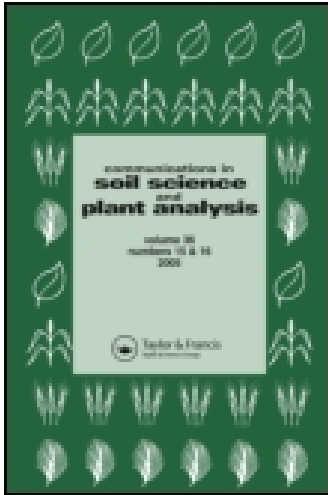


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Influence of Two Nitrification Inhibitors (DCD and DMPP) on Annual Ryegrass Yield and Soil Mineral N Dynamics after Incorporation with Cattle Slurry

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Abstract: Nitrogen (N) losses through nitrate leaching, occurring after slurry spreading, can be reduced by the use of nitrification inhibitors (NIs) such as dicyandiamide (DCD) and 3,4-dimethyl pyrazole phosphate (DMPP). In the present work, the effects of DCD and DMPP, applied at two rates with cattle slurry, on soil mineral N profiles, annual ryegrass yield, and N uptake were compared under similar pedoclimatic conditions. Both NIs delayed the nitrate formation in soil; however, DMPP ensured that the soil mineral N was predominantly in the ammonium form rather than in the nitrate form for about 100 days, whereas with DCD such effect was observed only during the first 40 days after sowing. Furthermore, the use of NIs led to an increase of the dry-matter (DM) yields in a range of 32–54% and of the forage N removal in a range of 34–68% relative to the slurry-only (SO) treatment (without NIs). A DM yield of 8698 kg ha⁻¹ was obtained with the DMPP applied at the greater rate against only 7444 kg ha⁻¹ obtained with the greater rate of DCD (4767 kg ha⁻¹ in the SO treatment). Therefore, it can be concluded that DMPP is more efficient as an NI than DCD when combined with cattle slurry.

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Keywords: Cattle slurry, dicyandiamide (DCD), 3,4-dimethyl pyrazole phosphate (DMPP), forage yield, Nitrification inhibitor, soil mineral nitrogen

INTRODUCTION

Animal feeding at intensive dairy farms in northwest Portugal is based on a double-cropping forage system with maize and annual ryegrass (*Lolium multiflorum* Lam). High rates of cattle slurry are generally applied to both crops, and this region is under a humid Mediterranean climate with rainfall concentrated in autumn and winter. As a consequence, important nitrogen (N) losses, leading to groundwater pollution and poor N-use efficiencies, are expected to occur by nitrate leaching following slurry spreading at sowing of the ryegrass in October.

An efficient solution to reduce N losses consists in maintaining the mineral N in the ammonium form (NH_4^+) and delaying the formation of nitrate (NO_3^-), which is considered to be the source of major processes of N losses as leaching or denitrification (Zerulla et al. 2001). Such result can be achieved by using nitrification inhibitors (NIs) combined with fertilizers (Boeckx, Xu, and Van Cleemput 2005; Gioacchini et al. 2006; Yu et al. 2007) or with animal manures (Schröder et al. 1993; Corré and Zwart 1995; Cameron and Di 2004; Hatch et al. 2005; Vallejo et al. 2005). The NIs have a specific influence on the first step of nitrification because the activities of *Nitrosomas* bacteria in soil are strongly depressed in the presence of NIs (Zerulla et al. 2001). Hence, the bacterial oxidation of NH_4^+ to NO_2^- (first step of the nitrification process) is delayed during the period of NI activity.

The main benefits of NI utilization for agriculture, and simultaneously for the environment, are a significant decrease of N losses by nitrate leaching from N fertilizers and N slurry (Serna et al. 2000; Zerulla et al. 2001) and better N utilization by plants, often leading to yield increases and a greater protein content (Sharma and Prasad 1996; Pasda, Hähndel, and Zerulla 2001).

