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Biochar from Various Sources at Different Application Rates Suppresses Nitrification Activity and N_2O Emissions in Soil

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BIOCHAR is a soil amendment widely used as a strategy to mitigate greenhouse gas emissions, such as nitrous oxide (N_2 O), especially by suppressing nitrification, a crucial first step in N_2O formation. The effectiveness of biochar in suppressing nitrification depends on its source and application rate. This research investigated the impacts of biochar application derived from corn stover (BC), rice husk (BR), or sawdust (BS) at rates of 0, 5, 10, and 20 tonnes ha^{-1} on nitrification activity and N_2O flux in soil. We conducted the soil incubation experiment for 14 days using the microcosm incubation method to investigate NH_4^+-N and NO_3^--N dynamics, N_2O emissions, and nitrification activity. BC20 had the lowest nitrification activity, with approximately 70% of NH_4^+-N remaining in the soil for 14 days. Biochar application had an inhibitory effect. More inhibition was observed on day 7 across all treatments (8-77%) compared to day 14, when only the BR20 treatment showed inhibition (69%). Compared to the control, it lowered the potential nitrification activity (PNA) by 48-92% decreasing from 597.12 to between 46.59 and311.17 mg NO_3^--N kg^{-1} soil h^{-1}. BC presented the lowest daily N_2O emissions, ranging from 581.38 to 1,765.31 µg N_2O kg^{-1} soil day^{-1}. The BC, BR, and BS treatments reduced cumulative N_2O emissions by 47%, 43%, and 41%, respectively. The results indicate that biochar types and rates, and their interaction, significantly reduced soil nitrification activity and N_2O emissions, with the BC application having stronger positive effects. This research underscores the effectiveness of targeted biochar application in reducing nitrogen losses in tropical agricultural systems.

Keywords: Pyrolysis, Inhibition, Ammonium, Nitrate, Greenhouse gas emissions.

1. Introduction

Climate change represents a significant global challenge, primarily driven by the rise in greenhouse gas (GHG) emissions in the atmosphere. The primary GHG emitted by agricultural systems are carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4). Over a 100-year period, N_2O and CH_4 exhibit 265 and 28 times stronger global warming potentials than that of CO_2, respectively. N_2 O is a highly powerful GHG that significantly plays a major role in stratospheric ozone depletion (IPCC, 2021). Synthetic nitrogen fertilizers, which are intensively used by farmers to support plant growth and development drive the increase of N_2O emission in agriculture. The manufacture of synthetic nitrogen fertilizers accounts for approximately 39% of total N_2O emissions in agricultural systems due to its application, unless urgent mitigation techniques are implemented (Menegat et al., 2022). This contribution is considered the primary source of N_2O emissions in agriculture (Zhang et al., 2023). Nitrous oxide emissions arise from microbial activities during nitrogen transformation. Nitrification is the primary source of N_2O emissions in oxygen-rich environments and generates nitrate (NO_3^-) as a precursor for denitrification, the principal source of N_2 O under anoxic circumstances (Liu et al., 2024).

Regulating the nitrification rate is a critical action, as nitrification promotes nitrogen loss and plays a role in N_2O production. Microbial enzymes such as hydroxylamine oxidoreductase and ammonia monooxygenase play a crucial role in the biological process of nitrification (Yin et al., 2023). Some plants, like sorghum, rice, and certain grasses, can stop nitrification by releasing special substances called biological nitrification inhibitors (BNIs) from their roots. However, only certain plant species and types can produce BNIs, which makes it difficult to use this natural method on a larger scale (Subbarao and Searchinger, 2021; Azizah et al., 2023a). Consequently, soil amendments such as biochar have gained attention as alternative tools for mitigating N_2O emissions. Biochar, a carbon-rich material produced by pyrolysing organic matter under limited oxygen conditions, is known to influence soil nitrogen dynamics and reduce GHG emissions (Elhakem et al., 2025). Previous research has demonstrated how biochar can effectively suppress N_2O production through diverse

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mechanisms. He et al. (2023) found biochar creates a sink for NH_3 uptake and absorption in the soil, leading to a decrease in NO_3^- concentration and a reduction in N_2O emissions. The capability of biochar for reversible absorption and desorption of nitrate suppresses N_2O production. Additionally, Zhang et al. (2025) demonstrated that adding biochar helps reduce NO_3^- to N_2 instead of N_2O by increasing the expression of N_2O reductase genes in denitrifiers (*NosZ*). Biochar can also mitigate N_2O emissions by creating phenolic compounds (PHCs) and polycyclic aromatic hydrocarbons (PAHs) that inhibit nitrification (Wang et al., 2013).

The makeup of biochar, which depends on how much cellulose, hemicellulose, and lignin it has from the original material, influences how well it works (Mansyur et al., 2022). Additionally, the impact of biochar on N_2O emissions has been shown to vary significantly with application rate, source type, and interactions with soil characteristics (Lan et al., 2019). While many studies have highlighted the potential of biochar in mitigating N_2O emissions, the specific role of biochar in suppressing nitrification activity remains underexplored, particularly in relation to biochar source and rate. This study seeks to address this knowledge gap by examining the effect of biochar derived from different source, applied at varying rates, on soil nitrification activity —a primary pathway driving N_2 O emissions. -. By focusing on the early-stage responses of nitrogen transformation processes in agricultural soils, this research aims to evaluate how biochar source and application rate can suppress nitrification—thereby contributing novel insights for sustainable fertilizer management and climate-resilient agriculture.

2. Materials and methods

2.1. Soil and biochar conditions

In this study, Cambisol soil was collected from agricultural soil in Sleman Regency, Special Region of Yogyakarta, Indonesia (7°44’47.9” S 110°25’47.1” E). The soil used in this study was collected from the same site, at the same time, and from the same bulk batch as in Azizah et al. (2023b). Therefore, the chemical properties—including pH (H_2O) of 5.94, a total carbon concentration of 0.372 g kg^{-1} (measured via the Walkley and Black methods), a bulk density of 1.11 g cm^{-3}, a cation exchange capacity (CEC) of 33.7 $mmol_+$ kg^{-1}, an available P concentration (Olsen method) of 9.83 mg kg^{-1}, an exchangeable K^+ concentration of 0.25 $cmol_+$ kg^{-1}, and a total N concentration of 0.016 g kg^{-1} —are identical and were reported previously (Azizah et al. 2023b). These properties were measured at the time of soil collection and before the incubation experiment. Three different biochar sources, i.e., corn stover, rice husk, and sawdust, were produced by slow pyrolysis at 550°C for 2–3 hours in a closed cylindrical batch pyrolysis chamber designed to limit oxygen availability. The physical and chemical properties of the biochar were characterized as follows: (1) corn stover biochar (BC): pH 9.95, moisture content 4.20%, total carbon concentration 6.573 g kg^{-1}, total N concentration 0.137 g kg^{-1}, surface area 4.199 m^2 g^{-1}, and pore volume 0.046 cc g^{-1}; (2) rice husk biochar (BR): pH 9.00, moisture content 3.29%, total carbon concentration 4.515 g kg^{-1}, total N concentration 0.10 g kg^{-1}, surface area 0.943 m^2 g^{-1}, and pore volume 0.004 cc g^{-1}; and (3) sawdust biochar (SD): pH 7.86, moisture content 4.56%, total carbon concentration 7.566 g kg^{-1}, total N concentration 0.061 g kg^{-1}, surface area 16.802 m^2 g^{-1}, and pore volume 0.234 cc g^{-1}.

