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ORIGINAL PAPER

G. Barth · S. von Tucher · U. Schmidhalter

Influence of soil parameters on the effect of 3,4-dimethylpyrazole-phosphate as a nitrification inhibitor

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Abstract Nitrification inhibitors specifically retard the oxidation of NH_4^+ to NO_2^- during the nitrification process in soil. In this study, the influence of soil properties on the nitrification-inhibiting effect of 3,4-dimethylpyrazole-phosphate (DMPP), a newly developed nitrification inhibitor, has been investigated. Based on short-term incubation experiments, where the degradation of DMPP could be largely disregarded, the oxidation of the applied NH_4^+ was more inhibited in sandy soils compared with loamy soils. The influence of soil parameters on the relative NO_2^- formation could be described by a multiple regression model including the sand fraction, soil H^+ concentration and soil catalase activity (R^2=0.62). Adsorption studies showed that the binding behaviour of DMPP was influenced markedly by soil textural properties, viz. the clay fraction (r^2=0.61). The adsorption of DMPP was found to be an important factor for the inhibitory effect on NH_4^+ oxidation in a short-term incubation (r^2=0.57). It is concluded that the evaluated soil properties can be used to predict the short-term inhibitory effect of DMPP in different soils. The significance of these results for long-term experiments under laboratory and field conditions needs further investigation.

Keywords Adsorption · 3,4-Dimethylpyrazole-phosphate · Nitrification inhibitor · Short-term incubation · Soil texture

Introduction

Nitrification is a key process of N transformation in soils. It converts a relatively immobile form of N, NH_4^+, into a mobile form, NO_3^-. NO_3^- is subjected to losses by leaching and gaseous emissions from denitrification. Nitrification inhibitors (NI) retard specifically the oxidation of NH_4^+, leading to an extended NH_4^+ phase. Consequently, N losses may be reduced and the efficiency of N-fertiliser use is increased.

3,4-dimethylpyrazole-phosphate (DMPP) is a new nitrification inhibitor developed by BASF (Ludwigshafen, Germany) (Zerulla et al. 2001). DMPP shows some advantages when compared to dicyandiamide (DCD). When using DMPP the amount of the NI can be markedly reduced, and it is applied as only 1% of the NH_4^+-N in the fertiliser (Zerulla et al. 2001). DCD may be subject to leaching (Abdel-Sabour et al. 1990), but no leaching of DMPP was observed in previous lysimeter experiments (Fettweis et al. 2001).

In field studies, DMPP retarded nitrification under different site conditions, but NH_4^+ was more persistent in a silty loam soil than in a loamy sand soil (Linzmeier et al. 1999). Differences in soil properties are therefore expected to influence the nitrification-inhibiting effect of DMPP. This is supported by the observation that positive effects of DMPP on crop yield were more pronounced in light than in heavy soils (Pasda et al. 2001). But so far, interactions between DMPP and soil properties are little understood. To predict the effect of DMPP on the nitrification of fertiliser NH_4^+, it is necessary to evaluate soil parameters which influence the behaviour of DMPP in soils.

This study investigates the influence of different soil parameters on the nitrification-inhibiting effect of DMPP. Therefore, a large number of soils was required for testing. However, field studies and classic incubation studies are time consuming. Therefore, a short-term incubation procedure has been adapted to demonstrate direct interactions between soil and DMPP. In addition, the short duration of the experiment (2 days) minimised the risk of DMPP decomposition.

Differential effects of DMPP in various soil types may be caused by DMPP adsorption to soil components. Thus, studies were carried out with several soils to investigate to what extent DMPP may be adsorbed to soil and which soil parameters correlate with DMPP adsorption. If parameters which influence the inhibition effect

G. Barth · S. von Tucher (✉) · U. Schmidhalter
Technical University Munich,
85350 Freising-Weihenstephan, Germany
e-mail: tucher@weihenstephan.de
Fax: +49-8161/71-4500

of DMPP are known, strategies can be developed to optimise the use of DMPP.

