

## Article

# Smaller Nano-Silica Particles Promote Ammonium Dominance and Mitigate N<sub>2</sub>O Emissions in Flooded Tropical Paddy Soil

Xiaomeng Sun<sup>1</sup>, Junjie Feng<sup>1</sup> , Rui Zhang<sup>1</sup>, Yu Zhang<sup>1</sup>, Yunxing Wan<sup>1</sup>, Tao Li<sup>1</sup>, Siyi Xu<sup>1</sup>, Mengru Kong<sup>1</sup>, Yanzheng Wu<sup>1</sup>, Lei Meng<sup>1,2</sup> , Jinbo Zhang<sup>2,3</sup> and Ahmed Salah Elrys<sup>1,3,4,\*</sup>

<sup>1</sup> School of Tropical Agriculture and Forestry, Hainan University, Haikou 570228, China; 24220951320200@hainanu.edu.cn (X.S.); 24220951320130@hainanu.edu.cn (J.F.); 24220951310190@hainanu.edu.cn (R.Z.); 25220951320029@hainanu.edu.cn (Y.Z.); 24110901000021@hainanu.edu.cn (Y.W.); 25110901000025@hainanu.edu.cn (T.L.); 25220951320020@hainanu.edu.cn (S.X.); 23220951320028@hainanu.edu.cn (M.K.); 993741@hainanu.edu.cn (Y.W.); menglei@hainanu.edu.cn (L.M.)

<sup>2</sup> School of Breeding and Multiplication, Hainan University, Sanya 572025, China; zhangjinbo@hainanu.edu.cn

<sup>3</sup> Liebig Centre for Agroecology and Climate Impact Research, Justus Liebig University, 35392 Giessen, Germany

<sup>4</sup> College of Agriculture, University of Al Dhaid, Al Dhaid, Sharjah P.O. Box 27272, United Arab Emirates

\* Correspondence: aselrys@hainanu.edu.cn

## Abstract

Silicon (Si)-based amendments can regulate soil nitrogen (N) cycling and reduce gaseous N losses, but the effect of nano-Si particle size on mineral N dynamics and nitrous oxide (N<sub>2</sub>O) emissions under flooded tropical paddy soil conditions remains unclear. This study aimed to elucidate how nano-Si particle size regulates mineral N dynamics and N<sub>2</sub>O emissions, with particular emphasis on whether smaller particles promote ammonium N (NH<sub>4</sub><sup>+</sup>-N) dominance and more effectively mitigate N<sub>2</sub>O emissions than larger particles. A 30-day flooded incubation experiment was conducted using tropical paddy soil amended with 15 or 50 nm nano-Si particles, each applied at 67 mg kg<sup>-1</sup> dry soil, alongside an unamended control. Mineral N dynamics, extracellular enzyme activities, N-cycling functional genes, and N<sub>2</sub>O emissions were evaluated. Compared with the control, 15 nm and 50 nm nano-Si significantly increased NH<sub>4</sub><sup>+</sup>-N concentration by 16.4% and 5.25% while reducing nitrate N (NO<sub>3</sub><sup>-</sup>-N) concentration by 40.8% and 23.0%, respectively. The NO<sub>3</sub><sup>-</sup>-N/NH<sub>4</sub><sup>+</sup>-N ratio decreased significantly by 49.8% and 22.1%, indicating a particle-size-dependent shift toward NH<sub>4</sub><sup>+</sup> dominance. The 15 nm treatment significantly enhanced β-N-acetylglucosaminidase and leucine aminopeptidase activities, supporting organic N turnover and NH<sub>4</sub><sup>+</sup>-N accumulation. It also significantly reduced the abundance of the nitrite reductase gene *nirS*, involved in denitrification, whereas the N<sub>2</sub>O reductase gene *nosZ*, responsible for N<sub>2</sub>O reduction, showed no consistent treatment-specific response. Consistently, cumulative N<sub>2</sub>O emissions decreased significantly by 33.6% and 18.2% under 15 nm and 50 nm treatments, respectively. These results indicate that both nano-Si treatments significantly shifted mineral N dynamics toward NH<sub>4</sub><sup>+</sup>-N dominance and reduced cumulative N<sub>2</sub>O emissions compared with the control. These responses were consistently stronger under the 15 nm treatment than under the 50 nm treatment, demonstrating that smaller nano-Si particles more effectively limit NO<sub>3</sub><sup>-</sup>-N accumulation, promote NH<sub>4</sub><sup>+</sup>-N retention, and mitigate N<sub>2</sub>O emissions in flooded tropical paddy soil. Nevertheless, direct measurements of N-transformation rates, comparisons with conventional Si sources, and field-scale validation are required before practical application.

**Keywords:** paddy soil; silica nanoparticles; enzyme activity; denitrification; nitrous oxide emissions



Received: 24 June 2026

Revised: 4 August 2026

Accepted: 12 August 2026

Published: 14 August 2026

**Copyright:** © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

## 1. Introduction

Silicon (Si) is one of the most abundant elements on Earth and is widely considered a beneficial growth-promoting element for crops [1,2], especially those grown in rice production systems [3]. Although Si is not universally classified as an essential plant nutrient, its application can enhance plant growth and development [4], improve plant resistance to both biotic and abiotic stresses, and regulate the physicochemical and biological characteristics of soil [5]. Paddy soils represent special environments in which Si fertilization can play an important role by improving soil structure, increasing soil organic matter (SOM) content, modifying soil pH [6], decreasing soil bulk density, and stimulating soil microbial biomass and extracellular enzyme activities [7]. These findings suggest that Si application may influence soil functioning not only through plant physiological regulation but also through its impact on soil biogeochemical processes [8,9].

Nitrogen (N) is essential for plant growth and soil fertility, and its adequate availability is critical for rice productivity. However, inefficient soil N cycling can promote substantial N losses, including gaseous emissions from flooded paddy soils [10]. Most N in soil is stored in organic forms, and plant-available N largely depends on microbial mineralization processes that produce ammonium-N ( $\text{NH}_4^+\text{-N}$ ) and nitrate-N ( $\text{NO}_3^-\text{-N}$ ) [11]. Oxygen diffusion is restricted under flooded anaerobic conditions, and thus soil  $\text{NH}_4^+\text{-N}$  becomes the predominant and relatively stable inorganic N form [12]. In contrast,  $\text{NO}_3^-\text{-N}$  is usually less persistent because nitrification is limited by low oxygen availability, while existing  $\text{NO}_3^-$  can be rapidly consumed through microbial reduction pathways, including denitrification [13]. Therefore, the balance between  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  is a key indicator of N retention, N transformation, and the potential risk of gaseous N losses in paddy soils [14–16].

Silicon application may directly or indirectly regulate soil N dynamics by modifying the physicochemical and biological conditions that control N retention and transformation [17,18]. The dissolution of silicate materials can consume protons and release alkaline cations, thereby increasing soil pH and altering the adsorption, availability, and microbial transformation of mineral N [19]. Silicon-induced changes in mineral surfaces and organo-mineral interactions may also affect soil organic carbon (SOC) stabilization and accessibility, while associated shifts in microbial biomass and extracellular enzyme activities can regulate organic matter decomposition and the supply of C substrates supporting heterotrophic N transformations [20]. These effects may be particularly important in flooded soils, where restricted  $\text{O}_2$  diffusion creates strongly reducing conditions and makes denitrification highly sensitive to changes in pH, C availability, mineral N supply, and microbial functional capacity. Through these interconnected pathways, Si amendments may regulate both the availability of  $\text{NO}_3^-$  for denitrification and the balance between its stepwise reduction to  $\text{N}_2\text{O}$  and the subsequent reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  [17,21]. Understanding this regulation therefore requires an integrated assessment linking mineral N dynamics and  $\text{N}_2\text{O}$  emissions with their physicochemical, biochemical, and microbial controls. However, whether and to what extent these coupled responses depend on the intrinsic properties of the applied Si material, particularly its particle size, remains poorly understood.

The rapid development of nanotechnology has led to the emergence of a novel type of Si fertilizer: nano-Si fertilizers. Compared with conventional Si fertilizers and relatively larger-sized particles, nano-Si particles usually possess larger specific surface areas, higher surface reactivity, a higher proportion of active functional groups, and stronger interactions with soil particles, soil nutrients, microbial cells, and extracellular enzymes [22,23]. Based on these characteristics, nano-Si fertilizers may exert stronger impacts on soil N dynamics than conventional Si fertilizers. However, nano-Si should not be treated as a single uniform material. Particle size is likely to be a key factor driving its behavior in soil. Smaller

nano-Si particles may dissolve more readily, provide more reactive surfaces, and interact more closely with soil colloids and microbial habitats than larger particles [24]. Therefore, different nano-Si particle sizes may produce various impacts on microbial enzyme activity, mineral N dynamics, denitrification processes, and greenhouse gas emissions [17]. Despite growing research on nano-Si fertilizer applications, the role of particle size in controlling the N cycle in soils still lacks comprehensive investigation [22]. To the best of our knowledge, no study has systematically compared the effects of different nano-Si particle sizes on mineral N dynamics, extracellular enzyme activities, denitrification-related functional genes, and N<sub>2</sub>O emissions under flooded tropical paddy soil conditions.

It is expected that smaller nano-Si particles will exert stronger effects on mineral N dynamics in flooded paddy soils than larger particles due to their higher surface reactivity and stronger interactions with soil components and microbial processes [25,26]. In flooded soils, smaller particles may stimulate the decomposition of SOM and the activity of nutrient-acquisition enzymes, thereby increasing organic N mineralization and NH<sub>4</sub><sup>+</sup> accumulation [27]. At the same time, smaller nano-Si particles may reduce NO<sub>3</sub><sup>−</sup> accumulation by shifting the soil N environment toward NH<sub>4</sub><sup>+</sup> dominance [28]. This could occur through several linked mechanisms, including enhanced NH<sub>4</sub><sup>+</sup> retention, limited nitrification under flooded conditions, and increased microbial consumption of NO<sub>3</sub><sup>−</sup> through reduction pathways [29]. As a result, the NO<sub>3</sub><sup>−</sup>-N/NH<sub>4</sub><sup>+</sup>-N ratio is expected to decrease in the presence of smaller nano-Si particles, not simply because NO<sub>3</sub><sup>−</sup> is unstable under flooded conditions, but because smaller particles may actively redirect mineral N transformation toward NH<sub>4</sub><sup>+</sup> accumulation and microbial NO<sub>3</sub><sup>−</sup> consumption.

Particle size may also regulate N<sub>2</sub>O emissions in paddy soils. Under flooded conditions, N<sub>2</sub>O is chiefly produced as an intermediate during denitrification, but whether it accumulates or is further reduced to N<sub>2</sub> depends strongly on denitrifying microbial communities and soil biochemical conditions [30,31]. Smaller nano-Si particles may reduce N<sub>2</sub>O emissions through two complementary mechanisms. First, by decreasing NO<sub>3</sub><sup>−</sup> availability and lowering the NO<sub>3</sub><sup>−</sup>/NH<sub>4</sub><sup>+</sup> ratio, smaller particles may reduce NO<sub>3</sub><sup>−</sup> substrate supply for incomplete denitrification [32]. Second, smaller nano-Si particles may stimulate extracellular enzyme activities and increase the availability of labile organic C, which provides electron donors for denitrification. Together with Si-induced changes in soil pH, this increased C availability may promote the final denitrification step—the reduction of N<sub>2</sub>O to N<sub>2</sub>—thereby decreasing the proportion of N<sub>2</sub>O emitted from the soil [33]. Hence, smaller particles are expected to cause the largest decrease in N<sub>2</sub>O emissions in flooded soil, not only because of reduced NO<sub>3</sub><sup>−</sup> availability, but also because of their potential positive influence on denitrification completion and N<sub>2</sub>O reduction.

The aim of this study is to determine the effect of nano-Si fertilizers with varying particle sizes on mineral N dynamics and N<sub>2</sub>O emissions under flooded tropical paddy soil conditions. The questions addressed by this study are as follows: (i) Does nano-Si particle size influence mineral N dynamics and shift the N balance in flooded soil toward NH<sub>4</sub><sup>+</sup>-N dominance? (ii) Do smaller nano-Si particles stimulate extracellular enzyme activities more effectively than larger particles? (iii) Can nano-Si particle size influence denitrification-related processes and reduce N<sub>2</sub>O emissions?

As stated above, we hypothesized that smaller nano-Si particles would exert stronger effects on soil N cycling than larger particles under flooded conditions. Specifically, smaller nano-Si particles were expected to (1) increase NH<sub>4</sub><sup>+</sup>-N accumulation while decreasing NO<sub>3</sub><sup>−</sup>-N accumulation and the NO<sub>3</sub><sup>−</sup>-N/NH<sub>4</sub><sup>+</sup>-N ratio; (2) more strongly enhance extracellular enzyme activities associated with organic matter decomposition and microbial nutrient acquisition; and (3) reduce N<sub>2</sub>O emissions by decreasing NO<sub>3</sub><sup>−</sup>-N availability for N<sub>2</sub>O-producing pathways and altering denitrification-related functional gene abundances.

