Inhibitory Effects of 3,4-Dimethylpyrazole Phosphate on CH$_4$ and N$_2$O Emissions in Paddy Fields of Subtropical China

Shan Yin$^{1,2,†}$, Xianxian Zhang$^{1,3,†}$, Zaidi Jiang$^{1,4}$, Penghua Zhu$^{1,2}$, Changsheng Li$^{1,4,‡}$ and Chunjiang Liu$^{1,2,*}$

$^1$ School of Agriculture and Biology and Research Centre for Low Carbon Agriculture, Shanghai Jiao Tong University, 800 Dongchuan Rd., Shanghai 200240, China; yinshan@sjtu.edu.cn (S.Y.); xixinzi01090@163.com (X.Z.); jzd930309@sjtu.edu.cn (Z.J.); penghuazhu@hotmail.com (P.Z.); cs_li_98@yahoo.com (C.L.)

$^2$ Shanghai Urban Forest Research Station, State Forestry Administration, Shanghai 200240, China

$^3$ Eco-Environmental Protection Research Institute, Shanghai Academy of Agricultural Sciences, 1000 Jinxi Road, Shanghai 201403, China

$^4$ Key Laboratory for Urban Agriculture, Ministry of Agriculture, 800 Dongchuan Rd., Shanghai 200240, China

* Correspondence: chjliu@sjtu.edu.cn; Tel.: +86-21-3420-6603

† These authors contributed equally to this work.

‡ Deceased.

Received: 22 August 2017; Accepted: 25 September 2017; Published: 5 October 2017

Abstract: 3,4-Dimethylpyrazole phosphate (DMPP) has been widely employed to reduce nitrogen leaching and greenhouse gas emissions in the soils of dry farmlands. However, the effects of DMPP on the dynamics of nitrogen in paddy fields remain unclear. For this study, treatments with 0%, 0.25%, 0.5%, 1%, or 1.5% DMPP levels of nitrogen fertilization plus urea were designed to determine the effects on greenhouse gas emissions in paddy fields of subtropical China. All DMPP treatments significantly reduced CH$_4$ and N$_2$O emissions, from 54% to 34%, and 94% to 39%, respectively, compared with a urea fertilizer treatment alone. The soil NH$_4^+$ content decreased and NO$_3^−$ increased more slowly with the application of DMPP. The crop yields under the various DMPP treatments showed no significant difference ($p < 0.05$). We concluded that the application of 0.5% and 1% DMPP may significantly reduce CH$_4$ and N$_2$O emissions in contrast to other treatments. This has important implications for the maintenance of rice yields, while reducing greenhouse gas emissions in paddy fields.

Keywords: DMPP; paddy field; urea fertilizer; greenhouse gas emission

1. Introduction

The application of nitrogenous fertilizers increases crop productivity but can cause serious environmental problems. For the last 30 years, nitrogenous fertilizer consumption in China has increased by 3.28-fold; however, nitrogen use efficiency is only 30–35%, which is much lower than the average value (40–60%) at the global scale [1,2]. Increased nitrogen losses through volatilization, leaching, runoff, and denitrification/nitrification are caused by excessive fertilization. In China, nitrogenous fertilization in paddy fields has led to a series of environmental problems, such as water pollution, soil acidification, and greenhouse gas (GHG) emissions [3–5].

Numerous potential methods have been employed to enhance nitrogen utilization, while reducing the GHG emissions that are related to nitrogen fertilizer use. For instance, nitrification inhibitors (NIs) and slow-release fertilizers have been added to chemical fertilizers in order to inhibit NH$_4^+$ conversion to NO$_3^−$ [6–8]. As new chemical compounds that are utilized in agriculture and horticulture, NIs
are effective in preventing the transformation of nitrogen to NO$_3^-$, thus they could increase both the content of NH$_4^+\text{-N}$ and the recovery of nitrogen efficiencies over long periods [9–11]. The application of commonly used NIs has been considered to be an effective strategy to increase crop yields and nitrogen use efficiencies, with mean increases of 7.5% and 12.9%, respectively [12].

As one of the highly effective NIs [13,14], 3,4-Dimethylpyrazole phosphate (DMPP) exhibits highly favorable attributes for optimal nitrification inhibition and non-toxicological or ecotoxicological side effects [13,15]. At a high soil water content (>80%), denitrification is the primary source of N$_2$O, and its emissions may be decreased by 23–45% with the use of DMPP [16]. The impacts of the application of DMPP have been investigated not only as it relates to nitrogen transformation [17], but also N$_2$O and CH$_4$ emissions from soils [18,19], NH$_3$ emissions [20], and nitrifiers and denitrifiers [21] in dry lands. Several experiments have also been conducted on crop yields [19,22].

Rice is an important staple in many parts of the world, and is a semi-aquatic species that grows primarily under flooded lowland conditions in paddies [23]. GHG emission from rice paddies is a major contributor to agricultural emissions. Hence, it is critical to identify and develop effective measures to reduce N$_2$O and CH$_4$ emissions in paddy soils. In the present study, a five-level DMPP experiment was designed for a rice-bean rotation system in an alluvial plain in the northern subtropical area of Central Eastern China. Our aim was to examine the effects of DMPP application on (1) rice yields, and (2) GHG emissions in subtropical China.