2.2. Soil incubation experiment

The soil incubation was carried out using the microcosm incubation method outlined by Lu et al. (2019). The soil microcosm was composed of 3 g of air-dried soil with a diameter of 2 mm, contained within a 50 mL glass bottle. The soil in a 50 mL glass bottle was treated with biochar derived from corn stover, rice husk, and sawdust at rates of 0, 1.5, 3, and 6 g biochar kg^{-1} of soil, which were equivalent to 0, 5, 10, and 20 tonnes biochar ha^{-1}, respectively, resulting in 10 combination treatments. Each treatment underwent three replicates. Incubation was set at three time points (0, 7, and 14 days) for each glass bottle, resulting in a total of 90 units. The control involved soil incubated without the addition of biochar (0 tonnes of biochar kg^{-1} of soil). The soil microcosm was incubated in darkness at 25°C for one week, with moisture levels maintained at 60% water-filled pore space (WFPS) using deionized water. After seven days of incubation 27 mM $(NH_4)_2SO_4$ (3.58 g kg^{-1}) was applied to the biochar amended soil as a nitrogen source and was subsequently incubated at 25°C for 14 days. Soil moisture was maintained at 60% WFPS using deionised water during incubation. The experimental design and workflow are summarized in the flowchart (Fig. 1).

2.3. Soil NH_4^+-N and NO_3^--N measurement

Destructive samples were collected to measure NH_4^+ and NO_3^- concentrations after 0, 7, and 14 days of incubation. Ammonium and nitrate in the soil, as inorganic nitrogen, were measured via the colorimetric determination method. The initial step involved extracting the soil sample using 1 M potassium chloride, followed by a 30-minute shaking period and subsequently filtering the mixture through the Whatman No. 2 filter paper. The mixture was then treated with reagents from Keeney and Nelson (1983) and Kempers and Zweers (1986). The NH_4^+ and NO_3^- concentrations were quantified colorimetrically with a UV-VIS spectrophotometer (Shimadzu A-06-22, Japan) at 655 and 540 nm, respectively. The soil nitrification rate was calculated from the

slope of the linear regression between NO_3^- concentration and incubation time. Nitrification inhibition was determined using NO_3^- concentrations in the treated and control soils, following Equation (1) from Bremner and McCarty (1989):

$$Nitrification\ inhibition = \frac{(C-A)}{C} \times 100\% \text{.....(1)}$$

Where C represents the concentration of NO3- generated in the $(NH_4)_2SO_4$ control (0 tonne biochar ha^{-1}) from day 0 to day 7 or day 14 (mg kg^{-1} soil), and A denotes the concentration of NO_3^- produced in biochar-amended soil between day 0 and day 7 or day 14 (mg kg^{-1} soil). A higher C value than A indicates that the biochar has nitrification inhibition capacity, whereas a lower C value indicates that the biochar has no nitrification inhibition capacity in the soil.

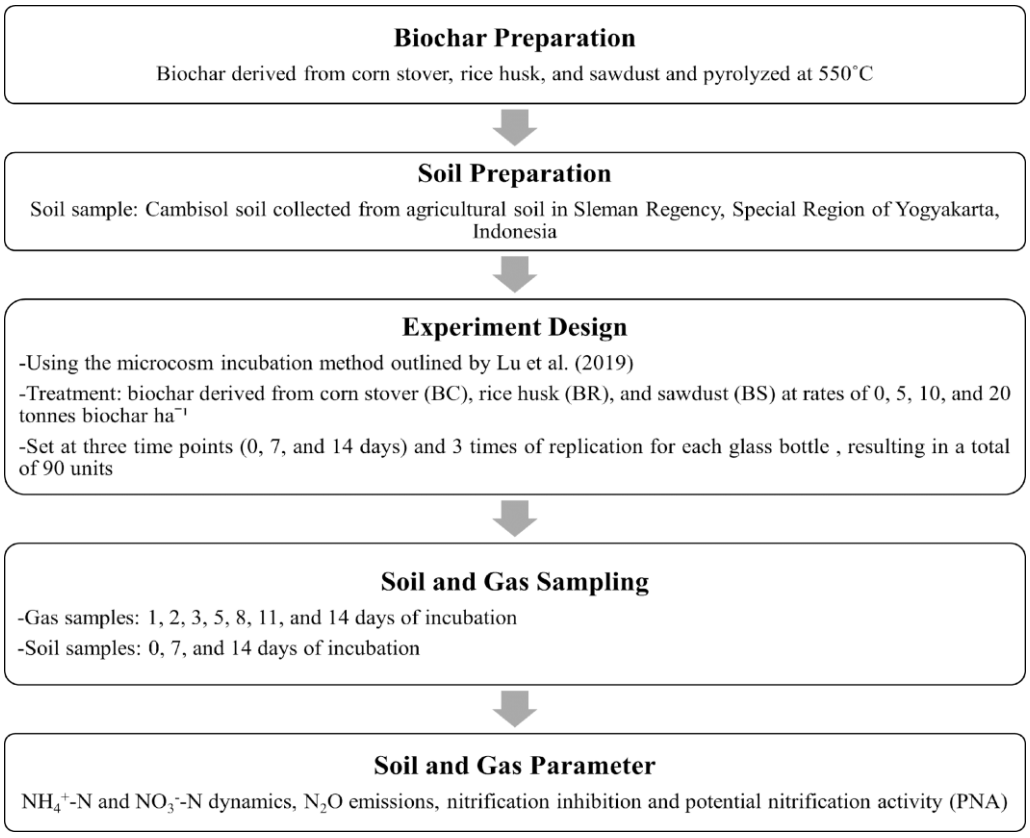

Fig. 1. Flowchart illustrating the experimental workflow used to evaluate the effects of different biochar sources and application rates on nitrification and N_2 O emissions.

