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NITRAPYRIN DELAYS DENITRIFICATION ON MANURED SOILS

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Excessive application of manure may lead to NO_3^- leaching to groundwater and fluxes of nitrogen oxides to the atmosphere. Nitrification inhibitors such as nitrapyrin (N-serve; 2-chloro-6-(trichloromethyl)pyridine) may help to conserve manure N in the root zone by limiting NO_3^- supply to denitrifiers. The objective of this study was to test the effect of nitrapyrin on the timing and amounts of denitrification and N_2O fluxes in manured soils under conditions favorable to denitrification. The study consisted of a laboratory incubation of soils under aerobic conditions. Three agricultural soils and a sand were included in the study, all with high moisture and initial NO_3^--N content. Each soil received three treatments: 1) manure plus nitrapyrin (190 mg nitrapyrin kg^{-1} soil), 2) manure alone (0.15 mg manure N g^{-1}), and 3) soil alone controls. Nitrapyrin was mixed with the manure before addition to soil. Destructive samplings were carried out weekly for 10 weeks. At each sampling, soil-extractable mineral N, microbial biomass N, denitrified N, and N_2O fluxes were measured. Nitrapyrin was effective in reducing nitrification, thus enhancing soil NH_4^+-N accumulation and possibly reducing the potential for nitrate leaching. Although nitrapyrin was effective in reducing nitrification in manured soils, the effect on soil mineral N and potential N supply to plants varied across soils because of the interaction between nitrification, denitrification, and N immobilization. Neither manure nor nitrapyrin consistently affected net N mineralization in the five different soil types. Microbial N immobilization and/or denitrification were strong sinks of N that reduced net N mineralization. Nitrapyrin did not affect cumulative denitrification, but some soils had delayed denitrification when nitrapyrin was added. Manure had a strong effect on N_2O fluxes and denitrified N in some soils, but the effects of nitrapyrin were inconsistent. Nitrapyrin significantly reduced microbial N immobilization in two agricultural soils. The observed reductions in microbial biomass may affect N availability beyond the time frame of the experiment because less N will be available for remineralization. (Soil Science 2005;170:350–359)

Key words: Manure, nitrapyrin, denitrification, nitrous oxide, nitrification.

DAIRY manure is an important source of N for crops. However, in the springtime manure is often applied when the soil is very moist because of the necessity to free up space in holding tanks and storage facilities. Because of this, more than 30% of the total N applied may be nitrified and denitrified resulting in nitrogen losses to the atmosphere and groundwater (Calderón et al., 2004; Harter et al., 2002; Lowrance et al., 1998).

Nitrapyrin hinders oxidation of NH_4^+-N by chemoautotrophic nitrifiers (Lopez et al., 2003) and thus has the potential to reduce N_2O emissions from aerobic soils (Bremner, 1997). However, the effects of nitrification inhibitors are not consistent for different soils and environmental conditions (McCarty and Bremner, 1990).

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Nitrapyrin has the potential to increase crop yields in manured soils by conserving manure N (McCarty and Bremner, 1990; McCormick et al., 1984). However, the positive effect on yield is not universal, since some studies have failed to show increased harvests in soils receiving nitrapyrin and manure (Randall et al., 1999; Schmitt et al., 1995). These unpredictable effects may be due to the fact that nitrapyrin may affect denitrification as well as nitrification.

Nitrapyrin has the potential to indirectly hinder denitrification by curtailing the nitrate supply to denitrifiers. Nitrapyrin may have the most beneficial impact in manured waterlogged soils, such as fields that have been left fallow during the winter in wet climates and are planted in the spring when soil moisture is high. In such systems, the manure may exacerbate the denitrification N losses because of the readily available C added with the manure, so nitrapyrin could be used to curtail NO_3^- supply to denitrifiers. However, previous studies have shown contrasting effects of nitrification inhibitors on denitrification on soils receiving mineral fertilizers. Notton and Watson (1979) observed that nitrapyrin stimulates denitrification in sandy soils, whereas others have shown that nitrapyrin applied at rates up to 50 mg kg^{-1} may reduce soil denitrification (Mills, 1984; Mills and McElhannon, 1983; Ronaghi et al., 1993).

An important question is whether C or NO_3^- limits denitrification in soils that receive both manure and nitrapyrin. Nitrapyrin may vary in the effect on soil denitrification according to C availability as well as nitrapyrin dosage (Bremner and Yeomans, 1986), indicating that the effect of nitrapyrin on manured soils deserves further scrutiny. Although many studies have examined the effect of nitrapyrin on soil denitrification, few studies have tested the effect of nitrapyrin on the denitrification timing and magnitude.

