Nitrous Oxide Production in an Eastern Corn Belt Soil: Sources and Redox Range

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Nitrous oxide derived from soils is a main contributor to the greenhouse gas effect and a precursor to ozone-depleting substrates; however, the source processes and interacting controls are not well established. This study was conducted to estimate the magnitude and source (nitrification vs. denitrification) of N_2O production as affected by the form of N fertilizer, soil water content, and redox potential (Eh). Soils from continuous corn (*Zea mays* L.) experimental plots with a history of eight consecutive years of either side-dressed urea–NH_4NO_3 (UAN) or fall liquid swine manure (FM) were collected and N_2O evolution was traced in both aerobic and anaerobic incubations using ^{15}N labeling. Partitioning results were highly variable but suggested that enhanced denitrification occurred after an extreme increase in soil water content (from 45 to 90% water-filled pore space [WFPS]) while a more coupled nitrification–denitrification process drove N_2O evolution at moderate water content (55% WFPS). Manured soils at high water contents registered shorter duration peaks but with higher overall N_2O production rates than those observed at moderate water content (7-d weighted average of 0.61 vs. 0.09 μg N_2O kg^{-1} soil h^{-1}). Under anoxic conditions, manured soils showed higher N_2O production rates than UAN soils (up to 336 and 145 μg N_2O kg^{-1} soil h^{-1}, respectively) shortly after flooding, which coincided with a sharp drop in Eh (from 575 to 466 mV). Irrespective of the N source, a narrow, consistent Eh range for N_2O production occurred under moderate reducing conditions (420–575 mV). These results indicate that soils receiving repeated manure application that are subject to intensive, recurrent soil rewetting events may be prone to higher N_2O emissions.

Abbreviations: DOC, dissolved organic carbon; DW, distilled water; Eh, redox potential; FM, fall liquid swine manure; OVDE, oven-dried equivalent; PP, preplant; RM, repeated measures; UAN, urea–ammonium nitrate; WFPS, water-filled pore space.

Nitrous oxide is a precursor to ozone-depleting substrates (Crutzen, 1981; Cicerone, 1987) and a very potent greenhouse gas (Mosier et al., 1996, 1998). Comparatively, N_2O possesses a global warming potential 298 and 12 times higher than CO_2 and CH_4, respectively, on a 100-yr time horizon basis (Intergovernmental Panel on Climate Change, 2007). Atmospheric concentrations of N_2O have increased during the last 250 yr from 270 to 319 nL L^{-1}, and the current annual rate of increase is approximately 0.25% (Intergovernmental Panel on Climate Change, 2007). As reviewed by Bremner (1997) and Kroeze et al. (1999), N_2O emitted from soils can largely account for these increases in atmospheric N_2O concentrations; agricultural soil management may contribute up to 78% of the anthropogenic N_2O sources (Johnson et al., 2007; USEPA, 2007).

Soils commonly produce N_2O during both nitrification and denitrification processes (Bremner and Blackmer, 1981; Firestone and Davidson, 1989; Davidson, 1992), with the possibility of both processes coexisting within the same soil aggregate due to microsite variability (Kuenen and Robertson, 1994; Renault and Stengel, 1994; Nielsen et al., 1996; Granli and Bockman, 1994). Nitrification is the biological oxidation of NH_4^+ catalyzed by both NH_4– and CH_4–oxidizing bacteria, whereas denitrification is predominantly performed by the heterotrophic bacterial reduction of NO_3^- or NO_2^- (Bremner, 1997; Sutka et al., 2006). Soil O_2 availability governs the relative contribution of these microbial pathways (Bremner, 1997); the primary drivers of N_2O source and flux are fluctuations of soil water content in unsaturated soils (Firestone and Davidson, 1989; Davidson, 1992; Paul et al., 1993) and the Eh status in saturated soils (Hou et al., 2000; Yu and Patrick, 2003), which reflects their close relationships to soil O_2 availability. When the soil water content limits O_2 availability, N_2O production via denitrification is normally enhanced (Takaya et al., 2003), whereas shortly after soil water content decreases, denitrification rates typically decline

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(Robertson and Tiedje, 1987) although nitrification continues (Khalil et al., 2004). Concomitantly, as soils undergo reduction shortly after submersion, O_2 is microbially consumed, leading to abrupt nitrification suppression, eventually followed by the cessation of denitrification following the consumption of preexisting NO_3^- (Letey et al., 1980, 1981; Jenkins and Kemp, 1984; Khalil et al., 2004).

In seasonally flooded soils, the dependency of N_2O production on Eh has been well established (Yu and Patrick, 2003, 2004; Yu et al., 2007); however, N_2O production has only rarely been assessed in soils that experience intermittent reduction but that have no history of seasonal flooding (Yu et al., 2001). Laboratory N_2O source-partitioning studies have produced inconsistent results, which may be a partial artifact of the wide variety of methods used to test hypotheses. In aerobic incubations, the major N_2O source has been identified as nitrification (Bremner and Blackmer, 1978, 1979; Robertson and Tiedje, 1987; Skiba et al., 1993; Ma et al., 2007), denitrification (Paul et al., 1993; Azam et al., 2002; Mørkved et al., 2006), or roughly equal partitioning between both pathways (Kester et al., 1997; Stevens et al., 1997; Stevens and Laughlin, 2001; Khalil et al., 2004; Carter, 2007). Furthermore, in field studies, both temporal (Panek et al., 2000) and spatial (Holtgrieve et al., 2006) shifts between the two microbial pathways of N_2O production have been observed and associated with soil water content fluctuations as well as inorganic N transformations. These divergent results indicate the need for further studies on N_2O source partitioning to identify the underlying causes for these temporal and ecosystem-to-ecosystem variations.

Single-factor studies conducted during more than three decades of intensive research have shown the prominent controlling role of N management on soil N_2O production (Dobbie et al., 1999). Most of the agriculturally sourced N_2O emissions have been linked to increasing N inputs in the form of either synthetic fertilizer or animal manure (Robertson and Grace, 2004; Lokupitiya and Paustian, 2006). Additional, critical information is still necessary, however, for better selection, design, and implementation of improved management practices in croplands. To date, few studies have simultaneously assessed N_2O production, source partitioning, and associated Eh changes as influenced by extreme increases in soil water content in soils with multiyear histories of differing N fertilizer sources. Thus, the objectives of this study were: (i) to examine the variation in both soil water content and Eh on soil N_2O evolution rates under both aerobic and anaerobic conditions; (ii) to estimate both the magnitude and sources of N_2O production during the ninth consecutive year of repeated N applications (fall liquid swine manure vs. side-dressed UAN) in a continuous corn cultivation system; and (iii) to assess the interactive effects of soil water content and type of N inputs on soil N_2O production.

