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# Dicyandiamide as nitrification inhibitor of pig slurry ammonium nitrogen in soil

## Dicianodiamida como inibidor da nitrificação do nitrogênio amoniacal de dejetos líquidos de suínos no solo

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**ABSTRACT**

*Inhibition of nitrification of ammoniacal nitrogen pig slurry after its application to the soil can mitigate nitrogen (N) losses by nitrate ($NO_3^-$) denitrification and leaching, with economical and environmental benefits. However, the use of this strategy is incipient in Brazil and, therefore, requires further assessment. The aim of this study was to evaluate the efficiency of dicyandiamide (DCD) nitrification inhibitor in slowing the nitrification of ammoniacal N applied to the soil with pig slurry (PS). For this, incubation was performed in laboratory, where nitrification was assessed by $NO_3^-$ accumulation in the soil. Rates of 2.8, 5.7 and 11.3kg DCD $ha^{-1}$ were compared, being applied to the soil during PS addition. Nitrification was inhibited by DCD, and inhibition magnitude and duration depended on DCD applied rate. At a dose of 11.3kg $ha^{-1}$ DCD, nitrification was completely inhibited in the first 12 days. During the first month after PS application, each 2.8kg of DCD increase applied per hectare promoted $NO_3^-$-N reduction in the soil of 13.3kg $ha^{-1}$, allowing longer ammoniacal N maintenance in the soil.*

**Key words**: *nitrogen oxidation, DCD, nitrogen dynamics.*

**RESUMO**

*A inibição da nitrificação do nitrogênio amoniacal dos dejetos de suínos, após sua aplicação no solo, poderá mitigar as perdas de nitrogênio (N) por desnitrificação e lixiviação de nitrato ($NO_3^-$), com benefícios econômicos e ambientais. Todavia, o uso dessa estratégia é incipiente no Brasil e, por isso, necessita ser avaliada. O objetivo deste trabalho foi verificar a eficiência do inibidor de nitrificação dicianodiamida (DCD) em retardar a nitrificação do N amoniacal aplicado ao solo com dejetos líquidos de suínos (DLS). Para isso, foi realizada uma incubação no laboratório, quando a nitrificação foi avaliada através do acúmulo de $NO_3^-$ no solo. Foram comparadas as doses de 2,8, 5,7 e 11,3kg de DCD $ha^{-1}$, aplicadas ao solo no momento da adição dos DLS. A nitrificação foi inibida pela DCD, sendo que a magnitude e a duração da inibição foram dependentes da dose de DCD aplicada. Na dose de 11,3kg de DCD $ha^{-1}$, a nitrificação foi completamente inibida nos primeiros 12 dias. Durante o primeiro mês após a aplicação dos dejetos, cada incremento de 2,8kg de DCD aplicado por hectare promoveu uma redução no aparecimento de N-$NO_3^-$ no solo de 13,3kg $ha^{-1}$, o que permite a manutenção de N amoniacal por mais tempo no solo.*

**Palavras-chave**: *oxidação de nitrogênio, DCD, dinâmica do nitrogênio.*

## INTRODUCTION

Pig slurry is rich in N and mainly found in ammoniacal form ($NH_4^+$ + $NH_3$) (YAGUE & QUÍLEZ, 2010), and its application as a source of this nutrient for crops is a typical practice in the same agricultural fields. Ammoniacal N applied to the soil with PS is rapidly oxidized to nitrate ($NO_3^-$) through nitrifying bacteria action (AITA et al., 2007). This rapid transformation of PS ammoniacal N to $NO_3^-$ in the soil has important implications, both from environmental and economic points of view. $NO_3^-$ is movable in the soil, which facilitates its transport to surface water sources and groundwater, possibly causing environmental contamination (VELTHOF & MOSQUERA, 2011) and fertilizer value reduction caused by nitrate output from the absorption zone of the roots. Moreover, some bacteria can use $NO_3^-$ in their catabolism (alternatively to $O_2$) during the

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denitrification process (SUBBARAO et al., 2006), generating nitrogen gas ($N_2$) and its by-product, nitrous oxide ($N_2O$), which is a gas that has 296 time more global warming potential than $CO_2$ (IPCC, 2007). Therefore, nitrification rate control is a key aspect of the N internal cycle, by improving fertilizer potential as well as reducing negative environmental impacts (ABALOS et al., 2014).