2. Materials and Methods

2.1. Experimental Site

The experimental site was located in the Experimental Farm of Shanghai Jiao Tong University (121.49° E, 31.04° N), Minhang District, Shanghai, China. The area is characterized by a humid subtropical climate according to a modified Köppen climate classification. During the experimental period of 2012–2013, the mean annual temperature and precipitation were 18.4 °C and 1242 mm, respectively. The soil was anthrosols according to FAO (Food and Agriculture Organization of the United Nations) classification, and the main properties starting from the surface down to a 10-cm depth were as follows: pH of 7.32, EC of 0.137 ms cm$^{-1}$, total nitrogen content of 1.39 g kg$^{-1}$, available phosphorus content of 9.45 mg kg$^{-1}$, total carbon content of 9.37 g kg$^{-1}$, and cation exchange capacity (CEC) of 17.5 cmol kg$^{-1}$.

2.2. Experimental Design

Three plots with dimensions of 8 m $\times$ 8 m were set for each treatment. The crop rotation was rice (Oryza Sativa L.)/faba bean (Vicia faba L.)/rice (June–October for rice, and November–May for beans).

The field experiment began in 2012 with different concentrations of the DMPP plus urea fertilizer. Urea was added to the fields at a traditional local level of 300 kg N ha$^{-1}$ during the rice growing season. In 2012, DMPP with four levels of nitrogen (0%, 0.5%, 1%, and 1.5%) was applied to the paddy fields. Prior to the rice being transplanted, phosphorus and potassium fertilization was conducted. Urea fertilizer alone, or urea plus DMPP fertilizer was applied early, on 28 June (day 1 following transplantation). The rice was harvested on 26 October of the same year. All plots were regularly irrigated up to a water depth of 10 cm, except for the paddy field drying period, which proceeded from day 14 to 17, and day 105 until the harvesting period.

Following the rice harvest, faba bean seeds were sown on 8 December, 2012, where no fertilizer was applied during the growing season. In May 2013, the faba beans were plowed into the fields without harvesting with green manure prior to rice transplantation.

In 2013, DMPP containing five levels of nitrogen was applied (0%, 0.25%, 0.5%, 1%, and 1.5%) to the fields. Urea with DMPP was applied on 6 June 2013, during the first day of rice transplantation. Additional agricultural managements were the same as those performed in 2012.
All experimental treatments were designed following a completely random order with three replications. Different treatments used in this research were labeled as CK (urea only), 0.25% DMPP, 0.5% DMPP, 1% DMPP, and 1.5% DMPP.

2.3. Measurement of CH$_4$ and N$_2$O Emissions and Crop Yields

GHG emissions were detected using the static chamber/GC system method [24,25]. During the day (9:00 a.m.–12:00 p.m.), gas samples were collected using syringes, transferred into 100-mL aluminum foil bags (Delin, Dalian, China), and immediately brought to the laboratory to analyze N$_2$O and CH$_4$ through configured gas chromatography (Agilent 6890N, Santa Clara, CA, USA). The fluxes of gases were calculated from the rate of gas concentration change during the sampling time. The calculation was as follows:

$$F = \left( \frac{dC}{dt} \right) \times \left( \frac{mP}{ART} \right) \times \left( \frac{mP}{RT} \right)$$  (1)

where \( \left( \frac{dC}{dt} \right) \) is acquired through the linear regression equation. The value \( m \) is the molecular weight of trace gas, \( P \) indicates the atmospheric pressure \( (P = 1.013 \times 10^5 \text{ Pa}) \), \( R \) is the gas constant \( (R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}) \), and \( T \) is the air temperature inside the chamber. \( V, H, \) and \( A \) are the volume, height, and area of the static chamber, respectively.

Before harvest, crop yields from the three selected 0.5 m $\times$ 0.5 m areas were measured for each treatment. Subsequent to drying at 105 °C, the rice biomass was weighed to calculate the crop yield.

2.4. Measurement of Soil NH$_4^+$ and NO$_3^-$ Concentrations

Soil samples were extracted from the plough layer (0–10 cm) once the rice was transplanted. Samples were transferred to the laboratory and stored in a refrigerator at $-20$ °C until they were analyzed. Inorganic N (NO$_3^-$ and NO$_3^-$) from the soil was measured on the extraction of moist field soil.

We removed inorganic N from the paddy soil by shaking 5.0 g of fresh soil into a 50-mL 2 mol L$^{-1}$ KCl solution, which remained therein for 1 h. Samples were then centrifuged and filtered through filter paper (11 µm) to remove particulates or clays, and the filtrate samples were preserved at 4 °C prior to analysis. NH$_4^+$ and NO$_3^-$ concentrations were analyzed by colorimetric determination method using a SmartChem Discrete Auto Analyzer with a detection limit of 0.001 mg/L (SmartChem 200, Alliance, France).

2.5. Data Analyses

The global warming potential (GWP, kg CO$_2$-eq ha$^{-1}$ on a 100-year scale) was calculated to estimate the potential greenhouse effects of CH$_4$ and N$_2$O emissions. This result indicated that CH$_4$ and N$_2$O emissions were converted into the CO$_2$ equivalents via the following equation:

$$\text{GWP} = 21 \times E_{\text{CH}_4} + 310 \times E_{\text{N}_2\text{O}}$$  (2)

where \( E_{\text{CH}_4} \) and \( E_{\text{N}_2\text{O}} \) are the accumulated CH$_4$ and N$_2$O emissions during the rice growing season, respectively. These were used to estimate the potential greenhouse effects of CH$_4$ and N$_2$O emissions by converting them into their CO$_2$ equivalents.

