

EFFECTS OF NITRIFICATION INHIBITORS ON MINERAL NITROGEN DYNAMICS IN AGRICULTURE SOILS

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Received: November 12, 2010/ Accepted: September 2, 2011

ABSTRACT

Experiments were conducted under laboratory conditions to elucidate the effect of three nitrification inhibitors viz, 3,4-dimethylpyrazole-phosphate (DMPP), 4-Chloromethylpyrazole (CIMP) and dicyandiamide (DCD) on mineral nitrogen dynamics of $(\text{NH}_4)_2\text{SO}_4$ in soil incubated at 25°C in soils. The quantitative determination of ammonium, nitrite and nitrate were carried out spectrophotometrically, while potential denitrification capacity (PDC) was measured gas chromatographically. DMPP, CIMP and DCD were used on recommended rates of 90 kg N ha⁻¹ corresponding to 0.36 µg DMPP; 0.25 µg CIMP and 10 µg DCD g⁻¹ dry soil. In all treatments, the influence of 1, 10, 50, 100, 250 and 500 times of the recommended-concentrations were examined. Results suggested that DMPP, CIMP and DCD applied at rates generally recommended for agricultural use may not be effective to inhibit nitrification. Thus even at the highest tested NIs-concentrations, nitrate and nitrite formation still occurred. Application of high concentrations of these chemicals up to 180 µg DMPP, 125 µg CIMP and 2500 µg DCD were needed for inhibiting nitrification completely. The three NIs began to inhibit PDC at 10 to 50 times recommended concentration and were more effective in sandy than in loamy or clay soils. CIMP influenced PDC at much lower concentration as DMPP or DCD.

Keywords: nitrification inhibitors, mineral nitrogen dynamics

INTRODUCTION

Optimization of agricultural resources for improved and sustainable agriculture involves the use of nitrification inhibitors. The application of these compounds that retard nitrification is used to improve N recovery and N use efficiency in

agriculture soils, while at the same time limiting the environmental impacts of N loss and thus improving sustainability (Fillery, 2007, Pasda *et al.*, 2001). Beneficial effects are reduction of nitrate leaching into ground water (Di and Cameron, 2004; 2005) and nitrous oxide emission to the atmosphere (Weiske *et al.*, 2001; Di and Cameron, 2006; Di *et al.*, 2007), as well as an affect on N retention in the root zone and microbial biomass and activity in the rhizosphere resulting in increased plant growth (Serna *et al.*, 2000; Wolt, 2004; Douma *et al.*, 2005; Malla *et al.*, 2005, Moir *et al.*, 2007). Nitrification inhibitors (NIs) can reduce NO₃-leaching by about 60%, in N₂O emissions by about 70% and an increase crop and pasture yield by more than 20% (Sahrawat, 2004; Douma *et al.*, 2005; Di *et al.*, 2007; Moir *et al.*, 2007; Singh and Verma, 2007). Nitrification results in the formation of highly mobile nitrate, which is susceptible to loss from root zone by leaching and/or gaseous emissions of di-nitrogen or nitrous oxide through denitrification. As the loss of soil N in solution or gaseous form can cause pollution as well as N deficiencies in crops and pastures, the prospect of actively regulating these soil processes has major implication for improving efficiency of fertilizer nitrogen in agriculture and for plant productivity (Chen *et al.*, 2008; Stark and Richards, 2008) especially in horticulture and rice (Pasda *et al.*, 2001; Zerulla *et al.*, 2001; Li *et al.*, 2008). The reduced nitrification can have significant impacts on the soil carbon cycle and, for example, decrease organic decomposition. N species (i.e. ammonium vs. nitrate) may a more important driver of carbon cycling and ecosystem functioning than the quantity of N present in the system (Austin *et al.*, 2006).

Nitrification inhibitors use in agriculture should be recommended in low concentration but capable to control nitrate supply while excess of nitrate is avoid. The inhibitor should inhibit the nitritation and not nitratation so that accumulation

can be avoided. The inhibitor should be *bacteriostatic* and not a *bactericide* killing certain microorganism in soils like *Nitrosobacter* sp, *Nitrosococcus* sp. Recently, more than 300 type of nitrification inhibitors have been well recognized and used in agriculture. Some of these NIs consisted of N-heterocyclic compounds, acetylene derivatives, sulphates and also various pesticides and herbicides (Regina *et al.*, 1998; Mc Carty, 1999). Ammonium recommended fertilizers are the most widely used source of N for crop production and keeping the applied fertilizer N in the NH_4^+ form. The use of nitrification inhibitors (NIs) is a well documented strategy for reducing N loss and to minimize negative environmental impacts of the fertilizer-use. Three compounds have been commercialized as NIs for agricultural use including (i) nitrapyrin (2-chloro-6-trichloromethyl-pyridin, trade name N-Serve), (ii) dicyandiamide or DCD (trade name Didin, Alzon or and Ensan), and (iii) more recently DMPP (a pyrazole derivative, 3,4-dimethylpyrazole phosphate; trade name ENTEC) (Weiske *et al.*, 2001; Zerulla *et al.*, 2001; Barth, 2006; 2008; Ali *et al.*, 2008).

The main objective of the present study was to evaluate under laboratory conditions the effect of three nitrification inhibitors viz., 3,4-dimethylpyrazole-phosphate (DMPP) 4-Chlormethylpyrazole (CIMP) and dicyandiamide (DCD) on mineral nitrogen dynamics (ammonium oxidation, nitrite, nitrate formation, denitrification) in three different type of soils incubated at 25°C. Using this information, patterns and recommendations for the use of fertilizers containing nitrification inhibitors can be identified.

