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

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List of Abbreviations

%	per cent
µg	microgram
AMO	ammonia monooxygenase
b	buffer capacity
$CaCl_2$	calcium chloride
CEC	cation exchange capacity
CMP	1-carbomyl-3-methylpyrazole
C_{org}	organic carbon
Cu	copper
d	days
DCD	dicyandiamide
D_e	effective diffusion coefficient
D_l	diffusion coefficient in water, cm^2*s^{-1}
DMP	3, 4-dimethypyrazole
DMPP	3, 4-dimethypyrazole phosphate
f	factor of resistance
H^+	proton
H_2O	water
H_2SO_4	sulphuric acid
H_3PO_4	phosphoric acid
hPa	hecto Pascal
HPLC	High performance liquid chromatography
KCl	potassium chloride
l	liter
M	molar
min	minute

MPa	Mega Pascal
MTBE	*tert*-buthyl methyl ether
N	nitrogen
NaCl	sodium chloride
$NaClO_4$	sodium chlorate
NaOH	sodium hydroxide
NH_4^+	ammonium
NH_4NO_3	ammonium nitrate
N_{min}	mineral nitrogen
NO_2^-	nitrite
NO_3^-	nitrate
N_t	total nitrogen
p	probability
pH	pH value
r	correlation coefficient
R^2	Coefficient of determination
rpm	rotation per minutes
UV	ultraviolet
v : v	volume per volume
θ	theta, water content per volume

1 Introduction

1.1 Nitrification in agriculture

Nitrification is a key process of nitrogen transformation in soils. It converts a relatively immobile form of nitrogen, ammonium (NH_4^+), into a mobile form, nitrate (NO_3^-). Nitrate is subjected to losses by leaching and gaseous emissions commonly described as denitrification. Nitrification consists of two steps carried out by two different bacteria groups. In the first step, *Nitroso*- bacteria (*Nitrosomonas*, *Nitrosococcus*, *Nitrosolobus*, *Nitrosospira*) oxidise ammonium to nitrite (NO_2^-). In the second step, *Nitro*- bacteria (*Nitrobacter*, *Nitrococcus*) oxidise the nitrite to nitrate (Schlegel 1992). In agriculture and horticulture, a considerable part of the nitrogen applied as fertiliser may be lost, and is not available to the cultivated plants. Amberger (1981a) mentioned 30 – 40% losses of nitrogen with mineral fertilisers and around 70 % with liquid organic N-fertilisers. Under most conditions, the predominant part of nitrogen is lost by nitrate leaching that amounts up to between 40 and 80 kg nitrogen per hectare and year depending on the environmental conditions. It can reach the ground water, and pollute the drinking water.

Denitrification is a further source for nitrogen losses. Under anaerobic conditions some facultative anaerobic bacteria, such as *Pseudomonas denitrificans, Thiobacillus denitrificans,* or *Bacillus licheniformis* can decompose nitrate (NO_3^-) to nitrite (NO_2^-), nitrogen oxide (NO), nitrous oxide (N_2O), and to atmospheric nitrogen (N_2). An other form of gaseous nitrogen losses is the emission of NH_3 predominantly from the storage and application of organic fertilisers (Schlegel 1992).

The application of nitrification inhibitors is one possibility to reduce nitrogen losses thereby increasing nitrogen fertiliser efficiency. Nitrification inhibitors specifically retard the oxidation of Ammonium (NH_4^+) leading to an extended NH_4^+ phase.

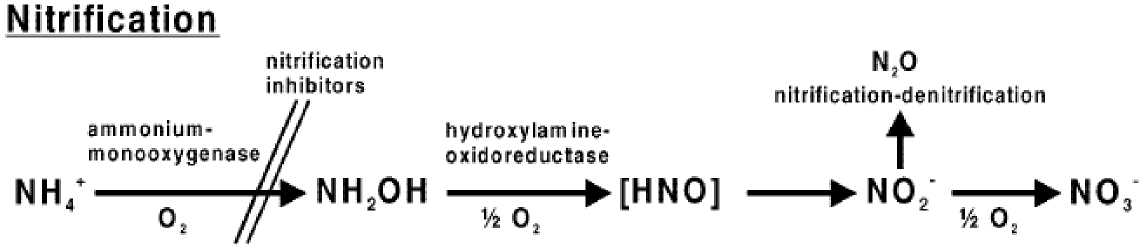

Fig. 1 Inhibition of nitrification by specific nitrification inhibitors (NIs) blocking the NH_3^- oxidation (ammonium monooxygenase).

Consequently, N - losses by nitrate leaching and gaseous emissions from denitrification may be reduced and the efficiency of N - fertiliser use is increased (Slangen and Kerkhoff 1984).

Moreover the extended ammonium phase allows to reduce the number of nitrogen fertiliser applications to crops. (Linzmeier et al. 2001a).

1.2 Nitrification inhibitors and their mode of action

The efficiency of nitrification inhibitors is influenced by the interaction with the ammonia monooxygenase (AMO) (Fig. 1) and by external factors like soil properties including temperature and water content. Three different kinds of interaction between nitrification inhibitors and AMO are known. For most compounds the inhibitory effect is due to a competition for the active site of the AMO. Others, such as acetylene, are oxidised by the normal catalytic cycle of AMO to highly reactive products which covalently bind the enzyme causing irreversible inhibition. A broad range of S-containing compounds inhibits AMO activity by binding with Cu within the active site. Heterocyclic N compounds, like Nitrapyrin (2-Chloro-6-(trichloromethyl)pyridine) or CMP (1-carbonyl-3-methylpyrazole) represent another important class of nitrification inhibitors whereby only little is known about their mode of action (McCarty 1999). Researches suggest that their inhibitory influence is closely related to the presence of adjacent ring N atoms (McCarty and Bremner 1989). The mode of action of dicyandiamide (DCD), an established nitrification inhibitor in Germany is unknown too. Amberger (1968, 1981b, 1983) suggests an uncoupling between respiration and transfer of energy. Presumably the effect is caused by the inhibition of the oxidative phosphorylation within the metabolism of *Nitrosomonas sp*.

1.3 Nitrification inhibitors in the current use

World-wide, different compounds are applied as nitrification inhibitors. Nitrapyrin (2-Chlor-6-trichlormethylpyridin), CMP (1-Carbomyl-3-methylpyrazole), acetylene (H_2C_2) and DCD (dicyandiamide) are part of them.

The solubility of nitrapyrin in water is low and in soil it is relatively persistent. Bundy and Bremner (1973) show that nitrapyrin is relatively strongly bound to the organic matter in the soil. Depending on the mode of application, it is bacteriostatic or bactericidal (Rodgers and Ashworth 1982). Nitrapyrin is predominantly used as nitrification inhibitor in the USA (McCarty and Bremner 1989).

Compared with nitrapyrin, CMP is similar in its efficiency of nitrification inhibition. It is more effective when nitrogen is applied as ammonium nitrogen than as urea nitrogen (Bremner and McCarty 1990).

Acetylene causes an irreversible inactivation of the ammonia monooxygenase (AMO). Nevertheless, it is bacteriostatic (Juliette et al. 1993). Acetylene is manly used in rice cropping.

DCD has a bacteriostatic effect on *Nitrosomonas sp.* and the activities of other soil micro-organisms are not influenced by DCD (Amberger 1983). DCD is well soluble in water and has to applied in high concentrations, but it is of low toxicity. In addition DCD consists of 67% nitrogen and hence is also a slow release nitrogen fertiliser (Amberger 1983). After repeated application on the same site DCD has been observed to lose in efficacy. Because the soil microflora adapts to the active ingredient the mineralisation of DCD is accelerated (Rajbahnshi et al. 1992).

1.4 DMPP as nitrification inhibitor

Between 1997 and 2001 DMPP has been tested and introduced as nitrification inhibitor by BASF AG in cooperation with different research groups. DMPP shows some advantages compared to another nitrification inhibitor like DCD. The amount of the nitrification inhibitor can be markedly reduced, and is applied at only 1 % of the ammonium-N in the fertiliser (Zerulla et al. 2001a). DCD may be subject to leaching (Abdel-Sabour et al. 1990). Fettweis et al. (2001) investigated the leaching of DMPP during several years in lysimeter studies with undisturbed monoliths of a gleyic cambisol soil with potatoes, winter wheat and winter barley. During the entire period, leaching of DMPP was always below 0.1 µg l^{-1}.

In field studies, DMPP retarded nitrification under different site conditions but compared to a sandy loam soil, NH_4^+ was more persistent in a loamy soil (Linzmeier et al. 1999). Differences in soil properties, e.g. texture, cation exchange capacity, water capacity or the content of organic carbon 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. (2001b) who have conducted a large number of field experiments under different climatic conditions in Western and Southern Europe confirm this effect. In these experiments various fertiliser strategies with different agricultural and horticultural crops were applied. But so far, interactions between DMPP and soil properties are little understood. To predict the effect of DMPP on the nitrification of the fertiliser ammonium, it is necessary to evaluate soil parameters, which affect the behaviour of DMPP in soils. Further factors, such as DMPP concentration, soil water content, application form and DMPP degradation can also influence the nitrification inhibitory effect of DMPP. In this study, the influence of external soil properties, soil matric potential, application form and the decomposition of DMPP in soil will be investigated.

1.4.1 Short term influence of soil parameters on the DMPP efficacy

One part of this study will investigate the influence of different soil parameters on the nitrification inhibitory effect of DMPP. For that, a large number of soils is required for testing. However, field studies and classical incubation studies with an incubation time between four and six weeks, are labour-intensive and time consuming. Consequently, a short - term incubation procedure (5 hours incubation time) has been applied, to investigate direct interactions between soil and DMPP. In addition, the short duration of the experiment (two days) minimised the risk of DMPP decomposition.

Different effects of DMPP in various soil types may be caused by DMPP adsorption to soil components. Therefore, a number of different soils were selected for DMPP adsorption studies and for evaluation of soil parameters which correlate with DMPP adsorption.

1.4.2 Long-term Influence of soil parameters on the DMPP efficacy

In classical long - term incubation experiments (32 d incubation time), the long term efficacy of DMPP was studied in two soils, a sandy loam and a loamy sand. DMPP was added in aqueous solutions at different concentrations and also applied in solid form formulated on ammonium nitrate fertiliser granules. When added in liquid form, the active substance will be more or less homogeneously distributed in the samples. In agricultural practice however, DMPP is commonly used formulated on fertiliser granules. Consequently, there are spots with high DMPP and NH_4^+ concentrations in the soil. The size of these spots and the substance concentrations, may be a result of the influence of soil moisture and soil type. To simulate these conditions and to examine the influence of different soil moisture levels on the nitrification inhibitory effect of DMPP, long - term incubation experiments were carried out with different soil matric potentials in two different soils.

1.4.3 DMPP decomposition

DMPP decomposition is a further factor affecting nitrification inhibition. This effect is investigated in selected samples of the long - term experiments.

If the soil parameters which influence the inhibitory effect of DMPP are known, and the interactions between DMPP, soil properties, soil water content and application form are investigated, strategies can be developed to optimise N fertilisation by using DMPP as nitrification inhibitor.

2 Material and Methods

2.1 Soils

Twenty-two different soils with a wide variation in soil characteristics were investigated (Tab. 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 carbon (C_{org}) was determined by elemental analysis on a LECO – Instrument CN 200 (Kirchheim, Germany), total nitrogen (N_t) by elemental analysis (Macro – N Elementar Analysensysteme GmbH, 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.

2.2 Influence of soil parameters on the nitrification inhibition of DMPP: short - term effects

2.2.1 Inhibition of nitrite formation in short - term experiments

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 was based on the same principle as the determination of potential nitrification (Belser and Mays 1980; Berg and Rosswall 1985). 2.36 mg ammonium sulphate, 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 mg kg^{-1} soil), were added to moist soil (5 g dry weight). The samples were water saturated and incubated at 25°C for five hours. 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 minutes at a rate of 250 movements min^{-1} (Köttermann GmbH, Uetze, Germany).

Afterwards, the soil samples were centrifuged for 10 minutes at 2,700 g with a Beckmann GS 6 centrifuge (Beckmann Instruments, Munich, Germany) and soil extract was obtained by filtration with a membrane filter (0,22 µm mesh size). Nitrite and nitrate 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 relatively to the control without DMPP. Correlation coefficients and multiple regressions where calculated for soil properties and relative NO_2^- formation at a concentration of 1 mg, 5 mg and 10 mg DMPP kg^{-1} dry soil. This were the most sensitive DMPP concentrations at which inhibition in all soils reached levels above 0 % and below 100 %. Coefficients of correlations and multiple regressions between soil parameters and the relative nitrite formation were calculated by using SAS® (SAS Institute Inc., Cary, NC, USA).

Table 1: Physical, chemical and biochemical properties of the investigated soils

Soil	**clay**	**silt**	**sand**	**CEC**[a]	**pH ($CaCl_2$)**	**C_{org}**	**N_t**	**catalase activity**	**potential nitrification**
		——— % ———		*meq 100 g^{-1}*		– *g kg^{-1}* –		*catalase number*	*mg NO_2^- - N kg^{-1} soil*
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	46	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	29	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	75	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	22	11.9	6.6	18.2	1.6	10.2	0.498
16	25	49	26	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] Cation exchange capacity was determined at pH 8.1

* Soils used in long - term incubation experiments

2.2.2 DMPP - Adsorption studies

100 ml of 0,01 M $CaCl_2$ solution with 0.2, 2.0 or 20.0 mg DMPP was added to moist soil (20 g fresh weight) and shaken for one hour at 40 rpm. The samples were centrifuged for 10 min at 2,700 g and subsequently filtered with folded filters (Schleicher & Schuell 595 ½, Dassel, Germany). 50 ml of the extract was transferred into a 250 ml separator funnel. After the addition of 25 g sodium chloride, 150 ml of 2.5 % ammonia solution and 80 ml of *tert*-buthyl methyl ether (MTBE), the mixture was horizontally shaken for two minutes at 250 movements min^{-1} and then allowed to settle. The aqueous phase was drained off into a second 250 ml separator funnel and 80 ml of MTBE were added. The organic phase was drained off into a 500 ml round bottom flask containing 25 ml of 0.1 M hydrochloric acid. 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 adjusted to pH 12 with 32% NaOH and quantitatively transferred into a Baker SPE C_{18} column (J. T. Baker, Phillipsburg NJ, USA) with 0.05 M NaOH. Thereafter the column was dried by placing it above silicate granulate over-night. DMPP was then eluted with 3 ml acid methanol (25 ml methanol plus 1 ml 1 M H_2SO_4) and measured by a HPLC (column: lichrosorb C_{18} 7 µm, 250 x 4 mm and precolumn 60 x 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).

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. As adsorption curves were linear throughout all three concentrations, mean values 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®.

2.3 Efficiency of DMPP in long - term incubation experiments as influenced by external factors

2.3.1 Fertiliser concentration and application form

On the basis of dry weight, 100 g of two different moist soils were placed in 500 ml plastic bottles. The study included ten treatments. Details are presented in table 2. In all treatments the water content was adjusted to 20 % (g/g) and the soil was homogenised by stirring. In treatments number eight (ENTEC™, NH_4NO_3 formulated with DMPP) and ten (NH_4NO_3 formulated with DCD) the fertiliser granules were added and covered with soil after adjusting water content and homogenising, whereas in all other treatments, NH_4^+, DMPP and DCD were added, dissolved in H_2O, before adjusting water content. The 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, NH_4^+, NO_3^-, NO_2^- and, in the DCD samples, DCD contents were determined. Subsamples (25 g soil dry weight) were used for DMPP analysis. Samples, which were not immediately analysed, were stored at -18°C.

Table 2: Treatments in the long - term incubation study with different DMPP concentrations (all additives given in 100g soil)

treatment	silty loam	loamy sand
1	without additives	without additives
2	Ammonium sulphate (10 mg NH_4^+ - N) in liquid form	Ammonium sulphate (10 mg NH_4^+ - N) in liquid form
3	Ammonium sulphate (10 mg NH_4^+ - N) and 0.071 mg DMPP in liquid form	
4	Ammonium sulphate (10 mg NH_4^+ - N) and 0.118 mg DMPP in liquid form	Ammonium sulphate (10 mg NH_4^+ - N) and 0.118 mg DMPP in liquid form
5	Ammonium sulphate (10 mg NH_4^+ - N) and 0.237 mg DMPP in liquid form	Ammonium sulphate (10 mg NH_4^+ - N) and 0.237 mg DMPP in liquid form
6	Ammonium sulphate (10 mg NH_4^+ - N) and 0.710 mg DMPP in liquid form	Ammonium sulphate (10 mg NH_4^+ - N) and 0.710 mg DMPP in liquid form
7		Ammonium sulphate (10 mg NH_4^+ - N) and 3.464 mg DMPP in liquid form
8	Ammonium sulphate nitrate (10 mg NH_4^+ - N) and 0.163 mg DMPP in solid form*	Ammonium sulphate nitrate (10 mg NH_4^+ - N) and 0.163 mg DMPP in solid form*
9	Ammonium sulphate (10 mg NH_4^+ - N) and 1,5 mg DCD in liquid form	Ammonium sulphate (10 mg NH_4^+ - N) and 1,5 mg DCD in liquid form
10		Ammonium sulphate nitrate (10 mg NH_4^+ - N) and 1.581 mg DCD in solid form

* ENTEC^{TM}, NH_4NO_3 formulated with DMPP

2.3.2 Influence of soil matric potential on the efficiency of DMPP

Ten treatments were prepared for each soil, five different soil matric potentials with fertiliser granules of ammonium sulphate nitrate formulated with or without DMPP.

The fertiliser granules contained 10 mg NH_4^+ - N. The DMPP formulated granules contained 15.15 µg DMPP (ENTEC™). The soils were adjusted to the following soil matric potentials: -600 kPa, -300 kPa, -100 kPa, -50 kPa and -5.8 kPa (loamy sand) or -3.4 kPa (silty loam). The corresponding gravimetric water contents were 0.07, 0.086, 0.11, 0.123, and 0.14 kg * kg^{-1} dry soil in the loamy sand and 0.21, 0.227, 0.235, 0.255 and 0.275 kg * kg^{-1} dry soil in the silty loam. Soil matric potentials were established one week before starting the incubation. At incubation start 100 g soil (dry matter basis) were placed in a 500 ml plastic bottle, fertiliser granules were incorporated into the soil and the samples 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.

2.3.3 Analysis of N_{min}, DCD and DMPP

200 ml 0.0125 molar $CaCl_2$ - solution were added to the samples (100 g soil dry weight) and shaken for one hour at 40 rpm. Part of the suspension was filtered with folded filter (Schleicher and Schüll 602 EH ½, Dassel, Germany). One ml was used for NO_3^-, NO_2^- and DCD determination by HPLC (Vilsmeier 1984). The rest of the filtrate and the filter were given back into the suspension. A 1 M KCl solution was obtained by adding 10.95 g KCl to the suspension. After shaking for one hour at 40 rpm the suspension was filtered with folded filter (Schleicher and Schüll 602 EH ½, Dassel, Germany) and the NH_4^+ concentration was measured by the indophenol blue method (Bernt and Bergmeyer 1970).

For the treatments 5 and 6 (2.4 and 7.1 mg DMPP *1 kg^{-1} soil, liquid form) soil DMPP content was determined after 0, 3, 5, 7, 11, 14, 18, and 25 incubation days. 50 ml of 1 % K_2SO_4 - solution were added to the samples and shaken horizontally for 30 minutes at a rate of 250 movements min^{-1} (Köttermann GmbH Uelzen Germany). The samples were centrifuged for 10 minutes at 2700 g (Beckmann GS 6 centrifuge, Beckmann Instruments, Munich, Germany), the supernatant was decanted and filtered. This procedure was carried out three times and the filtrates were combined. 50 ml of the solution were taken and DMPP was analysed as described in chapter *2.2.2*.

3 Results

3.1 Influence of soil parameters on the nitrification inhibition of DMPP: short - term effects

3.1.1 Inhibition of nitrite formation in short - term experiments

Short - term incubation experiments (5 hours), as conducted in this study enable the first step of the nitrification process - the formation of NO_2^- - to be examined. Figures 2 - 4 show the nitrite formation in three representative soils without and with DMPP in different concentrations. Results of all investigated soils are given in the appendix (Figure A1 - A22). Increasing DMPP concentrations reduce the nitrite formation in all soils but this effect is also soil specific.

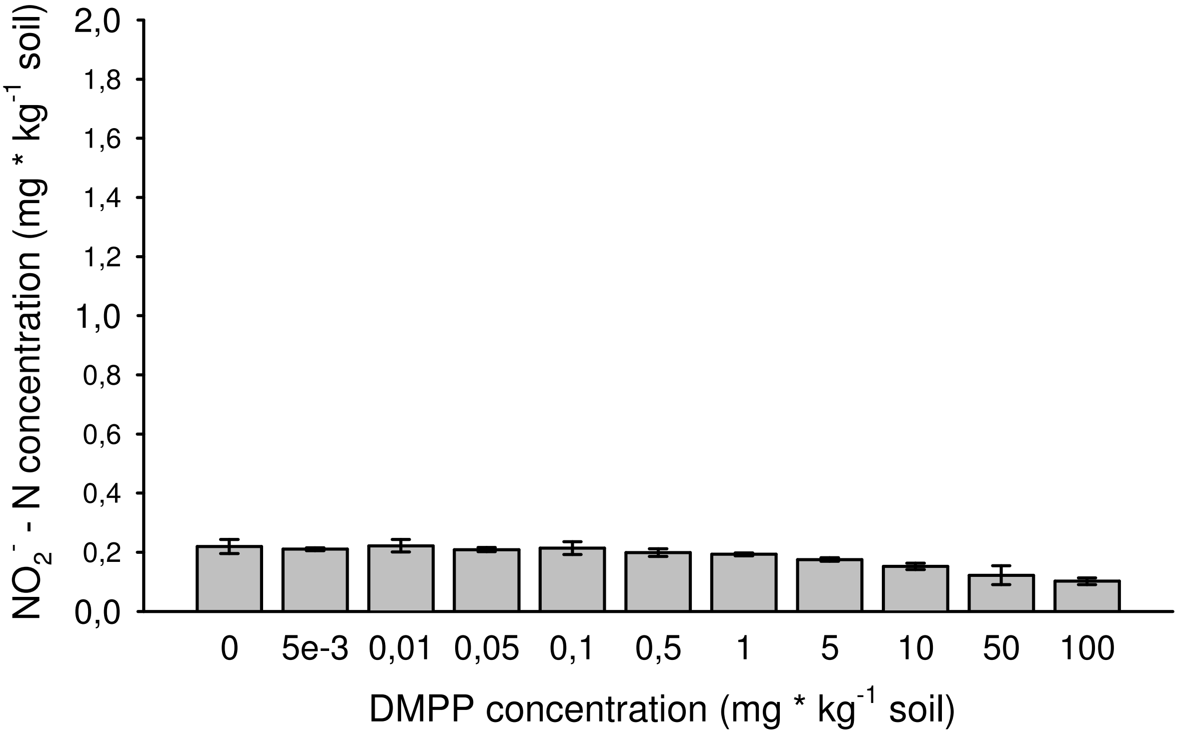

Fig. 2 Nitrite formation in soil 4 without DMPP and with different DMPP concentrations in short - term incubation experiments.
Error bars represent standard deviations.

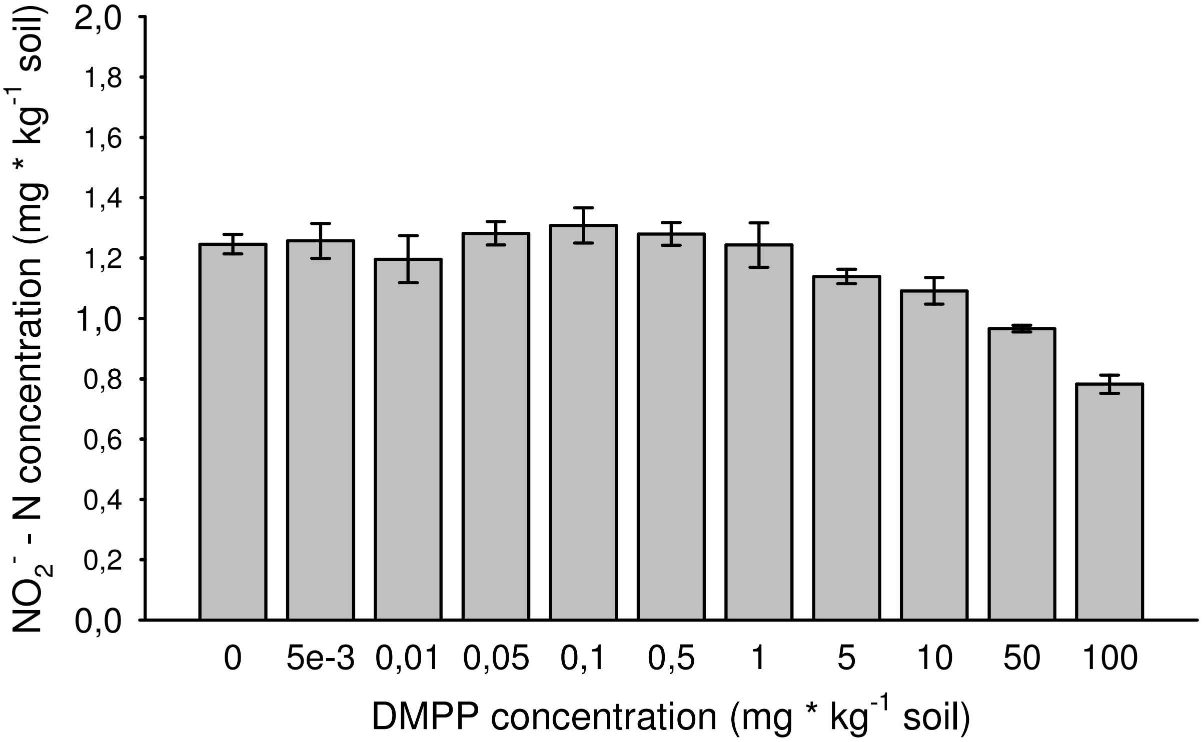

Fig. 3 Nitrite formation in soil 6 without DMPP and with different DMPP concentrations in short - term incubation experiments.
Error bars represent standard deviations.

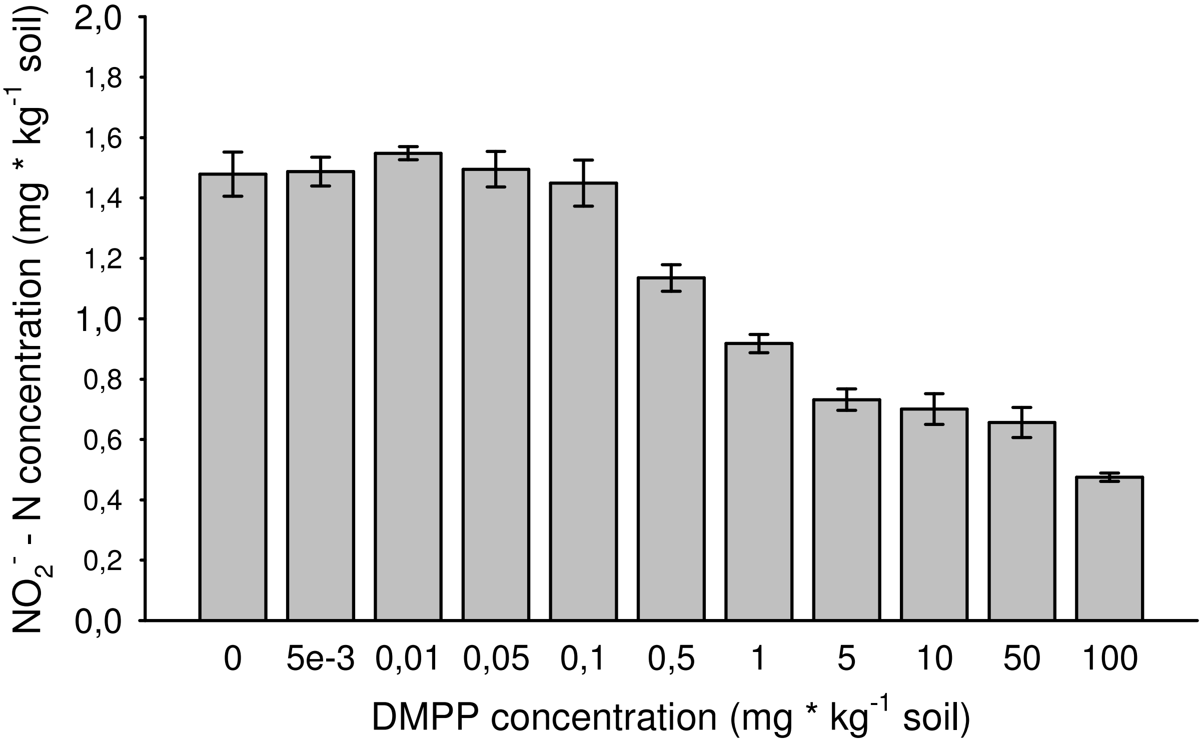

Fig. 4 Nitrite formation in soil 8 without DMPP and with different DMPP concentrations in short - term incubation experiments.
Error bars represent standard deviations.

The nitrite formation, also without DMPP markedly differs between the soils: while in soil 6 and 8 about 1.2 to 1.5 mg NO_2^- kg^{-1} soil are produced within the incubation period, the nitrite content in soil 4 is much lower. Such differences in nitrite formation are observed for the other soils as well. For this reason and to evaluate the specific effect of the nitrification inhibitor in different soil types, NO_2^- - formation in the presence of DMPP is expressed relative to the NO_2^- - formation without inhibitor. Low values of relative NO_2^- - formation indicate a strong reduction of nitrification. Fig. 5-7 shows the relative NO_2^- - formation with DMPP in the three representative soils (soil 4, 6 and 8). The lowest relative NO_2^- - formation is observed in a sandy soil (soil 8, Fig. 7), whereas nitrification is much less inhibited in a loamy soil (soil 6, Fig. 6). Differences between soils are most distinct at DMPP concentrations between 1 mg and 10 mg DMPP kg^{-1} dry soil. The relative nitrite formation of all soils is given in the appendix (Fig. A23 - A44).

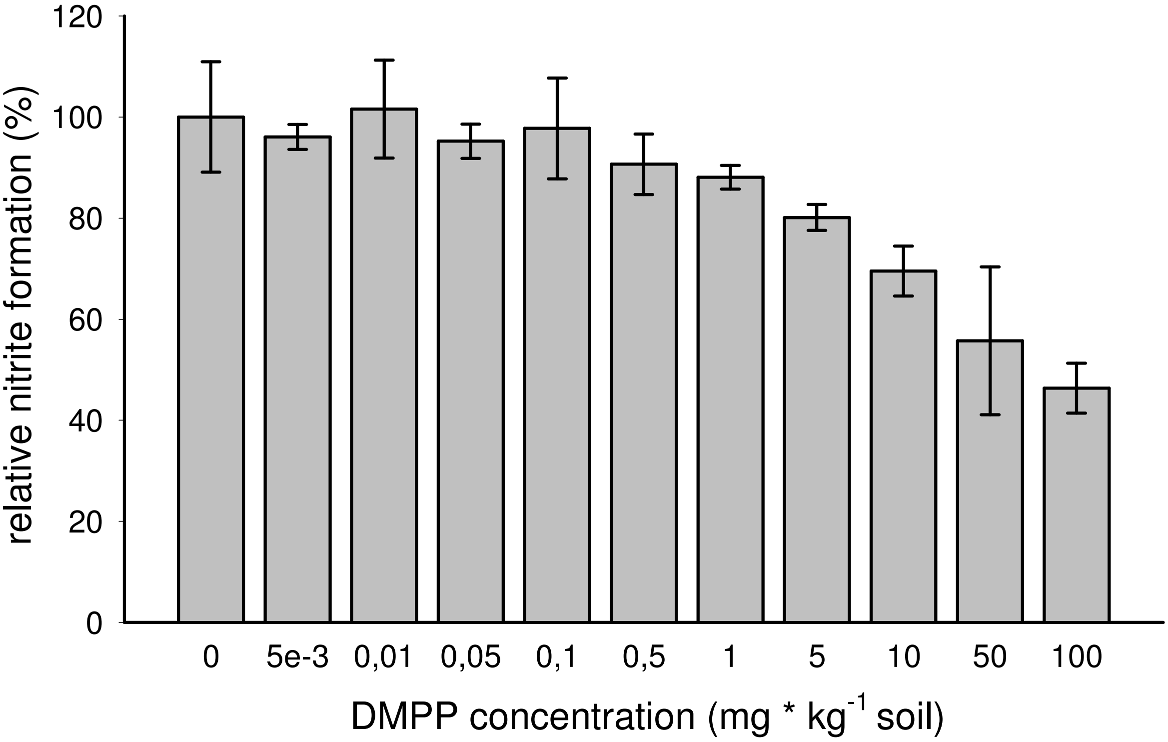

Fig. 5 Relative nitrite formation in soil 4 without DMPP and with different DMPP concentrations in short - term incubation experiments.
Error bars represent standard deviations.

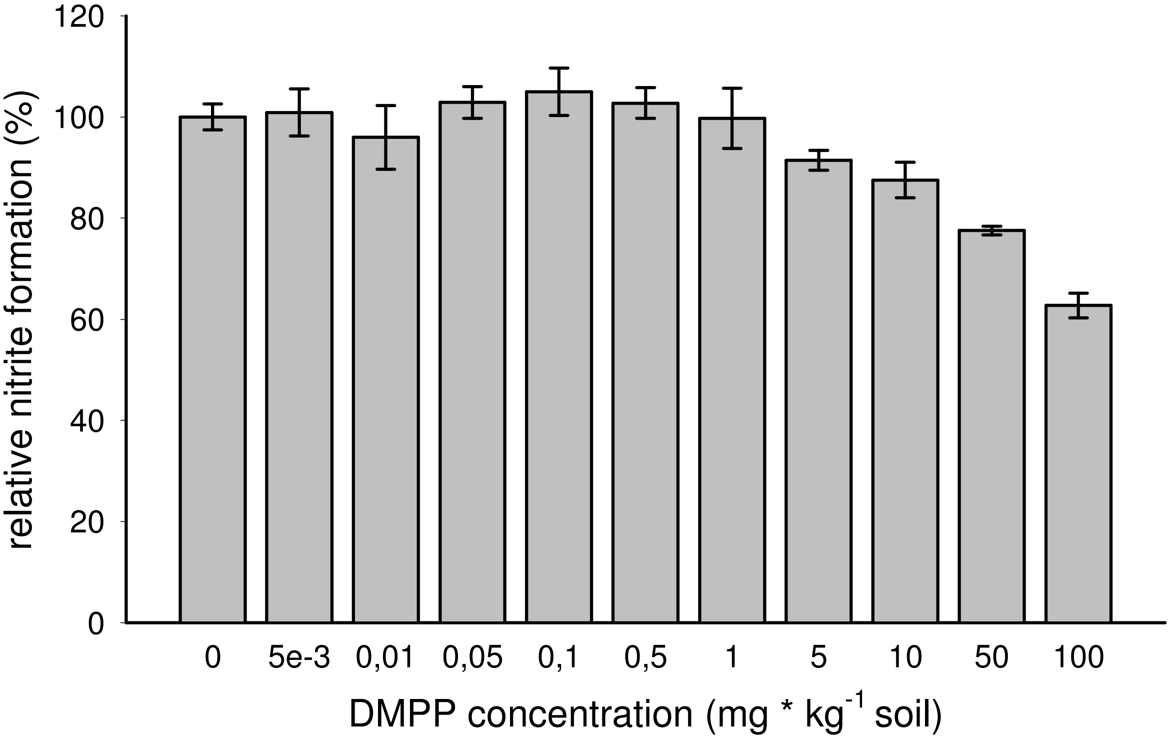

Fig. 6 Relative nitrite formation in soil 6 without DMPP and with different DMPP concentrations in short - term incubation experiments. Error bars represent standard deviations.

