Spanish Journal of Agricultural Research (2004) 2(4), 588-596

Effects of DMPP on the growth and chemical composition of ryegrass *(Lolium perenne* L.) raised on calcareous soil

E. Guillaumes and J. M. Villar*

Department of Environmental and Soil Science. University of Lleida. Rovira Roure, 191. 25198 Lleida. Spain

Abstract

This paper reports a 406 day outdoor experiment (performed in pots) to determine the influence of the new nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP), when added to pig slurry (PS), on the growth and chemical composition of ryegrass. Pots containing a loamy, calcareous soil were treated with either no PS or 73.7, 147.3 or 221 cm^3 pot^{-1}, with or without DMPP, prior to seeding with ryegrass. The greatest quantity of above ground dry matter was obtained with the highest dose of PS + DMPP (36.3 g pot^{-1}) – 7.4% greater than that obtained for the same treatment without DMPP, and 46.1% greater than with the no PS treatment. The plants treated with the high and medium doses of PS + DMPP absorbed 70% of total N during the first quarter of the experimental season (104 days). Without DMPP, N uptakes were 55.7% and 63% for the high and medium treatments respectively. The inhibition of nitrification with DMPP increased agronomic efficiency and reduced N leaching by 17%.

Key words: pig slurry, nitrification inhibitor.

Resumen

Efecto del DMPP sobre el crecimiento y la composición química del raigrás *(Lolium perenne* L.) en un suelo calcáreo

Se llevó a cabo un experimento de 406 días en macetas para evaluar el nuevo inhibidor de la nitrificación, 3,4-dimetilpirazol fosfato (DMPP), añadido a purines de cerdo. Se utilizaron macetas que contenían tierra franca calcárea que fueron sujetas a los siguientes tratamientos: sin purín, 73,7; 147,3 y 221 cm^3 de purín por maceta, todas con o sin tratamiento de DMPP. A los 18 días las macetas fueron sembradas con *Lolium perenne* L. El mayor rendimiento (36,3 g $maceta^{-1}$) se obtuvo para el tratamiento con la dosis superior de purín y DMPP, siendo un 7,4% superior al mismo tratamiento sin inhibidor y un 46,1% superior al tratamiento control. Las plantas tratadas con dosis alta y mediana, más el DMPP, absorbieron el 70% del total del N durante la primera fase del experimento (104 días) mientras que sin inhibidor absorbieron el 55,3 y el 62% respectivamente. Se observó una reducción significativa del 17% en el N lixiviado en los tratamientos sin cultivo al aplicar DMPP. El inhibidor aumentó significativamente la eficiencia agronómica del purín (g materia seca g^{-1} N aplicado).

Palabras clave: purín de cerdo, inhibidor de la nitrificación, experimento con macetas.

Introduction

Catalonia has a total agricultural area of 0.94 million hectares, 62% of which is destined to arable crops (more than 60% is given over to barley, wheat, maize and alfalfa) (DARP, 1999). As in most areas of Europe, the agricultural soils of this region of NE Spain have received large quantities of animal manure, mainly pig slurry (PS). These increase the soil concentration of N, P, Zn and Cu, but in some cases the N and P surplus per hectare can be large, increasing N losses by leaching, runoff, volatilisation and denitrification, and increasing P losses by the first two of these phenomena.

In Catalonia, nearly 12 million pigs are sacrificed every year (DARP, 1999). There are around 11,800 pig production farms, almost half of which (4,665) are located in nitrate vulnerable zones (NVZs) (DARP, 1999). Based on Catalonian agricultural census data, it is estimated that about 9 million m^3 of PS are produced every year, most of which is applied to the land. The present capacity of PS treatment plants is approximately 0.6 million m^3 per year (7% of total production). PS has been recognised as a valuable

* Corresponding author: jmvillar@macs.udl.es
Received: 13-02-04; Accepted: 07-10-04.

source of N and P for crops, but the amounts applied are sometimes greater than those that might be considered appropriate agronomic doses (20-50 and even up to 200 m^3 ha^{-1} $year^{-1}$). The Catalonian autonomous government has adopted several measures to prevent the overuse of animal manures in line with European legislation. In 1998 a Code of Best Management Practices was passed (DOGC, 1998), and in 2001 a law was brought into force that obliges farmers to draw up N management plans (DOGC, 2001). The calendar for PS application in Catalonia depends on the crop. For example, in maize fields, PS is spread between February (two months before the sowing period) and July. The amount of N applied as PS is limited to 210 kg N ha^{-1} per year (30 to 40 m^3 ha^{-1}).

There is strong social pressure to reduce environmental pollution, although farmers also want to diminish the use of nitrates in order to reduce costs. In recent years, technological innovations in the fertiliser industry have sought to produce «eco-fertilisers» that are more compatible with the natural environment. Nitrification inhibitors (NI) are compounds that delay the bacterial oxidation of ammonium ions to nitrite (and subsequently to nitrate) by suppressing the activity of *Nitrosomonas* spp. in the soil (Prasad and Power, 1995; Trenkel, 1997; Zerulla *et al*., 2001). NI have been used to increase yields and to reduce nitrate leaching in several crops (Malzer and Randall, 1985; Frye *et al*., 1989; Malzer *et al*., 1989; Walters and Malzer, 1990; Serna *et al*., 1994; Corré and Zwart, 1995; Davies and Williams, 1995; Martin *et al*., 1997; Trenkel, 1997; Ball-Coelho and Roy, 1999; Pasda *et al*., 2001).