Nitrification inhibition can occur by the addition of synthetic or natural products to the soil. Among synthetic products that are added to the soil to inhibit animal slurries ammoniacal N nitrification, one of the most used is dicyandiamide (DCD) (ZAMAN et al., 2009), which has bacteriostatic action, inhibiting the first step of nitrification, which consists of $NH_4^+$ oxidation to nitrite ($NO_2^-$) (SINGH et al., 2008b). Assessing the DCD potential in inhibiting PS ammoniacal N nitrification has been the object of a relatively small number of studies, whose results are contradictory and incipient (VALLEJO et al., 2005; AITA et al., 2014). Due to PS availability in southern Brazil and its high ammoniacal N content, in addition to the environmental problems resulting from this practice, studies that assess the potential of PS ammoniacal N nitrification inhibitors in the soil need to be intensified. Therefore, the present study was performed with the aim of verifying dicyandiamide efficiency, applied at different rates, in inhibiting pig slurry ammoniacal N nitrification process in the soil.

## MATERIALS AND METHODS

The study consisted of incubation conducted for 72 days at the Laboratório de Biotransformações do Carbono e Nitrogênio (LABCEN), Universidade Federal de Santa Maria (UFSM). Soil collected in the 0-10cm layer was classified as Typic Hapludalf Soil (Soil Survey Staff, 1999). After collection, the soil was sieved in a 4mm mesh and incubated on 19 August 2009. Some characteristics assessed in the soil during incubation were: $pH_{H2O}$ (1:1) = 5.0; organic matter (OM) = 2.6%; Clay=24%; phosphorus (P) = 3.0mg $dm^{-3}$; potassium (K) = 48mg $dm^{-3}$; aluminum (Al) = 2.0$cmol_c$ $dm^{-3}$; calcium (Ca) = 1.4$cmol_c$ $dm^{-3}$; magnesium (Mg) = 0.6$cmol_c$ $dm^{-3}$.

Pig slurry used was collected from a pig fattening facility where slurry is stored in an anaerobic storage pit. PS dry matter was determined after its drying in an incubator at 65°C, until constant weight. The C of this sample was assessed by combustion in a CHNS elemental analyzer (FlashEA1112 model, Thermo Finnigan). The pH and total N and ammoniacal N contents were determined according to TEDESCO et al. (1995). On a wet basis, slurry showed contents of (g $kg^{-1}$) 50.1 (dry matter), 20.3 (C), 3.59 (total N), 1.85 (ammoniacal N) and 1.74 (organic N). C/N ratio was of 5.7, and pH was of 7.2. Quantities added to the soil (mg $kg^{-1}$of soil) were 2.46 (dry matter), 996.0 (C), 176.0 (total N), 90.7 (ammoniacal N) and 85.3 (organic N). The substance assessed regarding its effectiveness to inhibit PS ammoniacal N nitrification was dicyandiamide (DCD), which is present in Agrotain® Plus (AP) (trade name) product since no product containing DCD alone was available in Brazil in 2009. AP is a synthetic compound that has 81% of DCD, 6.5% of N-(n-butyl) thiophosphoric triamide (NBPT) and 12.5% of inert substances in its formulation. DCD inhibits the first stage of nitrification, the oxidation of $NH_4^+$ to $NO_2$ by deactivating temporarily ammonium monooxygenase enzyme.