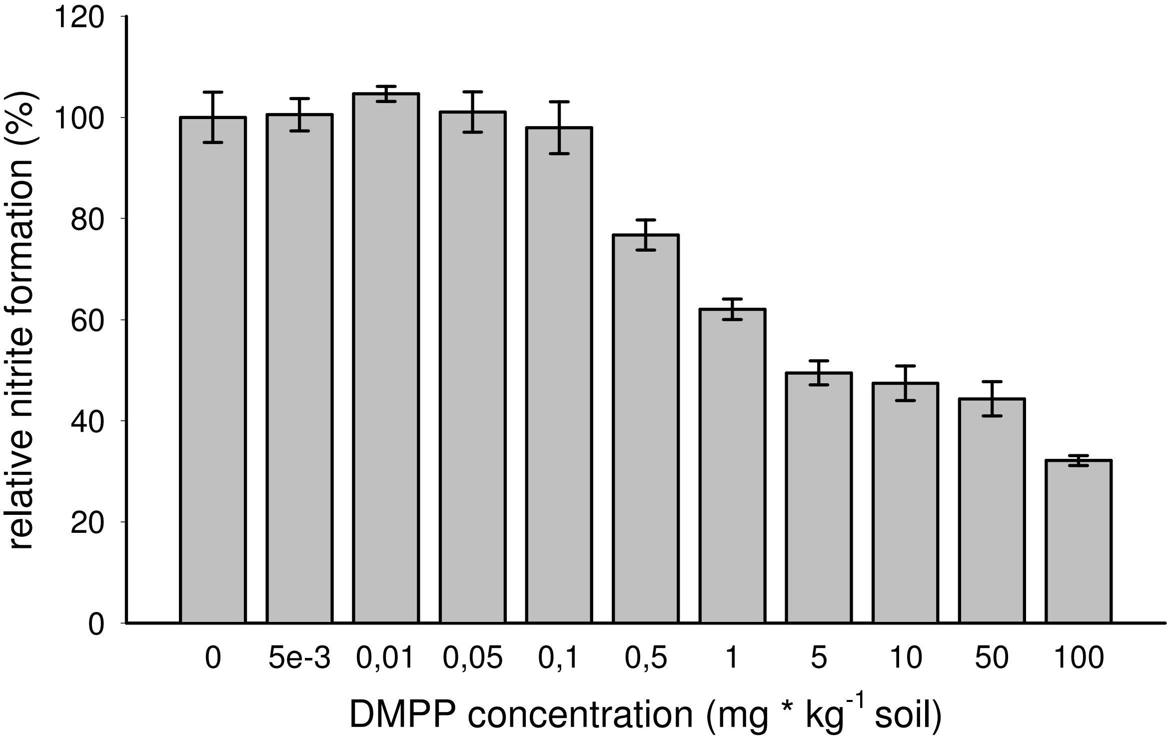

Fig. 7 Relative nitrite formation in soil 8 without DMPP and with different DMPP concentrations in short - term incubation experiments. Error bars represent standard deviations.

In Fig. 8 the three representative soils are directly compared so that the increase in the inhibition effect with increasing DMPP concentrations and the differences in the inhibition effect between the soils become clearly evident.

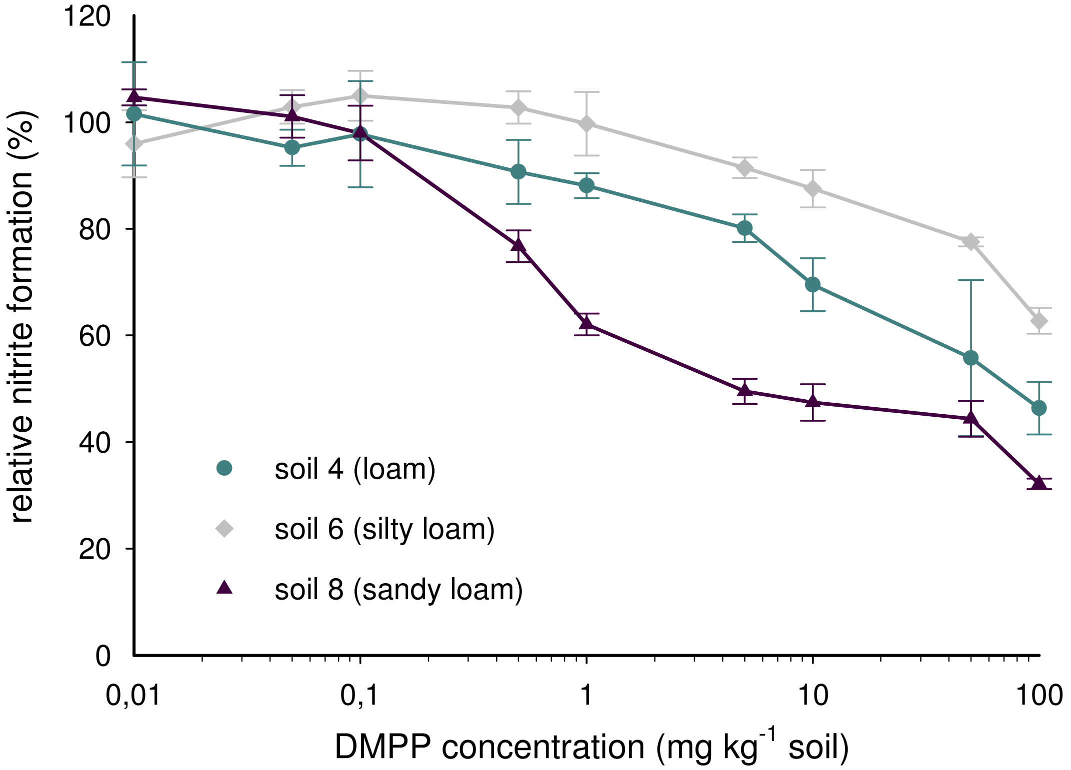

Fig. 8 Influence of different DMPP concentrations in short - term incubation experiments on the relative nitrite formation in three selected soils. Error bars represent standard deviations.

The relative NO_2^- - formation at the different DMPP concentrations is only moderately explained by a single soil parameter. The Person correlation coefficients for the DMPP concentrations 1, 5 and 10 mg DMPP kg^{-1} soil are given in Table 3. Significant and positive correlations are found between the relative NO_2^- - formation and silt, catalase activity, clay, N_t, CEC and C_{org}. With the exception of 10 mg DMPP kg^{-1} soil the highest, but negative correlation, is observed with sand (Table 3). The soil parameters proton concentration and potential nitrification, show no significant correlation.

Table. 3 Pearson correlation coefficients between soil parameters and relative NO_2^- - formation at 1 mg, 5 mg and 10 mg DMPP kg^{-1} soil.

	sand	**silt**	**catalase activity**	**clay**	**N_t**	**CEC**	**C_{org}**	**potential nitrification**	**proton concentration**
1mg DMPP kg^{-1} soil	**-0,60**	0,57	0,55	0,45	0,35	0,24	0,25	n.s.	n.s.
5mg DMPP kg^{-1} soil	**-0,65**	0,60	0,59	0,54	0,48	0,34	0,25	n.s.	n.s.
10mg DMPP kg^{-1} soil	**-0,60**	0,56	0,61	0,48	0,47	0,36	0,38	n.s.	n.s.

n.s. = correlation coefficient is not significant

The inhibitory effect of DMPP is better predicted by a multiple regression model. The influence of soil texture on the relative NO_2^- - formation in this model is best explained by the (single) correlation to the sand fraction with $R^2 = 0.43$, shown in Fig 9 for a DMPP concentration of 5 mg kg^{-1} soil. This value is significant ($p < 0,05$), but it is to low for a meaningful prognosis. The relationship is improved by including the catalase activity (Fig. 10) and the proton concentration to $R^2 = 0,62$ (Fig. 11). If potential nitrification is also included, the relation in the regression equation further improved (Fig. 12).

At 1 mg and 10 mg DMPP kg^{-1} the inhibitory effect of DMPP is also predicted by the regression model including sand, proton concentration and catalase activity (Fig 13 and Fig 14). But at these DMPP concentrations the relation is not so discernible as compared to 5 mg DMPP kg^{-1} soil.

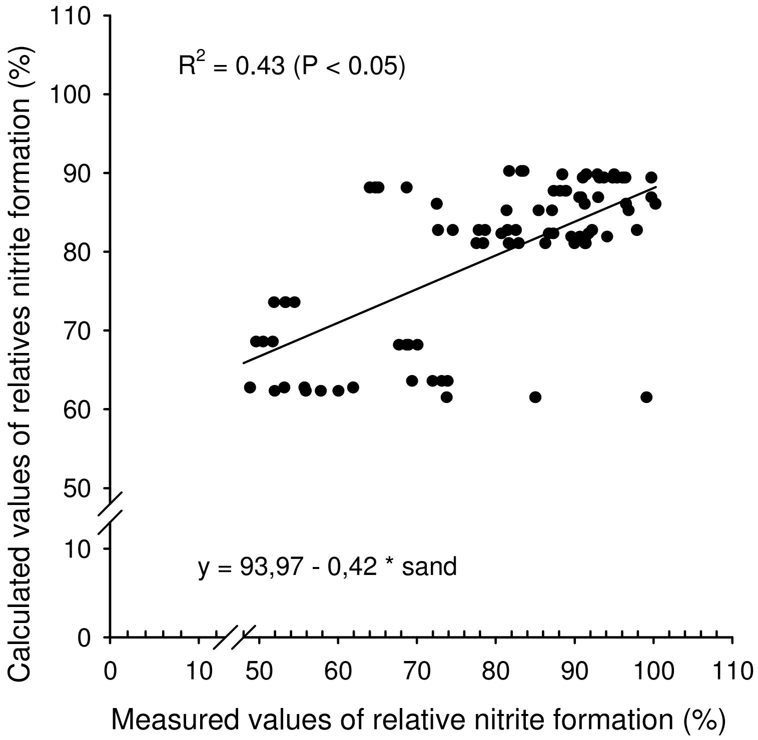

Fig. 9 Influence of soil parameters on the relative NO_2^- formation in short - term incubation experiments. Predicted versus measured values (DMPP concentration 5 mg kg^{-1} soil). Calculated only with sand.

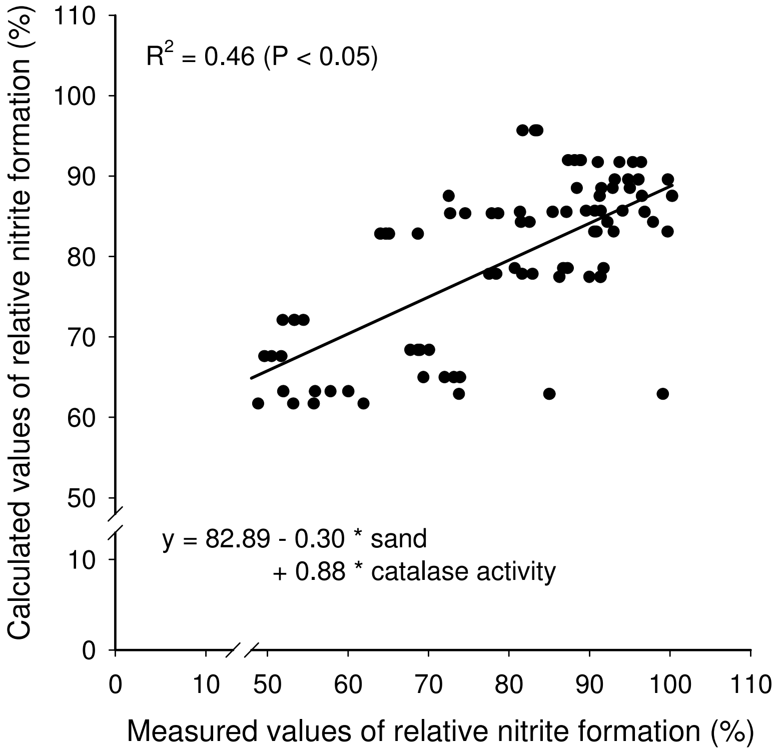

Fig. 10 Influence of soil parameters on the relative NO_2^- formation in short - term incubation experiments. Predicted versus measured values (DMPP concentration 5 mg kg^{-1} soil). Calculated only with sand and catalase activity.

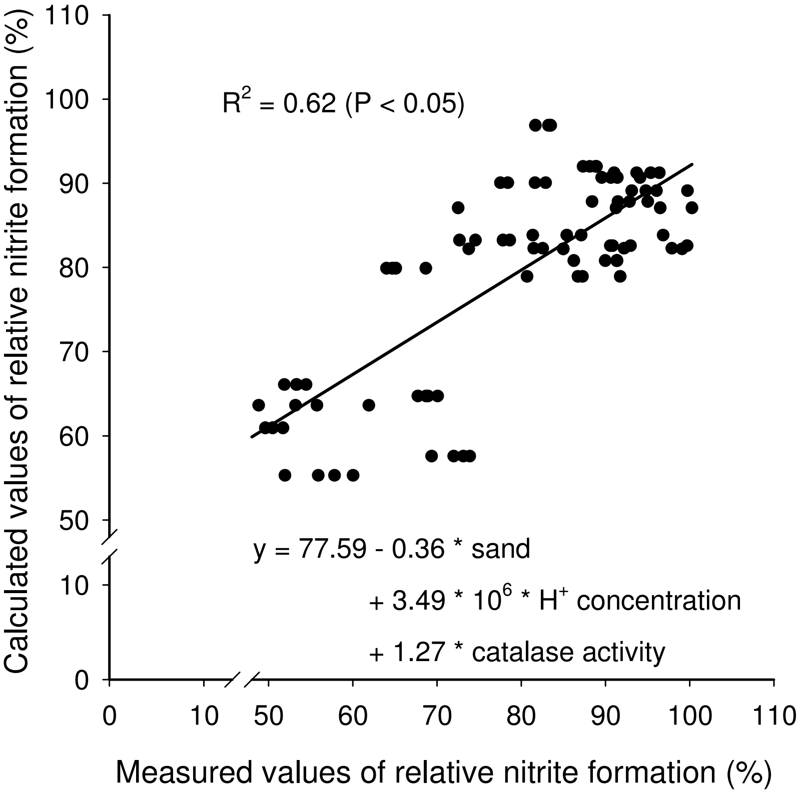

Fig. 11 Influence of soil parameters on the relative NO_2^- formation in short - term incubation experiments. Predicted versus measured values (DMPP concentration 5 mg kg^{-1} soil). Calculated with sand, H^+ concentration and catalase activity.

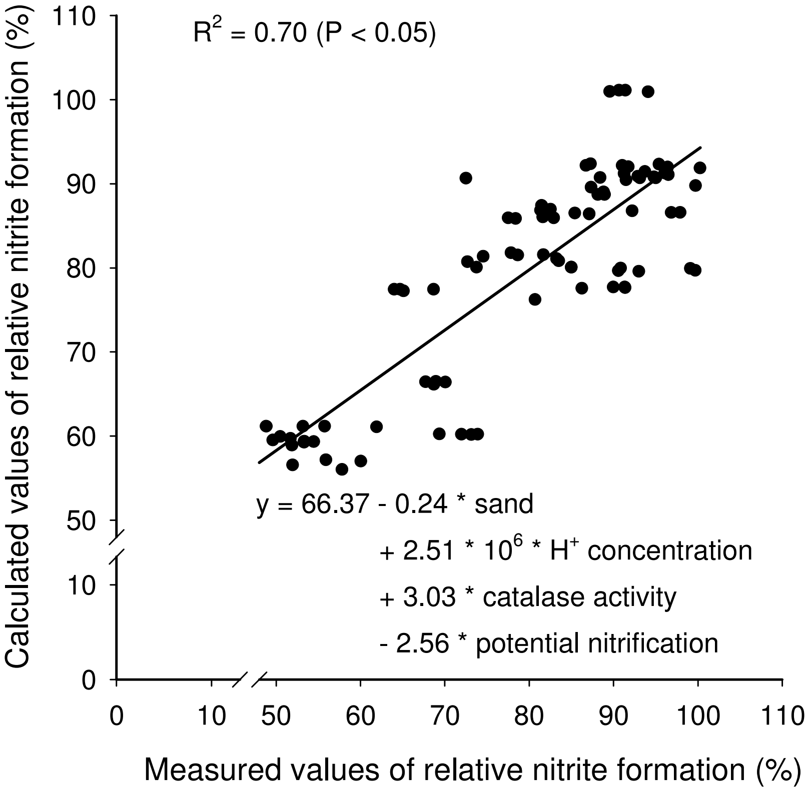

Fig. 12 Influence of soil parameters on the relative NO_2^- formation in short - term incubation experiments. Predicted versus measured values (DMPP concentration 5 mg kg^{-1} soil). Calculated with sand, catalase activity, H^+ concentration and potential nitrification.

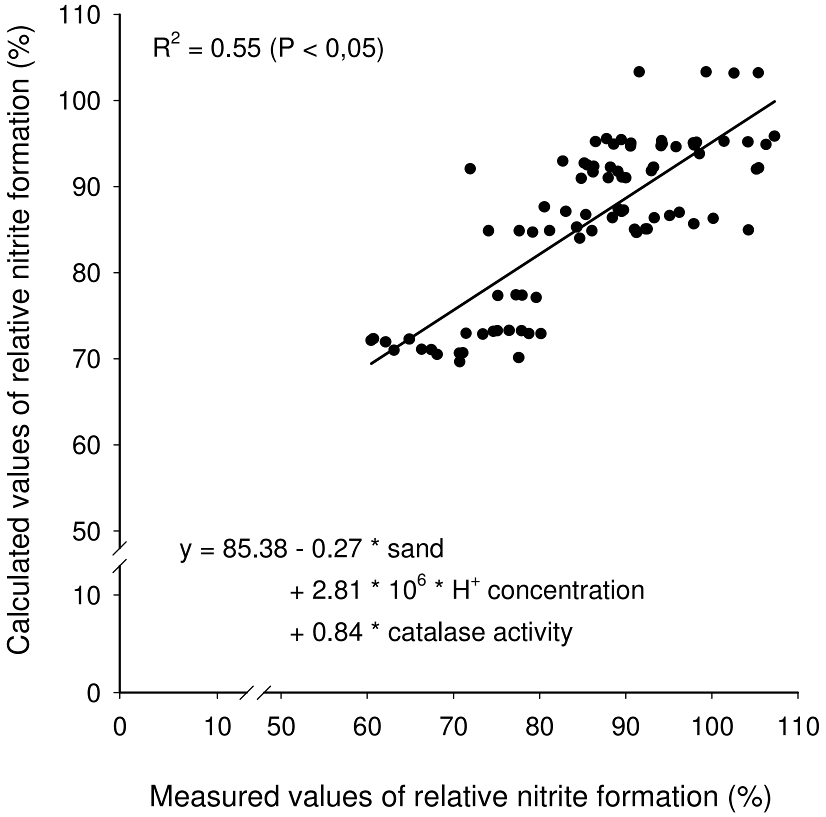

Fig. 13 Influence of soil parameters on the relative NO_2^- formation in short - term incubation experiments at 1 mg DMPP kg^{-1} soil. Predicted versus measured values.

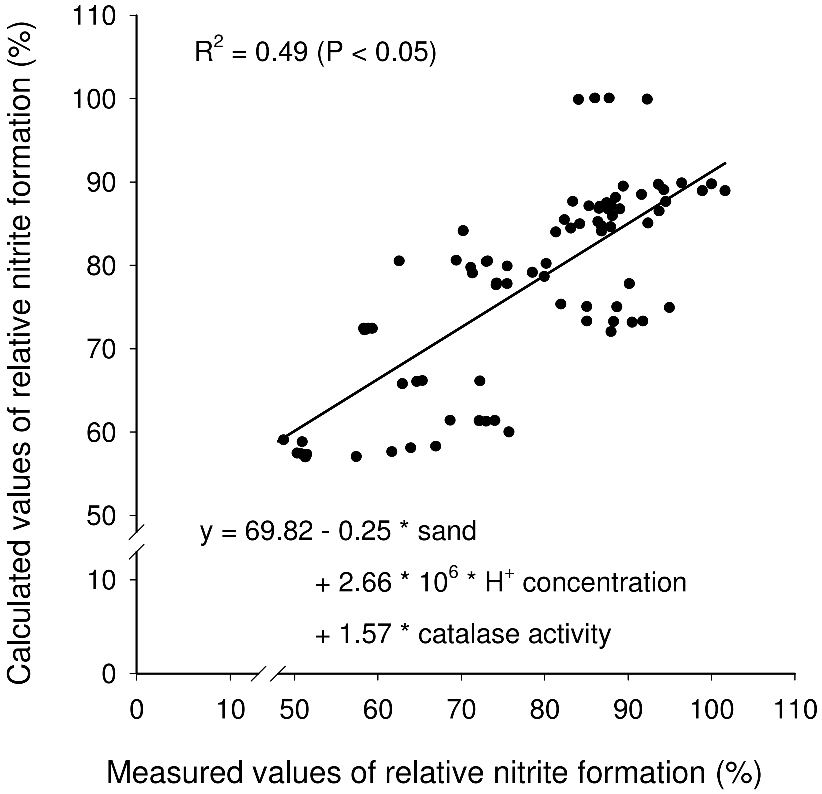

Fig. 14 Influence of soil parameters on the relative NO_2^- formation in short - term incubation experiments at 10 mg DMPP kg^{-1} soil. Predicted versus measured values.

3.1.2 DMPP - adsorption experiments

Variations in DMPP efficiency among soils may arise from differences in DMPP adsorption to soil components. Adsorption studies are carried out with several soils to verify this hypothesis.

Single correlation results from adsorption studies show a significant relationship between DMPP adsorption and soil texture like clay (r = 0.78), shown in Fig. 15, silt (r = 0.68), shown in Fig 16, and sand (r = -0.76), shown in Fig 17. Total nitrogen (r = 0.51) and organic carbon content (r = 0.49) are less suitable to explain DMPP adsorption (Fig. 18 and Fig. 19). The highest correlation is observed between DMPP adsorption and catalase activity (r = 0.85), shown in Fig. 20.

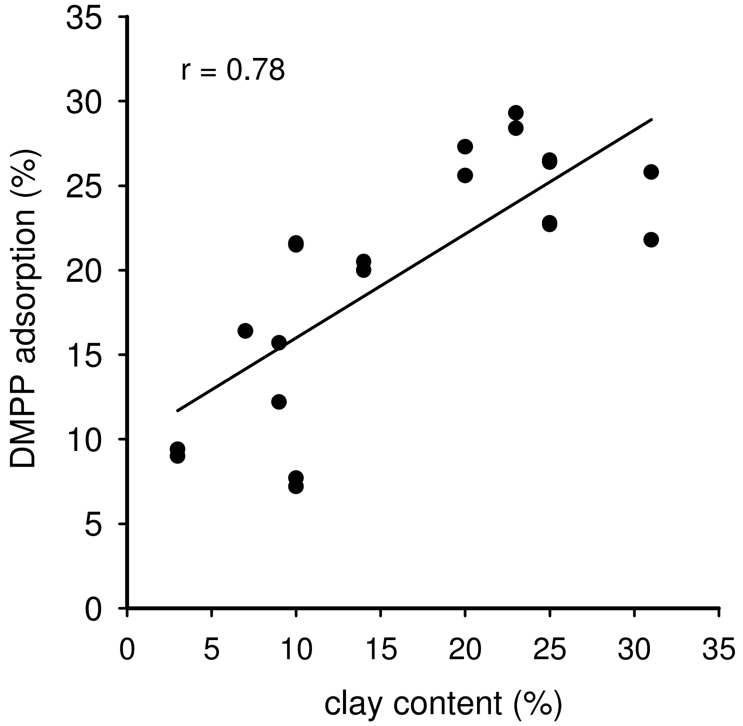

Fig. 15 Relation between clay content and DMPP adsorption in 11 different soils.

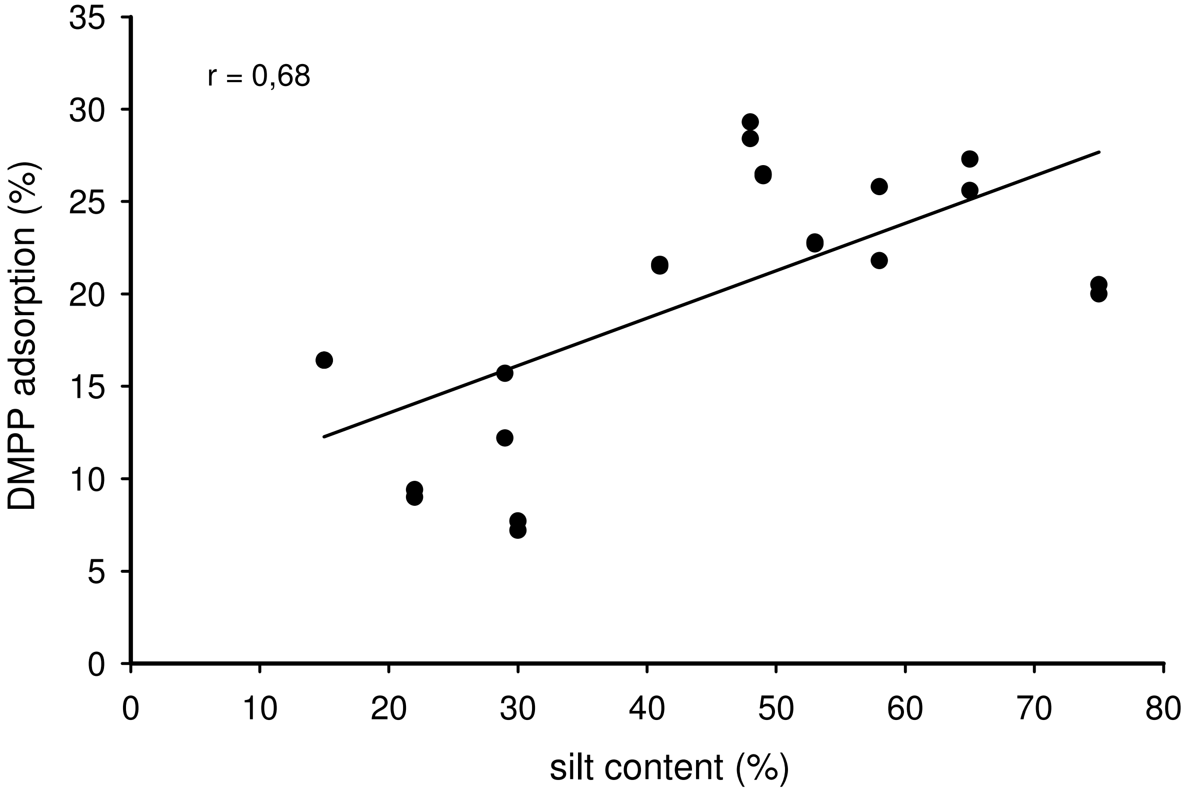

Fig. 16 Relation between silt content and DMPP adsorption in 11 different soils.

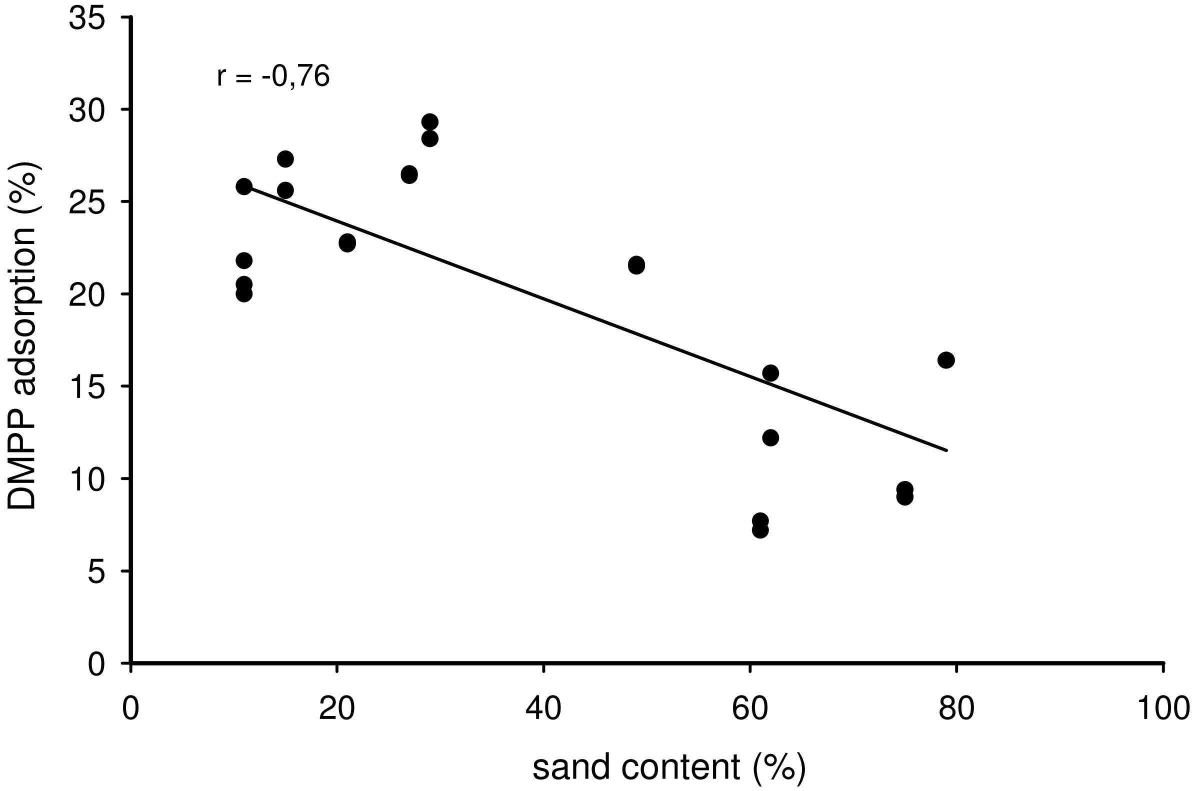

Fig. 17 Relation between sand content and DMPP adsorption in 11 different soils.

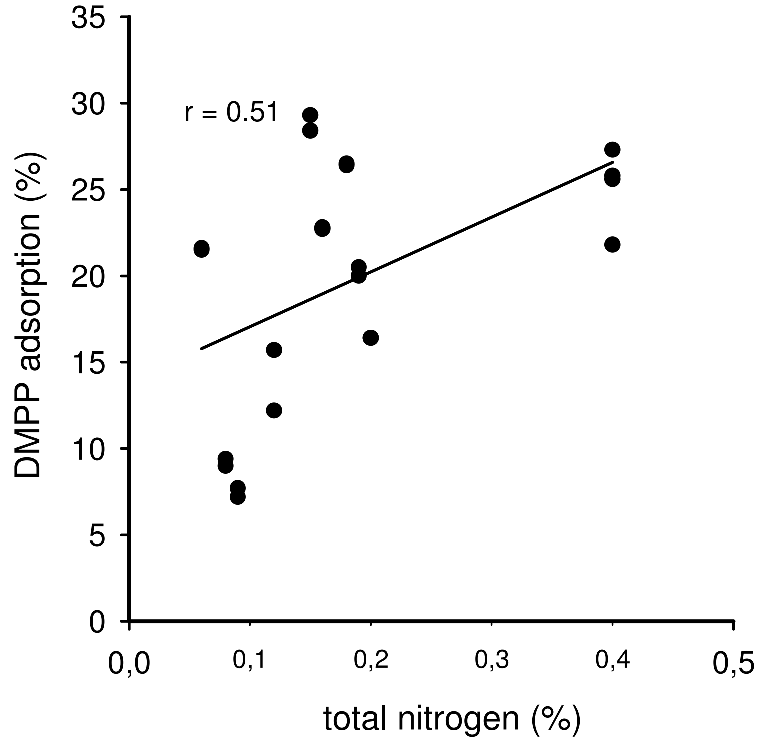

Fig. 18 Relation between total nitrogen content and DMPP adsorption in 11 different soils.

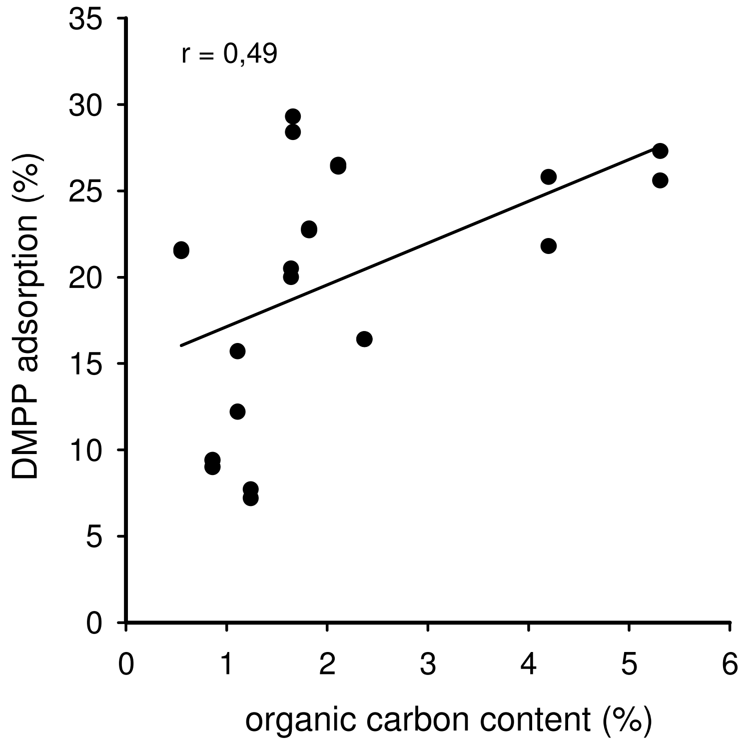

Fig. 19 Relation between organic carbon content and DMPP adsorption in 11 different soils.

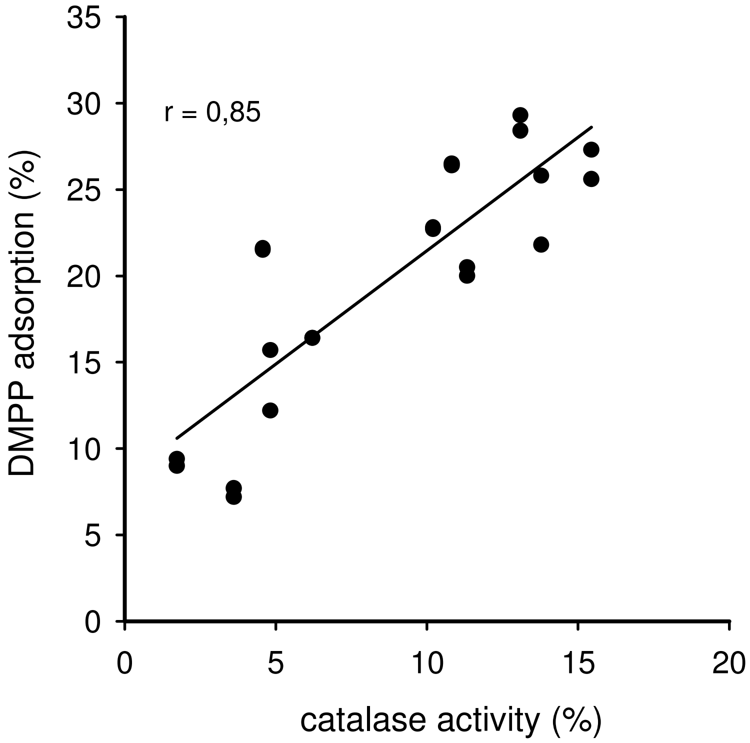

Fig. 20 Relation between catalase activity and DMPP adsorption in 11 different soils.

