agronomy

MDPI

Article

Combined Application of Lime and a Nitrification Inhibitor, 3,4-Dimethylpyrazole Phosphate Markedly Decrease Nitrous Oxide Emissions from an Acid Soil

Shilpi Das [1,2,3,*], Weijin Wang [1,2], Steven Reeves [2], Ram C. Dalal [1], Yash P. Dang [1] and Peter M. Kopittke [1]

[1] School of Agriculture and Food Sciences, The University of Queensland, St. Lucia, QLD 4072, Australia; weijin.wang@des.qld.gov.au (W.W.); r.dalal@uq.edu.au (R.C.D.); y.dang@uq.edu.au (Y.P.D.); p.kopittke@uq.edu.au (P.M.K.)

[2] Science and Technology Division, Department of Environment and Science (DES), GPO Box 2454, Brisbane, QLD 4001, Australia; steven.reeves@des.qld.gov.au

[3] Soil Science Division, Bangladesh Institute of Nuclear Agriculture, BAU Campus, Mymensingh 2202, Bangladesh

* Correspondence: shilpi.das@uq.net.au

Abstract: Nitrous oxide (N_2O) emissions from acid sugarcane (Saccharum officinarum) soils are high ($\geq$10% of N). We assessed the impact of lime, a nitrification inhibitor (NI), and copper (Cu) on N_2O emissions from an acid sugarcane soil in a laboratory experiment using (1) urea (U), (2) U + 3,4-dimethylpyrazole phosphate (U + DMPP), (3) U + $CuSO_4.5H_2O$ (U + Cu), and (4) U + DMPP + Cu. The treatments were applied to both an un-limed soil (pH 5.1) and a limed soil (pH 6.9) and incubated at 25 °C and 55% water holding capacity (WHC) for 28 d, and then increased to 90% WHC for another 8 d to favour denitrification. At 55% WHC, both the addition of the NI (U + DMPP) and the liming of the acid soil significantly decreased cumulative N_2O emissions, with this being due to significantly lower net nitrifications. Liming and DMPP decreased N_2O emissions by 79% and 90%, respectively. However, where lime and DMPP were applied together, N_2O emissions decreased by 94% compared to those in the un-limed (acid) U-treated soil. In contrast, the addition of Cu and water content to 90% WHC had no significant effect on N_2O emissions. Therefore, the combined use of lime and DMPP provides the best option to decrease N_2O emissions from an acid soil.

Keywords: nitrous oxide emissions; acid soil; lime; nitrification inhibitor; copper

check for updates

Citation: Das, S.; Wang, W.; Reeves, S.; Dalal, R.C.; Dang, Y.P.; Kopittke, P.M. Combined Application of Lime and a Nitrification Inhibitor, 3,4-Dimethylpyrazole Phosphate Markedly Decrease Nitrous Oxide Emissions from an Acid Soil. *Agronomy* **2022**, *12*, 1040. https://doi.org/10.3390/agronomy12051040

Academic Editor: Ward Smith

Received: 23 March 2022
Accepted: 24 April 2022
Published: 27 April 2022

1. Introduction

Nitrous oxide (N_2O) is an important greenhouse gas with a global warming potential 265 times that of carbon dioxide (CO_2) over a 100-year time horizon [1]. The rate of its release into the atmosphere has increased over the past century [2]. Soil ecosystems are considered the largest source of N_2O production, which accounts for almost 67% of total N_2O emissions [2,3], and it is anticipated that agricultural soil will still account for the majority of N_2O emissions (59%) by 2030 [4].

N_2O is predominantly released from agricultural soil during microbial nitrification and denitrification processes [4,5]. Among the factors regulating N_2O production, soil water is one of the most important as it regulates gas and substrate diffusion, aeration, and microbial functionality [6,7]. In general, N_2O is produced by nitrifying microorganisms under aerobic conditions, but by denitrifying microorganisms in anaerobic conditions [8–11]. The magnitude of N_2O production through denitrification is generally much higher than through nitrification [12]. Thus, an increase in soil water content can stimulate N_2O production [13], as this typically increases the activity of denitrifying organisms [14,15]. Soil pH is also an important regulator affecting most nitrogen (N) transformation processes [16]. Several studies have shown that soil pH regulates the abundance and structure of nitrogen-cycling genes as well as the rates of nitrification and denitrification and, thus, N_2O production [17–19]. In addition, crop production practices, such as the use of lime, nitrification inhibitors (NIs), and copper (Cu), may have non-target impacts on soil biological processes, including N_2O emissions. However, we are not aware of any studies investigating the combined effect of lime, an NI, and Cu in an acid soil.

Acidification is a common problem of soils in high rainfall regions used for production of sugarcane (*Saccharum officinarum*). The application of large N fertiliser rates in many sugarcane systems also further accelerates acidification processes [20]. Traditionally, liming materials are added to acid soils to neutralise soil acidity by increasing pH, but lime also has non-target effects on the soil's physical, chemical, and biological properties [21]. Given that soil pH has a strong impact on the concentrations and transformation processes of NH_3, NH_4^+, and NO_3^- in the soil ecosystem [22,23], with this affecting nitrification and denitrification processes [24], soil pH could also affect N_2O emissions. For example, Barton et al. [25] demonstrated that lime application to a cropped soil in a semiarid region in southwestern Australia decreased cumulative N_2O emissions. Similarly, other researchers have shown that increasing soil pH by liming may decrease N_2O emissions by limiting the availability of NO_2^- for reduction to N_2O [26] or chemical decomposition to N_2O [27]. Conversely, liming has been observed to increase N_2O emissions from an arable acidic soil and in acidic forest soils [26,28] by increasing NH_4^+ and NO_3^- production. Clough et al. [29] concluded that liming is most effective at mitigating N_2O emissions when soils are at field capacity. However, the precise effect of liming on N_2O emissions is often difficult to predict for different environments. However, further studies are required to understand the effect of liming in acidic sugarcane cropping soils to improve the understanding of the cause of N_2O production and suggest possible N_2O mitigation strategies.

Nitrogen fertilisers are critical for crop production. However, in sugarcane systems, N use efficiency is generally low, with as much as ~40–60% of the applied N lost from the plant–soil ecosystem [30,31]. This causes economic loss to the farmers as well as environmental pollution. The application of nitrification inhibitors is one option that can be used to reduce N losses (including via N_2O emission), enhance the efficiency of applied N fertiliser, and improve crop yield. Nitrification inhibitors slow the nitrification process by delaying the microbial oxidation of NH_4^+ to NO_2^- for several weeks or months [32]. This occurs due to suppression of the activity of the ammonia mono-oxygenase (AMO) enzyme [33,34], which is encoded by the *amoA* gene within ammonia-oxidising archaea (AOA) and ammonia-oxidising bacteria (AOB) [35,36]. However, the effectiveness of NIs depends upon various factors, including the soil N status, soil pH, soil texture, soil water content, soil microbial population, and soil temperature [37]. One commonly used NI is 3,4-dimethylpyrazole phosphate (DMPP) [38]. Many authors have reported that DMPP is effective in inhibiting nitrification [34,39,40] and decreasing N_2O emissions from soil [41,42]. However, only a limited number of studies have examined the impact of DMPP in limed acidic sugarcane soil [43,44].

The use of a metal cofactor, such as Cu, is another potential option for the mitigation of N_2O emissions from soil [45–47]. Under specific conditions, the last step in the denitrification process of the N cycle ($NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$) remains incomplete, thus N_2O is emitted into the atmosphere. Cu is a limiting factor in the denitrification process and is required by the N_2O reductase (*nosZ*) that reduces N_2O to N_2 gas [48,49]. When a soil is Cu-deficient, the catalytic function of the *nosZ* enzyme may be suppressed, and thereby N_2O is emitted by denitrifying bacteria [49,50]. Therefore, the addition of Cu in soil stimulates the *nosZ* gene expression and may decrease N_2O production and emissions from soils with Cu deficiency.

To date, there is no information on the combined effect of lime ($CaCO_3$), NI (DMPP), and metal co-factor (Cu) on soil N dynamics, particularly N_2O emissions from acid soils used for sugarcane cropping. Such information is important both from an agricultural perspective (for increasing profitability) and an environmental perspective (decreasing NO_3^- loss and N_2O emissions). The present study aimed to determine the combined effects of the lime, NI, and Cu on nitrification, denitrification, and N_2O emissions from a sugarcane-cropped acid soil. We used a microcosm experiment with a soil at two soil water conditions, aerobic (55% water holding capacity, WHC) and partly anaerobic (90% WHC), to influence the nitrification and denitrification processes. This study was conducted (1) to improve the understanding of the mechanisms by which lime application decreases N_2O emissions from acid soil, and (2) to assess the effectiveness of DMPP and Cu, both alone and in combination, to decrease N_2O emissions after liming acid soil used for sugarcane cropping.

