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Effectiveness of 3,4-Dimethylpyrazole Phosphate as Nitrification Inhibitor in Soil as Influenced by Inhibitor Concentration, Application Form, and Soil Matric Potential*[1]

G. BARTH, S. VON TUCHER*[2] and U. SCHMIDHALTER

Chair of Plant Nutrition, Technical University Munich, D-85350 Freising-Weihenstephan (Germany). E-mail: barth@wzw.tum.de

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ABSTRACT

The efficacy of nitrification inhibitors depends on soil properties and environmental conditions. The nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) was investigated in a sandy loam and a loamy soil to study its effectiveness as influenced by inhibitor concentration, application form, and soil matric potential. DMPP was applied with concentrations up to 34.6 mg DMPP kg^{-1} soil as solution or as ammonium-sulfate/ammonium-nitrate granules formulated with DMPP. DMPP inhibited the oxidation of ammonium in both soils, but this effect was more pronounced in the sandy loam than in the loamy soil. When applied as solution, increasing DMPP concentrations up to 7 mg DMPP kg^{-1} soil had no influence on the inhibition. The effectiveness of DMPP formulated as fertilizer granules was superior to the liquid application of DMPP and NH_4^+, particularly in the loamy soil. Without DMPP, a decline in soil matric potential down to −600 kPa decreased nitrification in both soils, but this effect was more pronounced in the sandy loam than in the loamy soil. DMPP was most effective in the sandy loam particularly under conditions of higher soil moisture, *i.e.*, under conditions favorable for nitrate leaching.

Key Words: 3,4-dimethylpyrazole phosphate, DMPP, fertilizer granules, nitrification inhibitor

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INTRODUCTION

The application of nitrification inhibitors offers the chance to reduce N losses and to increase fertilizer use efficiency (Slangen and Kerkhoff, 1984; Amberger, 1989; Xu *et al.*, 2002; Di and Cameron, 2004; Boeckx *et al.*, 2005). In recent years ammonium nitrate fertilizers with 3,4-dimethylpyrazole phosphate (DMPP) as a nitrification inhibitor have been introduced into agricultural practice in Europe, South America, North Africa, Australia, and different countries in Asia. The application of these N fertilizers was found to affect economical and ecological aspects of plant production. Under different site conditions, an increase in crop yield has been demonstrated, yet this effect was more pronounced in light textured soils (Pasda *et al.*, 2001; Linzmeier *et al.*, 1999). In pot experiments with DMPP amended ammonium nitrate fertilizers the number of fertilizer applications could be reduced and the fertilizer use efficiency was increased (Roco and Blu, 2006). DMPP decreased nitrogen losses as gaseous emissions of N_2O both from inorganic (Weiske *et al.*, 2001; Linzmeier *et al.*, 2001) and organic fertilizers (Dittert *et al.*, 2001; Macadam *et al.*, 2003; Hatch *et al.*, 2005; Merino *et al.*, 2005). By the application of DMPP the leaching of nitrate from ammonium nitrate fertilizers was reduced (Linzmeier *et al.*, 1999; Roco and Blu, 2006). A significantly lower formation of NO_3^--N was observed when DMPP was applied in combination with

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*[2]Corresponding author. E-mail: tucher@wzw.tum.de.

vegetable crop residues (Chaves *et al.*, 2006). Moreover, the application of DMPP improved crop quality by the reduction of nitrate contents in different vegetables (Pasda *et al.*, 2001; Xu *et al.*, 2005).

In general, the efficacy of nitrification inhibitors depends on internal factors such as molecular structure (McCarty and Bremner, 1989) and the mode of interaction with the ammonia monooxygenase in nitrifying bacteria (McCarty, 1999). But external factors like soil properties, soil temperature, and soil moisture affect the extent and the duration of the nitrification inhibition (McCarty and Bremner, 1990, Sachdev and Sachdev, 1995; Kpomblekou-A and Killorn, 1996). In short-term incubation experiments, decreasing sand content and pH value and increasing catalase activity reduced the efficacy of DMPP to retard the NH_4^+ oxidation (Barth *et al.*, 2001). It was shown that the adsorption of DMPP to inorganic soil constituents was a crucial factor under these conditions.