Data are scarce regarding the effect of nitrapyrin on microbial N immobilization in manured soils. Nitrapyrin addition usually results in an increase in soil NH_4^+-N, which is a preferred form of mineral N by soil microbes (McCarty and Bremner, 1992). Incorporation into microbial biomass, as determined by microbial biomass N, is an important variable for sustainable agriculture, since it represents an N pool that will eventually be available to crops. In manured soils, N immobilization will depend on the timing and availability of C as well as N.

In this study, we tested the effect of nitrapyrin on denitrification-prone soils receiving manure. We measured microbial N immobilization, denitrified N, soil mineral N dynamics, and soil N_2O gas fluxes. The experiment consisted of a laboratory incubation of four different soils of high moisture and initial NO_3^- content. Each soil was incubated for 10 weeks with manure, with manure and nitrapyrin, or with nothing added and was destructively sampled to carry out the measurements.

MATERIALS AND METHODS

All soils used for this experiment were collected from 0- to 30-cm depth and sieved (4.76 mm) to exclude rocks and coarse plant material. The sieved soils were stored at room temperature (10 to 16 weeks) before the start of the experiment, resulting in high initial soil NO_3^--N concentrations. The initial gravimetric moisture content of the soils was 24.3% for USA, 14.3% for USC, 16.8% for USS, and 13.9% for GCG. Initial soil organic matter and particle size distribution were analyzed by the Maryland Cooperative Extension Laboratory in College Park and are summarized in Table 1. The organic matter was analyzed using the method detailed by Pella (1990), whereas the particle size analysis was done with the hydrometer method (Gee and Bauder, 1986).

Before the incubation, the initial soil total C and total N contents were analyzed with an Elementar Variomax CNS (Hanau, Germany). Three agricultural soils (GCG, USA, and USC)

TABLE 1

Soil characteristics measured before the start of the incubation for four different soils (USA, USC, USS, and GCG)

	pH	O.M.	total C	total N	C/N	Sand %	Silt %	Clay %
GCG	4.3	42.0	23.90	1.96	12.17	58	22	20
USA	6.1	40.0	23.56	2.55	9.25	22	51	27
USC	5.7	40.0	24.13	2.42	9.98	60	27	13
USS	5.2	15.0	8.67	0.83	10.43	72	14	14

Total C, total N, and O.M. are in mg Kg^{-1}.

were included. GCG is a Meckesville silt loam soil (fine-loamy, mixed, active, mesic typic Fragiudults) collected from a soil under vegetable production in Garrett County, MD. USA is a Christiana silt loam soil (typic Normudults) collected from an alfalfa field at the Beltsville Agricultural Research Center (BARC) in Beltsville MD. USC is a Beltsville silt loam soil (fine-loamy, mixed, mesic typic Fragiudults) collected at BARC from a corn field. USS is a sandy soil collected near Beaver Dam Creek in BARC.

The manure used in this experiment was obtained from a milking herd of 4-year-old confined Holsteins on a protein-rich diet at the USDA Dairy facility in Beltsville, MD. The manure was 15.5% dry matter, 0.47% N, and 7.54% C on a fresh weight basis. The NO_3^--N content of the manure was 0.03 g kg^{-1}, and the NH_4^+-N content was 0.78 g kg^{-1} on a fresh weight basis. We have shown in previous experiments that this manure, with a C:N ratio of 16, leads to relatively high denitrification and N immobilization upon incubation in soil (Calderón et al., 2004). To homogenize the manure, fresh manure was ground by blending at high speed with dry ice (1:2 manure:dry ice, vol:vol). The manure/dry ice mix was placed at 4 °C overnight to allow for the sublimation of the CO_2.

The experiment included three treatments: 1) the manured (M) treatment received manure at a rate of 0.15 mg manure N g^{-1} dry soil and 2.4 mg manure C g^{-1} dry soil, 2) the nitrapyrin plus manure treatment (NM) received the same amount of manure as the M treatment, and nitrapyrin was mixed in with the manure to achieve a rate of 190 mg kg^{-1} soil of the active ingredient, 3) the control treatment (C) received no manure or nitrapyrin, but water was added to compensate for the moisture added with the manure in the other two treatments. The microcosms were prepared by packing soil (50 g dry basis) in plastic beakers to a density of 1 g cm^{-3}. Water was then added to each soil to achieve a uniform gravimetric moisture of 33.8% across soils. Previous studies with manured soils show that moisture contents of 16.8% (25.5% water-filled pore space) is favorable for relatively high denitrification N losses (Calderón et al., 2004). We chose 33.8% soil moisture contents for this experiment to simulate conditions where moisture and soil NO_3^- make the soils highly susceptible to N losses through denitrification. However, this soil moisture content is below field capacity, and we can expect that farmers will occasionally apply manure to soils under similar conditions. In the manured treatments, the manure (1.6 g fresh weight) was pipetted to the top of the packed soil.