2. Materials and Methods

2.1. Soil Collection and Preparation

Soil samples were collected from a sugarcane field in Mackay, Australia. Six surface soil samples (0–20 cm) were randomly collected from a field. Samples were mixed homogeneously to form a composite sample. Unwanted materials, such as roots and plant debris, were removed from the soil sample using tweezers before the soil was sieved to 4 mm and stored at 4 °C until the commencement of the experiment. The soil was a Chromosol in the Australian Soil Classification [51], corresponding to a Luvisol in the World Reference Base, having 45% coarse sand, 27% fine sand, 18% silt, and 18% clay, a pH of 5.1 (1:5 soil: water), a cation exchange capacity (CEC) of 6.96 $\text{cmol}_{(c)}\ \text{kg}^{-1}$, an exchangeable soil acidity of 0.18 $\text{cmol}_{(c)}\ \text{kg}^{-1}$, DTPA extractable copper concentration of 2.9 mg kg^{-1}, a total C content of 1.64%, and a total N content of 0.11% [52].

The water holding capacity (WHC) of the soil was determined by placing soil into plastic cylinders (the bottom of the cylinders was fitted with fine nylon cloth) before being immersed in water for 2 h. The plastic cylinder was then placed on a funnel to allow drainage for 2 h and the soil WHC was calculated from the soil moisture content determined gravimetrically [53,54]. The WHC of this soil was 37% (w/w). Two points on the water characteristic curve were determined: ~0.33 bar at 55% WHC and $<<$ 0.1 bar at 90% WHC. The water-filled pore space (WFPS) was determined as (gravimetric water content × soil bulk density)/soil porosity [55]. In the present study, 100% WHC was equivalent to 68% WFPS, with this being similar to previous studies [55–57].

2.2. Lime, DMPP Coated Urea (ENTEC), and Copper Sulphate

Three agrochemicals were used in this study. These were (1) urea granules (U) (Sigma Aldrich, St. Louis, MO, USA), which is a common N fertiliser; (2) ENTEC® (Incitec Pivot fertiliser), which is granulated urea coated with 0.35% of a nitrification inhibitor, 3,4-dimethylpyrazole phosphate (DMPP) [58]; (3) copper sulphate pentahydrate ($CuSO_4.5H_2O$) (Sigma Aldrich, St. Louis, MO, USA), which is a blue crystalline water-soluble powder; and (4) $CaCO_3$ (Sigma Aldrich, St. Louis, MO, USA), which is the most common liming material (often termed agricultural lime) used for correcting soil acidity [59].

2.3. Pre-Incubation and pH Adjustment

The lime requirement of the soil (to neutralise the acidity) was determined using Dunn titration curves [60], and lime added to the soil at rates of 0 and 3.4 g kg^{-1} soil (with the latter being equivalent to 4.4 t ha^{-1}). Aliquots of 200 g (equivalent to oven-dry soil) samples of un-limed or limed soil were each placed into two 250 mL plastic jars, with one-third of the soil placed in one jar and the remaining two-thirds placed in another jar. The lids of the plastic jars had two holes to maintain aerobic conditions. The soils were then pre-incubated for 7 d at 25 °C to stabilise the microbial population [61] and soil pH [62].

2.4. Experimental Design

We conducted a laboratory incubation experiment, with four treatments applied to the un-limed and limed soils (yielding to a total of eight treatments). The four treatments were (1) urea (U), (2) U + 3,4-dimethylpyrazole phosphate (U + DMPP), (3) U + $CuSO_4.5H_2O$ (U + Cu), and (4) U + DMPP + Cu. For the relevant treatments, urea or ENTEC (U + DMPP) granules were applied at 250 mg N kg^{-1} soil, and a $CuSO_4.5H_2O$ solution was applied at 8 mg Cu kg^{-1} soil.

The urea or U + DMPP granules were added to plastic jars that contained one-third of the soil, with the amendments (un-limed or limed) thoroughly mixed, the remaining two-thirds of the soil was added, and the jars were gently tapped on the bench to achieve a similar soil volume in each jar. Next, appropriate quantities of $CuSO_4.5H_2O$ were dissolved into double-distilled water (ddH_2O) and then added to the appropriate soils using a syringe. After the relevant agrochemicals were added to the soils, the plastic jars containing soil samples were placed into an incubator at 25 °C for 36 d. For this 36 d incubation, the soils were initially adjusted to 55% WHC for the first 28 d, then increased to 90% WHC for the remaining 8 d. Soil water content was maintained by adding ddH_2O with plastic droppers twice a week, as required. It was assumed that 55% WHC (equivalent to 38% WFPS) corresponded to aerobic conditions and would favour nitrification, and that 90% WHC (equivalent to 62% WFPS) corresponded to partly anaerobic conditions and would favour denitrification [11,26,41,55,63]. The plastic jars containing the soils were arranged in a completely randomised design with four replications and four destructive soil samplings, yielding a total of 128 experimental units for the eight treatments.

2.5. Gas Sampling and Measurement

Gas samples were collected at 0, 1, 2, 3, 4, 5, 7, 10, 14, 18, 23, 28, 29, 30, 31, 32, 33, 34, and 36 d. Soil samples were placed into sealed gas-collecting glass jars (1.6 L) that had previously been flushed with compressed air for 30 s. The gas samples were collected after the soil samples had been sealed in the jar enclosure for 8 h, except for the first gas sampling when the jars were enclosed for only 5 h. Before sampling the gas, the air inside the headspace was mixed by drawing and injecting the air three times using a gas-tight syringe (25 mL). Next, gas samples were collected using the syringe from the headspace of each jar and injected into a pre-evacuated glass vial (12 mL). At each sampling time, background air samples were also collected. The gas samples were analysed for N_2O using gas chromatography ((GC) (GC-2010, Shimadzu Co., Kyoto, Japan)). For the GC analysis, N_2 was used as a carrier gas. The cumulative N_2O production was calculated by assuming linear changes in gas production during the period between two successive days of gas sampling.

N_2O fluxes were calculated using the following equations:

$$N_2O \text{ flux } (\mu g\ N\ kg^{-1}\ d^{-1}) = \Delta N_2O/24.4 \times V/\Delta t \times 24/\text{soil weight} \times 28 \quad (1)$$

where ΔN_2O is the increase in headspace N_2O concentration ($\mu L\ L^{-1}$), V is the headspace volume (L), Δt is the closure time (h) of the incubated jar, 24.4 is the ideal gas constant at 25 °C, 28 is the molecular weight of N in N_2O (g N mol^{-1}), and 24 is the number of hours in one day.

2.6. Destructive Soil Sampling and Analysis

Following the collection of the gas samples, the soils were destructively sampled after 0, 7, 14, 28, and 36 d. Soil pH and electrical conductivity (EC) were determined in a 1:5 soil water extract (10 g soil in 50 mL water) [52]. Mineral N concentrations (NH_4^+ and NO_3^-) in the soils were determined from a 2 M KCl extract using colorimetric procedures [52]. Net nitrification and net mineralisation rates were determined by comparing the initial (0 d) and final (7, 14, 28, and 36 d) concentrations of NO_3^-–N and mineral N, respectively.

Net nitrification and net N mineralisation rates were calculated using the following equations:

$$\text{Net nitrification rate } (mg\ N\ kg^{-1}\ d^{-1}) = (M_2 - M_1)/(T_2 - T_1) \quad (2)$$

$$\text{Net N mineralisation rate } (mg\ N\ kg^{-1}\ d^{-1}) = (N_2 - N_1)/(T_2 - T_1) \quad (3)$$

where M_2 and M_1 are the NO_3^-–N concentrations after times T_2 and T_1 (d), respectively, and N_2 and N_1 are the mineral N concentrations (summation of NH_4^+–N and NO_3^-–N concentrations) after times T_2 and T_1 (d), respectively.

2.7. Statistical Analysis

An analysis of variance (ANOVA) was performed using STAR version 2.0.1 [64]. Mean comparisons of the treatments were conducted using the least significant difference (LSD) test with a level of significance of $p \leq 0.05$. A Pearson's correlation analysis among variables of various treatments was performed using SigmaPlot version 14.5 [65].

3. Results

3.1. Soil pH

Soil pH in the un-limed soil increased slightly at 7 d from 5.1 to 5.6 after wetting the soil to 55% WHC (Figure 1a), then steadily decreased at 14 d (pH 4.7) and 28 d (pH 4.4), before then remaining essentially unchanged as soil water was increased to 90% WHC for the final 8 d period (Figure 1a). The soil treated with U + DMPP and U + DMPP + Cu had significantly higher soil pH at 7 d and 14 d compared to the U treatment (Figure 1a).

