

A short-circuit of the nitrogen cycle during the mineralization of metals by *Geobacter* species

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The extensive use of nitrate fertilizers in agriculture disrupts the nitrogen (N) cycle, causing nitrogen losses to the atmosphere (nitrous oxide greenhouse gas) and water bodies (nitrate and nitrite leaching from soils). By converting nitrate and nitrite species to the less mobile ammonium form, the dissimilatory reduction of nitrate to ammonium (DNRA) “short-circuits” the N cycle and simultaneously mitigates the leaching of the mobile N species from soils and their conversion to nitrous oxide via denitrification. The prevalence of iron-reducing *Geobacter* bacteria, like *Geobacter lovleyi*, in DNRA-active soils and sediments led us to investigate their role in retaining nitrogen through nitrate ammonification. Using *G. lovleyi* SZ in lab experiments, we found that low nitrate concentrations (5 mM) support optimal growth via nitrate ammonification with acetate, the preferred electron donor. DNRA growth was not sustained with other electron donors such as formate and Fe(II), and was independent of the carbon to nitrate ratio (C/NO₃⁻), challenging prevailing beliefs. Notably, in DNRA-growing cells, RNA sequencing identified redundancies for the sequential reduction of nitrate to nitrite (Nap and Nar enzymes) and ammonium (Nrf enzymes) as well as DNRA-specific sensors and response regulators. Flagellar and chemotaxis genes were upregulated, consistent with adaptive responses for sensing and competing for the limited supply of nitrate. Increasing nitrate from 5 to 10 mM in the cultures led to further upregulation of all the enzymes in the DNRA pathway as well as increases in nitrite, but not nitrate, reductase activities. Still, nitrite accumulated in the 10 mM cultures to toxic levels and severely impacted growth efficiency. Yet, it was possible to stimulate the removal of nitrite and alleviate cytotoxicity by supplementing the cultures with H₂ to provide an excess source of electrons for the reduction of the nitrite intermediate. These findings provide much needed mechanistic understanding of DNRA and highlight the important roles that iron-reducing bacteria in the genus *Geobacter* play in N conservation in nitrate-limited environments. Importantly, they establish the *G. lovleyi* subclade as a model ecological system for understanding the environmental conditions that control the mobility of oxidized N species and the fate in soils impacted by agriculture.