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Revisiting the Effectiveness of Nitrification Inhibitors Under Different Soil Moisture Conditions and Mineral Nitrogen Extraction Methods: Further Experimental Work Considering the Pasda et al. (2023) Opinion Paper

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ABSTRACT

Background: In a recent opinion article, Pasda et al. indicated that the findings from our previous study (Guo et al.) were misleading due to the inappropriateness of the experimental work. The mentioned study focused on the effectiveness of four nitrification inhibitors (NIs) in reducing nitrous oxide (N₂O) emissions as affected by temperature and moisture.

Aims: We assessed to what extent the issues raised by Pasda et al. impact the findings presented by Guo et al. The points considered were (i) the lack of distinction between pure NIs, commercial formulations, and NI-treated fertilizers, (ii) unsuitable application rates of nitrogen (N) and NIs, and (iii) inappropriate soil NH₄⁺-N extraction method.

Methods: We conducted an incubation experiment to evaluate the efficacy of dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP) (pure NIs), ENTEC (DMPP-treated fertilizer), and PIADIN (commercial NI formulation) under 60% and 80% water-holding capacity (WHC). Application rates of N and NIs followed Guo et al. but were adjusted according to Pasda et al. comments. The measurements included periodic monitoring of soil NH₄⁺-N and NO₃⁻-N concentrations, as well as N₂O-N emissions. We compared 0.0125 M CaCl₂ and 1 M KCl extraction solutions to assess the effectiveness of NIs in regulating soil mineral N concentrations.

Results: The control treatments at the both 60% and 80% WHC exhibited highest N₂O-N emissions; however, emissions were higher at 80% WHC compared to 60%. NIs effectively reduced N₂O emissions although the efficiency was slightly lower 80% WHC. Furthermore, NI treatments also retained higher NH₄⁺-N levels and suppressed NO₃⁻-N accumulation. KCl extractions consistently yielded higher amount of NH₄⁺-N as compared to CaCl₂.

Conclusions: The study proves that the dilution of ENTEC significantly affected Guo et al.'s results. In the current study, ENTEC effectively reduced N₂O emissions, performing similar to DMPP, DCD, and PIADIN. The high efficiency of these inhibitors remains consistent and in line with the earlier findings. Although the KCl extraction is more precise for NH₄⁺-N measurement, CaCl₂ can still detect NI effects and may be suitable depending on the specific context of study.

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1 | Introduction

The application of NH_4^+ -based fertilizers is commonly the main driver of nitrous oxide (N_2O) emissions from fertilized agricultural soils (Ribeiro, Hamta, et al. 2024; Ribeiro, Singh, et al. 2024). The global warming potential (GWP) of N_2O is 273 times greater than CO_2 (Intergovernmental Panel on Climate Change [IPCC] 2021). Moreover, N_2O is mainly produced by nitrification and denitrification biochemical processes (Liu et al. 2019). Thus, nitrification inhibitors (NIs) have been suggested to be added to fertilizers because they retard the nitrification process by inhibiting the activity of ammonia-oxidizing bacteria, thus reducing soil nitrate (NO_3^-) concentration and, consequently, mitigating NO_3^- leaching and N_2O emissions (Yin et al. 2023).

Several factors, including soil moisture, temperature, and N availability, may influence the effectiveness of NIs (Tufail et al. 2023; Ribeiro et al. 2023). Thus, our previous study, Guo et al. (2022) assessed the effectiveness of four NIs under different soil temperature and moisture conditions. It was concluded that the NIs DMPP (3,4-dimethylpyrazole phosphate) and PIADIN (formulation with the NIs 3-methylpyrazole combined with 1,2,4-triazole, MP + TZ) were more effective than DCD (dicyandiamide) in reducing N_2O emissions. In addition, it was indicated that the concentration of the NI in ENTEC (DMPP-treated fertilizer) was insufficient to delay the nitrification process. Subsequently, the findings of this research article were questioned by Pasda et al. (2023), especially regarding the methodology due to (i) a lack of distinguishing between pure NIs, NI formulations, and NI-treated fertilizers, (ii) unsuitable NI application rates, and (iii) usage of inappropriate NH_4^+ -N extraction method, leading to misleading conclusions.

