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Autotrophic nitrate reduction coupled to oxidation of Fe(II) phases by a *Gallionellaceae* sp.-dominated microbial community enriched from a pyrite-rich aquifer

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Autotrophic denitrification coupled to pyrite oxidation is thought to drive natural attenuation of nitrate in aquifers. However, laboratory-based cultivation of autotrophic nitrate-reducing iron-oxidizing (NRFeOx) microorganisms is challenging, and so far little is known about the identity of subsurface-inhabiting NRFeOx bacteria, their physiology, metabolic makeup, and the mechanisms of oxidation of solid Fe(II) sources. Here, we used culture-dependent studies supported by NanoSIMS analysis, SEM imaging and reaction-modeling to evaluate the rates, the extent and mechanisms of $Fe^{2+}_{(aq)}$, siderite ($FeCO_3$), and pyrite (FeS_2) oxidation coupled to nitrate reduction by an autotrophic NRFeOx culture enriched from a fractured limestone aquifer containing Fe(II) minerals. Additionally, we applied metagenomics to elucidate putative electron transfer pathways in nitrate reduction and Fe(II) oxidation. During incubation with $Fe^{2+}_{(aq)}$, and siderite, the $NO_3^-{}_{reduced}/Fe(II)_{oxidized}$ ratios were close to a stoichiometric ratio of 0.2, suggesting that complete Fe(II)-dependent denitrification occurred. While growing on pyrite, only a fraction of nitrate (25.8%) was reduced due to direct oxidation of structural Fe(II), and the rest was coupled to the oxidation of S^0 present as a residue from pyrite synthesis. The 16S rRNA gene sequencing revealed that the culture was dominated (ca. 62% relative abundance) by a so far uncultured organism belonging to the family *Gallionellaceae* (known Fe(II)-oxidizers). The unclassified *Gallionellaceae* sp., apart from having a cytochrome Cyc2 gene, putatively involved in extracellular electron transfer from Fe(II), also possesses *narGHJI, nirKS*, and *norBC*, a complete set of genes necessary to reduce NO_3^- to N_2O. It lacks, however, the *nosZ* gene and genes involved in S-cycling (*sox*), suggesting that NRFe oxidation in pyrite-containing aquifers requires multiple inter-species metabolic hand-offs. These findings highlight the importance of chemolithoautotrophic *Gallionellaceae* in linking biogeochemical cycles of N and Fe and has implications for predicting the fate of nitrate in freshwater ecosystems poor in organic carbon.