In recent decades, dicyandiamide (DCD) has been the most common NI used in Europe and proved to be effective in reducing the risk of NO_3^- leaching (Zerulla et al. 2001). More recently, a new chemical compound, 3,4-dimethyl pyrazole phosphate (DMPP), has been used as an NI combined with fertilizers and seems to have very high potential (Serna et al. 2000; Zerulla et al. 2001). The main advantages and weaknesses of both NIs have been reviewed by Zerulla et al. (2001), but both NIs combined with slurry always have been studied separately. However, it is known that the NIs' efficiency depends on the soil and climate parameters (Barth, von Tucher, and Schmidhalter 2001; Zerulla et al. 2001). Therefore, a comparison of the influence of DCD and DMPP combined with cattle slurry under similar pedoclimatic conditions is necessary.

The aim of the present work was to compare the effects of DCD or DMPP applied at two rates to cattle slurry at the establishment of an annual ryegrass winter crop on soil mineral N profiles, forage yield, and N uptake and to assess both NIs' efficiency under a Mediterranean climate with rainfall concentrated in autumn and winter.

MATERIALS AND METHODS

Slurry and Soil

A field experiment was carried out at Braga in the northwest region of Portugal where the soil type was a deep, well-drained, sandy loam derived from granite and classified as humic cambisol. Cattle slurry from dairy cows was used in all slurry treatments with an application rate equivalent to approximately 102 kg total N ha⁻¹. The main characteristics of the soil and slurry are shown in Table 1.

Experiment Setup

The experiment was performed in a randomized block design with three replicates and six fertilization treatments: NS (no slurry), a control not fertilized; SO (slurry only), 50 m³ of cattle slurry ha⁻¹; S + DCD1, 50 m³

Table 1. Characteristics of soil and slurry used for surface application

Parameter	Value
Soil (0–30 cm)	
Organic matter (g kg ⁻¹)	3.0
P ₂ O ₅ (available Egner–Riehm P) (mg kg ⁻¹)	170
K ₂ O (available Egner–Riehm K) (mg kg ⁻¹)	120
pH (water)	5.7
Slurry	
Total N (kg m ⁻³)	2.04
NH ₄ ⁺ N (kg m ⁻³)	1.04
NO ₃ ⁻ N (kg m ⁻³)	<0.001
Total P (kg m ⁻³)	0.35
Total K (kg m ⁻³)	1.84
Total C (kg m ⁻³)	35.03
CaCl ₂ -soluble C (kg m ⁻³)	3.02
C:N ratio	17.17
pH	7.7
Dry matter (%)	5.6

of cattle slurry ha^{-1} + 10 kg DCD ha^{-1} ; S + DCD2, 50 m^3 of cattle slurry ha^{-1} + 20 kg DCD ha^{-1} ; S + DMPP1, 50 m^3 of cattle slurry ha^{-1} + 4 L of 25% DMPP solution ha^{-1} ; and S + DMPP2, 50 m^3 of cattle slurry ha^{-1} + 8 L of 25% DMPP solution ha^{-1} . The concentrations of both NIs used in treatments S + DCD1 and S + DMPP1 were based on the commercial recommendation of each NI, and concentrations were doubled in the other two treatments. The plots were 10 × 10 m, and replicates were separated by a 10-m space. DCD and DMPP were kindly provided by the companies ADP (Portugal) and COMPO (Spain), respectively. Before the beginning of the experiment, the soil received 100 kg P_2O_5 ha^{-1} as ordinary superphosphate and was plowed. The slurry was mixed with the nitrification inhibitors, DCD and DMPP, in the vacuum tank spreader immediately before soil application. Slurry was surface applied just before sowing and followed by superficial soil mobilization to reduce ammonia volatilization. Ryegrass (*Lolium multiflorum* Lam), cv. 'Carena,' was sown with a density of 30 kg ha^{-1} .

Soil samples from the 0- to 10- and 10- to 30-cm soil layers were collected during the crop growth period to assess the NO_3^- N and NH_4^+ N profiles; soil sampling was performed 0, 9, 23, 39, 70, 99, 127, and 159 days after sowing (DAS).

Ryegrass was harvested 167 days after sowing, and fresh samples were collected in each plot to assess dry-matter (DM) yields and forage N removal; fresh samples were then placed in an oven at 65 °C for 72 h for dry weight determination and further N content determination by Kjeldhal methodology.

