S(0) cycling in acidic springs dominated by *Acidithiobacillus* spp.

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The Iroquois National Wildlife Refuge in Basom, NY consists of emergent marsh and hardwood swamp, and is notable for its diverse natural springs. Many of the springs are acidic (pH < 2), anoxic, and rich in sulfide, sulfate, reduced iron, and methane. The high concentrations of sulfur compounds may have significant impacts on biogeochemical cycling in the refuge, as freshwater wetlands are typically limited in sulfate, which can inhibit key carbon cycling processes like methanogenesis through competition for organic substrates. Overall, our goal is to understand how these unique springs impact wetland sulfur and carbon cycling in the refuge. To that end, we have investigated the microbial community structure of two springs and isolated representative strains for additional lab-based analyses.

Both sulfur springs showed extremely low diversity, especially when compared against a nearby creek. Indeed, 16S data from the primary sulfur spring (hereafter, SS1) identified >99% of the community as members of the *Acidithiobacillales*. *Acidithiobacillales* comprised 4% of all sequences for the second spring (SS2), and only 0.01% of all sequences for the creek. Given the remarkably homogeneous SS1 community, we focused isolation efforts on targeting acidiophilic, chemoautotrophic sulfur oxidizers.

Three closely related *Acidithiobacillus* strains were isolated from SS2. These three strains tightly cluster with the top three *Acidithiobacillus* OTUs identified in SS1, and thus we consider them appropriate representatives of the *Acidithiobacillus* community in the springs. We have focused on one of these isolates, HP-6, which grows well with thiosulfate as a sole electron donor. Unlike other *Acidithiobacillus* spp., HP-6 does not grow well on supplemented elemental sulfur (S^0) as a sole electron donor. However, HP-6 processes thiosulfate into extracellular S^0 globules before further oxidizing S^0 to sulfate. It is unclear why HP-6 grows on its own S^0, but not commercial S^0. This preference, or lack thereof, may explain a substantial layer of accumulated S^0 sediment within SS1.

Continuing work will aim to characterize the sulfur cycling capacity of HP-6 and other isolates, with a focus on rates of S^0 production and consumption. In addition, genes from both the isolate and environmental samples will be examined to better understand the dominant pathways of sulfur and carbon cycling in the springs.