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Spatial Constraints on Microbial Metabolic Activity in Anaerobic Environments

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Spatial separation of metabolic substrates creates constraints for microorganisms in any soil environment, but is particularly relevant to microbial metabolic activity in anaerobic sediments. Insoluble particulate organic carbon and mineral bound terminal electron acceptors (TEAs), such as Fe(III) or U(VI), are often not homogeneously distributed, creating a spatial dilemma for metal reducers to physically access both the carbon substrate and TEAs. In naturally reduced zones (NRZs), the reductive immobilization of uranium is closely linked to the gradual degradation of organic matter carried out by consortia of different microorganisms. To evaluate the impact of spatial constraints on microbial syntrophy and metal/uranium fate in NRZs, we have developed a co-culture system with three bacteria representing key metabolic processes: *Ruminiclostridium cellulolyticum* (hydrolysis and fermentation of cellulose), *Geobacter sulfurreducens* (metal reduction), and *Desulfovibrio vulgaris* (sulfate reduction). This co-culture is grown in an electrochemical reactor system where both the carbon source (cellulose) and the fluorine-doped tin oxide (FTO) anode serving as TEA for *G. sulfurreducens*, are immobilized in spatially separated patterns at micro- to millimeter scale. With cellulose as the sole carbon source, both *G. sulfurreducens* and *D. vulgaris* are relying on fermentation products provided by *R. cellulolyticum* for growth. Increasing separation distances between carbon substrate and TEA put *G. sulfurreducens*, who is relying on an immobilized TEA, at an increasing disadvantage compared to *D. vulgaris*, who is using a soluble TEA (sulfate in the medium) and thus can position themselves close to the carbon source. By growing our bacterial model community on spatially patterned glass slides, we are able to microscopically visualize the spatial organization of the community with high resolution and precisely monitor the flow of electrons and carbon through the microbial community. Our experiments will help evaluate the biogeochemical relevance of spatial separation of different metabolic substrates, a constraint imposed on microbial function within NRZs (and other anaerobic environments). Such information is needed to understand the fate of carbon, sulfur, iron, uranium and other metals in NRZs and guide the development of spatially explicit reactive transport models.