MATERIALS AND METHODS

Soils and Treatments

Soil samples were collected from two agronomic treatments of an existing, long-term experiment located at West Lafayette, IN. The soil series at the experimental site are a Drummer silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquoll) and a Raub silt loam (fine-silty, mixed, superactive, mesic Aquic Argiudoll). Treatments were arranged in a randomized complete block design with four replicates and were in their ninth consecutive year of application at the time of sampling. The two selected treatments were in continuous corn cultivation but N management differed. One treatment received liquid swine manure (2:1 C/N ratio, 80% of N as NH_4^+) injected in the fall (FM) at 255 ± 24 kg N ha^{-1} yr^{-1}, while the other treatment received UAN (28% N) side-dressed at the V5 growth stage at 157 kg N ha^{-1} yr^{-1}. Both manure and UAN were placed at a depth of 0.10 m in the soil. The dates of manure and UAN additions that preceded sample collection were 14 Nov. 2005 and 14 June 2006, respectively. Additional information about treatment management, the experimental site, and weather parameters can be found in Hernandez-Ramirez et al. (2009b).

Soils were collected at preplant (PP, 3 May 2006, 42 d prior to UAN addition) and again at the V6 corn growth stage (21 June 2006, 7 d after UAN addition). Our V6 field sampling in the UAN-treated plots avoided an interrow area (0.30 m wide) centered on the injection band to avoid extreme inorganic N concentrations. At 18 random positions throughout each treatment plot, the top 0.15 m of soil was sampled using a hand probe (2.5-cm i.d.). The 18 cores were composited, stored at 4°C in the dark, sieved (8-mm mesh) within 2 d of collection, mixed, and stored again at 4°C in the dark. The average soil gravimetric water content was 169 ± 8 g kg^{-1} measured 1 wk after initiating storage. Within 2 wk after the V6 collection and immediately before initiating preincubation, the composite soil samples were sieved again to attain aggregates under 6.4-mm diameter. Soil aggregates were then hand packed to a bulk density (ρ_b) of 1.2 g cm^{-3} in the bottom of incubation containers. Preincubation consisted of wetting with distilled water (DW) to 45% WFPS and storing at 20°C in the dark for 3 d. The WFPS was calculated as:

$$\text{Fractional WFPS} = \frac{W}{(\text{OVDE soil}/\rho_b)\left(1-\rho_b/\rho_p\right)} \quad [1]$$

where W is the mass of water (g), OVDE is oven-dried equivalent soil weight (g), and ρ_p is the soil particle density, assumed to be 2.65 g cm^{-3}.

Before soil preincubation, NH_4–N and NO_3–N concentrations were determined in our soil samples following Prokopy (1997) and Wendt (1999). Soils were analyzed for organic C and total N (LECO CHN-2000 analyzer, LECO Corp., St. Joseph, MI) and pH (1:1 in water). Dissolved organic C (DOC) was extracted by agitating air-dried soil (20 g OVDE) in 100 mL of solution with 5 mmol L^{-1} $CaCl_2$ (horizontal, reciprocal shaker at 120 rpm for 30 min), followed by centrifugation (10,000 × g for 10 min) and filtration (Whatman no. 2, ~8 μm, Maidstone, UK). The supernatant was analyzed in a total organic C analyzer (TOC-V_{WS}, Shimadzu, Kyoto, Japan) and corrected by method blank.

Soil Incubations

Aerobic Nitrous Oxide Production

Aerobic incubations used microcosms of 90 g of OVDE soil in 0.97-L glass Mason jars. Soil N_2O evolution was measured in a full factorial design with soil water content (55 or 90% WFPS), N source (FM or UAN), and sampling time (PP or V6) as the main factors. Three laboratory replicates of 55 and 90% WFPS for each of the four field sample replicates were incubated for 7 d at 25°C in the dark following Maag and Vinther (1999) and Moran et al. (2005) with modifications. Headspace gas samples (20 mL) were withdrawn five times during the incubation (0, 3, 24, 96, and 168 h) using a gas-tight syringe inserted through a butyl rubber septum port. During the 24- and 96-h samplings, microcosms were opened briefly (20 min) to allow reequilibration with the room atmosphere; as needed, DW was added by weight to maintain the desired soil water content levels. When the microcosms were opened,

gas samples were collected both immediately before opening and after resealing the microcosms. Method blanks for background N_2O concentrations were duplicate Mason jars with 23 and 37 mL of DW.

Gas samples were stored at 4°C in soda glass vials (Exetainer, Labco, High Wycombe, UK) prepared as described in Laughlin and Stevens (2003). Within a week of collection, sample concentrations of N_2O and CO_2 were determined by gas chromatography (GC, Varian CP 3800, Sunnyvale, CA) equipped with electron capture and thermal conductivity detectors (Arnold et al., 2001). Certified standards (Airgas Specialty Gases, Chicago) were run for instrument calibration. Volumetric concentrations were converted to a mass basis using the ideal gas law and molecular weights. For each interval between successive samplings, N_2O and CO_2 production rates were calculated from the time elapsed, headspace concentration and volume, and soil mass. At the end of the incubation, NH_4–N and NO_3–N concentrations were measured again as described above but only for the soils maintained at 55% WFPS. Net N mineralization during the experiment was estimated as the sum of the net ammonification and nitrification calculated as the difference between the final and initial soil NH_4–N and NO_3–N, respectively.

