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Permalink

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Journal

Pedosphere, 34(6)

ISSN

1002-0160

Authors

Feng, WANG

ZHANG, Jing

Yanqiong, ZENG

et al.

Publication Date

2024-12-01

DOI

10.1016/j.pedsph.2023.09.008

Peer reviewed



Arsenic mobilization and nitrous oxide emission modulation by different nitrogen management strategies in a flooded ammonia-enriched paddy soil

Feng WANG¹, Jing ZHANG⁴, Yanqiong ZENG¹, Honghui WANG⁴, Xiyu ZHAO¹, Yilin CHEN¹, Huanhuan DENG¹, Liyun GE¹, Randy A. DAHLGREN^{1,3}, Hui GAO^{2,*} and Zheng CHEN^{1,4,*}

¹Zhejiang Provincial Key Laboratory of Watershed Science & Health, Wenzhou Medical University, Wenzhou 325035 (China)

²Key Laboratory of Fertility Preservation and Maintenance of Ministry of Education, Ningxia Medical University, Yinchuan 750004 (China)

³Department of Land, Air & Water Resources, University of California, Davis (UC Davis), Davis CA 95616 (USA)

⁴School of Environmental Science & Engineering, Tan Kah Kee College, Xiamen University, Zhangzhou 363105 (China)

(Received March 20, 2023; revised August 8, 2023; accepted September 7, 2023)

ABSTRACT

Elevated arsenic (As) mobilization and increased nitrous oxide (N₂O) emission are two primary environmental concerns existing in flooded paddy soils. In this study, dissolved As(III), N isotope-labeled Na¹⁵NO₃, and/or ¹⁴NH₄Cl were incorporated into a microcosm incubation to determine the effects of N fertilization regimes on As mobilization and N₂O emission in a flooded paddy soil. Because nitrate had a higher redox potential and comprised a higher proportion of N substrate, As(V) was preferentially formed due to enhanced nitrate-dependent microbial As(III) oxidation. Thus, As availability was correspondingly attenuated due to the improved production of less mobile and toxic As(V). After 2-d incubation, more than 90% and 98% of soluble As(III) were immobilized in the As + NH₄⁺-N + NO₃⁻-N and As + NO₃⁻-N treatments, respectively. Following nitrate depletion (after 2 d), microbial As(V) and Fe(III) reductions were gradually enhanced, which was attributed to stimulation of anaerobic ammonium oxidation (anammox) coupled to Fe(III) reduction, known as Feammox, by the abundance of NH₄⁺. By the end of the incubation period (10 d), the As + NO₃⁻-N treatment led to higher As immobilization of originally added As(III) (ca. 61%) than the As + NH₄⁺-N + NO₃⁻-N treatment (42%). The As + NH₄⁺-N + NO₃⁻-N treatment prominently mitigated N₂O emission compared to the As + NO₃⁻-N treatment, which was ascribed to anammox, inducing accumulation of byproducts from incomplete denitrification. High-throughput sequencing indicated that the relative abundances of denitrifiers (*e.g.*, *Azoarcus*, *Ochrobactrum*, and *Thiobacillus denitrificans*) increased in the As + NO₃⁻ treatment, whereas quantitative polymerase chain reaction results indicated higher 16S rRNA gene copy numbers for anammox and Feammox (Acidimicrobiaceae bacterium A6) bacteria in the As + NH₄⁺ + NO₃⁻ treatment. Collectively, the experimental results demonstrated that N fertilization can be a feasible As remediation strategy while providing an effective strategy for mitigating N₂O emission from paddy soils at the same time.

Key Words: anammox bacteria, Feammox bacteria, flooded paddy soil, microbe, ¹⁵N-isotope tracing technique, redox reaction

Citation: Wang F, Zhang J, Zeng Y Q, Wang H H, Zhao X Y, Chen Y L, Deng H H, Ge L Y, Dahlgren R A, Gao H, Chen Z. 2024. Arsenic mobilization and nitrous oxide emission modulation by different nitrogen management strategies in a flooded ammonia-enriched paddy soil. *Pedosphere*. 34(6): 1051–1065.

INTRODUCTION

Arsenic (As) pollution in paddy soils is of great global concern owing to its effects on food safety and human health (Hussain *et al.*, 2021). Arsenic exists in soils primarily as inorganic species (*e.g.*, HAsO₃²⁻ and HAs(V)O₄²⁻) and their existence is closely associated with iron (Fe) redox reactions through adsorption/coprecipitation on the surface of Fe(III)-(hydro)oxides (Gorny *et al.*, 2015). Compared with As(V), soluble As(III) possesses higher mobility, phytoavailability, and toxicity (Gorny *et al.*, 2015), enabling its pervasive uptake and accumulation by rice plants and greatly elevating its environmental risks (Dong *et al.*, 2021).

Due to long-term irrigation, paddy fields commonly sustain flooded-anoxic conditions that promote the metabolic activities of anaerobic microbes (Chen *et al.*, 2017). Under long-term anaerobic conditions, the reductive dissolution of

As(V) and Fe(III)-bearing minerals driven by metal-reducing bacteria fosters the release of As(III) from paddy soils (Qiao *et al.*, 2019), which further increases the environmental risks of As in paddy soils. The complicated biotic/abiotic factors and processes interacting in paddy fields play a key role in As (im)mobilization in flooded agricultural systems (Hussain *et al.*, 2021). Thus, developing simple, economically feasible, and environment-friendly approaches for mitigating As-induced risks and attenuating the mobility and bioavailability of As(III) in submerged soils are urgently required for the control and remediation of As-contaminated paddy soils (Pothier *et al.*, 2020; Yuan *et al.*, 2023).

Among the various factors/processes affecting As (im)mobilization, N cycling in paddy soils has several biogeochemical linkages to As cycling (Hussain *et al.*, 2021). To ensure abundant rice production, excessive applications

*Corresponding author. E-mail: gh2010@126.com, chenzheng_new@163.com.

of N fertilizers are often added to paddy soils. This inevitably leads to high residual soil N levels, which in turn may contribute to surface water eutrophication, groundwater contamination, and greenhouse effects *via* nitrous oxide (N₂O) production (Zhang *et al.*, 2013; Wang *et al.*, 2020). Organic and inorganic N fertilizers, such as amide-N (*e.g.*, urea which hydrolyzes to ammonia) and ammonical N (*e.g.*, ammonium chloride), are the primary agricultural N fertilizers used in rice production (Cai *et al.*, 2023). These inputs of N fertilizer are partially volatilized (as NH₃) and partly oxidized to nitrate *via* the ammoxidation reaction (Bi *et al.*, 2017).

Previous investigations demonstrated that distinct As mobilization dynamics were generated by microbial processing of different N species under anoxic conditions. For instance, As mobility was suppressed in the presence of nitrate whereby highly mobile As(III) is microbially oxidized into less mobile As(V) through a coupled denitrification process (NO₃⁻ → NO₂⁻ → N₂O → N₂) mediated by nitrate-dependent As(III)-oxidizing bacteria (illustrated by Eqs. 5–7 in Table I) (Li *et al.*, 2020; Wu *et al.*, 2021). Conversely, As(III) mobilization was facilitated due to enhanced reductive dissolution of Fe(III)-(hydro)oxides in which the anaerobic ammonium oxidation (anammox) coupled to Fe(III) reduction, known as Feammox (illustrated by Eqs. 2–4 in Table I), mediated by Feammox bacteria which utilize Fe(III) as an electron acceptor and NH₄⁺ as an electron donor in dissimilatory Fe(III) reduction (Li *et al.*, 2019). These examples demonstrate the contrasting effects of various N species on As (im)mobilization in flooded soils. Notably, little is known regarding how key microbes preferentially utilize a given N species for energy metabolism while concomitantly mediating As biogeochemical processes affecting As (im)mobilization.

China is the largest N fertilizer consumer in the world (Wang and Lu, 2020; Zhang *et al.*, 2021). Long-term excessive input of N fertilizer over the past several decades has led

to high levels of N accumulation in agricultural soils that may trigger elevated N₂O emission through denitrification processes (Richards *et al.*, 2016). Although nitrate-based fertilization generally mitigates As(III)-induced environmental risks in paddy soils (Feng *et al.*, 2023), the denitrification resulting from excessive nitrate input intensifies the emission of N₂O (Eqs. 5–13 in Table I) (Liu *et al.*, 2017). N₂O is a potent greenhouse gas emitted from paddy soils having a global warming potential of *ca.* 298 and *ca.* 12 times that of CO₂ and CH₄, respectively (Hu *et al.*, 2012; Kraus *et al.*, 2016). A recent survey confirmed that paddy soils were a major source of soil-derived N₂O emission and N fertilizers and accounted for an estimated 54% of the increased terrestrial N₂O emission in the past decade (Tian *et al.*, 2019). Notably, anammox resulting from application of ammonia-based fertilizers might provide a feasible avenue to effectively suppress N₂O production in paddy soils as the nitrite resulting from incomplete denitrification and ammonia are neutralized as N₂ (Eq. 1 in Table I) (Ding *et al.*, 2021). However, ammonia-based fertilizers could retrigger the release of As(III) from flooded paddy soils through the Feammox pathway (Xiu *et al.*, 2020). Thus, sole N addition of either nitrate or ammonia might conflict with the objectives of simultaneously mitigating As(III) mobilization and N₂O emission. Considering the opposing environmental effects of nitrate and ammonia on As(III) immobilization *vs.* N₂O emission, a combined nitrate-ammonia treatment might represent an alternative N fertilization strategy that could address both As(III) mobilization and N₂O emission objectives. Moreover, As (im)mobilization and N₂O emission responses to different N fertilizer types might be distinct due to interactions between bacteria, dynamic N redox transformations, and dissolved organic matter (DOM) bioavailability (*e.g.*, endogenous refractory and/or exogenous labile DOM) that create potential synergistic/antagonistic effects (Hussain *et al.*, 2021).

TABLE I

Summary of microbial N redox pathways identified by ¹⁵N-isotope labeling and their interactions with (im)mobilization of As and Fe as adapted from previous reports

Key microbes	Reaction formula(s)	Reference
Anammox bacteria	$^{14}\text{NH}_4^+ + ^{15}\text{NO}_2^- \rightarrow 2\text{H}_2\text{O} + ^{29}\text{N}_2$ (Eq. 1)	Ding <i>et al.</i> (2021)
Feammox bacteria	$6\text{Fe}(\text{OH})_3 + 10\text{H}^+ + 2^{14}\text{NH}_4^+ \rightarrow 6\text{Fe}^{2+} + 18\text{H}_2\text{O} + ^{28}\text{N}_2$ (Eq. 2)	Yi <i>et al.</i> (2019)
	$16\text{H}^+ + 2^{14}\text{NH}_4^+ + 3\text{Fe}_3\text{O}_4 \rightarrow 9\text{Fe}^{2+} + 12\text{H}_2\text{O} + ^{28}\text{N}_2$ (Eq. 3)	Ding <i>et al.</i> (2014)
	$^{14}\text{NH}_4^+ + 6\text{Fe}(\text{OH})_3 + 10\text{H}^+ \rightarrow ^{14}\text{NO}_2^- + 6\text{Fe}^{2+} + 16\text{H}_2\text{O}$ (pH < 6.5) (Eq. 4)	Zhou <i>et al.</i> (2016)
Nitrate-dependent As(III)-oxidizing bacteria	$\text{H}_2\text{AsO}_3^- + ^{15}\text{NO}_3^- \rightarrow ^{15}\text{NO}_2^- + \text{H}_2\text{AsO}_4^-$ (Eq. 5)	Chen <i>et al.</i> (2023)
	$4\text{H}_3\text{AsO}_3 + 2^{15}\text{NO}_3^- \rightarrow 4\text{H}_2\text{AsO}_4^- + ^{46}\text{N}_2\text{O} + \text{H}_2\text{O} + 2\text{H}^+$ (Eq. 6)	Zhu <i>et al.</i> (2019)
	$5\text{H}_3\text{AsO}_3 + 2^{15}\text{NO}_3^- \rightarrow 5\text{HAsO}_4^{2-} + 8\text{H}^+ + \text{H}_2\text{O} + ^{30}\text{N}_2$ (Eq. 7)	Li <i>et al.</i> (2020)
Nitrate-dependent Fe(II)-oxidizing bacteria	$2\text{Fe}^{2+} + 5\text{H}_2\text{O} + ^{15}\text{NO}_3^- \rightarrow 2\text{Fe}(\text{OH})_3 + 4\text{H}^+ + ^{15}\text{NO}_2^-$ (Eq. 8)	Sun <i>et al.</i> (2016)
	$4\text{Fe}^{2+} + 2^{15}\text{NO}_2^- + 5\text{H}_2\text{O} \rightarrow ^{46}\text{N}_2\text{O} + 4\text{FeO}(\text{OH}) + 6\text{H}^+$ (Eq. 9)	Jamieson <i>et al.</i> (2018)
	$15\text{Fe}^{2+} + 2^{15}\text{NO}_3^- + 14\text{H}_2\text{O} \rightarrow 28\text{H}^+ + 5\text{Fe}_3\text{O}_4 + ^{30}\text{N}_2$ (Eq. 10)	Chen <i>et al.</i> (2023)
Denitrifying bacteria	$2^{15}\text{NO}_3^- + 4\text{H}^+ + 4\text{e}^- \rightarrow 2\text{H}_2\text{O} + 2^{15}\text{NO}_2^-$ (Eq. 11)	Luo <i>et al.</i> (2021)
	$2^{15}\text{NO}_2^- + 6\text{H}^+ + 4\text{e}^- \rightarrow 3\text{H}_2\text{O} + ^{46}\text{N}_2\text{O}$ (Eq. 12)	Di Capua <i>et al.</i> (2019)
	$^{46}\text{N}_2\text{O} + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O} + ^{30}\text{N}_2$ (Eq. 13)	Di Capua <i>et al.</i> (2019)

