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**Assessing the Effectiveness of 3, 4-dimethylpyrazole  
phosphate (DMPP) Inhibitor in Mitigating N<sub>2</sub>O Emissions from Contrasting  
Cd-Contaminated Soils**

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## Abstract

Improving nitrogen use efficiency of chemical fertilizers is essential to mitigate the negative environmental impacts of nitrogen. Nitrification, the conversion of ammonium to nitrate via nitrite by soil microbes, is a prominent source of nitrogen loss in soil systems. The effectiveness of nitrification inhibitors in reducing nitrogen loss through inhibition of nitrification is well-documented, however, their efficacy in heavy metals-contaminated soils needs thorough investigations. The current study assessed the efficacy of nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) in reducing nitrous oxide (N<sub>2</sub>O) emissions in cadmium (Cd) contaminated paddy and red soils under lab-controlled environment. Obtained results indicated the substantial reduction in N<sub>2</sub>O emissions with DMPP in paddy and red soil by 48 and 35%, respectively. However, Cd contamination resulted in reduced efficacy of DMPP, thus decreased the N<sub>2</sub>O emissions by 36 and 25% in paddy and red soil, respectively. It was found that addition of DMPP had a significant effect on the abundance of ammonia oxidizing bacteria (AOB) and archaea (AOA). Notably, the reduction in N<sub>2</sub>O emissions by DMPP varied with the abundance of AOB. Moreover, Cd pollution resulted in a significant ( $P < 0.05$ ) reduction in the abundance of archaeal and bacterial *amoA* genes, as well as bacterial *nirK*, *nirS*, and *nosZ* genes. The combined treatment of Cd and DMPP had a detrimental impact on denitrifiers, thereby influencing the overall efficiency of DMPP. These findings provide novel insights into the application of DMPP to mitigate nitrification and its potential role in reducing N<sub>2</sub>O emissions in contaminated soils.

**Keywords:** Nitrification; Denitrification; Cd contamination; 3, 4-dimethylpyrazole phosphate (DMPP); N<sub>2</sub>O emissions; Microbial dynamics

## 1. Introduction

Nitrous oxide ( $\text{N}_2\text{O}$ ) is a hazardous gas that persists in the atmosphere for approximately 114 years. The greenhouse gas ( $\text{N}_2\text{O}$ ) possesses significantly alarming global warming potential, estimated to be around 300 times higher than that of the carbon-dioxide ( $\text{CO}_2$ ) (IPCC, 2013). It is estimated that agricultural activities accounts for 60% shares in collective  $\text{N}_2\text{O}$  emissions (Smith, 2017). Under favorable eco-physiological conditions, the process of microbial denitrification has the ability to decrease the levels of soil nitrate-nitrogen while simultaneously increasing the emissions of  $\text{N}_2\text{O}$ . However, the production of  $\text{N}_2\text{O}$  during nitrification and denitrification process is influenced by various soil properties, including moisture content, pH levels, temperature, aeration, organic matter content, carbon-to-nitrogen ratio (C/N ratio), available nitrogen, soil texture, and agricultural practices such as fertilizer application and crop rotation (Brentrup et al., 2000; Snyder et al., 2009). Nitrification presents promising results for minimizing nitrogen (N) losses in agricultural farmlands, thereby increasing the N utilization efficiency (Chen et al., 2010; Luo et al., 2016; Li et al., 2016). However, excessive use of N fertilizers can stimulate nitrification and denitrification processes, resulting in increased  $\text{N}_2\text{O}$  production by soil microbes ((Shcherbak et al., 2014). Therefore, it is of utmost importance to develop strategies aimed at mitigating  $\text{N}_2\text{O}$  emissions from agricultural soils. Such efforts will not only mitigate environmental concerns but also improve the N use efficiency, as well preventing other harmful consequences of excessive N in the ecosystem (Fan et al., 2019). In the past, 3, 4-dimethylpyrazole phosphate (DMPP) has shown effectiveness as a prominent nitrification inhibitor that reduces N losses by diminishing the ammonia oxidation due to the suppression of ammonia monooxygenase enzyme activity (Di and Cameron, 2012). The

tendency of DMPP to form complexes with membrane proteins contributes to the inhibition of ammonia oxidation, subsequently reducing the production of nitrate and nitrous oxide, which can readily be lost from soil (Florio et al., 2014). Moreover, previous studies have documented the positive influence of DMPP on the populations of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in croplands (Di and Cameron, 2011; Gong et al., 2013). For instance, DMPP application has shown a positive impact on the prevalence of the *nirS* gene in low fertile agricultural soils, which is associated with denitrifying microorganisms (Kastl et al., 2015). Similarly, short-term incubation study has indicated a reduction in AOB population (but not AOA) following DMPP application (Kleineidam et al., 2011; Di and Cameron, 2011). However, the impacts of nitrification inhibitors on ammonia-oxidizing and denitrifying microorganisms, in high-N soils with varying heavy metals contamination, remain unclear.

The presence of heavy metals in soil settings is characterized by their extensive distribution and long-lasting persistence, which have been observed to exert detrimental impacts on various living species (Zhao et al., 2022). The elevated levels of heavy metals in soils and water bodies have been ascribed to both geogenic and anthropogenic activities. Soil contamination by heavy metals is ranked third in Europe and Eurasia, fourth in North Africa, fifth in Asia, and ninth in Sub-Saharan Africa and Latin America (Haris et al., 2021). Similarly, it has been reported that 16% of all Chinese soils and 19.4% of agricultural soils are polluted with heavy metals (People's Republic of China (MEP and MLR), 2014). Among these heavy metals, cadmium (Cd) has seen a global emission of 743.77 tons (Zhao et al., 2022). Moreover, in China, agricultural soils have been partly contaminated by Cd, thus leading to reduced efficiency of farmlands (Zhou et al., 2017). A recent soil survey in China estimated around 7% of soil samples as Cd contaminated, mainly belonging to moderate and severe contamination

(MEP and MLR, 2014). However, due to increasing food demand, abandoning moderately or heavily Cd-polluted soils is not feasible. However, there has been a lack of comprehensive investigation into the emission of greenhouse gases, particularly  $N_2O$ , from soils contaminated with Cd. Furthermore, the precise effects of applying inhibitors on the emission of  $N_2O$  from soils that are contaminated remain uncertain.

Therefore, it is vital to analyze the reactive capacity of DMPP treatment in different contaminated soils for an effective role in agroecosystem. Therefore, to fill the research gap, this study aimed to investigate the efficacy of DMPP treatment in mitigating greenhouse gas emissions in Cd polluted soil. Based on the existing literature, our hypothesis include: (i) the soil pH is a key factor in dictating the efficiency of DMPP in regulating  $N_2O$  emission. (ii) DMPP may have variable effects on  $N_2O$  emissions in contrasting agricultural soils subjected to contamination. For this, microcosm equipment under controlled moisture and temperature levels were deployed using two different soil types. Moreover, employing real-time quantitative PCR (q-PCR), we aim to assess the comparative significance of biotic and abiotic factors in determining the effectiveness of DMPP. The primary research targets are (i) elucidating the individual effect of DMPP on  $N_2O$  emission in different soil types (ii) understanding the N transformation and associated  $N_2O$  emission affected by the presence of Cd, (iii) exploring the interactive effect between DMPP and Cd on  $N_2O$  emissions in contrasting soils, and (iv) shedding light on specific underlying microbial mechanisms that impact the DMPP treatment on  $N_2O$  emission from Cd contaminated soils.

## **2. Materials and methods**

### **2.1. Soil collection**

Two diverse soil types (paddy soil and red soil) with contrasting properties were collected and utilized during the microcosm incubation experiment. The paddy soil originated from an experimental field of Zhejiang University, Hangzhou (30°30' N, 120°09' E), while the red soil was obtained from Anfu county, Ji'an city, Jiangxi province (27°38' N, 114°62' E), China. From each location, a total of twelve soil cores were gathered from the upper 0-20 cm layer and subsequently combined to form a composite sample. This composite sample was then transferred to the laboratory for further procedures. The soil was air dried, followed by grinding and sieving through a 2 mm mesh in order to eliminate larger plant residues and stones. After this, the uniformly mixed and sieved samples were left to air-dry for an additional week, prior to their preparation for various physicochemical analysis. Any remaining soil was stored at room temperature until the commencement of the microcosm experiment.

## 2.2. Soil analysis

Measurement of different soil parameters was conducted to evaluate the initial soil conditions (Table 1). Soil pH was determined by mixing soil and water at a ratio of 1-part soil to 2.50 parts water (w/v, soil/water) and reading was noted with a pH meter (McClellan, 1982). Hydrometer method was used to determine texture of soil sample (Gee and Bauder, 1986). Soil organic carbon (SOC) was assessed by subjecting the soil samples to digestion using potassium dichromate (Nelson and Sommer, 1982). Furthermore, calorimetry method was used to determine soil total nitrogen (N), ammonium ( $\text{NH}_4\text{-N}$ ), and nitrate ( $\text{NO}_3\text{-N}$ ) in 2 M potassium chloride (KCl) extract (Mulveny, 1996). In brief, the soil total nitrogen (TN) was determined by digesting a specific quantity of soil with concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ), and the readings were recorded using a semi-automatic Kjeldahl apparatus. The soil moisture content was

determined using gravimetric method, which involves drying the soil in an oven at 105°C for 24 h, and the resulting weight loss was documented.

