Nitrous Oxide Emissions from Soils Amended with Polyphenols and Cowpea Residues

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Abstract

Polyphenols can influence the rate of N_2O 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_2O emissions and inorganic N dynamics in a tropical soil amended with cowpea residue. The results show that N_2O emission and mineral N concentrations in the sole cowpea amended soils were significantly higher than in all the polyphenol treatments. Decrease in N_2O 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_2O emissions and N concentrations even though tannic acid showed the highest numerical decrease. The tannic acid lowered N mineralisation and N_2O production through protein binding while ferulic and vanillic acids decreased N_2O 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_2O 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

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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_2O) 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_2O emitted from these high N residues amended soils were negatively correlated with residue polyphenol:N ratio and lignin + polyphenol:N content. Moreover, the ^{15}N-N_2O emission, which indicates the contribution of residue-^{15}N to measured N_2O 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_2O emissions from agricultural soils (Abdalla *et al.,* 2010). The effect of polyphenols on N_2O 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_2O 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_2O 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 *p*H (H_2O) 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-Ciolcateau 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

Biochemical characteristics	*Value*
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^{-1} 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^{-1} 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_2O and CO_2 analysis

Gas samples for N_2O and CO_2 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_2O concentration was done with a Perkin Elmer autosystem gas chromatograph fitted with an electron capture detector (ECD). CO_2 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. Linerality 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

Common name	*Chemical name*	*Formula mass*	*Source*
Ferulic acid	Trans-4-hydroxymethoxycinnamic acid($C_{10}H_{10}O_4$)	194.14	Aldrich, UK
Vanillic acid	4-hydroxy-3-methoxybenzoic acid($C_8H_8O_4$)	168.15	Aldrich, UK
Tannic acid	Penta-O-galloyl- D-glucose($C_7H_{52}O_{48}$)	1701.23	Aldrich, UK

calculated from a single determination at the end of the closure. Total N_2O and CO_2 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_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. Soil *p*H was analysed on a 1:5 soil:H_2O ratio on days 0, 1, 3, 7, 14 and 21 after incubation using a *p*H 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_2SO_4) 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:

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

where

$$Extracted\ C = TOC_{fumigated} - TOC_{non\text{-}fumigated}$$

$TOC_{fumigated}$ = Total organic carbon concentration from fumigated soil

$TOC_{non\text{-}fumigated}$ = 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_2O and mineral N concentrations, and to determine relationships between total N_2O and total CO_2 emissions, and concentrations of the polyphenols added.

Results

N_2O and CO_2 emissions

Total N_2O 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_2O 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_2O emission. Over the 30-day period, total N_2O 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_2O emitted was generally higher ($P < 0.05$) from the 0.1 and 0.25 g kg^{-1} treatments than from the corresponding 0.5 g kg^{-1} treatments.

Total CO_2 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_2 emitted from all the 0.5 g kg^{-1} polyphenol treatments were similar. Over the 30 days, total CO_2 emitted from the 0.1 and 0.25 g kg^{-1} tannic acid treatments were significantly lower than from the corresponding 0.1 and 0.25 g kg^{-1} vanillic and ferulic acid treatments. A strongly negative relationship ($r = -0.70$, $P < 0.05$) was observed between the polyphenol concentration and total N_2O emissions over the 30 days across all the treatments (data not shown).

Daily N_2O and CO_2 emissions

Daily N_2O emissions from all the treatments peaked on day 1 and decreased sharply thereafter until day 30 (Fig. 1). Peak N_2O flux measured from the sole cowpea treatment on day 1 (3.2 mg N_2O-N m^{-2} day^{-1}) was significantly higher ($P < 0.05$) than peak fluxes from all the polyphenol treatments. The N_2O emissions from the 0.1

