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Redox-active Mn loading enhances the electron-shuttling capacity of biochar to promote DNRA in soil

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Abstract

Enhancing dissimilatory nitrate reduction to $\text{NH}_4^+\text{-N}$ (DNRA) in agricultural soils provides environmental and agronomic benefits by improving nitrogen retention and reducing nitrogen losses. However, DNRA requires a higher electron input (8 e^- per NO_3^-) than denitrification (5 e^- per NO_3^-), which often results in denitrification dominating nitrate reduction in soils. Although biochar can function as an electron shuttle to facilitate DNRA, it remains unclear whether enhancing its electron-shuttling capacity can further stimulate this nitrogen-conserving pathway. Here, we prepared a biochar with enhanced electron-shuttling capacity by loading redox-active Mn phases onto its surface and evaluated its effects on DNRA in soil. Mn-loaded biochar markedly enhanced electron-shuttling capacity and increased soil DNRA rates by 2.9-fold compared with the control. The electron-shuttling capacity of biochar was positively correlated with surface redox-active Mn content, and both DNRA rates and their contribution to total nitrate reduction increased accordingly. In addition, Mn-loaded biochar increased the relative abundance of *nrfA*-type bacterial taxa (e.g., Bacteroidota, Ignavibacteriota, and Bacillota) and significantly upregulated *nrfA* gene expression. These results demonstrate that loading redox-active Mn onto biochar effectively enhances its electron-shuttling function. Reversible Mn redox cycling may facilitate electron transfer, thereby increasing electron availability for nitrate reduction to $\text{NH}_4^+\text{-N}$. Meanwhile, Mn-mediated mobilization of native soil dissolved organic carbon (DOC), without increasing carbon loss, may provide supplementary bioavailable electron donors, collectively promoting DNRA and enhancing soil nitrogen conservation, which was accompanied by lower N_2O emissions.

Highlights

- Mn-loaded biochar increased soil DNRA rates by 2.9-fold relative to the control.
- Reversible Mn(II)/Mn(III)/Mn(IV) redox cycling strengthened biochar's electron shuttle function.
- Mn-loaded biochar stimulated *nrfA*-type bacteria, enhancing soil N retention and mitigating N_2O emissions.

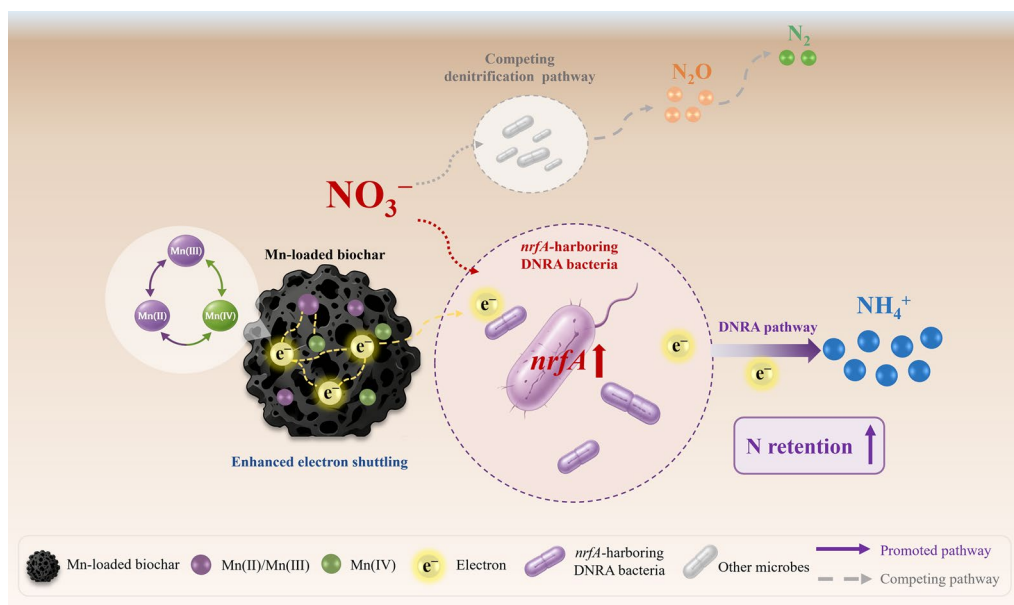
Keywords DNRA, Manganese redox cycling, Biochar, Electron shuttling, *nrfA* gene, Nitrogen retention

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Graphical Abstract



1 Introduction

Inefficient nitrogen (N) utilization in agriculture threatens both food security and environmental sustainability (Zhang et al. 2015). A substantial portion of applied fertilizer N is rapidly transformed to nitrate (NO₃⁻), which is then lost through leaching or via denitrification-driven gaseous emissions such as nitrous oxide (N₂O) and dinitrogen (N₂) (He and Löffler 2024; Zhao et al. 2025). Dissimilatory nitrate reduction to ammonium (NH₄⁺-N) (DNRA) is a key nitrogen-conserving pathway in the soil nitrogen cycle that converts NO₃⁻ to NH₄⁺, a form that is less mobile and more easily retained in soils, thereby reducing nitrogen loss and N₂O emissions (Putz et al. 2018; Yuan et al. 2022). However, denitrification is typically favored over DNRA in soils, because the DNRA pathway requires a greater supply of electrons to convert NO₃⁻ into NH₄⁺ (accepting eight electrons per NO₃⁻ molecule) than denitrification, which converts NO₃⁻ into N₂ (accepting five electrons per NO₃⁻ molecule) (Pandey et al. 2020). Thus, the availability and transfer of electrons are critical factors regulating DNRA activity in soils (Wen et al. 2025).

Biochar has attracted considerable attention as a soil amendment for regulating nitrogen cycling due to its redox-active properties (Harter et al. 2014; Zhou 2025). Previous studies have demonstrated that biochar can enhance soil NH₄⁺ accumulation by creating anaerobic microsites and by serving as an electron shuttle that

facilitates microbial nitrate reduction via the DNRA pathway (Yuan et al. 2022). This function is attributed to biochar's aromatic carbon matrix, conjugated π -electron systems, and surface redox-active functional groups, which collectively enable electron storage and transfer during microbially mediated redox reactions. However, the intrinsic electron-shuttling capacity of pristine biochar is limited, which may constrain its overall effectiveness in DNRA due to electron transfer limitation. This limitation highlights the need for modification strategies that enhance the redox activity and electron-transfer capacity of biochar in soil systems.

Enhancing the electron-shuttling capacity of biochar is a promising strategy to expand its environmental functions. Among available modification strategies, loading redox-active manganese (Mn) onto biochar has emerged as particularly effective (Liu et al. 2023; Zeng et al. 2025). Surface-associated Mn species, especially in their reduced forms (Mn(II) and Mn(III)), can substantially enhance both the electron-donating and electron-accepting capacities of biochar by establishing reversible Mn(II)/Mn(III)/Mn(IV) redox cycles (Xu et al. 2021; Li et al. 2021, 2022). In practice, Mn-loaded biochar has been widely applied to facilitate a range of redox-driven processes, including the removal or transformation of metal contaminants (e.g., As and U) and organic pollutants, through Mn-mediated electron-transfer pathways in soil and aqueous environments (Wang et al. 2015; Iqbal

et al. 2021; Liu et al. 2023). Despite these demonstrated advantages, whether and how Mn-loaded biochar regulates DNRA in paddy soils remains poorly understood.

Accordingly, we hypothesized that loading redox-active Mn phases onto biochar would enhance its electron-shuttling capacity and simultaneously increase the availability of electron donors, thereby promoting DNRA in paddy soils. To test this hypothesis, we systematically compared unmodified and Mn-loaded biochars in terms of their electron-transfer properties, evaluated their effects on DNRA rates and nitrate reduction pathways, and examined shifts in DNRA-related microbial communities and functional gene expression.

2 Materials and methods

2.1 Soil collection and analysis

Soil samples were collected from the sandy loam deposits of a paddy field at Fujian Agriculture and Forestry University, Fujian Province, China (26°5' N, 119°13' E). The site is located in the East Asian Monsoon region with a subtropical monsoon climate. The paddy field follows a water–land rotation system, with rice cultivated in the first year, followed by dryland crops such as corn (*Zea mays* L.) or peanuts (*Arachis hypogaea* L.) in the second year (Yuan et al. 2022). The field was prepared in March 2024 prior to the first rice season and remained submerged until the subsequent dryland crop planting in March 2025. Soil from the 0–20 cm layer was sampled in October 2024, during the tillering stage of the second-season rice, while the field remained flooded. Samples were placed in sterile plastic bags, kept on ice, and transported to the laboratory immediately. They were divided into two subsamples: one was air-dried and sieved (<2 mm) for physical and chemical analyses, and the other was stored at 4 °C for subsequent ¹⁵N soil slurry incubation experiments. The basic physical and chemical properties of the soil were as follows: pH 6.2 (soil:water, 1:5, w/v); dissolved organic carbon (DOC) 22.6 mg kg⁻¹, NO₃⁻-N 23.2 mg kg⁻¹, NO₂⁻-N 0.3 mg kg⁻¹, NH₄⁺-N 9.2 mg kg⁻¹, EC 104.1 mS cm⁻¹, organic matter 1.5 g kg⁻¹, and total nitrogen (TN) 0.1 g kg⁻¹.

