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Effectiveness of Nitrification Inhibitor in Reducing N_2O Emissions Depends on Soil Acidification Mitigation in Acid Soils

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Abstract

The addition of alkaline amendments is considered an important strategy to alleviate soil acidification, with profound impacts on soil nitrogen (N) transformations such as nitrification as well as greenhouse gas (GHG) nitrous oxide (N_2O) emissions. Nitrification inhibitors (NIs) have been widely recognized to effectively mitigate N_2O emissions by depressing the nitrification process. However, the effectiveness of NIs on N_2O emissions reduction under different alkaline amendments remains largely unknown, hindering our knowledge of the optimal soil acidification mitigation strategies. In this study, the effects of NIs in combination with different alkaline amendments on N_2O emissions were assessed on typical acid soils collected from four sites during a 28-day aerobic incubation experiment. Treatments included four alkaline amendments (quicklime, chicken manure, cow dung, biochar) and no amendment control, designated as CaO, CM, CD, BC, and CK, combined with a typical NI (3,4 dimethylpyrazole phosphate, DMPP) applied at 2 mg soil kg^{-1} or non-NI applied, respectively. Both individual amendments and their combination with DMPP significantly elevated the soil pH by 4.9–64.2% compared with the CK treatment, with the effectiveness ranking as CaO > CM ≈ CD > BC. Cumulative N_2O emissions were stimulated by the individual application of CaO, CM, and CD but were reduced by BC application compared with the CK treatment. Changes in N_2O emissions were positively correlated with the responses of the net N mineralization and nitrification rates to individual amendments, which were regulated by changes in the soil pH. The suppressive effects of NI combined with individual amendments on N_2O emissions were significant in the CaO treatment with a reduction ranging from 3.3% to 60.2%, which was attributed to decreased abundances of ammonia-oxidizing bacteria (AOB). Therefore, we concluded that the combined application of CaO and DMPP could be considered as a suitable mitigation strategy for addressing soil acidification through optimized N management. Additionally, BC can serve as a supplementary practice to simultaneously improve soil fertility. These insights are crucial for developing integrated fertilization management strategies to mitigate soil acidification with low N loss risks.

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Keywords: soil acidification mitigation strategies; N_2O emissions; nitrification inhibitors; nitrogen cycling microorganisms; enhanced-efficiency fertilizers

1. Introduction

Approximately 40–50% of the global arable area is acidic (pH < 5.5) [1], and this percentage continues to rise with a pH decrease, primarily due to increasing nitrogen (N) deposition and excessive chemical N fertilizer application [2]. The soil pH has declined by 0.63 and 0.50 units over two decades since the 1980s in grasslands and croplands in China, respectively [2,3]. This increasing soil acidification causes severe damage to soil health and food security, which includes decreased soil fertility with essential nutrient loss and enhanced toxic heavy metal accumulation, especially aluminum (Al^{3+}) and manganese (Mn^{2+}) [4]. Hence, mitigation strategies for soil acidification, with a high potential to improve the soil pH and maintain crop production, have been a worldwide priority [5,6]. Besides the alleviation of soil acidification, these strategies, such as alkaline substances, have shown profound impacts on soil biogeochemical processes, especially N cycling including N_2O emissions [5,7]. However, how N_2O emissions respond to divergent soil acidification mitigation strategies and the underlying mechanisms remain largely unknown, hindering the cognition of the optimal alleviation measures of soil acidification.

It is well-known that N_2O is one of the most important GHGs, with a warming potential 298 times that of carbon dioxide (CO_2) over a 100-year time horizon [8]. It has also been one of the substances destroying the stratospheric zone [9]. Globally, agricultural soils are the primary emitters of N_2O and account for 11% of anthropogenic GHG emissions [10]. Soil physicochemical properties, such as pH, soil organic carbon (SOC), soil N content, and microbial properties, play crucial roles in N_2O production and emissions [11]. These properties are significantly shifted by the amendments (mainly including lime, manure, and biochar) to alleviate soil acidification [12]. A growing number of research has been conducted to investigate the responses of N_2O emissions to soil acidification mitigation strategies, and the results are variable with regard to the various types and properties of the amendments as well as their extent to affect the soil properties [13,14]. For example, lime, the most common practice in which to raise the soil pH, has been found to increase both the N_2O and $N_2O/N_2 + N_2O$ product ratio under oxic conditions [13]. This observation is likely to be attributed to the elevated pH increases in soil nitrification and denitrification processes but unaffected relative transcriptions rates of denitrifying genes (*nosZ*), resulting in N_2O accumulation with high potential warming risks [15,16]. The application of manure could also alleviate soil acidification by adding organic matter and base cations [17]. Wei et al. demonstrated that although N_2O emissions were stimulated by the application of manure, the ratio of $N_2O/N_2 + N_2O$ decreased [14]. This reduction was attributed to the imbalance in the soil N pool and microbial activities caused by organic materials with a high C/N ratio, in addition to pH, as noted by Cheng et al. [18]. In comparison, biochar has been recognized as a promising amendment to mitigate N_2O emissions [19,20]. Its effectiveness in reducing N_2O emissions due to the modification in soil physical properties such as pore size and surface area depends on the morphological structure of biochar [21]. In contrast, biochar has also been reported to induce higher N_2O emissions by accelerating the nitrification rate [22]. Various amendments can alleviate soil acidification, making it essential to quantify and assess their effects, particularly those with a strong acidification alleviation effect and low N_2O emissions, to support effective mitigation strategies for acidic soils.

Biochemical N transformation processes, such as nitrification, denitrification, nitrifier denitrification, and dissimilatory nitrate reduction to ammonium, are common N_2O production pathways, especially the former two [23,24]. In the traditional view, nitrification is considered as a two-step process including ammonia (NH_3) oxidized to nitrite (NO_2^-N) via ammonia oxidizers (i.e., ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA)) and the subsequent oxidation of NO_2^-N to nitrate (NO_3^-N) by nitrite-oxidizing bacteria (NOB) [25]. However, this has been challenged by a recent discovery of complete ammonia oxidizers, which could directly oxidize ammonium (NH_4^+-N) to NO_3^-N and produce a low yield of N_2O [26,27]. Denitrification, including the production and reduction of N_2O, is subjected to the availability of NO_3^-N, the ultimate product of the nitrification process [28]. NIs, such as 3,4 Dimethylpyrazole phosphate (DMPP), nitrapyrin, and dicyandiamide (DCD), have been utilized to reduce nitrification by depressing AOA and AOB [17,29], thereby mitigating N_2O emissions via suppressing nitrification, and the decrease accumulation of NO_3^-N may also lead to a reduction in N_2O emissions via denitrification [30,31]. Generally, under oxic conditions, N_2O emissions have been observed to be positively correlated with the soil pH [32] because the nitrification rates often increase with increasing pH [33]. The utilization of alkaline amendments to raise soil pH may carry the risk of simultaneously stimulating soil N_2O emissions. Hence, combining soil acidification mitigation strategies with NIs could be a win-win measure to alleviate soil acidification and minimize concomitant N loss risks. Previous studies have generally individually assessed the impacts of soil acidification mitigation strategies or NIs on soil N_2O emissions in acid soils [13,34]. However, it is still unclear whether this combined measure is feasible, and which mitigation strategies for soil acidification would yield the most effective results when combined with NIs.