Herein, the effects of two N fertilization regimes (*i.e.*, mixture of nitrate and ammonia vs. nitrate) on the environmental behaviors of As (im)mobilization and N₂O emission were examined in flooded paddy soils having high background concentrations of NH₄⁺-N (0.49 ± 0.08 g kg⁻¹) and As (79.17 ± 6.59 mg kg⁻¹). Batch microcosm incubation incorporating ¹⁵N isotope-labeled nitrate was conducted to assess specific N redox transformations and trace the specific source of N₂O emission. Moreover, high-throughput 16S rRNA gene sequencing and gene quantification technologies were employed to identify potential microbial mechanisms responsible for As and N redox transformation processes. Results of this study inform practical N fertilizer strategies for remediating As pollution and mitigating N₂O emission in As-contaminated paddy soils.

MATERIALS AND METHODS

Soil collection

Arsenic-contaminated soil samples were collected in June 2022 from a flooded paddy field located in Shimen County (29°58' N, 111°38' E) in Changde City, Hunan Province, China. To obtain fresh anoxic paddy soils with sufficient microbial biomass to simulate *in-situ* conditions, soil cores (25 cm × 25 cm × 10 cm) were collected at the peak rice tillering stage from the 20–30 cm depth using a soil corer. Several subsamples were uniformly mixed to form a single composite sample, sealed in a sterile plastic bag, and transported to the lab for analysis. A subsample was naturally air-dried and sieved to < 2 mm for the determination of physicochemical properties (File S1, see Supplementary Material for File S1). Soils were typical acid (pH = 5.8) red clay soils with high total As (79.17 mg kg⁻¹) and As(V) (60.72 mg kg⁻¹) concentrations. The concentrations of available N, phosphorus, and potassium, total Fe, and organic C were 1.35, 0.49, 52.78, 32.11, and 13.67 g kg⁻¹, respectively (Table SI, see Supplementary Material for Table SI). Notably, the background NH₄⁺-N concentration was highly enriched (0.41 ± 0.08 g kg⁻¹) in the soil sample owing to long-term excessive application of N fertilizers and poor farm drainage that limited nutrient leaching. A fresh subsample of homogenized soil was sealed in polyethylene vinyl containers and stored at 4 °C in the dark to sustain the original environment for the survival of indigenous bacteria prior to the incubation trials (Chen *et al.*, 2017).

Soil incubation design

To investigate the influence (Table I) of N species (NH₄⁺

and NO₃⁻) on the mobilization of As from flooded paddy soils, batch anaerobic microcosm incubation was conducted in triplicate using sealed 105-mL serum bottles. Four biotic treatments were established by incubating 12.0 g of fresh (< 2 mm soil isolated using a nylon sieve) paddy soil and 36.0 mL of a given autoclaved culture solution. Following the experimental design of previous investigations (Li *et al.*, 2020; Feng *et al.*, 2023), dissolved As(III) was directly added into microcosms to evaluate As(III) immobilization behavior under simulated flooded As(III)-rich soil conditions. To ensure that direct and effective microbial metabolism regulated As (im)mobilization and N₂O emission, the substrates comprised of nitrate and/or ammonia, as well as acetate, were directly added to the culture solutions. Herein, ¹⁵N isotope-labeled Na¹⁵NO₃ (99 atom% ¹⁵N, Aladdin, USA) and ¹⁴NH₄Cl (100 atom% ¹⁴N, Beyotime, China) were used to trace N redox transformations. Therein, the four culture solutions added to soils consisted of 5.0 mmol L⁻¹ acetate (designated as the control, CK), 5.0 mmol L⁻¹ acetate + 0.1 mmol L⁻¹ As(III) (the As(III) treatment), 5.0 mmol L⁻¹ acetate + 0.1 mmol L⁻¹ As(III) + 10.0 mmol L⁻¹ ¹⁵NO₃⁻ (the As(III) + NO₃⁻-N treatment), and 5.0 mmol L⁻¹ acetate + 0.1 mmol L⁻¹ As(III) + 6.0 mmol L⁻¹ ¹⁵NO₃⁻ + 4.0 mmol L⁻¹ NH₄⁺ (the As(III) + NO₃⁻-N + NH₄⁺-N treatment). Prior to incubation, the culture solutions were autoclaved for 20 min at 120 °C. Anaerobic microcosms were incubated at 30 °C in the dark without shaking. To create an anoxic environment and purge atmospheric gases to allow for collection of microbially generated isotopically-labeled nitrogenous gases (N₂ and N₂O), high-purity helium gas was used for deoxygenation. Helium was bubbled into the liquid phase for 30 min and headspace for another 30 min before sealing the serum bottles. All experimental operations were performed in an anaerobic glove box to ensure anaerobic conditions further.

Determinations of As, Fe, and N species

Concentrations of total Fe (Fe(T)), Fe(III), Fe(II), total As (As(T)), As(V), and As(III) were determined to assess the (im)mobilization and redox transformations of Fe and As species as affected by N amendment in flooded paddy soils. Approximately 2.0 mL of incubated supernatant were extracted from each microcosm using a 2.5-mL sterilized syringe, filtered through a 0.22-μm sterile filter (nylon, Jinteng Co., China), acidified with 1% HNO₃ (volume/volume), and then stored in a 3.0 mL sterilized centrifuge tube. Supernatant samples were collected on days 0, 2, 4, 6, and 10 of the 10-d incubation. All sample processing was performed in an anaerobic glovebox. Concentrations of Fe(T) and Fe(II) were determined according to the phenanthroline colorimetric method with UV/VIS spectroscopy detection (UV-2802, UNICO, China) (Chen *et al.*, 2019a). Fe(III) concentration

was determined by the difference of Fe(T) and Fe(II). As(T) concentration was determined using an inductively coupled plasma-mass spectrometer (NexION 2000, PerkinElmer, USA) (Chen *et al.*, 2019b), whereas concentrations of As(III) and As(V) were determined using high-performance liquid chromatography-atomic fluorescence spectroscopy (SA-20, Jitian Inc., China) (Burguera and Burguera, 1997).

Dissolved inorganic N species (NH_4^+ , NO_3^- , and NO_2^-) were quantified in duplicate to reflect changes in N-derived electron donors and acceptors during the redox transformations of As and Fe (Liu *et al.*, 2019b). Specifically, the salicylate, hydrazine reduction, and sulfanilamide/N-1-naphthylethylene spectroscopy methods were applied for the determinations of NH_4^+ -N, NO_3^- -N, and NO_2^- -N, respectively, using a continuous-flow analyzer (Autoanalyser-3, Seal, Germany). To determine the abundances of $^{30}\text{N}_2$, $^{29}\text{N}_2$, and $^{30}\text{N}_2\text{O}$ in the N-amended assays, the headspace gas was collected using an aspirator pump to transfer a gas sample from the incubated soil microcosm to a 20-mL pre-evacuated headspace bottle. All gas samples were sealed with a butyl rubber stopper secured by a crimped aluminum closure. The abundances of $^{30}\text{N}_2$, $^{29}\text{N}_2$, and $^{46}\text{N}_2\text{O}$ in headspace gas were measured using an isotope ratio mass spectrometer with a $\pm 0.5\text{‰}$ precision for $\delta^{15}\text{N}$ (IRMS, Thermo Finnigan Delta V Advantage, Germany) (Fuchslueger *et al.*, 2019).

Characterization of dynamic changes in organic carbon (C) substrates

Dissolved organic C (DOC) and dissolved organic matter (DOM) were examined to characterize dynamic changes in organic C substrates. The DOC concentration was measured in the supernatant after passing through a 0.45- μm membrane filter (Millipore, USA) and then quantified using a total organic C (TOC) analyzer (TOC-L CPH, Shimadzu, Japan). Soil DOM fraction was extracted from a mixture of 2.0 g freeze-dried soil and 20.0 mL Milli-Q water after stirring at 200 r min^{-1} for 24 h at 25 °C in the dark, and the supernatant was then filtered through a 0.45- μm membrane filter (Millipore, USA) (Xu *et al.*, 2013). Fluorescence excitation-emission matrix (EEM) spectra were employed to characterize the composition of DOM extracts using a fluorescence spectrophotometer (FS5, Edinburgh, UK) (Chen *et al.*, 2016). The EEM spectra were scanned for the excitation (Ex) wavelengths of 200–400 nm with a 2-nm interval and emission (Em) wavelengths of 250–550 nm with a 5-nm interval. The scanning rate was set as 1 200 nm min^{-1} , and the slits for both Em and Ex were set at 10 nm. The EEMs were corrected for inner-filter effects, normalized Raman scattering, and a background blank (Kulkarni *et al.*, 2017). The EEMs corresponding to each treatment were further corrected through deducting the corresponding signals derived from Milli-Q water and were finally visualized using

the SigmaPlot 10.0. Fluorescence regional integration (FRI) analysis was implemented to quantify the abundances of representative fluorescent compounds in DOM, including soluble microbial byproduct-, humic acid-, fulvic acid-, and aromatic protein-like components (Table SII, see Supplementary Material for Table SII) (Chen *et al.*, 2019b). The FRI results were calculated according to Eq. S1 as reported in File S2 (see Supplementary Material for File S2).

Soil DNA extraction and 16S rRNA high-throughput sequencing

Total microbial DNA derived from the microcosms cultured for 10 d was extracted and subjected to high-throughput sequencing targeting the V4–V5 regions of the bacterial 16S rRNA genes (Chen *et al.*, 2017). Total DNA was extracted from a 0.5-g soil sample using the FastDNA spin kit for soil (MP Biomedicals, USA) following manufacturer's protocols. The 338F and 806R primers were used to amplify the V4–V5 regions of the 16S rRNA genes by polymerase chain reaction (PCR), and the PCR products were pooled into 2% agarose gels for electrophoresis. The resulting products were purified using an AxyPrep DNA gel extraction kit (Axygen Biosciences, USA) and quantified using QuantiFluor™-ST kit (Promega, USA). The purified amplicons were pooled at equal molar concentrations, and paired-end sequencing (2×250) was conducted on an Illumina MiSeq platform (Illumina, USA). The 16S rRNA gene sequence data were processed using the QIIME 1.8.0 tool kit. Details of the PCR amplification and sequencing analyses are reported in File S3 (see Supplementary Material for File S3). The 16S rRNA gene sequence data sets were submitted to NCBI Sequence Read Archive (BioProject accession number PRJNA940860).

