Nitrous oxide and carbon dioxide emissions from pelletized and nonpelletized poultry litter incorporated into soil

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Abstract

While several studies have shown that the addition of animal manures to soil can increase N_2O and CO_2 emissions, limited information is available on the effect that manure physical characteristics can have on these emissions. This study compared N_2O and CO_2 emissions from poultry litter incorporated as pellets (5.5 mm OD, 7 mm long) or fine particles (<0.83 mm) into Cecil soil samples. The soil-litter mixture was packed in acrylic plastic cylinders and adjusted to 55 or 90 % water-filled porosity (WFP). The cylinders were placed inside jars that were sealed and placed in an incubator at 25°C for 35 d, with periodic air samplings conducted for N_2O and CO_2 analyses. At 55 % WFP, cumulative emission of $CO₂$ was similar for both litter types, but cumulative emission of N₂O was slightly higher for pelletized (6.8 % of applied N) than for fine-particle litter (5.5 %). In contrast, at 90 % WFP, cumulative emission of N₂O was larger for fine-particle litter (3.4 % of applied N) than for pelletized litter (1.5 %). These results indicate that the effect of poultry litter physical characteristics on N_2O emissions from incorporated applications can be expected to vary depending on the soil water regime.

Introduction

Understanding the factors that control emissions of nitrous oxide (N_2O) and carbon dioxide (CO_2) from soils is important for a complete understanding of the nitrogen (N) and carbon (C) cycles. In addition, N_2O and $CO₂$ are implicated in global climate warming (Dickinson and Cicerone, 1986; Wang and Molnar, 1985) and $N₂O$ participates in the destruction of the ozone layer (Crutzen, 1976; Liu et al., 1976).

The main soil biogenic process responsible for the release of $CO₂$ into the atmosphere is the aerobic decomposition of organic compounds (Alexander, 1977). With regard to N_2O , the two main soil biogenic processes responsible for its release are nitrification (Poth and Focht, 1985) and denitrification (Knowles, 1982). Nitrification is a two-step aerobic process that involves the oxidation of $NH₄⁺$ to $NO₂⁻$, followed by the oxidation of $NO₂⁻$ to $NO₃⁻$. Nitrous

oxide is produced in significant amounts during the oxidation of NH_4^+ under low O₂ levels (Goreau et al., 1980; Klemedtsson et al., 1988). Anderson (1964) and Ritchie and Nicholas (1972) have suggested that N_2O is generated from an intermediate between $NH₂OH$ and NO_2^- during NH_4^+ oxidation. In addition, Poth and Focht (1985) have shown that under O_2 limiting conditions *Nitrosomonas europaea* can become a denitrifier, producing N_2O from NO_2^- .

Denitrification is typically considered as an anaerobic process in which bacteria utilize NO_3^- , NO_2^- , N_2O , or NO as electron acceptors, and produce NO, N_2O and/or N_2 as end products. The proportion of N_2O in the denitrification end products increases when $NO_3^$ concentrations increase because $NO₃⁻$ is a better electron acceptor than N_2O (Alexander, 1977). The proportion of N_2O also increases under microaerophilic conditions because of the O_2 sensitivity of the N₂Oreductase system (Knowles, 1982). Thus, N_2O pro-

duction by denitrifiers increases from anaerobic to microaerophilic conditions (Focht, 1974), whereas $N₂O$ production by nitrifiers increases from aerobic to microaerophilic conditions (Klemedtsson, 1988). As a result, the highest rates of $N₂O$ production from both nitrification and denitrification are expected under microaerophilic conditions.