Materials and methods

Soils

Twenty-two different soils with a wide variation in soil characteristics were investigated (Table 1). Soil texture was determined by the pipette method (Gee and Bauder 1986) and cation exchange capacity (CEC) according to Mehlich (1948) modified by Meiwes et al. (1984). Organic C (C_{org}) was determined by elemental analysis on a LECO-Instrument CN 200 (Kirchheim, Germany), total N (N_t) by elemental analysis (Macro-N Elementar Analysensysteme, Hanau, Germany) and catalase activity as described by Weigand et al. (1995). Soil pH was measured in a 1:2.5 soil/0.01 M $CaCl_2$ suspension. Potential nitrification was determined by means of a short-term incubation procedure as described below.

Short-term incubation

Short-term incubation experiments were carried out to investigate the influence of soil parameters on the effect of DMPP in various soils. The short-term incubation procedure is based on the same principle as the determination of potential nitrification (Belser and Mays 1980; Berg and Rosswall 1985). $(NH_4)_2\ SO_4$ (2.36 mg), 15.97 mg $NaClO_4$, and DMPP dissolved in distilled water at different concentrations (0, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 50.0, 100.0 µg g^{-1} soil), were added to moist soil (5 g dry weight). The samples were water saturated and incubated at 25°C for 5 h. After the incubation, 15 ml of 0.0125 M $CaCl_2$ solution was added to the water-saturated soil and the soil samples were shaken horizontally for 30 min at a rate of 250 movements min^{-1} (Köttermann, Uetze, Germany). Afterwards, the soil samples were centrifuged for 10 min at 2,700 *g* with a Beckmann GS 6 centrifuge (Beckmann Instruments, Munich) and the soil extract was obtained by filtration with a membrane filter (0.22-µm mesh). NO_2^- and NO_3^- concentrations were measured by HPLC (Vilsmeier 1984).

To eliminate effects of different levels of soil nitrifying potentials, NO_2^- formation of the soils was expressed relative to the control without DMPP. Correlation coefficients and multiple regressions were calculated for soil properties and relative NO_2^- formation at a concentration of 5 mg DMPP kg^{-1} dry soil. This was the most sensitive DMPP concentration at which inhibition in all soils reached levels >0% and <100%. Coefficients of correlations and multiple regressions between soil parameters and the relative NO_2^- formation were calculated by using SAS (SAS Institute, Cary, N.C.).

Adsorption studies

$CaCl_2$ solution (100 ml of a 0.01 M solution) with 0.2, 2.0 or 20.0 mg DMPP was added to moist soil (20 g fresh weight) and shaken for 1 h at 40 r.p.m. The samples were centrifuged for 10 min at 2,700 *g* and subsequently filtered with folded filters (Schleicher and Schuell 595 1/2, Dassel, Germany). A 50-ml subsample of the extract was transferred into a 250-ml separating funnel. After the addition of 25 g NaCl, 150 ml of 2.5% NH_3 and 80 ml *tert*-butyl methyl ether (MTBE), the mixture was horizontally shaken for 2 min at 250 movements min^{-1} and then allowed to settle. The aqueous phase was drained off into a second 250-ml separating funnel and 80 ml MTBE was added. The organic phase was drained off into a 500-ml round-bottom flask containing 25 ml of 0.1 M HCl. This partition step was repeated twice. The combined MTBE solution was rotary evaporated to the acid phase at a pressure of 0.045 MPa using a water bath temperature of 40°C. The solution was brought to pH 12 with 32% NaOH and quantitatively transferred into a Baker SPE C18 column (Baker, Phillipsburg, N.J.) with 0.05 M NaOH. Thereafter the column was dried by placing it above silicate granulate overnight. DMPP was then eluted with 3 ml acidic CH_4 (25 ml CH_4 plus 1 ml of 1 M H_2SO_4) and measured by HPLC (column, Lichrosorb C18 7 µm, 250×4 mm and precolumn 60×4 mm; eluent, acetonitrile in H_2O, 0.15:1 (v:v) with 1 ml of 85% H_3PO_4 l^{-1}; flow, 1 ml min^{-1}; UV detection at 220 nm).