These responses were expected to result in a stronger shift toward  $\text{NH}_4^+$ -dominated mineral N partitioning and lower  $\text{N}_2\text{O}$  emissions under the smaller particle-size treatment.

## 2. Materials and Methods

### 2.1. Site Description and Sample Collection

The study area is located in the basalt-developed paddy soil region of Jiuzhou Town, Haikou City, China's Hainan Province ( $19^\circ 45' 42.48''$  N,  $110^\circ 24' 42.69''$  E). This region exhibits a typical tropical maritime monsoon climate, characterized by warm and humid conditions throughout the year. The mean annual temperature is approximately  $24^\circ\text{C}$ , with warmer and humid conditions during summer (June–August;  $28$ – $32^\circ\text{C}$ ), and relatively cooler conditions during winter (December–February;  $15$ – $20^\circ\text{C}$ ). The mean annual precipitation is approximately  $1700$  mm, with rainfall concentrated mainly between May and October.

Soil used for the incubation experiment was collected after rice harvest in October 2025. To obtain sufficient and representative bulk soil for the laboratory incubation, surface soil was collected from the  $0$ – $20$  cm layer at multiple randomly selected locations within the rice field using a  $5$  cm diameter soil auger. Visible roots, stones, and plant residues were removed during sampling. The collected soil was thoroughly mixed to obtain a homogeneous bulk sample, placed in sealed plastic bags, transported to the laboratory in an ice box, and passed through a  $2$  mm sieve to remove coarse particles and remaining plant debris. The sieved soil was then air-dried at room temperature before being used to set up the incubation experiment. The initial physicochemical properties of the soil are shown in Table S1.

### 2.2. Preparation and Characterization of Nano-Silica Materials

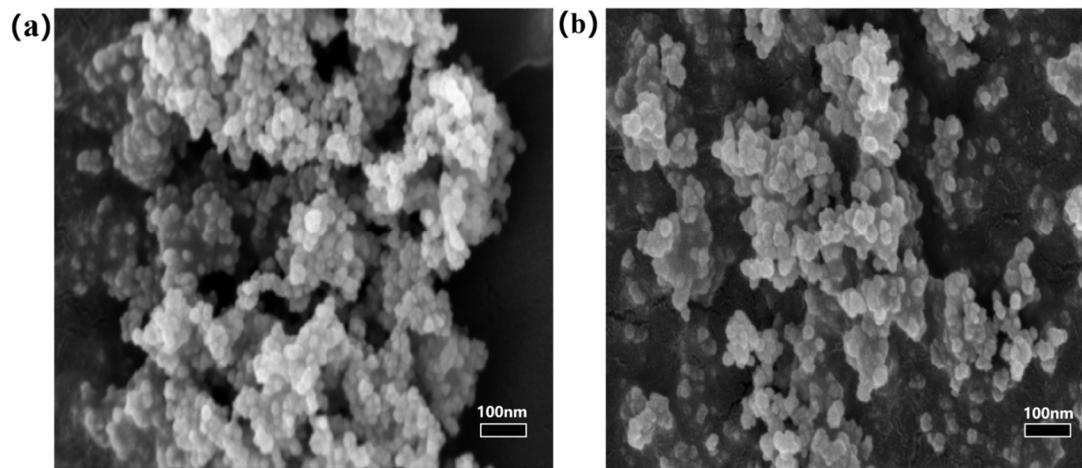
The nano-silica ( $\text{SiO}_2$ ) materials used in the experiment were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. Shanghai, China (product codes S861488 and S817567). According to the supplier, the two materials had nominal particle sizes of  $15 \pm 5$  nm and  $50 \pm 5$  nm, with purity  $\geq 99.0\%$  for both. The materials were supplied as white powders and were stored in sealed containers in a desiccator immediately after purchase to minimize moisture absorption and particle aggregation. The morphology and particle size distribution of the nano-silica particles were further characterized using field-emission scanning electron microscopy (FE-SEM; Hitachi SU8010, Tokyo, Japan), as described below:

**Sample preparation:** For FE-SEM analysis,  $10$  mg of nano-silica powder was dispersed in  $5$  mL of anhydrous ethanol to prepare a  $2$  mg  $\text{mL}^{-1}$  suspension. The suspension was ultrasonicated for  $15$  min at  $200$  W to reduce particle aggregation and improve dispersion. A  $20$   $\mu\text{L}$  aliquot of the dispersed suspension was then drop-cast onto a clean silicon wafer. The wafer was dried under gentle heating using an infrared/electric lamp until the ethanol had fully evaporated, and the dried particles were subsequently examined by FE-SEM.

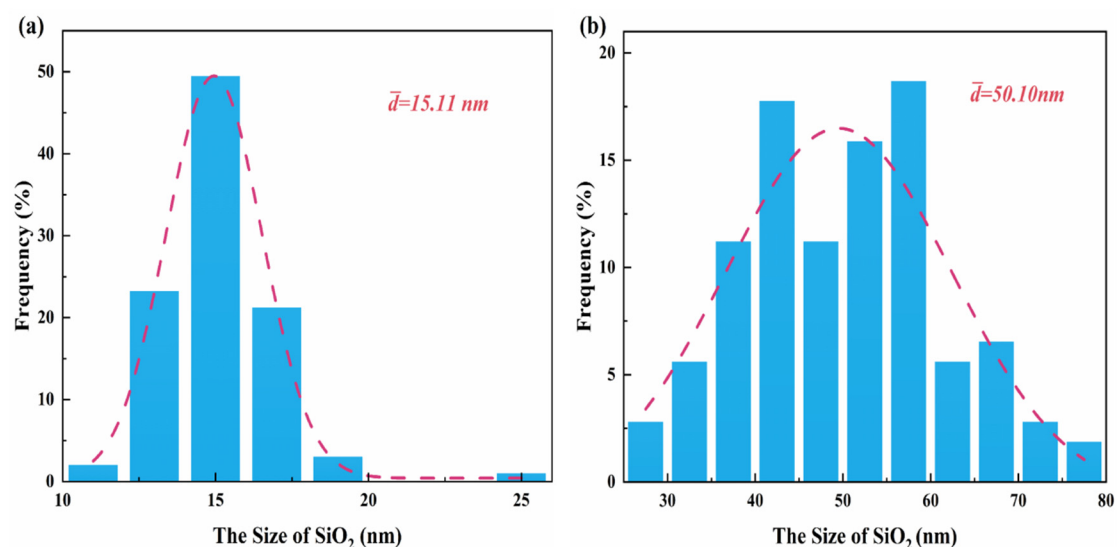
**Gold sputter coating:** Because  $\text{SiO}_2$  particles are non-conductive, the prepared samples were sputter-coated with gold before FE-SEM observation. Gold coating was performed using an ion sputter coater (Q150T ES; Quorum Technologies Ltd., Laughton, UK) at  $20$  mA for  $60$  s, resulting in an approximately  $5$  nm thick conductive layer on the sample surface.

**Electron microscopy observation and particle size analysis:** The gold-coated samples were mounted on the FE-SEM stage and placed in the microscope chamber (Figure 1). The chamber was evacuated to a vacuum pressure of  $\leq 1 \times 10^{-3}$  Pa. Images were acquired at an accelerating voltage of  $15$  kV and a working distance of approximately  $8$  mm. Representative micrographs were obtained at different magnifications, including  $10,000\times$ ,  $20,000\times$ , and  $50,000\times$ . Particle size analysis was performed using ImageJ software (version 1.53c).

At least 50 particles were randomly selected from the FE-SEM images of each sample, and their diameters were measured to calculate the mean particle size and standard deviation. Particle size distribution plots were generated using Origin 2024 to illustrate the size distribution of the nano-silica particles (Figure 2).



**Figure 1.** Field-emission scanning electron microscopy (FE-SEM) images showing the morphology and aggregation state of nano-silica ( $\text{SiO}_2$ ) materials with supplier-reported nominal particle sizes of (a)  $15 \pm 5$  nm and (b)  $50 \pm 5$  nm.



**Figure 2.** Particle size distributions of the small- (a) and large (b)-sized nano-silica ( $\text{SiO}_2$ ) particles based on FE-SEM image analysis. Bars represent the relative frequency of individually measured particles within each particle size interval. Dashed lines represent fitted Gaussian curves of particle size distribution. Particle diameters were measured using ImageJ, and the mean particle diameters were 15.11 nm and 50.10 nm, respectively.

### 2.3. Laboratory Soil Incubation Experiment

A laboratory flooded incubation experiment was conducted to evaluate the effects of nano-silica particle size on inorganic N dynamics, extracellular enzyme activities, N-cycling functional gene abundances, and gaseous N emissions in tropical paddy soil. The experiment included three treatments: an untreated control (CK), 15 nm nano-silica, and 50 nm nano-silica, with three replicates per treatment. Nano-silica was added to the 15 nm and 50 nm treatments at a rate of  $67 \text{ mg kg}^{-1}$  dry soil, which is nominally equivalent to approximately  $167.5 \text{ kg ha}^{-1}$  based on a 0–20 cm surface-soil mass of  $2.5 \times 10^6 \text{ kg ha}^{-1}$ .

This theoretical mass-based conversion is provided only to indicate the approximate application scale and should not be interpreted as a recommended field application rate. Before incubation, the nano-silica powder was thoroughly mixed with dry, sieved soil in sealed polyethylene bags to ensure uniform distribution and minimize the dispersion of fine particles. Soil moisture was then adjusted to 60% of water-holding capacity (WHC), and soils were pre-incubated for 7 days to stabilize microbial activity. Immediately after pre-incubation, the soils were flooded, and a 2–3 cm water layer was maintained above the soil surface throughout the subsequent 30-day incubation period to simulate flooded paddy conditions.

#### 2.4. Determination of Soil Biogeochemical Indicators

Following treatment application, soil samples were collected on days 1, 10, 20, and 30 to determine soil properties and the activities of extracellular enzymes. Soil pH was measured using a pH meter (HANNA HI2221, Padua, Italy) in a soil–water suspension at a soil ratio of 1:2.5. Soil  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N were extracted with 2 M KCl at a soil ratio of 1:5 and determined by UV spectrophotometry following the method described by [34]. The concentrations of SOC and total N were measured with a Sercon Integra 2 elemental analyzer (Sercon Limited, Crewe, UK). The concentrations of microbial biomass C (MBC) and N (MBN) were determined using the chloroform fumigation-extraction method [35]. The activities of four extracellular enzymes, including  $\beta$ -1,4-glucosidase (BG), leucine aminopeptidase (LAP),  $\beta$ -N-acetylglucosaminidase (NAG), and acid phosphatase (AP), were determined using a fluorometric microplate assay [36]. Briefly, fresh soil was extracted with sodium acetate buffer, and the soil suspension was incubated with enzyme-specific fluorogenic substrates. The activities of BG, NAG, LAP, and AP were assayed using 4-MUB- $\beta$ -D-glucoside, 4-MUB-N-acetyl- $\beta$ -D-glucosaminide, L-leucine-7-amido-4-methylcoumarin, and 4-MUB-phosphate, respectively. Fluorescence was measured using a microplate reader, and enzyme activities were calculated from standard curves and expressed as nmol product  $\text{g}^{-1}$  dry soil  $\text{h}^{-1}$ .

#### 2.5. Determination of Soil $\text{N}_2\text{O}$ Emissions

A 30-day laboratory flooded incubation experiment was conducted to evaluate the effects of nano-silica particle size on soil  $\text{N}_2\text{O}$  emissions. Three treatments were established: an untreated control (CK), 15 nm nano-silica, and 50 nm nano-silica, with three replicates per treatment. For each replicate, soil equivalent to 20 g dry weight was placed in a 125 mL polypropylene incubation container. Nano-silica was added to the 15 nm and 50 nm treatments at a rate of  $67 \text{ mg kg}^{-1}$  dry soil or Si-equivalent rate, while no nano-silica was added to the control. The nano-silica was thoroughly mixed with the soil to ensure uniform distribution. Before flooding, soil moisture was adjusted to 60% of WHC, and the bottles were loosely covered with perforated plastic film and pre-incubated in the dark at  $25^\circ\text{C}$  for 7 days to allow microbial activity to stabilize. After pre-incubation, flooded conditions were established by adding distilled water to maintain a 2–3 cm water layer above the soil surface. Water loss was compensated daily by weighing the bottles and adding distilled water as needed.

