https://doi.org/10.17221/71/2026-SWR

Effects of biochar-based composite amendments on nitrogen migration and enzymatic activities in high-nitrogen soils

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Citation: Chen Y., Zi W.Y., Wang F.J., Zou Y., Li Y.X., Shi J. (2026): Effects of biochar-based composite amendments on nitrogen migration and enzymatic activities in high-nitrogen soils. Soil & Water Res., XX:00–00.

Abstract: High nitrogen leaching from farmlands with excessive residual nitrogen in the Erhai Lake Basin, combined with a lack of effective in situ retention measures, threatens aquatic ecological security. Hence, efficient and regionally adaptable soil amendments are urgently needed. A pot experiment was conducted with cabbage grown in high-nitrogen soil from the western shore of Erhai Lake to evaluate biochar-based composite amendments. The composites consisted of biochar (corn straw, rice husk, or bamboo), bentonite, and humic acid at three ratios (1:1:1, 2:1:1, 3:1:1) and were applied at 2.5% and 5.0% (w/w). The optimal treatment was 5.0% corn straw biochar composite at a 3:1:1 ratio (coded CSB6). Compared to the unamended control, CSB6 significantly increased soil total N, NO_3^--N, and NH_4^+-N by 79.19%, 167.44%, and 200.73%, and reduced leachate total N, NO_3^--N, and NH_4^+-N by 48.8%, 44.9%, and 67.95%, respectively. Urease, leucine aminopeptidase, and peroxidase activities were significantly enhanced by 125.63%, 55.70%, and 262.24%. The enhanced N retention and reduced leaching were attributed to synergistic physical adsorption, cation immobilisation, and complexation-mediated slow release. This study elucidates the regulatory mechanisms and optimal formulation, providing theoretical and technical support for source control of agricultural non-point N pollution in high-nitrogen farmlands of plateau lake basins.

Keywords: bentonite; Erhai Lake basin; humic acid; nitrogen retention; non-point source pollution; soil enzyme activity

Nitrogen loss from intensive agricultural systems poses a significant threat to water quality. In 2020, agricultural non-point source pollution accounted for approximately 40% of the total nitrogen input into Erhai Lake (Zheng et al. 2021). Furthermore, watershed-scale nitrogen budget assessments indicate that vegetable, maize, and legume cultivation collectively contribute 53% of the total environmental nitrogen loss from crop production systems (Zou et al. 2023). This issue is particularly pronounced

Supported by the Yunnan Provincial Key Science and Technology Project (202102AE090005), Yunnan Provincial Science and Technology Talents and Platform Project (202205AF150017), Yunnan Provincial Science and Technology Plan Project Agricultural Joint Special Project (202101BD070001-106), Major Special Project of the Department of Science and Technology of Yunnan Province (202202AE090034-04), and Yunnan Provincial 'High level Talent Training Support Plan' (YNWR-QNBJ-2020-180).

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https://doi.org/10.17221/71/2026-SWR

in the lake basins of the Yunnan-Guizhou Plateau, where hydrological exchange is relatively restricted and environmental carrying capacity is low (Wang et al. 2023b; Xie et al. 2023).

The western shore of Erhai Lake is a core area of intensive vegetable cultivation. Long-term continuous cropping and excessive fertiliser input have led to a pronounced surplus of nitrogen in the plough layer, where total nitrogen in the topsoil generally exceeds 2 g/kg (He et al. 2023). Although high soil nitrogen reserves do not directly equate to leaching risks, the mobile inorganic nitrogen formed through the mineralisation and nitrification of residual organic nitrogen can migrate to deeper soil layers with rainfall and irrigation water. This increases the potential risk of nitrogen export to aquatic environments. Therefore, nitrogen management in nitrogen-rich farmlands urgently needs to shift from merely ensuring nutrient supply to simultaneously addressing crop nitrogen demand, soil nitrogen retention, and leaching mitigation, thereby reducing the environmental loss of agricultural nitrogen.

Due to its well-developed pore structure, large potential specific surface area, and abundant surface reactive sites, biochar can influence nitrogen retention and leaching processes through surface adsorption, ion exchange, and altering soil water transport characteristics. However, its regulatory effects exhibit significant material dependence and selectivity towards nitrogen forms. Gelardi et al. (2021) found that while all seven tested biochars were able to retain ammonium nitrogen (NH_4^+-N), only one exhibited effective retention capacity for nitrate nitrogen (NO_3^--N). The addition of biochar reduced ammonium leaching by 22–78%, whereas its impact on nitrate migration was relatively limited. Similarly, Sun et al. (2022) observed in an intensive cabbage cultivation system that biochar application decreased ammonium leaching losses by 20–30%, but did not significantly reduce nitrate leaching. This discrepancy is primarily attributed to the charge characteristics of the two inorganic nitrogen forms and their transport behaviours in soil. Ammonium nitrogen can be retained through cation exchange and surface adsorption. In contrast, as an anion, nitrate nitrogen lacks effective electrostatic attraction to the predominantly negatively charged surfaces of most biochars, making it more susceptible to transport with water. Therefore, the nitrogen regulation capacity of a single biochar is often constrained by its intrinsic physicochemical properties, making it difficult to simultaneously achieve effective retention and leaching mitigation for different inorganic nitrogen forms. Compounding biochar with mineral and organic materials that possess distinct structural and chemical properties offers a novel approach to expanding its nitrogen regulation functions. Bentonite, a layered clay mineral primarily composed of montmorillonite, features a high cation exchange capacity (CEC), interlayer ion exchange capability, and water-swelling properties. These characteristics enable it to influence nitrogen retention and migration by increasing the exchange sites for ammonium nitrogen and altering soil water retention characteristics (Li et al. 2026). Humic acid, on the other hand, is rich in active functional groups such as carboxyl and phenolic hydroxyl groups. It possesses strong surface reactivity, which enables it to influence the adsorption and release of soil nutrients and participate in nitrogen transformation by regulating microbial activity and enzymatic processes in the rhizosphere (Lumactud et al. 2022). Existing studies have provided evidence supporting the combined application of these two materials. Shen et al. (2020) found that under specific solution experimental conditions, the adsorption rate of NH_4^+ by humic acid-modified bentonite increased from 57.0% for unmodified bentonite to 96.4%. Furthermore, Zhou et al. (2019) demonstrated that the combined application of bentonite and humic acid can improve soil aggregate structure and water storage capacity. Thus, the porous structure and surface reactive sites of biochar, the cation exchange and water-retention properties of bentonite, and the active organic functional groups and biological regulatory roles of humic acid provide a complementary functional basis for developing composite materials with both nitrogen retention and soil environmental regulation capabilities. However, whether this functional complementarity can be translated into more effective leaching mitigation in actual soils, and specifically whether it can simultaneously improve the retention of both ammonium and nitrate nitrogen, remains to be verified.

However, existing studies have primarily focused on the application effects of single amendments or specific composite formulations. There remains a lack of systematic understanding regarding how the feedstock source of biochar, compounding ratios, and application rates collectively influence nitrogen retention and leaching processes in nitrogen-rich soils. Biochars derived from different feedstocks exhibit variations in pore structure, surface chemical proper-

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ties, and nutrient composition. Furthermore, changes in compounding ratios and application rates may further alter the nitrogen retention capacity of the amendments, as well as their impacts on soil water migration and nitrogen transformation processes. More importantly, changes in soil inorganic nitrogen content do not necessarily correspond to changes in nitrogen leaching losses; formulations that enhance NH_4^+ retention may not necessarily be effective in reducing NO_3^- migration. Currently, there is still insufficient understanding of the nitrogen form selectivity of various composite formulations, the relationship between soil nitrogen retention and leaching outputs, and the correlation between these responses and the activities of nitrogen cycle-related enzymes. Therefore, clarifying the formulation dependence of composite amendments and their differential impacts on the retention and migration of different nitrogen forms is a critical scientific issue for understanding their nitrogen regulation effects and screening optimal emission-reduction formulations.