Quantitative PCR (qPCR) examining the relative abundances of Feammox and anammox bacteria

The relative abundances of Feammox and anammox bacteria were quantified using real-time qPCR. At present, an uncultured Acidimicrobiaceae bacterium A6 has been identified as the only functional microorganism responsible for the Feammox reaction, although no bacterium has been isolated thus far (Huang and Jaff, 2015). For the detection of Feammox and anammox bacteria represented by 16S rRNA genes, the acm342f and acm439r primers were used for amplifying the relative abundance of Feammox bacteria (Acidimicrobiaceae bacterium A6) (Xiong *et al.*, 2022), whereas the Amx368F and Amx820R primers were used for amplifying the relative abundance of anammox bacteria (Nejidat *et al.*, 2018). In brief, amplification was performed in a 20 μL PCR reaction solution comprised of 1 μL of each primer ($10 \mu\text{mol L}^{-1}$), 10 μL of SYBR Premix Ex Taq (Promega, USA), 1 μL of DNA template (1–10 ng), and 7

μL of sterilized double-distilled water. More details on the methodology are provided by Yi *et al.* (2019). To normalize the relative abundances of anammox and Feammox bacteria, the corresponding primers were employed to quantify the 16S rRNA genes of total bacteria.

Data analysis

All data were processed/analyzed using Microsoft Excel 2016, Origin 2023, and SPSS 22.0. Data were presented as means $\pm$ standard deviations (SDs). Treatment effects were examined using analysis of variance (ANOVA). The least significant difference multiple range test was conducted to determine statistical significance at $P < 0.05$ among treatments using SPSS 22.0. Additionally, principal coordinate analysis (PCoA) and redundancy analysis (RDA) were performed using CANOCO 4.5 (Microcomputer Power, USA).

RESULTS AND DISCUSSION

(Im)mobilization of As and Fe

Previous studies demonstrated a poor As(III) mobilization capability in response to nitrate amendment (Li *et al.*, 2020; Feng *et al.*, 2023). In this study, N amendment induced differential impacts on the mobilization of As and Fe in biotic assays (Fig. 1). In the flooded paddy soil, dissolution of Fe(III) exhibited a similar tendency between the As(III) treatment and the control. Dissolved Fe(II) concentration increased with increasing incubation time and approached

the Fe(T) concentration of 1.8 mmol L^{-1} by the end of the incubation (Fig. 1a, b). These Fe dynamics are mainly ascribed to metal-reducing bacteria that assimilate substrates of labile organic C to produce electrons that thereafter modulate dissimilatory Fe(III) reduction under anaerobic conditions in the absence of nitrate (Chen *et al.*, 2018a). Markedly, N inputs produced opposite trends in Fe(III) reduction. Only a negligible concentration of Fe(II) ($< 0.12 \text{ mmol L}^{-1}$) was detected (Fig. 1b), whereas Fe(III) concentration (Fig. 1a) was similar to Fe(T) concentration in the As(III) + NO_3^- -N + NH_4^+ -N treatment after incubation for 10 d (Fig. 1c). In particular, a higher proportion of NO_3^- induced a greater release of Fe(III), with nearly a 1.6-fold higher maximum Fe(III) concentration detected in the As(III) + NO_3^- -N treatment vs. the As(III) + NO_3^- -N + NH_4^+ -N treatment. This promotion was ascribed to the higher redox potential of NO_3^- than Fe(III) (Borch *et al.*, 2010). Thus, nitrate-dependent microbial Fe(II) oxidation is more thermodynamically feasible than microbial dissimilatory Fe(III) reduction when amended with a higher proportion of nitrate under anoxic conditions (Bryce *et al.*, 2018; Liu *et al.*, 2019a).

Concerning As (im)mobilization, the reductive dissolution of As(V) displayed a similar trend to that of Fe(III) in the control (Fig. 1c, d, f). In the As(III) treatment, virtually no detectable As(V) was detected, whereas As(III) comprised the majority of As(T) (Fig. 1d–f). Fluctuations in As mobilization are affected by microbial activities and/or soil Fe(III)-bearing minerals that induce As coprecipitation (Gorny *et al.*, 2015). Release of As(III) increased to

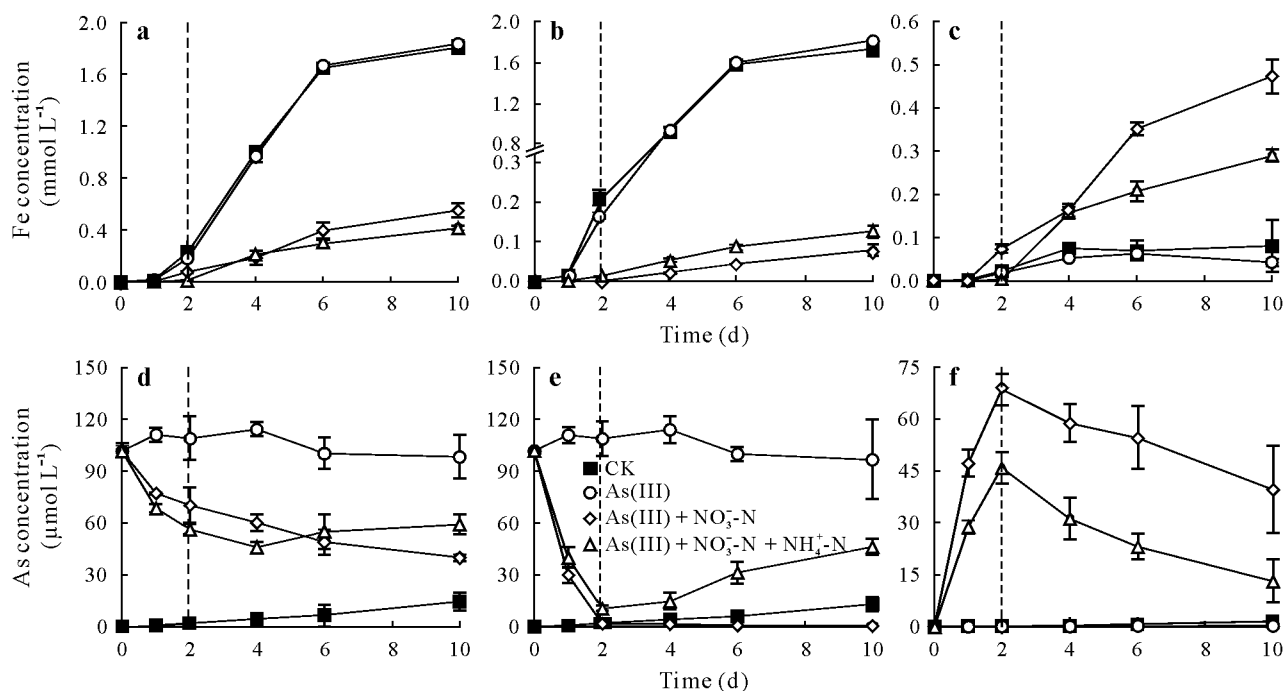


Fig. 1 Concentrations of total Fe (a), Fe(II) (b), Fe(III) (c), total As (d), As(III) (e), and As(V) (f) in an As-contaminated flooded paddy soil incubated with and without (control, CK) As(III) and/or N (NO_3^- -N and NH_4^+ -N) for 10 d. Vertical bars represent standard deviations of means ($n = 3$). Dashed lines indicate that the 2nd day is the optimal boundary of As(III) mitigation.

a maximum of $113 \mu\text{mol L}^{-1}$ on the 4th day and thereafter gradually decreased to $96 \mu\text{mol L}^{-1}$ at the end of the incubation period in the As(III) treatment (Fig. 1e). In contrast, N input caused rapid oxidation of soluble As(III) to As(V), resulting in a sharp decline of As(III) together with a concomitant increase of As(V) beginning on the 2nd day. The rapid increase of As(V) was attributed to nitrate-dependent microbial As(III) and Fe(II) oxidation being more competitive than the Feammox reaction, thereby suppressing microbial As(V) and Fe(III) reductive dissolution (Chen *et al.*, 2023). Since the couple $\text{NO}_3^-/\text{NO}_2^-$ has a higher redox potential than $\text{H}_2\text{AsO}_4^-/\text{H}_3\text{AsO}_3$ and $\text{Fe}^{3+}/\text{Fe}^{2+}$ (Desiredy and Pothanamkandathil Chacko, 2021), thermodynamic processes are preferentially directed to nitrate-dependent microbial As(III) and Fe(II) oxidation rather than the Feammox reaction, even though the surplus background NH_4^+ might favor the Feammox reaction. As the reaction continued, NO_3^- was completely consumed and the residual reductive NH_4^+ created a reductive environment conducive to microbial As(V) reduction (Fig. 1e) (Zhang *et al.*, 2022a; Chen *et al.*, 2023). By the end of the incubation period, residual As(V) concentrations reached 40 and $13 \mu\text{mol L}^{-1}$ in the As(III) + NO_3^- -N and As(III) + NO_3^- -N + NH_4^+ -N treatments, respectively. Since Fe(III) was released throughout the entire incubation period, the generation of Fe(III)-(hydro)oxides resulted in the coprecipitation and/or adsorption of As(III) or As(V) (Gorny *et al.*, 2015). The increased amounts of Fe(III) and As(V) were conducive to their coprecipitation/adsorption reactions

and thereby led to a prominent suppression of As(T) mobility during the later incubation period (Yuan *et al.*, 2023).

Redox transformations of N species

After 2-d incubation, NO_3^- -N concentrations in the control and the As(III) + NO_3^- -N + NH_4^+ -N treatment were nearly depleted (Fig. 2a), whereas denitrification-derived NO_2^- -N concentrations (Eqs. 5, 8, and 11 in Table I) were correspondingly increased to peak values of 3.8 and 2.3 mmol L^{-1} in these two treatments, respectively (Fig. 2b). The biotransformation performance of NO_3^- -N during the initial 2 d was strongly linked to microbial As(III) oxidation in the As(III) + NO_3^- -N and As(III) + NO_3^- -N + NH_4^+ -N treatments (Fig. 1d–f). This linkage revealed an apparent microbial-mediated nitrate-dependent As(III) oxidation process (Eqs. 5–7 in Table I).