The experiment was conducted in a completely randomized design with five treatments replicated four times and included: T1- soil (control) (S); T2- soil + pig slurry (S + PS); T3- soil + PS + 2.8kg $ha^{-1}$DCD (S + PS + 2.8 DCD); T4- soil + PS + 5.7kg $ha^{-1}$DCD (S + PS + 5.7 DCD); and T5- soil + PS + 11.3kg $ha^{-1}$DCD (S + PS + 11.3 DCD). Treatments were applied to the soil in acrylic containers with 5.0cm height and 5.1cm in diameter, in addition to 102mL capacity. Acrylic containers were packed in glass jars with 2.0L capacity. In order to avoid $O_2$ deficiency, which could limit slurry aerobic decomposition and nitrification, glass jars were opened periodically for a period of 15 minutes. The amount of soil placed in each acrylic container was of 139.9g with 14.3% humidity, being equivalent to 122.4g of dry soil. 6.0mL of distilled water was applied to the soil in the control treatment, being homogenized in order to maintain the soil moisture close to 80% of field capacity. In treatments with PS and PS + DCD addition, soil wetting occurred through liquid fraction, with the application of 6.0mL of slurry.

The DCD assessed rates (2.8, 5.7 and 11.3kg $ha^{-1}$) were defined according to the recommendation of 3-6kg $ha^{-1}$ AP mixed with animal slurries, which was suggested by Koch Agronomic Services Company. The PS was applied at a rate equivalent to 29.4$m^3$ $ha^{-1}$ (90.7mg $NH_4^+$-N $kg^{-1}$ soil). After the addition of PS and AP to the soil, the mixture was uniformly incorporated into the soil, with the soil being added to acrylic containers in two stages. An amount of 69.95g of soil was added in the first stage, where the acrylic flask was compacted up to a height of 2.5cm. In the second stage, the remaining soil (69.95g) was added and compacted up to a height of 5cm. Thus, the flask soil reached a final density of 1.2g $cm^{-3}$.

Treatments were maintained in an incubator at 25°C in the absence of light for a period of 72 days. Soil moisture was kept close to 80% of field capacity through periodic weighing of the units containing the samples. Mineral N contents in the soil ($NH_4^+$-N and $NO_2^-$-N + $NO_3^-$-N) were determined 3h after treatments application (time 0) and at 3, 6, 12, 20, 29, 37, 50 and 72 days after the beginning of incubation, according to the methodology described by TEDESCO et al. (1995). The effect of treatments on nitrification was assessed through $NH_4^+$ amounts and $NO_3^-$ accumulation reduction in the soil during incubation. Results were submitted to analysis of variance and means were compared using Tukey test at 5% probability level. In order to assess the effect of DCD rates on PS ammoniacal N nitrification, simple regression analysis was used.

## RESULTS AND DISCUSSION

The concentrations of $NH_4^+$-N in the control treatment were close to zero during the entire incubation period (Figure 1A). The gradual increase of soil $NO_3^-$-N content in the same treatment (Figure 1B) reveals that ammoniacal N from soil organic matter mineralization (basal mineralization) was quickly oxidized to $NO_3^-$ by nitrifying bacteria. The recovery of $NH_4^+$-N applied with PS during time zero was higher. Considering the ammoniacal N amounts that were added with PS (90.7mg $kg^{-1}$ soil) and originally present in the soil (1mg $kg^{-1}$ soil), it is observed in figure 1A that recovery ranged from 99% (89.7mg $kg^{-1}$ soil) in the treatment with exclusive PS use to 108% (99mg $kg^{-1}$ soil) in the mean of treatments with PS and DCD. Except for the first sampling, the treatment that received the highest DCD rate (11.3kg $ha^{-1}$) had the highest $NH_4^+$-N values throughout the sample period (Figure 1A). Comparing the rates of 2.8 and 5.7kg DCD $ha^{-1}$, which were added along with PS and the treatment with exclusive PS use, it was noted that $NH_4^+$-N amounts in the soil of treatments with DCD were significantly higher from 12 days until the end of the experiment. After 20 days, $NH_4^+$-N amounts found in the soil surpassed the S + PS treatment in seven, five and three times, in treatments in which rates of 11.3, 5.7 and 2.8kg DCD $ha^{-1}$ were added, respectively.