The points made by Pasda et al. (2023) are described in more detail in this paragraph. First, slightly different N application rates may have impacted the results because Guo et al. (2022) did not consider the varying N content in DMPP, DCD, PIADIN, and ENTEC. Second, Guo et al. (2022) utilized the NI rate of 5% of the applied NH_4^+ -N for all tested treatments (i.e., DMPP and DCD) without considering that EU regulations allow different concentrations for each NI. Therefore, these rates did not reflect what is implemented by fertilizer companies and commonly used by farmers, limiting the relevance of Guo et al. (2022) results for practical field conditions. Third, the NH_4^+ -N extraction method based on a 0.0125 M CaCl_2 solution was suggested to be insufficient for evaluating NI effectiveness, whereas a highly concentrated KCl or K_2SO_4 solution would be more appropriate.

Rather than discussing the abovementioned points on theoretical grounds, we carried out an incubation experiment to test if the conclusions drafted by Guo et al. (2022) would be changed when the points raised by Pasda et al. (2023) are considered. Thus, we hypothesized that (i) ENTEC was ineffective in Guo et al. (2022) because of an NI dilution effect due to using this fertilizer as simulating an inhibitor compound but ENTEC is effective under normal conditions; (ii) the high effectiveness of DMPP, PIADIN, and DCD will be confirmed and remain unaltered; and (iii) despite a highly concentrated KCl extraction solution being more accurate for measuring NH_4^+ -N soil availability, a low

concentrated CaCl_2 solution can also detect NIs impact on soil NH_4^+ -N concentrations.

2 | Materials and Methods

2.1 | Soil Description

The soil used in the experiment was collected from Schleswig Holstein, Germany (54°07'05.0" N 10°14'26.0" E). The soil was passed through a 2-mm sieve and mixed homogeneously. This soil was selected because it had characteristics similar to the one used by Guo et al. (2022) (Table 1).

2.2 | Experimental Design

A soil incubation experiment was carried out in a climatic chamber set at 25°C. The experimental design comprised two factors: N fertilization and soil moisture. The tested N fertilization factor included one NI-treated fertilizer (ENTEC), one NI formulation (PIADIN), and two pure NIs (DMPP and DCD). The soil moisture factors included moisture levels of 60% and 80% of the water-holding capacity (WHC). The soil moisture was maintained throughout the incubation period by weighting the pots twice a week and replenishing the water evaporation losses. Moreover, a completely randomized design with four replications was implemented. This setup was identical to the one presented in Guo et al. (2022), except for not including temperature as a factor. Temperature was not considered in the experimental design because the aim of the present study was not necessarily to replicate the entire setup of Guo et al. (2022) but to specifically address methodological issues raised by Pasda et al. (2023).

A summary of the NI rates used in Guo et al. (2022), the considerations made by Pasda et al. (2023), and the adjustments adopted in the present study are shown in Table 2. The table also includes a description of each treatment.

All treatments mentioned in Table 2 received 500 mg NH_4^+ -N kg^{-1} through ammonium sulfate. Moreover, as ENTEC is a commercial fertilizer that combines ammonium sulfate and ammonium nitrate, the treatments DMPP, DCD, and PIADIN also received 203 mg kg^{-1} of NO_3^- -N by adding calcium nitrate to compensate for the NO_3^- -N present in ENTEC. In other words, all treatments received 500 and 203 mg kg^{-1} of NH_4^+ -N and NO_3^- -N, respectively. Notably, the N content in DMPP, DCD, and PIADIN was not balanced for in the mentioned N rates because of their low application rate. Moreover, if N mineralized from NIs biodegradation affects their efficacy on reducing N_2O emissions, this is also part of the treatment effects to be assessed in this type of study. All other details regarding pot size, soil filling, fertilizer and NI application method, N_2O sampling, and measurement were done exactly as described in Guo et al. (2022).

