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Influence of Nitrification Inhibitor on Transformations and Recovery of N in Grassland Soil

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Abstract: The recovery of both mineralized and applied N in soil-plant system is low throughout the world. Leaching, denitrification and NH_3 volatilization are thought to be the major N loss mechanisms responsible for low N recovery. The aim of this study was to evaluate the effect of nitrification inhibitor nitrapyrin on N transformations, recovery and to prevent formation of NO_3^- -N which could be lost by leaching and denitrification. Intact soil cores were collected from a grassland field, incubated under aerobic conditions at 20°C with 70 percent WFPS. Amendments used were plus or minus nitrapyrin. Nitrapyrin was used as a nitrification inhibitor. The fate of mineralized and added NH_4^+ , accumulation of NO_3^- -N and apparent recovery of applied N were examined during the study. In the absence of nitrapyrin, more than 60 percent of the NO_3^- -N initially present was lost within 42 days period. The apparent recovery of applied N decreased significantly and more than 50 percent of applied N being lost from mineral pool. Accumulation of $60 \text{ mg } \text{NO}_3^- \text{ N kg}^{-1}$ soil in the surface 0-2.5 cm layer indicated that nitrification was an active process in the system. Addition of nitrapyrin decreased and delayed the extent of NH_4^+ disappearance, inhibited nitrification by approximately 73 percent and improved N recovery by 36 percent.

Key words: Grassland, nitrification inhibitor, nitrapyrin, N recovery, nitrification

Introduction

The yield potential of crops and productivity of forage species are often constrained by the supply of nitrogen (N). To overcome this limitation, N fertilizers are commonly applied to grasses, important food and cash crops. It has been suggested (Garret, 1993) that at least half the productivity of modern temperate grassland systems is due to the fertilizer N input. Supply of fertilizer N not only increases primary plant and subsequent animal production, but also allowed farmers a good deal of flexibility in the management of their enterprises. According to Munro and Davies (1973), in all temperate grassland regions N supply and pasture production are closely linked and yield is generally proportional to the supply of fertilizer N.

Nitrogen fertilizer is an expensive input and in many trials in Australia less than 50 percent of applied N is recovered in the crop and soil with the remainder being lost (Frenay *et al.*, 1993; Rochester *et al.*, 1996). The apparent recovery of N applied to grass grown in the United Kingdom varies widely, but a value in the range of 50-70 percent is frequently observed (Morrison *et al.*, 1980). A recovery of less than 45 percent from a single application of N fertilizer was reported by White (1985) due to abnormal weather conditions causing a large leaching or gaseous loss of N. Under wet conditions very low recoveries of applied N under 40 percent were reported by Van der Meer (1982). Fertilizer N recovery in plants and soil under upland conditions in India rarely exceeds 50 percent (Patra *et al.*, 1992). Increasing the amount of N applied does not increase the amount of N available to the crops, because the fertilizer N is lost before it can be assimilated by the crop (Frenay *et al.*, 1992).

Most of the fertilizer N applied to soils is largely in the form of NH_4^+ or NH_3 producing compounds such as urea and usually oxidized quite rapidly to nitrate. The NO_3^- formed through nitrification of added N by soil microorganisms is susceptible to loss by leaching and denitrification and may contribute to environmental and surface and ground waters pollution. Thus nitrification acts as a key process in determining fertilizer use efficiency by crops as well as N losses from soils. The adverse effects of oxidation of NH_4^+ to NO_3^- by the nitrifying microorganisms in soil have stimulated research to find compounds that can be used to retard nitrification of fertilizer N. Nitrification inhibitors have been used in agriculture to improve fertilizer efficiency and crop yields and minimize denitrification and/or leaching losses of NO_3^- , by delaying the biological oxidation of NH_4^+ to NO_2^- and thus reduces the availability of NO_3^- and maintaining applied fertilizer N in the soil as NH_4^+ -N (McTaggart *et al.*, 1997). A number of compounds have been shown to inhibit the nitrification process in soil

and the most commonly used is nitrapyrin (2-chloro-6-trichloromethyl) pyridine.

