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Groundwater ecosystem resilience to organic contaminations: microbial and geochemical dynamics throughout the 5-year life cycle of a surrogate ethanol blend fuel plume

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ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form
31 March 2015
Accepted 4 May 2015
Available online 9 May 2015

Keywords:
Phylogenetic
Taxonomic
Biofuel
Resilience
Metagenomic
Diversity

ABSTRACT

The capacity of groundwater ecosystem to recover from contamination by organic chemicals is a vital concern for environmental scientists. A pilot-scale aquifer system was used to investigate the long-term dynamics of contaminants, groundwater geochemistry, and microbial community structure (by 16S rRNA gene pyrosequencing and quantitative real-time PCR) throughout the 5-year life cycle of a surrogate ethanol blend fuel plume (10% ethanol + 50 mg/L benzene + 50 mg/L toluene). Two-year continuous ethanol-blended release significantly changed the groundwater geochemistry (resulted in anaerobic, low pH, and organotrophic conditions) and increased bacterial and archaeal populations by 82- and 314-fold respectively. Various anaerobic heterotrophs (fermenters, acetogens, methanogens, and hydrocarbon degraders) were enriched. Two years after the release was shut off, all contaminants and their degradation byproducts disappeared and groundwater geochemistry completely restored to the pre-release states (aerobic, neutral pH, and oligotrophic). Bacterial and archaeal populations declined by 18- and 45-fold respectively (relative to the time of shut off). Microbial community structure reverted towards the pre-release states and alpha diversity indices rebounded, suggesting the resilience of microbial community to ethanol blend releases. We also found shifts from O_2-sensitive methanogens (e.g., *Methanobacterium*) to methanogens that are not so sensitive to O_2 (e.g., *Methanosarcina* and *Methanocella*), which is likely to contribute to the persistence of methanogens and methane generation following the source removal. Overall, the rapid disappearance of contaminants and their metabolites, rebound of geochemical footprints, and resilience of microbial community unequivocally document the natural capacity of groundwater ecosystem to attenuate and recover from a large volume of catastrophic spill of ethanol-based biofuel.

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http://dx.doi.org/10.1016/j.watres.2015.05.003
0043-1354/

1. Introduction

Groundwater constitutes the largest terrestrial freshwater ecosystem, but still belongs to the least explored one on earth (Griebler et al., 2014). The capability of groundwater ecosystems to recover from pollution (such as fuel spills) is a vital concern for environmental scientists. As the most important mechanism (sometimes the only available one) for contaminant elimination in groundwater, biodegradation plays a key role for the resilience of groundwater ecosystems to contaminations (Loffler and Edwards, 2006). However, few studies have comprehensively investigated the temporal variability in microbial community structure and the long-term evidence for contaminant elimination and groundwater geochemistry evolution throughout the whole life cycle of a contaminant release, from initial plume expansion through its stabilization and eventual disappearance (Yagi et al., 2010). Improved understanding on dynamics of microbial community (supported by contaminants and geochemical monitoring data) throughout the entire life cycle of a contaminant plume would undoubtedly improve our understanding of the susceptibility and recovery of groundwater ecosystem to organic contaminations.

The growing use of ethanol as transportation biofuel is increasing the likelihood of encountering ethanol in current and future fuel releases (Ma et al., 2013b). Previous studies focused on microbial responses following the start of the ethanol blend fuel releases (Capiro et al., 2008; da Silva and Corseuil, 2012; Elazhari-Ali et al., 2013; Feris et al., 2008; Ma et al., 2013a; Nelson et al., 2010). However, few studies investigated microbial responses after the contaminant source was removed, which is usually the first and the most important step to remediate a contaminated site. Ma et al. used GeoChip to characterize the changes in functional gene structure in response to 2-year continuous releases of ethanol blend release and complete shut off of the release for 4 months (Ma et al., 2015a). That study showed that most functional genes returned to pre-release abundance levels, but the final functional structure still significantly differed from pre-release conditions (Ma et al., 2015a). As it acknowledged, 4 months of recovery time in that study was too short to fully understand the microbial response following source removal. It is still not known whether microbial community is able to completely restore to pre-release conditions following a longer recovery period.