2.4. Gas sampling and measurement

Gas samples were collected on days 1, 2, 3, 5, 8, 11, and 14 of incubation using a plastic syringe and transferred into a 10 mL vacuum tube. After each collection, the bottle was ventilated for five minutes by opening and closing the lid. Daily N_2 O emissions were measured using gas chromatography (Agilent Technology 7820A, USA) equipped with an electron capture detector (ECD).

2.5. Potential nitrification activity (PNA)

Potential nitrification activity was assessed using the shaken slurry method detailed by Hart et al. (1994). Initially, a phosphorus-nitrogen (PN) working solution was formulated by dissolving 0.2 M KH_2 PO_4 (27.22 g L^{-1}), 0.2 M K_2 HPO_4 (34.83 g L^{-1}), and 50 mM $(NH_4)_2$ SO_4 (6.60 g L^{-1}) in distilled water. The final working solution contained 50 mM PO_4^{3-} and 75 mM NH_4^+ at pH 7.2. Fifteen grams of air-dried soil (Ø 2 mm) mixed with biochar corn stover (BC), rice husk (BR), or sawdust (BS) was placed into a 100 mL Erlenmeyer flask and mixed with 100 mL of PN working solution. The slurries were kept at 25°C and continuously agitated at 180 rpm for 24 hours. Ten-millilitre aliquots were collected at 2, 4, 22, and 24-hour intervals, subsequent to which the nitrate concentration was assessed colorimetrically with a UV-VIS spectrophotometer (Shimadzu A-06-22, Japan) at 540 nm, based on a standard calibration curve. The potential nitrification activity was calculated based on the rate of nitrate accumulation, determined as the slope of the linear regression obtained using all incubation time points (2, 4, 22, and 24 h). The regression was computed numerically using the SLOPE function in Microsoft Excel, which ensures standardized and objective treatment across all samples. This method was

adopted to maintain comparability and reproducibility, especially when early nitrate increases are gradual and not visually linear.

2.6. Statistical analyses

R Core Team (2024) version 4.2.2 was used to conduct the statistical analyses. Significant differences in the data across the biochar source and rate treatments were assessed using analysis of variance (ANOVA), with the Tukey HSD test applied for multiple comparisons at a 5% significance level. Data exhibiting deviation from a normal distribution (Shapiro–Wilk test) were subjected to analysis via the Kruskal–Wallis test, subsequently followed by Dunn's multiple comparison test.

3. Results

3.1. Soil NH_4^+-N and NO_3^--N dynamics

The biochar source, biochar rate, and their interaction demonstrated significant effects on nitrification and N dynamics in the soil during 14 days of incubation, with different effects depending on the quality of each biochar source. Generally, NH_4^+-N concentration in the control gradually decreased over 14 days, while NO_3^--N concentration sharply rose by day 7, followed by a decline on day 14 (Table 1, Table 2). The NH_4^+-N concentration in the control decreased from 109.71 mg kg^{-1} soil to 80.91 kg^{-1} soil (a reduction of 28.8 mg kg^{-1} soil; 26%) by day 7 and continued to decrease to 74.04 mg kg^{-1} soil (a further reduction of 6.87 mg kg^{-1} soil; 8%) by day 14, with an overall decrease of approximately 33% (Table 1). This decrease was accompanied by an increase in NO_3^--N concentration from 2.5 mg kg^{-1} soil to 13.03 mg kg^{-1} soil on day 7, followed by a further decrease to 4.47 mg kg^{-1} soil (a 66% decline) by day 14 (Table 2).

Table 1. NH_4^+-N contents in soil amended with corn stover, rice husk, and sawdust biochar at 0, 7, and 14 days of incubation.

Treatments	NH_4^+-N (mg kg^{-1})		
	0 d	7 d	14 d(*)
W	109.71 ± 0.09^{a}	80.91 ± 0.36^{e}	74.04 ± 0.04^{Aa}
BC5	103.63 ± 0.00^{cde}	93.22 ± 0.76^{bc}	19.53 ± 0.37^{Bb}
BC10	100.96 ± 0.55^{e}	94.85 ± 0.64^{b}	25.38 ± 0.31^{Bb}
BC20	101.91 ± 0.78^{de}	91.71 ± 1.32^{c}	68.44 ± 0.12^{ABb}
BR5	106.25 ± 0.19^{bc}	91.14 ± 0.35^{c}	65.94 ± 0.27^{Bb}
BR10	101.99 ± 0.65^{de}	95.07 ± 0.68^{b}	21.97 ± 0.02^{Bb}
BR20	103.90 ± 0.64^{cd}	84.07 ± 0.65^{d}	24.26 ± 1.02^{ABb}
BS5	105.47 ± 0.34^{bc}	69.48 ± 0.75^{f}	57.00 ± 2.80^{Ba}
BS10	106.85 ± 1.13^{b}	83.68 ± 0.29^{de}	68.02 ± 0.22^{Ba}
BS20	104.64 ± 0.77^{bcd}	103.84 ± 0.74^{a}	69.73 ± 0.32^{ABa}
F-test	***	***	S**:R**

Notes: W, control (without biochar application); BC, corn stover biochar; BR, rice husk biochar; and BS, sawdust biochar. 5 (5 tonnes of biochar ha^{-1}), 10 (10 tonnes of biochar ha^{-1}), and 20 (20 tonnes of biochar ha^{-1}). 0 d refers to the initial soil condition before incubation, while 7 d and 14 d indicate days after incubation. The mean values and standard errors are presented (n=3). At 0 and 7 days, data were normally distributed and analyzed using two-way ANOVA followed by Tukey's HSD test. Significant interactions (S × R) were found; thus, lowercase letters indicate differences among biochar sources and uppercase letters among application rates within each incubation time ($p < 0.05$). At 14 days(*), data did not meet normality assumptions (Shapiro–Wilk test), so the Kruskal–Wallis test was used, followed by Dunn's multiple comparisons. Since no interaction was observed, lowercase letters indicate differences among biochar sources and uppercase letters indicate differences among application rates ($p < 0.05$), assessed separately. F-test results: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$; ns = not significant.