The aim of this investigation was to determine the effect of nitrification inhibitor nitrapyrin on N transformations and its impact on recovery of applied N in grassland soil under laboratory conditions.

Materials and Methods

The site, which was almost level and in permanent pasture, situated adjacent to Blaendolau playing fields about 1 km from Soil Science Unit, University of Wales Aberystwyth, UK. The soil was a Dystric Eutrochrept and classified as Conway series (Grid Ref. SN 597804) as described in detail in an earlier study (Abbasi and Adams, 1998).

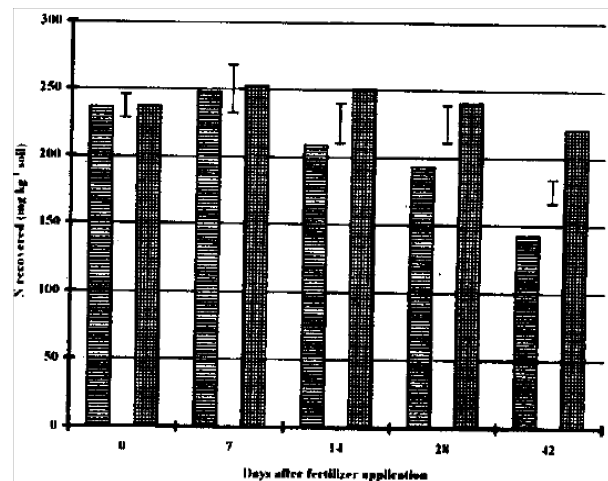


Fig. 1: Effect of nitrification inhibitor nitrapyrin on the recovery of N applied to intact soil cores during 42 days incubation. Vertical bars indicate least significant difference (LSD) between with and without nitrapyrin treatments at different time intervals.

Some physical and chemical characteristics of the soil used are presented in Table 1. Seventy intact soil cores (7.0 cm dia. x 7.5 cm deep) were collected according to the method described in Abbasi and Adams (1998). The moisture content of soil cores

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Table 1: Some chemical and physical properties of the soil used during the study Depth (cm)

Depth (cm)	pH (1:1 water)	OM (%)	N (%)	Bulk density (g cm ⁻³)	Particle size distribution (%)		
					sand	silt	clay
0-2.5	5.3	9.7	0.37	0.98	36	40	24
2.5-5.0	5.1	7.7	0.34	1.03	12	54	24
5.0-7.5	5.0	6.9	0.32	1.15	12	56	35
mean	5.1	8.1	0.34	1.05	20	50	30

Table 2: Effect of nitrification inhibitor nitrapyrin on the concentration of NH₄⁺-N and NO₃⁻-N in control soil without added N at different depth over a period of 42 days

Time (days)	without Nitrapyrin Depth (cm)				with Nitrapyrin Depth (cm)				
	-2.5	2.5-5.0.	5.0-7.5	LSD	-2.5	2.5-5.0.	5.0-7.5	LSD	
NH₄⁺-N mg kg⁻¹ soil					NH₄⁺-N mg kg⁻¹ soil				
0	24	15	8	4.1	20	9	8	9.5	
	19	12	8	18.1	57	15	13	28.0	
14	29	18	11	18.2	77	15	9	33.4	
28	30	16	13	18.5	106	22	8	13.3	
42	62	18	10	19.7	110	28	11	18.6	
LSD	23.3	11.7	4.6	-	24.5	8.8	6.9	-	
NO₃⁻-N mg kg⁻¹ soil					NO₃⁻-N mg kg⁻¹ soil				
0	2	1	1	-	1	1	1	-	
7	1	0	0	-	0	2	0	-	
14	6	0	1	-	3	1	1	-	
28	3	1	1	-	1	1	0	-	
42	15	8	5	-	1	2	2	-	
LSD	6.3	4.2	3.1	-	-	-	-	-	