Aerobic Nitrous Oxide Sources

A supplemental 24-h incubation with ^{15}N labeling was used to assess the proportion of N_2O derived from soil NO_3–N and NH_4–N. Two complete sets of microcosms (for a total of 64 with main factors of soil water content [55 or 90% WFPS], N source [FM or UAN], and sampling time [PP and V6]) were prepared as described above but they received additions of ^{15}N-labeled solution by pipette, containing either $(^{15}NH_4)_2SO_4$ or $K^{15}NO_3$ as tracers (Cambridge Isotope Laboratories, Andover, MA) following Stevens et al. (1997), Panek et al. (2000), and Master et al. (2005) with modifications. Labeled solution concentrations were 20% of the native NH_4–N and NO_3–N pools at 98 atom% excess. Among all microcosms, the N additions ranged from 7.95 to 34.0 μg $(^{15}NH_4)_2SO_4$–N and 148 to 238 μg $K^{15}NO_3$–N per microcosm. Microcosms were sealed and headspace gas samples (20 mL) were withdrawn (as described above) in duplicate after 24 h. Duplicate blanks of DW without the ^{15}N-labeled solutions provided the background for ^{15}N-N_2O isotopic composition analysis. Isotopic composition (^{15}N-N_2O atom% excess) was determined in a ThermoFinnigan Delta V continuous-flow III GC/IRMS (Thermo Fisher Scientific, Waltham, MA) after Röckmann et al. (2003). Calculations of N_2O source partitioning followed mixing equations outlined by both Panek et al. (2000) and Holtgrieve et al. (2006) and used measurements of the ^{15}N-N_2O atom% excess, the initial soil extractable NH_4–N and NO_3–N, and the changes in N_2O concentrations in the headspace of the microcosms with no labeling.

It should be noted that implicit in this approach to N_2O source partitioning are the following assumptions: (i) there was uniform labeling of the soil N pools, (ii) the natural abundance for the N_2O from the ambient atmosphere was 0.3663 atom% excess ^{15}N, (iii) the N_2O evolved from the soil was at the same isotopic enrichment as the soil N pools, and (iv) given the use of highly enriched isotopic tracers (98 atom% excess ^{15}N), the loss of ^{15}N from the soil into the headspace did not affect soil enrichment. Under this high isotopic enrichment of the soil N pools, we further assumed that there was no significant $^{15}N/^{14}N$ isotopic fractionation during the nitrification or denitrification processes. Finally, we assumed that the NH_4–N and NO_3–N pools in the microcosms were the only sources of N for nitrification and denitrification, respectively, and that a 24-h incubation was of sufficient duration to observe significant shifts in the isotopic composition due to these processes. This last assumption follows the methods of Laughlin and Stevens (2002), who successfully identified N_2O source partitioning in soils.

Anaerobic Nitrous Oxide Production

A 15-d anaerobic incubation was conducted where Eh, pH, and N_2O evolution were simultaneously monitored for the four factor combinations of N treatment (FM or UAN) and sample collection time (PP or V6) following Yu and Patrick (2004) with modifications. Sixty grams of OVDE soil from three of four field replicates (randomly selected) were packed into 0.25-L polycarbonate centrifuge bottles. Following the preincubation procedure described above, centrifuge bottles received 140 mL of an amendment solution prepared with degassed DW and KNO_3 to a standard level of 50 mg N kg^{-1} soil. The bottles were then tightly capped with modified rubber stoppers containing a platinum Eh combination electrode (Yellow Springs Instrument Co., Yellow Springs, OH), a pH combined glass electrode with an inner AgCl reference (Yellow Springs Instrument Co.), and a gas inlet and outlet with stopcock valves for sampling and for flushing the headspace with pure N_2 at a rate of 15 mL min^{-1}. To ensure the accuracy of pH and Eh measurements, all electrodes were calibrated and deviation among Eh electrodes was also prechecked (<15 mV) after Bohn (1971) and Owens et al. (2005). Throughout the incubation, electrodes were kept in permanent contact with the soil solution. Given that we used a Ag/AgCl reference electrode saturated in KCl, Eh readings were standardized to the H_2 electrode by adding 199 mV (Patrick et al., 1996). All Eh measurements were subsequently normalized to pH 7 using the Nernst equation (−59 mV per pH unit; Bohn, 1971; Yu and Patrick, 2004). The method blank for this incubation was a soilless centrifuge bottle with amendment solution. Room temperature was maintained at 19.8°C.

Headspace gas samples for estimation of N_2O production rates were taken when relatively large decreases in Eh (>15 mV) were observed; in total, 21 samplings were done during the 15-d incubation. At every sampling, three sequential subsamples (20 mL) were collected with a gas-tight syringe at 0, 15, and 30 min after sealing both gas inlet and outlet stopcock valves to close the purging gas. Withdrawn headspace gas was immediately replenished with pure N_2; this dilution effect was accounted for in N_2O production estimates. Between samplings, the headspace was constantly purged with pure N_2. When more than one gas sampling or pH–Eh measurement was done in a day, we reported the daily average. We calculated N_2O production rates using regression coefficients (β_1) derived by fitting curves to the measured headspace gas concentrations, plotted as a function of elapsed time for the three sequential gas sample collections (0–30 min). After Yu and Patrick (2003), curve fitting used simple linear least square regressions and assumed steady-state conditions (i.e., the diffusion rates were not changing with time). Rates were estimated as point measurements where

$$N_2O \text{ production rate} = \frac{\beta_1 \left(V_{hs} + V_{lp} \alpha \right)}{S} \qquad [2]$$

and V_{hs} is the net headspace volume (L), V_{lp} is the liquid phase volume (L), α is the Bunsen absorption coefficient in water (0.632 at 0.1 MPa and 20°C; Tiedje, 1982; Christensen and Tiedje, 1988), and S is the incubated soil mass (kg). The Bunsen coefficient accounts for N_2O dissolved in the liquid phase of the microcosms assuming equilibrium between headspace and liquid phases.

Statistical Analyses

We examined the association among variables using Pearson's product moment correlations (r) or, if the data were not normally distrib-

uted, we used nonparametric Spearman rank order correlations (ρ). We also performed linear regression analyses (PROC REG) among variables. Multicollinearity between potential explanatory variables was assessed using variance inflation factor criteria. Treatment effects on N_2O production rates were evaluated with repeated measures (RM) ANOVA models (PROC MIXED). Although Bartlett tests revealed nonconstant variance for the data, analyses performed on Box–Cox transformed data (PROC TRANSREG with offsetting) did not change the inferential outcomes; thus, untransformed results are reported here. Multiple comparisons among main factors and factor combination means were made with Tukey's honestly significant difference, simulation, or contrast tests ($\alpha = 0.05$ unless otherwise stated; SAS Version 9.1, SAS Institute, Cary, NC).