Figures 21 - 24 show the measured DMPP adsorption versus the calculated DMPP adsorption based on clay (Fig 21), silt (Fig 22), clay and silt (Fig. 23) and catalase (Fig 24) activity, respectively. Including clay into the regression model with catalase activity, improved the relation in the regression equation only marginally ($R^2 = 0.73$ with catalase activity and $R^2 = 0.74$ with catalase activity and clay (data not shown)).

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 short - term incubation was significantly explained by the adsorption behaviour of DMPP (Fig. 25).

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

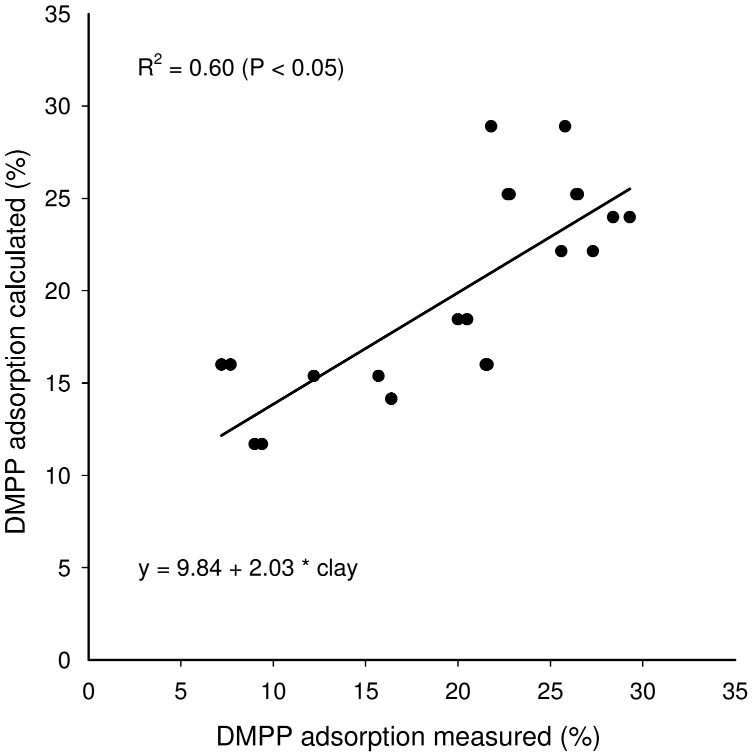

Fig. 21 Influence of clay on the DMPP adsorption in 11 different soils. Predicted versus measured values.

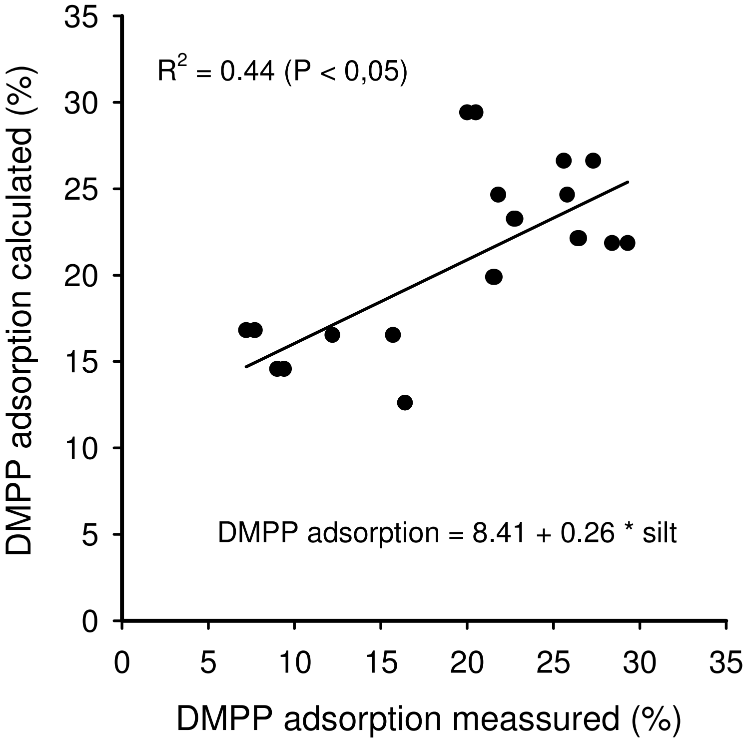

Fig. 22 Influence of silt on the DMPP adsorption in 11 different soils. Predicted versus measured values.

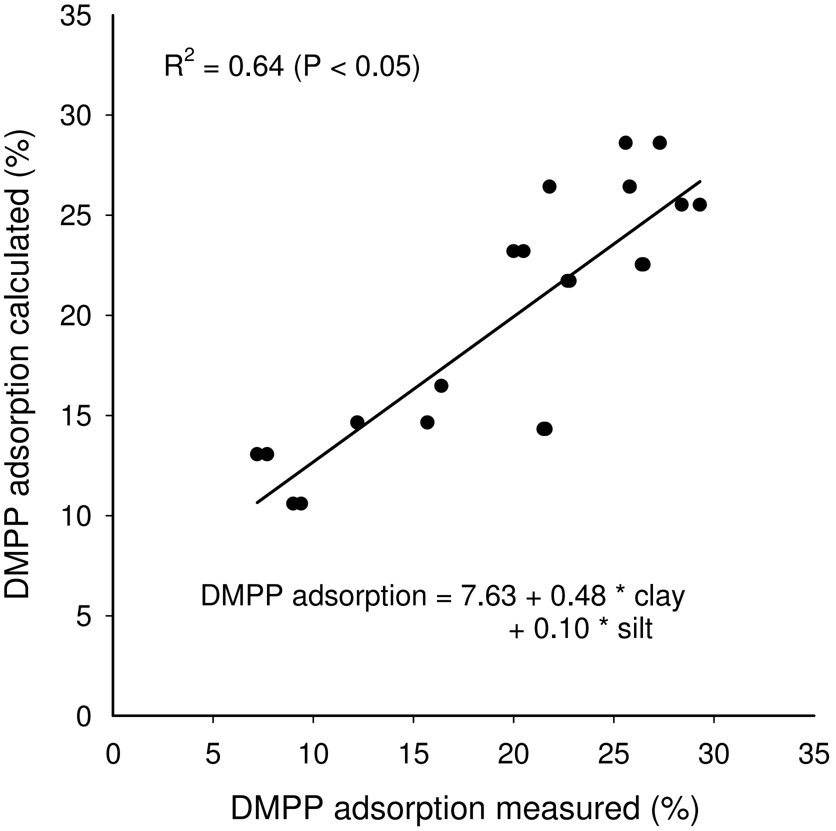

Fig. 23 Influence of clay and silt on the DMPP adsorption in 11 different soils. Predicted versus measured values.

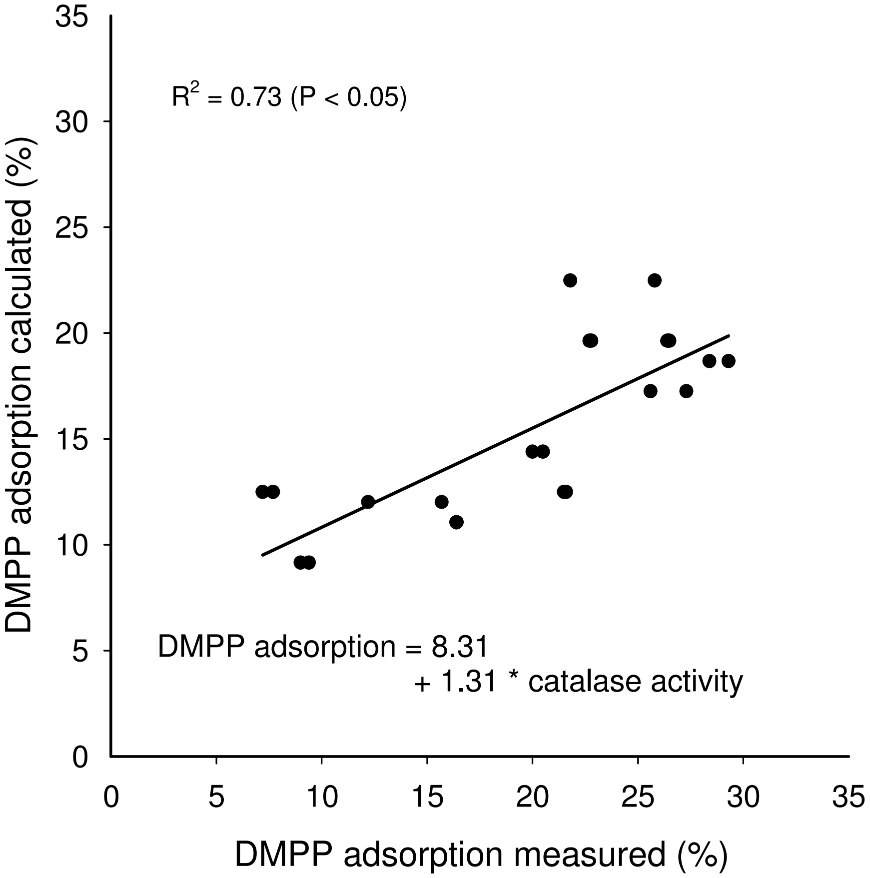

Fig. 24 Influence of catalase activity on the DMPP adsorption in 11 different soils. Predicted versus measured values.

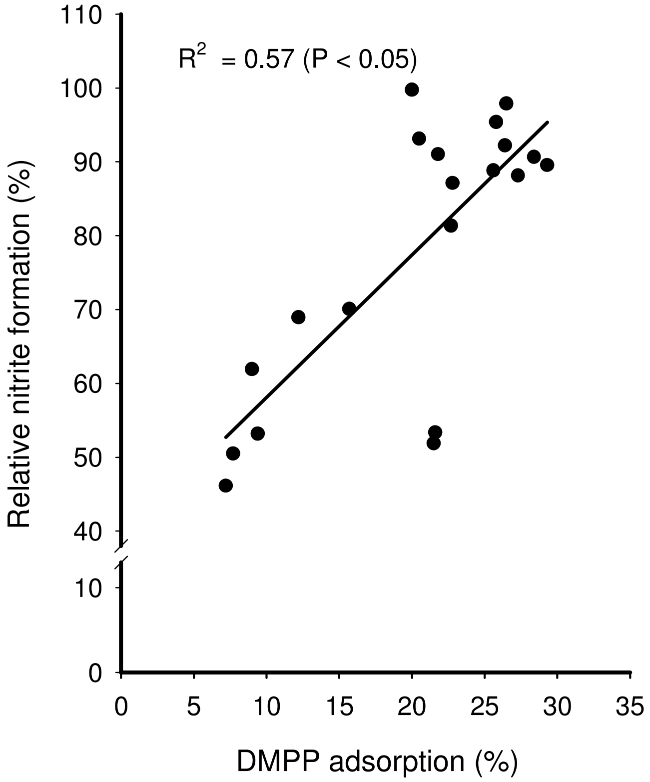

Fig. 25 Correlation between DMPP adsorption and relative nitrite formation at a DMPP concentration of 5 mg kg^{-1} soil.

3.2 Efficiency of DMPP in long - term incubation experiments as influenced by external factors

When DMPP is applied in agricultural or horticultural practise short - term mechanisms i.e. adsorption, potential nitrification will be influenced by additional factors. First, DMPP is not applied as a solution - homogeneously distributed in soil but broadcasted as fertiliser granules. This will lead to high concentrations of both fertiliser (NH_4NO_3) and DMPP in the vicinity of the granule. The extension of the affected zone will be influenced by external factors among which soil water content may play an important role. Second, during a period of efficacy of about several weeks, DMPP is subjected to decomposition. Consequently the DMPP concentration will be reduced.

In this study some of these factors, such as application form, DMPP concentration, DMPP decomposition, and soil matric potential have been investigated in classical incubation experiments over a period of 32 days. Two soils (soil no. 6 a silty loam and soil no. 20 a loamy sand) were included in this study.

3.2.1 Inhibitor concentration and application form

3.2.1.1 Nitrification inhibition with DMPP

In both soils all treatments with DMPP show an inhibited NH_4^+ oxidation in comparison to the control treatments without DMPP. NH_4^+ oxidation with and without DMPP is different between soils. In the loamy sand the DMPP effect is more pronounced than in the silty loam (Fig. 26).

In both soils an increase in DMPP - concentration (treatment 3 to 6, Table 2) applied in liquid form, has no effect on NH_4^+ decomposition. When DMPP and NH_4^+ are added as DMPP formulated fertiliser granules, a strong inhibition of nitrification is observed in the silty loam. In the loamy sand, the effect of the DMPP formulated fertiliser granules is much lower and similar to treatment 7 (table 2), applying a high DMPP concentration in liquid form (Fig. 26).

Fig. 27 shows the corresponding nitrate formation as dependent on the DMPP addition. These soil nitrate contents reflect the reversed image of the NH_4^+ contents. With the exception of small amounts at the start (<0,5 mg NO_2^- kg^{-1} soil) and at the end (<0,05 mg NO_2^- kg^{-1} soil) of the experiment no nitrite is observed during the whole incubation period (Table 4). Only in treatment 10 small amounts (<0,2 mg NO_2^-

kg^{-1} soil) of NO_2^- are observed during the whole incubation period (Table A5). The total mineral nitrogen (N_{min}) remained largely constant during the whole experiment (Fig. 28). The Figures 29 and 30 point out the difference in the efficiency of DMPP applied in liquid form or as fertiliser granules in both soils by a direct comparison of the ammonium degradation or the nitrate formation.

Table 4: NO_2^- - N concentration in soils of the beginning (day 0) and at the end (day 32) of the incubation experiments

incubation time (days)		**0**		**32**	
soil	**treatement**	**NO_2^-** mg*kg^{-1}	**standard derivation**	**NO_2^-** mg*kg^{-1}	**standard derivation**
silty loam	1	0,086	0,021	0,000	0,000
	2	0,498	0,007	0,000	0,000
	3	0,429	0,035	0,000	0,000
	4	0,377	0,015	0,000	0,000
	5	0,324	0,019	0,000	0,000
	6	0,215	0,010	0,000	0,000
	8	---	---	0,000	0,000
	9	0,323	0,044	0,000	0,000
loamy sand	1	0,088	0,007	0,000	0,000
	2	0,203	0,012	0,000	0,000
	4	0,113	0,003	0,000	0,000
	5	0,093	0,009	0,000	0,000
	6	0,083	0,011	0,025	0,043
	7	0,067	0,012	0,030	0,035
	8	0,160	0,010	0,038	0,046
	9	0,099	0,016	0,000	0,000
	10	0,191	0,005	0,023	0,047

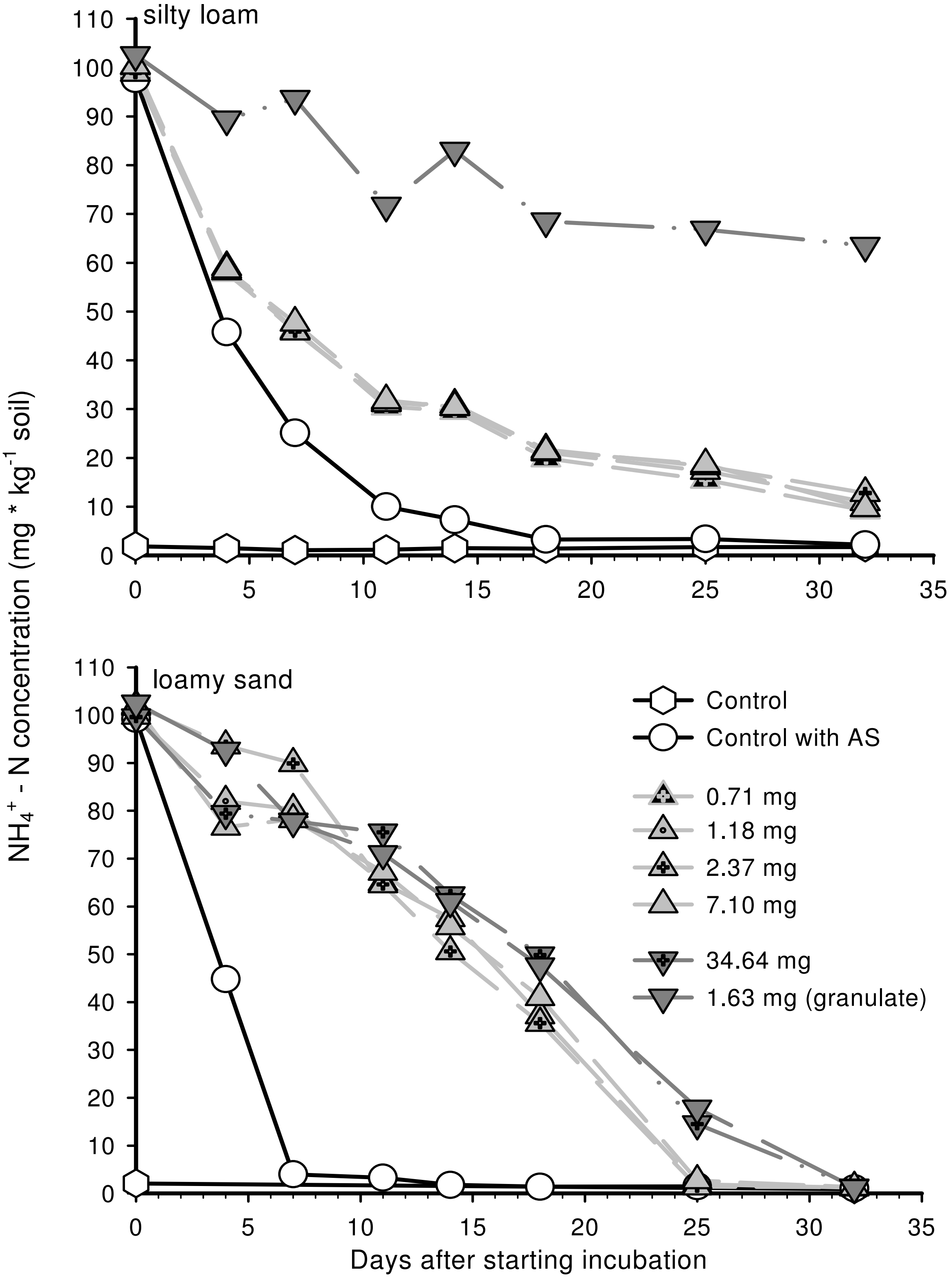

Fig. 26 Influence of different DMPP applications on the NH_4^+ content in a silty loam and a loamy sand. Treatments see also table 2.

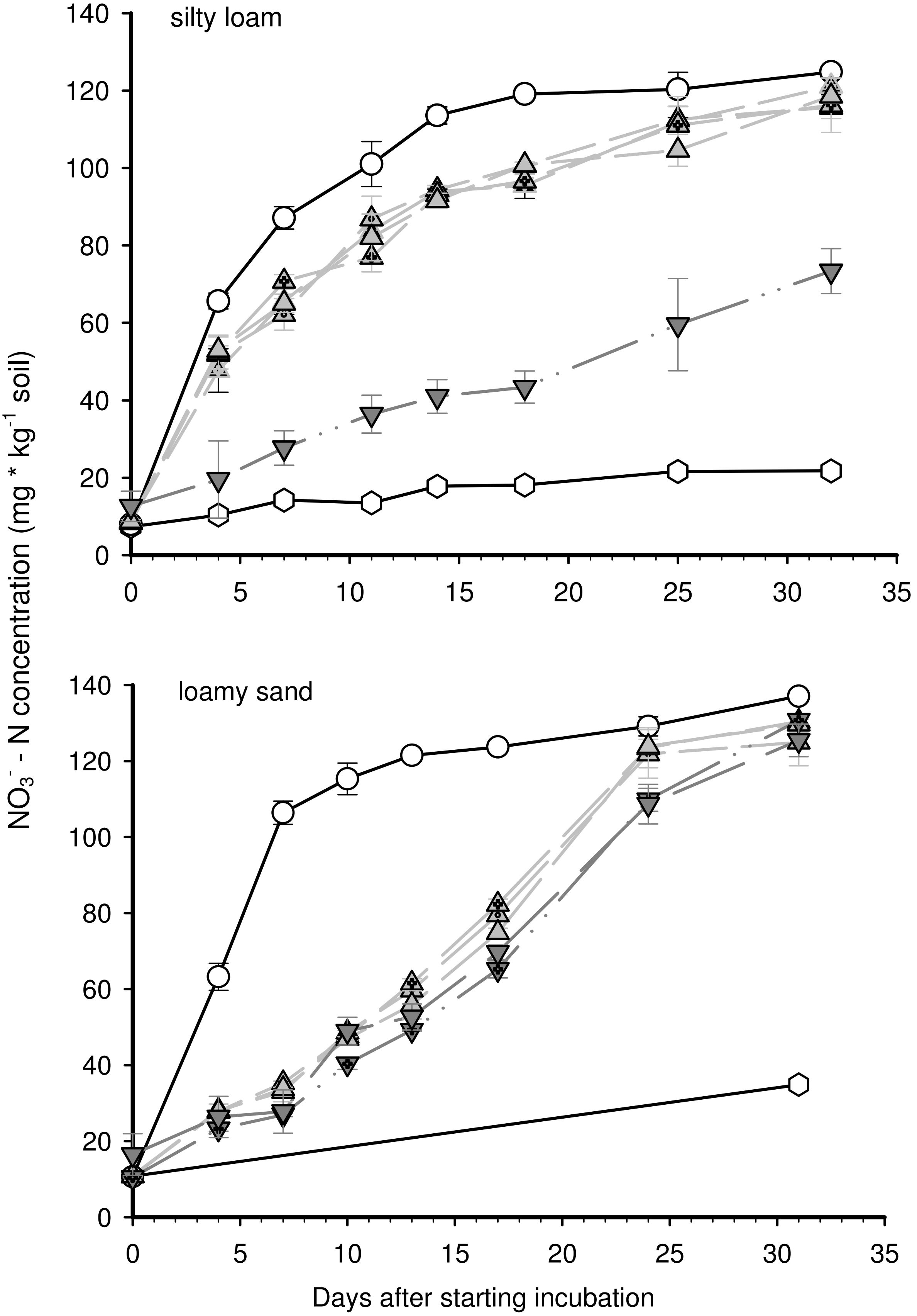

Fig. 27 Influence of different DMPP applications on the NO_3^- oxidation in a silty loam and a loamy sand.
Treatments see also table 2. Legend see Fig. 26

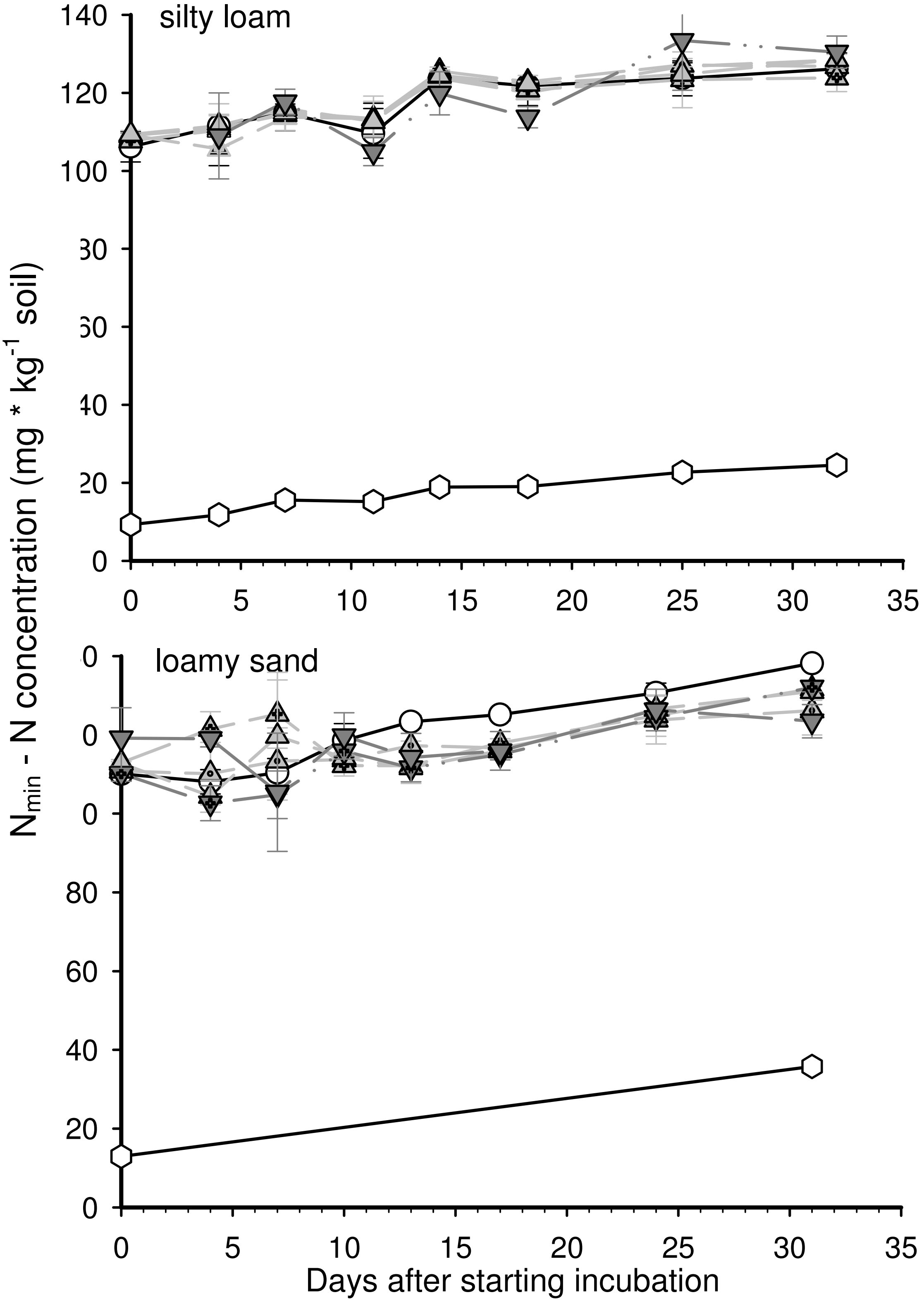

Fig. 28 Influence of different DMPP applications on the N_{min}^{-} concentration in a silty loam and a loamy sand. Treatments see also table 2. Legend see Fig. 26

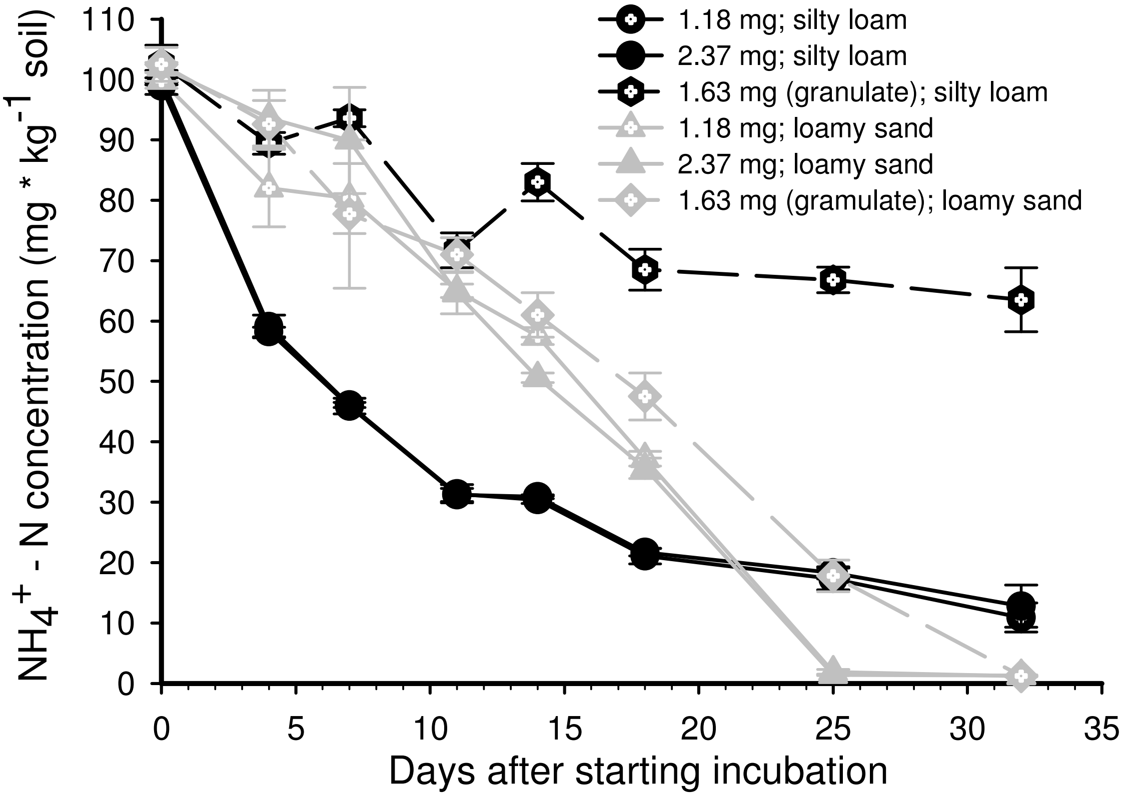

Fig. 29 Ammonium concentration in selected treatments with fertiliser and DMPP as solution or as granules in silty loam and loamy sand respectively.

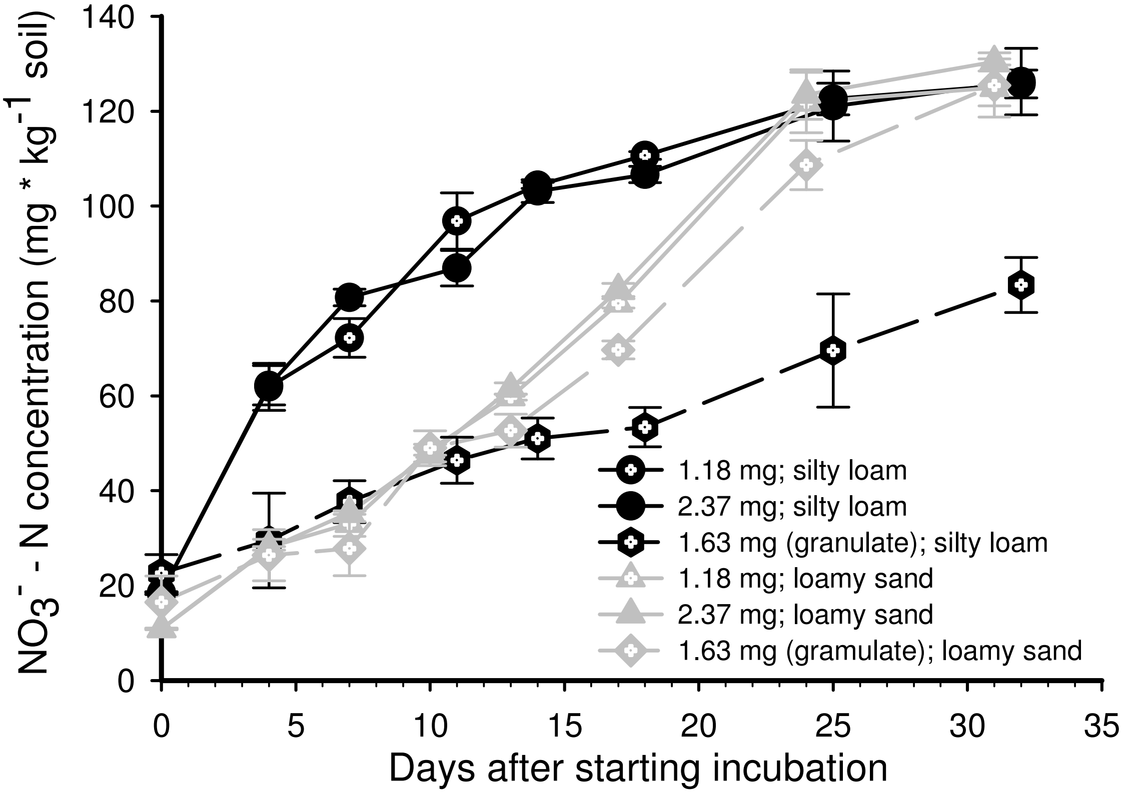

Fig. 30 Nitrate concentration in selected treatments with fertiliser and DMPP as solution or as granules in silty loam and loamy sand respectively.

3.2.1.2 Inhibitor effect of DMPP compared to DCD

The effect of DMPP is compared to DCD after an application in liquid form in both soils and with fertiliser granules in the sandy loam. For the liquid application inhibitor concentrations are selected that would correspond to the relation of ammonium to inhibitor near to what is recommended in practice. Under these conditions the effect of DCD (15 mg * kg^{-1} soil) is superior to DMPP (1.18 mg *kg^{-1} soil) in the silty loam (Fig. 31). In the loamy sand the efficacy of DCD and DMPP is similar until day 8 of the incubation. Thereafter, the oxidation of NH_4^+ with DMPP is slower compared to DCD (Fig. 32).

When DMPP and DCD were compared as fertiliser granules in the loamy sand there was not much difference in the effect on the NH_4^+ oxidation at the beginning of the incubation period, but after about 10 days the NH_4^+ content with DMPP was higher compared to DCD and DMPP was more efficient (Fig. 33).

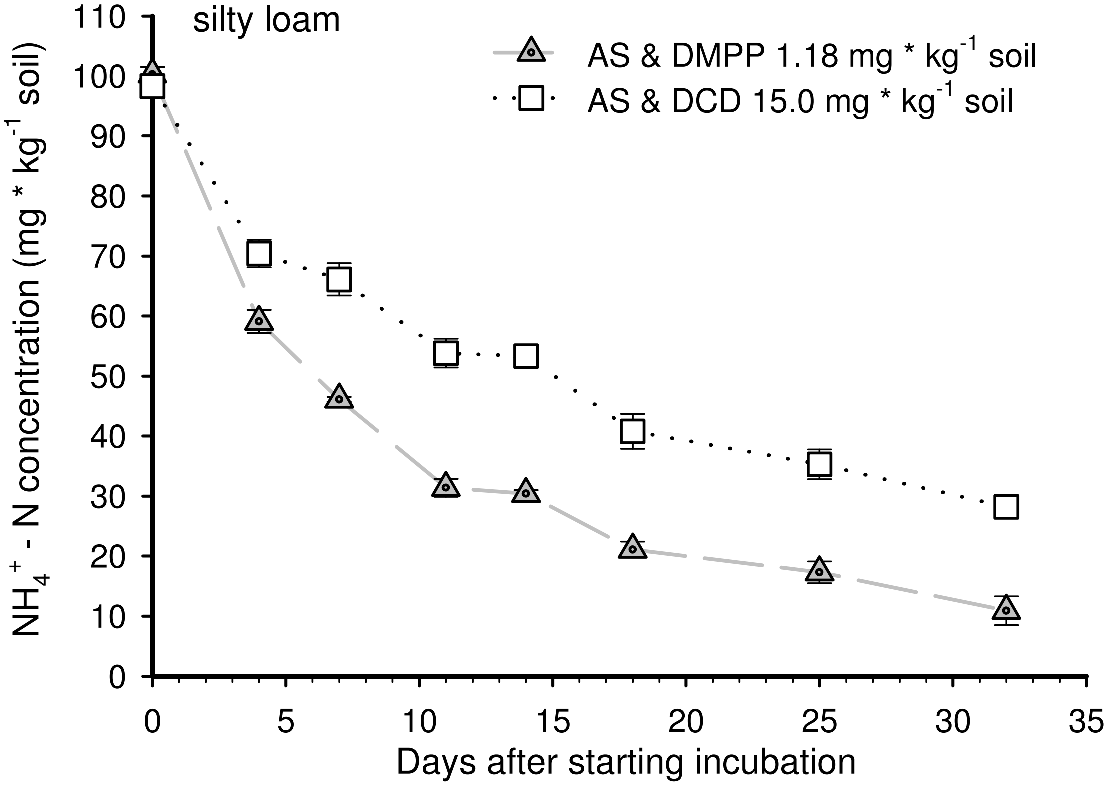

Fig 31 NH_4^+ decomposition in a silty loam soil influenced by DCD and DMPP applied in liquid form.
Error bars represent standard derivation.