MATERIALS AND METHODS

Soil Samples and Samples Preparation.

This model experiment was conducted in laboratory of Institute for Applied Microbiology, Justus Leibig University, Giessen, Germany in 2001 up to 2002. Agriculture soil samples in this study were clay, silt and sandy soils collected from the Soil Experiment Station, Institute for Agronomy and Plant Protection in Giessen Germany and a sandy soil from Agrochemical Experimental Station, Bayerische Acetylen Soda Fabrik (BASF SE, Limburgerhof, Germany). The respective soils were classified as Typic Udorthent (clay soil) Typic Kandudult (clay and loamy soil), and Typic Paleudult (sandy soil) according to Soil Taxonomy (Soil Survey Staff, 1999). The soils were analyzed physico-chemically (Table 1) by standard methods (Schlichting *et al.*, 1995) and used in the incubation experiments.

Nitrification Inhibitors Used

Nitrification inhibitors used in this study included 3,4dimethylpyrazole phosphate (DMPP, as pure 99,9 % active ingredient), 4-Chlormethylpyrazole (CIMP 99,7 %) both were produced by BASF SE, Ludwigshafen Germany) and dicyandiamide (DCD= Purity 96% produced by SKW Trotsberg Ag. Trotsberg Germany). In experiments utilizing these three NIs, stock solution of the inhibitor was prepared in distilled water by mixing the inhibitor in soil, whereas for experiment with NIs as pure active ingredient as well as the control.

Table 1. Chemical and physical properties of soils

Parameters	Soil		
	silty clay	silt	loamy sand
C_1 (%)	1.35	1.30	0.70
C_{H_2O} (%)	0.40	0.55	0.27
N_1 (%)	0.15	0.15	0.08
C/N	9.00	9.00	9.00
pH (H ₂ O)	6.00	7.00	7.00
pH (KCl)	6.30	5.50	6.40
CEC (cmol/c/kg)	20.10	12.00	8.40
mWHC (%) ¹⁾	46.00	40.00	26.00
$\text{NH}_4^+\text{-N}$ ($\mu\text{g g}^{-1}$ DS) ²⁾	1.00	4.30	3.00
$\text{NO}_3^-\text{-N}$ ($\mu\text{g g}^{-1}$ DS)	10.60	7.90	17.40
$\text{NO}_2^-\text{-N}$ ($\mu\text{g g}^{-1}$ DS)	0.06	0.10	0.10
Texture (%)			
Clay	51	24	6
Silt	41	46	19
sand	8	30	75

Remarks =¹⁾ mWHC = maximal Water Holding Capacity ²⁾ DS = dry soil

Portions of this stock solution were used to achieve the desired level in soil. These three NIs were applied at recommendation rates 0, 36 μg DMPP, 0, 25 μg CIMP and 10 μg DCD g^{-1} dry soil. These rates were equal to that incorporated in N-fertilizer for 90 kg N per Ha. The application rates used in the present study included 0, 1, 5, 10, 25, 50, 100, 250, and 500 times of recommended concentrations.

Incubation Trials

Incubations were carried out in glass incubation jars (Schott, 250 ml) using 100 gram soil. The soil was moistened to 60% WHC with a solution containing $(\text{NH}_4)_2 \text{SO}_4$ to provide $\text{NH}_4^+\text{-N}$ at 100 mg kg^{-1} . The bottles were covered with Parafilm that was perforated for gaseous exchange. Sufficient bottles were prepared for each treatment to allow the extraction four replicate bottles at each sampling interval for analysis of soil mineral N. To determine the NI-Inhibition on nitrification (ammonium decrease, nitrite and nitrate formation), the soil samples were treated with desired concentrations of DMPP, CIMP or DCD according to Beck (1983) and incubated at 25°C. Each sample (100 g moist soil) was placed in the respective jar (for every 5 incubation times and 4 parallels) and then thoroughly mixed with fixed concentrations of each NI and added in 10 ml of a 1%-ammonium-sulfate solution. One extra soil sample from each treatment was used as a blank. The samples were incubated at 60% of the M_{WHC} and the flask covered with perforated Parafilm (each 10 holes) (to ensure aerobic conditions and to reduce water evaporation) for 0-7-14-28 days at 25 ° C. Blank values, (without NI each with or without ammonium addition) served three flasks, which were immediately deep frozen at -18 ° C. One sample (20 g moist soil) was taken from each soil core and analyzed for inorganic N. The samples were mixed with 75 mL of 2 M KCl, shaken for 20 min, centrifuged, and the supernatants were stored at -20°C until analysis. At each sampling time, gravimetric moisture was determined after drying approximately 50 g of soil at 105°C for 48 h. The concentrations of ammonium-N, nitrite-N and nitrate-N were examined photo metrically according to the below mentioned methods. Forty g of soil samples were weighed in 500 ml plastic bottles and added 200 ml of a mixture of 1 N NaCl and 0.1 N CaCl_2 . Then the flasks were shaken for 30 minutes at 130 U-1 (VKS-shaker, Bühler,

Tübingen). The filtrate of ammonium, nitrite and nitrate (filter, 595 $\frac{1}{2}$ Schleicher & Schuell, Einbeck) were determined photo metrically (by U-3200 Hitachi, Japan).

Ammonium. $\text{NH}_4^+\text{-N}$ was quantified according to Dev (1983) 2ml up to 25 ml of the above Filtrate were mixed with 2 ml of a salicylate-citrate solution and filled with distilled water up to 25 ml of volume, then it determined spectrophotometrically (by U-3200 Hitachi, Japan) at 655 nm. The ammonium concentrations were taken from a calibration curve of NH_4SO_4 and printed in $\mu\text{g NH}_4^+\text{-N g}^{-1}$ dry soil.