2.2 Biochar preparation and characterization

Biochar was produced from wheat straw (Nanjing Biochar Co., China) via pyrolysis at 400 °C in a circulating N₂ system. To remove impurities, 2 g of biochar was suspended in 100 mL deionized (DI) water and stirred for 24 h, followed by three successive washes with DI water. The washed biochar was freeze-dried (–60 °C, 48 h) and stored in a desiccator for subsequent use and is hereafter referred to as BC. To prepare redox-active Mn-loaded biochar (MnBC), 2 g of BC was mixed with 100 mL of 0.2 M MnSO₄·H₂O solution and stirred for

1 h. Subsequently, 100 mL of 0.4 M KBH₄ solution was added dropwise at a rate of 2.5 mL min⁻¹. All modification procedures were conducted at room temperature (25 ± 2 °C). The mixture was continuously stirred until bubble formation ceased, indicating the completion of the reduction reaction. N₂ gas was continuously supplied throughout the reaction to prevent oxygen interference. The resulting suspension was washed three times with deoxygenated ultrapure water and ethanol (CH₃CH₂OH) to remove excess Mn. The solid product was separated, freeze-dried (–60 °C, 48 h), and stored in a vacuum desiccator (Zeng et al. 2025). To obtain biochars with different Mn loadings, 2.0 g of BC was mixed with MnSO₄·H₂O solutions at concentrations of 0.1, 0.2, 0.4, and 0.6 M, yielding 1MnBC, 2MnBC, 4MnBC, and 6MnBC, respectively. All subsequent preparation steps were identical to those described above.

The surface morphology of the prepared biochar materials (BC, MnBC, and biochars with different Mn loadings) was examined using a scanning electron microscope (SEM, ZEISS MERLIN Compact, Germany) coupled with an energy-dispersive spectrometer (EDS, Oxford X-Max N20 dual-detector system, UK). X-ray diffraction (XRD, Rigaku SmartLab 9, Japan) analysis was performed on dry powder samples scanned over a 2θ range of 10–100°. Surface functional groups of the biochars were characterized using Fourier transform infrared spectroscopy (FTIR, Nicolet iS5, Thermo Fisher Scientific, USA) over the range of 4000–400 cm⁻¹ and X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, Thermo Scientific, USA). FTIR absorption bands were assigned to –OH (3430 cm⁻¹), C=O (1590 cm⁻¹), C–O (1080 cm⁻¹), and Mn–O (543 cm⁻¹), consistent with previous reports (Liu et al. 2010; Yan et al. 2013; Rabeie et al. 2025; Tao et al. 2025). Binding energies of the XPS spectra were calibrated by setting the C 1 s peak to 284.6 eV. The C 1 s spectra were deconvoluted into C–C (284.7 eV), C–O (286.0 eV), and C=O (287.2 eV) components, each corresponding to a distinct binding energy (Huang et al. 2018). The Mn 2p spectra were deconvoluted into Mn(II), Mn(III), and Mn(IV) components centered at 641.5, 642.9, and 644.7 eV, respectively, and the relative abundances of different Mn valence states were estimated based on the fitted peak areas. Metal concentrations in the biochars were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent 720ES, USA) following microwave-assisted digestion using concentrated HNO₃ and H₂O₂ digestion. The elemental composition (C, H, N, and O) of the biochar materials was determined using an elemental analyzer (UNICUBE, Elementar Analysensysteme GmbH, Germany).

The electrical conductivity (EC) and pH of biochar suspensions (1:10, w/v, deionized water) were measured using a conductivity meter and a pH meter (Five Easy Plus, Mettler Toledo). Electrochemical properties, including cyclic voltammetry (CV), electron-donating capacity (EDC), and electron-accepting capacity (EAC), were evaluated using a three-electrode electrochemical workstation (CHI 660e, Chenhua Instrument, China). The specific capacitance (C_{sp} , $F\ g^{-1}$) was calculated from CV curves following the method of Sarkar et al. (2013). The EAC and EDC of biochar were determined using mediated electrochemical reduction (MER) and mediated electrochemical oxidation (MEO), respectively. Values of EAC and EDC ($mmol\ e^{-}\ g^{-1}$) were calculated by integrating the corresponding current peaks and normalizing to biochar mass.

$$EAC = \frac{\int \frac{I_{red}}{F} dt}{m} \quad (1)$$

$$EDC = \frac{\int \frac{I_{ox}}{F} dt}{m} \quad (2)$$

where I_{red} and I_{ox} (A) represent the baseline-corrected reductive and oxidative currents obtained from MER and MEO, respectively. F is the Faraday constant ($96,485\ s\ A\ mol^{-1}\ e^{-}$), and m (g) denotes the mass of biochar added. The electron exchange capacity ($EEC = EAC + EDC$; $mmol\ e^{-}\ g^{-1}$) was used as an integrated indicator of the total electron transfer capacity of biochar (Molstad et al. 2007).

2.3 Experimental design

2.3.1 Experiment 1: influence of Mn-loaded biochar on DNRA rate in paddy soil

To investigate the effect of Mn-loaded biochar on the DNRA rate in paddy soil, biochar was prepared and added into soil and the soil $NO_3^{-}-N$ and $NH_4^{+}-N$ concentrations, along with N_2O and N_2 emissions, were monitored as follows. Three treatments comprised: (1) a control with 10.0 g of fresh sieved soil (CK); (2) a biochar treatment with 9.8 g of fresh sieved soil and 0.2 g of original biochar (BC); and (3) a Mn-loaded biochar treatment with 9.8 g of fresh sieved soil and 0.2 g of Mn-loaded biochar (MnBC) prepared as described above. All treatments were provided with 10.0 ml of 4.0 mM $K^{15}NO_3$ (100 mg $^{15}N\ kg^{-1}$ dry soil; 98 atom% ^{15}N enrichment). All flasks were sealed with air-tight butyl rubber septa and aluminum caps, and the gas inside was replaced with a He (99.999%) atmosphere through five cycles of evacuation and refilling to establish an anaerobic environment (Yuan et al. 2026). The flasks were incubated in an incubator under static conditions at 25 °C for 22 days, with a

total of 54 flasks prepared (18 per treatment). Each treatment included three replicates, with six destructive sampling events and one group reserved for gas sampling. During incubation, three flasks per treatment were randomly selected to quantify N_2O and N_2 emissions. On days 0, 2, 5, 12, 17, and 22, three replicate flasks per treatment were sampled to determine $NO_3^{-}-N$ and $NH_4^{+}-N$ concentrations.

2.3.2 Experiment 2: influence of biochar with different Mn contents on DNRA rate in paddy soil

After Experiment 1 confirmed enhanced nitrate-derived NH_4^{+} production under MnBC using ^{15}N tracing, Experiment 2 was designed to examine whether increasing Mn loading was associated with corresponding changes in biochar electron-shuttling properties and DNRA-related NH_4^{+} accumulation. The experimental treatments, as previously described, included CK, BC, 1MnBC, 2MnBC, 4MnBC, and 6MnBC. For each treatment, 9.8 g of fresh sieved soil and 0.2 g of the given biochar were placed in a flask, except for CK, which received 10.0 g of fresh sieved soil alone. Each treatment was replicated three times. Each flask received 10.0 mL of 4.0 mM KNO_3 solution. To establish anaerobic conditions, all flasks were flushed with He, sealed, and incubated under the same conditions as in Experiment 1. $NO_3^{-}-N$ and $NH_4^{+}-N$ concentrations were analyzed at the end of incubation.

2.3.3 Experiment 3: influence of resolved Mn removal on DNRA rate in paddy soil from soil amended with biochar

During the incubation, Mn originally loaded within the biochar may leach into the soil solution. To evaluate the influence of dissolved Mn ions, which may participate in electron transfer and promote DNRA, we added sodium pyrophosphate ($Na_4P_2O_7$, SPP), a known complexing agent for Mn ions. The addition of SPP was intended to complex dissolved Mn species and thereby assess their potential contribution to the observed DNRA response (Chu et al. 2022). This approach was used as an operational test to assess the potential contribution of dissolved Mn species relative to Mn phases associated with the biochar surface. We further hypothesized that if Mn ions participate in electron transfer, adding SPP to the Mn-loaded biochar treatment would diminish its promoting effect on soil DNRA. The experiment included four treatments: (1) a control with 10.0 g of soil (CK), (2) a biochar treatment with 9.8 g of soil mixed with 0.2 g of original biochar (BC), (3) an Mn-loaded biochar treatment with 9.8 g of soil mixed with 0.2 g of Mn-loaded biochar (MnBC), and (4) an Mn-loaded biochar treatment with 9.8 g of soil mixed with 0.2 g of Mn-loaded biochar and 2.0 mM SPP (MnBC+SPP). Each treatment

was conducted in triplicate. Each flask received 10.0 mL of 4.0 mM KNO₃ solution, and incubation conditions followed those described in Experiment 1.

2.4 Analytical methods

2.4.1 N₂O and N₂ determination and soil mineral N, DOC

During incubation, concentrations of N₂O and N₂ in the flask headspace were monitored using an automated sampling system (Molstad et al. 2007). The robotized system consisted of a gas chromatograph (GC, Agilent 7890, USA) equipped with a peristaltic pump, an autosampler, and a thermostatic water bath. N₂O was quantified with an electron capture detector (ECD), whereas N₂ was measured with a thermal conductivity detector (TCD). Soil NO₃⁻-N, NH₄⁺-N, and DOC were extracted with 1.0 M KCl at both the start and end of the incubation. Extracted NO₃⁻-N concentrations were measured by the dual-wavelength spectrophotometry method (Norman and Stucki 1981; Norman et al. 1985). Extracted NH₄⁺-N was quantified by the indophenol blue colorimetric method (Dorich and Nelson 1983), and DOC was measured using a total organic carbon (TOC) analyzer (TOC-L CPH, Shimadzu, Japan). Soil pH was measured in water using a pH meter (Five Easy PlusTM, Mettler Toledo).