TABLE 3
Total N_2O and CO_2 emissions over 7 and 30 days periods

Treatment	*Total N_2O(mg N_2O-N m^{-2} 7/30 d^{-1})*		*Total CO_2(g CO_2-C m^{-2}7/30 d^{-1})*	
	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(0.1g kg^{-1})	8.14 ± 0.28 b	23.0 ± 0.98b	24.97 ± 0.91a	31.6 ± 1.46a
Cowpea + ferulic acid(0.25 g kg^{-1})	8.58 ± 0.23c	21.08 ± 0.53bc	27.61 ± 0.56a	33.58 ± 0.43a
Cowpea + ferulic acid(0.5 g kg^{-1})	5.01 ± 0.15d	17.37 ± 0.28d	6.74 ± 0.5b	12.6 ± 1.08d
Cowpea + tannic acid(0.1g kg^{-1})	8.92 ± 0.45 c	18.45 ± 0.67c	10.97 ± 1.7b	23.35 ± 1.8b
Cowpea + tannic acid(0.25 g kg^{-1})	7.61 ± 1.05c	17.41 ± 1.61bc	9.56 ± 0.29b	13.94 ± 1.2c
Cowpea + tannic acid(0.5 g kg^{-1})	5.04 ± 0.12d	12.47 ± 0.47d	5.52 ± 0.3b	12.64 ± 0.36c
Cowpea + vanillic acid(0.1 g kg^{-1})	8.75 ± 0.28c	21.07 ± 0.75b	23.51 ± 0.86a	30.54 ± 1.52a
Cowpea + vanillic acid(0.25 g kg^{-1})	8.09 ± 1.37c	20.54 ± 1.43bc	22.04 ± 0.34a	29.28 ± 1.07a
Cowpea + vanillic acid(0.5 g kg^{-1})	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 $P < 0.05$.