Each microcosm was placed in a sealed jar (3.8 L) to enable gas flux sampling as well as to minimize ammonia volatilization. This incubation method has been used previously to allow for headspace gas sampling during aerobic incubations of manured soils (Calderón et al., 2004). The jars are a closed system where N losses through ammonia volatilization are minimized because any ammonia in the headspace is available for reabsorption in soil. The microcosms were aerated weekly by opening the jars for 30 min. All measurements including soil mineral N, MBN, as well as N_2O fluxes and denitrification measurements were carried out on the same jars. The jars were destructively sampled at time zero and weeks 1, 3, 6, and 10. One week before each destructive sampling, 40 mL of acetylene was added to each jar to measure denitrified N. Each sample received the acetylene treatment only once, for a 1-week period before the destructive sampling. The role of the acetylene was to block N_2O reductases and provoke all the N reduced by denitrifiers to accumulate as N_2O, which gives an estimate of denitrification rates (Yoshinari and Knowles, 1976). Although low concentrations of acetylene also inhibit NO_3^- production from nitrification, our experiment design allowed for an initial uninterrupted period in which the natural interaction of nitrification-denitrification took place. Thereafter, there was a final period in which the acetylene block was used to measure denitrification. For example, the samples incubated for 10 weeks had a 9-week initial period in which no acetylene was present, followed by the measurement of denitrification with the soil NO_3^- present at the time.

At each destructive sampling, samples of soil (10 g) were obtained from each microcosm for extractable mineral N analysis. The samples were shaken in 50 mL of 2*M* KCl for 30 min on a wrist-action shaker. The sediments were allowed to settle 12 h at 4 °C, and the supernatants were stored in 20 mL screw-capped vials at 4 °C for no more than 24 h. Before the analysis, the extracts were filtered (Fisherbrand Serum Filter System, I.B. model, Fisher Scientific, Pittsburgh, PA) and then analyzed for NH_4^+-N, NO_2^--N, and NO_3^--N with an AutoAnalyzer 3 (Bran+Luebbe, Hamburg, Germany). Before the experiment, duplicate samples of the manure and manure plus nitrapyrin were analyzed for extractable mineral N, using the above procedure, with a ratio of 50 mL 2M KCl g^{-1} manure. The net N mineralization for the incubation was calculated as the final minus initial concentration of mineral N. Mineral N being the sum of soil NH_4^+-N, NO_2^--N, and NO_3^--N. The additional ammonified manure N for each microcosm was calculated as (NM − M)/added manure N, where NM is NH_4^+-N accumulation in the microcosms receiving manure and nitrapyrin, and M is the NH_4^+-N accumulation in the microcosms receiving manure alone.

The N_2O content of the jar headspace was determined 24 h, and 1 week, 3 weeks, 6 weeks, and 10 weeks after the start of the experiment. Gas samples (2 mL) were obtained with a syringe and injected into 22-mL vials with butyl rubber septa that were previously flushed with He. The N_2O concentration in the gas samples was measured with a Shimadzu GC-ECD (GC-8A, Shimadzu Scientific Instruments Inc., Columbia, MD) equipped with a Tekmar 7000 HT headspace autosampler (Tekmar Co., Cincinnati, OH). Hereafter, the N_2O fluxes from microcosms without acetylene will be the N_2O flux, whereas the N_2O fluxes from microcosms with added acetylene will be regarded as denitrified N. The cumulative denitrified N (acetylene added) and cumulative N_2O fluxes were calculated as the area under the curve using the trapezoid formula (Pruessner et al., 2003).

At week 10, four soil samples (20 g) from each manure × soil combination were analyzed for microbial biomass N (MBN), using the method of Horwath and Paul (1994). Briefly, the samples were fumigated with chloroform for 24 h, then incubated for 1 week at room temperature. At the end of the 1-week incubation, the soils were extracted with 2M KCl and the extracts analyzed for mineral N as detailed above. The NH_3 flush from the fumigated sample was multiplied by 1.47 to calculate the MBN (Horwath and Paul, 1994).

