

## **In situ demonstration of sustained adaptation of a natural microbial community to transform substrates**

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**Project Goals: ENIGMA (Ecosystems and Networks Integrated with Genes and Molecular Assemblies) uses a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods. For any microbe, it is possible to discover efficiently the genetic determinants of adaptation to life in dynamic environments in contact with other living members of its ecology and reveal hidden functional phenomena that are only possible by using a systems biology approach; For any environment, it is possible to predict the temporal changes in geochemistry with some precision, for some length of time, given geochemical and biological inputs.**

Here we aim to: (1) demonstrate the exposure history dependence of microbial mediated substrate transformation rates in groundwater at the field scale and (2) elucidate the microbial mechanism(s) which control the exposure history dependence of microbial mediated substrate transformation rates

Prior exposure of a natural microbial community to a substrate can result in the increased potential of the community to transform the substrate; this phenomenon is known as adaptation. Adaptation is thought to play an important role in biogeochemical cycling at the ecosystem scale and has been demonstrated at the laboratory scale. However, in situ demonstrations of the magnitude and duration of adaptation are lacking. Ethanol was used as a substrate and was injected into a groundwater well (substrate treatment) for six consecutive weeks to establish adaptation. A second well (substrate control) was not injected with ethanol during this time. The substrate treatment demonstrated adaptation for microbial-mediated oxidation of ethanol to acetate and reduction of nitrate and sulfate as evident by sequential and significant increases in zero-order reaction rates. Both wells were then monitored for six additional weeks under natural conditions. During the final week, ethanol was injected into both wells. The substrate treatment

demonstrated sustained adaptation as evident by significantly higher reaction rates than the substrate control. Surprisingly, the selective enrichment of a microbial community within the first six weeks of the substrate treatment was not sustained after the six-week absence of ethanol, as revealed by analysis of planktonic DNA. These results demonstrated that adaptation can be induced and sustained with no apparent enrichment of a select microbial community. This suggests that the predominant mechanisms of adaptation may exist at the enzymatic- and/or genetic-levels.

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