

Article

Biochar Silicon Content Divergently Regulates N₂O Emissions and Cadmium Availability in Acidic Soils

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Abstract

Acidic agricultural soils are frequently challenged by co-occurring heavy metal contamination and greenhouse gas (GHG) emissions. While biochar is widely used for integrated remediation, the specific role of silicon (Si) in modulating its effectiveness in cadmium (Cd) stabilization and nitrous oxide (N₂O) mitigation remains insufficiently understood. This study evaluated the co-remediation efficacy of two types of high-Si (bamboo leaves, ML; rice straw, RS) and two types of low-Si (*Camellia oleifera* leaves, CL; *Camellia oleifera* shells, CS) biochar, produced at 450 °C, within a Cd-contaminated and nitrogen-fertilized acidic soil. Results from a 90-day incubation showed that while all biochar effectively immobilized Cd, the low-Si CL biochar exhibited a superior stabilization efficiency of 66.2%. This enhanced performance was attributed to its higher soil organic carbon (SOC) and moderate dissolved organic carbon (DOC) release, which facilitated robust Cd²⁺ sorption and complexation. In contrast, high-Si biochar was more effective in mitigating cumulative N₂O emissions (up to 67.8%). This mitigation was strongly associated with an elevated abundance of the nosZ gene (up to 48.1%), which catalyzes the terminal step of denitrification. Soil pH and DOC were identified as pivotal drivers regulating both Cd bioavailability and N₂O dynamics. Collectively, low-Si biochar is preferable for Cd stabilization in acidic soils, whereas high-Si biochar is more effective at elevating pH and reducing N₂O emissions. These findings emphasize that optimizing co-remediation outcomes necessitates a targeted approach, selecting biochar based on the specific contamination profile and desired environmental benefits.

Keywords: silicon-rich biochar; denitrification; heavy metal immobilization; soil remediation; functional genes



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1. Introduction

Agricultural ecosystems face dual threats from heavy metal contamination and greenhouse gas (GHG) emissions, particularly nitrous oxide (N₂O) [1–3]. Cadmium (Cd), a highly toxic and mobile heavy metal, readily accumulates in crops, posing significant risks to food safety and human health [4,5]. This issue is particularly severe in the acidic agricultural soils of southern China, where intensive farming practices exacerbate Cd bioavailability and uptake [6,7]. Currently, agricultural soils are also a major source of nitrous oxide (N₂O), primarily produced by microbial nitrification and denitrification under high nitrogen (N) input [8]. The N₂O has a global warming potential approximately

273 times that of CO₂ over a 100-year horizon and contributes to stratospheric ozone depletion, representing a significant climate risk from agroecosystems [9]. These two forms of pollution frequently co-occur in acidic soils and can interact to amplify ecological risks. Acidic conditions enhance Cd solubility and bioavailability [1] while also influencing nitrogen transformation pathways and microbial functional groups, thereby increasing the potential for N₂O emissions [10]. Addressing either stressor in isolation is insufficient for improving environmental quality in acidic agroecosystems.

Biochar is a porous, carbon-rich material derived from pyrolysis of organic waste and has been widely applied in soil remediation owing to its low cost, environmental compatibility, and potential for resource recycling [11,12]. Its high surface area, cation exchange capacity, and abundance of alkaline functional groups enable biochar to enhance nutrient retention, buffer pH, stimulate microbial activity, and improve plant productivity [13]. With respect to heavy metal remediation, biochar has been shown to effectively reduce Cd mobility and bioavailability, thereby limiting crop uptake and mitigating Cd-induced phytotoxicity [13–15]. These effects are closely attributed to its capacity to increase soil pH and buffer acidic conditions, as well as to adsorb Cd via surface functional groups. In addition, biochar also influences soil N cycling and N₂O emissions by modifying the soil microenvironment and microbial community structure. Its alkaline properties and high C/N ratio can reshape microbial composition and activity, thereby regulating key denitrification genes. For instance, biochar amendments have been reported to increase *nosZ* gene abundance by improving soil pH and microbial metabolism, thereby enhancing complete denitrification and reducing the N₂O/(N₂O + N₂) ratio [16]. However, some studies have observed increased N₂O emissions after biochar application, possibly due to elevated carbon substrate availability stimulating nitrification or incomplete denitrification via enhanced *nirS* and *nirK* expression [16]. These contradictory findings suggest that biochar effects are context-dependent, varying with its properties, application conditions, and soil background. Therefore, it is necessary to study the effects of biochar on N₂O emissions in Cd-contaminated soils.

Although previous studies have largely focused on the effects of pyrolysis temperature and application rate on biochar functionality [17,18], the role of feedstock composition, particularly the influence of inorganic elements, remains insufficiently explored. In addition to carbon, silicon (Si) is a key inorganic component of biochar with well-documented ecological functions in agricultural systems [11,19]. Si can alleviate abiotic stress, mitigate heavy metal toxicity, and improve soil structure and microbial habitats [20,21]. However, Si release is typically limited in the acidic soils of southern China, and over 50% of cultivated land suffers from soluble Si deficiency, which constrains soil health and crop resilience [22]. Recent studies suggest that biochar derived from Si-rich plant materials possesses considerable Si-supplying potential [19]. The silicate fraction of biochar contributes to increased cation exchange capacity and pH buffering. For example, silicate modification has been shown to raise biochar surface area by 112%, introduce Si-functional groups, and significantly enhance Cd²⁺ adsorption [23]. Our previous study confirmed that biochar from rice straw and bamboo leaves contains higher total Si (30.4 and 25.8 g kg^{−1}) than that from *Camellia oleifera* (*C. oleifera*) leaves and shells (1.6 and 1.9 g kg^{−1}) [24]. In acidic *C. oleifera* soils, high-Si biochar increased available Si, reduced exchangeable Al, and alleviated soil acidity, accompanied by reduced N₂O emissions [25]. Moreover, Si may also regulate microbial-mediated N cycling. High-Si biochar has been reported to enhance the abundance of *nosZ* and other denitrification-related genes by improving soil microhabitats, thus promoting N₂O reduction [25,26]. Nevertheless, the mechanisms by which Si content drives the environmental functions of biochar remain poorly understood. In particular, under combined Cd contamination and nitrogen enrichment in acidic soils, the functional

differentiation of biochar with contrasting Si content and its microbial regulatory pathways have yet to be systematically elucidated.

Soils from *C. oleifera* plantations, prevalent in southern China, are characteristically acidic due to both natural pedogenesis and long-term fertilization. This acidity creates environmental hotspots where Cd mobilization threatens food safety and incomplete denitrification elevates N₂O emissions. Given this coupled challenge, we investigated the potential of biochar with contrasting Si contents to simultaneously immobilize Cd and mitigate N₂O production. Through a 90-day incubation of acidic soil with combined Cd and N additions, we aimed to elucidate the role of biochar-Si in regulating N transformations and the abundance of associated functional genes, thereby informing the design of targeted soil amendments. We hypothesized the following: (1) biochar functions are Si-dependent, with high-Si biochar being more effective at suppressing N₂O emissions and low-Si biochar superior for Cd immobilization; (2) this functional divergence is primarily driven by distinct impacts on soil pH and N transformation pathways, which in turn control Cd bio-availability and N₂O fluxes; and (3) these biogeochemical effects are underpinned by shifts in the abundance of key N-cycling genes, particularly the N₂O-reducing *nosZ* gene.