The statistical analyses were done separately for each soil, since each soil was treated as a separate experiment. To determine treatment and time effects, we performed analysis of variance (ANOVA) with the Proc GLM procedure of SAS version 8.2 (Cary, NC). Mean separations were determined with the least significant difference (LSD) test.

RESULTS

Nitrapyrin was effective in blocking nitrification for the entire 10-week period in all soils, as shown by the increase in soil NH_4^+-N in the NM treatment in all soils (Fig. 1). Nitrification activity in the M treatment did not ensue until 1 week after the start of the incubation. All M soils increased in their NH_4^+-N content during the first week of the incubation, then started a long gradual decline (Fig. 1).

Initial NO_3^--N was high for all soils, ranging from 32.8 mg kg^{-1} in the USS to 84.1 mg kg^{-1} in the USA (Fig. 2). The NM treatment consistently had the lowest final concentration in NO_3^--N for the incubation across all soils, whereas the C treatment had positive nitrification in all soils. The dynamics of soil NO_3^--N of the M treatment varied according to the soil. The M soils in the GCG and USA treatments had a net increase in

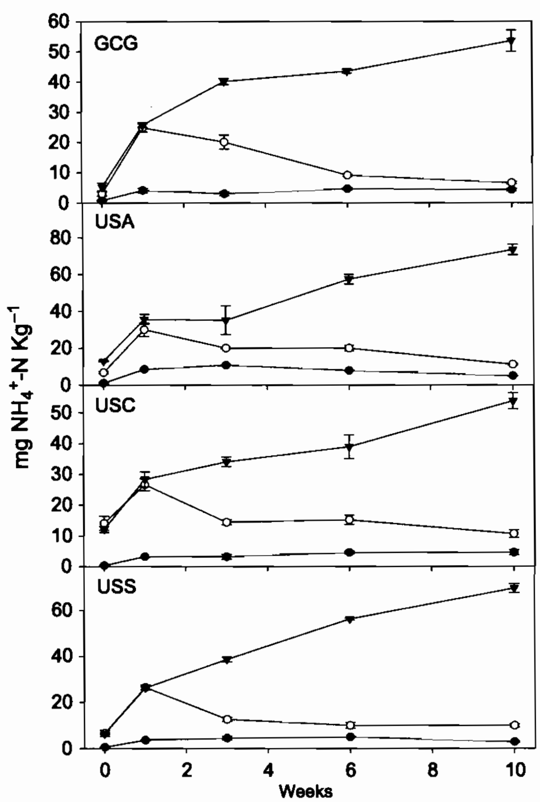

Fig. 1. Soil NH_4^+-N concentration in the control treatment (•), manured treatment (○), and manure plus nitrapyrin treatment (▼). The name of each soil is indicated in the upper left-hand corner of each graph. Each point is the mean (n = 4). Error bars are standard error of the mean. All manure treatment effects and week effects were significant according to ANOVA (P < 0.01).

NO_3^--N during the incubation (Fig. 2). In contrast to GCG and USA, the NO_3^--N in the M treatment of the USC and USS soils declined within the first week of the incubation and stayed below the initial level for the rest of the experiment (Fig. 2). The sharp decline in soil NO_3^--N observed in all treated soils during the first week of the incubation corresponds with a period of high denitrification activity (Fig. 3).

The additional ammonified manure N varied between soils: GCG had 29.78%, USC had 30.09%, USA had 37.44%, and USS had 39.83%. In all three agricultural soils, nitrapyrin increased the ratio of NH_4^+-N to NO_3^--N at the end of the incubation, causing NH_4^+-N to be the main form of soil mineral N instead of NO_3^--N. At week 10, the NH_4^+-N to NO_3^--N ratio in the M treatment ranged from 0.08 (GCG) to 0.71 (USC), whereas in the NM treatment, the ratio ranged from 1.55 (GCG) to