Several studies have shown that the addition of animal manures to soil can increase N_2O and CO_2 emissions (Stevens and Cornforth, 1974; Burford, 1976; Egginton and Smith, 1986). Limited information is available, however, on the effect that manure physical characteristics can have on these emissions. In recent work (Cabrera et al., 1994) we evaluated the effect of physical characteristics of poultry litter (fine particles versus pellets) on N_2O and CO_2 emissions from surface applications to soil. Poultry litter is a mixture of bedding material, excreta, and waste feed generated during poultry production. We found that when the soil was maintained at 90 % water-filled porosity (WFP), $CO₂$ and N₂O emissions did not differ between pelletized and fine-particle litter. When the soil was kept at 58 % WFP, however, N_2O losses amounted to 2.6 % of the applied N for pelletized litter and to only 0.2 % of the applied N for fine-particle litter. The present study was conducted to gather similar data with incorporated (rather than surface) applications of poultry litter. We compared N_2O and CO_2 emissions and inorganic N recovery from pelletized and fine-particle poultry litter incorporated into soil samples that were maintained 1) at 55 or 90 % WFP for 35 d, and 2) at 55 % WFP for the first 14 d and then changed to 90 % WFP for the remainder of 35 d. A 55 % WFP is optimum for nitrification and microbial respiration, whereas 90 % WFP is adequate for denitrification (Linn and Doran, 1984). A supplementary study was also conducted to determine the contributions of nitrification and denitrification to the maximum emission rates of N_2O at 55 % WFP.

Materials and methods

The soil used was collected moist from the upper 15 cm of an area mapped as Cecil loamy sand (clayey, kaolinitic, thermic, Typic Kanhapludults). The sample was passed through a 4-mm sieve, stored at 4°C, and before use wetted to 0.097 kg H_2O kg⁻¹ (- 0.02 MPa) with a N-free nutrient solution (100 mg Ca L^{-1} , 24 mg Mg L⁻¹, 113 mg S L⁻¹, 0.5 mg P L⁻¹, and 4 mg K L^{-1} added as KH_2PO_4 , K_2SO_4 , MgSO₄, and CaSO₄; $pH = 7$). The inorganic N content of the soil was low

 $(1 \text{ mg NH}_4^+$ -N kg⁻¹ and 5 mg NO₃ -N kg⁻¹) and was not increased so that it would be representative of inorganic N levels found before poultry litter applications. The soil had a pH in water of 7.03 (2 mL : 1 g soil) and contained 3.24 g C kg⁻¹ and 0.45 g total N kg⁻¹.

Poultry litter from a broiler house was passed through a 0.83-mm sieve to generate a fine fraction that was subsequently pelletized. The pelletized material contained $350.6 \text{ g C kg}^{-1}$, 56.9 total N kg⁻¹, 3.97 g NO_3^- -N kg⁻¹, 5.30 g NH₄⁺-N kg⁻¹, and 0.116 kg H₂O kg^{-1} . The fine material (hereafter called fine-particle litter) to be compared to pelletized litter was obtained by crushing the pelletized litter and passing it through a 0.83-mm sieve.

Three soil amendments (zero, pellets, fine-particle litter) and two levels of WFP (55 and 90 %) were combined to generate six treatments. Six replicates of each treatment were prepared, three to be extracted at 14 d and three at 35 d. To study the effect of a change in WFP, three extra replicates of all the treatments at 55 % WFP were prepared so that they could be changed from 55 to 90 % WFP at 14 d. These additional replicates were extracted with 1 M KCI at 35 d. The treatments were arranged in a completely randomized design.

The experimental units consisted of acrylic plastic cylinders (4.45 cm ID, 10 cm long) with one end plugged by a rubber stopper. Each cylinder held 151 g of dry weight equivalent soil packed to a bulk density of 1.8 g cm^{-3}, which is a common bulk density for the Ap horizon of naturally consolidated Cecil soils (Perkins, 1987). For the fine-particle litter treatments, 0.81 g dry-weight equivalent litter was mixed with soil before packing, whereas for the treatments with pelletized litter, three cylindrical pellets (5.5 mm OD, 7 mm long, 0.27 g dry weight each) were inserted into the soil after packing. For that purpose, a small cork borer (0.9 cm ID) was used to take a soil core from the center of the packed column to a depth of 4.5 cm (the total depth of packed soil was 5.4 cm). A pellet was placed at the bottom of the hole, soil was packed on top of it until a depth of 2.7 cm (measured from the soil surface) was reached, and a second pellet was placed in the hole. This pellet was covered with packed soil until a depth of 0.9 cm was reached, the third pellet was placed in the hole, and soil was packed on top of it until the hole was completely covered. The amount of litter applied was equivalent to 307 kg N ha⁻¹ on an area basis.