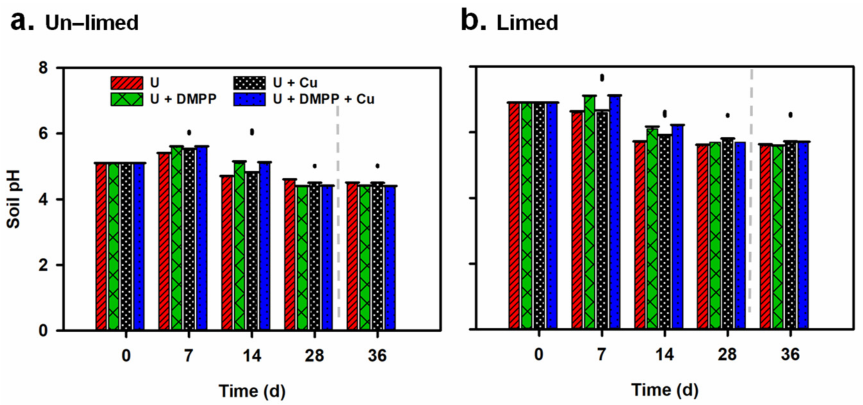

Figure 1. Changes in soil pH after application of urea, DMPP, and Cu in the (**a**) un-limed soil and (**b**) limed soil. The soil water was maintained at 55% WHC for the first 28 d then increased to 90% WHC for the remainder of the experimental period (as indicated by the grey broken vertical line). Data are the means of four replicates ± SE. The single vertical bars indicate the least significant differences among treatments ($LSD_{0.05}$).

As expected, the application of lime had a pronounced effect on soil pH, increasing values from 5.1 (un-limed soil) to 6.9 (limed soil) after the pre-incubation (Figure 1b). Soil pH remained high for the first 7 d of incubation, before then progressively decreasing to an average of 5.6 after 36 d (Figure 1b). Some slight differences were observed between the treatments in limed soil. Specifically, for the first 14 d of the incubation, both the U + DMPP and U + DMPP + Cu treatments had significantly higher pH values (6.1 and 6.2) than the U treatment (5.7) (Figure 1b). However, the increase in soil water content after 28 d (when the soil water content increased from 55 to 90% WHC) had no significant effect on soil pH (Figure 1b).

3.2. Soil EC

Soil EC increased progressively with time for the first 28 d in the un-limed soil in all the treatments (Figure 2a). However, the U + DMPP and U + DMPP + Cu treatments had significantly ($p \leq 0.05$) lower EC than the U treatment with this being observed from the beginning of the incubation up to 28 d. Over the final 8 d of the incubation, when soil water content was increased from 55 to 90% WHC, no significant differences in EC values were observed between any of the treatments in that un-limed soil (Figure 2a).

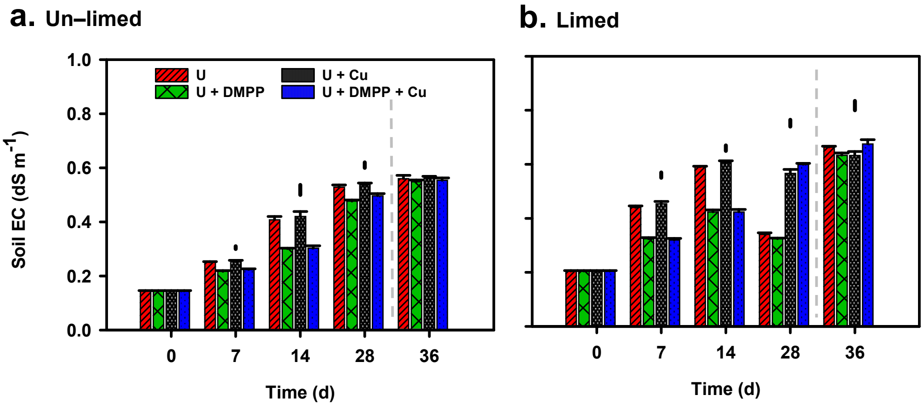

Figure 2. Changes in soil EC (1:5 soil: water) after application of urea, DMPP, and Cu in the (**a**) un-limed soil and (**b**) limed soil. The soil water was maintained at 55% WHC for the first 28 d then increased to 90% WHC for the remainder of the experimental period (as indicated by the grey broken vertical line). Data are the means of four replicates ± SE. The single vertical bars indicate the least significant differences among treatments ($LSD_{0.05}$).

For the limed soil, soil EC also significantly increased over time, specifically, for the first 14 d of the incubation period. Similar to the un-limed soil, both the U + DMPP and U + DMPP + Cu treatments had significantly lower EC values than the U treatment for the first 14 d (Figure 2b). However, after 28 d (when the soil water content increased from 55 to 90% WHC), U and U + DMPP + Cu treatments had significantly higher EC values than the other treatments, although the differences in EC values were comparatively small.

3.3. Changes in Soil Mineral N (NH_4^+ N and NO_3^-–N) Concentrations in Soil

Soil NH_4^+–N concentrations in both the un-limed and limed soils were significantly ($p \leq 0.05$) affected by the application of urea and DMPP (Figure 3a,b). Overall, NH_4^+–N concentrations increased rapidly in the treatments during the initial 7d then decreased for the remaining experimental period, with concentrations tending to be higher for the un-limed soil than the limed soil. However, it was observed that the U + DMPP and U + DMPP + Cu treatments had higher NH_4^+–N concentrations compared to the U treatment, with these higher concentrations persisting over the remaining incubation period for both the un-limed and limed soils. It is noteworthy that the NH_4^+–N concentrations remained relatively low for the final 8 d of the experimental period when the soil water content was increased from 55 to 90% WHC (Figure 3a,b).

For NO_3^-–N, concentrations tended to increase over time for both the un-limed and limed soils (Figure 4a,b). The NO_3^-–N concentrations tended to be higher for the limed than for the un-limed soil. However, the U + DMPP and U + DMPP + Cu treatments had significantly ($p \leq 0.05$) lower NO_3^-–N concentrations than the U treatment, with this being observed from the beginning of the incubation up to 28 d. Over the final 8 d of the incubation when the soil water content was increased from 55 to 90% WHC, NO_3^-–N concentrations remained relatively constant (Figure 4a,b).

3.4. Net Nitrification and Net Mineralisation Rates

In the un-limed soil, the U + DMPP and U + DMPP + Cu treatments significantly decreased net nitrification rates compared to the U treatment between 0 and 28 d (Table 1), with the net nitrification rate in the incubated soil ranging from 7.41 to 9.27 mg N kg^{-1} d^{-1}. However, following an increase in soil water content from 55 to 90% WHC after 28 d, the overall net nitrification rate decreased, ranging from −1.13 to 1.41 mg N kg^{-1} d^{-1} (28–36 d), with net denitrification recorded. For the limed soil, the U + DMPP and U + DMPP + Cu treatments also significantly decreased net nitrification for the initial 28 d, but the overall rate of net nitrification was almost 16% higher compared to the un-limed soil (Table 1).

Following an increase in water content from 55 to 90% WHC after 28 d, net denitrification was observed for all the treatments, although treatment effects were not significant.

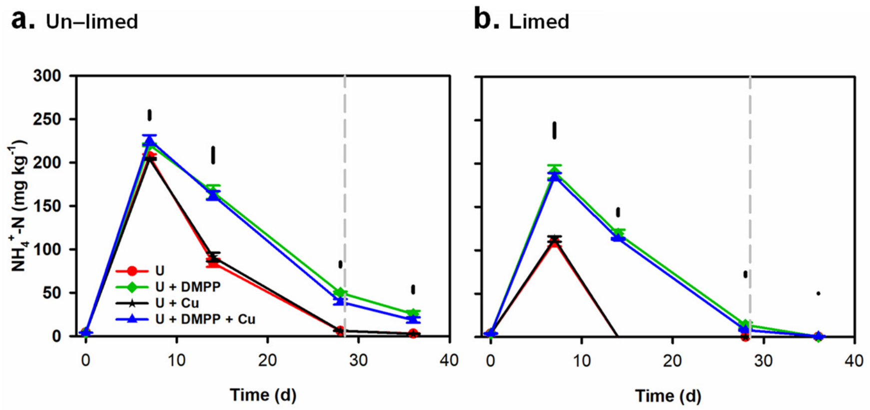

Figure 3. Effects of urea, DMPP, and Cu on soil $NH_4{}^+$–N concentrations in the (**a**) un-limed treatment and (**b**) limed treatment. The soil water was maintained at 55% WHC for the first 28 d then increased to 90% WHC for the remainder of the experimental period (as indicated by the grey broken vertical line). Data are the means of four replicates ± SE. The single vertical bars indicate significant differences among treatments ($LSD_{0.05}$).