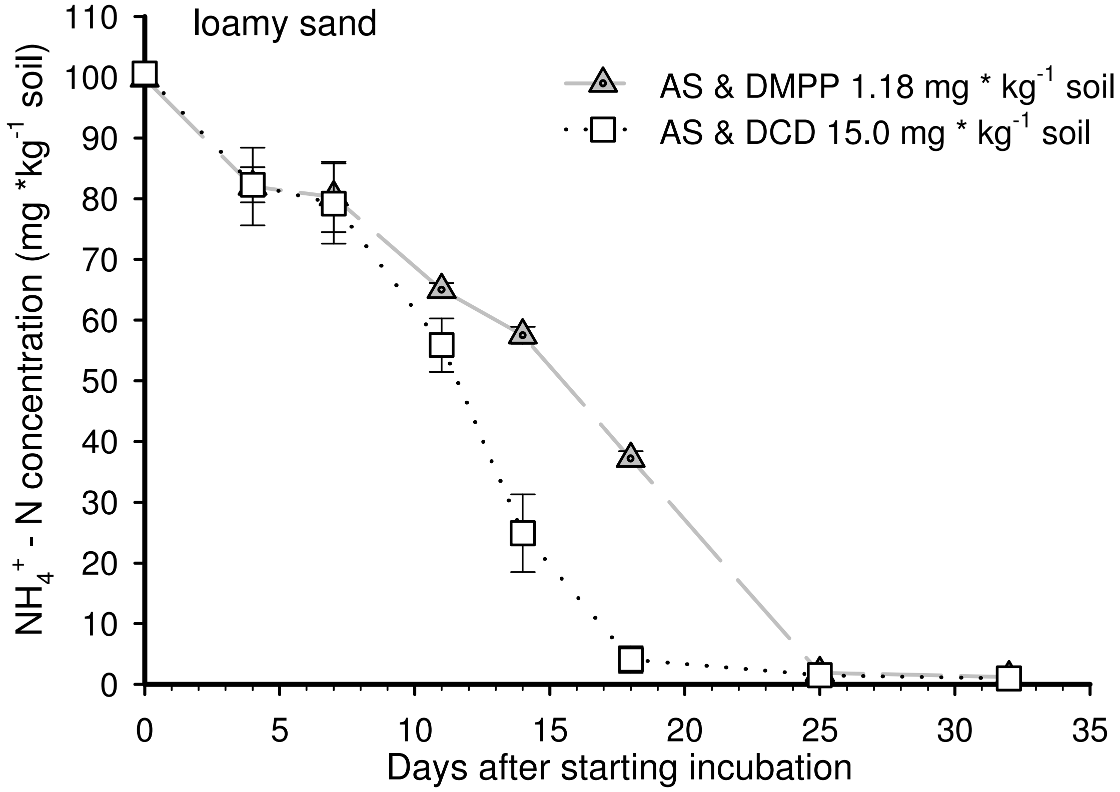

Fig 32 NH_4^+ decomposition in a loamy sand soil influenced by DCD and DMPP respectively.
Error bars represent standard derivation.

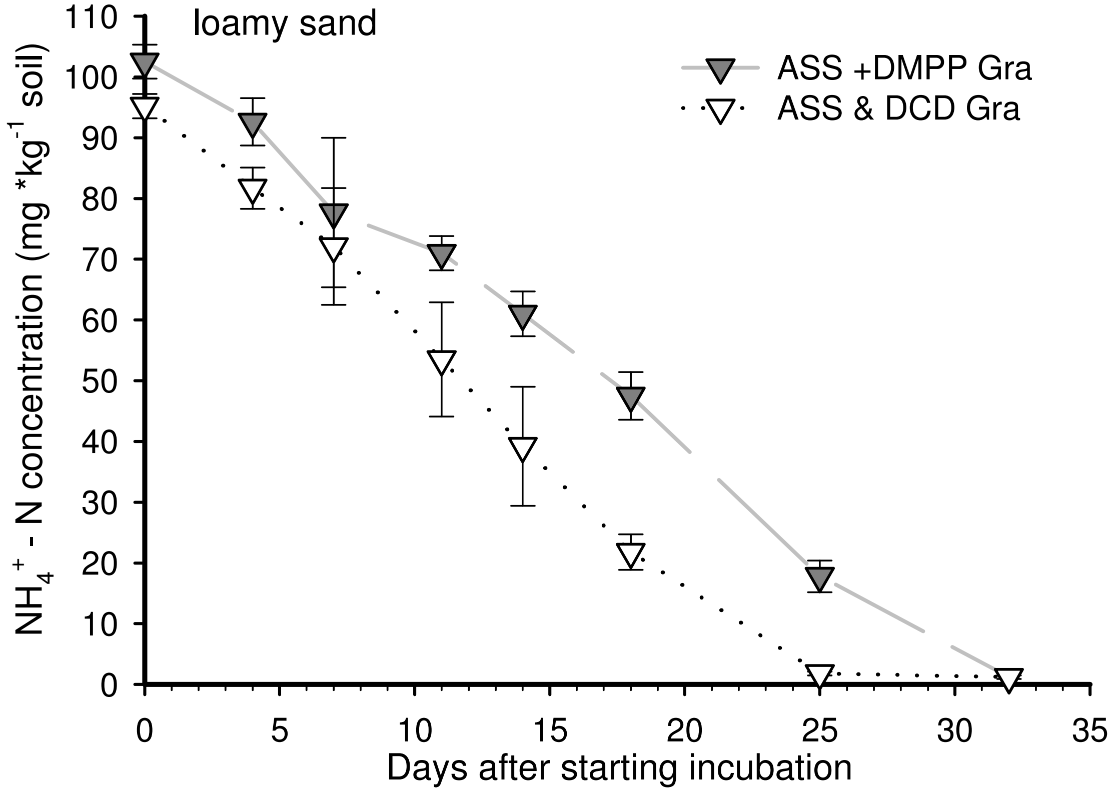

Fig 33 NH_4^+ decomposition in a loamy sand soil influenced by DCD granules and DMPP granules respectively.
Error bars represent standard derivation.

3.2.2 Decomposition of the nitrification inhibitors

3.2.2.1 Decomposition of DMPP

Samples of the treatments 5 and 6 (2.4 and 7.1 mg DMPP kg^{-1} soil; table 2) of the loamy sand (soil no. 20) are analysed for DMPP to follow the process of DMPP degradation. DMPP concentration added with 7.1 mg kg^{-1} soil decreases significantly until the 12th incubation day. In the second half of the incubation period, DMPP decomposition slows down. DMPP is still present at a concentration of about 1mg kg^{-1} soil (Fig. 34) at day 25.

For treatment 5 (2.4 mg DMPP kg^{-1} soil; table 2) DMPP concentration is determined at 3 dates (Fig. 35). As expected the DMPP concentration is distinctly lower compared to the addition of 7.1 mg kg^{-1} soil. After 18 days DMPP in the treatment 2.4 mg kg^{-1} soil is almost completely degraded.

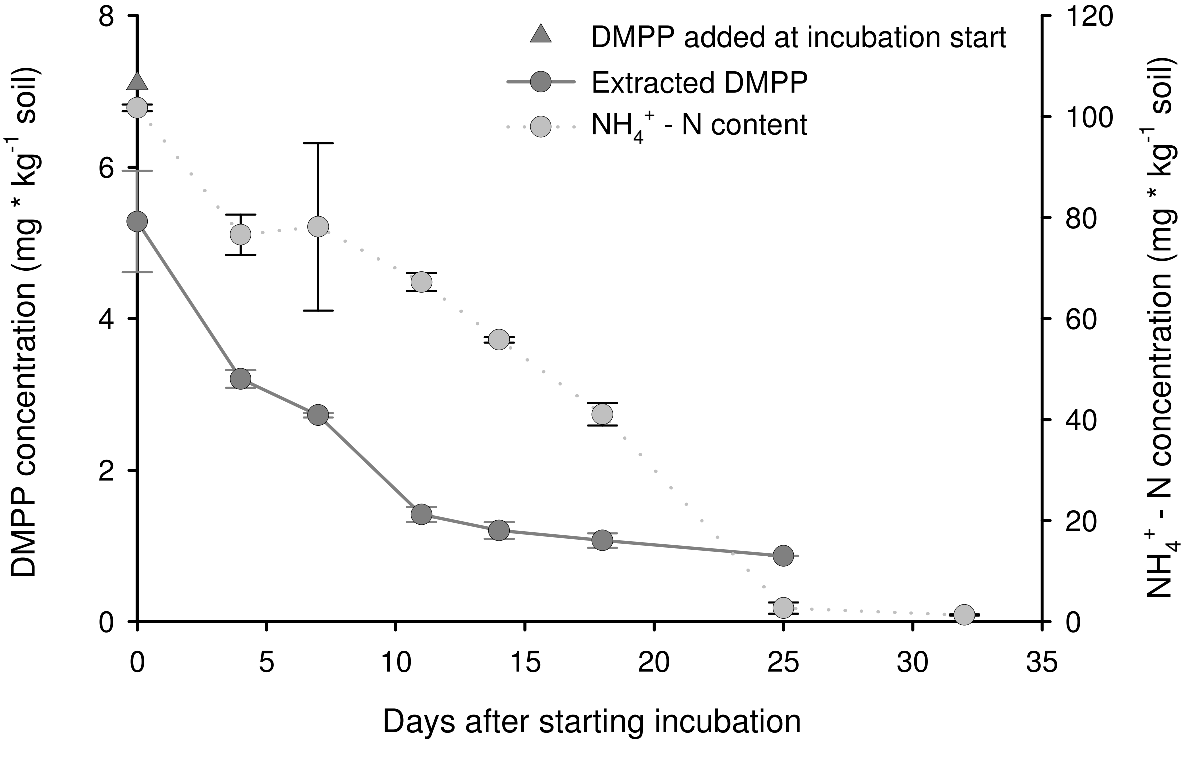

Fig. 34 Ammonium and DMPP degradation in loamy sand.
7.1 mg DMPP * kg^{-1} were added at the beginning of the incubation.
Error bars represent standard deviations.

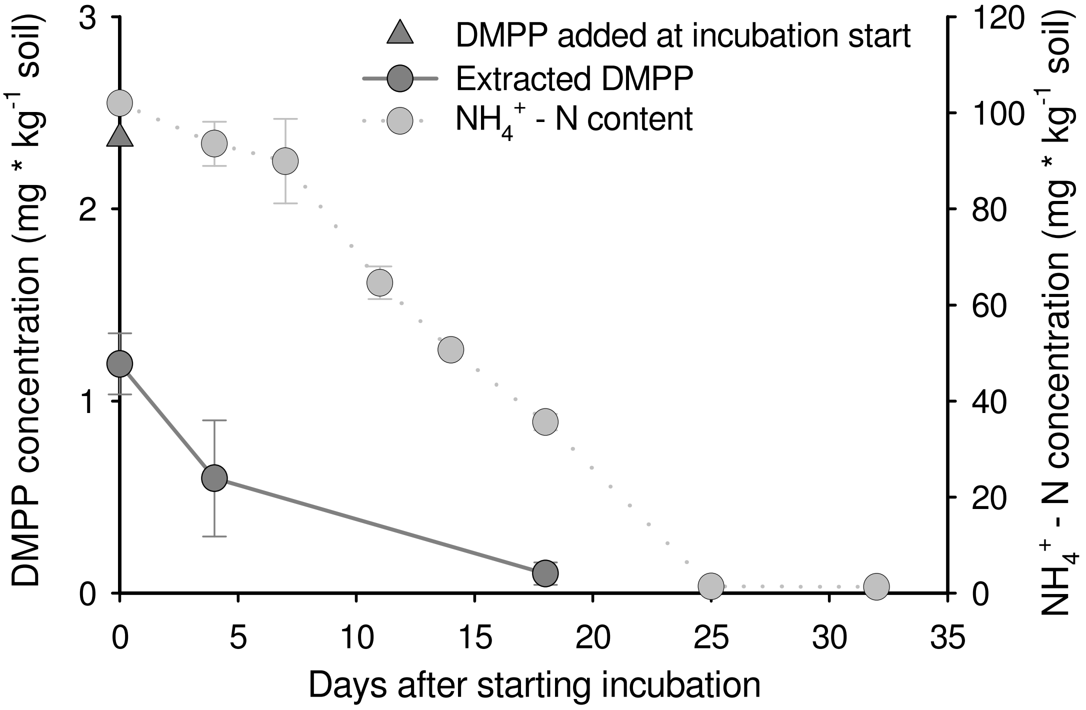

Fig. 35 Ammonium and DMPP degradation in loamy sand.
2.4 mg DMPP * kg^{-1} were added at the beginning of the incubation.
Error bars represent standard deviations.

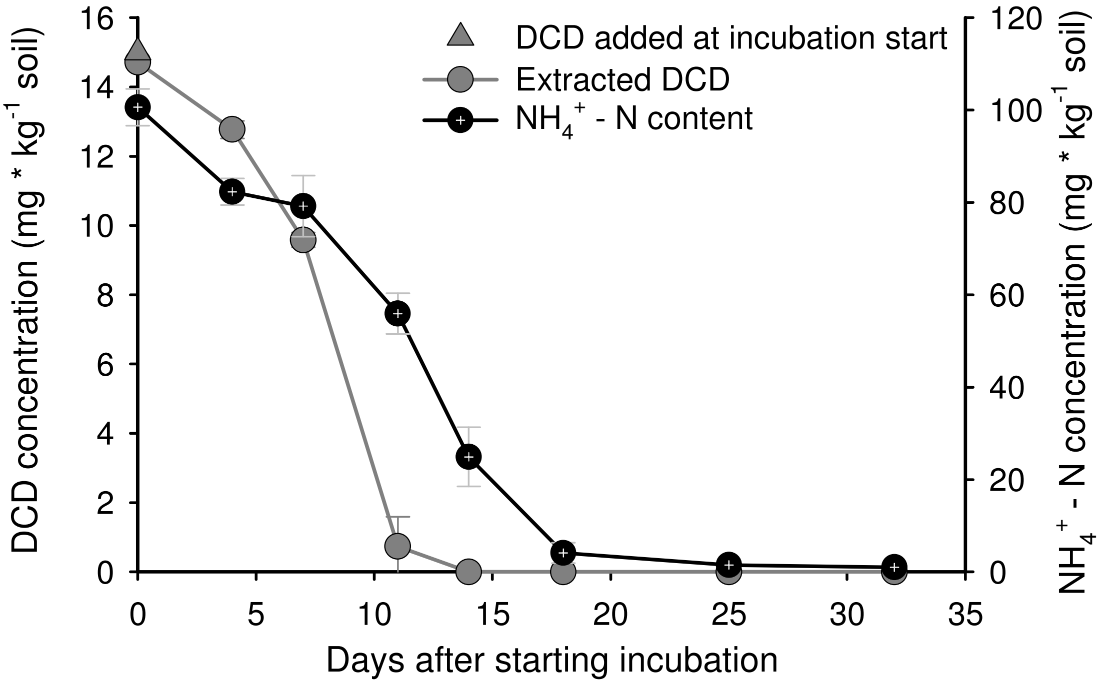

Fig. 36 Ammonium and DCD degradation in loamy sand.
15 mg DCD kg^{-1} were added at the beginning of the incubation.
Error bars represent standard deviations.

3.2.2.2 Decomposition of DMPP compared to DCD

The degradation of DCD started right from the beginning of the incubation period concomitantly with the turnover of ammonium (Fig 36).

The behaviour of the inhibitor degradation differed between DMPP and DCD (Fig. 37). DCD was rapidly and completely degraded within 14 days. Compared to this the decline in DMPP concentration particularly of the higher concentration of 7.1 mg kg^{-1} soil was much slower. Ammonium degradation was also slower in the DMPP treatments than in the DCD treatments (Fig. 34, 35 and 36).

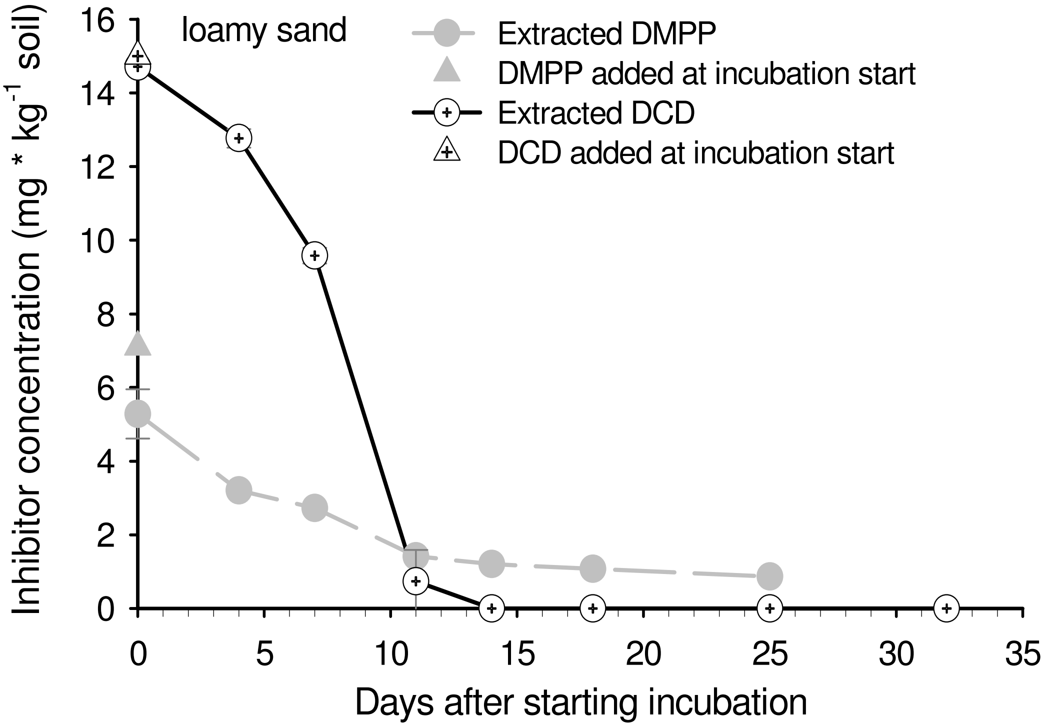

Fig.37 Comparison of DMPP and DCD decomposition in a loamy sand soil. 7.1 mg DMPP kg^{-1} and 15 mg DCD kg^{-1} were added at the beginning of the incubation.
Error bars represent standard derivation.

3.2.3 Influence of soil matric potential on the efficiency of DMPP

Soil matric potentials affect nitrification in the treatments without DMPP in both soils. Nitrification continuously decreass with a decline in soil matric potential. However this reduction is more pronounced in the loamy sand. In the silty loam, differences are smaller. In this soil even at the lowest soil matric potential a relatively high nitrification is observed. In general at comparable matric potentials, nitrification is considerably more reduced in the loamy sand than in the silty loam (Fig. 38 and 39).

With DMPP, nitrification is equally inhibited in all treatments of the silty loam soil. Soil matric potentials does not influence the efficacy of DMPP in this soil (Fig. 38). In the loamy sand with added DMPP, strong nitrification inhibition is observed at all soil matric potentials. The strongest nitrification inhibitory effect is found in the loamy sand soil at the highest soil matric potential (Fig. 39). In the silty loam DMPP induced nitrification is in about the same range at all soil matric potentials. The effect of soil matric potential on nitrification is still obvious. In the loamy sand nitrification inhibition is strong and independent of soil matric potential. Even at the highest level nitrification is low during the whole incubation period.

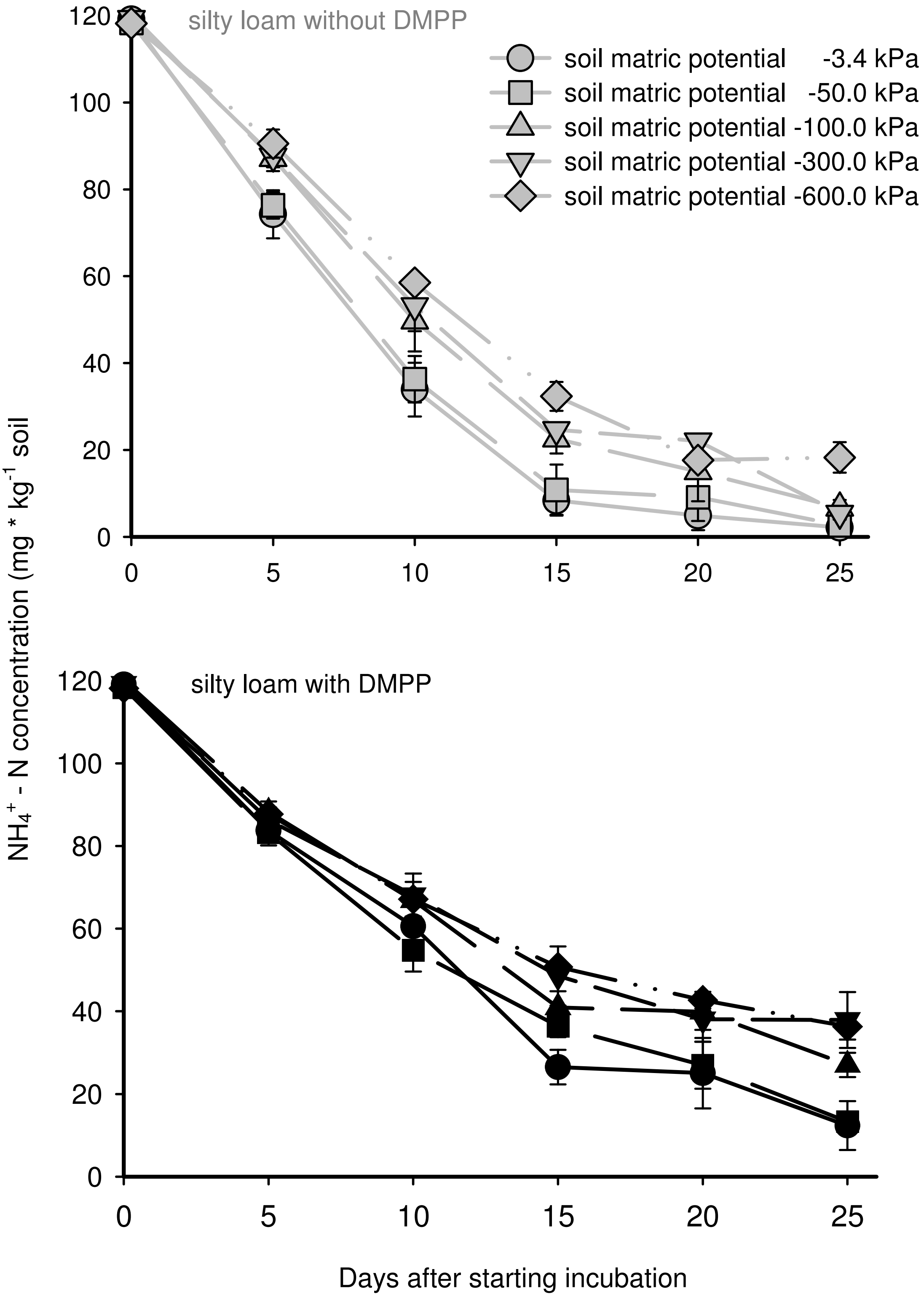

Fig. 38 Effect of soil matric potential on ammonium oxidation in soil 6 with and without the nitrification inhibitor DMPP.
Error bars represent standard deviations.

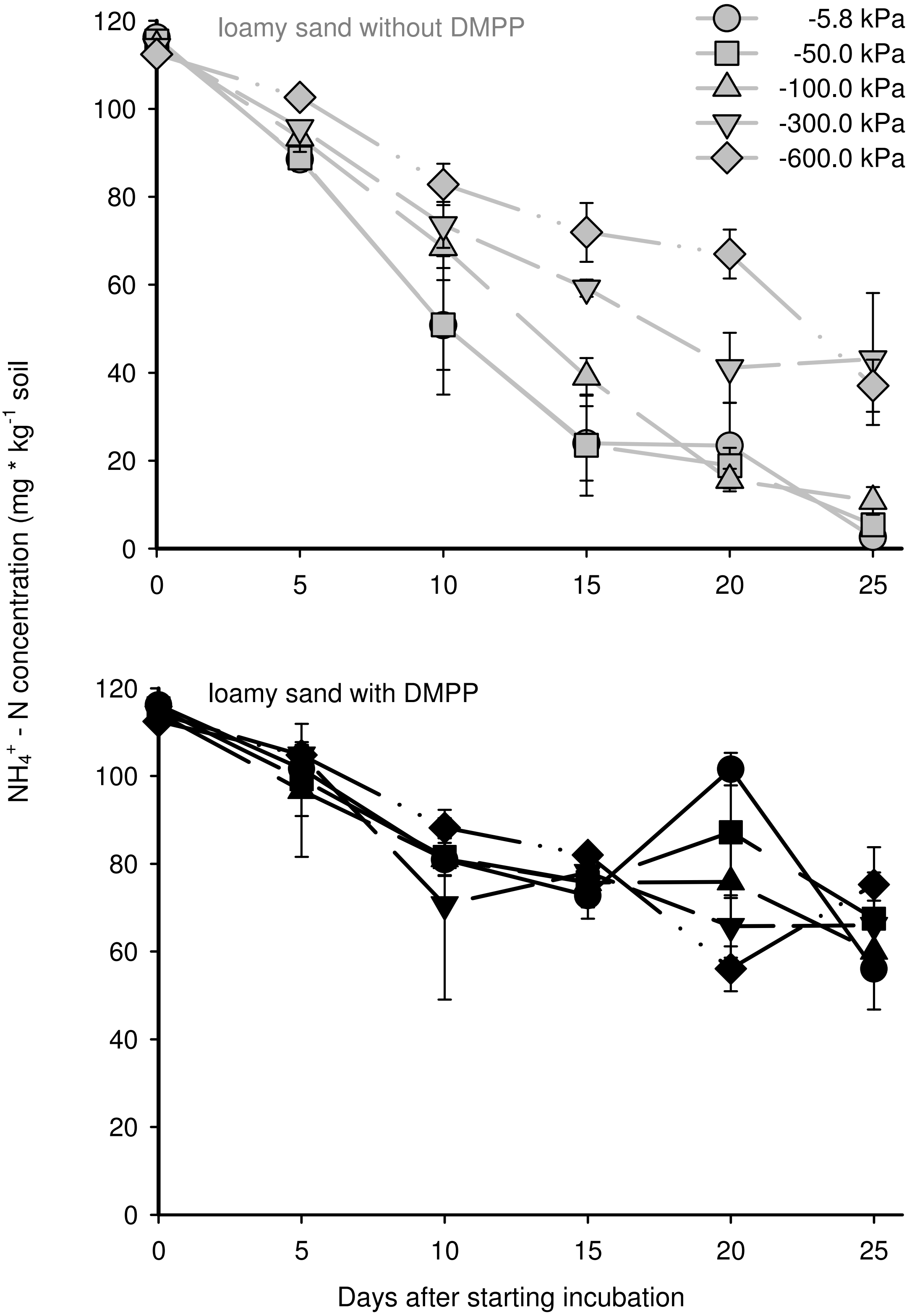

Fig. 39 Effect of soil matric potential on ammonium oxidation in soil 20 with and without the nitrification inhibitor DMPP.
Error bars represent standard deviations.

4 Discussion

These studies investigated the effect of DMPP on nitrification in 22 soils. The properties of these soils differed in soil texture, organic matter and biological activity. For investigating the efficacy of DMPP influenced by different soil parameters, short - term incubation experiments were carried out with all 22 soils. In two of these soils the effect of DMPP on nitrification was tested in long - term incubation experiments.

4.1 Short - term incubation

4.1.1 Inhibition of nitrite formation in short-term experiments

In the short - term incubation experiments DMPP inhibited the oxidation of added NH_4^+ to NO_2^- in all tested soils. Distinct differences in the inhibitory effect of DMPP were observed among the soils. The calculation of single correlations between relative NO_2^- formation and soil parameters, indicated a relation between soil texture and the efficacy of DMPP. At all investigated DMPP concentrations, sand was highly, and negatively correlated with the relative NO_2^- formation, so that the efficacy of DMPP increased, when soils were higher in sand content. This indicates, that properties of the silt or clay fraction impair the efficiency of DMPP. Correlation to catalase activity was similar to the soil texture, soil organic matter and CEC were of less significance. The potential ability of soils to nitrify ammonium (potential nitrification) obviously plays no role in the efficacy of DMPP.

The single correlations explained only partially the relationship between soil parameters and DMPP efficiency. Therefore, multiple regressions were calculated.

A multiple regression including sand fraction and catalase activity explained DMPP efficacy only marginally better than the sand fraction alone. Including soil H^+-concentration to the regression model, the correlation significantly improved. By including the parameter of potential nitrification into the regression, the model could be further improved with a coefficient of determination of $R^2 = 0.70$.

However, the last parameter represents a microbiological soil characteristic which is highly affected by the timing of soil collection (Staley et al. 1990) and possibly by the subsequent storage conditions. For this reasons, the potential nitrification seems not to be suitable to be included in a prognosis model. The model without potential nitrification already supplies a significant result.

In the actual 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 describe the inhibitory effect of DMPP in short - term incubation experiments should be interpreted in the context of adsorption properties of both the catalase enzyme and DMPP at soil surfaces. Fusi et al. (1989) and Calamai et al. (1991) showed that the catalase enzyme was adsorbed by 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 due to their similar binding behaviour on soil surfaces. Adsorption of DMPP in different soils proved to be an important factor in relative NO_2^- formation.

4.1.2 DMPP adsorption experiments

The adsorption behaviour of DMPP was markedly influenced by soil textural properties viz clay content. A regression model including only clay explained the DMPP adsorption with $R^2 = 0.60$. Adding silt fraction to this model, improved the correlation only slightly. 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, were more efficient in soils with low contents of clay, silt and organic carbon. The present study demonstrates that the efficacy of 3,4-dimethylpyrazole-phosphate (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 inhibitory effect. Nitrification was less inhibited in soils with higher clay and/or silt contents where DMPP may be adsorbed to inorganic soil constituents.

Correlations of DMPP adsorption with total soil N and organic matter were less relevant (N_t: $r = 0.54$, C_{org}: $r = 0.49$). Nevertheless, the role of organic matter in the adsorption of nitrification inhibitors has been documented for a phenyl pyrazole compound (Bobe et al. 1997), for nitrapyrin and XDE-474 (Kpomblekou and Killron 1996) and also for DCD (Zhang et al. 2004).

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). Soil adsorption behaviour of DMPP also implies, that, in contrast to dicyandiamide (Corre and Zwart 1995; Adbel-Sabour et al. 1990; Amberger and Vilsmeier 1988) a spatial separation of the active substance from the applied ammonium seems to be much less probable.

From these results, it can be concluded that the short-term inhibitory effect of DMPP was strongly 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 its concentration which is available to the nitrifying microorganisms over an extended period of time. During long-term conditions, additional factors, such as the degradation of the inhibitor, become more relevant.

4.2 Long - term incubation

4.2.1 Concentration, application form, and decomposition of DMPP

In the long - term incubation experiments, DMPP markedly retarded nitrification in both tested soils which differed mainly in their textural properties. This is in line with a broad efficacy of DMPP reported for different soils in the short-term incubation experiments and under field conditions (Linzmeier et al. 1999; Pasda et al. 2001a).

Distinct differences in the extent and duration in the inhibiting effect of DMPP among the soils may be due to the adsorption behaviour of DMPP. If adsorbed DMPP, as opposed to DMPP in soil solution, is better protected against microbial degradation and is then remobilized 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).

Nevertheless results from the DMPP degradation experiments indicate, that in the loamy sand with a low adsorption capacity, DMPP added with 7.1 mg kg^{-1} soil was not completely converted within 25 days. Presumably, the remaining amount of DMPP, of about 1 mg DMPP kg^{-1} soil, might still be able to delay the nitrification, when further NH_4^+ is added. In contrast to this, 2.4 mg DMPP kg^{-1} soil were completly degraded after about 20 days. However it might be argued that the extraction with 1% K_2SO_4 underestimated the DMPP content in the soil because the recovery at the beginning of the experiment was only about 50 - 70%.

When applied as solution, a moderate increase in the added amount (0,7-7,0 mg kg^{-1}) did not affect the efficiency of DMPP. McCarty and Bremner (1989) investigating a number of different pyrazoles, reported that all compounds capable to retard NH_4^+ oxidation (including 3,5 DMP), were more effective at higher inhibitor concentrations. The reason for this discrepancy may be associated with the distinctly lower range of concentrations used in the present experiments. But this also indicates, that DMPP was sufficiently efficacious at low concentrations. Moderately higher dosages were apparently unable to further reduce the activity of the nitrifiers beyond a soil specific extent of inhibition. For a higher inhibition effect most probably much higher DMPP concentrations would be necessary.

Compared to the application as solution, the inhibition of the nitrification in the silty loam was more pronounced when NH_4^+ and DMPP were applied together as fertiliser granules. Granules will create high local concentrations of NH_4^+ and DMPP, exceeding the concentrations applied as solution. Consequently the protection of NH_4^+ against oxidation is very effective. In addition, a water content of 0.2 kg kg^{-1} soil (corresponding to a soil matric potential of –600 kPa) probably delays the dissolution of the granule in the silty loam, while in the loamy sand, granules will be readily disintegrated at the same soil water content (soil matric potential -58 kPa). This results in a restricted microbiological accessibility to NH_4^+ and DMPP in the silty loam at -600 kPa. In consequence, the efficiency of the application of granules in the silty loam was higher than in the loamy sand, where granules show a similar effect compared to DMPP added as solution.

4.2.2 Influence of soil matric potential on the efficiency of DMPP

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 and inhibits 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 soil matric potential below -50 kPa decreased the nitrification of ammonium. This inhibition was further enhanced with lowered soil matric potentials down to -600 kPa. At this soil matric potential the decline in the nitrification rate is ascribed to cell dehydration and substrate limitation in rather equal shares (Stark and Firestone 1995). Above -600 kPa substrate limitation caused by the inhibition of the diffusion of uniformly distributed NH_4^+ possibly increased in importance. NH_4^+ diffusion would be expected to decrease in coarse-textured soils, as soil water contents at defined soil matric potentials (below field capacity) are much lower compared to fine textured soils. This is in line with the results presented here for the sandy loam soil, as the decrease in NH_4^+ oxidation was markedly more significant in this soil.

The system described in this study becomes more complex by the application of NH_4^+ as fertiliser granules, entailing aspects of spatial distribution of NH_4^+ around the granule. An estimation of the mean diffusion distance with the parameters $D_e = D_l * \theta$

* f * 1/b – buffer capacity (b) for NH_4^+ according to Anghinoni and Barber (1990), diffusion coefficient in solution (D_l) according to Teo et al. (1992), tortuosity factor (f) according to Barraclough and Tinker (1981) – indicates, that decreasing water content below 0,11 kg kg^{-1} soil (-100 kPa) in the loamy sand will markedly restrict the local distribution of NH_4^+ in the vicinity of the granule and thereby the spatial accessibility of NH_4^+ to the nitrifying bacteria. The resulting high initial NH_4^+ concentrations will further decrease the nitrification rate.

