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Efficacy of nitrate and biochar@birnessite composite microspheres for simultaneous suppression of As(III) mobilization and greenhouse gas emissions in flooded paddy soils

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ABSTRACT

Elevated As(III) pollution and greenhouse gas (GHG) emissions are two primary environmental concerns associated with flooded paddy soils. Herein, a novel biochar@birnessite composite microsphere was engineered using a biochar, birnessite and sodium alginate formulation. The microspheres were applied along with nitrate to examine their efficacy in suppressing As(III) mobilization and GHG emissions in an As-contaminated flooded paddy soil. After a 10-day incubation period, the combined nitrate + microsphere treatment achieved desirable remediation effects versus a nitrate-alone treatment, with mobile As(III) (initially 0.1 mM in flooded layer) completely immobilized and N₂O, CH₄ and CO₂ emissions declining by 89 %, 73 % and 31 %, respectively. As(III) immobilization was ascribed to oxidation/adsorption/coprecipitation by FeO_x/MnO_x regenerated from successive cycles of Feammox/Mnammo and nitrate-reduction coupled with Fe(II) oxidation (NRFO)/nitrate-reduction coupled with Mn(II) oxidation (NRMO). Moreover, NRFO/NRMO-derived full denitrification displayed high thermodynamic feasibility, leading to full denitrification with the generation of N₂ rather than N₂O. The co-occurrence of anaerobic oxidation of methane (AOM) driven by biochar-shuttling and coupled reduction of nitrate/FeO_x/MnO_x fostered anaerobic oxidation of CH₄ to CO₂. A portion of the resulting CO₂ was incorporated into poorly-soluble carbonate minerals leading to lower CO₂ emission and soil carbon sequestration. Metagenomic sequencing revealed that the nitrate + microsphere treatment enriched the abundances of key microorganisms linked to As/Fe/Mn oxidation and GHG mitigation (e.g., *Geobacter*, *Streptomyces*, *Cupriavidus* and *Chloroflexus*). Our findings document the efficacy of nitrate + biochar@birnessite microsphere treatment as an effective remediation strategy to simultaneously mitigate As(III) pollution and GHG emissions in flooded paddy soils.

1. Introduction

Elevated As(III) pollution and greenhouse gas (GHG) emissions are two primary environmental concerns associated with flooded, paddy-

field agriculture (Qian et al., 2023; Zhang et al., 2024). Under anoxic soil conditions, the predominant microbial processes contribute to As (III) mobilization and GHG emissions, which are regulated by coupled As/C/N biogeochemical cycles (Zeng et al., 2024; Zhang et al., 2022b). In terms of environmental risks from As pollutants and warming

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Abbreviation lists

GHG	greenhouse gas
AOM	anaerobic oxidation of methane
AOM-FeR	AOM coupled with Fe(III) reduction
AOM-MnR	AOM coupled with Mn(IV) reduction
AOM-AsR	AOM coupled with As(V) reduction
DAOM	denitrification coupled with AOM
NRAO	nitrate-reduction coupled with As(III) oxidation
NRFO	nitrate-reduction coupled with Fe(II) oxidation
NRMO	nitrate-reduction coupled with Mn(II) oxidation
ANME	anaerobic methanotrophic archaea

potentials of different GHGs, As(III) possesses higher mobility and toxicity than As(V) (Gorny et al., 2015), whereas the global warming potentials of CH₄ and N₂O are 28 and 298 times higher than that of CO₂ (Evans et al., 2019; Kraus et al., 2016). Notably, microbial remediation strategies commonly achieve environmentally friendly and economically effective merits for mitigating the environmental risk of As(III) and GHG when applied independently. For instance, the microbially-induced processes of nitrate-reduction coupled with As(III) oxidation (NRAO) or nitrate-reduction coupled with Fe(II) oxidation (NRFO) can contribute to the immobilization of mobile As(III) through direct oxidation to mitigate As(III) toxicity or indirect adsorption by Fe(III)-(hydr)oxides to attenuate the mobility of As(III) (Feng et al., 2023). Likewise, anaerobic oxidation of methane (AOM) can generate CO₂ through the microbial AOM process (Zhang et al., 2022b). Similarly, N₂O emission could be mitigated through microbially-mediated Anammox and/or full denitrification reaction wherein N₂O is eventually converted to N₂ under anoxic conditions (Pang and Wang, 2021; Wang et al., 2022).

Given the complex redox interactions among CH₄, N₂O and As(III) in flooded paddy fields, microorganisms often induce opposing environmental outcomes for greenhouse gas (CH₄ and N₂O) emissions versus As(III) mobilization. For example, diminished CH₄ emission could be achieved through microbial AOM coupled with the As(V) reduction (AOM-AsR) process. Therein, CH₄ is anaerobically oxidized into CO₂ accompanied by As(V) reduction to As(III) by anaerobic methanotrophic archaea (ANME) and bacteria or specific ANME-bacteria consortia (Shi et al., 2022). As a result, the AOM-AsR process results in a decrease of CH₄ production, but an increase in As(III) mobilization. Conversely, As(III) mobilization can be suppressed through the microbial NRAO pathway wherein As(III) is microbially oxidized into less mobile As(V) through an incomplete denitrification process leading to increased production of N₂O (Wang et al., 2024b). Unfortunately, there are currently no reliable microbial remediation technologies available to simultaneously alleviate As(III)-GHG concerns in paddy fields. Because paddy fields often contain elevated levels of As leading to As-enriched rice grain (Qian et al., 2023; Zhang et al., 2024) and paddy fields are a major source of GHG emissions, soil quality/health of paddy fields has a large influence on food safety and regional warming, thereby requiring urgent prevention and remediation strategies (Hou and Li, 2017). Hence, developing effective, economically feasible and environmentally friendly remediation strategies for attenuating As(III) risks and mitigating GHG emissions has tremendous implications for sustaining the agricultural and environmental functions of paddy fields.

Combined remediation strategies incorporating functionalized materials are a promising approach to address the inherent inefficiencies associated with microbial remediation alone (Chen et al., 2017b; Dong et al., 2020). Because birnessite can directly immobilize As(III) and is also highly bioavailable to different microorganisms (Dong et al., 2021; Yuan et al., 2023), it can actively participate in the coupled biogeochemical cycling of As/C/N. The integrated processes include As(III)

oxidation, AOM coupled with Mn(IV) reduction (AOM-MnR) and Anammox, which in combination may achieve both suppressed mobilization of As(III) and GHG emissions in flooded paddy soils (He et al., 2018; Wang et al., 2024a). Moreover, biochar synthesized from the anaerobic pyrolysis of waste biomass not only offers agronomic benefits but can also reduce atmospheric GHG emissions (Bamminger et al., 2018). Hence, the co-utilization of birnessite and biochar might be a promising technique for simultaneously addressing As(III) and GHG pollution in flooded paddy soils. However, the interactions among biochar, birnessite and bacteria related to As(III) mobilization and GHG emissions have not been rigorously examined.

Beyond the merits of easy access to raw synthesis materials, low-cost and facile synthesis (Wang et al., 2023), both biochar and birnessite demonstrate strong interactions with soil microorganisms that can be used to address the remediation of a wide range of soil pollutants (Yang et al., 2021). Owing to the high surface area and porosity, crystallinity, stability, and functional group diversity of biochar (Xia et al., 2016), several novel functionalized materials are synthesized by in-situ anchoring tailor-made functional groups on the surface of biochar for optimizing various pollution remediation strategies (Zhang et al., 2022a). Given the merits of birnessite and biochar contributing to suppression of As(III) mobilization and GHG emissions (Bamminger et al., 2018; He et al., 2018; Wang et al., 2024a), functionalized composited materials incorporating biochar and birnessite components might greatly surpass the inherent efficacy of the pristine materials. This would enable a higher efficiency for addressing the dual concerns of elevated As(III) pollution and GHG emissions. In particular, the application of a birnessite/biochar composite material may add synergist potential to paddy soil remediation (Chen et al., 2022; Zhao et al., 2022), as the pristine materials (biochar or birnessite) contribute their respective remediation advantages while offsetting their corresponding weaknesses. The incorporation of microorganisms as biofilm communities on the biochar/birnessite composite material is regarded as a “green remediation” technology. The co-application of microorganisms and composite materials is eco-friendly and holds great potential for simultaneously attenuating the hazardous effects of As(III) and climate warming impacts of GHG emissions from paddy fields.

In the present study, we fabricated a novel functionalized material (denoted as biochar@birnessite composite microspheres) that favors biofilm colonization of the microorganisms responsible for As/C/N biotransformation. Design of the functionalized birnessite/biochar composite comprised the architectural parameters related to how to assemble the composited material, and optimization of the biochar-to-birnessite ratio in the assembled material to achieve the desired remediation outcome. The objectives of this study were to (1) examine the effects of co-utilization of biochar@birnessite composite microspheres with and without nitrate additions to suppress As(III) mobilization and GHG emissions in flooded As-contaminated paddy soils, (2) clarify the biogeochemical mechanisms involved in As(III) immobilization and GHG mitigation, and (3) evaluate the application potential of contrasting biochar-birnessite composite materials. Overall, this work highlights the fabrication of a novel biochar/birnessite composite featuring the merits of high efficiency and suitable biocompatibility to achieve simultaneous attenuation of As(III) pollution to address food safety and GHG emissions to reduce climate warming. Moreover, the findings of this study provide a new perspective for resource utilization of agricultural wastes and minerals in fabricating functionalized materials to achieve desirable environmental remediation outcomes.

2. Materials and methods

2.1. Soil sampling and soil physicochemical characterization

As-contaminated soil was sampled from a flooded paddy field in Shimen County (29°58' N, 111°38' E), Hunan Province, China. The high content of As in the background soil was attributed to runoff

contamination from a nearby abandoned As mine (Guo et al., 2024). The soil samples collected from the site were used for the purpose of evaluating As environmental risks and developing potential remediation strategies. During the peak tillering stage of rice, multiple core samples ($25 \times 25 \times 10 \text{ cm}^3$) were extracted from the 10–30 cm soil depth. The samples were thoroughly mixed to form a composite sample, sealed in a polyethylene bag and then transported to the laboratory for experimental use. Prior to experimentation, the fresh soils were stored at 4°C in the dark. A subsample was air-dried at room temperature and passed through a 2-mm sieve for determination of physicochemical properties. Details of the analytical methods are described in supporting Method S1. Briefly, the As-contaminated paddy soil was a red clay soil with an acidic pH (5.8) that contained high levels of total As ($79.2 \pm 6.6 \text{ mg/kg}$) and oxidizable As ($60.7 \pm 8.1 \text{ mg/kg}$) (Table S1).

