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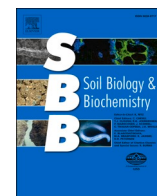
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Viruses enhance bacterial-mediated arsenic reduction processes by enriching rare functional taxa in flooded paddy soil

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ABSTRACT

Soil viruses are abundant and may play a role in arsenic (As) metabolic processes, which pose potential environmental risks in paddy field soils. However, little is known about the association of soil viruses with abundant and rare taxa and their ecological roles in regulating As transformation processes. Herein, we investigated the influence of soil viruses on bacterial-mediated As transformation and the response of abundant and rare taxa by inoculating with different soil viral concentrates (suspensions with no-viruses, extracellular free viruses, and mitomycin-C induced viruses) into sterilized soils containing a gradient of As concentrations. Results showed that both viral concentrates increased porewater As(III) concentrations, with extracellular free viruses increasing by 26.7 % and 37.1 % relative to treatment without viruses under low and high As conditions, while mitomycin-C induced viruses increased by 11.6 % and 23.4 %, respectively. Moreover, bacterial-generated As(III) displayed a significant positive correlation with Fe(II) concentrations even in the presence of viruses. Rare taxa were more susceptible than abundant taxa to virus inoculation. These changes in bacteria structure led to the enrichment of some rare functional taxa, especially those associated with potential Fe and As reduction functions. Evidence from qPCR analysis showed an increase in arsenic (*arsC*) and iron (*FeRB*) reduction genes, and these cumulative relative abundance of enriched rare functional taxa were significantly related to the increased As(III) concentrations. Overall, this study provides direct experimental evidence that viruses enrich rare functional taxa to enhance bacterial-mediated As reduction processes, highlighting a crucial role that viruses play in shaping soil ecosystem functions.

1. Introduction

Arsenic (As) is a toxic metalloid contaminant widely distributed in paddy soils. The proportion of rice produced on soils with As concentrations above 20 mg kg⁻¹ has increased from 13.0 % in 2000 to 17.1 % in 2020, and is projected to reach 18.3 % by 2040 in China (Zhang et al., 2024). Under flooded conditions, As is released into porewater through Fe(III) (hydr)oxides dissolution or arsenate (As(V)) reduction by soil microbiomes (Zobrist et al., 2000; Roberts et al., 2010). These microbial processes increase As mobility and facilitate its uptake by rice, thus threatening food safety and human health (Zhu et al., 2014). While both biotic and abiotic factors shape the composition of soil microbiomes, our understanding of biotic influences remains limited.

Viruses are the most abundant and genetically diverse biological entity in the biosphere with an estimated 4.80×10^{31} particles (Cobián Güemes et al., 2016). They can control host abundance and shape microbial community structure by inducing host mortality, and influence biogeochemical cycles via nutrient release through cell lysis or participating in host metabolisms with auxiliary metabolic genes (AMGs) (Jansson and Wu, 2023). To date, the potential role of viruses in carbon (Jin et al., 2019; Lee et al., 2021), phosphate (Goldsmith et al., 2015), and sulfur metabolism (Roux et al., 2016; Kieft et al., 2021) has been proposed in marine and soil ecosystems. Currently, virus-host interactions in metal-contaminated soil environments have garnered substantial interest owing to toxicity and food safety concerns.

Viral populations in As-contaminated soils exhibit the ability to

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confer their hosts with As resistance and detoxification (Sun et al., 2023; Huang et al., 2024). Genes involved in As resistance and metabolism, such as As(III)-responsive *trans*-acting repressor proteins (*arsR*), As(III) responsive transcriptional repressors (*arsD*), cytoplasmic arsenate reductases (*arsC*), and As(III) S-adenosylmethionine methyltransferases (*arsM*), have been detected in soil viromes, and the abundance of these genes in viruses increases with the As contamination level (Gao et al., 2023; Tang et al., 2023). Moreover, the introduction of prophages from arsenic-resistant bacteria carrying the *arsC* and *arsM* genes into arsenic-contaminated soil increased the abundance of *arsC* and *arsM* by 122- and 457-fold compared to soil without phage treatment, respectively (Tang et al., 2019). These results suggest soil viruses may facilitate the propagation of these genes during subsequent lysogenic infection stages, thereby assisting their hosts in adapting to As stress and contributing to As biotransformation processes.

Virus-host interactions and microbial-mediated ecological processes depend on their life strategies (lytic and lysogenic replication), which can be explained by two theories: kill-the-winner (KtW) and piggyback-the-winner (PtW) (Pratama and van Elsas, 2018). The “KtW” theory posits that the dominant bacteria are easily infected and lysed by viruses (Breitbart, 2012). In contrast, the “PtW” theory proposes that lysogenic infection is favored at high host densities as viruses exploit their hosts through lysogeny rather than killing them (Knowles et al., 2016). A recent study found a higher proportion of lysogeny under low-contamination conditions, where lysogenic viruses that aid host survival were more likely to be selected and maintained, supporting the “PtW” hypothesis (Xia et al., 2023). Although previous consensus indicates that viruses predominantly affect abundant taxa, rare taxa may also play critical roles in contaminated soil ecosystems, as they represent the bulk of bacterial diversity (Bouvier and Del Giorgio, 2007). Given these virus-host collaborations, we hypothesize that viruses are more likely to impact abundant than rare taxa due to their higher density and greater accessibility to viral infection. However, further studies are needed to examine how viruses interact with both abundant and rare taxa, as this is crucial for understanding the roles of viruses in metal-contaminated environments.

In this study, native soil concentrates of viruses were individually collected by filtration using a tangential flow filtration system (TFF) and induction from bacteria suspension with mitomycin-C (MC) solution. These viruses were then combined with the bacteria community by reinoculation into three sterilized As-contaminated soils (Control: no As addition, Low: 60 mg kg⁻¹, and High: 200 mg kg⁻¹) to allow for viruses to recolonize. We aimed to (1) determine which bacterial-mediated As transformation processes were affected by soil viruses, and (2) reveal how viruses effect on As biotransformation processes through interactions with either rare or abundant taxa under flooded soil conditions.

2. Materials and methods

2.1. Soil sampling and experimental soil preparation

Paddy soils were collected in March 2022 from a paddy field located in Jiaxing, Zhejiang, China (30° 50' N, 120° 43' E). Topsoil samples (0–20 cm) were randomly collected from 5 sampling points within the field and mixed to form a composite sample, then immediately transported to the laboratory on ice. Before experimental operations, soil samples were sieved through a 2-mm sieve (alcohol sterilized) and divided into two subsamples after removing plant residues. One subsample was stored at 4 °C for virus and bacteria extraction, while the other subsample was used to prepare the As-contaminated soils for subsequent incubations. The basic soil physicochemical properties in this study were described previously (Shen et al., 2022) and summarized as follows: pH: 6.76, organic C: 54.4 g kg⁻¹, total N: 2.1 g kg⁻¹ and total As concentration: 16.9 mg kg⁻¹, which was below the threshold established for As-contaminated soil (Zhao et al., 2015).