**Table 1.** Physio-chemical properties of two soils types selected for this study.

| Soil type | pH   | SOC (gkg <sup>-1</sup> ) | CEC (meq 100g <sup>-1</sup> ) | NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> ) | NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> ) | TN (g kg <sup>-1</sup> ) | Texture |      |      |
|-----------|------|--------------------------|-------------------------------|--|--|--------------------------|---------|------|------|
|           |      |                          |                               |  |  |                          | Clay    | Sand | Silt |
| Paddy     | 7.79 | 3.3                      | 15.8                          | 2.32   | 9.2  | 0.83                     | 36%     | 15%  | 48%  |
| Red       | 5.25 | 4.8                      | 12.3                          | 9.59   | 15.9   | 2.38                     | 21%     | 35%  | 46%  |

SOC= Soil organic carbon

CEC= Cation exchange capacity

NH<sub>4</sub>-N= Ammonium nitrogen

TN= Total nitrogen

#### 2.4. Soil microcosm experiment

The incubation period was initiated by pre-conditioning the collected soil sample at 15 % of their water holding capacity under ambient room conditions for one week. Afterward, 30 g of the pre-incubated soil (measured by dry weight) was placed into sealed 125 mL serum vials. The experiment consists of four treatments including: (i) a control treatment without any additional substance, (ii) alone Cd (Sigma Aldrich, 99 % pure, China) @ 2mg kg<sup>-1</sup> (Cd), (iii) alone DMPP (Maya R, China) (DMPP), and (iv) both DMPP and Cd (DMPP + Cd). During the experiment, urea was applied at a rate of 100 mg N kg<sup>-1</sup> soil in treatments (ii), (iii), and (iv), while treatment (iii) DMPP was applied at a rate of 2 % relative to the urea-N, aligning with the commercially recommended application rate. Moreover, soil moisture content of 60 % was achieved by adding

water at desired intervals. The experiment was conducted in triplicate using a fully randomized approach and measurements were taken on 0, 4, 14, 28, 36, and 48 days of the incubation period. All the vials were incubated in dark conditions at a constant temperature of 25 °C for two months.

## 2.5. N<sub>2</sub>O emission

Headspace gas samples were collected from 10 mL of the sealed bottles using a syringe through the septum and subsequent reading for N<sub>2</sub>O emission was noted using GC-2010 Plus instrument. Subsequently, bottles were opened for a 30 min ventilation before being resealed. Just after the gas samples, soil samples were destructively acquired and separated into two sub-sets, where one sub-set was utilized for soil ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) determination while, second sample was retained for DNA extraction (Mosier et al., 2006).

Formula for calculating N<sub>2</sub>O emission is as follows:

$$F = \frac{273 \times M \times 10^{-3} \times 60 \times 24}{0.13 \times (273 + T) \times \frac{V}{22.4} \times \frac{dc}{dt}}$$

where F denotes N<sub>2</sub>O (μg N<sub>2</sub>O kg<sup>-1</sup> day<sup>-1</sup>), M is the molecular weight of either N in molecular N<sub>2</sub>O (28 g mol<sup>-1</sup>), T represents air temperature (°C) during incubation in the jar, 24 shows the number of hours in a day, V is headspace volume (L), 60 is the number of minutes in an hour, 22.4 (L mol<sup>-1</sup>) is the mole volume of gas at 101 kPa and 273 K, c is the concentration of N<sub>2</sub>O (nL L<sup>-1</sup>), t is the time (min) of jar sealing, dc/ dt is the rate of change in the N<sub>2</sub>O (nL L<sup>-1</sup> min<sup>-1</sup>) concentration after jar sealing, and 0.13 is the dry weight (kg) of soil in the jar.

## 2.6. Potential nitrification rate (PNR)

The potential nitrification rate (PNR) was calculated using the methodology outlined by Rusk et al. (2004). In brief, 30 g of soil from each sample was pre-incubated to achieve a stable state before being employed in the experiment. Subsequently, a 5 g soil subsample was extracted from each container and transferred to individual 100 mL Erlenmeyer flasks. One subsample received  $(\text{NH}_4)_2\text{SO}_4$  to achieve a concentration of  $100 \text{ mg N kg}^{-1}$  dry soil, while other remained untreated, serving as the negative control. The soil-filled flasks were then placed in incubation at  $25^\circ\text{C}$  for 7 days. Subsequently, the soil samples from each treatment were combined with a 2 M KCl solution at a 5:1 ratio of solution to soil. These soil suspensions were vigorously shaken at 200 rpm for 1 h, followed by filtration to remove solid particles. After this, the filtered soil extracts underwent analysis to determine the levels of nitrite nitrogen ( $\text{NO}_2\text{-N}$ ) and nitrate nitrogen ( $\text{NO}_3\text{-N}$ ). Variations in  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations in the soil extracts were calculated to determine the potential nitrification rate.

## 2.7. DNA extraction and real-time quantitative PCR analysis

The DNA extraction from the soil samples (0.5 g) was initiated using Fast DNA SPIN Kit for soil (MP Biomedicals, OH, USA) following the instructions provided by the manufacturer, while the purity and amount of the extracted DNA was assessed using a Colibri Micro volume Spectrometer. The extracted DNA samples were retained at  $-20^\circ\text{C}$  prior to use for subsequent experiments. By using quantitative polymerase chain reaction (qPCR) assay, *nirS*, *nirK*, *nosZ*, AOA, and AOB genes were quantified. The Light Cycler 480 system (Roche Applied Science) was employed for qPCR analysis. The qPCR assays were performed in triplicate for each target gene to ensure the accuracy and reproducibility of the results (Cui et al., 2016; Yin et al., 2017).

Table S2 provides a detailed inventory of the specific primers and PCR conditions employed for amplifying the target genes. These primers are meticulously designed to selectively

target the desired genes for quantification. To facilitate this measurement, standard curves were constructed using a 10-fold sequential dilution of plasmids containing the target gene with known copy numbers. This particular method enables the measurement of target genes in soil samples by comparing the amplification signals to the standard curves. Typically, each reaction mixture contains total volume of 15  $\mu\text{L}$ , including 7.5  $\mu\text{L}$  of SYBR® Premix Ex Taq™, 2  $\mu\text{L}$  of template DNA, and 0.3 milligrams of each primer. SYBR® Premix Ex Taq™ is a widely utilized reagent for real-time PCR, encompassing the essential components necessary for DNA amplification and fluorescence detection.

## 2.8. Statistical analysis

SPSS Statistics 22 software package was used to conduct one-way analysis of variance (ANOVA) to assess the impact of treatments. For the detection of notable dissimilarities among treatment groups, post-hoc comparisons of means were conducted utilizing Duncan's multiple range test at a significance threshold of  $\alpha = 0.05$ . Graphical presentations were generated using Origin Pro 2023b.

## Results and discussion

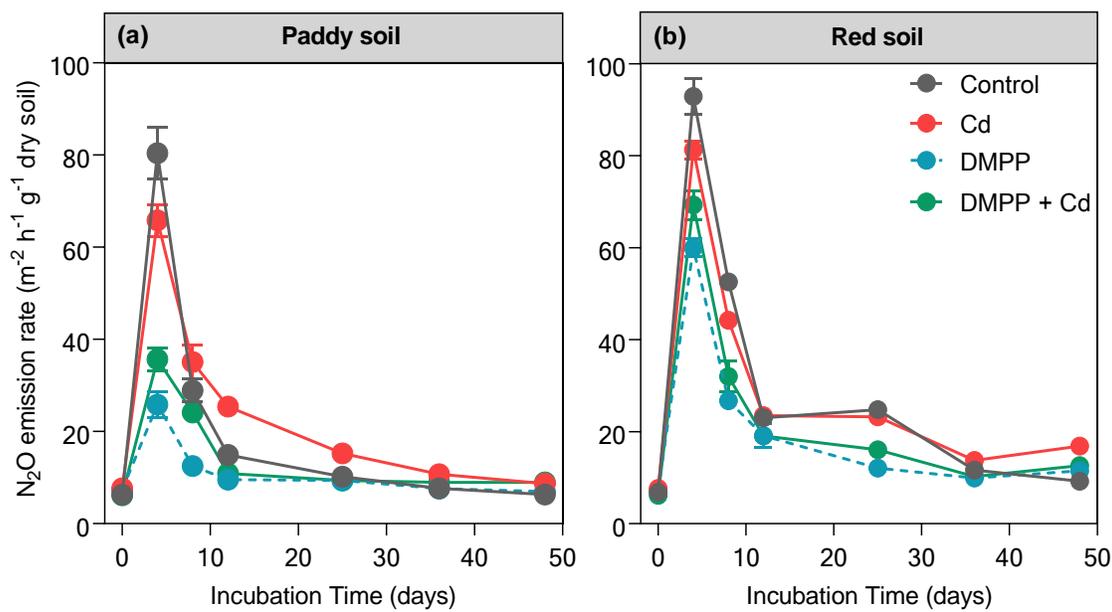
### 3.1. Changes in N<sub>2</sub>O emission

Changes in N<sub>2</sub>O emission from paddy and red soils resulting from treatments including control, Cd, DMPP, and DMPP + Cd throughout the incubation period are presented in Figure 1. It was found that, both soil type and Cd contamination had a significant impact on soil N<sub>2</sub>O emissions. Notably, there was a rapid increase ( $P < 0.05$ ) in N<sub>2</sub>O emission rates of both soils in control (80.3 in paddy and 92.8  $\text{mg m}^{-2} \text{h}^{-1}$  in red soil) and Cd treatment (65.8 in paddy and 81.3  $\text{mg m}^{-2} \text{h}^{-1}$  in red soil) depicting highest values on day 4 (Fig. 1). In contrast, during the first week of incubation, the application of DMPP alone substantially decreased the N<sub>2</sub>O emission from paddy

