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Deciphering microbial mechanisms for hexavalent chromium reduction in contaminated sediment and paddy soil

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Abstract

Hexavalent chromium [Cr(VI)] is a heavy metal that poses serious environmental risk. Microbial Cr(VI) reduction is a potential remediation approach to reduce the mobility and toxicity of this metal in the environment. However, the diversity and metabolic mechanisms of Cr(VI)-reducing bacteria (CrRBs) remain unknown. In this study, a combination of enrichment incubation and high-throughput sequencing was used to elucidate CrRBs and their associated metabolic pathways for Cr(VI) reduction. Enrichment incubation identified bacterial populations belonging to *Cellulomonas, Enterobacter, Rikenellaceae*, and *Citrifermentans* as putative CrRBs in the two Cr(VI)-contaminated sediments and paddy soils as they gradually dominated the microbial community. High-quality metagenome-assembled genomes (MAGs) associated with putative CrRBs were reconstructed, and functional genes responsible for Cr(VI) reduction were detected, suggesting that they are putative CrRBs. This study advances our understanding of CrRBs diversity and their underlying metabolic mechanisms.

1. Introduction

Chromium (Cr) is a toxic metal that is extensively distributed in the Earth's crust and has an approximate concentration of 100 mg kg^{-1} (Kazakis et al., 2015; Zhao et al., 2023). Due to its extensive use in industries, including the manufacture of refractory materials, production of chemical color pigments, and chrome plating, large amounts of Cr are released into the environment, resulting in the severe contamination of soil and water (Rahman and Thomas, 2021). Therefore, remediation of Cr contamination has attracted significant attention. Cr occurs in nature in different valence states ranging from -II to + VI. The two dominant species in the environment are Cr(VI) and Cr(III) (Zhong et al., 2017). Of these two states, Cr(VI) is extremely toxic and exhibits high mobility, whereas Cr(III) is less toxic and tends to form insoluble minerals at neutral pH values (Zhang et al., 2020; Bishop et al., 2019). Therefore, the reduction of Cr(VI) to Cr(III) contributes to the remediation of Cr-contaminated sites and rehabilitation of environments affected by Cr contamination.

Microbially mediated Cr(VI) reduction is an efficient remediation strategy that has attracted the interest of the scientific community due to its cost-effectiveness and environmental sustainability (Le et al., 2023; Min et al., 2024). In microbial Cr(VI) reduction, electron donors play an important role in the biogeochemical process, and various electron donors have been used, such as organic matter, CH_4, Fe(0), S(0), and H_2 (Lara et al., 2017; He et al., 2020; Lai et al., 2016; Long et al., 2017; Shi et al., 2019). In addition, several Cr(VI)-reducing bacteria (CrRBs) have been isolated from various habitats, including *Enterobacter cloacae* HO1 (Wang et al., 1989), *Cellulomonas tarbata* (Viti et al., 2003), *Pseudomonas* sp. G1DM21 (Desai et al., 2008), *Bacillus cereus* (He et al., 2010), *Lysinibacillus* sp. (Chen et al., 2021), and *Lactococcus lactis* AM99 (Akhzari et al., 2024). Most of these bacteria are heterogeneous because organics can support high microbial activity, making heterotrophic Cr(VI) reduction a promising remediation technology. However, the potential use of Cr(VI)-reducing bacteria, particularly indigenous microbial communities, in the remediation of Cr(VI)-contaminated environments may be underestimated because of the inherent limitations of culture-dependent analytical techniques.

Most studies have focused on identifying CrRBs and evaluating their chromate transport capability. For instance, *Anoxybacillus flavithermus* ABF1 was isolated as an alkaline Cr(VI)-reducing bacterium from a geothermal region, and was able to reduce 93.71% of Cr(VI) at an initial concentration of 20 mg L^{-1} in 72 hours (Yang et al., 2022). Members of the *Comamonadaceae* family have been identified in methane- and oxygen-based membrane biofilm reactor with the potential for chromate reduction, which could reduce Cr(VI) to Cr(III) at a rate of 89% (Long et al., 2017). Zhang et al. (2020) proposed a mechanism for the metabolic pathway of Cr(VI)-reducing bacteria in a sulfur-based mixotrophic system by determining the abundance of the *chrA* genes and the compounds involved in Cr(VI) reduction (Zhang et al., 2020). However, the reduction mechanisms of most Cr(VI)-reducing bacteria are not clearly understood because most of them have not been isolated, resulting in a lack of molecular biological evidence. Therefore, a better understanding of the metabolic mechanisms of Cr(VI)-reducing bacteria is required.

In this study, anaerobic cultures were established to enrich indigenous Cr(VI)-reducing bacteria using long-term Cr(VI)-contaminated sediments and paddy soils as inocula. A metagenomic approach was used to reconstruct the metagenome-assembled genomes (MAGs) of CrRBs from these complex microbial communities and predict the Cr(VI) reduction pathway. The aim of the current study was to investigate (i) which microorganisms are responsible for Cr(VI)-reducing bacteria in Cr(VI)-contaminated sediments and paddy soils and (ii) the metabolic mechanisms of CrRBs. These findings provide new insights into the diversity and metabolic potential of CrRBs for Cr(VI) reduction, which may contribute to the remediation of Cr(VI) in contaminated environments.