2.4.2 ¹⁵N tracer technique

Nitrogen transformations and gaseous products in Experiment 1 were quantified using a ¹⁵N tracer approach following K¹⁵NO₃ addition. Detailed sampling procedures and isotopic analyses are provided in Text S1 of the Supplementary Information.

2.4.3 16S rRNA gene sequencing and real-time quantitative PCR (qPCR)

In Experiment 1, soil samples were collected at the beginning (day 0) and end (day 22) of the incubation, transferred into 2 mL centrifuge tubes, and immediately frozen at -80 °C. The samples were subsequently sent to MAGI Gene Technology Co. (Guangdong, China) for analyses of microbial community composition, *nrfA*-type microbial diversity, and the transcription of DNRA (*nrfA*) and denitrification-related functional genes (*narG*, *nirK*, *nirS*, *nosZ-I*, and *nosZ-II*). The 16S V3–V4 region was amplified using the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTA CHVGGGTWTCTAAT-3'). The diversity of *nrfA*-type microorganisms was assessed by amplifying the target region using primers *nrfA*-F (5'-GCNTGYTGGWSNTGYAA-3') and *nrfA*-R (5'-TWNGGCATRTGRCAR TC-3'). The transcription levels of the DNRA gene (*nrfA*) and denitrification-related functional genes (*narG*, *nirK*, *nirS*, *nosZ-I*, and *nosZ-II*) were quantified using real-time

quantitative PCR (qPCR). The primers used for amplification are listed in Table S1, the reaction setup and qPCR conditions are detailed in Tables S2 and S3, and the RNA extraction, cDNA synthesis, and qPCR procedures are detailed in Text S4. Gene transcription levels were quantified using the standard curve method. The CK treatment was used as the reference control for transcriptional analysis. All measurements were conducted with three biological replicates and three technical replicates. The amplification efficiencies exceeded 75%, with *R*² values greater than 0.99, indicating robust and reliable quantification performance.

2.5 Statistical analysis

Statistical analyses were conducted using R (version 4.4.2). Data were first checked for normality and homogeneity of variance using Shapiro–Wilk and Levene's tests, respectively. One-way ANOVA was applied to evaluate treatment effects on soil physicochemical properties, N₂O and N₂ concentrations during incubation, microbial community composition, and gene expression (*P* < 0.05). Tukey's HSD post hoc test was used to identify pairwise differences between treatments. Linear regression analyses examining relationships among Mn loading level, electrochemical properties, and DNRA-related parameters were performed using the mean values of five biochar treatments (BC, 1MnBC, 2MnBC, 4MnBC, and 6MnBC). Each treatment consisted of three independent replicates, and regression analyses were conducted using the corresponding treatment means.

3 Results

3.1 Influence of Mn incorporation on biochar surface properties and electron-shuttling function

As shown in Table 1, Mn loading did not significantly affect the pH of the biochar (*P* > 0.05); the pH values of BC and MnBC were 7.68 and 7.52, respectively. In contrast, MnBC exhibited a markedly higher electrical conductivity (EC) than BC. Elemental composition analysis showed decreases in C, H, and O contents following Mn incorporation, accompanied by a pronounced increase in Mn content. The decrease in C content primarily reflected the incorporation of Mn-containing phases, which diluted the relative carbon proportion of the biochar. Accordingly, the increase in O/C ratio from 0.31 in BC to 0.56 in MnBC mainly reflected the lower relative C content after Mn incorporation rather than a substantial increase in O content, while the H/C ratio increased slightly from 0.52 to 0.84. Mn incorporation substantially altered the pore structure of biochar. The BET surface area increased from 13.5 to 121.7 m² g⁻¹, accompanied by an approximately 19-fold increase in total pore volume (from 0.023 to 0.435 cm³ g⁻¹) and an increase in

Table 1 The basic physicochemical properties of biochar materials

Property	BC	MnBC
pH	7.68	7.52
BET Surface area ($\text{m}^2 \text{g}^{-1}$)	13.51	121.72
Total pore volume ($\text{cm}^3 \text{g}^{-1}$)	0.023	0.435
Average pore diameter (nm)	8.21	14.31
Elements contents (%)		
C	67.77	38.91
N	0.76	0.43
H	2.92	2.73
O	21.26	21.85
Atomic ratio		
(O+N)/C	0.32	0.57
O/C	0.31	0.56
H/C	0.52	0.84

BC represents the pristine wheat-straw biochar. MnBC represents the Mn-loaded biochar containing approximately 29 wt% Mn used in Experiment 1. This material is equivalent to the 2MnBC treatment used in Experiment 2. All physicochemical properties were determined prior to soil incubation

average pore diameter from 8.2 to 14.3 nm (Table 1). The micropore surface area accounted for less than 1% of the total BET surface area, indicating that the increase in surface area was mainly associated with the development of external surfaces and mesoporous structures rather than micropore formation.

SEM-EDS analysis revealed distinct morphological and compositional differences between BC and MnBC (Fig. 1). BC exhibited a relatively smooth surface, whereas MnBC showed flocculent surface deposits primarily composed of manganese oxides, as confirmed by EDS spectra and elemental mapping. XRD analysis of MnBC revealed characteristic diffraction peaks at 33.5° , 37.0° , and 44.5° , corresponding to the (103), (211), and (220) planes of Mn_3O_4 (PDF 00-024-0734), respectively (Fig. S1a). An additional broad peak at 32.3° was indexed to the (104) plane of Mn_2O_3 (PDF 41-1442), indicating the presence of a secondary manganese oxide phase. These peaks indicate the presence of Mn in a face-centered cubic crystal structure (Zeng et al. 2025). FTIR spectra of MnBC exhibited a new absorption band near 543 cm^{-1} , attributed to Mn–O stretching vibrations (Fig. S1b). XPS analysis of the C 1s region showed no significant differences in carbon functional group composition between BC and MnBC, whereas Mn 2p spectra revealed the coexistence of Mn(II), Mn(III), and Mn(IV) species, with a higher proportion of lower-valence Mn species at increased Mn loadings (Fig. S2, S3).

Electrochemical measurements demonstrated that Mn loading markedly enhanced the electron transfer

properties of biochar. The specific capacitance and EEC of MnBC reached 6.5 F g^{-1} and $649.2 \mu\text{mol e}^- \text{g}^{-1}$, corresponding to approximately 2.4- and 1.8-fold increases relative to BC, respectively (Fig. 2b, c). The Mn content increased from undetectable levels in BC to 29.0 wt% in MnBC, and both specific capacitance and EEC were positively correlated with Mn content across the biochar series (Fig. 2d, e). The EEC, quantified through microbial electron reduction (MER) and oxidation (MEO), also increased substantially with Mn incorporation (Fig. 2c). Specifically, EEC increased from $364.0 \mu\text{mol e}^- \text{g}^{-1}$ for BC to $801.6 \mu\text{mol e}^- \text{g}^{-1}$ for 6MnBC, which exhibited the highest Mn loading, consistent with a positive relationship between Mn incorporation and electron exchange capacity (Fig. 2b, d, e).

3.2 Influence of Mn-loaded biochar on NH_4^+ -N production and N transformation in soil

In Experiment 1, both BC and MnBC significantly increased soil NH_4^+ -N concentrations during incubation compared with the CK treatment (Fig. 3b). The net NH_4^+ -N production rate was highest under MnBC, reaching $0.63 \text{ mg kg}^{-1} \text{ dry soil day}^{-1}$, followed by BC ($0.28 \text{ mg kg}^{-1} \text{ day}^{-1}$), representing 2.9-fold and 1.3-fold increases over CK ($0.22 \text{ mg kg}^{-1} \text{ day}^{-1}$) (Fig. S4). However, both BC and MnBC significantly reduced soil NO_3^- -N concentrations and N_2O emissions in the early stages of the incubation compared to the CK (Fig. 3a, c). The MnBC treatment achieved the strongest suppression of N_2O , with cumulative emissions of only $1.9 \text{ mg N kg}^{-1} \text{ dry soil}$, markedly lower than in BC ($7.4 \text{ mg kg}^{-1} \text{ dry soil}$) and CK ($17.6 \text{ mg kg}^{-1} \text{ dry soil}$) (Fig. 3c). No significant differences in N_2 emissions were observed among the treatments (Fig. 3d).

The ^{15}N tracing results indicated that both BC and MnBC enhanced the production of $^{15}\text{NH}_4^+$ -N derived from $^{15}\text{NO}_3^-$ -N reduction compared with CK. The fraction of $^{15}\text{NH}_4^+$ -N in the total reduced $^{15}\text{NO}_3^-$ -N increased from 2.0% in CK to 6.7% and 10.8% under BC and MnBC, respectively, and N_2 ($^{29}\text{N}_2 + ^{30}\text{N}_2$) accounted for 78.4%, 66.6%, and 67.5% of reduced $^{15}\text{NO}_3^-$ -N in CK, BC, and MnBC, respectively. (Fig. 4).