To prepare the experimental soils, Na₂HAsO₄·7H₂O solution was added to the soil to achieve final total As concentrations of 60 (Low) and 200 (High) mg kg⁻¹, while the unamended soil was designated as the control (CK). Soil moisture was adjusted to 60 % of field capacity and allowed to equilibrate for 1 month at room temperature (25 °C). Finally, the soils were air-dried and sterilized with 100 kGy γ-radiation for 24 h (Wei et al., 2021). To assess sterilization effectiveness, soil samples were diluted for plate counts to compare microbial populations before and after sterilization. Specifically, 0.5 g of soil samples were 10-fold diluted with sterile water, and the suspensions were plated onto tryptic soy broth agar and potato-dextrose agar media (Chen et al., 2017; Yan et al., 2017). No microbial growth was observed on agar plates associated with sterilized soils during the 7-day incubation period.

2.2. Preparation of native soil viral and bacterial suspensions

To prepare different soil virus treatments, the soil suspension was subjected to filtration by TFF (Braga et al., 2020; Tong et al., 2023) and MC induction assays (Liang et al., 2020; Tang et al., 2023). Specifically, the collected soil (5 kg) was washed with 5 L sterile saline magnesium buffer (SM; 5.8 g NaCl, 2 g MgSO₄, 50 mL Tris-HCl, pH 7.5) on a vortex at the maximum speed for 3 min and shaking for 2 h (4 °C, 250 rpm), before centrifuging the slurry at 4 °C for 20 min (2500 × g) (Tong et al., 2023). The supernatant was then passed through a 2-μm cellulose acetate filter before sequential filtering and concentrating using the TFF system (Cogent μScale, Merck, USA) with pore sizes of 0.22 μm and 100 kDa. The obtained retentates (>0.22 μm) were used as the bacteria community (BC), and the concentrated filtrates (100 kDa–0.22 μm) were used as extracellular free virus suspension (V1) and no-virus suspension (<100 kDa, SM buffer) (Braga et al., 2020; Tong et al., 2023). Finally, 750 mL of bacteria suspension (BC), 250 mL of extracellular free virus suspension (V1) and 4000 mL of no-virus suspension (SM) were collected for the following procedures.

Mitomycin-C (MC) is widely used in prophage induction assays (Williamson et al., 2007). Before the induction experiment, sterile water-soluble organic carbon (WSOC) solutions were prepared to supply carbon, energy and other nutrient resources essential for supporting viral production during prophage induction processes (Liang et al., 2020; Tang et al., 2023). Briefly, the collected soil was suspended in sterile deionized water and homogenized by vortexing at maximum speed for 3 min. Then, the soil slurry was centrifuged at 5000 × g for 20 min, and the supernatant was filtered through a 0.02 μm PVDF filter and autoclaved. Subsequently, 150 mL of the previously bacteria suspension (BC) was mixed with the prepared sterile WSOC and MC solutions (1 μg mL⁻¹ final concentration) in a sterile 500 mL flask, while another was prepared without MC. The mixture was incubated with shaking at 200 rpm for 24 h in the dark (room temperature) (Liang et al., 2020), and the MC-induced viruses were collected after passing through a 0.22 μm sterile filter (Thermo Scientific). After filtering, we further used Amicon Ultra filters (Millipore) to concentrate and collect the MC-induced viruses (V2) (Albright et al., 2022). Finally, 250 mL of extracellular free virus suspension (V1), 50 mL of MC-induced viral concentrate (V2) and 450 mL of bacteria suspension (BC) were stored at 4 °C before inoculating microbial suspensions into different microcosms within 24 h (Braga et al., 2020).

To ensure the presence and purity of each microbial community before inoculation, both viral and bacterial suspensions were verified and enumerated with epifluorescence microscopy (EFM) (See supplementary materials for detailed methodology). In this study, the virus abundance of V1 was 6.5 × 10⁷ VLPs g⁻¹ soil (Fig. S1A), while V2 exhibited a concentration of 1.5 × 10⁸ VLPs g⁻¹ soil (Fig. S1C). These concentrations were representative of indigenous virus concentrations found in soils, which typically ranged from 10³ to 10⁹ VLPs g⁻¹ soil (Williamson et al., 2017). Moreover, the abundance of bacteria in our experiments reached up to 2.7 × 10⁸ cells g⁻¹ soil (Fig. S1B).

2.3. Microcosm experimental design

To simulate the abundance of native virus and bacteria communities in the paddy soil environment, the microcosms were separately incubated with 1 mL viral suspensions (no-virus SM, V1 or V2) and 3 mL bacterial suspensions (at a ratio of 1:3 by volume, virus: bacteria) added to the sterile bottles containing 20 g of dry sterilized soil. The resulting four treatments were as follows: (1) 4 mL SM buffer, abiotic control (SM); (2) 1 mL SM buffer + 3 mL bacterial suspension, biotic treatment (BC); (3) 1 mL extracellular free virus suspension (V1) + 3 mL bacterial suspension, biotic treatment (V1BC); and (4) 1 mL MC-induced virus concentrate (V2) + 3 mL bacterial suspension, biotic treatment (V2BC) (Fig. S2). For each treatment, a homogenized inoculum was first prepared by adding viral and bacterial extracts, and then sterile water was added to simulate field flooding (2 cm above the soil surface). Finally, all bottles were sealed with a sterilized gas permeable sealing membrane that allowed the surface of the flooded water to contact atmospheric air during incubation. Each treatment was performed in triplicate resulting in a total of 180 individual microcosms (3 replicates $\times$ 3 arsenic levels $\times$ 4 treatments $\times$ 5 time points). All microcosms were incubated in a controlled environment room at 25 °C in the dark. Soil samples and porewater were collected on day 0, 3, 7, 14 and 30. Each soil sample was stored at -80 °C for DNA extraction and functional gene analysis. Soil porewater was collected to quantify total As, As species and Fe(II) concentrations.

2.4. Chemical analysis

Soil pH and Eh were determined using standard probes/meters. Porewater was collected by centrifugation at $3100 \times g$ for 8 min and mixed with 6 M HCl to prevent Fe oxidation (Tang et al., 2021). Fe(II) concentrations were measured using the phenanthroline-spectrophotometric method (Fadrus and Malý, 1975). Total As concentrations of porewater were determined by ICP-MS (NEXION300XX) (Tang et al., 2021). As species were measured by HPLC-ICP-MS (PerkinElmer Series 200 HPLC and NEXION300XX, ICP-MS) after chromatographic separation with 20 mM $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 6.5) as the mobile phase. Standard curves were constructed (5, 10, 20, 50, 100 and 200 $\mu\text{g L}^{-1}$) for As(III), As(V), dimethylarsinic acid (DMAs(V)), and monomethyl arsenic acid (MMAs(V)), respectively (Shi et al., 2020).

The contributions of bacterial-mediated As(III)/Fe(II) accumulation ($\Delta\text{B-As(III)/Fe(II)}$) and the contribution of virus-mediated As(III) accumulation ($\Delta\text{enriched As(III)}$) at experimental time points were calculated as:

$$\Delta\text{B-As(III)/Fe(II)} = \text{As/Fe}_{\text{SM}} - \text{As/Fe}_{\text{SM}}$$

$$\Delta\text{enriched As(III)} = \text{As}_v - \text{As}_{\text{BC}}$$

Where As/Fe are the As(III)/Fe(II) concentrations in the three biotic treatments (BC, V1BC, and V2BC) at each time point, $\text{As}_{\text{SM}}/\text{Fe}_{\text{SM}}$ are the As(III)/Fe(II) concentrations in the abiotic treatments (SM) at each time point, As_v is the As concentration in viral treatments (V1BC and V2BC) at each time point, and As_{BC} is the As concentration in the bacterial treatment (BC) at each time point.