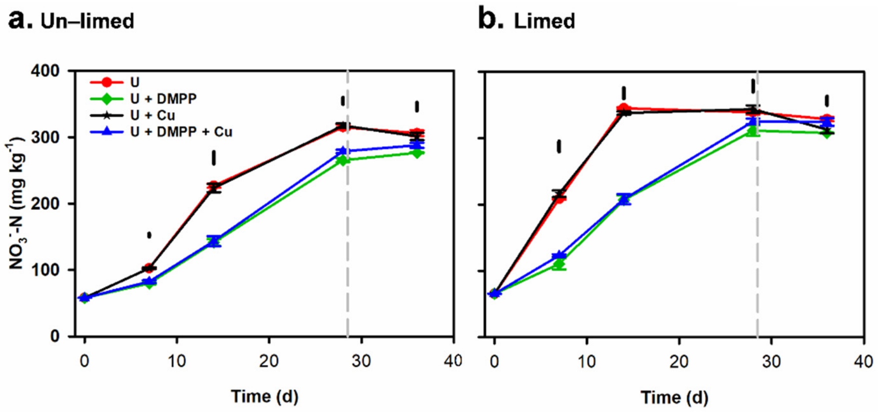

Figure 4. Effects of urea, DMPP, and Cu on soil $NO_3{}^-$–N concentrations in the (**a**) un-limed treatment and (**b**) limed treatment. The soil water was maintained at 55% WHC for the first 28 d then increased to 90% WHC for the remainder of the experimental period (as indicated by the grey broken vertical line). Data are the means of four replicates ± SE. The single vertical bars indicate significant differences among treatments ($LSD_{0.05}$).

3.5. Nitrous Oxide Emissions

For the un-limed soil, during the initial 28 d incubation period at 55% WHC, average daily N_2O emissions from the soil were comparatively high, ranging from 2 to 349 µg N kg^{-1} soil d^{-1} (Figure 5a). Of these treatments in the un-limed soil, the U and U + Cu treatments had higher N_2O emissions than the U + DMPP, and U + DMPP + Cu treatments during the first 17 d. Across all treatments, the N_2O emissions decreased to minimum levels by 28 d. However, after increasing the soil water content from 55 to 90% WHC after 28 d, there was a marked increase in average daily N_2O emissions for the U + DMPP and U + DMPP + Cu treatments where N_2O emissions increased approximately two-fold compared to that measured before 28 d (Figure 5a).

Table 1. Net nitrification and net mineralisation rates (mg N kg^{-1} soil d^{-1}) in the soil at different periods of incubation. Soils were maintained at 55% WHC for the first 28 d then increased to 90% WHC for the remainder of the experimental period. Data are the means of four replicates ± SE.

Treatment		Net Nitrification Rates (mg N kg^{-1} Soil d^{-1})		Net Mineralisation Rates (mg N kg^{-1} Soil d^{-1})	
		0–28 d (55% WHC)	**28–36 d (90% WHC)**	**0–28 d (55% WHC)**	**28–36 d (90% WHC)**
Un-limed	U	9.19 ± 0.38 Ab	−1.13 ± 0.78 Aa	9.26 ± 0.22 Aa	−1.52 ± 0.81 Aa
	U + DMPP	7.41 ±0.47 Cb	1.41 ± 0.47 Aa	9.05 ± 0.56 Aa	−1.66 ± 0.48 Aa
	U + Cu	9.27 ± 0.62 Ab	−2.03 ± 0.91 Aa	9.35 ± 0.27 Aa	−2.47 ± 0.90 Aa
	U + DMPP + Cu	7.88 ± 0.38 Bb	1.15 ± 0.49 Aa	9.15 ± 0.55 Aa	−1.47 ± 0.60 Aa
Limed	U	9.78 ± 0.14 Aa	−1.30 ± 0.13 Aa	9.64 ± 0.18 Aa	−1.29 ± 0.13 Aa
	U + DMPP	8.79 ± 0.74 Ca	−0.46 ± 1.00 Aa	9.14 ± 0.80 Aa	−2.16 ± 1.05 Aa
	U + Cu	9.94 ± 0.59 Aa	−3.83 ± 1.23 Aa	9.40 ± 0.57 Aa	−3.83 ± 1.23 Aa
	U + DMPP + Cu	9.28 ± 0.35 Ba	−0.10 ± 0.86 Aa	9.43 ± 0.47 Aa	−0.99 ± 0.80 Aa
p-values	Lime	<0.001	NS	NS	NS
	Urea, DMPP, Cu	<0.001	<0.001	0.02	NS
	Lime × urea × DMPP × Cu	0.007	NS	NS	NS

Means followed by the same letters within the same column and each treatment are not significantly different, the capital letters represent differences among four treatments within columns and small letters represent differences between un-limed and limed treatments. NS = non-significant. Lime represents un-limed or limed soil.

In both the un-limed and limed soils, the highest net mineralisation occurred in between 0 and 7 d followed by the 0–28 d (Tables 1 and S1). The U + DMPP treatment had lower net mineral N accumulation compared to the U treatment over the entire incubation period except for 7–14 d (Tables 1 and S1). Following an increase in water content from 55 to 90% WHC after 28 d, net mineral N accumulation was negative in all treatments (Table 1).

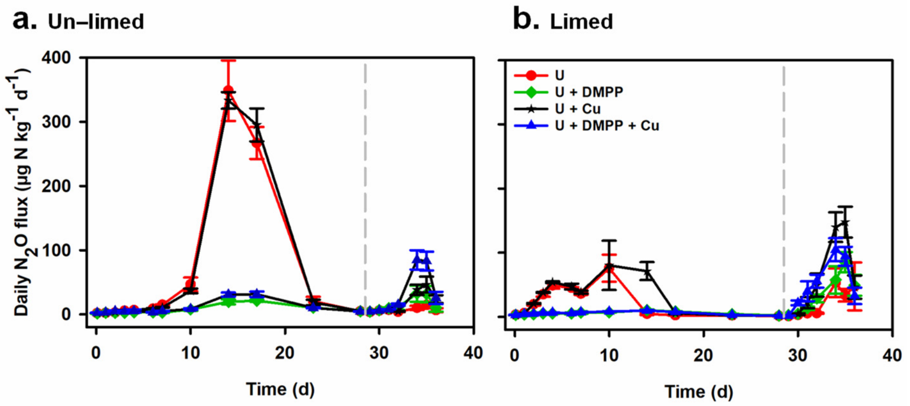

Figure 5. Effects of urea, DMPP, and Cu on N_2O emissions from an acidic sugarcane cropped soil in (**a**) un-limed treatment and (**b**) limed treatment. The soil water was maintained at 55% WHC for the first 28 d then increased to 90% WHC for the remainder of the experimental period (as indicated by the grey broken vertical line). Data are the means of four replicates ± SE.

For the limed soil, during the initial 28 d incubation period at 55% WHC, the U + DMPP, and U + DMPP + Cu treatments also significantly decreased average daily N_2O emissions compared to the U treatment (Figure 5b). During this period, the average daily N_2O emissions ranged from 3 to 80 µg N kg^{-1} soil d^{-1}. However, following an increase in water content from 55 to 90% WHC after 28 d, the rate of N_2O emissions tended to increase rapidly, with the sole exception being where U had been applied to the soil (Figure 5b).

Cumulative N_2O emissions were significantly greater ($p < 0.05$) in all the treatments in the un-limed soil compared to the limed soil (Table 2). During the initial 28 d period at 55% WHC, of the four treatments, the U treatment had the highest cumulative N_2O emissions (2778 µg N kg^{-1} soil), while the U + DMPP treatment had the lowest (288 µg N kg^{-1} soil) (Table 2). Therefore, the U + DMPP treatment decreased N_2O emissions by approximately 90%. However, following an increase in water content from 55 to 90% WHC after 28 d, the U + DMPP treatment tended to have higher emissions compared to the U treatment, although the differences were not significant.

Table 2. Cumulative N_2O emissions from the un-limed and limed sugarcane cropped soil. The soil water was maintained at 55% WHC for the first 28 d then increased to 90% WHC for the remainder of the experimental period. Data are the means of four replicates ± SE.

Treatment		**Cumulative N_2O Emissions (μg N kg^{-1} Soil)**	
		0–28 d (55% WHC)	**28–36 d (90% WHC)**
Un-limed (pH 5.1)	U	2778 ± 296 Aa	47 ± 8.0 Aa
	U + DMPP	288.0 ± 19 Ba	103 ± 32 Aa
	U + Cu	2803 ± 154 Aa	109 ± 20 Aa
	U + DMPP + Cu	387.0 ± 31 Ba	210 ± 31 Aa
Limed (pH 6.9)	U	582 ± 84 Ab	110 ± 25 Aa
	U + DMPP	170 ± 8.0 Ba	200 ± 38 Aa
	U + Cu	867 ± 97 Ab	389 ± 80 Aa
	U + DMPP + Cu	169 ± 9.0 Ba	349 ± 9.0 Aa
Mean of un-limed treatments (pH 5.1)		1564 ± 125 a	117 ± 23 b
Mean of limed treatments (pH 6.9)		447.0 ± 49.3 b	262 ± 50 a
p-values	Lime	<0.001	0.002
	Urea, DMPP, Cu	<0.001	<0.001
	Lime × urea × DMPP × Cu	<0.001	NS

Means followed by the same letters within the same column and each treatment are not significantly different; the capital letters represent differences among four treatments within columns and small letters represent differences between un-limed and limed treatments. NS = non-significant. Lime represents un-limed or limed soil.

