



RESEARCH ARTICLE

Efficacy of four nitrification inhibitors for the mitigation of nitrous oxide emissions under different soil temperature and moisture[#]

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Abstract

Background: Nitrification inhibitors (NI) may be used to inhibit nitrous oxide (N₂O) emissions from agricultural soils, but their efficacy could be strongly affected by soil temperature and moisture.

Aims: This study investigated how different NI behave in a sandy soil under variable temperature and moisture levels, which currently remains unclear.

Methods: Efficacies of four NI [dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP), nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) and active ingredients: 3.00–3.25% 1,2,4-triazole and 1.50–1.65% 3-methylpyrazole (PIADIN)] were investigated in an incubation experiment under two soil temperatures (15 and 25°C) and two moisture levels [60% and 80% water-holding capacity (WH)] for 60 days in a sandy soil. The soil received 0.5 g NH₄⁺-N kg⁻¹ soil and the inhibitors were applied at 5% of applied N. Total N₂O emissions were calculated based on gas samples collected throughout the incubation period.

Results: Increasing soil temperature from 15 to 25°C decreased N₂O emissions by 73–191% while increasing soil moisture from 60% to 80% WHC caused a 109–251% increase in the emissions. ENTEC followed by DCD were the least effective inhibitors to reduce N₂O emissions under all temperature and moisture combinations. Depending upon temperature and soil moisture, DMPP and PIADIN inhibited 85–100% of N₂O emissions. At 15°C, both DMPP and PIADIN almost completely inhibited N₂O emissions. At 25°C, the efficacy of PIADIN decreased to 88%, while that of DMPP ranged from 86% to 98%. Increased soil moisture content improved the efficacy of all the NI.

Conclusions: Overall, DMPP and PIADIN were more effective than DCD in a sandy soil under both the soil moisture levels and temperatures. DMPP was the most effective inhibitor, completely inhibiting N₂O emissions at 15–25°C and up to 80% WHC in this soil, and is therefore recommended for use in agricultural lands. The concentration of the NI contained in ENTEC was insufficient to affect nitrification at the both soil moisture levels and temperatures.

KEYWORDS

N₂O emissions, nitrification, nitrification inhibitors, soil moisture, soil temperature

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1 | INTRODUCTION

Nitrous oxide (N_2O) is a major greenhouse gas emitted to the atmosphere from soils, which has a 300 times stronger global warming potential than that of carbon dioxide (CO_2) (Carneiro et al., 2010). Moreover, N_2O also indirectly contributes to stratospheric ozone depletion. Worldwide emissions of N_2O increased by 9% from 1998 to 2010, with agricultural soils contributing more than 70% of the total global emissions (Carneiro et al., 2010). Nitrification of ammonium (NH_4^+) by microbes plays a key role in N_2O production (Lan et al., 2018). The nitrate (NO_3^-) resulting from nitrification later undergoes denitrification and N_2O is produced as an intermediate product. Under low-oxygen environments with a sufficient amount of NO_3^- and metabolizable organic carbon (C), considerable quantities of N_2O can be released to the environment. Ammonia-oxidizing archaea (AOA) and bacteria (AOB) are involved in the nitrification of NH_4^+ to NO_3^- in soil (Francis et al., 2005).

Nitrification inhibitors (NIs) can be used to reduce emissions of N_2O from soils and thereby improve N fertilizer use efficiency (Carneiro et al., 2010; Nair et al., 2020; Ruser & Schulz, 2015). These NIs decrease the nitrification rate (Barth et al., 2019) by blocking NH_3 oxidization, which is the initial and rate-limiting step of autotrophic nitrification (Di & Cameron, 2012). The use of NIs has been shown to effectively reduce N_2O emissions and NO_3^- leaching following application of mineral fertilizers to soil (Meijide et al., 2007). Akiyama et al. (2010) found a 31–44% reduction in N_2O emissions following the application of different NIs to grasslands and arable fields alongside different N fertilizers. Based upon these results, it could be concluded that use of NIs has considerable mitigation potential for reducing N_2O emissions.

It has been shown that the effect of NIs in reducing the nitrification rate can be strongly affected by environmental factors such as temperature and soil moisture (Lan et al., 2018). However, there are contradictory reports regarding the effect of these factors on N_2O emissions and efficiency of NIs. Puttanna et al. (1999) reported that the efficacy of NIs decreased from 62% to 6% with an increase in temperature from 10 to 30°C during a 30-day incubation study. Conversely, Menéndez et al. (2012) found that an increase in temperature from 10 to 20°C increased N_2O emission reduction efficiency of 3,4-dimethylpyrazole phosphate (DMPP) from 17% to 42%.

The effect of NIs is also influenced by the soil properties and soil parameters (Barth et al., 2001). Barth et al. (2008) found that DMPP was most effective in a sandy loam soil, particularly under conditions of higher soil moisture. Therefore, in this research, we only investigated a very sandy soil. However, we will also conduct research on the influence of soil type on the effect of NIs in the future.