In Experiment 2, all Mn-loaded biochars increased NH_4^+ -N concentrations at the end of incubation. Final NH_4^+ -N concentrations were 7.3, 10.0, 15.1, 20.2, 22.8, and $24.2 \text{ mg kg}^{-1} \text{ dry soil}$ for CK, BC, 1MnBC, 2MnBC, 4MnBC, and 6MnBC, respectively (Fig. 5b). Consistent with this trend, the apparent DNRA rate and apparent contribution of DNRA to total nitrate reduction increased with Mn loading, with the latter reaching 27.9% under 6MnBC, approximately 2.3 times that under BC (Fig. 5d, Table S4).

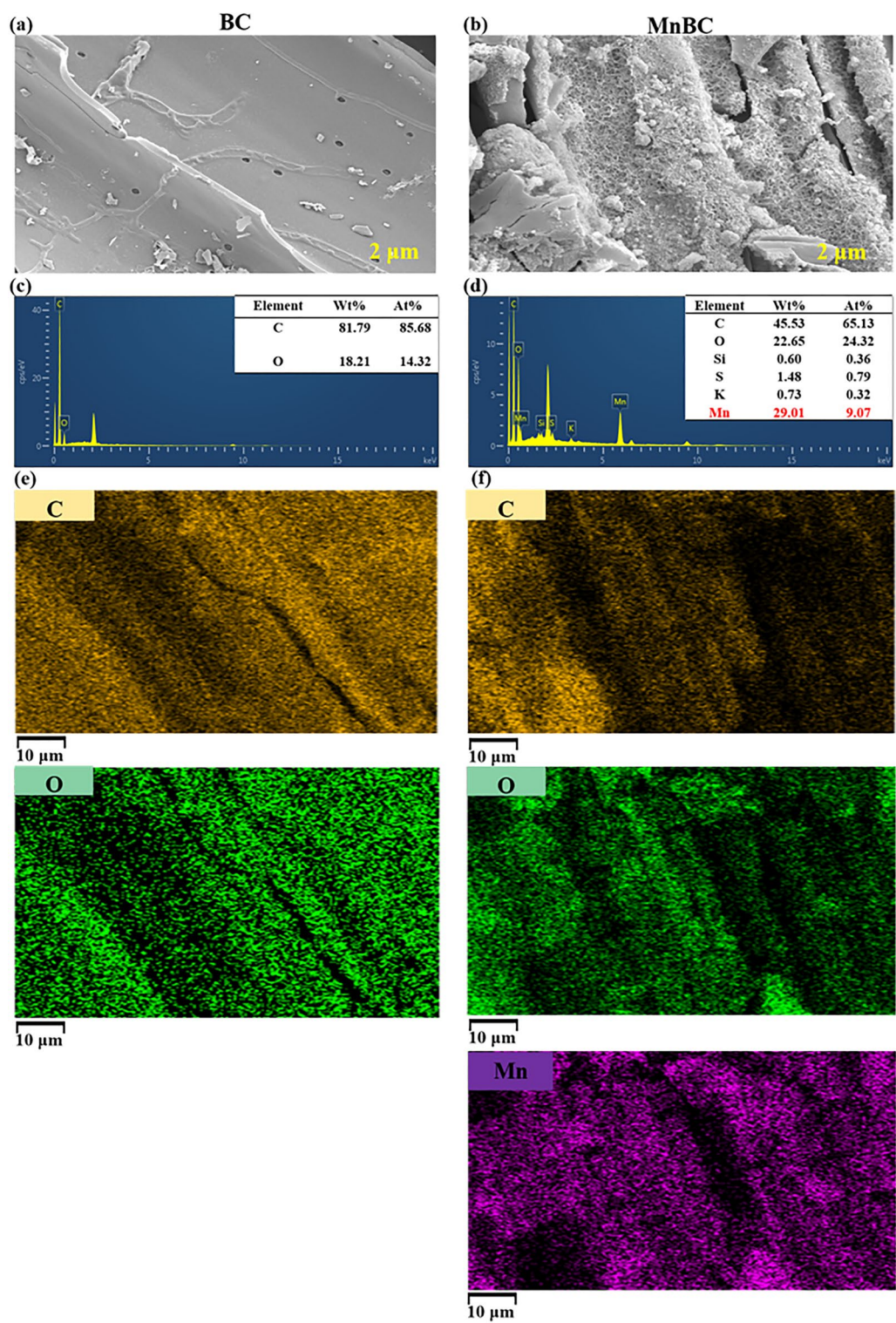


Fig. 1 Morphological characteristics and elemental composition of pristine biochar (BC) and Mn-loaded biochar (MnBC). **a, b** SEM images of BC and MnBC, respectively; **c, d** corresponding EDS spectra; **e, f** elemental mapping images. BC and MnBC denote biochar washed with deionized water and Mn-loaded biochar, respectively

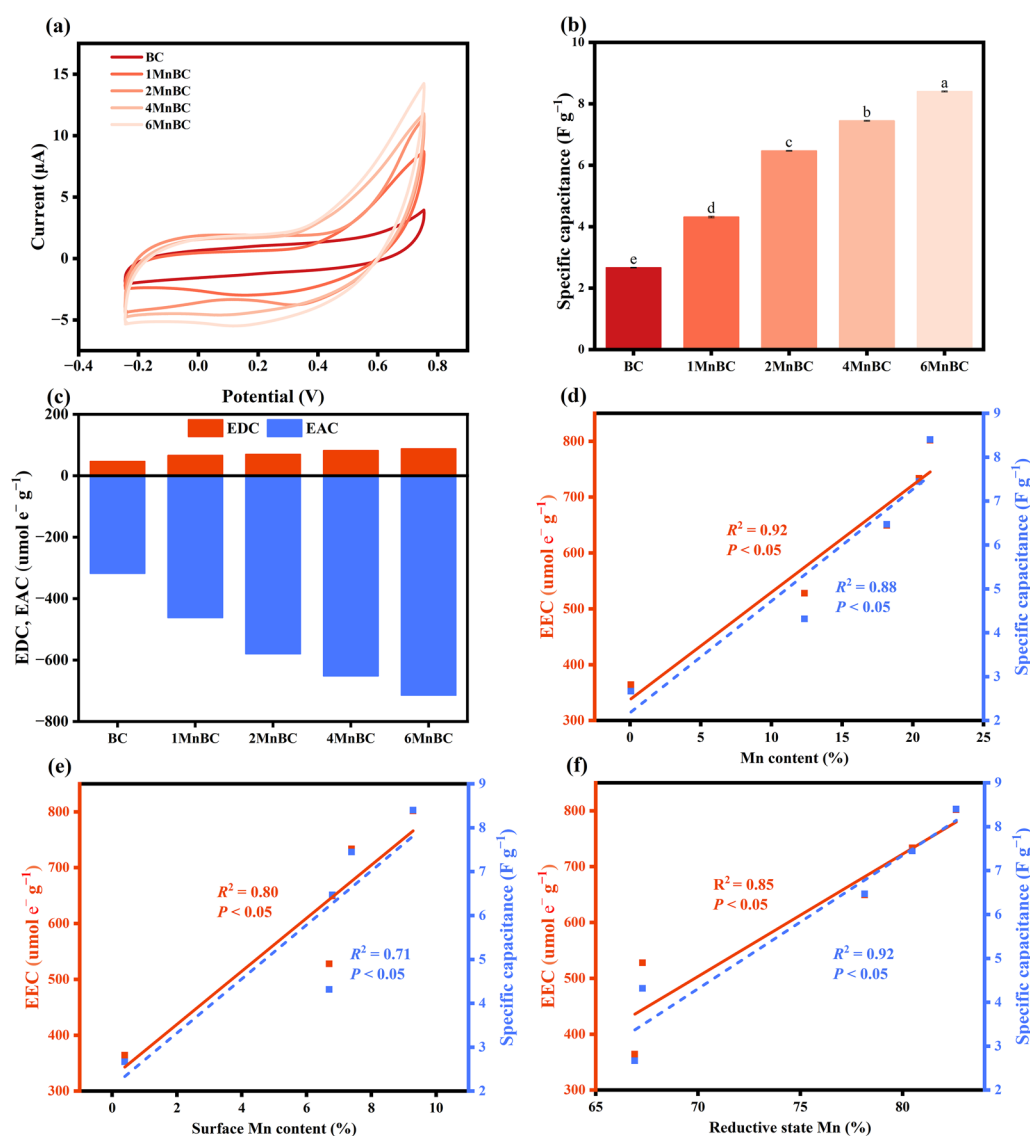


Fig. 2 Electrochemical properties of biochars with different Mn incorporation ratios. **a** Cyclic voltammetry curves; **b** specific capacitance; and **c** EEC. **d–f** Relationships of EEC (red) and specific capacitance (blue) with total Mn content, surface Mn content, and the fraction of reduced Mn species, respectively. EAC and EDC were quantified by mediated electrochemical reduction (MER) and oxidation (MEO), respectively. Total Mn content was determined by ICP-OES; surface Mn content and reductive state Mn were characterized by XPS. BC represents the original biochar addition treatment. 1MnBC, 2MnBC, 4MnBC, and 6MnBC represent different treatments with biochars pretreated with 0.1, 0.2, 0.4, and 0.6 M $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ solutions, respectively. Regression analyses in panels **d–f** were performed using treatment means from the five Mn-loading levels (BC, 1MnBC, 2MnBC, 4MnBC, and 6MnBC), with each mean derived from three independent replicates. Detailed regression statistics are provided in Table S6

In Experiment 3, consistent with the results of Experiments 1 and 2, the MnBC treatment significantly enhanced DNRA activity compared with the CK and BC treatments, as evidenced by higher $\text{NH}_4^+\text{-N}$ accumulation and DNRA rates. Notably, the addition of sodium pyrophosphate (SPP), which was applied to complex Mn potentially released from the Mn-loaded biochar, did not

significantly alter DNRA-related nitrogen transformations relative to the MnBC treatment alone (Fig. 6). Specifically, the soil $\text{NH}_4^+\text{-N}$ concentrations under CK, BC, MnBC, and MnBC + SPP treatments were 7.6, 12.9, 17.7, and 17.5 mg N kg⁻¹ dry soil, respectively, while the corresponding DNRA rates were 0.4, 0.5, 0.8, and 0.8 mg N kg⁻¹ dry soil day⁻¹ (Fig. 6a, b).

