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# EFFECT OF NITRIFICATION INHIBITORS APPLIED TO SLURRY ON SOIL MINERAL N.

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## INTRODUCTION

An important step in implementing the policy to minimise this greenhouse gas emissions is to study and understand how farm management and edaphoclimatic conditions control the fate of N in agroecosystems, but establishing relationships between management, soil moisture and temperature is complicated by the high spatial and temporal variability of microbial processes in general, and denitrification in particular (Groffman et al., 1991). $N_2O$ is a gas involved in both ozone destruction and global warming. It is released from the soil to the atmosphere during nitrification and denitrification processes, being agriculture the main source of $N_2O$ at European Union level with 41% of anthropogenic $N_2O$ emissions (Morard, 1999). In the Basque Country (north Spain) there is a high risk for N losses by denitrification due to its high rainfall (typically in excess of 1000 mm per year) and warm temperatures, specially in spring and autumn when fertilizer is applied (Estavillo et al., 1994). Nitrification inhibitors used in the field have been proposed as management alternatives to reduce both nitrate leaching and denitrification, and provide greater N availability to the sward (Frye et al., 1981). Dicyandiamide (DCD) seems to interfere with the respiratory electron transport system of bacteria responsible for the first step in nitrification. It has been evaluated in previous studies with fertilizers and slurry, proving to be effective in decreasing denitrification losses (Puttana et al., 1999). Inhibition of nitrification in field conditions has often not increased crop yields (Martin et al., 1993) or had an effect on total inorganic soil N (Martin et al., 1997).
The objective of this work was to evaluate the effect of DCD on mineral N in soil and, indirectly on $N_2O$ flux rates in a grassland soil treated with organic fertilizer.

## MATERIALS AND METHODS

In autumn 1998, a randomised complete block design with 4 replicates and 6 treatments was established on a pasture at the Basque Country. The soil was a poorly drained soil (2.26% fine sand, 41.59% coarse sand, 30.40% loam, 25.69% clay for the top 10cm, 5.74 of pH; 2.58 %OM; total N 0.15%; 9.48 C/N; a bulk density of 1.35 g $cc^{-3}$ and a potential nitrification of 9.07 kg N $ha^{-1}$ $day^{-1}$. The plots received, on 29th September, the treatments: **C**) Control; **M**) 80 kg N $ha^{-1}$ calcium ammonium nitrate (CAN); **M+D**) 80 kg N $ha^{-1}$ CAN+ DCD; **S**) 85 kg N-$NH_4^+$ $ha^{-1}$ Slurry; **S+D**); 85 kg N-$NH_4^+$ $ha^{-1}$ Slurry + DCD. Mineral N (N-$NH_4^+$ and N-$NO_3$) was measured weekly (0-10 cm depth).

Fresh soil extracts were used to measure $NH_4^+$ and $NO_3$. A soil subsample was used for moisture determination. Fluxes of $N_2O$ from the soil were measured using the closed chamber technique. Air samples were collected from the headspace of the chamber just following the covering of the chambers and after 40-60 minutes. The $N_2O$ concentration in these samples was measured by gas chromatography. A schedule for quantification of the $N_2O$ emission rates of the soil system was carried from 30th September to 27th November. During this period, rainfall was 346.9mm, air and soil temperature was measured on each measuring day. Fluxes were measured on day 1, 2, 3, 5, 7, 9, 13 and 20 after fertiliser application.

## RESULTS AND DISCUSSION

### *Soil Mineral N*

Soil ammonium and nitrate contents were significantly affected by DCD (Table 1). Soil ammonium content in DCD amended soils was significantly higher than the rest of treatments.Five days after fertilisation there was a rapid decline in soil N-$NH_4^+$ content (about 30 kg N $ha^{-1}$) in the M and S treatments that suggested a high nitrification activity. However, this decrease in N-$NH_4^+$ did not result in a proportional increase in the soil N-$NO_3^-$ content of M treatment related to S, indicating either immobilization of mineral N or either losses by denitrification or leaching. As during the first 5 days, N-$N_2O$ losses were for M treatments lower than for slurry, it seemed that most of the N-$NO_3$ had leached but due to the clayey texture of the soil, the $NH_4^+$ decrease is likely to result from microbial immobilisation. Nevertheless, the higher soil NO3- content in S treatment after 5 days with respect to M treatment suggests immobilization process is less limited due to the higher C/N. For slurry treatment, immobilization or $NH_4^+$ volatilization occurred from day 0. Until day 5, the inhibitors affected the soil N-$NH_4^+$ and N-$NO_3^-$ contents. The soil N-$NH_4^+$ content of DCD amended soils was higher respect to the rest of treatments 5 days after fertilisation. Soil N-$NO_3^-$ was higher with no DCD. However, since small amounts of N-$NO_3^-$ were added with the slurry, the increased in $NO_3^-$ supply was likely to be due to nitrification of derived N-$NH_4^+$. On day 13, N-$NH_4^+$ content in + DCD continued to be higher, and on day 29, declined to control levels (< 5kg N $ha^{-1}$).