2. Materials and Methods

2.1 Sample collection and characterization

Samples were collected from river sediments at depth of 0–20 cm in Xikuangshan, Hunan, China (27°44' N, 111°28' E, designated as XKS), and from flooded rice fields in Wanshan, Guizhou, China (27°31' N, 109°12' E, designated as WS), both located near the mining area. The collected samples were immediately transported to the laboratory on ice and stored at -20 ℃ for chemical analysis and microcosm incubation. To determine the Cr content, the samples were freeze-dried, digested with an HCl: HNO_3 solution (3:1, v/v), and measured by ICP-OES (Vista MPX, Varian). The samples of XKS and WS contained 237.3 mg kg^{-1} and 136.8 mg kg^{-1} of Cr, respectively.

2.2 Anaerobic enrichment cultures

Anaerobic enrichment cultures were established using the collected samples to detect potential Cr-reducing bacteria. Approximately 2 g of the sample was added to 100 mL of mineral salt medium (MSM; 7.9 g L^{-1} $Na_2HPO_4 \cdot 7H_2O$, 1.5 g L^{-1} KH_2PO_4, 0.3 g L^{-1} NH_4Cl, 0.1 g L^{-1} $MgSO_4 \cdot 7H_2O$, 10 mL L^{-1} vitamin solution, and 5 mL L^{-1} trace element solution) as inoculum (Zhang et al., 2015). The bottles were then purged with N_2 for 30 min, capped with butyl rubber septa and aluminum caps, and inoculated for three days at room temperature to degrade soluble organics and indigenous Cr in the original inoculum. Cr(VI)

was added to the cultures at a final concentration of 20 mg L^{-1} and 6 mM sodium acetate was used as the electron donor. In addition, abiotic controls were prepared using autoclaved samples to demonstrate that the biological activity mediates this process. All cultures were prepared in triplicates at each sampling time point and incubated at 30 ^{o}C. During incubation, the Cr(VI) concentration was monitored by taking 1 mL of supernatant from each culture for colorimetric measurement according to the established protocol (He et al., 2020). After the complete removal of Cr(VI), the cultures were spiked again with 20 mg L^{-1} of Cr(VI) (which was considered as one cycle), and duplicate cultures were destructively sampled for microbial community analysis. Three samples were analyzed for each case.

2.3 DNA extraction

Cultures collected from each enrichment cycle were centrifuged at 7,000 rpm for 10 min. Total genomic DNA was extracted from the cultures and raw samples using the DNeasy PowerSoil DNA Kit (QIAGEN, Germany) according to the manufacturer's protocol. DNA was not extracted from the sterile controls in this study.

2.4 Illumina MiSeq sequencing of 16S rRNA gene amplicons

To characterize the prokaryotic communities, the hypervariable region V4 of the 16S rRNA gene was amplified using extracted DNA as a template. The forward primer was 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and the reverse primer was 806R (5'-GGACTACNVGGGTWTCTAAT-3') (Klindworth et al., 2013). The amplification products were sequenced on an Illumina MiSeq platform at Personal Biotechnology (Shanghai, China). The raw amplicon sequencing data were analyzed using the QIIME2-202002 toolkit (Bolyen et al., 2019). Briefly, raw reads were merged, trimmed, and filtered. The filtered reads were clustered into amplicon sequence variants (ASV) using DADA2 (Callahan et al., 2016). Representative sequences were assigned to the SILVA 132 database (Quast et al., 2012).

2.5 Shotgun metagenomic sequencing and analysis

In the third cycle, DNA from triplicate cultures was combined to create a composite DNA sample because the amount of DNA extracted from each culture was insufficient for shotgun metagenomic sequencing. The DNA samples were then sequenced on an Illumina HiSeq 4000 platform (PersonalBio, Shanghai, China). Approximately 33,934,255 and 33,985,822 raw reads were generated from the XKS and WS metagenomes, respectively. All raw reads were trimmed using Trimmomatic 0.39 (Bolger et al., 2014) with the following arguments: "sliding window: 4:15, leading: 3, trailing: 3, and minilen: 80". Overlapping read pairs were identified and merged using FLASH with a minimum overlap of 15 bp and a maximum overlap of 100 bp. Clean datasets were assembled de novo into contigs using MEGAHIT (Li et al., 2015) (k = 21–141, step = 10). Metagenome-assembled genomes (MAGs) were retrieved from the assembled contigs by metagenome binning using MaxBin2 (Wu et al., 2016), MetaBAT2 (Kang et al., 2015), and CONCOCT (Alneberg et al., 2014). The obtained MAGs were refined and their quality was improved using DAS (Sieber et al., 2018). The MAGs were then filtered using an MDMcleaner (Vollmers

et al., 2022) and their quality was assessed using CheckM (Parks et al., 2015). Only MAGs with a completion rate of > 60% and contamination rate of < 10% were retained and subjected to taxonomic annotation with GTDB-TK (Chaumeil et al., 2019). The functional genes of the MAGs were annotated using KofamKOALA (Aramaki et al., 2020) with default parameters. The phylogeny of MAGs genomes was generated using MEGA (Tamura et al., 2021).

2.6 Data availability

Nucleic acid reads based on the 16S rRNA gene and metagenomic sequencing were submitted to the NCBI GenBank database under project number PRJNA847997.