Table 1. Inorganic N pools and net rates of inorganic N transformation of surface soil (0–15 cm depth) with four treatment combinations including N sources (fall liquid swine manure [FM] or side-dressed urea-NH_4NO_3 [UAN]) and times of field sampling (corn preplanting [PP] or growth stage V6) within an experimental year.† Data are means ± SE, $n = 4$.

Treatment and sampling time	Soil NH_4–N	Soil NO_3–N	Ammonification	Nitrification	Mineralization
	mg kg^{-1}		mg kg^{-1} d^{-1}		
FM-PP	0.74 ± 0.09	11.5 ± 0.59	0.49 ± 0.10	0.98 ± 0.12	1.48 ± 0.12
FM-V6	0.92 ± 0.11	11.3 ± 0.57	0.49 ± 0.14	1.12 ± 0.15	1.61 ± 0.18
UAN-PP	0.74 ± 0.12	10.6 ± 0.73	0.34 ± 0.04	0.88 ± 0.10	1.22 ± 0.11
UAN-V6	0.91 ± 0.14	10.4 ± 0.72	0.34 ± 0.08	1.01 ± 0.13	1.35 ± 0.17

† Net N transformation rates were estimated from change in N pools during aerobic incubation at moderate soil water content (55% water-filled pore space).

RESULTS

Initial soil NH_4–N and NO_3–N pools as well as N transformation rates under aerobic incubation at 55% WFPS identified NO_3^- production as the dominant transformation process. Across both N sources (FM and UAN) and sampling times (PP and V6), the average initial soil NO_3–N concentration was 10.9 mg kg^{-1}, 13 times greater than that of NH_4–N (Table 1); the average nitrification rate was 1.0 mg N kg^{-1} soil d^{-1}, 2.4 times the average ammonification rate ($P < 0.001$). Inorganic soil N pools and N transformations were not affected by either N source or sampling time. A contrast test revealed a significant difference in extractable DOC between FM and UAN soils, with 86.3 and 71.3 mg C kg^{-1} soil, respectively (Table 2). Similarly, FM soils exhibited significantly higher soil organic C and total N contents than UAN soils ($P < 0.05$).

Nitrous Oxide Production in Aerobic Incubation

The N_2O evolution rates from aerobically incubated soil were significantly affected by soil water content ($P = 0.024$; Fig. 1), while neither N source (FM vs. UAN, data not shown) nor sampling time (PP vs. V6, data not shown) were significant factors ($P > 0.05$; Table 3). The time-weighted mean rates of N_2O production were 0.54 and 0.24 μg N_2O kg^{-1} soil h^{-1} at 90 and 55% WFPS, respectively. This treatment difference was most pronounced during the first 96 h of the incubation ($P < 0.05$; Fig. 1). Specifically, N_2O evolution in the second interval (3–24 h) was 4.5 times greater with 90 than 55% WFPS. Both the factor combination and interaction of soil water content and N source were also significant ($P < 0.05$; Table 3). Analysis of mean separation by sampling interval among the four N source × water content treatments identified that the largest differences occurred between FM soils at 55 and 90% WFPS during the second and third incubation intervals with 10-fold or greater N_2O evolution by FM at 90% WFPS (Fig. 2). By the fourth sampling interval, however, the lowest N_2O evolution was observed in FM at 90% WFPS compared with the other N source and water content treatments. In contrast, N_2O production by UAN-treated soils was not altered by water content treatment and was relatively constant throughout the 7-d incubation.

In general, mean CO_2 evolution rates were relatively constant throughout the 7-d aerobic incubation but with differences across some experimental factors (data not shown). The time of field sampling did not affect soil respiration, but both N fertilizer source and WFPS treatment were significant main factors. The CO_2 production rates for 90 and 55% WFPS were 2.52 ± 0.15 and 1.87 ± 0.15 mg CO_2 kg^{-1} soil h^{-1}, respectively (averaged across N source and sampling time, $P = 0.003$); comparison of the two N sources found that CO_2 production rates with FM averaged 2.40 ± 0.15 mg CO_2 kg^{-1} soil h^{-1}, while those for UAN averaged 1.99 ± 0.16 mg CO_2 kg^{-1} soil h^{-1} (averaged across sampling time and WFPS treatment, $0.1 > P > 0.05$).

Proportion of Nitrous Oxide Derived from Nitrate and Ammonium Labeled Pools

A trend toward a greater percentage of evolved N_2O from the soil ^{15}N-labeled NO_3^- (assumed denitrification) vs. NH_4^+ (assumed nitrification) pools was observed in conjunction with 90% compared with 55% WFPS treatments (Fig. 3A). After the 24-h incubation at 90% WFPS, we quantified mean production rates of 0.63 and 0.24 μg N_2O kg^{-1} soil h^{-1} or 72 and 28% produced from the soil NO_3–N and NH_4–N pools, respectively. Likewise, FM-treated soils showed a trend toward greater N_2O evolution via denitrification when compared with UAN treatments (Fig. 3B). Statistical significance (RM ANOVA, $P > 0.05$) in the source pathway was not detected, however, for any of the three main factors (data not shown for sampling time).

Table 2. Selected properties of surface soil (0- 15 cm depth) with four treatment combinations including N sources (fall liquid swine manure [FM] or side-dressed urea-NH_4NO_3 [UAN]) and times of field sampling (corn preplanting [PP] or growth stage V6) within an experimental year. Data are means ± SE, $n = 4$.