Based on the aforementioned research gaps, this study focuses on nitrogen-rich farmland soils on the western shore of Erhai Lake. A cabbage pot experiment was conducted using a three-factor factorial design involving biochar feedstock, compounding ratio, and application rate. The experiment compared the nitrogen regulation effects of maize straw, rice husk, and bamboo-derived biochars compounded with bentonite and humic acid at mass ratios of 1 : 1 : 1, 2 : 1 : 1, and 3 : 1 : 1, applied at rates of 2.5% and 5.0%. By measuring soil inorganic nitrogen, various nitrogen forms in leachate, and the activities of nitrogen cycle-related enzymes, this study systematically evaluates the impacts of different composite formulations on soil nitrogen retention, leaching migration, and associated biological responses. Three hypotheses are proposed: (1) Biochar feedstock, compounding ratio, application rate, and their interactions collectively influence soil inorganic nitrogen retention and leaching characteristics; (2) Different composite formulations exert differential regulatory effects on NH_4^+-N and NO_3^--N, with the emission-reduction response for NO_3^--N leaching likely being more dependent on material formulation and application level; (3) Changes in the activities of nitrogen cycle-related enzymes are correlated with soil inorganic nitrogen status and leaching losses. By elucidating the response relationships among soil nitrogen retention, leaching migration, and biological indicators under different formulations, this study aims to deepen the understanding of the nitrogen regulation effects and formulation dependence of biochar-based composite amendments. Furthermore, it seeks to identify composite formulations with high potential for mitigating nitrogen loss, thereby providing a scientific basis for source emission reduction and nutrient management in nitrogen-rich farmlands within lake basins of the plateau region.

MATERIAL AND METHODS

Material

Test soil. The soil employed in this study was collected from the 0–20 cm plough layer of a vegetable field in Dazhuang Village, Xiaguan Town, Dali City, Yunnan Province (100°21'E, 25°40'N; elevation 1 976 m). The soil type is the vegetable garden soil developed from the flood-alluvial parent material on the west bank of Erhai Lake. According to IUSS Working Group WRB (2006), it is classified as Phaeozem and has a loam texture, and its fundamental physicochemical properties are presented in Table 1.

Test plant. The test plant employed in the experiment was cabbage (*Brassica oleracea* var. *capitata* L.), specifically the cultivar Tietou No. 2.

Soil amendments. Corn straw biochar, rice husk biochar, and bamboo biochar were all procured from Henan Lize Environmental Protection Technology Co., Ltd. (Henan, China). The corn straw and rice husk biochars were produced via pyrolysis at 500 °C under oxygen-limited conditions, whereas the bamboo biochar was produced at 700 °C. Bentonite (calcium-based) was obtained from Tianjin Huasheng Chemical Reagent Co., Ltd. (Tianjin, China). Humic acid was sourced from Rouwanwanrou E-commerce Co., Ltd. The physicochemical properties of the tested soil amendments are summarised in Table 2.

Table 1. Physical properties of the tested soil

pH	Soil organic matter	Total nitrogen	Total phosphorus	Total potassium	Available phosphorus	Available potassium
	(g/kg)				(mg/kg)	
6.98	34.67	2.18	3.10	7.58	200.98	242.85

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Table 2. Basic physicochemical characteristics of tested amendments (in g/kg)

	pH	Total nitrogen	Total phosphorus	Total potassium	Organic carbon
Bentonite	8.54	nd	1.95	2.25	35.40
Humic acid	4.85	4.73	4.37	0.59	55.56
Corn straw biochar	9.46	4.90	nd	nd	420.80
Rice husk biochar	9.86	3.20	nd	nd	486.20
Bamboo biochar	8.22	5.30	nd	nd	589.20

nd – not detected

Experimental design

Three biochar-based composite soil amendments were evaluated in this experiment: corn straw biochar + bentonite + humic acid (designated CSB), rice husk biochar + bentonite + humic acid (designated RHB), and bamboo biochar + bentonite + humic acid (designated BB). For each composite amendment, three ratios of biochar:bentonite:humic acid were established: 1 : 1 : 1, 2 : 1 : 1, and 3 : 1 : 1 (w/w/w). Each ratio was applied at two concentrations: 2.5% and 5.0% (w/w, calculated on an oven-dry soil weight basis). This design resulted in 3 biochar types × 3 ratios × 2 concentrations = 18 treatments. A control (CK) treatment with no conditioner application was included. Each treatment was replicated three times, resulting in a total of 57 pots (18 treatments × 3 replicates + 3 control pots).

The soil used in the pot experiment was collected from the 0–20 cm plough layer of a local agricultural field, air-dried, and subsequently packed into plastic pots (top diameter 20 cm, bottom diameter 15 cm, height 30 cm) at a rate of 3 kg soil per pot. Each pot was clearly labelled on its exterior with the treatment code, type, ratio, and concentration. Throughout the experimental period, soil moisture content was maintained at approximately 70% of field water-holding capacity via gravimetric adjustment. As the tested soil was classified as a high-nitrogen agricultural soil, its inherent nitrogen availability was considered sufficient to meet the nutritional

Table 3. Details of experimental treatments

Treatments	Types	Ratio	Concentration (%)
CK	blank control (no amendment application)		0
CSB1	corn straw biochar + bentonite + humic acid	1 : 1 : 1	2.5
CSB2	corn straw biochar + bentonite + humic acid	2 : 1 : 1	2.5
CSB3	corn straw biochar + bentonite + humic acid	3 : 1 : 1	2.5
CSB4	corn straw biochar + bentonite + humic acid	1 : 1 : 1	5.0
CSB5	corn straw biochar + bentonite + humic acid	2 : 1 : 1	5.0
CSB6	corn straw biochar + bentonite + humic acid	3 : 1 : 1	5.0
RHB1	rice husk biochar + bentonite + humic acid	1 : 1 : 1	2.5
RHB2	rice husk biochar + bentonite + humic acid	2 : 1 : 1	2.5
RHB3	rice husk biochar + bentonite + humic acid	3 : 1 : 1	2.5
RHB4	rice husk biochar + bentonite + humic acid	1 : 1 : 1	5.0
RHB5	rice husk biochar + bentonite + humic acid	2 : 1 : 1	5.0
RHB6	rice husk biochar + bentonite + humic acid	3 : 1 : 1	5.0
BB1	bamboo biochar + bentonite + humic acid	1 : 1 : 1	2.5
BB2	bamboo biochar + bentonite + humic acid	2 : 1 : 1	2.5
BB3	bamboo biochar + bentonite + humic acid	3 : 1 : 1	2.5
BB4	bamboo biochar + bentonite + humic acid	1 : 1 : 1	5.0
BB5	bamboo biochar + bentonite + humic acid	2 : 1 : 1	5.0
BB6	bamboo biochar + bentonite + humic acid	3 : 1 : 1	5.0

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requirements of cabbage throughout the entire growth cycle. To avoid confounding effects from extraneous nitrogen inputs and to enable precise elucidation of the direct regulatory influence exerted by the biochar-based composite amendments on soil nitrogen pools and nitrogen-cycling enzyme activities, no supplemental fertilisation was applied during the experiment.

The pot experiment was conducted from September to December 2024 in a naturally illuminated greenhouse at the Dian-Tai Centre of Yunnan Agricultural University. The greenhouse was maintained at a diurnal temperature range of 18–24 °C with a photoperiod of approximately 10 h/d. Cabbage was established via direct seeding and cultivated for a duration of 60 days prior to harvest.

Sample collection and analytical determinations

Determination of soil properties. The total nitrogen in the soil (S-TN), nitrate nitrogen (S-NO_3^--N), ammonium nitrogen (S-NH_4^+-N) and soil organic matter (SOM) were determined according to the scheme described in Soil and Agricultural Chemistry Analysis (Bao 2000).