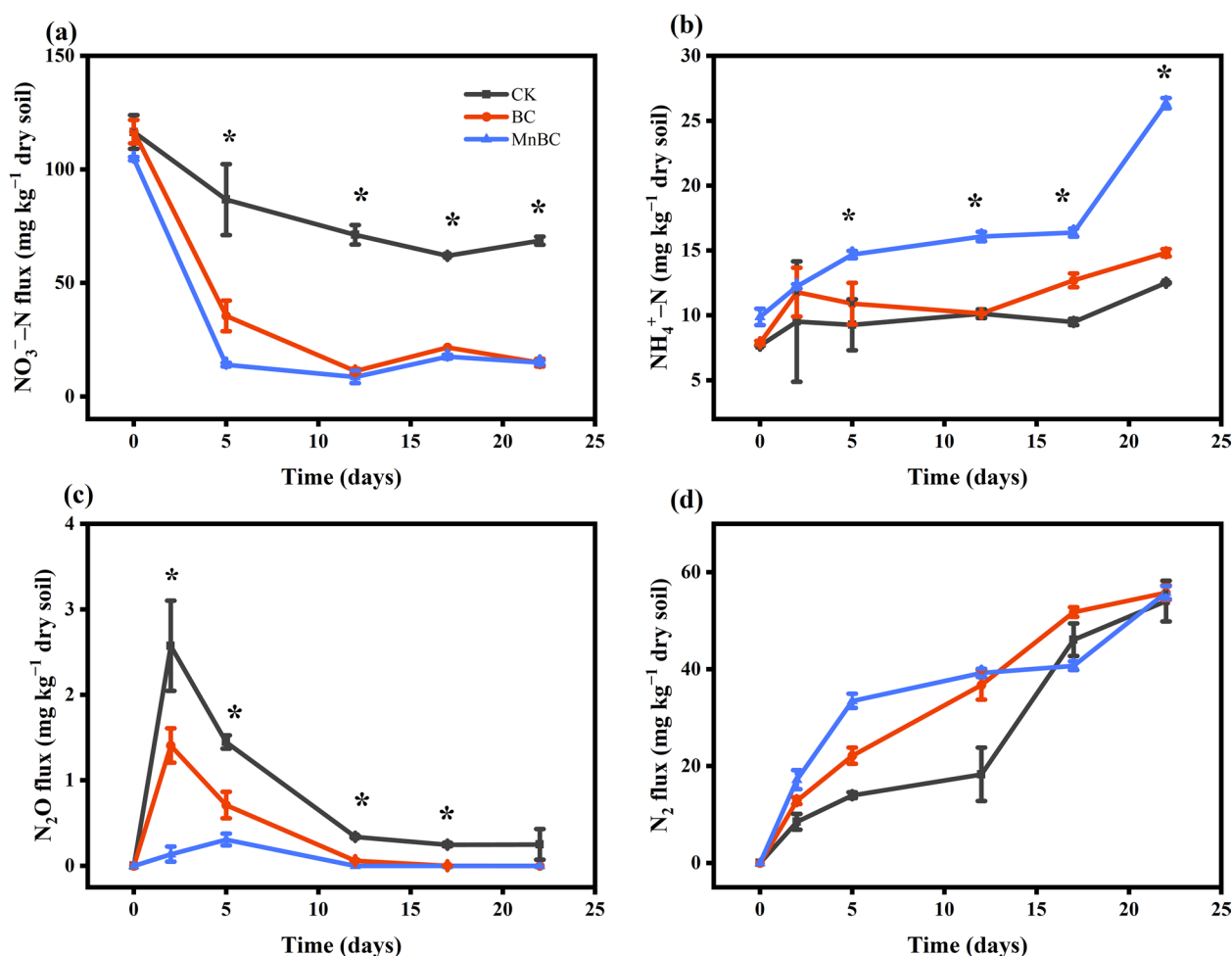


Fig. 3 Temporal dynamics of inorganic nitrogen species and gaseous N products during incubation (Experiment 1). Concentrations of soil NO_3^- -N (a) and NH_4^+ -N (b), and headspace concentrations of N_2O (c) and N_2 (d) over time under different treatments. CK, BC, and MnBC represent the control (no biochar), pristine biochar, and Mn-loaded biochar treatments, respectively. Error bars indicate standard deviation ($n = 3$). *Asterisks indicate significant differences among treatments at the corresponding sampling time (* $P < 0.05$). Note the different y-axis scales

3.3 Influence of Mn-loaded biochar on microbial community composition and DNRA functional gene expression

The soil microbial communities across all treatments were mainly composed of the phyla Pseudomonadota, Bacillota, and Actinomycetota. Throughout the incubation period, the MnBC treatment significantly increased the relative abundance of Bacillota (29.6%), while decreasing that of Thermodesulfobacteriota, which mainly comprises sulfate-reducing bacteria, from 14.5% at the initial stage to 5.4% at the end of incubation (Fig. 7a).

The *nrfA*-type DNRA bacterial group accounted for 63.8–78.1% of the total microbial community. During the incubation, the MnBC treatment increased the relative abundances of several DNRA-associated phyla, including Bacteroidota, Ignavibacteriota, and Bacillota, compared

with BC and CK. In contrast, Thermodesulfobacteriota showed a decreasing trend under MnBC. By the end of the incubation, Bacteroidota accounted for 18.6%, 0%, and 0.08% of the microbial community in the MnBC, BC, and CK treatments, respectively (Fig. 7b).

Consistent with these compositional changes, the expression level of the DNRA functional gene *nrfA* was significantly higher ($P < 0.05$) under MnBC than that under BC and CK at the end of incubation (Fig. 7c). These results indicate that Mn-loaded biochar stimulated the enrichment of DNRA-associated taxa such as Bacteroidetes and enhanced *nrfA* gene activity, thereby promoting nitrate reduction through the DNRA pathway. In contrast, MnBC did not significantly increase the relative transcription levels of the denitrification-related genes *narG*, *nirK*, *nirS*, or *nosZ-II* compared with CK or BC (Fig. S6). The transcription level of

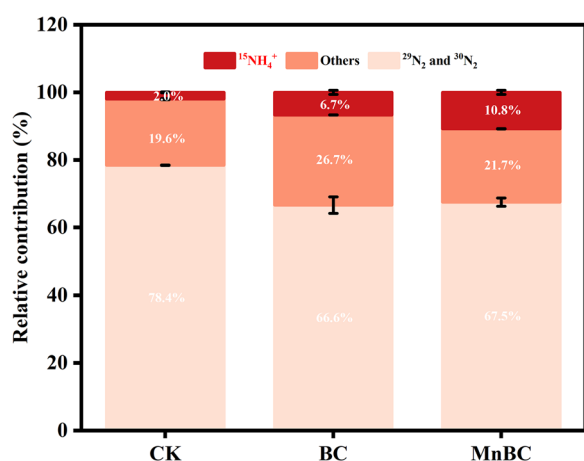


Fig. 4 Distribution of ^{15}N among different nitrogen pools following incubation (Experiment 1). Changes in the relative sizes of N pools between day 0 and day 22, expressed as percentages of total $^{15}\text{NO}_3^-$ -N reduced and partitioned into $^{15}\text{NH}_4^+$ -N, N_2 ($^{29}\text{N}_2$ and $^{30}\text{N}_2$), and other pools (including residual NO_3^- -N and unmeasured pools such as microbial biomass N). CK, BC, and MnBC represent the no biochar, pristine biochar, and Mn-loaded biochar treatments, respectively. Error bars indicate standard deviation ($n = 3$)

nosZ-I was significantly lower under MnBC than under BC, while not differing significantly from CK.

4 Discussion

4.1 Redox-active Mn loading enhances the capacity of biochar to promote soil DNRA

Our results demonstrate that MnBC markedly enhanced the DNRA process, resulting in increased NH_4^+ -N production in soil. The net DNRA rate under MnBC reached $0.67 \text{ mg } \text{NH}_4^+\text{-N kg}^{-1} \text{ dry soil day}^{-1}$, which was 2.9-fold and 1.3-fold higher than those observed in the CK and BC treatments, respectively (Fig. 3b; Fig. S4). Given that all biochars were extensively washed to neutral pH and showed low electrical conductivity before soil amendment, the substantial accumulation of NH_4^+ cannot be attributed to biochar-derived ash components or readily soluble carbon fractions.