Nitrite. $\text{NO}_2\text{-N}$ was determined by using a photometer according to Dev (1981) with a-naphthylamine Sulfanilacid solution. For this purpose, 40 ml of filtrate in a flask was mixed with 2 ml of a-naphthylamine and Sulfanilacid solution. Nitrite reacts with sulfanilic acid in acidic solution of a red diazonium salt, the absorbance of the red color complex was measured at 535 nm spectrophotometrically. The nitrite concentration was taken from a calibration curve with NaNO_2 solution and expressed in $\mu\text{g NO}_2\text{-N g}^{-1}$ dry soil.

Nitrate. The concentration of nitrate ($\text{NO}_3\text{-N}$) in the above Filtrate was determined by self-absorption directly (Navone, 1964). Five to 25 ml of the filtrate were filled up with distilled H_2O up to 25 ml of volume and mixed well homogenized with 1 ml H_2SO_4 (10%). The self-absorption of nitrate after 1 h was determined photo metrically at 210 nm. After reduction of nitrate by copper-plated zinc granule (about 3 days) the same sample again was measured and made a correction to the first reading to the absorption of the filtrate. The nitrate concentration was calculated net of a blank (salt solution) from a NaNO_3 -calibration series and expressed in $\mu\text{g NO}_3\text{-N g}^{-1}$ dry soil.

pH. Ten grams of air-dried soil in 50 ml mixed with 25 ml distilled water or a 0.01 M CaCl_2 solution, and shaken for 1h (VKS shaker, Bühler, Tübingen). After equilibration (30 min) the pH was measured by potentiometric (Microprocessor pH-Meter 535, Multi-Cal, Weilheim).

Potential Denitrification Capacity.

Denitrification as measured via the acetylene-inhibition-method. This method can be used to determine either actual denitrification under field conditions or potential denitrification under optimized laboratory conditions (anaerobiosis, addition of substrates, optimum temperatures). Acetylene was used to block the conversion

from nitrous oxide to dinitrogen, which means that all the denitrified nitrogen can be measured as nitrous oxide by gas chromatography. Under O₂-deficient condition nitrate can be used as an alternative electron for denitrification (N₂O, N₂ emission) by many soil bacteria and measured therefore as a potential denitrification capacity (PDC, Pell *et al.*, 1998).

The experimental flasks were fitted for gas sampling with screw caps, including Gummisepten (Verneret, France). Each sample was mixed with 20 ml of a KNO₃-glucose solution (50 g NO₃-N and 300 µg glucose-C g⁻¹ dry soils). After gas-tight closure, the bottles were flushed for 2 minutes with N₂ gas (Messer-Griesheim, Darmstadt) and 2% volume of the bottles-atmosphere was replaced by acetylene (99.9%, Messer-Griesheim, Darmstadt, Germany). Acetylene blocks the N₂O reductase so that potential denitrification capacity (PDC) (i.e. N₂O +N₂) can be determined. Later, the samples were incubated in the dark for 24 h and 48 h at 25 ° C. By using gas-tight Plastipak syringes (Becton Dickinson, Ireland), 50 ml of gas was injected into a gas chromatograph with an electron capture detector (ECD). With an external standard, the PDC printed in µg N₂O-N g⁻¹ dry soil h⁻¹. At the end of the experiment, the concentrations of NH₄+N, NO₂-N NO₃-N in the soil samples were measured photo metrically according to the above methods.

Statistical Analysis

One-way ANOVA and Duncan's test for comparison of means were performed using Sigma Plot and Sigma Stat. Unless otherwise stated, the level of significance referred to in the results was P<0.05. (Gomez and Gomez, 1984). Results are reported as means of three replicates and are expressed on the soil dry weight basis.

RESULTS AND DISCUSSIONS

The Effect of Increasing Nitrification Inhibitors on Ammonium Oxidation, Nitrite and Nitrate Formation

Silty Clay

In Figure 1 is shown the effect of increasing NI-concentrations (DMPP, CIMP and DCD) on the ammonium oxidation, nitrite or nitrate formation and on the pH gradient in the clayey soil. The ammonium oxidation is inhibited with increasing DMPP-concentrations, apparently at the base concentration which shows a rapid decrease of

ammonium. A complete inhibition of ammonium oxidation in the clay soil is reached within 1-2 weeks earlier at 500 times the base concentration (0.36 µg DMPP g⁻¹ dry soils, Figure. 1). The nitrite formation was still observed up to 500-time DMPP base concentration because the formation of nitrate is not completely inhibited. After 28 days (end of the experiment) is about 30 -50% more nitrate was formed in the control (with ammonium fertilization; Figure 1). The pH values on DMPP concentration in all areas in incubation times (especially after three weeks) were significantly dropped as a result of nitrification. A complete inhibition of ammonia oxidation by CIMP in over a period of 1 to 3 weeks was observed only at 500-fold of the base concentration. Compared to DMPP, CIMP thus seems to inhibit the oxidation of ammonium much stronger. The pH values in the clay soil decreased less than that of DMPP. The initial pH values decreased as a result of nitrate formation after 4 weeks by about 0,2 of pH units. Compared to DMPP and CIMP, DCD resulted in inhibition of ammonium oxidation in the first two weeks at the 50-fold of DCD-based concentration (equals to 500 µg DCD g⁻¹ dry soil). It may be caused by the microbial hydrolysis of DCD (contains ~ 66% N) and release of ammonium in to the soil.