Gas sampling was conducted on days 1, 2, 4, 6, 9, 12, 15, 19, 26, and 30 after the start of flooded incubation. Before each gas sampling event, the serum bottles were sealed airtight with rubber stoppers for 45 min to allow gas accumulation in the headspace. A 110 mL headspace gas sample was then collected using a gas-tight syringe and transferred to pre-evacuated vials for analysis. After gas collection, the bottles were reopened or covered again with perforated film to maintain incubation conditions until the next sampling time.

N<sub>2</sub>O concentrations were determined using a gas chromatograph (Shimadzu GC-2014, Kyoto, Japan) equipped with a <sup>63</sup>Ni electron capture detector (ECD).

#### 2.6. DNA Extraction and Quantitative PCR Analysis

Soil DNA was extracted from 0.5 g of fresh soil using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and purity were determined using a micro-volume UV spectrophotometer, and the extracted DNA was diluted to an appropriate concentration prior to qPCR analysis. The abundances of ammonia-oxidizing bacteria (AOB) and archaea (AOA), *nirK*, *nirS*, and *nosZ* genes were quantified by quantitative real-time PCR (qPCR) using an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Each reaction was performed in a total volume of 30 µL containing 15 µL of qPCR Mix (Vazyme Biotech Co., Ltd., Nanjing, China; Cat. No. Q321), 0.5 µL of each primer (10 µM), 2 µL of template DNA, and nuclease-free water to volume. The amplification program consisted of an initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, annealing for 20 s, and extension at 72 °C for 20 s. The annealing temperature was 57 °C for *Bac-amoA*, *AOA*, *nirK*, and *nirS*, and 62 °C for *nosZ*. Standard curves were generated using 10-fold serial dilutions of linearized plasmids containing the corresponding target gene fragments. Gene copy numbers were calculated from the standard curves and expressed as copies g<sup>-1</sup> dry soil. Primer sequences, amplification efficiencies, and coefficients of determination (R<sup>2</sup>) are shown in Table S4.

#### 2.7. Statistical Analysis

All data were first checked for normality and homogeneity of variance before statistical analysis. Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was assessed using Levene's test. When necessary, data were log- or square-root-transformed to meet the assumptions of parametric analysis. If the assumptions were not satisfied after transformation, appropriate non-parametric tests were used. Statistical analyses were conducted using R software (version 4.3.0). The effects of nano-silica particle size treatment, incubation time, and their interaction were analyzed using linear mixed-effects models. Treatment and incubation time were considered fixed factors, and incubation replicate was included as a random factor where appropriate to account for the experimental structure. When significant treatment effects were detected, pairwise comparisons were performed using Tukey's honestly significant difference (HSD) test.

Pearson correlation analysis was performed to examine relationships among soil inorganic N forms, extracellular enzyme activities, microbial gene abundances, SOM, and N<sub>2</sub>O emissions. Principal component analysis (PCA) was conducted using standardized variables to evaluate the overall association among N transformation-related indicators, enzyme activities, microbial variables, and N<sub>2</sub>O emissions across treatments. Statistical significance was set at *p* < 0.05. Figures were prepared using Origin 2024, and the N transformation flowchart was generated using Adobe Illustrator CC 2019.

### 3. Results

#### 3.1. Nano-Silica Particle-Size Effects on Soil Physicochemical Properties

After incubation, nano-silica particle-size treatments significantly affected soil pH, SOC and MBC (Table 1). Compared with CK, the 15 nm treatment significantly increased soil pH from 5.70 to 5.82, whereas the 50 nm treatment had no significant effect. Both 15 nm and 50 nm treatments significantly increased SOC content by 6.7% and 8.0%, respectively, but reduced MBC content by 19.5% and 16.1%, respectively. No significant differences in soil total N or MBN were observed among treatments.

**Table 1.** Properties of soil after incubation with different nano-Si particle sizes.

| Parameter                       | CK                       | 15 nm                    | 50 nm                    |
|---------------------------------|--------------------------|--------------------------|--------------------------|
| pH                              | 5.70 ± 0.01 <sup>b</sup> | 5.82 ± 0.02 <sup>a</sup> | 5.72 ± 0.02 <sup>b</sup> |
| Total N (g N kg <sup>−1</sup> ) | 1.40 ± 0.06 <sup>a</sup> | 1.49 ± 0.06 <sup>a</sup> | 1.51 ± 0.07 <sup>a</sup> |
| SOC (g C kg <sup>−1</sup> )     | 15.6 ± 0.31 <sup>b</sup> | 16.7 ± 0.30 <sup>a</sup> | 16.9 ± 0.41 <sup>a</sup> |
| MBC (mg kg <sup>−1</sup> )      | 566 ± 29.0 <sup>a</sup>  | 455 ± 9.90 <sup>b</sup>  | 475 ± 61.5 <sup>b</sup>  |
| MBN (mg kg <sup>−1</sup> )      | 32.9 ± 3.68 <sup>a</sup> | 45.0 ± 3.54 <sup>a</sup> | 35.1 ± 1.70 <sup>a</sup> |

Values are presented as mean ± standard deviation ( $n = 3$ ). Different lowercase letters within the same row indicate significant differences among treatments at  $p < 0.05$ . CK, untreated control; SOC, soil organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.

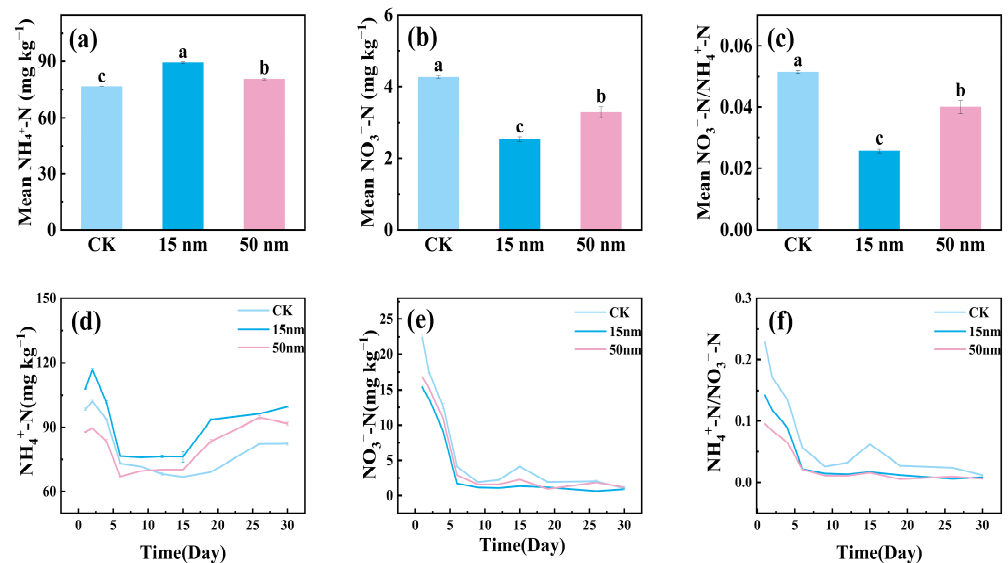
### 3.2. Nano-Silica Particle-Size Effects on Soil Mineral N

Nano-silica particle size significantly influenced soil inorganic N during the flooded incubation period (Figure 3). The most pronounced changes occurred during Days 1–4, with rapid declines in  $\text{NO}_3^-$ -N and the  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratio, followed by greater  $\text{NH}_4^+$ -N accumulation, particularly under the 15 nm treatment. Time-weighted mean concentrations of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N were calculated to characterize the overall inorganic N status during incubation. The time-weighted mean  $\text{NH}_4^+$ -N concentration differed significantly among treatments (Figure 3a). Relative to CK, the 15 nm and 50 nm treatments increased  $\text{NH}_4^+$ -N concentration by 16.4% and 5.25%, respectively.  $\text{NH}_4^+$ -N concentration in the 15 nm treatment was significantly higher than that in the 50 nm treatment, suggesting that smaller nano-Si particles had a stronger effect on  $\text{NH}_4^+$ -N accumulation under flooded conditions. The time-weighted mean  $\text{NO}_3^-$ -N concentration showed the opposite pattern (Figure 3b). Compared with CK, the 15 nm and 50 nm treatments significantly reduced  $\text{NO}_3^-$ -N concentration by 40.8% and 23.0%, respectively. The reduction was more pronounced in the 15 nm treatment, where  $\text{NO}_3^-$ -N concentration was 23.1% lower than that in the 50 nm treatment. Consistent with these responses, nano-silica application significantly reduced the  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratio, with the lowest value observed in the 15 nm treatment (Figure 3c). Compared with CK, this ratio decreased by 49.8% and 22.1% in the 15 nm and 50 nm treatments, respectively. These treatment-level responses were further supported by the temporal dynamics of inorganic N (Figure 3d–f). During most of the incubation period, the 15 nm treatment maintained higher  $\text{NH}_4^+$ -N concentrations but lower  $\text{NO}_3^-$ -N concentrations and  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratios than CK and the 50 nm treatment, further indicating that smaller nano-silica particles favored  $\text{NH}_4^+$ -N accumulation and reduced  $\text{NO}_3^-$ -N availability under flooded conditions.

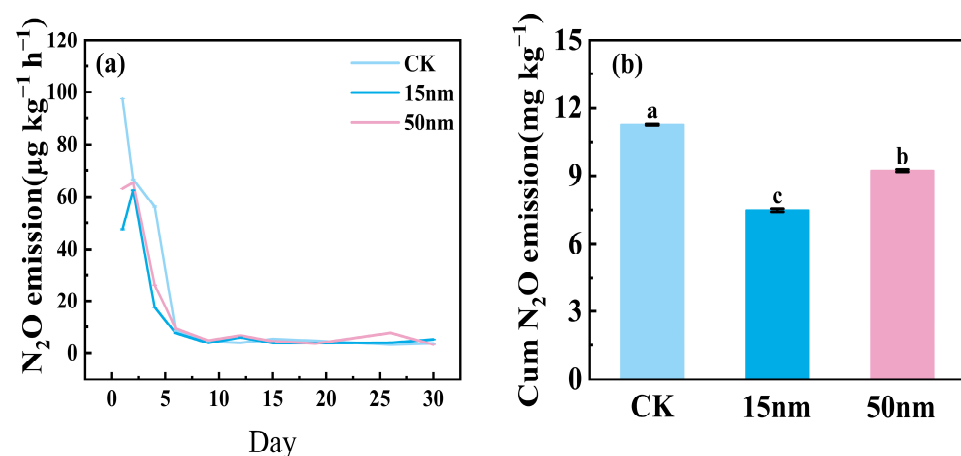
### 3.3. Nano-Silica Particle-Size Effects on $\text{N}_2\text{O}$ Emissions

Nano-silica particle size significantly influenced  $\text{N}_2\text{O}$  emissions from flooded paddy soil during the incubation period (Figure 4). Cumulative  $\text{N}_2\text{O}$  emissions differed significantly among treatments, with the highest value observed in CK, followed by the 50 nm treatment, whereas the lowest value was recorded in the 15 nm treatment (Figure 4a). Compared with CK, the 15 nm and 50 nm treatments significantly reduced cumulative  $\text{N}_2\text{O}$  emissions by 33.6% and 18.2%, respectively. Cumulative  $\text{N}_2\text{O}$  emission in the 15 nm treatment was 18.8% lower than that in the 50 nm treatment, indicating that smaller nano-Si particles had a stronger effect in reducing  $\text{N}_2\text{O}$  release under flooded soil conditions. The temporal dynamics of  $\text{N}_2\text{O}$  fluxes further supported the cumulative emission pattern (Figure 4b).  $\text{N}_2\text{O}$  fluxes were highest at the beginning of incubation and then declined rapidly, remaining at relatively low levels after day 6. During the early incubation stage, both nano-silica treatments reduced  $\text{N}_2\text{O}$  fluxes compared with CK, with the 15 nm treatment showing the lowest flux. Thereafter,  $\text{N}_2\text{O}$  fluxes remained low across all treatments, but the consistently lower cumulative emission in the 15 nm treatment indicated a stronger

overall reduction in  $\text{N}_2\text{O}$  loss. These results suggest that nano-silica application reduced  $\text{N}_2\text{O}$  emissions from flooded paddy soil in a particle-size-dependent manner, with the 15 nm particles showing the greatest mitigation effect.



**Figure 3.** Particle-size-dependent effects of nano-Si on soil mineral N dynamics during flooded incubation. Panels (a–c) show time-weighted mean  $\text{NH}_4^+\text{-N}$ , time-weighted mean  $\text{NO}_3^-\text{-N}$ , and the  $\text{NO}_3^-\text{-N}/\text{NH}_4^+\text{-N}$  ratio under CK, 15 nm, and 50 nm treatments. Panels (d–f) show temporal changes in  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and  $\text{NO}_3^-\text{-N}/\text{NH}_4^+\text{-N}$  ratio over the incubation period. Different lowercase letters indicate significant differences among treatments at  $p < 0.05$ .