The addition of NI to different types of slurry has been the subject of numerous studies. Schröder *et al*. (1993) observed that the addition of dicyandiamide (DCD) to cattle slurry did not sufficiently improve N recovery by silage maize to justify its recommendation, and concluded that the risk of pollution could only be limited by reducing the dose of N to rates below economically optimum levels. DCD has also been added to cattle slurry (Corré and Zwart, 1995), to pig slurry (Tittarelli *et al*., 1997) and to the urine of dairy cattle (Di *et al*., 1998). Schmitt *et al*. (1995) confirmed the results reported by McCormick *et al*. (1984), indicating that the effect of nitrapyrin on maize yield, when added to different types of manure (dairy and swine), was inconsistent. These yields increased at some sites while no response was seen at others where nitrapyrin was added to manure applied at different rates. Amberger (1990) recommended the addition of a NI to prevent groundwater pollution, especially if slurry is applied in late autumn or winter.

In the USA, nitrapyrin is the most extensively used NI (Walters and Malzer, 1990), but in Europe the most used is probably DCD (Zerulla *et al*., 2001).

The compound 3,4-dimethylpyrazole phosphate (DMPP) is a new nitrification inhibitor developed by BASF (Germany) in cooperation with a number of universities and research institutes (Conrad, 2000; Zerulla *et al*., 2001).

The main aim of this work was to assess the agronomic and environmental effects of DMPP when added to PS. The variables studied were the above ground dry matter yield of ryegrass grown in pots, the concentration of nutrients in these plants, the quantity of N volatilised during the growth season, total absorbed nutrients, and the amount of nitrogen leached. A number of nitrogen use efficiency ratios were also calculated.

This work was part of a project to assess the behaviour of DMPP in calcareous soils in nitrate-sensitive areas using field and pot experiments (Carrasco and Villar, 2001).

Material and Methods

The outdoor trial was performed in pots over a period of approximately one year (June 2001 to September 2002). The pots were 25 cm in diameter and 23.5 cm deep, and were filled with 12.25 g of a loamy, calcareous soil taken from the surface layer of an arable area (see Table 1 for soil properties). These pots were assigned to receive one of the following treatments: no PS or either 73.7, 147.3 or 221 cm^3 pot^{-1}, with or without

Table 1. Properties of the soil used in the experiment

Variable	
Organic C	11.6 g kg^{-1}
Organic matter	20 g kg^{-1}
pH (H_2O)	8.3
Electrical conductivity (1:5)	0.29 dS m^{-1} 25ºC
Calcium carbonate	340 g kg^{-1}
NO_3^--N	22 mg kg^{-1}
Extractable P (Olsen met.)	49 mg kg^{-1}
Extractable K (NH_4OAc)	133 mg kg^{-1}
Texture-USDA	Loam
Sand	51.3%
Silt	28.5%
Clay	20.2%

DMPP. These quantities of PS corresponded to 57, 114, and 171 kg NH_4-N ha^{-1}, and 72, 144, and 216 kg N total ha^{-1} respectively. The EC Nitrate Directive limits the annual application rate of organic manure to 170 kg N ha^{-1} (European Community, 1991). A volume of 0.02 ml of a 25% DMPP solution was added to the assigned pots. The PS, with and without DMPP, was buried 5 cm under the soil surface 18 days (14th June) before sowing ryegrass seeds (*Lolium perenne* L.; 3.26 g pot^{-1}; 2nd July 2001) in the pots. Two more treatments were prepared but without the ryegrass: the low dose PS with and without DMPP. Each treatment was replicated four times according to a completely randomised design.

The PS was collected from a collective slurry storage tank; Table 2 shows its composition. During the experimental period, the total rainfall was 405 mm; 380 mm of water were also supplied by irrigation.

The ryegrass crop was hand-harvested 51, 78, 104, 208, 263, 356 and 406 days after sowing (22nd Aug., 19th Sept., 15th Oct., 12th Des., 19th Apr., 26th Jul. and 3rd Sept.).

Plant samples were dried for 48 h at 65°C to determine their dry matter content (yield). Total N was analysed by the Kjeldahl method. Total P and K were determined by the ICP method, but only for the last four harvests. The above ground N, P and K uptakes were calculated by multiplying nutrient concentrations by total plant dry mass. Soil samples were collected on 3rd September 2002 to determine the residual nutrient content. Leachates were sampled to determine nitrate concentrations at different periods after irrigation. Nitrate concentrations were measured using the Nitrachek® meter quick test (Merck, Darmstadt, Germany).