2.5. DNA extraction, qPCR and sequencing

Soil DNA was extracted using the Fast DNA SPIN Kit for soil (QBIOM Gene, USA) following the manufacturer's instructions. The final extracted DNA volume was adjusted to 80 μL and divided into two subsamples for subsequent quantitative polymerase chain reaction (qPCR) and MiSeq (Illumina) sequencing. Functional genes were quantified using qPCR (LightCycler 480II; Roche, Switzerland). 16S rRNA gene for total bacteria, As reduction gene (*arsC*), and iron reduction gene (*FeRB*) were selected for analysis. Detailed information concerning the primers and amplification procedures was provided in Supplementary

Table S1. Plasmids containing the target genes were used as standards to calculate gene copy numbers; standard curves were constructed from 10-fold serial dilutions (1.69×10^{10} to 1.69×10^3 copies μL^{-1} for *arsC* gene, 1.70×10^{10} to 1.70×10^3 copies μL^{-1} for 16S rRNA gene and 1.75×10^{10} to 1.75×10^3 copies μL^{-1} for *FeRB* gene). Negative controls were prepared for each assay using sterilized H_2O .

For sequencing, the bacterial V3-V4 region of the 16S rRNA gene was amplified with primers 341F and 806R, which were labeled with unique barcodes to enable the discrimination of different samples during sequencing (Thijs et al., 2017). Each sample was amplified in triplicate. After verification and purification, the samples were pooled together and prepared for sequencing, which was performed on an Illumina MiSeq platform (2 $\times$ 300 bp) at Novogene Bioinformatics Technology (Beijing, China). Raw sequences were processed using the Quantitative Insight into Microbial Ecology (QIIME 2, v2020.2) pipeline (<http://view.qiime2.org>). Briefly, the reads were quality controlled, chimera filtered and denoised based on DADA2 to generate amplicon sequence variants (ASVs) (Callahan et al., 2016). The bacteria taxonomy of ASVs was assigned using the naive Bayes classifier in QIIME2 and SILVA 132 database (<http://www.arb-silva.de/download/archive/qiime/>). Overall, we obtained a total of 7,090,858 high-quality sequences (average = 53,718; range = 43,498–65,354). Finally, all samples were rarefied to 42,000 sequences for further analysis. The original sequence data were deposited into the National Center for Biotechnology Information Sequence Read Archive database under project ID PRJNA985387.

2.6. Statistical analysis

Bacteria taxa were classified into abundant (AT) and rare (RT) taxa according to the method described by Jiao et al. (2020). Thresholds for the relative abundance of ASVs were <0.01 % for "RT" and >0.1 % for "AT", while the remainder were considered as "intermediate" ASVs (MT). Since our main objective was to focus on the abundant and rare taxa, we did not further consider the "intermediate" taxa. Species richness of the bacterial community was assessed with the Chao1 index using the "vegan" packages (Xu et al., 2022). A difference analysis was performed to determine sensitive taxa ($p < 0.05$) using the "DESeq2" package (version 1.31.13) and visualized using a volcano plot (Yang et al., 2023).

Co-occurrence networks were built with Pearson's correlation (r) greater than 0.7 and $p < 0.05$ (Jiang et al., 2023). Each node represented an individual ASV, and each edge denoted the pairwise correlation between nodes. Multiple topological properties (i.e., nodes, edges and average degree) were calculated and visualized using the *igraph* package (Fan et al., 2021). Random forest-based models were used to identify key microbial predictors for porewater As(III) concentrations enriched by virus inoculation, and they were ranked in the order of feature importance and visualized by percentage increases in the MSE (mean squared error); higher MSE% values indicate more important features (Jiao et al., 2018). All statistically significant differences, such as the concentrations of total As, As species, Fe(II), functional gene abundance and species richness, were determined by one-way analysis of variance (ANOVA) with Tukey's post-Hoc test in SPSS 19.0.

3. Results

3.1. Changes of porewater As concentrations with virus inoculation

Soil pH increased, while Eh decreased over the 30 day incubation period (Fig. S3). Porewater total As concentrations were significantly higher in biotic treatments (BC, V1BC, and V2BC) than in the abiotic treatment (SM) (Fig. S4, $p < 0.05$). Three As species (i.e., As(V), As(III), and DMA) were detected in porewater, with As(III) being the predominant fraction from day 7–30 (Fig. 1A and B). As(III) concentrations in V2BC were lower than BC under low As conditions from day 3–7

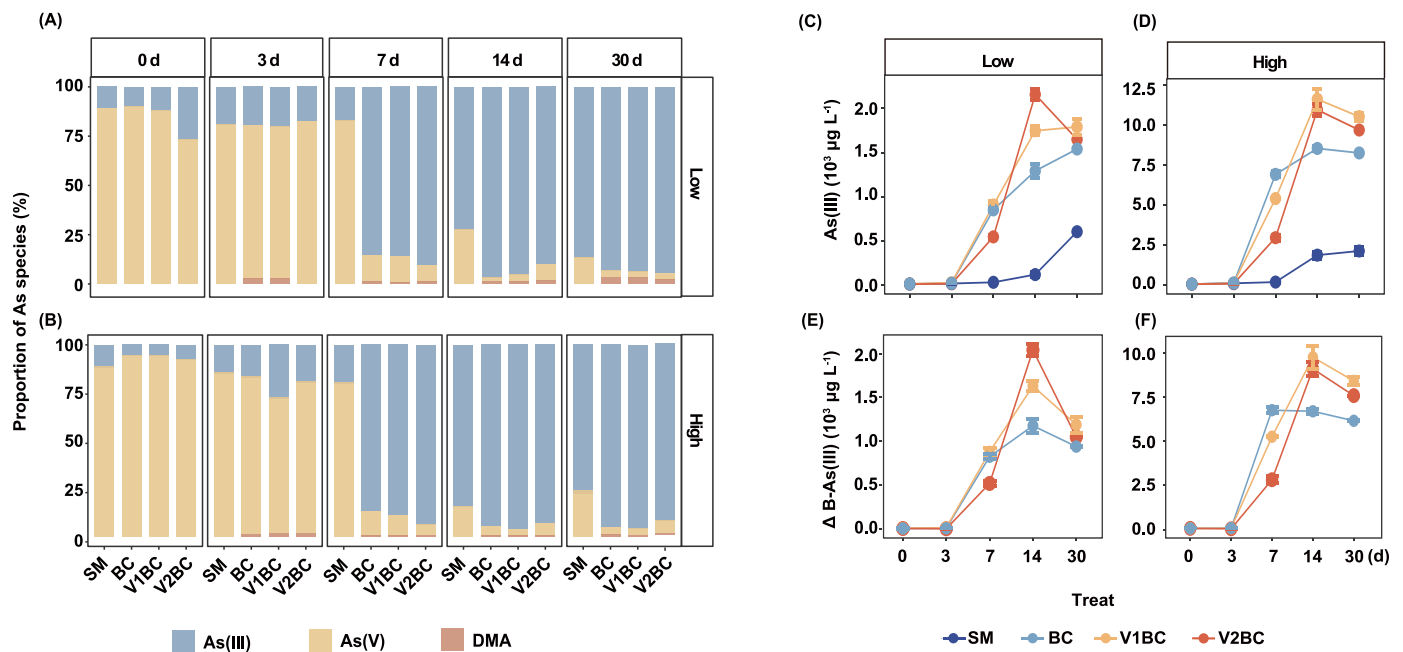


Fig. 1. As transformations in soil porewater with or without soil virus inoculation under flooded conditions. The proportion of As species (A and B), As(III) concentrations (C and D), and Δ B-As(III) concentrations (E and F) in low and high As-contaminated soils. Arsenic species: As(III), arsenite; As(V), arsenate; DMA, dimethylarsinic acid. Δ B-As(III) represents bacteria-mediated As(III) concentrations, which are calculated by subtracting As(III) concentrations in the abiotic treatment (SM) from each of the three biotic treatments, respectively. Error bars represent standard deviations of three replications. SM, only SM buffer (abiotic); BC, SM buffer and bacteria (biotic); V1BC, bacteria and V1 virus filtrate (biotic); V2BC, bacteria and V2 virus filtrate (biotic). Low and High refer to total As concentrations of 60 and 200 mg kg⁻¹ soil, respectively.