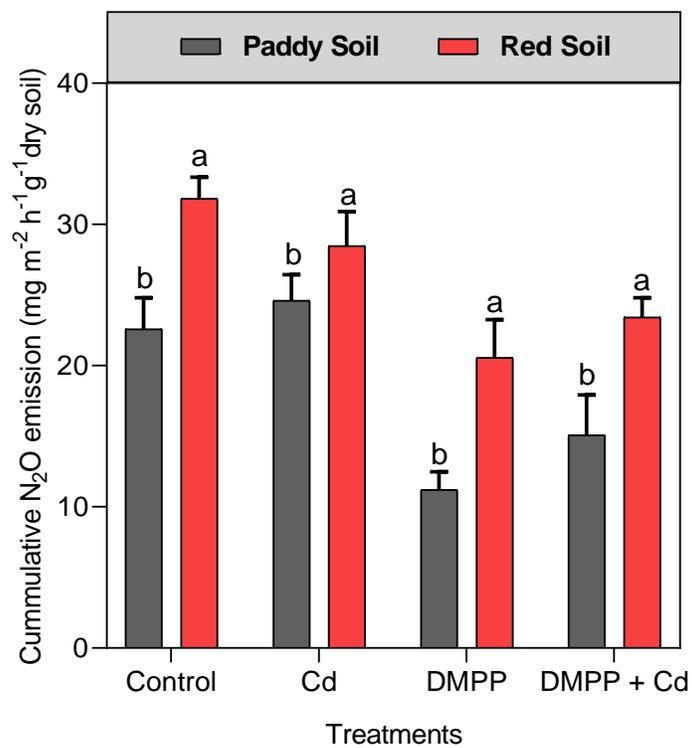
and red soils ( $17.5$  and  $59.2 \text{ mg m}^{-2} \text{ h}^{-1}$ ), respectively. The showed that DMPP had an inhibitory impact of on  $\text{N}_2\text{O}$  emission, reducing it by 35 and 48 % in red and paddy soils, respectively (Fig. 2). Moreover, the combined application of Cd and DMPP also effectively reduced the  $\text{N}_2\text{O}$  emission by 36 and 25 % in paddy and red soil, respectively, however this reduction was lower than that of alone DMPP treatment. It was noticed that soil type had significant impact on both  $\text{N}_2\text{O}$  emissions and efficiency of DMPP in limiting the release of gas. Moreover, different soil types exhibited distinct response to DMPP treatment, showing a varying effect on nitrification process. DMPP was more effective in soil with high pH and active nitrification process. DMPP is an effective inhibitor of nitrification activity, which is usually more pronounced in alkaline soils. the average inhibitory effect of DMPP on  $\text{N}_2\text{O}$  emission ranged between 43 to 70%, presenting least efficiency in acidic (pasture soil) as compared to slightly alkaline soil (vegetable soil) (Shi et al., 2016). Tariq et al. (2022) also observed a positive effect of DMPP in reducing  $\text{N}_2\text{O}$  emission. On the other hand, Cd may also play a role in modifying  $\text{N}_2\text{O}$  emission from soil, however, its ability to diminish or lift  $\text{N}_2\text{O}$  discharge depends on the incubation stage. In this study, Cd treatment stimulated the  $\text{N}_2\text{O}$  emission, which in turn impacted the soil microbial population involved in mitigating  $\text{N}_2\text{O}$  release. However, the impact of Cd on  $\text{N}_2\text{O}$  emission became insignificant with passage of time which might be owed to the changes in soil microbial communities (Afzal et al., 2019). Moreover, the combined treatment of DMPP and Cd may potentially suppress the efficiency of DMPP, hence affecting the growth and functionality of nitrifiers, resulting in inhibited denitrification process (Liu et al., 2018).

A principal component analysis (PCA) using  $\text{NO}_3$ ,  $\text{NH}_4$ , PNR, and functional genes was performed to further clarify the relationship between treatments and  $\text{N}_2\text{O}$  emissions (Fig. 3). PC1 and PC2 accounted for 50.6 and 21.5 % in paddy and 55.5 and 28.2 % in red soil of the

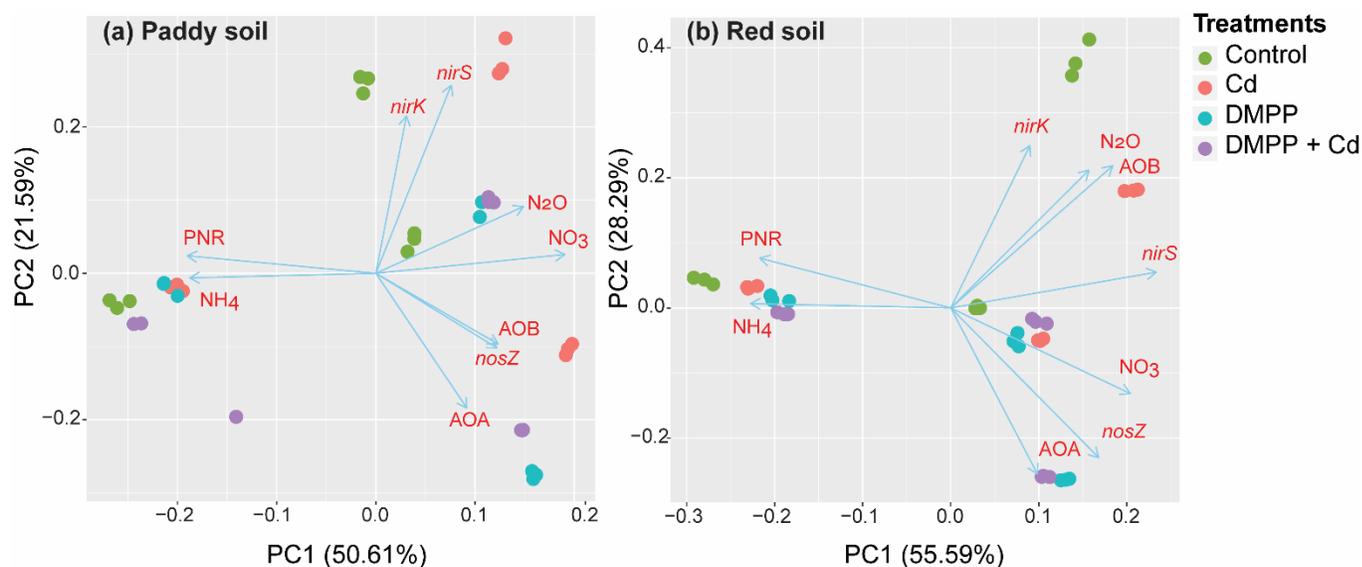
investigated variation, respectively. PC1 had a greater influence on  $\text{NO}_3\text{-N}$  and functional genes, while  $\text{NH}_4\text{-N}$  and PNR were more inclined by PC2. At the same time, each treatment had a good degree of differentiation.



**Fig. 1.** Changes in  $\text{N}_2\text{O}$  emission rates (a, b) across two soils (paddy and red) receiving four treatments (Control, Cd, DMPP and DMPP + Cd) during the experimental incubation. Bars are standard errors of the mean ( $n = 3$ ).



**Fig. 2.** Cumulative N<sub>2</sub>O emission across two soils (paddy and red) receiving four treatments (Control, Cd, DMPP and DMPP + Cd) during the experimental incubation. Bars are standard errors of the mean (n = 3).