Soil water content (WC) was measured gravimetrically using the cutting ring method. Soil pH was determined with a glass electrode pH meter (Leici PHS-3C pH meter, INESA Scientific Instrument Co., Ltd., Shanghai, China) at a soil-to-water ratio of 1 : 2.5 (w/v). Soil electrical conductivity (EC) was measured using a conductivity meter (AIPUO DDS-11, AIPUO, China) at a soil-to-water ratio of 1 : 5 (w/v).

At harvest, cabbage leaves were rinsed with deionised water, deactivating-dried at 105 °C for 30 min, and then oven-dried at 65 °C to a constant weight. The dried samples were ground and passed through a 0.25 mm sieve. Plant total nitrogen (NCA) was determined by the Kjeldahl method following H_2SO_4-H_2O_2 digestion, with the digestion, distillation, and titration procedures carried out according to Bao (2000).

Determination of soil enzyme activities. Soil urease (URE) activity was determined using the urea colourimetric method (May & Douglas 1976). Soil leucine aminopeptidase (LAP) activity was assayed using a chromogenic p-nitroanilide (pNA) substrate-based kit provided by Suzhou Grace Biotechnology Co., Ltd. (Suzhou, China) (Singh et al. 2023). Peroxidase (POD) activity was determined using the pyrogallol colourimetric method (Zhong et al. 2023).

Determination of nitrogen species in aqueous samples. Leachate samples were collected weekly from the drainage outlet at the bottom of each pot. Each pot was fitted with a perforated base and a pre-weighed collection bottle. After 24 h of natural drainage following each irrigation event, leachate volume was recorded, and samples were filtered through 0.45 μm membrane filters prior to analysis. Total nitrogen in the leachate (L-TN) was quantified via alkaline potassium persulfate oxidation followed by ultraviolet spectrophotometry (Bao 2000). Ammonium-nitrogen (L-NH_4^+-N) concentrations were determined using Nessler's reagent spectrophotometry at a wavelength of 420 nm. Nitrate-nitrogen (L-NO_3^--N) was extracted with a 1 mol/L KCl solution and subsequently quantified by dual-wavelength spectrophotometry at 220 nm and 275 nm. Both methods were performed according to the Water and Wastewater Monitoring and Analysis Methods (Agency 1989). All analyses were performed immediately upon sample collection; in instances where immediate determination was not feasible, leachate samples were stored at 4 °C pending analysis.

Quality assurance and quality control. Quality control measures were implemented throughout all analytical procedures to ensure data reliability. Procedural reagent blanks were included in each sample batch to subtract background interference. All samples were analysed in triplicate, with the RSD of replicate determinations controlled below 5%. Matrix spike recoveries were conducted for soil, cabbage and leachate matrices by adding known concentrations of standard nitrogen solutions, with overall recoveries ranging from 91% to 106%. All instruments were calibrated before use, and standard calibration curves were freshly prepared for each analytical batch with a coefficient of determination (R^2) ≥ 0.999.

Data processing

Data were organised using Microsoft Excel 2024, and all statistical analyses were performed with SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA). To evaluate the overall efficacy of the biochar-based composite amendments, all 18 treatment groups were compared against the CK. One-way analysis of variance (ANOVA) was conducted, followed by Dunnett's post hoc test to identify treatment means that differed significantly from the CK. To examine the effects of the experimental factors among the 18 amendment treatments (excluding CK), a three-way ANOVA was performed with concentration (two levels), type (three levels), and ratio (three levels) as fixed factors.

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The model included all main effects as well as their respective two-way and three-way interactions (concentration × type, concentration × ratio, type × ratio, and concentration × type × ratio). Where significant main effects were detected, multiple comparisons were conducted using Tukey's honestly significant difference (HSD) test. In instances of significant interaction effects, simple main effects analyses were subsequently performed. Bar charts, correlation matrices, and principal component analysis (PCA) plots were generated using OriginPro 2024b (OriginLab Corp., Northampton, MA, USA) and its associated extensions. All data are presented as the mean ± standard deviation (SD) of three replicates.

RESULTS

Effects of different treatments on soil physicochemical properties and cabbage nitrogen content

Table S1 in the Electronic Supplementary Material (ESM) presents the variation in other soil physicochemical properties. The application of biochar-based soil amendments markedly increased soil pH across all treatments. Compared with CK (pH 6.56), soil pH values ranged from 6.74 to 7.31.CSB showed the strongest amelioration effect, with the highest pH of 7.31 ± 0.01 recorded under the CSB5 treatment. ANOVA results indicated that soil pH was significantly influenced by the three-way interaction among biochar type, concentration, and ratio. EC was substantially altered by biochar addition, with EC values of all treatments ranging from 426.67 to 564.33 μS/cm. The CSB treatments maintained the overall highest EC, and the CSB5 treatment reached 564.33 ± 3.06 μS/cm. RHB and BB exhibited similar EC levels, both lower than those of CSB. Main effects and interactive effects of material type, concentration and ratio all reached an extremely significant level ($P < 0.001$).

For SOM, most treatments exhibited no significant difference compared with CK (25.44 ± 3.35 g/kg). Only CSB3 (33.79 ± 0.82 g/kg) and CSB6 (35.87 ± 1.82 g per kg) had significantly higher SOM content than CK ($P < 0.001$). The main effect of ratio and the interactive effect between type and ratio were extremely significant for SOM ($P < 0.001$). In terms of soil water content, a significant difference was only detected in the CSB4 treatment ($P < 0.01$), and no statistical difference was found in other treatments. The main effect of type was significant ($P < 0.01$), suggesting type dominated the variation of soil water content.

Biochar amendments significantly affected nitrogen accumulation in cabbage. Compared with the CK (2.58% ± 0.003%), most treatments increased cabbage nitrogen content. CSB consistently enhanced nitrogen content, with the highest value of 4.28% ± 0.009% recorded in the CSB5 treatment. RHB and BB differed in their promotional efficiency and optimal application rate. Analysis of variance revealed that biochar type, concentration, ratio, and their three-way interaction all exerted highly significant effects on cabbage nitrogen content ($P < 0.001$).

Effects of different treatments on soil nitrogen pools

The application of biochar-based composite amendments markedly enhanced soil nitrogen retention capacity, as reflected in concurrent increases in S-TN, S-NO_3^--N, and S-NH_4^+-N concentrations. The effects were jointly modulated by type, ratio, and concentration (Figure 1).

Dunnett's test confirmed that the CSB composite consistently outperformed RHB and BB across all three soil N fractions, with the CSB6 treatment (5% concentration, 3 : 1 : 1 ratio) producing the largest significant increases relative to CK ($P < 0.001$). RHB and BB showed significant effects only under high ratio or high concentration conditions.

Three-way ANOVA further clarified the main effects and interactions of the experimental factors (Tables S2–S6 in the ESM). For S-TN, significant main effects were found for type ($F_{2,36} = 51.976$, $P < 0.01$) and ratio ($F_{2,36} = 18.68$, $P < 0.01$), whereas the type × ratio interaction was not significant ($P = 0.843$). This indicates that the contributions of the optimal type (corn straw biochar) and the optimal ratio (3 : 1 : 1) to total nitrogen retention were independent and additive (Figure 2A, B).

In contrast, for the more labile inorganic nitrogen fractions – S-NO_3^--N and S-NH_4^+-N – the concentration × type interaction was significant ($P < 0.05$). Simple main effects analysis revealed that the CSB amendment responded most strongly to changes in concentration. Increasing the concentration from 2.5% to 5.0% significantly raised S-NO_3^--N and S-NH_4^+-N concentrations by 10.13 and 6.56 mg/kg, respectively ($P < 0.01$), whereas the rice husk and bamboo biochar-based amendments showed relatively stable performance across the two concentrations. Overall, the corn straw biochar group yielded mean S-NO_3^--N (107.20 mg/kg) and S-NH_4^+-N (25.74 mg per kg) concentrations that were significantly higher than those of the other two types ($P < 0.05$; Figure 2C, E).