(Fig. 1C, Fig. S5A, $p < 0.05$), whereas As(III) concentrations in V1BC and V2BC were both lower under high As conditions (Fig. 1D, Fig. S5B, $p < 0.05$). From day 14–30, As(III) concentrations in V1BC and V2BC exceeded those of BC under high As condition (Fig. 1D, Fig. S5B), while only V1BC was higher than BC under low As condition (Fig. 1C, Fig. S5A, $p < 0.05$). Moreover, As(III) concentrations in V1BC were higher than that in V2BC under high As condition on day 30 (Fig. 1D, Fig. S5B, $p < 0.05$).

The Δ B-As(III) concentration patterns (Fig. 1E and F) resembled the changes in As(III) concentration (Fig. 1C and D). In the low As condition, Δ B-As(III) was highest on day 14 and treatments followed: V2BC ($2042 \pm 67 \mu\text{g L}^{-1}$) > V1BC ($1630 \pm 53 \mu\text{g L}^{-1}$) > BC ($1175 \pm 79 \mu\text{g L}^{-1}$) (Fig. 1E, Fig. S6A, $p < 0.05$). Under the high As condition, a similar trend was observed, but with much higher values: V1BC ($9743 \pm 669 \mu\text{g L}^{-1}$) > V2BC ($9081 \pm 375 \mu\text{g L}^{-1}$) > BC ($6669 \pm 142 \mu\text{g L}^{-1}$) (Fig. 1F, Fig. S6B, $p < 0.05$). Compared with BC on day 30, V1 virus increased As(III) concentrations by 26.7 % and 37.1 % relative to treatment without viruses under low and high As conditions, whereas V2 virus increased As(III) concentrations by 11.6 % and 23.4 %, respectively (Fig. S6).

3.2. Porewater Fe(II) concentrations and relationships with As(III)

Porewater Fe(II) concentrations were higher in biotic treatments (BC, V1BC, and V2BC) than in the abiotic treatment (SM) (Fig. 2, Fig. S7). Maximum Fe(II) concentrations were observed on day 14 for V2BC ($13.2 \pm 0.5 \text{ mg L}^{-1}$) in CK soil (Fig. 2A, Fig. S7A), V1BC ($10.3 \pm 0.5 \text{ mg L}^{-1}$) under low As condition (Fig. 2B, Fig. S7B) and V1BC ($8.0 \pm 0.4 \text{ mg L}^{-1}$) under high As condition (Fig. 2C, Fig. S7C, $p < 0.05$). Positive correlations between Δ B-Fe(II) and Δ B-As(III) were observed under both low and high As conditions for V1BC and V2BC treatments (Fig. 2E and F, $p < 0.001$).

3.3. Virus inoculation effects on abundant (AT) and rare (RT) bacteria subcommunities

A total of 33,138 and 126 ASVs were identified as RT and AT, respectively (Fig. S8C). Only RT showed significant differences in Chao1 index, with V2BC increasing the index more than BC in both CK and low As-contaminated soils (Fig. S9C, $p < 0.05$). Virus inoculation also affected the relative abundance of the phyla in RT (Fig. S10B). Under As stress, the number of nodes (N) in co-occurrence networks increased for V1BC and V2BC, while the average degree (D) decreased compared to BC (Fig. 3A). The proportion of positive links in the networks increased under both low and high As-contaminated soils compared to CK (Fig. 3B). Among the co-occurrence networks, the proportion of nodes for RT increased in the low and high As-contaminated networks, particularly in the V1BC and V2BC treatments (Fig. 3C).

RT showed greater susceptibility to virus inoculation compared to AT (Fig. 4A). The most significantly enriched or depleted taxa in V1BC versus BC and V2BC versus BC belonged to RT. Among the total differential species for all treatments, the proportions of RT were higher under low and high As stress than CK (Fig. 4B). Specifically, the proportions of RT were 92.3 % (CK), 97.2 % (Low) and 96.6 % (High) for V1BC, versus 72.9 % (CK), 94.1 % (Low) and 92.3 % (High) for V2BC. Additionally, the cumulative relative abundance of RT in the V1BC and V2BC treatments showed a positive correlation with Δ B-As(III) under low and high As conditions (Fig. 5, $p < 0.05$).

3.4. Functional profiles for enriched rare taxa with virus inoculation

Virus inoculation increased the abundance of bacteria (Fig. S11) and *arsC* (Fig. 6A and B) and *FeRB* genes (Fig. 6C and D). Compared to previously published research, most enriched rare taxa were associated with potential Fe and As reduction functions (Table S2, Fig. S12). Moreover, Fe reduction taxa were positively correlated with Δ enriched As(III) for V1BC (Fig. 6E, $p < 0.05$), while the As reduction taxa were positively correlated with Δ enriched As(III) for V2BC (Fig. 6F, $p < 0.05$).