In the short-term incubation experiments the degradation of the inhibitor was insignificant and water availability and temperature were in an optimum range. Under field conditions these factors may vary substantially and have to be considered. The effect of temperature on the efficacy of DMPP has been investigated in an incubation study (Irigoyen *et al.*, 2003). At 10 °C addition of DMPP stabilized the NH_4^+ content in soil over a period of more than 100 days. At 20 °C and even more at 30 °C NH_4^+ degradation markedly accelerated with half-lives of NH_4^+-N of 18 and 8 days, respectively. However, to our knowledge no published data are available about the effect of water availability on the efficacy of DMPP.

Besides soil water content, different concentrations of DMPP may affect nitrification inhibition. This is of particular interest with respect to the inhibitor concentration that is necessary to achieve a certain degree of reduction in NH_4^+ oxidation. But the inhibitor concentration will also play an important role when DMPP and NH_4^+ fertilizers are applied to the soil as a liquid, *i.e.*, as fertigation or in combination with liquid organic fertilizers or in solid form as fertilizer granules. The application as a liquid may lead to a more uniform distribution of NH_4^+ and DMPP within the soil compared to an application as fertilizer granules, where it can be supposed that fertilizer and inhibitor only react within a smaller area in the vicinity of the granule.

The present study therefore aimed to evaluate the influence of the inhibitor concentration, the role of the application form as well as the effect of the soil matric potential on the effectiveness of DMPP during a long-term incubation experiment.

MATERIALS AND METHODS

For the incubation studies, topsoils, a sandy loam and a loam (both Eutric Cambisols, Driessen *et al.*, 2001), were collected from arable land around Freising near Munich, Germany. Soil parameters are listed in Table I. Details for the determination of the soil properties are given elsewhere (Barth *et al.*, 2001). The soils were slightly dried before being homogenized by sieving (2-mm mesh size) and stored at 4 °C.

TABLE I

Physical, chemical, and biochemical properties of the investigated soils

Soil	Clay	Silt	Sand	CEC[a)]	pH ($CaCl_2$)	Organic carbon	Total nitrogen	Potential nitrification
	g kg^{-1}			$cmol_c$ kg^{-1}		g kg^{-1}		mg NO_2^--N kg^{-1} soil h^{-1}
Loamy soil	230	480	290	11.9	5.7	1.66	0.15	0.250
Sandy loam	90	290	620	6.4	6.0	1.11	0.12	0.196

a) Cation exchange capacity was determined at pH 8.1.

DMPP concentration and application form

Nitrogen (100 mg N kg^{-1} soil) and different concentrations of DMPP (0.71–7.1 mg kg^{-1} for the loamy soil and 1.18–34.64 mg kg^{-1} for the sandy loam) either dissolved in water or as $(NH_4)_2SO_4/NH_4NO_3$

fertilizer granules formulated with DMPP corresponding to 1.63 mg DMPP kg^{-1} soil were applied to the 100 g soils on a dry weight basis. The granules have the trade name ENTEC 26 and were developed by BASF Agricultural Center, Limburgerhof, Germany. The samples were adjusted to a soil water content of 200 g kg^{-1} soil and homogenized by stirring. The fertilizer granules were added to the soil immediately after adjusting the water content and covered with soil. In the other treatments, $(NH_4)_2SO_4$ and DMPP were added as mixed solution before finally adjusting the water content. The 500 mL plastic bottles were closed by cling film, which prevented water loss but allowed gas exchange. The samples were incubated in the dark at 25 °C. After 0, 3, 5, 7, 11, 14, 18, 25, and 32 days the NH_4^+ contents were determined. Subsamples (25 g on soil dry weight basis) were used for DMPP analysis as described later. Samples that were not analysed immediately were stored at −18 °C.

Soil matric potential on the effectiveness of DMPP

Ten treatments were prepared for each soil. Five different soil matric potentials were established and fertilizer granules of $(NH_4)_2SO_4/NH_4NO_3$ formulated with or without DMPP were added. The fertilizer granules were selected for uniform diameter and contained about 10 mg of NH_4^+-N per granule. The DMPP formulated granules contained about 15.15 μg DMPP. The soils were adjusted to the following soil matric potentials: −600, −300, −100, −50, and −5.8 kPa (sandy loam) or −3.4 kPa (loam). The corresponding gravimetric water contents were 70, 86, 110, 123, and 140 g kg^{-1} dry soil in the sandy loam and 210, 227, 235, 255, and 275 g kg^{-1} dry soil in the loamy soil. Soil matric potentials were established one week before starting the incubation. At the start of the incubation 100 g soil on a dry matter basis was weighed into a 500 mL plastic bottle and the fertilizer granules were incorporated into the soil. Then the bottles were closed by cling film and incubated in the dark at 25 °C. After 5, 10, 15, 20, and 25 days NH_4^+ contents were determined.

