx P., Van Cleemput O. and Hofman G.** (2005). The effect of mixing organic biological waste materials and high-N crop residues on the short-time N<sub>2</sub>O emission from horticultural soil in model experiments. *Biology and Fertility of Soils* **41**: 411-418.
- Constantinides M. and Fownes J. H.** (1994). Nitrogen mineralisation from leaves and litter of tropical plants: relationship to nitrogen, lignin and soluble polyphenol concentrations. *Soil Biology and Biochemistry* **26**: 49-55.
- Cowan M. M.** (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12**: 564-582.
- De Neve S., Saez G. S., Daguiar B. C., Sleyel S. and Hofman G.** (2004). Manipulating N mineralisation from high N crop residues using on and off-farm organic materials. *Soil Biology and Biochemistry* **36**: 127-134.
- FAO** (1998). World Reference Base for soil resources No. 84. *World Soil Resources Report*. FAO, Rome, Italy.
- Fierer N., Schimel J. P., Cates R. G. and Zou J.** (2001). Influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. *Soil Biology and Biochemistry* **33**: 1231-1243.
- Frimpong K. A. and Baggs E. M.** (2010). Does combined application of crop residues and inorganic N fertiliser decrease N<sub>2</sub>O emissions? *Soil Use and Management*. Doi 10.1111/j.1457-2743.2010.00293.
- Gamba C., Piovanelli C., Papini R., Pezzarora B. and Ceccarini L.** (2005). Microbial characteristics and mineral N availability as affected by olive oil waste water applied to cultivated soil. *Communications in Soil Science and Plant Analysis* **35**: 937-950.
- Garcia-Montiel D. C., Stuedler P. A., Piccolo M., Melilo J., Neill C. and Cerri C. C.** (2001). Controls on soil nitrogen oxide emission from forest and pastures in the Brazilian Amazon. *Global Biogeochemical Cycles* **4**: 1021-1030.
- Hagerman A. E.** (2002). *The Tannin Handbook*. <http://www.users.muohio.edu/hagermae> (accessed 15th April 2010).
- Handayanto E., Cadisch G. and Giller K. E.** (1997). Regulating N mineralisation from plant residues by manipulating quality. In *Driven by Nature: Plant Litter Quality and Decomposition*. (G. Cadisch, K. E. Giller, eds), pp. 175-185. CAB International, Wallingford, UK.
- Haslam E.** (1989). *Practical Polyphenols: From structure to Molecular Recognition and Physiological Action*. Cambridge University Press, Cambridge.
- Hättenschwiler S. and Vitousek P. M.** (2000). The role of polyphenol in terrestrial ecosystem nutrient cycling. *Tree* **15**: 238-243.
- Indejit S. and Malik A. U.** (1997). Effect of phenolic compounds on selected soil properties. *Forest Ecology and Management* **92**: 11-18.



- Jan M. T., Roberts P., Tonheim S. K. and Jones D. L.** (2009). Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. *Soil Biology and Biochemistry* **41**: 2272–2282.
- Joshua P. S., Rex G. C. and Roger R.** (1998). The role of balsam poplar secondary chemicals in controlling soil nutrient dynamics through succession in the Alaskan taiga. *Biogeochemistry* **42**: 221.
- Kawamoto H., Nakatsubo F. and Murakami K.** (1996). Stoichiometric studies of tannin-protein coprecipitation. *Phytochemistry* **41**: 1427–1431.
- Kirk T. K.** (1971). Effects of microorganisms on lignin. *Annual Reviews in Phytopathology* **9**: 182–210.
- Kraus T. E. C., Zasoski R. J. and Dahlgren R. A.** (2004). Fertility and pH effects on polyphenol and condensed tannin concentrations in foliage and roots. *Plant and Soil* **262**: 95–109.
- Lopez-Amoros M. L., Hernandez T. and Estrella I.** (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition Analysis* **19**: 277–283.
- Mafongoya P. L., Giller K. E. and Palm C. A.** (1998). Decomposition and nitrogen release patterns of tree prunings and litter. *Agroforestry Systems* **38**: 77–97.
- Martin J. P. and Haider K.** (1980). Lignin Biodegradation. In *Microbiology, Chemistry and Potential Applications* (T. K. Kirk, T. Higuichi, H. Chang, eds), pp. 77–100. Vol. I. CRC Press, Michigan, USA.
- Millar N. and Baggs E. M.** (2004). The chemical composition or quality of agroforestry residues influences N<sub>2</sub>O emissions after their addition to soils. *Soil Biology & Biochemistry* **36**: 935–943.
- Mole S. and Waterman P. G.** (1986). Tannic acid and proteolytic enzymes. Enzyme inhibition or substrate deprivation? *Phytochemistry* **26**: 99–102.
- Moorhead G., Douglas P., Morrice N., Scarabel M., Aitken A. and Markintosh C.** (1996). Phosphorylated nitrate reductase from spinach leaves is inhibited by 14-3-3 proteins and activated by fusicoccin. *Current Biology* **6**: 1104–1113.
- Mutabaruka R., Hairah K. and Cadisch G.** (2007). Microbial degradation of hydrolysable and condensed tannin polyphenols in protein complexes in soil from different land use histories. *Soil Biology & Biochemistry* **39**: 1479–1472.
- Myers R. K. J., Palm C. C., Cueva E., Gunatileke I. U. N. and Brossard M.** (1994). The synchronisation of nutrient mineralisation and plant nutrient demand. In *The Biological Management of Tropical Soil Fertility*. (P. L. Woome, Swift M. J., eds), pp. 81–116. Wiley, Chichester, UK.
- Palm C. A. and Sanchez P. A.** (1991). Nitrogen release from leaves of some tropical legumes as affected by their lignin and polyphenolic contents. *Soil Biology & Biochemistry* **23**: 83–88.
- Rahn C. R., Bending G. D., Turner M. K. and Lillywhite R. D.** (2003). Management of N mineralization from crop residues of high N content using amendment materials of varying quality. *Soil Use and Management* **19**: 193–200.
- Schimel J. P., Van Cleve K., Cates R. G., Clausen T. P. and Reichardt P. B.** (1996). Effects of balsam poplar v (*Populus balsamifera*) tannins and low molecular phenols on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. *Canadian Journal of Botany* **74**: 84–90.
- Siebert K. J.** (1999). Effect of protein-polyphenol interactions on beverage haze stabilisation and analysis. *Journal of Agriculture and Food Chemistry* **47**(2): 353–362.
- Sugai S. F. and Schimel J. P.** (1993). Decomposition and biomass incorporation of <sup>14</sup>C-labeled glucose and phenolics in taiga forest-floor: effect of substrate quality, successional state and season. *Soil Biology and Biochemistry* **25**: 1379–1389.
- Talbot J. M. and Finzi A. C.** (2008). Differential effects of sugar maple, reed oak and humlock tannins on carbon and nitrogen cycling in temperate forest soil. *Oecologia*, **155**: 583–592.
- Tiedje J. M., Sextone A. J., Parkin T. B., Revbec N. P. and Shelton D. R.** (1984). Anaerobic processes in soils. *Plant and Soil* **76**: 117–212.
- Vance E. D., Brookes P.C. and Jenkinson D. S.** (1987). An extraction method for measuring soil microbial biomass. *Soil Biology and Biochemistry* **19**: 703–707.
- Zibliske L. M. and Bradford J. M.** (2007). Oxygen effects on carbon, polyphenol and nitrogen mineralisation potential in soil. *Soil Science Society of America Journal* **71**: 133–139.