The organic matter content of the initial soil sample was determined by the volumetric method (Walkey-Black). Soil pH was measured using a 1:2.5 soil/water

Table 2. Chemical composition of the pig slurry

Variable	
Dry matter	70 g kg^{-1}
pH	8.6
Total N Kjeldahl	4.83 g kg^{-1}
NH_4-N	3.81 g kg^{-1}
P	2.31 g kg^{-1}
K	4.48 g kg^{-1}

weight ratio. P was determined by the Olsen method, using an UV-VIS spectrophotometer. K was extracted using ammonium acetate and measured by the ICP method. Nitrates were extracted using a 1:5 soil/water ratio and colorimetrically analysed using an ICA Autoanalyzer. P and K in PS were determined by the ICP method; total N also was determined by the Kjeldahl method. N-NH_3 volatilisation was measured using open static chambers (with oxalic acid traps); this began on the same day the PS was applied and continued for five days (Teira-Esmatges *et al*., 2004).

Several nitrogen efficiency indices were used to evaluate plant response to the PS applied and to the nitrification inhibitor (Table 3). The apparent recovery of PS N by the ryegrass (NREC) was calculated as described by Greenwood and Draycott (1989). Physiological efficiency (PE) and agronomic efficiency (AE) were calculated as described by Yadvinder-Singh *et al*. (2004).

The nitrogen balance was calculated based on the principle of the conservation of mass:

$$\textit{N inputs} - \textit{N outputs} = \\ = \textit{the change in N content in the pot}$$

N inputs were taken as the N levels in the soil at the beginning (N_{min} initial) and at the end of the experiment (N_{min} residual) plus the nitrogen applied with the PS

Table 3. Definition of N efficiency indices

Indices	Definition	Calculated as
NREC	Apparent recovery of PS N (%)	$\frac{\text{N uptake}_{\text{fertilised crop}} - \text{N uptake}_{\text{unfertilised crop}}}{\text{N applied}} \times 100$
PE	Physiological efficiency (g biomass g^{-1} N uptake)	$\frac{\text{Yield}_{\text{fertilised crop}} - \text{Yield}_{\text{unfertilised crop}}}{\text{N uptake}_{\text{fertilised crop}} - \text{N uptake}_{\text{unfertilised crop}}}$
AE	Agronomic efficiency (g biomass g^{-1} N applied)	$\frac{\text{Yield}_{\text{fertilised crop}} - \text{Yield}_{\text{unfertilised crop}}}{\text{N applied}}$

All variables are expressed in g pot^{-1}. N applied was considered as total N PS (analysed by the Kjeldahl method).

Table 4. Average dry matter yield, average N leaf concentration, total N uptake, and partial N, P and K uptake (Mean ± SD, n = 4)

Treatment	Yield (g pot^{-1})	N Conc. (%)	N uptake in the first 104 days *vs.* total N uptake (%)	Uptake			
				In the 406 days	In the last 302 days		
				N (g pot^{-1})	N (g pot^{-1})	P (g pot^{-1})	K (g pot^{-1})
No PS	24.8 ± 2.4	3.16 ± 0.10	64.8 ± 6.2	0.78 ± 0.07	0.28 ± 0.07	0.047 ± 0.007	0.29 ± 0.07
L	24.5 ± 3.0	3.38 ± 0.08	62.8 ± 9.4	0.83 ± 0.11	0.32 ± 0.11	0.042 ± 0.011	0.28 ± 0.10
L + DMPP	28.9 ± 3.8	2.66 ± 0.12	66.0 ± 5.6	0.77 ± 0.07	0.26 ± 0.07	0.057 ± 0.014	0.30 ± 0.09
M	28.4 ± 4.7	3.32 ± 0.02	63.0 ± 9.6	0.94 ± 0.16	0.36 ± 0.16	0.045 ± 0.013	0.32 ± 0.13
M + DMPP	33.3 ± 3.1	3.01 ± 0.05	70.3 ± 5.3	1.00 ± 0.08	0.30 ± 0.08	0.067 ± 0.011	0.36 ± 0.08
H	33.8 ± 2.6	3.38 ± 0.17	55.7 ± 5.4	1.14 ± 0.11	0.51 ± 0.11	0.055 ± 0.006	0.39 ± 0.11
H + DMPP	36.3 ± 6.1	3.12 ± 0.12	70.9 ± 9.5	1.13 ± 0.15	0.34 ± 0.15	0.067 ± 0.021	0.38 ± 0.15

L: low. M: medium. H: high.

(N applied with irrigation was negligible). N outputs were taken to be N uptake, N volatilised after slurry application, and leached N. The N unaccounted for corresponds to mineralised N (input) and N_2O emissions (output).

The data were examined by analysis of variance (ANOVA) and orthogonal contrast. All statistical analyses were undertaken using the Statistical Analysis System (SAS Institute, 1999).

Results

Above ground dry matter yield

The mean dry matter yields were 6.3, 5.6, 3.8, 3.2, 2.7, 4.3, and 3.1 g pot^{-1} for the seven harvests respectively. A positive response to the application of PS was seen. The accumulated dry matter yield ranged from 24.8 g pot^{-1} for the no PS treatment to 33.8 g pot^{-1} for the high PS dose without DMPP treatment (Table 4) – an increase of 36%. The effect of adding the DMPP was also positive. The greatest amount of above ground dry matter was obtained with the highest dose of PS + DMPP (36.3 g pot^{-1}), 7.4% greater than the same treatment without DMPP and 46.1% greater than the no PS treatment. The quantity obtained with the medium PS dose + DMPP (33.3 g pot^{-1}) was 17.4% higher than the same treatment without DMPP. Finally, the low PS dose with DMPP (28.9 g pot^{-1}) produced 17.8% more above ground dry matter than the same treatment without DMPP. Statistical analysis showed the effect PS to be significant (see Table 5).