Following the addition of N in the ammoniacal form to the soil, $NH_4^+$ amount reduction depends on the occurrence of physicochemical (volatilization) and biological (immobilization and nitrification) processes. In the present study, there was no wind action during incubation and soil initial pH was relatively low (5.0), as well as PS pH (7.2). Moreover, incubation temperature was kept constant (25°C), and PS was uniformly incorporated to the soil, which greatly reduces $NH_3$ volatilization (DELL et al., 2012). As for N microbial immobilization, it consumes $NH_4^+$ especially in environments with recent addition of organic materials that have high C/N ratio (GIACOMINI et al., 2009), although this was not the case in this study. Thus, N immobilization must also not have been the main cause of $NH_4^+$ levels sharp decline in PS treatments. The dominant process on the $NH_4^+$-N content reduction in the soil of treatments with slurry application was nitrification in this study, as evidenced by the results in figures 1A and 1B. Additionally, it was observed that in treatments with PS, $NH_4^+$-N content decrease was accompanied by a respective increase in $NO_3^-$-N amounts, showing the occurrence of oxidation from $NH_4^+$ to $NO_3^-$ through nitrifying bacteria action. However, by applying the same PS amounts in the four treatments, it was observed in figure 1B that $NO_3^-$-N accumulation kinetics in the soil was different between treatments. These results show the effects of DCD rates on nitrifying bacteria action.

The inhibitory effect of DCD on nitrification is best evidenced by the results found in figures 1C and 1D, where the ratio between $NH_4^+$-N (Figure 1C) and $NO_3^-$-N (Figure 1D) in each sample can be seen. Furthermore, it was observed in figure 1D that after 29 days nearly all PS ammoniacal N had already been nitrified, since the ratio of mineral N in the soil that was in N-$NO_3^-$ form was close to 100%. On this same assessment date, the $NO_3^-$-N ratio was of 81% in the lowest DCD rate (2.8kg $ha^{-1}$), 60% in the intermediate rate (5.8kg $ha^{-1}$) and only 26% in the highest inhibitor rate (11.3kg $ha^{-1}$). Therefore, DCD was able to maintain 75% of PS N in $NH_4^+$ form, even one month after slurry addition (Figure 1C). In the study by VALLEJO et al. (2005), under field conditions, PS were injected into the soil along with DCD, and the inhibitor kept high $NH_4^+$-N levels in the 0-10cm soil layer within the period of 7 to 20 days after application.

Control treatment values were subtracted from $NO_3^-$-N values found in the soil (Figure 1B), with linear regression equations being adjusted only where there was PS addition, whose angular coefficients represent the $NO_3^-$-N accumulation rate. Therefore, $NO_3^-$-N values shown in figure 2A represented $NO_3^-$-N net accumulation, which was