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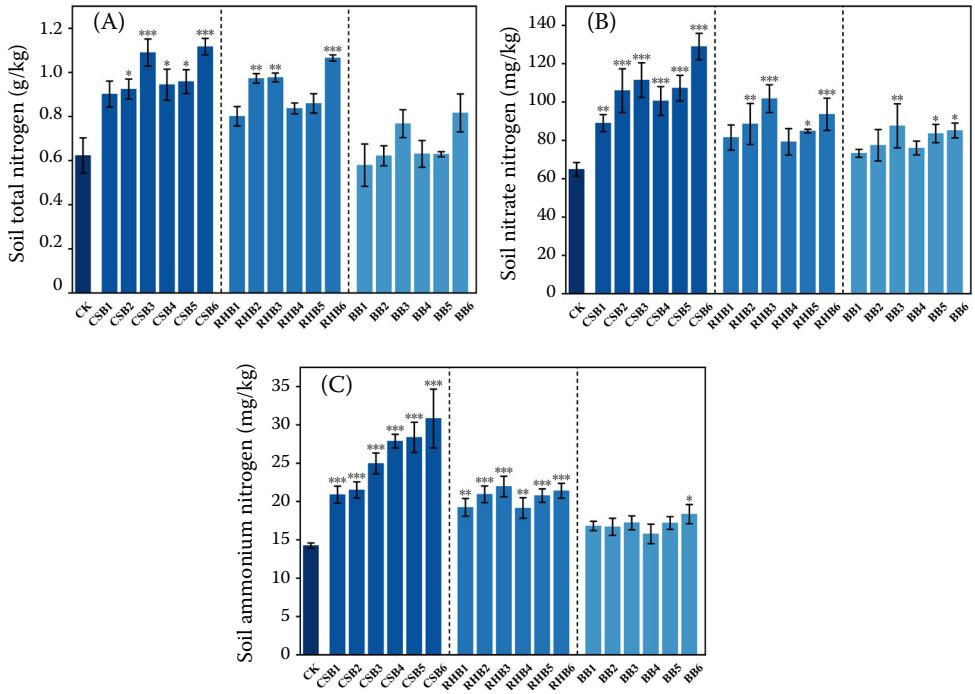


Figure 1. Effects of different biochar-based amendments on various nitrogen contents in soil: S-TN (A), S-NO_3^--N (B), S-NH_4^+-N (C)

For treatment abbreviations explanation see Table 3; all data are presented as mean ± SD, with error bars indicating standard deviation (n = 3); *,**,***significant differences compared with CK at P < 0.05, 0.01, 0.001 (according to Dunnett's test: one group is set as the control and compared with all other groups)

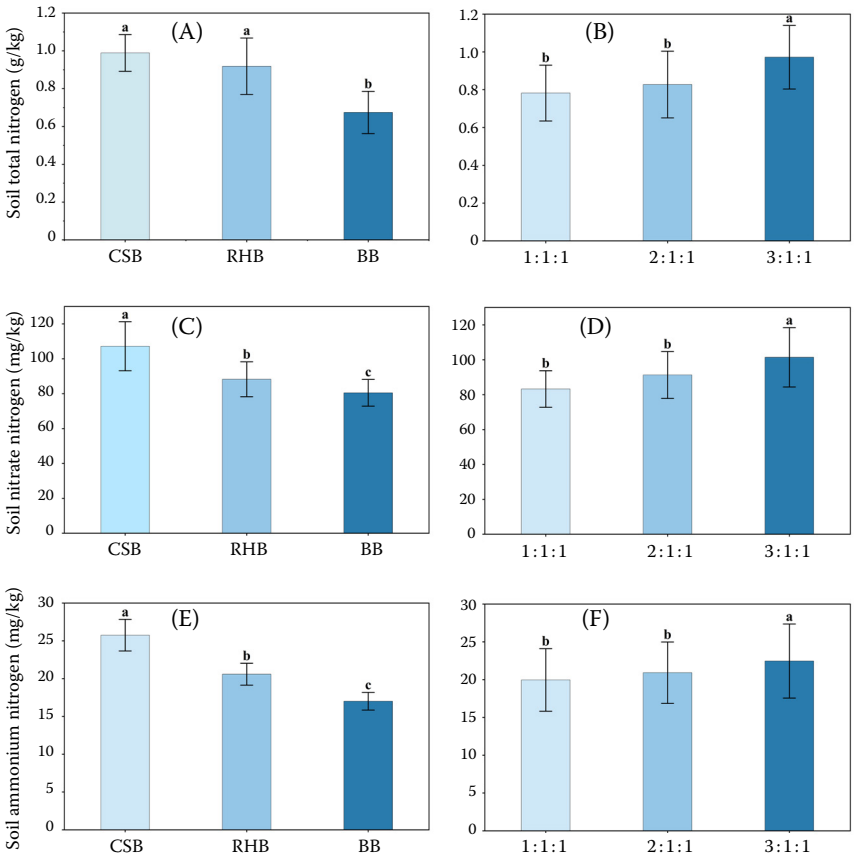


Figure 2. Statistical results of soil total nitrogen content under different biochar types and ratios: S-TN, S-NO_3^--N, S-NH_4^+-N under different amendments (A, C, E), corresponding nitrogen forms under different compounding ratios (B, D, F)

CSB – corn straw biochar + bentonite + humic acid; RHB – rice husk biochar + bentonite + humic acid; BB – bamboo biochar + bentonite + humic acid; all data are expressed as the mean ± standard deviation (SD); error bars indicate the standard deviation (n = 3); different letters indicate significant differences at P < 0.05

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Moreover, regardless of type and concentration, the 3:1:1 ratio – containing the highest biochar proportion – consistently maximised inorganic nitrogen retention (Figure 2D, F).

Nitrogen leaching under different treatments

Effects of different treatments on nitrogen leaching. Figure 3 presents the temporal dynamics of L-TN, L-NO_3^--N, and L-NH_4^+-N concentrations in leachate under different treatments. Throughout the cabbage growth period, all amendment treatments yielded lower nitrogen concentrations in leachate than the CK. Among the types, the CSB consistently reduced leachate nitrogen concentrations more than the RHB and BB composites. The CSB6 treatment achieved the strongest leaching inhibition among all formulations, with significantly greater reduction than RHB and BB treatments ($P < 0.05$).

Temporally, leachate N concentrations peaked in the seedling stage (0–2 weeks), declined through the rapid growth stage, showed a secondary peak at the heading stage (4–6 weeks), and decreased