Apparent nitrogen recovery (ANR) was calculated for each amended treatment using the following formula:

$$\text{ANR}(\%) = \frac{(\text{NT}_i - \text{NT}_0)}{N_{\text{app}}} \times 100$$

with (NT_i) as value of forage N removal in treatment i , (NT_0) as value of forage N removal in the control, and N_{app} as value of total organic N applied.

Rainfall and temperature were measured daily at the experimental location, and values are shown in Figure 1.

Analytical Methods

The N content of the slurry was determined using a modified Kjeldahl method based on a sulfuric acid/potassium sulfate digestion and with copper selenium catalyst, using a Kjeldahl thermo digestion unit and a compact distillation unit.

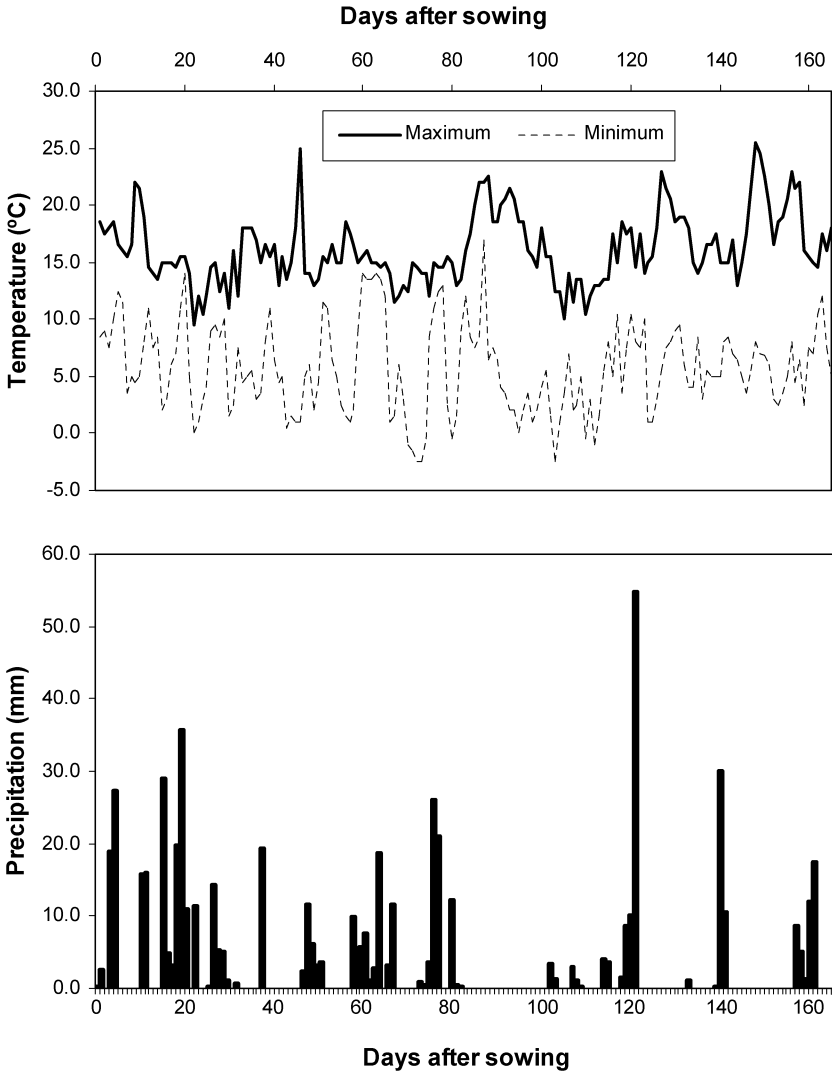


Figure 1. Daily precipitation and temperature observed at the experimental location during the study.

Mineral N content of slurry was extracted with 2M potassium chloride (KCl) in a 1:10 slurry-extractant ratio. NH_4^+ and NO_3^- contents of the extracts were determined by an automated colorimetric procedure (Houba, Van der Lee, and Novozamsky 1995). The segmented flow analyzer (ScanPlus, Skalar, Breda) was equipped with dialyzers to prevent interferences from color or suspended solid particles in the extracts.

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The organic matter (OM) content in soil and slurry was calculated by the loss of mass on ignition at 450 °C for 7 to 8 h. Based on the assumption that soil OM is 58% carbon (C), the C content in slurry was estimated by dividing the OM by a factor of 1.724.

