Groundwater microbial communties response to simulated spills of hydraulic fracturing-related fluids

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Shale gas is being considered as a complementary energy resource to other fossil fuels. Its exploitation requires using advanced drilling techniques and hydraulic stimulation (fracking), i.e. the injection of fluids at high pressures into the formations, to create fractures and fissures, and thus to release gas from the source rock into the wellbore. The injected fluid partly remains in the formation, while up to 40% of these fluids flow back to the surface, together with reservoir waters, sometimes containing dissolved hydrocarbons, high salt concentrations, etc. As microbial communities may play a key role in the natural attenuation of potential fracking-related groundwater contamination, the aim of our study was to investigate the response of groundwater microbial communities to a potential spill along a contamination plume, and their resilience.

Laboratory experiments under in situ conditions (i.e. at *in situ* temperatures, with high pressure, etc.) were conducted using groundwater samples from different locations. Series of microcosms (3 of each kind) containing R2 broth medium or groundwater spiked with either single frac chemicals, frac fluids, artificial reservoir water, NaCl, oil, or different mixtures of reservoir water and frac fluid (to simulate flowback) were incubated in the dark. Controls included non-amended and non-inoculated microcosms. Classical microbiological methods and molecular analyses were used to assess the effects in the microbial abundance and community structure during and after the exposure to different concentrations of the above mentioned fluids.

Microbial communities were quite halotolerant and their growth benefited from low concentrations of reservoir waters or salt, but they were negatively affected by higher concentrations of formation waters, salt, biocides, frac fluids or simulated flowback. They could recover after the exposure to artificial formation waters, or flowback, but not after the exposure to biocides or frac fluids. Changes on the microbial community structure could be detected by T-RFLP. Single frac components like guar gum or choline chloride were used as substrates, while others like triethanolamine or light oil distillate hydrogenated prevented microbial growth in groundwaters. Ongoing work will provide information on potential transformations of frac or geogenic chemicals by groundwater microbiota and their lifetime.