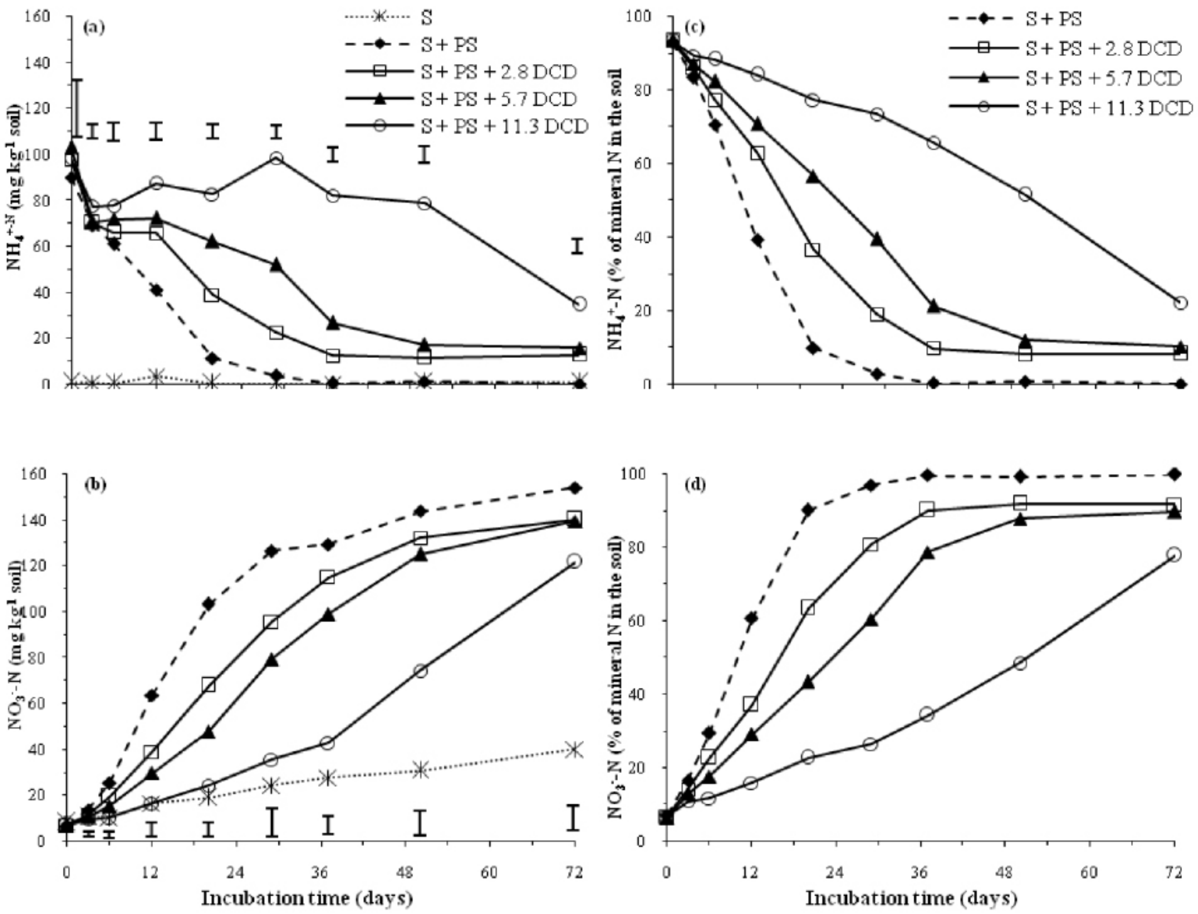


Figure 1 - $NH_4^+$-N (a) and $NO_3^-$-N (b) amounts and N-$NH_4^+$-N (c) and $NO_3^-$-N (d) ratios in the soil, on each assessment date, in control (S), treatments with and without pig slurry (PS) addition and in treatments with dicyandiamide (DCD), at rates of 2.8, 5.7 and 11.3kg $ha^{-1}$. Vertical bars represent the minimum significant difference (Tukey at 5%) among treatments.

produced by the nitrification of the ammoniacal N applied to the soil with PS.

Typically, $NO_3^-$-N gross production in the soil overcomes the net production, since produced $NO_3^-$-N can be immobilized by soil microbial population and/or reduced to N gaseous forms by denitrifying bacteria. However, in the incubation conditions used (without the addition of materials with high C/N ratio and relative moisture content of 80% field capacity), it can be considered that the $NO_3^-$-N disappearance by these two biological processes was minimal. In figure 2A, an increase in $NO_3^-$-N accumulation in the soil occurred linearly and at constant rates in the S + PS treatment and in treatments in which DCD was applied along with slurry, at rates of 2.8 and 5.7kg $ha^{-1}$. What differed between these treatments was the $NO_3^-$-N net accumulation rate, which decreased from 3.8mg $kg^{-1}$ soil $day^{-1}$ in the treatment in which slurry was applied without DCD to 2.3 and 2.1mg $kg^{-1}$ soil $day^{-1}$, in treatments with 2.8 and 5.7kg of DCD $ha^{-1}$, respectively. These results showed that the nitrification process was slowed by DCD use, and the magnitude of this effect was directly related to the inhibitor applied rate. The nitrification inhibitory effect here is attributed to the DCD action in blocking the enzymatic system involved in the oxidation from ammonia to nitrite (SINGH et al., 2008a; AITA et al., 2014).