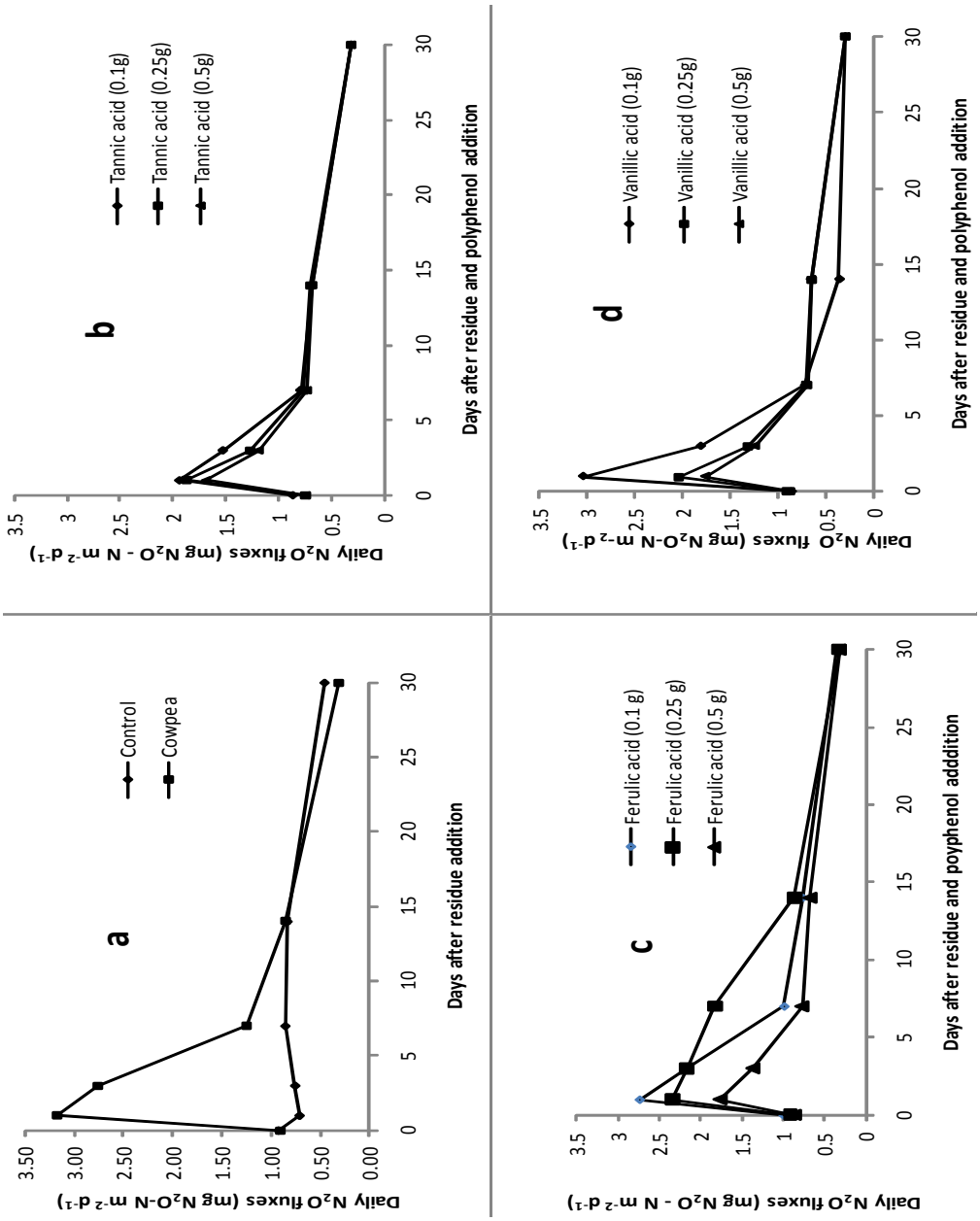

Fig. 1. Pattern of daily N_2O emissions from the amended soils

and 0.25 g kg^{-1} 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^{-1} treatments, but N_2O fluxes measured on days 14 and 30 were similar in all the treatments.

On day 0, no significant difference was found between the CO_2 fluxes measured from any of polyphenol treatments and the sole cowpea treatment (Fig. 2). Daily CO_2 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^{-1} 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_2 fluxes from all the tannic acid treatments were less than 2 g CO_2- C m^{-2} day^{-1} throughout the 30 days and were significantly lower.

Mineral N concentration

Available NH_4^+ 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_4^+ concentration of 25.6 mg kg^{-1} soil was measured in the sole cowpea treatment on day 1 after residue incorporation. The available NH_4^+ concentrations in all the polyphenol treatments decreased from day 0 to day 30 but, by day 21, NH_4^+ concentrations in all the polyphenol treatments were < 2 mg N kg^{-1} soil. The NH_4^+ concentrations in the tannic acid treatments were generally lower.

The highest ($P < 0.05$) NO_3^- concentration of 42.6 mg kg^{-1} 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_3^- concentration from day 0 to a peak on day 14. The NO_3^- concentration in the sole cowpea was significantly higher ($P < 0.05$) than the polyphenol treatments. While the NO_3^- 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_2O and CO_2 emissions

Over the 30 days, positive and significant correlations were found between log N_2O emissions and mineral N concentrations (NH_4^+, NO_3^- and available N) for ferulic and vanillic acid treatments (Table 4). However, NO_3^- concentration was not significantly correlated with tannic acid treatment. CO_2 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^{-1} vanillic, tannic and ferulic acid treatments than their corresponding 0.5 g kg^{-1} 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