Soil moisture has been reported to increase N_2O emissions by influencing NH_3 oxidizing and denitrifying microbial communities, with the functional genes increasing with high soil moisture (Di et al., 2014). The effects of different NIs on N_2O production under different moisture conditions remain unclear (Lan et al., 2018). In 4-year multi-location trials in Germany, it was found that emission of N_2O was stimulated under higher soil moisture conditions, and the PIADIN (active ingredients: 3.00–3.25% 1,2,4-triazole and 1.50–1.65% 3-methylpyrazole) was

ineffective to control the emission under these circumstances (Marcus Rohwer, personal communication). In contrast, Puttanna et al. (1999) reported that benzotriazole was equally effective under soil moisture conditions ranging from 40% to 80% of the water-holding capacity (WHC) of the soil. Although several studies have considered the individual effects of different temperatures and moisture levels on the efficacy of NIs, to the best of our knowledge, the interactive effect of the temperature, moisture and four NIs has not been studied yet.

In this study, therefore, we conducted an incubation experiment to elucidate the effects of different combinations of temperature and soil moisture levels on the efficacy of four NIs to reduce N_2O emissions and affect the dynamics of NH_4^+ and NO_3^- in an N-fertilized sandy soil. We hypothesized that: (1) high temperature will lead to a reduction in the efficacy of NIs; (2) high soil moisture will also lead to a reduction in the efficacy of NIs; and (3) DMPP is the most effective inhibitor under all the temperature and moisture combinations in our sandy soil. The findings of this research could help us to improve the use of soil fertilizer and reduce emission of N_2O from agricultural sandy soils.

2 | MATERIALS AND METHODS

2.1 | Collection, preparation and characterization of soil

The upper 0–20 cm layer of a sandy soil was collected from Grevenkrug, Schleswig-Holstein, Germany (54°11'09.1"N, 10°00'36.6"E). Visible plant residues and stones were carefully removed. The soil was passed through a 2-mm sieve and homogeneously mixed. A representative subsample of the experimental soil was analyzed for particle size distribution (sand and silt), pH, total C and N contents, NH_4^+ -N and NO_3^- -N concentrations and bulk density following standard methods (Table 1).

2.2 | Incubation experiment

Four NIs namely, dicyandiamide (DCD, $\text{C}_2\text{H}_4\text{N}_4$), (C₅H₁₁N₂O₄P), ENTEC (nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer: 7.5% NO_3^- -N, 18.5% NH_4^+ -N, and 13.0% sulphur, 0.15% DMPP) and PIADIN (active ingredients: 3.00–3.25% 1,2,4-triazole, and 1.50–1.65% 3-methylpyrazole) were tested under all possible combinations of two soil temperatures (15 and 25°C) and two soil moisture levels (60% and 80% of WHC). For comparison, a control without NI addition was also included under each combination of moisture and temperature. Thus, the following four temperature and soil moisture combinations were tested for four NIs and a control (without NIs):

1. 15°C with 60% WHC: Control; DCD, DMPP, ENTEC and PIADIN.
2. 15°C with 80% WHC: Control; DCD, DMPP, ENTEC and PIADIN.
3. 25°C with 60% WHC: Control; DCD, DMPP, ENTEC and PIADIN.
4. 25°C with 80% WHC: Control; DCD, DMPP, ENTEC and PIADIN.

TABLE 1 Basic characteristics of the soil used in this experiment

Silt (%)	Sand (%)	pH _(CaCl2)	Total carbon (g kg ⁻¹)	Total N (g kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	Bulk density (g cm ⁻³)
5	95	6.5	11.6	0.8	2.16	4.80	1.40

Each treatment combination had four replicates, giving 80 experimental units (pots) in total.

The soil was packed into cylindrical pots (15 cm diameter and 33 cm length, the bottom of each pot was completely sealed) to achieve a bulk density of 1.4 g cm⁻³ with 20 cm depth. All pots were fertilized with 0.5 g NH₄⁺-N kg⁻¹ soil using ammonium sulphate [(NH₄)₂SO₄] salt. The NIs were applied at 5% of applied NH₄⁺-N (i.e., 25 mg kg⁻¹ soil). The application rate of nitrification inhibitors that we used in our study is the same as that used by most of the farmers. The required quantities of both the N fertilizer and NIs were dissolved in 100 mL deionized water. To achieve an even distribution in the soil, N fertilizer plus NIs solution was applied through a small, perforated tube, which was inserted in the centre of the soil pot. This allowed the solution to uniformly diffuse in the soil and reach throughout the pot. Following this, deionized water was added into the tube in order to maintain the soil moisture levels to 60% or 80% of the WHC for the respective moisture treatments. The control treatment under each combination of temperature and moisture was applied with fertilizer and deionized water only. Deionized water was added each day to maintain the soil WHC. Following treatment application, pots were incubated for a period of 57 days inside an incubation chamber (controlled temperature) under the specified conditions.