2.2. Synthesis of BC@MnO₂ microspheres

Synthesis of birnessite was conducted using the method described by Yang and Wang (2001). Detailed information regarding the synthesis of birnessite is provided in Method S2. The biochar (0.1–1.0 mm) was pyrolyzed from waste rice straw and supplied by Jun Meng's research group at Zhejiang University of Science and Technology (China). The rice straw-derived biochar was prepared according to the protocol described in Chen et al. (2016). Briefly, rice straw was slowly pyrolyzed at 550°C under a N_2 atmosphere for 3 h, and then rinsed three times with Milli-Q water in an anoxic glove box to harvest the precipitate before finally drying at 100°C . The properties of biochar comprising the elemental composition and FTIR and XRD characterization are listed in Table S2 and Fig. S1.

The functionalized composite materials (denoted as BC@MnO₂ microspheres) were fabricated using sodium alginate (SA) as a binding agent for the in-situ immobilization of birnessite particles on the biochar surface. To examine the effects of contrasting birnessite-to-biochar ratios on remediation efficacy, different ratios of biochar and birnessite suspensions were subjected to in-situ polymerization with an equal amount of SA. For brevity, the BC@MnO₂ microspheres assembled with biochar-to-birnessite ratios of 1.0:0.0, 0.2:0.8, 0.5:0.5, 0.8:0.2 and 0.0:1.0 (mass:mass) are subsequently denoted as Microsphere A,

Microsphere B, Microsphere C, Microsphere D and Microsphere E, respectively.

The synthesis reaction was conducted in a 1000-mL beaker with 125 mL of KOH (1.0 M), 125 mL of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.6 M) and continuous magnetic stirring and N_2 bubbling. Next, the designated amount of biochar particles for assembling a given microsphere formulation was rapidly added to the above Mn-mixture to achieve the desired mass ratios of biochar in the microspheres. After reacting for 20 min, 250 mL KMnO_4 (0.1 M) was added to the resulting biochar-containing suspensions, stirred for 30 min, and then transferred to an oven for aging at 70°C for 8 h. The resulting aged suspensions were rinsed with Milli-Q water to remove dissolved constituents until the pH value of the final solution remained in the 9–10 range. The solids were dried at 80°C and then ground to a final diameter of $\sim 0.83 \text{ mm}$.

To shape the microspheres, 50 mL of 2 % wt SA was added to the biochar/birnessite to produce a suspension that was fully dispersed by manual stirring to form a mushy colloidal suspension. The colloidal suspension was dripped into 500 mL of 5 % wt CaCl_2 solution above the 20 cm of liquid using a 1 mL syringe and then immersed with CaCl_2 solution to form microspheres (5 mm) for 24 h (Fig. 1a). Finally, the microspheres were separated, sprayed with ethyl alcohol (75 % wt), washed twice with Milli-Q water, and stored dried in a sterilized 50 mL-centrifuge tube for subsequent experimental use. Scanning electron microscopy was employed to characterize the morphology of the synthesized microspheres (Fig. 1a.)

2.3. Soil microcosm experiments

We used anoxic microcosms to simulate the redox conditions in As-contaminated paddy soils. All reactions were performed in batch experiments using 50-mL serum bottles. Each microcosm system was incubated with 8.0 g of fresh paddy soil (<2-mm fraction), 24.0 mL reaction solution (0.1 mM As(III) with/without nitrate) and BC@MnO₂ microspheres. The experimental design in this work was comprised of two parts. Part I experiments consisted of the following treatment groups: (1) control (CK), (2) 20 mM nitrate alone, (3) 0.4 g microspheres alone and (4) 20.0 mM nitrate+0.4 g microspheres. Herein, microsphere C (biochar/birnessite ratio of 0.5:0.5) was firstly applied to examine its

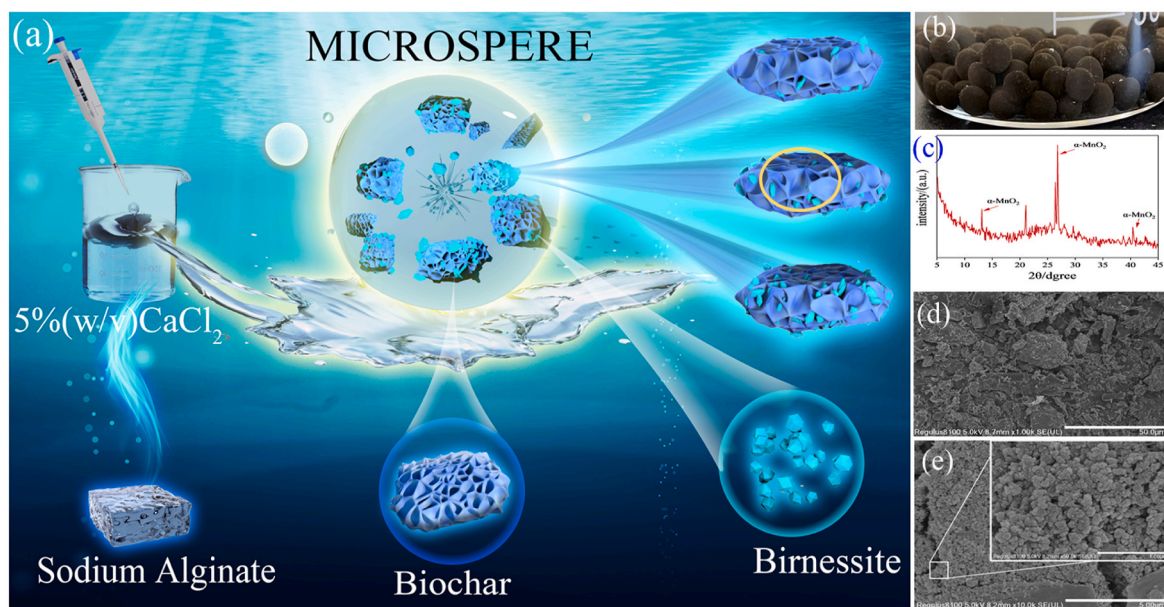


Fig. 1. Schematic illustration (a) for the stepwise fabrication of biochar@birnessite composite microspheres (b) composed of biochar, birnessite and sodium alginate (SA) through assembling with variable mixed biochar-to-birnessite ratios (1.0:0.0, 0.2:0.8, 0.5:0.5, 0.8:0.2 and 0.0:1.0 in mass/mass). XRD confirmed the presence of birnessite anchored on the microsphere surfaces (c). Morphological characterization by SEM for pristine biochar particles (d) and fabricated biochar@birnessite composite microspheres (e).

effects together with nitrate on As(III) immobilization and GHG emissions. Part II experiments utilized combined treatments of nitrate and microspheres with a range of biochar-to-birnessite ratios (microspheres A, B, C, D and E) to evaluate suppression of As(III) mobilization and GHG emissions. Each treatment was conducted in triplicate with the detailed experimental design shown in Table S3.

Prior to incubation, all microcosms were deoxygenated by purging with helium for 60 min (30 min for liquid phase and 30 min for headspace) and thereafter tightly sealed with a butyl rubber septum and aluminum caps. All reagents, serum bottles and incubation tools were sterilized in an autoclave at 121 °C for 20 min prior to use. All microcosms were incubated at 30 °C in the dark. Subsets of each treatment group were destructively harvested for analysis after 2, 4, 6, 8, and 10 days of incubation. The supernatant, soil, headspace gas, and microsphere components were collected. All experimental procedures were carried out in an anoxic glove box.

2.4. Analytical methods

2.4.1. Determination of Fe/As/Mn species

To quantify changes in the forms of Fe, As, and Mn throughout the incubation period, sampling was conducted after 2, 4, 6, 8, and 10 days of microcosm cultivation. Speciation changes of As/Fe/Mn/O/C adsorbed on the surface of the microspheres were assessed by XPS (ESCALAB 250Xi, Thermo, USA) (Dong et al., 2021). The supernatant was filtered through a sterile 0.22-μm membrane filter (nylon; Jinteng, China) for determination of dissolved constituents. Concentrations of dissolved Fe (T) (total iron) and Fe(II) were measured using a UV–visible spectrophotometer (UV-2802, UNICO, China) and phenanthroline colorimetric method (Chen et al., 2019b). Dissolved Fe(III) was calculated by difference: Fe(III)=Fe(T)-Fe(II). As(T) (total arsenic) concentration was quantified using ICP-MS (NexION, 2000; PerkinElmer, USA) (Chen et al., 2018). The concentrations of As(III) and As(V) were measured by high-performance liquid chromatography-atomic fluorescence spectrometry (HPLC-AFS, SA-20, Jitian, China) (Chen et al., 2019a). The Mn (II) concentration was determined using the potassium periodate oxidation spectrophotometric method (Liu et al., 1995) to examine the reductive dissolution of MnO₂ involved in the Mnammox process.

2.4.2. Analyses of C and N involved in redox transformations

To investigate microbial transformations involving C and N, both C-related parameters (TOC, DOM, CH₄ and CO₂) and N-related parameters (NH₄⁺-N, NO₃⁻-N, NO₂⁻-N and N₂O) were quantified. Soil derived NH₄⁺-N, NO₃⁻-N and NO₂⁻-N were extracted with 1M KCl (Kodithuwakku et al., 2024) and quantified using the salicylate, hydrazine reduction and sulfanilamide/N-1-naphthylethylene spectroscopy methods, respectively, with a continuous flow analyzer (Autoanalyser-3, Seal, Germany) (Alsaaffar et al., 2020). Concentrations of dissolved organic carbon (DOC) in the flooded layer were determined using a TOC analyzer (Shimadzu, TOC-L CPH, Japan) to assess DOC conversion to CH₄ and CO₂ (Zheng et al., 2024). Gas samples were extracted into 25-mL vacuum headspace bottles with a syringe. The concentrations of CH₄, CO₂ and N₂O were measured using a gas chromatograph (GC, Agilent Technologies 7890B, USA) equipped with a TCD to measure CH₄ and CO₂ and an ECD and Poropak Q (80/100) column to quantify N₂O (Cao et al., 2023).

We assessed changes in the composition of dissolved organic matter (DOM) (Chen et al., 2017a), including aromatic proteins, fulvic acid, humic acid, and microbial byproducts. The supernatant was filtered through a 0.45 μm membrane (Millipore, USA), and the DOM composition was characterized using fluorescence excitation-emission matrix (EEMs) spectroscopy (FS5, Edinburgh, UK). The EEM spectrum was scanned with excitation (Ex) wavelengths ranging from 200 to 400 nm at 2-nm intervals and emission (Em) wavelengths from 250 to 550 nm at 2-nm intervals. The scanning rate was set at 1200 nm/min, with both Ex and Em slit widths of 5 nm. Corrections for inner filter effects, normalized Raman scattering, and background blanks were applied. Further,

fluorescence regional integration (FRI) analysis was conducted to assess the abundance of representative fluorescent DOM compounds, with FRI results calculated using the formula described in supporting Method S3.