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

In contrast to the loamy sand, the effect on nitrification inhibition was less sensitive to soil matric potential in the silty loam. Diffusions experiments with a silty clay soil, published from Azam et al. (2001), show an influence of moisture on the diffusion behaviour of DMPP and NH_4^+, too. DMPP and NH_4^+ diffuse faster at a higher soil water content.

4.3 General discussion

The investigations were carried out under constant environmental conditions, but in the field frequent changes in temperature and soil moisture occur. These changes influence the activity and growth of the nitrifying bacteria (Gödde and Conrad 1999) and also potentially impact the efficiency of DMPP.

For a better understanding of the behaviour of DMPP in soils, the mode of action of DMPP on the nitrifying bacteria must be understood. But neither the passage of DMPP through the membranes of micro-organisms nor the interaction of DMPP with the ammonia monooxigenase enzyme is known. Even studies about the direct effect of different DMPP concentrations on i.e. *Nitrosomonas sp.* in solution cultures are still missing.

There is limited knowledge about the influence of DMPP on other soil organisms, as microbes, fungi and soil fauna. Studies on the influence of DMPP on soil respiration and dehydrogenase activity in two different soils showed no effect of DMPP on soil respiration and dehydrogenase activity (Pasda, 1999 personal communication).

Our degradation studies showed that DMPP in even moderate concentrations was not completely decomposed within the incubation time which was confirmed by other studies (Pasda, 1999 personal communication). Further research has to determine the lifetime of DMPP in soils. Other investigations should examine the accumulation of adsorbed DMPP or its metabolites in the soil, during continuous long-term DMPP application on the same field for several years.

By long - term applications, it cannot be fully excluded that resistances of the nitrifiers against the nitrification inhibitor might occur. So far, this has not yet been described for DMPP, but Deni and Penninckx (1999) found, that nitrifying bacteria developed resistance and adapted to hydrocarbon - polluted soils. Further more, it was found that often repeated applications the efficacy of nitrification inhibitors, i.e. DCD, is reduced because of an accelerated microbial degradation of the inhibitor (Rhajbanshi et al. 1992). It might be hypothesised that this could also be true for DMPP.

The mode of degradation of DMPP in soils is unknown and this is also true for most of its metabolites. In an unpublished incubation study (Pasda, 1999 personal communication) with ^{14}C labelled DMPP there were three metabolites extractable and the formation of carbon dioxide was detected. Two of the metabolites were determined as 3-methyl-1H-pyrazole-4-carboxylic acid and pyrazole-3, 4-dicarbonic acid-3 (4)-monomethylester. The third compound was unknown. It was found in only low concentrations and was no longer detected after 60 incubation days. The unextractable radioactively labelled fraction reached maxima of more than 50 % during the incubation time. The composition of the unextractable compounds and their DMPP proportion is unknown.

The long-term potential accumulation of DMPP or unknown catabolic products, should be studied.

The influence of temperature on the efficacy of DMPP was not the subject of the present study. In laboratory experiments, Sachdev and Sachdev (1995) show that the efficiency of DCD markedly decrease at higher temperature and Puttana et al. (1999) show, the same effect for different nitrification inhibitors. The efficiency of DMPP also decrease with increasing temperature (Irigoyen et al. 2003). They postulate an effective use of DMPP mainly under cold and temperate climate conditions.

Results from the present investigation and from some field studies show the potential of DMPP to reduce N - losses in agriculture. Chaves et al. (2006) could show that DMPP was able to inhibit the nitrification of nitrogen from crop residues incorporated in the soil over a longer time period. A number of studies show a significant reduction of nitrate leaching (Banuls et al. 2001, Fettweis et al. 2001, Linzmeier et al. 2001a, Linzmeier et al. 2001b, Serna et al. 2000, Wissemeier et al. 2001, Zerulla et al. 2001a, Zerulla et al. 2001b).

Others have shown that the use of DMPP reduces the loss of nitrogen through gaseous emissions in the form of N_2O and NO_x. Linzmeier et al. (2001a), Linzmeier et al. (2001b) and Linzmeier et al. (1999) could demonstrate this in field with different crops and different mineral fertilisers. In these experiments the nitrogen fertiliser as well as the nitrification inhibitor were applied in both liquid and granular form. Weiske et al. (2001a), Weiske et al. (2001b), Weiske et al. (2001c) and Wissemeier et al. (2001) confirmed a reduction in N_2O losses using mineral fertiliser as well. Dittert et al. (2001) and Merino et al (2005) were showed a reduction in the loss of gaseous nitrogen from organic fertilisers when using DMPP as a nitrification inhibitor. Menendez et al (2006) studied gaseous nitrogen losses on intensively cultivated grassland. Here also, a significant reduction in the N-loss was shown with DMPP both as a mineral and organic fertiliser. Hatch et al. (2005) observed a reduction of gaseous nitrogen losses under the influence of DMPP in laboratory experiments. In thas study it was also demonstrated that the production of methane in the soil is not significantly increased after the application of DMPP. Fan und Tsuruta (2004) studied N_2O emissions under varying levels of soil moisture. With DMPP the N_2O emission was lower in all cases. The reduction of nitrogen looses leads to a higher nitrogen utilisation for agricultural and horticultural crops. Effects on yield and the nitrate levels in plants have been described in different publications (Banuls et al. 2001, Xu et al. 2005, Pasda et al. 1999, Pasda et al. 2001a, Pasda et al. 2001b, Serna et al. 2000, Zerulla et al. 2001a, Zerulla et al. 2001b). While the influence of DMPP on the yield was not consistent in these studies, all authors point out a reduction of NO_3^- in the crop products.

It is concluded that the application of DMPP is benefical to increase fertilise use efficiency and to reduce nitrigen losses into atmosphere and hydrosphere.

5 Conclusions

In short - term incubation experiments, the adsorption of DMPP to inorganic soil constituents mostly explained the extent of nitrification inhibition. 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.

In long - term incubation experiments efficiency of DMPP is influenced by soil properties as well. Further more, the water regime of the soil and the application form, as DMPP solution or fertiliser granules, affected its efficacy as nitrification inhibitor. The effect of temperature was not included in the present study.

Nevertheless, based on the results presented here, a high efficiency of DMPP can be expected particularly when it is formulated on fertiliser granules, and when applied in coarse textured soils espacilly under conditions of higher rainfall.

6 Summary

Nitrification inhibitors specifically retard the oxidation of ammonium to nitrite during the nitrification process in soil. The efficiency of nitrification inhibitors is dependent on the effect of the NH_3 - monooxygenase in nitrifying bacteria. Further more, external factors such as inhibitor concentration, soil properties, soil moisture and temperature affect the efficiency of nitrification inhibitors. In this study the influence of soil properties, inhibitor concentration, soil matric potential and application form on the nitrification inhibitory effect of 3,4-dimethylpyrazole-phosphate (DMPP), a recently developed nitrification inhibitor has been investigated.

Based on short - term incubation experiments which allow to largely disregard the degradation of DMPP, the oxidation of the applied ammonium was more inhibited in sandy soils compared with loamy soils. The influence of soil parameters on the relative nitrite formation were best described by a multiple regression model including the sand fraction, soil H^+-concentration and soil catalase activity ($R^2 = 0.62$). The adsorption of DMPP to soil components or constituents was found to be an important factor for the inhibitory effect on the ammonium oxidation in short - term incubation studies ($r^2 = 0.57$). Adsorption studies showed, that the binding behaviour of DMPP was remarkably influenced by soil textural properties viz. the clay fraction ($r^2 = 0.61$).

In long - term incubation experiments the efficiency of DMPP in two different soils as dependent on inhibitor concentration, soil matric potential and application form (liquid or formulated on fertiliser granules of NH_4NO_3) was investigated. Generally, the efficacy of DMPP was higher in the loamy sand soil compared to the silty loam soil. When applied as solution, increasing DMPP concentration did not influence the inhibitory effect, determined as decrease of NH_4^+ concentration in both soils. Based

on equal amounts applied, the efficacy of DMPP, formulated on fertiliser granules was superior to the liquid application of DMPP and NH_4^+, particularly in the silty loam soil.

Without nitrification inhibitor, a decline in soil matric potential decreased nitrification in both soils but the reduction was more pronounced in the loamy sand soil. DMPP formulated on fertiliser granules almost completely inhibited nitrification in the loamy sand soil independent of the soil matric potential. In the silty loam soil, DMPP reduced the nitrification rate at all soil matric potentials by nearly the same amount compared with the treatments without DMPP.

Based on these results, DMPP may be a successful nitrification inhibitor, especially in moist coarse textured soils.

7 Zusammenfassung

Nitrifikationsinhibitoren hemmen während des Nitrifikationsprozesses im Boden spezifisch den ersten Schritt, die Oxidation von Ammonium zu Nitrit. Die Wirksamkeit von Nitrifikationsinhibitoren ist abhängig von deren Einfluss auf die NH_3 - Monooxygenase in nitrifizierenden Bakterien. Zusätzlich beeinflussen äußere Faktoren, wie Bodeneigenschaften, Wirkstoffkonzentration, Bodenfeuchtigkeit und Temperatur die Effizienz von Nitrifikationshemmstoffen.

In der vorliegenden Arbeit wurde der Einfluss von Bodeneigenschaften, Wirkstoffkonzentration, Bodensaugspannung und der Anwendungsform auf die nitrifikationshemmende Wirkung von 3,4 - Dimethylpyrazol Phosphat (DMPP), einem neu entwickelten Nitrifikationshemmstoff, untersucht.

In Kurzzeit - Inkubationsversuchen (mit einer Inkubationsdauer von 5 Stunden), bei denen der DMPP Abbau vernachlässigbar ist, wurde der Abbau des zugegebenen Ammoniums in den sandigen Böden stärker gehemmt als in lehmigen Böden. Der Einfluss der Bodeneigenschaften auf die relative Nitritbildung konnte durch eine multiple Regressionsgleichung (R^2 = 0,62) beschrieben werden. In diese Gleichung gingen die Parameter Sand, Protonenkonzentration und Katalasezahl ein. Adsorptionsstudien zeigten, dass das Bindungsverhalten von DMPP deutlich von der Bodentextur, vornehmlich der Tonfraktion (r^2 = 0,61) beeinflusst wird. Die Adsorption von DMPP war ein bedeutender Einflussfaktor für die Hemmwirkung von DMPP auf die Ammoniumoxidation in den Kurzzeit Inkubationsversuchen (r^2 = 0,57).

In Langzeitinkubationsversuchen wurde die Wirksamkeit von DMPP in zwei verschiedenen Böden, unter dem Einfluss verschiedener Hemmstoffkonzentrationen, Bodenmatrixpotentiale und Anwendungsformen (als Lösung oder auf

Düngergranalien formuliert) untersucht. Grundsätzlich war die Wirksamkeit von DMPP in lehmigem Sand höher verglichen mit schluffigem Lehm. In gelöster Form beeinflusst eine zunehmende DMPP Konzentration in keinem der Böden die Hemmwirkung, gemessen als Abnahme der NH_4^+ Konzentration. Auf Düngergranalien formuliert, wirkte DMPP stärker als bei Anwendung in gelöster Form. Dieser Unterschied zeigte sich vor allem in einem schluffigen Lehm.

Ohne Nitrifikationshemmstoff verringert eine Abnahme des Bodenmatrixpotentials in beiden Böden die Nitrifikation, im lehmigen Sand jedoch deutlicher. Bei Formulierung von DMPP auf Düngergranalien, ist die Wirkung des Hemmstoffes in lehmigem Sand sehr hoch und vom Bodenmatrixpotential unabhängig. Im schluffigen Lehm reduziert DMPP den Ammoniumabbau bei allen untersuchten Matrixpotentialen um annähernd denselben Betrag gegenüber den Varianten ohne DMPP Einsatz.

Die Ergebnisse zeigen, dass DMPP auf leichten Böden bei hoher Bodenfeuchte seine beste Wirkung entwickelt.

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The content of the following publication is a part of this doctoral thesis.

Barth M, von Tucher S, Schmidhalter U (2001) Influence of soil parameters on the effect of 3,4-dimethylpyrazole-phosphate as a nitrification inhibitor. Biol Fertil Soils 34: 98 - 102

9 Appendix

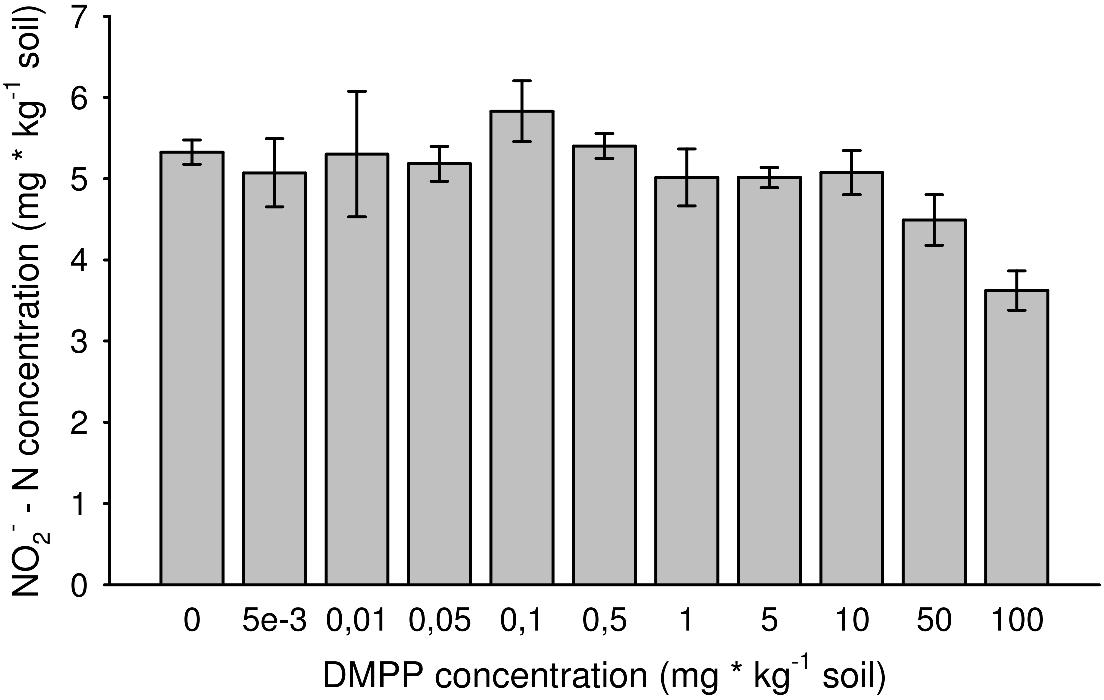
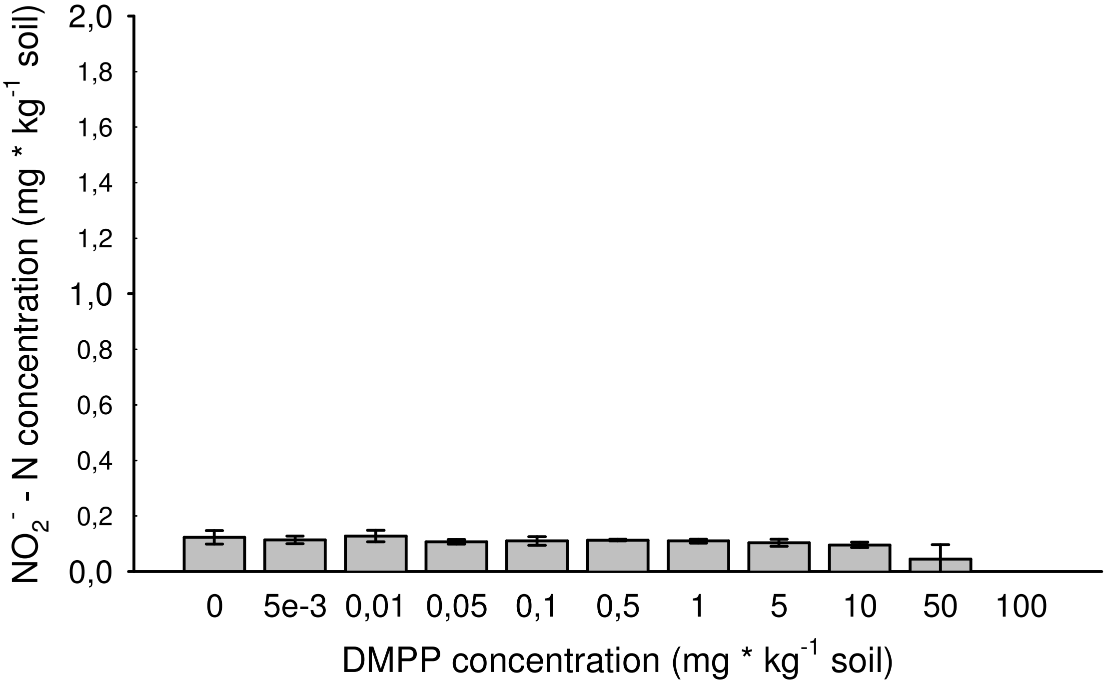


Fig. A1 Nitrite formation in short - term incubation studies in soil 1 with different concentrations of DMPP. Error bars represent standard deviations.



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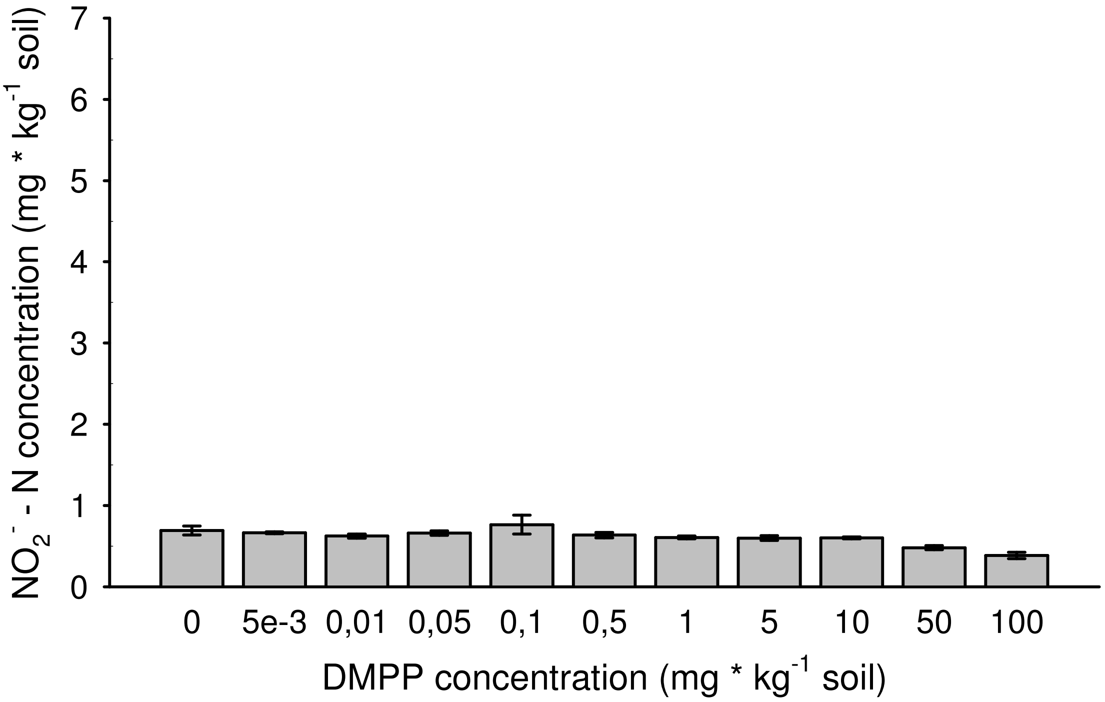

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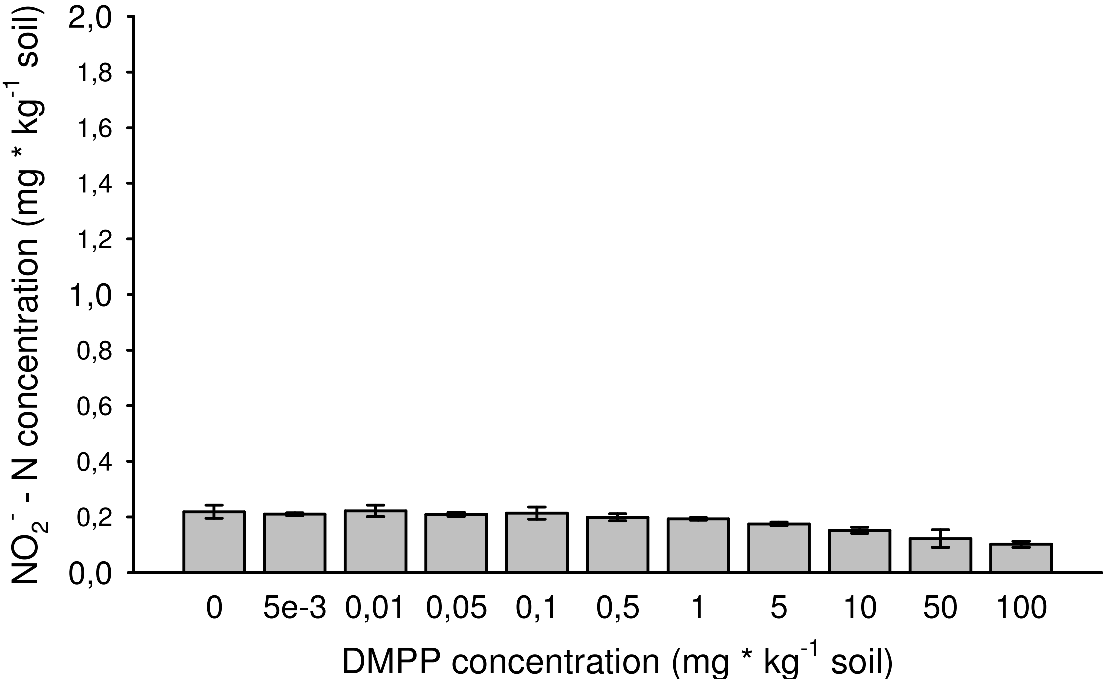

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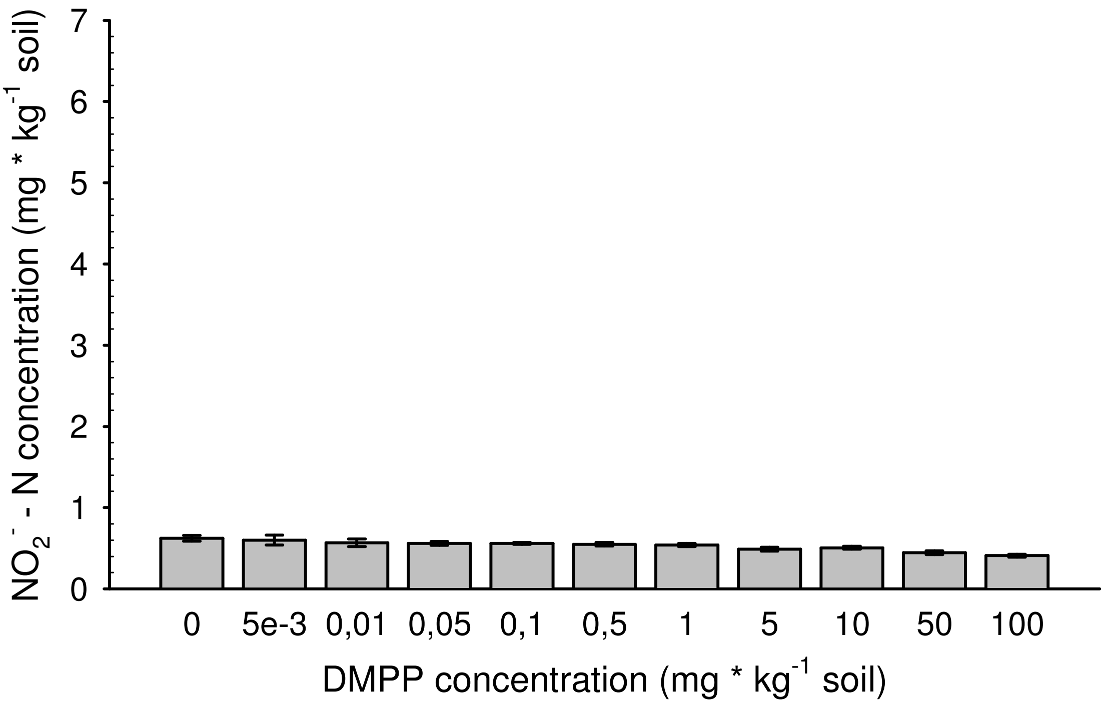

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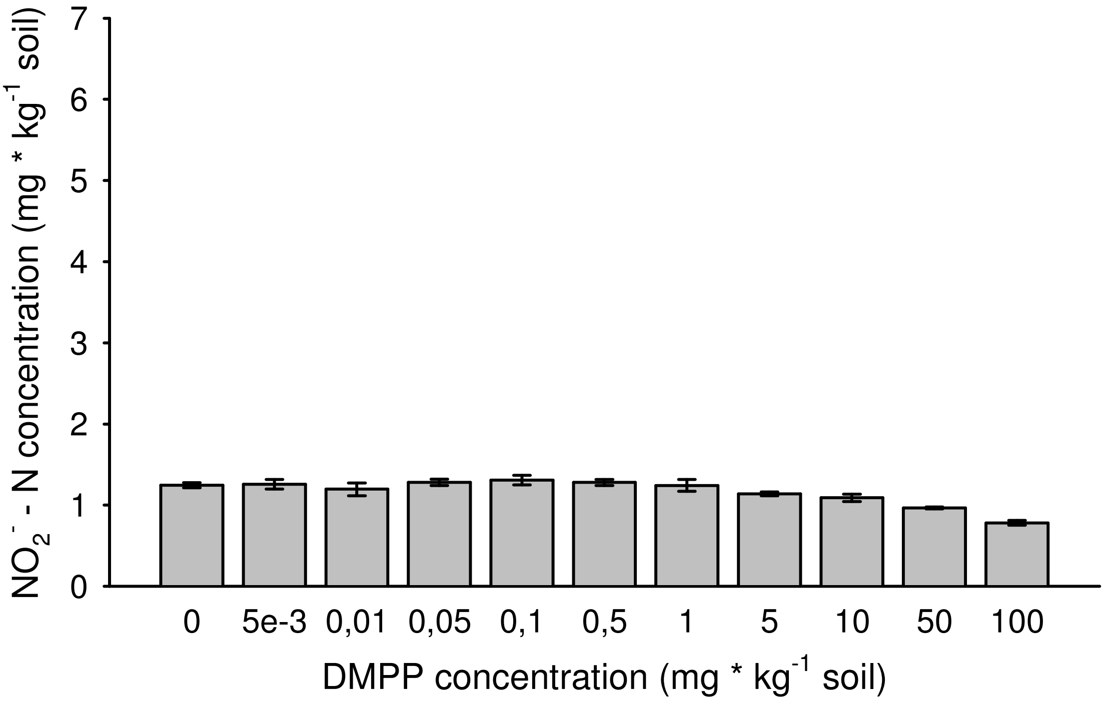

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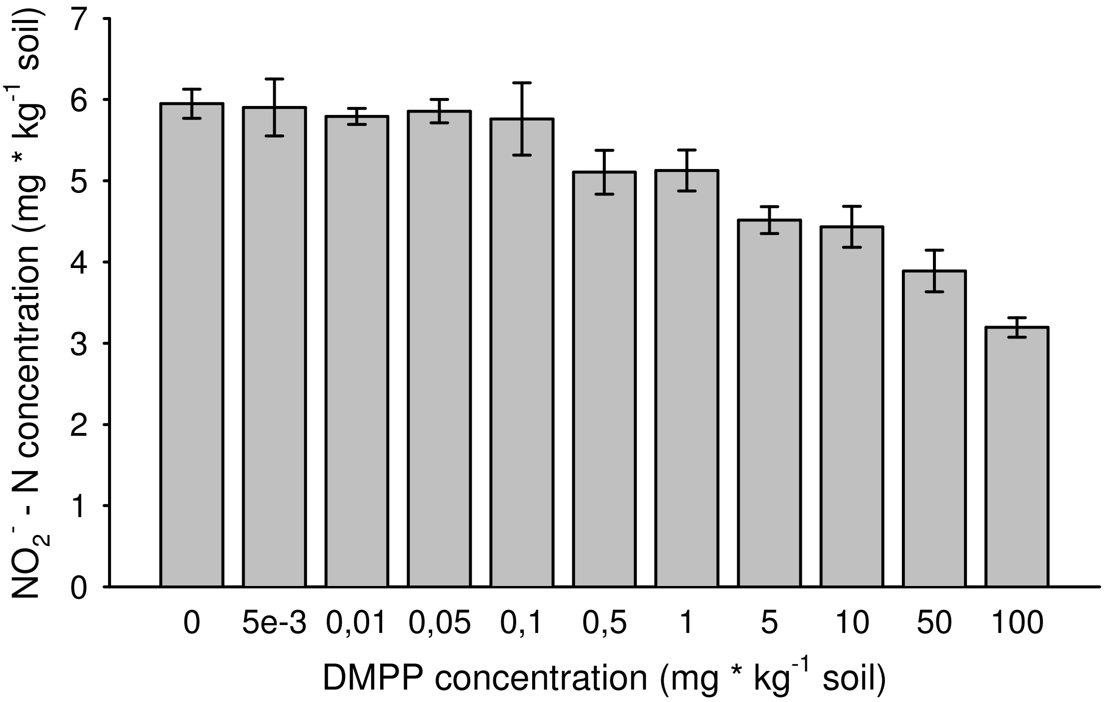

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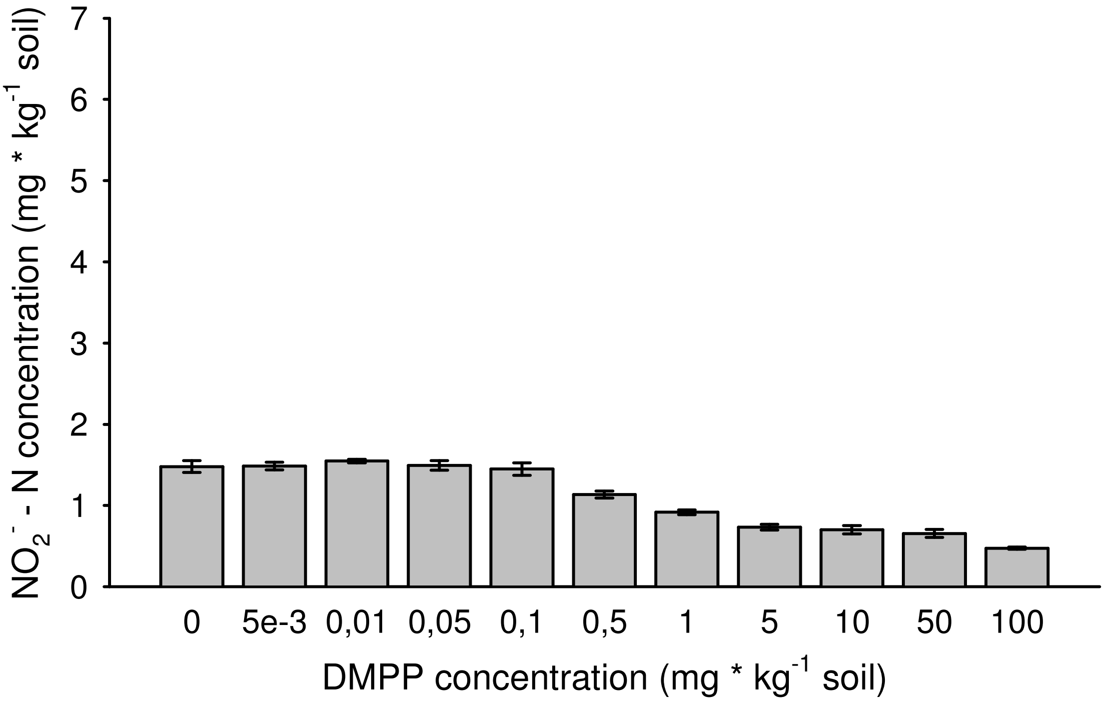
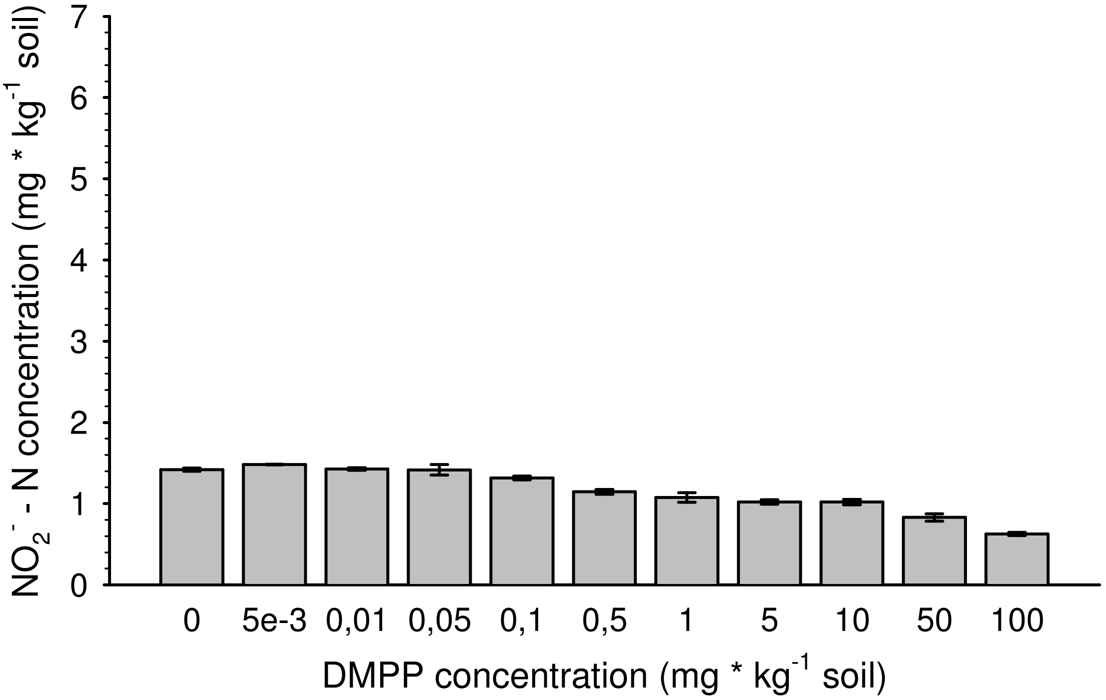


Fig. A8 Nitrite formation in short - term incubation studies in soil 8 with different concentrations of DMPP. Error bars represent standard deviations.