2.3 | Collection and measurement of emitted N₂O

The N₂O samples were collected once a day during the first 7 days, once after 2 days during the second 7 days and once after 3 days during the last days of the incubation period. Air-tight lids were placed on top of the pots. Each lid had one rubber membrane, which was the contact between the sample collecting syringe and the incubated environment. The pots were closed first and then gas samples were collected every 20 min for 1 h (0, 20, 40, and 60 min). A 10-mL syringe, placed in pre-evacuated 2 mL headspace glass vials, was used to collect the gas samples using a hypodermic needle. The glass vials had a chloro-butyl rubber septum. Gas samplings were carried out between 09:00 and 11:00 am. Except for the times when samples were taken, the pots were left open. The concentration of N₂O in the gas samples was measured by gas chromatography (Agilent 7890A GC, Agilent, CA, USA). Total N₂O emission during the experimental period was calculated from the daily emitted amount of gas. The rate of N₂O emission from each pot was calculated from the total amount of N₂O emitted and the duration of lid closure, using a linear relation between the N₂O concentration and time.

2.4 | Analysis of soil NH₄⁺ and NO₃⁻

To determine the dynamics of NH₄⁺-N and NO₃⁻-N in the soil, 50 g soil samples were collected carefully from 0–20 cm depth on days 1, 15, 29, 43, and 57 of incubation without disturbing the soil. Each soil sample was divided into two subsamples; the first was oven-dried at 105°C for 8 h to calculate the water content while the other was used for determination of NH₄⁺-N and NO₃⁻-N. For this purpose, 10 g of each fresh soil sample was mixed with 40 mL of 0.0125 M CaCl₂ solution (1:4) and shaken for 1 h. The suspensions so obtained were centrifuged for 10 min and the extracts were then filtered through Whatman filter paper no. 40. The NH₄⁺ and NO₃⁻ concentrations in the filtered extracts were determined using a continuous flow autoanalyzer [San⁺⁺ automated wet chemistry analyzer-continuous flow analyzer (CFA), Skalar, The Netherlands]. The autoanalyzer determines the concentrations of NH₄⁺-N and total mineral nitrogen (NH₄⁺-N and NO₃⁻-N) in the soil extract separately, and NO₃⁻-N is obtained by subtracting NH₄⁺ from the total mineral N.

2.5 | Statistical analysis

Data were verified for normal distribution and the treatment means for total N₂O emission were compared using three-way analysis of variance. The NH₄⁺-N and NO₃⁻-N concentrations in the soil were compared using one-way analysis of variance. Differences between individual treatments were determined using Tukey's honest significant difference test. The statistical analyses were performed by R statistical software (R Core Team, 2013; Oakland, CA, USA) at a confidence level of 95% ($p \leq 0.05$).

3 | RESULTS

3.1 | Rate of N₂O emission

The rate of N₂O emission was affected by temperature, moisture and applied NIs (Figure 1). Without NIs application, the N₂O emissions were significantly higher during the first 20 days of the incubation period for all combinations of temperature and moisture levels. Irrespective of soil moisture content, the peak rate of N₂O emission in the control soil occurred during day 22 of incubation at 15°C and earlier in beginning of day 15 at 25°C, and thereafter it declined progressively. In NI-treated soils, a range of different N₂O emission rate trends were recorded. For example, in soils that received DMPP and PIADIN, the

