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The fate of injected CH_4 in shallow groundwater monitored through a geochemical, isotopic and next generation DNA sequencing approach

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Recent increases in natural gas and oil extraction from unconventional reservoirs such as shale have raised concerns over potential impacts of fugitive gas migration on the quality of shallow groundwater. The situation is made more complex by the fact that methane (CH_4) may sometimes occur in aquifers not through migration from deeper geological strata; but rather as a result of *in-situ* microbial activity within the aquifer itself. Although the presence of CH_4 in shallow groundwater is well documented, reliable scientific data concerning the occurrence and mechanisms of stray gas migration is limited, and the vulnerability of shallow groundwater to natural gas contamination from below is often unclear. The objective of this study was to investigate the migration and persistence of CH_4 in groundwater as well as the response to CH_4 contamination by *in-situ* microbial communities via a shallow aquifer CH_4 injection field experiment and complementary laboratory microcosm studies.

Methane (~ 36.4 kg) was injected over 72 days into a shallow, freshwater, sand aquifer at the CFB Borden, Ontario, Canada, over a 72-day period. The impact on groundwater quality and the fate of the gas was monitored for 254 days through a multidisciplinary approach involving aqueous and stable isotope geochemistry, alongside microbial DNA sequencing techniques, among others. For microbiological analyses, groundwater samples were collected at 2, 4, 6 and 8m depth from a multilevel monitoring well 1m from the CH_4 injection point. Sampling was performed 1 day before CH_4 injection commenced, as well as after 45, 125 and 254 days after injection. Groundwater samples from the aquifer were also used as inoculum in a laboratory bioreactor enrichment culture to further characterize the methane-oxidizing microbial communities.

Following methane injection, CH_4 efflux rate calculations indicated that approximately 30% of the injected methane was released into the atmosphere (Cahill *et al*., 2017). Furthermore, methane migration in the shallow aquifer was rapid and widespread resulting in an extensive, continuous, dispersed zone of dissolved methane in the groundwater. Free and dissolved-phase CH_4 persisted in the groundwater over the duration of the study. The $\delta^{13}C$ values of dissolved groundwater CH_4 during injection approached values similar to the carbon isotope ratio of the injected gas phase methane (-42‰) and remained unchanged after 245 days of monitoring. The $\delta^{13}C$-CH_4 values recorded indicated that the methane was not significantly degraded within the aquifer and that natural attenuation via microbial methane oxidation was relatively ineffective during the observation period according to chemical and isotopic monitoring data (Cahill *et al*., 2017).

Amplicon sequencing of 16S ribosomal RNA genes from the samples, however, revealed that the methane release strongly altered the microbial community structure in the aquifer. CH_4 availability stimulated the growth of aerobic methanotrophic (*Methylococcaceae*) and methylotrophic (*Methylophilaceae*) bacteria despite low oxygen concentrations. The relative sequence abundance of both bacterial groups increased substantially from <2 % up to 65% of the overall microbial community at 2 m and 4 m depths (Fig. 1). Oxygen limitation was more evident at 6 and 8 m depths where a lower abundance of methanotrophs was observed, and methane release eventually led to anoxic conditions. This, coupled with no detection of taxa capable of anaerobic oxidation of methane (AOM), explained why microbial methane oxidation was relatively ineffective in the aquifer during the monitoring period. Additional laboratory enrichment culture experiments showed that under oxygen limitation, electron acceptors such as NO_3^- could facilitate the growth and abundance of *Methylococcaceae* such as *Methylomonas*, able to perform methane oxidation coupled to nitrate reduction. Consequently, enrichment of ^{13}C in the remaining CH_4 and potential water quality changes can be expected to occur over longer time scales beyond the duration of the field experiment.

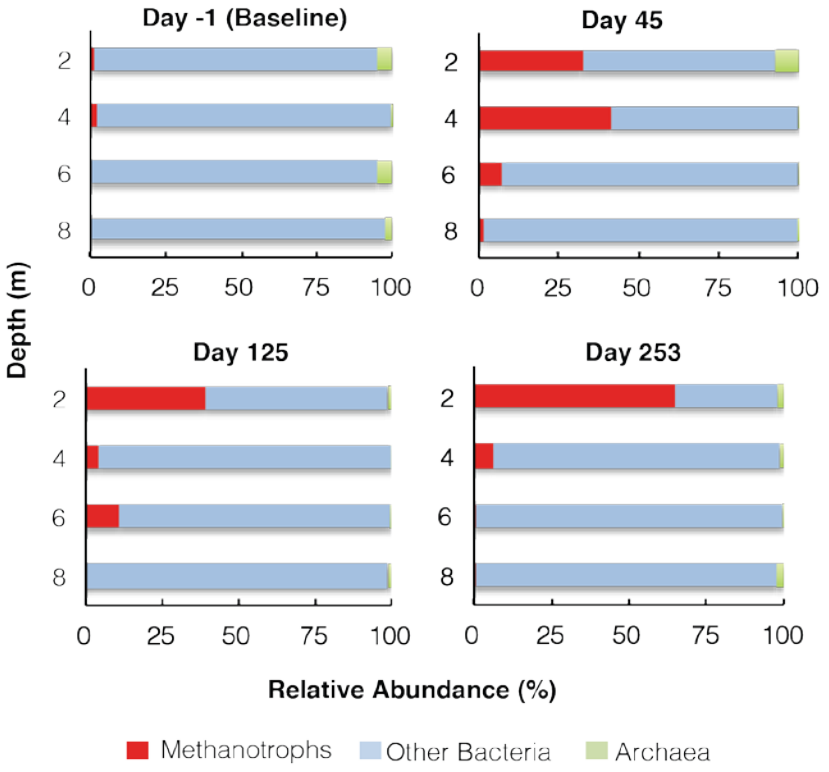

Fig. 1. Relative DNA sequence abundance of domain level microbial communities per depth at the study site. The methane oxidizing bacteria are highlighted in red.

Our study, therefore, demonstrates that through a multidisciplinary approach that combines traditional geochemical and stable isotope techniques, with microbiological analyses including next generation DNA sequencing tools, the impact, and fate of fugitive CH_4 in groundwater can be

detected more conclusively than was previously possible. This approach could potentially lead to remediation opportunities for CH_4-contaminated shallow aquifers.

Reference

Cahill, A. G., Steelman, C. M., Forde, O., Kuloyo, O., Ruff S. E., Mayer, M., Mayer, K. U., Strous, M., Ryan, C. M., Cherry, J. C. & Parker, B. L. Mobility and persistence of methane in groundwater in a controlled-release field experiment. *Nature Geoscience* **10**, 289-294 (2017).