3. Results

3.1 Microbial reduction of Cr(VI)

Anaerobic enrichment cultures were established using two Cr-contaminated samples as the inocula. Cr(VI) was added for three cycles as the sole electron acceptor to enrich the Cr(VI)-reducing bacteria. The concentration of aqueous Cr(VI) was monitored during the incubation period (Fig. 1). The Cr(VI) concentration constantly decreased in the two live treatments inoculated with the XKS sediment and WS paddy soil. In contrast, the Cr(VI) concentration in the sterile control initially decreased slightly and then reached a metastable state. These observations suggest that the reduction of Cr(VI) was driven by microorganisms. Additionally, the Cr(VI) reduction rates were significantly different between the two live treatments. Approximately 20 mg L^{-1} Cr(VI) was reduced to 0 mg L^{-1} within 6 days in the XKS treatment, while 12 days were required for the complete reduction of Cr(VI) in the WS treatment, suggesting a higher reduction potential of Cr(VI) in the XKS treatment than in the WS treatment. The different microbial communities in the two treatments may have contributed to this outcome, or the XKS treatment may have resulted in a higher abundance of potential Cr(VI)-reducing bacteria.

3.2 Enrichment of Cr(VI) reducing bacteria (CrRB)

Similar to the original inoculum, the microbial communities in both cultures were characterized at the end of each cycle. Different microbial taxa were enriched in XKS and WS cultures, as shown in Fig. 2. At the phylum level, the relative abundance of *Bacteroidota* in the XKS cultures gradually increased during the incubation period and reached the level of the dominant population (36%) after three cycles. At the same time, the relative abundance of *Desulfobacterota* in the XKS cultures increased from 2% to 16%. During incubation, the relative abundances of *Actinobacteriota* and *Proteobacteria* in the WS cultures were 7% and 11%, respectively, whereas those in the original inoculum were 42% and 36%, respectively. Additionally, *Proteobacteria* and *Firmicutes* were the dominant bacteria in the XKS and WS cultures, accounting for 64% and 65% of the total abundance, respectively, while their abundance decreased to 18% and 13%, respectively, at the end of incubation. These findings suggest that distinct microbial communities mediate Cr(VI) reduction and reveal that Cr(VI) exerts a strong selective pressure on different microorganisms.

At the genus level, uncultured *Rikenellaceae* became the most abundant taxon in the XKS enrichment cultures, with a relative abundance of 21% in the third cycle compared to 0.4% in the original inoculum, followed by *Citrifermentans* with a relative abundance of 7% **(**Fig. 3**)**. After three cycles of incubation, bacteria associated with *Cellulomonas* and *Enterobacter* gradually dominated, with relative abundances of 36% and 28%, respectively, in the WS cultures. In addition, the relative abundances of other genera, such as *Thiobacillus*, *Brevundimonas*, *Clostridium*, and *Desulfitobacterium*, decreased with incubation time. These findings suggest that uncultured *Rikenellaceae*, *Citrifermentans*, *Cellulomonas*, and *Enterobacter* may be responsible for Cr(VI) reduction.

3.3 Metagenomic analysis of CrRBs

Metagenomic binning was performed to retrieve genomic information on the proposed CrRBs and further elucidate their metabolic potential. A total of 21 metagenomic-assembled genomes (MAGs) with > 60% completeness and < 10% contamination were obtained from the two metagenomes. These MAGs were categorized into seven different bacterial phyla: *Halobacteriota*, *Acidobacteriota*, *Chloroflexota*, *Bacteroidota*, *Proteobacteria*, *Desulfobacterota*, and *Actinobacteriota* **(**Fig. 4**)**. Among the recovered MAGs, seven were taxonomically linked to putative CrRB, including *Rikenellaceae_uncultured* (bin.5 and bin.8), *Citrifermentans* (bin.9 and bin.11), *Cellulomonas* (bin.4 and bin.5) and *Enterobacter* (bin.1), which were selected for further investigation.

Functional genes of MAGs associated with CrRBs were identified **(**Fig. 5**)**. Key genes involved in chromate reduction (*chrR*) and chromate transport (*chrA*) (He et al., 2018; Lu et al., 2020) were detected in all putative CrRB-associated MAGs except for *Cellulomonas*-associated MAGs. In addition, other genes potentially involved in Cr(VI) reduction have been examined in these MAGs, including *ssuE*, *azoR*, *rutE*, *nfsA*, *ribF*, *nemA*, *nrfA*, and *modA* (Xia et al., 2018; Xia et al., 2021). These observations suggested that these bacteria have the potential to reduce Cr(VI). In addition, putative CrRB-associated MAGs harbored diverse genes related to metal resistance, such as *czcABCD*, arsC, arsR, cusB, and znuABC, suggesting their genetic potential to adapt to metal-contaminated habitats. All seven CrRB-associated MAGs harbored genes encoding for six major carbon fixation pathways, indicating their ability to grow with acetic acid. In addition, some genes involved in nitrogen and sulfur metabolism were detected in the CrRB-associated MAGs.

4. Discussion

Microbial-driven Cr(VI) reduction has important environmental implications for the remediation of Cr-contaminated environments and has been widely studied in natural habitats, such as soils (Hou et al., 2020; Fu et al., 2021) and groundwater (Shi et al., 2020). In this study, the diversity and metabolic potential of Cr(VI)-reducing microbial communities in sediments and paddy soils were investigated using anaerobic enrichment cultures and metagenomics.

4.1 Potential Cr(VI)-reducing bacteria

Biological Cr(VI) reduction has been observed in the cultures inoculated with Cr-contaminated sediment and paddy soil from two sites whose aqueous Cr(VI) reduced in 6 and 12 days, respectively. Four bacteria, *Rikenellaceae*, *Citrifermentans*, *Cellulomonas*, and *Enterobacter*, were identified as putative CrRBs based on their relative abundances in the cultures, which increased significantly with incubation time. After incubation, the dominant taxa in the cultures among these CrRBs were *Rikenellaceae*, *Cellulomonas*, and *Enterobacter*, with relative abundances of more than 20%.