2. Materials and Methods

2.1. Soil Sampling and Biochar Preparation

The experimental soil was collected from the cultivated topsoil layer (0–20 cm) of a *C. oleifera* plantation (21-year-old) located in a representative acidic soil region in Jiangxi Province, China (28°31' N, 116°37' E). The soil in this region is classified as Quaternary red soil. The local climate is characterized as a subtropical monsoon climate, exhibiting significant seasonal variations, with a mean annual temperature of 17.7 °C and a mean annual precipitation of approximately 1600 mm. In June 2023, soil samples were randomly collected from 16 points within the 0–20 cm soil layer during the peak fruiting period of *C. oleifera*. The collected samples were thoroughly homogenized, immediately placed into insulated boxes filled with ice packs, and transported promptly to the laboratory for further processing. Impurities such as stones, plant and animal residues, and roots were meticulously removed, after which soil samples were sieved through a 2 mm mesh. Basic soil physicochemical properties were as follows: soil pH of 4.39 ± 0.02 , soil organic carbon (SOC) of $7.01 \pm 0.10 \text{ g kg}^{-1}$, total nitrogen (TN) of $0.65 \pm 0.03 \text{ g kg}^{-1}$, ammonium nitrogen (NH₄⁺-N) of $4.73 \pm 0.25 \text{ mg kg}^{-1}$, nitrate nitrogen (NO₃⁻-N) of $0.50 \pm 0.04 \text{ mg kg}^{-1}$, and dissolved organic carbon (DOC) of $16.25 \pm 0.32 \text{ mg kg}^{-1}$. Additionally, total Cd (TCd) and available Cd (ACd) concentrations were $0.02 \pm 0.00 \text{ mg kg}^{-1}$ and $0.004 \pm 0.00 \text{ mg kg}^{-1}$, respectively, while available Si (ASi) was $294.59 \pm 7.30 \text{ mg kg}^{-1}$.

Four agricultural residues differing in Si content were selected as biochar feedstocks, including low-Si materials (*C. oleifera* leaves and shells) and high-Si materials (bamboo leaves and rice straw). Specifically, *C. oleifera* leaf (CL) and shell (CS) biochar feedstocks were obtained directly from the plantation at the sampling site. Bamboo leaf biochar (ML) feedstock was collected from bamboo forests located in Lushan, Jiangxi Province, while rice straw biochar (RS) was produced from rice straw collected near the sampling site. Feedstocks were air-dried at room temperature, pulverized, and pyrolyzed at 450 °C under oxygen-limited conditions for 1 h. This temperature was selected to optimize the balance between biochar stability and surface functionality, which could develop sufficient porosity while retaining a higher abundance of oxygen-containing functional groups compared to higher temperatures [27]. After pyrolysis, the biochar samples were ground and passed through a 0.25 mm sieve prior to subsequent use. Basic physicochemical properties of biochar are presented in Table 1.

Table 1. Basic physicochemical properties of the biochars derived from *Camellia oleifera* leaves (CL), *Camellia oleifera* shells (CS), Moso bamboo leaves (ML), and rice straw (RS). Values are presented as the mean $\pm$ standard error (SE), with a sample size of $n = 4$ for all measurements.

Factors	ML Biochar	RS Biochar	CL Biochar	CS Biochar
pH	8.50 $\pm$ 0.01	9.98 $\pm$ 0.00	8.39 $\pm$ 0.02	10.49 $\pm$ 0.03
SOC (g kg ⁻¹)	456.68 $\pm$ 14.54	410.75 $\pm$ 6.36	526.01 $\pm$ 4.61	453.40 $\pm$ 16.22
TN (g kg ⁻¹)	75.81 $\pm$ 8.07	38.46 $\pm$ 3.24	42.26 $\pm$ 1.27	9.74 $\pm$ 0.72
DOC (mg kg ⁻¹)	426.17 $\pm$ 37.92	2231.33 $\pm$ 125.00	269.55 $\pm$ 14.52	1358.04 $\pm$ 148.02
NH ₄ ⁺ -N (mg kg ⁻¹)	12.47 $\pm$ 0.07	3.45 $\pm$ 0.11	2.82 $\pm$ 0.22	2.79 $\pm$ 0.11
NO ₃ ⁻ -N (mg kg ⁻¹)	0.46 $\pm$ 0.05	0.09 $\pm$ 0.01	0.18 $\pm$ 0.02	0.63 $\pm$ 0.01
TCd (mg kg ⁻¹)	0.15 $\pm$ 0.00	0.04 $\pm$ 0.00	0.06 $\pm$ 0.00	0.013 $\pm$ 0.00
ACd (mg kg ⁻¹)	0.02 $\pm$ 0.00	0.01 $\pm$ 0.00	0.007 $\pm$ 0.00	0.003 $\pm$ 0.00
TSi (g kg ⁻¹)	24.03 $\pm$ 0.23	31.37 $\pm$ 0.38	4.43 $\pm$ 0.29	5.90 $\pm$ 0.40

2.2. Experimental Design and Laboratory Incubation

A full-factorial randomized design was conducted to investigate the interactive effects of biochar with different Si content, N addition, and Cd contamination on N₂O emissions and heavy metal stabilization in acidic agricultural soils. The experiment followed a full-factorial design with three factors: N addition (control vs. N addition), Cd contamination (control vs. Cd addition), and biochar treatment (con vs. biochar addition), yielding a total of 20 (2 $\times$ 2 $\times$ 5) treatments. The five biochar treatments comprised a control, two types of high-Si biochar (derived from ML and RS) and two types of low-Si biochar (derived from CL and CS). Each treatment had four replicates, resulting in a total of 80 experimental units (2 $\times$ 2 $\times$ 5 $\times$ 4 = 80). Furthermore, N was applied as NH₄NO₃ at a rate of 200 mg N kg⁻¹ soil. Cd was introduced as CdCl₂ at a concentration of 30 mg Cd kg⁻¹ soil. Biochar was incorporated into the soil at a rate of 3% (*w/w*, based on dry soil weight). For each replicate, 30 g of air-dried soil was transferred into a 250 mL Erlenmeyer flask. Soil moisture was maintained at 60% of the maximum field water-holding capacity, and all samples were incubated aerobically at 25 °C in the dark for 90 days. This duration was chosen to capture the complete dynamic process of N transformation (from rapid turnover to stabilization).

To accommodate measurements of indicators at multiple time points, six parallel sets of samples were established, yielding a total of 480 incubation samples (80 $\times$ 6 = 480). One set (80 samples) was dedicated to greenhouse gas measurements. Soil N₂O fluxes and cumulative emissions were determined on days 3, 6, 11, 15, 22, 28, 37, 48, 60, 75, and 90. The remaining five sets were used for the assessment of soil physicochemical properties, including pH, NH₄⁺-N, and NO₃⁻-N contents on days 6, 22, 37, 60, and 90. At the peak of N₂O emission, subsamples were collected from the gas monitoring set for quantitative analysis of microbial functional genes associated with the N₂O process, including ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), *nirS*, *nirK*, and *nosZ*. After incubation, all samples were analyzed for TN, SOC, DOC, TCd, and ACd. The ACd ratio was calculated to comprehensively evaluate the regulatory effects of different biochar treatments on soil N dynamics and Cd behavior.