After the packing was complete, 9.6 mL of N-free nutrient solution was added dropwise with a syringe and needle to all the treatments that were to have 90 % WFP. Nutrient solution was used, rather than water, to prevent soil dispersion. This extra addition of nutrients to treatments at 90 % WFP should not have affected N_2 O and CO_2 emissions because the N-free nutrient solution was very dilute and did not include substrates that could enhance nitrifier or denitrifier populations. The water potential at 90 % WFP was not measured, but should have been very close to zero.

Each cylinder was placed inside a 0.95-L glass jar that was sealed with a screw-cap lid fitted with a rubber septum. All jars were placed in an incubator at 25°C. Periodically, 5-mL air samples of the headspace of three replicates of each treatment were taken for $N₂O$ and $CO₂$ analyses; then the jars were flushed with atmospheric air and resealed. This process was carried out every 8 h during the first 3 d to ensure that the $CO₂$ pressure inside the jars would not exceed 1 kPa. After the first 3 d, gas samples were taken every 24 to 32 h. The cylinders were weighed periodically and deionized water was added when needed to replenish (by weight) any water lost by evaporation.

At 14 and 35 d after treatment application, three replicates of each treatment were extracted with 1 M KC1 for inorganic N determinations. The packed soil was pushed out of the acrylic cylinder and into a 1.9-L glass jar, 1.5 L of 1 *M* KCl was added, and the jar was placed in a reciprocating shaker at 120 oscillations min^{-1} for 30 min. After the extraction, a sample of the extract was centrifuged and an aliquot of the supernatant volume was frozen for later analysis.

A supplementary study was conducted to determine the contributions of nitrification and denitrification to the maximum, initial emission rates of N_2O at 55 % WFP. Three levels of C_2H_2 (0, 10 Pa, and 10 kPa) were applied to three replicates of treatments with fineparticle or pelletized litter, and the emission of $N₂O$ was measured during the following 8 h. A preliminary study had shown that C_2H_2 at 10 Pa was sufficient to inhibit nitrification in the soil used. In addition, Davidson et al. (1986) found that 10 Pa C_2H_2 had little or no effect on the reduction of N_2O to N_2 during short incubations. A partial pressure of 10 kPa C_2H_2 was used to inhibit the reduction of N_2O to N_2 (Davidson et al., 1986) and thus measure total denitrification losses. Nitrous oxide emission at the three levels of C_2H_2 was measured at the times of maximum, initial emission rates (16-24 h for fine-particle litter and 40-48 h for pelletized litter).

Nitrous oxide concentration in the gas samples was determined with a Tracor 550 GC (Trametrics Analytical Division, Austin, Texas) equipped with an electron capture detector at an operating temperature of 340° C. A mixture of Ar: $CH₄$ (95:5) was used as carrier gas at 25 mL min^{-1}, and a 1.8-m Porapak O 80/100 column (Alltech Associates, Inc., Deerfield, Illinois) at 50°C was used to separate N_2O . Carbon dioxide concentrations were determined using a Varian 3700 GC (Varian Analytical Instruments, Sugarland, Texas) equipped with a thermal conductivity detector operating at a temperature of 200 $^{\circ}$ C. Helium at 20 mL min⁻¹ was used as carrier gas, and a 1.8-m Porapak Q 80/100 column at 75° C was used to separate CO₂. The volume of air in the jars was calculated by subtracting the volume occupied by soil and water from the air volume of a jar containing an empty acrylic cylinder (with a rubber stopper at the bottom). All values were corrected for the solubility of N_2O and CO_2 in water, as described by Tiedje (1982).

Carbon and N contents of the soil and litter materials were determined by dry combustion (Nelson and Sommers, 1982) in samples ground to pass through a 0.149-mm sieve. Inorganic N in the litter was extracted by placing 1 g dry-weight equivalent litter into a 50-mL centrifuge tube, adding 40 mL of 1 M KC1, shaking for 30 min, and subsampling the supernatant volume. Nitrite + nitrate-N was determined by the Griess-Ilosvay technique (Keeney and Nelson, 1982), after reduction of NO_3^- to NO_2^- with a Cd column. Ammonium-N was determined by the salicylatehypochlorite method (Crooke and Simpson, 1971).