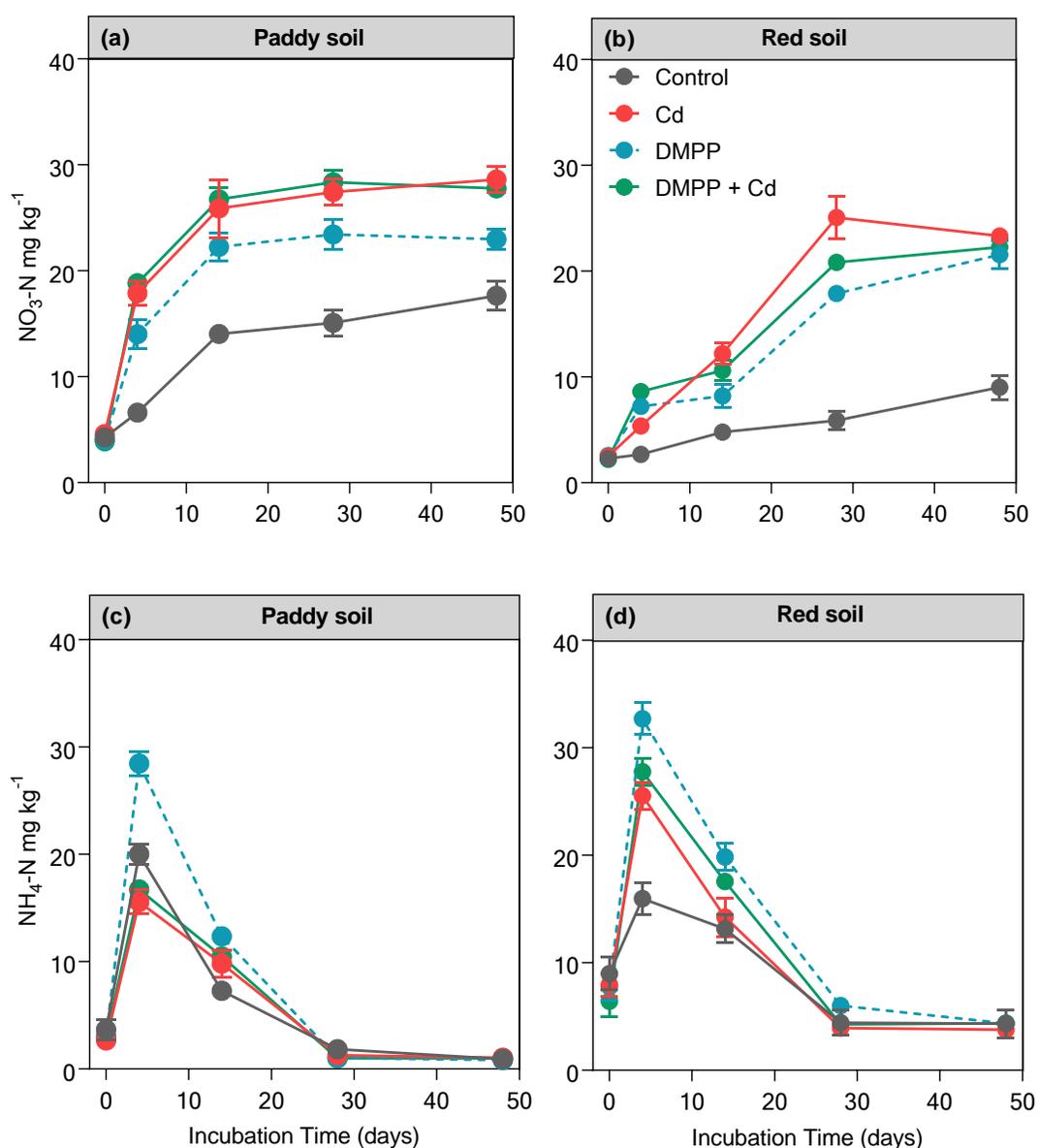
**Fig. 3.** Principle component analysis across two soils (paddy and red) receiving four treatments (Control, Cd, DMPP and DMPP + Cd) during the experimental incubation.

### 3.2. Soil N availability

Figure 4 demonstrated the impact of DMPP and Cd on N availability in tested soils. A gradual increase in  $\text{NO}_3\text{-N}$  concentration was observed over time in both soils in control and Cd treatment with the highest value ( $14$  and  $25 \text{ mg kg}^{-1}$ , respectively) found in paddy soil (Fig.4a). These findings illustrate the presence of the nitrification process, which is responsible for converting ammonium into nitrate. It was observed that DMPP treatment initially increased the  $\text{NO}_3\text{-N}$  release which stabilized after 14 days of incubation, showing the effectiveness of DMPP application in slowing down the nitrification process, resulting in a relative stability of  $\text{NO}_3\text{-N}$  in both soils. In contrast, Cd application in combination with DMPP was helpful in increasing the nitrate availability as compared to alone DMPP treatment. These findings suggest that Cd addition may affect the nitrate availability in soil, ultimately influencing the microbial processes, leading to improved nitrate production. A negative correlation was observed between nitrate and  $\text{N}_2\text{O}$  in both soils (Fig. 7)

Regarding the  $\text{NH}_4^+$ -N content, the soil  $\text{NH}_4^+$ -N levels in Cd treated microcosm showed similar pattern as that of control, which decreased after day 4 and become undetectable after 14 days of incubation. However, DMPP treatment resulted in higher  $\text{NH}_4^+$ -N concentrations after 4 days of incubation which declined gradually over time. The slower fall observed in the alone DMPP treatment suggests that it has a more effective inhibitory effect compared to the combined application (Fig. 4b). These results suggest that DMPP effectively delayed the nitrification process, thus preserving more  $\text{NH}_4^+$ -N in reduced form. Interestingly, in red soil, DMPP exhibited a higher  $\text{NH}_4$ -N content when compared to the control until day 14, implying a pronounced influence of DMPP in maintaining the elevated ammonium levels in red soil which continued till the end of experiment. Positive correlation was observed between ammonia and  $\text{N}_2\text{O}$  in both soils (Fig. 7). The initial rise in  $\text{NH}_4^+$ -N concentration might be attributed to the disruption of microbial processes related to nitrification and denitrification caused by Cd treatment (Afzal et al., 2019). Similar results were reported by (Tessier and Turner, 1995) who reported an inverse relationship between nitrification rates and metals concentration in soil. Similarly, a batch experiment reported an inhibition of  $\text{NH}_4^+$ -N oxidation approximately by 88 % with Cd application (Park and Ely, 2008). Furthermore, Liu et al. (2014) observed a significant decrease in nitrification by a factor of eight when the paddy soil was subjected to a concentration of  $1.4 \text{ mg Cd kg}^{-1}$  (Liu et al., 2014). Our study indicated a decline in nitrifying microorganisms due to a correlation between Cd bioavailability and  $\text{NH}_4^+$ -N concentration, leading to lowering the  $\text{NH}_4^+$ -N oxidation. This might be owed to the Cd contamination which has the potential to hinder the activity of ammonium oxidizers, resulting in a decreased conversion of  $\text{NH}_4$ -N to  $\text{NO}_3$ -N (Afzal et al., 2019). Furthermore, it is worth noting that soil pH counteracts the suppressive effects of DMPP on ammonia oxidation, a phenomenon clearly discerned in our

findings within the context of acidic soil. Furthermore, the existence of Cd pollution can significantly affect the effectiveness of DMPP, potentially causing major changes in the process of nitrification, which in turn, might affect the availability of  $\text{NH}_4\text{-N}$  in the soil (Afzal et al., in 2019). These relationships between soil pH, DMPP, and Cd contamination offer fertile ground for further investigations, with potential implications for optimizing ammonia oxidation and ensuring sustainable soil health.



**Fig. 4.** The dynamics of  $\text{NO}_3^-$  (a–b) and  $\text{NH}_4^+$  (c–d) concentrations within the two test soils (paddy and red) receiving Four treatments (Control, Cd, DMPP and DMPP + Cd) during the incubation period. Bars represent the standard errors of the mean ( $n = 3$ ).

### 3.3. Potential nitrification rate (PNR)

The variations in rates of potential nitrification in paddy and red soil during the incubation period with the application of different treatments are presented in Table 2. Our results showed that control and Cd had the highest PNR throughout the incubation period of both soils, while DMPP treatment led to reduced nitrification rate ( $P < 0.05$ , Table 2) of both soils within first 14 days of incubation. After 4 days of incubation, DMPP application had a significant inhibitory effect by 56 and 30% reduction in paddy and red soil, respectively. This effectiveness of nitrification with DMPP treatment depends on the activity of nitrifying bacteria aimed at converting ammonium to nitrate soil biota. The nitrification inhibitors, specifically DMPP has the capacity to improve N fertilizer use efficiency, regulate nonpoint-source pollution, and reduce soil  $\text{N}_2\text{O}$  emissions (Kong et al., 2017). Previously, a number of mechanisms have been proposed to enlighten the function of DMPP in inhibiting the soil nitrification, including abundance of ammonia-oxidizing archaea (AOA) amoA gene and ammonia-oxidizing bacteria (AOB) amoA gene (Liu et al., 2015) and by affecting the specific microbial cell activities without significant impact on growth of AOA and AOB (Kleineidam et al., 2011; Zhang et al., 2018). Furthermore, it was shown that DMPP suppressed the process of nitrification in the soils by reducing the AOB abundance. Since, paddy soil used in this study has high pH (7.79) and water content, it produces an anaerobic environment, thus limiting the activity of nitrifying bacteria. This type of favorable environment results in the inhibition of the activity of nitrifying bacteria, ensuing a decrease in potential nitrification rates. DMPP treatment decreases the potential nitrification rates by further reducing the activity of nitrifying bacteria.

Our results highlighting the vulnerability of  $\text{NH}_4\text{-N}$  oxidation to the stress induced by Cd. Moreover, it has been previously documented that Cd might induce the  $\text{NH}_4\text{-N}$  oxidation in the soil by affecting the activity and/or functions of involved microbes (Park and Ely, 2008). In this study, combined treatment of Cd and DMPP substantially reduced the PNR in both soils due to reduced activity of nitrifying bacteria (ammonia-oxidizing bacteria (AOB), which are crucial for the process of nitrification. Cd interacts with ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO), which are essential for the conversion of ammonium ( $\text{NH}_4^+$ ) to nitrate ( $\text{NO}_3^-$ ), resulting in a decrease in PNR (Liu et al., 2014). DMPP has the ability to remain in soil for an extended duration, leading to the suppression of AOB activity, ultimately reducing PNR in soil.