The As(III) + NO_3^- -N + NH_4^+ -N and As(III) + NO_3^- -N treatments induced a differential response in the accumulations of $\delta^{15}\text{N}$ -labeled gas products ($^{29}\text{N}_2$, $^{30}\text{N}_2$, and $^{46}\text{N}_2\text{O}$, Fig. 2d–f). Higher abundances of $^{30}\text{N}_2$ and $^{46}\text{N}_2\text{O}$ were found in the As(III) + NO_3^- -N treatment, which was attributed to the higher proportion of $^{15}\text{NO}_3^-$ substrate ($10 \text{ mmol L}^{-1} \text{ NO}_3^-$ -N vs. $4 \text{ mmol L}^{-1} \text{ NO}_3^-$ -N) that favored denitrification processes (Eqs. 5–13 in Table I). Conversely, the abundance of $^{29}\text{N}_2$ was higher in the As(III) + NO_3^- -N + NH_4^+ -N treatment than the As(III) + NO_3^- -N treatment. This phenomenon might be attributed to the surplus NH_4^+ and NO_2^- in the microcosm in the former treatment providing

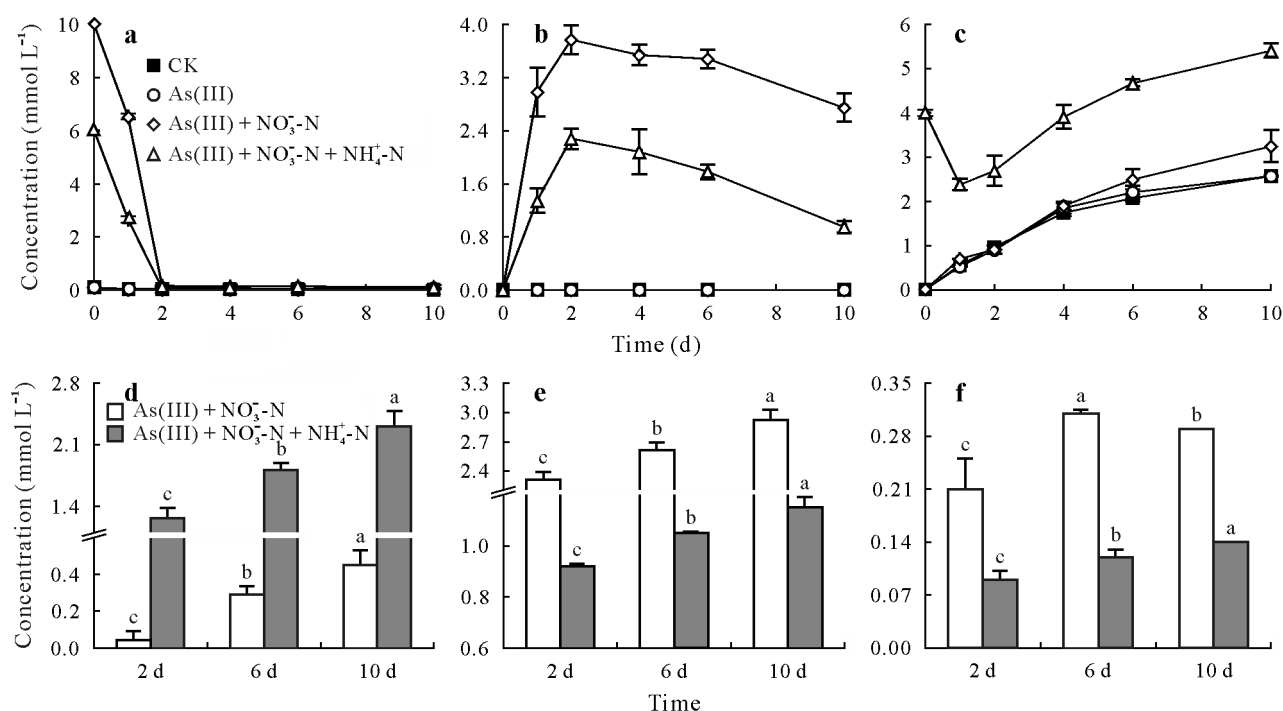


Fig. 2 Dynamic concentrations of NO_3^- -N (a), NO_2^- -N (b), NH_4^+ -N (c), $^{29}\text{N}_2$ (d), $^{30}\text{N}_2$ (e), and $^{46}\text{N}_2\text{O}$ (f) in an As-contaminated flooded paddy soil incubated with and without (control, CK) As(III) and/or N (NO_3^- -N and NH_4^+ -N) for 10 d. Vertical bars represent standard deviations of means ($n = 3$). In panels d–f, bars with different letters indicate significant differences among different incubation time for each treatment at $P < 0.05$.

sufficient substrates for the anammox reaction (Eq. 1 in Table I), as there was a 5.2 times higher abundance of $^{29}\text{N}_2$ in the $\text{As(III)} + \text{NO}_3^- \text{-N} + \text{NH}_4^+ \text{-N}$ treatment than the $\text{As(III)} + \text{NO}_3^- \text{-N}$ treatment.

As the incubation progressed, soil background-derived NH_4^+ was released to the liquid phase by microbial mineralization (Fig. 1c) and was thus available to participate in Feammox and anammox reactions (Eqs. 1–7). This inference was supported by the detectable Fe(II) and As(III) levels ($0.02\text{--}0.13 \text{ mmol L}^{-1}$ and $10\text{--}46 \text{ }\mu\text{mol L}^{-1}$, respectively, Fig. 1b, e) and the increased abundance of $\delta^{29}\text{N}_2$ ($1.3\text{--}2.3 \text{ mmol L}^{-1}$, Fig. 2d) on days 2–10. Moreover, the decline in NO_2^- concentration (Fig. 2b) likely results from its participation in the biotic and/or abiotic oxidation of Fe(II) produced from the Feammox reaction (Jakus *et al.*, 2021). This reasoning was supported by the higher production of Fe(III) ($0.21\text{--}0.35 \text{ mmol L}^{-1}$) than Fe(II) ($0.04\text{--}0.09 \text{ mmol L}^{-1}$) (Fig. 1b–c) on day 6 in the $\text{As(III)} + \text{NO}_3^- \text{-N}$ and $\text{As(III)} + \text{NO}_3^- \text{-N} + \text{NH}_4^+ \text{-N}$ treatments. Through the end of the incubation, the amount of $^{46}\text{N}_2\text{O}$ accumulated in the $\text{As(III)} + \text{NO}_3^- \text{-N} + \text{NH}_4^+ \text{-N}$ treatment was only 0.47 times that of the $\text{As(III)} + \text{NO}_3^- \text{-N}$ treatment (Fig. 2f), suggesting a suppression of N_2O emission from the input of NH_4^+ . It should be noted that the incidence of dissimilatory nitrate reduction to ammonia (DNRA) is lower than denitrification because the DNRA reaction requires a higher electron donor capacity and a relatively high C/N ratio (van den Berg *et al.*, 2016, 2017). Moreover, DNRA facilitates the mobilization of As(III) whereas denitrification-derived As(III) oxidation suppresses As(III) mobilization. In this study, the decline of As(III) concentration (Fig. 1e) during the initial 2 d of incubation displayed a similar pattern to those of NO_3^- and TOC consumption (Figs. 2b and 3a). This infers that limited amounts of acetate and nitrate participated in denitrification linked to As(III) oxidation rather than DNRA.

Dynamic changes in organic C substrates

The quantity and quality of organic C substrates are reported to strongly affect the bioavailability of As in paddy soils (Chen *et al.*, 2018b; Hussain *et al.*, 2021). Under flooded conditions, electron donors (*e.g.*, acetate) are abundant and available to various microbes, contributing to the energy metabolism responsible for microbial As(III) oxidation and As(V) reduction. During the 10-d incubation, 61% and 67% DOC were removed in the control and the As(III) treatment, respectively (Fig. 3a). This decrease was attributed primarily to increased consumption of acetate, which serves as an electron donor for microbial reductive-dissolution of As(V) and Fe(III) that are bound together in Fe(III)-(hydro)oxides (Chen *et al.*, 2018b). Higher DOC consumption was observed in the As(III) treatment compared to the control, which

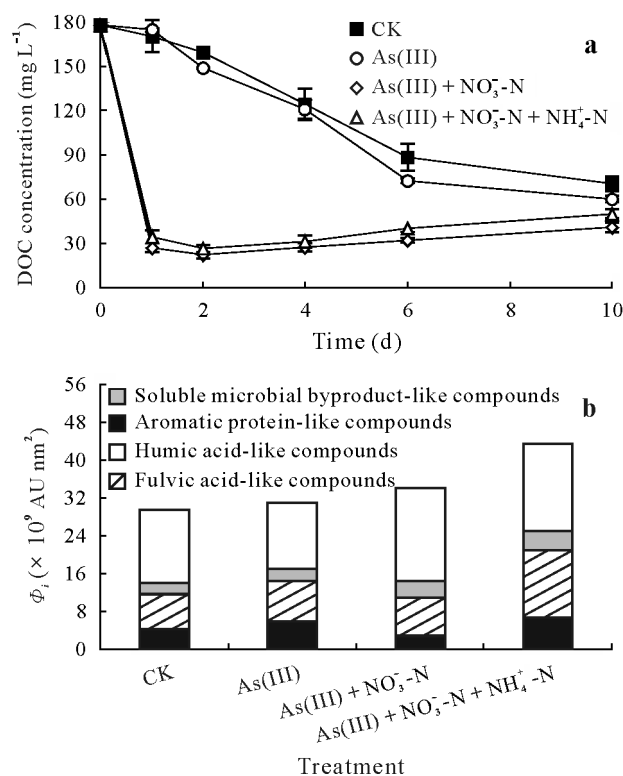


Fig. 3 Removal of dissolved organic C (DOC) (a) and fluorescence regional integration for dissolved organic matter on day 10 (b) in an As-contaminated flooded paddy soil incubated with and without (control, CK) As(III) and/or N ($\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$) for 10 d. In panel a, vertical bars represent standard deviations of means ($n = 3$). Φ_i = the volume beneath region i ; AU = absorbance unit, indicating fluorescence intensity.

might be due to the As(III)-induced microbial metabolic detoxication (Bertin *et al.*, 2011).

In the context of N inputs, rapid DOC consumption occurred in the initial two days of the incubation. This large consumption might result from labile organic C substrates (*e.g.*, acetate) serving as an energy source for heterotrophic microbial metabolism that participate in nitrate-dependent As(III) oxidation processes (Fig. 1f). The lower DOC consumption in the $\text{As(III)} + \text{NO}_3^- \text{-N} + \text{NH}_4^+ \text{-N}$ treatment than the $\text{As(III)} + \text{NO}_3^- \text{-N}$ treatment was ascribed to enhanced anammox/Feammox-derived autotrophic bacterial metabolisms that rely on ammonia-assimilation, thereby attenuating the need for heterotrophic organic C-dependent energy processes (Gonzalez-Cabaleiro *et al.*, 2015). Notably, DOC levels in the N-amended treatments gradually increased during the subsequent 8 d of the incubation. This phenomenon was attributed to N-induced promotion of microbial activities that results in solubilization and leaching of DOM from the solid-phase soil (Yang *et al.*, 2015), as further inferred from the results of EEM and FRI analyses.

Compared with the control and the As(III) treatment, a pair of fluorescence peaks attributed to soluble microbial byproduct-like compounds were more pronounced in EEM spectra in two N-amended treatments (Fig. 4). Moreover,

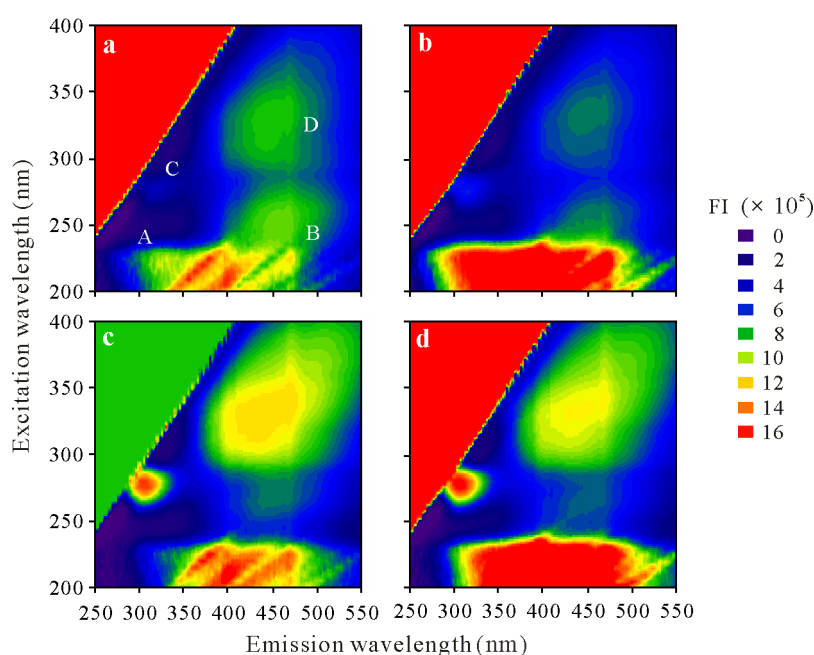


Fig. 4 Fluorescence excitation-emission matrix spectra of dissolved organic matter in liquid phase derived from an As-contaminated flooded paddy soil incubated with and without As(III) and/or N (NO_3^- -N and NH_4^+ -N) for 10 d. Peaks A–D refer to the regions of aromatic protein-, fulvic acid-, soluble microbial byproduct-, and humic acid-like compounds, respectively. FI = fluorescence intensity.