Slurry and soil pH values were determined after 1 h of contact with occasional agitation in a slurry–water (1:5 w/v) suspension and in a soil–water (1:2.5 w/v) suspension, respectively. Soluble C in slurry was determined in a elemental analyzer (Formac, Skalar) after extraction with 0.01 M calcium chloride (CaCl₂) (1:10 w/v) by combustion at 850 °C followed by Near Infrared detection.

Phosphorus (P) and potassium (K) content were determined by the ammonium lactate–acetic acid method (Egner, Riehm, and Domingo 1960). The total P content in the extract was assessed by a colorimetric procedure and total K by flame emission spectroscopy.

Slurry DM was calculated after drying 50 g of slurry in an oven at 103 °C ± 2 °C.

For soil NO₃⁻ and NH₄⁺ measurement, 6 g of soil of each sample were shaken with 30-mL 2 M KCl for 1 h. The suspension was then centrifuged during 10 min at 3000 rpm, and the supernatant was analyzed for NH₄⁺ and NO₃⁻ by automated segmented-flow spectrophotometric methods (Houba, Van der Lee, and Novozamsky 1995). The remaining soil was used to determine the soil moisture content (drying at 105 °C, 24 h).

Statistical Analysis

Data obtained for each treatment were statistically analyzed. Differences between treatments were tested by analysis of variance (ANOVA) using the statistical program STATISTIX 7 (Analytical Software, Tallahassee, FL). Multiple comparisons among the means were made using the least squares difference (LSD) test. Significant differences are expressed at $P < 0.05$, unless otherwise stated.

RESULTS AND DISCUSSION

Soil Mineral N Dynamics

Figure 2 shows the time course of the amount of NH₄⁺ N and NO₃⁻ N in the 0- to 10- and 10- to 30-cm soil layers observed on the different treatments studied. Amounts of NH₄⁺ N and NO₃⁻ N were generally greater in the 10- to 30-cm soil layer in all treatments, but differences between soil layers were statistically significant ($P < 0.05$) only in the case

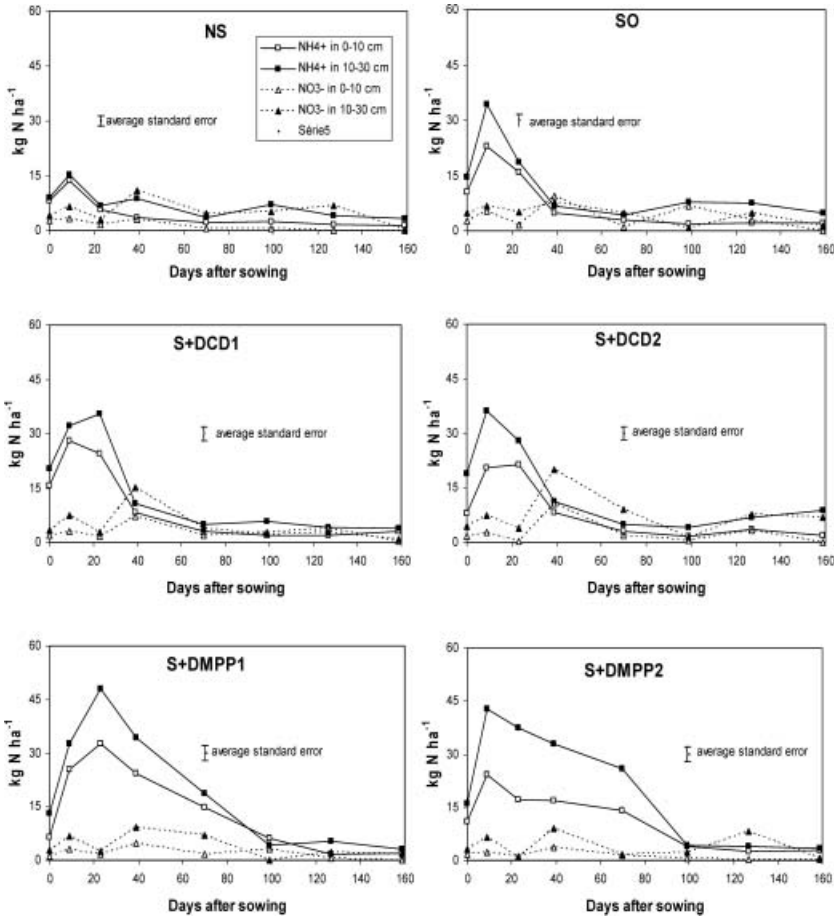


Figure 2. Time course of the amount of N-NH₄⁺ and N-NO₃⁻ in the 0- to 10- and 10- to 30-cm soil layers observed on the different treatments studied; mean values of three replicates; standard error bars were removed for clarity.