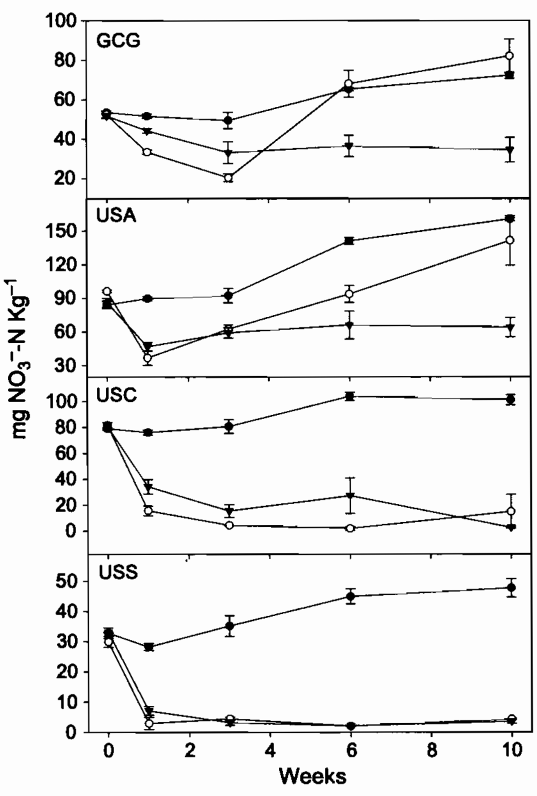

Fig. 2. Soil NO_3^--N concentration in the control treatment (•), manured treatment (○), and manure plus nitrapyrin treatment (▼). The name of each soil is indicated in the upper left-hand corner of each graph. Each point is the mean (n = 4). Error bars are standard error of the mean. All manure treatment effects, week effects, and week × treatment effects were significant according to ANOVA ($P < 0.01$).

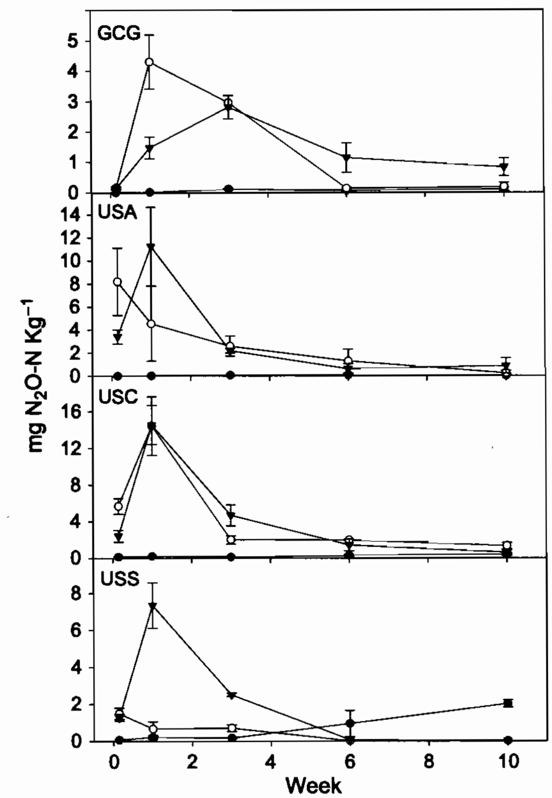

Fig. 3. Denitrified N fluxes from the control treatment (•), manured treatment (○), and manure plus nitrapyrin treatment (▼), as measured by acetylene block. The name of each soil is indicated in the upper left-hand corner of each graph. Each point is the mean (n = 4). Error bars are standard error of the mean. Data are on a per-week basis. All manure treatment effects and week effects were significant according to ANOVA ($P < 0.01$).

20.57 (USC). NO_2^--N was low in all soils staying below 0.7 mg kg^{-1} (data not shown). There were no consistent differences in NO_2^--N between treatments, but small increases in NO_2^--N in the manured soils were observed during the first week of the incubation in the USA and USC soils.

The N mineralization values ranged widely across soils and treatments (Table 2). The addition of manure in the M and NM treatments did not always result in increased N mineralization. In the USC soil, the M and NM treatments had negative N mineralization, whereas the control had positive N mineralization. Likewise, the addition of nitrapyrin did not have a consistent effect on N mineralization across the different manured soils. For the GCG, and USA soils, there was no statistical difference between the M and NM treatments. In the USC and USS

TABLE 2

N mineralization during the 10-week incubation of soils incubated alone (C), with manure (M), or with manure plus nitrapyrin (NM)

	N mineralization†		
	C	M	NM
GCG	22.25ª (1.09)	33.22ª (7.55)	31.04ª (8.47)
USA	79.86ª (4.30)	49.39ª (22.20)	38.23ª (6.98)
USC	26.29ª (4.32)	−70.31ᵇ (13.33)	−36.59ᶜ (5.60)
USS	17.29ª (2.47)	−22.18ᵇ (1.68)	33.50ᶜ (2.63)

Four different soils are shown (USA, USC, USS, and GCG). Within each row, values not sharing a letter are significantly different ($p < 0.05$) according to *t*-test.
†Units are mg N kg^{-1} soil. Values are mean (SEM). n = 4.

soils, the N mineralization was significantly higher in the NM relative to the M treatment.