FRI results (Fig. 3b) indicated that the As(III) + NO_3^- -N + NH_4^+ -N treatment contained the greatest amounts of heavy-molecular-weight DOM components (*e.g.*, fulvic acid- and humic acid-like compounds) among all treatments. These findings revealed that N inputs changed microbial communities by altering C substrates and redox dynamics, thereby modulating the bioavailability of As and Fe in paddy soils. Therefore, selective functional microbes appear to respond to the availability of different N species to alter electron transfer pathways that correspondingly modulate As and Fe bioavailability through preferential utilization of alternative organic C substrates (Olaniran *et al.*, 2013; Zhao *et al.*, 2022).

Diversity of microbial communities resulting from N amendment

High-throughput sequencing revealed that the most dominant phyla of bacterial communities were affiliated with Actinobacteriota (15%–20%), Proteobacteria (18%–34%), and Firmicutes (16%–32%) in all treatments at the end of the 10-d incubation (Fig. 5a). Compared with the control, As(III), NO_3^- -N, and NH_4^+ -N resulted in distinct enrichment for phyla of Planctomycetota, Proteobacteria, and Verrucomicrobiota. In particular, N amendment led to a prominent increase in the relative abundance of Proteobacteria (26%–34% in the control *vs.* 18%–20% in the As(III) treatment), whereas there was a distinct decrease in the relative abundance of Firmicutes (15%–19% in the control *vs.* 26%–32% in the As(III)-amended treatments). Therein, the relative

abundance of Proteobacteria was significantly increased with the increase in the proportion of nitrate, indicating that nitrate gives a boost to the enrichment of Proteobacteria. To further evaluate the influence of As(III), NO_3^- -N, and NH_4^+ -N on microbial communities, PCoA based on the weighted Fast UniFrac metric at the genus level was employed to examine patterns of microbial diversity among treatments (Fig. 6). The PCoA 1 represented 59.3% of the variations among samples. The striking overlap region revealed a close similarity at the genus level between the As(III) + NO_3^- -N and As(III) + NO_3^- -N + NH_4^+ -N treatments.

The relative abundances of several representative genera responsible for metal redox reactions (Fig. 5b) were compiled for a statistical assessment of N amendment on As (im)mobilization. Differences in microbial diversity were apparent between treatments with and without N amendment. In the absence of N amendment, metal-reducing bacteria (*e.g.*, *Bacillus*, *Anaeromyxobacter*, *Geobacter*, and *Halomonas*) were able to take full advantage of the non-nitrogen electron acceptors (*i.e.*, endogenous As(V) and Fe(III) in soil minerals) through extracellular electron transfer processes (Soda *et al.*, 2006; Qiao *et al.*, 2018, 2019; Zhang *et al.*, 2022a). These metal-reducing bacteria comprised a predominant status, representing 23%–25% of total bacterial abundance (Table SIII, see Supplementary Material for Table SIII) in the anaerobic microcosms. Notably, the relative abundances of *Geobacter* and *Halomonas* demonstrated opposing distributions between the control and the As(III) treatment. Specifically, the control had an elevated relative

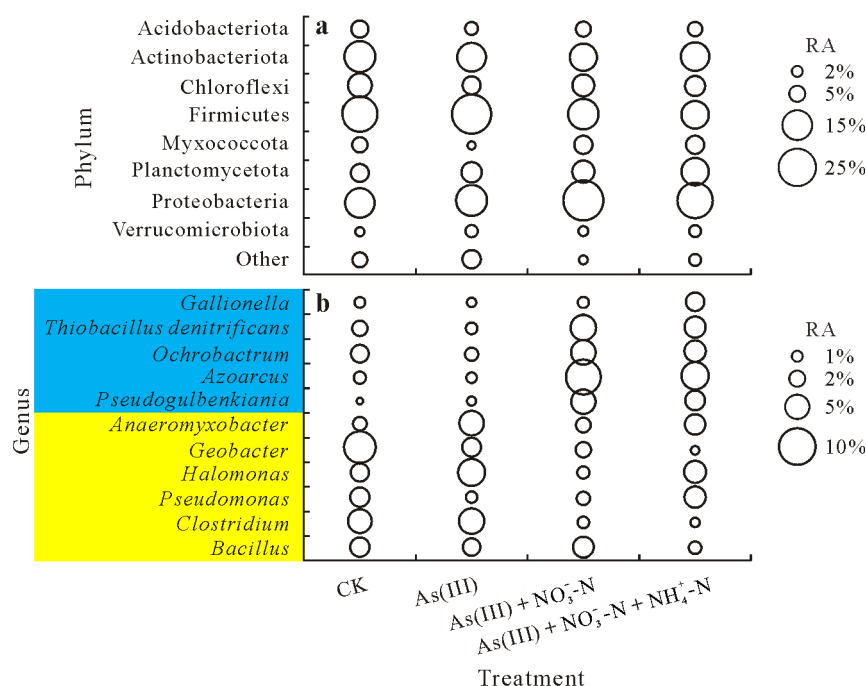


Fig. 5 Relative abundance (RA) of bacterial community at phylum (a) and genus (b) levels in an As-contaminated flooded paddy soil incubated with and without (control, CK) As(III) and/or N (NO_3^- -N and NH_4^+ -N) for 10 d. Only microbial members with RA greater than 1% are displayed. In panel b, the genera located in the blue and yellow regions are affiliated with the putative Fe(II)/As(III) oxidizers and Fe(III)/As(V) reducers, respectively. The larger the bubble size, the higher the RA.

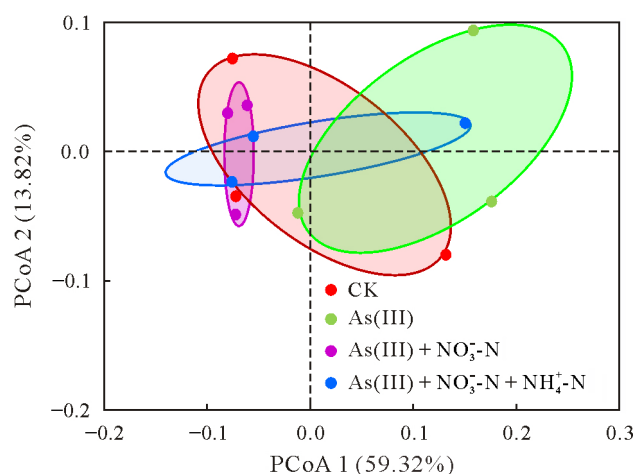


Fig. 6 Weighted UniFrac principal coordinate analysis (PCoA) of microbial communities in an As-contaminated flooded paddy soil incubated with and without (control, CK) As(III) and/or N (NO_3^- -N and NH_4^+ -N) based on 16S rRNA high-throughput sequencing.

abundance of *Geobacter*, whereas the As(III) treatment had a greater relative abundance of *Halomonas*. The dominance of *Halomonas* in the As(III) treatment was consistent with recent findings that *Halomonas* is a representative As(III)-defensive bacterium that can adapt to As(III)-rich environments (Zhang *et al.*, 2022a). The putative genera responsible for biological As(III)/Fe(II) oxidation and denitrification were compared to the immobilization of As in N-amended treatments using correlation analysis. An increased pro-

portion of nitrate in treatments was positively correlated with the total abundance of metal-oxidizing bacteria (Beller *et al.*, 2013; Li *et al.*, 2020; Xue *et al.*, 2022), namely the oxidizers *Azoarcus* (7%–11%), *Ochrobactrum* (4%–7%), and *Thiobacillus denitrificans* (4%–8%), which were enriched in the two treatments amended with NO_3^- -N. Thus, the aforementioned results indicate that nitrate serves as a key driver modulating the overall distribution of those bacteria participating in As(III) oxidation processes in paddy soils.

The qPCR assays were further conducted to determine the abundances of anammox and Feammox bacteria (Fig. 7). The As(III) + NO_3^- -N + NH_4^+ -N treatment led to a distinct enrichment of anammox and feanammox bacteria. At the end of the 10-d incubation, abundances of anammox and feanammox bacteria encoded by gene copy numbers increased by 2.4 and 0.3 times in the As(III) + NO_3^- -N + NH_4^+ -N treatment, respectively, compared with the control (Fig. 7a). In contrast, the As(III) + NO_3^- -N treatment led to a decrease in abundances of anammox and Feammox bacteria, with decreases in gene copy numbers by 0.53 and 0.45 times, respectively, compared with the control (Fig. 7b). The enrichment of anammox and Feammox bacteria responded to ammonia-activation, which supports the aforementioned enhanced microbial dissolution of Fe(III) and As(V) (Fig. 1c, f) and concomitant decrease of N_2O emission (Fig. 2f).

Interactions between microbes and C, N, Fe, and As cyclings

Redundancy analysis illustrated the biogeochemical

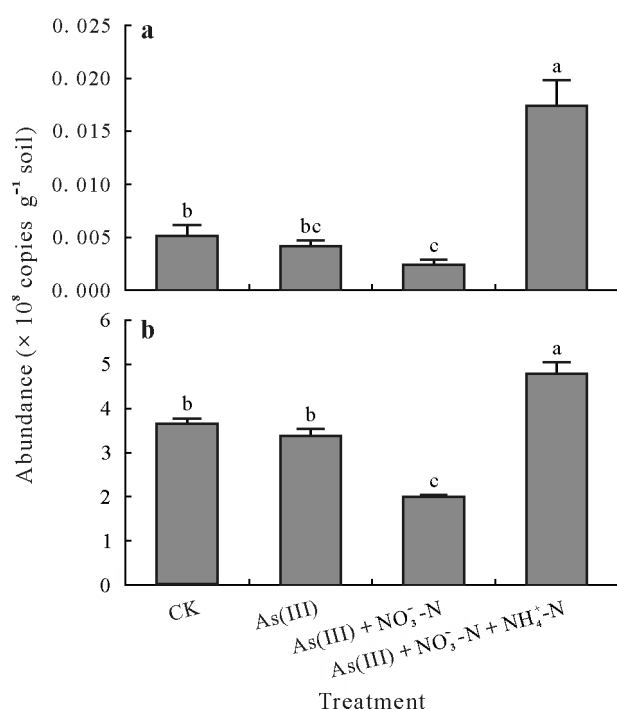


Fig. 7 Abundances of anammox (a) and Feammox (b) bacteria in an As-contaminated flooded paddy soil incubated with and without (control, CK) As(III) and/or N (NO_3^- -N and NH_4^+ -N) for 10 d. Vertical bars represent standard deviations of means ($n = 3$). Bars with different letters indicate significant differences among different treatments at $P < 0.05$.

complexities governing the fate and transport of C, N, As, and Fe compounds after incubation (Fig. 8). The abundances of key genera (e.g., *Anaeromyxobacter*, *Bacillus*, and *Clostridium*) were positively correlated with the concentrations of As(III) and Fe(II). In contrast, *Halomonas* displayed the highest positive correlation with concentrations of NH_4^+ -N and As(III), thereby inferring its crucial role in the reduc-

tive dissolution of As(V) in ammonia-enriched paddy soils. Moreover, a positive correlation occurred between refractory humic acid and fulvic acid-like compounds and anammox and Feammox bacteria, as well as microbial Fe(III) reduction.

To further examine the effects of N and DOC on As mobilization and N_2O emission, Pearson correlation analysis (Fig. S1, see Supplementary Material for Fig. S1) was conducted based on the results on days 2, 6, and 10. With the inputs of NO_3^- -N and NH_4^+ -N, the crucial factors associated with N_2O emission were aromatic protein-like ($R^2 = -0.90$, $P < 0.01$), soluble microbial byproduct-like ($R^2 = -0.84$, $P < 0.01$), humic acid-like ($R^2 = -0.85$, $P < 0.01$), and fulvic acid-like ($R^2 = 0.90$, $P < 0.01$) compounds (Fig. S1a). These results suggest that the bioavailability of endogenous organic C compounds is strongly linked to N_2O emission. This relationship might be related to the active metabolism of anammox bacteria and their energy acquisition from N redox transformations stimulated by NO_3^- -N and NH_4^+ -N amendment in which humic acid-like compounds reinforce electron shuttling for energy conversion (Yi et al., 2019; Tong et al., 2021).