Treatment and sampling time	pH	Extractable dissolved organic C	Organic C	Total N	C/N ratio
		mg C kg^{-1} soil	g kg^{-1} soil		
FM-PP	6.4 ± 0.2	82.0 ± 4.9	25.4 ± 1.4	2.03 ± 0.11	13 ± 1
FM-V6	6.3 ± 0.2	90.7 ± 5.5	24.9 ± 1.4	1.99 ± 0.11	13 ± 1
UAN-PP	6.6 ± 0.1	64.2 ± 5.1	22.8 ± 0.6	1.83 ± 0.04	12 ± 0
UAN-V6	6.5 ± 0.1	78.3 ± 6.2	24.3 ± 1.0	1.84 ± 0.09	13 ± 1
Contrast			P > F		
FM vs. UAN	0.112	0.046	0.023	0.014	0.238

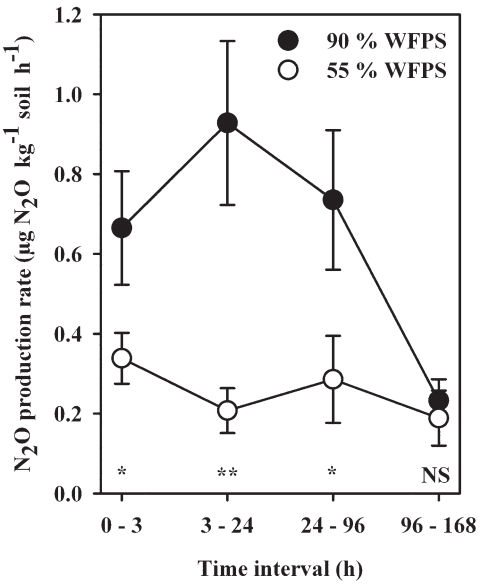

Fig. 1. Nitrous oxide production rates as a function of incubation time interval under aerobic conditions at two soil water contents (55 or 90% water-filled pore space [WFPS]). Within each time interval, * and ** indicate significant differences between soil water content levels at the 0.05 and 0.01 probability levels, respectively; NS indicates not significant at the 0.05 level. Error bars are ± SE, *n* = 48.

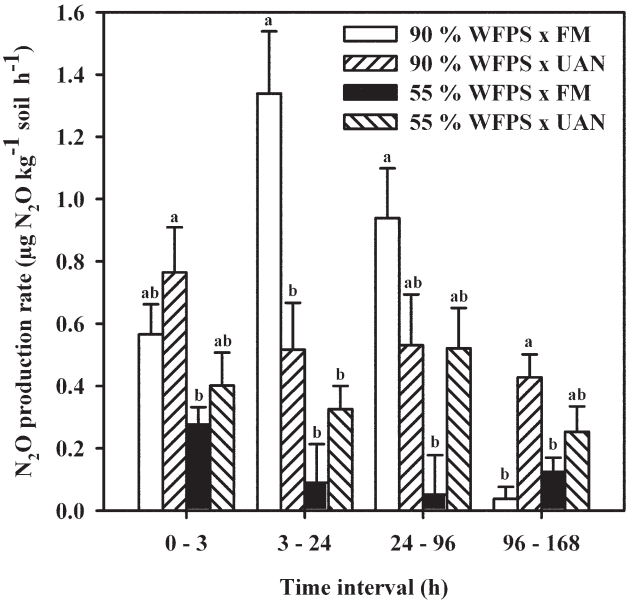

Fig. 2. Nitrous oxide production rates in a 168-h aerobic soil incubation for two-factor combinations: soil water content (90 or 55% water-filled pore space [WFPS]) × N source (fall liquid swine manure [FM] or side-dressed urea-NH_4NO_3 [UAN]). Within each time interval, factor combinations labeled by the same letter are not significantly different based on Tukey's honestly significant difference test (α = 0.05). Error bars are ±SE, *n* = 24.

The proportions of N_2O derived from both soil NO_3–N and NH_4–N labeled pools were highly variable. The isotopic composition (^{15}N-N_2O atom% excess) of gas samples taken from ^{15}N-NH_4^+ labeled microcosms varied from 2.4 to 5.7% with a CV of 17%, while ^{15}N-NO_3^- labeled microcosms ranged from 2.5 up to 11.9% with a CV of 37%. Given that initial soil N concentrations were normalized and corrected for headspace N_2O concentration changes from experimental artifact (described above), the variability of our source-partitioning estimates (CV = 64%) reflect large sample-to-sample variation in both 24-h N_2O evolution (CV = 51%) and 24-h changes in the inorganic N pool sizes (CV = 46 and 20% for NH_4–N and NO_3–N, respectively).

Table 3. Analysis of variance results of N_2O production rates during aerobic incubation with repeated measures (intervals of headspace sampling) for main factors, interactions, and factor combinations of interest, *n* = 384.

Sources of variation	df	*F*	*P* > *F*
ANOVA with main factors and their interactions			
Soil water content (W)	1	6.07	*
N source (N)	1	0.35	NS
Time of field sampling (T)	1	0.03	NS
W × N	1	4.67	*
W × T	1	2.36	NS
N × T	1	0.04	NS
W × N × T	1	0.09	NS
ANOVA with factor combination†			
Soil water content × N source	3	4.12	**

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† ANOVA after removing main factors (soil water content, N fertilizer source, and time of field sampling) and their interactions terms from the model and replacing them with factor combinations of soil water content × N source (90% water-filled pore space [WFPS] × fall liquid swine manure [FM], 90% WFPS × urea–NH_4NO_3 [UAN], 55% WFPS × FM, and 55% WFPS × UAN).

Nitrous Oxide Production Rates during Anaerobic Incubation

With anaerobic incubation, the rate of N_2O evolution sharply increased from time zero (1 h after flooding) to Day 2, when the maximum rates were observed for both N fertilizer sources (Fig. 4A). The maximum rate in FM-treated soils was 336 μg N_2O kg^{-1} soil h^{-1}, which was significantly greater than the 146 μg N_2O kg^{-1} soil h^{-1} observed in the UAN soils (RM ANOVA, $P = 0.018$; simulation test, $P = 0.041$). From Days 2 to 7, N_2O production rates gradually declined back to baseline levels. The

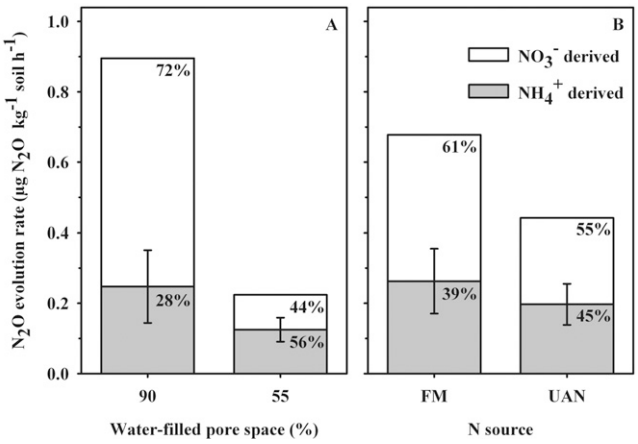

Fig. 3. Soil N_2O evolution rates as produced by isotopically labeled NO3–N and NH4–N pools after 24 h of aerobic incubation with two main factors: (A) soil water content and (B) N source (fall liquid swine manure [FM] or side-dressed urea-NH4NO3 [UAN]). Values (%) inside the columns for N_2O source partitioning in this study. Error bars are ±SE for the N_2O source-partitioning, *n* = 12.