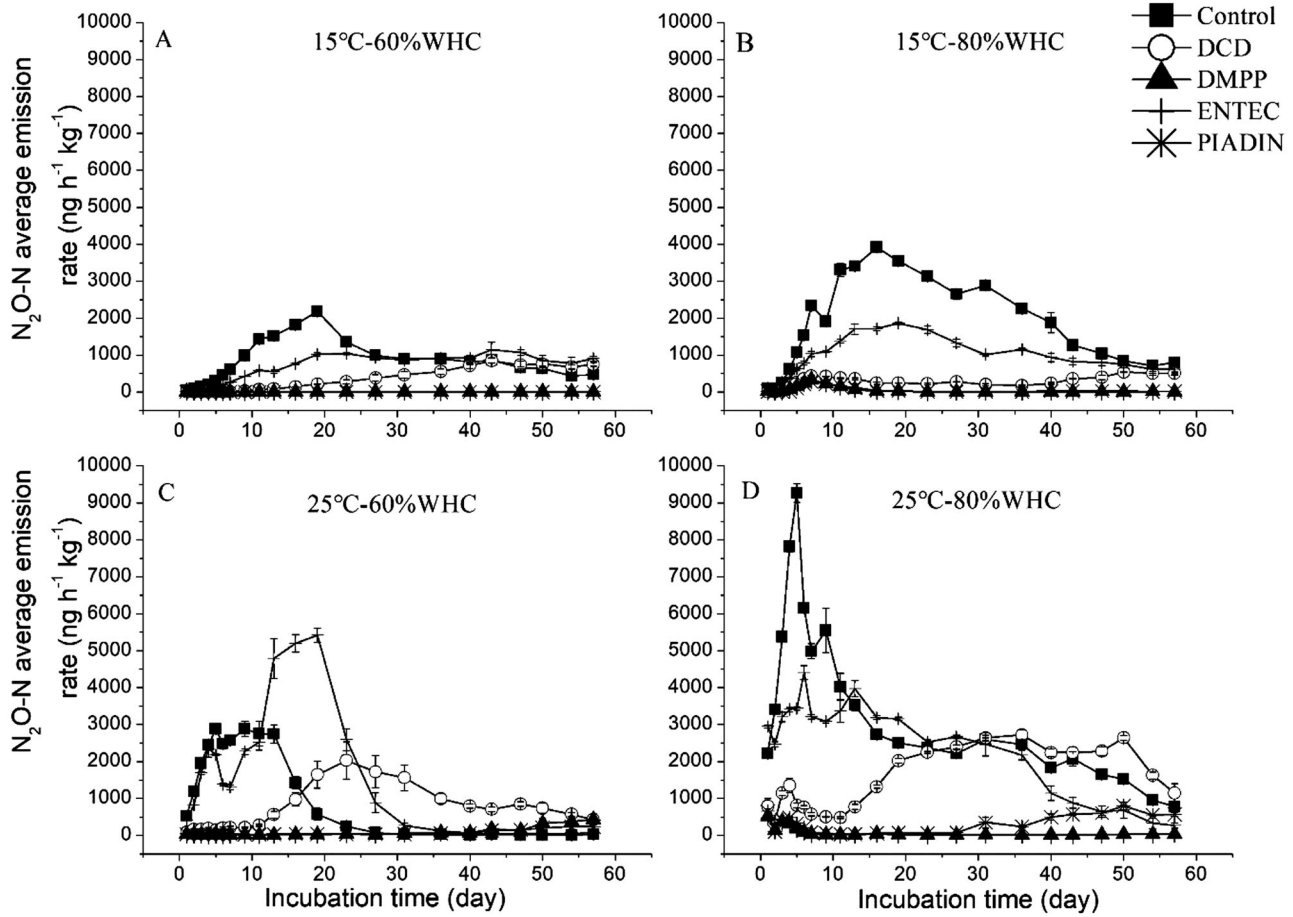


FIGURE 1 Rate of N_2O emissions as affected by dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP), nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) and 3-methylpyrazole (PIADIN) under all possible combinations of two moisture levels [60% and 80% water-holding capacity (WHC)] and two incubation temperatures (15 and 25°C). (A) 15°C and 60% WHC, (B) 15°C and 80% WHC, (C) 25°C and 60% WHC, and (D) 25°C and 80% WHC. The data values are means of four independent pot replicates and error bars represent the standard error of the mean ($n = 4$)

rate of N_2O emission throughout the incubation period was negligible at 15°C and both WHCs, whereas the rate of emission was slightly higher at 25°C than at 15°C, especially for PIADIN-treated soil at 80% WHC. Overall, DMPP and PIADIN maintained the lowest N_2O emission rate throughout the incubation period. ENTEC behaved similar to the control, whereby a peak emission rate occurred during day 15 or 22 of incubation and thereafter emissions declined. This peak was reached faster at 25°C than at 15°C. In general, except for DCD and ENTEC at 25°C and 60% WHC, the NIs decreased the N_2O emission rate.

3.2 | Total N_2O emissions

Without NI application, total emission of N_2O during a period of 60 days at 15°C was 191% and 73% higher than at 25°C under 60% and 80% WHC, respectively (Table 2). In contrast, compared with 60% WHC, total emission of N_2O at 80% WHC was 109% and 251% higher at 15 and 25°C, respectively. The effect of the NIs was dependent on soil moisture as well as incubation temperature. At 15°C under 60% WHC, all the NIs decreased N_2O emission, with DMPP and PIADIN

almost completely inhibiting N_2O emission, whereas DCD and ENTEC reduced it by 56% and 25%, respectively. At 15°C under 80% WHC, the NIs exhibited almost the same response, in which both DMPP and PIADIN decreased N_2O emission by 98%, whereas DCD and ENTEC decreased it by 85% and 43%, respectively. At 25°C and 60% WHC, DCD and ENTEC increased N_2O emission by 32% and 102%, respectively, whereas DMPP and PIADIN decreased it by 85% and 87%, respectively. At 25°C and 80% WHC, the NIs decreased N_2O emission, the decreased N_2O emission results show that DMPP (98%) and PIADIN (88%) were more effective than DCD (35%) and ENTEC (21%) in a sandy soil at 25°C and 80%.