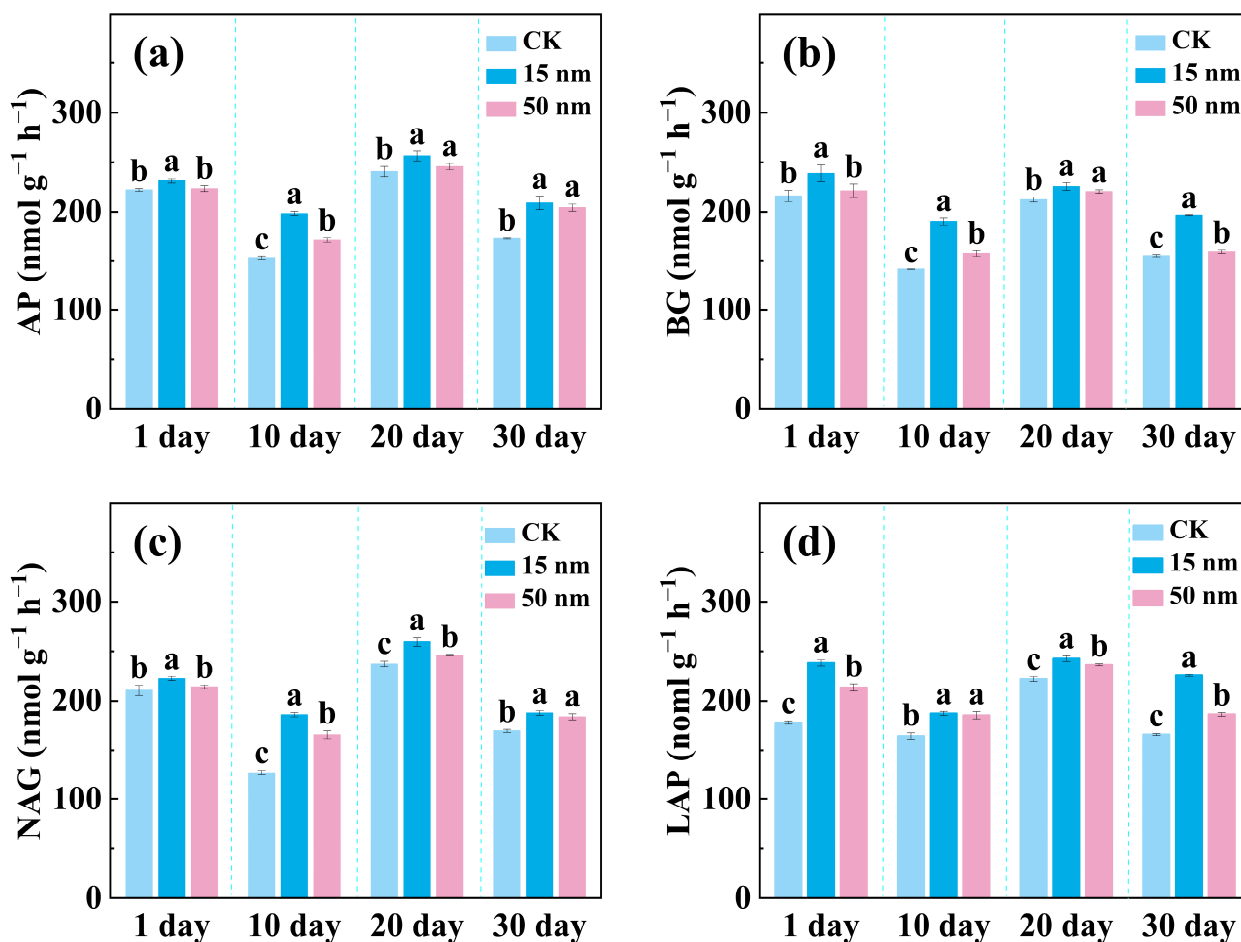


**Figure 4.** Effects of nano-silica particle size on soil  $\text{N}_2\text{O}$  emissions during flooded cultivation. (a) Temporal variation in  $\text{N}_2\text{O}$  emission flux; (b) cumulative (Cum)  $\text{N}_2\text{O}$  emissions. Data are expressed as mean  $\pm$  standard deviation ( $n = 3$ ). Different lowercase letters above the bar charts indicate significant differences among treatments ( $p < 0.05$ ).

### 3.4. Nano-Silica Particle-Size Effects on Soil Extracellular Enzyme Activities

Nano-silica particle size significantly affected soil extracellular enzyme activities during flooded incubation (Figures 5 and S1). Treatment, incubation time, and their interaction significantly influenced AP, BG, NAG, and LAP activities (Table S2), indicating that the response of extracellular enzymes to nano-silica addition depended on both particle size and incubation stage. Across the incubation period, the four enzymes showed broadly similar temporal dynamics. Enzyme activities generally declined from day 1 to day 10, increased sharply by day 20, and then decreased again by day 30 (Figure S1). This pattern

was observed across all treatments, suggesting that extracellular enzyme activities changed dynamically during flooded incubation rather than remaining constant over time.

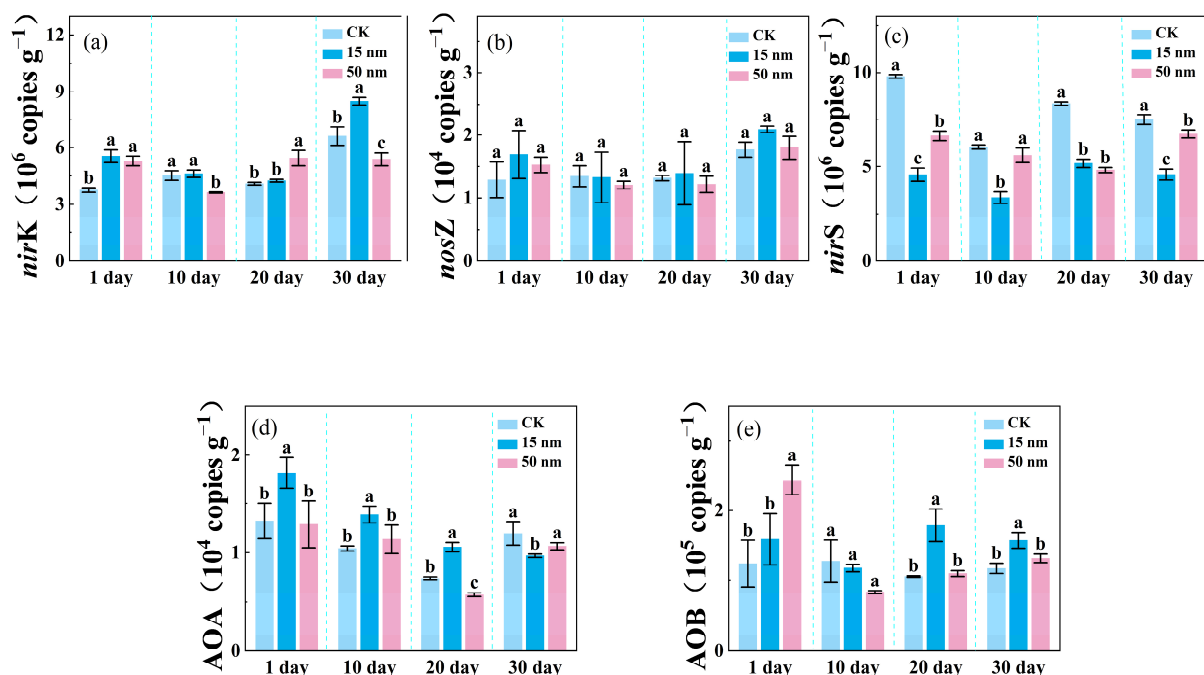


**Figure 5.** Effects of nano-silica particle size on soil extracellular enzyme activities during flooded incubation: (a) acid phosphatase (AP), (b)  $\beta$ -1,4-glucosidase (BG), (c)  $\beta$ -N-acetylglucosaminidase (NAG), and (d) leucine aminopeptidase (LAP). CK, control; 15 nm, small-sized nano-silica treatment; 50 nm, large-sized nano-silica treatment. Different lowercase letters indicate significant differences among treatments at the same sampling time at  $p < 0.05$ .

Nano-silica addition generally increased extracellular enzyme activities compared with CK, but the magnitude of the response differed between particle sizes and sampling times (Figure 5). The 15 nm treatment showed the strongest overall stimulatory effect and significantly increased AP, BG, NAG, and LAP activities relative to CK at most sampling times. The 50 nm treatment also enhanced enzyme activities in several cases, although its effect was generally weaker and less consistent than that of the 15 nm treatment. The particle-size effect was particularly evident for LAP, where the 15 nm treatment maintained significantly higher activity than CK throughout the incubation period and was also higher than the 50 nm treatment at days 1, 20, and 30. For AP, BG, and NAG, the 15 nm treatment generally produced the highest activities, but the difference between 15 nm and 50 nm was not always significant, especially at day 20 or day 30 for some enzymes. Therefore, the results indicate that nano-silica enhanced extracellular enzyme activities under flooded conditions, with the smaller particle size generally producing a stronger response.

### 3.5. Nano-Silica Effect on Nitrification- and Denitrification-Related Functional Genes

Nano-Si particle size altered the abundance of several N-cycling functional genes during flooded incubation, although the response patterns differed among genes and sampling times (Figure 6). Linear mixed-effects models showed that nano-Si treatment, incubation time, and their interaction significantly affected *nirS* and *nirK* abundances ( $p < 0.001$ ; Table S3), indicating that the effects of nano-Si particle size varied across incubation stages. The abundance of *nirS* showed the clearest treatment response. Across most sampling times, *nirS* abundance was highest in CK and lowest under the 15 nm treatment. On day 1, *nirS* abundance followed the order CK > 50 nm > 15 nm. A similar pattern was observed on day 30, whereas on days 10 and 20, the 15 nm treatment remained significantly lower than CK. In contrast, *nosZ* abundance showed no consistent treatment-specific differences, and all treatments shared the same statistical grouping at each sampling time. However, *nosZ* abundance varied over the incubation period, with relatively higher values observed toward the end of incubation. The *nirK* gene displayed a different pattern from *nirS*. On day 1, both nano-Si treatments significantly increased *nirK* abundance relative to CK. On day 10, CK and the 15 nm treatment did not differ significantly, whereas the 50 nm treatment showed significantly lower *nirK* abundance. On day 20, the 50 nm treatment showed the highest *nirK* abundance, whereas on day 30, the 15 nm treatment had the highest value, followed by CK and then 50 nm.



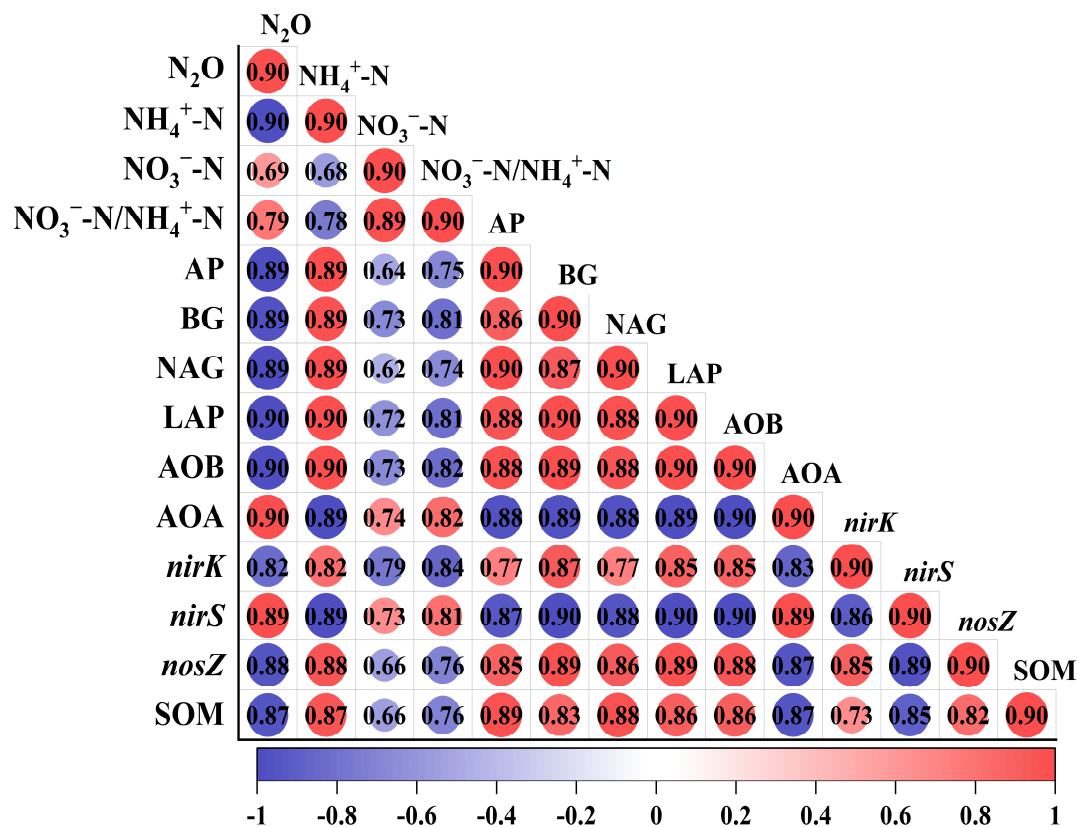
**Figure 6.** Effects of nano-silica particle size on soil N-cycling functional genes during flooded incubation. (a) *nirK*; (b) *nosZ*; (c) *nirS*; (d) AOA; (e) AOB. Data represent mean  $\pm$  standard deviation ( $n = 3$ ). Different lowercase letters indicate significant differences among treatments at the same incubation time ( $p < 0.05$ ).