In the three agricultural soils, average cumulative N_2O fluxes were higher in the M and NM treatments relative to the control, although the difference was statistically significant only for the USA soil (Table 3). The M and NM cumulative N_2O fluxes were statistically indistinguishable from each other in all soils. As with the denitrified N, the highest N_2O fluxes from the manured agricultural soils occurred between weeks 0 and 4 of the incubation (Fig. 4).

In all three agricultural soils, the NM and M treatments had higher denitrification N losses than the C treatment during the first 2 to 4 weeks of the experiment (Fig. 3). As a result, the cumulative denitrified N was higher for the N and NM treatments relative to the control in all soils except USS, where only the NM treatment was higher that the control (Table 3). However, the cumulative denitrified N in the M and NM treatments was statistically indistinguishable in the three agricultural soils. For GCG and USA, the peaks in denitrification activity were delayed up to 2 weeks relative to the M treatment (Fig. 3).

Manure addition had a positive effect on the MBN of most soils. However, the effects of the M and NM treatments on MBN were not consistent across the different soils (Table 4). In the USC and GCG soils, nitrapyrin annulled the positive effect of manure on MBN, whereas in the USA treatment, the M and NM treatments were statistically indistinguishable and both higher than the C treatment. The USS soils had an opposing trend in MBN, since the manured treatments had less MBN than the C at the end of the incubation.

DISCUSSION

In this experiment, we have shown that adding nitrapyrin while manuring moist soils may affect the timing of the denitrification flux by as much as 2 weeks. This effect, however, was not uniform across soils and further studies should explore why. Our results also suggest that adding nitrapyrin together with manure causes no measurable change in total soil N_2O fluxes relative to adding manure alone. Nitrapyrin did curtail nitrification in manured soils but did not reduce denitrified N after soil application. Nitrapyrin increased net N mineralization relative to the manure alone treatment in two soils. This shows that in some instances, nitrapyrin may increase N supply to crops when applied with manure. Adding nitrapyrin to manured soils may prevent nitrogen losses through leaching from manured soils, since nitrapyrin addition results in NH_4^+-N as the predominant form of mineral N rather than NO_3^--N. However, losses of N through NH_4^+-N volatilization become a concern because of the marked accumulation of NH_4^+-N in soils receiving manure and nitrapyrin. However, at the neutral to acidic soil pH range in this study, ammonia volatilization should not be an important factor.

In our study, soil NH_4^+-N in the manure only treatment increased during the first week in all soils, showing an initial lag in nitrification. In the absence of nitrapyrin, manured soils suffered

TABLE 3

Cumulative denitrified N (acetylene added) and N_2O fluxes (no acetylene) of soils incubated alone (C), with manure (M), or with manure plus nitrapyrin (NM)

	N_2O flux†		
	C	M	NM
GCG	0.11ª (0.02)	2.05ª (0.35)	3.16ª (1.80)
USA	0.62ª (0.42)	3.71ᵇ (0.42)	4.80ᵇ (1.11)
USC	3.57ª (1.37)	7.14ª (1.54)	6.32ª (0.63)
USS	2.90ª (1.48)	7.96ᵇ (1.74)	4.32ªᵇ (0.74)
	Denitrified N†		
	C	M	NM
GCG	0.60ª (0.19)	10.49ᵇ (1.47)	9.10 ᵇ (4.23)
USA	1.02ª (0.42)	16.54ᵇ (4.59)	28.56ᵇ (4.83)
USC	4.19ª (2.33)	26.64ᵇ (5.43)	32.10ᵇ (1.90)
USS	4.53ª (1.86)	5.94ª (2.27)	13.54ᵇ (2.78)

Four different soils are shown (USA, USC, USS, and GCG). Values not sharing a letter within each row are significantly different ($p < 0.05$) according to *t*-test.
†Units are mg N_2O-N kg^{-1} soil. Values are mean (SEM). n = 4.