The nitrification rate is the intensity at which ammonium is transformed into nitrate. The biochar corn stover treatment at 20 tonnes ha^{-1} (BC20) resulted in the lowest nitrification rate, with approximately 70% of NH_4^+-N remaining in the soil for 14 days, whereas BC5 and BC10 showed <25% of NH_4^+-N remaining in the soil (Table 1). On day 0, BC20 showed the highest NO_3^--N concentration (3.46 mg kg^{-1} soil), but by day 7, it produced the lowest amount (5.92 mg kg^{-1} soil), and on day 14, its NO_3^--N level remained lower than that at the lower application rates (5.77 mg kg^{-1} soil) (Table 2). Contrary to BC, BR5 demonstrated a slower decrease in

nitrification rate (approximately 30%) than did BR10 and BR20 (no inhibition effect) over 14 days. Although BR5 had the lowest nitrification rate compared with those of BR10 and BR20, it presented the highest NO_3^--N production (0.15-8.68 mg kg^{-1} soil), especially on day 7 (Table 1). Compared with the BC and BR treatments, the BS treatment resulted in a slower nitrification rate during the 14-d incubation, with approximately 50-70% of NH_4^+-N remaining in the soil on day 14, regardless of the application rate. Under BS treatment, higher biochar application rates resulted in lower nitrification rates (Table 1), which were followed by lower NO_3^--N production, varying from 2.66 to 7.31 mg kg^{-1} soil for BS5, 1.66-5.53 mg kg^{-1} soil for BS10, and 1.23-5.48 mg kg^{-1} soil for BS20 (Table 2).

Table 2. NO_3^--N contents in soil amended with corn stover, rice husk, and sawdust biochar at 0, 7, and 14 days of incubation.

Treatments	NO_3^--N (mg kg^{-1})		
	0 d	7 d	14 d
W	2.50 ± 0.05^{a}	13.03 ± 0.24^{ab}	4.47 ± 0.15^{e}
BC5	2.54 ± 0.52^{a}	6.74 ± 0.08^{f}	6.93 ± 0.34^{bc}
BC10	2.33 ± 0.13^{a}	10.21 ± 0.26^{c}	6.05 ± 0.10^{cd}
BC20	3.46 ± 0.08^{a}	5.92 ± 0.10^{g}	5.77 ± 0.11^{d}
BR5	0.15 ± 0.01^{a}	8.68 ± 0.22^{d}	8.02 ± 0.48^{a}
BR10	1.36 ± 0.18^{a}	6.93 ± 0.19^{fg}	4.62 ± 0.15^{e}
BR20	4.70 ± 1.53^{a}	7.61 ± 0.34^{e}	5.31 ± 0.27^{de}
BS5	2.66 ± 1.61^{a}	14.11 ± 0.26^{a}	7.31 ± 0.09^{ab}
BS10	1.66 ± 0.08^{a}	7.47 ± 0.28^{ef}	5.53 ± 0.11^{de}
BS20	1.23 ± 0.15^{a}	11.61 ± 0.87^{bc}	5.48 ± 0.14^{de}
F-test	ns	***	***

Notes: W, control (without biochar application); BC, corn stover biochar; BR, rice husk biochar; and BS, sawdust biochar. 5 (5 tonnes of biochar ha^{-1}), 10 (10 tonnes of biochar ha^{-1}), and 20 (20 tonnes of biochar ha^{-1}). The mean values and standard errors are presented (n=3). 0 d refers to the initial soil condition before incubation, while 7 d and 14 d indicate days after incubation. At 0 days$^{(*)}$, data did not follow a normal distribution (Shapiro–Wilk test) and were analyzed using the Kruskal–Wallis test; no significant differences were found among treatments. At 7 and 14 days, data were normally distributed and analyzed using two-way ANOVA followed by Tukey's HSD test. Significant interactions (S × R) were observed at both times; thus, lowercase letters indicate significant differences among biochar sources and uppercase letters among application rates within each incubation time ($p < 0.05$). F-test results: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$; ns = not significant.

3.2. Nitrification inhibition

The higher NO_3^- concentration in the control compared to the biochar-treated samples suggests that biochar inhibited nitrification. The lower NO_3^- concentration in the control indicates that biochar did not inhibit nitrification in the soil. This study revealed that biochar treatment inhibited nitrification on day 7 (8–77%) in all treatments except BS5 (Fig. 2). Only BR20 continually had an inhibitory effect (69–72%) throughout the 14 days, whereas BS5 consistently showed no inhibitory effect (23–217%). BC presented the greatest range of inhibitory effects (25.2%-76.6%), followed by BR (19.0-72.4%) and BS (8.1-44.8%) on day 7. Higher biochar application rates generally showed stronger inhibitory effects on day 7 and weaker promoting effects on day 14. Furthermore, no inhibition effect was observed under BS (96.3-254%), followed by BR (65-278%) and BC (24-104.2%).

3.3. Potential nitrification activity (PNA)

In this study, a 24-h incubation system with continuous shaking of soil treated with biochar revealed that biochar source, biochar rate, and their interaction reduced the PNA by 48-92% compared to the control (from 597.12 mg NO3--N kg-1 soil h-1 to 46.59-311.17 mg NO3--N kg-1 soil h-1) (Fig. 3). The lowest range of PNA was demonstrated by BS (57.13-74.94 mg NO3--N kg-1 soil h-1), followed by BR (45.59-258.01 mg NO3--N kg-1 soil h-1) and BC (110.87-311.17 mg NO3—N kg-1 soil h-1) (Fig. 3). The biochar applications demonstrated that BR10 (551.54 mg NO3--N kg-1 soil h-1) reduced PNA in the soil the most, whereas BC10 (285.96 mg NO3--N kg-1 soil h-1) did so the least (Fig. 3).

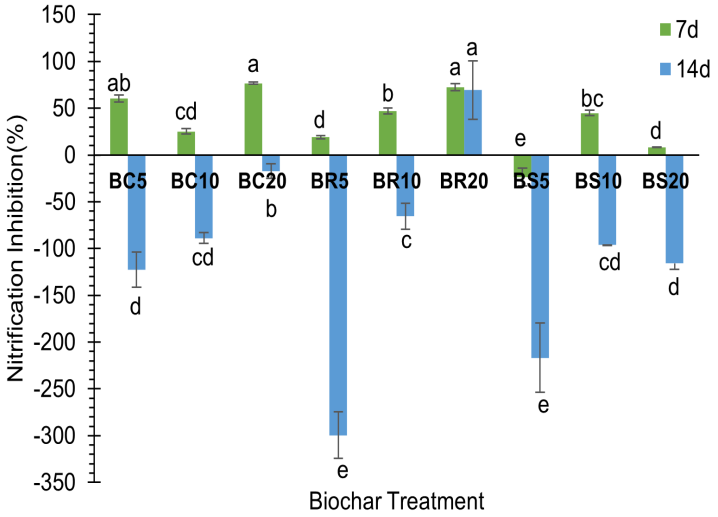

Fig. 2. Nitrification inhibition (%) after 7 and 14 days of incubation under different biochar types and application rates. BC (corn stover biochar), BR (rice husk biochar), and BS (sawdust biochar). 5 (5 tonnes of biochar ha^{-1}), 10 (10 tonnes of biochar ha^{-1}), and 20 (20 tonnes of biochar ha^{-1}). The control treatment (0 tonne ha^{-1}) is used as a reference in the nitrification inhibition calculation and is not shown as a separate bar, in accordance with Equation (1) (Bremner and McCarty, 1989). Negative values indicate no inhibition or enhanced nitrification. Negative values indicate that NO_3^--N concentrations in biochar-treated soils exceeded those in the control (no biochar), suggesting stimulation rather than inhibition of nitrification. The bars denote the standard error of triplicate data. Letters denote significant differences at $p < 0.05$ among the treatments, as determined by the Kruskal–Wallis test and Dunn's test.