2.4.3. Determination of enzyme activities and biomass attached to microspheres

Activities of key enzymes influencing the generation of N₂O and CH₄ were quantified (Li et al., 2023). Therein, we assessed the key denitrifying enzymes (NAR [NO₃⁻→NO₂⁻], NIR [NO₂⁻→NO], NOR [NO→N₂O] and NOS [N₂O→N₂]) (Wang et al., 2024a) and the Mcr-enzyme mediating catalytic processes involved in the reverse-methanogenesis pathway occurring in denitrification coupled with AOM (DAOM) by ANME (Yu et al., 2022). The activities of NAR, NIR, NOR, NOS and Mcr were determined using an ELISA Kit (Jiangsu Meimian Industrial, China) to assess the impacts of the nitrate with/without microsphere treatments on the generation of N₂O and CH₄ (Li et al., 2023). Additionally, the bicinchoninic acid (BCA) assay was employed to quantify biomass incorporated into biofilms on the microspheres following the protocols for the BCA Protein Quantification Kit (Yeast, China) (Chen et al., 2024).

2.4.4. DNA extraction, library construction, and metagenomic sequencing

After 8 days of incubation, soil samples from each treatment group were collected for metagenomic sequencing. Total genomic DNA was extracted from 0.5 g soil using the Mag-Bind® Soil DNA Kit (Omega Bio-tek, USA) according to manufacturer's instructions, with triplicate samples for each treatment (n = 3). DNA concentration and purity were measured using a NanoDrop 2000, and the quality and integrity of the extracted DNA were assessed on 1 % agarose gels. DNA extracts were sheared to an average size of ~400 bp using a Covaris M220 (Gene Company, China) for paired-end library construction. Paired-end libraries were constructed using the NEXTFLEX® Rapid DNA-Seq Kit (Bioo Scientific, USA). Adapters containing complementary sequences to the sequencing primer binding sites were ligated to the blunt ends of the DNA fragments. Sequencing was performed on an Illumina NovaSeq platform (Illumina, USA) with the NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles) according to manufacturer's protocol (www.illumina.com) at Majorbio Bio-Pharm Technology (Shanghai, China).

Metagenomic data were assembled using MEGAHIT (version 1.1.2). Analysis was conducted on the Majorbio Cloud Platform (www.majorbio.com). In brief, paired-end Illumina reads were trimmed to remove adapters, and low-quality reads (length <50 bp, quality score <20, or containing ambiguous bases) were filtered out using fastp (version 0.23.0). Each sample yielded approximately 13 Gbp of clean reads. Filtered metagenomic reads were classified using Kraken2 and Bracken against nr-derived databases, including Greengenes, SILVA, and the Ribosomal Database Project (RDP). Contigs longer than 300 bp were selected as the final assembly results for further gene prediction and annotation. Functional profiling was performed using Diamond against the UniRef90 database to identify gene families (alignment length ≥25 amino acids, identity ≥80 %, e-value ≤ 1e-5). RPKM (reads per kilobase per million mapped reads) was used to estimate the abundance and gene expression at the genus level. Gene families were annotated to KEGG pathways and modules, EC categories, eggNOG, Pfam domains, and GO terms using built-in scripts in Humann. Detailed information on the metagenomic sequencing protocol is provided in Method S4.

2.5. Data analysis

All parameters are presented as mean ± standard deviation unless otherwise noted. Data were analyzed using one-way analysis of variance (ANOVA) with SPSS 26 software at a 95 % confidence level (P < 0.05). Data visualization was performed using Origin 2024b and Microsoft Excel 2016, with error bars representing the standard deviation from triplicate analyses.

3. Results and discussion

3.1. Characterizations of synthesized BC@MnO₂ microspheres

The morphologies of pristine biochar particles and synthesized microspheres were characterized by SEM imaging (Fig. 1). The pristine biochar particles were 3–20 μm in diameter, which allowed for in-situ immobilization of birnessite on the biochar surface. XRD analysis (Fig. 1c) confirmed the formation of birnessite on the microspheres (Grygar et al., 2004). The biochar and birnessite particles were agglomerated into small particles ranging in size from 30 to 200 nm following the hydro-thermal synthesis and material aging steps (Fig. 1e). The surface of the synthesized microspheres was prominently wrinkled creating a large surface area for possible biofilm colonization.

3.2. Fe and As mobilization/immobilization effects

The control group (no amendments) displayed higher Fe and As mobilization with maximum concentrations of 0.05 mM and 104.02 μM on the 10th day, respectively (Fig. 2). The effects of nitrate, microsphere and nitrate + microsphere treatments on As and Fe concentrations varied considerably. Microsphere treatment alone displayed a stronger Fe mobilization than the control (CK) group (0.08 vs. 0.05 mM for Fe(T), Fig. 2a), whereas the nitrate and nitrate + microsphere amendments exhibited nearly complete suppression of Fe mobilization. The composition and proportion of Fe species varied with incubation time in the microsphere amendment, wherein Fe mainly existed as Fe(II) (69–76 % of Fe(T)) during the initial 4 days of incubation, and Fe(III) became dominant (70–74 % of Fe(T)) during the 6–10 day incubation period. The promotion of reductive dissolution of Fe(III) in the microsphere amendment during the initial 4 days (Fig. 2b) was ascribed to the electron shuttling effect of biochar that fosters the microbial reduction

of Fe(III)-bearing minerals in anoxic sediments (Chen et al., 2016). Thereafter, Fe(III) was generated in the flooded layer, which was likely due to enhanced Fe(II) oxidation by MnO₂ (supported by the pronounced mobilization of Mn(II); Fig. S2). As nitrate possesses a high oxidative potential (0.71 V), it favors the microbial oxidation of Fe (II)/Mn(II) to FeO_x/MnO_x through the NRMO and NRFO processes (Sun et al., 2016; Swathi et al., 2017). The FeO_x/MnO_x regenerated from the nitrate + microsphere treatment effectively suppresses Fe mobilization from the anoxic sediments (Fig. 2a to c).

An effective As(III)-remediation strategy for flooded paddy fields must ensure As(III) immobilization in the flooded layer, as well as suppression of the reductive dissolution of As(V)/Fe(III) in the sediments (Chen et al., 2018). The As(T) concentration rapidly decreased with increasing incubation time for all treatments apart from the control group (Fig. 2d). With respect to As(III) immobilization, the treatment effects were ranked as nitrate + microsphere (0.4 μM) > nitrate (1.3 μM) > microsphere (9.0 μM) on the 10th day (Fig. 2e). The stronger As(III) immobilization induced by nitrate versus microspheres was ascribed to the nitrate treatment providing greater suppression of reductive Fe(III) dissolution (Fig. 2a to c) and the suppressed reductive dissolution of Fe(III)-(hydr)oxides attenuating the reductive dissolution of As(V) from sediment (Chen et al., 2017a). Furthermore, As(III) immobilization may be reinforced by NRAO together with oxidation/adsorption by MnO_x/FeO_x in the nitrate + microsphere treatment. These mechanisms may explain the strong As(III) immobilization by the nitrate + microsphere treatment (Fig. 2d to f).

3.3. Nitrogen redox biotransformations

In flooded paddy soil microcosms, As(III) is an electron donor available for autotrophic denitrification. The combined nitrate + microsphere treatment led to stronger nitrate consumption than the

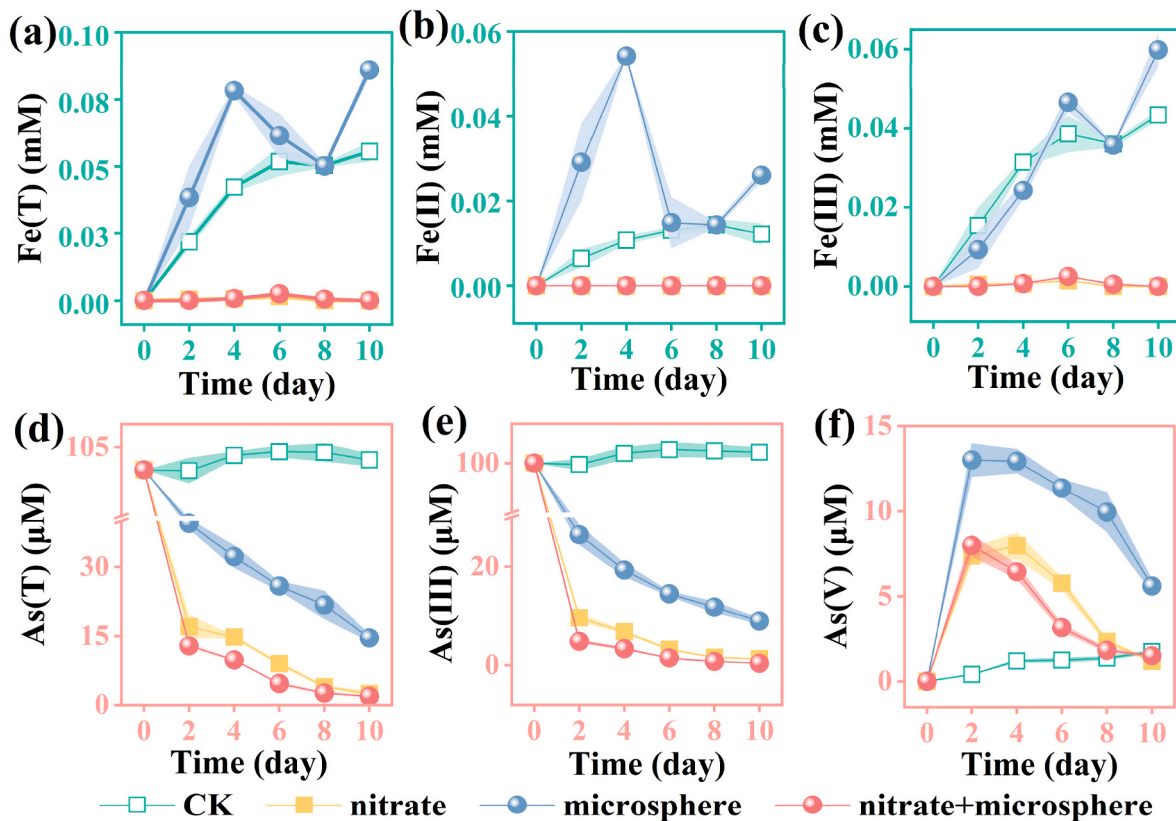


Fig. 2. Dissolved Fe(T) (a), Fe(II) (b), Fe(III) (c), As(T) (d), As(III) (e) and As(V) (f) in flooded layer of microcosms ($n = 3$, shaded area indicates SD, and CK denotes the control group).

nitrate treatment alone (4.0 vs. 5.3 mM retained on the 10th day) (Fig. 3a). This could contribute to the greater production of NO_2^- and N_2O through incomplete denitrification associated with the NRAO pathway. However, the combined nitrate + microsphere treatment diminished the production of nitrite and N_2O when compared to the nitrate treatment alone (Fig. 3b to c). Specifically, the production of NO_2^- -N on the 2nd day and N_2O emission on the 4th day for the combined treatment was only 38 % and 56 % that of the nitrate alone treatment. These results infer that the combined nitrate + microsphere treatment suppressed denitrification. These contradicting results raise the question of whether the greater loss of nitrate in the combined treatment was due to denitrification pathways other than NRAO.