The mobilization of As(T) was highly affected by the input of NO_3^- -N ($R^2 = -0.88$, $P < 0.01$) (Fig. S1b). Since the redox potential of nitrate is substantially more positive than that of Fe(III) and As(V) (Fanning, 2000), denitrification occurs preferentially to microbial reduction of Fe(III)/As(V); thereby preventing electron transfer for Fe(III) and As(V) reduction. In addition, microbial interactions between Fe and organic C substrates could be another factor promoting As(III) immobilization (Fig. S1b). These results are supported by the significant negative correlations of As(T) with Fe(T) ($R^2 = -0.88$, $P < 0.01$) and DOC ($R^2 = -0.79$, $P < 0.05$) as well as the significant positive correlations of

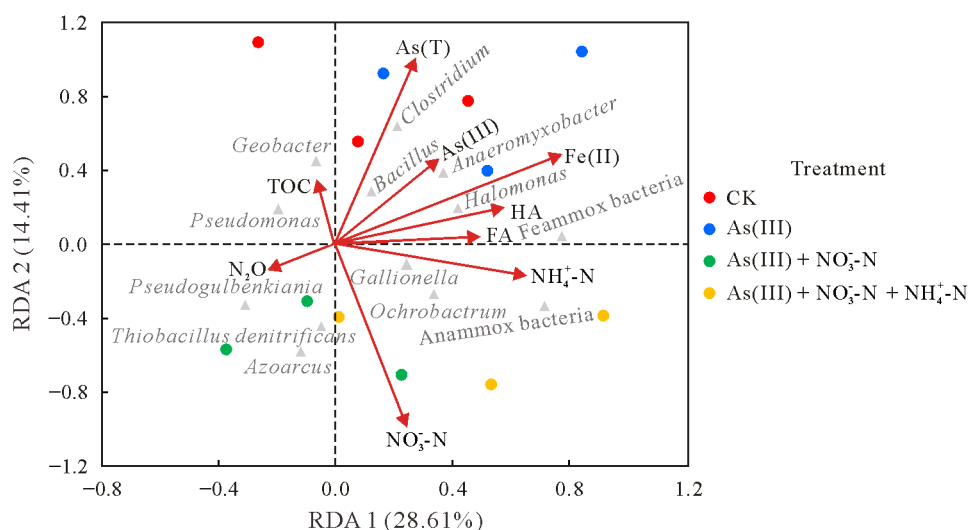


Fig. 8 Redundancy analysis (RDA) of main chemical constituents related to As, Fe, C, and N cyclings and the key bacterial species related to putative functional genera responsible for As and Fe redox transformations in an As-contaminated flooded paddy soil incubated with and without (control, CK) As(III) and/or N (NO_3^- -N and NH_4^+ -N). As(T) = total As; TOC = total organic C; HA and FA = humic acid- and fulvic acid-like compounds, respectively.

As(T) with aromatic protein-like ($R^2 = 0.77$, $P < 0.05$) and fulvic acid-like ($R^2 = 0.84$, $P < 0.01$) compounds. Labile organic C might be preferentially used as an energy source by heterotrophic As(III)/Fe(II) oxidizers (*e.g.*, *Thiobacillus denitrificans* and *Azoarcus*) (Fig. 5b) (Durante-Rodríguez *et al.*, 2019; Zhang *et al.*, 2022b). The preferential utilization of labile organic substrates was consistent with the stronger correlation of DOC with aromatic protein-like compounds in the As(III) + NO_3^- -N treatment ($R^2 = -0.92$, $P < 0.01$) than the As(III) + NO_3^- -N + NH_4^+ -N treatment ($R^2 = -0.71$, $P < 0.01$) (Fig. S1a). Thus, different organic C sources can alter the bioavailability of As in association with N redox reactions through strengthening electron donor capacity

and/or electron shuttling processes. To further validate the mechanisms by which organic C sources interact with the biogeochemical N/Fe/As cyclings, future studies employing C isotopes are warranted to identify co-occurrence patterns among these interacting processes.

Proposed pathways for As mobilization and N_2O emission as affected by C/N dynamics

A conceptual diagram illustrated the primary factors affecting As mobilization and N_2O emission for two different N input scenarios, including C/N substrate- and microbial-mediated mechanisms (Fig. 9). The contrasting N pools (NO_3^- -N vs. NO_3^- -N + NH_4^+ -N) serve as a key factor regulating redox transformations among C, Fe, and As, as mediated

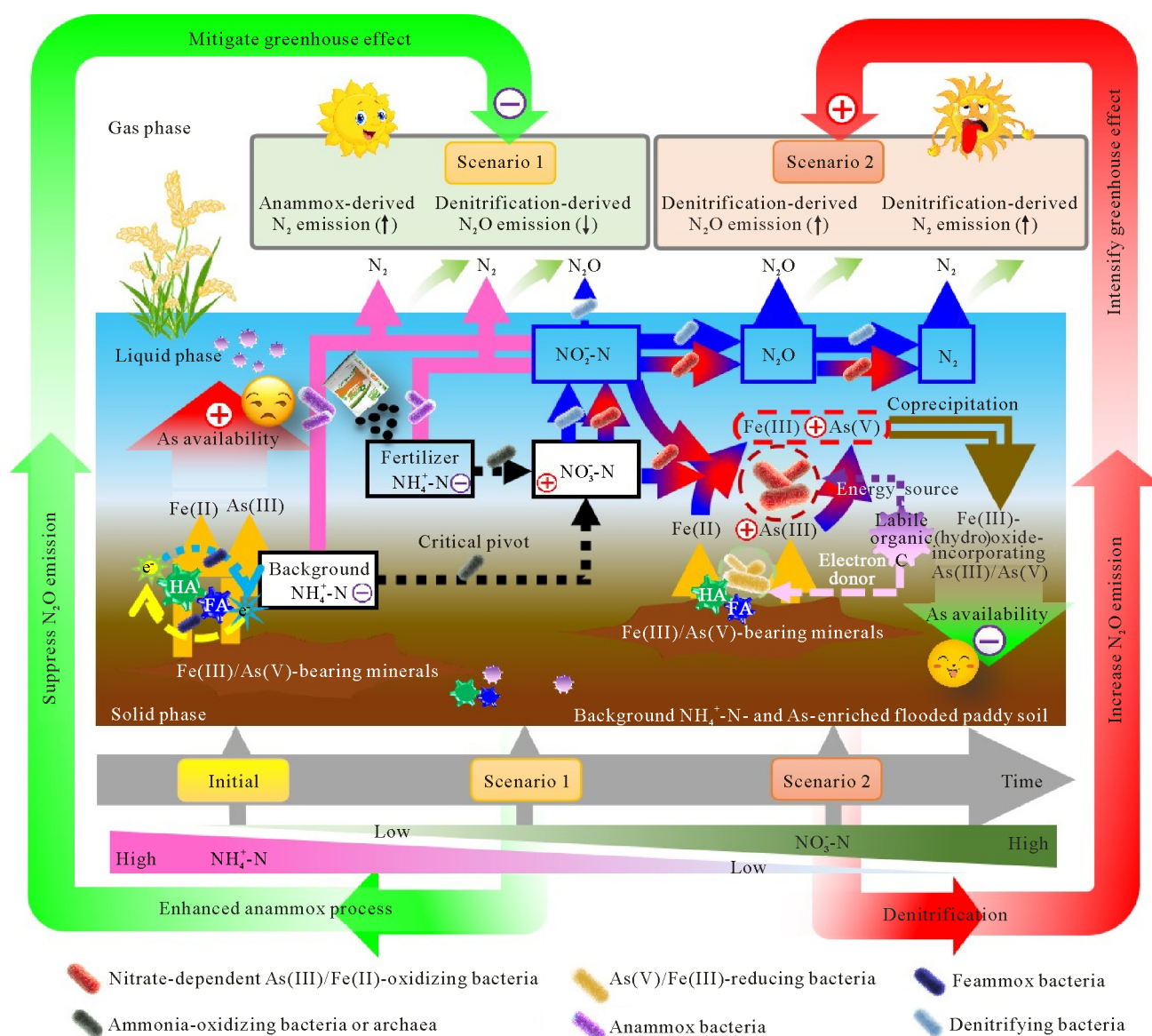


Fig. 9 Conceptual diagram illustrating the behavior of As mobilization and N_2O emission in the context of N amendment resulting from interactions of organic C substrates and functional microbe-mediated mechanisms based on the synthesis of this study and literature reports (Yi *et al.*, 2019; Li *et al.*, 2020; Zhang *et al.*, 2022a). FA and HA refer to fulvic acid- and humic acid-like compounds, respectively. The black dotted lines denote the simplified nitrification process. Red and green arrows indicate negative and positive regulations. The symbols of + and – indicate the increasing and decreasing effects, respectively.

by microbial interactions at different spatial and temporal scales. In the context of N inputs, the mechanisms relating to As mobilization and N₂O emission result from interactions between organic C substrates and key microbes as follows. i) High background ammonia concentration in paddy soils has the potential to provide abundant substrates for Feammox bacteria that enhance reductive dissolution of humic acid- and fulvic acid-like compounds (Xiu *et al.*, 2020), thereby increasing As(III) availability. These reactions are especially promoted by humic acid- and fulvic acid-like compounds that induce electron shuttling (Yi *et al.*, 2019). ii) Due to ammonia-oxidizing bacteria/archaea-mediated nitrification, an increased proportion of nitrate in the inorganic N pool enables microbial nitrate-dependent As(III) oxidation. Moreover, surplus background/fertilizer-derived ammonia and nitrite resulting from incomplete denitrification provide substrates for the anammox reaction that contributes to a higher production of N₂ (*i.e.*, complete denitrification) by blocking the conversion of nitrite to N₂O during denitrification. Results revealed that an appropriate mixture of ammonia and nitrate in flooded paddy soils not only increases As(III) immobilization but also suppresses N₂O emission, providing a desirable fertilizer management strategy to attenuate adverse environmental effects. iii) A sufficient input of nitrate is beneficial for the enrichment of nitrate-dependent As(III)/Fe(II)-oxidizing bacteria. Enrichment of these oxidizing bacteria promotes beneficial As(III)/Fe(II) oxidation that attenuates the mobility, toxicity, and bioavailability of As in paddy soils. Soluble As species are largely immobilized through adsorption and coprecipitation with Fe(III)-(hydro)oxides (Gorny *et al.*, 2015). Correspondingly, denitrification-derived N₂O emission is aggravated due to the dominant mechanism driven by electron hopping.

Overall, this study indicates that N fertilizer amendment to anaerobic paddy soils can indirectly regulate As availability, as well as N₂O emission, especially in soils with high background ammonia concentration. Considering the critical need for C sequestration/mitigation strategies (Chi *et al.*, 2021), a mechanistic understanding of factors and processes driving N and As cyclings is needed to inform specific mitigation strategies for N₂O emission and As immobilization in soils. This work found that the combined amendment of NO₃⁻ and NH₄⁺ in paddy soils can contribute to microbial As(III) oxidation processes while substantially attenuating N₂O emission from incomplete denitrification (Xu *et al.*, 2023). Addressing soil pollution, including metal pollutants and greenhouse gas emissions, is a critical human health and environmental issue requiring the development of prevention and remediation strategies (Hou and Li, 2017). The present study validated the hypothesis that an appropriate mixed application of N fertilizers (NO₃⁻ and NH₄⁺) can realize a win-win outcome for suppressing As(III) mobilization and

N₂O emission in flooded paddy soils. We fully acknowledged that the present study was based on a controlled laboratory incubation of a limited scale, but it provided proof-of-concept as a simple remediation strategy. Thus, a critical next step is to design field trials to determine the impacts of various N fertilization strategies based on different stoichiometric proportions, as well as different treatments with different C/N ratios, on managing As mobility/bioavailability and N₂O emission at the more complex and larger scale of flooded paddy soils (Yin *et al.*, 2021; Lin *et al.*, 2022). Since CH₄ is a more serious greenhouse gas contributor from paddy soils than N₂O and rice grain yield is the most prominent concern related to soil quality/health (Jiang *et al.*, 2020; Rani *et al.*, 2021), future investigations targeting the effects of different N regimes on the combined impacts of total greenhouse gas (CO₂, CH₄, and N₂O) emissions and grain yield/quality in paddy fields are strongly warranted.