Anaerobic fermentation bacteria associated with the *Rikenellaceae* family have been found in anaerobic environments including swamps (Su et al., 2014) and anaerobic activated sludge (Yan et al., 2021). Some studies have shown that members of the *Rikenellaceae* are enriched during the decomposition and digestion of organic matter (Tian et al., 2019; Chen et al., 2022), suggesting that they may have the ability to utilize acetate. It has been reported that *Rikenellaceae* are potentially syntrophic bacteria that can directly transfer electron with methanogens through iron oxide minerals (Lee et al., 2019). In addition, slight enrichment of *Rikenellaceae* was observed in the reduction of Cr(VI) and nitrate driven by the microbiome and iron oxide minerals (Hu et al., 2021), suggesting that *Rikenellaceae* may have the potential to reduce or tolerate Cr(VI). However, the ability of *Rikenellaceae* spp. to reduce Cr(VI) has not yet been reported.

Enterobacter spp. have been previously identified as Cr-reducing bacteria. *Enterobacter cloacae* HO1 was the first strain to have the ability to reduce Cr(VI) (Wang et al., 1989). Various members of *Enterobacter* have been isolated, including *Enterobacter aerogenes* T2 (Panda and Sarkar, 2012), *Enterobacter cloacae* B2-DHA (Rahman et al., 2015), and *Enterobacter* sp. SL (Sun et al., 2020). *Enterobacter cloacae* B2-DHA can resist 1000 mg L^{-1} of Cr(VI) (Rahman et al., 2015), and *Enterobacter* sp. DU17 reduced Cr(VI) by 100 mg L^{-1} over 16 h using glucose as an electron donor (Rahman and Singh, 2014), suggesting its strong ability to reduce Cr(VI). In addition, Cr(VI) reduction was effectively observed when *Enterobacter cloacae* HO1 was enriched with acetate compared to glucose, citrate, or lactate (Komori et al., 1989), suggesting that acetate is an efficient electron donor for *Enterobacter cloacae* HO1 to reduce Cr(VI). In agreement with our current study, *Enterobacter* dominated with increasing incubation time when acetate was used as the electron donor.

Cellulomonas spp. are known to reduce Cr(VI) and some of their members have been isolated from various habitats (Brookshier et al., 2018; Viamajala et al., 2007b). For example, *Cellulomonas tarbata* isolated from Cr-contaminated soil was resistant to Cr(VI) concentrations up to 12 mM (Viti et al., 2003). In another study, *Cellulomonas* WS01 reduced 0.2 mM of Cr(VI) in 70 hours (Sani et al., 2002). These results indicated that bacteria associated with *Cellulomonas* can reduce Cr(VI). Additionally, *Cellulomonas* ES6 was isolated from Cr-contaminated sediment reduced anaerobically with Cr(VI) using acetate as an electron donor (Viamajala et al., 2007b), similar to the conditions used in this study.

Citrifermentans are a rarely studied group of anaerobic bacteria and only three strains have been isolated from this genus: *Citrifermentans bemidjiense* (Nevin et al., 2005), *Citrifermentans bremense*, and *Citrifermentans pelophilum* (Straub and Buchholz-Cleven, 2001). These three bacteria have been

previously identified as Fe(III)-reducing bacteria (Straub and Buchholz-Cleven, 2001; Aklujkar et al., 2010; Neal et al., 2004), indicating their potential for metal reduction. Although some members of the *Geobacteraceae* family have been reported to reduce Cr(VI) (Shi et al., 2019; He et al., 2019), identification of the genus *Citrifermentans* has not yet been reported.

4.2 Metabolic Potentials of CrRB-Associated MAGs

The genetic traits responsible for Cr(VI) reduction are of particular significance as they may represent some of the most significant characteristics for the bioremediation of Cr. Metagenomic binning was employed to retrieve bins associated with putative Cr(VI)-reducing bacteria, thereby enabling the exploration of the underlying mechanisms involved in Cr(VI) reduction. Various gene families hypothesized to be involved in Cr(VI) reduction were selected to examine the presence of these genes within the MAGs linked to putative Cr(VI)-reducing bacteria. C*hrR*, which belongs to the flavodoxin superfamily, is a chromate reductase (Eswaramoorthy et al., 2012). A reductase in the NADPH-dependent flavoprotein family, ChrA, also plays a key role in Cr(VI) reduction and resistance (Ramirez-Diaz et al., 2008). During reduction, ChrR transfers NADH electrons to Cr(VI) and directly reduces Cr(VI), whereas ChrA serves as a chromate transporter that exports Cr(III) into the extracellular environment. In this study, *chrR* was detected in MAGs associated with *Citrifermentans* and *Enterobacter*, whereas *chrA* was detected in MAGs from *Rikenellaceae*, *Citrifermentans*, and *Enterobacter*, suggesting that either or both enzymes may be used to reduce Cr(VI). Neither *chrR* nor *chrA* were detected in *Cellulomonas*-associated MAGs, which may be attributed to incomplete metagenomic binning that did not provide the whole genomes of these bacteria. The genomes of the *Cellulomonas* sp. strains K38 and B12 were annotated using chromate reductases (ChrRs) (Brookshier et al., 2018).