Analysis of NH_4^+ and DMPP

Two hundred milliliters of 1 mol L^{-1} KCl solution were added to 100 g dry soil and shaken for 1 h at 40 r min^{-1}. The suspension was filtered through a folded filter (Schleicher and Schüll 602 EH 1/2, Dassel, Germany) and the NH_4^+ concentration of the liquid was measured by an indophenol blue method (Bernt and Bergmeyer, 1970).

In the sandy loam the DMPP content was determined in the treatment with the DMPP concentration of 7.1 mg kg^{-1} soil after 0, 3, 5, 7, 11, 14, 18, and 25 days of incubation. Fifty milliliters of 10 g L^{-1} K_2SO_4 solution were added to the samples and shaken horizontally for 30 min at a rate of 250 r min^{-1} (Köttermann GmbH Uelzen, Germany). Then the samples were centrifuged for 10 min at 2 700 × *g* (Beckmann GS6 centrifuge, Beckmann Instruments, Munich, Germany) and the supernatant was decanted and filtered. This procedure was carried out three times and the filtrates were combined. From 50 mL of the filtrate DMPP was transferred into *tert*-buthyl methyl ether. After several steps of concentration and purification DMPP was measured by high performance liquid chromatography (column: lichrosorb C^{18} 7 μm, 250 mm × 4 mm and precolumn 60 mm × 4 mm; eluent: acetonitrile in H_2O 0.15:1 (v:v) with 1 mL 85% H_3PO_4 L^{-1}; flow: 1 mL min^{-1}; UV detection at 220 nm). For more details see Barth *et al.* (2001).

RESULTS

DMPP concentration and application form

In both soils DMPP decreased the rate of NH_4^+ oxidation in all treatments compared to the control without DMPP (Fig. 1). When DMPP was applied as solution, nitrification was more inhibited in the sandy loam than in the loamy soil. In liquid form, the concentration of DMPP had no effect on the NH_4^+ oxidation, except for the sandy loam, where at day 25, slightly more NH_4^+ was found with the highest DMPP concentration of 34.6 mg kg^{-1} soil. In this soil, the effectiveness of DMPP formulated as fertilizer granules (1.63 mg DMPP kg^{-1} soil) was similar to that of the application of DMPP as solution during the first 15 days of the experiment. In the later stage around day 25, the NH_4^+ content in the treatment with DMPP formulated fertilizer granules was temporarily somewhat higher than that with the liquid application up to 7.1 mg DMPP kg^{-1} soil and comparable to the liquid application of 34.6 mg DMPP kg^{-1} soil. By contrast, the efficacy of DMPP granules was markedly higher in the loamy soil and by far superior to the liquid application of DMPP (0.71 up to 7.1 mg kg^{-1}) in this soil.