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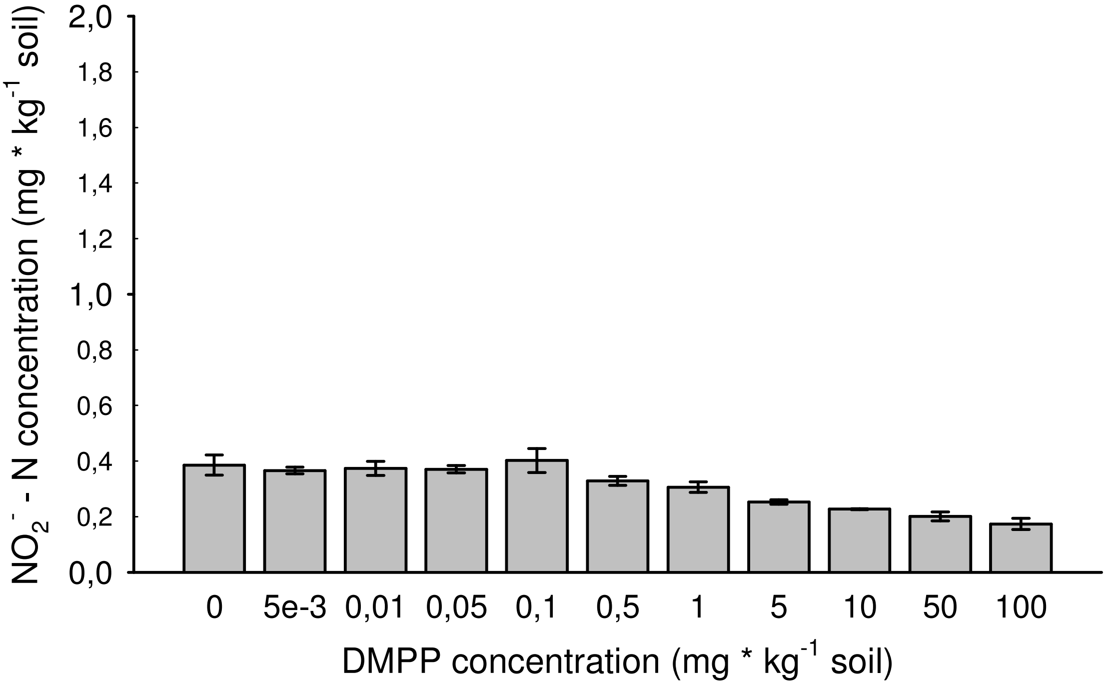

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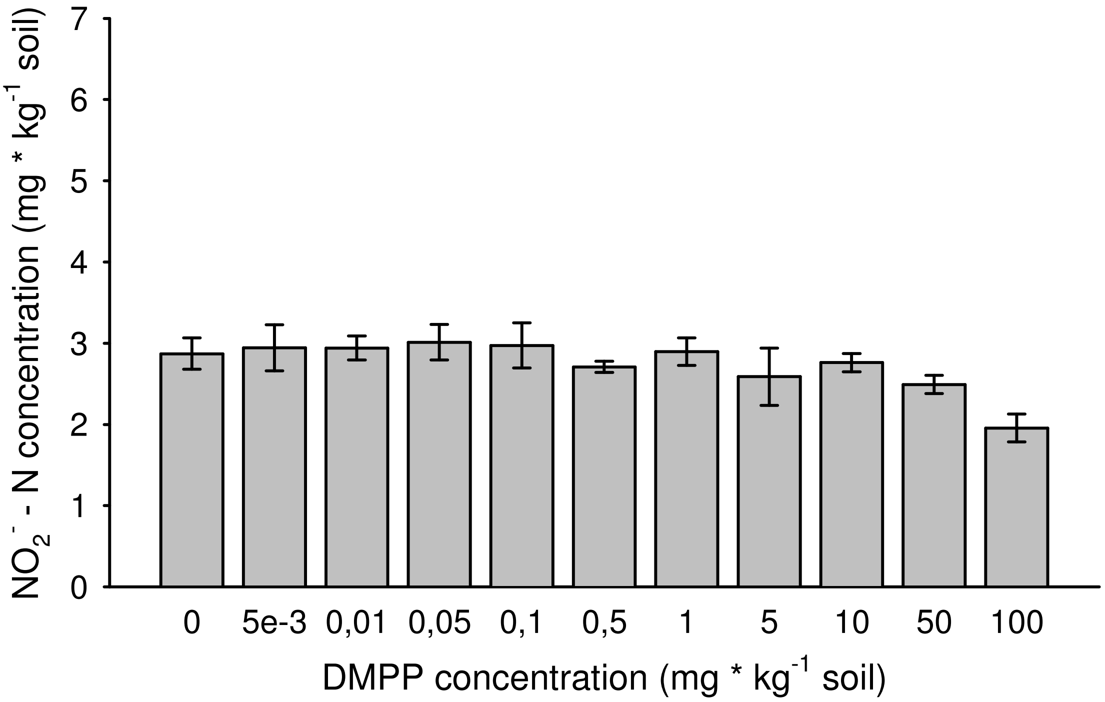

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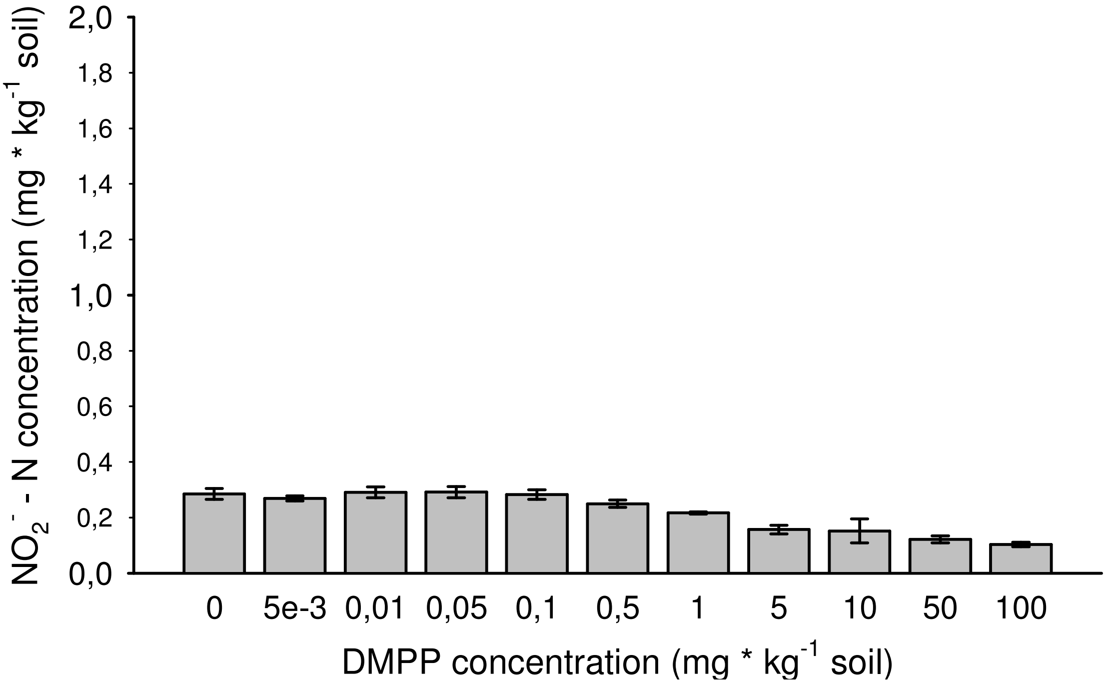

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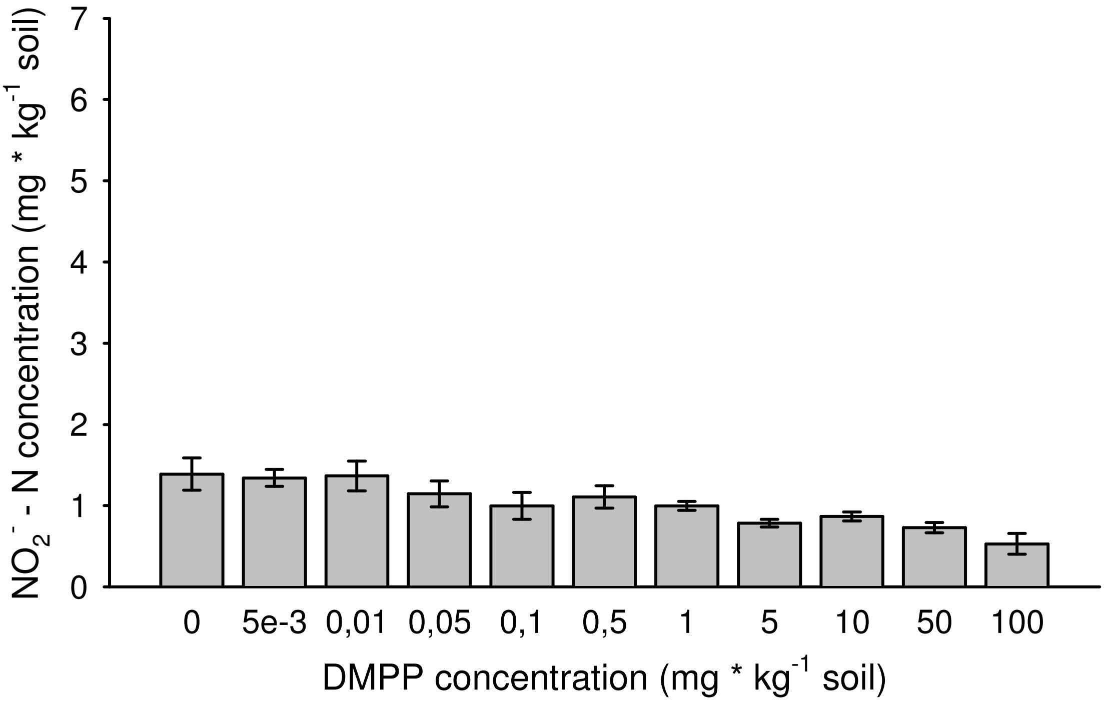

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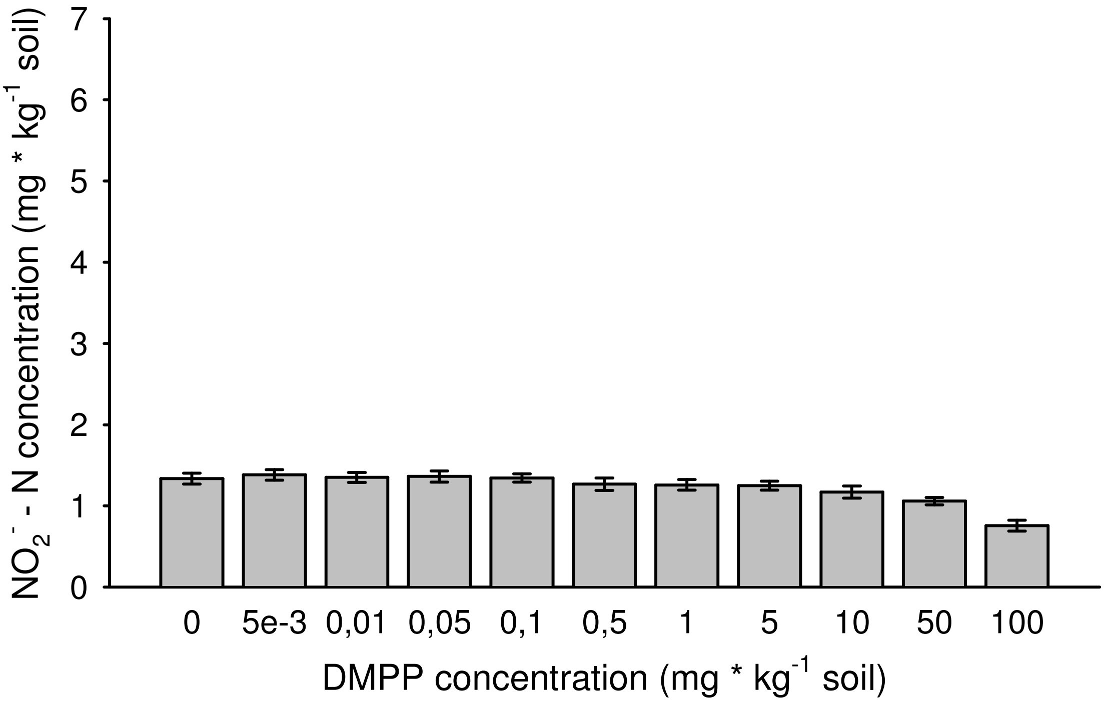

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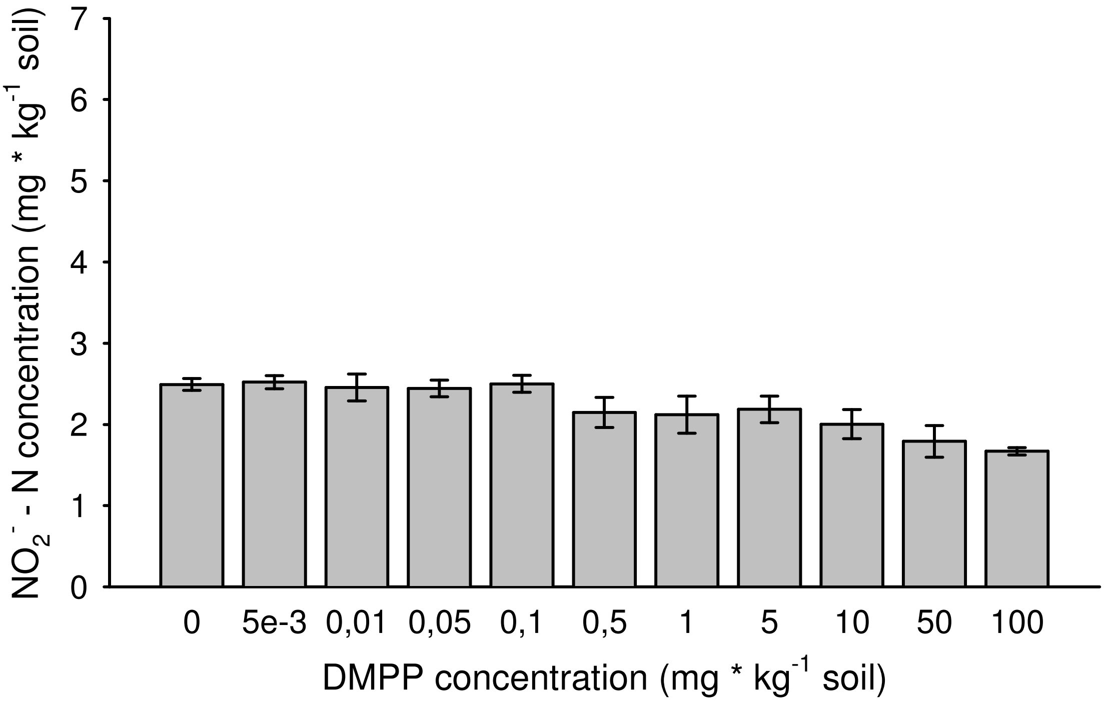

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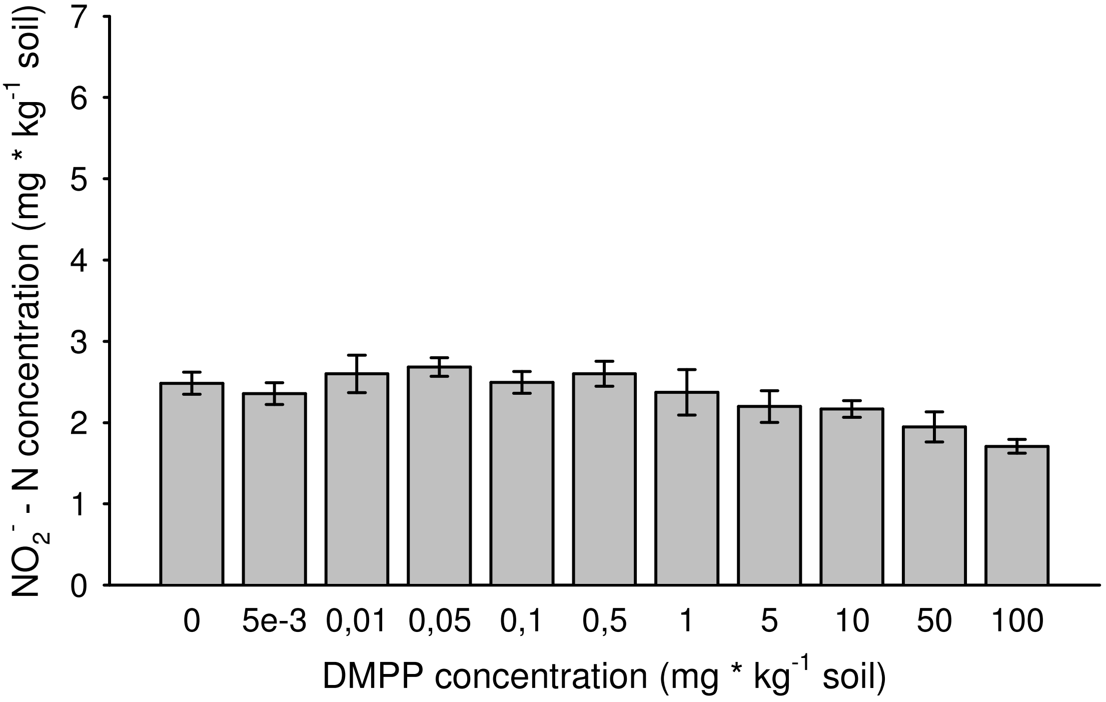

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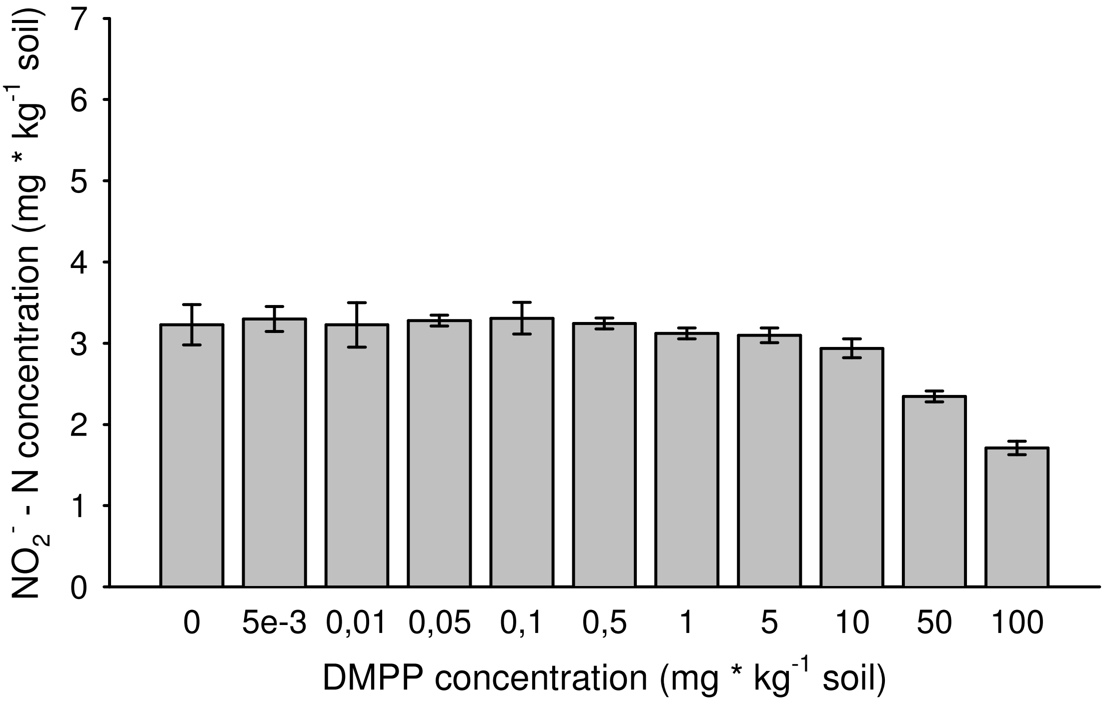

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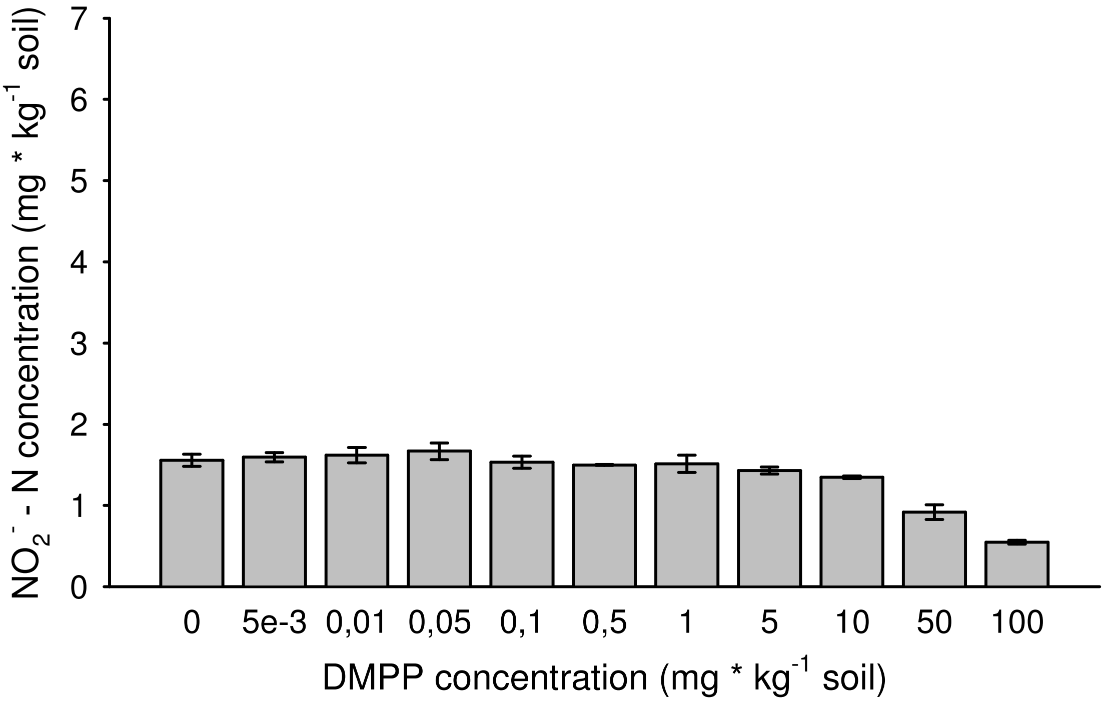

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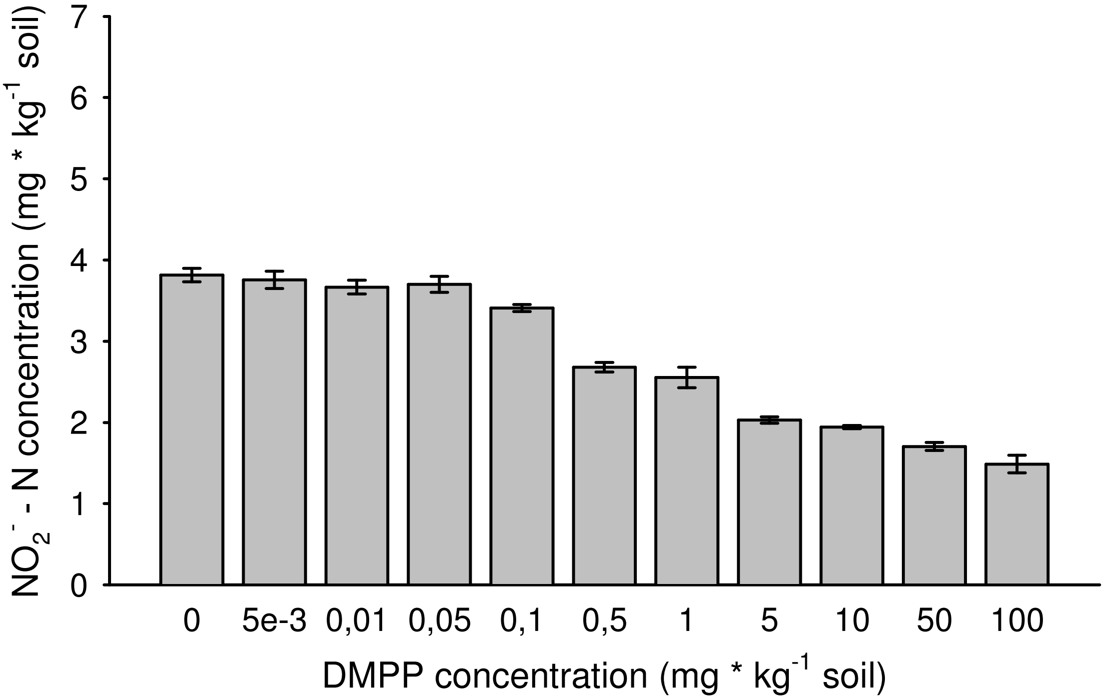

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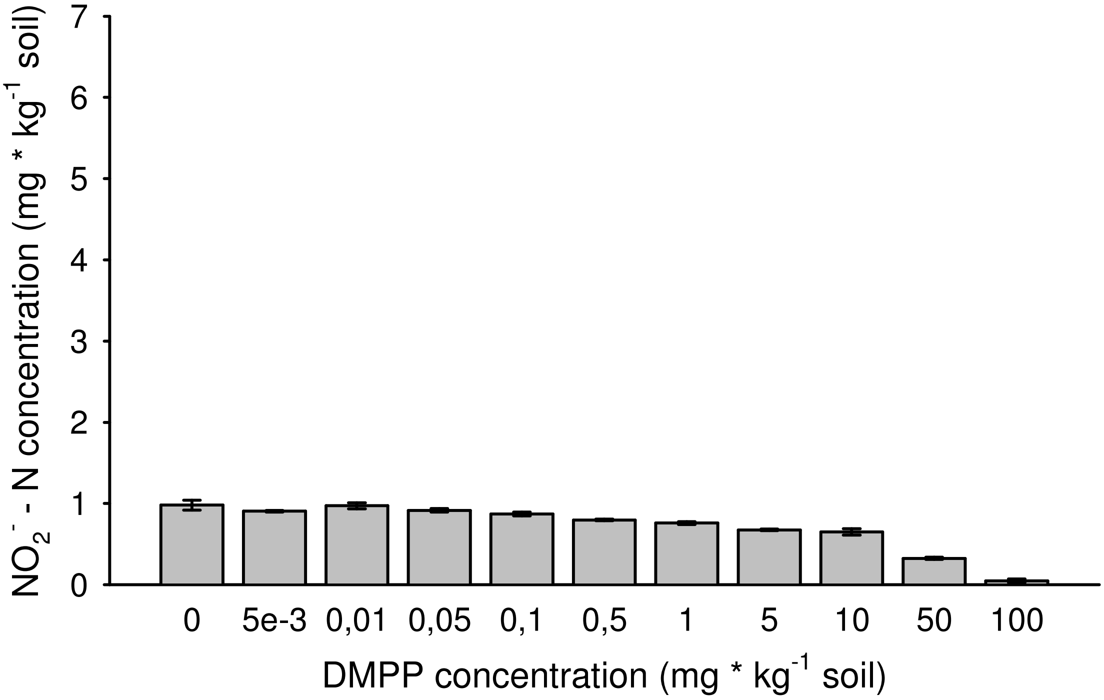

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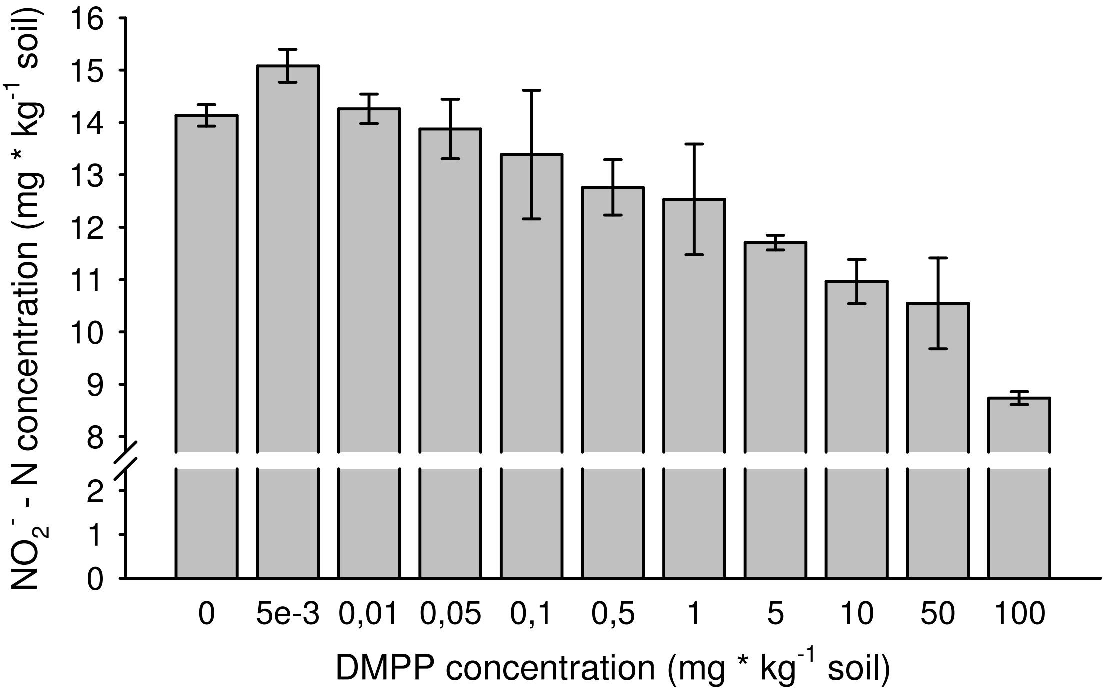

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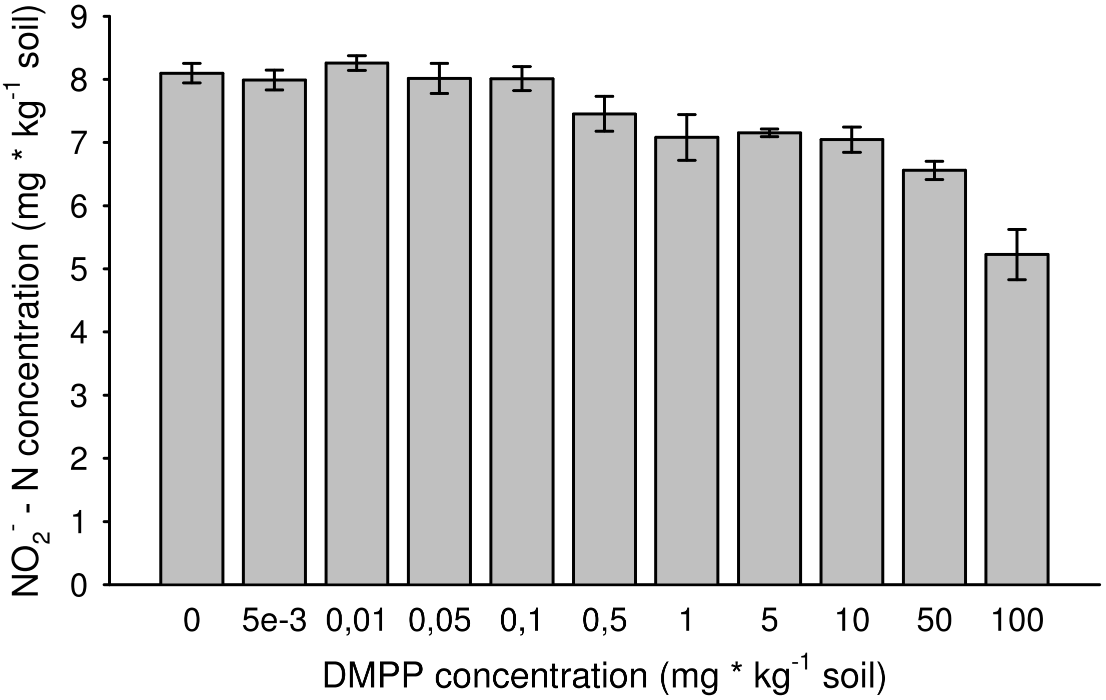

Fig. A22 Nitrite formation in short - term incubation studies in soil 22 with different concentrations of DMPP. Error bars represent standard deviations.

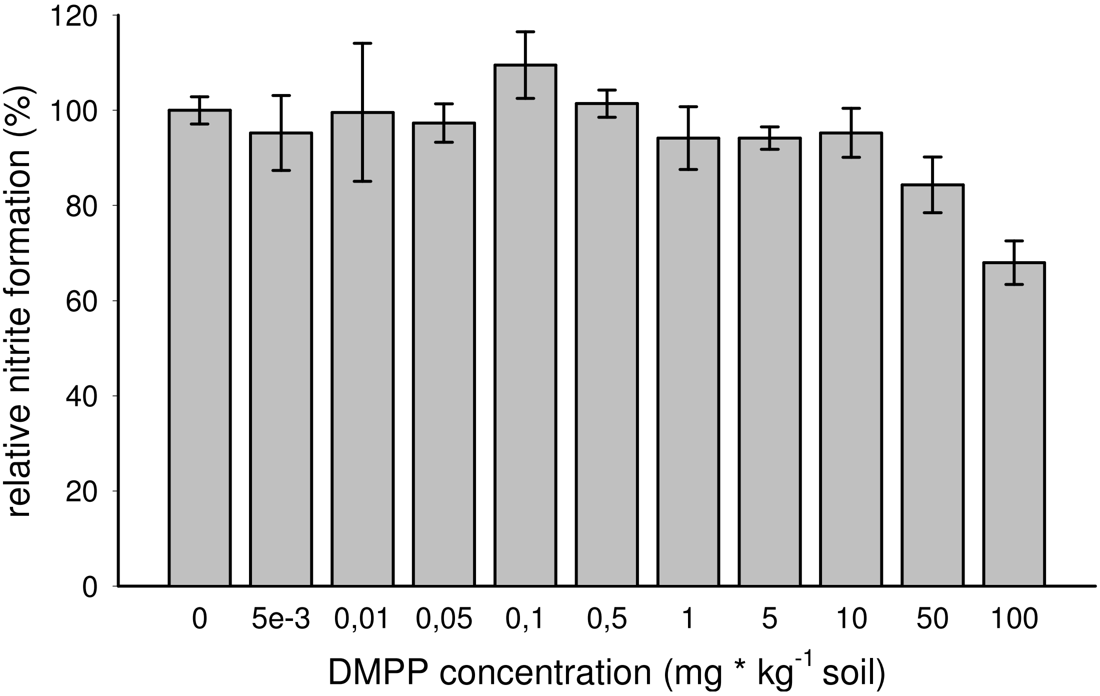

Fig. A23 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 1. Error bars represent standard deviations.

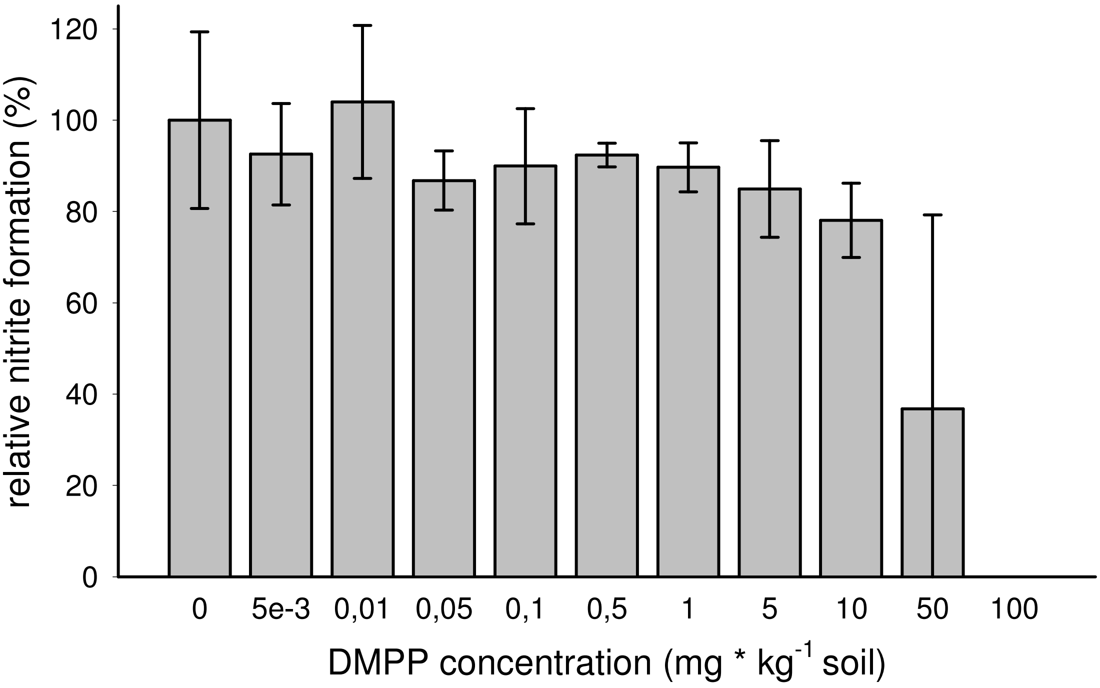

Fig. A24 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 2. Error bars represent standard deviations.