2.3. Measurement of N₂O Emissions

Prior to gas sampling, all incubation flasks containing soil samples were aerated using a blower to ensure aerobic conditions. Each flask was then sealed with a rubber stopper equipped with a three-way valve. A 50 mL gas-tight syringe was used to inject 40 mL of ambient air into the flask. To ensure homogeneity, the headspace gas was mixed thoroughly by repeatedly drawing and expelling the gas within the syringe. After mixing, 40 mL of gas were immediately withdrawn to serve as the initial (pre-incubation) sample. The sealed flask was then incubated in a dark growth chamber at 25 °C for 4 h. After incubation, another 40 mL gas sample was collected to represent the post-incubation gas. Gas samples were analyzed for N₂O concentration using an Agilent 7890B gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with an electron capture detector (ECD). The rate and cumulative N₂O emissions were calculated using the following Equations (1) and (2), respectively.

$$N_2O_{\text{rate}} = P \times V \times (\Delta c / \Delta t) \times (1/RT) \times M \times (1/m) \quad (1)$$

where N_2O_{rate} represents the N₂O emission rate (ng g⁻¹ h⁻¹), P is the standard atmospheric pressure (Pa), V is the headspace volume of the flask (cm³), Δc denotes the N₂O concentration, Δt is the duration of gas collection (h), R is the universal gas constant (8.314 Pa m³·mol⁻¹ K⁻¹), T is the absolute temperature (K), M is the molecular weight of N₂O (g mol⁻¹), and m is the dry weight of soil (g).

$$N_2O_{\text{cumulative}} = \sum_{i=1}^n \frac{(F_i + F_{i+1})}{2} \times (t_{i+1} - t_i) \times 24 \quad (2)$$

where $N_2O_{\text{cumulative}}$ is the cumulative N₂O emissions, F_i and F_{i+1} represent the N₂O emission fluxes measured at the i th and $(i + 1)$ th consecutive sampling points, respectively; $t_{i+1} - t_i$ is the time interval between two consecutive sampling time points, and n is the total number of sampling intervals during the incubation period; and 24 is a conversion factor used to calculate daily emissions.

2.4. Determination of Soil Physicochemical Properties and Microbial Functional Genes

Soil physicochemical properties were determined following the classical procedures [28]. Soil pH was measured using the suspension method with specific material-to-water ratios: 1:2.5 for soil, 1:20 for biochar, and 1:5 for plant materials. Measurements were performed using a pH meter (PHS-3C, INESA, Shanghai, China). SOC was determined using the external heat potassium dichromate oxidation method, and TN was quantified with an elemental analyzer (AA3, Seal Analytical, Norderstedt, Germany). NH₄⁺-N and NO₃⁻-N were extracted with 2 mol L⁻¹ KCl solution, followed by filtration and quantification via ultraviolet spectrophotometry at specific wavelengths. DOC was extracted using deionized water with sample-to-water ratios of 1:4 for soil and 1:8 for both biochar and plant materials. The mixture was shaken thoroughly, filtered, and analyzed using a total organic carbon analyzer (Multi N/C 3100, Jena, Germany). TCd and ACd concentrations in soil were determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent Technologies, Santa Clara, CA, USA). Total Si content was measured following digestion with nitric and perchloric acids. The SiO₂ was isolated by ignition at 800 °C, and the mass loss method was employed to calculate ASI.

Furthermore, total soil microbial DNA was extracted using the FastDNATM Spin Kit for Soil (MoBio Laboratories, Carlsbad, CA, USA) according to the instructions of the manufacturer. The abundance of functional genes related to N transformation processes, including AOA, AOB, *nirS*, *nirK*, and *nosZ*, was quantified by real-time quantitative PCR

(qPCR). The qPCR reactions were performed using SYBR® Premix Ex Taq™ (Takara, Tokyo, Japan), and the primer sequences and amplification conditions are provided in Table S1. Each reaction was conducted in triplicate for technical replication. Amplification specificity was confirmed by analyzing the melting curves and amplification profiles. Standard curves were used to calculate gene copy numbers, which were subsequently used to represent the relative abundance of target genes.

2.5. Statistical Analyses

Statistical analyses were performed using R software (version 4.3.1) and SPSS 28.0 (Chicago, IL, USA), with data visualization conducted in OriginPro 2025 (OriginLab, Northampton, MA, USA). A two-way ANOVA was employed to assess the effects of biochar type, Cd addition, N addition, and their interactions on soil pH, Cd activation rate, N₂O emissions, and functional gene abundances. A significance level of $p < 0.05$ was considered, with Duncan's test used for multiple comparisons. Pearson correlation analysis was applied to explore relationships between soil properties, N₂O emissions, and Cd removal. Redundancy analysis (RDA) was conducted using the vegan package in R to examine multivariate relationships among soil physicochemical properties, microbial functions, and N₂O dynamics.

3. Results

3.1. Soil pH and N Transformation Dynamics Under Biochar, N, and Cd Addition

Soil pH and mineral N dynamics exhibited significant variations under different biochar and Cd addition treatments (Figure 1). Soil pH values in all treatment groups consistently declined from the initial incubation stage until the end of the incubation period (Figure 1a,b). Biochar application significantly increased soil pH (Table S2). Particularly under the no N addition condition, the pH in the RS treatment group remained relatively high, with an average value of 5.80 ± 0.13 . This represented a significant increase of 30.38–31.82% compared to the control group (Con_Con, 4.41 ± 0.09), an effect surpassed only by the CL and CS treatments (Figure 1a). A similar trend was observed under N addition conditions. Furthermore, the dynamics of mineral nitrogen revealed that NH₄⁺-N content gradually decreased over the incubation period across all treatments, while NO₃[−]-N progressively increased (Figure 1c–f). N addition significantly enhanced the levels of both NH₄⁺-N and NO₃[−]-N in the soil (Table S2). Among these, the ML treatment group exhibited significantly higher contents of both N forms compared to other treatments. In contrast, the RS treatment group showed significantly lower NO₃[−]-N content, reduced by 72.10–81.03% relative to other treatments (Figure 1e,f). Significant correlations existed between soil pH and nitrogen transformation processes under the combined treatments of different biochar, N addition, and Cd addition (Figure S1). Specifically, soil pH was significantly negatively correlated with the cumulative NNR (Figure S1a; $R^2 = 0.10$, $p < 0.05$) but significantly positively correlated with the cumulative NMR (Figure S1c,d, $R^2 = 0.11/0.14$, $p < 0.05$). Further analysis revealed significant differences in NNR and NMR among treatment groups: N addition significantly stimulated both N transformation processes. The NNR in the high-Si biochar (ML) treatment group was significantly higher than that in the control and low-Si biochar treatment groups (Figure S1; $p < 0.05$). Conversely, under N addition conditions, the NMR in the low-Si biochar (CL and CS) treatment groups was significantly higher than that in the control and high-Si biochar treatment groups (Figure S1, $p < 0.05$).