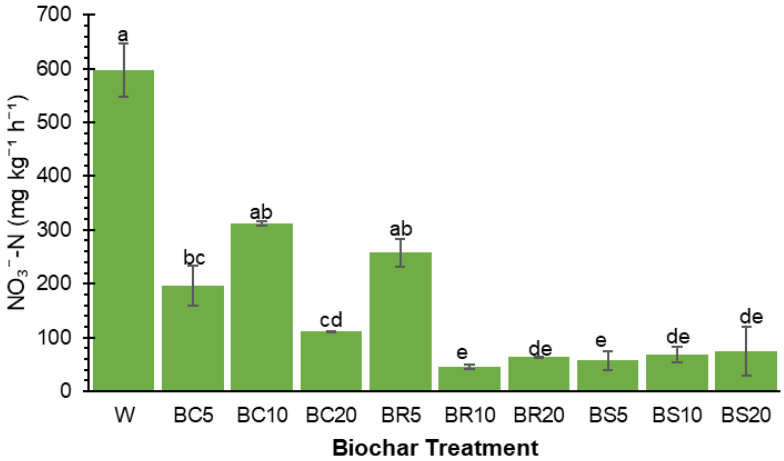

Fig. 3. Potential nitrification activity (PNA) of biochar-amended soil using the slurry shaking method. Potential nitrification activity was based on NO_3^- production in soils during 24 h of incubation. W, control (without biochar application), BC (corn stover biochar), BR (rice husk biochar), and BS (sawdust biochar). 5 (5 tonnes of biochar ha^{-1}), 10 (10 tonnes of biochar ha^{-1}), and 20 (20 tonnes of biochar ha^{-1}). The *bars* denote the standard error of triplicate data. *Letters* represent significant differences ($p < 0.05$) among treatments, as determined by one-way ANOVA followed by Tukey's HSD test.

3.4. N_2 O emission rate

Successive biochar amendments caused a significant decline in daily N_2 O productions compared to the control over the 14-day incubation period (Fig. 4). The biochar source exhibited a greater impact than the biochar rate, with BC resulting in the greatest reduction in N_2O emissions regardless of the application rate. The biochar-treated soil exhibited fluctuating trends over the 14 days and reached peaks at 5d (2,121.63 µg N_2O kg^{-1} soil day^{-1}) and 7d (2,020.64 µg N_2O kg^{-1} soil day^{-1}), with further reductions until 14 d. In contrast to those in the biochar-amended soil, the N_2O emissions in the control samples peaked at day 2 (4,058.30 µg N_2O kg^{-1} soil day^{-1}), sharply decreased at day 3, and continuously decreased until day 14 (Fig. 4a). Compared with the control, biochar addition suppressed daily N_2O emissions by up to 76% during 14 days of incubation, and the suppression effectiveness decreased over time.

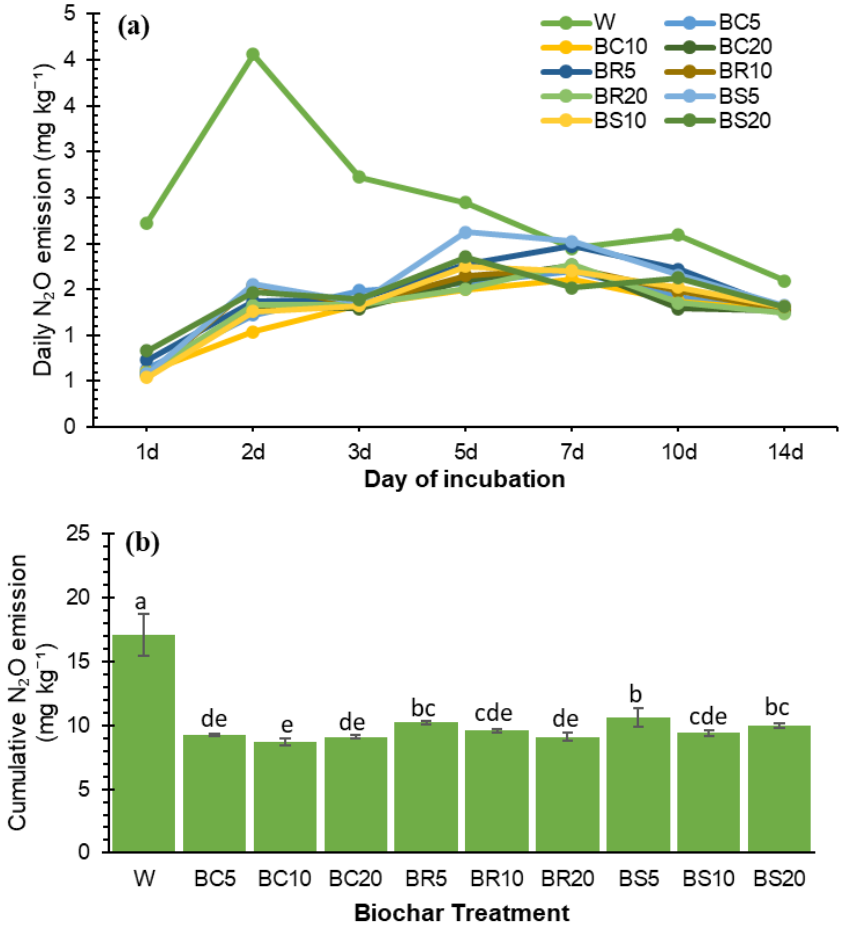

Fig. 4. (a) Daily N_2O emissions and (b) cumulative N_2O emissions of biochar-amended soil during 14 days of incubation. W, control (without biochar application), BC (corn stover biochar), BR (rice husk biochar), and BS (sawdust biochar). 5 (5 tonnes of biochar ha^{-1}), 10 (10 tonnes of biochar ha^{-1}), and 20 (20 tonnes of biochar ha^{-1}). The *bars* represent the standard error of triplicate data. *Letters* represent significant differences ($p < 0.05$) among treatments, as determined by one-way ANOVA followed by Tukey's HSD test.