*LSD represent the least significant difference (p≤0.051 appropriate to comparisons between the values within each column and rows

Table 3: Effect of nitrification inhibitor nitrapyrin on the concentration of NH₄⁺-N and NO₃⁻-N in NH₄⁺ added soil at different depth over a period of 42 days

Time (days)	without Nitrapyrin Depth (cm)				with Nitrapyrin Depth (cm)				
	-2.5	2.5-5.0.	5.0-7.5	LSD	-2.5	2.5-5.0.	5.0-7.5	LSD	
NH₄⁺-N mg kg⁻¹ soil					NH₄⁺-N mg kg⁻¹ soil				
0	673	24	7	33.6	676	23	8	36.4	
7	698	19	15	71.2	710	22	18	78.1	
14	536	43	15	46.8	670	59	15	70.6	
28	432	29	10	54.9	564	110	20	79.1	
42	277	35	12	10.4	517	106	22	23.1	
LSD	68.5	24.1	21.1	-	93.7	24.9	12.6	-	
NO₃⁻-N mg kg⁻¹ soil					NO₃⁻-N mg kg⁻¹ soil				
0	4	1	0	4.3	4	1	0	2.0	
7	7	1	2	5.7	6	0	2	8.5	
14	24	2	1	24.1	4	4	0	5.8	
28	62	36	12	38.0	13	12	3	10.4	
42	50	38	16	18.5	8	8	3	2.2	
LSD	29.3	15.3	10.5	-	8.2	6.8	2.2	-	

*LSD represent the least significant difference (p<0.05) appropriate to comparisons between the values within each column and rows

held within brass sampling rings, was adjusted at 70 percent WFPS by saturating and then equilibrating at -5 kPa over 3 days on a tension table. The soil cores were arranged into four treatments i.e. T₁ = control, T₂ = control with nitrapyrin, T₃ = NH₄⁺ amended cores without nitrapyrin and T₅ = NH₄⁺ amended cores with nitrapyrin. Five ml solution of [(NH₄)₂HPO₄] at a rate equivalent to 200 mg N kg⁻¹ (on moist soil basis) and 2 ml solution of 2-chloro-6- (trichloromethyl) pyridine known commonly as nitrapyrin at a rate of 100 mg kg⁻¹ soil were uniformly applied on cores surface. Cores were placed in an incubator at 20°C in the dark for 42 days. Small amount of distilled water was added after every two days if necessary to restore its initial weight.

Triplicate cores from each treatment were removed from the incubator at the beginning and after 7, 14, 28 and 42 days of

incubation and sectioned into 0-2.5, 2.5-5.0 and 5.0-7.5 cm layers. Each section was broken down separately and mixed thoroughly. The concentration of total mineral N and NH₄⁺-N in soil samples were determined by shaking 40 g sub-samples of fresh soil for 1 h with 200 ml of 2 M KCl followed by steam distillation with MgO and Oevarada's alloy and titration (Keeney and Nelson, 1982). NO₃⁻-N was determined by subtracting NH₄⁺-N from total mineral N. Total N in the soil was determined following the Kjeldahl method of Bremner and Mulvaney (1982). Organic matter was estimated as weight loss on ignition at 400°C (Ball, 1964). Soil pH was measured in a water suspension (1:1 v/v). Particle size distribution was determined by the pipette method described by Avery and Bascomb (1974). Soil moisture content was determined gravimetrically after drying of sub-samples at 105°C for 24 h. Water-filled pore space

(WFPS), synonymous with relative moisture content, was calculated following the method of Aulakh and Bijay-Singh (1996). $WFPS = [(gravimetric\ water\ content \times soil\ bulk\ density / total\ soil\ porosity)]$, where total soil porosity $= [1 - (soil\ bulk\ density / 2.65)]$ and 2.65 was the assumed particle density of soil. All data in Table 2, 3 and Fig. 1 were statistically analysed by multifactorial analysis of variance (ANOVA) using the software package StatGraphics Manugistics Inc (1992).