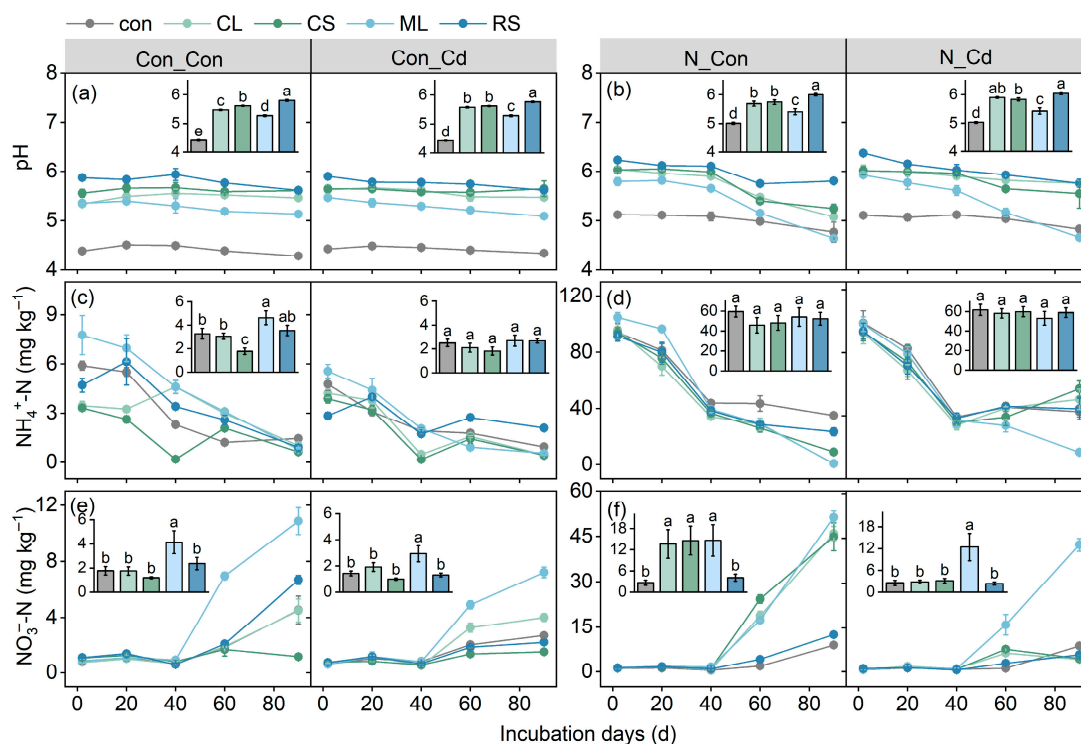


Figure 1. Temporal dynamics of soil pH and mineral nitrogen (N) under biochar, N, and cadmium (Cd) treatments over a 90-day incubation. (a,b) Soil pH time courses under control (no N) and N addition, respectively. (c,d) Temporal dynamics of ammonium ($\text{NH}_4^+\text{-N}$); (e,f) temporal dynamics of nitrate ($\text{NO}_3^-\text{-N}$) under Con and N, respectively. The five biochar levels include a control (Con, no biochar) and biochar derived from *Camellia oleifera* leaves (CL), *Camellia oleifera* shells (CS), Moso bamboo leaves (ML), and rice straw (RS). All data points and bars represent the mean $\pm$ standard error (SE) of four replicates ($n = 4$). Different letters above the inset bars indicate statistically significant differences among treatments as determined by a Tukey HSD test ($p < 0.05$).

3.2. N_2O Emissions and Functional Gene Responses to Biochar, N, and Cd Addition

Under treatments involving biochar, N, and Cd addition, N_2O emission rates exhibited significant differences over the incubation period (Figure 2a–d). Without N addition, biochar application significantly reduced the peak N_2O emission rates. Following N addition (N_Con, N_Cd), emission rates generally increased, with the timing and magnitude of peaks influenced by biochar type. Furthermore, N addition significantly increased cumulative N_2O emissions, a process significantly modulated by biochar type. Within the no-N group under Cd addition, the RS treatment showed significantly lower cumulative N_2O emissions than the control. After N addition, biochar application significantly decreased cumulative N_2O emissions by 11.59–80.46%, with high-Si biochar exhibiting superior mitigation effects. However, under Cd addition, cumulative N_2O emissions from high-Si biochar were significantly higher than the Con treatment; only the CS treatment retained a mitigating effect (Figure 2e). In the initial incubation phase (Figure S2), N addition significantly affected the abundances of AOA, AOB, and denitrification functional genes (*nirS*, *nirK*, and *nosZ*) (Table S3). Following N addition, AOA and AOB gene abundances increased significantly across all biochar treatments. For denitrification genes, *nirS*, *nirK*, and *nosZ* copy numbers generally decreased compared to the control. Conversely, without N addition, biochar treatments significantly increased denitrifier abundance and the *nosZ*/(*nirS* + *nirK*) ratio, with high-Si biochar showing particularly pronounced effects (Table S3). In the later incubation phase (Figure S2), AOA and AOB abundances significantly increased in the control, while N fertilization significantly reduced their copy

numbers; denitrification gene abundances generally declined. For the *nosZ* gene, abundances in high-Si biochar treatments remained significantly higher than in the control. Cd addition induced more pronounced fluctuations in gene abundances.