During the 14 days of incubation, BC showed the lowest daily N_2O emission emissions, ranging from 581.38 to 1,765.31 µg N_2O kg^{-1} soil day^{-1}, followed by BR, ranging from 564.91 to 1,971.90 µg N_2O kg^{-1} soil $day^{-1,}$ and BS, ranging from 539.13 to 2,121.63 µg N_2O kg^{-1} soil day^{-1}. BS5 (579.05–2,121.63 µg N_2O kg^{-1} soil day^{-1}) presented the highest daily N_2O emissions during 14 days of incubation, and BC10 (605.57–1,614.16 µg N_2O kg^{-1} soil day^{-1}) presented the lowest. The control (C) emitted significantly higher N_2 O than all other treatments when total emissions were evaluated over the 14-day incubation period (Fig. 4b). The biochar application significantly suppressed N_2O production by up to 49% relative to the control, decreasing from 17,078.14 to 8,728.39 µg N_2O kg^{-1} after 14 days of incubation. The biochar source, application rate, and their interaction strongly influenced cumulative N_2O emissions. The BC, BR, and BS treatments decreased cumulative N_2O emissions by 47% (by a mean of 8,041.55 µg N_2O kg^{-1} soil day^{-1}), 43% (by a mean of 7,426.67 µg N_2O kg^{-1} soil day^{-1}, and 41% (by a mean of 7,067.70 µg N_2O kg^{-1} soil day^{-1}), respectively.

4. Discussion

4.1. The effects of biochar application on N dynamics

In the present study, a positive effect on N dynamics was observed, considering the different qualities of BC, BR, and BS under varying application rates, including the impacts of their interactions. The addition of biochar in soil influences nitrogen dynamics via its physicochemical interactions with soil (Li et al. 2020). The application rate and soil characteristics are considerably two key factors driving the dynamics of nitrogen (Palansooriya et al., 2019). In addition, Hu Tian et al. (2023) reported that total nitrogen and ammonium levels increased by as much as 32% and 59%, respectively, due to the application of biochar to soil while reducing nitrate levels by up to 12.5%. The BS treatment showed a slower rate of nitrification than the BC and BR treatments over the 14-day incubation period, whereas between 50% and 70% of NH_4^+-N remained in the soil on day 14, regardless of the biochar application rate. Higher biochar rates in BS treatment resulted in lower nitrification rates (Table 1) and consequently reduced NO_3^--N production, with ranges of 2.66-7.31 mg kg^{-1} soil for BS5, 1.66-5.53 mg kg^{-1} soil for BS10, and 1.23-5.48 mg kg^{-1} soil for BS20 (Table 1). These results showed that biochar application reduced nitrification activity in soil through inhibiting the production of NO_3^- and N_2O emission from NH_4^+. The critical factor in suppressing ammonium and nitrate concentration in the soil is the application rate of biochar. The evidence shows that using biochar either takes in NH_4^+, NO_3^-, and dissolved organic nitrogen or slows down the breakdown of organic material in the soil (Song et al., 2019). Furthermore, Yan et al. (2024) declared that biochar source also affects its ability to lower NH_4^+ and NO_3^- levels. In addition, Pidlisnyuket al. (2025) reported that woody waste biochar was more effective than agricultural waste at improving the NH_4^+ and lowering the NO_3^- levels in the soil. One proposed mechanism behind this difference is the varying electrostatic sorption capacities of these biochar sources. The biochar application could lead the lower levels of NH_4^+ and NO_3^- resulting to less inorganic N available for nitrification activity in the soil.

Numerous studies have highlighted the role of microbial biomass nitrogen (MBN) in regulating the transformation and cycling of nitrogen in soils. Although MBN was not measured in this study, previous findings offer relevant insights into potential microbial responses to biochar. For example, in northern Swedish forest soils, biochar increased the development of microorganisms, according to Ge et al. (2023); conversely, Qu et al. (2022) reported a decrease in the same growth. Numerous factors impact this fluctuation, including nutrient levels, soil texture, starting microbial populations, and biochar characteristics (Hu Tian et al., 2023). In this study, compared to the control, the biochar-amended soil transformed the NH_4^+ concentration less at 7 days of incubation and more significantly at 14 days. The NO_3^- concentration in soil with biochar amendments was consistently lower than that of the control at both 7 and 14 days of incubation. The findings demonstrate that biochar suppresses nitrification and diminishes NO_3^- loss from soil via various mechanisms. According to He et al. (2023), the decreased NO_3^- concentration might result from biochar serving as a potential sink in the soil that enables NH_3 uptake and extraction. The capability of biochar to alternately adsorb and release nitrate (He et al., 2023) and its liming effect, which shifts the NH_4^+/NH_3 equilibrium and encourages the release of NH_3, are expected to facilitate the reduction in soil N_2O emissions and NO_3^- leaching (Liu et al., 2024). Additionally, biochar can lower N_2O emissions by releasing polycyclic aromatic hydrocarbons and phenolic compounds, which inhibit nitrification (Hale et al., 2012).

4.2. The effect of biochar application on nitrification

This study demonstrated that biochar application exhibited an inhibitory effect of up to 77%. Previous studies have demonstrated that NH_4^+ levels in soil decrease during the incubation process, with biochar-amended soil exhibiting a lower reduction than the control (Fontes et al., 2023). The decreased level might be attributed to the biochar capacity to (i) retain NH_4^+ from its surface area and function as a controlled release source for NH_4^+ and (ii) stimulate urease activity in soil, thereby reducing nitrification (Zhao et al., 2022 and Parasar and Agarwala, 2025). The capability of biochar to inhibit nitrification varies depending on its amount and quality. Over 14 days, only BR20 consistently demonstrated an inhibitory effect (69–72%), whereas BS5 consistently demonstrated a promoting effect (23–217%). Bacteria with the ability to oxidise ammonia and nitrite typically responsible to nitrification. Ammonia-oxidising microorganisms are crucial in facilitating ammonia oxidation, which occurs as the first and slowest step in the nitrification process (Hu Tian et al.; 2023). The soil characteristics and the quantity and type of biochar influence how the soil microbial community changes over time (Dai et al. 2021). Hu Tian et al. (2023) stated that application of biochar initially increases the soil bacterial community before its activity decreases, which suggests that the specific quantity of biochar will either encourage or suppress the development of particular species of bacteria, leading to variations in the composition of the soil bacterial community. The soil characteristics and the quantity of biochar significantly influence the migration, distribution, and leaching of soil nitrogen, leading to changes in its composition of the microbial community (Bandara et al.,2022).