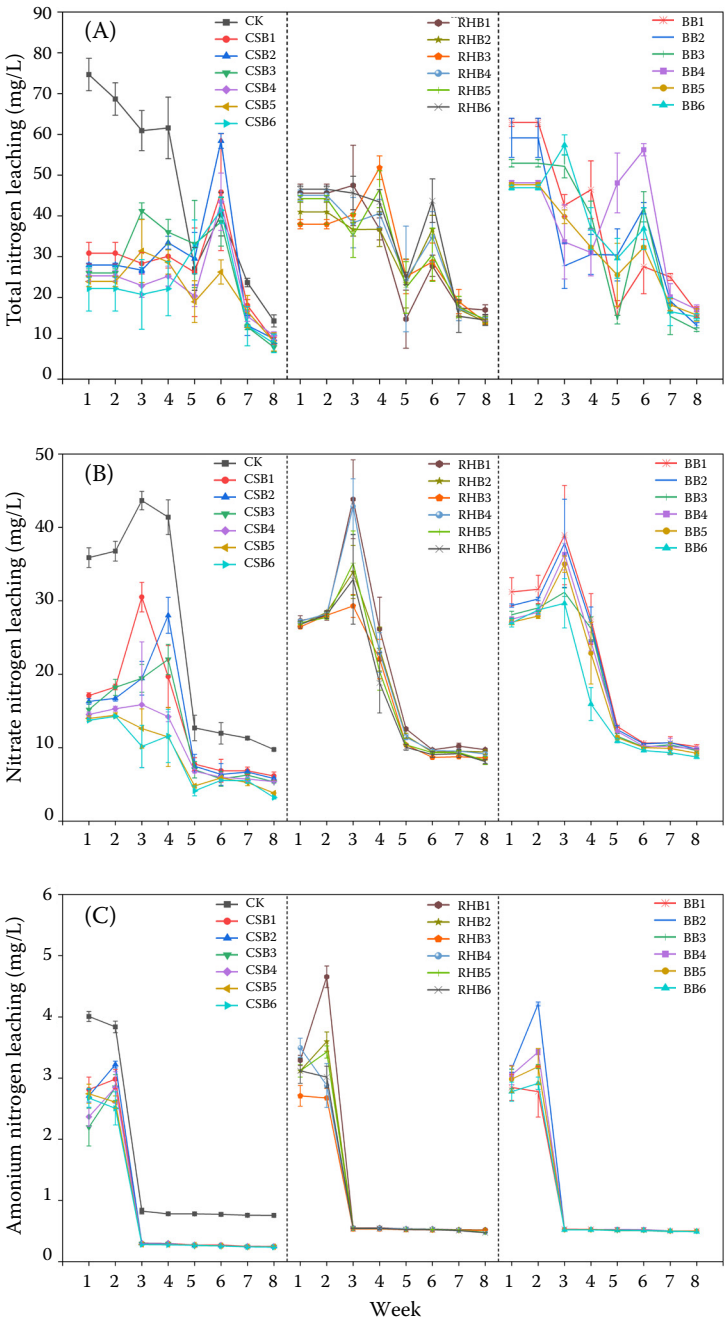


Figure 3. Concentration changes of different nitrogen forms in leachate from potted plants: the concentration variation of L-TN during cabbage growth (A), L-NO_3^--N (B), L-NH_4^+-N (C)

For treatment abbreviations explanation see Table 3

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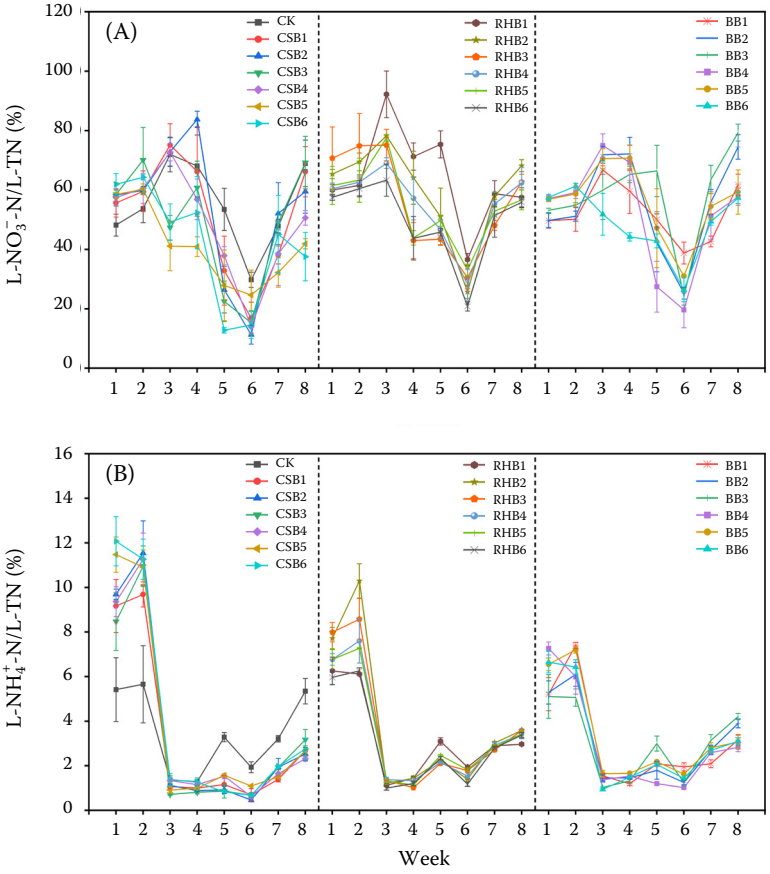


Figure 4. Changes in the morphological proportion of leached nitrogen during the growth period of cabbage: the ratio of L-NO_3^--N to L-TN (A), the ratio of L-NH_4^+-N to L-TN (B)
For treatment abbreviations explanation see Table 3

to the lowest level at maturity. The CSB treatment maintained lower leachate concentrations than RHB and BB throughout the cultivation period.

The ammonium nitrogen leaching concentration rose to its peak in the second week and then dropped sharply. From the third week onward, all treatments maintained a low and stable level.

Ammonium nitrogen to total nitrogen ratio and nitrate nitrogen to total nitrogen ratio in leachate. Throughout the cabbage growth period (Figure 4A, B), nitrate-nitrogen dominated the leached nitrogen pool, accounting for a markedly higher proportion than ammonium-nitrogen and indicating that it was the primary form of nitrogen leached. The proportion of nitrate-nitrogen in the leachate fluctuated considerably over the growth period.

Under the three types of biochar-based composite amendments, the proportion of nitrate-nitrogen in the leachate showed varying degrees of increase or decrease from week 0 to week 5, with most treatments peaking during weeks 3–5. This proportion then declined sharply from week 5 to week 6, reaching its minimum at week 6, after which it rebounded from week 6 to week 8. Notably, the CSB and RHB types recovered to levels comparable to those in the seedling stage (0–2 weeks).

Regarding the proportion of ammonium-nitrogen leached, the CSB type exhibited a higher value than the control (CK) during the seedling stage (0–2 weeks), whereas the BB and RHB types remained close to CK. Across all types, this proportion peaked in week 2, then declined markedly from week 3 to week 4, reaching its lowest point of the entire growth period. A slight rebound occurred in week 5, followed by another decrease in week 6, and a substantial upward trend was observed from week 6 to week 8.

Effects of different treatments on soil enzyme activities

The application of biochar-based composite amendments significantly enhanced the activities of soil urease (URE), leucine aminopeptidase (LAP), and peroxidase (POD), with the magnitude of these effects modulated by type, ratio, and concentration (Figure 5).

Dunnett's test indicated that most amendment treatments significantly elevated URE, LAP and POD activities relative to CK ($P < 0.001$), with CSB treat-

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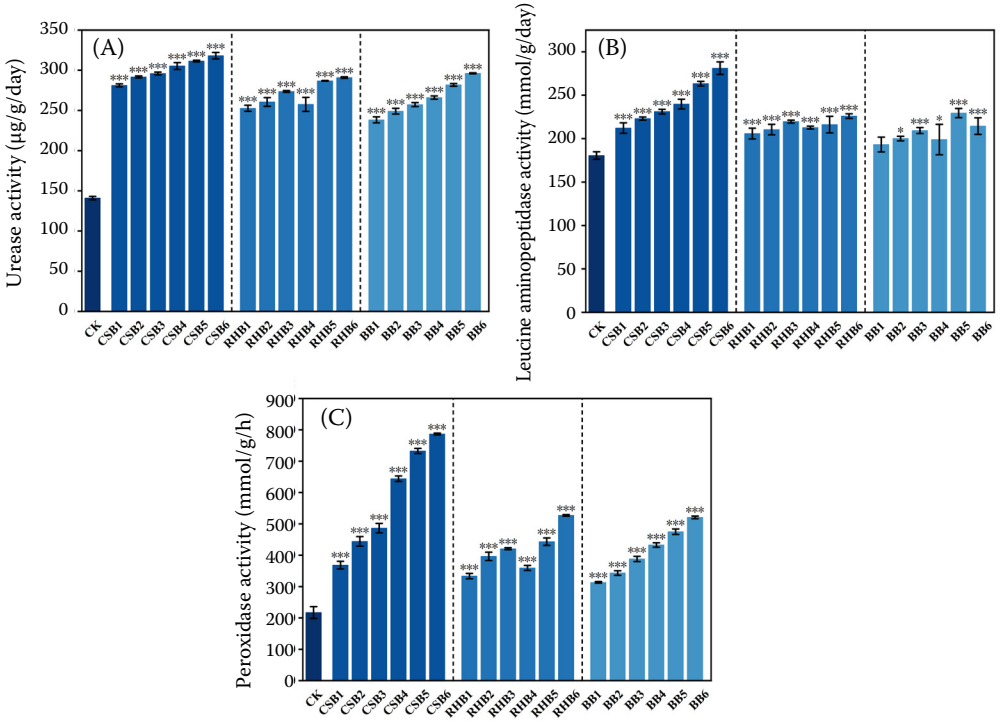


Figure 5. Effects of different biochar-based amendments on soil urease activity: URE (A), LAP (B), POD (C)
For treatment abbreviations explanation see Table 3; all data are presented as mean ± SD, with error bars indicating standard deviation (n = 3); *,**,***significant differences compared with CK at P < 0.05, 0.01, 0.001 (according to Dunnett's test: one group is set as the control and compared with all other groups)

ments consistently showing the strongest enhancement. Low-ratio BB treatments had no significant effect on LAP activity.

