# Identification of mechanisms of microbial nitrate-dependent Fe(II) oxidation by N-isotope analysis

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Nitrate-reducing Fe(II)-oxidizing bacteria are commonly found in the environment and may play a major role in the remediation of nitrate-contaminated sites [1,2]. Moreover, coupled nitrate reduction coupled to Fe(II) oxidation appears to be an important $N_2O$ production pathway [3]. The mechanisms of nitrate-dependent Fe(II) oxidation, however, are still not fully understood. For example, it remains unclear to what extents, respectively, Fe(II) oxidation is enzymatically driven or caused by an abiotic reaction of reactive nitrogen (N) species (e.g. nitrite) that are formed during heterotrophic denitrification [3]. Previous studies showed that enzyme-catalyzed reactions can be distinguished from abiotic reactions based on differences in reaction-dependent isotope effects [5]. Therefore, we conducted time-series experiments with several nitrate-dependent Fe(II)-oxidizers: *Acidovorax* sp. strains BoFeN1 and 2AN, and *Pseudogulbenkiania ferrooxidans* sp. strain 2002. Concentrations of nitrate, nitrite, acetate, Fe(II), Fe(III), as well as the N-isotopic composition of denitrification substrates and intermediates (i.e., $NO_2^-$, $N_2O$) in the presence and absence of Fe(II) were measured over time and compared to abiotic experiments with nitrite and Fe(II). Additionally, we are analyzing the concentration and isotopic composition of $N_2O$ produced in the different setups. We will discuss the obtained N-isotope data in the context of their potential to assess the relative importance of biotic vs. abiotic nitrate-dependent Fe(II) oxidation, Ultimately, such data will not only allow evaluating the role of these processes in the natural environment, they may also aid in our efforts to test and establish viable nitrate-mitigating biotechnological applications.

[1] Wang et al. (2015) *Geomicrobiology Journal, in press*. [2] Zhu et al. (2013) *Environmental Science & Technology* **47**, 8970-8977. [3] Jones et al. (2015) *Environmental Science and Technology* **49**, 3444–3452 [4] Klueglein and Kappler (2013) *Geobiology* **11,** 180-190. [5] Granger et al. (2008) *Limnology and Oceanography* **53**, 2533-2545.