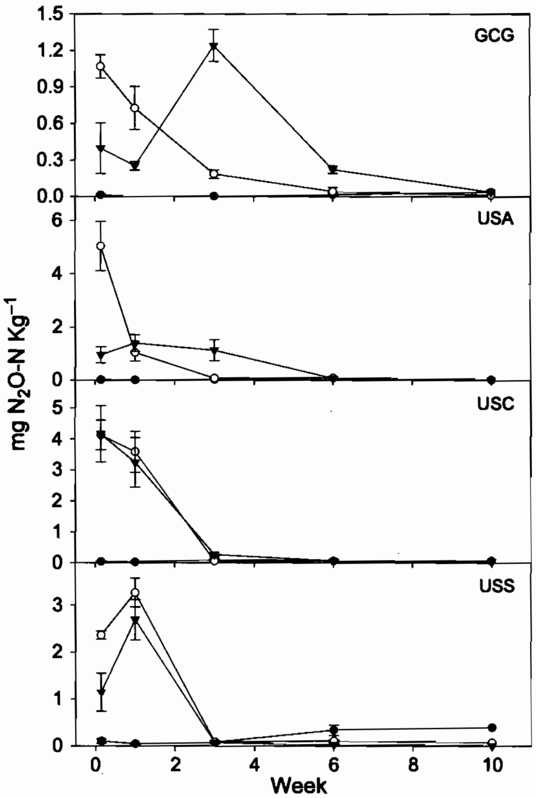

Fig. 4. N_2O-N fluxes from the control treatment (•), manured treatment (○), and manure plus nitrapyrin treatment (▼). The name of each soil is indicated in the upper left-hand corner of each graph. Each point is the mean (n = 4). Error bars are standard error of the mean. Data are on a per-week basis. All manure treatment effects and week effects were significant according to ANOVA ($P < 0.01$).

a net decline of NH_4^+-N that started after the first week of the incubation, showing that the moisture or pH conditions during the incubation did not preclude nitrifier activity. It has been shown that nitrapyrin may delay the onset of nitrifying activity in manured soils (Sawyer et al., 1990). The effect of nitrapyrin may be long-lived, with residual effects lasting through entire growth seasons (Shi and Norton, 2000). In this experiment, the NH_4^+-N accumulation shows that nitrapyrin was effective in blocking nitrification for up to 10 weeks of incubation in all four soils. Previous studies have shown that the intensity of the nitrapyrin effect is dependent on environmental conditions (Lopez et al., 2003; Randall et al., 1999). In our study, we also show that soils varied in the extent of NH_4^+-N accumulation when nitrapyrin was included with the manure, as illustrated with the variation in additional ammonified manure N.

In this study, the manure and nitrapyrin effects on N mineralization were inconsistent across soils. Others have shown that potentially mineralizable N in manured soils is affected by soil type (Eneji et al., 2002). In the USA and USC soils, the large increases in NH_4^+-N in the NM treatment were offset by declines in soil NO_3^--N, resulting in net N mineralization values that were below those of the controls. Manure may have supplied available C, NO_3^--N, and NH_4^+-N and could have favored denitrifier populations (Azam et al., 2002). Thus, the reductions between N mineralization in the M and NM treatments in USA and USC soils may be explained primarily by the increased loss of N through denitrification and secondarily by N immobilization by soil microbes.

Previous studies have shown that nitrapyrin addition can result in increased N recovery by crops (Freney et al., 1993). In our study, two of the soils did receive a benefit from the addition of nitrapyrin when the NM soils are compared with the M soils. In the USC and USS soils, mineralizable N was higher in the NM relative to the M treatments, indicating that nitrapyrin may increase N supply to crops in some manured soils. The USC and USS manured soils suffered large declines in soil NO_3^--N throughout the incubation, possibly due to the large denitrification NO_3^- sink (see above). Yet, the blockage of nitrification caused by the nitrapyrin in the USC and USS soils allowed for the accumulation of NH_4^+-N. This could prevent the eventual denitrification of some mineralized manure N in the USC and USS soils.

Nitrification and denitrification are important contributors to N_2O emissions from soil

TABLE 4

Microbial Biomass N† (MBN) of soils incubated alone (C), with manure (M), or with manure plus nitrapyrin (NM)

	C	M	NM
GCG	16.8^{ab} (1.4)	23.4^{b} (2.1)	10.6b^{a} (3.6)
USA	47.8^{a} (2.6)	65.3^{b} (3.7)	64.0^{b} (2.7)
USC	30.9^{a} (1.4)	46.7^{b} (1.6)	33.9^{a} (2.0)
USS	22.1^{a} (1.2)	14.9^{b} (2.4)	12.9^{b} (1.8)

Four different soils are shown (USA, USC, USS, and GCG). Values not sharing a letter are significantly different ($p > 0.05$) according to t-test.

†Units are mg N kg^{-1} soil. Values are mean (SEM). n = 4.

(Bremner, 1997). In this study, despite the marked effect of nitrapyrin on soil mineral N dynamics, addition of nitrapyrin did not have a discernible effect on the total N_2O fluxes of the different manured soils included in the experiment. However, manure increased the average N_2O fluxes relative to control soils, although this effect was not statistically significant in all soils.