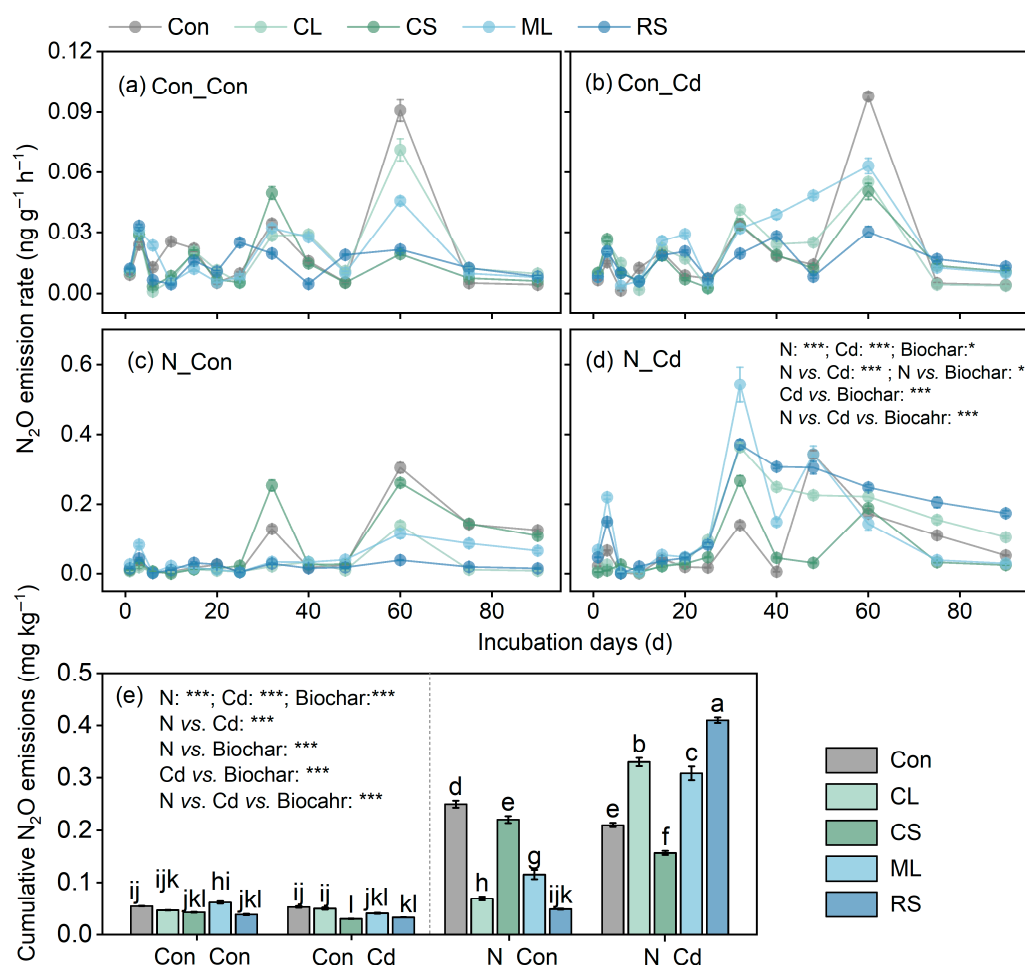


Figure 2. The effects of biochar, nitrogen (N), and cadmium (Cd) on nitrous oxide (N₂O) emissions. (a–d) N₂O emission rates (ng g⁻¹ h⁻¹) under four experimental backgrounds: (a) control N and control Cd (Con_Con), (b) control N and Cd addition (Con_Cd), (c) N addition and control Cd (N_Con), and (d) N and Cd addition (N_Cd). (e) Cumulative N₂O emissions (mg kg⁻¹) over the 90-day incubation period. Lines and bars are the mean ± standard error (SE) of four replicates (n = 4). Statistical analysis of cumulative emissions was performed using a three-way ANOVA. Different letters above the bars denote significant differences among biochar levels within each background, as determined by a post-hoc Tukey HSD test (p < 0.05). The inset text reports the significance of main effects and their interactions from the ANOVA: * p < 0.05, *** p < 0.001.

Further analysis revealed coupling between changes in N₂O emission rates and functional gene copy numbers. Variations in AOA and AOB gene copy numbers showed a linear, significant negative correlation with N₂O emission rates (Figure 3a,b; p < 0.05). The total abundance of *nirS* + *nirK* and the *nosZ* gene abundance exhibited quadratic relationships with emission rates, reflecting the synergistic regulation of denitrification genes in the N₂O production-reduction processes (Figure 3).

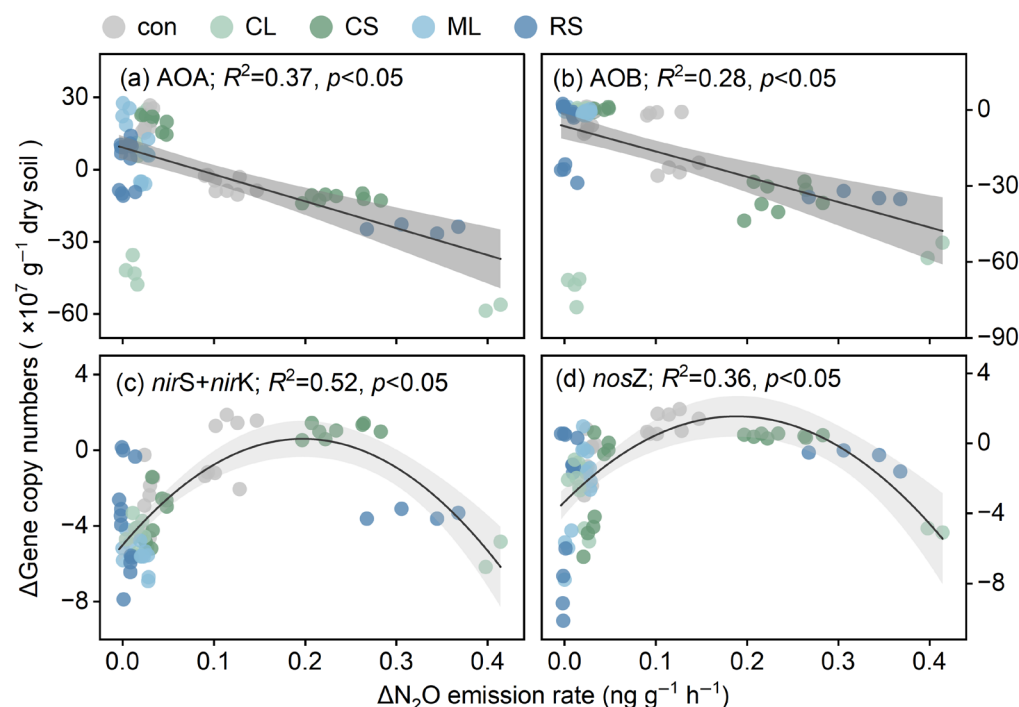


Figure 3. Relationships between N_2O emission rates and the abundance of nitrogen-cycling functional genes. The scatterplots show correlations between the N_2O emission rate ($\text{ng g}^{-1} \text{h}^{-1}$) and the copy numbers of (a) ammonia-oxidizing archaea (AOA), (b) ammonia-oxidizing bacteria (AOB), (c) *nirS* + *nirK* (sum), and (d) *nosZ* gene. Each data point represents the mean of a treatment group ($n = 4$). The solid lines represent the best-fit linear or quadratic models, with the shaded areas indicating the 95% confidence interval (CI). The coefficient of determination (R^2) for each model is reported on the respective panel.

3.3. Effects of Biochar on Cd Immobilization and Removal Efficiency

Cd activation rate, immobilization, and removal efficiency were all significantly influenced by biochar type (Figure 4). Application of both low-Si (CL, CS) and high-Si (ML, RS) biochar significantly decreased soil available Cd content by 34.2–63.3% and reduced Cd activation rate by 34.2–74.9% (Figure 4a,b). Among these, low-Si biochar, particularly CL, exhibited the strongest remediation effect on Cd contamination. Furthermore, all biochar treatments significantly enhanced Cd immobilization, with low-Si biochar demonstrating superior performance (Figure 4c). Regarding Cd removal efficiency, low-Si biochar (e.g., CL, CS) was significantly higher (by 11.89–99.33%) than high-Si biochar (ML, RS) (Figure 4d), indicating more effective Cd remediation by low-Si biochar in the soil. A significant coupling effect was observed between cumulative N_2O emissions and Cd removal efficiency under biochar treatments. Under conditions of N and Cd addition, both parameters exhibited a negative correlation trend (Figure S3; $R^2 = 0.16$ for N addition group; $R^2 = 0.12$ for Cd addition group). In high-Si biochar treatments (ML, RS), increased N_2O emissions were accompanied by improved Cd removal efficiency, a coupling relationship particularly pronounced under Cd addition.