The index of yield-scaled CO$_2$-eq (GWPI, kg CO$_2$-eq kg$^{-1}$ yield) was calculated to indicate the amount of GHG that was consumed during the rice growing period, and to evaluate the environmental effects on crop output. The equation of GWPI is as follows:

$$\text{GWPI} = \frac{\text{GWP}}{\text{Yield}}$$  (3)

All statistical analyses were conducted using OriginPro 8.5.1 (Systat Software Inc., San Jose, CA, USA) and SPSS16.0 (IBM Co., Armonk, NY, USA).
3. Results

3.1. Methane Emissions

During the rice growing season, the CH$_4$ emissions under all DMPP treatments were significantly reduced ($p < 0.05$) compared with the values under the CK treatment (Table 1). CH$_4$ emissions decreased by 33.5–53.9% and 3–94% following the application of DMPP in 2012 and 2013, respectively (Table 1).

Table 1. Seasonal cumulative CH$_4$ and N$_2$O emissions under different treatments in the paddy fields in 2012 and 2013.

| Treatments  | Rice Growing Season | Faba Bean Growing Season | Fallow Season |
|-------------|---------------------|--------------------------|---------------|
|             | 2012 Reduction (%) | 2013 Reduction (%)      | 2012–2013     | Flooded     |
| CH$_4$ emissions (kg C ha$^{-1}$) |                      |                          |               |
| 0.25% DMPP  | -                   | 318.23 ± 6.50 b          | 33.5          | -           |
| 0.5% DMPP   | 250.08 ± 3.64 c     | 220.26 ± 6.52 e          | 53.9          | -           |
| 1% DMPP     | 246.96 ± 4.36 c     | 271.69 ± 8.27 c          | 43.7          | 0.07 ± 0.06 b - |
| 1.5% DMPP   | 297.18 ± 6.06 b     | 253.57 ± 6.49 d          | 47.0          | 0.22 ± 0.06 c - |
| CK          | 464.97 ± 8.36 a     | 478.23 ± 6.05 a          | -             | 0.49 ± 0.07 a 101.51 ± 4.35 |

N$_2$O emissions (kg N ha$^{-1}$)

| Treatments  | Rice Growing Season | Faba Bean Growing Season | Fallow Season |
|-------------|---------------------|--------------------------|---------------|
|             | 2012                 | 2013 Reduction (%)      | 2012–2013     | Flooded     |
| CH$_4$ emissions (kg C ha$^{-1}$) |                      |                          |               |
| 0.25% DMPP  | -                   | 0.056 ± 0.006 b          | 71.6          | -           |
| 0.5% DMPP   | 0.101 ± 0.01 b       | 0.056 ± 0.005 b          | 71.6          | 0.025 ± 0.01 c - |
| 1% DMPP     | 0.050 ± 0.009 c      | 0.011 ± 0.007 d          | 94.4          | 0.052 ± 0.01 b - |
| 1.5% DMPP   | 0.059 ± 0.012 a      | 0.030 ± 0.006 c          | 84.8          | 0.050 ± 0.012 b - |
| CK          | 0.165 ± 0.005 a      | 0.197 ± 0.008 a          | -             | 0.099 ± 0.009 a 0.022 ± 0.011 |

Note: Rice growing seasons were from 29 June to 26 October and 14 June to 11 October in 2012 and 2013, respectively. The faba bean growing season was from 1 November 2012 to 17 April 2013. After the faba beans were harvested, the field was flooded from 3 May to 31 May 2013. The different letters represent a significant difference ($p < 0.05$) among all the treatments. DMPP: 3,4-Dimethylpyrazole phosphate.

During the period of continuous flooding, the CH$_4$ emissions gradually increased; however, they rapidly dropped to almost zero after a few days, due to midseason aeration. The CH$_4$ emissions increased again subsequent to re-flooding. A strong seasonal variation was characterized by two pronounced higher values. The first peak occurred in the early growing period (June to July), whereas the second peak occurred during the reproduction stage of rice plants in August (Figure 1). Most of CH$_4$ emissions in the atmosphere were observed during the rice growing season, and were rarely observed during the faba bean growing season.
3.2. Nitrous Oxide Emissions

For all of the treatments, a similar variable pattern of N\textsubscript{2}O emissions was observed throughout the year (Figure 2). Following the first week of flooding/fertilization, N\textsubscript{2}O was observed to decrease from 11.98 g N ha\textsuperscript{-1} d\textsuperscript{-1} to 0 g N ha\textsuperscript{-1} d\textsuperscript{-1}, which quickly increased at the onset of the midseason aeration, and then just as quickly decreased. The N\textsubscript{2}O emissions decreased with the addition of DMPP during the entire season. All treatments with DMPP, particularly at the 1% level, demonstrated lower N\textsubscript{2}O emissions than the control. The cumulative N\textsubscript{2}O emissions at 0.25%, 0.5%, 1%, and 1.5% DMPP levels accounted for approximately 28.4%, 28.4%, 5.6%, and 15.2% of the CK treatments, respectively (Table 1).

More N\textsubscript{2}O was released into the ambient atmosphere from the paddy field during the rice growing season, in contrast with the faba bean growing season. The addition of DMPP resulted in lower levels of N\textsubscript{2}O emissions compared to the control (Table 1).
3.3. Crop Yield

Under all treatments, the differences in the mean yields were not statistically significant (Table 2). Compared with CK, the treatments with 0.5% and 1% DMPP showed higher yields. GWPI indicated the yield-scale warming potential, as shown in Table 2. The GWPI was decreased by 33.3%, 56.9%, 47.1%, and 47.7% with the application of 0.25%, 0.5%, 1%, and 1.5% DMPP compared with the CK treatment, respectively.