2.3 | Gas Sampling

The gas samples were taken at intervals of 0, 20, 40, and 60 min. The pots were kept open except for the time of gas sampling.

TABLE 1 | Basic characteristics of the soil used in this experiment.

Clay (%)	Silt (%)	Sand (%)	pH _{CaCl2}	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	Bulk density (g cm ⁻³)
7.4	13.3	79.3	5.9	1.22	0.21	1.40

The samples were taken with a clean syringe each time to avoid cross contamination. N₂O samples were collected daily during the first 7 days, every 2nd day during the following 7 days, and every 3rd day during the rest of the experiment. The samples were transferred to gas chromatography immediately after collection. N₂O concentrations were measured with an electron capture detector at 300°C with N₂ as carrier gas. The calibration for GC was performed with certified gas standards before each measurement. The rate of N₂O emissions was calculated using headspace volume and the linear relationship between gas concentration and time during lid closure. The following equation was used to calculate N₂O-N emission rates.

$$EN_2O - N = \frac{R \times 60 \times V_{gas} \times AR}{W_{soil} \times V_m} \times 2 \times 1000$$

where EN_2O is the rate of emission of N₂O calculated in nanograms per hour per kg soil; R stands for the rate of N₂O emissions from each pot (ppm min⁻¹); V_{gas} is the volume of gas in each pot (L); W_{soil} is the weight of dry soil in pot (kg); AR is the relative atomic mass of N, that is, 14; and V_m is the molar volume of gas at 25°C. The emissions for non-sampling days were estimated through linear interpolation between adjacent sampling points. Subsequently, cumulative N₂O-N emissions for the entire 63-day period were calculated by summing the estimated daily emissions.

2.4 | Sampling and Measurement of Soil NH₄⁺-N and NO₃⁻-N Concentrations

The soil samples for NH₄⁺-N and NO₃⁻-N analysis were taken from all pots at days 0, 15, 30, 45, and 60 of the experiment. The soil mineral N analysis was done by taking 10 g of fresh soil, mixing it with 1 M KCl solution (1:10), and shaking it for an hour. The same extraction procedure was repeated for samples from control and DCD treatments by using 0.0125 M CaCl₂ solution to compare the NH₄⁺-N extraction efficiency of both extractants. The treatments were chosen to compare the extractants because they represent unstabilized- and stabilized-NH₄⁺-N conditions. A continuous flow analyzer (CFA) of Skalar (San++ Automated Wet Chemistry Analyzer-CFA, Breda, the Netherlands) was used for measuring NH₄⁺-N and NO₃⁻-N.

2.5 | Statistical Analysis

The Shapiro–Wilk normality test was used to check data distribution. In addition, the treatment means for total N₂O-N emissions were compared using a two-way analysis of variance. The Tukey test was used to determine differences between treatment means ($p \leq 0.05$). Statistical analyses were performed using Statistix 10 software.

3 | Results

3.1 | Daily N₂O-N Emissions

The control treatment showed the highest N₂O-N emission rates at 80% WHC during the experiment (Figure 1). There was a noticeable increase in N₂O-N emissions on the 7th day, which peaked on the 17th day at around 9000 ng h⁻¹ kg⁻¹. After the 17th day, emissions started to decrease but remained higher than in other treatments. All the NIs considerably decreased N₂O-N emissions for the first 30 days of the experiment. Moreover, DCD caused a small N₂O-N peak on the 7th day that declined slowly until the 17th day. All the NIs showed small increases after the 30th day toward the end of the experiment at 80% WHC.

The control treatment also showed the highest N₂O-N emissions compared to all the treatments at 60% WHC, although the emissions were not as high as at 80% WHC. The highest peak of approximately 2000 ng h⁻¹ kg⁻¹ was observed on the 31st day. The N₂O-N emissions remained low and steady during the entire experiment in all the NI treatments at 60% WHC.