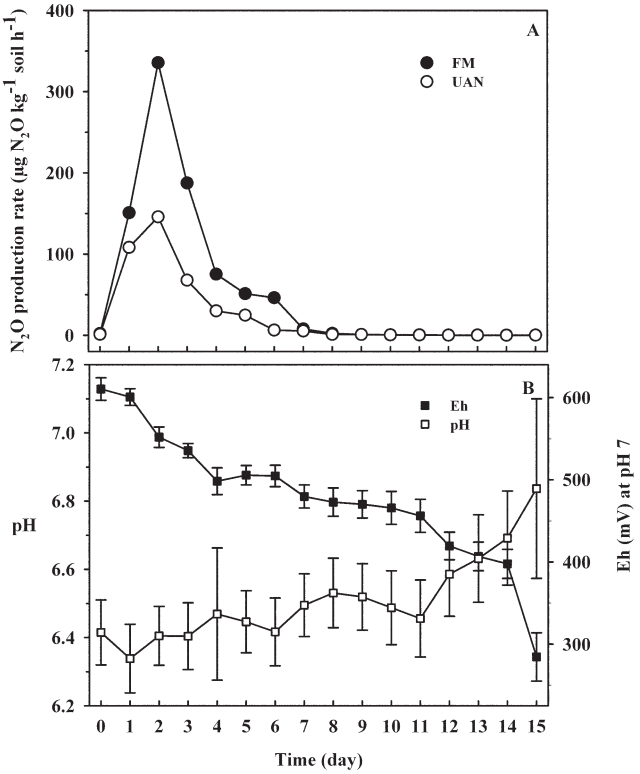

Fig. 4. (A) Nitrous oxide production rates by N source (fall liquid swine manure [FM] or side-dressed urea-NH_4NO_3 [UAN]), and (B) pH and redox potential (Eh) patterns during a 15-d anaerobic soil incubation. Error bars for pH and Eh are ±SE, *n* = 12. Standard errors for N_2O production rates are not shown for clarity.

15-d mean production rate for the FM soil was 53.9 ± 18.9 μg N_2O kg^{-1} soil h^{-1}, which was not significantly different from the 15-d mean of the UAN soil (24.4 ± 9.2 μg N_2O kg^{-1} soil h^{-1}). Mean mass N losses as N_2O in FM and UAN were 25 and 11%, respectively, of the initial 50 mg NO_3–N kg^{-1} soil. Sampling time (PP vs. V6) and its interaction with N source did not significantly affect anaerobic N_2O production rates.

Nitrogen fertilizer source or sampling time had no effect on either Eh or pH during the 15-d incubation. As expected, the mean soil solution Eh dropped relatively sharply from 575 mV on Day 0 to 466 mV on Day 4 (Fig. 4B). Solution pH increased incrementally with decreases in Eh, especially after Day 4, and Eh and pH were significantly correlated ($r = -0.89$, $P < 0.001$). Linear regression found that variation in pH accounted for 78% of the variation in Eh (Eh = 3400 − 453pH) with a highly significant regression coefficient and intercept ($P < 0.001$).

Production rates of N_2O were positively rank correlated with Eh ($\rho = 0.78$, $P < 0.001$); thus the overall, interactive pH–Eh–N_2O pattern was decreasing Eh associated with both increasing pH and declining N_2O production rates (Fig. 5). The Eh ranges of N_2O production were approximately 470 to 575 and 420 to 550 mV for FM and UAN, respectively, and N_2O production rates decreased exponentially as a function of decreasing Eh (Fig. 6). In simple linear regressions for N_2O production as a function of either Eh (N_2O production rates = −0.309 + 0.000936Eh) or pH (N_2O production rates = 3.18 − 0.47pH), regression coefficients (β_1) and intercepts (β_0) for both functions were statistically significant and different from zero ($P < 0.05$) with no significant difference in β_1 between N sources. Multiple regression of

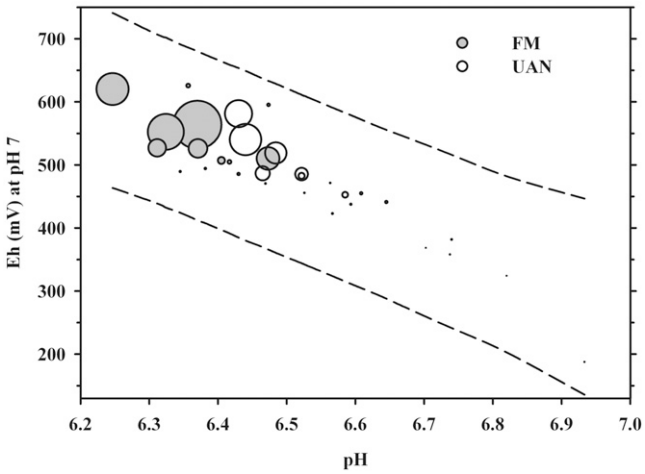

Fig. 5. Nitrous oxide production rates by N source (fall liquid swine manure [FM] or side-dressed urea-NH_4NO_3 [UAN]) as a function of pH–Eh coordinates during an anaerobic incubation. Areas of circles are proportional to production rates (μg N_2O kg^{-1} soil h^{-1}). Confidence interval (α = 0.10) predicts the pH–Eh location of any new observed N2O production rate within the Eh ranges of this study after pooling the two data subsets (FM and UAN); *n* = 6.

