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Anoxic nitrogen cycling in a hydrocarbon and ammonium contaminated aquifer

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ABSTRACT

Nitrogen fate and transport through contaminated groundwater systems, where N is both ubiquitous and commonly limits pollutant attenuation, must be re-evaluated given evidence for new potential microbial N pathways. We addressed this by measuring the isotopic composition of dissolved inorganic N (DIN = NH_4^+, NO_2^-, and NO_3^-) and N functional gene abundances (*amoA*, *nirK*, *nirS*, *hszA*) from 20 to 38 wells across an NH_4^+, hydrocarbon, and SO_4^{2-} contaminated aquifer. In-situ N attenuation was confirmed on three sampling dates (0, +6, +12 months) by the decreased [DIN] (4300 - 40 μM) and increased δ^{15}N-DIN (5‰–33‰) over the flow path. However, the assumption of negligible N attenuation within the plume was complicated by the presence of alternative electron acceptors (SO_4^{2-}, Fe^{3+}), both oxidizing and reducing functional genes, and N oxides within this anoxic zone. Active plume N cycling was corroborated using an NO_2^- dual isotope based model, which found the fastest (~10 day) NO_2^- turnover within the N and electron donor rich central plume. Findings suggest that N cycling is not always O_2 limited within chemically complex contaminated aquifers, though this cycling may recycle the N species rather than attenuate N.

1. Introduction

Pressure to clean up contaminated groundwater resources is increasing along with demand on water resources. However, the multiple groundwater pollutants found associated with industrial sites present a complex problem as contaminant interactions can alter expected attenuation rates (Meckenstock et al., 2015). The fact that N is both an ubiquitous groundwater pollutant (from agriculture, sewage, coke ash) and a regulator of the biological activity that drives natural attenuation of, e.g., hydrocarbons, makes accurate assessment of in-situ N reaction rates fundamental to the development of viable remediation plans (Kleinsteuber et al., 2012).

Standard remediation models assume that nutrient (C, N, P, S) rich plumes drive complete O_2 consumption, effectively immobilizing N as NH_4^+. This confines N attenuation to a narrow fringe where O_2 diffusing back from oxygenated downgradient water leads to the autotrophic nitrification of NH_3 to NO_2^- (NH_3 oxidation) and NO_3^- (NO_2^- oxidation) and enables heterotrophic reduction of NO_3^- to N_2 (with NO_2^- and N_2O as intermediates) (Christensen et al., 2001; Rivett et al., 2008). However, this understanding is increasingly challenged by evidence for both divergent biological N oxidation and reduction pathways and that they can be driven by alternative electron donors and acceptors (Burgin and Hamilton, 2007; Jones et al., 2017; Jørgensen et al., 2009; Yang et al., 2012).

Potential confounding processes include anaerobic NH_4^+ oxidation (anammox; autotrophic conversion of NO_2^- and NH_4^+ to N_2), which can attenuate N without C consumption or antecedent NO_3^- production (Kartal et al., 2013; Moore et al., 2011; Smith et al., 2015), and the heterotrophic dissimilatory reduction of NO_3^- to NH_4^+ (DNRA) (Henson et al., 2017). The latter effectively switches NO_3^- reduction from a removal to a recycling process. Chemolithotrophic N cycling may also be favored in the absence of O_2 and labile C (Baker et al., 2012; Henson et al., 2017; Kumar et al., 2017). Chemodenitrification reduces NO_3^- to N_2 by oxidizing Fe^{2+} to Fe^{3+} or S^{2-} to SO_4^{2-} (Buchwald et al., 2016; Coby et al., 2011; Jørgensen et al., 2009; Vaclavkova et al., 2014), or to NH_4^+ by oxidizing S^{2-} to SO_4^{2-} (Burgin and Hamilton, 2008; Hayakawa et al., 2013).

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Oxidation of NH_4^+ to N_2, NO_2^-, or NO_3^- by anammox can also co-occur with Fe^{3+} reduction (Crowe et al., 2012; Ding et al., 2014). Beyond directly driving process, S and Fe availability may also regulate partitioning between denitrification (NO_3^- reduced to N_2) and DNRA (NO_3^- reduced to NH_4^+) (Cheng et al., 2016; Plummer et al., 2015). These chemolithotrophic processes are potentially of major importance in hydrocarbon contaminated sites, where sharp redox gradients can alter the bioavailability of the geologic Fe and Mn in the sediment matrix (Cozzarelli et al., 2001; Ziegler et al., 2015). So although new evidence for anammox in NH_4^+ plumes is important (Moore et al., 2011; Robertson et al., 2012; Smith et al., 2015), the balance of possible anoxic N pathways in contaminated groundwater is unknown.

This paucity of information on groundwater N cycling is in part due to the difficulty measuring them, which can require costly ^{15}N additions and/or ex-situ experiments. Natural abundance stable isotope measurements could present a powerful, yet relatively inexpensive, means of assessing groundwater N transformations. This approach is based on the knowledge that the isotopic composition of N species are predictably affected by biological and chemical reactions (Eq. (1)):

$$\frac{C}{C_0} = \left(\frac{R}{R_0}\right)^{\alpha-1} \tag{1}$$

where the proportion of the initial N pool (C/C_0) is related to the parallel change in the pool's isotopic composition (R/R_0) by a process-specific fractionation factor (α). Although DNRA α values are unknown, the established NO_3^-, NO_2^-, and NH_4^+ fractionation during oxidation v. reduction is sufficient to constrain N pathways (Bourbonnais et al., 2015; Wells et al., 2016).