3.2 | Total N₂O-N Emissions

The total N₂O-N emissions showed significant variations among treatments and moisture conditions during the course of the experiment. The N₂O-N emissions observed at 60% WHC and 80% WHC were 3.57 and 8.08 mg kg⁻¹, respectively, indicating a significant increase in N₂O emissions under high soil moisture (Table 3).

All NIs significantly reduced N₂O emissions compared to the control. The reductions ranged from 96% to 98% at 60% WHC and 79%–88% at 80% WHC. Overall, the NIs efficiency was slightly lower under 80% WHC but remained significant (Table 3).

3.3 | Soil NH₄⁺-N and NO₃⁻-N Concentrations

At both 80% and 60% WHC in the control treatments NH₄⁺-N were initially high but decreased over time, whereas NO₃⁻-N increased indicating active nitrification. The decrease in NH₄⁺-N and increase in NO₃⁻-N were more rapid at 60% WHC. On the other hand, at 80% NH₄⁺-N persisted longer and accumulation of NO₃⁻-N was slower as compared to 60% WHC. The treatments with NIs consistently retained higher NH₄⁺-N levels and suppressed NO₃⁻-N accumulation in contrast to control at both moisture levels, with a more pronounced effect at 80% WHC (see Figure S1).

With regard to the investigated mineral N extraction methods, the KCl solution consistently extracted more NH₄⁺-N than CaCl₂ across all the treatments and moisture levels (Figure 2). For

TABLE 2 | Description of DMPP (3,4-dimethylpyrazole phosphate), DCD (dicyandiamide), PIADIN (formulation with the nitrification inhibitor (NIs) 3-methylpyrazole combined with 1,2,4-triazole, MP + TZ), and ENTEC (DMPP-treated fertilizer) rates utilized in Guo et al. (2022) and in the present study following Pasda et al. (2023) recommendations.

Treatment	Description	NI maximum and minimum rates according to EU regulations as indicated by Pasda et al. (2023)	NI rate (% of applied NH_4^+ -N) in Guo et al. (2022)	NI rate (% of applied NH_4^+ -N) used in the present short communication
ENTEC ^a	18.5% NH_4 -N, 7.5% NO_3 -N (ammonium sulfate + ammonium nitrate), and 1% DMPP	0.8% and 1.6%	0.008	1
DMPP	Pure DMPP	0.8% and 1.6%	5	1
DCD	Pure DCD	2.5% and 4.5%	5	4.5
PIADIN ^a	4.7% of MP + TZ (1.575% 3-methylpyrazole + 3.125% triazole) in urea ammonium nitrate solution	0.2% and 1% of the combination MP + TZ	0.23	0.23

^aThe NI rates presented for ENTEC and PIADIN refer to the active ingredients DMPP and MP + TZ included in the commercial products, respectively.

TABLE 3 | Total emission of N_2O -N from soil with no added NI (control) and soil treated with DMPP (3,4-dimethylpyrazole phosphate), DCD (dicyandiamide), PIADIN (formulation with the nitrification inhibitor (NIs) 3-methylpyrazole combined with 1,2,4-triazole, MP + TZ), and ENTEC (DMPP-treated fertilizer) under 60% and 80% water-holding capacity (WHC) for 63 days.

Treatment	60% WHC (mg kg^{-1})	80% WHC (mg kg^{-1})
Control	3.57 ± 0.32 B	8.08 ± 0.77 A
DMPP	0.14 ± 0.01 D (-96%)	1.23 ± 0.08 C (-85%)
DCD	0.11 ± 0.02 D (-97%)	1.69 ± 0.12 C (-79%)
PIADIN	0.12 ± 0.01 D (-97%)	0.79 ± 0.15 CD (-88%)
ENTEC	0.08 ± 0.01 D (-98%)	1.39 ± 0.07 C (-83%)

Note: Values (\pm SE) are means of four independent pot replicates. The coefficient of variation (CV) for the dataset is 36.11%. The values within parentheses represent the percentage decrease in N_2O emissions with nitrification inhibitors over their respective controls. Means indicated by the same letter are not significantly different at the $p \leq 0.05$ level according to the Tukey honest significant difference test.

