Using Molecular Biological Tools to Address Nitrogen Transformation in Groundwater

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Background/Objectives. Microorganisms have demonstrated a remarkable ability to bioremediate sites worldwide. They are instrumental in preserving the safety of our groundwater as well as water destined for environmental and industrial use. Molecular biological tools (MBTs) such as QuantArray-BGC, a suite of 25 quantitative polymerase chain reaction (qPCR) assays, can be used to comprehensively assess the native genetic potential for biogeochemical processes in groundwater and monitor the microbial response to treatment over time. The ability to compare concentrations of key microbial populations spatially, temporally, and even across sites can provide context and influence decision making to optimize treatments and maximize cost-savings. With a comprehensive review of pivotal steps in nitrogen cycling such as ammonification, nitrification and denitrification, this presentation will illuminate the organisms and enzymes involved in the nitrogen cycle that can be quantified using qPCR and provide their detection frequencies and median concentrations based on a database of qPCR results from over 65,000 groundwater samples.

Approach/Activities. Using case studies, we will build a deeper understanding of how MBTs can be utilized to assess the diverse microbial communities that carry out biogeochemical processes in groundwater, including the nitrogen cycle, sulfate reduction, sulfur oxidation, iron reduction, metal oxidation, fermentation, acetogenesis, and methanogenesis. This presentation will illustrate ways qPCR data can be incorporated into routine monitoring plans with the goal of driving better management decisions at sites where nitrate is a concern. Drawing from a database of over 65,000 groundwater samples, statistical analysis will provide insight into how often relevant microbial groups such as denitrifiers, nitrite oxidizers, nitrogen fixers, and ammonia oxidizers, among others, are detected in water samples and their median concentrations.

Results/Lessons Learned. QuantArray-BGC results from two petroleum sites will be shared. At the first site, moderate to high concentrations of three different genes related to denitrification were detected along with sulfate reducers, iron reducers, and methanogens. At the second site, aerobic microbial groups predominated with high concentrations of ammonia oxidizing bacteria, nitrite oxidizing bacteria, and metal oxidizing while denitrification genes were below the detection limit. Through analysis of the database of qPCR results, detection frequencies and median concentrations of key organisms behind pivotal steps in nitrogen cycling as well as other biogeochemical processes of interest will be shared to add context to the site data. For example, the *nirK gene* related to denitrifying bacteria was detected in 50% of the 1,897 samples analyzed for this target with a median concentration of 7.88E+03 cells/mL. Nitrite oxidizing bacteria were detected in 27% of water samples analyzed (n=1,254), and the median concentration was 1.92E+02 cells/mL. Nitrogen fixing bacteria were identified in 75% of samples analyzed (n=1,309) with a median concentration of 1.93E+04 cells/mL. Ammonia oxidizing bacteria were identified in 309 water samples with 23% detection and median of 8.20E+01 cells/mL. Establishing these metrics is beneficial for comparing microbial results between sites and developing context for successful remediation of groundwater. Although microbial concentrations do not have to be "better than average" to ensure biotransformation, elucidating biogeochemical interactions via qPCR analysis can be helpful to assess the state of the site and develop a plan to shift redox conditions if needed.