CONCLUSIONS

In this study, simulations for N fertilizers were conducted to clarify how and to what extent the N pools influence the mobility of As in a flooded paddy soil and to evaluate the potential to attenuate N₂O emission. Distinct As mobility linked to the redox transformations of different N species was documented in the paddy soil studied. The N-redox transformation prominently shifted the microbial community composition and activated bacterial interactions between DOM, As, Fe, and N. With the combined amendment of NO₃⁻ and NH₄⁺ to the flooded NH₄⁺-enriched paddy soil, As mobility was affected by two main pathways. The NO₃⁻/NO₂⁻ couple has a higher redox potential than H₂AsO₄⁻/H₃AsO₃ and Fe³⁺/Fe²⁺, making nitrate-dependent microbial As(III) and Fe(II) oxidation more thermodynamically favorable than the Feammox reaction. A higher proportion of nitrate ensured the preferential occurrence of nitrate-dependent microbial As(III) and Fe(II) oxidation and readily attenuated the mobility of dissolved As(III). When nitrate was subsequently substantially depleted, the reductive dissolution of As(V)/Fe(III) was gradually enhanced due to surplus background and/or fertilizer-derived ammonia providing sufficient substrate for promoting the anammox and Feammox reactions. Therein, N₂O emission was effectively suppressed due to the neutralization (NO₂⁻ + NH₄⁺ → N₂) induced by the anammox reaction. In contrast, the nitrate treatment contributed to microbial-mediated oxidation of As(III)/Fe(II) under anaerobic conditions in which nitrate serves as an electron acceptor together with a concomitant denitrification producing more N₂O. This study provided valuable insights into the effects of interactions between native soil/fertilizer N and DOM on As mobilization and N₂O emission.

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.

ACKNOWLEDGEMENT

This work was supported by the National Natural Science Foundation of China (No. 41807035), the Public Welfare Technology Application Research Project of Zhejiang Province, China (No. LGF22E080002), the Higher Education Institution Scientific Research Project of Ningxia, China (No. NYG2024118), the Natural Science Foundation of Ningxia, China (No. 2024AAC05063), the Natural Science Foundation of Xiamen, China (No. 3502Z20227321), and the Municipal Science and Technology Bureau of Wenzhou, China (Nos. X20210033 and X20210096).

SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

CONTRIBUTION OF AUTHORS

Feng Wang and Jing Zhang contributed equally to this work.

REFERENCES

Beller H R, Zhou P, Legler T C, Chakicherla A, Kane S, Letain T E, O'Day P A. 2013. Genome-enabled studies of anaerobic, nitrate-dependent iron oxidation in the chemolithoautotrophic bacterium *Thiobacillus denitrificans*. *Front Microbiol.* **4**: 249.

Bertin P N, Heinrich-Salmeron A, Pelletier E, Goulhen-Chollet F, Arsène-Ploetze F, Gallien S, Lauga B, Casiot C, Calteau A, Vallenet D, Bonnefoy V, Bruneel O, Chane-Woon-Ming B, Cleiss-Arnold J, Duran R, Elbaz-Poulichet F, Fonknechten N, Giloteaux L, Halter D, Koehler S, Marchal M, Mornico D, Schaeffer C, Smith A A T, Van Dorsselaer A, Weissenbach J, Médigue C, Le Paslier D. 2011. Metabolic diversity among main microorganisms inside an arsenic-rich ecosystem revealed by meta- and proteo-genomics. *ISME J.* **5**: 1735–1747.

Bi Q F, Chen Q H, Yang X R, Li H, Zheng B X, Zhou W W, Liu X X, Dai P B, Li K J, Lin X Y. 2017. Effects of combined application of nitrogen fertilizer and biochar on the nitrification and ammonia oxidizers in an intensive vegetable soil. *AMB Express.* **7**: 198.

Borch T, Kretzschmar R, Kappler A, Van Cappellen P, Ginder-Vogel M, Voegelin A, Campbell K. 2010. Biogeochemical redox processes and their impact on contaminant dynamics. *Environ Sci Technol.* **44**: 15–23.

Bryce C, Blackwell N, Schmidt C, Otte J, Huang Y M, Kleindienst S, Tomaszewski E, Schad M, Warter V, Peng C, Byrne J M, Kappler A. 2018. Microbial anaerobic Fe(II) oxidation—Ecology, mechanisms and environmental implications. *Environ Microbiol.* **20**: 3462–3483.

Burguera M, Burguera J. 1997. Analytical methodology for speciation of arsenic in environmental and biological samples. *Talanta.* **44**: 1581–1604.

Cai S Y, Zhao X, Pittelkow C M, Fan M S, Zhang X, Yan X Y. 2023. Optimal nitrogen rate strategy for sustainable rice production in China. *Nature.* **615**: 73–79.

Chen G N, Du Y H, Fang L P, Wang X Q, Liu C P, Yu H Y, Feng M, Chen X, Li F B. 2023. Distinct arsenic uptake feature in rice reveals the importance of N fertilization strategies. *Sci Total Environ.* **854**: 158801.

Chen Z, Dong G W, Chen Y B, Wang H H, Liu S R, Chen Z J, Yang C H, Shang X, Dahlgren R. 2019a. Impacts of enhanced microbial-photochemical and suppressed dark microbial reductive dissolution on the mobility of As and Fe in flooded tailing soils with zinc sulfide. *Chem Eng J.* **372**: 118–128.

Chen Z, Dong G W, Gong L B, Li Q B, Wang Y P. 2018a. The role of low-molecular-weight organic carbons in facilitating the mobilization and biotransformation of As(V)/Fe(III) from a realgar tailing mine soil. *Geomicrobiol J.* **35**: 555–563.

Chen Z, Li H, Ma W D, Fu D, Han K Z, Wang H T, He N, Li Q B, Wang Y P. 2018b. Addition of graphene sheets enhances reductive dissolution of arsenic and iron from arsenic contaminated soil. *Land Degrad Dev.* **29**: 572–584.

Chen Z, Liu Y, Zhang C K, Pan Y J, Han R W, Chen Y B, Shang X, Dong G W, Zhang J L. 2019b. Titanium dioxide nanoparticles induced an enhanced and intimately coupled photoelectrochemical-microbial reductive dissolution of As(V) and Fe(III) in flooded arsenic-enriched soils. *ACS Sustain Chem Eng.* **7**: 13236–13246.

Chen Z, Wang Y P, Jiang X L, Fu D, Xia D, Wang H T, Dong G W, Li Q B. 2017. Dual roles of AQDS as electron shuttles for microbes and dissolved organic matter involved in arsenic and iron mobilization in the arsenic-rich sediment. *Sci Total Environ.* **574**: 1684–1694.

Chen Z, Wang Y P, Xia D, Jiang X L, Fu D, Shen L, Wang H T, Li Q B. 2016. Enhanced bioreduction of iron and arsenic in sediment by biochar amendment influencing microbial community composition and dissolved organic matter content and composition. *J Hazard Mater.* **311**: 20–29.

Chi Y Y, Liu Z R, Wang X, Zhang Y Y, Wei F. 2021. Provincial CO₂ emission measurement and analysis of the construction industry under China's carbon neutrality target. *Sustainability.* **13**: 1876.

Desireddy S, Pothanamkandathil Chacko S. 2021. A review on metal oxide (FeO_x/MnO_x) mediated nitrogen removal processes and its application in wastewater treatment. *Rev Environ Sci Bio/Technol.* **20**: 697–728.

Di Capua F, Pirozzi F, Lens P N L, Esposito G. 2019. Electron donors for autotrophic denitrification. *Chem Eng J.* **362**: 922–937.

Ding B J, Zhang H, Luo W Q, Sun S Y, Cheng F, Li Z K. 2021. Nitrogen loss through denitrification, anammox and Feammox in a paddy soil. *Sci Total Environ.* **773**: 145601.

Ding L J, An X L, Li S, Zhang G L, Zhu Y G. 2014. Nitrogen loss through anaerobic ammonium oxidation coupled to iron reduction from paddy soils in a chronosequence. *Environ Sci Technol.* **48**: 10641–10647.

Dong G W, Han R W, Pan Y J, Zhang C K, Liu Y, Wang H H, Ji X L, Dahlgren R A, Shang X, Chen Z, Zhang M H. 2021. Role of MnO₂ in controlling iron and arsenic mobilization from illuminated flooded arsenic-enriched soils. *J Hazard Mater.* **401**: 123362.

Durante-Rodríguez G, Fernández-Llamosas H, Alonso-Fernandes E, Fernández-Muñoz M N, Muñoz-Oliveras R, Díaz E, Carmona M. 2019. ArxA from *Azoarcus* sp. CIB, an anaerobic arsenite oxidase from an obligate heterotrophic and mesophilic bacterium. *Front Microbiol.* **10**: 1699.

Fanning J C. 2000. The chemical reduction of nitrate in aqueous solution. *Coordin Chem Rev.* **199**: 159–179.

Feng M, Du Y H, Li X M, Li F B, Qiao J T, Chen G N, Huang Y M. 2023. Insight into universality and characteristics of nitrate reduction coupled with arsenic oxidation in different paddy soils. *Sci Total Environ.* **866**: 161342.

Fuchslueger L, Wild B, Mooshammer M, Takriti M, Kienzl S, Knoltsch A, Hofhansl F, Bahn M, Richter A. 2019. Microbial carbon and nitrogen cycling responses to drought and temperature in differently managed mountain grasslands. *Soil Biol Biochem.* **135**: 144–153.

González-Cabaleiro R, Ofiteiru I D, Lema J M, Rodríguez J. 2015. Microbial catabolic activities are naturally selected by metabolic energy harvest rate. *ISME J.* **9**: 2630–2641.

Gorny J, Billon G, Lesven L, Dumoulin D, Madé B, Noiriel C. 2015. Arsenic behavior in river sediments under redox gradient: A review. *Sci Total Environ.* **505**: 423–434.