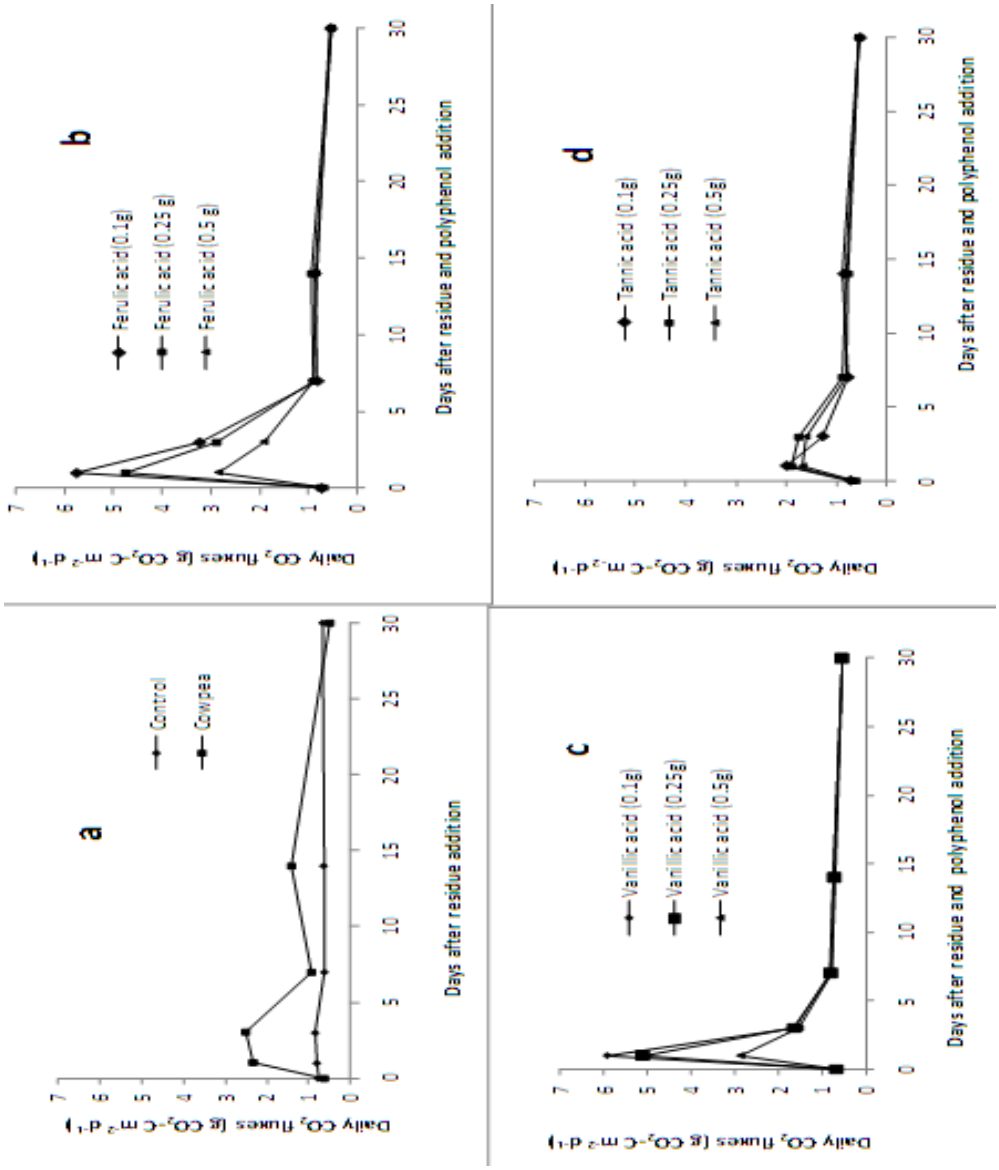

Fig. 2 Pattern of daily CO_2 emissions from the amended soils

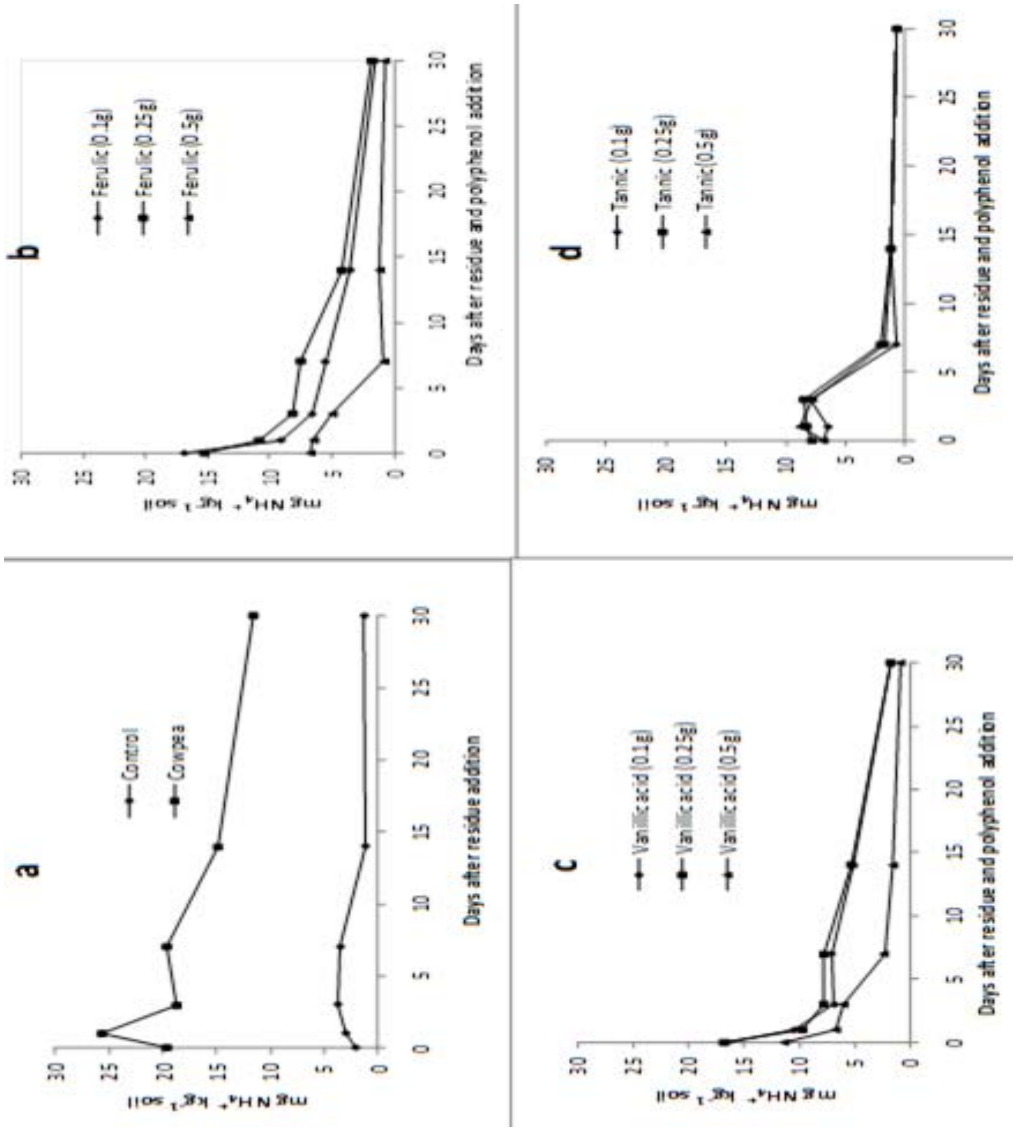

Fig. 3. NH_4^+ concentration in the amended soils

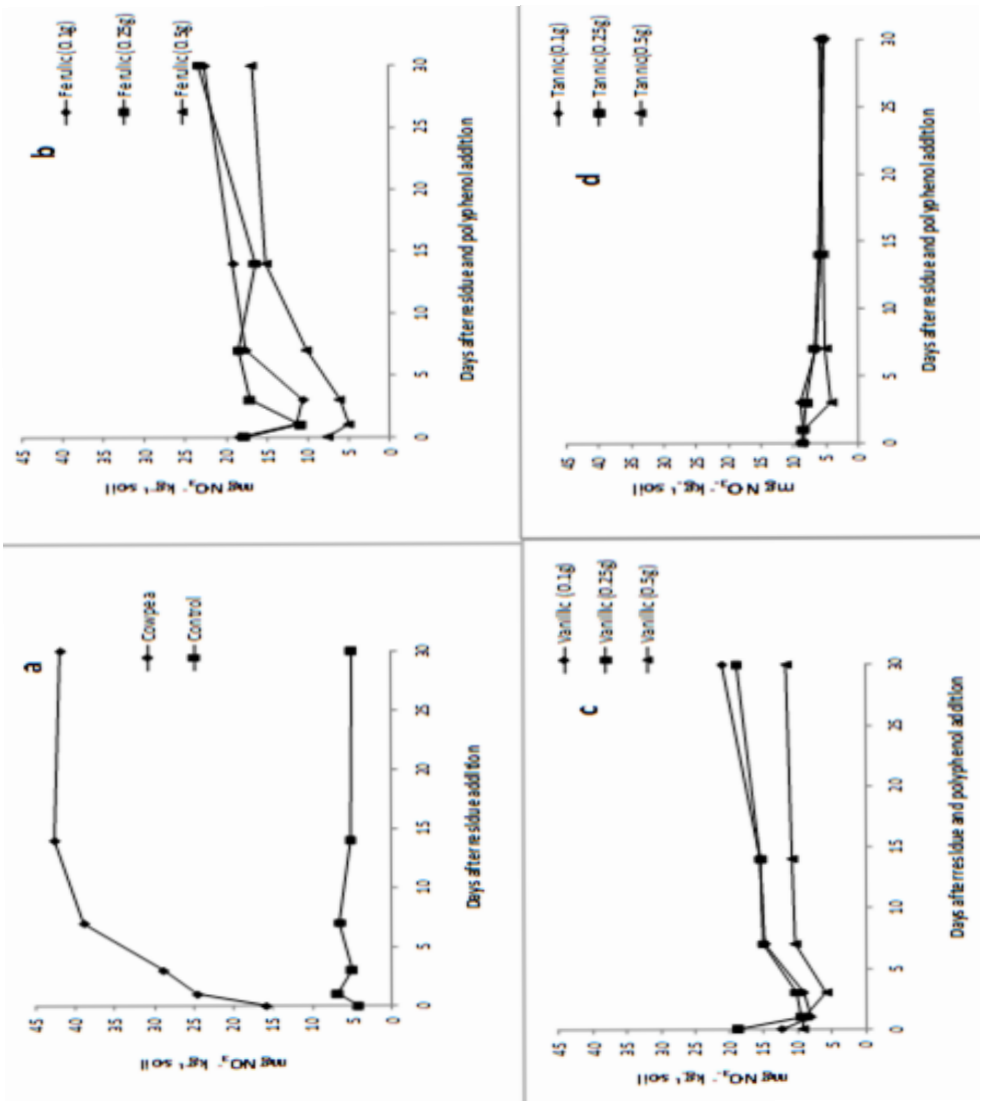

Fig. 4. *NO_3^- concentration in the amended soils*

TABLE 4
Relationships between mineral N concentrations and N_2O and CO_2 emissions

Polypehnol	*Mineral N concentrations (mg kg^{-1} soil)*		
Ferulic acid	*NH_4^+*	*NO_3^-*	*Available N (NH_4^+ + NO_3^-)*
log N_2O	0.6 *	0.45 *	0.75 **
CO_2	0.45	0.39	0.6
Vanillic acid			
log N_2O	0.75 **	0.54 *	0.74 **
CO_2	0.57 *	0.52 *	0.68 **
Tannic acid			
log N_2O	0.61 *	0.13 ns	0.58 *
CO_2	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^{-1} ferulic and vanillic acid treatments.

Pooling the results from all the treatments together, a strong positive relationship was found between MBC and both N_2O (r = 0.80; $P < 0.001$) and CO_2 (r = 0.86, $P <$ 0.001) measured on day 1 (Fig. 6).

Discussion

Influence of polyphenol type on N_2O emission and N mineralization

The N_2O 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_2O production via nitrification and denitrification because by day 30 inorganic N (NH_4^+ and NO_3^-) 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_3^- 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_3^- 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_4^+ and NO_3^- supply for the nitrification and denitrification processes that are mainly responsible for soil N_2O 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_3^- concentrations after the addition of 0, 80 and 160 kg ha^{-1} of high polyphenol olive oil waste. Similarly, Millar & Baggs (2004) reported a negative correlation between N_2O fluxes and both NO_3^- and NH_4^+ in a Kenyan oxisol amended with high polyphenol residues from some tropical agroforestry species (*Calliandra calothyrsus, Sesbania sesban, Macroptillum atropurpureum* and *Crotolaria grahamiana*).