The inhibition of nitrification also resulted in significant increases in yield (Table 5). The differences between the low and the medium and high PS doses were significant, both with and without DMPP.

Table 5. Statistical analysis using contrasts

Contrast	Yield (g pot^{-1})	N Conc. (%)	N uptake in the first 104 days *vs.* total N uptake (%)	Uptake			
				In the 406 days	In the last 302 days		
				N (g pot^{-1})	N (g pot^{-1})	P (g pot^{-1})	K (g pot^{-1})
No PS *vs.* PS	**	ns	ns	***	ns	ns	ns
DMPP *vs.* No DMPP	**	***	**	ns	*	**	ns
L *vs.* M & H	**	ns	ns	**	ns	ns	ns
M *vs.* H	ns	ns	ns	**	ns	ns	ns
L + DMPP *vs.* M + DMPP & H + DMPP	**	***	ns	***	ns	ns	ns
M + DMPP *vs.* H + DMPP	ns	ns	ns	ns	ns	ns	ns

*, **, *** Significant at the 0.1, 0.05 and 0.01 probability levels, respectively. ns: not significant at $\alpha = 0.10$.

N uptake and interaction with other nutrients

The application of PS with or without DMPP significantly increased N uptake (Table 5). This ranged from 0.78 g pot^{-1} for the no PS treatment to 1.14 g N pot^{-1} for the highest PS dose without DMPP (Table 4), an increase of 46%. The increments associated with the high PS dose compared to the low and medium doses were 37% and 21% respectively. The inhibition of nitrification did not influence the total accumulated N uptake. However, when the low dose was compared with the medium and high dose, the differences were even more significant when DMPP was present. Moreover, when the velocity of N absorption was analysed, the influence of nitrification inhibition was clearly evident. The high and medium dose treatments of PS + DMPP absorbed 70% of the total N by the third harvest (i.e., during the first 104 days of the experiment), while the treatments without DMPP absorbed 55.7% and 63% respectively (Table 4).

The inhibition of nitrification significantly affected the plant N concentration. This concentration ranged from 3.3 to 3.4% in treatments without DMPP, and from 2.7 to 3.1% in treatments with DMPP. The lower values in the DMPP treatments were due to a dilution effect. In all cases, an increase in yield due to the inhibition of nitrification was associated with a lower plant N concentration.

Phosphorus uptake during the last 302 days of the experiment was significantly greater in the nitrification inhibitor treatments (Tables 4 and 5). No significant effect was observed on potassium uptake.

Table 6. N use efficiency indices: apparent recovery of pig slurry N by ryegrass (NREC), agronomic efficiency (AE), and physiological efficiency (PE). These indices were calculated from accumulated values

Treatment	NREC (%)	AE (g biomass g^{-1} N uptake)	PE (g biomass g^{-1} N uptake)
No PS	—	—	—
L	13	–1	23
L + DMPP	–5	11	16
M	22	5	16
M + DMPP	31	12	39
H	34	8	25
H + DMPP	32	11	32

Table 7. Statistical analysis using contrasts

Contrast	NREC	AE	PE
DMPP *vs.* No DMPP	ns	**	ns
L *vs.* M & H	ns	ns	ns
M *vs.* H	ns	ns	ns
L + DMPP *vs.* M + DMPP & H + DMPP	**	ns	ns
M + DMPP *vs.* H + DMPP	ns	ns	ns

*, **, *** Significant at the 0.1, 0.05 and 0.01 probability levels, respectively. ns: not significant at $\alpha = 0.10$.

Nitrogen use efficiencies

The NREC of PS N increased with the amount of N applied, ranging from 13% to 34% in treatments without DMPP (Table 6). These values are relatively low, probably because of initially high soil N levels (Table 1). The low N uptake in the low dose + DMPP treatment (0.77 g pot^{-1}) resulted in a negative NREC. The inhibition of nitrification had no effect on NREC (Table 7). NREC increased significantly with the medium PS dose + DMPP and the high dose + DMPP compared to the low dose + DMPP. The agronomic efficiency (AE) was similar (10.7 – 11.9 g biomass g^{-1} N uptake) among DMPP treatments. The addition of DMPP significantly affected agronomic efficiency (Table 7). Physiological efficiency (PE) also varied among treatments but the differences were not significant.

Residual nutrients in the soil

The soil N, P and K contents were measured at the end of the experiment (Table 8). Only the no PS treatment had a significantly lower soil N content (Table 9). This

Table 8. Residual soil N, P and K at 7th October 2002

Treatment	N (g pot^{-1})	P (g pot^{-1})	K (g pot^{-1})
No PS	0.067	0.659	1.868
L	0.096	0.575	1.876
L + DMPP	0.177	0.626	2.072
M	0.142	0.713	2.269
M + DMPP	0.065	ND	ND
H	0.132	0.625	2.421
H + DMPP	0.091	0.627	2.202

ND: no data.