In addition to *chrR* and *chrA*, Cr(VI) reduction has been observed to be mediated by flavoprotein reductase (RibF) in Cr(VI)-reducing *Serratia* sp. S2 (Dong et al., 2018). This gene was observed in all the putative CrRB-associated MAGs. Some genes have been reported to be responsible for Cr(VI) reduction in various bacteria including *nrfA*, *ssuE*, *rutE*, *azoR*, *nemA*, and *nfsA* (Yang et al., 2022; Bhattacharya et al., 2015; Chen et al., 2021; Xia et al., 2018). These genes were detected in putative CrRB-associated MAGs, indicating that putative CrRBs may use various pathways for Cr(VI) reduction. These bacteria were further verified as putative Cr(VI) reducers by identifying potential functional genes for Cr(VI) reduction.

In addition, putative CrRB-associated MAGs harbored several genes involved in resistance to different metals. For instance, most MAGs harbor the *arsR* and *arsC* genes, both of which are involved in As and Sb resistance and their detoxification pathways (Ji et al., 1992). Genes encoding Cd, Zn, Cu, and Mn exporters were identified in all MAGs, suggesting that CrRBs can resist or transform these metals, because the samples used in this study were contaminated with various metals.

5. Conclusions

In this study, enrichment incubation and metagenomics were combined to enrich CrRBs and to elucidate the metabolic pathways involved in Cr(VI) reduction. After three enrichment cycles, *Cellulomonas*,

Enterobacter, *Rikenellaceae*, and *Citrifermentans* dominated Cr-contaminated sediments and paddy soils, with abundances of 36, 28, 21, and 7%, respectively, indicating that these were putative CrRBs. Among these, *Rikenellaceae* and *Citrifermentans* are novel chromium-reducing bacteria. The reconstructed genomes of the four putative CrRBs encoded key genes (*chrA* and *chrR*) for chromate reduction, indicating their ability to reduce chromate. This study expands the known diversity of Cr(VI)-reducing bacteria and improves our understanding of their metabolism.

Declarations

Authors contribution

Xin Zhang: Writing - Original Draft, Investigation, Data curation,Formal analysis,Methodology. **Zexin Wang**: Data curation,Investigation, Writing - Original Draft,Writing - Review & Editing. **Muhammad Usman Ghani**:Investigation,Data curation,Writing - Review & Editing. **Tianle Kong**:Investigation,Methodology,Validation.**Weimin Sun**: Formal analysis,Funding acquisition, Writing - Review & Editing.**Xiaoxu Sun**:Funding acquisition, Writing - Review & Editing. **Baoqin Li**: Visualization,Writing - Review & Editing. **Zewen Tan**: Data curation, Writing - Review & Editing. **Geng Yan** :Conceptualization,Funding acquisition,Supervision,Writing - Review & Editing.

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Data Availability

The datasets obtained during the current study are available from the corresponding author on reasonable request.

Ethics Approval Not applicable.

Consent for Publication This manuscript is approved by all authors for publication in Water, Air, & Soil Pollution.

Competing interests The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Figures