Although soil organic matter (SOM) mineralization can serve as a potential alternative source of NH_4^+ -N (Ameloot et al. 2014; Zheng et al. 2016; Jiang et al. 2019), the observed carbon dynamics do not support enhanced soil C mineralization under MnBC. Specifically, MnBC exhibited significantly higher dissolved organic carbon (DOC) concentrations but lower CO_2 emissions compared with BC and CK (Fig. S5). This result suggests that Mn-loaded biochar may alter microbial carbon utilization and electron allocation pathways, rather than stimulating overall microbial respiration. Redox-active Mn(III/IV) oxides on the biochar surface may destabilize mineral-associated organic matter and disrupt organo-mineral

associations involving Fe/Al oxides, releasing previously protected organic carbon into the dissolved phase (Tang et al. 2013; Li et al. 2021; Ao et al. 2025). These surface-mediated processes could increase DOC availability without stimulating complete mineralization. Therefore, the elevated DOC under MnBC is more plausibly attributed to Mn-mediated mobilization of native soil organic carbon rather than enhanced mineralization. This interpretation is supported by the concurrent accumulation of DOC, lower CO_2 emissions, and the absence of detectable changes in total soil carbon (Fig. S5c), suggesting that DOC mobilization did not lead to measurable carbon loss during the incubation period. The mobilized DOC may subsequently serve as an electron donor for nitrate reduction, while Mn-associated redox cycling facilitates electron transfer toward DNRA. The released organic carbon may also contain relatively humified and redox-active fractions derived from the disruption of organo-mineral associations. Such carbon pools are likely to be released and utilized more gradually, thereby providing a sustained electron supply that better matches the high electron demand of DNRA and potentially contributing to electron-shuttling processes in soil. This interpretation is further supported by the ^{15}N tracing results. The increase in total NH_4^+ production under the MnBC treatment ($16.4 \text{ mg kg}^{-1} \text{ dry soil}$) closely aligned with the increase in ^{15}N -labeled NH_4^+ derived from $^{15}\text{NO}_3^-$ ($14.0 \text{ mg kg}^{-1} \text{ dry soil}$). This close agreement suggests that SOC mineralization made only a minor contribution to the observed NH_4^+ accumulation, indicating that the enhanced NH_4^+ -N pool was predominantly derived from nitrate reduction via the DNRA pathway.

Application of the ^{15}N tracer approach allowed the fraction of NO_3^- -N reduction attributable to DNRA to be quantified explicitly. Compared with CK and BC, MnBC markedly increased the conversion of $^{15}\text{NO}_3^-$ -N to $^{15}\text{NH}_4^+$ -N, with DNRA contributing 2.0%, 6.7%, and 10.8% of total $^{15}\text{NO}_3^-$ -N reduction in the CK, BC, and MnBC treatments, respectively (Fig. 4). As a result, DNRA represented a substantially greater share of nitrate reduction in the MnBC treatment, 5.3 times higher than in CK and 1.6 times higher than in BC. Together, these findings indicate that MnBC increased the contribution of DNRA to nitrate reduction, thereby promoting the retention of nitrate-derived N as NH_4^+ . In contrast, MnBC did not significantly enhance the transcription of denitrification-related functional genes (Fig. S6). Nevertheless, because N_2 remained the dominant nitrate-reduction product, changes in denitrification product partitioning may also have contributed to the lower N_2O accumulation and cannot be excluded. As a highly reductive process, DNRA requires the transfer of eight electrons for the complete reduction of NO_3^- to NH_4^+ (Liu

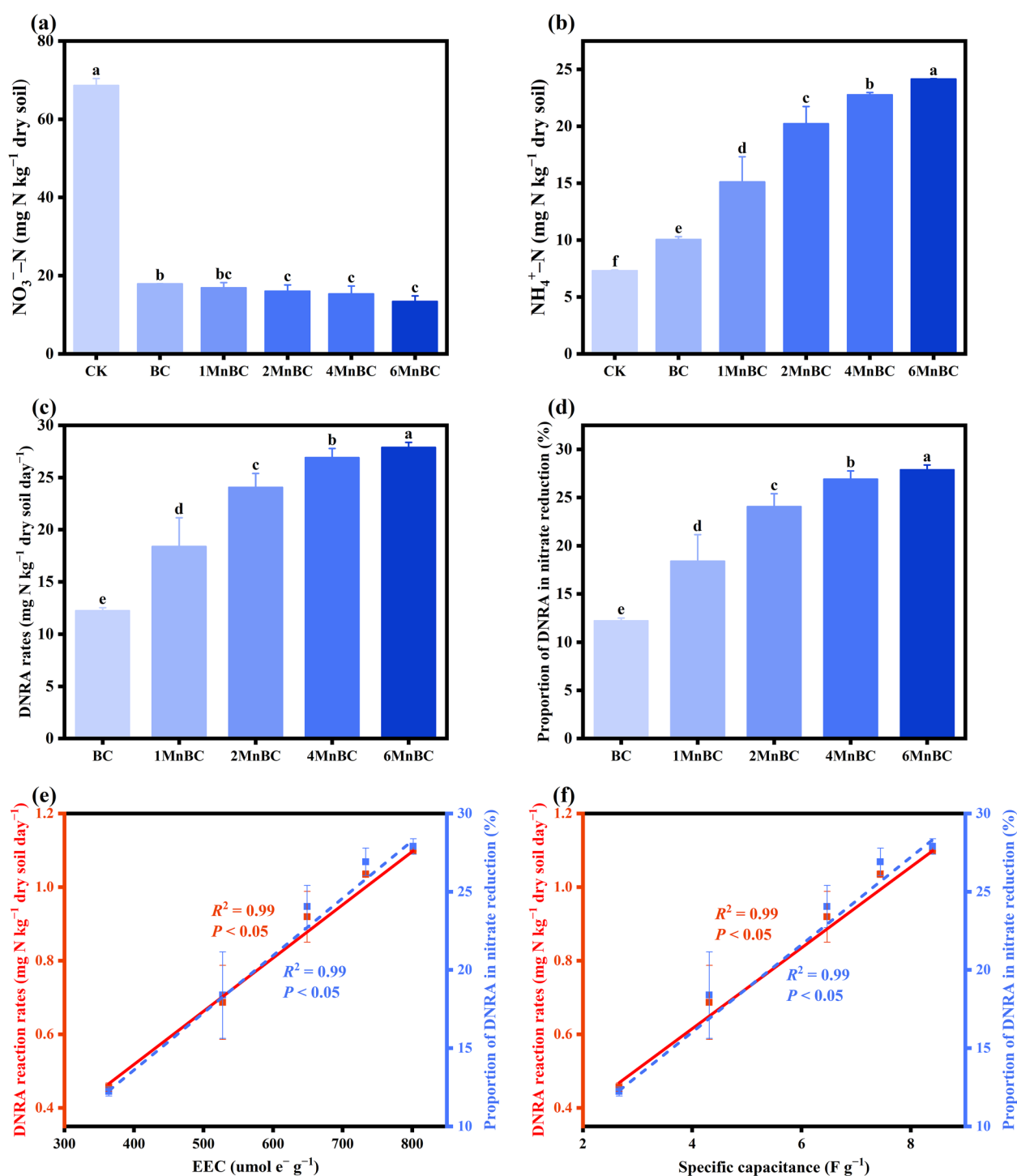


Fig. 5 DNRA activity and its relationship with biochar electrochemical properties at the end of incubation (Experiment 2). Concentrations of NO_3^- -N (a) and NH_4^+ -N (b), DNRA rates (c), and the proportion of DNRA in total nitrate reduction (d) under different treatments. Error bars represent standard deviation ($n=3$). Different lowercase letters indicate significant differences among treatments ($P < 0.05$). **e, f** Correlations of EEC and specific capacitance with DNRA rates (red) and the proportion of DNRA (blue). DNRA rates were calculated based on changes in NH_4^+ -N concentrations over the incubation period. BC represents the original biochar addition treatment. 1MnBC, 2MnBC, 4MnBC, and 6MnBC represent different treatments with biochars pretreated with 0.1, 0.2, 0.4, and 0.6 M $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ solutions, respectively. Regression analyses in panels **e–f** were performed using treatment means from the five Mn-loading levels (BC, 1MnBC, 2MnBC, 4MnBC, and 6MnBC), with each mean derived from three independent replicates. Detailed regression statistics are provided in Table S6