**Table 2.** Changes in Potential nitrification rate across two soils (paddy and red) receiving four treatments (Control, Cd, DMPP and DMPP + Cd) during the experimental incubation. Bars are standard errors of the mean (n = 3).

| Soil  | Day | Control       | Cd            | DMPP           | DMPP + Cd      |
|-------|-----|---------------|---------------|----------------|----------------|
| Paddy | 0   | 4.49a ± 0.13  | 4.2ab ± 0.12  | 3.4a ± 0.3     | 4.06a ± 0.14   |
|       | 4   | 2.1b ± 0.06   | 2.4a ± 0.16   | 0.9b ± 0.2     | 2.4b ± 0.42    |
|       | 14  | 2.24a ± 0.21  | 2.3a ± 0.27   | 0.64bc ± 0.02  | 1.8b ± 0.26    |
|       | 28  | 2.5a ± 0.17   | 1.3b ± 0.10   | 0.83b ± 0.06   | 1.2bc ± 0.08   |
|       | 48  | 1.18a ± 0.09  | 0.5b ± 0.09   | 0.41c ± 0.05   | 0.53c ± 0.03   |
| Red   | 0   | 3.03a ± 0.06  | 2.42ab ± 0.08 | 1.69c ± 0.044  | 1.99bc ± 0.104 |
|       | 4   | 2.38a ± 0.03  | 1.93b ± 0.089 | 1.62bc ± 0.045 | 1.82b ± 0.09   |
|       | 14  | 1.02ba ± 0.02 | 0.62bc ± 0.07 | 0.78b ± 0.094  | 0.80b ± 0.066  |
|       | 28  | 0.76a ± 0.04  | 0.61b ± 0.074 | 0.47bc ± 0.04  | 0.53bc ± 0.081 |
|       | 48  | 0.44b ± 0.044 | 0.58a ± 0.03  | 0.22c ± 0.078  | 0.32bc ± 0.093 |

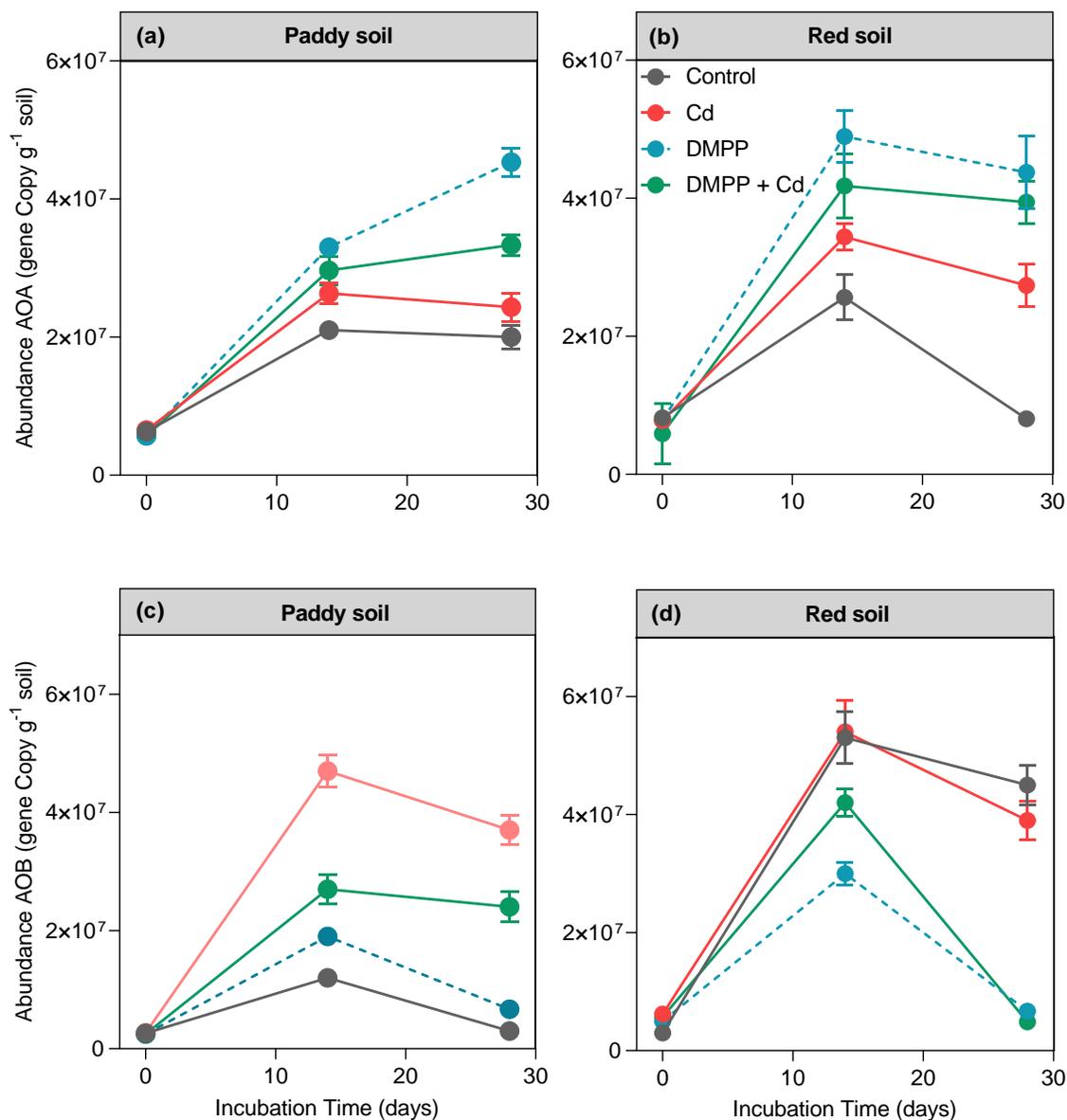
Means (significance tested by LSD;  $P < 0.05$ ) followed by SE the same letter(s) within each column are not significantly different among treatments ( $P < 0.05$ ).

### 3.4. Impact on bacterial amoA and archaeal amoA genes

The AOA abundance remains  $1.5 \times 10^7$  in paddy and  $2.9 \times 10^7$  in red soil, while AOB were found as  $6.8 \times 10^7$  in paddy and  $5.3 \times 10^7$  copies per gram of soil in red soil (Fig. 5a-d). It was noted that AOA and AOB significantly increased to  $6.3 \times 10^3$  to  $4.8 \times 10^7$  and  $3.8 \times 10^7$  to  $5.4 \times 10^7$  copies per gram of soil, specifically in control and Cd treatment, respectively (Fig. 5a-d). Similarly, application of DMPP treatment resulted in a significant increase in AOA abundance from day 14 to 28 in both soils ( $P < 0.05$ , Fig. 5a-b). However, DMPP treatment tends to a significant decrease in the AOB abundance (Fig.5c-d) till 14 days of incubation as compared to Cd alone. In addition, the inhibitory effects of DMPP on AOB were more visible in paddy soil than in red soil.

The impact of inhibitor application on AOB and AOA differs between the two soil types. It was evidenced that DMPP exhibited inhibitory impacts on the growth of AOB in paddy soil as compared to red soil, indicating its efficiency in targeting the N oxidation process mediated by AOB (Di and Cameron, 2011). This study observed an increase in AOA abundance with DMPP treatment as compared to control (Fig. 4a-d) which were in contradiction with an earlier study, which reported which indicated either no influence or a decrease in the AOA population with the use of inhibitors (Duan et al., 2017). However, Hink et al. (2017) documented a substantial increase in proliferation of AOA, while suppressing AOB activity, which aligns with the results of our study. The niche differentiation between soil AOA and AOB might be attributed to several factors such as ammonia limitation, mixotrophy, and pH (Fan et al., 2019).

The above data suggests that AOA may experience enhanced growth in conditions with relatively high  $\text{NH}_4^+$  (ammonium) concentrations, particularly when AOB are inhibited. These results are consistent with Hink et al. (2017), who reported similar patterns for AOA and AOB growth. Moreover, presence of organic compounds may potentially promote the growth of AOA (Tourna et al., 2011). However, although having similar functions, the genetic variations of *amoA* genes between bacterial and archaeal species indicate physiological discrepancies in corresponding *amoA* enzyme (Nicol and Schleper, 2006). Our results also indicated that addition of Cd ensue a decrease in number of *amoA* copies and subsequently decreased the nitrification after 14 days of incubation period. These findings suggest that the reduction in  $\text{NH}_4\text{-N}$  oxidation in Cd-treated soil (Fig. 4) may help in reduced *amoA* gene copies (Wang et al., 2018; Xing et al., 2015). AOB are generally more prone to Cd toxicity as compared to AOA, however, the underlying mechanisms behind the differential sensitivity of AOA and AOB to Cd are not yet fully understood.



**Fig. 5.** Changes in Gene abundance of AOA (a and b) and AOB (c and d) across two soils (paddy and red) receiving four treatments (Control, Cd, DMPP and DMPP + Cd) during the experimental incubation. Bars are standard errors of the mean (n = 3).