Table 2. Rice yield and GWPI under different treatments in the paddy fields in 2012 and 2013.

| Treatments | Crop yields (kg ha\(^{-1}\)) | GWP (kg CO\(_2\)-eq ha\(^{-1}\)) | GWPI (kg CO\(_2\)-eq kg\(^{-1}\) yield) |
|------------|-----------------------------|----------------------------------|--------------------------------------|
|            | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 |
| 0.25% DMPP | 8764.24 ± 300.86 a | - | 8937.72 | - | 1.020 | - |
| 0.5% DMPP  | 8825.00 ± 116.59 a | 9365.13 ± 337.33 a | 7051.44 | 6194.56 | 0.799 | 0.661 |
| 1% DMPP    | 9047.32 ± 378.76 a | 9336.67 ± 288.95 a | 6939.24 | 7612.68 | 0.767 | 0.815 |
| 1.5% DMPP  | 9002.43 ± 207.31 a | 8880.77 ± 441.30 a | 8349.78 | 7114.57 | 0.928 | 0.801 |
| CK         | 8993.97 ± 100.03 a | 8820.87 ± 254.56 a | 13099.54 | 13486.41 | 1.456 | 1.529 |

Note: Mean ± standard error of three replicates is shown in the table. The different letters represent a significant difference (\(p < 0.05\)) among all the treatments. GWPI: The index of yield-scaled CO2-eq; GWP: The global warming potential.

3.4. Soil Inorganic N Concentration

Higher soil NH\(_4\)^+ concentrations existed under urea + DMPP treatments compared to the urea only treatment. Following the application of urea fertilizer, the soil NH\(_4\)^+ content decreased, and
NO$_3^-$ increased more slowly with the DMPP application (Figure 3). In 2012, the mean soil NH$_4^+$ concentration was 2.79 mg kg$^{-1}$, 1.75 mg kg$^{-1}$, 3.00 mg kg$^{-1}$, and 1.96 mg kg$^{-1}$ for 0.5%, 1%, and 1.5% DMPP and CK treatments, respectively; the mean soil NO$_3^-$ concentration was 2.89 mg kg$^{-1}$, 3.88 mg kg$^{-1}$, 3.65 mg kg$^{-1}$, and 1.84 mg kg$^{-1}$ for the four treatments, respectively (Figure 3). In 2013, the mean soil NH$_4^+$ concentration was 4.61 mg kg$^{-1}$, 5.37 mg kg$^{-1}$, 4.68 mg kg$^{-1}$, 4.22 mg kg$^{-1}$, and 4.22 mg kg$^{-1}$ for 0.25%, 0.5%, 1%, and 1.5% DMPP and CK treatments, respectively; the mean soil NO$_3^-$ concentration was 3.44 mg kg$^{-1}$, 4.67 mg kg$^{-1}$, 5.53 mg kg$^{-1}$, 4.41 mg kg$^{-1}$, and 6.34 mg kg$^{-1}$ (Figure 3).

**Figure 3.** Variation of soil inorganic N concentration under different DMPP treatments.

CH$_4$ emissions for all treatments had negative correlations with soil NH$_4^+$ concentrations, and positive correlations with soil NO$_3^-$ concentrations (Table 3). There was no significant relationship between N$_2$O emissions and soil inorganic N concentrations.

**Table 3.** Pearson correlation between greenhouse gas emissions and soil inorganic N concentrations in the paddy fields.

|          | CH$_4$ | N$_2$O | NH$_4^+$  | NO$_3^-$ |
|----------|--------|--------|-----------|----------|
| CH$_4$   | 1      | -0.154 | -0.570 ** | 0.439 ** |
| N$_2$O   | 1      | 0.068  | -0.161    |          |
| NH$_4^+$ | 1      | -0.323 |          |          |
| NO$_3^-$ | 1      |        |          |          |

Note: ** Correlation is significant at the 0.01 level (two-tailed); * Correlation is significant at the 0.05 level (two-tailed).

4. Discussion

4.1. Seasonal Variation of CH$_4$ and N$_2$O Emissions

Our results indicated that there was an evident variation in the CH$_4$ and N$_2$O emissions from paddy fields during the rice growing season in contrast to the faba bean growing season, with higher emission rates compared to those reported in previous studies. For instance, the highest and lowest CH$_4$ emission values observed in Japan were 4.25 and 0.0062 kg C ha$^{-1}$ day$^{-1}$, respectively [26]. Other research [27] showed that CH$_4$ emissions ranged from 0.17 kg C ha$^{-1}$ day$^{-1}$ to 0.63 kg C ha$^{-1}$ day$^{-1}$ during the rice growing season in Hubei Province, China. With three rotations, the relatively lower CH$_4$ emission, which occurred during the non-rice periods, accounted...
for 16–49% of the total annual emissions [28]. Nitrous oxide emissions from dry farmlands or paddy fields varied from 0.0017 g N ha\(^{-1}\) day\(^{-1}\) to 0.0296 g N ha\(^{-1}\) day\(^{-1}\) during the upland crop season, and the net average of \(\text{N}_2\text{O}\) emissions during the rice growing season was 0.0119 g N ha\(^{-1}\) day\(^{-1}\) [29]. For paddy fields, 25–39% of \(\text{N}_2\text{O}\) was generated during the rice growing season, with the remainder being formed during the off season [30,31].