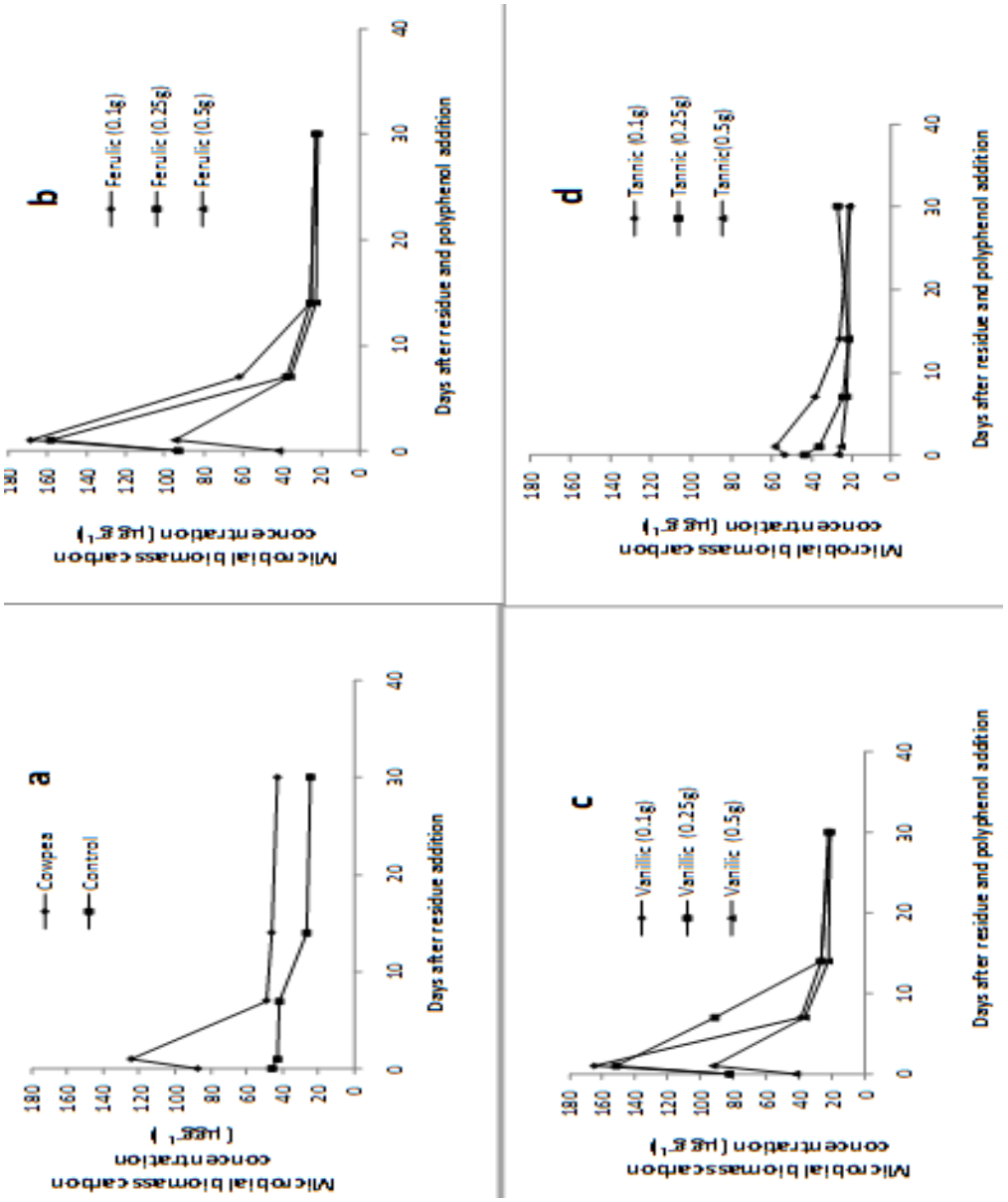

Fig. 5. Microbial biomass carbon in the amended soils

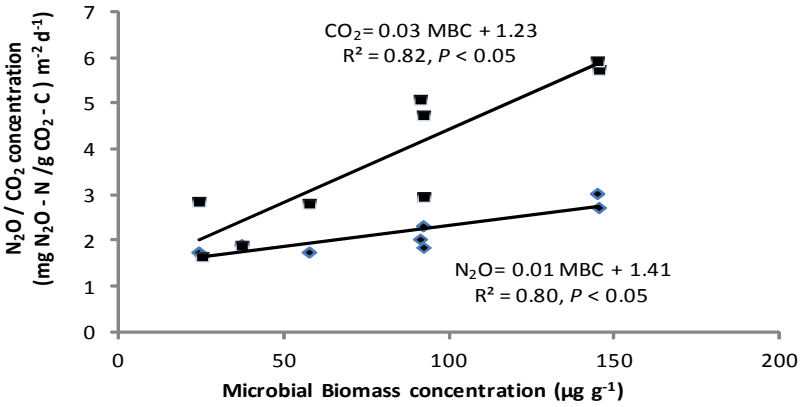

Fig. 6. Relationship between MBC and N_2O and CO_2

Over the 30 days, available NH_4^+ concentrations in all the polyphenol treatments were lower ($P < 0.05$) than in the sole cowpea treatment. The declining NH_4^+ concentration in the sole cowpea treatment was associated with increasing NO_3^- concentration, indicative of net N nitrification, but the decreasing NH_4^+ concentration in the polyphenol treatments did not translate into increases in NO_3^- concentration. This observation further confirms the potential of the added polyphenol compounds to lower N mineralisation and lower inorganic N availability for N_2O 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_2O 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 0.25g kg^{-1} 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^{-1} 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 *p*H (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_2 and N_2O fluxes from the 0.1 and 0.25 g kg^{-1} 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_2O 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_2O 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_4^+ 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_3^- concentration in these treatments could be due to decrease in nitrification following NH_4^+ substrate depletion through immobilisation. In contrast, Castells (2004) concluded that low NO_3^- 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 vanilic acid treatments. This was in good agreement with Sugai & Schimel (1993), who found that 90% of the polyphenols (hydroxybenzoic acid and salicyclic 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_2O emitted from the 0.1 and 0.25 g kg^{-1} of each polyphenol treatment was significantly higher ($P < 0.05$) than from their corresponding 0.5 g kg^{-1} treatments, but there was no significant difference between the total N_2O emitted from the 0.1 g kg^{-1} and 0.25 g kg^{-1} 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^{-1}) treatments decreased N_2O emitted over the 30 days period by up to 41% whilst their corresponding 0.5 g kg^{-1} treatments decreased N_2O 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^{-1}, the polyphenols, particularly the tannic acid, possibly provided