The initial increase in ammonium formation (maximum after about seven days) is reflected in an increased nitrate formation (especially in increase of the 250 - and 500-fold of base concentration). The nitrification was highest in the two controls (with and without ammonium addition; Figure 1). The results showed that DCD in the high concentration range (about 50 to 500 times the base concentration) used as N-source for the heterotrophic microorganisms and less acts as a nitrification inhibitor for the nitrifiers. The increase of NH₄⁺ formation derived from the DCD-hydrolysis is also evident from the pH level.

Silty soil

Figure 2 showed the effect of increasing concentrations of DMPP on the decrease of ammonium, nitrite, nitrate formation and the pH change in the loamy soil. Compared to Figure 1 (clayey soil), it showed as an almost identical influences on all measured parameters. Even in the loamy soil DMPP, inhibition of ammonium oxidation only at about 500-fold of the base concentration in about 2 weeks effective could be reached. Thus, the minimum inhibitory

concentration for DMPP was to temporarily block the nitrification at $180 \mu\text{g DMPP g}^{-1}$ dry soil. A rapid decrease of ammonium still took place at the base concentration ($0.36 \mu\text{g DMPP g}^{-1}$ dry soil). Compared to the control (with added ammonium), nitrite and nitrate formation was inhibited with increasing concentrations of DMPP. The inhibitory effect was already about 56% and 76% of control for nitrite and nitrate formation. The pH values were relatively constant in the first 2 weeks in all DMPP concentrations, indicating indirectly to delay of nitrate enrichment. The rapid pH drop after 14 days would indicate a decline in the inhibitory effect and on nitrate accumulation in a loamy soil. Figure 2 is also shown the influence of increasing CIMP-concentrations on the corresponding N dynamics well as on the pH values in loamy soil. As in the clayey soil (Figure 1) we found that a complete inhibition of ammonia oxidation over a period of 1 to 3 weeks in first place of the 500 fold of base concentration ($180 \mu\text{g CIMP g}^{-1}$ dry soil). The effect of CIMP on nitrification was similar to how DMPP (Figure 2), as compared to DMPP; it seems that CIMP affected the ammonium oxidation slightly more negative in this soil. And changes of the pH values were similar to those of the DMPP treatment (Figure 1).

In contrast to the clayey soil (Figure 1) DCD could inhibit ammonium oxidation in loamy soil

already over a 14 days period in 250-500 folds of base concentrations. A portion of the added DCD was hydrolyzed but, then obviously strong and rash converted into nitrate. The pH values increased slowly in all DCD-concentration within 2 weeks significantly (DCD hydrolysis), but then decreased again. The decrease of pH (from pH 6.0 to about pH 5.5) was found in the two controls (with and without ammonium addition). It showed indirectly an intense on nitrification and indicated as an acidification process.

Loamy Sand

The influence of increasing DMPP concentrations on the ammonium nitrite or nitrate formation and to the pH gradients in the sandy soil is presented in Figure 3. Ammonium oxidation was inhibited completely even at 500 times the DMPP-base concentration. The nitrite and nitrate concentrations of the untreated controls (with Ammonium) were at the highest. The differences between the concentrations were relatively low in terms of nitrite and nitrate formation. The simple base concentration had only a limited influence on the decrease in the ammonium concentration. The pH values were always significantly in the higher concentrations of DMPP over those of the control.

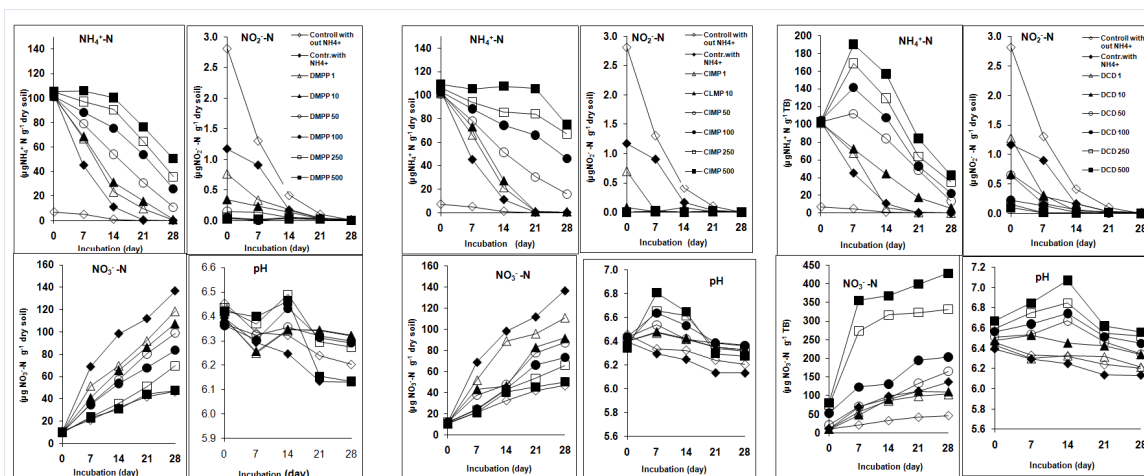


Figure 1. Effect of increasing DMPP, CIMP and DCD concentrations on the ammonium oxidation, nitrite and nitrate formation and on the pH (H₂O) values in the clayey soil during an incubation period of 28 days (60% MWC, adding 100 mg Ammonium-N g^{-1} dry soil, at 25 °C). The recommended concentration is $0.36 \mu\text{g DMPP}$, $0.25 \mu\text{g CIMP}$ and $10 \mu\text{g DCD g}^{-1}$ dry soil.