In this context, four typical soil acidification mitigation strategies (i.e., lime, chicken manure, cow dung, and biochar) and the most effective synthetic nitrification inhibitor (DMPP) were applied in an aerobic experiment using acidic soils collected from four sites in China. The objective was to evaluate the effectiveness of individual soil acidification mitigation strategies and their combination with NIs on the reduction in N_2O emissions, pursuing suitable measures based on optimal N management. We hypothesized that the effectiveness of NIs in reducing N_2O emissions would vary among the different soil acidification mitigation strategies, and that the NIs would exhibit superior N_2O emissions suppression effects in lime-treated soils compared with other approaches, attributed to the fact that a stronger increase in pH by lime results in a greater stimulation of N_2O emissions.

2. Materials and Methods

2.1. Site Description and Soil Sampling

Soil samples (0–20 cm) were collected in July 2022 from tea plantations in Xuancheng (XC), Jurong (JR), Xinyang (XY), and Quanzhou (QZ) across China (Figure S1). The detailed geographical information of the sampling sites is shown in Table S1. For each site, samples were collected and mixed into one composite sample. After removing visible stones and leaf pieces, fresh soils were passed through a 2 mm sieve and stored at 4 °C immediately until the start of the incubation experiment. These four sites were selected as representative acid soils in China, with a pH ranging from 3.76 to 4.27, and other basic soil physicochemical properties are shown in Table 1.

Table 1. Physical and chemical properties of typical acid soils in China.

Sites	pH	SOC	TN	C/N	NH_4^+-N	NO_3^--N	Clay	Silt	Sand
		(g kg^{-1})			(mg kg^{-1})			(%)	
XC	3.76 ± 0.07 b	23.3 ± 0.5 b	2.22 ± 0.03 c	10.55	17.4 ± 0.1 c	55.4 ± 1.1 b	6.8 ± 0.1 b	55.8 ± 0.3 a	37.4 ± 0.3 c
JR	4.27 ± 0.04 a	22.8 ± 0.9 b	2.46 ± 0.02 a	9.27	19.9 ± 0.6 b	89.9 ± 1.1 a	4.4 ± 0.1 c	47.9 ± 0.7 d	47.8 ± 0.7 a
XY	4.37 ± 0.05 a	12.2 ± 0.3 c	1.53 ± 0.01 d	7.94	36.0 ± 0.3 a	30.5 ± 0.83 c	4.6 ± 0.1 c	53.7 ± 0.6 b	41.7 ± 0.63 b
QZ	3.79 ± 0.18 b	32.0 ± 0.6 a	2.31 ± 0.03 b	13.65	2.5 ± 0.4 d	19.3 ± 0.2 d	15.1 ± 0.1 a	52.0 ± 0.1 c	32.9 ± 0.01 d

Note: Values are expressed as the means ± standard deviation (n = 3). Different letters indicate significant differences ($p < 0.05$). pH, soil pH; SOC, soil organic carbon; TN, total nitrogen; C/N, SOC to TN ratio; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou.

2.2. Incubation Experiment

An incubation experiment was conducted to evaluate the effectiveness of mitigation strategies for soil acidification and NIs on N_2O emissions. The treatments of the incubation experiment comprised the untreated control (CK); quicklime (CaO); chicken manure (CM); cow dung (CD); maize straw-derived biochar (BC); untreated control plus DMPP (CK + DMPP); quicklime plus DMPP (CaO + DMPP); chicken manure plus DMPP (CM + DMPP); cow dung plus DMPP (CD + DMPP); and maize straw-derived biochar (BC + DMPP). The NI utilized in this study was DMPP, which can effectively suppress nitrification at a low rate and has been approved for common use with a functional duration of about 4–10 weeks [31,35]. The basic properties of the amended materials are shown in Table S2. The application rate of CaO and DMPP were 3.5 g kg^{-1} soil and 2 mg kg^{-1} soil, respectively [36,37]. The amount of CM, CD, and BC was applied at an equal N level of 200 mg N kg^{-1} soil. Fresh soil (ranging from 22.4 g to 22.7 g, equivalent to 20 g dry weight) was pre-incubated at 25 °C in the dark for 1 day and then mixed thoroughly with the amended materials in 250 mL flasks according to the treatments. Three replicates were performed for each treatment. The moisture content of each mixed sample was adjusted to 60% water holding capacity (WHC) and maintained constantly every three days by weighing. All flasks were sealed with plastic wrap with small holes punched in the plastic wrap to ensure air circulation. Finally, the flasks were placed in dark incubators at 25 °C, with a total of 720 Erlenmeyer flasks (that is, 4 (sampling sites), ×10 (treatments), ×3 (incubation replicates) ×6 (sampling time)).

Soil sampling was carried out at 0.5 h, 1, 3, 7, 14, and 28 days after the incubation, and the samples were extracted with 100 mL KCl solution by shaking at 250 rpm for 1 h to measure the concentrations of NH_4^+-N and NO_3^--N. After 1, 3, 7, 14, and 28 days of incubation, gas samples were taken during a 6-h sealed incubation from the headspace of the flasks to determine the concentration of N_2O and CO_2. At the end of gas sampling, fresh air was pumped into the flasks to balance the pressure immediately. Moreover, the soil microbial properties (abundance of bacteria, fungi, AOA *amoA*, AOB *amoA*, and denitrifiers (*nirS*, *nirK*, and *nosZ*) were also measured at the end of the incubation.

2.3. Analyses

Soil pH was measured using a pH meter (Thermo Eutech pH 700, Waltham, MA, USA) at a soil-to-KCl ratio of 1: 5 (w/v). Soil organic carbon (SOC) and total N (TN) were measured by wet digestion with H_2SO_4-$K_2Cr_2O_7$ and semi-micro Kjeldahl digestion, respectively (Tables 1 and S2). Soil texture was determined with a Mastersizer-3000 laser particle characterization analyzer (Malvern Company, Worcestershire, UK). A continuous flow analyzer measured the concentrations of NH_4^+-N and NO_3^--N in the soil extracts at each sampling time (San^{++}, Skalar, Breda, The Netherlands) [37]. The N_2O and CO_2 concentrations at each sampling time were simultaneously measured using gas chromatography

(Agilent 7890, Santa Clara, CA, USA) [37]. All measured properties were converted based on oven-dried soil weight.

Microbial DNA was extracted from 0.5 g fresh soil following the instructions using the FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) for all soil samples at the end of the incubation period. The DNA contents were determined using a Nano Drop^{TM} 2000 spectrophotometer (Thermo Scientific, Waltham, USA) and stored at −80 °C before molecular analysis. The abundances of bacteria, fungi, and N-cycling genes were quantified with the CFX96 Real-time System (Bio-Rad Laboratories Inc., Hercules, CA, USA). The primer pairs and thermal profile are shown in Table S3. The copies of all genes were transformed to the Log_{10} value based on the oven-dried soil weight.