Cumulative amounts of N_2O and CO_2 evolved and amounts of inorganic N present were expressed as percentages of the amounts of N and C applied with the litter by subtracting control treatments. Analyses of variance and comparisons of N_2O , CO_2 , and inorganic N measurements were done with procedures available in SAS (SAS Institute Inc., 1985).

Results and discussion

Maximum rates of $N₂O$ emission occurred within the first 2 d both at 55 and 90% WFP (Figs. 1 and 2). At 55% WFP, fine-particle litter showed significantly faster emission of N_2O and CO_2 than pelletized litter (Fig. 1). The faster emission of $CO₂$ from fine-particle litter was due to its homogeneous distribution in the soil, which made it more available to heterotrophic microorganisms. In contrast, pelletized litter was concentrated in three discrete zones at the center of each column, which restricted access by microorganisms.

Fig. 1. Cumulative N_2O and CO_2 emissions (mean \pm std error) for treatments at 55% WFP and for treatments switched from 55 to 90% WFP after 14 days ($C55$ = control at 55% WFP; $F55$ = Fine-particle litter at 55% WFP; P55 = Pelletized litter at 55% WFP; C55- > 90, F55- > 90, P55- > 90 = treatments switched from 55 to 90% WFP at 14 days. Where error bars do not appear, symbols were larger than the error).

Maximum rates of $N₂O$ emission were not significantly different for samples with and without 10 Pa C₂H₂ in both fine-particle litter (278 μ g N₂O-N kg^{-1} h⁻¹) and pelletized litter (121 μ g N₂O-N kg h^{-1}). Because a concentration of 10 Pa C_2H_2 in the atmosphere inhibits nitrification, these results indicate that nitrification was not contributing significantly to the initial emission of N_2O at 55% WFP. In addition, the emission of N_2O at 10 kPa C_2H_2 was higher for fine-particle litter (421 μ g N₂O-N kg⁻¹ h⁻¹) than for pelletized litter (107 μ g N₂O-N kg⁻¹ h⁻¹), indicating that total denitrification losses were higher with fine-particle litter. Therefore, the lower emission of $N₂O$ observed with pelletized litter was due to a lower denitrification rate, which may have been due to the lower emission of $CO₂$ (Fig. 1) and its consequent production of fewer anaerobic microsites adequate for denitrification (Parkin, 1987). An association between

Fig. 2. Cumulative N₂O and CO₂ emissions (mean \pm std error) for treatments at 90% WFP (C90 = control at 90% WFP; F90 = Fine-particle litter at 90% WFP; P90 = pelletized litter at 90% WFP. Where error bars do not appear, symbols were larger than the error).

rate of $CO₂$ production and rate of denitrification was observed by Aulakh et al. (1991) in a study in which crop residues were mixed with soil and incubated at 60% WFE Similarly, Rice et al. (1988) found that the rate of CO₂ production was related to the rate of denitrification in a soil that received injections of organic waste.

After the high, initial rates of N_2O emission (at 55% WFP) subsided, the cumulative amount of N_2O evolved increased very slowly up to day 14 with both types of poultry litter (Fig. 1). Cumulative emissions of CO_2 and N₂O after 14 d of incubation at 55% WFP were significantly higher for fine-particle litter than for pelletized litter (Table 1). No differences were found, however, in the amounts of inorganic N recovered from both litters (\sim 60% of applied N).

Between days 14 and 35 there was an increase in $N₂O$ emission for both types of poultry litter at 55% WFP (Fig. 1), although the increase was much larger with pelletized than with fine-particle litter. The fact that the N_2O emission increased as the emission of CO_2 subsided suggests that the increase was in response to

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higher levels of O_2 in the soil. Although it is not possible from our results to identify the process directly responsible for the increased N_2O emission, a possible explanation is that the increased $NO₃$ availability (Fig. 3) and the decreased $CO₂$ emission (Fig. 1) led to conditions that favored the release of a larger proportion of the denitrification losses as $N₂O$. Similar reasons were proposed by Comfort et al. (1990) to explain an increase in $N₂O$ emission observed 16 d after injecting liquid dairy manure into soil. An alternative explanation for the increased $N₂O$ evolution is that the decreased $CO₂$ emission allowed nitrifiers to operate in microaerophilic microsites with the consequent production of N_2O . Regardless of the process responsible for the increased N_2O production, it seems that nitrification was involved in some manner because the highest $N₂O$ increase was observed in treatments with pelletized litter (Fig. 1), which also had the highest nitrification rate (Fig. 3).