The ammonia-oxidizing groups also responded differently to nano-silica particle size. AOA abundance was significantly lower under both nano-silica treatments than under CK on Days 1 and 10. On Day 20, the 15 nm treatment showed the lowest AOA abundance, while on Day 30 it remained significantly lower than CK, with the 50 nm treatment showing an intermediate value (Figure 6d). AOB abundance also varied with incubation time and treatment. On Day 1, CK showed significantly higher AOB abundance than both nano-silica treatments, with no significant difference between the 15 and 50 nm treatments. On Day

10, the 50 nm treatment did not differ significantly from CK, whereas the 15 nm treatment showed lower AOB abundance. On Days 20 and 30, both nano-silica treatments showed significantly lower AOB abundance than CK, with no significant difference between the two particle sizes (Figure 6e). Overall, these results indicate that the responses of ammonia-oxidizing microbial groups to nano-silica were gene-specific and time-dependent, with particle-size-dependent differences occurring at selected incubation stages.

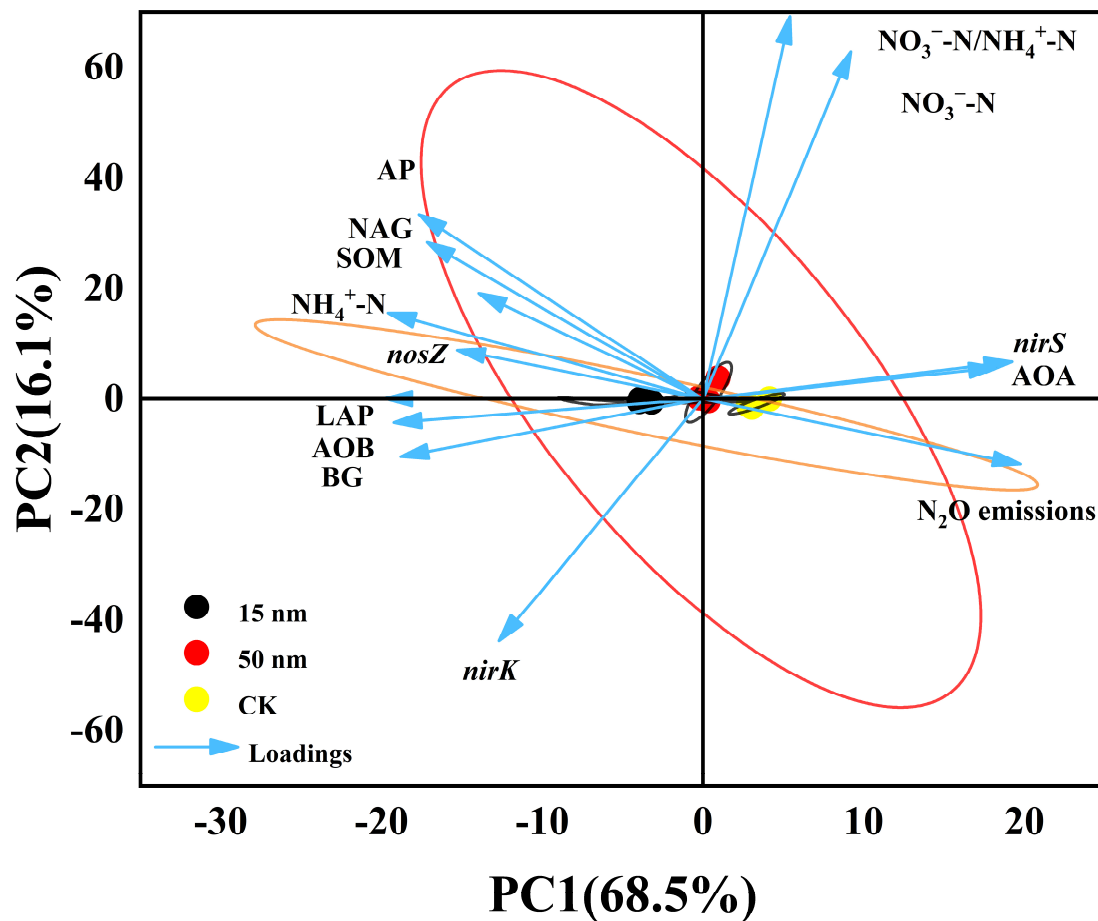
### 3.6. Integrated Relationships Among Soil N Variables, Enzyme Activities, Functional Genes, and N<sub>2</sub>O Emissions

Pearson correlation analysis showed that N<sub>2</sub>O emissions were positively correlated with NO<sub>3</sub><sup>-</sup>-N, the NO<sub>3</sub><sup>-</sup>-N/NH<sub>4</sub><sup>+</sup>-N ratio, AOA, and *nirS*, but negatively correlated with NH<sub>4</sub><sup>+</sup>-N, SOM, AP, BG, NAG, LAP, AOB, *nirK*, and *nosZ* (Figure 7). NH<sub>4</sub><sup>+</sup>-N was positively correlated with SOM, extracellular enzyme activities, AOB, *nirK*, and *nosZ*, whereas NO<sub>3</sub><sup>-</sup>-N and the NO<sub>3</sub><sup>-</sup>-N/NH<sub>4</sub><sup>+</sup>-N ratio showed generally opposite relationships with these variables. These results indicate that N<sub>2</sub>O emissions during flooded incubation were more closely associated with NO<sub>3</sub><sup>-</sup>-N availability and selected nitrification- and denitrification-related genes, particularly AOA and *nirS*. However, these relationships represent statistical associations rather than direct evidence of specific N transformation processes.



**Figure 7.** Pearson correlation heatmap showing the relationships among cumulative N<sub>2</sub>O emissions, soil mineral N variables, extracellular enzyme activities, N-cycling functional genes, and soil organic matter (SOM) at the end of the flooded incubation. Values in the circles represent Pearson correlation coefficients (r). Red circles indicate positive correlations, whereas blue circles indicate negative correlations. Circle size and color intensity represent the absolute magnitude of the Pearson correlation coefficient (|r|), with larger and darker circles indicating stronger correlations. AP, acid phosphatase; BG, β-1,4-glucosidase; NAG, β-N-acetylglucosaminidase; LAP, leucine aminopeptidase; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; *nirK* and *nirS*, nitrite reductase genes; *nosZ*, nitrous oxide reductase gene; SOM, soil organic matter.

Principal component analysis further supported the correlation patterns. The first two principal components explained 84.6% of the total variation, with PC1 and PC2 accounting for 68.5% and 16.1%, respectively (Figure 8). The treatments were clearly separated along PC1. The 15 nm nano-SiO<sub>2</sub> treatment was mainly distributed on the negative side of PC1 and was associated with higher NH<sub>4</sub><sup>+</sup>-N, SOM, extracellular enzyme activities, and *nosZ* abundance. In contrast, N<sub>2</sub>O emissions, NO<sub>3</sub><sup>-</sup>-N, the NO<sub>3</sub><sup>-</sup>-N/NH<sub>4</sub><sup>+</sup>-N ratio, AOA, and *nirS* were positioned on the positive side of PC1. This separation indicates that the response to the smaller nano-SiO<sub>2</sub> particles was characterized by NH<sub>4</sub><sup>+</sup>-dominated mineral N partitioning and enhanced enzyme-associated organic matter turnover, together with lower NO<sub>3</sub><sup>-</sup>-N accumulation and N<sub>2</sub>O emissions.



**Figure 8.** Principal component analysis (PCA) of soil N-related variables and cumulative N<sub>2</sub>O emissions under CK, 15 nm nano-silica, and 50 nm nano-silica treatments at the end of the flooded incubation. The PCA was performed using standardized day-30 data, including inorganic N forms and their ratio, extracellular enzyme activities, N-cycling functional gene abundances, soil organic matter (SOM), and cumulative N<sub>2</sub>O emissions. Points represent individual replicate samples ( $n = 3$ ). Blue arrows indicate variable loadings; their direction and length reflect the relationships with PC1 and PC2 and the relative contributions of variables, respectively, while colored ellipses indicate treatment-level grouping trends. SOM was calculated from soil organic carbon (SOC) using the conventional Van Bemmelen conversion factor:  $SOM = SOC \times 1.724$ . AP, acid phosphatase; BG,  $\beta$ -1,4-glucosidase; NAG,  $\beta$ -N-acetylglucosaminidase; LAP, leucine aminopeptidase; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; *nirK* and *nirS*, nitrite reductase genes; *nosZ*, nitrous oxide reductase gene.

## 4. Discussion

### 4.1. Particle-Size-Dependent Regulation of Inorganic N Partitioning Under Flooded Conditions

A central finding of this study is that nano-Si did not merely alter the absolute concentrations of mineral N, but changed the relative partitioning of inorganic N between  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N under flooded conditions. This distinction is important because flooded paddy soils are typically characterized by restricted oxygen diffusion, low redox potential, and strong spatial heterogeneity in aerobic and anaerobic microsites [37]. Under such conditions,  $\text{NH}_4^+$  is generally the dominant mineral N form, whereas  $\text{NO}_3^-$  accumulation is correlated with the balance between localized nitrification in oxidized microsites and  $\text{NO}_3^-$  consumption through denitrification, dissimilatory  $\text{NO}_3^-$  reduction to  $\text{NH}_4^+$  (DNRA), microbial immobilization, or other reduction processes [38]. Therefore, the simultaneous increase in  $\text{NH}_4^+$ -N, decrease in  $\text{NO}_3^-$ -N, and decline in the  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratio under the 15 nm nano-Si treatment indicates a directional shift in the mineral N environment toward  $\text{NH}_4^+$  dominance rather than  $\text{NO}_3^-$  accumulation [39].

This shift cannot be explained by flooding alone, because all treatments were incubated under the same flooded conditions. Instead, the stronger response under the 15 nm treatment suggests that nano-Si influenced mineral N partitioning in a particle-size-dependent manner. Compared with the 50 nm treatment, the smaller 15 nm particles likely had greater surface reactivity because of their larger specific surface area and higher density of reactive surface sites [40]. Such properties may increase the interaction of nano-Si with SOM, mineral surfaces, dissolved substrates, and microbial microhabitats [41]. In flooded soils, where N transformations are highly sensitive to micro-scale changes in substrate availability and redox conditions, these particle-size-dependent interactions may be sufficient to alter the balance between  $\text{NH}_4^+$  accumulation and  $\text{NO}_3^-$  formation or persistence [42].

The increase in  $\text{NH}_4^+$ -N under the 15 nm treatment may reflect several non-exclusive processes. First, the higher activities of NAG and LAP suggest greater enzymatic potential for the decomposition of N-containing organic substrates, while the increase in BG indicates enhanced organic C turnover and microbial energy acquisition [43]. These enzyme responses may have increased the potential release of mineral N from organic substrates, thereby contributing to  $\text{NH}_4^+$ -N accumulation [44]. Second, the slight but significant increase in soil pH under the 15 nm treatment may have improved the biochemical environment for enzyme activity and organic matter turnover [45]. Third, reactive nano-Si surfaces may have contributed to the retention or stabilization of  $\text{NH}_4^+$ -N in the soil matrix, although this process was not directly measured [41,46]. Thus, the observed  $\text{NH}_4^+$ -N enrichment should be interpreted as the net outcome of altered organic N turnover potential,  $\text{NH}_4^+$  retention, and reduced conversion or persistence of  $\text{NO}_3^-$  under flooded conditions.