2.4. Calculations and Statistical Analyses

The net N mineralization rate (NMRs was calculated as the difference between the final and initial mineral N concentrations (NH_4^+-N and NO_3^--N) divided by 28 days. The net nitrification rates (NNR) was calculated in the same way as the daily mean accumulation of NO_3^--N. The average rates of N_2O and CO_2 emissions (represented as soil respiration) in each incubation interval were calculated from the increasing concentrations during that period. The cumulative N_2O emissions and soil respiration were calculated as follows:

$$E = \sum \left[\left(\frac{F_i + F_{i+1}}{2} \right) \times (t_{i+1} - t_i) \times 24 \right]$$

where F indicates the fluxes of gas (N_2O or CO_2), i is the ith measurement, and $(t_{i+1} - t_i)$ is the time interval (d) between two adjacent measurements.

Statistical analysis was performed using SPSS 22.0. A two-way analysis of variance (ANOVA) was conducted to determine the effects of the acidification mitigation measures and NIs on the soil N transformation rates, N_2O emissions, soil respiration, and microbial properties at each site. Statistical significance was set at a p value < 0.05 using the least significant difference (LSD) test. Regression analysis was also conducted to determine the associations between the soil properties, soil N transformation rates, N_2O emissions, and microbial properties using standardized data at each site to eliminate the differences in the magnitude of indicators. The variable importance on projection value (VIP value) was obtained using the partial least squares regression (PLSR) in SIMCA 14.1 (Umetrics, Malmo, Sweden) to indicate the importance of an independent variable with regard to a dependent variable. Variables with a VIP value above 1 were considered as important for the predictive variable.

3. Results

3.1. Soil pH and Mineral N Concentrations

All soil acidification mitigation strategies significantly increased the soil pH, regardless of DMPP application, across all four sites, compared with the CK treatment (average pH = 3.46). The effectiveness in raising the soil pH was ranked as follows: CaO (average pH = 5.68) > CM (average pH = 3.83) ≈ CD (average pH = 3.80) > BC (average pH = 3.63) (Figure S2 and Figure 1). In the CaO treatment at the XC and XY sites as well as all treatments at the JR site, the pH was significantly higher with the DMPP application than those without DMPP (Figure 1).

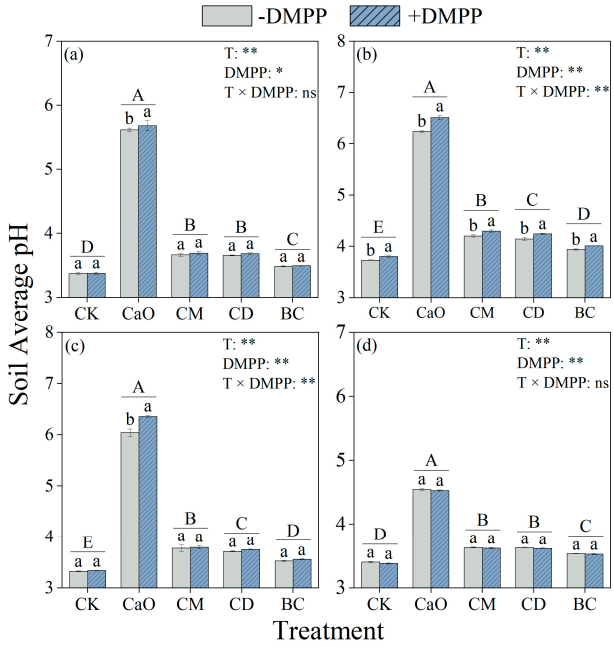

Figure 1. Changes in the soil average pH over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP at the XC (**a**), JR (**b**), XY (**c**), and QZ (**d**) sites. Different uppercase letters indicate significant differences at $p < 0.05$ among different acidification mitigation strategies at each site, and different lowercase letters indicate significant differences at $p < 0.05$ between treatments with and without DMPP application with a certain acidification mitigation strategy. Bars represent the standard deviation (n = 3). Symbols *, **, and ns following the capital letters T (acidification mitigation strategies), DMPP, and T × DMPP (interaction of the acidification mitigation strategies and DMPP) indicate the statistical significance for the main effects of the acidification mitigation strategies, DMPP, and their interaction effect at $p < 0.05$; $p < 0.01$, and no significant differences, respectively. CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou.

Besides the CaO + DMPP treatment, the NH_4^+-N concentrations in all treatments decreased within the first 7 days and stabilized or slightly increased until the end of the incubation (Figure S3), while the NO_3^--N concentrations exhibited an increasing trend during all incubation periods (Figure S4). DMPP application exhibited a higher NH_4^+-N concentration but a lower NO_3^--N concentration compared with those without DMPP application during the incubation (Figures S3 and S4). Soil NH_4^+-N/NO_3^--N significantly increased in the CaO treatment but decreased in the CM, CD, and BC treatments compared with the CK treatment at the XC and XY sites (Figures S5 and S6). The application of DMPP significantly increased the soil NH_4^+-N/NO_3^--N in all of the treatments at the XC and XY sites and decreased it at the JR site. However, no significant impact was observed at the QZ site, except for the CaO treatment (Figure S6).

3.2. Soil N_2O Emissions

Generally, the N_2O emission rates reached the highest within the first 3 days of incubation, except for the CaO + DMPP and CM + DMPP treatments at the XY site and the CM treatment at the QZ site, and then slightly fluctuated and decreased throughout the rest of the incubation period (Figure S7). Cumulative N_2O emissions over the 28-day incubation were largely enhanced by the CM and CD treatments at the XC site, the CaO and CM treatments at the XY site, and the CM and CD treatments at the QZ site, but decreased with the BC treatment at all four sites compared with the CK treatment (Figure 2). Moreover, due to the application of DMPP, the cumulative N_2O emissions significantly

declined in the CK, CaO, CM, and CD treatments at the JR site as well as the CM treatment at the QZ site compared with the respective non-DMPP applied treatments. However, the cumulative N_2O emissions increased in the CK, CM, and CD treatments at the XC sites and the CM and BC treatments at the XY and QZ sites, respectively, compared with the corresponding non-DMPP applied treatments (Figure 2). In addition, the suppressive effects of NI combined with individual amendments on N_2O emissions were significant in the CaO treatment compared with the other amendments, with a reduction ranging from 3.3% to 60.2% (Figure 2).