### 3.5. Impact on the abundance of *nirK*, *nirS*, and *nosZ* in soil

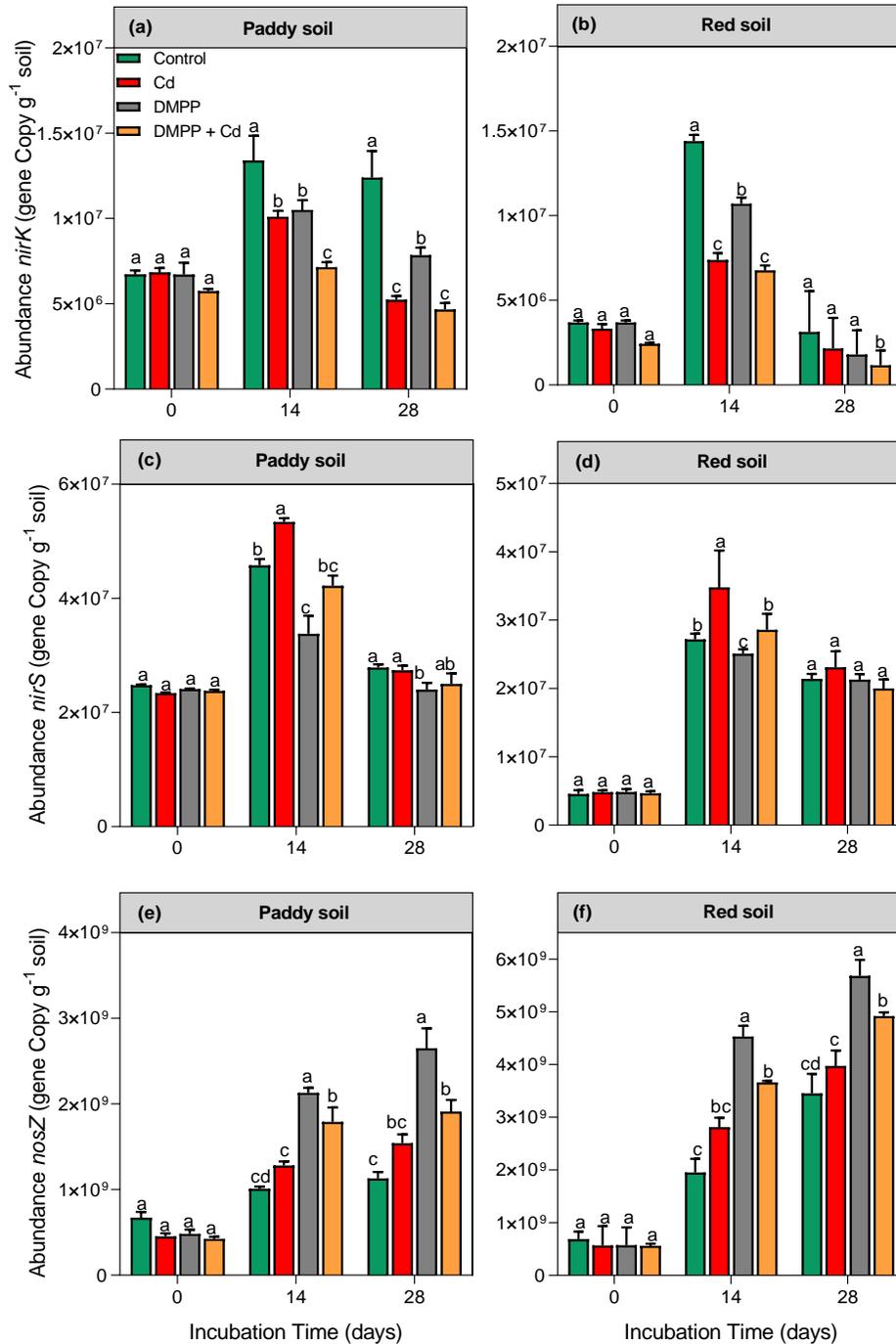
The changes in the abundance of specific genes including *nirK* (a and b), *nirS* (c and d), and *nosZ* (e and f) were observed in both soil types following the application of treatments (Fig. 6a-f). The initial pyramid of the *nirK* gene in control remained  $2.4 \times 10^6$  and  $1.6 \times 10^7$  copies in paddy and red soil, respectively (Fig. 6a and 4b). Cd treatment steadily reduced ( $P < 0.05$ ) the

*nirK* gene abundance from  $1.04 \times 10^7$  at day 14 to  $5.2 \times 10^6$  at day 28 in paddy soil and  $7.38 \times 10^6$  to  $2.3 \times 10^6$  in red soil. Moreover, sole DMPP and combined with Cd effectively decreased the *nirK* gene abundance from  $7.8 \times 10^6$  in paddy and  $1.8 \times 10^6$  in red soil after 28 days of incubation (Fig. 6a and 6b). After 28 days of incubation, the abundance of *nirS* gene was shown to be higher in control, showing values of  $2.7 \times 10^7$  and  $2.1 \times 10^7$  copies  $g^{-1}$  for paddy soil and red soil, respectively. However, the abundance of *nirS* gene was initially enhanced by the Cd treatment, but subsequently decreased by the end of experiment. The results of combined application of DMPP and Cd depicted a significant increase in *nirS* gene within 14 days but exposed a drop till the end of incubation period.

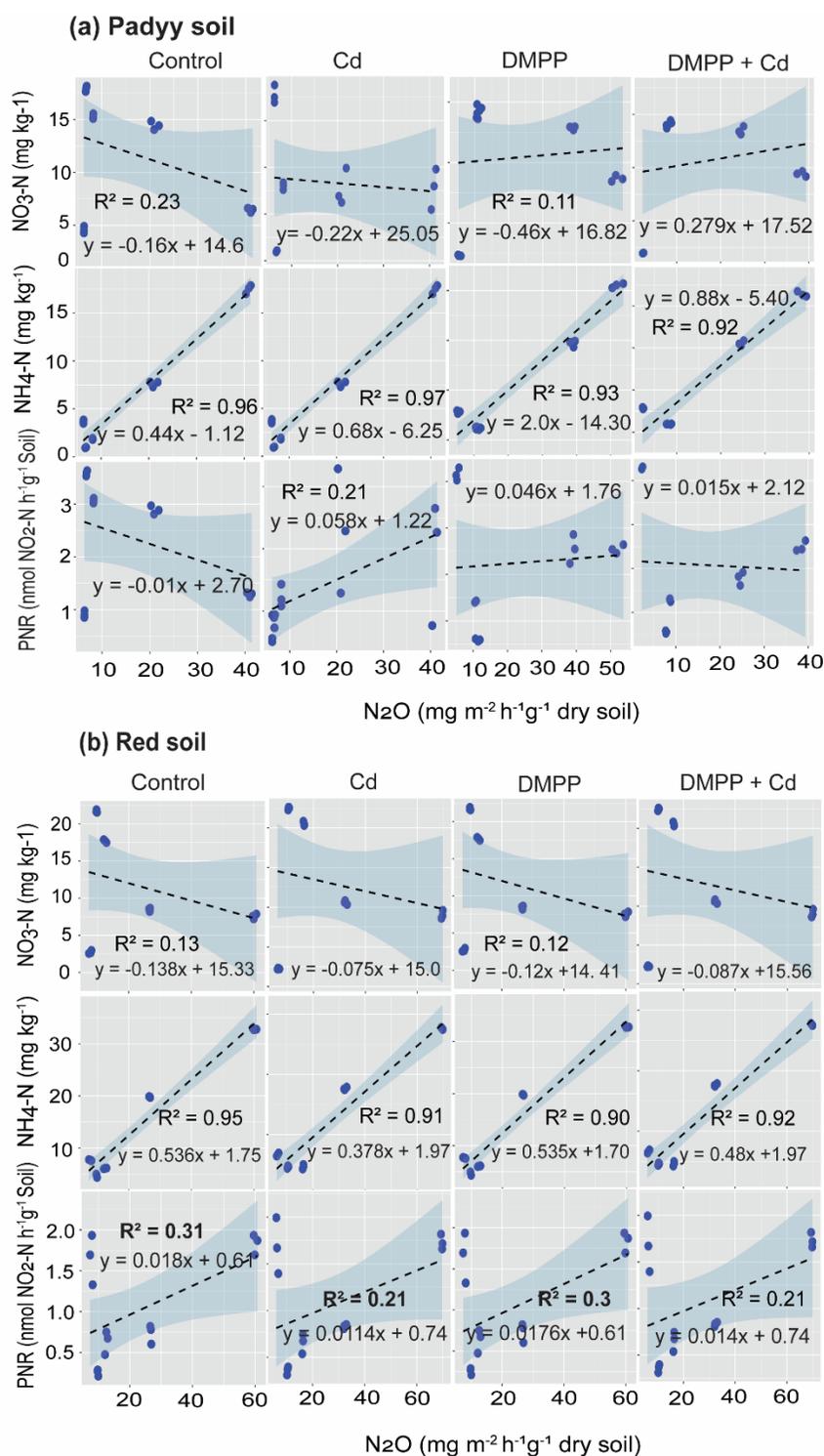
Moreover, the effectiveness of DMPP treatment on *nosZ* gene abundance was also observed in both soils, where DMPP + Cd treatment enriched the copy number of *nosZ* gene (up to  $4 \times 10^9$  and  $6 \times 10^9$  in paddy soil and red soil, respectively). However, it was found that the *nosZ* gene abundance was lower in combined treatment than DMPP alone, which might be owed to the harmful impacts of Cd on microbes. The *nosZ*-type was found to be more abundant as compared to *nirK* and *nirS*-type denitrifiers in both soils, indicating its importance in denitrification process. On the other hand, DMPP alone treatment substantially decreased the *nirK* and *nirS* genes in both soils, causing a decrease in the availability of  $NO_3-N$  (Fan et al., 2019). Denitrification mainly relies on the availability of  $NO_3-N$ , thus the inhibition of nitrification with DMPP leads to lowering the conversion of ammonium to nitrate, ultimately reducing the substrate availability to denitrifiers. Hence, the diminished prevalence of the *nirK* and *nirS* genes due to DMPP treatment indicates a direct influence of DMPP on the population of denitrifiers (Fan et al., 2019). In addition, DMPP may reduce the profusion of *nirK* and *nirS* genes by suppressing the nitrification process. These findings highlight the need to understand

the dynamics of denitrifiers and their gene abundance in response to environmental factors such as nitrification inhibitors.