For the limed soil, cumulative N_2O emissions in all the treatments were 71% lower than the un-limed soil during the 0–28 d period (Table 2). During this period, the U + DMPP treatment (170 μg N kg^{-1} soil) and U + DMPP + Cu treatment (169 μg N kg^{-1} soil) had significantly lower emissions than the U treatment (582 μg N kg^{-1} soil) (Table 2). Therefore, the U + DMPP and U + DMPP + Cu treatments decreased N_2O emissions by 70.7% and 71.1%, respectively. However, after 28 d (90% WHC), the limed treatment increased N_2O emissions by 55% compared to the initial 28 d, although the flux of N_2O was low and we did not find any significant treatment effects on N_2O emissions (Table 2). In contrast to the DMPP and liming, the addition of Cu as a metal co-factor had no significant effect on N_2O emissions (Table 2).

There was a significant ($p < 0.01$) interaction between the amendments (liming, urea, DMPP and Cu) on N_2O emissions for all the incubation periods except 28–36 d (Tables 2 and S2), which showed that urea, DMPP, and Cu affect emissions, but the pattern of this effect depended upon whether the soils were limed or not. For instance, for the U treatment, N_2O emissions were 79% lower in the limed soil than the un-limed soil (N_2O emissions from 2778 to 582 μg N kg^{-1} soil) during the initial 28 d (Table 2). The decrease in the cumulative N_2O emissions that occurred in the U + DMPP, and U + DMPP + Cu treatments was much smaller following liming the soil (41%, from 288 to 170 μg N kg^{-1} soil and 56%, from 387 to 169 μg N kg^{-1} soil, respectively). However, the combined lime and DMPP resulted in the largest N_2O emissions reduction from 2778 μg N kg^{-1} soil for the acid soil (un-limed soil) applied with U to 170 μg N kg^{-1} for the limed soil applied with U + DMPP during the 0–28 d period, a total reduction of 94% (Table 2).

3.6. Correlation Analysis

Pearson's correlation (r) analysis showed that in the un-limed soil, there were strong positive correlations between N_2O emissions and EC, NO_3^-–N concentrations, and net nitrification rates for the initial 28 d when the soil water content was maintained at 55% WHC (Table 3). In contrast, for the limed soil maintained at 55% WHC, averaged daily N_2O emissions were negatively correlated with NH_4^+–N concentrations during the initial 28 d (Table 3). However, from 28 to 36 d (90% WHC), average daily N_2O emission rates were positively correlated with pH in the limed soil (Table 3).

Table 3. Pearson's correlation among variables of various treatments in the un-limed and limed sugarcane cropped soil. The soil water was maintained at 55% WHC for the first 28 d then increased to 90% WHC for the remainder of the experimental period. Data are the means of four replicates ± SE.

Variables	N_2O	Soil pH	Soil EC	NH_4^+–N	NO_3^-–N	Net Nitrification	Net Mineralisation	N_2O	Soil pH	Soil EC	NH_4^+–N	NO_3^-–N	Net Nitrification	Net Mineralisation
Un-limed soil	0–28 d (55% WHC)							28–36 d (90% WHC)						
N_2O	–							–						
Soil pH	-0.95^{ns}	–						-0.45^{ns}	–					
Soil EC	0.99^{**}	-0.93^{ns}	–					-0.56^{ns}	0.95^{ns}	–				
NH_4^+–N	-0.99^{**}	0.95^{*}	-0.99^{**}	–				0.49^{ns}	-0.99^{**}	-0.99^{**}	–			
NO_3^-–N	0.99^{**}	-0.95^{*}	0.99^{**}	-0.99^{**}	–			-0.46^{ns}	0.99^{ns}	0.94^{ns}	-0.98^{**}	–		
Net nitrification	0.99^{**}	-0.95^{*}	0.99^{**}	-0.99^{**}	0.99^{**}	–		0.53^{ns}	-0.91^{*}	-0.99^{**}	0.96^{ns}	-0.89^{ns}	–	
Net mineralisation	0.94^{ns}	-0.79^{ns}	0.97^{*}	-0.94^{ns}	0.94^{ns}	0.94^{ns}	–	0.15^{ns}	-0.35^{ns}	-0.58^{ns}	0.48^{ns}	-0.31^{ns}	0.69^{ns}	–
Limed soil														
N_2O	–							–						
Soil pH	-0.81^{ns}	–						0.99^{**}	–					
Soil EC	0.85^{ns}	-0.59^{ns}	–					-0.13^{ns}	-0.01^{ns}	–				
NH_4^+–N	-0.93^{*}	0.96^{*}	-0.76^{ns}	–				0.21^{ns}	0.33^{ns}	0.75^{ns}	–			
NO_3^-–N	0.94^{ns}	-0.96^{*}	0.79^{ns}	-0.99^{**}	–			-0.29^{ns}	-0.19^{ns}	0.89^{ns}	0.36^{ns}	–		
Net nitrification	0.93^{ns}	-0.96^{*}	0.76^{ns}	-0.99^{**}	0.99^{**}	–		-0.41^{ns}	-0.31^{ns}	0.58^{ns}	0.76^{ns}	0.25^{ns}	–	
Net mineralisation	0.91^{ns}	-0.97^{*}	0.75	-0.99^{**}	0.99^{**}	0.99^{**}	–	-0.48^{ns}	-0.37^{ns}	0.86^{ns}	0.73^{ns}	0.67^{ns}	0.88^{ns}	–

* $p \leq 0.05$; ** $p \leq 0.01$ (n = 4); ns, not significant.

4. Discussion

4.1. Co-Application of DMPP with Urea to an Acidic Soil Markedly Decreases Nitrous Oxide Emissions

Cumulative N_2O emissions from urea application to the acidic soil (pH 5.1) were relatively high (2778 µg N kg^{-1} soil) during the 0–28 d period (Table 2), corresponding to approximately 1% of applied N (assuming background mineral N concentration was 62 mg kg^{-1} soil), similar to the IPCC [66] default value for agricultural soils. Several studies have shown that soil pH plays an important role in the abundance and structure of N-cycling genes [18,19,67]. The N_2O reductase enzyme (NOR gene) is essential for the reduction of N_2O to N_2, which is very sensitive to low pH. Thus, in an acidic soil, N_2O that is produced is not reduced to N_2 gas, and N_2O emissions increase [68,69].

Of the various treatments investigated in the present study, the addition of DMPP (a NI) was found to have the most pronounced effect on N_2O emissions. Indeed, the co-application of DMPP with urea (U + DMPP) to the un-limed, acid soil decreased N_2O emissions by 90% compared to the U treatment during the 0–28 d period (Table 2). In this regard, the nitrification inhibitor (DMPP) reduced the net nitrification rate by more than 19% (Table 1). Thus, the NH_4^+–N concentration remained higher and the NO^-–N concentration remained lower for the U + DMPP treatment (Figures 3a and 4a), resulting in the marked decrease in N_2O emissions from the acidic soil during the nitrification process. Our findings are in accordance with the previous studies that have demonstrated that DMPP acts as an effective nitrification inhibitor by delaying the microbial oxidation from NH_4^+ to NO_2^- (several weeks) [32,70,71], and thus effectively decreases N_2O emissions from agricultural soils when applied with urea fertiliser [72,73]. Other researchers have also found that the application of urea–DMPP in cereal cropping systems can decrease N_2O emissions by more than 50% compared to the urea fertiliser [74,75]. DMPP interferes with AMO or hydroxylamine oxidoreductase enzymes to block the NH_4^+–N conversion to NO_2^-–N in the soil. In addition, soil-nitrifying microbes may have been inhibited by either osmotic effects or specific ion effects in the DMPP-treated soil [76]. In this regard, DMPP-treated soil showed lower EC values over the 28 d incubation period (Figure 2a). From the Pearson's correlation, N_2O emissions were significantly and positively correlated with EC and nitrification rates (Table 3).

Although DMPP-treated acidic soil had lower N_2O emissions in the initial 28 d period (when WHC was 55%), we did not find any significant N_2O decrease from the DMPP-treated soil after 28 d (at which point soil water content increased from 55 to 90% WHC) (Figure 5a and Table 2). The inability of DMPP to decrease N_2O emissions at the higher water content was probably because nitrification is inhibited under anaerobic conditions. Soil water is a vital factor that can directly impact nitrification inhibitors, for instance, inhibition of nitrification by DCD reduced from 52% to 32% inhibition when soil water content increased from 40 to 80% WHC [77]. Rose et al. [78] also found an inconsistent effect of DMPP on N_2O emissions from an Australian rice field, where DMPP–urea significantly decreased N_2O emissions in the 2013–14 season (aerobic conditions); however, it had no effect in 2014–15, possibly due to wet conditions, which favoured denitrification following fertilisation.