As a result of the large increase in N_2O emission observed with pelletized litter, the total amount of N_2O evolved in 35 d was slightly but significantly higher for pelletized litter (6.8% of N applied) than for fine-particle litter (5.5%, Table 1). Cumulative $CO₂$ -C losses in 35 d did not differ between litter types and represented 42.4 and 43.1% of the C applied in fine-particle and pelletized litters, respectively. These $CO₂-C$ losses agree with those obtained by Gale and Gilmour (1986) in a study in which poultry litter was surface applied to soil and incubated at 25°C for 34 days.

The inorganic N recovered at 55% WFP did not change between days 14 and 35 for fine-particle litter but decreased for pelletized litter (Table 1). This loss was due to a decrease in NH4 which was not followed by a similar increase in $NO₃$ (Fig. 3). Among possible reasons for the decrease in N recovery are N immobilization and N losses through NH₃ volatilization, denitrification, and nitrification. In a study on N mineralization from pelletized and ground manures mixed with soil and incubated at 60% water-holding capacity, Hadas et al. (1983) also observed that between 7 and 30 d after application there was a decrease in total inorganic N recovered from poultry manure pellets. They postulated that the loss may have been due to NH3 volatilization, although immobilization and denitrification were not excluded. These results indicate that the recovery of inorganic N from poultry manure pellets mixed with soil and incubated under aerobic conditions can be expected to be lower than that from ground manure.

Fig. 3. Inorganic N content (mean \pm std dev) at 14 and 35 days in treatments maintained at 55% WFP (a), in treatments switched from 55 to 90% WFP at 14 days (b) and in treatments maintained at 90% WFP (c) (Where error bars do not appear, symbols were larger than the error).

Fourteen days after treatment application, a set of samples was switched from 55 to 90% WFP and maintained at that water content until day 35. As a result, most of the $NO₃$ present at 14 d was lost by day 35 in both litter treatments (Fig. 3). The most plausible explanation for this large loss of $NO₃$ at 90% WFP is denitrification (Linn and Doran, 1984). In comparison to the treatments that remained at 55% WFP, the switch to 90% WFP increased N_2O emission from fineparticle litter and decreased N_2O emission from pelletized litter (Fig. 1). The increased N_2O emission from fine-particle litter was probably due to an increase in

Table 1. Cumulative N_2O and CO_2 evolved and inorganic N recovered (25 C) at 14 and 35 days after incorporation of pelletized or fine-particle poultry litter into soil samples maintained at either 55 or 90% WFE and after incorporation of pelletized or fine-particle poultry litter into soil samples maintained at 55% WFP for the first 14 days and then changed to 90% WFP for the remainder of 35 days

	$0-14$ days			$0-35$ days		
		Litter N_2O^2 Inorg. N^2 CO_2 ^y N_2O Inorg. N CO_2				
	55% WFP (0-35 days)					
Pellet		1.5 ^x 59.7 ^a	31.6b		6.8b $51.3b$	43.1a
Fine	3.9a	60.3a	38.5a		5.5b $60.3a$	42.4a
	55% WFP (0-14 days) and 90% WFP (15-35 days)					
Pellet	1.5 _b	59.7a	31.6b		$2.3b$ 32.8a	35.4a
Fine	3.9a	60.3a	38.5a		$10.8a$ 45.2a	40.3a
	90% WFP (0-35 days)					
Pellet	0.7a	57.6a	10.9 _b		1.5b 59.0a	29.2a
Fine	0.6а —	50.7b	20.1a		3.4a 35.8b	37.3a

^z Expressed as percentage of the N applied.

Y Expressed as percentage of the C applied.

x Within each water content regime, means within a column followed by the same letter are not significantly different according to Fisher's LSD at a 0.05 probability level.

denitrification, although some $N₂O$ emission through nitrification cannot be completely excluded. Because the fine-particle litter was homogeneously distributed within the soil, the $NO₃$ generated from it should also have been homogeneously distributed. Thus, when the switch to 90% WFP created conditions adequate for denitrification, some of the $NO₃$ should have been present in microaerophilic microsites, in which denitrification would have led to production of N_2O . In contrast to the fine-particle litter, pelletized litter was present in three discrete zones in the soil, causing a localized concentration of NO₃. When the water content was changed from 55 to 90% WFP these zones became anaerobic, which would have led to the reduction of $NO₃$ to $N₂$, thus decreasing the emission of $N₂O$.