Table 9. Statistical analysis using contrasts

Contrasts	N_{soil}	P_{soil}	K_{soil}	$N_{leached}$	$N_{unaccount}$
No PS *vs.* PS	*	ns	*	***	***
DMPP *vs.* No DMPP	ns	ns	ns	ns	ns
L *vs.* M & H	ns	ns	ns	***	**
M *vs.* H	ns	ns	ns	ns	ns
L + DMPP *vs.* M + DMPP & H + DMPP	**	ns	ns	**	***
M + DMPP *vs.* H + DMPP	ns	ND	ND	ns	**

*, **, *** Significant at the 0.1, 0.05 and 0.01 probability levels, respectively. ns, not significant at $\alpha = 0.10$. ND: no data.

implies that PS had a clear effect on residual N in the soil. The residual amounts of N in the soil at the end of the experiment were 0.11 g N pot^{-1} on average, 55% less than at the beginning of the experiment (0.25 g N pot^{-1}). Similar results were found for residual K. Unlike N and K, the levels of residual P at the end of the experiment did not differ between treatments.

Leached nitrogen

When ryegrass was present, the amount of N leached was significantly lower in the no PS treatment than when PS was added. The quantities leached increased with PS dose (Tables 9 and 10). The N leached increased until reaching approximately 15 mm (measured linearly) of the cumulative drainage volume corresponding to the first six leachates. From this point, the nitrate content in the drainage volume diminished abruptly. A dose-dependent effect of PS was clear (Fig. 1).

Of the available N, 22.5% was leached in the no PS treatment, 14.5% in the low dose treatment, 16.3% in the medium dose treatment, and 13.4% in the high dose treatment. However, the effect of the nitrification inhibitor was only apparent with the high PS dose. The N leached was equivalent to 11.8% of available N for the high dose + DMPP – a reduction of some 15% compared to the high dose without DMPP. The apparent N leached from the PS applied ([$N_{leached\ fert.\ crop}$ – $N_{leached\ for\ unfert.\ crop}$]*100 / N applied) was 11.9% for the high dose and 9.3% for the high dose + DMPP.

The concentrations of nitrate in the first six leachates were high. The highest values ranged from 479 mg NO_3^- L^{-1} in the no PS treatment to 1,124 mg NO_3^- L^{-1} in the high PS dose treatment. At the end of the sampling period, nitrate concentrations were below 10 mg NO_3^- L^{-1} for treatments with ryegrass and above 100 mg NO_3^- L^{-1} for treatments without the crop.

It is important to highlight the values obtained for the treatments in which there was no ryegrass, especially the low dose PS without DMPP (Table 11). The N leached was 75% of that available (applied + initial mineral soil N content) for the low dose without the ryegrass but only 15% for the low dose with the crop. The N leached was reduced to 63% of the available N for the low dose + DMPP without the crop. This implies a significant reduction of 17% in the N leached when using the nitrification inhibitor (Table 11).

Nitrogen balance

Table 10 shows a simplified N balance. On average, the initial soil N content was 0.24 g N pot^{-1} (22 ppm NO_3-N). The available nitrogen (applied + initial mineral soil N content) increased with increasing PS dose. Mean ammonia emissions were 0.003 g N pot^{-1} (equivalent to less than 1 kg N ha^{-1}). The residual N in

Table 10. Nitrogen mass balance for a 406 day period expressed as g N pot^{-1}

Treatment	N_{soil}	N_{slurry}	N_{volat}	N_{uptake}	$N_{leached}$	$N_{residual}$	$N_{unaccounted}$
No PS	0.24	0	0.0020	0.78	0.054	0.067	–0.662
L	0.24	0.36	0.0028	0.83	0.087	0.096	–0.419
L + DMPP	0.24	0.36	0.0026	0.77	0.098	0.177	–0.447
M	0.26	0.71	0.0022	0.94	0.130	0.142	–0.250
M + DMPP	0.24	0.71	0.0040	1.00	0.153	0.065	–0.272
H	0.24	1.07	0.0025	1.14	0.181	0.132	–0.149
H + DMPP	0.24	1.07	0.0033	1.13	0.154	0.091	–0.064