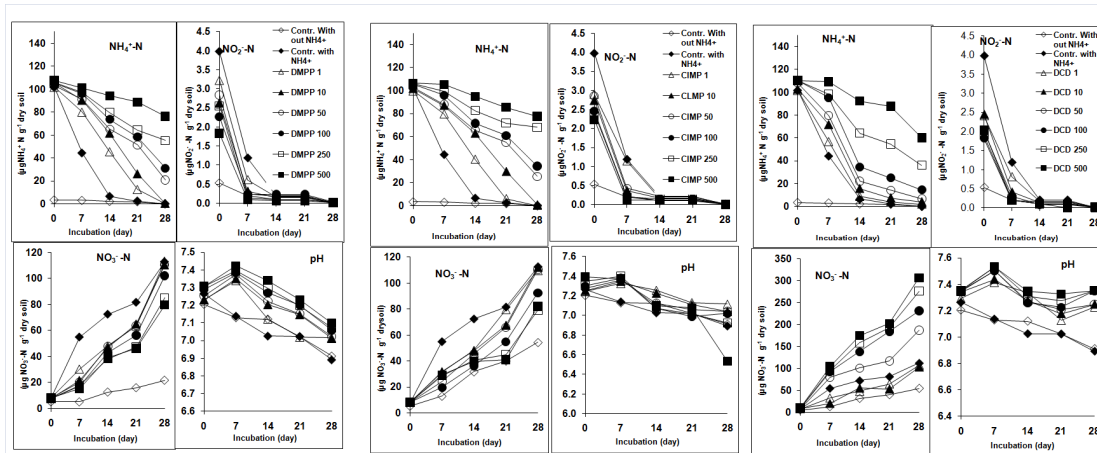


Figure 2: Effect of increasing DMPP, CIMP and DCD concentrations on the ammonium oxidation, nitrite and nitrate formation and on the pH (H₂O) values in the silty soil during an incubation period of 28 days (60% MWC, adding 100 mg Ammonium-N g⁻¹ dry soil, at 25 °C). The recommended concentration equivalent to 0.36 μg DMPP, 0.25 μg CIMP and 10 μg DCD g⁻¹ dry soil.

The influence of increasing CIMP concentrations on the inorganic nitrogen transformations and on the pH values in the loamy sand is graphically summarized in Figure 3. The effects of CIMP were similar to those of DMPP (Figure 3). The pH values were increased at the end of the experiment, however, this indicate which indirectly to enhanced the nitrate formation.

The influence of DCD concentrations on the inorganic N transformations and on the pH gradients in the sandy soil is finally summarized in Figure 3. DCD inhibited ammonium oxidation in the 500 times of the base concentrations of in about a 7 days period to completely. As in clay and loamy soil (Figure 1 and 2), DCD occurred to facilitate more intensive nitrate formation, confirming the incomplete inhibition of ammonium oxidation at the 100 -500 fold of base concentration. Apparently, part of the added DCD was rash degraded by microbial nitrification and speedily transformed into nitrate. The pH values were decreased significantly as a result of unbridled Nitratation. Overall, it came to conclude that DMPP and CIMP began to inhibit the ammonium oxidation completely in over a period of 1 to 2 weeks at the 500-fold base concentration (minimum inhibitory concentration at 180 μg DMPP g⁻¹ and 125 μg CIMP g⁻¹ dry soils).

Influence of Nitrification Inhibitors on the Potential Denitrification Capacity (PDC)

The influence of NIs- concentrations; DMPP, CIMPP or DCD on the potential denitrification capacity (PDC) and the NH_4^+ , NO_2^- and NO_3^- concentrations in the three study soils were presented in Figure 4-6 and in Table 2. In clay soil occurred only an inhibitory effect on the PDC at about 10 to 50-fold of recommended concentration of DMPP, CIMPP or DCD (Figure 5). This No Observable Effect Level (NOEL- values) were for CIMPP already at 2.5 μg g⁻¹ dry soil, DMPP at 18 μg and DCD 250 μg g⁻¹ dry soil. The Effective Dose of 50 % inhibition (ED₅₀- values) of CIMPP, DMPP and DCD were at about 187 μg , 270 μg and 2500 μg g⁻¹ dry soil. CIMPP affected the denitrification at much lower concentration than as DMPP or DCD.

In the silty soil (Figure 5), inhibitory effects of the three NIs on PDC occurred earlier as than in clay soil at 10 times of recommended concentration. The NOEL values for DMPP CIMPP and DCD were 3.6 μg and 2.5 μg , 100 μg DCD g⁻¹ dry soil. At the concentrations of 105 μg CIMPP, 193 μg DMPP or 2049 μg DCD g⁻¹ dry soil, the ED₅₀ values were achieved. Compared to the clay soil inhibitory effects of three NIs in silty soil was reached at lower concentrations.