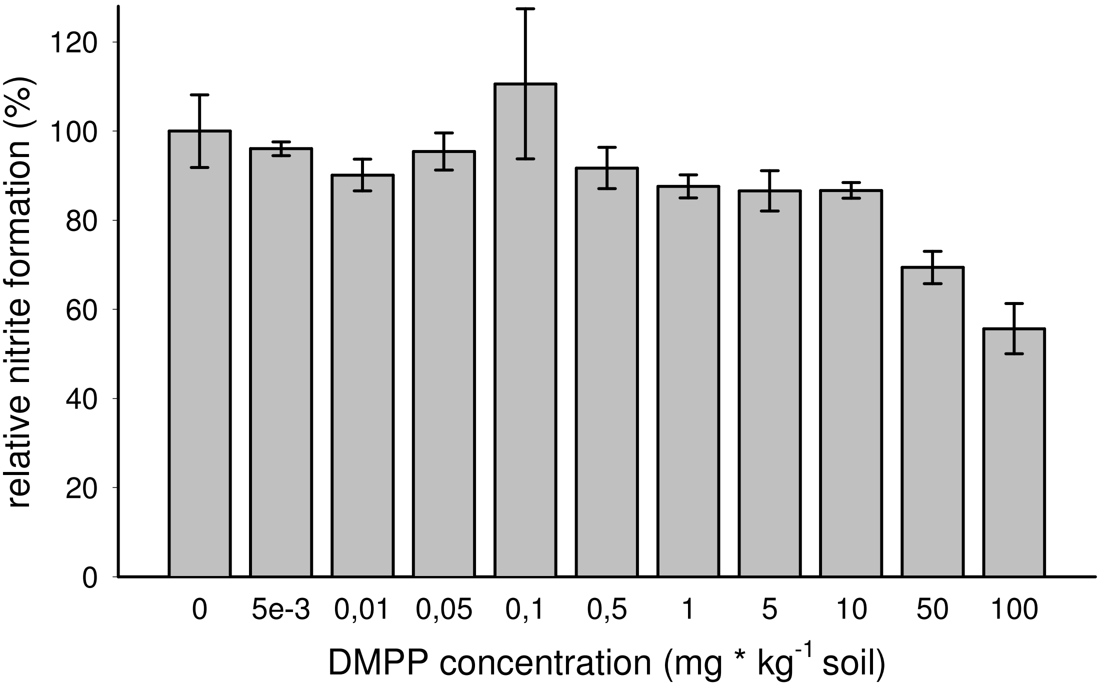

Fig. A25 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 3. Error bars represent standard deviations.

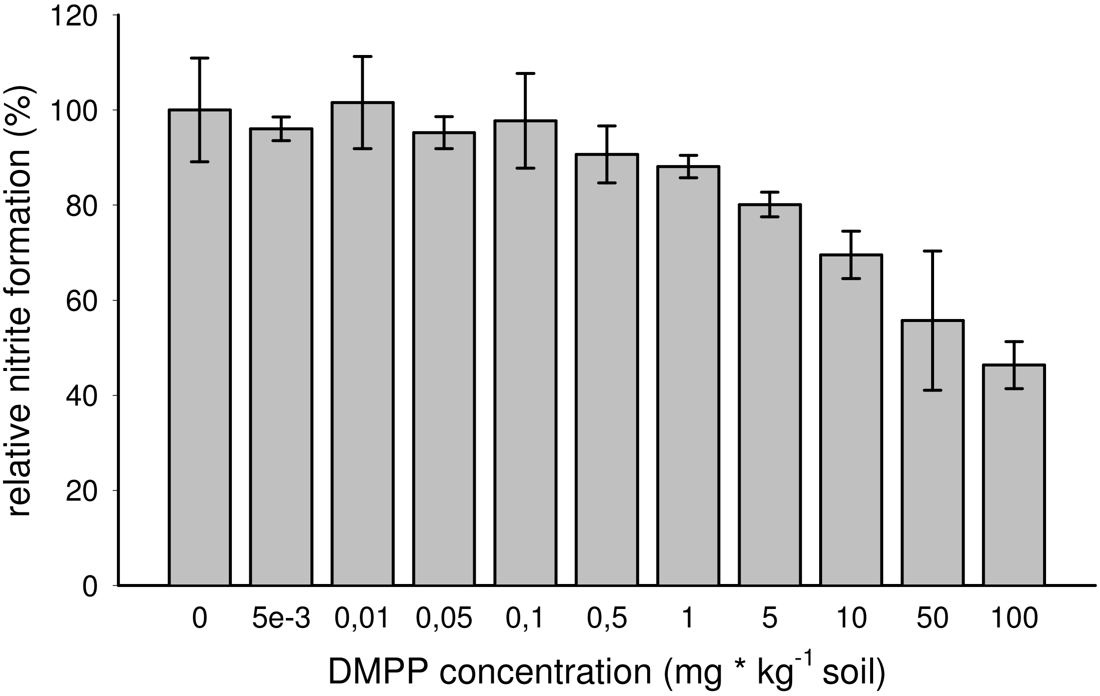

Fig. A26 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 4. Error bars represent standard deviations.

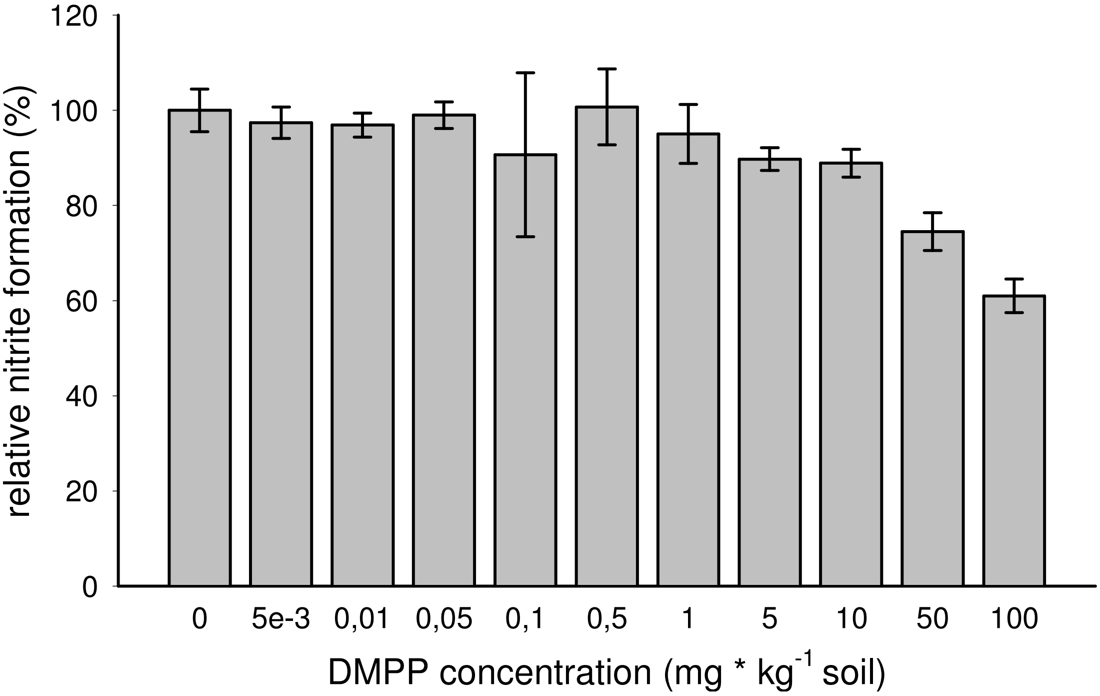

Fig. A27 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 5. Error bars represent standard deviations.

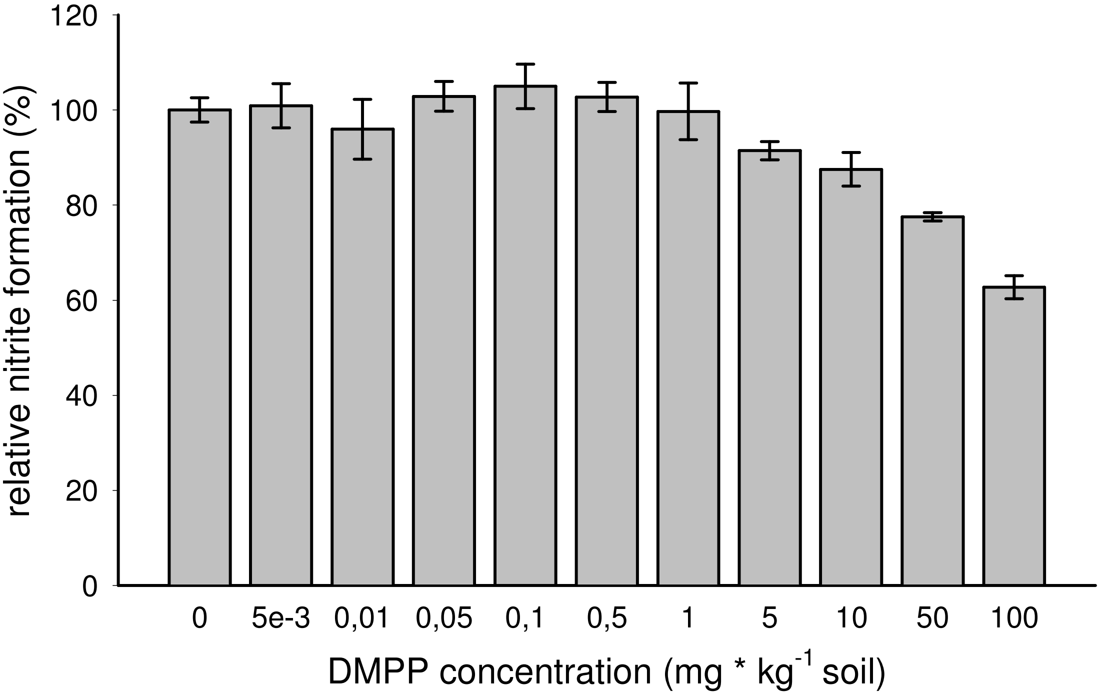

Fig. A28 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 6. Error bars represent standard deviations.

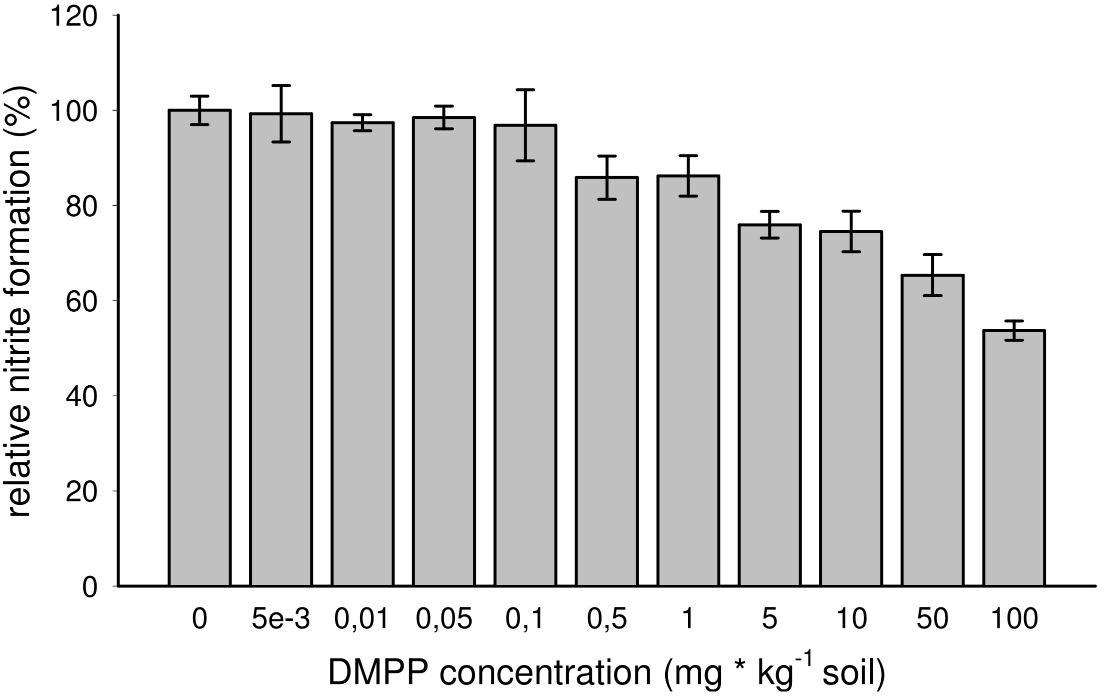

Fig. A29 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 7. Error bars represent standard deviations.

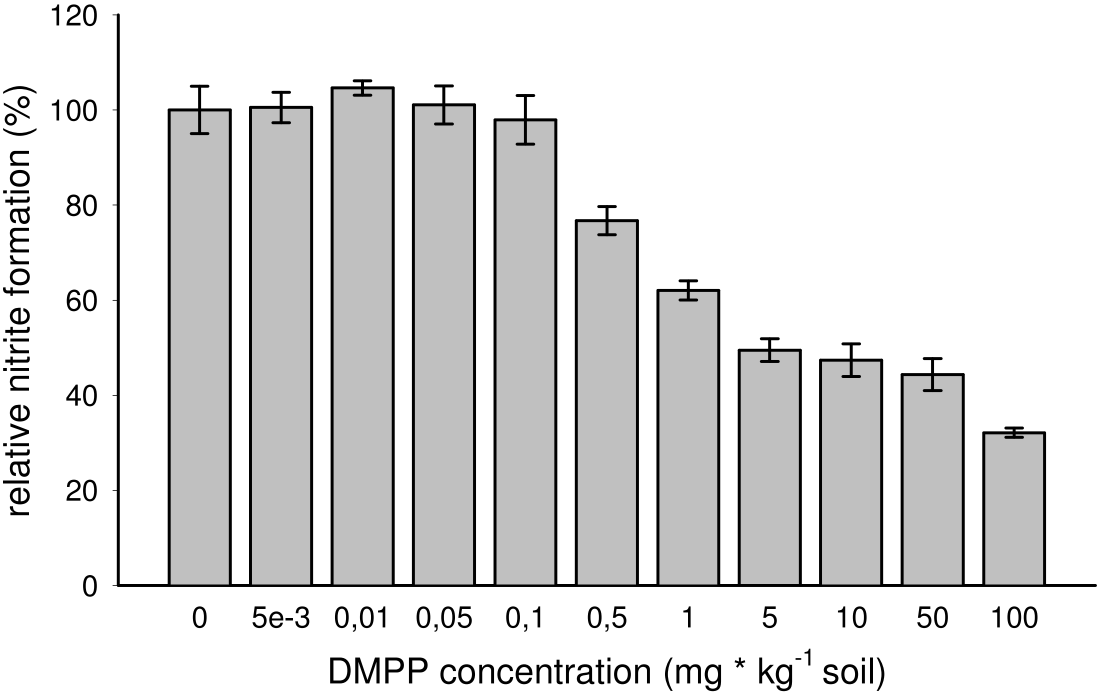

Fig. A30 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 8. Error bars represent standard deviations.

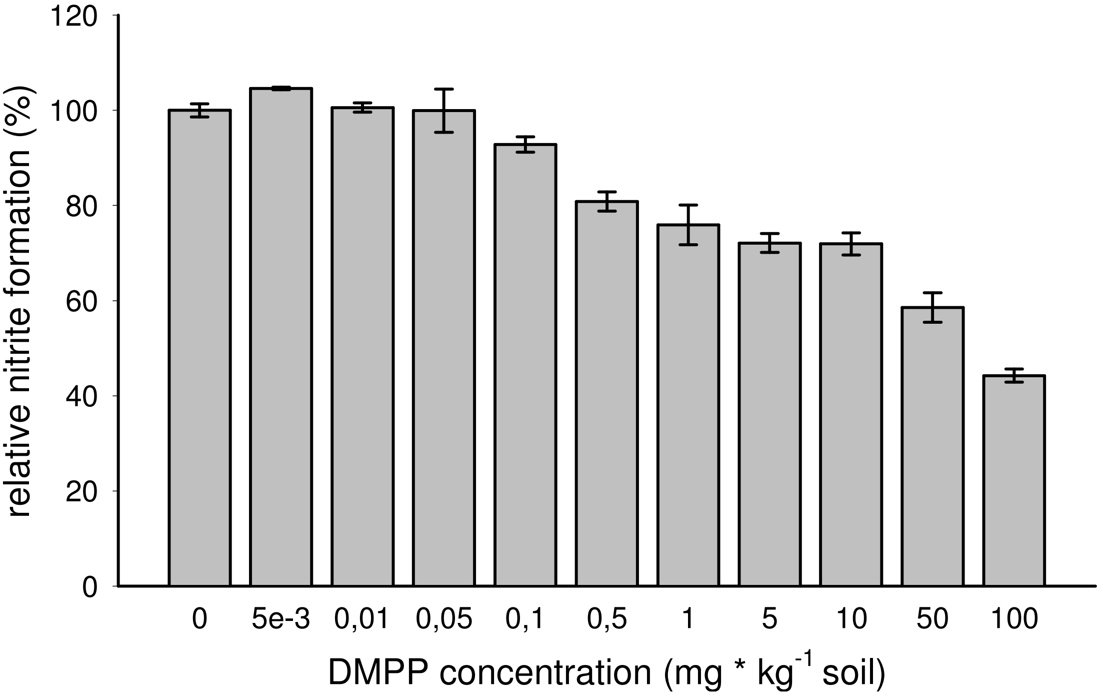

Fig. A31 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 9. Error bars represent standard deviations.

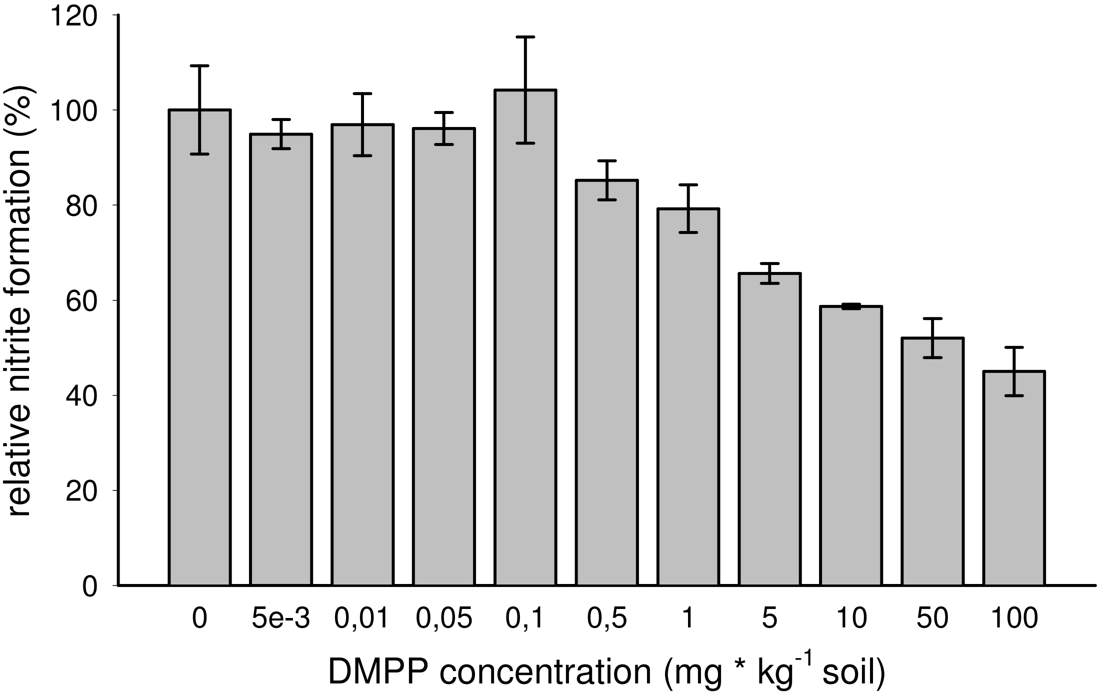

Fig. A32 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 10. Error bars represent standard deviations.

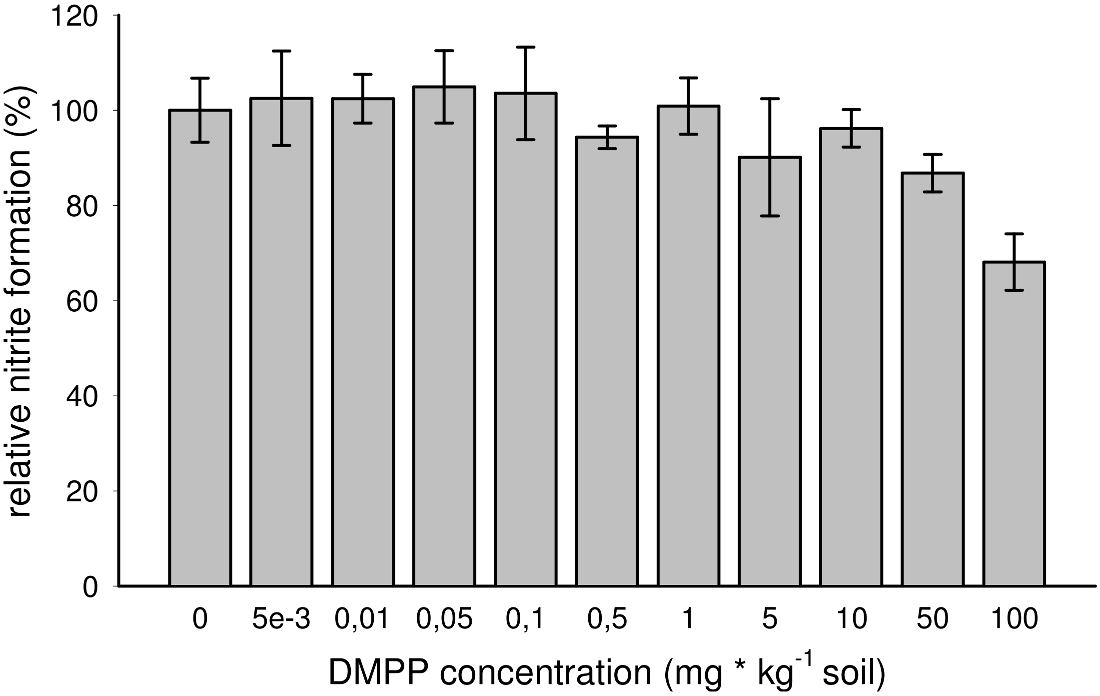

Fig. A33 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 11. Error bars represent standard deviations.

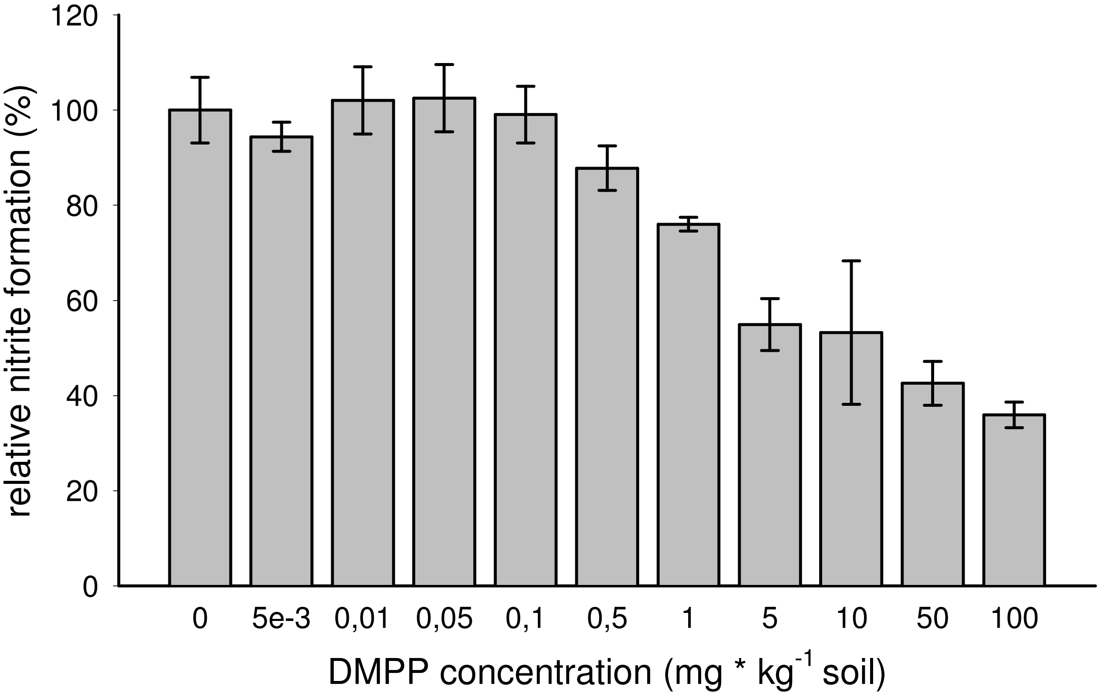

Fig. A34 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 12. Error bars represent standard deviations.

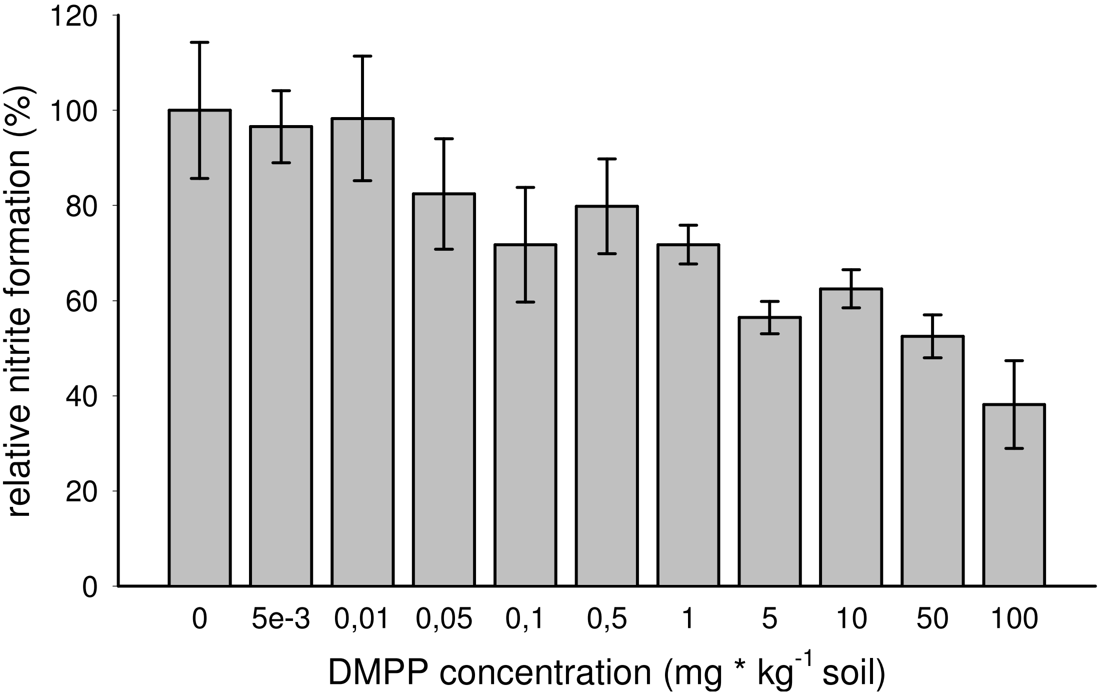

Fig. A35 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 13. Error bars represent standard deviations.

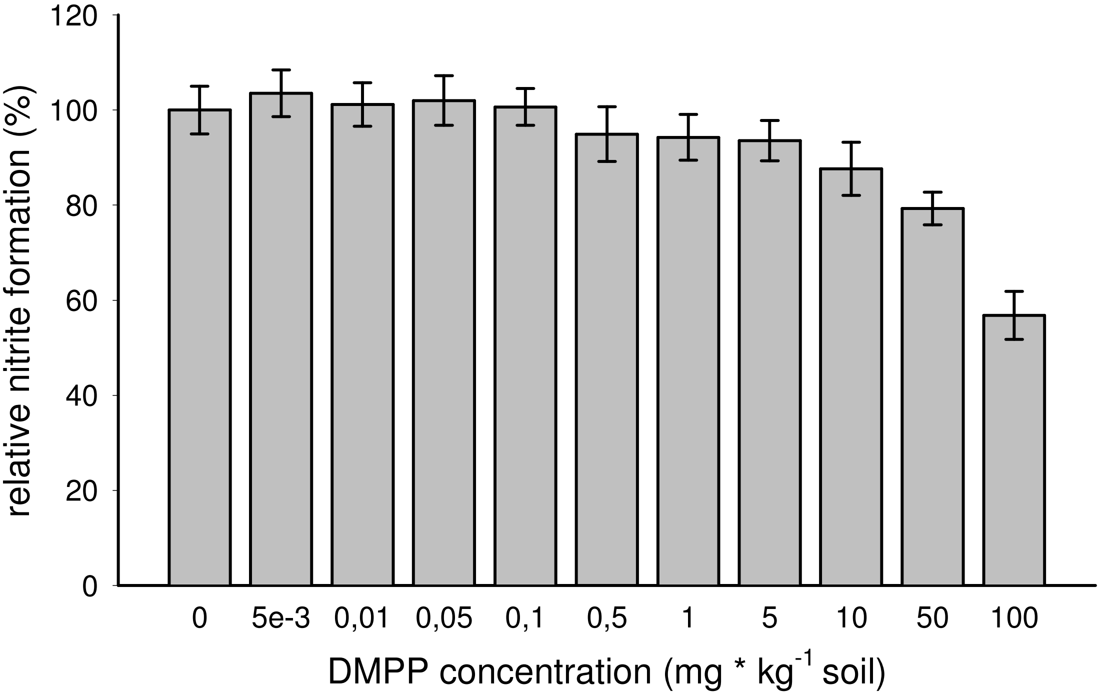

Fig. A36 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 14. Error bars represent standard deviations.

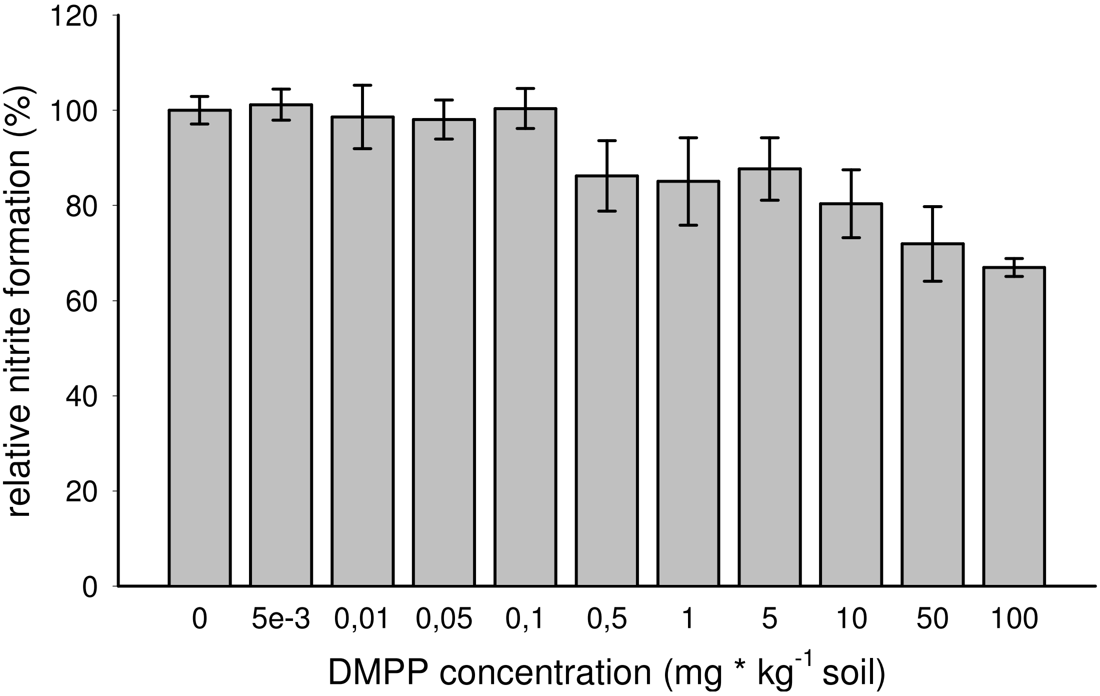

Fig. A37 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 15. Error bars represent standard deviations.

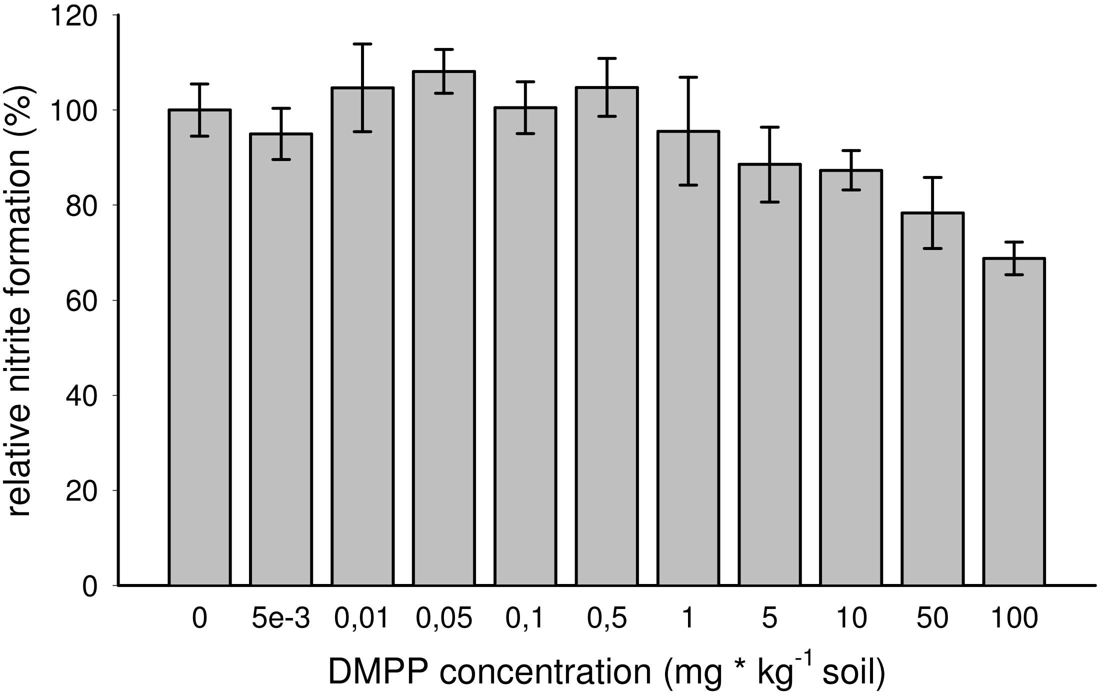

Fig. A38 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 16. Error bars represent standard deviations.