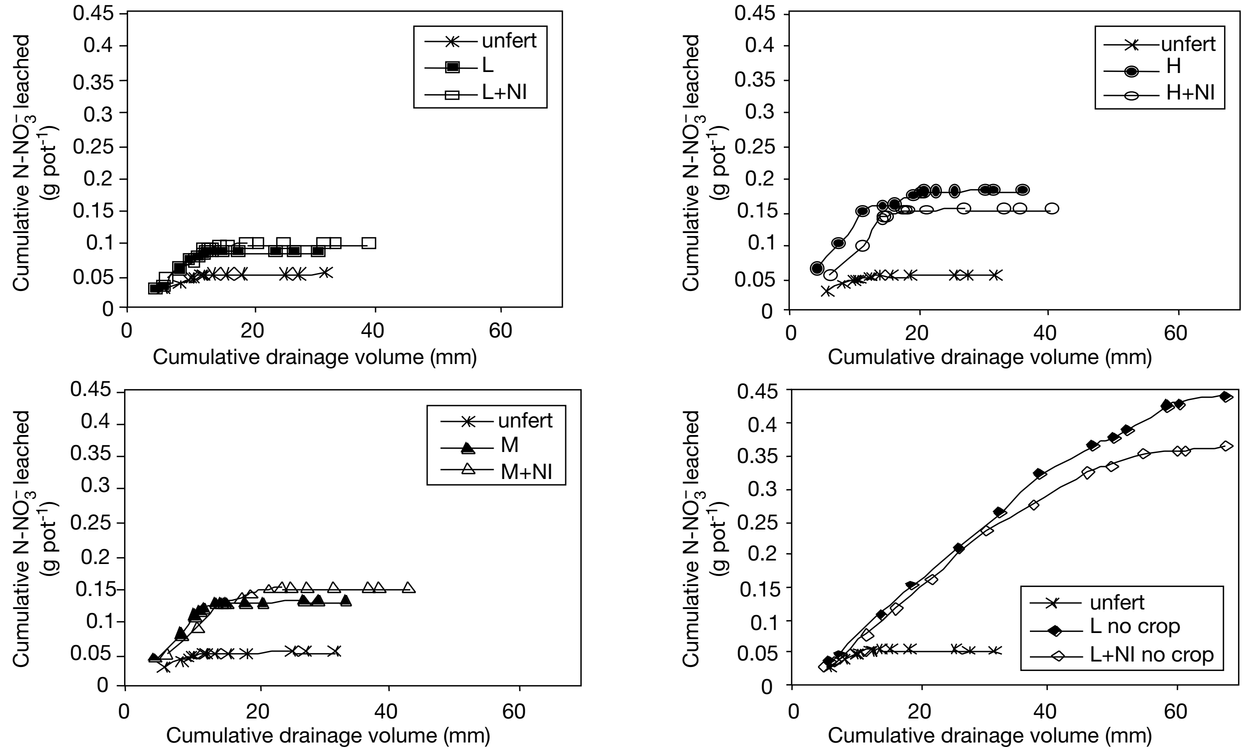

Figure 1. Cumulative $N\text{-}NO_3^-$ leached in cumulative drainage volume from different treatments.

the soil was 0.11 g N pot^{-1}. The levels of unaccounted N depended on the treatments in question. The negative values for unaccounted N imply that some input N was not taken into account, e.g., the mineralisation or denitrification of organic matter.

Discussion

Dry matter yield significantly increased with increasing PS dose. This confirms the observations of Adeli *et al.* (2003) who worked with swine effluent and Bermuda grass. Pasda *et al.* (2001) performed several field-experiments under different soil and climatic conditions and with different crops, and concluded that DMPP had a positive effect on yields. Possible explanations for the higher yields obtained with NH_4^+-N fertilisers supplemented with NI should be understood in terms of the reduction in N leaching and volatilisation losses, the partial nutrition of plants with NH_4^+, and the improved N supply resulting from fertiliser application. Williamson *et al.* (1998), who worked with perennial ryegrass, observed an increase in dry matter yield that accounted for the N prevented from leaching due to the use of DCD.

Table 11. Accumulated N leached expressed as g N pot^{-1}

Treatment	$N_{leached}$
L	0.087 c
L + DMPP	0.098 c
L (without crop)	0.436 a
L + DMPP (without crop)	0.364 b

Values followed by the same letter do not differ significantly ($P < 0.1$) (Duncan's multiple range test).

Nitrous oxide emissions produced by denitrification were not measured in this study. Ditter *et al.* (2001) showed that when DMPP is added to cattle slurry it efficiently reduces N_2O emissions. Emissions from the slurry pool ranged from 0.93 kg N_2O ha^{-1} (without DMPP) to 0.50 kg N_2O ha^{-1} (with DMPP), equivalent to 1.45 and 0.78% of the slurry applied respectively. N_2O emissions can also be reduced by using N fertilisers with DMPP (Linzmeier *et al.*, 2001; Weiske *et al.*, 2001).

The velocity of N absorption was enhanced with DMPP. Williamson *et al.* (1998) observed that the inhibition of nitrification contributes to the enhancement of plant N uptake. Greater P uptakes due to DMPP (an indirect advantage of its use) might be explained by the fact that DMPP improves the mobilisation and uptake of phosphates from the rhizosphere, probably due to the presence of ammonium ions in the soil solution (which might reduce soil pH) (Pasda *et al.,* 2001). However, an unknown mechanism may also be at work.

Adding DMPP to the medium dose PS significantly increased NREC (from 22 to 31%), but no such effects were associated with other doses. Schröder *et al.* (1993) observed an increase in NREC following the addition of DCD to cattle slurry. Williamson *et al*. (1998) found that 32% of the N applied in farm effluent was recovered, compared to 42% of the same effluent when DCD was added.

There were no significant differences between treatments in terms of soil residual phosphorous at the end of the experiment because the initial P content was very high (49 ppm, equivalent to 0.543 g P pot^{-1}). The quantities of P applied ranged from 0.168 g P pot^{-1} to 0.508 g P pot^{-1}. At the end of the experiment, the mean P content was 57 ppm (0.632 g P pot^{-1}).