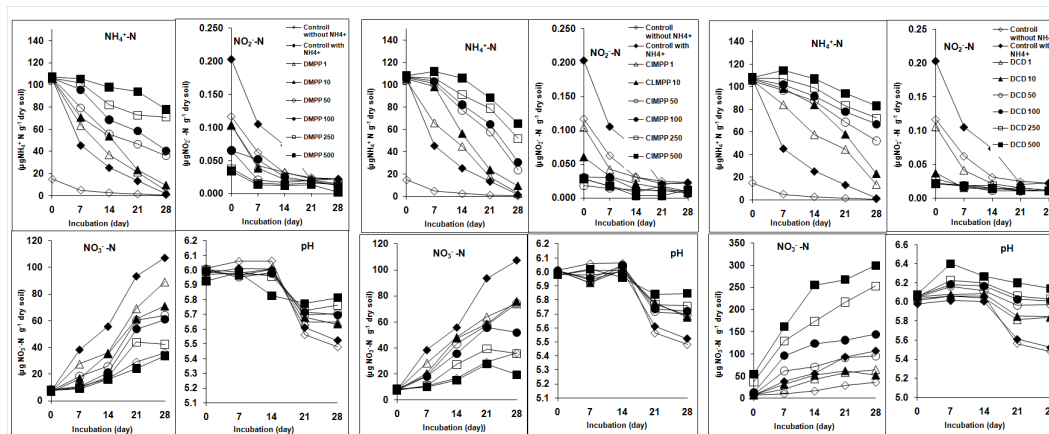


Figure 3. Effect of increasing DMPP, CIMPP and DCD concentrations on the ammonium oxidation, nitrite and nitrate formation and on the pH (H₂O) values in loamy sand during an incubation period of 28 days (60% MWC, adding 100 mg Ammonium-N g⁻¹ dry soil, at 25 ° C). The recommended concentration equivalent to 0.36 μg DMPP, 025 μg CIMPP and 10 μg DCD g⁻¹ dry soil

Similar to the loamy soil, the inhibition of PDC began at about 10 times the recommended concentration of DMPP and CIMPP in the loam sandy soil (Figure 6). The NOEL-values of PDC in sandy soil were 1.25 μg CIMPP and at 3.6 μg DMPP g⁻¹ dry soil, while by the DCD has already reached 100 μg g⁻¹ dry soils, therefore at much lower concentrations than in clay and loamy soil. The ED50-values of CIMPP, DMPP and DCD are at about 98 μg , 150 μg or 1724 μg g⁻¹ dry soil. A comparison of these values with those in Figure 1 (clay) and Figure 2 (loam) showed that the inhibitory effects of CIMPP, DMPP and DCD decreased significantly in the three soil types in the ranking CIMPP > DMPP >> DCD.

The influence of increasing concentrations of DMPP, CIMPP and DCD on the PDC and the concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the clay, silty and sandy soils were summarized in Table 2. It was clear that the nitrate formation decreased in the three experimental soils after the NI-treatment only in low mass, while the NH_4^+ levels increased and easily entered in the three soil types in comparison to the control. This confirms a slight inhibition of the PDC and ammonium oxidation. The $\text{NO}_2^-\text{-N}$ formation looks similar under NI application in the three test soils in comparison to the control (Table 2).

As the evidence available laboratory test data, the threshold of NIs-concentrations to provide a complete blockage of Nitration over a period of 1-2 weeks for CIMPP was on average at 125 μg

CIMPP and approximately 180 μg DMPP g⁻¹ dry soil and DCD was about 500 μg up to 1000 μg DCD g⁻¹ dry soil (depending on soil types). The minimal inhibitory concentration with homogenous distribution in soils was around 15 times of recommended concentration for CIMPP or about 11 times for DMPP). These confirmed the relatively high specific effect of the two new nitrification inhibitors (DMPP and CIMPP). These thresholds inhibitory concentrations from the laboratory were resulted initially a contradiction to the recommended dose for ENTEC in practice. The recent experiment reported that the effect of DMPP in practice was depending on the N-Fertilizing intensity. Further, DMPP in concentrations of 0.5 to 1.5 kg of active substance per ha seems was sufficient disincite to inhibit nitrification in the field over a period 4 up to 10 weeks (Zerulla *et al.*, 2001). DMPP-concentration would be applied by homogeneous distribution in a soil depth of 20 cm at rate about 0.36 to 0.50 μg g⁻¹ dry soil. The equivalent terms for CIMPP concentrations would around 0.25 μg up to 0.35 μg CIMPP g⁻¹ dry soil. Such concentrations of DMPP or CIMPP would be lower in comparison to the above threshold inhibitory concentrations (250 to 500-fold recommended concentrations). With homogeneous incorporation into the topsoil DMPP should therefore in the recommended concentrations (0.5 to 1.5 kg DMPP ha⁻¹) inhibit the nitrification hardly efficient. DCD was applied in the field equal to about 10% of the fertilized N or at a dose of 90 kg N ha⁻¹ in the topsoil or equals to 10 g

DCD g^{-1} dry soil. This DCD concentration would not reach even with an intensive N fertilization up to 200-300 kg N ha^{-1} , in order to reach the above mentioned minimum inhibitory concentrations from the laboratory tests. Thus, there is a contradiction dose between the laboratory and in field applications.

In practice, however, DMPP applied as aggregates formulated on ammonium-N (with a grain diameter of about 4mm), therefore it would not in a homogeneous distribution (Azam, *et al.*, 2001; Zerulla, *et al.*, 2001, Di and Cameron, 2006). It can be assumed that the granules of ENTEC (N-fertilizer and DMPP) after rain hydrolyzed gradually with the result that ammonium and possibly DMPP (also CIMPP) to diffuse rapidly among the granules into the soil.

It took consequently in the field, temporally and spatially different concentration gradients of DMPP (or CIMPP) and ammonium, which for DMPP between 0 and about 100 $\mu\text{g g}^{-1}$ dry soil hat

a granulate distance (in the 0-5 mm zone of the granule center) are expected (Azam *et al.*, 2001).