Abbreviation: WHC, water-holding capacity.

example, in the control treatment at 80% WHC on day 60, the KCl solution extracted 30% more NH_4^+ -N kg^{-1} soil as compared to CaCl_2 . A similar trend was observed at 60% WHC, where KCl extracted 51% more NH_4^+ -N kg^{-1} soil than CaCl_2 .

The two studied extraction solutions effectively detected the impact of NIs on keeping higher soil NH_4^+ -N concentrations. For instance, the KCl extraction method evidenced 50%, 9%, 17%, and 27% higher NH_4^+ -N on DCD-treated soil relative to the control treatments under 80% WHC. Similarly, the CaCl_2 extraction solution also showed 18%, 67%, 46%, and 22% higher NH_4^+ -N due to the DCD application compared to the control treatments, considering the same conditions.

4 | Discussion

4.1 | Impact of NI and N Application Rates

This study addressed the comments of Pasda et al. (2023) on our previous study (Guo et al. 2022) experimental work, which

assessed the effectiveness of four NIs under different moisture and temperature conditions. The present study indicated that DMPP, DCD, and PIADIN effectively reduced nitrification and N_2O -N emissions in a 63-day incubation experiment under 60% and 80% WHC despite their rates being lowered according to EU fertilizer regulations in relation to the application rates adopted by Guo et al. (2022). Interestingly, Guo et al. (2022) indicated DCD was less effective than PIADIN and DMPP, whereas the present study did not find differences between the mentioned inhibitors. The soil used in this study had slightly lower sand content than the one used by Guo et al. (2022). Previous work has suggested that the higher the sand content, the lower the effectiveness of DCD due to the polar nature of this compound, which eases its spatial separation from NH_4^+ -N. Likewise, DCD effectiveness tends to be lower than PIADIN and DMPP in coarse-textured soils (Ribeiro, Pitann, et al. 2024).

Moreover, we are aware that ENTEC is a nitrogenous fertilizer, and it was utilized as a simulation of an inhibitor compound by Guo et al. (2022). We acknowledge that this issue was not

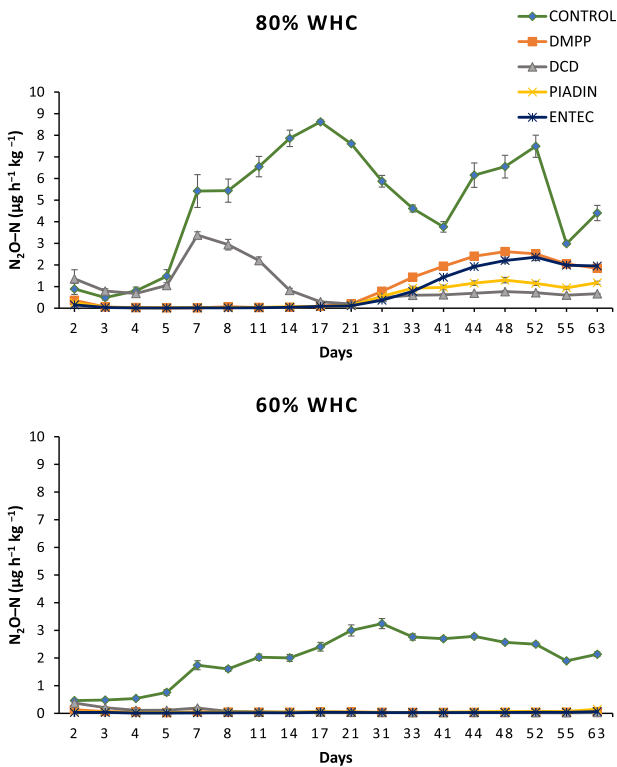


FIGURE 1 | Daily N_2O-N emissions from soil with no added NI (control) and soil treated with DMPP (3,4-dimethylpyrazole phosphate), DCD (dicyandiamide), PIADIN (formulation with the NIs 3-methylpyrazole combined with 1,2,4-triazole, MP + TZ), and ENTEC (DMPP-treated fertilizer) under 60 and 80% WHC for 63 days. WHC, water-holding capacity.