3.3 | Soil NH_4^+-N and $NO_3^- -N$ concentrations

In the control treatment, soil NH_4^+-N concentration decreased over time, whereas that of $NO_3^- -N$ increased (Figures 2–5). However, the typical crossover of the trend lines for the two N forms occurred earlier at 25°C than at 15°C. Within the tested combinations of temperatures and moisture contents, the range of NH_4^+-N at 57 incubation days was

TABLE 2 Total emission of N_2O from soils treated with nitrification inhibitors dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP), nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) and 3-methylpyrazole (PIADIN) and incubated under different combinations of two soil moisture levels and temperatures for 57 days

Moisture level	Treatment	N_2O -N emission ($mg\ kg^{-1}$ soil $60\ d^{-1}$)	
		15°C	25°C
60% WHC	Control	1.31 ± 0.08f	0.45 ± 0.03d
	DCD	0.59 ± 0.07 (−55.5)d	0.60 ± 0.11 (+31.8)d
	DMPP	0.00 ± 0.00 (−99.7)a	0.07 ± 0.02 (−85.2)b
	ENTEC	0.99 ± 0.08 (−24.5)e	0.92 ± 0.05 (+101.9)e
	PIADIN	0.01 ± 0.00 (−99.4)a	0.06 ± 0.02 (−86.9)b
80% WHC	Control	2.74 ± 0.05g	1.58 ± 0.02f
	DCD	0.42 ± 0.03 (−84.6)d	1.02 ± 0.02 (−35.4)e
	DMPP	0.05 ± 0.00 (−98.3)b	0.03 ± 0.00 (−98.2)a
	ENTEC	1.56 ± 0.11 (−43.1)f	1.26 ± 0.04 (−20.5)f
	PIADIN	0.06 ± 0.00 (−97.6)b	0.19 ± 0.01 (−88.2)c

Note: Values (\pm SE) are means of four independent pot replicates. The values in parenthesis represent the percentage increase (+) or decrease (−) in N_2O emissions with nitrification inhibitors over their respective controls. WHC stands for water-holding capacity. Means indicated by the same lowercase letter are not significantly different at the $p \leq 0.05$ level according to the Tukey honest significant difference test.

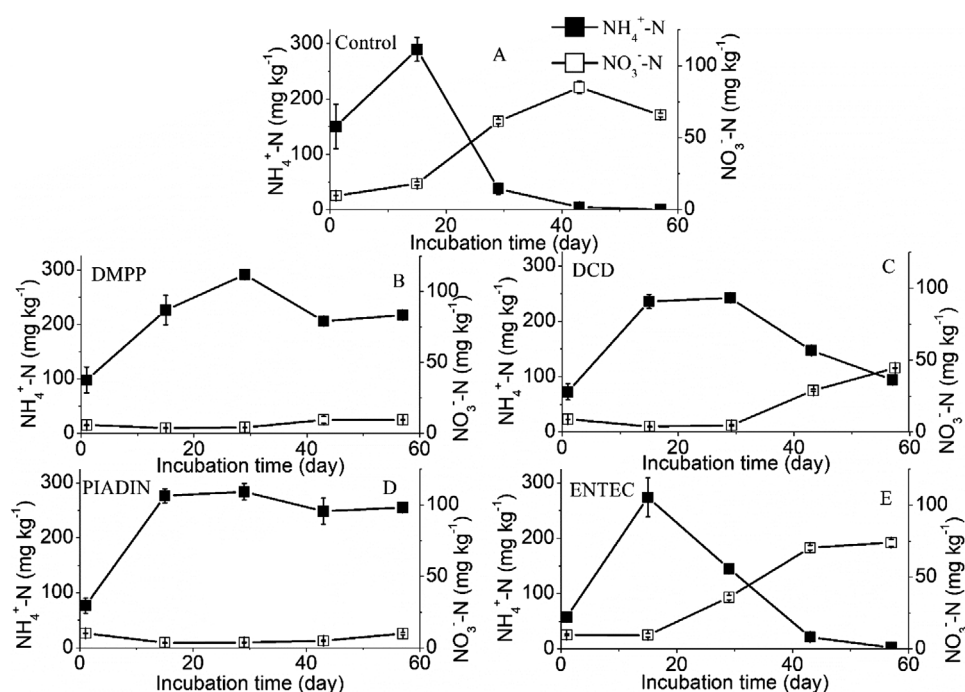


FIGURE 2 Changes in the NH_4^+ -N and NO_3^- -N concentrations in soils treated with control (A) and nitrification inhibitors 3,4-dimethylpyrazole phosphate (DMPP) (B), dicyandiamide (DCD) (C), 3-methylpyrazole (PIADIN) (D), and nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) (E) and incubated at 15°C and 60% water-holding capacity for 57 days. The data values are the mean of four independent pot replicates and the error bars represent the standard error

0.1–2 $mg\ kg^{-1}$ soil, whereas that of NO_3^- -N was 9.6–86 $mg\ kg^{-1}$ soil in the control treatment. The concentration of NO_3^- -N on day 57 of the control treatment was less at 80% WHC than at 60% WHC. Compared with the control, higher NH_4^+ -N concentrations were recorded in treatments with NIs application (Figures 2–5). In soils treated with

DMPP and PIADIN, NH_4^+ -N remained fairly stable throughout the course of the experiment at low temperature (15°C). In contrast, at 25°C, the decline in NH_4^+ -N concentration was recorded but it was very slow compared with the control treatment. The NO_3^- -N concentration was not significantly affected by NI addition. However,