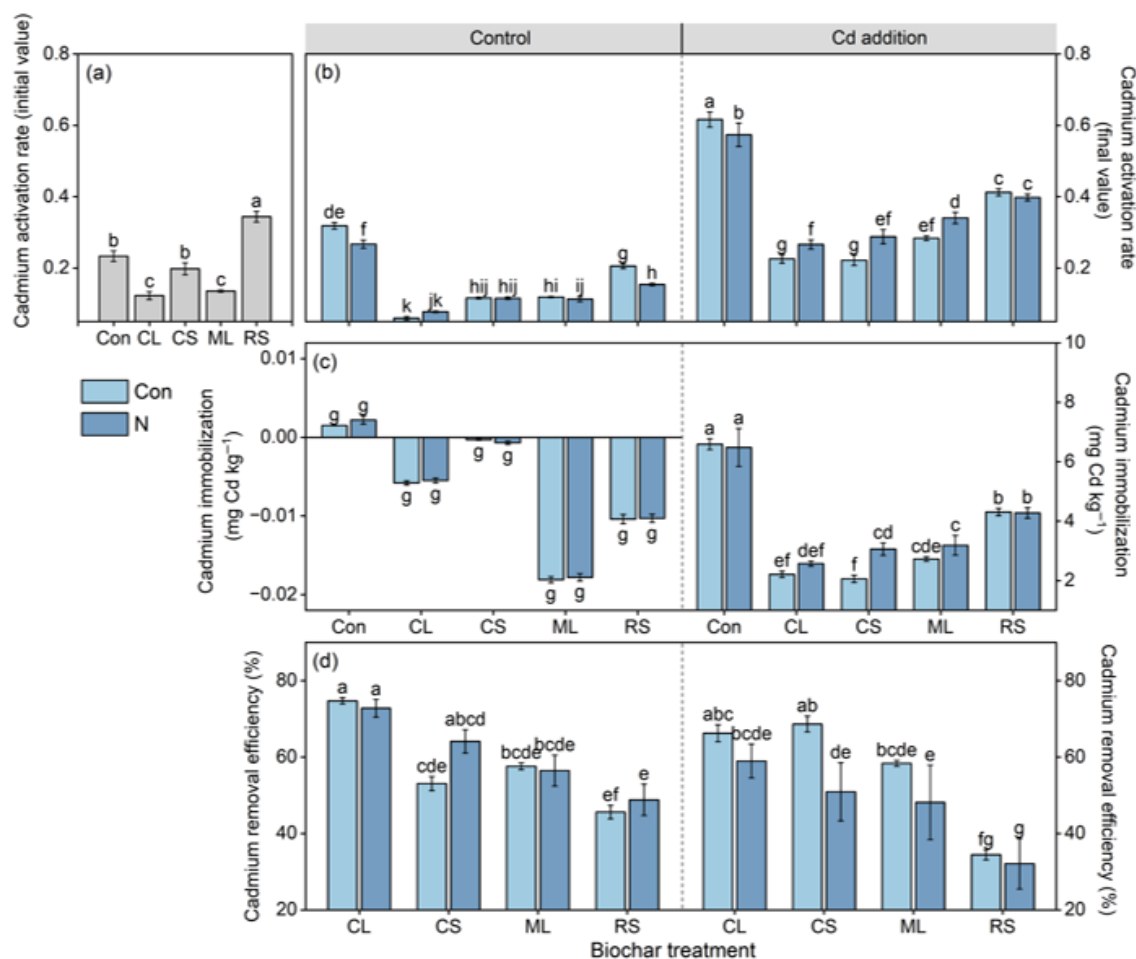


Figure 4. The effects of biochar on cadmium (Cd) geochemistry and its relationship with N₂O mitigation. (a) The baseline Cd activation rate (ratio of available Cd to total Cd, ACd/TCd) before incubation. (b) The final Cd activation rate (%) after 90 days. (c) The total amount of Cd immobilized (mg Cd kg⁻¹ soil; right y-axis) and the corresponding change in activation rate (left y-axis). (d) The efficiency of Cd removal/immobilization (%) by each biochar relative to the control. All data are presented as the mean $\pm$ standard error (SE) of four replicates ($n = 4$). Statistical significance for end-point responses was determined by a three-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Different letters (if present on the figure) would indicate significant differences among treatments.

3.4. Integrated Variable Relationships of N₂O Emissions and Cd Mobilization

RDA further revealed that both cumulative N₂O emissions and Cd removal efficiency were closely associated with soil pH and the abundance of functional genes involved in N transformation. Under no N addition (Figure 5a), RDA explained 94.79% of the total variance. Cumulative N₂O emissions were significantly associated with NH₄⁺-N content, AOB abundance, and Cd activation rate; pH, DOC, and NO₃⁻-N content were primary factors influencing variable distribution. The association pattern between cumulative N₂O emissions and Cd immobilization efficiency in high-Si biochar differed distinctly from the low-Si and the control, highlighting the influence of Si content on the regulatory effect. Under N addition (Figure 5b), RDA collectively explained 91.77% of the variance. The direction of the association between cumulative N₂O emissions and Cd immobilization efficiency shifted, and their coupling relationships with microbial factors (AOA, AOB, *nirK* + *nirS*) and soil properties (pH, DOC) were reconfigured. N addition strengthened the associations between microbial functional genes (e.g., AOA, *nosZ*) and N₂O emissions or Cd behavior.