To address this question, we examined the NH_4^+ -N and TN concentrations of the soil and flooded layers in the soil microcosms. We found that consumption of soil-phase NH_4^+ -N and flooded layer TN was stronger for the combined nitrate + microsphere treatment than that of the nitrate treatment alone (Fig. 3d and e). These phenomena might be attributed to enhanced Mnammox caused by birnessite in the microspheres and soil-derived Feammox reactions producing Mn(II) and Fe(II) that take part in Mn(II)/Fe(II)-dependent denitrification (i.e., NRMO and NRFO) (Wang et al., 2024a). Successive cycles of Mnammox/-Feammox together with NRMO/NRFO play a pivotal role in the regeneration of $\text{MnO}_x/\text{FeO}_x$ and achieve efficient TN removal wherein the intermediates formed during NRMO/NRFO processes were denitrified to gaseous N_2 (Eq. (15) and Eq. (16) in Table 1) (Desireddy and Pothanankandathil Chacko, 2021). This reaction pathway is supported by the suppressed mobilization of Mn(II) and Fe(II), as well as the diminished TN concentration in the combined nitrate + microsphere treatment (Fig. S1, Figs. 2b and 3e). Because NRMO and NRFO are more thermodynamically favorable than NRAO (Table 1), a substantial portion of nitrate in the combined treatment might participate in full denitrification by the NRMO and NRFO pathways, thereby promoting complete denitrification by NRAO and reducing N_2O production. Additionally, dissolved As(III) might be immobilized through adsorption/oxidation by the regenerated $\text{MnO}_x/\text{FeO}_x$ (Dong et al., 2021; Wang et al., 2024a).

3.4. Organic carbon biotransformations

During incubation, labile DOM was largely consumed by methanogenesis during the initial 4 days, as CH_4 generation peaked on the 4th day. Subsequently, a pattern of increasing TOC concentrations was observed in the flooded layer (Fig. 4a). This phenomenon was ascribed to the solubilization of endogenic heavy-molecular-weight DOM driven by microbial mineralization in the sediment (Li et al., 2018). Notably, the nitrate + microsphere treatment had higher DOC mobilization than that of the nitrate treatment, inferring stronger microbial mineralization in the nitrate + microsphere treatment. Moreover, the DOC content was sustained at a higher level in the combined treatment, which was attributed to microsphere-derived biochar components attenuating the mineralization of native DOM in the soils and/or some DOM leaching from biochar components (Riaz et al., 2017).

Since no exogenous labile organic C substrates (e.g., acetate) were supplied to the microcosms, CH_4 dynamics were largely influenced by the balance between methanogenesis relying on microbial fermentation of endogenous labile DOM (positive) and the AOM process (negative) (Zhang et al., 2023). Among a variety of electron acceptors available for AOM, nitrate is considered as the most competitive electron acceptor owing to its high oxidative potential (0.71 V) and correspondingly favorable Gibbs free energy in AOM (-765 kJ/mol ; Table 1). Thus, AOM (Eq. (8) in Table 1) was prominently active in the nitrate-supplied microcosms as CH_4 concentration progressively declined with increasing incubation time (Fig. 4b). Furthermore, we found a sharp decline in CH_4 emission (73 %) in the combined treatment by the 10th day compared to the nitrate treatment alone. This indicates that the combined treatment promotes the AOM reaction. Given stimulated AOM in the combined treatment, the emission of CO_2 should theoretically increase. However, CO_2 emission from the combined treatment was suppressed, with CO_2 emission only 70 % that of the nitrate treatment on the 10th day.

EEM characterization (Fig. 4d) confirmed that the nitrate + microsphere treatment resulted in a pronounced fluorescence peak located in the humic acid-like region. Similarly, the FRI analysis (Fig. 4e) showed the proportion of humic acid-like compounds was higher in the

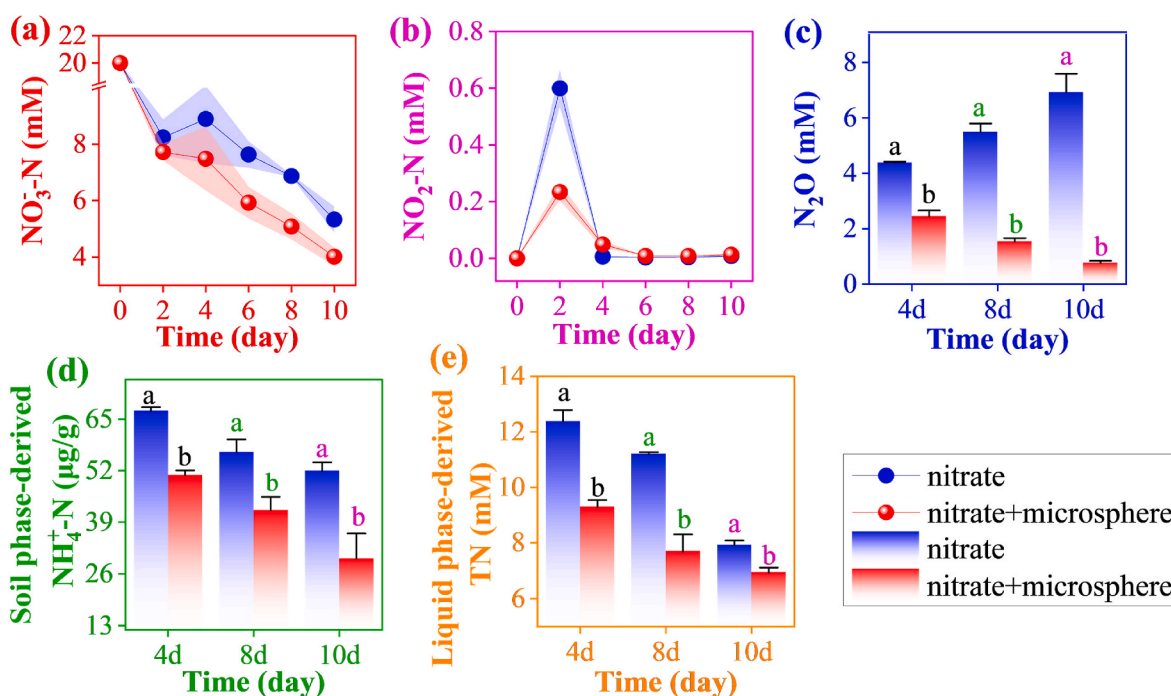


Fig. 3. Concentrations of dissolved NO_3^- -N (a), NO_2^- -N (b), N_2O production (c), solid-phase NH_4^+ -N (d), and liquid-phase TN (e) in the nitrate + microsphere combined treatment and nitrate-alone treatment. Different lower-case letters indicate treatments are significantly different at $P < 0.05$. ($n = 3$, error bars and shaded area are SD, and CK denotes the control group).

Table 1

Summary of reaction equations and related standard Gibbs free energy for AOM and denitrification reactions as regulated by different electron donors/acceptors.

Process	Electron acceptor/ donor	Reaction equation	Eq.	ΔG^0 (kJ/mol)	Ref.
Electron acceptor					
Anammox	NO_2^-	$\text{NH}_4^+ + \text{NO}_2^- \rightarrow 2 \text{H}_2\text{O} + \text{N}_2$	1	−335	Ding et al. (2021)
Feammox	Fe(III)	$11 \text{FeOOH} + \text{NH}_4^+ + 23 \text{H}^+ \rightarrow 11 \text{Fe}^{2+} + \text{NO}_3^- + 19 \text{H}_2\text{O}$	2	−38	Yi et al. (2019)
	Fe(III)	$9 \text{FeOOH} + \text{NH}_4^+ + 19 \text{H}^+ \rightarrow 9 \text{Fe}^{2+} + \text{NO}_2^- + 16 \text{H}_2\text{O}$	3	−71	Desireddy and Pothanamkandathil Chacko (2021)
	Fe(III)	$6 \text{FeOOH} + 10 \text{H}^+ + 2 \text{NH}_4^+ \rightarrow 6 \text{Fe}^{2+} + 12 \text{H}_2\text{O} + \text{N}_2$	4	−233	Desireddy and Pothanamkandathil Chacko (2021)
	Mn(IV)	$4 \text{MnO}_2 + \text{NH}_4^+ + 6 \text{H}^+ \rightarrow 4 \text{Mn}^{2+} + \text{NO}_3^- + 5 \text{H}_2\text{O}$	5	−269	Cheng et al. (2022)
Mnammx	Mn(IV)	$3 \text{MnO}_2 + \text{NH}_4^+ + 4 \text{H}^+ \rightarrow 3 \text{Mn}^{2+} + \text{NO}_2^- + 4 \text{H}_2\text{O}$	6	−195	Cheng et al. (2022)
	Mn(IV)	$3 \text{MnO}_2 + 2 \text{NH}_4^+ + 4 \text{H}^+ \rightarrow 3 \text{Mn}^{2+} + \text{N}_2 + 6 \text{H}_2\text{O}$	7	−553	Cheng et al. (2022)
	Mn(IV)	$3 \text{MnO}_2 + 2 \text{NH}_4^+ + 4 \text{H}^+ \rightarrow 3 \text{Mn}^{2+} + \text{N}_2 + 6 \text{H}_2\text{O}$	7	−553	Cheng et al. (2022)
AOM	Electron acceptor				
	NO_3^-	$5 \text{CH}_4 + 8 \text{NO}_3^- + 8 \text{H}^+ \rightarrow 5 \text{CO}_2 + 4 \text{N}_2 + 14 \text{H}_2\text{O}$	8	−765	Yao et al. (2024)
	NO_2^-	$3 \text{CH}_4 + 8 \text{NO}_2^- + 8 \text{H}^+ \rightarrow 3 \text{CO}_2 + 4 \text{N}_2 + 10 \text{H}_2\text{O}$	9	−928	Raghoebarsing et al. (2006)
	N_2O	$\text{CH}_4 + 4 \text{N}_2\text{O} \rightarrow \text{CO}_2 + 4 \text{N}_2 + 2 \text{H}_2\text{O}$	10	−1232	Cheng et al. (2019)
	Fe(III)	$\text{CH}_4 + 8 \text{Fe}^{3+} + 3 \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + 8 \text{Fe}^{2+} + 9 \text{H}^+$	11	−418	Beal et al. (2009)
	Mn(IV)	$\text{CH}_4 + 4 \text{MnO}_2 + 7 \text{H}^+ \rightarrow \text{HCO}_3^- +$	12	−494	He et al. (2018)