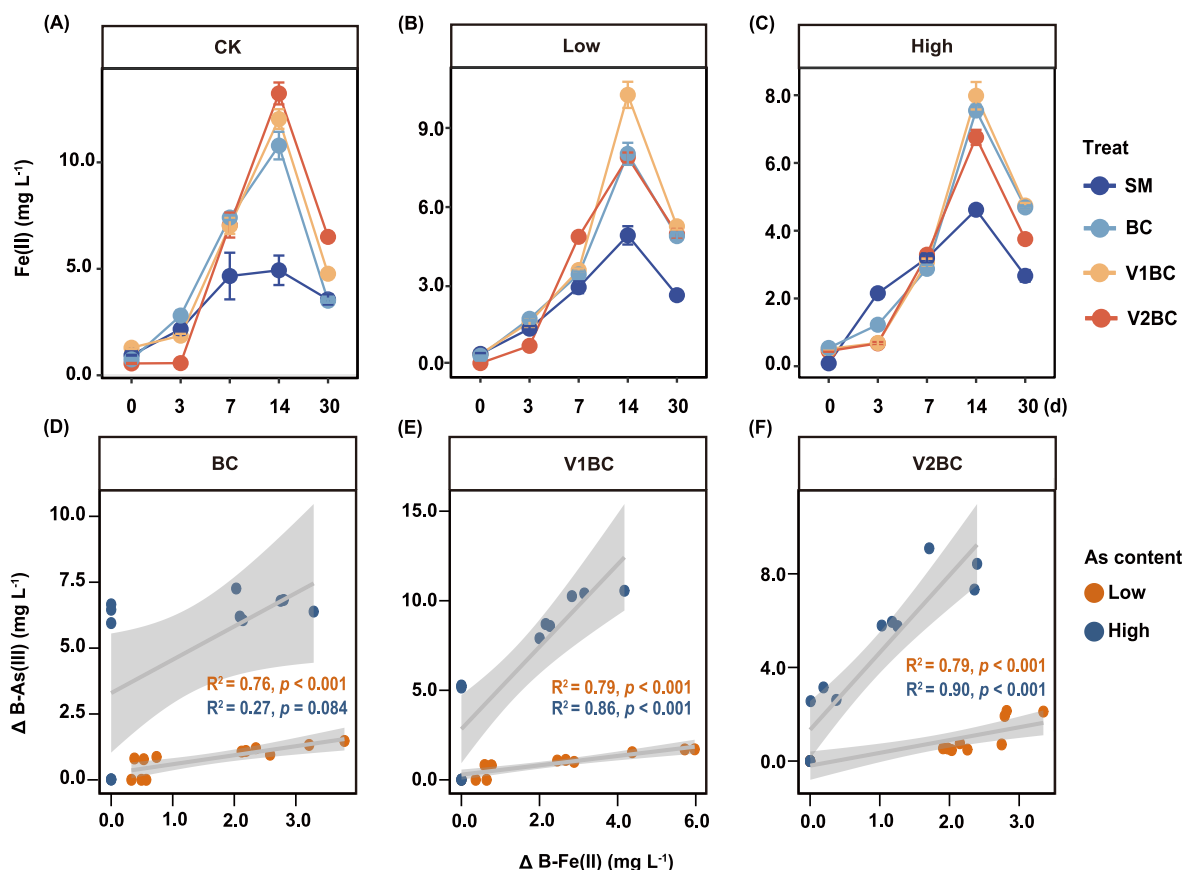


Fig. 2. Fe(II) concentrations of soil porewater and associated linear regressions for $\Delta B\text{-As(III)}$ to $\Delta B\text{-Fe(II)}$ with or without soil virus inoculation under flooded conditions. Porewater Fe(II) concentrations in CK (A), low (B), and high (C) As-contaminated soils. Linear regressions of the $\Delta B\text{-As(III)}$ to $\Delta B\text{-Fe(II)}$ without virus (D), with V1 virus (E), and V2 virus (F) inoculation in low and high As-contaminated soils. $\Delta B\text{-As(III)}$ and $\Delta B\text{-Fe(II)}$ represent bacteria-mediated As(III)/Fe(II) concentrations, which are calculated by subtracting As(III)/Fe(II) concentrations in the abiotic treatment (SM) from each of the three biotic treatments, respectively. Error bars represent standard deviations of three replications. SM, only SM buffer (abiotic); BC, SM buffer and bacteria (biotic); V1BC, bacteria and V1 virus filtrate (biotic); V2BC, bacteria and V2 virus filtrate (biotic). Low and High refer to the total As concentrations of 60 and 200 mg kg⁻¹ soil, whereas the unamended As soil is designated as the control (CK).

0.01).

Enriched rare functional taxa in V1BC and V2BC mainly belong to the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* and *Firmicutes* (Fig. 6G and H). Random forest analysis revealed that the key microbial predictor for As reduction was *Geobacter* in the V1BC treatment (Fig. 6G), whereas *Micromonospora* was the principal genus contributing to As reduction in the V2BC treatment (Fig. 6H).

4. Discussion

4.1. Soil viruses increased porewater As(III) and Fe(II) concentrations

Previous studies revealed the presence of genes associated with As uptake, reduction and methylation in the virus genomes, as well as complex virus-host linkages, implying the potential involvement of viruses in As biogeochemical processes (Gao et al., 2023; Tang et al., 2023; Yu et al., 2023). The results of this study provide direct experimental evidence that soil viruses enhance bacterial-mediated As reduction processes (Fig. 1). The increments of bacterial-generated As(III) concentrations were positively associated with Fe(II) concentrations (Fig. 2), implying that the accumulation of As(III) in porewater occurred in association with the microbial reductive dissolution of Fe(III), even in the presence of the viruses. Many studies report that microbial-mediated Fe(III) reduction can increase porewater As(III) concentrations in flooded soils (Weber et al., 2010; Ohtsuka et al., 2013). This follows from iron minerals being the primary solid-phase component responsible for

adsorbing arsenic in soils, wherein microbial-mediated reductive dissolution of iron (hydr)oxides leads to the release of sorbed As to porewaters (Zobrist et al., 2000). Thus, the virus-mediated changes of porewater As concentrations are explained, in part, by their regulation of As-Fe reduction interactions.

Viruses impact nearly all organisms on Earth, encompassing not only microbial communities but also their associated biogeochemical processes (Jansson and Wu, 2023). In the V1BC treatment, the higher As(III) concentrations may be due to bacterial mortality caused by viral lysing, as lytic viruses appeared to constitute a “majority” of the extracellular free virus suspensions. The substrates released from the lysed cells may further boost the growth of existing bacteria that participate in As reduction processes (Shiah et al., 2022; Tong et al., 2023), which is supported by the increased arsenic-related functional genes in the V1BC treatment (Fig. 6A–D). Furthermore, prior research has demonstrated that similar treatments with viral filtrates significantly accelerated the mineralization of dissolved organic matter, highlighting the potential influence of virus-mediated host predation on microbial functions (Wang et al., 2022).

The higher As(III) concentrations observed in the MC-induced virus suspension (V2BC) may be attributed to the abundance of prophages (Wang et al., 2025). Prophages serve as mediators for gene transfer among various bacterial hosts, conferring arsenic biotransformation capabilities to their hosts (Canchaya et al., 2003). Given the widespread existence of the *arsC* gene, the conversion of As(V) to As(III) and its subsequent expulsion is an efficient mechanism employed by native soil