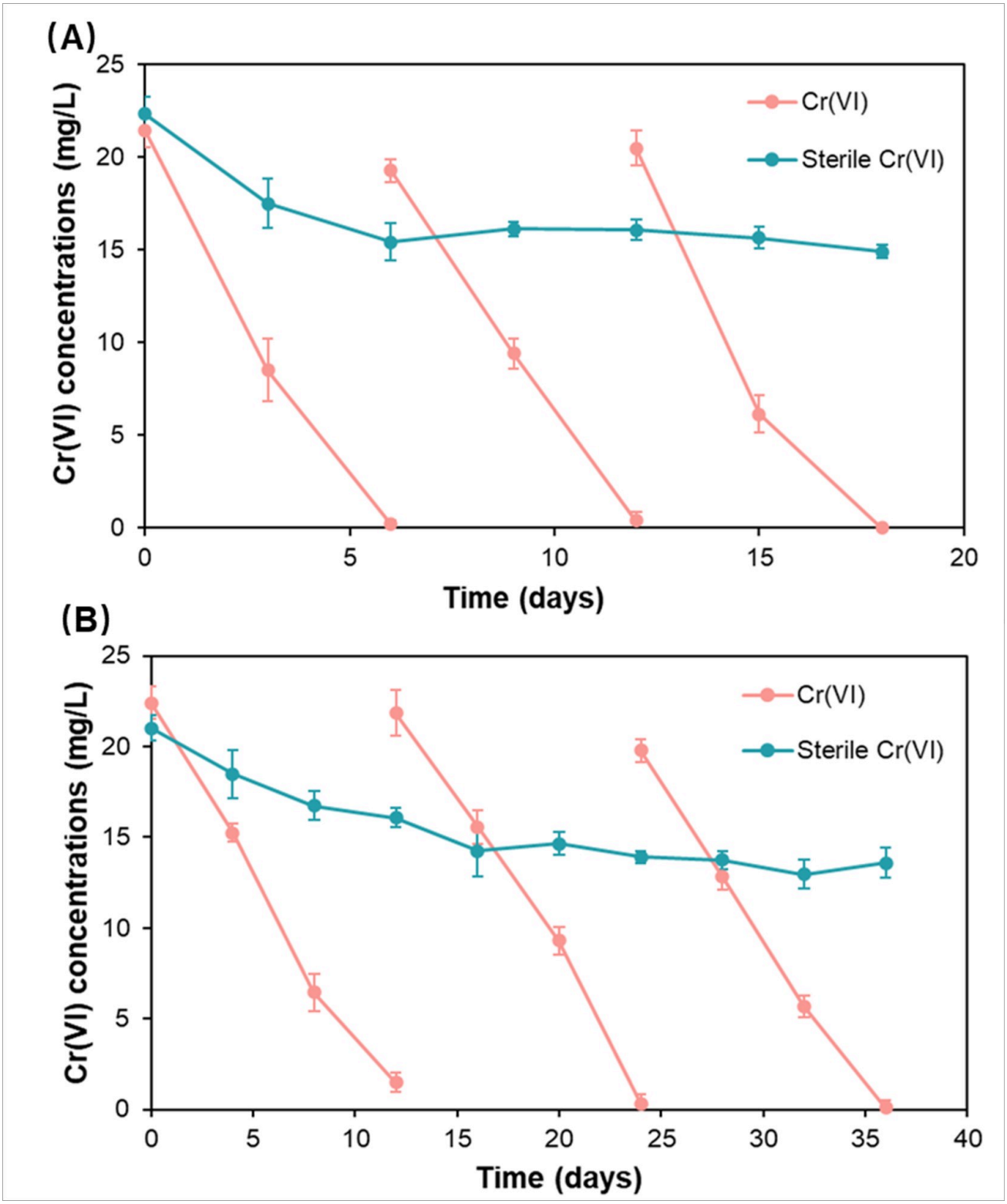


Figure 1

Concentrations of Cr(VI) in the live treatment and sterile control inoculated from the XKS sediment (A) and WS paddy soil (B).