- Hou D Y, Li F S. 2017. Complexities surrounding China's soil action plan. *Land Degrad Dev.* **28**: 2315–2320.
- Hu Z, Lee J W, Chandran K, Kim S, Khanal S K. 2012. Nitrous oxide (N₂O) emission from aquaculture: A review. *Environ Sci Technol.* **46**: 6470–6480.
- Huang S, Jaffé P R. 2015. Characterization of incubation experiments and development of an enrichment culture capable of ammonium oxidation under iron-reducing conditions. *Biogeosciences.* **12**: 769–779.
- Hussain M M, Bibi I, Niazi N K, Shahid M, Iqbal J, Shakoor M B, Ahmad A, Shah N S, Bhattacharya P, Mao K, Bundschuh J, Ok Y S, Zhang H. 2021. Arsenic biogeochemical cycling in paddy soil-rice system: Interaction with various factors, amendments and mineral nutrients. *Sci Total Environ.* **773**: 145040.
- Jakus N, Mellage A, Höschen C, Maisch M, Byrne J M, Mueller C W, Grathwohl P, Kappler A. 2021. Anaerobic neutrophilic pyrite oxidation by a chemolithoautotrophic nitrate-reducing iron(II)-oxidizing culture enriched from a fractured aquifer. *Environ Sci Technol.* **55**: 9876–9884.
- Jamieson J, Prommer H, Kaksonen A H, Sun J, Siade A J, Yusov A, Bostick B. 2018. Identifying and quantifying the intermediate processes during nitrate-dependent iron(II) oxidation. *Environ Sci Technol.* **52**: 5771–5781.
- Jiang M D, Xu P, Zhou W, Shaaban M, Zhao J S, Ren T, Lu J W, Hu R G. 2020. Prior nitrogen fertilization regulates CH₄ emissions from rice cultivation by increasing soil carbon storage in a rapeseed-rice rotation. *Appl Soil Ecol.* **155**: 103633.
- Kraus D, Weller S, Klatt S, Santabárbara I, Haas E, Wassmann R, Werner C, Kiese R, Butterbach-Bahl K. 2016. How well can we assess impacts of agricultural land management changes on the total greenhouse gas balance (CO₂, CH₄ and N₂O) of tropical rice-cropping systems with a biogeochemical model? *Agr Ecosyst Environ.* **224**: 104–115.
- Kulkarni H V, Mladenov N, Johannesson K H, Datta S. 2017. Contrasting dissolved organic matter quality in groundwater in Holocene and Pleistocene aquifers and implications for influencing arsenic mobility. *Appl Geochem.* **77**: 194–205.
- Li H, Su J Q, Yang X R, Zhou G W, Lassen S B, Zhu Y G. 2019. RNA stable isotope probing of potential feammox population in paddy soil. *Environ Sci Technol.* **53**: 4841–4849.
- Li X M, Qiao J T, Li S, Häggblom M M, Li F B, Hu M. 2020. Bacterial communities and functional genes stimulated during anaerobic arsenite oxidation and nitrate reduction in a paddy soil. *Environ Sci Technol.* **54**: 2172–2181.
- Lin S Y, Wang W Q, Vancov T, Yuk Fo Lai D, Wang C, Wiesmeier M, Jin Q, Liu X Y, Fang Y Y. 2022. Soil carbon, nutrients and their stoichiometry decrease in relation to paddy field degradation: Investigation in a subtropical region. *Catena.* **217**: 106484.
- Liu S W, Lin F, Wu S, Ji C, Sun Y, Jin Y G, Li S Q, Li Z F, Zou J W. 2017. A meta-analysis of fertilizer-induced soil NO and combined NO+N₂O emissions. *Glob Change Biol.* **23**: 2520–2532.
- Liu T X, Chen D D, Li X M, Li F B. 2019a. Microbially mediated coupling of nitrate reduction and Fe(II) oxidation under anoxic conditions. *FEMS Microbiol Ecol.* **95**: f030.
- Liu T X, Chen D D, Luo X B, Li X M, Li F B. 2019b. Microbially mediated nitrate-reducing Fe(II) oxidation: Quantification of chemodenitrification and biological reactions. *Geochim Cosmochim Acta.* **256**: 97–115.
- Luo X H, Jiang X X, Xue S G, Tang X J, Zhou C H, Wu C, Qian Z Y, Wu K K. 2021. Arsenic biomineralization by iron oxidizing strain (*Ochrobactrum* sp.) isolated from a paddy soil in Hunan, China. *Land Degrad Dev.* **32**: 2082–2093.
- Nejidat A, Diaz-Reck D, Massalha N, Arbiv A, Dawas A, Dosoretz C, Sabbah I. 2018. Abundance and diversity of anammox bacteria in a mainstream municipal wastewater treatment plant. *Appl Microbiol Biot.* **102**: 6713–6723.
- Olaniran A O, Balgobind A, Pillay B. 2013. Bioavailability of heavy metals in soil: Impact on microbial biodegradation of organic compounds and possible improvement strategies. *Int J Mol Sci.* **14**: 10197–10228.
- Pothier M P, Lenoble V, Garnier C, Misson B, Rentmeister C, Poulain A J. 2020. Dissolved organic matter controls of arsenic bioavailability to bacteria. *Sci Total Environ.* **716**: 137118.
- Qiao J T, Li X M, Li F B. 2018. Roles of different active metal-reducing bacteria in arsenic release from arsenic-contaminated paddy soil amended with biochar. *J Hazard Mater.* **344**: 958–967.
- Qiao J T, Li X M, Li F B, Liu T X, Young L Y, Huang W L, Sun K, Tong H, Hu M. 2019. Humic substances facilitate arsenic reduction and release in flooded paddy soil. *Environ Sci Technol.* **53**: 5034–5042.
- Rani V, Bhatia A, Kaushik R. 2021. Inoculation of plant growth promoting-methane utilizing bacteria in different N-fertilizer regime influences methane emission and crop growth of flooded paddy. *Sci Total Environ.* **775**: 145826.
- Richards M, Metzel R, Chirinda N, Ly P, Nyamadzawo G, Duong Vu Q, de Neergaard A, Oelofse M, Wollenberg E, Keller E, Malin D, Olesen J E, Hillier J, Rosenstock T S. 2016. Limits of agricultural greenhouse gas calculators to predict soil N₂O and CH₄ fluxes in tropical agriculture. *Sci Rep.* **6**: 26279.
- Soda S O, Yamamura S, Zhou H, Ike M, Fujita M. 2006. Reduction kinetics of As(V) to As(III) by a dissimilatory arsenate-reducing bacterium, *Bacillus* sp. SF-1. *Biotechnol Bioeng.* **93**: 812–815.
- Sun J, Chillrud S N, Mailloux B J, Stute M, Singh R, Dong H L, Lepre C J, Bostick B C. 2016. Enhanced and stabilized arsenic retention in microcosms through the microbial oxidation of ferrous iron by nitrate. *Chemosphere.* **144**: 1106–1115.
- Tian H Q, Yang J, Xu R T, Lu C Q, Canadell J G, Davidson E A, Jackson R B, Arneeth A, Chang J F, Ciais P, Gerber S, Ito A, Joos F, Lienert S, Messina P, Olin S, Pan S F, Peng C H, Saikawa E, Thompson R L, Vuichard N, Winiwarter W, Zaehle S, Zhang B W. 2019. Global soil nitrous oxide emissions since the preindustrial era estimated by an ensemble of terrestrial biosphere models: Magnitude, attribution, and uncertainty. *Glob Change Biol.* **25**: 640–659.
- Tong H, Zheng C J, Li B, Swanner E D, Liu C S, Chen M J, Xia Y F, Liu Y H, Ning Z P, Li F B, Feng X B. 2021. Microaerophilic oxidation of Fe(II) coupled with simultaneous carbon fixation and As(III) oxidation and sequestration in karstic paddy soil. *Environ Sci Technol.* **55**: 3634–3644.
- van den Berg E M, Boleij M, Kuenen J G, Kleerebezem R, van Loosdrecht M C M. 2016. DNRA and denitrification coexist over a broad range of acetate/N-NO₃⁻ ratios, in a chemostat enrichment culture. *Front Microbiol.* **7**: 1842.
- van den Berg E M, Elisário M P, Kuenen J G, Kleerebezem R, van Loosdrecht M C M. 2017. Fermentative bacteria influence the competition between denitrifiers and DNRA bacteria. *Front Microbiol.* **8**: 1684.
- Wang M L, Hu R G, Ruser R, Schmidt C, Kappler A. 2020. Role of chemodenitrification for N₂O emissions from nitrate reduction in rice paddy soils. *ACS Earth Space Chem.* **4**: 122–132.
- Wang Y C, Lu Y L. 2020. Evaluating the potential health and economic effects of nitrogen fertilizer application in grain production systems of China. *J Clean Prod.* **264**: 121635.
- Wu Y F, Chai C W, Li Y N, Chen J, Yuan Y, Hu G, Rosen B P, Zhang J. 2021. Anaerobic As(III) oxidation coupled with nitrate reduction and attenuation of dissolved arsenic by *Noviherbaspirillum* species. *ACS Earth Space Chem.* **5**: 2115–2123.
- Xiong Y J, Du Y, Deng Y M, Ma T, Wang Y X. 2022. Feammox in alluvial-lacustrine aquifer system: Nitrogen/iron isotopic and biogeochemical evidences. *Water Res.* **222**: 118867.
- Xiu W, Lloyd J, Guo H M, Dai W, Nixon S, Bassil N M, Ren C, Zhang C R, Ke T T, Polya D. 2020. Linking microbial community composition to hydrogeochemistry in the western Hetao Basin: Potential importance of ammonium as an electron donor during arsenic mobilization. *Environ Int.* **136**: 105489.
- Xu C, Zhu H S, Wang J, Ji C, Liu Y B, Chen D Y, Zhang H, Wang J D, Zhang Y C. 2023. Fertilizer N triggers native soil N-derived N₂O emissions by priming gross N mineralization. *Soil Biol Biochem.* **178**: 108961.
- Xu H C, Yu G H, Yang L Y, Jiang H L. 2013. Combination of two-dimensional correlation spectroscopy and parallel factor analysis to characterize the binding of heavy metals with DOM in lake sediments. *J Hazard Mater.* **263**: 412–421.
- Xue S G, He X, Jiang X X, Pan W S, Li W C, Xia L B, Wu C. 2022. Arsenic biotransformation genes and As transportation in soil-rice

- system affected by iron-oxidizing strain (*Ochrobactrum* sp.). *Environ Pollut.* **314**: 120311.
- Yang X R, Li H, Nie S A, Su J Q, Weng B S, Zhu G B, Yao H Y, Gilbert J A, Zhu Y G. 2015. Potential contribution of anammox to nitrogen loss from paddy soils in southern China. *Appl Environ Microb.* **81**: 938–947.
- Yi B, Wang H H, Zhang Q C, Jin H, Abbas T, Li Y, Liu Y M, Di H J. 2019. Alteration of gaseous nitrogen losses via anaerobic ammonium oxidation coupled with ferric reduction from paddy soils in southern China. *Sci Total Environ.* **652**: 1139–1147.
- Yin X L, Peñuelas J, Xu X P, Sardans J, Fang Y Y, Wiesmeier M, Chen Y Y, Chen X X, Wang W Q. 2021. Effects of addition of nitrogen-enriched biochar on bacteria and fungi community structure and C, N, P, and Fe stoichiometry in subtropical paddy soils. *Eur J Soil Biol.* **106**: 103351.
- Yuan C L, Li Q, Sun Z Y, Zhang W J, Chen J R, Chen Z, Na S, Sun H W. 2023. Chemical oxidation of arsenic in the environment and its application in remediation: A mini review. *Pedosphere.* **33**: 185–193.
- Zhang M M, Kolton M, Häggblom M M, Sun X X, Yu K, He B, Yuan Z J, Dong Y R, Su X F, Chen Z Y, Li H, Xiao T F, Xiao E Z, Sun W M. 2022. Anaerobic ammonium oxidation coupled to arsenate reduction, a novel biogeochemical process observed in arsenic-contaminated paddy soil. *Geochim Cosmochim Acta.* **335**: 11–22.
- Zhang R C, Chen C, Xu X J, Lee D J, Ren N Q. 2022. The interaction between *Pseudomonas* C27 and *Thiobacillus denitrificans* in the integrated autotrophic and heterotrophic denitrification process. *Sci Total Environ.* **811**: 152360.
- Zhang W F, Dou Z X, He P, Ju X T, Powlson D, Chadwick D, Norse D, Lu Y L, Zhang Y, Wu L, Chen X P, Cassman K G, Zhang F S. 2013. New technologies reduce greenhouse gas emissions from nitrogenous fertilizer in China. *Proc Natl Acad Sci USA.* **110**: 8375–8380.
- Zhang X, Zhang Y, Shi P, Bi Z L, Shan Z X, Ren L J. 2021. The deep challenge of nitrate pollution in river water of China. *Sci Total Environ.* **770**: 144674.
- Zhao C Y, Zhang J, Chen Z, Li J, Shu L L, Ji X L. 2022. Effective constructions of electro-active bacteria-derived bioelectrocatalysis systems and their applications in promoting extracellular electron transfer process. *Prog Chem* (in Chinese). **34**: 397–410.
- Zhou G W, Yang X R, Li H, Marshall C W, Zheng B X, Yan Y, Su J Q, Zhu Y G. 2016. Electron shuttles enhance anaerobic ammonium oxidation coupled to iron(III) reduction. *Environ Sci Technol.* **50**: 9298–9307.
- Zhu X B, Zeng X C, Chen X M, Wu W W, Wang Y X. 2019. Inhibitory effect of nitrate/nitrite on the microbial reductive dissolution of arsenic and iron from soils into pore water. *Ecotoxicology.* **28**: 528–538.