Table 1 (continued)

Process	Electron acceptor/ donor	Reaction equation	Eq.	ΔG^0 (kJ/mol)	Ref.
Electron acceptor					
Denitrification	As(III) [−]	$4 \text{Mn}^{2+} + 5 \text{H}_2\text{O} + \text{CH}_4 + 4 \text{HAsO}_4^{2-} + 3 \text{H}^+ \rightarrow \text{HCO}_3^- + 4 \text{H}_2\text{AsO}_3^- + \text{H}_2\text{O}$	13	−300	Shi et al. (2020)
	Electron donor				
	As(III)	$5 \text{H}_3\text{AsO}_3 + 2 \text{NO}_3^- \rightarrow 5 \text{HAsO}_4^{2-} + 8 \text{H}^+ + \text{H}_2\text{O} + \text{N}_2$	14	−9	Li et al. (2020)
	Fe(II)	$11 \text{Fe}^{2+} + 16 \text{H}_2\text{O} + 2 \text{NO}_3^- \rightarrow 11 \text{FeOOH} + 21 \text{H}^+ + \text{N}_2$	15	−483	Sun et al. (2016)
	Mn(II)	$5 \text{Mn}^{2+} + 2 \text{NO}_3^- + 4 \text{H}_2\text{O} \rightarrow 5 \text{MnO}_2 + \text{N}_2 + 8 \text{H}^+$	16	−143	Swathi et al. (2017)
	CH_4	$5 \text{CH}_4 + 8 \text{NO}_3^- + 8 \text{H}^+ \rightarrow 5 \text{CO}_2 + 4 \text{N}_2 + 14 \text{H}_2\text{O}$	17	−765	Cui et al. (2015)

combined versus nitrate treatment (74 % vs. 52 %). Notably, humic substances possess electron-shuttling quinone groups that can serve as a bridge to enlarge the extracellular electron transfer-connected network. This network favors the co-occurrences of enhanced dissimilatory Fe (III)/Mn(IV) reduction and humus-mediated AOM (Chen et al., 2016; Liu et al., 2023). The microspheres are comprised of birnessite and biochar that can fuel AOM through AOM-MnR and humus-mediated AOM (Valenzuela et al., 2019; Xie et al., 2024). This infers that the enhanced AOM performance in the combined treatment (Fig. 4b) was mainly attributed to the incorporation of AOM-MnR and that humus-mediated AOM promoted the DAOM pathway dominant in the nitrate treatment.

A large amount of Fe(II) and Mn(II) might be generated from the pathways involved in AOM coupled with Fe(III) reduction (AOM-FeR)/AOM-MnR and/or dissimilatory Fe(III)/Mn(IV) reduction mediated by humus (Dang et al., 2021; Valenzuela et al., 2019). The generated Fe(II) and Mn(II) might coprecipitate with carbonate formed from the CO_2 generated by AOM to form poorly-soluble carbonate precipitates (e.g., FeCO_3 and MnCO_3) (Valenzuela et al., 2019). This mechanism is supported by the suppressed Fe(II) and Mn(II) concentrations (Fig. 2b and Fig. S2), as well as the diminished CO_2 emission exhibited in the combined treatment (Fig. 4c). The suppressed CO_2 emission from the combined treatment might also result from the microsphere-derived biochar components decreasing organic C mineralization and/or enhancing microbial carbon-use efficiency (CUE) (Riaz et al., 2017). Overall, these findings confirm that the combined treatment achieved diminished CH_4 and CO_2 emissions through promoting integrated AOM pathways and

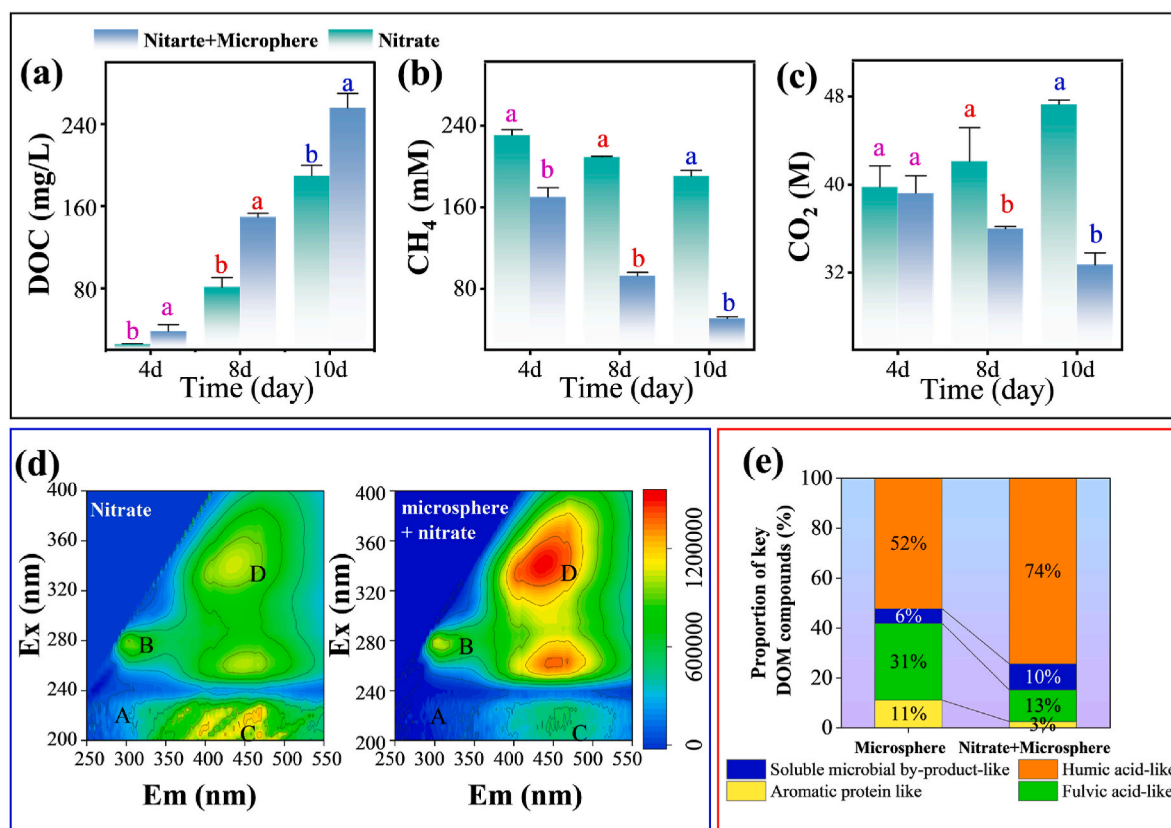


Fig. 4. The biogeochemical cycle of C comprising the concentrations of residual TOC (a), CH₄ emission (b) and CO₂ emission (c), and EEM fluorescence spectroscopy sampled on the 8th day of incubation (d), as well as the related fraction for FRI values of DOM in corresponding EEMs (e) derived from different treatments. The letters of A to D in the EEMs represent the key DOM compounds (Table S4). Different lower-case letters with the same color indicate treatments are significantly different at $P < 0.05$; $n = 3$; error bars indicate SD. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

enhancing C sequestration.

3.5. Activities of key enzymes involved in metal- and CH₄-dependent denitrification

Denitrification involves electrons produced from oxidation of electron donors by a series of denitrifying reductases (NAR, NIR, NOR and NOS) associated with the different steps of nitrate reduction (Wang et al., 2024a). Meanwhile, the activity of Mcr largely influences the oxidation of CH₄ in the reverse methanogenesis pathway of DAOM-associated denitrification by ANME (Yu et al., 2022). In this work, As(III) and CH₄ were the key available electron donors present to

drive the processes of autotrophic and heterotrophic denitrification (i.e., NRAO and DAOM). Specifically, the combined nitrate + microsphere treatment led to suppression of key enzyme activities consisting of NAR, NIR, NOR, NOS and Mcr (Fig. 5). This implies that an inefficient transfer of electron flux occurred in NRAO/DAOM-associated denitrification processes that would theoretically lead to larger amounts of nitrate and CH₄ in the combined versus nitrate treatment. However, we found lower amounts of residual nitrate (Fig. 3a) and CH₄ (Fig. 4b) in the combined treatment, which conflicts with this prediction.

Therein, we posit that some nitrate was consumed in the combined treatment for replenishing MnO_x through nitrate-reducing Mn(II)/Fe(II) oxidation (NRMO/NRFO) pathways (Desireddy and Pothanamkandathil

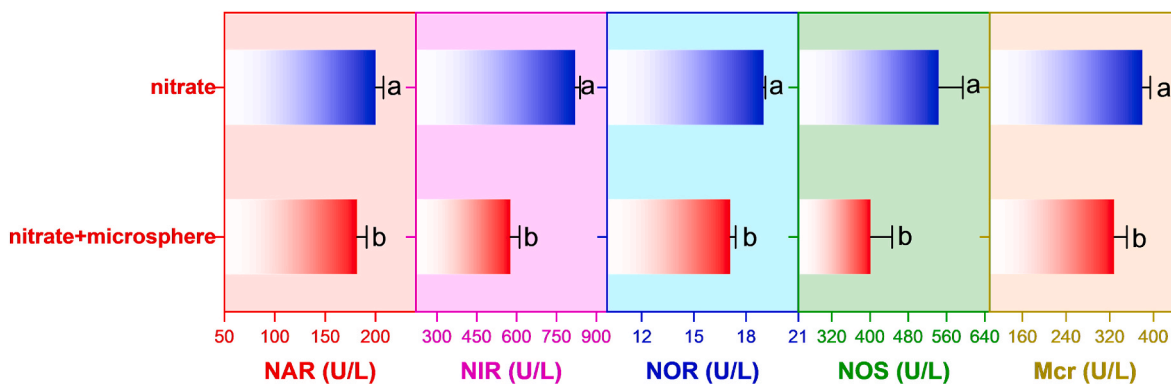


Fig. 5. Activities of the denitrifying enzymes (a–d) and Mcr (e) under different treatment conditions ($n = 3$; error bars indicate SD). Different lower-case letters indicate treatments are significantly different at $P < 0.05$.