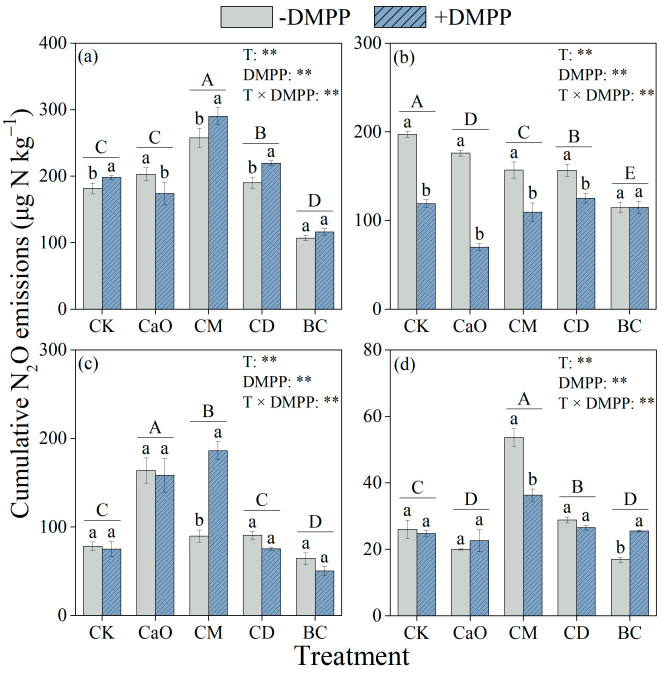

Figure 2. Changes in cumulative N_2O emissions over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP at the XC (**a**), JR (**b**), XY (**c**), and QZ (**d**) sites. Different uppercase letters indicate significant differences at $p < 0.05$ among the different acidification mitigation strategies at each site, and different lowercase letters indicate significant differences at $p < 0.05$ between treatments with and without DMPP application with a certain acidification mitigation strategy. Bars represent the standard deviation (n = 3). Symbol ** following the capital letters T (acidification mitigation strategies), DMPP, and T × DMPP (interaction of the acidification mitigation strategies and DMPP) indicates the statistical significance for the main effects of the acidification mitigation strategies, DMPP, and their interaction effect at $p < 0.01$. CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou.

3.3. Related N Transformation Rates and Microbial Properties

The addition of CaO, CM, and CD significantly increased the NMR, regardless of the DMPP application, compared with the CK treatment (Figure 3a–d) across all sites. In contrast, the NMR in the BC treatment was significantly lower than in the CK treatment (Figure 3a–d). The application of DMPP in the CaO treatment at the XC, JR, and XY sites as well as the CM treatment at the XC site exhibited significant influences on the NMR (Figure 3a–d). Changes in the NNR by the soil acidification mitigation strategies and DMPP were similar to their effects on the NMR, which was increased by the CaO, CM, and CD treatments at all sites (Figure 3e–h). The effects of BC treatment on the NNR were lower than the other treatments and varied across sites: it increased at the XC and XY sites, decreased at the QZ site, and remained unchanged at the JR site (Figure 3e–h). The application of DMPP significantly decreased the NNR in all treatments at the XC and JR

sites as well as the CaO and CD treatments at the XY site, but had no significant influences at the QZ site (Figure 3e–h).

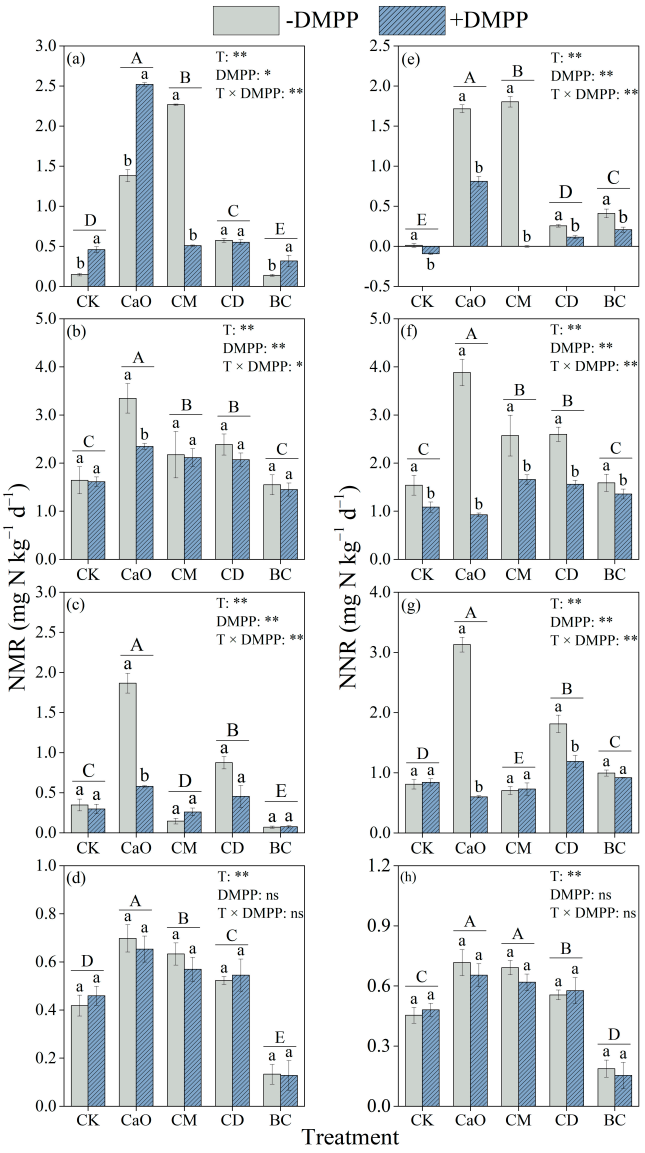

Figure 3. Changes in NMR (**a**–**d**) and NNR (**e**,**f**) over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP at the XC (**a**,**e**), JR (**b**,**f**), XY (**c**,**g**), and QZ (**d**,**h**) sites. Different uppercase letters indicate significant differences at $p < 0.05$ among different acidification mitigation strategies at each site, and different lowercase letters indicate significant differences at $p < 0.05$ between treatment with and without DMPP application with a certain acidification mitigation strategy. Bars represent the standard deviation (n = 3). Symbols *, **, and ns following the capital letters T (acidification mitigation strategies), DMPP, and T × DMPP (interaction of acidification mitigation strategies and DMPP) indicate the statistical significance for the main effects of the acidification mitigation strategies, DMPP, and their interaction effect at $p < 0.05$; $p < 0.01$, and no significant differences, respectively. NMR, net N mineralization rates; NNR, net nitrification rates; CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou.

The soil acidification mitigation strategies increased the soil cumulative respiration compared with the CK treatment, with the CaO treatment showing significant effects (Figures S8 and S9). Overall, the CaO treatment generally tended to increase the abundances of soil bacteria, fungi, and N-cycling genes compared with the CK treatment, regardless of the DMPP application (Figure 4). The application of CM also increased the microbial

abundances at the XC, JR, and XY sites, except for fungi and AOB *amoA* at the JR site. However, the microbial abundances were generally decreased at the QZ site, except for bacteria (Figure 4). Within the CD-treated soil, the abundances of denitrifiers increased significantly compared with those in the CK treatment (Figure 4). The abundance of AOB *amoA* significantly increased in the BC treatment, except for the XC site. In contrast, the denitrifiers increased at the XC and XY sites but decreased at the JR and QZ sites after the BC treatment. The abundances of AOA *amoA* increased in the DMPP treatment at the XC, JR, and XY sites but was decreased at the QZ site. On the other hand, the AOB *amoA* abundance decreased (Figure 4). The abundances of denitrifiers were slightly shifted in the DMPP treatment across the XC, JR, and XY sites but significantly decreased in the CM, CD, and BC treatments at the QZ site (Figure 4). The CaO treatment significantly decreased the ratio of AOA/AOB but increased the ratio of *nirS* + *nirK*/*nosZ* at all sites (Figure S10). The application of DMPP decreased the ratio of F/B, increased the ratio of AOA/AOB, and showed slight effects on the ratio of *nirS* + *nirK*/*nosZ* (Figure S10).