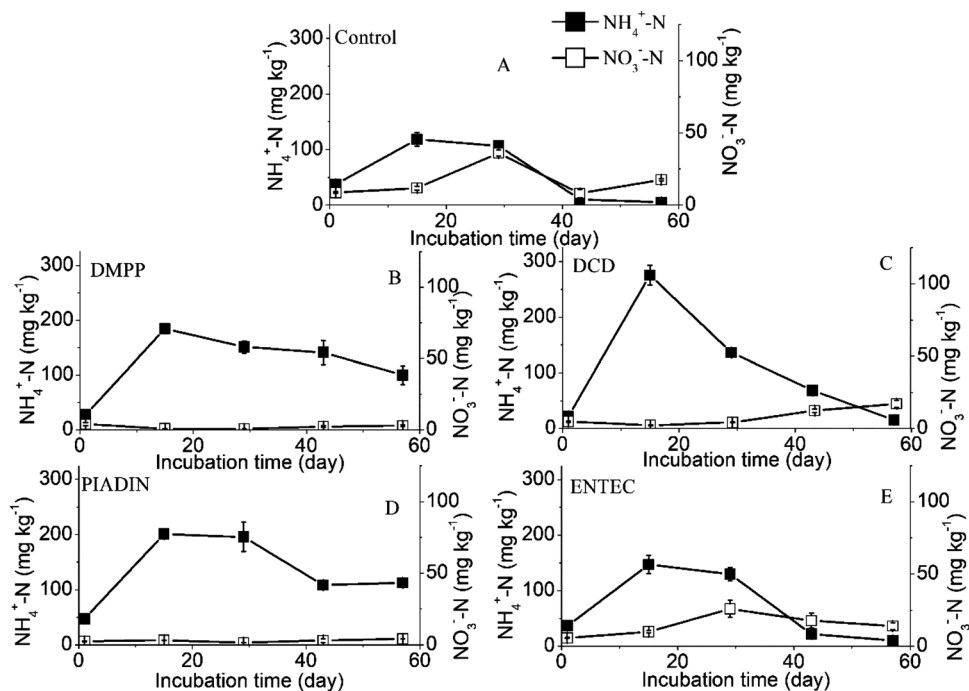


FIGURE 3 Changes in the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in soils treated with control (A) and nitrification inhibitors 3,4-dimethylpyrazole phosphate (DMPP) (B), dicyandiamide (DCD) (C), 3-methylpyrazole (PIADIN) (D), and nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) (E) and incubated at 15°C and 80% water-holding capacity for 57 days. The data values are the mean of four independent pot replicates and the error bars represent the standard error

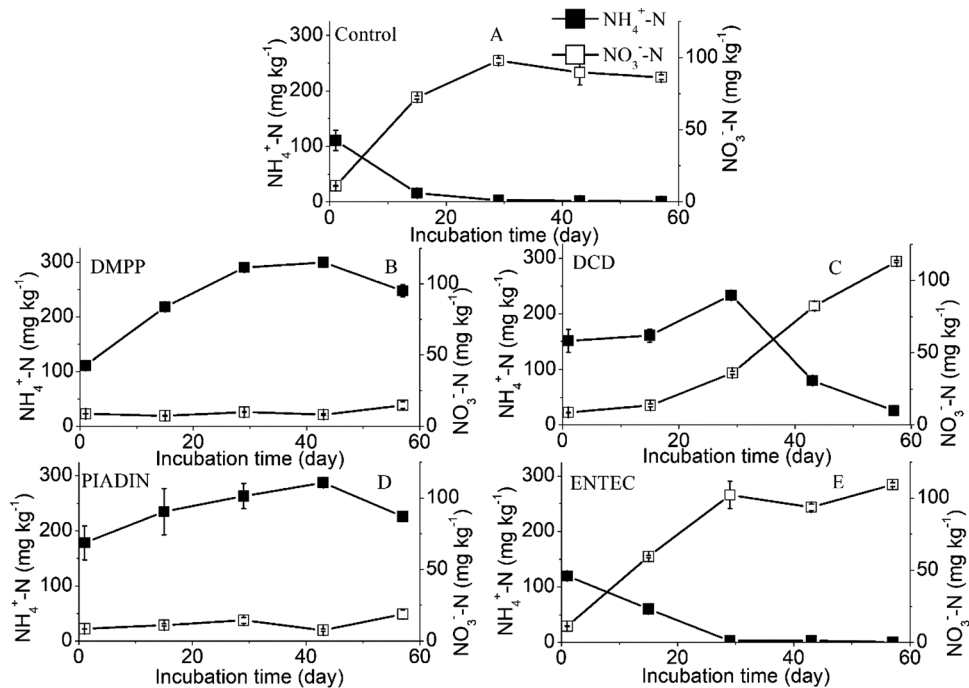


FIGURE 4 Changes in the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in soils treated with control (A) and nitrification inhibitors 3,4-dimethylpyrazole phosphate (DMPP) (B), dicyandiamide (DCD) (C), 3-methylpyrazole (PIADIN) (D), and nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) (E) and incubated at 25°C and 60% water-holding capacity for 57 days. The data values are the mean of four independent pot replicates and the error bars represent the standard error