The initial soil K content was low (133 ppm, equivalent to 1.47 g K pot^{-1}). The quantities of K applied ranged from 0.327 g K pot^{-1} to 0.985 g K pot^{-1}; at the end of the experiment the average K content was 187 ppm (2.08 g K pot^{-1}).

More N was leached in the treatments without the ryegrass than in the treatments with the crop. Beckwith *et al*. (1998) also reported that the presence of a growing crop reduced N leaching following the application of manure.

As expected, in treatments with the ryegrass, the highest PS dose led to the leaching of more accumulated N. However, if the percentage of leached N is compared to the N applied, the former appears to diminish with increasing PS dose (from 24% to 17%). Nevertheless, inhibiting nitrification appeared to have no effect except with the high dose treatment, and even then it was small, as Corré and Zwart (1995) found with DCD. Shepherd (1996), using liquid digested sludge, found that the addition of DCD decreased N leachate losses from the October application.

The effect of PS on the net amount of N mineralised from the soil organic matter was unknown; this represented an important amount of N that remained unaccounted for in the experiment. Unaccounted N (N_2O emission and N mineralised from organic matter) decreased with increasing PS dose.

Contrary to the «priming effect», the simplified apparent N mass balance for the 406 day period shows that the addition of PS (with or without DMPP) seems to reduce the net amount of N mineralised from the soil.

The results of this experiment provide preliminary information on the performance of DMPP in irrigated calcareous soils. Further research is needed to confirm and extend these interesting agronomic and environmental results.

Acknowledgements

The authors would like to thank the Ministry of Science and Technology of Spain for its financial support of this work (projects AGL2000-1363 and AGL2001-1323). E. Guillaumes was the recipient of a pre-doctoral grant from the University of Lleida. Dr. I. Romagosa (University of Lleida) made invaluable comments regarding the statistics used. The authors express their gratitude to Mr. Israel Carrasco of COMPO Agricultura S.L., who provided the DMPP solution, Dr. Rosa Teira (University of Lleida), who measured the ammonia emissions, and Dr. Pere Villar and Mr. Miquel Aran from the Soil Fertility and Analysis Laboratory (*Diputació de Lleida*) for their comments.

References

ADELI A., VARCO J.J., ROWE D.E., 2003. Swine effluent irrigation rate and timing effects on Bermudagrass growth, nitrogen and phosphorus utilization, and residual soil nitrogen. J Environ Qual 32, 681-686.

AMBERGER A., 1990. Use of organic wastes as fertilizers and its environmental implications. In: Fertilization and the Environment (Merckx, R., Vereecken, H., Vlassak, K. ed). Leuven University Press, Louvain, Belgium. pp. 314-329.

BALL-COELHO B.R., ROY R.C., 1999. Enhanced ammonium sources to reduce nitrate leaching. Nutr Cycl Agroecosys 54, 73-80.

BECKWITH, C.P., COOPER, J., SMITH, K.A., SHEPHERD, M.A., 1998. Nitrate leaching loss following application of organic manures to sandy soils in arable cropping. I. Effects of application time, manure type, overwinter crop cover and nitrification inhibition. Soil Use Manage 14, 123-130.

CARRASCO I., VILLAR J.M., 2001. Field evaluation of DMPP as a nitrification inhibitor in the area irrigated by the Canal d'Urgell (Northeast Spain). In: Plan nutrition-food security and sustainability of agro-ecosystems. Developments in Plant and Soil Sciences Vol. 92 (W.J. Horst *et al*., eds). Ed. Kluwer Academic Publishers, Dordrecht, Netherlands. pp. 764-765.

CONRAD J., 2000. Eco-efficient fertilizers: an environmental innovation organised by a corporation and promoted by technology policy. Forschungsstell für Umweltpolitik-report 00-03. Frei Universität Berlin, Germany.

CORRÉ W.J., ZWART K.B., 1995. Effects of DCD addition to slurry on nitrate leaching in sandy soils. Neth J Agr Sci 45, 195-204.

DARP, 1999. Estadístiques agràries i pesqueres de Catalunya. Departament d'Agricultura Ramaderia i Pesca. Generalitat de Catalunya. Spain.

DAVIES D.M., WILLIAMS P.J., 1995. The effect of the nitrification inhibitor dicyandiamide on nitrate leaching and ammonia volatilization: A UK nitrate sensitive areas perspective. J Environ Manage 45, 263-272.

DI H.J., CAMERON K.C., MOORE S., SMITH N.P., 1998. Nitrate leaching and pasture yield following the application of dairy shed effluent or ammonium fertilizer under spray or flood irrigation: results of a lysimeter study. Soil Use Manage 14, 209-214.

DITTERT K., BOL R., CHADWICK D., HATCH D., 2001. Nitrous oxide emissions from injected ^{15}N-labelled cattle slurry into grassland soil as affected by DMPP nitrification inhibitor. In: Plan nutrition-food security and sustainability of agro-ecosystems. Developments in Plant and Soil Sciences (W.J. Horst *et al*. ed.) Vol. 92. Ed. Kluwer Academic Publishers, Dordrecht, Netherlands. pp. 768-769.

DOGC, 1998. Ordre 22/10/98. Codi de Bones Pràctiques Agràries. Diari Oficial de la Generalitat de Catalunya No. 2761, 9/11/1998.