Three-way ANOVA revealed highly significant main effects of concentration, type, and ratio on the activities of all three enzymes (P < 0.001; Tables S7, S9, S11 in the ESM). The three-way interaction (concentration × type × ratio) exerted a significant or highly significant influence on URE ($F_{4,36}$ = 6.507, P < 0.001), LAP ($F_{4,36}$ = 3.021, P < 0.05), and POD ($F_{4,36}$ = 5.834, P < 0.001) activities, indicating that the interactive pattern between concentration and type varied as a function of the ratio. Accordingly, stratified simple main effects analyses were conducted separately within each of the three ratio levels (Tables S8, S10, S12 in the ESM; Figure 6).

Across all three ratios (1 : 1 : 1, 2 : 1 : 1, and 3 : 1 : 1) and at both 2.5% and 5.0% concentrations, the CSB treatments exhibited significantly higher URE, LAP, and POD activities than the corresponding RHB and BB treatments (P < 0.05). In contrast, no significant differences were detected between the RHB and BB treatments under most conditions evaluated (Figure 6). Regarding the effect of concentration, enzyme activities in the CSB treatments increased significantly as the concentration was raised from 2.5% to 5.0%. Analysis of the main effect of ratio further revealed a consistent trend whereby URE, LAP, and POD activities all increased incrementally with a greater ratio of biochar in the composite blend.

Multivariate analysis of nitrogen transformation indicators across the soil–plant–leachate continuum under different treatments

Principal component analysis (PCA) showed that the first two principal components (PC1 and PC2) together explained 76.0% of the total variance in soil and environmental parameters, with PC1 alone accounting for 69.0% and thus representing the primary axis of differentiation among treatments (Figure 7A). The PCA ordination plot revealed clear spatial separation among treatment groups: samples from the CSB treatments clustered distinctly in the positive region of PC1, while those from the BB treatments aggregated in the negative region, resulting in complete separation along the PC1 axis. Samples from the

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CK and the RHB treatments were located primarily near the PC1 centroid, although some dispersion was observed along the PC2 axis.

Analysis of the environmental factor loadings showed that the positive direction of PC1 had strong positive correlations with basic soil physicochemical properties (pH, EC, SOM, WC), NCA, and the activities of hydrolytic enzymes involved in nitrogen transformation (URE and LAP). These parameters were consistently associated with the CSB treatments, indicating that this type markedly improved soil fertility, enhanced soil water retention, and effectively promoted the hydrolytic conversion of soil nitrogen. Conversely, the negative direction of PC1 exhibited strong positive associations with soil inorganic nitrogen concentrations (S-NO_3^--N and S-NH_4^+-N), leachate nitrogen fractions (L-TN, L-NO_3^--N, and L-NH_4^+-N), and POD activity. These variables were predominantly aligned with the BB treatments, suggesting that this type fostered the accumulation of soil available nitrogen while concurrently increasing the risk of nitrogen leaching losses.

In summary, PCA effectively differentiated the integrated effects of the different biochar-based composite amendments, revealing the clear superiority of the CSB treatment in promoting a beneficial soil nitrogen cycle while also highlighting the tendency of the BB treatment to increase the risk of soil nitrogen loss.

To further examine linear associations among selected indicators, Spearman correlation coefficients were calculated and presented as a heatmap (Figure 7B). The analysis revealed extensive, statistically significant positive correlations among soil nitrogen fractions (S-TN, S-NO_3^--N, S-NH_4^+-N), key enzyme activities (URE, LAP, POD), and soil chemical properties (pH, EC). All these variables also showed highly significant positive correlations with NCA. In marked contrast, leaching indicators (L-TN, L-NO_3^--N, L-NH_4^+-N) were significantly negatively correlated with all the aforementioned soil and

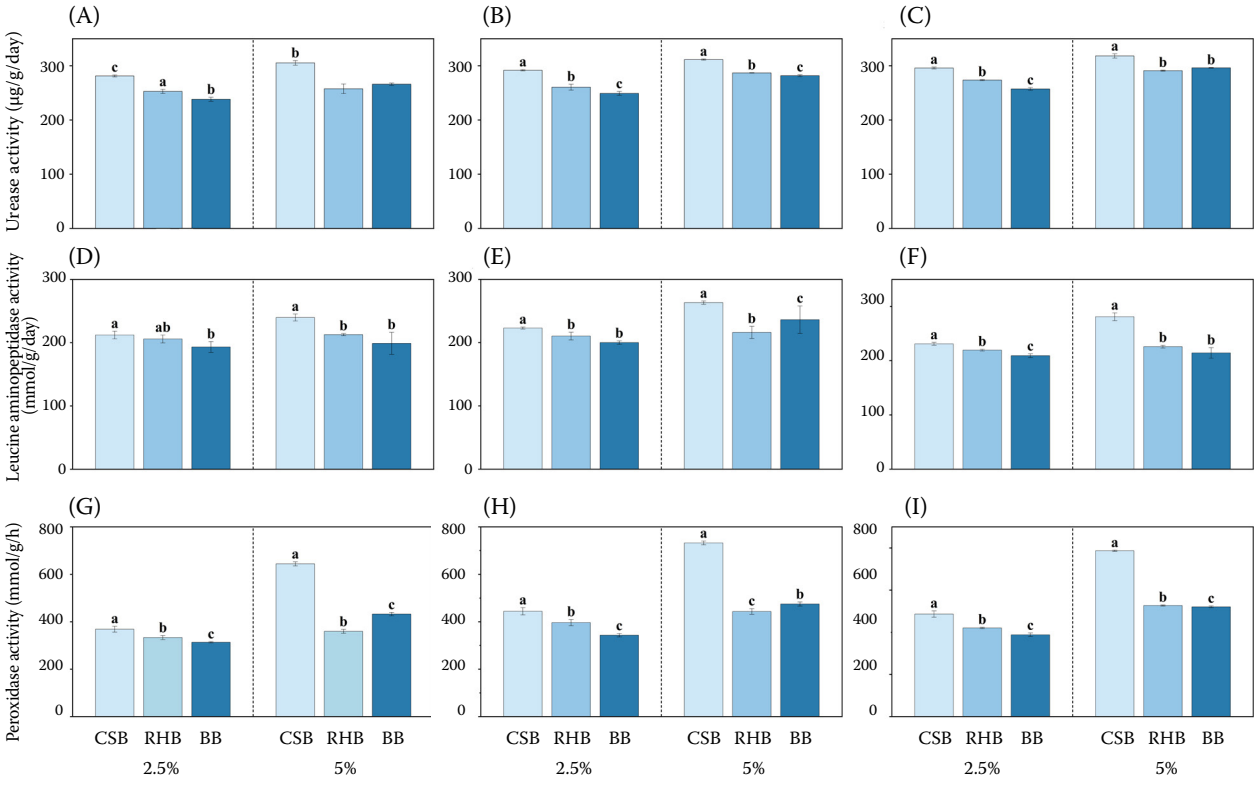


Figure 6. Effects of concentration and material type on soil enzyme activities across compounding ratios: subpanels represent urease (A–C), leucine aminopeptidase (D–F), and peroxidase (G–I) at mass ratios of 1 : 1 : 1, 2 : 1 : 1, and 3 : 1 : 1, respectively
Bars represent mean ± standard error (n = 3); within the same ratio and concentration, different lowercase letters indicate significant differences among materials ($P < 0.05$)
CSB – corn straw biochar + bentonite + humic acid; RHB – rice husk biochar + bentonite + humic acid; BB – bamboo biochar + bentonite + humic acid