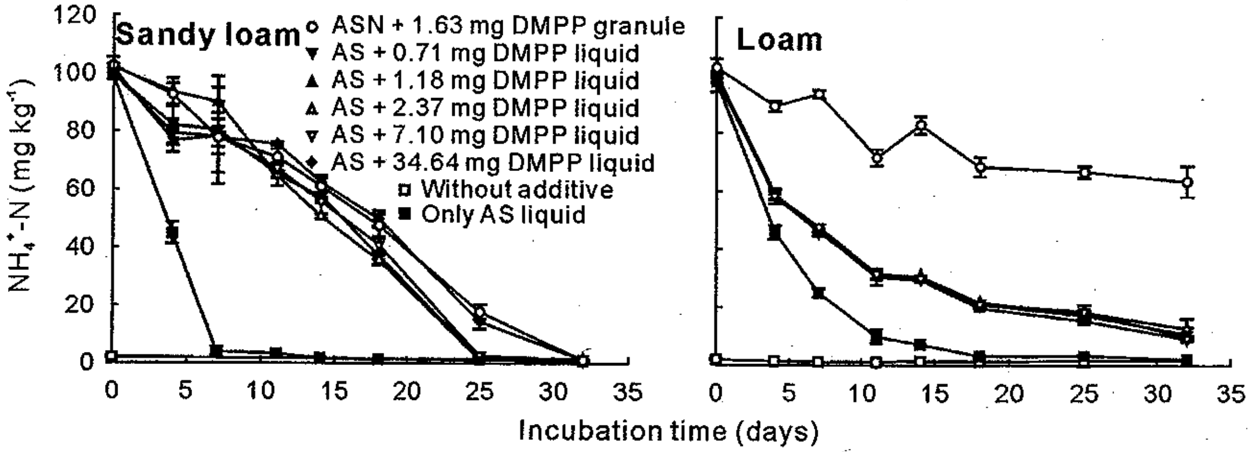

Fig. 1 Influence of different 3,4-dimethylpyrazole phosphate (DMPP) applications on the NH_4^+ oxidation in a sandy loam and a loamy soil. NH_4^+ was added as $(NH_4^+)_2SO_4$ (AS) or $(NH_4^+)_2SO_4/NH_4NO_3$ (ASN). DMPP addition is indicated in mg kg^{-1} soil. Vertical bars represent standard deviations.

DMPP decomposition

Samples of the sandy loam with 7.1 mg DMPP kg^{-1} soil were analysed for DMPP to follow the process of DMPP degradation. DMPP concentrations decreased significantly until the 12th day of incubation. In the second half of the incubation period, the DMPP decomposition slowed down. Small amounts of DMPP were still detectable at the end of the experiment (Fig. 2).

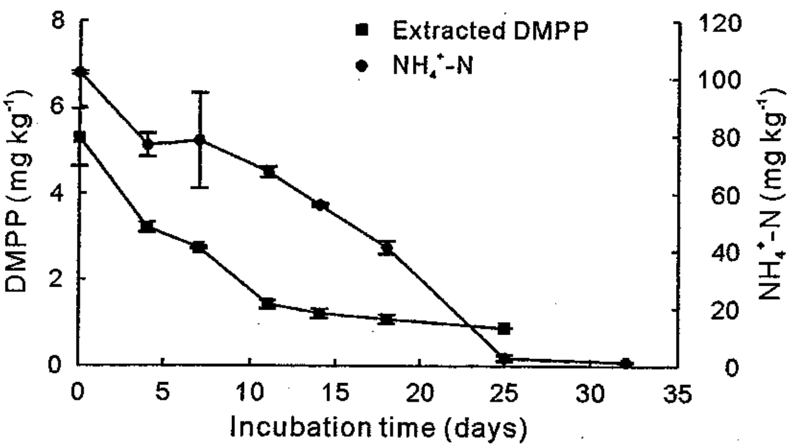

Fig. 2 3,4-Dimethylpyrazole phosphate (DMPP) degradation in sandy loam. 7.1 mg DMPP kg^1 was added at the beginning of the incubation. Vertical bars represent standard deviations.

Influence of soil matric potential on the effectiveness of DMPP

As microbial activity is significantly influenced by water availability, the effect of soil moisture on the effectiveness of DMPP was investigated at equal soil matric potentials. Without DMPP decreasing soil matric potentials from −50 down to −600 kPa reduced nitrification in both soils (Fig. 3). Compared to the loamy soil this decrease in nitrification under drier conditions was much more pronounced in the sandy loam, where the oxidation of NH_4^+ was markedly reduced at −600 kPa compared to −50 kPa.

However, a smaller reduction in nitrification with decreasing soil matric potential was also observed in the loamy soil.

