

## Metagenomes Reveal Interplay of Microbes in Nitrate-Reducing As(III) and Fe(II) Oxidation in Anoxic Paddy Soil

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Microbially nitrate ( $\text{NO}_3^-$ ) reduction coupling arsenite (As(III)) and ferrous iron (Fe(II)) oxidation (NR-AsFeO) is pivotal in mediating the transformation of nitrogen, arsenic and iron elements under anoxic conditions. However, the functional microbial community involved in the processes are still unclear. In this study, the kinetics of NR-AsFeO and the associated microbial community in paddy soils were studied using anaerobic microcosms supplied with acetate as the carbon source. The results showed that As(III) and Fe(II) oxidation were observed in the NR-AsFeO culture, however, no significant Fe(II) or As(III) oxidation was observed in the treatments without  $\text{NO}_3^-$ . While  $\text{NO}_3^-$  reduction was observed in all  $\text{NO}_3^-$  amendments, the As(III) addition did not affect  $\text{NO}_3^-$  reduction, but the Fe(II) addition inhibited  $\text{NO}_3^-$  reduction. In order to evaluate the metabolic interdependencies of the microorganisms involved in the NR-AsFeO processes, 12 near-complete genomes were retrieved from the metagenomes of the NR-AsFeO microcosms using an ultra-deep sequencing and binning procedures. These microorganisms included representatives of *Azospira oryzae*, *Dechloromonas* sp., *Geobacter metallireducens*, *Bacillus* sp., *Azoarcus* sp., *Herbaspirillum* sp. and *Pseudogulbenkiania* sp.. Genera affiliated with *Bacillus* sp. were predicted to be the major As(III) oxidizing microorganisms in the presence of  $\text{NO}_3^-$ . Fe(II) oxidation may be stimulated chemically by  $\text{NO}_2^-$  generated from  $\text{NO}_3^-$  reduction or by  $\text{NO}_3^-$  reducing Fe(II) oxidizing bacteria, such as *Azospira oryzae* and *Pseudogulbenkiania* sp.. This study expanded our knowledge of the interactions among nitrogen, arsenic and iron transformation, and shed light on the microorganisms involved in the NR-AsFeO processes in flooded paddy soils.

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