Chacko, 2021). In particular, 4.0 mM nitrate remained on the 10th day of incubation (Fig. 3a) promoting the occurrence of NRMO/NRFO, which is consistent with the lower dissolved Mn(II)/Fe(II) concentrations found in the combined treatment (Fig. S2 and Fig. 2b). The regenerated $\text{MnO}_x/\text{FeO}_x$ might further participate in AOM-MnR/AOM-FeR leading to a decline in CH_4 emission for the combined treatment (Fig. 4b). Since the occurrences of AOM-MnR/AOM-FeR and Mnammox/Feammox are thermodynamically feasible (Table 1), the AOM-MnR/AOM-FeR and/or Mnammox/Feammox reactions could supply the Mn(II)/Fe(II) precursors required for the subsequent NRMO/NRFO reactions. Finally, the redox recycling reactions involved in the formation of $\text{MnO}_x/\text{FeO}_x$ within the combined treatment resulted in CH_4 removal through the AOM-MnR and AOM-FeR pathways.

3.6. Assessment of nitrate + BC@ MnO_2 microspheres on simultaneous suppression of As(III) mobilization and GHG emissions

XPS characterization was employed to identify the speciation changes of As/Fe/Mn/O/C occurring on the surface of the microspheres in the nitrate + microsphere treatment after incubating for 10 days. For the As 3d spectrum (Fig. 6a), the integral area for the As(V) fitted peak at 45.6 eV was significantly larger than the peak for As(III) at 44.8 eV (86 % vs. 14 %, Table S5) (Dong et al., 2021). This confirms the effective

oxidation of mobile As(III) to As(V) by birnessite. Likewise, the generation of a Fe(III) peak (710.8 eV) in the Fe 2p spectrum with an integral area of $\sim 48\%$ (Fig. 6b) supports the oxidation of Fe(II) that formed from the microbial reduction of sediment-derived Fe(III)-oxides. Notably, representative peaks for Mn(II) and Mn(III) located at 641.6 and 641.9 eV (Dong et al., 2021) were present in the Mn 2p spectrum (Fig. 6c), indicating that Mn(III) species were produced during redox reactions with Mn(IV)/Mn(II). Peaks for C-H, C-O, C=O and CO_3^{2-} were located at 284.8, 286.6, 288.3 and 289.1 eV (Prucek et al., 2013) in the C1s spectrum (Fig. 6d), respectively, revealing generation of organic C compounds and carbonate deposits on the surface of the microspheres. The O1s spectra indicated generation of H_2O (30 %), -OH (38 %) and O^{2-} (32 %) groups at 531.9, 531.2 and 530.0 eV (Hou et al., 2019) (Fig. 6e), implying that these oxygen-bearing groups might participate in As/Fe/Mn immobilization or organic C sequestration through formation of Mn/Fe-(hydr)oxides or mineral-DOM complexes (Hou et al., 2019).

SEM images demonstrated that the microsphere granules were coated with abundant flocculent precipitates after incubating for 10 days (Fig. 1e vs. Fig. 6f). The overlapping precipitates are likely aggregated biofilms formed during incubation, inferring that the combined treatment favors the colonization of biofilms that are likely enriched with the key functional microorganisms involved in As/Fe/Mn/C/N biotransformations. According to experiment I, we preliminarily

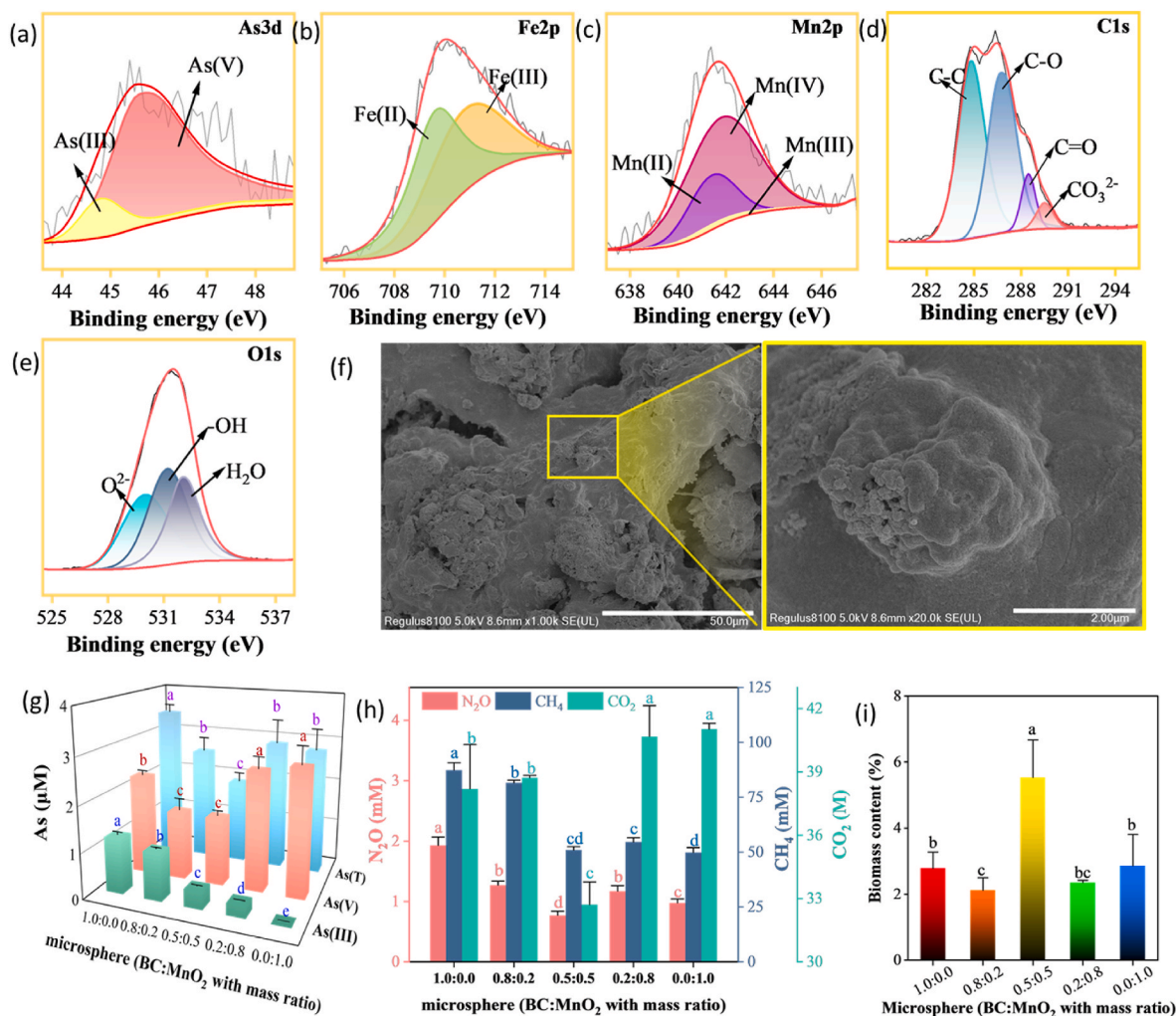


Fig. 6. XPS spectra showing speciation of adsorbed As (a), Fe (b), Mn (c), C (d) and O (e) on the BC@ birnessite microspheres separated from the nitrate + microsphere treatment. SEM characterization (f) of microsphere morphology separated after incubation. As(III) immobilization (g), GHG emissions (h) and residual microbial biomass (i) on microspheres of variable biochar-to-birnessite ratios following a 10-day incubation.

conclude that the combined nitrate + BC@MnO₂ microsphere treatment achieved the desirable objectives of simultaneously suppressing As(III) mobilization and GHG emissions in flooded paddy soils based on the 10-day incubation results. Next, we assessed the effects of the BC-to-birnessite ratio of the microspheres (Experiment II) on the performance of the combined treatment to suppress As(III) mobilization and GHG emissions.

Because of the strong adsorption and oxidation of As(III) by MnO₂, the microspheres assembled with greater amounts of birnessite commonly led to lower concentrations of As(III) and larger concentrations of As(V) in the flooded layer (Fig. 6g). As estimated from the residual As(T) concentration after incubating for 10 days (Fig. 6g), microsphere C exhibited the optimum composition for suppressing As(III) mobilization (only 1.89 μM As(T) remained) among all nitrate + microsphere treatments (2.56–3.44 μM As(T) remained in other formulations). Likewise, the suppression of GHG production (CO₂, CH₄ and N₂O) was most effective for the combined treatment with microsphere C (Fig. 6h). Emissions of CO₂, CH₄ and N₂O derived from the nitrate + microsphere C treatment declined by 14–20 %, 6–42 % and 21–60 % when compared with other microsphere compositions, respectively.

Given the crucial role that microorganisms play in modulating As(III) immobilization and GHG emissions, BCA assays were conducted to estimate the extent of biofilm colonization on different microspheres. BCA assays (Fig. 6i) confirmed that microsphere C accumulated the largest microbial biomass among all microsphere formulations, suggesting that the habitat formed by the biochar-birnessite mixture with a mass ratio of 1:1 was the most efficient for promoting biofilm colonization. Thus, the nitrate + microsphere treatment promotes biofilm formation that, in part, contributes to suppression of As(III) mobilization and GHG

emissions. Hence, the composition and key gene expression of the microbial community present in the biofilms should reflect the overall remediation effect.

3.7. Composition and functional genes of microbial communities

3.7.1. Sensitivity of microbial community toward nitrate and/or BC@MnO₂ microspheres

Metagenomic sequencing revealed that *Pseudomonadota* (20–77 %), *Actinomycetota* (10–13 %), *Bacillota* (7–25 %) and *Thermodesulfobacteriota* (5–14 %) were the dominant phyla among bacteria, whereas *Euryarchaeota* (20–29 %) incorporating the majority of methanogens was the dominant phylum for archaea across all treatments (Fig. 7a). Specifically, *Pseudomonadota* and *Bacillota* were highly enriched in assays with nitrate, whereas the abundance of *Actinomycetota* decreased in the presence of microspheres. Further, abundances of key genera in the archaea and bacteria domains were analyzed based on their role in regulating processes associated with C/N/Fe/Mn/As cycling (Fig. 7b). The bubble plot of RPKM abundances for genera revealed that the microsphere treatment led to an enrichment of several metal-reducing bacteria (e.g., *Bacillus*, *Geobacter* and *Desulfuromonas*) responsible for As(V)/Fe(III)/Mn(IV) reduction. This might be ascribed to the biochar particles aggregated in the microsphere that have been shown to foster the enrichment of metal-reducing bacteria (Chen et al., 2016).

