

Natural Occurrence of Feammox Conditions and Anammox Microbiota within a PFAS Plume at the Groundwater-to-Surface Water Interface

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Background/Objectives. Anaerobic ammonia oxidation coupled to Fe(III) reduction, known as Feammox, is a recently documented nitrogen-cycling process of particular interest to remediation practitioners due to the presence of lithoautotrophic bacteria within the genus *Acidimicrobiaceae* (Strain A6, or A6 for short) reportedly capable of breaking the carbon-fluorine (C-F) bond in per- and polyfluoroalkyl substances (PFAS). The cleaving of the C-F bond is substantiated by identification of multiple dehalogenase genes in A6. The majority of research performed to date on Feammox and A6 has been in vitro using controlled laboratory settings, although field-documentation of Feammox conditions is reported. Here, we document both in situ Feammox modalities and a complex microbial Anammox consortium within an iron-enriched groundwater seep adjacent to a river in western Michigan. Objectives were to 1) identify Feammox indicators, 2) document and map the microbiological signature of the seep soils, and 3) document the relationship between the Feammox condition and PFAS chemistry, if any, if possible.

Approach/Activities. Field reconnaissance during the summer of 2022 identified a prospective study location comprising approximately 150 square feet and adjacent to a western Michigan tributary. Soil and porewater sampling was performed at three separate locations within the main study area. Soil analytes included: field redox using resazurin/methylene blue assay, 16s rRNA V3-V4 microbiomics, shotgun metagenomic sequencing, PFAS-28 list, ammonia, nitrate, nitrite, total iron, ferrous iron and pH. Porewater analytes included: pH, dissolved oxygen, oxidation reduction potential, PFAS-28 list, and ammonia, nitrate, nitrite, total iron, ferrous iron and pH. Porewater extraction was implemented using low-flow techniques via stainless-steel push-point samplers. Next generation sequencing and microbiomic reporting were performed by Zymo Research, of Irvine, California. Iron mineralogy was confirmed using X-ray diffraction (XRD).

Documentation of Feammox conditions were largely based on indicators and chemical ratios identified by Jaffé, Huang, Ruiz-Urigüen and others.

Results/Lessons Learned. Next generation sequencing indicates that bacteria residing in seep locations are primary nitrogen cyclers (*Nitrospira*, *Hyphomicrobium*, *Gaiella*, and *Methylomirabilis*), iron-reducers (*Leptothrix* and *Acidiferrobacter*) and ammonia oxidizers (*Nitrospira* and *Denitratisoma*) suggestive of Feammox conditions. Two of the genera identified, *Hyphomicrobium* and *Anaeromyxobacter*, are known to be encoded with dehalogenase genes. Seep geochemistry including the ratio of iron to ammonia are Feammox-like with higher iron content than typically found in reported Feammox cells. Analysis of the shotgun metagenomic sequencing and PFAS data are on-going as well as relationship of PFAS to Feammox conditions.