A comparison of the inhibitory concentrations for further inhibition of nitrification in the laboratory showed that a complete inhibition of ammonium oxidation was temporally and spatially under the pellets and around them is around given the least, especially since about 80% of DMPPs remains in a loamy soil over a 10-days period in around 0-5 mm of the granules (Azam *et al.*, 2001). However, it remains very difficult to know the real, locally and time and the process of granules to inhibit nitrification in the field, because the concentrations of DMPP (or CIMPP) and ammonium because of the different diffusion rates should change continuously. It is likely that DMPP and CIMPP in soils with usual pH values from 5 to 6.5 predominantly active cation (diffuse through Protonation) and due to their molecular sizes significantly slower than the ammonium.

Table 2. The effects of nitrification inhibitors on potential denitrification capacity (PDC in $\mu\text{g N}_2\text{O g}^{-1}$ dry soil 6 h^{-1}) in clayey, silty and loamy sand

Time of Recommended Concentration	Dose of Nitrification inhibitor ($\mu\text{g g}^{-1}$ ds)	PDC ($\mu\text{g N}_2\text{O g}^{-1}$ dwt.s 6h^{-1})		
		silty clay	silt	loamy sand
DMPP				
0 = Controll	0.0	3.87	1.886	3.96
1	0.4	3.93	1.871	3.94
10	3.6	3.86	1.730	3.48
50	18.0	3.73	1.657	3.37
100	36.0	2.96	1.281	2.48
250	90.0	2.60	0.985	2.52
500	180.0	2.41	0.760	2.15
CIMPP				
0 = Controll	0.0	3.87	1.886	3.96
1	0.3	3.88	1.807	3.94
10	2.5	3.65	1.640	3.43
50	12.5	3.10	1.577	2.79
100	25.0	2.70	1.239	2.48
250	62.5	2.43	0.954	2.32
500	125.0	2.36	0.737	1.58
DCD				
0 = Controll	0.0	3.87	1.886	3.96
1	10.0	3.89	1.909	3.89
10	100.0	3.93	1.719	3.94
50	500.0	3.64	1.537	3.15
100	1000.0	2.57	1.427	3.06
250	2500.0	1.81	1.016	1.09
500	5000.0	0.87	0.314	0.94

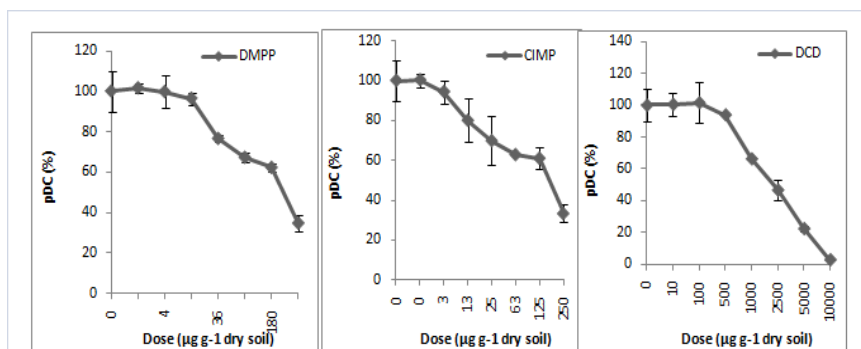


Figure 4. The effect of increasing the concentration of NIs; DMPP, CIMP and DCD on potential denitrification capacity (% of control) in clayey soil. Recommendation dosage was 0.36 $\mu\text{g DMPP}$; 0.25 $\mu\text{g DCD}$; CIMP and 10 $\mu\text{g DCD g}^{-1}$ dry soil

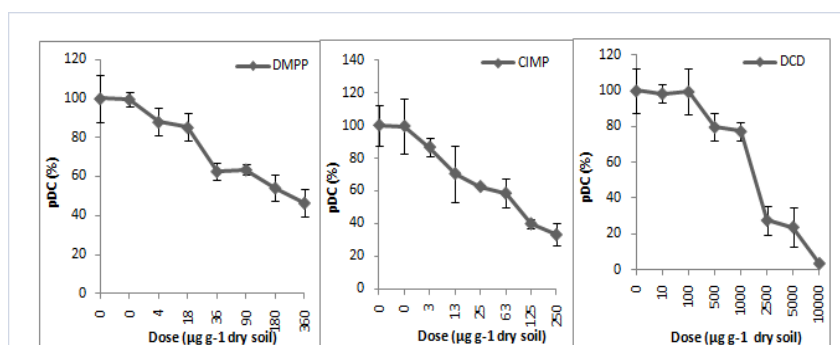


Figure 5. The effect of increasing the concentration of NIs; DMPP, CIMP and DCD on potential denitrification capacity (% of control) in silty soil. Recommendation dosage was 0.36 $\mu\text{g DMPP}$; 0.25 $\mu\text{g DCD}$; CIMP and 10 $\mu\text{g DCD g}^{-1}$ dry soil

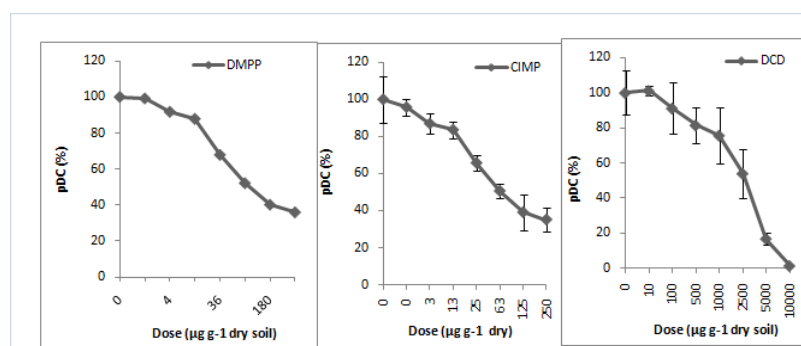


Figure 6. The effect of increasing the concentration of NIs; DMPP, CIMP and DCD on potential denitrification capacity (% of control) in loamy sand. Recommendation dosage was 0.36 $\mu\text{g DMPP}$; 0.25 $\mu\text{g DCD}$; CIMP and 10 $\mu\text{g DCD g}^{-1}$ dry soil