N_2O production rate as a function of both pH and Eh, however, identified moderate multicollinearity (variance inflation factor ≥ 4.5) between explanatory variables, suggesting that Eh and pH were similarly predictive and redundant.

DISCUSSION

Soil Water Content and Manure Application Controls on Nitrous Oxide Production

Soil production of N_2O was enhanced by the interaction between high soil water content (90% WFPS) and fall liquid swine manure (Fig. 2). Our study corroborated the previously known role of soil water content as a primary driver of N_2O production (Bremner and Blackmer, 1979; Davidson, 1992; Paul et al., 1993; Holtgrieve et al., 2006) (Fig. 1); however, the synergy between

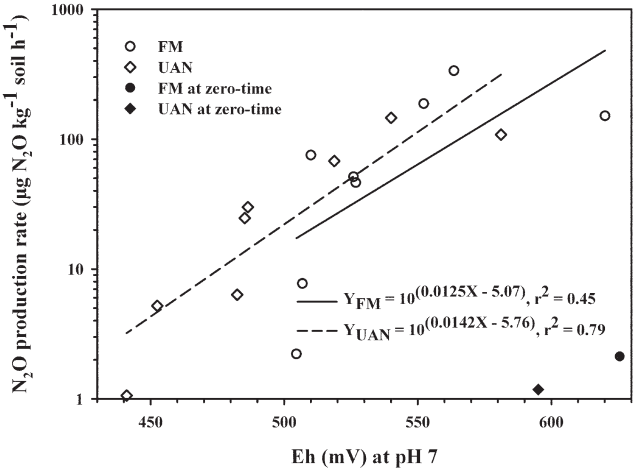

Fig. 6. Nitrous oxide production rates for two N sources (fall liquid swine manure [FM] or side-dressed urea-NH_4NO_3 [UAN]) as a function of soil solution redox potential (Eh) under anaerobic soil incubation. The closed symbols are zero-time data points corresponding to N_2O production rates measured 1 h after the incubation started. Lines are the exponential declines in N_2O production rates with linear decrease in Eh for the two data subsets, but excluding the respective zero-time data points. Points are means of six treatment replicates (microcosms).

annual applications of liquid swine manure and increased soil water content, which resulted in notably greater N_2O production than in UAN soils, has not been previously reported. Manure's addition of water ($\sim$63 m^3 ha^{-1} yr^{-1}) and easily oxidizable C (510 ± 52 kg C ha^{-1} yr^{-1}) was associated with a 1.2-fold greater DOC (Table 2); net soil respiration was higher in the FM than in the UAN treatment and in the 90% than in the 55% WFPS treatments. Furthermore, in a related study, Hernandez-Ramirez et al. (2009a) reported an approximately 50% higher fine particulate organic matter fraction in the FM- vs. UAN-treated soils. Collectively, these results suggest that the FM soils contain more available, labile C than the UAN soils. Increased soil water content restricts O_2 diffusion into and throughout soils (McKenney et al., 2001; Takaya et al., 2003), favoring reducing conditions and therefore denitrification (Bremner, 1997), while manure applications may provide available organic C (Paul and Beauchamp, 1989; Maag and Vinther, 1999), which acts as an e^- donor for heterotrophic denitrifier populations (Tiedje et al., 1984; Azam et al., 2002). Manure addition may further favor denitrification by causing increased microsite anaerobiosis via generalized enhancement of the respiratory activity of the whole soil microbial community (Burford and Bremner, 1975; Granli and Bockman, 1994; Stevens and Laughlin, 2001; Azam et al., 2002; Mørkved et al., 2006).

The temporal pattern of N_2O evolution from the FM soils at 90% WFPS also differed from other treatments by exhibiting a more pronounced pulse early in the incubation followed by an abrupt decline by the incubation's end (Fig. 2). Rapid pulses and abrupt cessation of N_2O production in manured soils following extreme increases in soil moisture have been previously documented (Davidson, 1992; Paul et al., 1993). In our study, the N_2O production decline in the 90% WFPS FM treatment after 96 h was probably due to depletion of the soil NO_3–N pool by denitrification (Paul et al., 1993) and concurrent anoxia, leading to cessation of NH_4^+ nitrification or to a favoring of the last step of the denitrification process (N_2O to N_2 gas) (Khalil et al., 2004). In a related field study, Hernandez-Ramirez et al. (2009b) associated fluctuating rainfall and consequent soil rewetting cycles with sudden, high pulse N_2O emissions in corn fields. Such stronger, shorter lived pulses from manured soils than from soils receiving other N fertilizers suggest the need for intensification of sampling schemes when measuring field-scale N_2O emissions with higher frequency and intensity of wet–dry cycles.

Partitioning Nitrous Oxide Sources: Nitrification vs. Denitrification

The tendency for denitrification to be the predominant pathway for N_2O production in our soils when the water content was high (Fig. 3A) is in keeping with the hypothesis of a synergistic interaction between high soil moisture and manure application driving large pulse emissions. The differences among treatments in source partitioning were not significant, however, and this is probably at least a partial reflection of a high degree of field replicate variability that was further propagated in the laboratory. Specifically, we used soil aggregates in our incubations; Khalil et al. (2004) suggested that nonuniform diffusion of ^{15}N labeling solutions among and within aggregates may confound source-partitioning results. Certainly others have encountered similar high levels of measurement variability when attempting to partition N_2O pathways. Studies on soil aggregates (Khalil et al., 2004), urine patches (Carter, 2007), soil moisture along rainfall gradients in forests (Holtgrieve et al., 2006), and soil moisture fluctuation in irrigated wheat (*Tritucum aestivum* L.; Panek et al., 2000) all report high variability in source-partitioning measurements as a function of the main experimental factor. Thus, resolving current disagreements among the results of field studies measuring N_2O flux (reviewed by Matson, 1997) and improving associated local, regional, and global N_2O budgets requires a better understanding of experimental artifacts and important covariates than has been achieved in research conducted to date.

Regardless of the lack of significance in the main factor effect on source partitioning, our results clearly demonstrate that both pathways contributed to N_2O production under all the experimental conditions we examined. Coexistence of nitrification and denitrification in soils with adequate drainage is spatially possible as a function of microsite diversity in aeration status within soil aggregates (Parkin, 1987; Renault and Stengel, 1994) and temporally possible in conjunction with extreme rainfall events and soil moisture cycling (Skiba et al., 1993; Panek et al., 2000). Coupled nitrification–denitrification has been previously observed in manured soils (Nielsen et al., 1996).