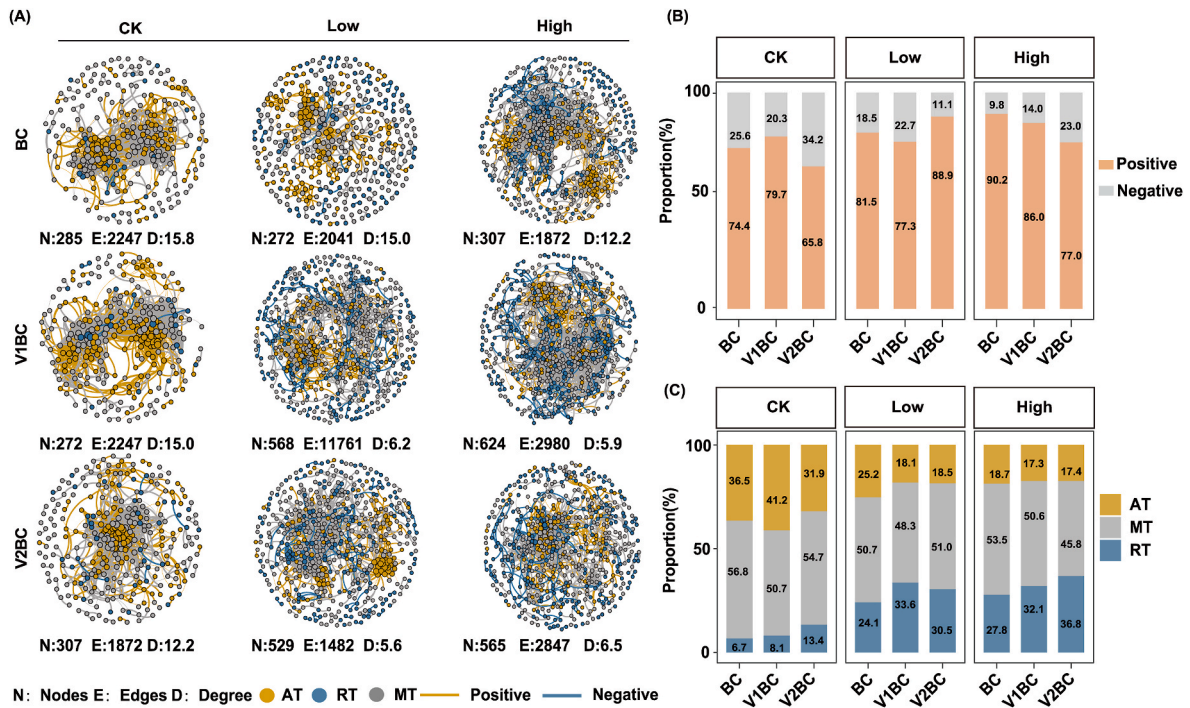


Fig. 3. Co-occurrence network analysis for investigating the interactions of the different taxa with or without virus inoculation under As-gradient stress. (A) Bacterial co-occurrence networks of without virus (BC), with V1 virus (V1BC), and with V2 virus (V2BC) inoculation in CK, low and high As-contaminated soils. Orange, blue and gray nodes represent the abundant (AT), rare (RT) and intermediate taxa (MT), respectively. A connection stands for a strong (Pearson's $r > 0.7$) and significant ($p < 0.05$) correlation. (B) Proportion of the edge properties in nine networks. (C) Proportion of the node amount that was affiliated with abundant (AT), rare (RT) and intermediate taxa (MT) in the nine networks. BC, SM buffer and bacteria (biotic); V1BC, bacteria and V1 virus filtrate (biotic); V2BC, bacteria and V2 virus filtrate (biotic). Low and High refer to the total As concentrations of 60 and 200 mg kg⁻¹ soil, whereas the unamended As soil is designated as the control (CK). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

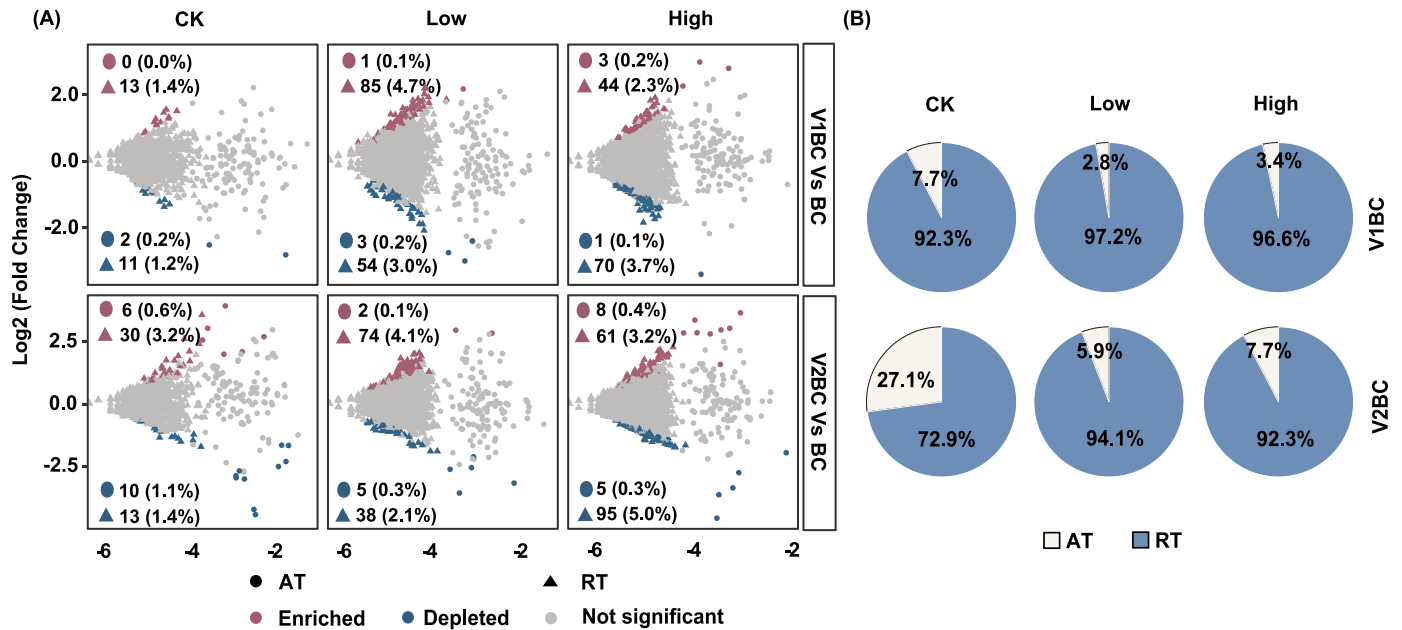


Fig. 4. Differences in rare (RT) and abundant (AT) taxa due to virus inoculation under As-gradient stress. (A) Volcano plots illustrate the enrichment and depletion patterns of the rare and abundant taxa in each soil with the inoculation of V1 (V1BC) and V2 (V2BC) virus. Red point represents a virus-enriched ASV, and blue points represent virus-depleted ASVs. Circles and triangles referred to abundant (AT) and rare (RT) taxa, respectively. (B) Pie charts are the proportions of total significantly different AT and RT based on the volcano plots under CK, low and high As conditions. BC, SM buffer and bacteria (biotic); V1BC, bacteria and V1 virus filtrate (biotic); V2BC, bacteria and V2 virus filtrate (biotic). Low and High refer to the total As concentrations of 60 and 200 mg kg⁻¹ soil, whereas the unamended As soil is designated as the control (CK). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