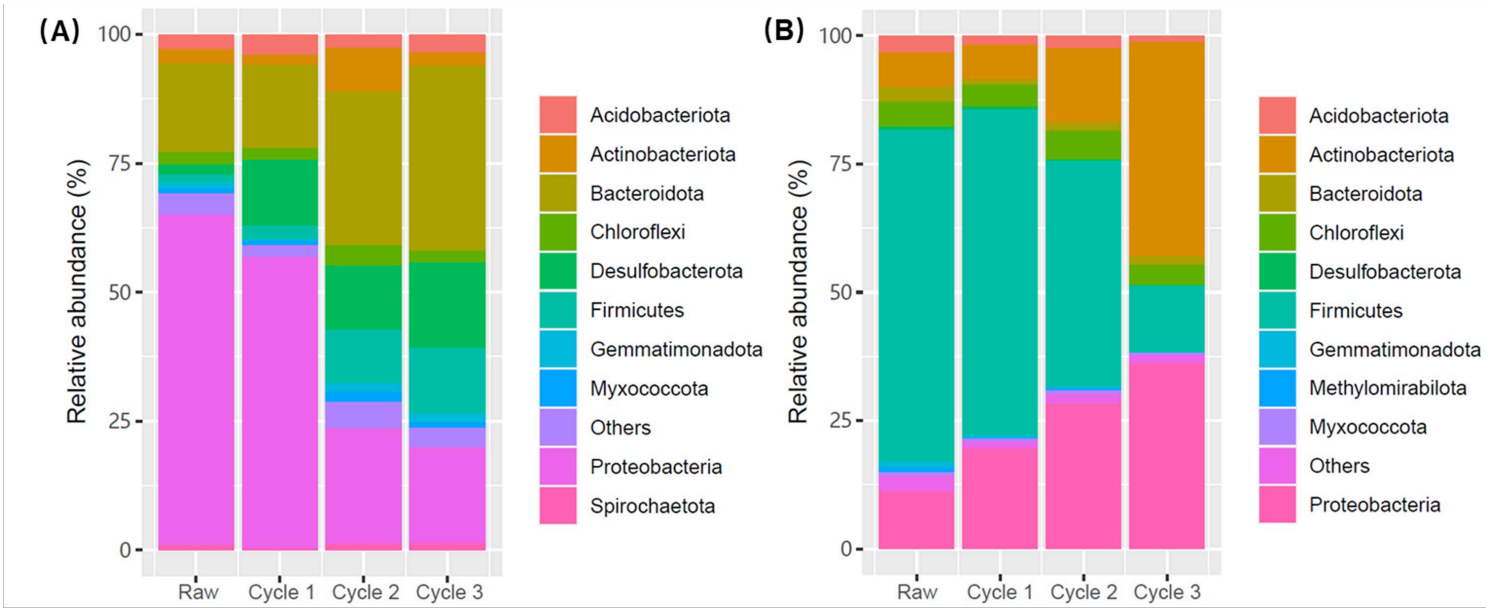


Figure 2

Microbial communities at the phylum level in the XKS (A) and WS (B) treatments

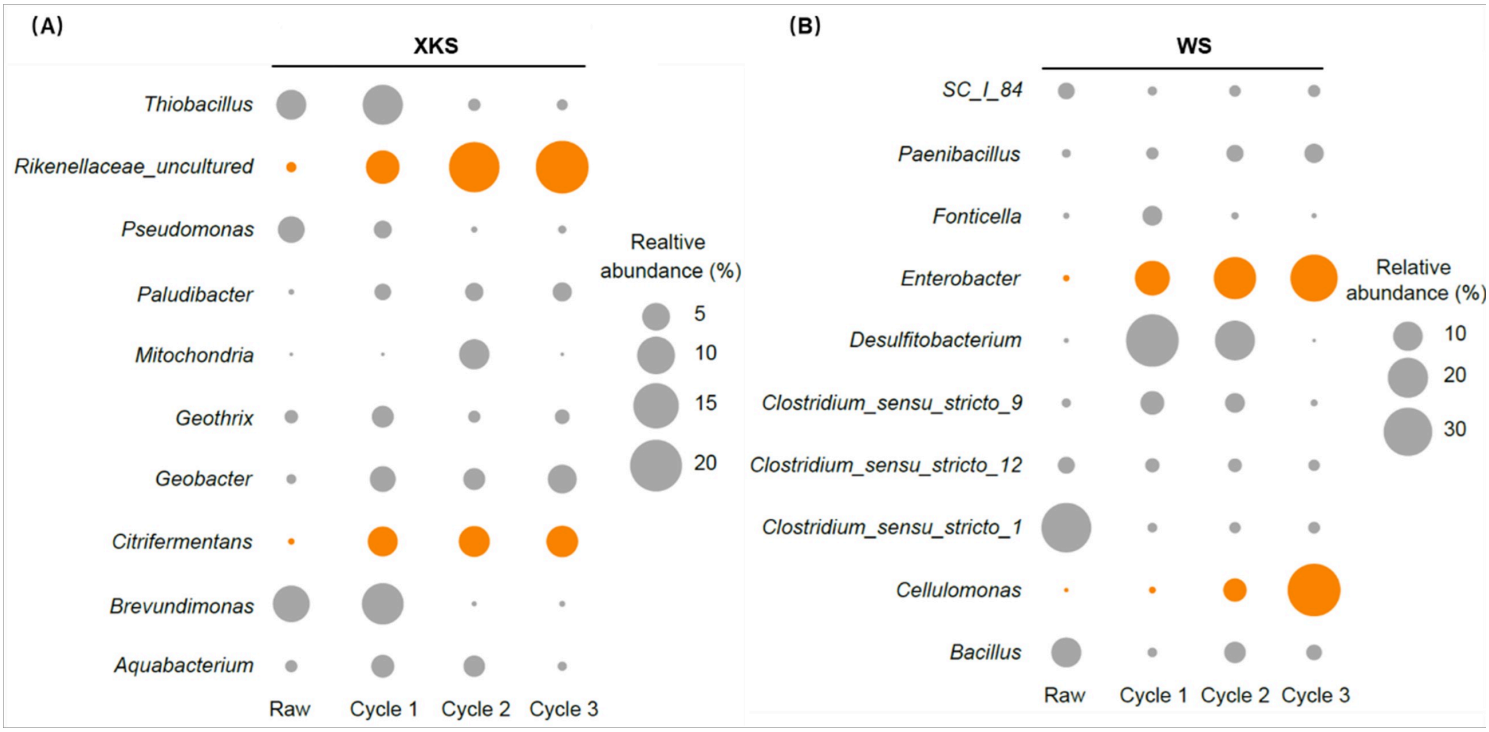


Figure 3

Relative abundance of domain genera in the XKS (A) and WS (B) treatments.