4.2. Lime Application to an Acidic Soil also Reduces Nitrous Oxide Emissions

In the present study, cumulative N_2O emissions from the limed soil (average pH of 6.5) were much lower than the un-limed soil (average pH of 5.2) during the initial 0–28 d period. For example, for the U treatment, N_2O emissions were 79% lower in the limed soil than the un-limed soil (Table 2). Therefore, liming could be used as a strategy for decreasing N_2O emissions from an acid soil. This was also observed by Barton et al. [25] for semiarid Western Australian soils, even though nitrification activity increased, apparently due to the increased activity of NO_2^- oxidisers [26]. The magnitude of the effect of liming was smaller for the treatments where DMPP was co-applied (41% reduction in N_2O emissions) given that the co-application of the DMPP itself had also decreased N_2O emissions by

90% from the un-limed soil (Table 2). Nevertheless, the combined application of lime plus DMPP for the initial 0–28 d period decreased N_2O emissions from 2778 µg N kg^{-1} in the un-limed soil with the U treatment to 170 µg N kg^{-1} in the limed soil for the U + DMPP treatment, being a total reduction of 94% (Table 2). These results agree with earlier studies, which reported that N_2O emissions decreased following the application of lime [25,62,79]. Qu et al. [69] also reported that a small increase in soil pH increased the activity of the NOR enzyme, subsequently decreasing N_2O emissions. Therefore, our results suggest that application of lime probably stimulated NOR enzyme activity due to the increased soil pH, subsequently decreasing N_2O emissions. Although liming of the acidic soil decreased N_2O emissions in the initial 28 d period when WHC was 55% (aerobic conditions), in the subsequent 8 d when the soil water content was increased to 90% WHC (partly anaerobic conditions), we did not find any significant effect of any of the agrochemicals on N_2O reduction (Figure 5b and Table 2). Thus, our data suggest that liming is effective for decreasing N_2O emissions in soils favouring nitrification [25,29], but not for soils with higher water contents where anaerobic conditions likely prevail.

This decrease in N_2O emissions in the limed soil was associated with a higher concentration of NO_3^-–N (Figure 4b) and lower concentration of NH_4^+–N (Figure 3b) compared to the un-limed soil, suggesting that increasing soil pH may decrease the proportion of nitrified N lost as N_2O during nitrification. In this regard, it is likely that nitrite reductase genes (*nirK*, *nirS*) are less effective in their activity to produce N_2O with increasing soil pH. The higher NO_3^-–N concentrations in the limed soil may also be due to a lower rate of denitrification, which would also subsequently decrease N_2O emissions [25,79,80]. In the U + DMPP treatment, however, NH_4^+–N oxidation was inhibited, and nitrification rates were lower than the U treatment in both the un-limed and limed soils (Table 1) because nitrifiers were adversely affected by the NI–DMPP under aerobic conditions.

4.3. Ineffectiveness of Cu on N_2O Emissions

In contrast to DMPP and liming, the addition of Cu as a metal co-factor had no significant effect on N_2O emissions (Table 2). Possibly it was either adsorbed on the organic matter or the amount applied was not sufficient to promote the function of *nosZ* gene in this soil. This is inconsistent with the previous studies [46,47], where Cu application resulted in the reduction of N_2O emissions. Further research is required on different soil types and higher rates of Cu to confirm our findings.

5. Conclusions

This microcosm study has shown that, overall, the addition of the nitrification inhibitor (U + DMPP) and the liming of the acid soil significantly decreased cumulative N_2O emissions. This was largely due to significantly lower net nitrification rates during the initial 28 d aerobic incubation period. Liming and DMPP decreased N_2O emissions by 79% and 90%, respectively, compared to the un-limed U treatment. However, the combined effect of lime and DMPP resulted in the largest reduction in N_2O emissions (94%) compared to those in the un-limed (acid) U-treated soil. After 28 d, when the water content was increased to 90% WHC, we did not find any significant effect of DMPP and lime on N_2O decrease, possibly due to denitrifying conditions. In contrast to DMPP and liming, the addition of Cu as a metal co-factor had no significant effect on N_2O emissions from this soil. Therefore, the combined use of lime and DMPP provides a promising management strategy to effectively decrease N_2O production and emissions from sugarcane-cropped acid soil.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12051040/s1, Table S1: Net nitrification and net mineralisation rates (mg N kg^{-1} soil d^{-1}) in the soil at different periods of incubation. The soils were maintained at 55% WHC for the first 28 d then increased to 90% WHC for the remainder of the experimental period. Data are the means of four replicates ± SE.; Table S2: Cumulative N_2O emissions from the un-limed and limed sugarcane cropped soil in different incubation period. The soil water was maintained at 55% WHC for the first 28 d then increased to 90% WHC for the remainder of the experimental period. Data are the means of four replicates ± SE.

Author Contributions: S.D.: conceptualisation, methodology, formal analysis, investigation, visualisation, data curation, software, writing—original draft. W.W.: conceptualisation, methodology, visualisation, formal analysis, data curation, project administration, writing—review and editing. R.C.D.: conceptualisation, methodology, formal analysis, visualisation, data curation, writing—review and editing. S.R.: data curation, investigation, software, writing—review and editing. Y.P.D.: visualisation, data curation, writing—review and editing. P.M.K.: visualisation, data curation, Formal analysis, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Additional data will be provided by the corresponding author for an authentic reason.

Acknowledgments: We are grateful to the Bangabandhu Science and Technology Fellowship Trust (BSTFT) for the support of the first author through a PhD scholarship. The authors acknowledge the assistance of Wayne Vickers (land owner) and Mark Sugars from the Queensland Department of Resources (DoR, Australia) for the soil sampling. The authors also acknowledge the assistance of Marijke Heenan and Siok Yo from the Queensland Department of Environment and Science (DES, Australia) for laboratory analyses.

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

References

1. IPCC. *Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; IPCC: Geneva, Switzerland, 2014; p. 151.
2. Tian, H.; Yang, J.; Xu, R.; Lu, C.; Canadell, J.G.; Davidson, E.A.; Jackson, R.B.; Arneth, A.; Chang, J.; Ciais, P.; et al. Global soil nitrous oxide emissions since the preindustrial era estimated by an ensemble of terrestrial biosphere models: Magnitude, attribution, and uncertainty. *Glob. Chang. Biol.* **2019**, *25*, 640–659. [CrossRef] [PubMed]
3. Tian, H.; Xu, R.; Canadell, J.G.; Thompson, R.L.; Winiwarter, W.; Suntharalingam, P.; Davidson, E.A.; Ciais, P.; Jackson, R.B.; Janssens-Maenhout, G.; et al. A comprehensive quantification of global nitrous oxide sources and sinks. *Nature* **2020**, *586*, 248–256. [CrossRef] [PubMed]
4. Hu, H.-W.; Chen, D.; He, J.-Z. Microbial regulation of terrestrial nitrous oxide formation: Understanding the biological pathways for prediction of emission rates. *FEMS Microbiol. Rev.* **2015**, *39*, 729–749. [CrossRef]
5. Zhang, J.; Lan, T.; Müller, C.; Cai, Z. Dissimilatory nitrate reduction to ammonium (DNRA) plays an important role in soil nitrogen conservation in neutral and alkaline but not acidic rice soil. *J. Soils Sediments* **2015**, *15*, 523–531. [CrossRef]
6. Granli, T.; Bøckman, O.C. Nitrous oxide (N_2O) emissions from soils in warm climates. *Fertil. Res.* **1995**, *42*, 159–163. [CrossRef]
7. Linn, D.M.; Doran, J.W. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled Soils. *Soil Sci. Soc. Am. J.* **1984**, *48*, 1267–1272. [CrossRef]
8. Bremner, J.M. Sources of nitrous oxide in soils. *Nutr. Cycl. Agroecosyst.* **1997**, *49*, 7–16. [CrossRef]
9. Stevens, R.J.; Laughlin, R.J. Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils. *Nutr. Cycl. Agroecosyst.* **1998**, *52*, 131–139. [CrossRef]
10. Khalil, K.; Mary, B.; Renault, P. Nitrous oxide production by nitrification and denitrification in soil aggregates as affected by O_2 concentration. *Soil Biol. Biochem.* **2004**, *36*, 687–699. [CrossRef]
11. Firestone, M.K.; Davidson, E.A. Microbiological basis of NO and N_2O production and consumption in soil. *Exch. Trace Gases Between Terr. Ecosyst. Atmos.* **1989**, *47*, 7–21.
12. Harris, E.; Diaz-Pines, E.; Stoll, E.; Schloter, M.; Schulz, S.; Duffner, C.; Li, K.; Moore, K.L.; Ingrisch, J.; Reinthaler, D.; et al. Denitrifying pathways dominate nitrous oxide emissions from managed grassland during drought and rewetting. *Sci. Adv.* **2021**, *7*, eabb7118. [CrossRef] [PubMed]
13. Hofstra, N.; Bouwman, A.F. Denitrification in agricultural soils: Summarizing published data and estimating global annual rates. *Nutr. Cycl. Agroecosyst.* **2005**, *72*, 267–278. [CrossRef]