sufficiently explained and strongly influenced the interpretation and conclusions of Guo et al. (2022). In fact, considering the use of ENTEC to simulate an inhibitor, it resulted in an extremely diluted DMPP application rate (0.008%) in Guo et al. (2022), which is far below the minimum required for its utilization in agriculture and what is present in ENTEC (1%). The present study data confirmed ENTEC as an efficient NI-treated fertilizer in reducing N_2O-N release. Moreover, as expected, there was no difference between ENTEC (commercial fertilizer containing DMPP) and pure DMPP added to ammonium sulfate salt as the DMPP rate was kept at the same level.

The increase in soil $NO_3^- -N$ and decrease in $NH_4^+ -N$ concentration at the 60% WHC evidenced active nitrification for control and DCD treatments (Figure 2). However, N_2O emissions from these treatments were much lower than emissions at 80% WHC (Table 3). This is probably because denitrification was limited due to anaerobic conditions at 60% WHC, whereas more anaerobic microsites were formed at 80% WHC, leading to conditions conducive to denitrification and large N_2O emission peaks. At the same time, oxygen availability was still adequate for nitrification at 80% WHC. These results agree with previous literature showing that the highest N_2O emissions originating from both nitrification and denitrification processes occur at nearly 80% of water-filled pore space (Wang et al. 2023).

4.2 | Impact of Using KCl and $CaCl_2$ on Extracting $NH_4^+ -N$ to Assess the Efficacy of NIs

Concentrated 2 or 1 M KCl solution is internationally accepted as a standard method for extracting $NH_4^+ -N$ and $NO_3^- -N$ (Li et al. 2023; Song et al. 2023). Especially for $NH_4^+ -N$, the utilization of a highly concentrated salt solution is based on the principle of ion exchange between the added cation and $NH_4^+ -N$ adsorbed to negatively charged soil colloids (Li et al. 2012). Comparatively, a low-concentrated $CaCl_2$ solution has been shown not to provide sufficient cations to displace $NH_4^+ -N$ from the surface of fertilized soils and consequently underestimates the available $NH_4^+ -N$. Moreover, both extraction solutions show similar accuracy for $NO_3^- -N$ (Li et al. 2012).

Considering the assessment of NI effectiveness by using KCl and $CaCl_2$, our data suggested that both solutions detected the impact of NIs on increasing soil $NH_4^+ -N$ levels, as mentioned in Section 3.3. Thus, keeping in mind that a low-concentrated $CaCl_2$ solution may underestimate the soil $NH_4^+ -N$ availability relative to KCl, it can detect changes in soil $NH_4^+ -N$ levels caused by NIs. Thus, it might be used considering the possibility of easing other measurements (i.e., soil pH), depending on the objectives of the study. For instance, it is not recommended when the objective is to estimate plant N requirements and soil $NH_4^+ -N$ levels are likely to be significant. However, it might be used to assess relative changes in $NH_4^+ -N$ availability caused by fertilizers and NIs or soil amendments (Yang et al. 2023), when $NO_3^- -N$ is the dominant N form as in most soils (Heinemann and Schmidhalter 2021), and when it is of interest to measure multiple nutrients and/or soil pH in the same extract (Houba et al. 2000).

4.3 | Additional Considerations

The opinion article by Pasda et al. (2023) included other comments regarding the scientific rigor of the Guo et al. (2022) study. First, Pasda et al. (2023) pointed out that Guo et al. (2022) did not test four NIs because ENTEC was a nitrogenous fertilizer containing DMPP, and PIADIN is an NI formulation containing two NIs, whereas only DCD and DMPP were pure NIs. We acknowledge that Guo et al. (2022) did not sufficiently differentiate between pure NIs, fertilizers containing NIs, and NI formulations were included by Guo et al. (2022), but this has been clarified in the present study.