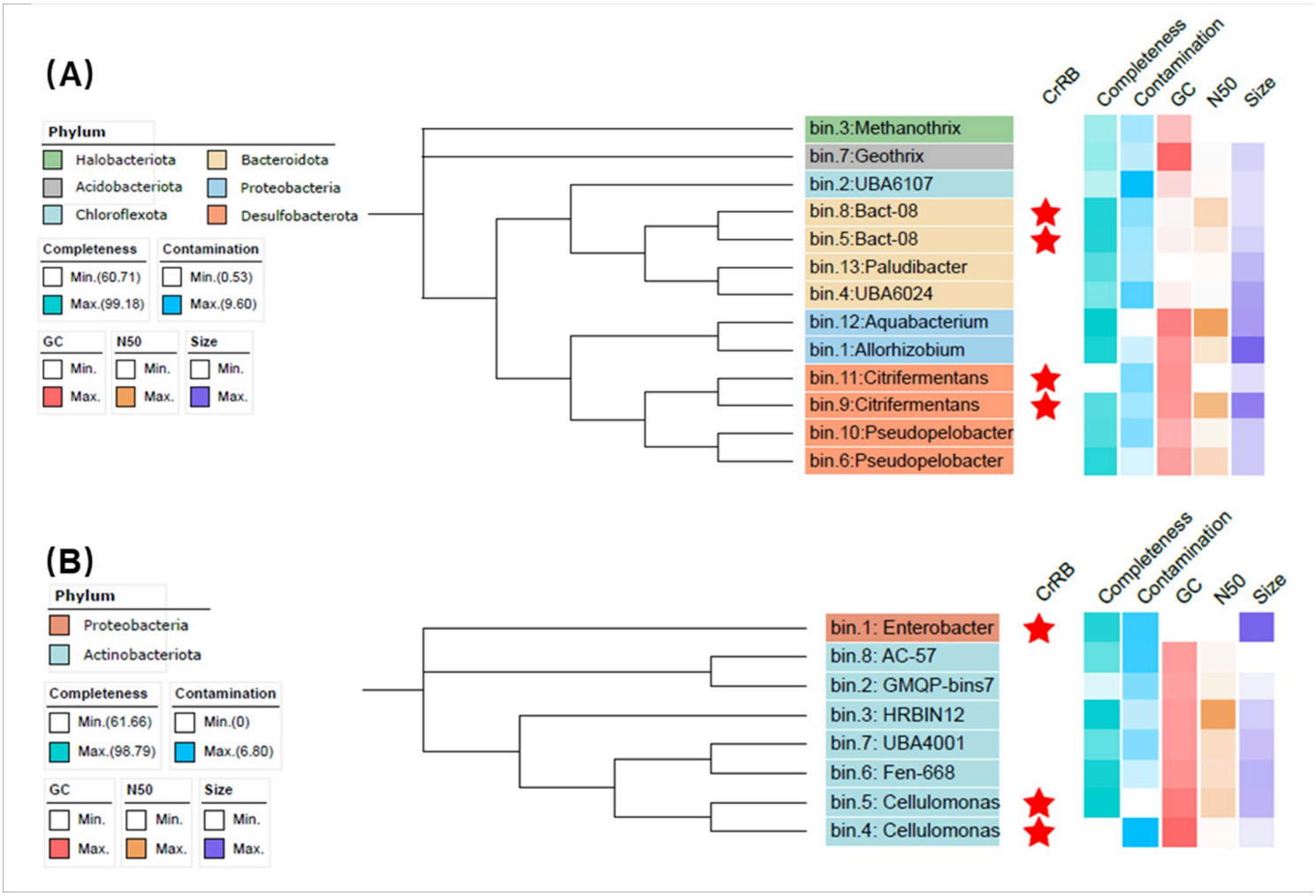


Figure 4

Phylogenetic affiliations of the retrieved genomic bins from XKS (A) and PD (B) samples and the bin quality (i.e., completeness, redundancy, GC content, N50, and size).

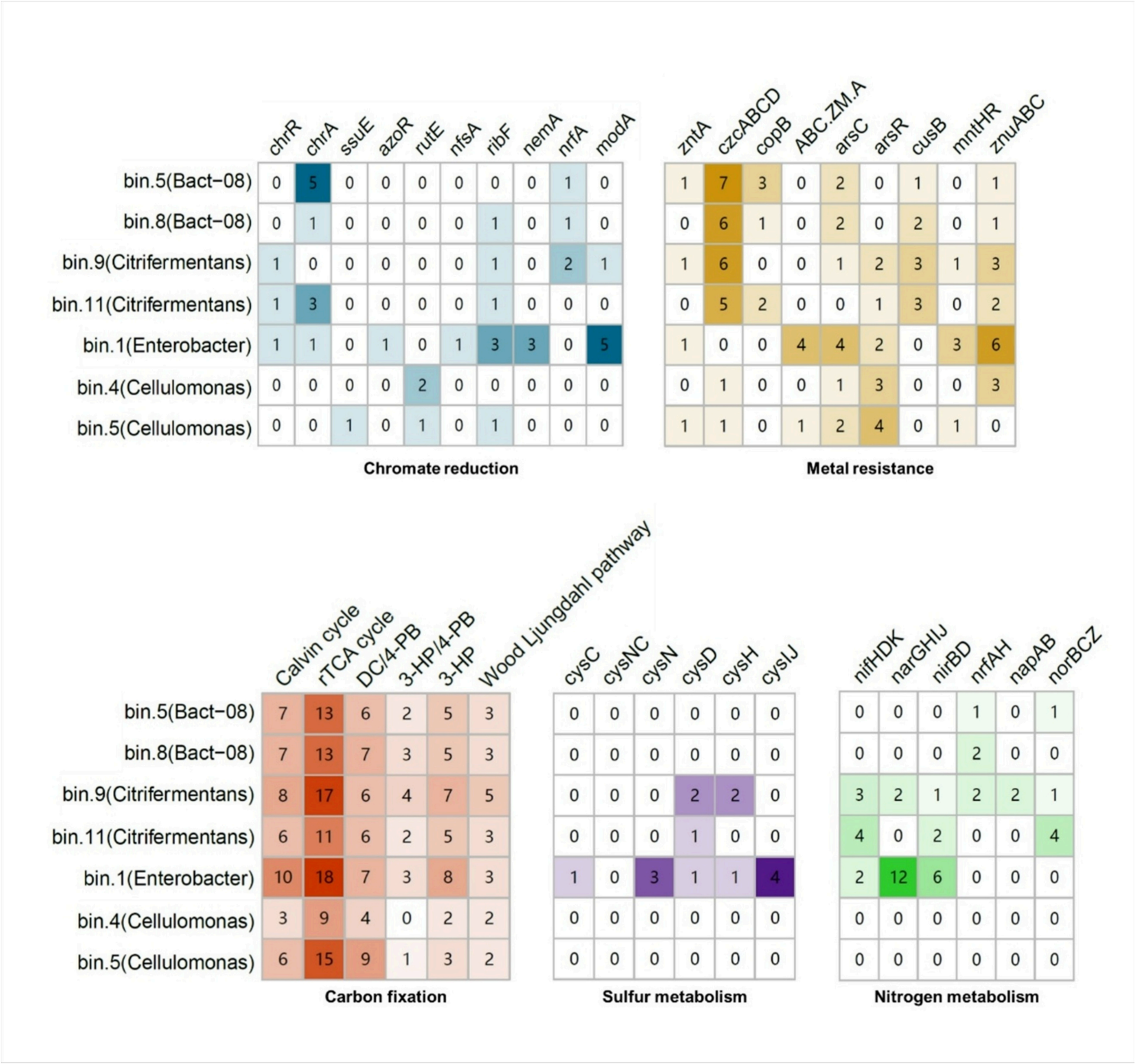


Figure 5

Heat map of genes involved in chromate reduction, metal resistance, carbon fixation, sulfur metabolism, and nitrogen metabolism in putative CrRB-associated MAGs.