When discussing strategies to regulate nitrification in agricultural systems, SUBBARAO et al. (2006) mentioned that rates from 10 to 50mg DCD $kg^{-1}$ soil are normally required to inhibit nitrification. Notably, it was observed in figure 1 that even at the lowest DCD rate used, the presence of this inhibitor preserved a higher N amount in $NH_4^+$-N form in the soil than in the PS treatment, although without DCD. The DCD amount applied with the lowest rate was

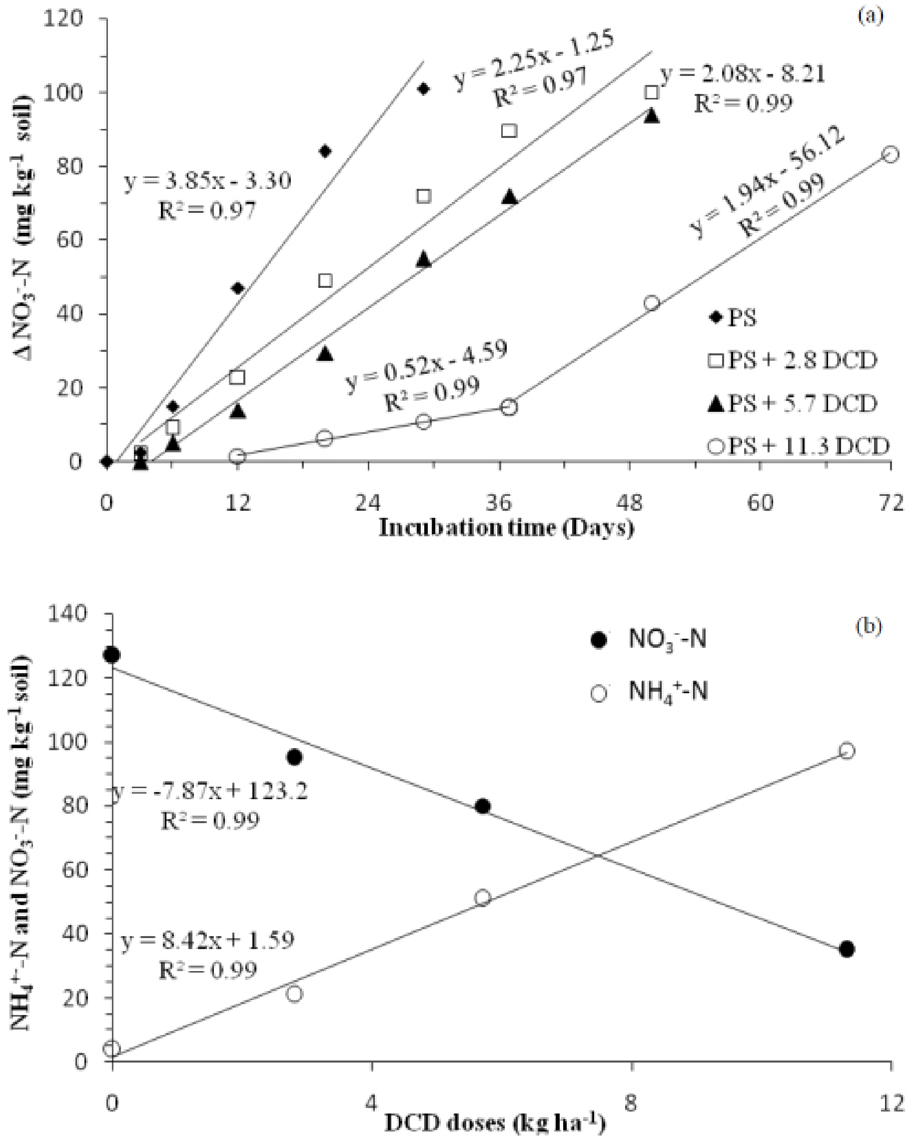


Figure 2 - Linear equations adjusted to the values observed for $NO_3^-$-N net production (a) in the first 29 days for the treatment with pig slurry (PS) only, in 50 days for treatments that received dicyandiamide (DCD) rates (2.8 and 5.7kg ha$^{-1}$) and up to 72 days in treatments with the highest DCD applied rate (11.3kg DCD ha$^{-1}$). In addition, the figure also shows the ratio between DCD rates and $NH_4^+$-N and $NO_3^-$-N amounts in the soil at 29 days of experiment (b).

of 4.7mg DCD kg$^{-1}$ soil, which is 2.1 times lower than the lower limit specified by SUBBARAO et al. (2006) for nitrification inhibitory efficiency by DCD.