Compared with the distribution of metal-oxidizing bacteria, we found that abundances of key autotrophic denitrifiers, such as *Streptomyces*, *Pseudomonas*, *Methylobacterium* and *Cupriavidus*, capable of utilizing nitrate as an electron acceptor for As(III)/Fe(II)/Mn(II) oxidation (Wang et al., 2024a) increased with the addition of nitrate and their

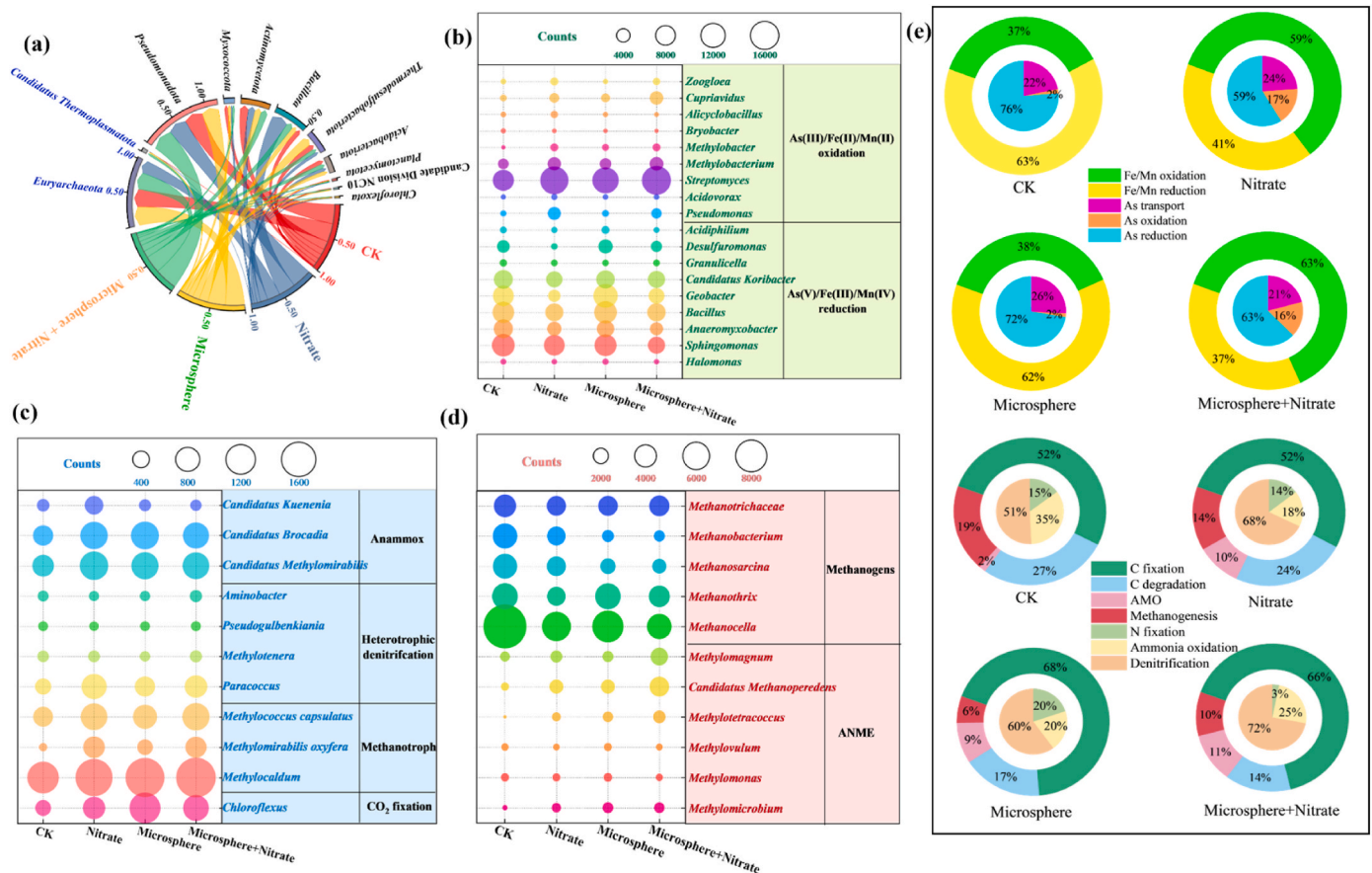


Fig. 7. Composition of microbial communities at phylum level (a), RPKM (reads per kilobase million) abundances of key genera involved in As/Fe/Mn cycling (b) and C/N cycling within the bacterial domain (c), as well as methanogenesis and AOM processes within the archaeal domain (d). The proportions of functional genes (e) were calculated for a defined microbial metabolism pathway associated with As-Fe-Mn-C-N cycling.

abundances were highest in the nitrate + microsphere treatment. The enrichment of these autotrophic denitrifiers in the nitrate + microsphere treatment promoted autotrophic full denitrification by utilizing Fe (II)/Mn(II) as available electron donors, thereby attenuating N₂O production. The key metal-oxidizing genera involved in the immobilization of As/Fe/Mn were sensitive to additions of nitrate and microspheres providing a mechanistic explanation for attenuating As(III) immobilization in the nitrate + microsphere treatment.

Emissions of N₂O and CH₄ were strongly linked to the bioavailability of nitrate and As(III)/Fe(II)/Mn(II) by different microorganisms (Fig. 7b to d). The high abundance of anammox bacteria (e.g., *Candidatus Methyloirambis* and *Candidatus Brocadia*, Fig. 7c) infer the possibility of anammox for attenuating N₂O emission. Moreover, we found that the abundances of heterotrophic denitrifiers were generally low. This was attributed to the lack of organic substrates necessary for ensuring the metabolism of heterotrophic denitrifiers, which further contributes to the lack of full denitrification by the heterotrophic denitrifiers.

In flooded paddy soils, CH₄ emission is influenced by both methanogenesis and AOM. The addition of nitrate and/or microspheres decreased the abundance of methanogens (Fig. 7d) based on the decrease in the overall abundance of archaeal genera. This phenomenon might be ascribed to the biochar and nitrate amendments decreasing methanogenic archaea abundance in flooded paddy soils (Luo et al., 2021; Qi et al., 2021). AOM is carried out by ANME and bacteria or specific ANME-bacteria consortia that can utilize various electron acceptors (Fan et al., 2021). Interestingly, the addition of nitrate and/or microspheres boosted the enrichment of several methanotrophic bacteria that can perform DAOM alone (Fig. 7c) or ANME (Fig. 7d) via a syntrophic partnership between ANME and metal-reducing bacteria/denitrifiers or through mediation by humic substances (Valenzuela et al., 2019; Xie et al., 2024). The most abundant ANME genus in the nitrate + microsphere treatment was *Candidatus Methanoperedens* (ANME-2d). It is capable of catalyzing nitrate/Fe(III)/Mn(IV)-dependent AOM via a syntrophic microbial consortium (Cai et al., 2018; Leu et al., 2020), highlighting its role in regulating Mn/Fe/nitrate cycling linked to CH₄ consumption. The microsphere-derived treatments (with/without nitrate) led to an enrichment of *Chloroflexus*, which is responsible for autotrophic CO₂ fixation (Freches and Fradinho Joana, 2024), thereby providing a pathway for CO₂ sequestration. Overall, the enrichment of key microorganisms in specific ANME-bacteria consortia of the nitrate + microsphere treatment was essential to fuel CH₄ and N₂O consumption in the anoxic paddy soils.

Fig. S3 illustrates the composition of microbial communities in soils resulting from the nitrate + microsphere treatments receiving microspheres of differing BC-to-birnessite ratios. The combined treatment with a greater content of birnessite appeared to promote enrichment of metal-oxidizing bacteria (e.g., *Streptomyces*, *Pseudomonas*), while decreasing the abundance of some methanogens (e.g., *Methanocella*, *Methanotrichaceae*). Among all the nitrate + microsphere treatments, microsphere C was most favorable in promoting the growth of key microorganisms involved in NRFO/NRMO and AOM, and in inhibiting the growth of methanogens. The difference in biodiversity reflects the specific bio-affinity induced by the biochar and birnessite components.

3.7.2. Abundances of key functional genes involved in As/Fe/Mn/C/N cycling

Relative abundances of key genes encoding As/Fe/Mn/C/N cycling functions (Table S6, Fig. S3 and Fig. S4) as annotated by the KEGG Orthologs database were collectively analyzed (Fig. 7e). Therein, the abundances of genes involved in Fe/Mn oxidation (e.g., *ydb*, *cot*, *mox*, *mnx* and *mco*; Fig. S4) were commonly lower in nitrate-free microcosms than nitrate-supplemented microcosms (37–38 % vs. 59–63 %, Fig. 7e). Likewise, the proportions of As oxidation genes (i.e., *aox* in Fig. S4) were also higher in nitrate-supplemented microcosms, accounting for 16–17 % of total As functional genes. These examples highlight that nitrate serves as a key factor in sustaining beneficial conditions for microbial

As/Fe/Mn oxidation processes. As/Fe/Mn (im)mobilizations are commonly correlated with C/N cycling processes that are regulated by autotrophic/heterotrophic microorganisms (Couture et al., 2015). With respect to N metabolism, we found that the abundances of key genes encoding for ammonia oxidation and denitrification processes in the nitrate + microsphere treatment (25 % and 72 %) were the highest among all treatments. This infers that MnammoX/Feammox are active to produce Mn(II)/Fe(II) and successively coupled to nitrate reduction via the NRMO/NRFO pathways to regenerate MnO_x/FeO_x. These integrated processes are essential for sustaining As(III) immobilization and ensuring full denitrification to mitigate N₂O emission.

Concerning microbial participation in C cycling processes, the addition of nitrate and/or microspheres resulted in up-regulation to the transcript level of the *pmo* gene responsible for AOM as compared to the control group (9–11 % vs. 2 %, Fig. 7e). Enrichment of the *pmo* gene as modulated by nitrate and/or microsphere treatments enhances the AOM performance to attenuate CH₄ emission. Moreover, C fixation genes (e.g., *fdh*, *ato*, *pyc*, *frd*, etc.; Fig. S4) represented a dominant proportion (52–68 %) of C functional genes among all treatments. The related abundances were higher (66–68 %) in the microsphere-supplemented microcosms than the microsphere-free microcosms (~52 %). This highlights the crucial role of biochar components in promoting C fixation and possible carbon sequestration (Zheng et al., 2022). This may further explain the mechanisms contributing to suppressed CO₂ emission in nitrate + microsphere treatments versus the treatment with nitrate alone or microspheres alone.