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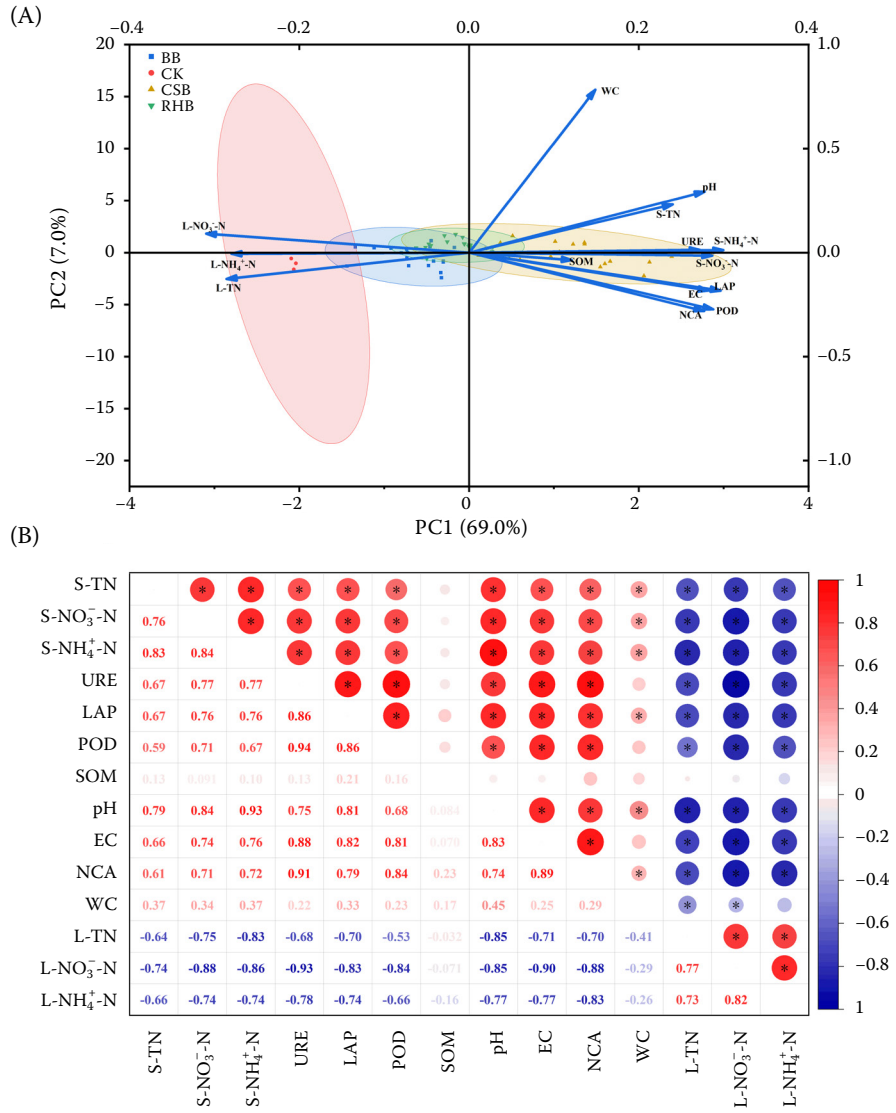


Figure 7. Principal component analysis (A) and Spearman correlation heatmap (B) of nitrogen transformation indicators
*a correlation between the indicators at $P < 0.05$
BB – bamboo biochar + bentonite + humic acid; CK – control; CSB – corn straw biochar + bentonite + humic acid; RHB – rice husk biochar + bentonite + humic acid; S-TN – total nitrogen in the soil; URE – urease; POD – peroxidase; SOM – soil organic matter; EC – electrical conductivity; NCA – plant total nitrogen; WC – water content; L-TN – total nitrogen in the leachate

plant parameters. Notably, the negative associations between L-NO_3^--N and both S-NO_3^--N and URE activity were particularly strong. By comparison, SOM and WC exhibited relatively weak correlations with most other variables, reflecting a more independent pattern of variation.

In summary, soil nitrogen fractions, key nitrogen-cycling enzyme activities, and soil chemical properties such as pH and EC were closely and synergistically interrelated. Together, these factors jointly enhanced nitrogen acquisition by cabbage while suppressing nitrogen leaching losses to the environment. URE activity appeared to play a central role in these interconnected processes. Under the experimental conditions, SOM and WC exhibited relatively weak direct associations with the other measured parameters

DISCUSSION

Restructuring effects of biochar-based composite amendments on soil nitrogen pool and physicochemical environment in high-nitrogen

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farmland of the Erhai Lake watershed. The variable N retention effects across formulations arise from the degree of functional coupling among biochar, bentonite and humic acid, rather than a simple additive effect of individual components.

The three-way ANOVA results further demonstrated that different components of formulation exerted distinct influences on soil nitrogen fractions. Soil total nitrogen was mainly affected by biochar type and blending ratio, whereas inorganic nitrogen retention showed stronger responses to the interaction between amendment type and application concentration. These findings indicate that increasing overall nitrogen storage and regulating readily available nitrogen pools may require different formulation strategies. Specifically, the selection of biochar feedstock and blending ratio may determine the capacity of the amendment to modify soil nitrogen pools, whereas application concentration may regulate the extent of nitrogen retention under specific amendment compositions. Unlike most existing studies that focus on single biochar or biochar–fertiliser interactions, this study reveals from the level of ternary composite system the rule that "type – ratio – concentration" jointly determine the nitrogen retention effect, which provides more direct experimental evidence for the screening of regionally targeted formulations (Ibrahim et al. 2020). From the perspective of the mechanism of action, the biochar-bentonite-humic acid ternary system constructed in this study performs better than single materials or binary combinations, which may be related to the functional complementarity of its component materials. Biochar provides porous structure and surface functional groups, undertaking physical adsorption and surface entrapment of soluble nitrogen; bentonite, with its high cation exchange capacity, enhances exchange fixation of ammonium nitrogen; and humic acid reduces the instantaneous release intensity of nitrogen through complexation and slow-release effects (Shen et al. 2020; Luo et al. 2025; Xing et al. 2025). These three components do not simply sum their functions; rather, they jointly form a synergistic retention system of physical adsorption – ion exchange – complexation and slow release, thereby effectively shrinking the pool of soluble inorganic nitrogen that is prone to migration and leaching in the soil.

Among the different biochar feedstocks, corn straw biochar performed the best, indicating that the regulatory effect of the ternary system is highly dependent on the structural compatibility of biochar as the core carrier. Compared with the other two, corn straw biochar possesses a more suitable pore structure, more abundant surface oxygen-containing functional groups, and better hydrophilicity, making it more conducive to forming stable coupling with bentonite and humic acid, thereby enhancing nitrogen interception and slow-release capacity (Ren et al. 2020). In contrast, rice husk biochar is limited by its relatively low specific surface area and susceptibility to pore blockage, resulting in relatively weak adsorption and loading capacity (Huang et al. 2020); bamboo biochar, due to its high degree of aromatisation after high-temperature pyrolysis and weak surface polarity, tends to be unfavourable for efficient synergy with other components, ultimately leading to lower overall nitrogen fixation and soil improvement effects (Kumar et al. 2025). This difference essentially originates from the differences in the physicochemical properties of the feedstocks and the surface structures after pyrolysis, and is consistent with existing research conclusions on the suitability and environmental functional differences of biochars from different feedstocks (Ji et al. 2022; Xu et al. 2022).