of the NH₄⁺ N amounts observed in the S + DMPP1 and S + DMPP2 treatments during the first 70 days of the experiment. Furthermore, the amount of NH₄⁺ N and NO₃⁻ N followed similar trends in the 0- to 10-cm and 10- to 30-cm layers in all treatments during the entire experiment.

In all treatments, most of the soil mineral N was in the NH₄⁺ form in the few days after slurry application, which is in agreement with the speciation of mineral N in slurry present almost exclusively in the NH₄⁺ form. As can be seen in treatment SO with no NIs, the amounts of NH₄⁺ N decreased rapidly after slurry application, and simultaneously the amounts of NO₃⁻-N increased slowly but remained lower. Both NIs allowed a reduction in nitrate formation and maintained greater NH₄⁺ N

amounts in soil during the first 30 DAS. Similarly, Linzmeier, Gutser, and Schmidhalter (2001) and Zerulla et al. (2001) showed clear inhibition of NH_4^+ oxidation during the 4 weeks after NI application. However, at 39 DAS, the amounts of nitrate were greater than the amounts of ammonium in treatments SO, S + DCD1, and S + DCD2, whereas the opposite was still observed in treatments S + DMPP1 and S + DMPP2. Therefore, the main difference between NIs is that DMPP effects lasted longer because even at 70 DAS, the amount of NH_4^+ was significantly greater ($P < 0.05$) than the amount of NO_3^- in treatments with DMPP. Furthermore, levels of NH_4^+ in treatment S + DMPP1 (DMPP at low application rate) were always significantly greater ($P < 0.05$) than in treatments with DCD. The longer effect promoted by DMPP should be mainly because of its low degradability, which allow nitrification inhibition continuing at a fast rate during the first 100 days of the experiment (Zerulla et al. 2001). According to Barth, von Tucher, and Schmidhalter (2001), the inhibitory effect of DMPP depends on many factors, such as the adsorption of the active substance and the degradation of the NI, which can be more significant than the NI concentration available in soil.

Accumulation of large amounts of NO_3^- N in the soil was not observed during the experiments, excepts at 39 DAS, when an increase of nitrate levels occurred, especially in the 10- to 30-cm soil layer, in all treatments including those with DMPP. This large increase may be due to the rainfall that occurred the days before (see Figure 1), and after this day, the high rainfall values observed throughout the experimental period probably contributed to nitrate leaching rapidly after its formation. Furthermore, the low values of nitrate N observed after 80 DAS may also be related to the active uptake of this N form by the crop.

It is to note that the greater mineral content in DCD-treated soils compared to that in the slurry-only treatment might be partly explained by the release of N from the degradation of the DCD, which has a N content of about 67% (Chaves et al. 2005).

Another parameter studied in the present study was the influence of the NI rate of application. Indeed, to be commercially competitive, an NI has to be efficient at the lowest possible application rates because NIs are generally expensive. In terms of NH_4^+ concentration, no significant differences ($P > 0.05$) between rates were observed with DCD, but greater amounts were observed when DMPP was used at the higher rate on 21, 39, and 70 DAS. However, no effect of the NI rate was observed on the amount of NO_3^- in soil during the entire experiment. In the case of DCD, it was expected that an increase of the DCD concentration led to a reduction of nitrification, as explained by Chaves et al. (2005). Meanwhile, this was not observed in the present study, indicating that the lower rate of DCD applied was enough to prevent nitrification. Also, the

observation that doubling the application rate of DMPP did not significantly influence the inhibitory effect of DMPP supports the finding of Zerulla et al. (2001) that DMPP is effective at very low concentrations (0.5–1.5 kg ha⁻¹).