We hypothesize that denitrification was strongly C limited in all control agricultural soils. This is illustrated by the USC soil, which had high NO_3^--N in the C treatment but also had much less denitrification activity than the manured M and NM treatments. Manure contains significant amounts of readily available C, and manure addition may increase soil denitrification activity (Calderón et al., 2004; Lowrance et al., 1998; Tenuta et al., 2000). In this study, all manured treatments in the three agricultural soils had significantly higher cumulative denitrification than the nonmanured controls. Denitrification was a noticeable sink of NO_3^--N in manured soils as shown by the decline in soil NO_3^--N in all NM microcosms. However, the manure alone treatments in the GCG and USA soils did show increases in soil NO_3^--N toward the end of the experiment, indicating that nitrate demand by denitrifiers declined after week 6 in these soils, and/or mineralization-nitrification of manure N increased toward the end of the experiment in the absence of nitrapyrin.

Other studies simulating injection of diluted manure slurry have shown that nitrification inhibitors do not consistently affect denitrification in soils amended with dairy manure (Comfort et al., 1990). Bremner and Yeomans (1986) found that in soils receiving no manure, nitrapyrin can retard denitrification when applied at a rate of 50 mg kg^{-1} but can stimulate denitrification when applied at a rate of 100 mg kg^{-1}. Our results show that nitrapyrin, when applied at the relatively high rate of 190 mg kg^{-1}, may delay denitrification activity in some manured soils while not affecting the total amounts of N denitrified. In contrast, Comfort et al. (1990) did not find an effect of nitrapyrin on the timing of the denitrification flux in a manured silt loam, supporting our results that the effects of nitrapyrin in manured soils are not uniform across soil types. In agricultural soils, total N losses through denitrification in the NM treatment were not different from the manure alone treatments when averaged over several weeks. The initially high NO_3^--N availability in some M and NM soils rendered nitrification less important for the adequate NO_3^--N supply to denitrifiers. However, the delay in peak denitrification in the GCG and USA treatments suggests that in these two soils, denitrification may rely on nitrification of manure NH_4^+-N in microsites rather than on the NO_3^--N supplied initially by the soil. It is possible that in soils with low initial soil NO_3 levels, nitrapyrin may reduce denitrification losses relative to soils receiving manure alone.

To our knowledge, this is the first study that measured differences in microbial biomass N between soils receiving nitrapyrin and manure and soils receiving manure alone. Our results show that nitrapyrin can reduce the MBN of some manured soils. When comparing NM and M treatments, nitrapyrin decreased MBN in two of the soils, whereas no statistical differences were observed in the rest. This may have important management implications, since the microbial biomass is a reservoir of N that could be remineralized in subsequent growing seasons. Soil microbes generally prefer NH_4^+ to NO_3^- as N sources for growth (McCarty and Bremner, 1992). The observed negative effect of nitrapyrin on the MBN of the GCG and USC soils occurred despite the high amounts of NH_4^+-N throughout most of the incubation in the NM soils. The aerobic status of the microcosms may also have played a role in limiting MBN in some instances. For example, the negative effect of manure on the MBN of the USS treatment may be explained by an increase in anaerobicity exacerbated by the addition of manure C, which resulted in the decline in the mostly aerobic microflora that existed in the sand before the experiment.

Our results show that the soil responses to nitrapyrin vary with soil type. This suggests that it would be beneficial to test soils before the addition of nitrapyrin and manure to better predict the effects of the nitrification inhibitor. The variation in responses to nitrapyrin may be caused by soil physical, chemical, and microbiological factors. Hendrikson and Keeney (1979) showed that several soil properties including soil organic matter may affect nitrapyrin decomposition in soil.

In conclusion, this study shows that nitrapyrin can affect microbial N immobilization as well as the timing of the denitrification flux after manure application. These results have implications for sustainable agriculture as well as for scientific research. This experiment also shows that adding nitrapyrin when manuring moist soils has the potential to reduce N leaching from soils by reducing the relative amount of NO_3^- in the soil-extractable mineral N. However, no consistent benefit in N_2O emissions to the atmosphere or N mineralization were found under the high moisture conditions used in the experiment. Nevertheless, some soils did accumulate more extractable mineral N when nitrapyrin was added together with the manure, warranting further studies to determine what factors modulate the effect of nitrapyrin on manure N mineralization. Future studies should address how the nitrapyrin dosage, mode of application, as well as a variety of soil conditions determine the benefits or disadvantages of nitrapyrin addition to manured soils.

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