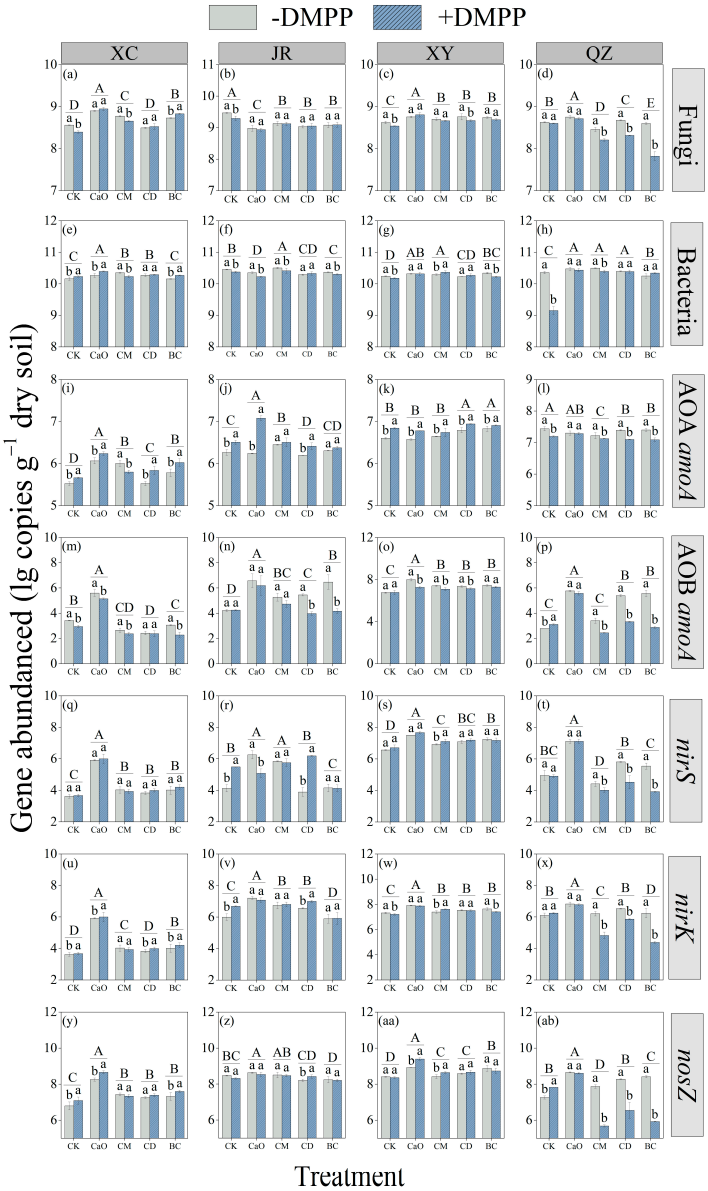

Figure 4. (**a**–**ab**) Changes in microbial properties over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP of different sampling sites. Different uppercase letters indicate significant differences at $p < 0.05$ among the different acidification mitigation

strategies at each site, and different lowercase letters indicate significant differences at $p < 0.05$ between treatment with and without DMPP application at with a certain acidification mitigation strategy. Bars represent the standard deviation (n = 3). CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; AOA, ammonia-oxidizing archaea; AOB, 48 ammonia-oxidizing bacteria; nirK and nirS, nitrite reductases; nosZ, nitrous oxide reductase; XC, 49 Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou.

3.4. Relationships Between N_2O Emissions and Soil Properties, N Transformation Rates, and Functional Gene Abundances

The partial least squares results showed that the cumulative N_2O emissions were regulated by the NMR and NNR, and AOA /AOB, nirK, and NH_4^+-N/NO_3^--N under both non-DMPP and DMPP treatments, respectively (Figure 5). Notably, positive relationships between cumulative N_2O emissions and NMR as well as NNR were observed exclusively under the non-DMPP treatment (Figure 6a,b). The soil NH_4^+-N/NO_3^--N was positively correlated with the cumulative N_2O emissions under the DMPP-applied condition (Figure 6c). Additionally, AOA/AOB was significantly correlated with cumulative N_2O emissions, regardless of the DMPP application (Figure 6d). The relationships between pH and NMR were significant irrespective of the DMPP application. In contrast, the NNR was correlated with pH only under the non-DMPP applied condition (Figure 6e,f). In addition, AOA/AOB was positively correlated with the pH and NH_4^+-N/NO_3^--N under the non-DMPP applied condition, while the opposite was observed under the DMPP-applied condition (Figure 6g,h).

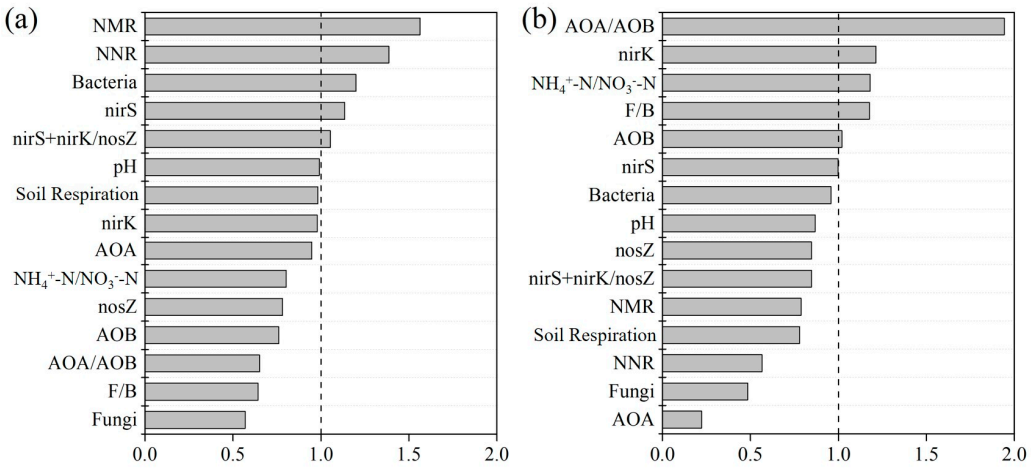

Figure 5. VIP values of the effects of the soil properties on cumulative N_2O emissions without (**a**) and with DMPP (**b**) application under the acidification mitigation strategies. VIP = 1.0 indicates a threshold above which the predictors are considered important for predictive purposes. VIP, variable importance in projection; NMR, net N mineralization rates; NNR, net nitrification rates; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; nirK and nirS, nitrite reductases; nosZ, nitrous oxide reductase; F/B, the ratio of Fungi to Bacteria; AOA/AOB, the ratio of AOA amoA to AOB amoA; nirS + nirK/ nosZ, the ratio of the sum of nirS and nirK to nosZ; NH_4^+-N/NO_3^--N, the ratio of soil NH_4^+-N to NO_3^--N.

