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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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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, (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

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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 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.

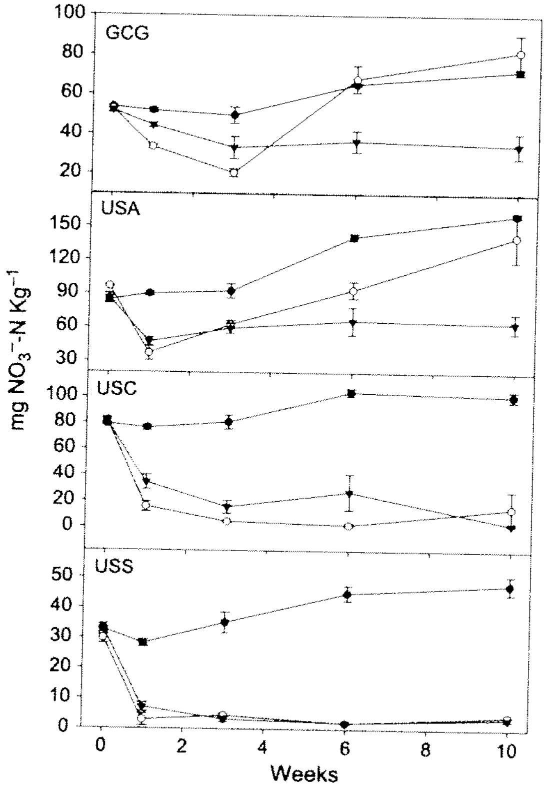

Fig. 2. Soil NO_3^--N concentration in the control treatment (●), manured treatment (○), and manure plus nitrapyrin treatment (▼). The name of each soil is indicated in the upper left-hand corner of each graph. Each point is the mean ($n = 4$). Error bars are standard error of the mean. All manure treatment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

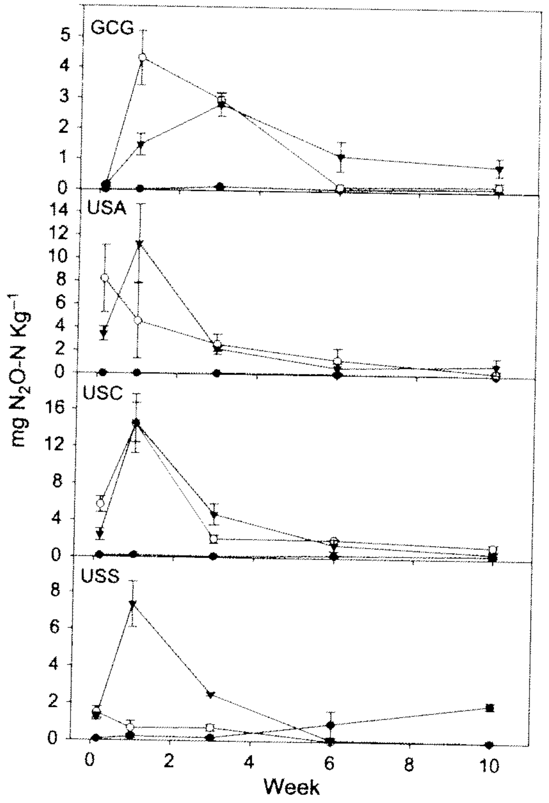

Fig. 3. Denitrified N fluxes from the control treatment (●), manured treatment (○), and manure plus nitrapyrin treatment (▼), as measured by acetylene block. The name of each soil is indicated in the upper left-hand corner of each graph. Each point is the mean ($n = 4$). Error bars are standard error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

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