The adsorption of DMPP was calculated as the difference between the initial DMPP concentrations and the equilibrium DMPP concentrations, and related to soil dry weight. Since adsorption curves were linear throughout all three concentrations, mean val-

Table 1 Physical, chemical and biochemical properties of the investigated soils. *CEC* Cation exchange capacity, C_{org} organic C, N_t total N

Soil	Clay	Silt	Sand	CEC[a] (mEq 100 g^{-1})	pH ($CaCl_2$)	C_{org}	N_t	Catalase activity (Catalase number)	Potential nitrification (mg NO_2^-–N kg^{-1} soil)
	(%)					(g kg^{-1})			
1	31	58	11	26.3	7.5	42.0	4.0	13.8	1.066
2	8	14	78	7.2	5.1	26.3	2.5	4.1	0.024
3	26	46	28	9.8	5.8	9.2	1.0	4.7	0.138
4	23	47	31	7.9	5.3	9.4	1.0	4.9	0.044
5	23	46	31	8.5	5.6	8.9	1.0	4.5	0.102
6	23	48	29	11.9	5.7	16.6	1.5	13.1	0.250
7	23	50	27	13.0	7.5	23.1	0.9	12.0	1.190
8	10	30	61	9.0	6.6	12.4	0.9	3.6	0.296
9	9	18	73	8.9	6.9	8.0	0.8	4.7	0.284
10	25	61	14	10.6	6.4	10.4	0.9	4.7	0.078
11	15	66	19	11.8	6.4	13.9	1.4	11.8	0.574
12	3	22	75	11.0	5.5	8.6	0.8	1.7	0.058
13	6	19	76	5.0	6.9	11.7	0.8	3.8	0.278
14	27	56	17	11.5	6.0	11.8	1.2	6.0	0.268
15	25	53	21	11.9	6.6	18.2	1.6	10.2	0.498
16	25	49	27	12.7	6.7	21.1	1.8	10.8	0.496
17	14	75	11	11.9	6.5	16.4	1.9	11.3	0.912
18	13	77	10	8.3	6.4	8.8	1.3	9.8	0.312
19	10	41	49	10.3	6.9	5.5	0.6	4.6	0.762
20	9	29	62	6.4	6.0	11.1	1.2	4.8	0.196
21	40	51	9	25.3	7.2	38.0	3.7	17.6	2.826
22	20	65	15	27.8	7.3	53.1	4.0	15.4	1.620

[a] CEC was determined at pH 8.1

ues of all DMPP concentrations were calculated. Correlations between soil parameters and DMPP adsorption, as well as between results from the short-term incubation experiments and DMPP adsorption were calculated by using SAS.

All statistical values in the short-term incubation and adsorption studies were significantly different at $P<0.05$.

Results

Short-term incubation

Short-term incubation experiments (5 h) as conducted in this study enable the first step of the nitrification process – the formation of NO_2^- – to be examined. To evaluate the specific effect of the nitrification inhibitor in different soil types, NO_2^- formation in the presence of DMPP was expressed relative to the NO_2^- formation without inhibitor. Low values of relative NO_2^- formation indicate a strong reduction of nitrification. Figure 1 shows the relative NO_2^- formation with DMPP in three representative soils (soils 4, 6 and 8). The lowest relative NO_2^- formation was observed in a sandy soil, whereas nitrification was much less inhibited in a loamy soil. Differences between soils were most distinct at DMPP concentrations between 1 mg and 10 mg DMPP kg^{-1} dry soil.

The relative NO_2^- formation was only moderately explained by a single soil parameter. Significant and positive correlations were found between relative NO_2^- formation and silt (r=0.60), catalase activity (r=0.59), clay (r=0.54), N_t (r=0.48), C_{org} (r=0.37), and CEC (r=0.34). The highest, but negative correlation, was observed with sand (r=–0.65). The inhibitory effect of DMPP was better predicted by a multiple regression model (Fig. 2). The influence of soil texture on the relative NO_2^- formation in this model was best explained by the correlation to the sand fraction (R^2=0.43). The relationship was improved by including the H^+ concentration (R^2=0.55) and catalase activity (R^2=0.62). Potential nitrification further improved the regression to give R^2=0.70 (data not shown).

Adsorption experiments

The variations in DMPP efficiency among soils may be caused by differences in DMPP adsorption to soil components. Adsorption studies were carried out with several soils to verify this hypothesis.