Moreover, most of previous taxonomic studies rely on finger printing tools such as denaturing gradient gel electrophoresis (DGGE) (Capiro et al., 2008; Elazhari-Ali et al., 2013), automated ribosomal intergenic space analysis (ARISA) (Nelson et al., 2010), and quantitative real-time PCR (qPCR) (Beller et al., 2008; Capiro et al., 2008; da Silva and Corseuil, 2012; Feris et al., 2008; Nelson et al., 2010). These approaches generally detect only predominant microbial groups whereas contaminant biodegradation is usually carried out by a complex microbial food web (de Lorenzo, 2008), and smaller populations that fill important niches may remain undetected (Osborn and Smith, 2005). Ma et al. used 454 pyrosequencing to characterize microbial community in impacted aquifer, however, this study only detected 1000–2000 16S rRNA gene sequences per sample which was too low to have complete coverage (Ma et al., 2013a). A more in-depth characterization (e.g., >10,000 16S rRNA gene sequences/sample) of microbial community would undoubtedly reveal previously unrecognizable level of biodiversity, thus providing a more complete and accurate picture of microbial ecology in impacted sites.

In a pilot-scale model aquifer system, 16S rRNA gene pyrosequencing and qPCR was used to characterize microbial successions throughout the 5-year life cycle of a surrogate ethanol blend fuel plume. The pilot-scale experiments are unique in that they are of sufficient scale such that more realistic three-dimensional contaminant plumes can be established, but at a small enough scale to provide sufficiently controlled experimental conditions. Contaminant concentrations (ethanol, benzene, toluene, methane, acetate, and butyrate) and groundwater geochemical parameters (temperature, pH, redox potential, and dissolved oxygen) were monitored throughout the plume life cycle to provide a comprehensive perspective of impacts and dynamics of the blend release.

2. Materials and methods

2.1. Pilot-scale tank aquifer

An 11-m^3 (3.7 m × 1.8 m × 1.2 m) pilot-scale continuous-flow tank packed with fine grain southeast Texas sand (Circle Sand; Houston, Texas) was used in this study (Fig. S1 in the supporting information). Tap water was added from the "inlet" (Fig. S1) at 170 L/day (average seepage velocity of 0.76 m/day) to obtain a water table elevation of about 70 cm from the bottom of the tank. The influent water contained around 5.5 mg/L of dissolved oxygen (DO). The groundwater retention time in this model tank was around 4 days. The total aquifer thickness was 115 cm and the depth of the water table was 45 cm below ground surface. The surrogate ethanol blend solution was a water solution containing 10% (v/v) ethanol, 50 mg/L benzene, 50 mg/L toluene and 24,000 mg/L of sodium bromide (NaBr). The blend solution was continuously injected into the tank from the ethanol blend injection well (22.5 cm below the water table) at a rate of 0.4 L/day. NaBr was added as a conservative tracer, and to maintain a solution density to reach neutral buoyancy with the flowing groundwater. The added NaBr was diluted by the tank flow to less than 2000 mg/L (measured at groundwater sampling port, see Fig. 1), which was within the typical tolerance range of soil bacteria (Atlas and Bartha, 1997). The groundwater sampling well was at the same depth as the ethanol blend injection well (22.5 cm below the water table). Details on the tank construction and packing methods can be found in Ma et al. (2011) and Ma et al. (2012).

2.2. Release stages and plume life cycle

This pilot-scale release experiment lasted for 5 years, which can be divided into 4 experimental stages (6 time points) (Fig. 1). General information for each stage can be found in Table 1. Stage 1 was the pre-release baseline. Stage 2 began with the continuous ethanol blend release (10% ethanol + 50 mg/L benzene + 50 mg/L toluene) and lasted 2