Abundance of various denitrifier genes can provide insights into the potential involvement of different denitrifier groups in denitrification and N<sub>2</sub>O emission. Cd addition may negatively impact the predominance of denitrifier genes, thus harboring the *nirK*, *nirS*, and *nosZ* genes (Shi et al., 2016). However, it was observed that Cd contamination in paddy soil did not have an impact on the frequency of *nirK* gene ( $P < 0.05$ ) (Fig. 6a-b). The variation in these results may be due to the diversity in soil type as well as levels of Cd contamination between two studies. Recently, Afzal et al., (2019) also reported a decrease in *nirK* due to the bioavailability of Cd in soil in the first half of the incubation. Moreover, the abundance of *nirK* gene due to DMPP treatment might occur because of the changes in AOB abundance, which directly influence the nitrification process in soils while indirectly influence denitrification (Torrallbo et al. 2017). Our results confirm the elevated levels of *nosZ* gene with DMPP treatment, while showed a decline in Cd treated soil. Further investigations are required to determine the optimal field conditions for the effective utilization of inhibitor compounds in order to provide appropriate N management guidance in agricultural output and also to enhance our understanding that how inhibitors affect the activity of soil microbes, involved in N<sub>2</sub>O production in field conditions.



**Fig. 6.** Changes in gene abundance of *nirK* (a and b), *nirS* (c and d) and *nosZ* (e and f) across two soils (paddy and red) receiving four treatments (Control, Cd, DMPP and DMPP + Cd) during the experimental incubation. Means (significance tested by LSD;  $P < 0.05$ ) followed by SE the same letter(s) within each column are not significantly different among treatments ( $P < 0.05$ ).



**Fig. 7.** Scatterplot of the relationship between N<sub>2</sub>O emission and different parameters across two soils (paddy and red) receiving four treatments (Control, Cd, DMPP and DMPP + Cd) during the experimental incubation.

#### **4. Conclusion**

This study aimed to investigate the efficacy of DMPP treatment in mitigating greenhouse gas emissions in Cd polluted soils. The findings revealed the positive contribution of DMPP application on significant reduction of N<sub>2</sub>O emissions from two contrasting soils. The presence of Cd contamination in soil system has been observed to compromise the inhibitory role of DMPP due to its adverse effect on microbial dynamics. The obtained results also suggest that utilization of nitrification inhibitors, such as DMPP in contaminated soils could offer a potential solution for reducing N<sub>2</sub>O emissions. The results of this study reveal the promising impact of utilizing DMPP under field circumstances under the growing emphasis on sustainable agriculture and the implementation of environmental regulations. Therefore, further studies are needed at field level to better understand the role of DMPP in diverse environmental conditions. Thus, continuous research and geographical considerations may, however, play a crucial role in shaping its future role in agriculture.

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**References**

- Afzal, M., Yu, M., Tang, C., Zhang, L., Muhammad, N., Zhao, H., Feng, J., Yu, L., Xu, J. 2019. The negative impact of cadmium on nitrogen transformation processes in a paddy soil is greater under non-flooding than flooding conditions. *Environment International*, 129(April), 451–460. <https://doi.org/10.1016/j.envint.2019.05.058>.
- Brentrup, F., Küsters, J., Lammel, J., Kuhlmann, H. 2000. Methods to estimate on-field nitrogen emissions from crop production as an input to LCA studies in the agricultural sector. *The International Journal of Life Cycle Assessment*, 5, 349–357.
- Chen, Z., Luo, X., Hu, R., Wu, M., Wu, J., Wei, W. (2010). Impact of long-term fertilization on the composition of denitrifier communities based on nitrite reductase analyses in a paddy soil. *Microb Ecol* 60:850–861.
- Cui, P., Fan, F., Yin, C., Song, A., Huang, P., Tang, Y., Zhu, P., Peng, C., Li, T., Wakelin, S. A., Liang, Y. 2016. Long-term organic and inorganic fertilization alters temperature sensitivity of potential N<sub>2</sub>O emissions and associated microbes. *Soil Biology and Biochemistry*, 93, 131–141. <https://doi.org/https://doi.org/10.1016/j.soilbio.2015.11.005>.
- Di, H. J., Cameron, K. C. 2011. Inhibition of ammonium oxidation by a liquid formulation of 3, 4-dimethylpyrazole phosphate (DMPP) compared with a dicyandiamide (DCD) solution in six New Zealand grazed grassland soils. *J Soils Sediments*. 11:1032–1039.
- Di, H. J., Cameron, K. C. 2012. How does the application of different nitrification inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in grazed pastures? *Soil Use Manag.* 28:54–61.
- Duan, Y.-F., Kong, X.-W., Schramm, A., Labouriau, R., Eriksen, J., Petersen, S. O. 2017. Microbial N transformations and N<sub>2</sub>O emission after simulated grassland cultivation: Effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). *Applied and*

- Environmental Microbiology, 83(1). <https://doi.org/10.1128/AEM.02019-16>.
- Fan, X., Yin, C., Chen, H., Ye, M., Zhao, Y., Li, T., Wakelin, S. A., Liang, Y. 2019. The efficacy of 3,4-dimethylpyrazole phosphate on N<sub>2</sub>O emissions is linked to niche differentiation of ammonia oxidizing archaea and bacteria across four arable soils. *Soil Biology and Biochemistry*, 130(May 2018), 82–93. <https://doi.org/10.1016/j.soilbio.2018.11.027>.
- Florio, A., Clark, I. M., Hirsch, P. R., Jhurrea, D., Benedetti, A. 2014. Effects of the nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) on abundance and activity of ammonia oxidizers in soil. *Biol. Fertil. Soils* 50, 795–807.
- Gee, G.W. and Bauder, J. W. 1986. Particle-size analysis. In: Klute A (ed) *Methods of soil analysis. Physical and mineralogical methods. Agronomy monograph, vol 9, 2nd edn.* American Society of Agronomy, Madison, pp 383–411.
- Gong, P., Zhang, L., Wu, Z., Chen, Z., Chen, L. 2013. Responses of ammoniaoxidizing bacteria and archaea in two agricultural soils to nitrification inhibitors DCD and DMPP: a potexperiment. *Pedosphere* 23:729–739.
- Haris, M., Hamid, Y., Usman, M., Wang, L., Saleem, A., Su, F., Guo, J., Li, Y. 2021. Crop-residues derived biochar: Synthesis, properties, characterization and application for the removal of trace elements in soils. *Journal of Hazardous Materials*, 416, 126212. <https://doi.org/https://doi.org/10.1016/j.jhazmat.2021.126212>.
- Hink, L., Nicol, G. W., Prosser, J. I. 2017. Archaea produce lower yields of N<sub>2</sub>O than bacteria during aerobic ammonia oxidation in soil. *Environmental Microbiology*, 19(12), 4829–4837. <https://doi.org/10.1111/1462-2920.13282>.
- IPCC, A. R. 2013. *Climate change 2013: the physical science basis. Contribution of Working*

Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, 1535.

Kastl, E. M., Schloter, H. B., Buegger, F., Schloter, M. 2015. Impact of fertilization on the abundance of nitrifiers and denitrifiers at the root–soil interface of plants with different uptake strategies for nitrogen. *Biol Fertil Soils* 51:57–64.

Kleineidam, K., Košmrlj, K., Kublik, S., Palmer, I., Pfab, H., Ruser, R., Fiedler, S., Schloter, M. 2011. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on ammonia-oxidizing bacteria and archaea in rhizosphere and bulk soil. *Chemosphere*, 84(1), 182–186. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2011.02.086>.

Kong, X., Duan, Y., Schramm, A., Eriksen, J., Holmstrup, M., Larsen, T., Bol, R., Petersen, S. O. 2017. Mitigating N<sub>2</sub>O emissions from clover residues by 3,4-dimethylpyrazole phosphate (DMPP) without adverse effects on the earthworm *Lumbricus terrestris*. *Soil Biology and Biochemistry*, 104, 95–107. <https://doi.org/https://doi.org/10.1016/j.soilbio.2016.10.012>.

Li, J., Luo, J., Shi, Y., Li, Y., Ma, Y., Ledgard, S., Wang, L., Houlbrooke, D., Lindsey, S. 2016. Dung and farm dairy effluent affect urine patch nitrous oxide emissions from a pasture. *Anim Prod Sci* 56:337–342.

Liu, R., Hayden, H., Suter, H., He, J., Chen, D. 2015. The effect of nitrification inhibitors in reducing nitrification and the ammonia oxidizer population in three contrasting soils. *Journal of Soils and Sediments*, 15(5), 1113–1118. <https://doi.org/10.1007/s11368-015-1086-6>.

Liu, Y., Liu, Y., Ding, Y., Zheng, J., Zhou, T., Pan, G., Crowley, D., Li, L., Zheng, J., Zhang, X., Yu, X., Wang, J. 2014. Abundance, Composition and Activity of Ammonia Oxidizer and

- Denitrifier Communities in Metal Polluted Rice Paddies from South China. PLOS ONE, 9(7), e102000. <https://doi.org/10.1371/journal.pone.0102000>.
- Liu, J., Zhang, P., Li, H., Tian, Y., Wang, S., Song, Y., Zeng, G., Sun, C., Tian, Z. 2018. Denitrification of landfill leachate under different hydraulic retention time in a two-stage anoxic/oxic combined membrane bioreactor process: Performances and bacterial community. *Bioresource Technology*, 250, 110–116. <https://doi.org/10.1016/j.biortech.2017.11.026>.
- Luo, J., Ledgard, S., Wise, B., Lindsey S. 2016. Effect of dicyandiamide (DCD) on nitrous oxide emissions from cow urine deposited on a pasture soil, as influenced by DCD application method and rate. *Anim Prod Sci* 56:350–354.
- McClellan, E.O. 1982. Soil pH and lime requirement, pp 209–233. In: Page AL, Miller RH, Keeny DR (eds) *Methods of soil analysis. Part 2, 2nd edn, vol 9*. American Society of Agronomy, pp 199–208.
- MEP, M. L. R. 2014. China National Soil Pollution Gazette. Ministry of Environmental Protection China Ministry of Land and Resources China.
- Mosier, A. R., Halvorson, A. D., Reule, C. A., Liu, X. J. 2006. Net Global Warming Potential and Greenhouse Gas Intensity in Irrigated Cropping Systems in Northeastern Colorado. SDA Agricultural Research Service {Lincoln, Nebraska. <http://digitalcommons.unl.edu/usdaarsfacpub/271>.
- Mulveny, R., L. 1996. Nitrogen inorganic forms. In: Sparks DL et al (eds) *Methods of soil analysis. Part 2. Chemical properties*. SSSA Book Ser. 5. Soil Science Society of America, Madison, pp 1123–1184.