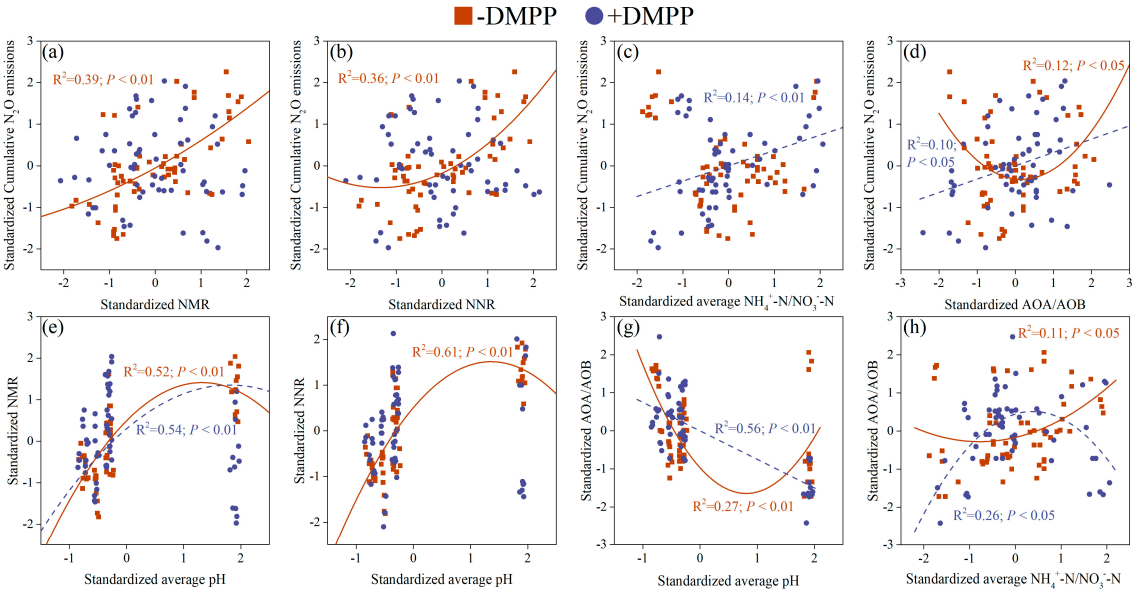

Figure 6. Relationships between the cumulative N_2O emissions and soil NMR (**a**), soil NNR (**b**), soil NH_4^+-N/NO_3^--N (**c**), and AOA/AOB (**d**), between the soil pH and NMR (**e**), NNR (**f**), and AOA/AOB (**g**), and between the soil NH_4^+-N/NO_3^--N and AOA/AOB (**h**) with and without DMPP application, respectively. NMR, net N mineralization rates; NNR, net nitrification rates; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; AOA/AOB, the ratio of AOA to AOB; NH_4^+-N/NO_3^--N, the ratio of soil NH_4^+-N to NO_3^--N.

4. Discussion

Soil acidification, driven by unreasonable anthropogenic activities such as excessive fertilization, represents a significant threat to food production and human health security [4]. Strategies such as crop residues, manure, biochar as well as lime (alkaline substances) application are effective in mitigating soil acidification by increasing the pH [5,7]. However, this pH increase may stimulate nitrification or denitrification processes, thereby posing significant environmental pollution risks through the production of N_2O [37,38]. Therefore, we integrated soil acidification mitigation strategies with the application of NIs to achieve optimal acidification mitigation control while reducing the GHG risks. Our findings provide valuable insights into selecting effective measures for mitigating soil acidification in consideration with optimal N management.

4.1. Effect of Elevated pH Under Soil Acidification Mitigation Strategies on N Transformation Rates

The soil pH is a general indicator of soil acidification, reflecting the concentration of active protons in the soil [39]. However, changes in pH are typically smaller than the proton inputs due to soil's acid buttering capacity [40] Therefore, even a slight change in pH reflects a significant shift in soil acid neutralizing capacity. In this study, the selected four strategies significantly increased the soil pH by 0.17–2.05 units, effectively mitigating the acidification of extremely acidic soils (pH < 4.5), regardless of DMPP application (Figure 1). Previous long-term field experiments have also reported an increase in pH following the application of manure and quicklime [11,41], and the acidification ameliorating effects of BC have also generated widespread attention [1,42]. Generally, manure and BC are rich in alkaline substances and possess a high pH buffering capacity, allowing them to directly neutralize acidogenic ions [43]. The pH of applied manure and BC materials exceeds 7.0 (Table S2), which is 2.5 units higher than acid soil (Table 1). Furthermore, cations such as Ca and Mg combined with organic anions in manure or formed carbonates during the pyrolysis process of BC production are effective in reacting with H^+ and monomeric

Al species and further decreasing the soil exchangeable acidity [44,45]. In addition, the application of high C/N ratio materials, such as BC with a C/N ratio exceeding 40, can promote the immobilization of soil NO_3^--N [46,47]. This process can further increase the soil pH, as the assimilation of one mole NO_3^--N assimilation results in a net consumption of 1 mol H^+ [48].

This study demonstrated that the NMR and NNR of soil acidification mitigation strategies, excluding the BC treatment, significantly increased by 45–58% and 58.5–76.5%, respectively, compared with the CK treatment, regardless of DMPP application (Figure 3). The changes in the NMR were significantly regulated by the soil pH regardless of DMPP application. In contrast, the effects of pH on the NNR were evident only under the non-DMPP treatment (Figure 6e,f). Previous studies have associated the soil N mineralization rate, the key process regulating soil N availability through the release of mineral N from organic N, with changes in soil pH changes. However, the relationships between soil acidification and the NMR rates remain inconsistent. It has been found that the NMR could increase [49], decrease [50], or remain unchanged [47] with an increase in pH in different soils. Such discrepancies can be attributed to the varying responses of soil gross mineralization and the NH_4^+-N immobilization rates to pH changes among studies. A ^{15}N label study demonstrated that the NH_4^+-N immobilization rates increased with the soil pH [11]. While these increased the immobilized N in MBN due to the increase in the soil pH, it was positively correlated to the gross N mineralization rate. This relationship suggests that immobilized N is temporarily conserved and could be subsequently re-mineralized, as previously highlighted in a global meta-analysis [51].

Furthermore, the pH is thought to influence the NNR via its impact on microbial communities [52]. We observed significant changes in the microbial abundances following the adoption of soil acidification mitigation strategies. Generally, an increase in pH could enhance ammonia-oxidizing microbes. In this case, we found that AOB *amoA*, rather than AOA *amoA*, was significantly shifted by the mitigation strategies for soil acidification and the application of DMPP (Figure 4; Table S4). This suggests that AOB may be primarily for ammonia oxidation, the first step of nitrification [53]. Moreover, the DMPP is a more effective inhibitor of AOB, and the attribution of AOB to ammonia-oxidizing activity can reach approximately 50% in acid arable soil [54]. Aside from nitrifiers, denitrifiers have also been found to be affected by pH changes (Table S4) [55]. The production of NO_3^--N through nitrification and the consumption of NO_3^--N via denitrification occur simultaneously, resulting in a complex relationship between the NNR and pH. This complexity arises from the differing impacts of pH on the gross nitrification and denitrification rates (Figure 6f).