Calculation of correlations from adsorption studies showed a strong relationship between DMPP adsorption and soil texture (Fig. 3): clay (r=0.78), silt (r=0.68), and

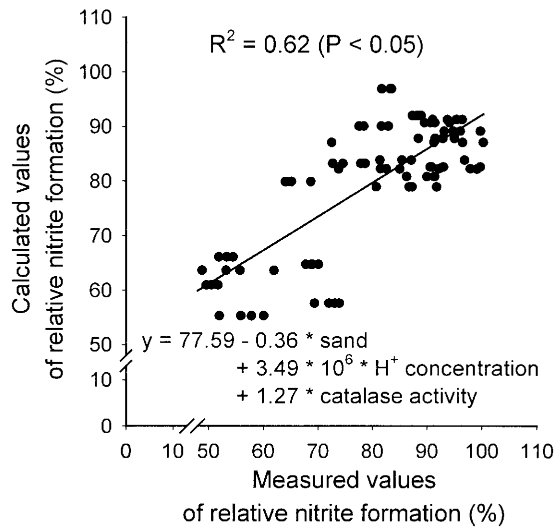

Fig. 2 Predicted versus measured values of relative NO_2^- formation in short-term incubation experiments at 5 mg DMPP kg^{-1} soil

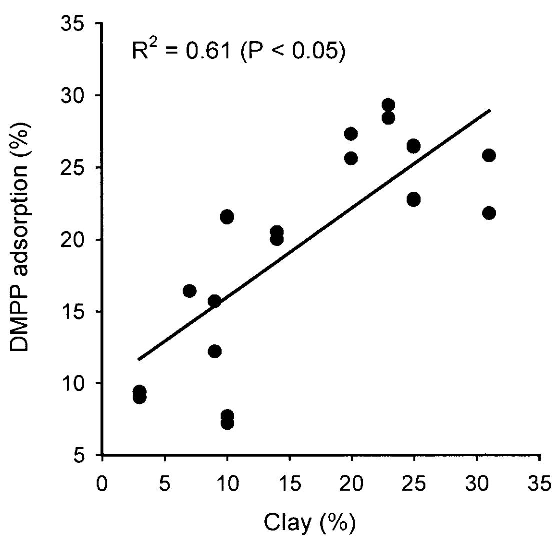

Fig. 1 Influence of different 3,4-dimethylpyrazole-phosphate (*DMPP*) concentrations in short-term incubation experiments on the relative NO_2^- formation in three selected soils. *Error bars* represent SDs

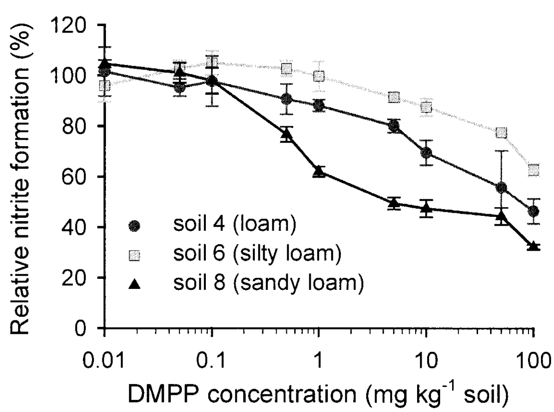

Fig. 3 Correlation between DMPP adsorption and clay content in soils

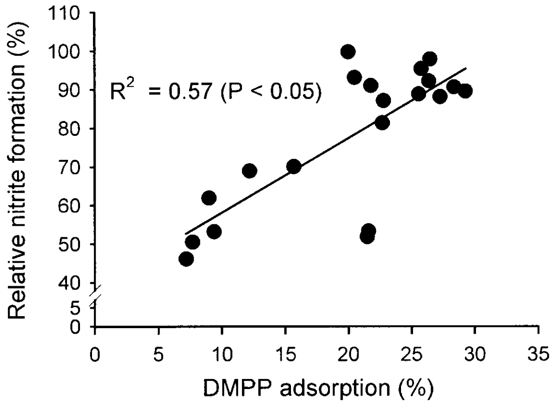

Fig. 4 Correlation between DMPP adsorption and relative NO_2^- formation at a DMPP concentration of 5 mg kg^{-1} soil

sand (r=–0.76). N_t (r=0.51) and C_{org} content (r=0.49) were less suitable indicators of DMPP adsorption. The highest correlation was observed between DMPP adsorption and catalase activity (r=0.85). Relative NO_2^- formation and DMPP adsorption were closely correlated to each other (r=0.76), which indicated that the inhibitory effect of DMPP in the short-term incubation was significantly explained by the adsorption behaviour of DMPP (Fig. 4).