Results and Discussion

Effect of inhibitor on mineral N of the control soil: Initially at the start of the experiment the concentration of NH_4^+ in the control soil without nitrapyrin was in the range of 8-24 mg kg^{-1} soil with greater concentration in the surface 0-2.5 cm layer. Table 2 indicated that with one exception at day 42, NH_4^+ did not show significant changes ($p < 0.05$) either with time or depth. Thus changes in NH_4^+ -N did not show any consistent pattern in control soil throughout the incubation. It was, however, clear that the surface 0-2.5 cm contained 2-3 times more NH_4^+ -N than the other two layers. The higher level of NH_4^+ -N in the surface layer attributed the variation in mineralization potential within depth which may be due to more labile organic N compounds, a higher level of microbial biomass and their activity and decrease organic C with depth which would limit the microbial populations from mineralizing organic N (Bolton *et al.*, 1990). In the soil to which nitrapyrin was added, a significant ($p \leq 0.05$) increase in NH_4^+ concentration was observed both with time and depth (Table 2). Values were generally higher in the 0-2.5 cm layer compared to the 2.5-5.7 cm layers. Over 42 days the concentration of NH_4^+ -N in the 0-2.5 cm layer of nitrapyrin added soil was 2 fold greater than the soil without nitrapyrin. This showed that in the soil without nitrapyrin a substantial amount of NH_4^+ -N resulted from the mineralization of organic N disappeared/lost from the system as observed in our previous study (Abbasi and Adams, 1998). The pattern of changes in NO_3^- -N in the control soil with and without nitrapyrin was similar to that of NH_4^+ -N (Table 2). Results indicated that the changes in NO_3^- -N during incubation were dependent mainly on the presence and availability of NH_4^+ . A maximum of 15 mg NO_3^- -N kg^{-1} was accumulated in the surface 0-2.5 cm of soil without nitrapyrin indicating that the soil at 70 percent WFPS exhibited a substantial nitrification potential. However, even within this moisture range, chances of loss of accumulated NO_3^- -N through denitrification or leaching are there so, determination of exact rate of nitrification under such conditions became difficult. In the soil that received the inhibitor, NO_3^- -N was never exceeded 2 mg kg^{-1} . The lower NO_3^- concentration in T_2 compared to the T_1 , treatment showed that inhibitor was effective in inhibiting the oxidation of NH_4^+ which increased the level of NH_4^+ in the soil.

Effect of inhibitor on mineral N of N added soil: In the soil without nitrapyrin, NH_4^+ -N was lost continuously from the surface 0-2.5 cm layer from the time of application till the end (Table 3). About 60 percent of NH_4^+ -N initially present was lost within 42 days of incubation. In lower layers concentration of NH_4^+ -N increased to some extent but the difference within incubation periods was not significant. Similar results were also found in our previous study (Abbasi and Adams, 1998). However, the extent of loss of NH_4^+ -N in this study was less because of the differences in moisture level between the two experiments. Results revealed that when moisture contents were above 80 percent WFPS, 84 percent of NH_4^+ initially present was lost from the mineral N pool compared with 60 percent loss in the present study having 70 percent WFPS. This showed that moisture is an important factor in N recovery and loss. In the soil cores to which nitrapyrin was added, the concentration of NH_4^+ in the 0-2.5 cm layer decreased slowly with time but the rate and amount of NH_4^+ disappearance was much smaller and a substantial amount was left till the end of incubation. Most of the NH_4^+ disappeared from the surface layer moved down to the lower layers as a significant ($p > 0.005$)

increase in NH_4^+ -N was found in 2.5-7.5 cm layers. Comparison between the two treatments revealed that nitrapyrin had a significant effect on the persistence and availability of NH_4^+ , NH_4^+ concentration of the inhibitor treatment was significantly ($p < 0.005$) greater than that in the treatment without nitrapyrin. In the soil without nitrapyrin, after taking into account the NH_4^+ -N in control soil, 55 percent of the added NH_4^+ -N was unaccounted for at the end of study relative to 10 percent of the nitrapyrin added soil. Results showed that nitrapyrin proved to be very effective in retarding oxidation of NH_4^+ and thus allowed N to remain in the soil for longer period for crop use. These results were in agreement with previous findings of Freney *et al.* (1992); Chen *et al.* (1994) and Abbasi and Adams (1998).