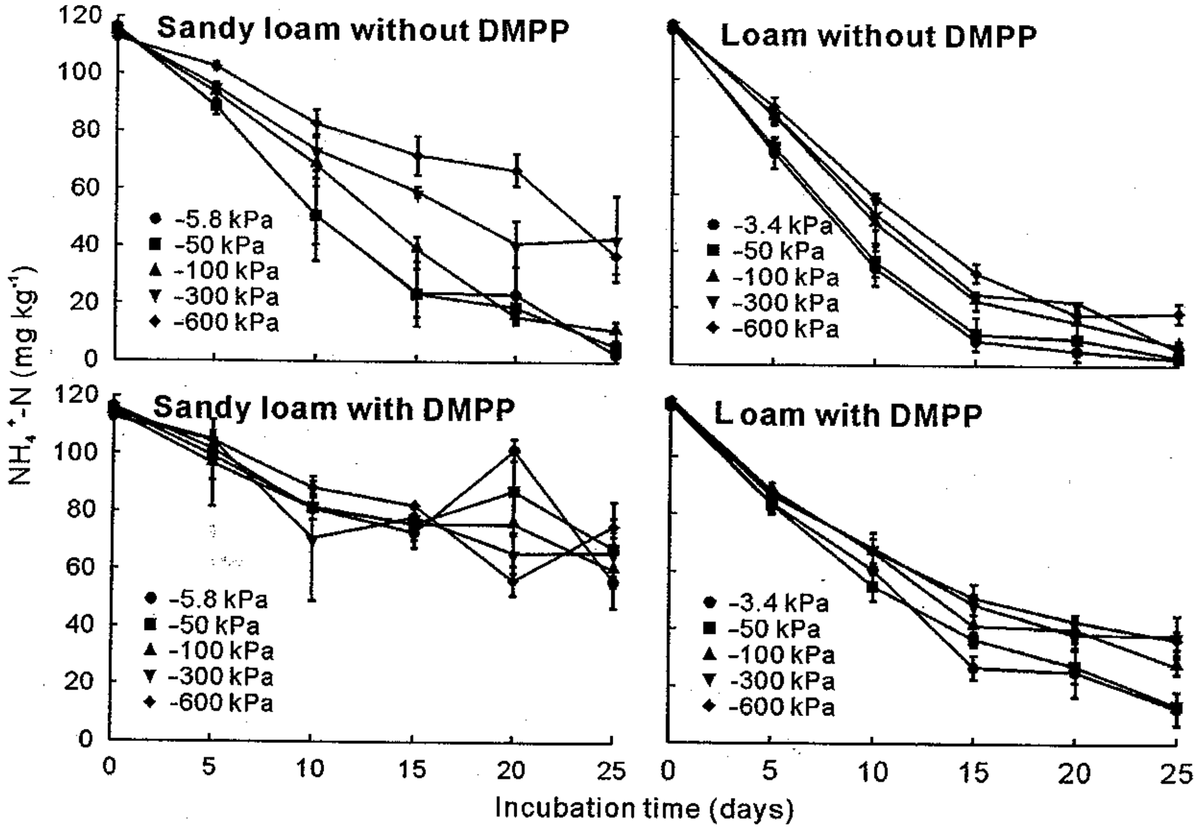

Fig. 3 Effect of soil matric potential on the NH_4^+ oxidation in a sandy loam and a loamy soil with and without addition of 3,4-dimethylpyrazole phosphate (DMPP). Vertical bars represent standard deviations.

With DMPP in the loamy soil the differences in the NH_4^+ oxidation depending on a soil matric potential between −50 and −600 kPa were similar to those without DMPP. Only the level of the NH_4^+ content at each of the different soil matric potentials was higher with DMPP compared to without DMPP. In the sandy loam the differences in the NH_4^+ levels between the soil matric potentials were much lower with DMPP compared to without DMPP. As a result, DMPP very efficiently inhibited the nitrification even at the higher soil matric potentials of −50 and −5.8 kPa.

DISCUSSION

In the incubation experiments the nitrification inhibitor DMPP markedly retarded the nitrification in two soils that differed mainly in their soil texture. This is in line with a broad efficacy of DMPP reported for different soils in short-term incubation experiments (Barth *et al.*, 2001) and under field conditions (Linzmeier *et al.*, 1999; Pasda *et al.*, 2001).

However, distinct differences in the extent and duration of the DMPP inhibition effect between the soils were observed. In the loamy soil, DMPP delayed NH_4^+ oxidation less efficiently than in the sandy loam. Soil related differences in the effect of pyrazole based nitrification inhibitors were also described by McCarty and Bremner (1989). Pyrazole compounds, including 3,5-dimethylpyrazole (3,5-DMP), were more efficient in soils with low contents of clay, silt and organic carbon. More recent studies demonstrated that the efficacy of 3,4-DMPP was closely related to soil inorganic constituents and that the adsorption of DMPP to the soil clay fraction played a major role in controlling the inhibition effect (Barth *et al.*, 2001). Results presented here supported this assumption. Nitrification was less inhibited in the soil with the higher clay and silt content, where DMPP may have been adsorbed.

When applied as solution, increasing the amount of DMPP (0.7 up to 7.1 mg kg^{-1}) to the soil did not lead to a change in the ammonium levels. This observation was supported by data from Azam and

Müller (2005) and Chaves *et al.* (2006), who used comparable DMPP concentrations. McCarty and Bremner (1989), investigating a number of different pyrazoles, reported that all compounds capable of retarding the NH_4^+ oxidation (including 3,5-DMP) were more effective at higher concentrations. The reason for this discrepancy may be associated with the distinctly lower range of concentrations used in the present experiment. But this also indicated that DMPP was efficacious at low concentrations. It might therefore be assumed that the DMPP concentration of about 1 mg kg^{-1} that remained in the sandy loam at the end of the incubation might still have been able to delay nitrification if further NH_4^+ was added. Our data also indicate that, when applied as solution, much higher dosages than tested here would be necessary to further reduce the activity of the nitrifiers.