Discussion

This study investigated the effect of DMPP on nitrification in 22 different soils in short-term incubation experiments. DMPP inhibited the oxidation of added NH_4^+ to NO_2^- in all the soils which were tested. Distinct differences in the inhibitory effect of DMPP were observed among the soils. The relative NO_2^- formation decreased and the efficacy of DMPP increased when soils were higher in sand content. This behaviour was only partially explained by single correlations. Therefore, a multiple regression was calculated including the sand fraction, soil H^+ concentration, and catalase activity, which significantly improved the relationship.

In this calculation, the soil H^+ concentration represents a suppression variable (Velicer 1978; Lutz 1983), the inclusion of which improved the prediction. This parameter was not correlated to the relative NO_2^- formation but a weak correlation existed with catalase activity, thereby indicating the influence of pH on this parameter.

The significance of catalase activity to the inhibitory effect of DMPP in short-term incubation experiments should be interpreted in the context of adsorption properties of both catalase and DMPP to soil surfaces. Fusi et al. (1989) and Calamai et al. (1991) showed that catalase was adsorbed to clay minerals. The adsorbed catalase is protected against microbial degradation (Stotzky 1986). This leads to more reproducible values in measured catalase activities despite different effects of season, or sample preparation (Beck 1971). The correlation between catalase activity and DMPP adsorption may be the result of their similar binding behaviour on soil surfaces. Adsorption of DMPP in different soils proved to be an important factor in relative NO_2^- formation (Fig. 4).

By including the parameter of potential nitrification in the regression, the model was further improved with a coefficient of determination of R^2=0.70. This parameter, however, represents a microbiological soil characteristic, which is highly affected by the timing of soil collection (Staley et al. 1990) and possibly the subsequent storage conditions.

The adsorption behaviour of DMPP was markedly influenced by soil textural properties, viz. clay content (Fig. 3). Correlations of DMPP adsorption with soil N_t and organic matter were less significant (N_t, r=0.54; C_{org}, r=0.49). Nevertheless, the role of organic matter in the adsorption of pyrazoles has been documented for a phenyl pyrazole compound (Bobe et al. 1997).

Results from the adsorption studies suggest that DMPP is hardly subjected to translocation within the soil profile and the risk of DMPP leaching is low. This is consistent with results from lysimeter studies, where DMPP could not be detected in the leachate and the major part of the applied radioactivity in the pyrazole ring remained in the upper part of the topsoil (Fettweis et al. 2001). The soil-adsorption behaviour of DMPP also implies, that, in contrast to DCD (Amberger and Vilsmeier 1988; Adbel-Sabour et al. 1990; Corre and Zwart 1995), a spatial separation of the active substance from the applied NH_4^+ seems to be much less probable.

From these results, it can be concluded that the short-term inhibitory effect of DMPP was markedly influenced by the adsorption of the active substance, especially to inorganic soil constituents. Yet, one of the most decisive factors for the efficacy of DMPP as a nitrification inhibitor is the concentration at which it is available to the nitrifying microorganisms over an extended period of time. During a long-term incubation and under field conditions, additional factors, such as the degradation of the inhibitor, become more relevant. If adsorbed DMPP, as opposed to DMPP in soil solution, is better protected against microbial degradation and is then remobilised at a sufficiently high equilibrium concentration, this may ultimately result in an extended inhibitory effect in soils with higher adsorption capacities. Field experiments conducted with a DMPP-stabilised N fertiliser (ENTEC) showed that in a silty loam nitrification was inhibited for a longer time than in a loamy sand (Linzmeier et al. 1999). In that study, ENTEC was applied in formulation with fertiliser granules. This may have important implications on the adsorption, as well as the degradation of DMPP, as high concentrations of both NH_4^+ and the inhibitor will be present in the vicinity of the granule.

In conclusion, in short-term incubation experiments, the adsorption of DMPP to inorganic soil constituents mostly explained the extent of the inhibition of nitrification. This binding behaviour could be described by certain soil parameters: sand content, H^+ concentration and catalase activity. These factors can be used for the prediction of the short-term efficiency of DMPP. The implications of these results for long-term experiments under laboratory and field conditions, including the degradation of the inhibitor and the effect of applied fertiliser, formulated as granules, need further investigation.

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