The concentration of NO_3^- -N in the soil without nitrapyrin increased slowly during the first 14 days of incubation (Table 2). Thereafter, concentration increased significantly ($p \leq 0.005$) and a maximum of 60 mg NO_3^- -N kg^{-1} was accumulated at day 28 in the surface 0-2.5 cm. The increase in NO_3^- -N in surface layer of N added soil compared with the control one indicated that nitrification was occurring. The pattern of increase in NO_3^- -N in lower layers was similar but the rate and amount of increase was significantly less compared with the surface 0-2.5 cm. The effect of depth on nitrification can be anticipated, since the supply of NH_4^+ from organic N mineralization and from added N decreased with depth and depth may also affect nitrifiers population (Hadas *et al.*, 1986). The accumulation of NO_3^- -N in the soil of the inhibitor treatment was significantly ($p \leq 0.05$) lower than that in the soil without nitrapyrin and a maximum of 13 mg NO_3^- -N kg was found in the 0-2.5 cm at day 28. On an average the NO_3^- concentration of the NH_4^+ added soil without nitrapyrin was greater at least 5-fold relative to soil with nitrapyrin. It revealed that nitrapyrin became effective in blocking biological oxidation of NH_4^+ to NO_2^- and thus reduces the availability of NO_3^- -N and maintaining applied fertilizer N in the soil as NH_4^+ -N.

Effect of inhibitor on recovery of applied N: Data regarding the recovery of applied N (Fig. 1) showed that in the soil cores without nitrapyrin added N was lost continuously after day 7 till the end of incubation. By that time, 53 percent of the N initially present was lost from the system. The rate of N loss was greatest at day 14 and 42, equivalent to more than 1 $kg^{-1} day^{-1}$. By contrast, 17 percent of the applied N was unaccounted for from the soil cores treated with nitrapyrin. Taking these losses into account, over 42 days about 83 percent of the added N was recovered in the nitrapyrin treatment compared with 47 percent recovery in soil cores without nitrapyrin. Nitrapyrin increased the apparent recovery of applied N and of the total amount of N applied 36 percent more N was recovered relative to soil without nitrapyrin. Previous work had shown that ammonia volatilization and leaching were not important pathways for N loss (Abbasi *et al.*, 1997). Consequently it was concluded that major losses were due to denitrification of accumulated NO_3^- -N after nitrification of added or mineralized NH_4^+ and that the use of nitrification inhibitor reduced NO_3^- production and therefore denitrification.

Nitrogen is usually the plant nutrient most limiting in arid and semiarid ecosystems that have a limited input of N and must rely on a controlled and efficient N cycle for continued plant growth and ecosystem function (Bolton *et al.*, 1990). The fact that nitrapyrin conserved NH_4^+ for a considerable time and improved the recovery of applied N showed that it can be used and practiced in Pakistan agriculture as well. A substantial area of Pakistan is under rice where denitrification and NO_3^- movement and leaching might be major mechanism of N loss. Similarly, under normal agriculture, use of heavy irrigation after fertilizer application created conditions conducive to denitrification. Under such circumstances use of nitrification inhibitors can be proved very effective to increase the fertilizer use efficiency as well as improved the apparent recovery of applied N both in crops and soil. However, in Pakistan because

of high soil pH and temperature, reduced nitrification could also increase N losses via NH₃ volatilization. To obtain full benefits from inhibitors, techniques are needed to predict the optimum time and methods for their application.

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