Following the harvesting of rice, paddy fields serve as a minor source of \(\text{CH}_4\), which contribute only ~1% of the total \(\text{CH}_4\) emissions during the rice growing season. The fields become a significant source of \(\text{N}_2\text{O}\), accounting for 40–50% of annual emissions [32].

### 4.2. Inhibition of DMPP on \(\text{CH}_4\) and \(\text{N}_2\text{O}\) Emissions

Our results clearly showed that DMPP substantially inhibited \(\text{CH}_4\) and \(\text{N}_2\text{O}\) emissions, with reductions of 34–54% and 39–94%, respectively, compared with the control treatment during the rice growing season. According to a meta-analysis (111 records from 39 studies), DMPP is effective in reducing \(\text{N}_2\text{O}\) emissions, with the highest inhibitory effect of 40% across all land-types, and 27% in paddy fields [33]. These data suggest that the application of DMPP in paddy fields is a feasible way to reduce GHG emissions, while enhancing the efficiency of nitrogen fertilizers. The basic mechanism is that DMPP can not only inhibit the first step of nitrification, but also can slow down the rate of \(\text{NH}_4^+\) oxidation, and delay the transformation of \(\text{NH}_4^+\) to \(\text{NO}_3^-\) in the soil. This is because DMPP can repress the activities of \textit{Nitrosomonas} bacteria [13,34] and inhibit the growth of ammonium-oxidizing bacteria (AOB) and ammonium-oxidizing archaea (AOA) [35–38].

In flooding paddy fields, oxygen is present at the floodwater/surface soil interlayer and in the rice rhizosphere [39,40]. In these areas, \(\text{N}_2\text{O}\) emissions may be observed via the nitrification of ammonium and the denitrification of accumulated nitrate subsequent to the application of nitrogen fertilizers (e.g., urea) [41–44]. The NI (DMPP) used in this study demonstrated some specific effects on nitrogen-molecule transformation, and thus influenced the GHG emissions.

Numerous controversial reports regarding the effects of nitrification-inhibited \(\text{CH}_4\) emissions exist. \(\text{CH}_4\) emissions caused by DMPP treatments are significantly lower than that caused by the treatment without DMPP, which may be attributed to the significant effect of DMPP on \(\text{CH}_4\) oxidation [19]. However, some reports revealed that DMPP exerted no obvious effect on \(\text{CH}_4\) emissions [16]. Another study observed that DMPP positively influenced the reduction of \(\text{CH}_4\) emissions [14,45]. In this study, lower emissions of \(\text{CH}_4\) occurred after treatment with DMPP + urea, compared with that observed with the urea treatment alone.

We observed the impact of DMPP on \(\text{CH}_4\) emissions in paddy fields and found a significant reduction in emissions. The application of urea may promote \(\text{NH}_4^+\) and \(\text{NO}_3^-\) content in irrigated rice paddies. In this study, soil \(\text{NH}_4^+\) content decreased, whereas \(\text{NO}_3^-\) increased more slowly with the application of DMPP, with similar results under the application of NIs (e.g., dicyandiamide, neem, and nimin) plus urea, compared with those observed with the application of urea alone [11,46]. It is likely that \(\text{NH}_4^+\) inhibited the emission of \(\text{CH}_4\) and elevated \(\text{CH}_4\) oxidation caused by fertilization [6,47]. In this study, inorganic soil N concentration had a strong relationship with \(\text{CH}_4\) emissions, particularly a negative relationship for \(\text{NH}_4^+\) concentrations and \(\text{CH}_4\) emissions, and a positive relationship for \(\text{NO}_3^-\) concentrations and \(\text{CH}_4\) emissions. Urea acts as an electron donor that increases the methanotrophic microbial population, while simulating the oxidation of \(\text{CH}_4\) [48]. As observed in the present study, Bodelier [47,49] indicated that \(\text{NH}_4^+\)-promoting methane oxidation dominated the rice ecosystem.

### 4.3. Optimal Quantity of DMPP Application

Our results suggested that there was maximal reduction in \(\text{CH}_4\) (39%) and \(\text{N}_2\text{O}\) (34%) emissions with 0.5% and 1% DMPP of nitrogen fertilizers in the paddy fields. Compared with other experiments, there was an evident variation in the quantity of DMPP applied with regard to soil type, climate conditions, crops, reduction of \(\text{CH}_4\) and \(\text{N}_2\text{O}\), etc. For instance, the application of DMPP with
0.5% urea-N in greenhouse vegetable soils significantly reduced N$_2$O emissions and acted to delay ammonia oxidation [50]. Within a specific temperature range (5–35 °C), DMPP with 0.39% urea-N (1.84 kg t$^{-1}$ urea) had the capacity to inhibit N$_2$O emissions, with a 14–76% reduction in pasture soils and a 19–99% reduction in Pin Gin, Mackay, and Dookie soils; the effectiveness in reduction of the experiments decreased with increasing temperatures [51]. With 0.42% nitrogen fertilizer as an active ingredient (3 mg kg$^{-1}$ soil DMPP with 715 mg N kg$^{-1}$ soil), DMPP was observed to slow NH$_4^+$ oxidation considerably, and reduced N$_2$O emissions by 83–95% under both 40% and 60% WFPS (water-filled poresize) [18]. The addition of 1% DMPP decreased the cumulative N$_2$O emissions of soils by 73.4% [52]. DMPP with mineral fertilizers, and at a low concentration of 1%, specifically inhibited nitration and stabilized NH$_4^+$ for several weeks [13]. Thus, the optimal quantity of DMPP application is contingent on soil type, crops, fertilizers, and climate.