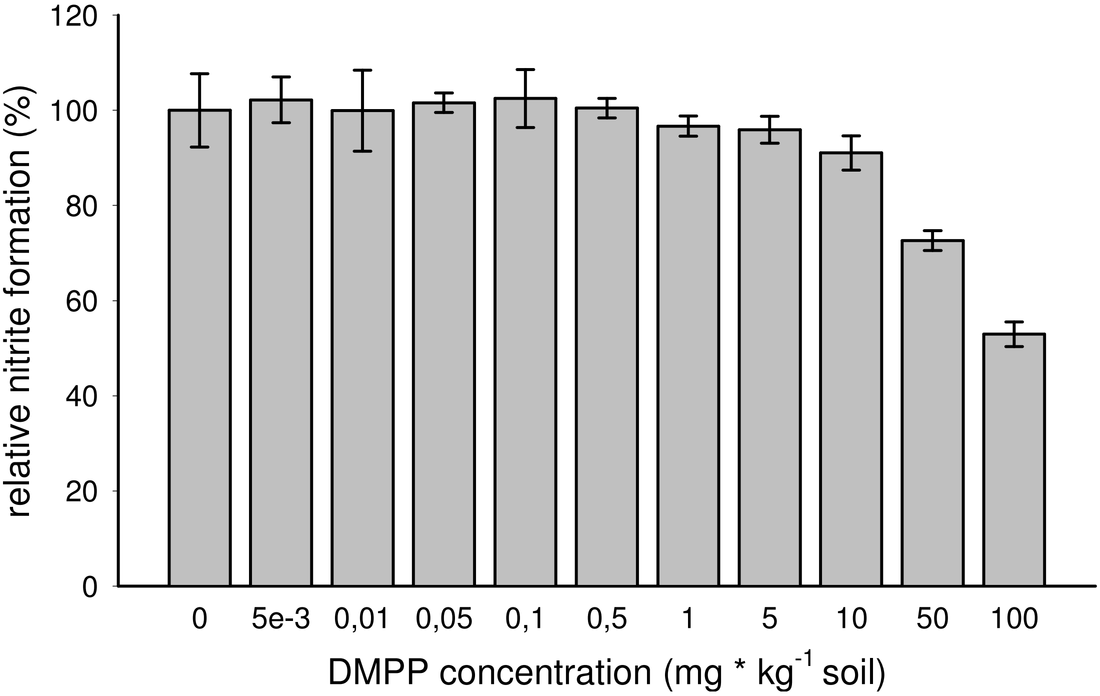

Fig. A39 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 17. Error bars represent standard deviations.

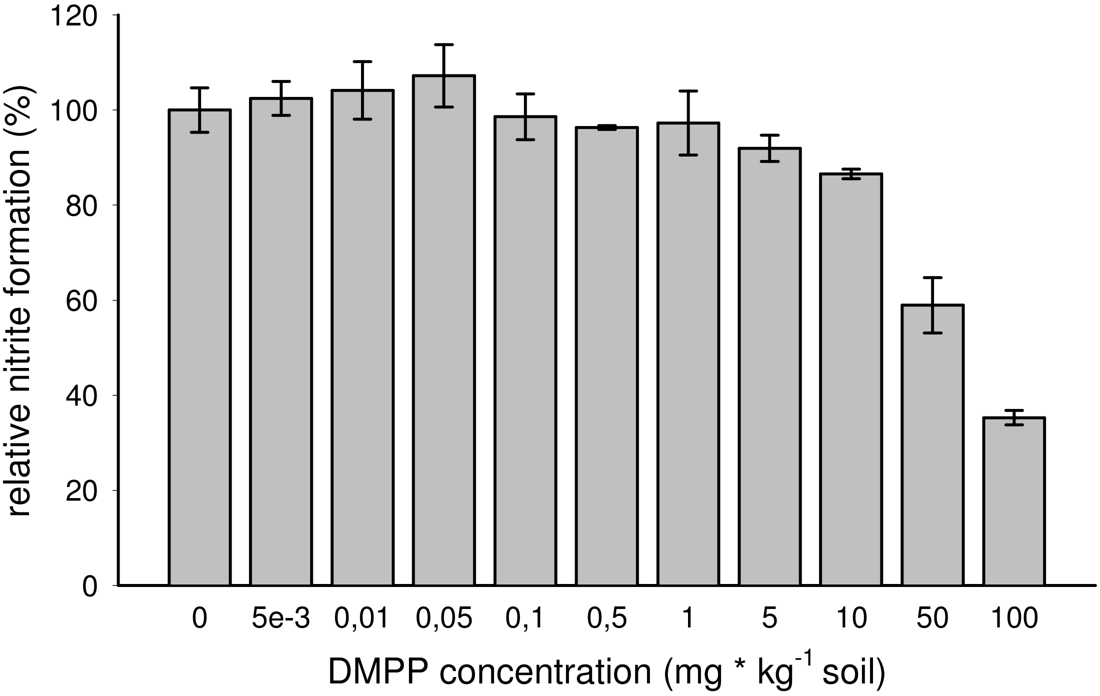

Fig. A40 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 18. Error bars represent standard deviations.

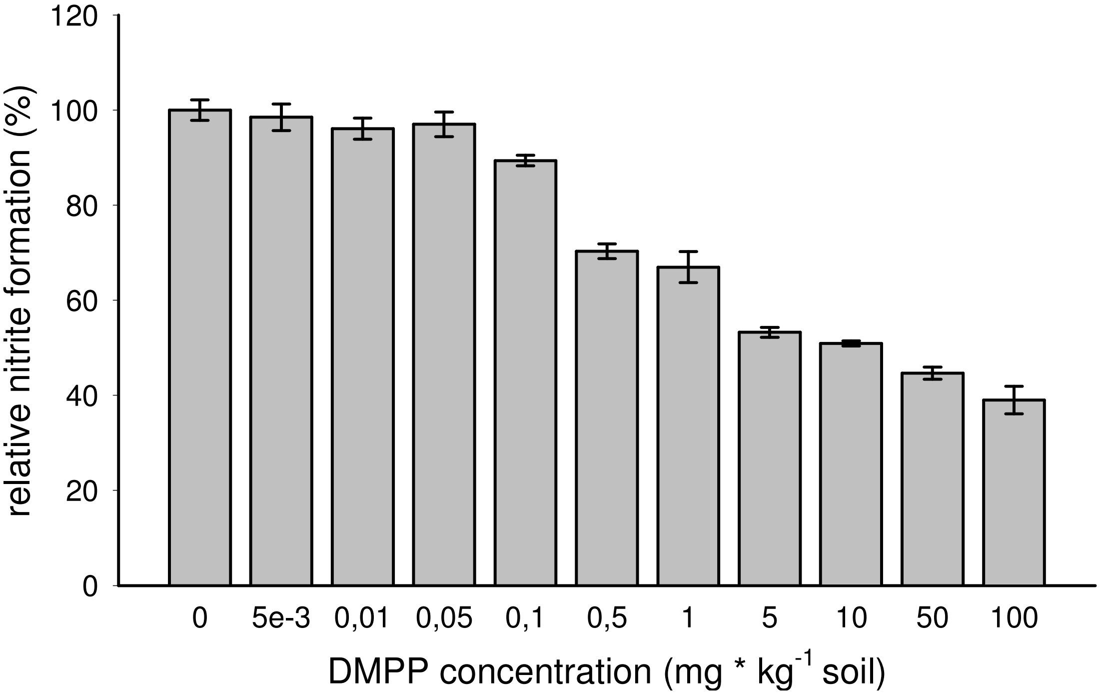

Fig. A41 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 19. Error bars represent standard deviations.

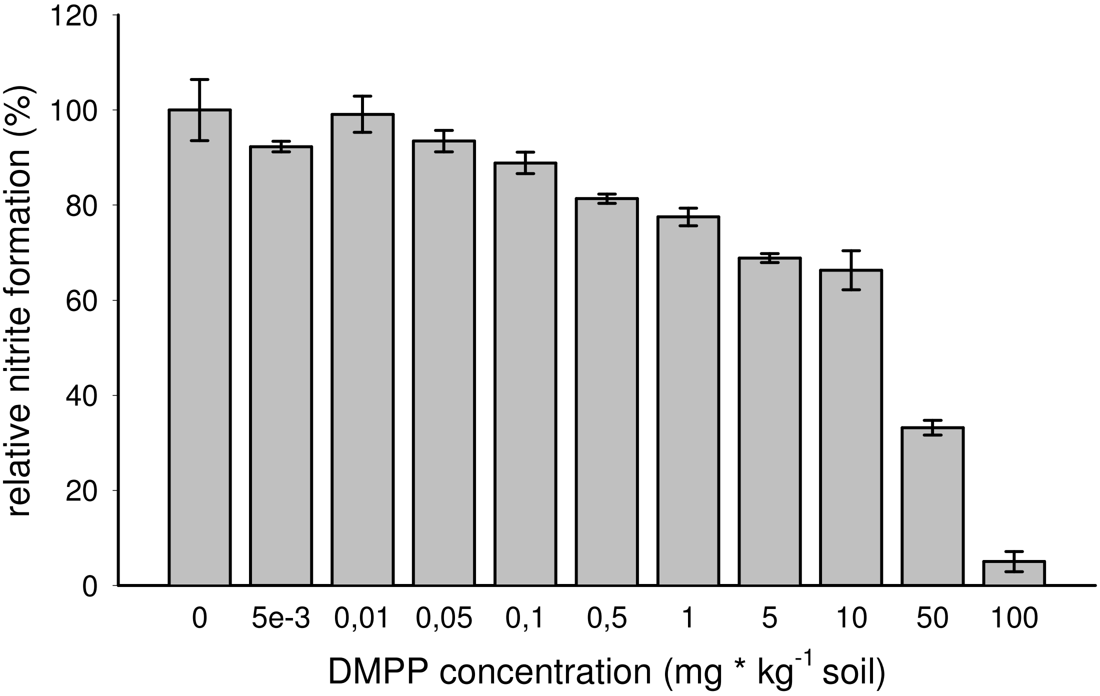

Fig. A42 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 20. Error bars represent standard deviations.

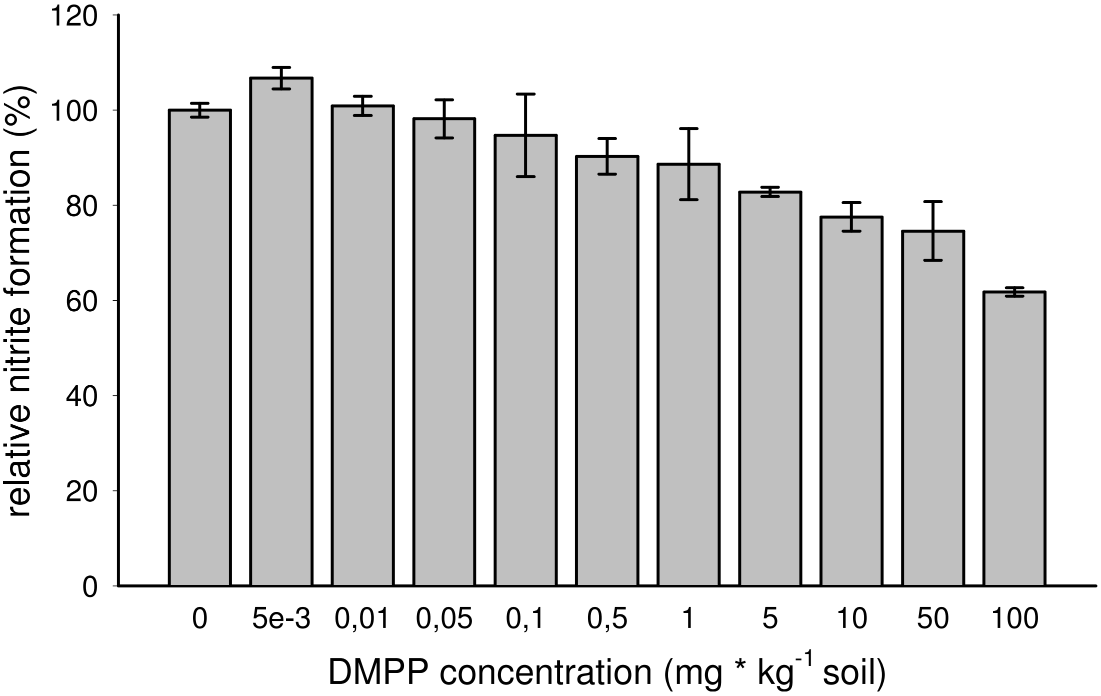

Fig. A43 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 21. Error bars represent standard deviations.

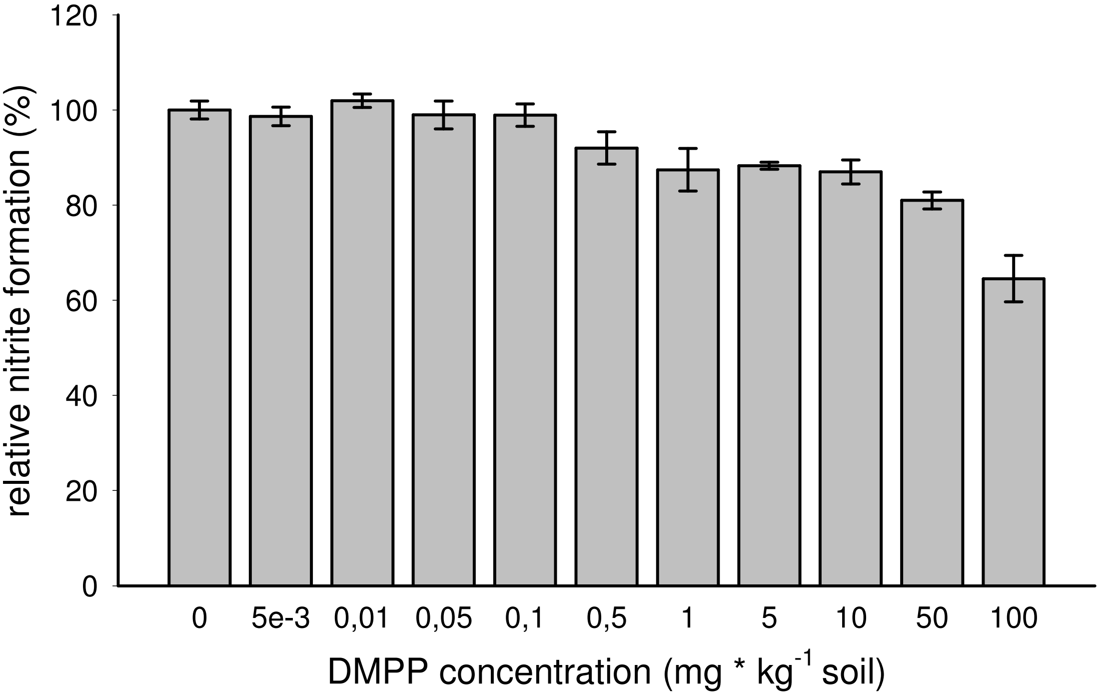

Fig. A44 Relative nitrite formation with DMPP concentration in short - term incubation experiments in soil 22. Error bars represent standard deviations.

Table A1: Nitrite formation of the short-term incubation study in all 22 investigated soils (NO_2^- - N in mg * kg^{-1} soil)

Soil No.	**Soil 1**		**Soil 2**		**Soil 3**		**Soil 4**		**Soil 5**		**Soil 6**	
DMPP (mg * kg^{-1} soil)	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**
0,000	5,326	0,151	0,123	0,024	0,692	0,056	0,219	0,024	0,624	0,037	1,246	0,032
0,005	5,073	0,421	0,114	0,014	0,665	0,011	0,210	0,005	0,601	0,062	1,257	0,058
0,010	5,303	0,772	0,128	0,021	0,624	0,025	0,222	0,021	0,568	0,049	1,196	0,078
0,050	5,184	0,215	0,107	0,008	0,660	0,029	0,209	0,007	0,561	0,023	1,282	0,039
0,100	5,831	0,373	0,110	0,015	0,765	0,116	0,214	0,022	0,561	0,013	1,308	0,058
0,500	5,401	0,154	0,113	0,003	0,635	0,032	0,199	0,013	0,550	0,023	1,280	0,038
1,000	5,016	0,351	0,110	0,007	0,606	0,018	0,193	0,005	0,541	0,021	1,243	0,074
5,000	5,014	0,125	0,104	0,013	0,599	0,031	0,175	0,006	0,490	0,023	1,139	0,024
10,000	5,074	0,273	0,096	0,010	0,600	0,012	0,152	0,011	0,507	0,016	1,091	0,044
50,000	4,491	0,311	0,045	0,052	0,480	0,025	0,122	0,032	0,446	0,023	0,966	0,011
100,000	3,622	0,242	0,000	0,000	0,385	0,039	0,102	0,011	0,409	0,019	0,782	0,030

Table A1(continued): Nitrite formation of the short-term incubation study in all 22 investigated soils (NO_2^- - N in mg * kg^{-1} soil)

Soil No.	**Soil 7**		**Soil 8**		**Soil 9**		**Soil 10**		**Soil 11**		**Soil 12**	
DMPP (mg * kg^{-1} soil)	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**
0,000	5,948	0,179	1,479	0,073	1,418	0,020	0,386	0,036	2,871	0,193	0,285	0,020
0,005	5,903	0,352	1,487	0,048	1,483	0,004	0,366	0,012	2,944	0,285	0,269	0,009
0,010	5,792	0,099	1,548	0,022	1,427	0,014	0,374	0,025	2,941	0,147	0,291	0,020
0,050	5,858	0,143	1,495	0,059	1,417	0,064	0,371	0,013	3,013	0,218	0,292	0,020
0,100	5,760	0,445	1,449	0,076	1,316	0,023	0,402	0,043	2,973	0,279	0,283	0,017
0,500	5,107	0,270	1,135	0,044	1,146	0,029	0,329	0,016	2,708	0,069	0,250	0,013
1,000	5,127	0,252	0,918	0,030	1,077	0,059	0,306	0,019	2,896	0,170	0,217	0,004
5,000	4,517	0,166	0,732	0,035	1,022	0,028	0,253	0,008	2,587	0,353	0,157	0,016
10,000	4,433	0,254	0,701	0,051	1,020	0,033	0,227	0,002	2,762	0,113	0,152	0,043
50,000	3,888	0,256	0,656	0,050	0,830	0,044	0,201	0,016	2,492	0,113	0,122	0,013
100,000	3,195	0,121	0,475	0,014	0,627	0,020	0,174	0,020	1,956	0,170	0,103	0,008

Table A1(continued): Nitrite formation of the short-term incubation study in all 22 investigated soils (NO_2^- - N in mg * kg^{-1} soil)

Soil No.	**Soil 13**		**Soil 14**		**Soil 15**		**Soil 16**		**Soil 17**		**Soil 18**	
DMPP (mg * kg^{-1} soil)	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**
0,000	1,389	0,199	1,335	0,067	2,492	0,072	2,483	0,136	3,228	0,249	1,557	0,073
0,005	1,342	0,105	1,382	0,066	2,521	0,081	2,358	0,134	3,299	0,155	1,595	0,056
0,010	1,366	0,182	1,351	0,061	2,456	0,166	2,599	0,229	3,226	0,275	1,621	0,094
0,050	1,145	0,161	1,362	0,070	2,443	0,102	2,684	0,115	3,279	0,066	1,669	0,102
0,100	0,997	0,167	1,344	0,051	2,501	0,105	2,494	0,135	3,308	0,196	1,535	0,075
0,500	1,109	0,138	1,268	0,077	2,149	0,184	2,601	0,152	3,243	0,066	1,500	0,006
1,000	0,997	0,056	1,258	0,065	2,119	0,228	2,372	0,281	3,121	0,068	1,514	0,105
5,000	0,784	0,048	1,249	0,057	2,185	0,164	2,198	0,196	3,097	0,091	1,432	0,043
10,000	0,868	0,056	1,170	0,075	2,003	0,178	2,167	0,103	2,938	0,116	1,347	0,016
50,000	0,729	0,062	1,059	0,046	1,792	0,195	1,946	0,186	2,345	0,066	0,918	0,091
100,000	0,530	0,128	0,758	0,067	1,669	0,047	1,707	0,085	1,709	0,083	0,550	0,024

Table A1(continued): Nitrite formation of the short-term incubation study in all 22 investigated soils (NO_2^- - N in mg * kg^{-1} soil)

Soil No.	Soil 19		Soil 20		Soil 21		Soil 22	
DMPP (mg * kg^{-1} soil)	NO_2^- - N	standard deviation	NO_2^- - N	standard deviation	NO_2^- - N	standard deviation	NO_2^- - N	standard deviation
0,000	3,813	0,083	0,980	0,063	14,134	0,206	8,098	0,155
0,005	3,756	0,106	0,905	0,011	15,083	0,317	7,989	0,158
0,010	3,665	0,085	0,972	0,037	14,262	0,283	8,258	0,114
0,050	3,700	0,099	0,916	0,022	13,876	0,570	8,014	0,236
0,100	3,409	0,043	0,871	0,022	13,388	1,228	8,012	0,190
0,500	2,681	0,059	0,798	0,010	12,760	0,528	7,453	0,276
1,000	2,554	0,126	0,760	0,018	12,531	1,056	7,080	0,363
5,000	2,031	0,040	0,675	0,009	11,705	0,139	7,152	0,060
10,000	1,943	0,020	0,650	0,040	10,963	0,422	7,045	0,202
50,000	1,703	0,050	0,325	0,015	10,545	0,870	6,559	0,145
100,000	1,488	0,110	0,050	0,021	8,736	0,124	5,226	0,396

Table A2: Relative nitrite formation of the short-term incubation study in all 22 investigated soils (NO_2^- - N in %)

Soil No.	**Soil 1**		**Soil 2**		**Soil 3**		**Soil 4**		**Soil 5**		**Soil 6**	
DMPP (mg * kg^{-1} soil)	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**
0,000	100,000	2,844	100,000	19,332	100,000	8,128	100,000	10,908	100,000	4,469	100,000	2,568
0,005	95,245	7,899	92,568	11,113	96,049	1,543	96,050	2,467	97,403	3,289	100,890	4,652
0,010	99,557	14,488	104,000	16,772	90,146	3,562	101,552	9,696	96,905	2,514	95,956	6,295
0,050	97,333	4,045	86,757	6,482	95,415	4,141	95,219	3,389	99,002	2,785	102,858	3,136
0,100	109,481	6,999	89,934	12,622	110,612	16,807	97,746	9,968	90,671	17,241	104,962	4,691
0,500	101,398	2,888	92,346	2,613	91,714	4,631	90,668	5,985	100,697	7,974	102,732	3,036
1,000	94,171	6,590	89,660	5,383	87,599	2,582	88,102	2,346	95,022	6,187	99,713	5,977
5,000	94,139	2,352	84,909	10,583	86,612	4,536	80,118	2,568	89,739	2,406	91,430	1,938
10,000	95,268	5,131	78,061	8,148	86,705	1,760	69,521	4,964	88,884	2,939	87,516	3,515
50,000	84,319	5,847	36,764	42,453	69,407	3,624	55,737	14,633	74,533	3,958	77,542	0,848
100,000	67,998	4,547	0,000	0,000	55,673	5,622	46,354	4,920	60,999	3,546	62,731	2,418

Table A2 (continued): Relative nitrite formation of the short-term incubation study in all 22 investigated soils (NO_2^- - N in %)

Soil No.	**Soil 7**		**Soil 8**		**Soil 9**		**Soil 10**		**Soil 11**		**Soil 12**	
DMPP (mg * kg^{-1} soil)	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**
0,000	100,000	3,009	100,000	4,965	100,000	1,379	100,000	9,301	100,000	6,719	100,000	6,905
0,005	99,252	5,921	100,525	3,225	104,595	0,272	94,906	3,067	102,534	9,915	94,389	3,088
0,010	97,389	1,668	104,636	1,512	100,588	0,976	96,925	6,521	102,430	5,114	102,035	7,029
0,050	98,496	2,406	101,066	3,986	99,927	4,515	96,104	3,392	104,941	7,580	102,507	7,053
0,100	96,851	7,483	97,949	5,124	92,814	1,633	104,195	11,172	103,550	9,731	99,054	5,943
0,500	85,859	4,542	76,731	2,980	80,833	2,042	85,208	4,093	94,339	2,386	87,786	4,679
1,000	86,204	4,239	62,038	2,028	75,952	4,170	79,247	5,032	100,887	5,931	76,024	1,471
5,000	75,939	2,797	49,488	2,389	72,098	1,983	65,623	2,082	90,130	12,311	54,924	5,470
10,000	74,533	4,271	47,403	3,426	71,953	2,316	58,710	0,468	96,200	3,934	53,259	15,046
50,000	65,362	4,311	44,346	3,357	58,554	3,106	52,025	4,100	86,799	3,933	42,590	4,621
100,000	53,711	2,032	32,129	0,970	44,245	1,378	45,019	5,077	68,131	5,932	35,943	2,723

Table A2 (continued): Relative nitrite formation of the short-term incubation study in all 22 investigated soils (NO_2^- - N in %)

Soil No.	**Soil 13**		**Soil 14**		**Soil 15**		**Soil 16**		**Soil 17**		**Soil 18**	
DMPP (mg * kg^{-1} soil)	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**
0,000	100,000	14,294	100,000	5,001	100,000	2,890	100,000	5,484	100,000	7,715	100,000	4,689
0,005	96,556	7,587	103,503	4,915	101,184	3,265	94,964	5,416	102,196	4,797	102,430	3,565
0,010	98,302	13,083	101,166	4,565	98,581	6,678	104,675	9,216	99,926	8,511	104,092	6,055
0,050	82,445	11,621	102,001	5,242	98,060	4,094	108,113	4,629	101,589	2,033	107,200	6,564
0,100	71,742	12,040	100,644	3,851	100,369	4,204	100,475	5,450	102,480	6,076	98,579	4,812
0,500	79,835	9,962	94,941	5,755	86,228	7,392	104,747	6,104	100,458	2,053	96,310	0,381
1,000	71,776	4,052	94,254	4,836	85,054	9,168	95,531	11,314	96,676	2,104	97,244	6,736
5,000	56,438	3,426	93,538	4,254	87,689	6,568	88,533	7,883	95,934	2,809	91,949	2,763
10,000	62,485	4,020	87,654	5,586	80,369	7,146	87,304	4,144	91,025	3,601	86,536	1,005
50,000	52,500	4,488	79,289	3,410	71,935	7,833	78,363	7,475	72,630	2,054	58,933	5,822
100,000	38,174	9,220	56,784	5,044	66,999	1,872	68,768	3,434	52,957	2,585	35,318	1,537

Table A2 (continued): Relative nitrite formation of the short-term incubation study in all 22 investigated soils (NO_2^- - N in %)

Soil No.	**Soil 19**		**Soil 20**		**Soil 21**		**Soil 22**	
DMPP (mg * kg^{-1} soil)	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**	**NO_2^- - N**	**standard deviation**
0,000	100,000	2,172	100,000	6,405	100,000	1,454	100,000	1,908
0,005	98,489	2,771	92,283	1,125	106,717	2,246	98,648	1,949
0,010	96,102	2,224	99,094	3,800	100,903	1,999	101,964	1,412
0,050	97,034	2,587	93,467	2,257	98,173	4,030	98,960	2,918
0,100	89,396	1,122	88,854	2,255	94,722	8,690	98,931	2,352
0,500	70,318	1,543	81,344	1,000	90,276	3,737	92,025	3,413
1,000	66,983	3,294	77,506	1,856	88,657	7,470	87,421	4,480
5,000	53,256	1,061	68,860	0,962	82,813	0,984	88,313	0,737
10,000	50,956	0,533	66,287	4,097	77,563	2,984	86,987	2,492
50,000	44,662	1,302	33,197	1,560	74,606	6,156	80,990	1,790
100,000	39,025	2,880	5,052	2,140	61,807	0,878	64,534	4,887

Table A3: NH_4^+ - N concentration in long tern incubation experiments (mg * kg^{-1} soil)

incubation time (days)		0		4		7		11		14		18		25		32	
soil	treatement	NH_4^+	standard deviation	NH_4^+	standard deviation	NH_4^+	standard deviation	NH_4^+	standard deviation	NH_4^+	standard deviation	NH_4^+	standard deviation	NH_4^+	standard deviation	NH_4^+	standard deviation
silty loam	1	1,854	0,186	0,396	0,137	0,719	0,180	0,677	0,118	0,351	0,046	0,492	0,115	0,176	0,194	0,193	0,386
	2	97,719	3,464	51,878	2,383	72,599	1,483	87,677	2,379	90,372	0,099	94,451	0,460	94,277	0,307	95,533	0,260
	3	99,862	0,495	42,004	2,210	53,418	0,678	69,409	2,709	70,232	0,593	79,974	0,386	84,316	0,358	90,690	0,670
	4	100,290	1,161	41,209	1,901	54,186	0,369	68,922	1,515	69,903	0,575	79,204	1,275	82,967	1,773	89,415	2,410
	5	98,897	1,416	40,706	0,842	53,019	1,305	67,671	1,133	68,030	0,262	77,234	0,648	80,598	0,963	86,136	3,542
	6	100,398	0,214	41,761	1,470	52,711	0,783	68,550	1,696	69,975	0,893	79,115	1,025	81,672	2,231	90,679	0,780
	8	102,647	3,075	13,209	1,767	9,008	1,425	30,905	2,919	19,666	3,064	34,109	3,352	35,896	2,087	39,098	5,255
	9	98,255	1,763	27,824	2,309	32,149	2,671	44,450	2,373	44,984	0,240	57,411	2,898	62,957	2,503	70,075	1,127
loamy sand	1	2,092	0,458													0,920	0,044
	2	99,207	1,476	44,808	3,669	3,255	0,126	1,825	0,094	1,413	0,177	1,523	0,169	0,176	0,139	1,079	0,078
	4	99,808	1,323	82,018	6,398	65,004	1,135	57,500	1,394	37,175	1,239	1,890	0,366	0,219	0,135	1,204	0,087
	5	102,010	0,826	93,565	4,641	64,603	3,421	50,642	0,844	35,611	1,710	1,388	0,090	0,149	0,127	1,306	0,068
	6	101,710	0,654	76,598	4,008	67,211	1,779	55,812	0,531	41,040	2,219	2,688	1,097	0,406	0,140	1,318	0,136
	7	99,607	1,831	79,388	4,537	75,527	0,725	62,353	1,351	49,782	2,825	14,523	2,519	1,807	1,219	1,295	0,141
	8	102,518	2,836	92,600	3,891	70,963	2,841	61,029	3,671	47,488	3,905	17,812	2,645	2,146	1,530	1,226	0,013
	9	100,609	0,383	82,321	2,902	55,854	4,406	24,863	6,379	4,104	2,187	1,482	0,110	0,159	0,134	1,045	0,064
	10	95,219	1,962	81,727	3,366	53,479	9,418	39,223	9,820	21,761	2,863	1,822	0,335	0,217	0,150	0,904	0,647

Table A4: NO_3^- - N concentration in long tern incubation experiments (mg * kg^{-1} soil)

incubation time (days)		0		4		7		11		14		18		25		32	
soil	treatement	NO_3^-	standard deviation	NO_3^-	standard deviation	NO_3^-	standard deviation	NO_3^-	standard deviation	NO_3^-	standard deviation	NO_3^-	standard deviation	NO_3^-	standard deviation	NO_3^-	standard deviation
silty loam	1	17,352	0,322	20,371	0,728	24,460	1,003	24,022	0,705	27,410	0,662	27,706	0,325	31,053	0,480	32,881	0,826
	2	17,949	0,538	75,624	2,049	99,718	2,909	109,551	5,786	126,287	2,218	128,561	0,574	130,292	4,418	133,982	0,824
	3	18,860	0,302	57,710	5,625	77,268	3,326	92,905	1,720	104,065	0,607	110,118	3,321	121,198	1,228	129,208	2,204
	4	18,364	0,239	61,856	4,964	78,343	4,102	91,890	5,914	105,165	0,647	111,702	0,816	119,826	3,363	125,857	2,929
	5	18,456	0,204	62,215	4,143	79,130	1,729	92,165	3,731	103,398	2,403	109,062	1,712	115,051	7,394	121,045	7,009
	6	18,718	0,477	62,918	1,159	78,269	2,352	90,776	6,033	104,030	1,947	110,919	0,746	116,044	4,126	128,806	1,843
	8	22,618	3,918	29,521	9,982	34,000	4,445	43,180	4,879	46,885	4,342	55,209	4,147	76,622	11,908	76,844	5,801
	9	17,875	0,075	47,377	2,664	56,792	2,673	67,615	0,475	76,587	1,010	75,956	9,670	96,826	3,130	106,352	2,776
loamy sand	1	10,767	0,175													34,871	1,125
	2	10,645	0,295	63,261	3,535	106,392	3,074	115,328	4,159	121,487	1,577	123,699	1,398	129,127	2,485	137,059	1,466
	4	10,871	0,165	28,103	1,688	33,072	2,722	48,668	1,148	59,746	0,639	79,466	0,965	121,843	6,370	124,920	6,142
	5	10,832	0,068	27,840	0,365	35,342	0,331	47,588	1,502	61,517	1,249	82,307	1,388	123,485	5,237	130,384	1,932
	6	10,984	0,567	27,802	0,343	33,799	0,407	46,979	0,478	55,861	3,693	74,887	1,130	123,866	1,855	129,695	2,126
	7	10,448	0,116	23,243	0,626	26,928	0,463	40,364	1,456	49,233	0,353	65,209	2,232	109,796	3,014	130,728	1,079
	8	16,500	5,485	26,382	5,407	27,687	5,705	48,754	3,659	53,212	3,478	68,420	1,878	108,493	5,206	123,454	4,319
	9	10,735	0,253	25,561	1,381	33,102	0,849	68,039	4,708	102,367	7,359	126,533	4,048	137,777	1,923	143,140	5,851
	10	16,547	2,289	25,998	3,081	37,663	4,727	67,760	9,698	87,719	11,066	107,568	4,927	133,872	6,988	139,806	5,932

Table A5: NO_2^- - N concentration in long tern incubation experiments (mg * kg^{-1} soil)

incubation time (days)		0		4		7		11		14		18		25		32	
soil	treatement	NO_2^-	standard deviation	NO_2^-	standard deviation	NO_2^-	standard deviation	NO_2^-	standard deviation	NO_2^-	standard deviation	NO_2^-	standard deviation	NO_2^-	standard deviation	NO_2^-	standard deviation
silty loam	1	0,086	0,021	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	2	0,498	0,007	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	3	0,429	0,035	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	4	0,377	0,015	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	5	0,324	0,019	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	6	0,215	0,010	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	8	---	---	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	9	0,323	0,044	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
loamy sand	1	0,088	0,007	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	2	0,203	0,012	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	4	0,113	0,003	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	5	0,093	0,009	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	6	0,083	0,011	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,025	0,043
	7	0,067	0,012	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,030	0,035
	8	0,160	0,010	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,038	0,046
	9	0,099	0,016	0,000	0,000	0,000	0,000	0,290	0,393	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	10	0,191	0,005	0,074	0,003	0,047	0,032	0,118	0,005	0,000	0,000	0,000	0,000	0,075	0,112	0,023	0,047