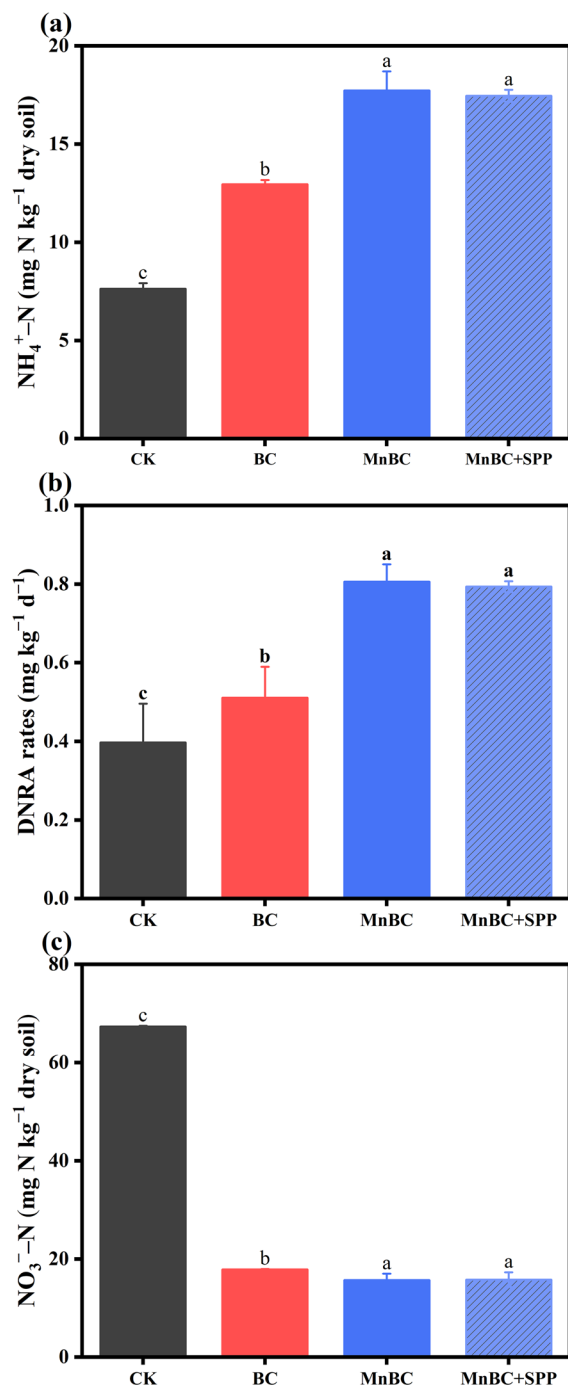


Fig. 6 Effects of sodium pyrophosphate addition on DNRA-related nitrogen transformations at the end of incubation (Experiment 3). Concentrations of NH_4^+-N (a), DNRA rates (b), and NO_3^--N (c) under different treatments. CK, BC, MnBC, and MnBC + SPP represent the no biochar, pristine biochar, Mn-loaded biochar, and Mn-loaded biochar amended with sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$, SPP) treatments, respectively. Error bars indicate standard deviation ($n = 3$)

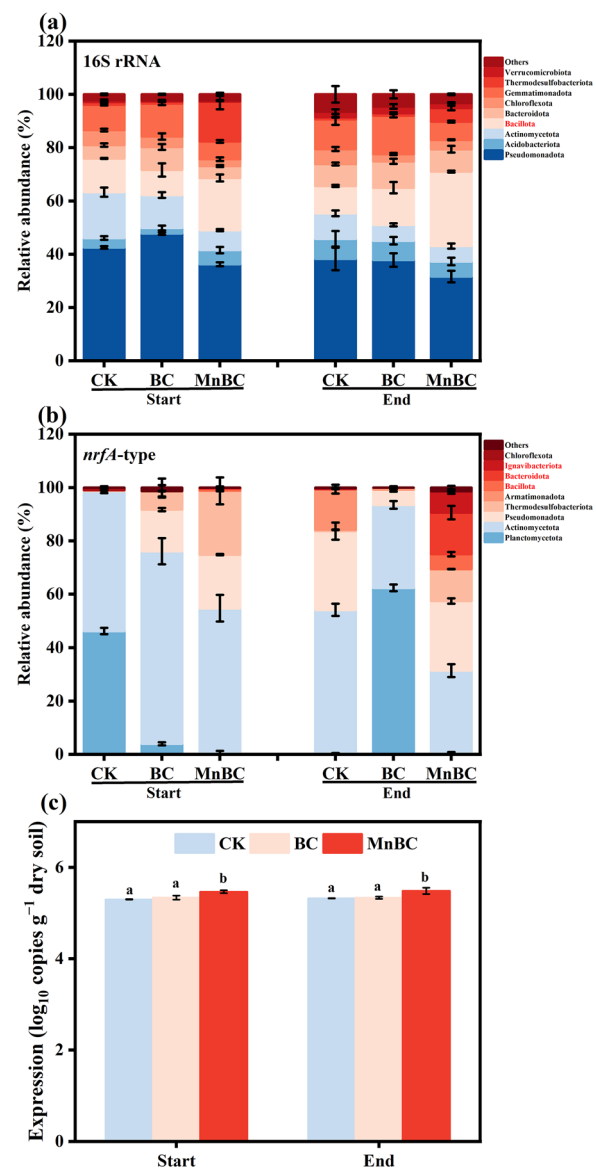


Fig. 7 Microbial community composition and DNRA functional gene expression under different treatments. a Overall soil microbial community composition; b composition of *nrfA*-type DNRA-associated microbial communities; and c relative expression levels of the DNRA functional gene *nrfA*. "Start" and "End" denote the beginning and end of incubation in Experiment 1, respectively. CK, BC, and MnBC represent the no biochar, pristine biochar, and Mn-loaded biochar treatments. Error bars indicate standard deviation ($n = 3$). Different lowercase letters indicate significant differences among treatments ($P < 0.05$)

et al. 2021; Rahman et al. 2024). However, energy conservation during DNRA depends on the coupling between electron donor oxidation and nitrate/nitrite reduction rather than on electron donor availability alone. Therefore, the stronger stimulation of DNRA observed under MnBC suggests that Mn loading not only increased the

availability of dissolved organic carbon as a potential electron donor source, but also enhanced the efficiency of electron transfer from organic carbon oxidation to nitrate reduction through its strengthened electron-shuttling capacity. By facilitating electron flow toward nitrate/nitrite reduction, MnBC may have increased the proportion of electrons utilized by DNRA microorganisms, thereby promoting nitrogen retention as NH_4^+ .

4.2 Mn loading strengthens biochar's electron shuttle function to facilitate Soil DNRA

Earlier research indicated that pristine biochar mediates electron transfer, contributing to DNRA in soils (Yuan et al. 2022). The electron transfer capacity of biochar is largely governed by redox-active components, including quinone/hydroquinone moieties and redox-active metals such as Mn (Tang et al. 2013; Yuan et al. 2026). During field aging, Mn can gradually accumulate on biochar surfaces, which may further enhance its electron transfer capacity over time (Li et al. 2022). Building on this understanding, the present study intentionally applied a high Mn loading (~29 wt%), far exceeding background levels, to actively and substantially enhance the electron transfer capacity of biochar. Material characterization confirmed that Mn was successfully incorporated into biochar, predominantly in redox-active forms. XRD analysis confirmed the presence of crystalline Mn_3O_4 and Mn_2O_3 phases in MnBC (Wang et al. 2015; Tan et al. 2021). FTIR and XPS analyses further verified the formation of Mn–O bonds on the biochar surface. XPS analysis of the Mn 2p spectra revealed the coexistence of Mn(II), Mn(III), and Mn(IV) species on the biochar surface, with approximately 78% of surface Mn present in reduced states (Mn(II)/Mn(III)) and the remaining ~22% as Mn(IV) (Fig. S1b, Fig. S3). The coexistence of Mn(II), Mn(III), and Mn(IV) species on MnBC indicates the presence of redox-active Mn phases with the potential to undergo valence transformations, which may contribute to the enhanced electron-shuttling capacity observed in MnBC. In addition to altering Mn speciation, Mn loading substantially modified the pore architecture of biochar. Compared with BC, MnBC exhibited markedly higher BET surface area, total pore volume, and average pore diameter (Table 1). The increase in surface area may be related to structural modification of the carbon matrix during the KBH_4 -mediated impregnation–reduction process, which could increase the accessibility of previously inaccessible pores (Ateş 2025; Yang et al. 2025). Because BC was not subjected to an identical Mn-free impregnation–reduction procedure, some of these structural changes may also have arisen from the modification process itself rather than Mn incorporation alone. Meanwhile, SEM images revealed that Mn oxides were present

as highly dispersed flocculent deposits rather than dense pore-blocking aggregates (Fig. 1a, b). These structural features likely increased the exposure of redox-active Mn sites and strengthened interactions among biochar surfaces, microorganisms, and electron acceptors, thereby facilitating interfacial electron transfer and contributing to the enhanced electron exchange capacity of MnBC (Yang et al. 2020; Gao et al. 2023).

Despite this limitation, the consistent Mn-dependent changes in electrochemical properties across the Mn-loading gradient provide additional support for a close association between Mn incorporation and enhanced electron-shuttling capacity. With increasing Mn content, the biochar exhibited significant increases in specific capacitance, EAC, EDC, and total EEC (Fig. 2a–c, Table S4). These electrochemical parameters (specific capacitance and EEC) were strongly positively correlated with total Mn content and, in particular, with the abundance of reduced Mn species (Mn(II)/Mn(III)) (Fig. 2d–f). This supports a close association between redox-active Mn phases and the enhanced electron-shuttling capacity of biochar, with reductive Mn (Mn(II)/Mn(III)) playing a central role. Compared with previously reported Fe- or quinone-modified biochars that promote electron transfer through different redox-active components, Mn-loaded biochar introduces redox-active Mn phases that may provide additional electron-relay sites within the conductive carbon matrix. The coexistence of multiple Mn oxidation states suggests the potential for Mn valence transformations, which may facilitate interfacial electron transfer under reducing paddy-soil conditions. Mn species may couple with the conductive carbon network of biochar to form a more efficient electron-transfer pathway, wherein the graphitic and π -conjugated carbon matrix provides long-range electron conduction while Mn valence cycling supplies localized electron relay and buffering sites. Such synergistic coupling likely contributed to the enhanced electron-shuttling capacity and electron exchange capacity observed in MnBC.