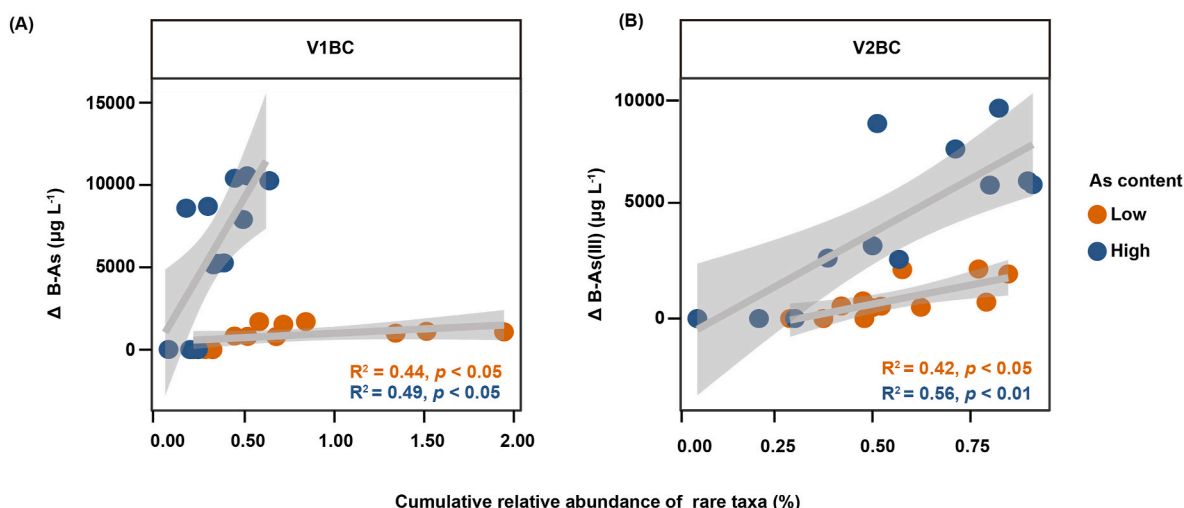


Fig. 5. Linear regression analysis between $\Delta B\text{-As(III)}$ and cumulative relative abundance of rare taxa in (A) V1BC and (B) V2BC treatment. $\Delta B\text{-As(III)}$ represents the increase in As(III) concentrations due to inoculation of microbiome, calculated by subtracting concentrations in the abiotic treatment (SM) from each of the three biotic treatments. Each orange and blue point represents the sample in low and high As contaminated soils, respectively. V1BC, bacteria and V1 virus filtrate (biotic); V2BC, bacteria and V2 virus filtrate (biotic). Low and High refer to total As concentrations of 60 and 200 mg kg^{-1} soil, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

microorganisms to cope with arsenic stress. Therefore, viruses in the V2BC treatment likely facilitate *arsC* gene transfer to enhance host survival (Tang et al., 2021). Previous studies have also demonstrated that MC-induced viruses carry *arsC* genes, leading to an increased abundance of this gene and consequently elevated As(III) concentrations in soils (Tang et al., 2019). This finding aligns with our observations of increased *arsC* (Fig. 6A and B) in the MC-induced treatment. Thus, MC-induced prophages retained their lysogenic functions and may have acquired arsenic resistance genes. This facilitates the transduction of these genes among bacteria, promoting As reduction. Collectively, this evidence underscores the multifaceted role of viruses in influencing biogeochemical processes in arsenic-contaminated soils.

4.2. Greater susceptibility of rare taxa to virus inoculation than abundant taxa

Both AT and RT play crucial roles in the overall functioning and dynamics of soil microbial communities (Jiao and Lu, 2020; Xu et al., 2022), with AT contributing significantly to soil microbial biomass, and RT being essential for microbial diversity, functional redundancy, niche specialization, and adaptability (Pedrós-Alió, 2012; Lynch and Neufeld, 2015; Jousset et al., 2017). Previous studies reported that RT displayed narrow niche breadths and higher sensitivity to environmental perturbations than AT, such as changes in soil pH and heavy metal contents (Jiao and Lu, 2020; Dong et al., 2021; Xu et al., 2021).

Our results showed that RT were more susceptible to the virus addition than AT in As contaminated soils, which was contrary to our hypothesis that virus addition would mainly affect the AT. We found the percentages of nodes belonging to RT within the networks were elevated after virus inoculation (Fig. 3), and RT were more sensitive to virus inoculation under both low and high As conditions (Fig. 4). Moreover, the cumulative abundance of RT in the V1BC and V2BC treatments were positively related to porewater $\Delta B\text{-As(III)}$ concentrations (Fig. 5), providing evidence that RT potentially contributed to As reduction. Under flooding conditions, RT may play a more active role due to their hardiness and continuous regrowth. This enables the RT with a prominent metabolic potential under appropriate conditions (Ye et al., 2023). Accordingly, we postulate that the RT were the initiators of changes in bacterial community structure and function in soils after virus inoculation.

Bacterial population dynamics are shaped by viruses via different

models. One viral mediation strategy on bacteria communities is the density-dependent theory “KtW”, which posits that abundant bacteria as winners are easily preyed upon by viruses owing to higher probability of encountering the host. Thus, low abundance is an advantage against predators (Lynch and Neufeld, 2015). We found contrasting results with RT being more sensitive than AT. This occurred because the most significantly enriched or depleted taxa all belonged to RT, especially in As-contaminated soils (Fig. 4A). Likewise, previous studies revealed that viruses appeared to reproduce by infecting rare but susceptible taxa (Middelboe et al., 2001; Bouvier and Del Giorgio, 2007).

We believe that the preferential impacts on RT in the V1BC treatment are likely consistent with the “KtW” hypothesis. The vulnerability of hosts to infection is not only determined by their population density but also by other host characteristics, such as higher competition for resources, higher growth rates and resistance to environmental stress (Bouvier and Del Giorgio, 2007). That is to say that RT may emerge as “dominant taxa” that are the winners in resource competition or have higher activity in flooded and/or contaminated environments (Hennes et al., 1995; Winter et al., 2005; Bouvier and Del Giorgio, 2007). Therefore, the RT influenced by extracellular free viruses may exhibit higher activity when compared to AT under As stress, making them the “winners” within bacterial communities, but they are also vulnerable targets for the free viruses.

Our findings also provide novel insight into the “PtW” theory. Most viewpoints about “PtW” posit that lysogenic infection is the pathway by which prophage is integrated into the genomes of hosts (Howard-Varona et al., 2017; Pratama and van Elsas, 2018; Jansson and Wu, 2023), whereby they tend to infect high abundance “winners” (Xia et al., 2023; Wang et al., 2024). Notably, more RT were found in the MC-induced viruses (V2BC) when exposed As stress (Fig. 4B), indicating microbial abundance is not the sole criterion for infection. Therefore, although RT display low abundance, they might be more susceptible than AT when viruses are present.

The significant response of RT to virus addition may also be related to the viral particles themselves, as viral particles can serve as a crucial nutritional source for soil microbial communities, particularly supplying essential elements such as carbon and phosphorus (Cobián Güemes et al., 2016). These nutrients are potentially utilized by RT in soils, potentially leading to reshaping of the soil microbial community structures and alterations in their As reduction capabilities. Therefore, the underlying mechanisms governing how RT respond to the addition of