Second, Pasda et al. (2023) suggested that the 5% NI application rate used by Guo et al. (2022) was unsuitable when considering EU regulations or rates used in practical agriculture. We reiterate that the aim of that work was not to provide practical information on how to use NIs but to identify how soil moisture and temperature could affect the efficacy of NI over a 60-day period. For this purpose, we considered important to use an equivalent application rate for all inhibitors, and this should be considered when interpreting that study. For the specific case of ENTEC, we acknowledge that a more detailed description of the application rates and its implications should have been highlighted. In this context, the information indicating that ENTEC had low efficiency in Guo et al. (2022) may be misleading because the 5%

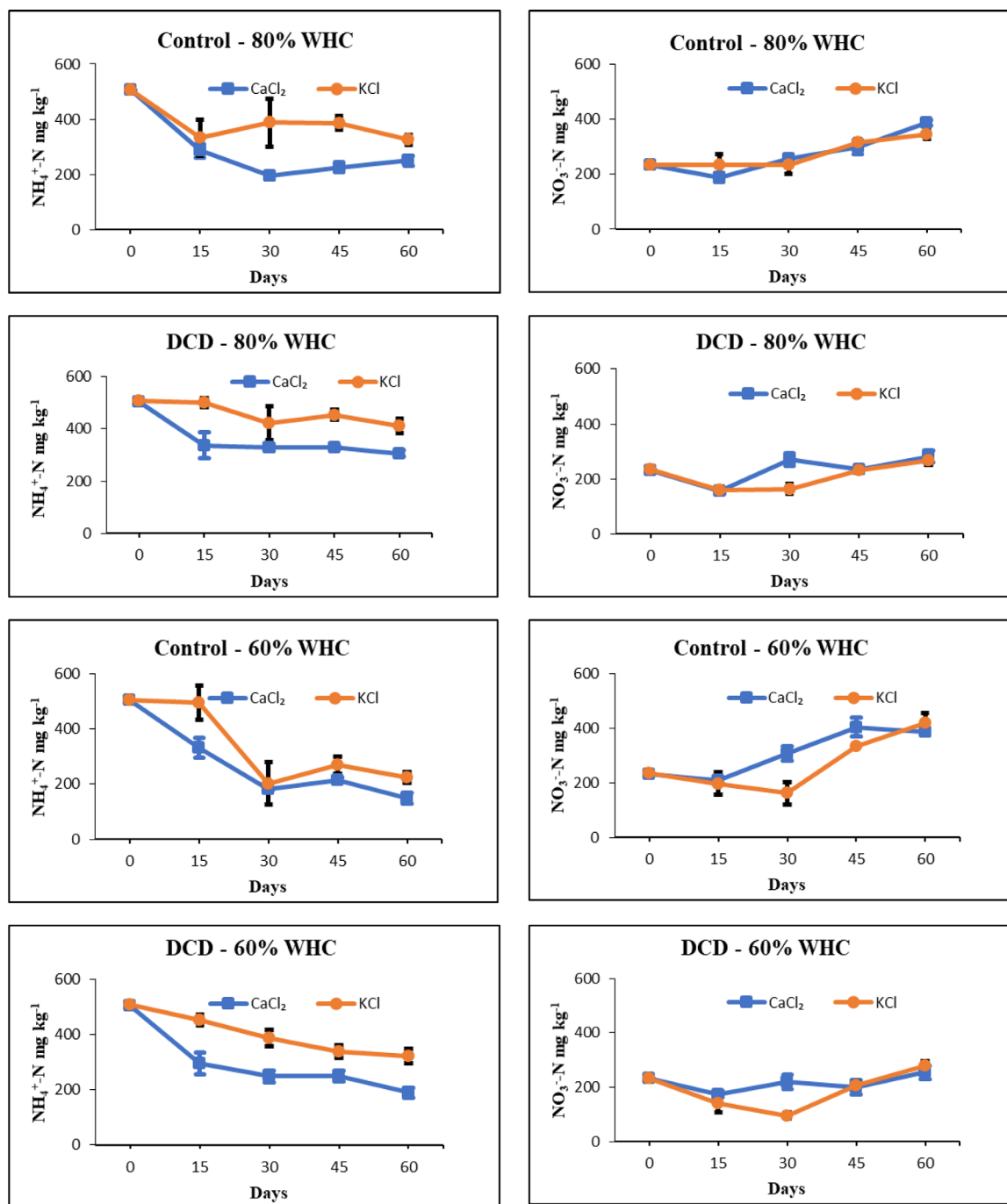


FIGURE 2 | Temporal dynamics of soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations extracted with 1 M KCl and 0.0125 M CaCl_2 solutions from soil with no added NI (control) and soil treated with dicyandiamide (DCD) subjected to 60 and 80% WHC. WHC, water-holding capacity.

application rate was considering the commercial product and not the active ingredient. Therefore, the DMPP concentration in that treatment was only 0.008% of the added $\text{NH}_4^+\text{-N}$, which is below the minimal concentration used in commercial fertilizers (1%) and recommended by EU regulations (Table 2). In the present study, when DMPP concentration was unchanged regarding the ENTEC commercial formulation (1%), the product was effective and, as expected, presented efficacy equivalent to the DMPP treatment, where pure DMPP was applied with ammonium sulfate. For the other treatments, namely, DMPP, DCD, and PIADIN, the data presented in this short communication evidenced that the

conclusions drawn by Guo et al. (2022) remained unchanged after considering Pasda et al. (2023) comments.

Third, Pasda et al. (2023) recommended the retraction of the statement indicating that PIADIN did not reduce $\text{N}_2\text{O-N}$ emissions under field conditions with high soil moisture because it was based only on a “personal communication.” However, this type of citation is widely accepted in scientific communication for information that is not publicly available. Interestingly, one of the references indicated by Pasda et al. (2023) to reinforce the proven efficacy of PIADIN (Ruser and Schulz 2015) includes