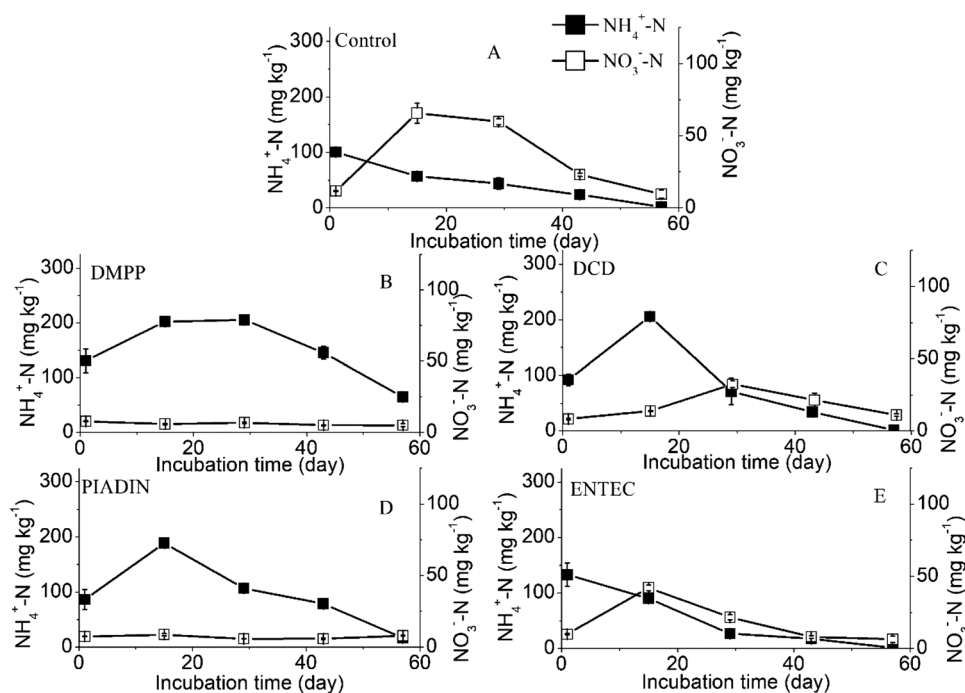


FIGURE 5 Changes in the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in soils treated with control (A) and nitrification inhibitors 3,4-dimethylpyrazole phosphate (DMPP) (B), dicyandiamide (DCD) (C), 3-methylpyrazole PIADIN (D), and nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) (E) and incubated at 25°C and 80% water-holding capacity for 57 days. The data values are the mean of four independent pot replicates and the error bars represent the standard error

DCD- and ENTEC-treated soils showed a comparatively fast decline in $\text{NH}_4^+\text{-N}$ concentration and emission of $\text{NO}_3^-\text{-N}$ at the fast increase rate (Figures 2–5). All added NIs were least effective in sustaining $\text{NH}_4^+\text{-N}$ in soil at 25°C and 80% WHC.

4 | DISCUSSION

This study investigated the efficacy of four NIs (DMPP, DCD, ENTEC, and PIADIN) to reduce N_2O emissions from an N-fertilized soil incubated under different combinations of temperature and soil moisture levels in a sandy soil. Our result showed that total N_2O emission decreased with the increase in temperature, whereas it increased with the increase in soil moisture level (Table 2) in the control treatment. However, the peak N_2O emission rate was achieved earlier at 25°C than at 15°C, which suggests that the nitrification rate was higher at higher temperature (Figure 1). Accordingly, compared with that at 15°C, $\text{NH}_4^+\text{-N}$ concentration declined, and $\text{NO}_3^-\text{-N}$ concentrations increased more rapidly in the control treatment at 25°C (Figures 3 and 5). The temperature-induced increase in nitrification rate, as found in this study, is in accordance with earlier findings reporting a promoting effect of temperature on nitrification in soil (Lan et al., 2018). Sahrawat (2008) reported that the nitrification rate was generally higher with an optimum temperature ranging from 25 to 35°C. This may be attributed to a positive effect of temperature on the activity of ammonia oxidizers (Gubry-Rangin et al., 2017). Despite the higher nitrification rate at the higher temperature

(25°C), total N_2O emissions were higher at the lower temperature (15°C) (Table 2). This may be explained by the higher shift of denitrified $\text{NO}_3^-\text{-N}$ toward formation of nitrogen gas (N_2) at the higher temperature.

N_2O emission was higher at 80% WHC than at 60% WHC at both the incubation temperatures under the control treatment (Table 2). Accordingly, $\text{NO}_3^-\text{-N}$ concentration at the end of the experiment under 80% soil WHC was significantly lower than those under 60% soil WHC (Figures 3 and 5) in the control. Similar results were found by Senbayram et al. (2009), who reported that N_2O emissions were higher at 85% than at 65% WHC. Denitrification dominates N_2O emissions from soils with a high moisture content (Diba et al., 2011) and subsequently N_2O emissions usually increase under wet environments as the soils become more anaerobic (Dobbie & Smith, 2001). Denitrifying enzymes are produced under anaerobic conditions and their activities are inhibited when the oxygen concentration in the soil is high (van Spanning et al., 2007). Zhu et al. (2013) also found that growth of AOB communities (Di et al., 2014) responsible for producing denitrifying enzymes was promoted with increasing soil moisture content or decreasing oxygen concentration.