In contrast to the results obtained with samples maintained at 55% WFP, the total emission of N_2O from samples switched from 55 to 90% WFP was larger for fine-particle litter (10.8% of applied N) than for pelletized litter (2.3%, Table 1). These results indicate that the effect of litter physical characteristics on $N₂O$ emission is strongly determined by the soil water regime.

At 90% WFP, the initial emissions of N_2O and CO_2 were similar for fine-particle and pelletized litter (Fig. 2). Although we did not evaluate the contributions of nitrification and denitrification for the initial N_2O emissions at 90% WFP, it is likely that denitrification was the main process involved because the environmental conditions (90% WFP, C and $NO₃$ availability) would favor denitrification over nitrification (Tiedje, 1988). In a laboratory study, Klemedtsson et al. (1988) measured very low rates of N_2O formation by nitrification during the first 2 d after the application of $NH₄⁺$ and $NO₃$ to soil incubated at 90% water-holding capacity and 15°C.

The cumulative amounts of $N₂O$ evolved by day 14 at 90% WFP were similar for both litter treatments, but the cumulative amount of $CO₂$ evolved was larger for fine-particle (20.1%) than for pelletized litter (10.9%, Table 1). Inorganic N recovery by day 14 was slightly larger for pelletized (57.6%) than for fine-particle litter (50.7%, Table 1).

Between days 14 and 35 there was an increase in cumulative $N₂O$ emission from samples maintained at 90% WFP (Fig. 2), with the increase being much larger for fine-particle litter than for pelletized litter. It should be noted that nitrification was also faster with fine particles than with pellets (Fig. 3). The higher nitrification rate with fine-particle litter was likely due to its homogeneous distribution in the soil, which would have caused some of the $NH₄⁺$ released from the litter to be near the soil surface where aeration conditions should have been better for nitrification. In contrast, pelletized litter was concentrated in localized zones in the center of the soil columns, where O_2 levels would be expected to be lower than near the soil surface.

As in the samples maintained at 55 $%$ WFP, the increase in N_2O emission observed after 14 d at 90 % WFP may have been due to a higher availability of $NO₃$ (Fig. 3) and a decreased $CO₂$ emission (Fig. 2), which would have caused a larger proportion of the end products of denitrification to be released as $N₂O$. On the other hand, the increased N_2O emission could also have been due to increased nitrification in microaerophilic sites. Klemedtsson et al. (1988) observed significant nitrification at 90 % water-holding capacity and found that the rate of N_2O production by nitrification was initially small but increased almost 70-fold in a 15-d incubation.

Due to the large increase observed in N_2O emission from fine-particle litter (between days 14 and 35), the cumulative amount of N_2O evolved in 35 d was larger for fine-particle litter $(3.4 \% \text{ of applied N})$ than for

pellets (1.5 %, Table 1). In addition, the inorganic N recovery was much lower for fine-particle (35.8 %) than for pelletized litter $(59.0 %)$, an effect which may have been due to denitrification losses.

In summary, the cumulative emission of $N₂O$ in 35 d of incubation at 55 % WFP was slightly but significantly higher for pelletized litter (6.8 % of applied N) than for fine-particle litter (5.5%) . At 55 % WFP there were no differences in cumulative $CO₂$ emissions by day 35, but pelletized litter showed lower inorganic N recovery (51.3 % of applied N) than fine-particle litter (60.3 %). Switching from 55 to 90 % WFP at 14 d resulted in higher cumulative $N₂O$ evolved by day 35 with fine-particle litter (10.8 % of applied N) than with pelletized litter (2.3 %). Similarly, cumulative N_2O emission in 35 d of incubation at 90 % WFP was larger for fine-particle litter (3.4 % of applied N) than for pelletized litter (1.5 %). These findings indicate that the effect of poultry litter physical characteristics on $N₂O$ emissions from incorporated applications can be expected to vary depending on the soil water regime.

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