“personal communication” citation itself when explaining the mode of action of DMPP and DCD. Furthermore, we agree with Pasda et al. (2023) that the citation of peer-reviewed studies should be preferred. In this context, several published studies have shown that PIADIN, DCD, and DMPP are effective, as indicated by meta-analyses (Fan et al. 2022; Ribeiro et al. 2023; Thapa et al. 2016; Yang et al. 2016). However, there is also plenty of evidence, mainly from field experiments, that these inhibitors might not be effective under specific conditions, which are yet not fully understood. For example, PIADIN did not reduce the annual N₂O-N release in a vegetable production system (Scheer et al. 2017), DCD was ineffective in grasslands, especially at high temperatures (Barneze et al. 2015; Mazzetto et al. 2015), and DMPP did not affect N₂O-N emissions in multilocation field trials in Australia (Nauer et al. 2018). This clearly highlights the relevance of carrying out experiments under controlled and semi-controlled conditions to identify the main soil factors regulating the effectiveness of NIs.

5 | Conclusions

It was confirmed that the dilution of DMPP in ENTEC-treated soil drastically affected the findings reported by Guo et al. (2022). The present study confirmed that ENTEC is an effective DMPP-treated fertilizer in terms of mitigation of N₂O-N emissions under the investigated conditions, without showing differences relative to DMPP, DCD, and PIADIN. Moreover, the high efficacy of DMPP, PIADIN, and DCD was confirmed and remained almost unchanged in relation to Guo et al. (2022). Finally, this study also showed that despite a highly concentrated KCl extraction solution being more accurate for measuring NH₄⁺-N soil availability, a low-concentrated CaCl₂ solution can also detect the effect of NI on soil NH₄⁺-N concentrations and can be used to assess its effectiveness depending on the study context.

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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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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Information file 1: jpln70044-sup-0001-SuppMat1.pdf **Supporting Information file 2:** jpln70044-sup-0002-SuppMat2.xlsx