Influence of NIs on Annual Ryegrass Yields, Forage N Removal, and Apparent N Recovery (ANR)

Values of DM yield and forage N removal obtained with the different treatments studied are shown in Table 2.

Significant differences ($P < 0.05$) were observed among treatments on annual ryegrass DM yields. The use of slurry without NIs resulted in values of DM yields similar to those obtained when no slurry was applied (treatments NS and SO). The use of an NI led to a ryegrass yield increase of at least 2 tons of DM (more than 40%), and the greatest value was reached when slurry was amended with the greater rate of DMPP. In this case, an increase of 70% of DM yield was obtained relative to treatment SO. However, results obtained with DCD (both rates of application) and DMPP at the lower rate were not statistically different ($P > 0.05$). It can be concluded that the rate of DCD application has no effect on DM yield, whereas with DMPP, significantly greater DM yields ($P < 0.05$) were obtained with the higher rate.

Results of forage N removal led to similar conclusions to those obtained for DM yields. Indeed, the greatest values for N removal were found for the DMPP treatments with an emphasis on S + DMPP2 treatment, which reached the value of 138 kg N ha⁻¹.

The ANR values obtained in the present work (Table 2) clearly showed that a better N uptake by ryegrass occurred when NIs are used because the value obtained in the SO treatment is 2%, whereas in all NI treatments, this value was more than 34%. Furthermore, it appears that

Table 2. Effect of treatments under study on forage DM yield and forage N removal (mean values of three replicates)

Treatment	DM yield (kg ha ⁻¹)	Forage N removal (kg ha ⁻¹)	Apparent N recovery (%)
NS	4059 ^{d*}	64 ^c	
SO	4767 ^d	66 ^c	2 ^c
S + DCD1	6272 ^c	98 ^b	34 ^b
S + DCD2	7444 ^b	118 ^{ab}	53 ^{ab}
S + DMPP1	7310 ^b	106 ^b	41 ^b
S + DMPP2	8698 ^a	138 ^a	72 ^a

*Data followed by the same letters do not differ at the $P < 0.05$ level, LSD test.

DMPP at the higher rate, with an ANR value of 72%, led to better N uptake by the plants relative to DCD. Pasda, Hähndel, and Zerulla (2001) also showed that DMPP combined with fertilizers may increase the mean crop yield. Weiske et al. (2001) observed no significant effects of DCD and DMPP on the grain yield of summer barley, maize, and winter wheat. The greater DM yield, N removal, and ANR of S + DMPP2 is possibly related to the most favorable soil N profile (Figure 2) in the last and more active period of crop growth (100 to 160 days) with greater amounts of nitrate in the 10- to 30-cm soil layer.

The reduction of N losses by leaching and denitrification may explain the better crop yields obtained in treatments with NIs even if the amounts of N lost were not enough to explain the differences between treatments with and without an NI (Pasda, Hähndel, and Zerulla 2001).

The increase of crop yields in the NI treatments can still be due to other factors induced directly or indirectly by NI employment such as the partial NH_4^+ nutrition of the plants or the decrease of the soil pH in the rhizosphere, which should improve the availability of other nutrients, especially micronutrients, for plant uptake (Pasda, Hähndel, and Zerulla 2001).

CONCLUSIONS

The present study showed that the application of cattle slurry combined with DMPP or DCD under a humid Mediterranean climate allowed maintaining more mineral N in soil as ammonium when compared to slurry-only application. However, DMPP's effect is more intense because it led to greater levels of NH_4^+ N in the soil and lasted longer than with DCD. No evidence of the influence of the rate of NI used on the mineral N profile was observed in the case of the DCD, but DMPP used at the higher rate had a longer effect in time. In terms of DM yield, forage N removal, and ANR, both NI treatments led to greater values than the slurry-only treatment, and the best yields were obtained with DMPP. Nevertheless, the high costs of NI may not be compensated by the benefits obtained with the yield increase, and the economical aspects of NI use have to be considered more accurately.

It can be concluded that for these rainfall and drainage conditions, DMPP combined with cattle slurry is more efficient than DCD to prevent nitrate formation and led to greater crop yields.

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