14. Davidson, E.A.; Swank, W.T. Environmental parameters regulating gaseous nitrogen losses from two forested ecosystems via nitrification and denitrification. *Appl. Environ. Microbiol.* **1986**, *52*, 1287. [CrossRef] [PubMed]
15. Huang, T.; Gao, B.; Hu, X.-K.; Lu, X.; Well, R.; Christie, P.; Bakken, L.R.; Ju, X.-T. Ammonia-oxidation as an engine to generate nitrous oxide in an intensively managed calcareous Fluvo-aquic soil. *Sci. Rep.* **2014**, *4*, 3950. [CrossRef] [PubMed]
16. Mørkved, P.T.; Dörsch, P.; Bakken, L.R. The N_2O product ratio of nitrification and its dependence on long-term changes in soil pH. *Soil Biol. Biochem.* **2007**, *39*, 2048–2057. [CrossRef]
17. Hu, H.-W.; Zhang, L.-M.; Dai, Y.; Di, H.-J.; He, J.-Z. pH-dependent distribution of soil ammonia oxidizers across a large geographical scale as revealed by high-throughput pyrosequencing. *J. Soils Sediments* **2013**, *13*, 1439–1449. [CrossRef]
18. He, J.-Z.; Shen, J.-P.; Zhang, L.-M.; Zhu, Y.-G.; Zheng, Y.-M.; Xu, M.-G.; Di, H. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. *Environ. Microbiol.* **2007**, *9*, 2364–2374. [CrossRef]
19. Shen, J.-p.; Zhang, L.-m.; Zhu, Y.-g.; Zhang, J.-b.; He, J.-z. Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. *Environ. Microbiol.* **2008**, *10*, 1601–1611. [CrossRef]
20. Guo, J.H.; Liu, X.J.; Zhang, Y.; Shen, J.L.; Han, W.X.; Zhang, W.F.; Christie, P.; Goulding, K.W.T.; Vitousek, P.M.; Zhang, F.S. Significant acidification in major Chinese croplands. *Science* **2010**, *327*, 1008. [CrossRef]
21. Goulding, K.W.T. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. *Soil Use Manag.* **2016**, *32*, 390–399. [CrossRef]
22. Kirchmann, H.; Witter, E. Ammonia volatilization during aerobic and anaerobic manure decomposition. *Plant Soil* **1989**, *115*, 35–41. [CrossRef]
23. Thangarajan, R.; Bolan, N.S.; Tian, G.; Naidu, R.; Kunhikrishnan, A. Role of organic amendment application on greenhouse gas emission from soil. *Sci. Total Environ.* **2013**, *465*, 72–96. [CrossRef] [PubMed]
24. ŠImek, M.; Cooper, J.E. The influence of soil pH on denitrification: Progress towards the understanding of this interaction over the last 50 years. *Eur. J. Soil Sci.* **2002**, *53*, 345–354. [CrossRef]
25. Barton, L.; Gleeson, D.B.; Maccarone, L.D.; Zúñiga, L.P.; Murphy, D.V. Is liming soil a strategy for mitigating nitrous oxide emissions from semi-arid soils? *Soil Biol. Biochem.* **2013**, *62*, 28–35. [CrossRef]
26. Feng, K.; Yan, F.; Hütsch, B.W.; Schubert, S. Nitrous oxide emission as affected by liming an acidic mineral soil used for arable agriculture. *Nutr. Cycl. Agroecosyst.* **2003**, *67*, 283–292. [CrossRef]
27. van Cleemput, O. Subsoils: Chemo-and biological denitrification, N_2O and N_2 emissions. *Nutr. Cycl. Agroecosyst.* **1998**, *52*, 187–194. [CrossRef]
28. Martikainen, P.J. Nitrous oxide emission associated with autotrophic ammonium oxidation in acid coniferous forest soil. *Appl. Environ. Microbiol.* **1985**, *50*, 1519. [CrossRef]
29. Clough, T.J.; Kelliher, F.M.; Sherlock, R.R.; Ford, C.D. Lime and soil moisture effects on nitrous oxide emissions from a urine patch. *Soil Sci. Soc. Am. J.* **2004**, *68*, 1600–1609. [CrossRef]
30. Vallis, I.; Catchpoole, V.; Hughes, R.; Myers, R.; Ridge, D.; Weier, K. Recovery in plants and soils of 15N applied as subsurface bands of urea to sugarcane. *Aust. J. Agric. Res.* **1996**, *47*, 355–370. [CrossRef]
31. Prasertsak, P.; Freney, J.R.; Denmead, O.T.; Saffigna, P.G.; Prove, B.G.; Reghenzani, J.R. Effect of fertilizer placement on nitrogen loss from sugarcane in tropical Queensland. *Nutr. Cycl. Agroecosyst.* **2002**, *62*, 229–239. [CrossRef]
32. Prasad, R.; Power, J.F. Nitrification inhibitors for agriculture, health, and the environment. In *Advances in Agronomy*; Sparks, D.L., Ed.; Academic Press: Cambridge, MA, USA, 1995; Volume 54, pp. 233–281.
33. 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. [CrossRef]
34. 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]
35. Di, H.J.; Cameron, K.C.; Shen, J.P.; Winefield, C.S.; O'Callaghan, M.; Bowatte, S.; He, J.Z. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nat. Geosci.* **2009**, *2*, 621–624. [CrossRef]
36. Gubry-Rangin, C.; Nicol, G.W.; Prosser, J.I. Archaea rather than bacteria control nitrification in two agricultural acidic soils. *FEMS Microbiol. Ecol.* **2010**, *74*, 566–574. [CrossRef] [PubMed]
37. Barth, G.; von Tucher, S.; Schmidhalter, U. Influence of soil parameters on the effect of 3,4-dimethylpyrazole-phosphate as a nitrification inhibitor. *Biol. Fertil. Soils* **2001**, *34*, 98–102. [CrossRef]
38. Subbarao, G.V.; Ito, O.; Sahrawat, K.L.; Berry, W.L.; Nakahara, K.; Ishikawa, T.; Watanabe, T.; Suenaga, K.; Rondon, M.; Rao, I.M. Scope and strategies for regulation of nitrification in agricultural systems—Challenges and opportunities. *Crit. Rev. Plant Sci.* **2006**, *25*, 303–335. [CrossRef]
39. Barth, G.; Von Tucher, S.; Schmidhalter, U. Effectiveness of 3, 4-dimethylpyrazole phosphate as nitriflcation inhibitor in soil as influenced by inhibitor concentration, application form, and soil matric potential. *Pedosphere* **2008**, *18*, 378–385. [CrossRef]
40. Huang, Y.; Li, Y.; Yao, H. Nitrate enhances N_2O emission more than ammonium in a highly acidic soil. *J. Soils Sediments* **2014**, *14*, 146–154. [CrossRef]
41. Zhang, M.; Wang, W.; Tang, L.; Heenan, M.; Xu, Z. Effects of nitrification inhibitor and herbicides on nitrification, nitrite and nitrate consumptions and nitrous oxide emission in an Australian sugarcane soil. *Biol. Fertil. Soils* **2018**, *54*, 697–706. [CrossRef]