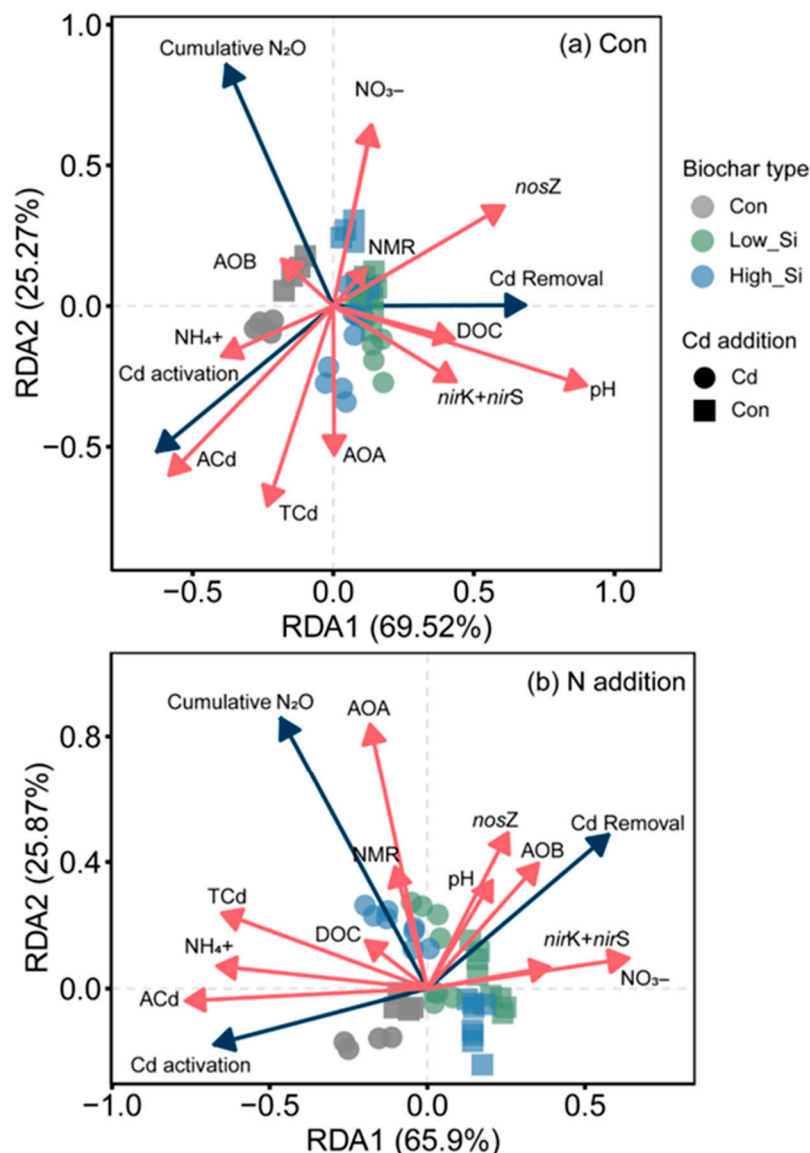


Figure 5. Redundancy analysis (RDA) of the relationships between environmental variables, microbial predictors, cumulative N₂O emissions, and cadmium (Cd) availability. The ordinations are shown for (a) control (no N) and (b) nitrogen (N) addition backgrounds. Each point represents the centroid of a treatment group ($n = 4$). Colors distinguish biochar silicon categories: gray for control (no biochar), green for low-Si biochars (CL, CS), and blue for high-Si biochars (ML, RS). Symbol shapes distinguish Cd treatments (circles for control, triangles for Cd addition). The percentage of constrained variance explained by each canonical axis is shown. Environmental vectors shown are significant ($p < 0.05$). The significance of the overall RDA model and its axes was assessed using a permutation ANOVA.

4. Discussion

Our results demonstrated that biochar addition significantly altered N transformation pathways and N₂O emissions, particularly under N input and Cd addition. Notably, biochar differing in Si content (high-Si vs. low-Si) exerted distinct regulatory effects on soil N transformations and N₂O fluxes. Variations in Si content, surface chemistry, and buffering capacity directly modified soil pH, microbial activity, and N cycling [29,30]. It should be noted that the regulatory effects of high-Si biochar are likely synergistic. While the high Si content contributes significantly to alkalinity and pH buffering, these chemical traits co-vary with the distinct physical structures inherent in Si-rich feedstocks [31]. In this study, high-Si biochar (e.g., ML, RS) significantly reduced soil N₂O emissions, especially under N

addition, which may be attributed to its stronger pH-buffering capacity. High-Si biochar had dense porosity and exhibited larger specific surface area ($>492.6 \text{ cm}^2 \text{ g}^{-1}$) and greater alkalinity, effectively alleviating soil acidification and elevating pH values [11]. Higher pH values could favor denitrification pathways that complete the reduction to N_2 , thereby reducing N_2O formation. This was consistent with previous studies showing that Si-rich biochar ameliorates soil acidity and lowers the $\text{N}_2\text{O}/(\text{NO}_2^- + \text{NO}_3^-)$ ratio during nitrification, enhancing its abatement effect on N_2O [25]. In addition, the higher surface negative charge and average pore diameter of high-Si biochar could provide effective adsorption sites and efficiency [32], which may promote ion exchange with NH_4^+ and NO_3^- to form relatively stable associations, hence reducing the bioavailability of these N substrates by 33.0–89.2%, suppressing nitrification, and facilitating denitrification [20,33]. Consistently, we found significant positive correlations between soil pH and both NNR and NMR; under high-Si treatments (ML, RS), elevated pH stimulated mineralization (e.g., NMR). Together, these patterns indicated that high-Si biochar might optimize N transformations primarily by increasing soil pH and, through increases in SOC, promote mineralization, thereby accelerating N turnover while reducing N_2O production. Moreover, microbial functional genes further modulated N_2O dynamics. For instance, the abundance of *nosZ*, which mediates the process of N_2O production [34,35], increased significantly with high-Si biochar, strengthening the soil capacity to reduce N_2O to N_2 and thus mitigating N_2O emissions by 39% [36]. These findings are concordant with reports that high-Si biochar elevates *nosZ* abundance and restructures denitrifier communities to suppress N_2O [25]. Under N addition, low-Si biochar (particularly CS) exhibited weaker surface negative charge, lower hydrophilicity, and poorer pH regulation than high-Si materials. Consequently, it tended to promote nitrification, diminishing its N_2O -mitigation potential. With low-Si biochar, soil pH changed little, and denitrification was not evidently enhanced. Instead, low-Si additions significantly increased NNR, leading to NO_3^- accumulation and greater substrate availability for N_2O production, consistent with previous study that observed higher N_2O associated with insufficient suppression of N pathways by low-Si biochar [30]. In our study, the regulation of N_2O emissions was not solely dependent on *nosZ* but closely linked to the balance between nitrification and denitrification genes. We observed significant correlations between ammonia-oxidizing genes (AOA and AOB) and N_2O dynamics (Figure 3). High-Si biochar suppressed AOB abundance under N addition, likely slowing the initial rate of ammonia oxidation and providing fewer substrates for subsequent denitrification. Conversely, low-Si treatments increased *nirS* and *nirK* abundances, indicating an enhanced potential for nitrite reduction to N_2O [34]. However, N_2O reduction capacity did not increase commensurately, thereby elevating emissions. Our finding suggested that low-Si biochar may enhance N_2O emissions by promoting nitrification and denitrification processes without simultaneously enhancing the reduction of N_2O to N_2 , which represents a key limitation of low-Si materials. Overall, biochar significantly influences N_2O emissions and N transformations by modulating soil pH, enhancing denitrification completeness, and restructuring microbial communities. Owing to stronger ion-exchange capacity and buffering, high-Si biochar plays a more favorable role in directing soil N cycling toward lower N_2O , particularly under N-enriched conditions. However, this mitigation potential appears to be context-dependent. Under combined Cd contamination, the efficacy of high-Si biochar was attenuated, suggesting that complex interactions between heavy metal toxicity and microbial communities may override the benefits of pH buffering. Hence, biochar selection should be context-dependent in soil amendment and pollution-control strategies according to the global studies [14,15,31]. These findings provide a mechanistic basis for GHG mitigation and soil remediation across heterogeneous soils and offer operational guidance for the precise application of biochar.