An important feature of our results is that the increase in extracellular enzyme activities occurred despite a decline in MBC under nano-Si addition. This indicates that the response was not simply associated with an increase in microbial biomass. Instead, nano-Si may have changed microbial functional expression, enzyme production per unit microbial biomass, substrate accessibility, or extracellular enzyme stabilization on mineral or nano-Si surfaces [47]. Such a response is plausible because extracellular enzymes can remain active outside microbial cells and may be protected or stabilized through interactions with mineral surfaces and organic matter [48]. Therefore, the higher enzyme activities under the 15 nm treatment should be viewed as evidence of enhanced enzymatic potential for organic matter and nutrient turnover, rather than as direct evidence of increased microbial biomass or measured gross N mineralization.

The lower  $\text{NO}_3^-$ -N concentration under the 15 nm treatment should not be interpreted simply as suppression of ammonia-oxidizing microorganisms. In fact, AOA and AOB abundances showed time-dependent responses to nano-Si addition, and in some cases

increased under the smaller particle-size treatment. This apparent contrast highlights an important point:  $\text{NO}_3^-$ -N concentration represents a net pool, determined by both  $\text{NO}_3^-$  production and  $\text{NO}_3^-$  consumption [49,50]. Therefore, higher AOA or AOB abundance does not necessarily lead to greater  $\text{NO}_3^-$  accumulation, especially under flooded conditions where oxygen limitation can restrict nitrification activity and where newly produced  $\text{NO}_3^-$  may be rapidly consumed through denitrification, DNRA, microbial immobilization, or other reduction pathways [51].

The different relationships of  $\text{NO}_3^-$ -N with AOA and AOB further suggest that ammonia-oxidizing archaea and bacteria did not contribute equally to  $\text{NO}_3^-$  dynamics in this system [52]. AOA and AOB often occupy different ecological niches and may respond differently to  $\text{NH}_4^+$  availability, oxygen status, pH, and microsite conditions [53]. Thus, the increase in AOA or AOB abundance under nano-Si addition should be regarded as a change in nitrification-related functional potential rather than direct evidence of increased nitrification activity. In the present study, the decline in  $\text{NO}_3^-$ -N and the  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratio under the 15 nm treatment indicates that  $\text{NO}_3^-$  accumulation was reduced at the system level, even though some ammonia-oxidizer groups increased in abundance.

The PCA showed that the 15 nm treatment was positioned closer to  $\text{NH}_4^+$ -N, SOM, extracellular enzyme activities, and *nosZ*, whereas  $\text{NO}_3^-$ -N, the  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratio, AOA, *nirS*, and  $\text{N}_2\text{O}$  emissions were oriented away from the 15 nm treatment. This suggests that the 15 nm treatment was characterized by an  $\text{NH}_4^+$ -dominated mineral N environment rather than by  $\text{NO}_3^-$  accumulation. The correlation analysis provides additional variable-level support, but it should not be interpreted as treatment-specific evidence. Across all samples,  $\text{NO}_3^-$ -N and the  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratio were more closely associated with some nitrification- and denitrification-related indicators, especially AOA and *nirS*, whereas their relationships with AOB differed. This difference indicates that AOA and AOB may have played distinct roles or occupied different microsite niches under flooded conditions [54]. Therefore, the decline in  $\text{NO}_3^-$ -N under 15 nm nano-Si should be interpreted as reduced net  $\text{NO}_3^-$  accumulation at the system level, rather than as direct evidence that all ammonia-oxidizing groups were suppressed.

Collectively, the change in mineral N partitioning under the 15 nm treatment may have resulted from interacting chemical, biochemical, and biological mechanisms. Chemically, the reactive surfaces of nano-Si and the slight increase in soil pH may have enhanced  $\text{NH}_4^+$  retention and altered mineral N stability [41,45,46]. Biochemically, the higher NAG, LAP, and BG activities indicated greater potential for organic N depolymerization and organic matter turnover, which may have increased  $\text{NH}_4^+$ -N supply [43,44]. Biologically, oxygen limitation under flooded conditions may have constrained nitrification, whereas  $\text{NO}_3^-$ -N could have been consumed through denitrification, DNRA, and microbial immobilization [38,51]. Therefore, the lower  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratio under the 15 nm treatment likely reflected the combined effects of increased  $\text{NH}_4^+$ -N production or retention and reduced  $\text{NO}_3^-$ -N formation or persistence, rather than a single N-transformation pathway.

Taken together, these results support a particle-size-dependent N partitioning framework. Under flooded conditions, 15 nm nano-Si appears to shift the soil mineral N pool toward  $\text{NH}_4^+$  dominance by enhancing enzymatic organic matter turnover potential, modifying microbial functional potential, and suppressing  $\text{NO}_3^-$  accumulation. This does not prove that nano-Si directly inhibited nitrification or stimulated DNRA or denitrification, but it provides a coherent explanation for the observed changes in  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, enzyme activities, functional gene associations, and multivariate separation. Future studies using  $^{15}\text{N}$  tracing and direct measurements of gross mineralization, nitrification, DNRA, denitrification, and  $\text{NH}_4^+$  retention are needed to verify the specific pathways underlying this particle-size-dependent regulation of inorganic N partitioning.

#### 4.2. Reduced N<sub>2</sub>O Emissions Under Small-Sized Nano-Si: Association with NO<sub>3</sub><sup>−</sup>-N Availability, Enzyme Activities, and Gene Abundances

N<sub>2</sub>O emissions from waterlogged paddy soil depend on mineral N availability, redox state, and processes related to nitrification and denitrification [55]. While waterlogging restricts oxygen supply and thus minimizes the occurrence of extensive nitrification in the soil, small aerobic microsites may still support NH<sub>4</sub><sup>+</sup> oxidation [56]. On the other hand, neighboring anaerobic microsites may favor NO<sub>3</sub><sup>−</sup> reduction and potential N<sub>2</sub>O formation [13]. In this regard, NO<sub>3</sub><sup>−</sup>-N availability becomes crucial because it is an important precursor for denitrification-associated N<sub>2</sub>O generation. Changes in NO<sub>3</sub><sup>−</sup>-N concentration and the NO<sub>3</sub><sup>−</sup>-N/NH<sub>4</sub><sup>+</sup>-N ratio may therefore strongly influence potential N<sub>2</sub>O emissions under waterlogged soil conditions [57].

In the present study, both nano-Si treatments contributed to reduced N<sub>2</sub>O emissions. However, the mitigation effect of the 15 nm treatment was more pronounced than that of the 50 nm treatment. As shown in Section 4.1, this difference was related to the fact that the 15 nm treatment was associated with the largest reduction in NO<sub>3</sub><sup>−</sup>-N concentration and the NO<sub>3</sub><sup>−</sup>-N/NH<sub>4</sub><sup>+</sup>-N ratio. In this respect, the reduction in cumulative N<sub>2</sub>O emissions can be linked to lower NO<sub>3</sub><sup>−</sup>-N availability under small-sized nano-Si [58]. It seems that nano-Si did not inhibit all N transformations in the soil, but rather altered mineral N partitioning toward NH<sub>4</sub><sup>+</sup>-N prevalence, thereby restricting the mineral N substrate required for NO<sub>3</sub><sup>−</sup>-dependent N<sub>2</sub>O generation under flooded conditions.

Nevertheless, mineral N availability is not the only factor that determines N<sub>2</sub>O emissions. The final N<sub>2</sub>O flux from soil is the result of a complex balance between processes generating and consuming this greenhouse gas [59]. The *nirK* and *nirS* genes encode nitrite reductases that mediate the reduction of NO<sub>2</sub><sup>−</sup> to NO, an important step in denitrification that may subsequently contribute to N<sub>2</sub>O formation, whereas the *nosZ* gene encodes N<sub>2</sub>O reductase and catalyzes N<sub>2</sub>O reduction to N<sub>2</sub> [60]. In the present study, N<sub>2</sub>O emissions were positively correlated with *nirS* abundance and negatively correlated with *nosZ* abundance. In other words, reduced N<sub>2</sub>O emissions under the 15 nm treatment may be associated not only with lower NO<sub>3</sub><sup>−</sup>-N availability, but also with a different microbial functional potential related to N<sub>2</sub>O production and reduction [61]. However, caution should be exercised when interpreting qPCR-based abundance data, since gene abundance does not necessarily reflect actual gene expression or process activity.

The enzyme data mainly provide a biochemical explanation for NH<sub>4</sub><sup>+</sup>-N enrichment rather than a direct explanation for NO<sub>3</sub><sup>−</sup>-N depletion. Higher BG, NAG, and LAP activities under the 15 nm treatment indicate greater potential for organic matter and organic N turnover, which could increase the supply of NH<sub>4</sub><sup>+</sup>-N [62]. Under flooded conditions, however, this additional NH<sub>4</sub><sup>+</sup>-N does not necessarily accumulate as NO<sub>3</sub><sup>−</sup>-N because oxygen limitation restricts nitrification activity and any NO<sub>3</sub><sup>−</sup> produced may be rapidly consumed through denitrification, DNRA, immobilization, or other reduction processes [38]. Thus, the combination of enhanced enzymatic turnover potential and reduced net NO<sub>3</sub><sup>−</sup> accumulation suggests that 15 nm nano-Si shifted the system toward NH<sub>4</sub><sup>+</sup> retention rather than NO<sub>3</sub><sup>−</sup> persistence, which is consistent with the observed reduction in N<sub>2</sub>O emissions [58].

The apparent discrepancy between lower NO<sub>3</sub><sup>−</sup>-N accumulation and the time-dependent increases in AOA or AOB abundance under nano-Si addition is important for interpreting the N<sub>2</sub>O response. NO<sub>3</sub><sup>−</sup>-N concentration should not be treated as a direct proxy for nitrifier abundance, because it represents a net balance between NO<sub>3</sub><sup>−</sup> production and NO<sub>3</sub><sup>−</sup> consumption [54,63]. Under flooded conditions, ammonia oxidizers may persist or increase in small aerobic microsites, but their activity may still be constrained by O<sub>2</sub> availability, and any NO<sub>3</sub><sup>−</sup> produced may be rapidly consumed through denitrification, DNRA, microbial immobilization, or other reduction pathways [64]. Therefore, the de-

cline in  $\text{NO}_3^-$ -N under the 15 nm treatment does not necessarily indicate suppression of ammonia-oxidizing communities. Rather, it suggests reduced persistence of  $\text{NO}_3^-$  in the mineral N pool at the whole-system level. This interpretation is consistent with the broader PCA and correlation patterns. These analyses do not prove a specific pathway, but they show a separation between a  $\text{NO}_3^-$  /  $\text{N}_2\text{O}$ -oriented soil state and an  $\text{NH}_4^+$ -dominated soil state. In this framework, AOA and AOB responses should be interpreted as changes in nitrification-related potential, whereas the lower  $\text{NO}_3^-$ -N concentration reflects the net balance between  $\text{NO}_3^-$  formation and  $\text{NO}_3^-$  consumption under flooded conditions [12,13,65]. Thus, the key effect of 15 nm nano-Si was not the simple inhibition of nitrifiers, but the reshaping of mineral N partitioning toward  $\text{NH}_4^+$  retention and reduced  $\text{NO}_3^-$  persistence, which provides a more coherent explanation for the lower  $\text{N}_2\text{O}$  emissions observed under this treatment.

Overall, the lower  $\text{N}_2\text{O}$  emissions under the 15 nm nano-Si treatment were associated with reduced  $\text{NO}_3^-$ -N availability, a lower  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratio, and generally lower *nirS* abundance. These findings suggest that reduced substrate availability for denitrification and changes in denitrifier functional potential may have contributed to the observed decrease in  $\text{N}_2\text{O}$  emissions. However, direct measurements of nitrification, denitrification, DNRA, and  $\text{N}_2\text{O}$  reduction rates are required to verify these potential mechanisms.

#### 4.3. Implications and Limitations Perspectives of Nano-Si in Paddy Soils

The findings indicate that nano-Si particle size regulates two linked outcomes in flooded paddy soil: retention of mineral N in the  $\text{NH}_4^+$  pool and mitigation of  $\text{NO}_3^-$ -linked  $\text{N}_2\text{O}$  loss. The 15 nm treatment increased time-weighted  $\text{NH}_4^+$ -N, decreased  $\text{NO}_3^-$ -N and the  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratio, enhanced extracellular enzyme activities, and produced lower cumulative  $\text{N}_2\text{O}$  emissions than the 50 nm treatment. This coordinated response suggests that smaller nano-Si particles may shift flooded soil N dynamics toward a more  $\text{NH}_4^+$ -dominated mineral N pool and a lower  $\text{N}_2\text{O}$  emission potential. The stronger effect of the 15 nm particles may be related to their larger specific surface area and higher surface reactivity, which could increase their interactions with SOM, mineral surfaces, dissolved substrates, and microbial microenvironments [66,67]. These results have important implications for tropical paddy soils, where high temperature, high humidity, and periodic flooding can intensify N transformations and increase the risk of gaseous N losses [68]. The results indicate that the effect of nano-Si on paddy soil N cycling depends not only on whether nano-Si is applied, but also on its particle size. In this study, the smaller 15 nm nano-Si particles produced greater changes in inorganic N partitioning and  $\text{N}_2\text{O}$  mitigation than the 50 nm particles. This suggests that particle-size selection should be considered when evaluating nano-Si as a potential amendment for regulating N dynamics in flooded rice soils. However, these implications should be interpreted within the context of the controlled incubation system and validated under field conditions before being translated into practical recommendations.