*Table 1. Soil ammonium and nitrate contents measured at different dates after fertilizer application.*

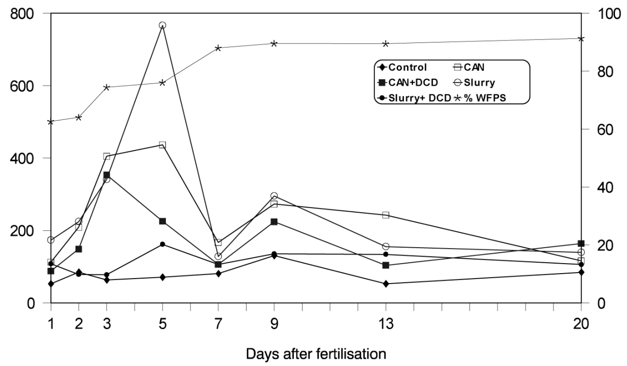
| Treat | kg $NH_4^+$-N $ha^{-1}$ | | | | kg $NO_3^-$-N $ha^{-1}$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 13 | Day 29 | Day 1 | Day 5 | Day 13 | Day 29 |
| Control | 3.2 c | 3.5 b | 4.8 a | 3.5 a | 7.4 b | 12.0 b | 14.8 b | 10.1a |
| CAN | 38.5 a | 4.4 b | 5.5 a | 3.1 a | 38.8 a | 33.5 a | 48.8 a | 11.8 a |
| CAN+DCD | 32.4 a | 23.5 a | 14.1 a | 3.7 a | 39.2 a | 19.9 ab | 22.6 b | 21.0 a |
| Slurry | 36.6 a | 2.3 b | 3.1 a | 2.9 a | 8.6 b | 38.6 a | 25.0 b | 9.8 a |
| Slurry+DCD | 32.6 b | 27.7 a | 13.6 a | 4.8 a | 9.8 b | 10.5 b | 16.8 b | 26.0 a |

† Means in a column followed by the same letter are not statistically different according to Duncan´s multiple range test at $p < 0.05$

### $N_2O$ emissions

As nitrate accumulated in soils occurs when there is C limitation to denitrifiers. The lower fluxes in M treatment may, therefore, have been the result of reduced C availability while the C added with the slurry may affect denitrification rates by providing an energy source for the denitrifying bacteria. The larger $N_2O$ emission from the slurry than from mineral fertiliser indicates that nitrification was by far the major source of $N_2O$ for slurry fertilization as it contains large quantities of $NH_4^+$ (fig.1).

*Fig. 1. $N_2O$ emissions related to treatments along the studied period*




This observation is supported by parallel incubation, where nitrification was the main source of $N_2O$-N. In addition, organic C will increase $O_2$ consumption, which joined with the increased soil water contents in plots fertilised with slurry (80.5% WFPS), would have enhanced the development of anaerobic zones in which denitrification could occur. The high rainfall registered led to 76% WFPS 5 days after fertilisation. As the WFPS generally exceeded 60%, the majority of $N_2O$ measured coming from anaerobic denitrification. Thereafter emission rates declined 2 days after due to an increase to 90% WFPS. In this situation, $N_2$ is the main gaseous product of denitrification when the ability of the soil to reduce $N_2O$ is greater than the rate of $N_2O$ production.

**M+D and S+D vs M and S**

The persistence of DCD estimated by $N_2O$ measurements or soil mineral N content, indicated that after 1 month, soil $NH_4^+$-N content does not differ between +DCD and -DCD treatments. Application of both mineral and organic fertilisers increased the $N_2O$ flux from grassland and addition of DCD to the slurry almost eliminated this effect. The fluxes of $N_2O$ from the different fertilizers peaked after 5 days after fertilizer application. The treatments S+DCD and C (those registering the lowest $N_2O$ emissions) were different to the rest of treatments 2 days after fertilisation. $N_2O$ varied considerably in magnitude, with slurry stimulating the greatest flux; maximum of 284 µg $m^{-2}$ $h^{-1}$, following in descending order by M, S+A, M+I and S+D; maximum of 113 µg $m^{-2}$ $h^{-1}$. However, a week after there was not differences between treatments. DCD was effective in reducing 57.3% $N_2O$ flux rates from grassland receiving slurry, registering 284.3 and 113.6 $\mu$g $N_2O$-N $m^{-2}$ $h^{-1}$ from treatments without DCD and with DCD respectively. The effect of DCD observed on mineral treatments was a 43.6% reduction. Maximum denitrification rates in the slurry treated plots were found 5 days after fertilisers application, when soil N-$NO_3^-$ contents had increased, may be a result of nitrification of slurry derived N-$NH_4^+$.

Fluxes were lower for mineral fertilisers and there were small differences between this treatment with or without DCD with mean values of 192 and 252.1 $\mu$g $N_2O$-N $m^{-2}$ $h^{-1}$ respectively, which was surprising, given that a mineral source available for nitrification losses (13% N-$NO_3$ and 13% N-$NH_4^+$) was provided. If enough carbon is available the $NO_3$ is the limiting factor for denitrification as the addition of DCD inhibited $NO_3$ supply coming from nitrification, then denitrification rates are reduced by 90%, but if carbon is low, denitrification rates were limited by available carbon rather than nitrate, and inhibiting nitrification rates by adding DCD therefore had no effect on denitrification rates. 124.2mg $N_2O$-N $m^{-2}$ were emitted from the CAN treatment and 71.5 from the CAN+DCD treatment. Thus, from a denitrification standpoint, a nitrification inhibitor may be effective in initially curtailing N-gas losses, by minimizing the supply of $NO_3$ during fast $O_2$ consumption by heterotrophic respiration.

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