In Laboratory experiments, which were carried out under standard conditions and at different temperatures (4, 15, 25 o C) and soil moisture (18 or 20% of the $m\text{WHC}$), let the example

of a silty clay showed that only 5-15% of DMPPs after 10 days were in the 25 -40 mm zone around the granules, which confirmed the very low mobility DMPPs (Azam *et al.*, 2001, Di *et al.*, 2007). In soils,

the ratio of ammonium was changed to DMPP in the course of the time probably constantly. The inhibitions of Nitritation in the field were depending on such conditions, which in model experiment are hardly possible to create them. Thus far, the potential side effects of nitrification inhibitors on the soil ecosystem have not been established in detail. The existing literature suggests that their use does not negatively affect the soil microbial community, and there is evidence that nitrification inhibitors have no effects on microbial biomass, respiration and enzymatic activities (Muller *et al.*, 2002; Ali *et al.*, 2009; Di *et al.*, 2007; 2010). Molecular analysis of the soil bacterial community indicated that application of nitrification inhibitor (dicyandiamide :DCD) to soil did not affect the composition of the predominant bacterial phyla present in soil (Callaghan *et al.*, 2010). In agreement with these findings, Egamberdiyeva *et al.* (2001) reported increased numbers of oligonitrophilic bacteria and cellulose degradation activity and a decrease in the number of nitrifying and denitrifying bacteria after application of potassium oxalate as nitrification inhibitor, while at the same time availability of fertilizer N to plants was increased. They concluded that the combination of potassium oxalate and mineral fertilization showed promising potential concerning nitrification inhibition. While their study did not assess the specific effects of synthetic nitrification inhibitors on soil microbial populations. Austin *et al.* (2006) showed that reduced nitrification can have significant impacts on the soil carbon cycle and, for example, decrease organic matter decomposition, when applying nitrapyrin to an undisturbed semi-arid steppe. Their results indicated that N species (i.e. ammonium vs. nitrate) may be a more important driver of carbon cycling and ecosystem functioning than the quantity of N present in the system. This underlines the significance of different forms of N in terms of carbon turnover in soils and highlights the need for further studies into the effects of chemical nitrification inhibitors on all nutrient turnover processes and their interactions in soil ecosystems.

More recent studies demonstrated that the efficacy of DMPP was closely related to soil organic constituent and that the adsorption of DMPP to the soil fraction played a major role in controlling inhibition effect (Austin, *et al.*, 2006; Barth, *et al.*, 2001; Barth, 2006:2008). Once the nitrification inhibitor is in the soil, it may be gradually broken down by soil microbes and its efficacy slowly disappears. A timely nitrification inhibition is wished

and an incubation of DMPP in the sandy loam showed that after 12 days of incubation the DMPP-concentration significantly decreased and at day 35 only small amounts of DMPP were still detectable. A major role hereby may play the temperature and availability of organic carbon (Irigoien *et al.*, 2003; Sahrawat, 2004). However, performance of NIs can be highly variable in different agro ecosystems. Granulated DMPP-fertilizer application is apparently superior to liquid DMPP-application and under wet conditions, favorable for nitrate leaching, most effective in the sandy loam (Barth, 2008; Irigoien *et al.*, 2003; Li *et al.*, 2008). From the above considerations can be concluded that the nitrification inhibition must take place substantially in close contact with the DMPP-granules. To clear up this point, some more field experiments in different soil types should be conducted.

CONCLUSIONS

In all tested agriculture soil, the applied NIs; DMPP, CIMP and DCD inhibited ammonium oxidation at recommended concentration only partially. On the average up to 125µg CIMP, 180µg DMPP and 2500 µg DCD g⁻¹ dry soil were needed to inhibit the nitrification completely. The recommended applications rate of 0.5 kg - 1.5 kg DMPP ha⁻¹ which corresponding to 0.36 µg DMPP - 0.50 DMPP µg g⁻¹ dry soil were far below the concentration level, that inhibits ammonium oxidation completely in the three investigated soils. Thus even at highest tested inhibitor concentrations, nitrate and nitrite formation still occurred. A decrease in the soil pH was observed in all experiments but highest in control plots.

Effect of various concentrations of inhibitors nitrifications on potential denitrification capacity has been occurred clearly at 50 times of the recommended dose on clayey soil equals to 18 µg DMPP, 12.5 µg CIMP and 500 µg DCD g⁻¹ dry soil, and 10 times of recommended dose on loamy and sandy soils or equals to 3.6 µg DMPP, 2.5 µg CIMP and 100 µg DCD g⁻¹ dry soil.

Generally, CIMP exhibited the strongest influence on soil mineral nitrogen dynamics in the three soils compared to DMPP and DCD. The NIs was generally the most effective in sandy soils than in clay or loamy soil.

ACKNOWLEDGMENTS

We highly regret that our supervisor and teacher Prof Johannes CG Ottow, who significantly

contributed to this paper, unreckoned died on August 20, 2011. Ferisman Tindaon gratefully acknowledges Prof Dr. JCG Ottow's cooperativeness and a Ph.D scholarship provided by Deutscher Akademischer Austauschdienst (DAAD), Bonn, Germany. This work was supported by the German Ministry for Education and Research (BMBF; Grant Nr. 0339812).

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