Several limitations should be acknowledged. First, this study was conducted under controlled laboratory flooded incubation conditions and did not include rice plants. Therefore, processes such as root N uptake, rhizosphere oxygen release, root exudation, plant-microbe interactions, fluctuating water regimes, and seasonal field variation were not represented. These factors may substantially influence mineral N partitioning and  $\text{N}_2\text{O}$  emissions under field paddy conditions [69]. Second, the mechanistic interpretation was inferred from coordinated changes in  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, extracellular enzyme activities, functional gene abundances,  $\text{N}_2\text{O}$  emissions, PCA, and correlation patterns. Although these variables provide useful mechanistic clues, they do not directly quantify gross N mineralization, nitrification, DNRA, denitrification, or  $\text{N}_2\text{O}$  reduction rates. Third, qPCR-based

gene abundance reflects microbial functional potential, but not necessarily gene expression, enzyme activity, or in situ process rates. Finally, although  $67 \text{ mg kg}^{-1}$  is nominally equivalent to approximately  $167.5 \text{ kg ha}^{-1}$  based on a surface-soil mass of  $2.5 \times 10^6 \text{ kg ha}^{-1}$ , this conversion should not be interpreted as a field recommendation. Field-equivalent rates vary with bulk density, incorporation depth, and application efficiency, while the economic feasibility of nano-silica at this rate remains untested. Moreover, because no conventional Si source was included, the present findings isolate particle-size effects but do not establish an agronomic or economic advantage of nano-silica over conventional Si fertilizers. Field trials incorporating conventional Si sources, multiple application rates, crop responses, and cost–benefit analyses are therefore required.

Future studies should therefore test whether the particle-size-dependent effects observed here persist in planted rice systems and under field conditions. Experiments combining nano-Si application with rice cultivation, dynamic water management, and seasonal monitoring would help determine the practical relevance of these findings. In addition,  $^{15}\text{N}$  isotope tracing, direct measurements of gross N transformation rates, and gene expression analyses are needed to verify the pathways through which nano-Si particle size regulates  $\text{NH}_4^+$  retention,  $\text{NO}_3^-$  turnover, and  $\text{N}_2\text{O}$  production and reduction. Such work will be essential for evaluating whether small-sized nano-Si can be developed as a targeted strategy for improving N retention and reducing  $\text{N}_2\text{O}$  emissions in tropical paddy soils.

## 5. Conclusions

This study demonstrates that nano-Si particle size controls mineral N dynamics and  $\text{N}_2\text{O}$  emissions in flooded tropical paddy soil. First, nano-Si shifted mineral N availability in a particle-size-dependent manner, with the 15 nm treatment producing the strongest increase in  $\text{NH}_4^+$ -N retention, the largest decrease in  $\text{NO}_3^-$ -N accumulation, and the lowest  $\text{NO}_3^-$ -N/ $\text{NH}_4^+$ -N ratio. Second, the smaller particles enhanced N-acquiring extracellular enzyme activities and produced gene-specific, time-dependent changes in nitrification- and denitrification-related functional genes. Third, cumulative  $\text{N}_2\text{O}$  emissions were reduced most strongly under the 15 nm treatment. The gene abundance patterns also indicate that the decline in  $\text{NO}_3^-$ -N should not be interpreted simply as suppression of ammonia-oxidizing microorganisms, because AOA and AOB responses were time-dependent. The reduction in  $\text{N}_2\text{O}$  emissions under the 15 nm treatment appears to be mainly associated with lower  $\text{NO}_3^-$ -N availability and reduced *nirS*-associated  $\text{N}_2\text{O}$  production potential, rather than with a consistent increase in *nosZ*-mediated  $\text{N}_2\text{O}$  reduction. Together, these findings suggest that small-sized nano-Si reshaped the soil N environment toward  $\text{NH}_4^+$  dominance and lower  $\text{NO}_3^-$ -linked  $\text{N}_2\text{O}$  emission potential. Overall, the results provide evidence that 15 nm nano-Si may have greater potential than larger particles for improving N retention and mitigating  $\text{N}_2\text{O}$  emissions in flooded paddy soils. However, the findings are based on laboratory incubation without rice plants, rhizosphere processes, or field water fluctuations. Therefore, future studies should combine planted rice experiments, field validation, direct N transformation rate measurements,  $^{15}\text{N}$  tracing, and gene expression analyses to confirm the mechanisms by which nano-Si particle size regulates N retention and gaseous N loss in tropical paddy systems.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture16161739/s1>. Figure S1: Time-dependent changes in soil extracellular enzyme activity during flooding cultivation; Table S1: Initial physicochemical properties of the soil; Table S2: Primers used for qPCR analysis of nitrogen-cycling functional genes; Table S3: Results of normality and homogeneity of variance tests for variables used in statistical analyses; Table S4. Effects of nano-Si treatment, incubation time, and their interaction on soil mineral

N dynamics, extracellular enzyme activities, and N-cycling functional gene abundances based on linear mixed-effects models.

**Author Contributions:** Conceived and designed the experiments: A.S.E., L.M. and X.S.; Performed the experiments: X.S., J.F., R.Z., Y.Z., Y.W., T.L., S.X. and M.K.; Contributed reagents/materials/analysis tools: X.S., J.F., R.Z., Y.Z., Y.W. (Yunxing Wan), T.L., S.X., M.K. and Y.W. (Yanzheng Wu); Analyzed the data: X.S.; Wrote the paper: X.S., L.M. and A.S.E.; Revised the paper: Y.W. (Yanzheng Wu), L.M., A.S.E. and J.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by National Natural Science Foundation of China (RZ2400002277).

**Data Availability Statement:** The data presented in this study are available in the article and Supplementary Material.

**Acknowledgments:** An AI-based large language model (ChatGPT, GPT-5.6 Sol; OpenAI, San Francisco, CA, USA) was used to assist with language refinement, clarity, coherence, readability, and flow in selected sections of the manuscript. All AI-assisted suggestions were reviewed, revised, and verified by the authors. No AI tool was used to generate data, analyses, figures, tables, references, interpretations, or scientific conclusions. All cited references were selected and verified by the authors, who take full responsibility for the final manuscript.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Yan, B.H.; Wang, Y.N.; Xian, R.Y.; Chen, X.F. Research on the application of silicon in improving plant drought resistance. *Agric. Eng.* **2023**, *13*, 139–144.
2. Sharma, B.; Kumawat, K.C.; Tiwari, S.; Kumar, A.; Dar, R.A.; Singh, U.; Cardinale, M. Silicon and plant nutrition—Dynamics, mechanisms of transport and role of silicon solubilizer microbiomes in sustainable agriculture: A review. *Pedosphere* **2023**, *33*, 534–555. [[CrossRef](#)]
3. Hu, W.B. Effects of Bio-silicon Fertilizer Application at Different Stages on Rice Yield. *Seed Sci. Technol.* **2025**, *43*, 16–18.
4. Luo, X.F.; Cao, Y.N.; Li, Y. Application of nano-silicon fertilizer in remediation of farmland soil contaminated by heavy metals. *Chin. J. Ecol.* **2025**, *44*, 619–626.
5. Elrys, A.S.; Uwiragiye, Y.; Zhang, Y.; Abdel-Fattah, M.K.; Chen, Z.-X.; Zhang, H.-M.; Meng, L.; Wang, J.; Zhu, T.-B.; Cheng, Y.; et al. Expanding agroforestry can increase nitrate retention and mitigate the global impact of a leaky nitrogen cycle in croplands. *Nat. Food* **2023**, *4*, 109–121. [[CrossRef](#)] [[PubMed](#)]
6. Booth, M.S.; Stark, J.M.; Rastetter, E. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecol. Monogr.* **2005**, *75*, 139–157. [[CrossRef](#)]
7. Fu, J.-R.; Ando, H.; Kakuda, K. Nitrogen mineralization in two paddy soils as affected by silica source addition. *Acta Agric. Zhejiangensis* **2001**, *13*, 133–140.
8. Cui, J.; Li, Y.; Jin, Q.; Li, F. Silica nanoparticles inhibit arsenic uptake into rice suspension cells via improving pectin synthesis and the mechanical force of the cell wall. *Environ. Sci. Nano* **2020**, *7*, 162–171. [[CrossRef](#)]
9. Yang, X.H.; Dong, Y.S.; Qi, Y.C.; Geng, Y.B.; Geng, H.L. Research progress on soil nitrogen transformation processes in grassland ecosystems. *Chin. J. Grassl.* **2004**, *2*, 55–63.
10. 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](#)]
11. Paul, E.A.; Robertson, G.; Groffman, P. Chapter 14—Nitrogen Transformations. In *Soil Microbiology, Ecology and Biochemistry*; Academic Press: Cambridge, MA, USA, 2015; pp. 421–446.
12. Keeney, D.; Sahrawat, K. 2. Nitrogen transformations in flooded rice soils. *Fertil. Res.* **1986**, *9*, 15–38. [[CrossRef](#)]
13. Ishii, S.; Ikeda, S.; Minamisawa, K.; Senoo, K. Nitrogen cycling in rice paddy environments: Past achievements and future challenges. *Microbes Environ.* **2011**, *26*, 282–292. [[CrossRef](#)] [[PubMed](#)]
14. Cai, Z. Ammonium transformation in paddy soils affected by the presence of nitrate. *Nutr. Cycl. Agroecosyst.* **2002**, *63*, 267–274. [[CrossRef](#)]
15. Yang, Y.; Meng, T.; Qian, X.; Zhang, J.; Cai, Z. Evidence for nitrification ability controlling nitrogen use efficiency and N losses via denitrification in paddy soils. *Biol. Fertil. Soils* **2017**, *53*, 349–356. [[CrossRef](#)]
16. Yang, Y.; Zhang, H.; Shan, Y.; Wang, J.; Qian, X.; Meng, T.; Zhang, J.; Cai, Z. Response of denitrification in paddy soils with different nitrification rates to soil moisture and glucose addition. *Sci. Total Environ.* **2019**, *651*, 2097–2104. [[CrossRef](#)] [[PubMed](#)]