We hypothesized that the abundant electron acceptors and donors found in groundwater underlying industrial sites would create diverse microbial pathways for N attenuation. This hypothesis was tested using patterns in the N and O stable isotope composition of the inorganic N (DIN) species NO_2^-, NO_3^-, and NH_4^+ to identify spatial and temporal variations in N cycling across a contaminant plume, and comparing these findings to the presence of relevant redox species and functional genes.

2. Materials & methods

2.1. Site description

Work was conducted in contaminated groundwater underlying a 100 + year old industrial site in Leuna, Germany (Fig. 1). The saturated zone is comprised of highly permeable alluvial gravel/sand deposit (2–4 m thick) underlain by an impermeable clay layer (UBA, 2003). Outside the influence of the contaminated site, the groundwater is characterized by $[Ca^+]$ ~5 mM, [Mn] ~7 μM, [Fe] ~3 μM, and $[Na^+]$ ~2 mM (Tessema et al., 2013). Natural groundwater flow NW-SE is modified by hydraulic barriers that create a W-E ~0.13 m d^{-1} flow. The shallow reducing zone directly under the industrial site contains high $[NH_4^+]$ (from historic septic leaks and NH_3 synthesis), plus SO_4^{2-} and hydrocarbon (BTEX + MTBE >80% of the organic C pool) concentrations (Martienssen et al., 2006; Seeger et al., 2011; Tischer et al., 2013). There is a small localized contaminant spike downgradient from the main plume (B, Fig. 1). Monitoring wells were installed below the water table along the flowpath in 2001 to track contaminant movements (Martienssen et al., 2006), and long-term data from these wells confirmed a persistent anoxic contaminant zone at the west of the site (E. Hasselwander, unpublished data).

2.2. Sample collection

Water was collected from 38 wells in Oct-2013. Historical contaminant concentrations (GUT Merseberg) were used to categorize these wells as either plume ($n = 10$), fringe ($n = 13$), downgradient ($n = 13$), or the downgradient 'B' ($n = 2$) (Fig. 1). Twenty wells were resampled the following spring (Mar-2014) and autumn (Oct-2014). Wells were pumped at <10 L min^{-1}, and

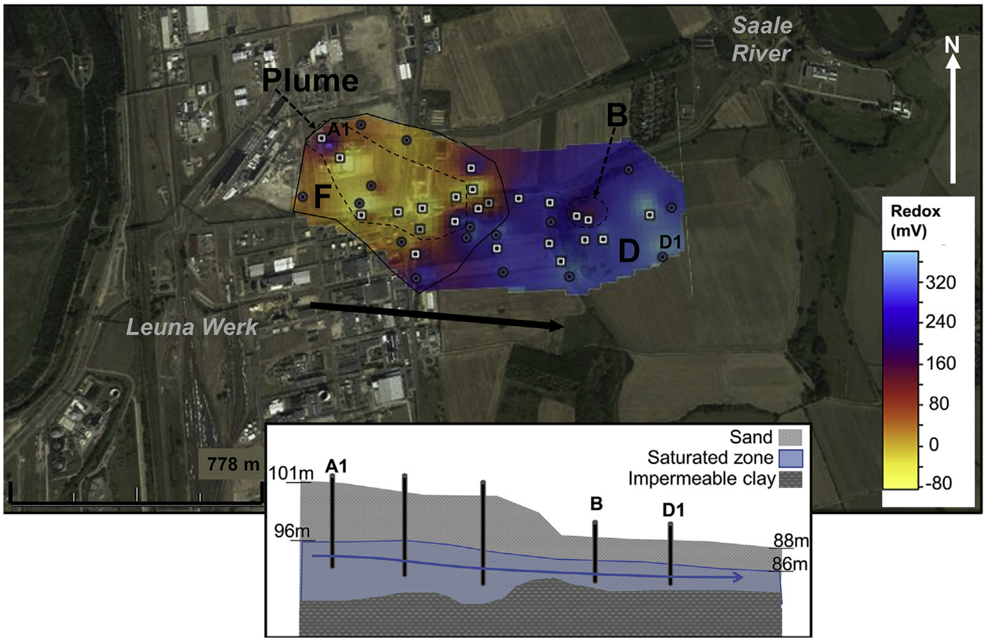

Fig. 1. Sampling wells (points) were located along the W-E groundwater flow path (black arrow) in the shallow sand aquifer, which is underlain by an impermeable clay layer (inset). White squares indicate wells sampled on all three sampling dates and black circles wells sampled only in Oct-2013. Contaminant (NH_4^+, SO_4^{2-}, MTBE, BTEX) sources are located at the west of the sampled area. Wells were distributed across the plume, fringe transition (F), and downgradient (D) sampling zones, and a secondary contaminant source (B), which were identified using site monitoring data and the Oct-2013 measured redox conditions (shown).