more protein binding sites, thereby, lowering N substrate availability for N_2O production more than when it was incorporated at 0.1 or 0.25 g kg^{-1}. Talbot & Finzi, (2008) reported that the addition of humic and tannic acids at 200–4000 mg kg^{-1} 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^{-1} of humic and tannic acid. The highest concentration in this experiment, (0.5 g kg^{-1}) 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_2O 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_2O appeared to be due to different mechanisms, which are dependent on their molecular weight and the complexity of their chemical structure. CO_2 fluxes from the 0.1 and 0.25 g kg^{-1} vanillic and ferulic acid treatments were higher than from the 0.5 g kg^{-1} treatments during days 0 and 1, but the increased CO_2 fluxes were short-lived such that up to 87% of the total CO_2 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^{-1}) treatments were significantly higher ($P < 0.05$) than in the corresponding 0.5 g kg^{-1} treatment on day 1, and pooling results from all the treatments together, a strong positive relationship was found between MBC and both N_2O ($R^2 = 0.80$; $P < 0.001$) and CO_2 ($R^2 = 0.82$ $P < 0.001$) measured on day 1. This observation supports reports by Garcia-Montiel *et al*. (2001) that higher CO_2 emissions indicate greater microbial activity, which is likely to favour the creation of anaerobic conditions favourable for N_2O production.

Conclusion

The study demonstrated that application of polyphenol compounds decreased soil mineral N concentration and N_2O emissions, but the decreases in N_2O 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_2O production through protein binding while the lower molecular weight ferulic and vanillic acids decreased N_2O production through N immobilisation by stimulating microbial activity. However, the extent to which the polyphenol compounds decreased mineral N concentration and N_2O 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^{-1} soil resulted in a greater decline in mineral N concentration and N_2O 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_2O emission from soils

amended with cowpea residue, but the magnitude of N_2O emission reduction was dependent on the type and concentration of the polyphenol added.

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