4.2. Effect of Shifted Soil Physicochemical and Microbial Properties Under Soil Acidification Mitigation Strategies on N_2O Emissions

Through an integrated analyses of the soil properties, N transformation rates, and functional gene abundances, it was observed that the responses of N_2O emissions to soil acidification mitigation strategies were closely correlated with the NMR and NNR the under non-DMPP applied condition (Figures 5a and 6a,b). As one of the major GHGs, a numbers of studies have focused on the production and consumption mechanisms of N_2O in various ecosystems [56,57]. Despite the multiple and complex pathways of N_2O emissions, the nitrification (including ammonia oxidation and nitrifier denitrification) and heterotrophic denitrification pathways have been considered the dominant routes of N_2O emissions from soils [57,58]. The mineralization of organic N was stimulated by increased pH under various soil acidification mitigation strategies, releasing NH_4^+-N, the native source of nitrification, while the end product of the nitrification process, NO_3^--N, was the substrate of denitrification, leading to the production of N_2O [57]. A recent ^{15}N tracing study revealed that the potential N_2O emissions from native soil N was closely correlated with the

gross mineralization rate and nitrifier (AOB)-mediated N_2O-production process [59]. We observed that the abundances of AOB *amoA* were more sensitive to the various soil acidification mitigation strategies, indicating that changes in the nitrification process induced by AOB may play a more significant role in the responses of N_2O emissions to soil acidification mitigation strategies under the non-DMPP treatment (Figures 5 and 6a,b). This also explains why the correlations between the N transformation rates and N_2O emissions were blurred under the DMPP treatment, as DMPP is an effective inhibitor of AOB (Figure 6a,b). Moreover, although the denitrifiers responded positively to the soil acidification mitigation strategies (Table S4), previous studies have suggested that the elevated pH may alleviate the depression of the *nosZ* gene, leading to the production of N_2, rather than N_2O as the terminal product of heterotrophic denitrification [15,38,56]. Therefore, the changes in cumulative N_2O emissions can be attributed to the balance between the production of N_2O emissions via nitrification and denitrification processes and the consumption of N_2O to N_2 via denitrification, leading to inconsistent changes in N_2O emissions among the different soil acidification mitigation strategies.

When combining the soil acidification mitigation strategies and NIs, we revealed that the related gene ratio and mineral N concentrations were predominant factors affecting N_2O emissions (Figures 5b and 6c,d). The increased expression of AOB after the soil acidification mitigation strategies was depressed by the application of DMPP (Figure 4m–p). Previous studies have emphasized that AOA plays a more important role in ammonia oxidation than AOB in highly acidic soils [60]. Therefore, we conjectured that the N_2O emission pathways via nitrification under the DMPP treatment could be dominantly mediated by AOA, as its counterpart AOB was depressed. This may explain the positive relationships between the N_2O emissions and the ratio of AOA/AOB when DMPP was applied (Figure 6d). Generally, a high NH_4^+-N/NO_3^--N ratio indicates a conservative N cycling in the ecosystem [51]. However, we found that cumulative N_2O emissions were positively correlated with NH_4^+-N/NO_3^--N under the DMPP condition (Figure 6c), which suggests that the consumption of NO_3^--N with N_2O production via the denitrification process could provide a reasonable explanation when the AOB-mediated N_2O-production process was inhibited. Previous studies have emphasized that heterotrophic denitrification and nitrifier denitrification processes produce a large amount of N_2O in soils with low pH [18]. This could be supported by the predominant influence of the denitrifying gene *nirK* on N_2O emissions under the DMPP treatment (Figure 5b). Besides the abundances of N functional genes, how responses of the diversity, composition, and interaction among those microorganisms to acidification mitigation and NIs affect N_2O emissions via high-throughput sequencing should also be taken into consideration and investigated in further studies.

4.3. Soil Acidification Mitigation Strategies Based on Optimal N Management

As a dominant threat to food production, soil acidification has been accelerated by cultivation management, especially the application of ammonium-based chemical fertilizers [2,61,62]. Considering the ultimate target of crop production and sustainable agriculture, suitable soil acidification mitigation strategies should be proposed based on optimal N management, increasing the N availability, and decreasing N loss risks via N_2O emissions and nitrate leaching. Hence, we propose the combination of CaO and DMPP as an optimal soil acidification mitigation strategy, with a relatively high pH and NMR but low NNR and N_2O emissions (Figure 7). Aside from the pH, the efficacy of NIs on N_2O emissions could also be affected by the soil properties. As a type of heterocyclic compound, DMPP can be absorbed into soil particles, particularly in soils with high organic matter [34], which may explain its lower effectiveness in reducing the N_2O emissions in treatments with carbon inputs (Table S2). Previous studies have demonstrated that when the pH is less

than 5.5, the poor soil fertility and toxicity caused by increasing levels of Al^{3+} induced by soil acidification lead to a decrease in crop yield [61]. In this case, the initial step involves increasing the pH through the application of alkaline substances such as CaO. However, applying CaO also has drawbacks including increased soil compaction, decreased soil bulk density, and associated yield reductions [12,63]. Moreover, the soil nitrification process was also accelerated by the CaO application (Figure 3b). Theoretically, when 1 mol of NH_4^+-N converts to NO_3^--N via nitrification, 2 mol of H^+ is produced, probably leading to potential re-acidification and NO_3^--N leaching risks following CaO application [64]. Therefore, a combination of a nitrification inhibitor with CaO application could be a positive solution to balance pH elevation and nitrification stimulation. However, although applying CaO and DMPP could alleviate soil acidification, it may enhance fertilizer N losses because it stimulates urease activity. The large amount of Ca^{2+} provided by CaO compete with NH_4^+-N for absorption sites in soil, leading to a decrease in the soil's capability to retain NH_4^+-N [65–67]. In addition, another possible way to alleviate N loss risks following the implementation of soil acidification mitigation strategies is to promote the immobilization of mineral N by microbes [48,68]. In this study, we revealed that the application of BC decreased the N_2O emissions and showed a slight influence on the N transformation rates (Figure 7), probably due to the adsorption of NH_4^+-N on its surface [69]. It has also been found that applying organic materials with a high C/N ratio (over 18) enhances the microbial NO_3^--N immobilization, indicating that BC could be a potential strategy to elevate soil fertility and decrease the N loss risks. Hence, a soil acidification mitigation strategy comprising of the application of CaO, DMPP, and BC was formulated, aiming to decrease soil acidification, improve fertility, and optimize N management.