- Nelson, D.W., Sommer, I. E. 1982. Total carbon, organic carbon and organic matter. In: Page AL, Miller RH, Keeny DR (eds) *Methods of soil analysis part 2*, 2nd edn. American Society of Agronomy, Madison, pp 539–577.
- Nicol, G. W., Schleper, C. 2006. Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? *Trends in Microbiology* 14, 207–212.
- Park, S., Ely, R. L. 2008. Candidate stress genes of *Nitrosomonas europaea* for monitoring inhibition of nitrification by heavy metals. *Applied and Environmental Microbiology*, 74(17), 5475–5482. <https://doi.org/10.1128/AEM.00500-08>.
- Rusk, J.A., Hamon, R.E., Stevens, D.P., McLaughlin, M. J. 2004. Adaptation of Soil Biological Nitrification to Heavy Metals. *Environmental Science & Technology*, 38(11), 3092–3097. <https://doi.org/10.1021/es035278g>.
- Shcherbak, I., Millar, N., Robertson, G. P. 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N<sub>2</sub>O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences of the United States of America*, 111. <https://doi.org/10.1073/pnas.1322434111>.
- Shi, X., Hu, H., He, J., Chen, D., Suter, H. C. 2016. Effects of 3,4-dimethylpyrazole phosphate (DMPP) on nitrification and the abundance and community composition of soil ammonia oxidizers in three land uses. *Biology and Fertility of Soils*, 52(7), 927–939. <https://doi.org/10.1007/s00374-016-1131-7>.
- Smith, K. A. 2017. Changing views of nitrous oxide emissions from agricultural soil: key controlling processes and assessment at different spatial scales. In *European Journal of Soil Science* (Vol. 68, Issue 2). <https://doi.org/10.1111/ejss.12409>.
- Snyder, C. S., Bruulsema, T. W., Jensen, T. L., & Fixen, P. E. (2009). Review of greenhouse gas

- emissions from crop production systems and fertilizer management effects. *Agriculture, Ecosystems & Environment*, 133(3–4), 247–266.
- Tariq, A., Larsen, K.S., Hansen, L.V., Jensen, L.S., Bruun, S. 2022. Effect of nitrification inhibitor (DMPP) on nitrous oxide emissions from agricultural fields: Automated and manual measurements. *Science of the Total Environment*. 847: 157650.
- Tessier, A., Turner, D. R. 1995. *Metal speciation and bioavailability in aquatic systems*. Wiley Chichester.
- Torralbo, F., Menendez, S., Barrena, I., Estavillo, J.M., Marino, D., Gonzalez-Murua, C. 2017. Dimethyl pyrazol-based nitrification inhibitors effect on nitrifying and denitrifying bacteria to mitigate N<sub>2</sub>O emission. *Scientific Reports*. 7: 13810.
- Wang, Y., Guo, J., Vogt, R. D., Mulder, J., Wang, J., Zhang, X. 2018. Soil pH as the chief modifier for regional nitrous oxide emissions: New evidence and implications for global estimates and mitigation. *Global Change Biology*, 24(2), e617–e626.  
<https://doi.org/10.1111/gcb.13966>.
- Xing, Y., Si, Y.-X., Hong, C., Li, Y. 2015. Multiple Factors Affect Diversity and Abundance of Ammonia-Oxidizing Microorganisms in Iron Mine Soil. *Archives of Environmental Contamination and Toxicology*, 69(1), 20–31. <https://doi.org/10.1007/s00244-015-0144-9>.
- Yin, C., Fan, F., Song, A., Fan, X., Ding, H., Ran, W., Qiu, H., Liang, Y. 2017. The response patterns of community traits of N<sub>2</sub>O emission-related functional guilds to temperature across different arable soils under inorganic fertilization. *Soil Biology and Biochemistry*, 108, 65–77. <https://doi.org/10.1016/j.soilbio.2017.01.022>.
- Zhang, M., Wang, W., Bai, S. H., Zhou, X., Teng, Y., Xu, Z. 2018. Antagonistic effects of nitrification inhibitor 3,4-dimethylpyrazole phosphate and fungicide iprodione on net

nitrification in an agricultural soil. *Soil Biology and Biochemistry*, 116, 167–170.

<https://doi.org/10.1016/j.soilbio.2017.10.014>.

Zhao, H., Wu, Y., Lan, X., Yang, Y., Wu, X., and Du, L. 2022. Comprehensive assessment of harmful heavy metals in contaminated soil in order to score pollution level. *Scientific Reports*, 12(1), 1–13. <https://doi.org/10.1038/s41598-022-07602-9>.

Zhou, Z. F., Wang, M. X., Zuo, X. H., Yao, Y. H. 2017. Comparative investigation of bacterial, fungal, and archaeal community structures in soils in a typical oilfield in Jiangnan, China. *Arch. Environ. Contam. Toxicol.* 72, 65–77.

#### Credit author statement

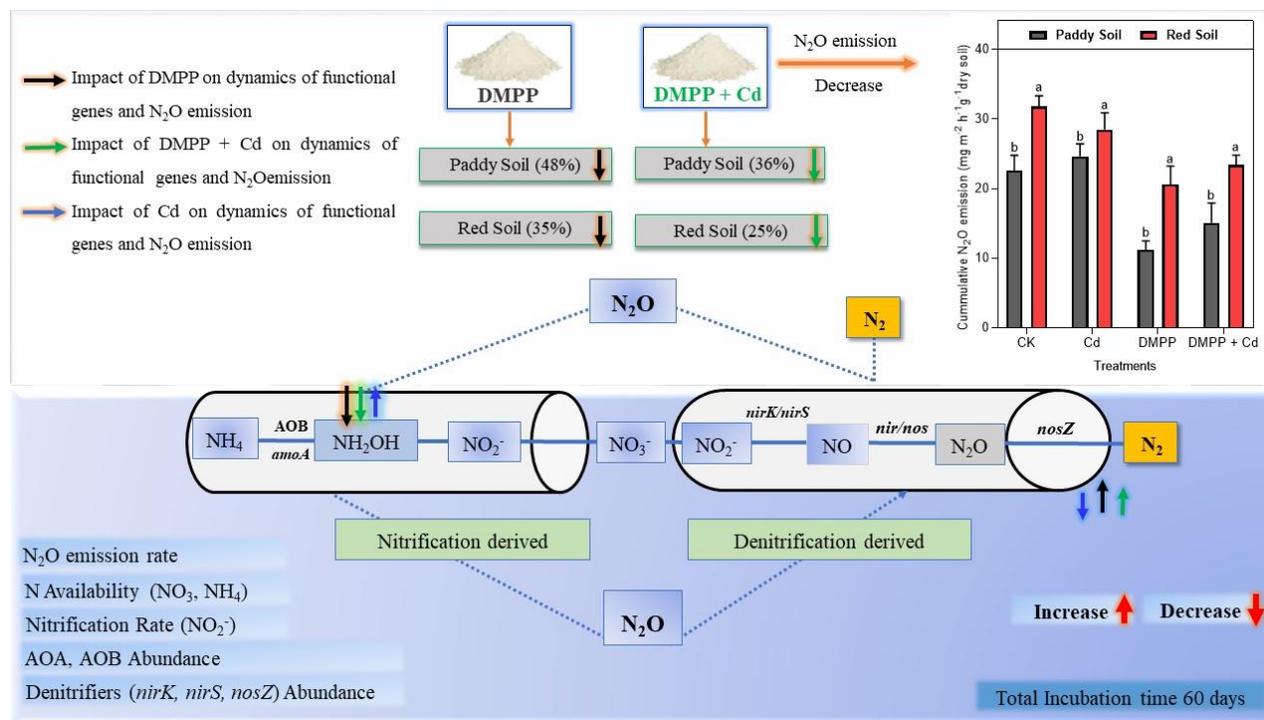
Muhammad Laeeq ur Rehman Hashmi: Methodology, Formal analysis, Original draft writing, Yasir Hamid: Reviewing and editing, Muhammad Usman: Reviewing and editing, Jipeng Luo: Conceptualization, Sangar Khan: Formal analysis, Tang Sheng: Formal analysis, Nabila Bano: Conceptualization, Talha Tufail Bhatti: Reviewing and editing, Tingqiang Li: Supervision, funding acquisition, review and editing, Tingqiang Li:

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

#### Graphical abstract



## Highlights

- DMPP effectively reduced N<sub>2</sub>O emission in paddy soil compared to red soil.
- AOB abundance was reduced in sole DMPP treatment while it increased AOA richness.
- Cd contamination had a negative impact on both AOB and *nosZ* abundance.
- Complex microbial mechanisms explained the differing impacts of DMPP on N<sub>2</sub>O emission in polluted soils.