17. Song, A.; Fan, F.; Yin, C.; Wen, S.; Zhang, Y.; Fan, X.; Liang, Y. The effects of silicon fertilizer on denitrification potential and associated genes abundance in paddy soil. *Biol. Fertil. Soils* **2017**, *53*, 627–638. [\[CrossRef\]](#)
18. Włodarczyk, T.; Balakhnina, T.; Matichenkov, V.; Brzezińska, M.; Nosalewicz, M.; Szarlip, P.; Fomina, I. Effect of silicon on barley growth and N<sub>2</sub>O emission under flooding. *Sci. Total Environ.* **2019**, *685*, 1–9. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Wang, W.; Sardans, J.; Lai, D.Y.; Wang, C.; Zeng, C.; Tong, C.; Liang, Y.; Peñuelas, J. Effects of steel slag application on greenhouse gas emissions and crop yield over multiple growing seasons in a subtropical paddy field in China. *Field Crops Res.* **2015**, *171*, 146–156. [\[CrossRef\]](#)
20. Das, S.; Lee, J.G.; Cho, S.R.; Song, H.J.; Kim, P.J. Silicate fertilizer amendment alters fungal communities and accelerates soil organic matter decomposition. *Front. Microbiol.* **2019**, *10*, 2950. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Hénault, C.; Bourennane, H.; Ayzac, A.; Ratié, C.; Saby, N.P.; Cohan, J.-P.; Eglin, T.; Le Gall, C. Management of soil pH promotes nitrous oxide reduction and thus mitigates soil emissions of this greenhouse gas. *Sci. Rep.* **2019**, *9*, 20182. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Yan, G.; Huang, Q.; Zhao, S.; Xu, Y.; He, Y.; Nikolic, M.; Nikolic, N.; Liang, Y.; Zhu, Z. Silicon nanoparticles in sustainable agriculture: Synthesis, absorption, and plant stress alleviation. *Front. Plant Sci.* **2024**, *15*, 1393458. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Bhat, J.A.; Rajora, N.; Raturi, G.; Sharma, S.; Dhiman, P.; Sanand, S.; Shivaraj, S.M.; Sonah, H.; Deshmukh, R. Silicon nanoparticles (SiNPs) in sustainable agriculture: Major emphasis on the practicality, efficacy and concerns. *Nanoscale Adv.* **2021**, *3*, 4019–4028. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Zhu, P.; Chen, S.; Shi, Y. Assessment of nano silicon fertilizer effects on soil nutrients, enzyme activities, and microbial communities. *Environ. Sci. Nano* **2024**, *11*, 3124–3136. [\[CrossRef\]](#)
25. Haider, F.U.; Virk, A.L.; Zhou, S.; Ul Ain, N.; Aguila, L.C.R.; Siddique, K.H.; Farooq, M.; Li, Y. Impact of silicon nitride nanoparticles on soil organic carbon dynamics in subtropical evergreen forest ecosystems of China: An incubation study. *Sci. Total Environ.* **2025**, *965*, 178682. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Kukreti, B.; Sharma, A.; Chaudhary, P.; Agri, U.; Maithani, D. Influence of nanosilicon dioxide along with bioinoculants on Zea mays and its rhizospheric soil. *3 Biotech.* **2020**, *10*, 345. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Min, K.; Lynch, L.; Zhu, X.; Chen, F.; Liang, C. Ecotypes shape extracellular enzyme stoichiometries via microbial resource allocation. *Appl. Soil Ecol.* **2024**, *204*, 105744. [\[CrossRef\]](#)
28. Yang, J.; Liu, L.; Wang, K.; Zheng, R.; Wu, J.; Fan, L.; Jiang, P.; Wang, J. Regulation of Soil Nitrogen Turnover and N<sub>2</sub>O Emissions by Silicon in Intensively Managed Phyllostachys edulis (Carrière) J.Houz. Forests. *Forests* **2026**, *17*, 482. [\[CrossRef\]](#)
29. Reddy, K.; Patrick, W.; Broadbent, F. Nitrogen transformations and loss in flooded soils and sediments. *Crit. Rev. Environ. Sci. Technol.* **1984**, *13*, 273–309. [\[CrossRef\]](#)
30. Butterbach-Bahl, K.; Baggs, E.M.; Dannenmann, M.; Kiese, R.; Zechmeister-Boltenstern, S. Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philos. Trans. R. Soc. B Biol. Sci.* **2013**, *368*, 1621. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Ha, T.K.T.; Maeda, M.; Fujiwara, T.; Nagare, H.; Akao, S. Effects of soil type and nitrate concentration on denitrification products (N<sub>2</sub>O and N<sub>2</sub>) under flooded conditions in laboratory microcosms. *Soil Sci. Plant Nutr.* **2015**, *61*, 999–1004. [\[CrossRef\]](#)
32. Senbayram, M.; Chen, R.; Budai, A.; Bakken, L.; Dittert, K. N<sub>2</sub>O emission and the N<sub>2</sub>O/(N<sub>2</sub>O+ N<sub>2</sub>) product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. *Agric. Ecosyst. Environ.* **2012**, *147*, 4–12. [\[CrossRef\]](#)
33. Rummel, P.S.; Well, R.; Pausch, J.; Pfeiffer, B.; Dittert, K. Carbon availability and nitrogen mineralization control denitrification rates and product stoichiometry during initial maize litter decomposition. *Appl. Sci.* **2021**, *11*, 5309. [\[CrossRef\]](#)
34. Bao, S.; Lu, R.; Jiang, S. BOOK REVIEW: *Analytical Methods for Soil and Agro-Chemistry*; Wiley: Hoboken, NJ, USA, 2000.
35. Wu, J.; Joergensen, R.; Pommerening, B.; Chaussod, R.; Brookes, P. Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. *Soil Biol. Biochem.* **1990**, *22*, 1167–1169. [\[CrossRef\]](#)
36. Bell, C.W.; Fricks, B.E.; Rocca, J.D.; Steinweg, J.M.; McMahon, S.K.; Wallenstein, M.D. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *J. Vis. Exp.* **2013**, *81*, 50961. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Wei, Z.; Senbayram, M.; Zhao, X.; Li, C.; Jin, K.; Wu, M.; Rahman, M.M.; Shan, J.; Yan, X. Biochar amendment alters the partitioning of nitrate reduction by significantly enhancing DNRA in a paddy field. *Biochar* **2022**, *4*, 44. [\[CrossRef\]](#)
38. Nojiri, Y.; Kaneko, Y.; Azegami, Y.; Shiratori, Y.; Ohte, N.; Senoo, K.; Otsuka, S.; Isobe, K. Dissimilatory nitrate reduction to ammonium and responsible microbes in Japanese rice paddy soil. *Microbes Environ.* **2020**, *35*, ME20069. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Pandey, A.; Suter, H.; He, J.-Z.; Hu, H.-W.; Chen, D. Nitrogen addition decreases dissimilatory nitrate reduction to ammonium in rice paddies. *Appl. Environ. Microbiol.* **2018**, *84*, e00870-18. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Xu, Z.; Tsang, D.C. Mineral-mediated stability of organic carbon in soil and relevant interaction mechanisms. *Eco-Environ. Health* **2024**, *3*, 59–76. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Cornelis, G.; Hund-Rinke, K.; Kuhlbusch, T.; Van den Brink, N.; Nickel, C. Fate and bioavailability of engineered nanoparticles in soils: A review. *Crit. Rev. Environ. Sci. Technol.* **2014**, *44*, 2720–2764. [\[CrossRef\]](#)

42. Wang, J.; Lv, L.; Hu, R.; Ma, H.; Liu, B.; Zhang, W.; Wu, L. Patterns and determinants of nitrification and denitrification potentials across 24 rice paddy soils in subtropical China. *Agric. Ecosyst. Environ.* **2024**, *361*, 108799. [\[CrossRef\]](#)
43. Sinsabaugh, R.L.; Lauber, C.L.; Weintraub, M.N.; Ahmed, B.; Allison, S.D.; Crenshaw, C.; Contosta, A.R.; Cusack, D.; Frey, S.; Gallo, M.E.; et al. Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* **2008**, *11*, 1252–1264. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Buresh, R.J.; Ramesh Reddy, K.; Van Kessel, C. Nitrogen transformations in submerged soils. *Nitrogen Agric. Syst.* **2008**, *49*, 401–436. [\[CrossRef\]](#)
45. Acosta-Martinez, V.; Tabatabai, M. Enzyme activities in a limed agricultural soil. *Biol. Fertil. Soils* **2000**, *31*, 85–91. [\[CrossRef\]](#)
46. Nieder, R.; Benbi, D.K.; Scherer, H.W. Fixation and defixation of ammonium in soils: A review. *Biol. Fertil. Soils* **2011**, *47*, 1–14. [\[CrossRef\]](#)
47. Allison, S.D. Soil minerals and humic acids alter enzyme stability: Implications for ecosystem processes. *Biogeochemistry* **2006**, *81*, 361–373. [\[CrossRef\]](#)
48. Wallenstein, M.D.; Burns, R.G. Ecology of extracellular enzyme activities and organic matter degradation in soil: A complex community-driven process. *Methods Soil Enzymol.* **2011**, *9*, 35–55. [\[CrossRef\]](#)
49. Giles, M.; Morley, N.; Baggs, E.M.; Daniell, T.J. Soil nitrate reducing processes—drivers, mechanisms for spatial variation, and significance for nitrous oxide production. *Front. Microbiol.* **2012**, *3*, 407. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Wu, L.; Tang, S.; Hu, R.; Wang, J.; Duan, P.; Xu, C.; Zhang, W.; Xu, M. Increased N<sub>2</sub>O emission due to paddy soil drainage is regulated by carbon and nitrogen availability. *Geoderma* **2023**, *432*, 116422. [\[CrossRef\]](#)
51. Patrick, J.W.; Reddy, K. *Nitrification-Denitrification Reactions in Flooded Soils and Water Bottoms: Dependence on Oxygen Supply and Ammonium Diffusion*; Report No.: 0047-2425; Wiley Online Library: Hoboken, NJ, USA, 1976.
52. Ke, X.; Angel, R.; Lu, Y.; Conrad, R. Niche differentiation of ammonia oxidizers and nitrite oxidizers in rice paddy soil. *Environ. Microbiol.* **2013**, *15*, 2275–2292. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Prosser, J.I.; Nicol, G.W. Archaeal and bacterial ammonia-oxidisers in soil: The quest for niche specialisation and differentiation. *Trends Microbiol.* **2012**, *20*, 523–531. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Wang, B.; Zhao, J.; Guo, Z.; Ma, J.; Xu, H.; Jia, Z. Differential contributions of ammonia oxidizers and nitrite oxidizers to nitrification in four paddy soils. *ISME J.* **2015**, *9*, 1062–1075. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Verhoeven, E.; Decock, C.; Barthel, M.; Bertora, C.; Sacco, D.; Romani, M.; Sleutel, S.; Six, J. Nitrification and coupled nitrification-denitrification at shallow depths are responsible for early season N<sub>2</sub>O emissions under alternate wetting and drying management in an Italian rice paddy system. *Soil Biol. Biochem.* **2018**, *120*, 58–69. [\[CrossRef\]](#)
56. Watanabe, I.; Padre, B.C.; Santiago, S.T. Quantitative study on nitrification in flooded rice soil. *Soil Sci. Plant Nutr.* **1981**, *27*, 373–382. [\[CrossRef\]](#)
57. Lan, T.; Han, Y.; Roelcke, M.; Nieder, R.; Cai, Z. Effects of the nitrification inhibitor dicyandiamide (DCD) on gross N transformation rates and mitigating N<sub>2</sub>O emission in paddy soils. *Soil Biol. Biochem.* **2013**, *67*, 174–182. [\[CrossRef\]](#)
58. Wang, M.; Hu, R.; Zhao, J.; Kuzyakov, Y.; Liu, S. Iron oxidation affects nitrous oxide emissions via donating electrons to denitrification in paddy soils. *Geoderma* **2016**, *271*, 173–180. [\[CrossRef\]](#)
59. Graf, D.R.; Jones, C.M.; Hallin, S. Intergenomic comparisons highlight modularity of the denitrification pathway and underpin the importance of community structure for N<sub>2</sub>O emissions. *PLoS ONE* **2014**, *9*, e114118. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Henry, S.; Bru, D.; Stres, B.; Hallet, S.; Philippot, L. Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. *Appl. Environ. Microbiol.* **2006**, *72*, 5181–5189. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Hoffmann, M.; Diaz, O.R.M.; Zentgraf, I.; Al-Hamwi, W.; Dubbert, M.; Stein, M.; Holz, M.; Schaller, J. Amorphous silica reduces N<sub>2</sub>O emissions from arable land at the field plot scale. *Front. Environ. Sci.* **2025**, *13*, 1522700. [\[CrossRef\]](#)
62. Moorhead, D.L.; Sinsabaugh, R.L.; Hill, B.H.; Weintraub, M.N. Vector analysis of coenzyme activities reveal constraints on coupled C, N and P dynamics. *Soil Biol. Biochem.* **2016**, *93*, 1–7. [\[CrossRef\]](#)
63. Hu, H.-W.; Zhang, L.-M.; Yuan, C.-L.; Zheng, Y.; Wang, J.-T.; Chen, D.; He, J.-Z. The large-scale distribution of ammonia oxidizers in paddy soils is driven by soil pH, geographic distance, and climatic factors. *Front. Microbiol.* **2015**, *6*, 938. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Shan, J.; Zhao, X.; Sheng, R.; Xia, Y.; Ti, C.; Quan, X.; Wang, S.; Wei, W.; Yan, X. Dissimilatory nitrate reduction processes in typical Chinese paddy soils: Rates, relative contributions, and influencing factors. *Environ. Sci. Technol.* **2016**, *50*, 9972–9980. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Kirk, G.; Kronzucker, H. The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: A modelling study. *Ann. Bot.* **2005**, *96*, 639. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Luyima, D.; Egyir, M.; Wacal, C. Effects of silicon (silicic acid)-enriched biochar amendments on emissions of methane and nitrous oxide from paddy soil. *Sci. Afr.* **2025**, *30*, e02968. [\[CrossRef\]](#)
67. Li, F.; Hou, Y.; Chen, L.; Qiu, Y. Advances in silica nanoparticles for agricultural applications and biosynthesis. *Adv. Biotechnol.* **2025**, *3*, 14. [\[CrossRef\]](#) [\[PubMed\]](#)

68. Tan, X.; Shao, D.; Gu, W. Effects of temperature and soil moisture on gross nitrification and denitrification rates of a Chinese lowland paddy field soil. *Paddy Water Environ.* **2018**, *16*, 687–698. [[CrossRef](#)]
69. LaHue, G.T.; Chaney, R.L.; Adviento-Borbe, M.A.; Linquist, B.A. Alternate wetting and drying in high yielding direct-seeded rice systems accomplishes multiple environmental and agronomic objectives. *Agric. Ecosyst. Environ.* **2016**, *229*, 30–39. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.