In comparison to the control, the biochar treatment decreased PNA by 48-92% (from 597.12 to 46.59-31.1 mg NO_3^--N kg^{-1} soil h^{-1}). These findings are similar to those of earlier research by Hu Tian et al. (2023), which reported that the addition of biochar decreased the soil nitrate concentration, with greater reductions observed at higher biochar application rates ranging from 0.5% to 8%. According toZhao et al., 2022 and Parasar and Agarwala (2025), the incorporation of biochar decreases PNA through several of mechanisms, including (i) absorbing NH_4^+ on its surface and acting as a slow-release agent for NH_4^+; (ii) promoting urease activity in the soil, which reduces nitrification; and (iii) altering soil chemistry.

Compared with BC and BR, BS presented the lowest PNA potential, which indicates that BS produces the least amount of NO_3^-. This result might be because BS has a greater surface area (16.802 m^2 g^{-1}) and pore volume (0.234 cc g^{-1}) compared to BC (surface area: 4.199 m^2 g^{-1} and pore volume: 0.046 cc g^{-1}) and BR (surface area: 0.943 m^2 g^{-1} and pore volume: 0.004 cc g^{-1}), which supports the capacity of BS to absorb NH_4^+ as a substrate for nitrification. Fontes et al. (2023) demonstrated that the biochar characteristic of high surface area promoted ammonium adsorption, ultimately leading to a higher amount of residual ammonium in the soil than in the control. Biochar with high porosity and functional groups contributed to improving soil capacity to absorb cations, resulting in the high retention of ammonium in the soil and the low conversion of ammonium to nitrate (Xu et al., 2025). Biochar produced at high temperatures (600°C–700°C) has a lower CEC than does low-temperature biochar (300°C–500°C) (Tu et al. 2022). In this study, a low temperature of 550°C was used to produce all batches of biochar. Low-temperature biochar may have a greater capacity for ammonium adsorption on the surface because of its high polarity (ratio of O/C) and the transformation of acidic to neutral or basic functional groups as oxygen-containing functional groups are lost, particularly carboxyl groups. The biomass source used in biochar production influences the primary adsorption of either nitrate or ammonium. According to Pidlisnyuk et al. (2025), grassy biochar outperforms woody biochar in ammonium adsorption, primarily due to its higher surface carboxylic functional group content.

The soil microenvironment and the changes in soil nutrients closely influence the development of soil microorganisms. Alterations in soil nutrients, water availability, oxygen level, and other characteristics can influence the diversity and composition of soil microbiota (Zhou et al., 2019). Adding biochar increases organic C, nitrogen content, and microbial C and N biomass—all of which are correlated with soil enzyme activity—according to Song et al. (2019), thus improving soil quality. Additionally, Hu Tian et al. (2023) revealed that ammonium, nitrate, and total nitrogen are the main soil components that influence bacterial communities. The abundance of the genes encoding nitrite and nitric oxide reductase and hydroxylamine synthase is related to the presence of specific bacteria engaged in the nitrogen cycle.

PNA in the soil was slowed the most by BR10 (by 551.54 mg NO_3^--N kg^{-1} soil h^{-1}) and the least by BC10 (by 285.96 mg NO_3^--N kg^{-1} soil h^{-1}) (Fig. 2). A small amount of biochar showed a significant effect in increasing nitrogen available in the soil. The proposed mechanism is by suppressing the expression of genes encoding ammonia monooxygenases and hydroxylamine dehydrogenase, which play a role in ammonia oxidation and influence the proliferation and behavior of ammonia-oxidizing bacteria (AOB) in the soil. A high application rate of biochar enhances the expression of nitric oxide reductase and nitric oxide synthase, thereby improving nitrogen utilisation efficiency (Hu Tian et al., 2023).

4.3. The effects of biochar application on N_2O emissions

This finding showed that biochar diminished daily N_2O emissions by as much as 76% over a 14-day period, surpassing the 38% reduction noted by Borchard et al. (2019). Gao et al. (2025) documented a 45%-80% reduction with rice straw biochar, while Yuan et al. (2024) observed a reduction of up to 92% with wheat straw biochar. The efficacy of biochar for suppressing daily N_2 O production diminished over time. This outcome variation is attributed to different biochar sources, which result in different biochar qualities, particularly in terms of their ability to change edaphic conditions, such as soil inorganic nitrogen availability and moisture. Different biochar rates and incubation system conditions, such as different soil types, also contribute to the variation. Research by Zhong et al. (2025) the biochar quantity application into the soil determines its impact on N_2O emissions. At higher application rates, the data followed a nonlinear polynomial regression, revealing a characteristic inverted U-shaped curve. Compared with the 1%, 2%, and 8% biochar applications, the addition of 4% biochar showed the highest inhibition ratio, which reduced N_2O emissions by 36–52%.

In this study, the application of 10 tonnes of BC and BS per ha resulted in the lowest daily N_2O emissions compared with 5 and 20 tonnes per ha, respectively. The only treatment that demonstrated a linear relationship between reduced daily N_2O emissions and a relatively high biochar application level was BS. In accordance with Nissen et al. (2021), various types and concentrations of organic compounds in biochar have the potential to be biotoxic at a particular level. Its significant effect on the makeup of the bacterial community is crucial for

limiting the capacity of biochar to mitigate N_2O emissions. Zhao et al. (2024) found biochar can enhance bacterial diversity by indirectly elevating soil total carbon and total nitrogen. Doses primarily influence the impact of biochar on microbial composition. For most soil microorganisms, biochar concentrations ranging from 2% to 5% w/w exert the most significant influence on soil bacterial beta-diversity.The role of biochar in reducing N_2O emissions has been widely studied. Bai et al. (2025) reported alterations in microbial diversity and population size are key factors through which biochar reduces N_2 O emissions in soil. Biochar influences N_2O emissions and modifies microbial communities by promoting the expression of denitrifier N_2O reductase genes (*nosZ*). According toWang et al. (2015), one way to increase the activity of nitrifying bacteria is by adsorption of phenolic compounds. These compounds can lower or inhibit nitrifier activity. Additionally, Zhong et al. (2025) demonstrated that biochar contributes to the increase of soil pH, cation exchange capacity, and ash content. These correlated to the reduce of N_2O emissions by promoting microbial growth and nitrogen immobilisation. Wang et al. (2013) identified PAHs in biochar as key contributors to N_2 O reduction because of their bactericidal properties. In addition to PAHs, Spokas and Reicosky (2009) reported that fresh biochar may also produce ethylene, which may be related to reduced N_2O production. These various impacts of biochar on the microbial communities and enzymes involved in N transformation in soil suggest that it has a priming effect on urease activity, which may prevent excess NH_4^+ from acting as a nitrification substrate. These effects are attributed to the ability of biochar to serve as a slow-release agent for surplus NH_4^+. As a result, NO_2 accumulation in the biochar treatment may have been prevented, resulting in a reduction in N_2O emissions in the biochar-enriched soil relative to those in the control soil.