5. Conclusions

Our results indicated that the application of DMPP with nitrogen fertilizers is a feasible way to reduce N$_2$O and CH$_4$ emissions in paddy fields, where the effectiveness of the reduction is contingent on the levels of DMPP that are applied. The 0.5–1% DMPP nitrogen fertilizer was found to be optimal in consideration of the reduction of CH$_4$ and N$_2$O emissions, as well as GWPI. These results have important implications in agricultural management as a strategy to mitigate GHG emissions.

Acknowledgments: This research was co-funded by the National Natural Science Foundation of China (31400605 and 71333010), The National Key Research and Development Plan of China (2017YFD0800204), and Shanghai Landscaping and City Appearance Administrative Bureau (G171206).

Author Contributions: Chunjiang Liu, Shan Yin and Xianxian Zhang conceived and designed the experiments; Shan Yin, Xianxian Zhang and Penghua Zhu performed the experiments; Shan Yin, and Xianxian Zhang analyzed the data; Shan Yin, Xianxian Zhang, Zaidi Jiang and Changsheng Li wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Fan, M.; Shen, J.; Yuan, L.; Jiang, R.; Chen, X.; Davies, W.J.; Zhang, F. Improving crop productivity and resource use efficiency to ensure food security and environmental quality in China. J. Exp. Bot. 2011, 63, 13–24. [CrossRef] [PubMed]
2. Zhang, F.; Wang, J.; Zhang, W.; Cui, Z.; Ma, W.; Chen, X.; Jiang, R. Situation and counter measures of nutrient utilization efficiency for major cereal crops in China. Acta Pedol. Sin. 2008, 45, 915–924.
3. Fageria, N.; Baligar, V. Enhancing nitrogen use efficiency in crop plants. Adv. Agron. 2005, 88, 97–185.
4. Ju, X.; Liu, X.; Zhang, F.; Roelcke, M. Nitrogen fertilization, soil nitrate accumulation, and policy recommendations in several agricultural regions of China. Ambio A J. Human Environ. 2004, 33, 300–305. [CrossRef]
5. Liu, G.; Wu, W.; Zhang, J. Regional differentiation of non-point source pollution of agriculture-derived nitrate nitrogen in groundwater in northern China. Agric. Ecosyst. Environ. 2005, 107, 211–220. [CrossRef]
6. Xu, X.; Boeckx, P.; Van Cleemput, O.; Zhou, L. Urease and nitrification inhibitors to reduce emissions of CH$_4$ and N$_2$O in rice production. Nutr. Cycl. Agroecosyst. 2002, 64, 203–211. [CrossRef]
7. Yu, Q.; Chen, Y. Effect of nitrification inhibitor 3,4-dimethylpyrazole phosphate on nitrogen transformation in vegetable soil. J. Soil Water Conserv. 2010, 24, 123–126.
8. Yu, Q.; Chen, Y. Influences of nitrification inhibitor 3,4-dimethylpyrazole phosphate on nitrogen transformation and potential runoff loss in rice fields. China Environ. Sci. 2010, 30, 1274–1280.
9. Slagen, J.; Kerkhoff, P. Nitrification inhibitors in agriculture and horticulture: A literature review. Fertil. Res. 1984, 5, 1–76. [CrossRef]
10. Freney, J.; Chen, D.; Mosier, A.; Rochester, I.; Constable, G.; Chalk, P. Use of nitrification inhibitors to increase fertilizer nitrogen recovery and lint yield in irrigated cotton. Fertil. Res. 1993, 34, 37–44. [CrossRef]
11. Majumdar, D.; Kumar, S.; Pathak, H.; Jain, M.; Kumar, U. Reducing nitrous oxide emission from an irrigated rice field of north India with nitrification inhibitors. Agric. Ecosyst. Environ. 2000, 81, 163–169. [CrossRef]
12. Abalos, D.; Jeffery, S.; Sanz-Cobena, A.; Guardia, G.; Vallejo, A. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agric. Ecosyst. Environ.* 2014, 189, 136–144. [CrossRef]

13. Zerulla, W.; Barth, T.; Dressel, J.; Erhardt, K.; von Locquenghien, K.H.; Pasda, G.; Rädle, M.; Wissemeyer, A. 3,4-dimethylpyrazole phosphate (DMPP)—A new nitrification inhibitor for agriculture and horticulture. *Biol. Fertil. Soils* 2001, 34, 79–84. [CrossRef]

14. Weiske, A.; Benckiser, G.; Herbert, T.; Ottow, J. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. *Biol. Fertil. Soils* 2001, 34, 109–117.

15. Zerulla, W.; Pasda, G.; Hähndel, R.; Wissemeyer, A. The new nitrification inhibitor DMPP (ENTEC®) for use in agricultural and horticultural crops—An overview. *Plant Nutr.* 2001, 92, 754–755.

