

# Nitrate-Reducing Fe(II)- Oxidizing microorganisms: linking Fe, C and N Cycles in subsurface environments

Cristina Escudero Parada<sup>‡</sup>, Andreas Kappler<sup>‡</sup>

<sup>‡</sup> University of Tuebingen, Tuebingen, Germany

Corresponding author: Cristina Escudero Parada ([c.escudero@uni-tuebingen.de](mailto:c.escudero@uni-tuebingen.de))

## Abstract

The discovery of the Nitrate-Reducing Fe(II)-Oxidizing (NRFeOx) microbial metabolism, which couples the oxidation of Fe(II) to the reduction of nitrate ( $\text{NO}_3^-$ ) using organic matter or carbon dioxide ( $\text{CO}_2$ ) as carbon source, was a major milestone in microbial ecology (Straub et al. 1996). NRFeOx microorganisms play an essential role on a global scale in three of the most important biogeochemical cycles: iron (Fe), carbon (C) and nitrogen (N) (Kappler et al. 2021, Huang et al. 2021). In addition, these organisms participate in the mobilization or stabilization of organic carbon, as well as in  $\text{CO}_2$  fixation, thus contributing to the reduction of atmospheric  $\text{CO}_2$  (Kappler et al. 2021). Finally, the activity of these microorganisms is key to remove the pollutant  $\text{NO}_3^-$  from aquifers, which is one of the major worldwide environmental issues since many environments exceed the maximum regulatory concentration ( $50 \text{ mg L}^{-1}$ ) (Kazakis et al. 2020).

A plethora of NRFeOx microorganisms have been described in the last decades. However, most of these microorganisms have been reclassified as chemodenitrifiers. That is to say, Fe(II) is not enzymatically oxidized but indirectly by the reactive nitrogen species produced during denitrification (Fig. 1). In fact, only in three cultures so far, named KS, BP and AG, has the presence of true NRFeOx metabolism been unequivocally demonstrated (Straub et al. 1996, Huang et al. 2021b, Jakus et al. 2021b).

Cultures KS, BP and AG have been studied thoroughly in the past years, analyzing the rate and mechanism by which these communities carry out autotrophic NRFeOx. Different omics studies have revealed that cultures KS, BP and AG consist of a mixture of bacterial species, which collaborate in order to grow under autotrophic NRFeOx conditions. Each culture is dominated by a novel candidate species of the genus *Ferrigenium* (Huang et al. 2022) capable of fixing  $\text{CO}_2$  and oxidizing Fe(II), but which requires flanking species to complete denitrification (Huang et al. 2021b, He et al. 2016, Huang et al. 2021a).

Interestingly, these communities not only carry out NRFeOx using dissolved Fe(II) as energy source (Straub et al. 1996, Huang et al. 2021b, Jakus et al. 2021b), but they can

also oxidize Fe(II) minerals, the main form in which Fe(II) can be found in the Earth's crust (Huang et al. 2021). In fact, Fe(II)-bearing minerals are thought to be the main drivers of  $\text{NO}_3^-$  reduction in subterranean environments (Huang et al. 2021), which has additional ecological consequences. NRFeOx microorganisms can trigger the turnover of the Fe(II)-bearing minerals, resulting in the mobilization of mineral structural elements such as S, P, C or contaminant heavy metals and the precipitation of Fe(III) minerals at circumneutral pH (Weber et al. 2001, Jakus et al. 2021a).

Here, we will present a review of the insights learned from the three NRFeOx autotrophic cultures and discuss their ecological role, their importance in biogeochemical cycles, and their potential biotechnological applications.

## **Keywords**

geomicrobiology, biogeochemical cycles, bioremediation

## **Presenting author**

Cristina Escudero Parada

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## **Conflicts of interest**

The authors have declared that no competing interests exist.

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## Types of interactions between nitrate-reducers and Fe(II)

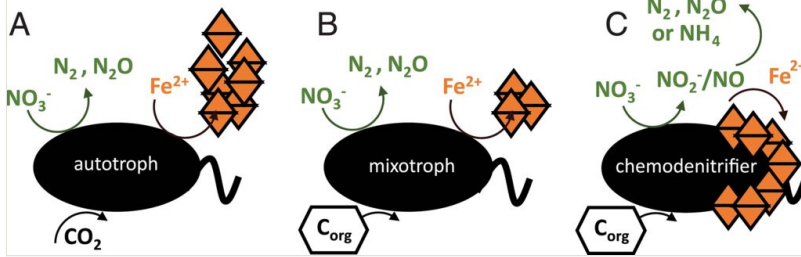


Figure 1.

Overview of the three different types of interaction between nitrate-reducing bacteria and Fe(II). (A) Autotrophic N<sub>R</sub>FeOx obtain carbon from CO<sub>2</sub> and oxidize Fe(II) enzymatically. (B) Mixotrophic N<sub>R</sub>FeOx require additional organic carbon as a carbon source, and Fe(II) oxidation has some enzymatic component (although there may also be some abiotic component). (C) Chemodenitrifiers require organic carbon and have no enzymatic component of Fe(II) oxidation. The position of the minerals (orange) relative to cells (black) indicates whether or not cell encrustation is expected. Image from Bryce et al. (2018).