After 14 days of incubation, the biochar treatment dramatically reduced the cumulative production of N_2O by up to 47%, in the order BC> BR> BS, compared with the control, which produced 17,078 µg N_2O kg^{-1}. The negative effects of applying biochar at high rates compared with those at low rates were noticeably greater for surface soil inorganic N. Due to the physical properties of biochar, N retention caused by biochar may exceed the adverse role in microbial nitrogen transformations in cultivated soils at low biochar rates (10 tonnes ha^{-1}). In this study, the treatment with 10 tonnes ha^{-1} BC and BS produced the lowest amount of N_2O per day compared to the treatment with 5 and 20 tonnes ha^{-1} BC and BS. Additionally, short-term studies have shown a more pronounced negative impact of biochar on the N cycle in agriculture, whereas longer-term (more than a year) investigations have shown a largely attenuated effect. According to Yang et al. (2022), this may be attributed to the in situ ageing of biochar or the quick degradation of any labile C introduced by biochar. The neutral effect observed in studies with biochar remaining in soil for over a year is further explained by the fact that biochar gradually reaches its full adsorption potential as it accumulates organic and mineral compounds on its surface. Liu et al. (2019) reported that the application of long-term (aged) biochar resulted in a reduction of total carbon mineralization in soil, whereas fresh biochar led to an increase in this process. Fresh biochar enhances microbial biomass, while the application of aged biochar significantly reduces the microbial metabolic quotient. This indicates that biochar amendment may enhance carbon usage efficiency and carbon sequestration. While greenhouse or laboratory studies frequently show that biochar negatively impacts soil nitrogen, field studies reveal many variations, with some showing no effect.

Overall, the differential effects of biochar source on nitrification rates and N_2O emissions highlight the importance of tailoring biochar application strategies based on source-specific characteristics. Unlike many previous studies that primarily focus on total nitrogen or soil pH changes, this work demonstrates how source-specific biochar distinctly influence the early-phase nitrogen transformation processes, particularly nitrification inhibition and ammonium retention. These findings deepen our understanding of biochar's short-term regulatory role in soil nitrogen cycling and offer a more nuanced approach to leveraging biochar for mitigating greenhouse gas emissions.

This study discusses mechanisms of microbe to elucidate the dynamic of nitrification and N_2O emission. However, this is important to acknowledge that microbial community composition and gene expression were not directly measured. The mechanism interpretation only was based on the functional responses and supported by previous research by Zhong et al. (2025). This study offers a conceptual interpretation of the potential influence of biochar on microbial-mediated processes. The proposed conceptual mechanism (Figure 5) delineates the potential connections among biochar characteristics, NH_4^+ and NO_3^- availability, nitrification inhibition, and subsequent N_2O emission reduction, adopted from proven microbiological and biogeochemical models. The choice to exclude microbial sequencing stemmed from resource constraints and the focus on short-term soil chemical transformations. Future investigations that include microbial profiling, gene-level analyses, or enzyme assays are essential to confirm and broaden these suggested mechanisms.

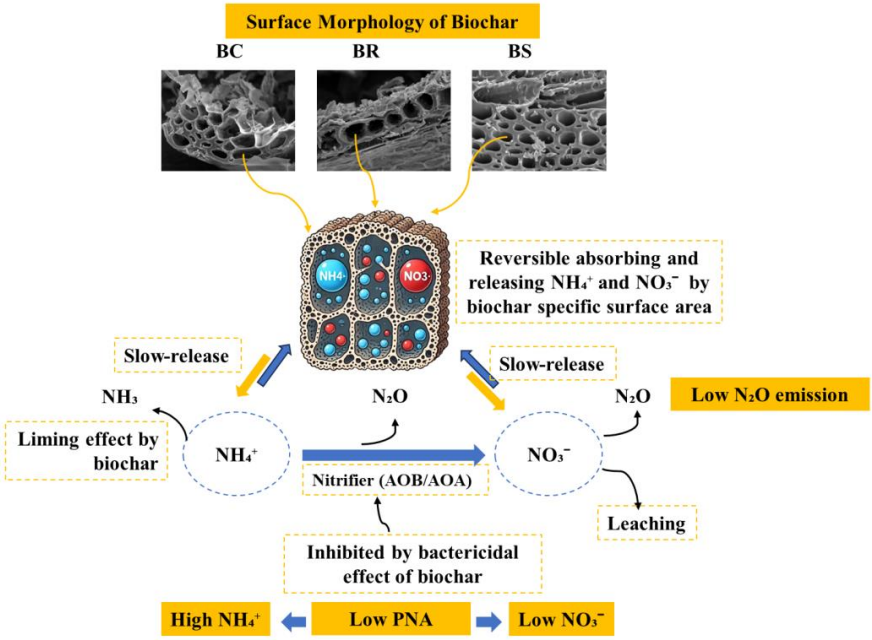

Fig. 5. Conceptual mechanism illustrating the role of biochar (BC: corn stover biochar, BR: rice husk biochar, BS: sawdust biochar) in nitrogen transformation pathways and nitrous oxide (N_2O) emissions suppression. The biochar structure, as shown in SEM images, provides specific surface areas for reversible adsorption and slow release of NH_4^+ and NO_3^-. Biochar may suppress nitrification by inhibiting ammonia-oxidizing microorganisms (AOB and AOA) through its liming and bactericidal effects, resulting in lower potential nitrification activity (PNA), lower NO_3^- concentration, and reduced N_2O emission. Microbial inhibition components were conceptualized based on (Zhong et al., 2025).

5. Conclusions

This study provides new evidence that the effects of biochar on soil nitrification and N_2O emissions are highly dependent on both the biochar source and application rate as well as some of their interactions. Across all treatments, biochar significantly slowed nitrification and enhanced ammonium retention in soil, leading to reduce NO_3^--N production and suppress daily and cumulative N_2O emission during the 14-day incubation. Biochar derived from corn stover showed the strongest inhibitory effect, followed by rice husk and sawdust biochar. These results demonstrate that biochar can act as an effective short-term nitrification inhibitor, likely through reversible ammonium/nitrate retention and alteration of nitrogen transformation pathways. By linking chemical characteristics of biochar to its impact on nitrogen dynamics, this study contributes to a mechanistic understanding of how biochar modulates nitrification and greenhouse gas emissions. These findings are especially relevant for designing source-specific biochar strategies to mitigate N_2O emissions and improve nitrogen retention in soils. Future research should focus on the long-term effects and microbial mechanisms underlying these processes under field conditions.

Declarations

Ethics approval and consent to participate

Consent for publication: The article contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

Availability of data and material: Not applicable.

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