DOGC, 2001. Decret 220/2001. Gestió de les dejeccions ramaderes. Diari Oficial de la Generalitat de Catalunya No. 3447, 7/8/2001.

EUROPEAN COMMUNITY, 1991. Council Directive of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. 91/6776/ECC, Legislation 1375/1-1375/8, European Community.

FRYE W.W., GRAETZ D.A., LOCASCIO S.J., REEVES D.W., TOUCHTON J.T., 1989. Dicyandiamide as a nitrification inhibitor in crop production in the southeastern USA. Commun Soil Sci Plant Anal 20, 1969-1999.

GREENWOOD D.J., DRAYCOTT A., 1989. Experimental validation of a N-response model for widely different crops. Fertil Res 18, 153-174.

LINZMEIER W., GUTSER R., SCHMIDHALTER U., 2001. Nitrous oxide emission from soil and from a nitrogen-15-labelled fertilizer with the new nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). Biol Fertil Soils 34, 103-108.

MALZER G.L., KELLING K.A., SCHMITT M.A., HOEFT R.G., RANDALL G.W., 1989. Performance of dicyandiamide in north central states. Commun Soil Sci Plant Anal 20, 2001-2022.

MALZER G.L., RANDALL G.W., 1985. Influence of nitrification inhibitors, N source, and time of N application on yield and N utilization of maize. J Fert Issues 2, 117-123.

MARTIN H.W., GRAETZ D.A., LOCASCIO S.J., HENSEL, D.R., 1997. Dicyandiamide effects on nitrification and total inorganic soil nitrogen in sandy soils. Commun Soil Sci Plant Anal 28, 613-633.

McCORMICK R.A., NELSON D.W., SUTTON A.L., HUBER D.M., 1984. Increased N efficiency from nitrapiryn added to liquid swine manure used as a fertilizer for maize. Agron J 76, 1010-1014.

PASDA G., HÄHNDEL R., ZERULLA W., 2001. Effect of fertilisers with the new nitrification inhibitor DMPP (3,4-dimetilpirazole phosphate) on yield and quality of agricultural and horticultural crops. Biol Fertil Soils 34, 85-97.

PRASAD R., POWER J.F., 1995. Nitrification inhibitors for agriculture, health, and the environment. Adv Agron 54, 233-281.

SAS INSTITUTE, 1999. SAS user's guide. Version 8. SAS Inst. Cary, NC.

SCHMITT M.A., EVANS S.D., RANDALL G.W., 1995. Effect of liquid manure application methods on soil nitrogen and maize grain yields. J Prod Agric 8, 186-189.

SCHRÖDER J.J., TEN HOLTE L., VAN KEULEN H., STEENVOORDEN J.H.A.M., 1993. Effects of nitrification inhibitors and time and rate of slurry and fertilizer N application on silage maize yield and losses to the environment. Fertil Res 34, 267-277.

SERNA M.D., LEGAZ F., PRIMO-MILLO E., 1994. Efficacy of dicyandiamide as a soil nitrification inhibitor in citrus production. Soil Sci Soc Am J 58, 1817-1824.

SHEPHERD, M.A., 1996. Factors affecting nitrate leaching from sewage sludges applied to a sandy soil in arable agriculture. Agric Ecosyst Environ 58, 171-185.

TEIRA-ESMATGES M.R., PAGANS-MIRÓ E., VILLARMIR J.M., 2004. Effect of 3,4-dimethylpyrazole phosphate (DMPP) addition on ammonia volatilisation from organic residues applied to soil. Biol Fertil Soils (submitted).

TITTARELLI F., CANALI S., BERTI C., BENEDETTI A., 1997. Effects of dicyandiamide on nitrification in soil amended with animal slurries. Agr Med 127, 44-48.

TRENKEL M.E., 1997. Improving fertilizer use efficiency: controlled-released and stabilized fertilizers in agriculture. International Fertilizer Industry Association, Paris. 151 pp.

YADVINDER-SINGH, BIJAY-SINGH, LADHA J.K., KHIND C.S., KHERA T.S., BUENO C.S., 2004. Effects of residue decomposition on productivity and soil fertility in rice-wheat rotation. Soil Sci Soc Am J 68, 854-864.

WALTERS D.T., MALZER G.L., 1990. Nitrogen management and nitrification inhibitors effects on nitrogen-15 urea: II. Nitrogen leaching and balance. Soil Sci Soc Am J 54, 122-130.

WEISKE A., BENCKISER G., HERBERT T., OTTOW J.C.G., 2001. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. Biol Fertil Soils 34, 109-117.

WILLIAMSON J.C., TAYLOR M.D., TORRENS R.S., VOJVODIC-VUKOVIC M., 1998. Reducing nitrogen leaching from dairy farm effluent-irrigated pasture using dicyandiamide: a Lysimeter study. Agric Ecosyst Environ 69, 81-88.

ZERULLA W., BARTH T., DRESSEL J., ERHARDT K., HORCHER VON LOCQUENGHIEN K., PASDA G., RÄDLE M., WISSEMEIER A.H., 2001. 3,4-dimethylpirazole phosphate (DMPP) – a new nitrification inhibitor for agriculture and horticulture. Biol Fertil Soils 34 (2), 79-84.