16. Menéndez, S.; Barrena, I.; Setien, I.; González-Murua, C.; Estavillo, J.M. Efficiency of nitrification inhibitor dmpp to reduce nitrous oxide emissions under different temperature and moisture conditions. *Soil Biol. Biochem.* 2012, 53, 82–89. [CrossRef]

17. Yu, Q.; Chen, Y.; Ye, X.; Tian, G.; Zhang, Z. Influence of the DMPP (3,4-dimethylpyrazole phosphate) on nitrogen transformation and leaching in multi-layer soil columns. *Chemosphere* 2007, 69, 825–831. [CrossRef] [PubMed]

18. Chen, D.; Suter, H.C.; Islam, A.; Edis, R. Influence of nitrification inhibitors on nitrification and nitrous oxide (N₂O) emission from a clay loam soil fertilized with urea. *Soil Biol. Biochem.* 2010, 42, 660–664. [CrossRef]

19. Maris, S.C.; Teira-Esmatges, M.R.; Arbones, A.; Rufat, J. Effect of irrigation, nitrogen application, and a nitrification inhibitor on nitrous oxide, carbon dioxide and methane emissions from an olive (*Olea europaea* L.) orchard. *Sci. Total Environ.* 2015, 538, 966–978. [CrossRef] [PubMed]

20. Menéndez, S.; Merino, P.; Pinto, M.; González-Murua, C.; Estavillo, J. 3,4-dimethylpyrazol phosphate effect on nitrous oxide, nitric oxide, ammonia, and carbon dioxide emissions from grasslands. *J. Environ. Q.* 2006, 35, 973–981. [CrossRef] [PubMed]

21. Li, H.; Liang, X.; Chen, Y.; Lian, Y.; Tian, G.; Ni, W. Effect of nitrification inhibitor DMPP on nitrogen leaching, nitrifying organisms, and enzyme activities in a rice-oilseed rape cropping system. *J. Environ. Sci.* 2008, 20, 149–155. [CrossRef]

22. Rowlings, D.W.; Scheer, C.; Liu, S.; Grace, P.R. Annual nitrogen dynamics and urea fertilizer recoveries from a dairy pasture using 15N; effect of nitrification inhibitor DMPP and reduced application rates. *Agric. Ecosyst. Environ.* 2016, 216, 216–225. [CrossRef]

23. Koegel-Knabner, I.; Amelung, W.; Cao, Z.; Fiedler, S.; Frenzel, P.; Jahn, R.; Kalbitz, K.; Koelbl, A.; Schloter, M. Biogeochemistry of paddy soils. *Gardenera* 2010, 157, 1–14. [CrossRef]

24. Zheng, X.; Xu, Z.; Wang, Y.; Han, S.; Huang, Y.; Cai, Z.; Zhu, J. Determination of net exchange of CO₂ between paddy fields and atmosphere with static poaque-chamber-based measurements. *J. Appl. Ecol.* 2002, 39, 1240–1244.

25. Wang, Y.; Wang, Y. Quick measurement of CH₄, CO₂ and N₂O emissions from a short-plant ecosystem. *Adv. Atmos. Sci.* 2003, 20, 842–844.

26. Kanno, T.; Miura, Y.; Tsuruta, H.; Minami, K. Methane emission from rice paddy fields in all of Japanese prefecture. *Nutr. Cycl. Agroecosyst.* 1997, 49, 147–151. [CrossRef]

27. Li, C.; Zhou, D.; Kou, Z.; Zhang, Z.; Wang, J.; Cai, M.; Cao, C. Effects of tillage and nitrogen fertilizers on CH₄ and CO₂ emissions and soil organic carbon in paddy fields of central China. *PLoS ONE* 2012, 7, e34642.

28. Jiang, C.; Wang, Y.; Zheng, X.; Zhu, B.; Huang, Y.; Hao, Q. Methane and nitrous oxide emissions from three paddy rice based cultivation systems in southwest China. *Adv. Atmos. Sci.* 2006, 23, 415–424. [CrossRef]

29. Xing, G. N₂O emission from cropland in China. *Nutr. Cycl. Agroecosyst.* 1998, 52, 249–254. [CrossRef]

30. Xing, G.; Zhao, X.; Xiong, Z.; Yan, X.; Xu, H.; Xie, Y.; Shi, S. Nitrous oxide emission from paddy fields in China. *Acta Ecol. Sin.* 2009, 29, 45–50. [CrossRef]

31. Liu, S.; Qin, Y.; Zou, J.; Liu, Q. Effects of water regime during rice-growing season on annual direct N₂O emission in a paddy rice–winter wheat rotation system in southeast China. *Sci. Total Environ.* 2010, 408, 906–913. [CrossRef] [PubMed]
32. Liang, W.; Shi, Y.; Zhang, H.; Yue, J.; Huang, G. Greenhouse gas emissions from northeast China rice fields in fallow season. *Pedosphere* **2007**, *17*, 630–638. [CrossRef]

33. Gilsanz, C.; Baez, D.; Misselbrook, T.H.; Dhanoa, M.S.; Cardenas, L.M. Development of emission factors and efficiency of two nitrification inhibitors, DCD and DMPP. *Agric. Ecosyst. Environ.* **2016**, *216*, 1–8. [CrossRef]

34. Arth, I.; Frenzel, P. Nitrification and denitrification in the rhizosphere of rice: The detection of processes by a new multi-channel electrode. *Biol. Fertil. Soils* **2000**, *31*, 427–435. [CrossRef]

35. Nicolaisen, M.H.; Risgaard-Petersen, N.; Revsbech, N.P.; Reichardt, W.; Ramsing, N.B. Nitrification–denitrification on nitrifying and denitrifying bacteria. *Geoderm* **2017**, *303*, 1–8. [CrossRef]