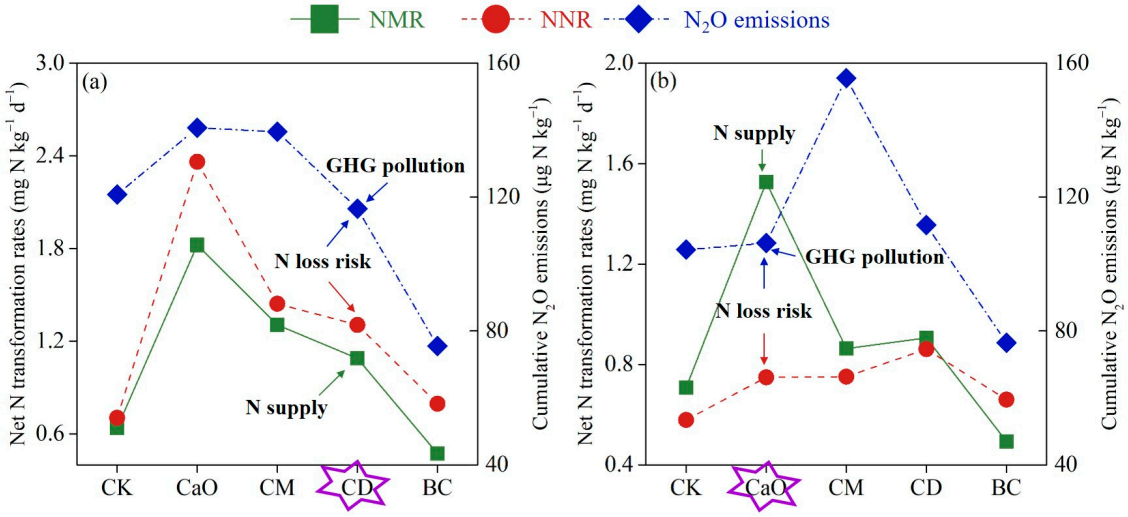

Figure 7. Diagram for the impacts of the acidification mitigation strategies on the soil N_2O emissions and related N transformation rates, and optimal acidification mitigation measures marked with a purple star, without (**a**) and with (**b**) DMPP application, respectively. NMR, net N mineralization rates; NNR, net nitrification rates; GHG pollution, greenhouse gas pollution.

5. Conclusions

This study explored optimal soil acidification mitigation strategies in consideration of effectively increasing the pH and mineralizing the soil organic N while minimizing N_2O emissions. Our results revealed that CaO application was the most effective measure to elevate pH in highly acid soils, followed by manure and BC application. The increases in the net N transformation rates triggered by soil acidification mitigation strategies stimulated N_2O emissions, except for the BC treatment. The interaction between NIs and soil acidification mitigation strategies effectively reduces the abundance of nitrifying functional

genes, and the suppressive effects on N_2O emissions were more telling than the CaO treatment. We concluded that combining CaO and NIs is a suitable measure to alleviate soil acidification and reduce the accompanying N loss. On this basis, BC could also be regarded as a supplementary practice to improve soil fertility with slight effects on N_2O emissions. Our results highlight the prospects of the combined use of NIs and alkaline amendments in alleviating soil acidification with the consideration of proper N management. The conclusions in this study are based on the incubation experiment with limited acidification mitigation strategies in soils with similar pH. Further research is needed to explore the effectiveness of these optimal soil acidification mitigation strategies in different soil types or pH ranges based on the field experiment, with the crop N use efficiency also being an important consideration.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15071536/s1, Table S1: Geographical information of the soil sampling sites; Table S2: Physical and chemical properties of acidification mitigation materials; Table S3: Primers and thermal profile; Table S4: Correlations between pH and microbial abundances; Figure S1: Sampling sites in China; Figure S2: Changes in soil pH over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP at the XC (a), JR (b), XY (c), and QZ (d) sites. CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou; Figure S3: Changes in soil NH_4^+-N concentrations over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP at the XC (a), JR (b), XY (c), and QZ (d) sites. CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou; Figure S4: Changes in soil No_3^--N concentrations over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP at the XC (a), JR (b), XY (c), and QZ (d) sites. CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou; Figure S5: Changes in soil NH_4^+-N/No_3^--N over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP at the XC (a), JR (b), XY (c), and QZ (d) sites. NH_4^+-N/No_3^--N, the ratio of soil NH_4^+-N to No_3^--N; CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou; Figure S6: Changes in soil average NH_4^+-N/No_3^--N over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP at the XC (a), JR (b), XY (c), and QZ (d) sites. Different uppercase letters indicate significant differences at $p < 0.05$ among the different acidification mitigation strategies at each site, and different lowercase letters indicate significant differences at $p < 0.05$ between with and without DMPP application treatment with a certain acidification mitigation strategy. Bars represent the standard deviation (n = 3). Symbols ** following the capital letters T (acidification mitigation strategies), DMPP, and T × DMPP (interaction of acidification mitigation strategies and DMPP) indicate the statistical significance for the main effects of acidification mitigation strategies, DMPP, and their interaction effect at the $p < 0.01$ level. NH_4^+-N/No_3^--N, the ratio of soil NH_4^+-N to No_3^--N; CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou; Figure S7: Changes in N_2O emission rates over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP at the XC (a), JR (b), XY (c), and QZ (d) sites. CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou; Figure S8: Changes in soil respiration rates over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP at the XC (a), JR (b), XY (c), and QZ (d) sites. CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou; Figure S9: Changes in soil respiration over a 28-day incubation period with and without the application of acidification mitigation strategies

and DMPP at the XC (a), JR (b), XY (c), and QZ (d) sites. Different uppercase letters indicate significant differences at $p < 0.05$ among the different acidification mitigation strategies at each site, and different lowercase letters indicate significant differences at $p < 0.05$ between with and without DMPP application treatment with a certain acidification mitigation strategy. Bars represent the standard deviation (n = 3). Symbols **, and ns following the capital letters T (acidification mitigation strategies), DMPP, and T × DMPP (interaction of acidification mitigation strategies and DMPP) indicate the statistical significance for the main effects of acidification mitigation strategies, DMPP, and their interaction effect at $p < 0.01$ and no significant differences, respectively. CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou; Figure S10: Changes in gene ratio over a 28-day incubation period with and without the application of acidification mitigation strategies and DMPP of different sampling sites. Different uppercase letters indicate significant differences at $p < 0.05$ among the different acidification mitigation strategies at each site, and different lowercase letters indicate significant differences at $p < 0.05$ between with and without DMPP application treatment with a certain acidification mitigation strategy. Bars represent the standard deviation (n = 3). CaO, quicklime; CM, chicken manure; CD, cow dung; BC, biochar; DMPP, 3,4-dimethylpyrazole phosphate; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; nirK and nirS, nitrite reductases; nosZ, nitrous oxide reductase; F/B, the ratio of fungi to bacteria; AOA/AOB, the ratio of AOA amoA to AOB amoA; nirS + nirK/ nosZ, the ratio of the sum of nirS and nirK to nosZ; XC, Xuancheng; JR, Jurong; XY, Xinyang; QZ, Quanzhou.

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