At lower temperature (15°C), all the NIs reduced N_2O emissions, DMPP and PIADIN were able to almost completely inhibit N_2O emissions at both moisture levels (Table 2). At lower temperature (15°C), the efficacy of DCD and ENTEC increased by 29% and 19%, respectively, with an increase in soil moisture from 60% to 80% WHC. Similarly, at 25°C, the efficacy of all the NIs increased with an increase in moisture content. DMPP completely inhibited N_2O emissions with

the same efficacy at 25°C and 15°C at 80% WHC (Table 2). The efficacy of DCD, ENTEC and PIADIN was lower at 25°C compared with that at 15°C (Table 2). The present study showed that the efficacy of all the NIs was negatively affected by increasing temperature from 15 to 25°C, but the magnitude of the effect differed among the NIs. DCD and ENTEC completely lost their nitrification inhibition ability at 25°C and 60% WHC, and also showed a higher decline in activity at 80% WHC. Menéndez et al. (2012) and Di et al. (2014) also found a decline in N₂O emission inhibition efficacy of DMPP and DCD with increasing soil temperature. The variation in the effect of temperature on the efficacy of NIs may be attributed to different sensitivity of NIs to temperature-dependent degradation and decomposition by microorganisms. At higher temperatures, previous studies have reported hastened degradation of NIs in soil (Di et al., 2014; Menéndez et al., 2012; Puttanna et al., 1999) and accelerated microbiological degradation, with the maximum values reached at 25–33°C.

This is the novelty of the present work to report that DMPP followed by PIADIN had the higher inhibition efficiency, whereby they sustained or maintained higher NH₄⁺-N concentrations and kept NO₃⁻-N concentrations at a low level at both studied temperatures and moisture levels (Figures 2–5). These results also imply that DMPP followed by PIADIN were resistant to temperature dependent degradation and microbial decomposition. Barth et al. (2019) found that NIs have a better performance in reducing NO₃⁻-N formation in sandy soils compared with that of the loam and clay textured soils, which was supported by our results. Lan et al. (2018) reported that DMPP generally reduced N₂O emission more than DCD and 3MP + TZ at 15 and 35°C, whereas DCD performed better than DMPP and 3-methylpyrazol and 1H-1,2,4-triazol (3MP + TZ) at 25°C. When temperature does not exceed 15°C, DCD has been reported to inhibit nitrification to a variable extent and for period ranging from 20 days to at least 6 months (Chaves et al., 2006; Guiraud & Marol, 1992; Vallejo et al., 2005). Above this temperature, its efficacy has been shown to decrease substantially. Similar to the results obtained in our study, Weiske et al. (2001) observed a larger inhibition in N₂O emissions under field conditions after DMPP application than after DCD application. It could thus be concluded that irrespective of soil moisture and temperature, DMPP is the most effective NI for use in controlling N₂O emissions from agricultural sandy soils. Similarly, Barth et al. (2008) found that DMPP inhibited the oxidation of NH₄⁺ in two soils, but this effect was more pronounced in a sandy loam than in a loamy soil. Most NIs retard microbial oxidation of NH₄⁺ by depressing the activities of nitrifiers in soil (Wolf et al., 2014) through deactivating the ammonia monooxygenase enzyme (Di et al., 2010). Hence, NI application slows down the conversion of NH₄⁺ to NO₃⁻ (Lan et al., 2018) and prevents against loss via leaching or emission as N₂O (Kirschke et al., 2019). In our research, we did not investigate the AOA and AOB, which play an important role during the soil nitrification and denitrification. Therefore, future studies should focus on the microbial changes with incubation time under different temperature and moisture levels with application of the four NIs.

5 | CONCLUSIONS

The findings of this study reveal that N₂O emission is decreased with the increase in temperature, whereas they increased with the increase in soil moisture level without nitrification inhibitors. DMPP and PIADIN were more effective than DCD for nitrification inhibition in a sandy soil at both 15 and 25°C as well as 60% and 80% WHC. The concentration of the NI contained in ENTEC was insufficient to affect nitrification at both soil moisture levels and temperatures. The efficacies of DMPP, PIADIN and DCD improved with the increase in soil moisture to 80% WHC, whereas increasing temperature had various negative effects in our sandy soil. The NIs could be used to maintain the soil fertilizer by maintaining NH₄⁺-N concentration at a high level and NO₃⁻-N concentration at a low level at both 15 and 25°C as well as 60% and 80% WHC. Atmospheric pollution and underground water pollution could thus be reduced by decreasing the N₂O emissions and NO₃⁻-N leaching, respectively.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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