42. Wang, W.; Salter, B.; Reeves, S.; Brieffies, T.; Perna, J. Nitrous oxide emissions from a sugarcane soil under different fallow and nitrogen fertiliser management regimes. In Proceedings of the Australian Society of Sugar Cane Technologists, Palm Cove, Australia, 1–4 May 2012; pp. 1–8.
43. Xue, Y.; Wu, Z.-J.; Zhang, L.; Gong, P.; Dong, X.-X.; Nie, Y.-X. Inhibitory effect of DMPP on soil nitrification as affected by soil moisture content, pH and organic matter. *Chin. J. Appl. Ecol.* **2012**, *23*, 2663–2669.
44. Kaveney, B.; Condon, J.; Doran, G.; Galea, F.; Rigg, J. The distribution of soil nitrifiers and efficacy of 3,4-dimethylpyrazole phosphate changes with soil depth and calcium carbonate application. *Soil Biol. Biochem.* **2020**, *150*, 108009. [CrossRef]
45. Signor, D.; Cerri, C.E.P. Nitrous oxide emissions in agricultural soils: A review. *Pesqui. Agropecu. Trop.* **2013**, *43*, 322–338. [CrossRef]
46. Thomson, A.J.; Giannopoulos, G.; Pretty, J.; Baggs, E.M.; Richardson, D.J. Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2012**, *367*, 1157–1168. [CrossRef]
47. Shen, W.; Xue, H.; Gao, N.; Shiratori, Y.; Kamiya, T.; Fujiwara, T.; Isobe, K.; Senoo, K. Effects of copper on nitrous oxide (N_2O) reduction in denitrifiers and N_2O emissions from agricultural soils. *Biol. Fertil. Soils* **2020**, *56*, 39–51. [CrossRef]
48. Richardson, D.; Felgate, H.; Watmough, N.; Thomson, A.; Baggs, E. Mitigating release of the potent greenhouse gas N2O from the nitrogen cycle—Could enzymic regulation hold the key? *Trends Biotechnol.* **2009**, *27*, 388–397. [CrossRef]
49. Felgate, H.; Giannopoulos, G.; Sullivan, M.J.; Gates, A.J.; Clarke, T.A.; Baggs, E.; Rowley, G.; Richardson, D.J. The impact of copper, nitrate and carbon status on the emission of nitrous oxide by two species of bacteria with biochemically distinct denitrification pathways. *Environ. Microbiol.* **2012**, *14*, 1788–1800. [CrossRef]
50. Granger, J.; Ward, B.B. Accumulation of nitrogen oxides in copper-limited cultures of denitrifying bacteria. *Limnol. Oceanogr.* **2003**, *48*, 313–318. [CrossRef]
51. Isbell, R. *The Australian Soil Classification*; CSIRO Publishing: Clayton, Australia, 2016.
52. Rayment, G.E.; Lyons, D.J. *Soil Chemical Methods: Australasia*; CSIRO Publishing: Melbourne, Australia, 2011.
53. Choudhary, M.I.; Shalaby, A.A.; Al-Omran, A.M. Water holding capacity and evaporation of calcareous soils as affected by four synthetic polymers. *Commun. Soil Sci. Plant Anal.* **1995**, *26*, 2205–2215. [CrossRef]
54. Wang, W.J.; Dalal, R.C.; Moody, P.W.; Smith, C.J. Relationships of soil respiration to microbial biomass, substrate availability and clay content. *Soil Biol. Biochem.* **2003**, *35*, 273–284. [CrossRef]
55. Lan, T.; Han, Y.; Roelcke, M.; Nieder, R.; Cai, Z. Processes leading to N_2O and NO emissions from two different Chinese soils under different soil moisture contents. *Plant Soil* **2013**, *371*, 611–627. [CrossRef]
56. Vargas, V.P.; Cantarella, H.; Martins, A.A.; Soares, J.R.; do Carmo, J.B.; de Andrade, C.A. Sugarcane crop residue increases N_2O and CO_2 emissions under high soil moisture conditions. *Sugar Tech.* **2014**, *16*, 174–179. [CrossRef]
57. Wang, L.; Cai, Z. Nitrous oxide production at different soil moisture contents in an arable soil in China. *Soil Sci. Plant Nutr.* **2008**, *54*, 786–793. [CrossRef]
58. Janke, C.K.; Moody, P.; Bell, M.J. Three-dimensional dynamics of nitrogen from banded enhanced efficiency fertilizers. *Nutr. Cycl. Agroecosyst.* **2020**. [CrossRef]
59. Calcino, D.; Schroeder, B.; Panitz, J.; Hurney, A.; Skocaj, D.; Wood, A.; Salter, B. *Australian Sugarcane Nutrition Manual*; Sugar Research Australia: Indooroopilly, Australia, 2018; p. 114. Available online: https://sugarresearch.com.au/sugar_files/2017/02/GCM2307-Sugar-Research-Australia-Publication-Layout-F-Web.pdf (accessed on 23 February 2022).
60. Dunn, L. Lime-requirement determination of soils by means of titration curves. *Soil Sci.* **1943**, *56*, 341–352. [CrossRef]
61. Wu, J.; Brookes, P.C.; Jenkinson, D.S. Evidence for the use of a control in the fumigation-incubation method for measuring microbial biomass carbon in soil. *Soil Biol. Biochem.* **1996**, *28*, 511–518. [CrossRef]
62. Mkhabela, M.S.; Gordon, R.; Burton, D.; Madani, A.; Hart, W. Effect of lime, dicyandiamide and soil water content on ammonia and nitrous oxide emissions following application of liquid hog manure to a marshland soil. *Plant Soil* **2006**, *284*, 351–361. [CrossRef]
63. Hütsch, B.W.; Wang, X.; Feng, K.; Yan, F.; Schubert, S. Nitrous oxide emission as affected by changes in soil water content and nitrogen fertilization. *J. Plant Nutr. Soil Sci.* **1999**, *162*, 607–613. [CrossRef]
64. STAR. *Biometrics and Breeding Informatics*, Version 2.0.1. PBGB Division. International Rice Research Institute: Los Baños, Philippines, 2014.
65. *SigmaPlot*, Version 14.5; Systat Software Inc.: San Jose, CA, USA, 2020.
66. IPCC. *Guidelines for National Greenhouse Gas Inventories*; Institute for Global Environmental Strategies: Geneva, Switzerland, 2006; p. 54.
67. Weslien, P.; Kasimir Klemedtsson, Å.; Börjesson, G.; Klemedtsson, L. Strong pH influence on N_2O and CH_4 fluxes from forested organic soils. *Eur. J. Soil Sci.* **2009**, *60*, 311–320. [CrossRef]
68. Liu, B.; Mørkved, P.T.; Frostegård, Å.; Bakken, L.R. Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. *FEMS Microbiol. Ecol.* **2010**, *72*, 407–417. [CrossRef]
69. Qu, Z.; Wang, J.; Almøy, T.; Bakken, L.R. Excessive use of nitrogen in Chinese agriculture results in high $N_2O/(N_2O + N_2)$ product ratio of denitrification, primarily due to acidification of the soils. *Glob. Chang. Biol.* **2014**, *20*, 1685–1698. [CrossRef]
70. Zerulla, W.; Barth, T.; Dressel, J.; Erhardt, K.; Horchler von Locquenghien, K.; Pasda, G.; Rädle, M.; Wissemeier, A. 3,4-Dimethylpyrazole phosphate (DMPP)—A new nitrification inhibitor for agriculture and horticulture. *Biol. Fertil. Soils* **2001**, *34*, 79–84. [CrossRef]

71. Mukhtar, H.; Lin, Y.-P. Soil nitrification potential influences the performance of nitrification inhibitors DCD and DMPP in cropped and non-cropped soils. *Agronomy* **2019**, *9*, 599. [CrossRef]
72. Gilsanz, C.; Báez, D.; Misselbrook, T.H.; Dhanoa, M.S.; Cárdenas, L.M. Development of emission factors and efficiency of two nitrification inhibitors, DCD and DMPP. *Agric. Ecosyst. Environ.* **2016**, *216*, 1–8. [CrossRef]
73. Zhu, G.; Ju, X.; Zhang, J.; Müller, C.; Rees, R.M.; Thorman, R.E.; Sylvester-Bradley, R. Effects of the nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) on gross N transformation rates and N_2O emissions. *Biol. Fertil. Soils* **2019**, *55*, 603–615. [CrossRef]
74. Scheer, C.; Rowlings, D.W.; De Antoni Migliorati, M.; Lester, D.W.; Bell, M.J.; Grace, P.R. Effect of enhanced efficiency fertilisers on nitrous oxide emissions in a sub-tropical cereal cropping system. *Soil Res.* **2016**, *54*, 544–551. [CrossRef]
75. De Antoni Migliorati, M.; Scheer, C.; Grace, P.R.; Rowlings, D.W.; Bell, M.; McGree, J. Influence of different nitrogen rates and DMPP nitrification inhibitor on annual N_2O emissions from a subtropical wheat–maize cropping system. *Agric. Ecosyst. Environ.* **2014**, *186*, 33–43. [CrossRef]
76. Yan, N.; Marschner, P.; Cao, W.; Zuo, C.; Qin, W. Influence of salinity and water content on soil microorganisms. *Int. Soil Water Conserv. Res.* **2015**, *3*, 316–323. [CrossRef]
77. Puttanna, K.; Gowda, N.M.N.; Rao, E.V.S.P. Evaluation of nitrification inhibitors for use under tropical conditions. *Commun. Soil Sci. Plant Anal.* **1999**, *30*, 519–524. [CrossRef]
78. Rose, T.J.; Morris, S.G.; Quin, P.; Kearney, L.J.; Kimber, S.; Van Zwieten, L. The nitrification inhibitor DMPP applied to subtropical rice has an inconsistent effect on nitrous oxide emissions. *Soil Res.* **2017**, *55*, 547–552. [CrossRef]
79. Khaliq, M.A.; Tarin, M.W.K.; Jingxia, G.; Yanhui, C.; Guo, W. Soil liming effects on CH_4, N_2O emission and Cd, Pb accumulation in upland and paddy rice. *Environ. Pollut.* **2019**, *248*, 408–420. [CrossRef]
80. Yan, D.; Wang, Q.; Mao, L.; Ma, T.; Li, Y.; Ouyang, C.; Guo, M.; Cao, A. Interaction between nitrification, denitrification and nitrous oxide production in fumigated soils. *Atmos. Environ.* **2015**, *103*, 82–86. [CrossRef]