Application of MnBC markedly enhanced both the absolute DNRA rate and the fraction of nitrate reduction proceeding via DNRA relative to BC and CK treatments (Fig. S4, Fig. 4). Across the Mn-loading gradient, the apparent DNRA indicators were positively associated with biochar electron-shuttling capacity, as reflected by specific capacitance and EEC (Fig. 5d–f). These estimates were based on net NH_4^+ –N accumulation in Experiment 2 and may therefore also reflect contributions from organic N mineralization and microbial NH_4^+ immobilization (Chen et al. 2015). Taken together, these observations are consistent with a role of enhanced electron shuttling in promoting DNRA under MnBC treatment.

Notably, Mn-active environments have been reported to enrich microbial communities associated with manganese redox cycling and extracellular electron transfer (Gotore et al. 2025), while soluble Mn(II) or Mn(III) can promote DNRA by stimulating the metabolism of specific microorganisms such as *Hyphomicrobium* (Jiang et al. 2024). However, the enhancement of DNRA observed in the present study appears to be more closely associated with Mn phases retained on the biochar surface than with dissolved Mn species. FTIR spectra of MnBC showed a new absorption band near 543 cm^{-1} , attributable to Mn–O stretching vibrations (Fig. S1b), suggesting that Mn was chemically associated with the biochar surface, which may have constrained Mn release into the soil solution. Consistently, only minimal Mn leaching was detected at the end of incubation (Table S5), and the addition of sodium pyrophosphate (SPP), which was included to complex dissolved Mn species and distinguish their effects from those of biochar-associated Mn phases, did not significantly alter $\text{NH}_4^+\text{--N}$ accumulation or DNRA rates compared with the MnBC treatment alone (Fig. 6). Taken together, the minimal Mn leaching and the lack of a significant response to SPP addition suggest that dissolved Mn was unlikely to be the sole factor responsible for the enhanced DNRA under MnBC. However, because pyrophosphate can alter the speciation and reactivity of dissolved Mn, particularly Mn(III), the SPP treatment cannot unequivocally distinguish the contributions of dissolved Mn species from those of biochar-associated Mn phases. Therefore, these results are interpreted as supporting, rather than definitive, evidence for the involvement of biochar-associated Mn in the observed DNRA enhancement. Future studies incorporating soluble Mn amendments and post-incubation Mn speciation analyses would help further resolve the relative contributions of different Mn pools and their transformations during soil incubation.

4.3 Mn-loaded biochar regulates DNRA-associated microbial communities and functional genes

Soil microbial analyses revealed that Mn-loaded biochar altered both community structure and DNRA-related functional potential (Fig. 7). Pseudomonadota, Bacillota, and Actinomycetota dominated the microbial communities across all treatments, and these phyla encompass a majority of known electrotrophic microorganisms capable of directly utilizing electrons from solid surfaces (Rumora et al. 2023). Notably, MnBC application shifted community composition toward a higher relative abundance of Bacillota, a group frequently associated with enhanced nitrogen retention under anaerobic conditions typical of paddy soils, particularly when organic carbon availability is elevated (Xue et al. 2024).

Further examination of DNRA-associated microorganisms showed that *nrfA*-harboring taxa accounted for a large fraction (64–78%) of the total microbial community (Fig. 7b). The MnBC treatment specifically increased the relative abundance of Bacteroidota, Ignavibacteriota, and Bacillota within the *nrfA*-type bacteria. Bacteroidota, Ignavibacteriota, and Bacillota are recognized as direct DNRA functional bacteria that play a crucial role in nitrate reduction and nitrogen retention (Wang et al. 2024). Concurrently, MnBC reduced the abundance of Thermodesulfobacteriota, which are mainly involved in sulfate reduction, an electron-donor–competing pathway with DNRA (Cai et al. 2021). This shift indicates that MnBC suppressed the enrichment of sulfate-reducing microorganisms, potentially alleviating competition for electron donors and thereby creating a more favorable microenvironment for nitrate reduction processes.

Consistent with these community-level changes, a significant upregulation of the DNRA marker gene *nrfA* ($P < 0.05$) in MnBC amended soils at the end of incubation (Fig. 7c). Collectively, these findings demonstrate that MnBC not only enriches *nrfA*-type bacteria (particularly Bacteroidetes) but also enhances the transcriptional activity of the DNRA pathway, thereby actively supporting the DNRA process in soil.

4.4 Mechanistic framework for Mn-loaded biochar mediated enhancement of soil DNRA

Based on the observed coexistence of Mn(II), Mn(III), and Mn(IV) species and the enhanced electrochemical properties of MnBC, we propose that surface-associated Mn phases may participate in electron-transfer processes through valence transformations among different Mn oxidation states (Fig. 8). Such Mn redox transformations could provide additional electron-relay sites on the biochar surface, thereby facilitating interfacial electron transfer between electron donors and nitrate-reducing microorganisms. Under reducing soil conditions, higher-valence Mn species may be reduced by soil organic matter or other reductants, whereas lower-valence Mn species may subsequently participate in electron-transfer reactions. Although these transformations were not dynamically monitored during incubation, this proposed mechanism is consistent with the coexistence of multiple Mn oxidation states on MnBC and its substantially enhanced electron-exchange capacity. Together with the increased availability of DOC, these properties may alleviate electron limitation of DNRA microorganisms and thereby favor nitrate reduction to $\text{NH}_4^+\text{--N}$.

Although the present study provides evidence that Mn-loaded biochar can enhance DNRA under controlled anaerobic conditions, the broader applicability of this strategy remains to be established. Future studies should

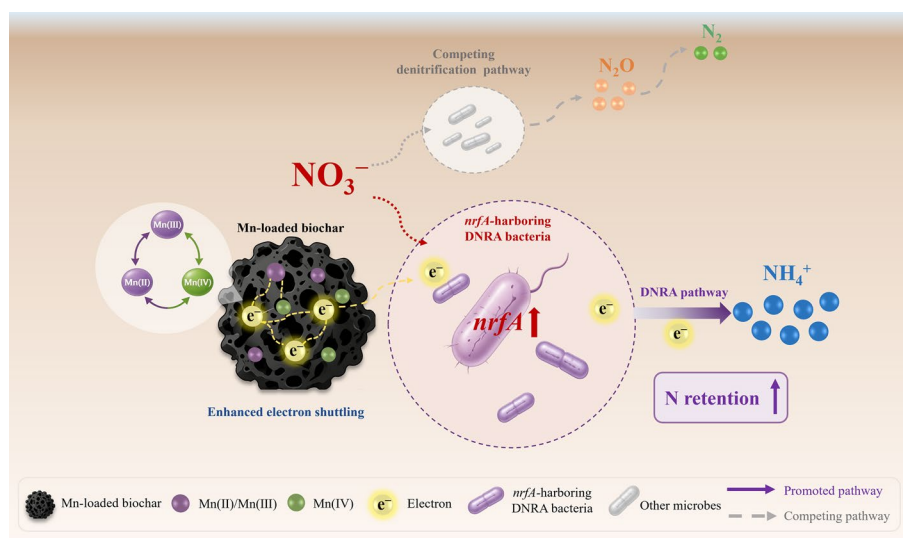


Fig. 8 Proposed mechanistic framework for Mn-loaded biochar mediated enhancement of electron transfer and DNRA in paddy soil

evaluate its performance across diverse soil types and moisture regimes and determine its long-term effects on soil carbon dynamics, microbial communities, and greenhouse gas emissions under field conditions. Such efforts will help assess the robustness and practical applicability of Mn-loaded biochar as a strategy for sustainable nitrogen management in agricultural soils.

5 Conclusion

This study demonstrates that Mn loading enhances the electron-shuttling capacity of biochar and is associated with enhanced DNRA in paddy soil, thereby increasing electron availability for nitrate reduction to NH_4^+-N . Concomitantly, Mn-associated redox processes may promote the mobilization of native soil dissolved organic carbon, providing an additional pool of bioavailable electron donors that further supports DNRA. As a result, the improved electron transfer capacity alleviates electron limitation in DNRA microorganisms, leading to a 2.3-fold higher DNRA rate and a doubling of its contribution to nitrate reduction compared with pristine biochar. This response was accompanied by an increased abundance of *nrfA*-type bacteria, elevated *nrfA* gene expression, and enhanced electron-exchange capacity associated with Mn loading. Collectively, these findings demonstrate that Mn-loaded biochar is an effective strategy for enhancing NH_4^+-N retention and mitigating N_2O emissions, likely through promoting DNRA via coupled regulation of electron transfer and carbon–nitrogen interactions. Although MnBC effectively promoted DNRA during the short-term incubation, future studies should assess its long-term

field performance, the fate and transformation of Mn under dynamic soil redox conditions, and the relative contributions of dissolved and surface-associated Mn to nitrate reduction processes.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s42773-026-00653-5>.

Additional file 1

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Author contributions

Jiao Yuan conducted the experiments, performed data analysis and visualization, and drafted the manuscript. Dan Yuan conceived and designed the study, supervised the research, acquired funding, and revised the manuscript. Shuping Qin supervised the research, acquired funding, and revised the manuscript. Chunsheng Hu supervised the project and acquired funding. Xinhui Liu contributed to software application and figure preparation. Lin Ma supervised the research and revised the manuscript. Ping Wu contributed to research supervision and guidance. All authors read and approved the final manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declare that they have no competing interests.

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