Compared to the application as solution, the inhibition of the nitrification in the loamy soil was more pronounced when NH_4^+ and DMPP were applied together as fertilizer granules. Granules will create high local concentrations of NH_4^+ and DMPP (Azam *et al.*, 2001), greatly exceeding the concentrations applied as solution, and consequently protecting NH_4^+ against oxidation very effectively. In addition, a water content of 200 g kg^{-1} (corresponding to a soil matric potential of less than -600 kPa) probably delays the dissolution of the granule in the loamy soil, while in the sandy loam, granules will be readily disintegrated at the same soil water content, which results in a soil matric potential of above -5.8 kPa. A low dissolution of the granules may also result in a restricted microbial accessibility to NH_4^+ in the loamy soil below -600 kPa. Consequently, the effectiveness of DMPP applied as granules seemed to be higher in the loamy soil than that in the sandy loam, where the effect of the granules was similar to that of the DMPP added as solution.

Nitrification is affected by soil water content (Grundmann *et al.*, 1995). Depending on the soil texture, equal gravimetric soil water contents result in different soil matric potentials. This implies changes in the availability of water to microorganisms, affecting the activity of the nitrifying bacteria at low water contents either by cell dehydration or by substrate limitation (Stark and Firestone, 1995). In the absence of DMPP, a reduction of the soil matric potential below -50 kPa decreased the nitrification of ammonium. This inhibition was further enhanced with decreasing soil matric potentials down to 600 kPa, particularly in the sandy loam. Under these conditions the decline in nitrification rates can be ascribed to cell dehydration and substrate limitation in rather equal shares (Stark and Firestone, 1995). Stark and Firestone (1995) further concluded that above -600 kPa substrate limitation caused by the inhibition of the diffusion of uniformly distributed NH_4^+ was of growing importance as the limiting factor. Ammonium diffusion will be expected to decrease in more coarse-textured soils, where soil water contents at defined soil matric potentials (below field capacity) are much lower compared to fine textured soils. This is in agreement with the results for the sandy soil, where the decrease in NH_4^+ oxidation is markedly more significant than that in the loamy soil. A distinct reduction of the nitrification with decreasing soil matric potentials from -33 to $-1\,000$ kPa in a sandy loam was also found by Yadvinder-Singh *et al.* (2001).

The system presented here was complicated by the application of NH_4^+ as fertilizer granules, entailing aspects of spatial distribution of NH_4^+ around the granule. Estimation of the mean diffusion distance $D_e = (D_1\theta f)/b$ with b representing buffer capacity for NH_4^+ according to Anghinoni and Barber (1990); D_1 diffusion coefficient in solution according to Teo *et al.* (1992); f tortuosity factor according to Barraclough and Tinker (1981); θ volumetric soil water content, indicates that decreasing water contents below 110 g kg^{-1} (-100 kPa) in the sandy loam will markedly restrict the local distribution of NH_4^+ in the vicinity of the granule and thereby the spatial accessibility of NH_4^+ to nitrifying bacteria. The resulting high initial NH_4^+ concentrations will further decrease the nitrification rate.

In the sandy loam, soil matric potential dependent differences in the NH_4^+ oxidation almost disappeared in the presence of DMPP. DMPP displayed a significant inhibition effect particularly at high water contents, where the microbial activity and the spatial availability of NH_4^+ would not be limited. This demonstrates the high efficacy of DMPP under conditions of high water availability and low DMPP adsorption to soil constituents. This will be important for the inhibition of the nitrification also under field conditions. Pasda *et al.* (2001) demonstrated that the effect of DMPP containing fertilizers on yield parameters was more pronounced under conditions of less fertile soils and higher rainfall.

CONCLUSIONS

3,4-Dimethylpyrazole phosphate (DMPP) was capable of inhibiting the nitrification of ammonium in varying soil types. Differences in the effectiveness of DMPP were related to soil properties, such as the contents of clay and silt, and environmental conditions like soil water content. DMPP strongly inhibited nitrification in the sandy soil particularly at high soil matric potentials. This will be advantageous with respect to the preservation of NH_4^+ under field conditions favorable for NO_3^- leaching. The application form, as DMPP/NH_4^+ granules or more uniformly distributed as solution, also affected the NH_4^+ oxidation. Granules were found to be more efficient, particularly in the fine textured soil.

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