The activation rate of soil Cd increased with biochar Si content, leading to higher toxicity and greater plant uptake [23]. Cd activation rate, therefore, can generally be used as an indicator to evaluate the effectiveness of biochar with different Si contents in remediating Cd-contaminated soils. Our results demonstrated that biochar addition significantly influenced Cd activation rates in acidic soils, with significant differences observed between biochar types. Both high-Si and low-Si biochar exhibited significant immobilization effects on effective Cd, indicating that both types of biochar play a dominant role in remediating Cd-contaminated soils. Among these, low-Si biochar (e.g., CL and CS) showed stronger Cd immobilization abilities under Cd addition, suggesting that Cd remediation effectiveness is not solely dependent on biochar Si content but also influenced by factors such as feedstock type, functional group composition, pyrolysis temperature, and soil adaptability [37,38]. Without Cd, all biochar types reduced the effective Cd concentration (low-Si biochar reduced by 55.9–73.3%, high-Si biochar by 47.4–57.1%), but no significant differences were observed. This could be attributed to the low background Cd levels in the soil, which were insufficient to drive adsorption reactions and form concentration gradients. However, low-Si biochar with Cd addition exhibited a significant increase in Cd fixation efficiency, particularly CL biochar. This is attributed to its higher SOC (526.01 g kg⁻¹) and moderate DOC levels (269.55 mg kg⁻¹). Previous studies have indicated that biochar produced at ~450 °C retains abundant oxygen-containing functional groups (e.g., carboxyl and phenolic hydroxyl groups), which can provide reactive surface sites and contribute to Cd²⁺ immobilization via surface complexation [27]. Unlike high-Si biochar, the moderate DOC release from low-Si biochar likely formed insoluble complexes with Cd rather than facilitating its mobility, thereby reducing Cd bio-availability under acidic conditions. This finding challenges the classical view that high-Si biochar could be more effective in fixing heavy metals [11]. A previous study has found that biochar derived from high-temperature pyrolysis of *C. oleifera* shells, when Si-modified, showed better Cd pollution remediation effects with higher Si content [23]. However, under the acidic soil conditions and pyrolysis temperature of 450 °C in this study, the performance of high-Si biochar did not meet expectations [11,39]. High-Si types of biochar such as ML and RS had compact structures at this temperature [16], with poorly developed pore structures and low exposure of functional groups, which limited their physical and chemical Cd fixation capabilities [37]. Moreover, high-Si biochar (e.g., ML and RS) showed lower Cd removal (34.2–40.0%), despite higher pH-buffering capacity, likely due to the excessive release of DOC. In particular, the RS biochar released significantly higher DOC (2231.3 mg kg⁻¹, Table 1) compared to other treatments. High DOC concentrations can act as carriers, forming soluble Cd-DOC complexes that maintain Cd in the soil solution. Although high-Si biochar increased soil pH significantly (RS: 9.98, CS: 10.49), this indirect pH-buffering mechanism did not dominate Cd immobilization under high Cd loadings, particularly given the instability of Si compounds under acidic conditions. These results were consistent with the RDA analysis, which identified DOC and pH as key drivers of Cd stabilization, with low-Si biochar showing negative correlations between Cd activation and both parameters. Although the direct Cd immobilization effect of high-Si biochar was weaker, its strong pH buffering capacity helped reduce Cd toxicity [40]. ML and RS significantly elevated soil pH, which contributed to decreased Cd bioavailability through chemical environment modulation rather than physical sorption [41]. This pH-buffering effect is particularly beneficial in highly acidic soils, indirectly mitigating metal toxicity. Although the biochar was classified based on TSi content, it is important to note that the remediation efficiency is largely driven by the bio-available fraction of Si and the associated alkalinity. The high-Si biochar in this study released significantly higher amounts of available Si and exhibited higher pH, which synergistically promoted the formation of silicate precipitates and Cd

immobilization. Therefore, TSi serves as a proxy for the potential capacity of the biochar to provide these active components. Importantly, we observed that Cd immobilization and N₂O emissions were significantly negatively correlated under Cd and N addition. While high-Si biochar exhibited limited Cd immobilization, its influence on microbial communities and soil pH may have contributed to reduced N₂O emissions, suggesting a synergistic effect in co-regulating heavy metal stabilization and GHG mitigation. In summary, low-Si biochar demonstrated greater efficacy in Cd stabilization, primarily due to its higher SOC content and moderate DOC release, while high-Si biochar was more effective in pH regulation and indirect toxicity mitigation. Biochar selection for co-remediation should consider pyrolysis temperature, feedstock characteristics, and soil properties to optimize outcomes for both heavy metal immobilization and N₂O emission mitigation.

Although this study demonstrated the potential of biochar for simultaneous GHG mitigation and Cd remediation, there are certain limitations. The experiment was restricted to a short-term (90-day) incubation, and the long-term stability and effectiveness of biochar, particularly across diverse ecological contexts, require further validation. Additionally, biochar types and application rates were not fully explored in this study. The trade-offs between Cd stabilization and N₂O emissions observed at the 3% application rate may shift with varying dosages. For high-Si biochar, increasing the application rate could amplify DOC release, thereby exacerbating Cd mobilization even if N₂O mitigation is enhanced by higher pH. Conversely, for low-Si biochar, higher dosages might compensate for its weaker pH-buffering capacity, potentially unlocking its N₂O mitigation potential without compromising Cd immobilization. Determining the optimal application threshold to balance these opposing processes is a critical next work. Moreover, this study primarily examined the roles of pH and microbial communities in regulating N₂O emissions and Cd immobilization. However, in field conditions, additional variables such as soil moisture, temperature, redox potential, and carbon availability may interact with biochar properties to affect its performance. Future research should incorporate a broader set of environmental and management factors to better reflect field-relevant complexity. Furthermore, while this study elucidated the regulatory effects of biochar on Cd and N₂O via soil physicochemical properties and functional gene abundances, direct spectroscopic evidence (e.g., FTIR, XPS) and Cd fractionation data were not included. Future research should employ these advanced characterization techniques to visually verify surface functional group evolution and quantitatively partition Cd speciation mechanisms. Collectively, from a practical standpoint, converting agricultural residues (e.g., rice straw, bamboo leaves) into biochar not only remediates soil but also offers a cost-effective strategy for crop waste management, reducing the environmental burden of open burning.

5. Conclusions

This study compared low-Si biochar (CL, CS) and high-Si biochar (ML, RS), all pyrolyzed at 450 °C, for their effects on N₂O emissions and Cd immobilization in a typical acidic red soil. Both classes reduced soil-available Cd and modulated N transformations, but their functional emphases diverged. Under Cd addition, low-Si biochar exhibited stronger Cd immobilization. High-Si biochar more effectively suppressed N₂O emissions and mitigated acidification by elevating soil pH and increasing N₂O-reduction capacity (e.g., *nosZ*). However, despite this significant pH elevation, its efficiency in Cd stabilization was lower than that of low-Si biochar. This suggests that the pH-induced immobilization was insufficient to counteract the Cd mobilization effect driven by the excessive release of dissolved organic carbon (DOC) from high-Si biochar. Concordant shifts in NNR and NMR, together with functional-gene responses, indicated coupled regulation of N₂O and Cd via physicochemical and microbial pathways. For dual-objective management, deployment

should be tailored to site conditions and biochar properties: prioritize low-Si biochar for rapid heavy-metal stabilization and high-Si biochar for GHG mitigation and pH amelioration; blending or post-modification can further optimize synergy. Given the sensitivity of outcomes to pyrolysis temperature, soil type, and nutrient context, longer-term, scenario-based validation is warranted to support precise application in acidic agroforestry soils.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy16010075/s1>: Figure S1: Soil pH–N transformation linkages and treatment effects on net rates; Figure S2: Functional gene responses to biochar, N and Cd addition across incubation stages. Initial stage (blue) and later stage (green) are shown side-by-side for each background (left: no Cd; right: Cd addition); Figure S3: Coupling between cumulative N₂O emissions (mg kg^{−1}) and Cd removal efficiency (%) across biochar types under (e) N addition and (f) Cd addition; lines are OLS fits with 95% CI, annotated with R²; Table S1: Fluorescent real-time quantitative PCR amplification primer information; Table S2: Effects of nitrogen (N), Cd and biochar (B) treatments and their interactions on soil AOA, AOB, *nirS*, *nirK*, and *nosZ* (gene copy numbers (×10⁷ g^{−1} dry soil); Table S3: Effects of nitrogen (N), Cd and biochar (B) treatments and their interactions on soil pH, NH₄⁺-N, NO₃[−]-N, DOC, total Cd (TCd), ACd (available Cd), and Cd activation rate.

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