

Compound Metabolism Insights Gained by a Holistic Testing Regime

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Background/Objectives. Anthropogenic nitrate, ammonium, and breakdown products, such as nitrous oxide, have substantial negative environmental effects. Nitrate and nitrite are toxic to humans and are regulated in drinking water. Ammonium and nitrate contribute to eutrophication of natural water bodies. Nitrous oxide (N₂O) can be produced by incomplete denitrification, and comprises approximately 6% of global warming emissions. Nitrogen compound contamination of surface water and groundwater originates from many sources including fertilizer application and production, human and livestock waste, and mining operations. Microbial processes can convert problematic nitrogen compounds into inert nitrogen gas, primarily via denitrification and anammox. Nitrification is also critical, functioning in tandem with denitrification to convert ammonium to nitrogen gas. Dissimilatory reduction of nitrate to ammonium (DRNA) can reduce nitrogen mass in water or soil via volatilization of ammonia. To understand and optimize nitrogen compound remediation it is important to monitor not only the forms of nitrogen and geochemistry but also microbial groups and functional genes critical to nitrogen metabolism.

Approach/Activities. Sites with nitrogen compound contamination in groundwater were assessed using a “multiple lines of evidence approach” to assess the status of nitrogen compound remediation and potential biological conversion pathways. Nitrate species measured were ammonium and nitrate, nitrite and Total Kjeldahl Nitrogen (TKN). Electron donors, important for denitrification, were measured using total organic carbon and volatile fatty acids analysis. The conversion of nitrogen compounds was differentiated from non-bond breaking processes, such as dilution, using compound specific isotope analysis (CSIA) methods that quantify increasing ¹⁵N:¹⁴N ratios. The presence of microorganisms capable of metabolic conversion of nitrogen compounds was assessed by molecular biological tools quantifying the presence of microbial taxa and functional genes involved in nitrification, denitrification, anammox and dissimilatory nitrate reduction to ammonium (DNRA) via quantitative polymerase chain reaction (qPCR) and next generation sequencing of 16S rRNA amplicons.

Results/Lessons Learned. A former dry blend fertilizer plant, adjacent to the Cape Fear River in North Carolina, was characterized using a suite of molecular, analytical, and isotopic tests to assess intrinsic nitrogen removal processes. The site was contaminated with petroleum hydrocarbons, naphthalene, metals, and ammonia in groundwater at concentrations above regulatory standards. Ammonia exceeded the NC state standard of 1.5 mg/L in all samples, with concentrations as high as 30.8 mg/L detected. The presence of nitrification, denitrification and anammox functional genes in groundwater at the site was confirmed by qPCR tests indicating the potential for multiple pathways for nitrogen mass removal that varied according to location. The potential for transformation of ammonium was confirmed by the detection of nitrification functional genes and anammox bacteria and was confirmed by ¹⁵N enrichment using CSIA. Even though nitrate was undetected, denitrification genes were detected at high abundance as were electron donors, providing significant evidence for denitrification. The data set provided compelling evidence that ammonia remediation was occurring at the site, most likely using multiple biological pathways.