Redox Potential and Nitrous Oxide Production

Under anaerobic conditions, N_2O production was greater in fall-manured soils than in soil receiving UAN, and, in keeping with the general results of previous studies (Letey et al.,1981; Khalil et al., 2001; Yu and Patrick, 2004), we found that most N_2O was produced within the first 4 d of our incubation (Fig. 4A). Our observation that enhanced, early, short-lived N_2O production coincided with a sharp drop in Eh (Fig. 4B) was previously reported (Flessa and Beese, 1995; Yu and Patrick, 2004). Given that NO_3^- was initially abundant (50 mg N kg^{-1} soil) and flooding and flushing with N_2 excluded O_2 from the incubators, we infer that N_2O production became C limited early in our incubation; hence, higher responses occurred in manured soils relative to soils receiving UAN. Soon after incubation initiation, the greater availability of organic C (extractable DOC; Table 2) serving as electron donors (Burford and Bremner, 1975) in our manured soils may have enhanced pulse N_2O production via denitrification (Azam et al., 2002) under moderate reducing conditions (400–600 mV). Later in the incubation period (>8 d), as soils with both manure and UAN underwent further reduction (<400 mV), electrons would be generally more abundant, favoring the reduction of N_2O to N_2 (Murakami et al., 1987). In a late stage of our incubation, N_2O production might also have been substrate limited, as the initial NO_3^- supply had been utilized.

The extent to which our results agree with or differ from previously published results can be explained by several critical experimental factors. Our range of N_2O production rates (up to 336 and 145 μg N_2O kg^{-1} soil h^{-1} in FM and UAN, respectively) closely agreed with values reported by Yu et al. (2001), particularly for their two well-drained soils cropped with wheat and corn, respectively. Rates two- to three-fold higher than ours, however, have been reported in paddy rice (*Oryza sativa* L.) fields (Yu and Patrick, 2003, 2004) and in tropical, acidic (pH in H_2O of 3.8–4.4) soils under moderate drainage conditions (Khalil et al., 2001). While paddy soils may have inherently high-

er anaerobic N_2O emission potential due to their seasonal flooding history (Tsuruta et al., 1997; Kirk, 2004), the Yu and Patrick (2003, 2004) studies also followed a substantively different experimental protocol. Specifically, they did not use preincubation, they amended their soils with rice straw, and they agitated the soil solutions throughout incubation. In our study, substantial N_2O may have been emitted during preincubation, while a lack of residue amendment and agitation may have resulted in relatively lower C availability. In the anaerobic incubations by Khalil et al. (2001), tropical soils with high natural acidity may initially emit greater N_2O than observed in our study as a consequence of inhibition of the last step of the denitrification process (N_2O reductase; Firestone et al., 1980) and a consequent increased N_2O/N_2 ratio (Burford and Bremner, 1975; Firestone, 1982).

The N fertilizer sources caused minimal differences in the Eh ranges of N_2O production (Fig. 6). In general, N_2O production in our experiment took place in a narrow portion of the Eh range (420–575 mV) that can be observed in soils as they proceed from well-aerated (400–700 mV) to permanently flooded (approximately −300 mV) conditions. Our Eh ranges for N_2O production are in general agreement with Yu et al. (2001, 2007) and Yu and Patrick (2003, 2004). Furthermore, N source did not correspond with differences in pH–Eh–N_2O patterns. The major N_2O production rates in FM took place at a slightly lower pH than for UAN, however, which closely agrees with the somewhat lower native pH in soils receiving manure than UAN as an N source (Table 2). Although pH and Eh were statistically redundant predictor variables for N_2O production rates, the correlation between these two variables may enhance our ability to identify Eh prediction intervals for new observations that occur with altered soil pH.

CONCLUSIONS

Our soils, cropped with continuous corn for 9 yr, were net N_2O producers under all assessed conditions, but with different degrees of response to changes in both water content and Eh status. Our results suggest that both abrupt soil rewetting events (i.e., from 45 to 90% WFPS) and sharp drops in Eh shortly after flooding can trigger abundant, short-lived soil N_2O production but to a greater extent in manured soils than in soils receiving synthetic N fertilizer (i.e., UAN). Irrespective of N fertilizer source, this dynamic, episodic N_2O production phase appeared to be confined to a window of 0 to 4 d immediately after the occurrence of either extreme increases in soil water content or flooding events.

While our study suggests that the most probable cause of enhanced N_2O production in manured soil is the increased organic C availability, other potential causes for this response may exist and need to be examined. For example, residual antibiotics incorporated in soils by repeated manure additions might inhibit microbial synthesis of N_2O reductase, and, hence, potentially favor N_2O emissions. Further studies on N_2O production in manured soils are also needed as the variability of both animal diets and manure characteristics can be expected to confound results and limit the strength of general conclusions.

For field conditions, our results imply that the shape, size, and duration of pulse N_2O emissions could be driven by the magnitude and incidence of soil denitrification resulting from abrupt, frequent soil rewetting events that occur in conjunction with continual manure additions. Therefore, an increased use of manure in Corn Belt soils subjected to extreme fluctuations in soil moisture (e.g., low landscape positions or floodplains) may further raise regional N_2O emissions; optimal manure management to mitigate N_2O emissions may entail manure application patterns that avoid locations prone to recurrent saturation. Furthermore, as denitrification appeared to be the predominant pathway during intensive, episodic N_2O production events in our soils, a rational, well-timed use of NH_4^+–based fertilizers (e.g., liquid manure and partly UAN) vs. the nitric form would perhaps diminish soil N_2O emissions. Our study also indicated, however, that a coupled nitrification– denitrification process at moderate soil moisture (i.e., 55% WFPS) can produce more constant, modest N_2O emissions in keeping with observed field flux measurements. Thus, further research should encompass a much wider variety of N fertilizer forms, placement, and application timing. Future observations should include field, soil aggregate, or microsite scales in contrasting cropping systems and be made in a manner that permits assessment of the influence of plant growth (e.g., root exudates) on the quantity and sources of N_2O production.

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