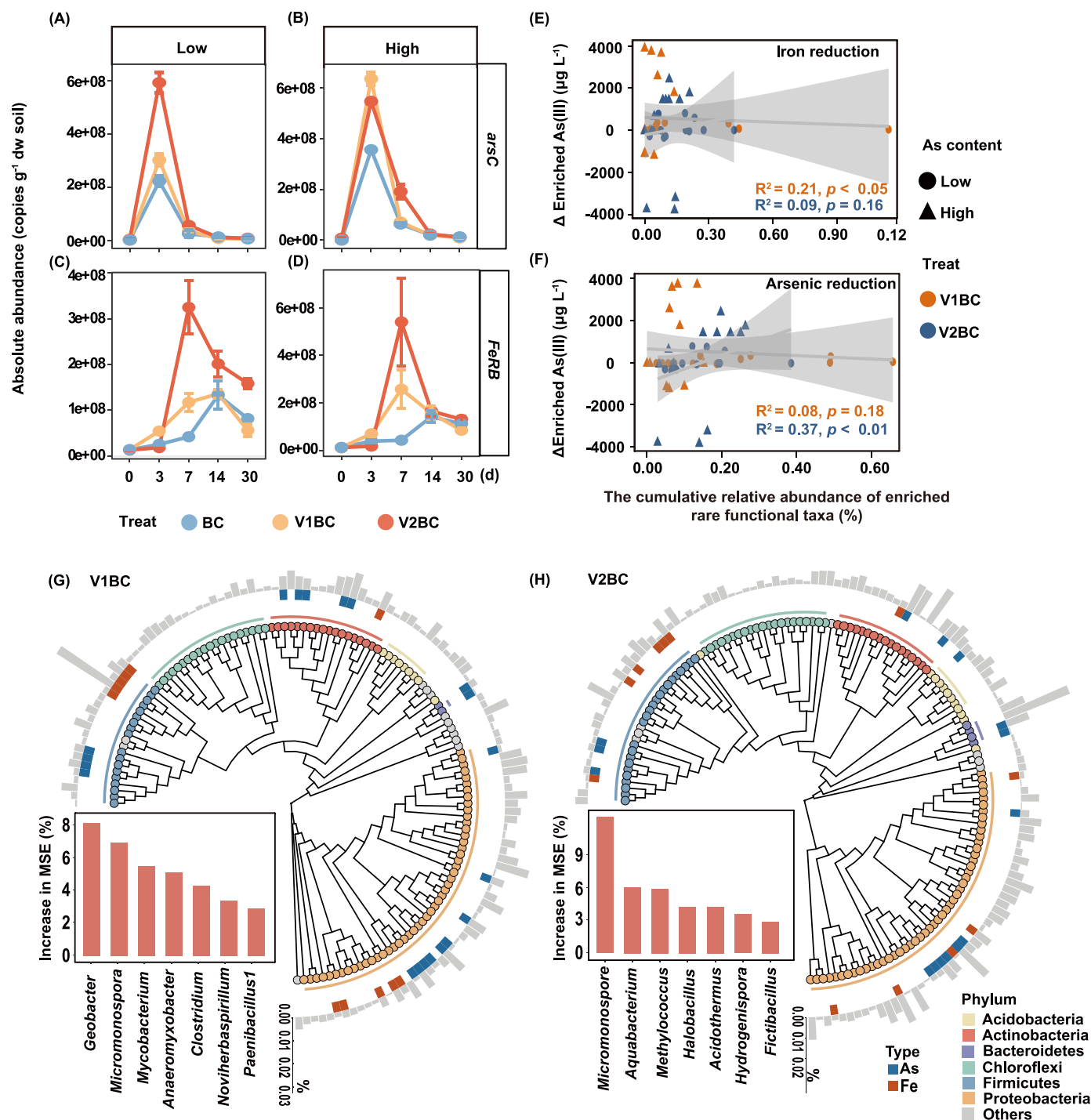


Fig. 6. Functional gene abundance and the association between Δ enriched As(III) and the enriched rare taxa following with virus inoculation. (A) and (B) Absolute abundance of arsenic reduction gene (*arsC*), and (C) and (D) iron reduction (*FeRB*) function gene with or without virus inoculation in low and high As-contaminated soils. (E) and (F) Linear regression analysis between Δ enriched As(III) and cumulative relative abundance of enriched rare functional taxa having potential arsenic and iron reduction functions for different virus inoculation treatments in low and high As-contaminated soils. Δ enriched As(III) represents the increase in As(III) concentrations due to virus inoculation, which was calculated by subtracting concentrations in no-virus treatments from the active virus treatments. (G) and (H) Phylogenetic trees and random forest analyses of the enriched rare taxa. The affiliation of ASVs at the phylum level is indicated by different colors on the internal ring. The Fe and As types refer to rare functional taxa with potential Fe and As reduction functions, respectively. The random forest analyses were bacteria predictors of enriched rare genus for increased As(III) concentrations. BC, SM buffer and bacteria (biotic); V1BC, bacteria and V1 virus filtrate (biotic); V2BC, bacteria and V2 virus filtrate (biotic). Low and High refer to total As concentrations of 60 and 200 mg kg⁻¹ soil, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

soil viruses, including alterations to biological and abiotic soil factors, require additional direct evidence to deepen our understanding of these complex interacting processes.

4.3. Soil viruses enriched rare functional taxa to enhance As reduction

Rare taxa often present a diversity of microorganisms with high-stress tolerance and the capacity to degrade recalcitrant compounds (Gao et al., 2021). For the extracellular free viruses (V1BC), the cumulative abundance of enriched RT, which possess potential Fe reduction functions, was significantly related to porewater As(III) concentrations (Fig. 6E). However, the MC-induced viruses (V2BC) mainly led to the enrichment of RT with potential As reduction functions, and their abundance was significantly related to porewater As(III) concentrations (Fig. 6F). In particular, we found both extracellular free viruses and MC-induced viruses could increase the abundance of *arsC* and *FeRB* genes under both low and high As conditions (Fig. 6A–D). This suggests that viruses can interact with different rare functional taxa to influence porewater As(III) concentrations. In particular, the genus *Geobacter* (Fig. 6G) and *Micromonospora* (Fig. 6H) were the most prominent taxa responsible for the increased porewater As(III) concentrations in extracellular free viruses (V1BC) and MC-induced viruses (V2BC), respectively. It has been reported that *Geobacter* can induce the reductive dissolution of Fe(III) (hydr)oxides (Islam et al., 2005), while *Micromonospora* possess arsenate reductase (*arsC* genes) and/or an arsenite efflux pump (*arsB* genes) (Corsini et al., 2010). Collectively, these findings demonstrate that the extracellular free viruses and MC-induced viruses tend to interact with rare functional taxa associated with potential Fe reduction and As reduction, respectively, to mediate porewater As concentrations.

Although viruses can influence the As reduction process through interactions with RT, we are unable to unambiguously describe virus-host collaborations due to the lack of genomic information for the two types of viruses. Future studies will address specific information on virus-host collaborations, using techniques such as stable isotope probing (SIP) combined with viral metagenomics, to assess active virus populations and unlock this vast virus biorepository.

5. Conclusion

Our results indicated that soil viruses increased bacterial-mediated As reduction processes under flooded soil conditions. The increments of increased porewater As(III) concentration were the result of virus-influenced, bacterial-mediated As–Fe interactions. These processes were primarily due to the response of rare taxa to virus inoculation under As stress. Moreover, soil viruses enriched rare taxa associated with As reduction, allowing them to directly participate in As transformation, as well as enriched rare taxa related to Fe reduction that are indirectly involved in As transformations through Fe reductive dissolution. To our knowledge, this is the first study to directly demonstrate the influence of soil viruses on As transformation through enriching different functional rare taxa. These findings enhance our understanding of the role viruses play in soil ecological interactions and As biogeochemical cycling. This knowledge has important implications for ecological conservation, remediation of As-contaminated soils and the development of sustainable agricultural practices in arsenic-contaminated soil environments.

CRedit authorship contribution statement

Youjing Wang: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Di Tong:** Methodology. **Haodan Yu:** Methodology. **Xueling Yang:** Methodology. **Xinwei Song:** Methodology. **Randy A. Dahlgren:** Writing – review & editing. **Jianming Xu:** Writing – review & editing, Writing – original draft, Supervision, Data curation.

Data availability

The original sequence data were deposited into the National Center for Biotechnology Information Sequence Read Archive database under project ID PRJNA985387.

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.soilbio.2025.109783>.

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