Moreover, figure 2A showed that DCD applied at a rate of 11.3kg ha$^{-1}$ differed from the other rates, and N-$NO_3^-$ net production in the soil of this treatment could not be detected during the first 12 days. After this period (12 to 36 days), nitrate net accumulation rate was lower than 1.0 (0.52mg kg$^{-1}$ soil day$^{-1}$). However, nitrate accumulation was evident after this period, with rates of 1.9mg $NO_3^-$-N kg$^{-1}$ soil day$^{-1}$. These results reflect DCD inhibitory power, which decreased over time according to its decomposition by soil microorganisms. According to SUBBARAO et al. (2006), decomposition is faster in soils with relatively high organic matter levels since, in this condition, heterotrophic microorganisms use the N present in DCD (65%). However, even in the period with the highest N-$NO_3^-$ accumulation rates, the value found in the treatment with the highest DCD rate was still 51.4% lower than the S + PS treatment. Lower value showed the

DCD prolonged effect when inhibiting nitrification under the experimental conditions used.

Another aspect that may have contributed to reduce DCD efficiency is the temperature used in this study (25°C). Although there is no consensus in the literature, the temperature has been listed as one of the main factors responsible for DCD degradation in the soil and gradual loss of its effectiveness in inhibiting nitrification. This aspect was assessed in an incubation carried out by DI & CAMERON (2004), by adding two DCD rates (7.5 and 15kg $ha^{-1}$) to the soil. These authors found that half-life ($t_{1/2}$) decreased from 111 to 116 days, when the soil temperature was 8°C, to only 18 to 25 days, when the temperature was 20°C. In the present study, the highest DCD rate used (11.3kg $ha^{-1}$, equivalent to 18.9mg $kg^{-1}$ soil) was intermediate for rates evaluated by DI & CAMERON (2004), and temperature was kept at 25°C throughout incubation. Thus, comparing the results for nitrification inhibition at the highest DCD rate in the present study (Figure 1) to the results of DI & CAMERON (2004), it is clear that DCD persistence in the soil was higher than the one reported by those authors.

The effect of DCD rate on direct amounts $NH_4^+$-N and $NO_3^-$-N detected in the soil was also illustrated in figure 2B, where linear regression equations were adjusted to the values observed for these two variables. For this ratio, only the sampling performed at 29 days was considered, as virtually all ammoniacal N of the PS treatment had been nitrified to that date (Figure 1). Notably, it can be observed in figure 2B that at each increment of 2.8kg of DCD, inhibition occurred in the appearance of $NO_3^-$-N in the soil equivalent to 22.1mg $kg^{-1}$ soil (13.3kg $NO_3^-$-N $ha^{-1}$) and maintenance of N in the form of $NH_4^+$-N equivalent to 24.1mg $kg^{-1}$ soil (14.3kg $NH_4^+$ -N$ha^{-1}$).

These results show that DCD inhibits the nitrification of ammoniacal N from the PS applied to the soil, and that this effect magnitude depends on the rate of DCD applied. At a rate of 11.3kg DCD $ha^{-1}$, nitrification was completely inhibited during the first 12 days after slurry application. However, it is important to consider that DCD is soluble in water and can be percolated into the soil. Depending on rainfall amount and its intensity, the inhibitor may leave the zone explored by the crops root system and have its effectiveness reduced (SUBBARAO et al., 2006). This does not occur in closed systems, such as the incubation carried out in this study, where DCD remained with the N applied with PS, acting in inhibiting nitrification until being degraded.

## CONCLUSION

Dicyandiamide inhibits PS ammoniacal N nitrification, and this effect magnitude is directly related to the rate applied to the soil. DCD use in the highest rate assessed (11.3kg $ha^{-1}$) retards PS ammoniacal N oxidation by 50%, and consequently, $NO_3^-$ production in the soil is also retarded up until 50 days after its incorporation into the soil.

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