The proportion of functional genes for defined microbial metabolism pathways associated with As/Fe/Mn/C/N cycling followed a somewhat similar pattern among all nitrate + microsphere treatments (Fig. S5 and Fig. S6), suggesting mediation by birnessite and biochar together. Nevertheless, the nitrate + microsphere C treatment achieved the greatest microbial biomass (Fig. 6i) and enrichment of key functional microorganisms (Fig. S3) among all the combined treatments, thereby achieving the highest performance for suppressing As(III) mobilization and GHG emissions.

3.8. Merits of combined nitrate + microsphere treatment for simultaneous suppression of As(III) mobilization and GHG emissions

In this study, we fabricated a novel functionalized biochar@birnessite composite microsphere for use with and without nitrate to simultaneously mitigate As(III) pollution and GHG emissions from flooded paddy soils (Fig. 8a). The nitrate + microsphere treatment promoted the colonization of biofilms enriched with key microbial taxa responsible for As/Fe/Mn/C/N biotransformation on the microsphere surface. Moreover, the competitive resource utilization strategies among the diverse and metabolically active microorganisms optimized the biogeochemical pathways associated with As(III) immobilization and GHGs emission by attenuating overlap in resource use (Wu et al., 2024). In flooded paddy soils, As(III) pollution is dynamically controlled by the turnover of As(III) mobilized in flooded layer and reductive dissolution of As(V) in the sediment. Consequently, As-remediation strategies require a two-step approach involving an initially rapid immobilization of As(III) followed by sequestration of As(V) over long time periods. Notably, As(III) immobilization relying on abiotic adsorption or microbial NRAO is generally short term (<4 days) (Wang et al., 2024a). Thus, for long-term As(III) remediation success, the removal of As(III) through abiotic adsorption/oxidation or NRAO treatment alone is not a sustainable strategy (Fig. 8b). The full denitrification derived from NRFO/NRMO is more thermodynamically competitive than the incomplete denitrification derived from NRAO, and also results in effective As(III) immobilization through adsorption/oxidation by FeO_x/MnO_x. In contrast, NRAO results in an appreciable fraction of incomplete denitrification generating N₂O emission. Moreover, the regenerated FeO_x/MnO_x inhibits reductive dissolution of As(V)/Fe(III) in sediments wherein FeO_x/MnO_x derived from Feammox/MnammoX contributes to

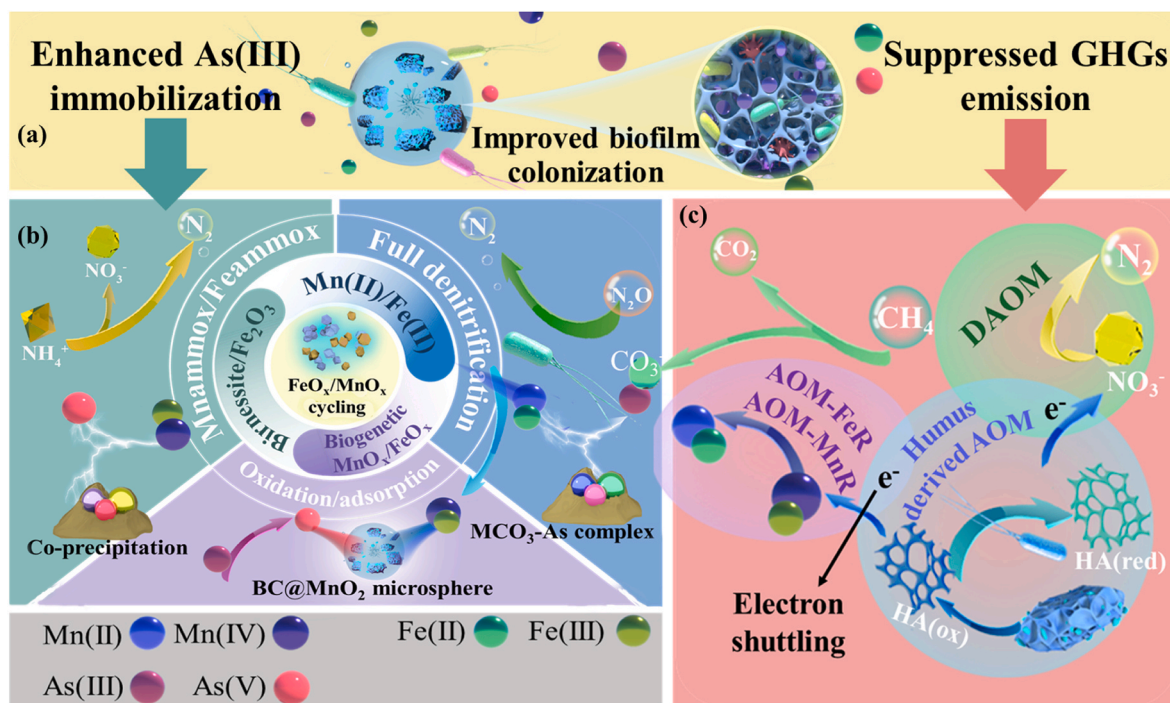


Fig. 8. A Schematic diagram illustrating the functions (a) of the composite microsphere components derived from nitrate + microsphere treatment with respect to suppressing As(III) mobilization (b) and GHG emissions (c).

As(III) immobilization.

In addition to suppression of N_2O emission through NRFO/NRMO-derived full denitrification and/or anammox pathways, the nitrate + microsphere treatment attenuated CH_4 emission and promoted CO_2 sequestration (Fig. 8c). Because nitrate is a thermodynamically favorable electron acceptor for AOM, the presence of nitrate effectively decoupled the AOM-AsR pathway, thereby eliminating the negative feedback of As(III) mobilization and promoting AOM. The regenerated components from the biochar-derived humus and MnO_x in the microspheres also participated in AOM (Xie et al., 2024), providing multiple-linkages within the AOM network to promote CH_4 consumption. Some of the CO_2 produced by AOM was precipitated in the form of carbonate minerals (confirmed by the existence of CO_3^{2-} in XPS characterization in Fig. 6d), possibly coprecipitated with dissolved Fe(II) , Ca(II) and/or Mg(II) to form poorly-soluble carbonate precipitates. As suggested by the previous investigations (Han et al., 2007; Neuberger and Helz, 2005), some carbonates may potentially form immobile As(III)-carbonate complexes as well.

The nitrate + microsphere treatment further fostered the colonization of biofilm communities on the surface of the microspheres that are enriched with functional microorganisms that contribute to AOM-MnR/AOM-FeR and NRMO/NRFO. The enrichment of functional microorganisms and coexistence with nitrate ensured successive regeneration/recycling of $\text{FeO}_x/\text{MnO}_x$ contributing to As(III) immobilization in the flooded layer. Moreover, the decreased C/N ratio and C mineralization associated with the nitrate and biochar components in the combined treatment could improve microbial CUE, which correspondingly enhances CO_2 sequestration (Sinsabaugh et al., 2016). Overall, the combined nitrate + biochar@birnessite treatment created a favorable microbial and chemical habitat to promote the complex interplay among microbial interactions linked to C/N/Mn/Fe cycling, which in turn provided an effective remediation strategy to suppress As(III) mobility and GHG emissions in flooded paddy soils.

There is paucity of the information concerning remediation strategies that simultaneously address As(III) mobilization and GHG (CO_2 , CH_4 and N_2O) emissions from flooded paddy soils. Compared to previous findings that targeted As(III) pollution or $\text{N}_2\text{O}/\text{CH}_4$ emission alone

(Shi et al., 2022; Wang et al., 2024a, 2024b; Yang et al., 2022), our study highlights the merits of biochar@birnessite and its combined effects with nitrate to concurrently suppress As(III) immobilization and $\text{CO}_2/\text{CH}_4/\text{N}_2\text{O}$ emissions in flooded paddy soils. Our study clearly demonstrates the efficacy of the combined treatment to achieve the desirable remediation effects with mobile As(III) completely immobilized and N_2O , CH_4 and CO_2 emissions declining by 89 %, 73 % and 31 % after incubating for 10 days, respectively. Given the considerable concerns raised by elevated As(III) pollution and GHG emissions from flooded paddy soils in recent years, the combined treatment developed in our study is worthy of further field evaluation as an effective, alternative remediation strategy to address food safety and climate warming associated with flooded paddy soils.

4. Conclusions

In this study, novel functionalized biochar@birnessite composite microspheres were fabricated through assembling with biochar, birnessite and SA components. Subsequently, the effects and the mechanisms of nitrate + microsphere treatment on suppressing As(III) mobilization and GHG emissions from flooded paddy soils were systematically examined. Therein, an appropriate amount of nitrate supplied in the combined treatment was crucial to achieve a reliable remediation effect. Meanwhile, the combined treatment fosters the colonization of biofilms enriched with functional microorganisms contributing to microbial oxidation of $\text{Fe(II)}/\text{Mn(II)}$ and AOM. The combined treatment allows the regeneration of $\text{MnO}_x/\text{FeO}_x$ via coupling with Mnamox/Feammox and NRMO/NRFO under NH_4^+ -enriched conditions. The regenerated $\text{MnO}_x/\text{FeO}_x$ facilitated the immobilization of mobile As(III) in flooded layer via oxidation/adsorption/coprecipitation as well as attenuated the bioreduction of $\text{Fe(III)}/\text{As(V)}$ in sediments. Additionally, the combined treatment activated the microbial transformations involved in NRMO/NRFO-derived full denitrification and AOM (such as DAOM, humus-derived AOM, AOM-MnR and AOM-FeR) reactions, wherein N_2O was completely reduced to N_2 while CH_4 was oxidized to CO_2 . Further, some of resulting CO_2 was sequestered by the formation of poorly-soluble carbonate minerals that might further

react with As(III) to form insoluble carbonate-As(III) complexes. Overall, the remediation strategy developed in this study highlights the advantages of exploiting an environmental-friendly functionalized remediation material for concurrently addressing As pollution and GHG emissions in flooded paddy soils, while also realizing the benefits of easy access to raw materials, facile synthesis and high remediation efficiency attributes.

CRedit authorship contribution statement

XiYu Zhao: Writing – original draft, Investigation. **Yilin Chen:** Methodology, Investigation. **Jiehua Hu:** Resources, Project administration. **Honghui Wang:** Conceptualization. **Zilu Ye:** Formal analysis. **Jing Zhang:** Software, Resources. **Jun Meng:** Resources, Conceptualization. **Jiale Li:** Data curation, Conceptualization. **Randy A. Dahlgren:** Writing – review & editing, Validation, Supervision. **Shuyun Zhang:** Formal analysis. **Hui Gao:** Supervision, Funding acquisition. **Zheng Chen:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2025.121757>.

Data availability

Data will be made available on request.

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