The significance of the composite amendment in this study lies not in adding extra nitrogen, but in transforming free, readily leachable inorganic nitrogen into relatively stable soil-retained nitrogen through in-situ retention and pool transformation, thereby improving soil nitrogen conservation capacity and reducing the risk of export to surrounding water bodies without increasing external nitrogen input. This is highly consistent with the regional management requirement for agricultural non-point source pollution control in the Erhai Lake basin, which emphasises both source reduction and process interception, and also avoids the additional ecological burden that some conventional nutrient-type amendments may bring (Lin et al. 2021; He et al. 2023); this enhanced in-situ retention capacity arising from nitrogen pool restructuring further explains the subsequent significant decrease in leaching flux.

Inhibitory effects of biochar-based composite amendments on nitrogen leaching, differences among nitrogen forms, and temporal dynamics. The reduction in N leaching is directly driven by enhanced in-situ retention of inorganic N in the soil matrix, with distinct underlying mechanisms for ammonium and nitrate.

From the perspective of responses of different nitrogen forms, nitrate was always the dominant form

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of nitrogen leaching throughout the entire cabbage growth period, while the extent of inhibition by the composite amendment on ammonium was markedly greater than that on nitrate. This difference has a clear electrochemical and hydrological migration basis. This is because nitrate carries a negative charge and experiences electrostatic repulsion with the mostly negatively charged soil colloids, making it difficult to be effectively adsorbed; at the same time, its strong water solubility allows it to more readily migrate downward with infiltrating water driven by irrigation and rainfall. In contrast, ammonium carries a positive charge and can be immobilised through surface adsorption onto soil colloids and cation exchange, so its inherent leaching potential is lower and it is more readily subject to enhanced retention by materials such as biochar and bentonite (Liu et al. 2017). The stronger inhibition of ammonium leaching by CSB6 compared to nitrate in this study precisely indicates that this ternary system has a more direct structural advantage in ammonium retention. In comparison, nitrate loss is more controlled by soil water movement and solute convection; the composite amendment regulates it mainly through indirect pathways, such as improving water retention, reducing macropore preferential flow, and delaying the conversion of nitrogen to the nitrate pool; hence, the extent of inhibition is relatively limited.

Apart from the differences in nitrogen forms, this study also showed that nitrogen leaching exhibited clear stage-dependent characteristics during the growing period, i.e., an overall declining trend with a slight rebound in the mid-to-late stage.

The seedling stage constitutes a high-risk window for nitrogen leaching mainly for two reasons. First, the soil background inorganic nitrogen is high while the crop root system and its uptake capacity are not yet fully developed, a pattern consistent with reports that leaching peaks concentrate in the early growth stage before root establishment (Karhu et al. 2021).

For highly intensive cropping areas such as the Erhai Lake basin, the transplanting period is often accompanied by high basal fertiliser application rates, and the combination of frequent irrigation during the seedling stage and concentrated local rainfall further amplifies the risk of nitrogen downward migration (Peng et al. 2024). These results suggest, as a hypothesis warranting field validation, that advancing amendment application before transplanting, combined with moderate control of seedling-stage irrigation and basal N and with split topdressing, may help improve the temporal matching between N supply and crop demand.

Regulation of soil enzyme activities by biochar-based composite amendments and its ecological significance in nitrogen transformation and retention. The elevated activities of N-cycling enzymes represent a key biochemical pathway through which the composite amendment regulates soil N transformation and retention, complementing direct physicochemical adsorption (Uwituze et al. 2022; Tang et al. 2023).

In this study, the maize straw biochar-based composite treatment showed the most significant enhancement in key enzyme activities, which is consistent with its advantages in improving soil physicochemical properties. On the one hand, the composite amendment increased soil pH, electrical conductivity, and organic matter content, thereby providing a more suitable habitat for nitrogen-cycling microorganisms and creating more favourable conditions for enzyme secretion and stable existence (Li et al. 2023; Daughtridge & Margenot 2024). On the other hand, the well-developed pore structure of biochar can serve as space for microbial colonization and substrate enrichment; bentonite can regulate the ionic environment and slow nutrient loss; and humic acid can enhance the stability of enzyme molecules through interactions with enzyme proteins and organic molecules, reducing the risk of inactivation and degradation (Roque et al. 2023; Yang et al. 2025). Therefore, the gradual enhancement of enzyme activities with increasing biochar proportion and application rate in this study is not simply caused by the increase of a single component, but represents the combined performance of the ternary system synergistically optimizing the soil biochemical environment.

However, enhanced nitrogen transformation in this study does not imply increased leaching. If only mineralization and hydrolysis were accelerated without concurrent adsorption, exchange, and slow-release mechanisms, the newly formed inorganic nitrogen could exacerbate losses; however, the ternary composite system in this study provided such a synchronous retention capacity. That is, enzymatic actions increase the efficiency of nitrogen transformation from inert to active forms, while biochar, bentonite, and humic acid can rapidly intercept, exchange-fix, or complex-slowly-release these newly generated inorganic nitrogen, thereby preferentially retaining them in the soil rather than entering the leaching flux (Shen et al. 2020; Li et al. 2026).

https://doi.org/10.17221/71/2026-SWR

Research limitations and prospects. Although this study systematically revealed the effects of biochar-based composite amendments on nitrogen retention, leaching inhibition, and enzyme activity regulation in high-N farmland in the Erhai Lake watershed, several limitations remain. The greenhouse pot experiment with controlled water and fertilizer supply cannot replicate field variability in the Erhai watershed (e.g., uneven rainfall, tillage, and intercropping), so the amendment's field-scale performance may differ. Moreover, only nitrogen leaching was monitored, while gaseous losses (NH_3 volatilization and N_2O emission) were not considered, resulting in an incomplete nitrogen budget. The microbial mechanism was only inferred from soil enzyme activities, without examining its effects on N-cycling microbial community structure and functional genes, which limits mechanistic understanding. Additionally, only cabbage was tested, so the applicability to locally dominant crops (rice, faba bean, maize) remains uncertain. Future research should conduct multi-year, multi-site field trials on typical high-N farmland in the Erhai watershed to assess long-term stability and optimize techniques integrated with local tillage and irrigation. A comprehensive N budget covering leaching, gaseous losses, and crop uptake is needed to clarify trade-offs among loss pathways, determine net environmental benefits, and develop N retention specifications for high-N farmland in plateau lake regions. Research should be extended to dominant rotation systems in the Erhai watershed to reveal long-term effects on soil nitrogen balance and water ecological protection, thereby providing theoretical and technical support for regional green agriculture transformation and plateau lake ecological management.

CONCLUSION

The biochar-based composite amendments examined in this study markedly enhanced in situ nitrogen retention in high-nitrogen soils while substantially reducing nitrogen leaching losses. These effects were jointly determined by amendment type, ratio, and application rate. Among the tested formulations, the corn straw biochar composite exhibited the optimal overall performance, significantly increasing soil nitrogen pools and concurrently decreasing leachate nitrogen concentrations. The activities of key nitrogen-cycling enzymes – urease, leucine aminopeptidase, and peroxidase – were also significantly elevated, with the corn straw composite consistently outperforming the rice husk and bamboo counterparts; enzyme activities increased progressively with higher ratios and application rates. The notable improvement in nitrogen retention and leaching reduction is attributed to a ternary synergistic mechanism that integrates physical adsorption by biochar, cation retention by bentonite, and complexation-mediated slow release by humic acid. These findings provide a theoretical foundation and material basis for source-oriented nitrogen abatement in high-nitrogen croplands of plateau lake watersheds.

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Received: June 5, 2026
Accepted: October 15, 2026
Published online: November 2, 2026