36. Gilsanz, C.; Baez, D.; Misselbrook, T.H.; Dhanoa, M.S.; Cardenas, L.M. Development of emission factors and efficiency of two nitrification inhibitors, DCD and DMPP. *Agric. Ecosyst. Environ.* **2016**, *216*, 1–8. [CrossRef]

37. Arth, I.; Frenzel, P. Nitrification and denitrification in the rhizosphere of rice. *Biol. Fertil. Soils* **2013**, *49*, 23–30. [CrossRef]

38. Di, H.J.; Cameron, K.C. 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 Sediment.* **2011**, *11*, 1032–1039. [CrossRef]

39. Arth, I.; Frenzel, P. Nitrification and denitrification in the rhizosphere of rice: The detection of processes by a new multi-channel electrode. *Biol. Fertil. Soils* **2000**, *31*, 427–435. [CrossRef]

40. Nicolaisen, M.H.; Risgaard-Petersen, N.; Revsbech, N.P.; Reichardt, W.; Ramsing, N.B. Nitrification–denitrification dynamics and community structure of ammonia oxidizing bacteria in a high yield irrigated philippine rice field. *FEMS Microbiol. Ecol.* **2004**, *49*, 359–369. [CrossRef] [PubMed]

41. Arth, I.; Frenzel, P.; Conrad, R. Denitrification coupled to nitrification in the rhizosphere of rice. *Biol. Fertil. Soils* **1999**, *30*, 509–515. [CrossRef]

42. Buresh, R.; De Datta, S. Denitrification losses from puddled rice soils in the tropics. *Biol. Fertil. Soils* **1990**, *9*, 1–13. [CrossRef]

43. Weiske, A.; Benckiser, G.; Ottow, J.C. Effect of the new nitrification inhibitor DMPP in comparison to DCD on nitrous oxide (N$_2$O) emissions and methane (CH$_4$) oxidation during 3 years of repeated applications in field experiments. *Nutr. Cycl. Agric. Ecosyst.* **2001**, *60*, 57–64. [CrossRef]

44. Arth, I.; Frenzel, P.; Conrad, R. Denitrification coupled to nitrification in the rhizosphere of rice. *Biol. Fertil. Soils* **2000**, *31*, 427–435. [CrossRef] [PubMed]

45. Arth, I.; Frenzel, P. Nitrification and denitrification in the rhizosphere of rice plants as determined by new methods of discrimination. *Ann. Microbiol.* **2000**, *51*, 225–257. [CrossRef]

46. Weiske, A.; Benckiser, G.; Ottow, J.C. Effect of the new nitrification inhibitor DMPP in comparison to DCD on nitrous oxide (N$_2$O) emissions and methane (CH$_4$) oxidation during 3 years of repeated applications in field experiments. *Nutr. Cycl. Agric. Ecosyst.* **2001**, *60*, 57–64. [CrossRef]

47. Buresh, R.; De Datta, S. Denitrification losses from puddled rice soils in the tropics. *Biol. Fertil. Soils* **1990**, *9*, 1–13. [CrossRef]

48. Arth, I.; Frenzel, P.; Conrad, R. Denitrification coupled to nitrification in the rhizosphere of rice. *Biol. Fertil. Soils* **2000**, *31*, 427–435. [CrossRef] [PubMed]

49. Bodelier, P.L.; Hahn, A.P.; Arth, I.R.; Frenzel, P. Effects of ammonium-based fertilization on microbial processes involved in methane emission from soilsplanted with rice. *Biogeochemistry* **2000**, *51*, 225–257. [CrossRef]

50. Bodelier, P.L.; Hahn, A.P.; Arth, I.R.; Frenzel, P. Effects of ammonium-based fertilization on microbial processes involved in methane emission from soilsplanted with rice. *Biogeochemistry* **2000**, *51*, 225–257. [CrossRef]

51. Bodelier, P.L.; Hahn, A.P.; Arth, I.R.; Frenzel, P. Effects of ammonium-based fertilization on microbial processes involved in methane emission from soilsplanted with rice. *Biogeochemistry* **2000**, *51*, 225–257. [CrossRef] [PubMed]

52. Mohanty, S.; Kollah, B.; Sharma, V.K.; Singh, A.B.; Singh, M.; Rao, A.S. Methane oxidation and methane driven redox process during sequential reduction of a flooded soil ecosystem. *Anna. Microbiol.* **2014**, *64*, 65–74. [CrossRef]

53. Mohanty, S.; Kollah, B.; Sharma, V.K.; Singh, A.B.; Singh, M.; Rao, A.S. Methane oxidation and methane driven redox process during sequential reduction of a flooded soil ecosystem. *Anna. Microbiol.* **2014**, *64*, 65–74. [CrossRef] [PubMed]

54. Mohanty, S.; Kollah, B.; Sharma, V.K.; Singh, A.B.; Singh, M.; Rao, A.S. Methane oxidation and methane driven redox process during sequential reduction of a flooded soil ecosystem. *Anna. Microbiol.* **2014**, *64*, 65–74. [CrossRef] [PubMed]
51. Suter, H. Reducing N2O emissions from nitrogen fertilizers with the nitrification inhibitor DMPP. In Proceedings of the 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia, 1–6 August 2010.

52. Wang, Z.; Kong, T.; Hu, S.; Sun, H.; Yang, W.; Kou, Y.; Mandlaa; Xu, H. Nitrification inhibitors mitigate earthworm-induced N2O emission—a mesocosm study. *Biol. Fertil. Soils* **2015**, *51*, 1005–1011.

© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).