ground water

In Situ Rates of Sulfate Reduction in Response to Geochemical Perturbations

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Abstract

Rates of in situ microbial sulfate reduction in response to geochemical perturbations were determined using Native Organism Geochemical Experimentation Enclosures (NOGEEs), a new in situ technique developed to facilitate evaluation of controls on microbial reaction rates. NOGEEs function by first trapping a native microbial community in situ and then subjecting it to geochemical perturbations through the introduction of various test solutions. On three occasions, NOGEEs were used at the Norman Landfill research site in Norman, Oklahoma, to evaluate sulfate-reduction rates in wetland sediments impacted by landfill leachate. The initial experiment, in May 2007, consisted of five introductions of a sulfate test solution over 11 d. Each test stimulated sulfate reduction with rates increasing until an apparent maximum was achieved. Two subsequent experiments, conducted in October 2007 and February 2008, evaluated the effects of concentration on sulfate-reduction rates. Results from these experiments showed that faster sulfate-reduction rates were associated with increased sulfate concentrations. Understanding variability in sulfate-reduction rates in response to perturbations may be an important factor in predicting rates of natural attenuation and bioremediation of contaminants in systems not at biogeochemical equilibrium.

Introduction

Geochemical perturbations are inherent in dynamic natural systems, occurring in response to a variety of environmental changes. For example, perturbations can occur in natural systems as a result of seasonal variations in hydrology (e.g., groundwater discharge to a wetland), which can cause water masses to migrate and mix. Mixing of water masses is important for biodegradation processes as it provides a fresh supply of electron acceptors and donors that constrain degradation rates (Scholl et al. 2005; Bekins et al. 2005). Geochemical perturbations also occur as a result of various anthropogenic activities, including the introduction of contaminants as well as scientific investigations such as push-pull tests, which often repeatedly introduce test solutions to the subsurface (Istok et al. 1997; Schroth et al. 2001; McGuire et al. 2002; Kneeshaw et al. 2007).

At a process level, microbial activity is affected by geochemical perturbations as it is limited by the supply of electron acceptors and donors in the subsurface (Oya and Valocchi 1998; Cirpka et al. 1999) and is strongly influenced by their concentration distribution within water masses (Smith 1997; Chapelle et al. 2009). Thus, native microbial communities must readily adapt and respond to perturbations that alter electron acceptor and donor supply and concentration. In turn, as native microbial communities adapt to new geochemical conditions, they alter the resulting geochemical concentrations (Harris et al. 2007). As a result, many natural systems are in a continual state of disequilibrium, making it a challenge to estimate reaction rates.

There are also other challenges in studying natural systems, including heterogeneities in physical properties and variability in sampling scale (Beeman and Suflita 1990; Adrian et al. 1994; Barlaz and Borden 1999;

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Cozzarelli et al. 2000; Cazull et al. 2006). To date, in situ studies have relied largely on (1) geochemical analyses of dissolved solutes to infer the activity of native microbial communities (Beeman and Suflita 1987; Cozzarelli et al. 1999; Christensen et al. 2001; Cazull et al. 2006), and (2) characterization of native microbial communities through analyses of sediments and water (Martino et al. 1998; Bekins et al. 1999; Bjerg et al. 1999; Weiss and Cozzarelli 2008). These two approaches, though valuable, have an inherent problem in that water and sediment samples are either collected at different spatial or different temporal scales. This disconnect may be problematic due to natural spatial and temporal heterogeneities.

Field experiments to capture or grow native microorganisms in situ have been used to evaluate native microbial populations and reaction kinetics (Bengtsson 1989; Ekendahl and Pedersen 1994; Rogers et al. 1998; Poindexter et al. 2000; Biggerstaff et al. 2007; Baldwin et al. 2008) and results from these investigations have proven valuable for evaluating complex linkages between geochemistry and microbiology. Difficulties, however, remain due to heterogeneities at the field scale and lack of experimental control. Laboratory results provide evidence of microbial response and allow for the quantification of microbial reaction rates with greater experimental control (Wilson et al. 1983; Chapelle et al. 1996; Cozzarelli et al. 2000), but replication of natural conditions is difficult and laboratory and field-rate measurements often vary by many orders of magnitude (Chapelle et al. 1996).

Several studies have used in situ microcosms as a valuable alternative to quantify microbial degradation rates and to evaluate microbial activity with greater experimental control (Mandelbaum et al. 1997; Bjerg et al. 1999; Godsy et al. 1999; Robador et al. 2009; Cozzarelli et al. 2010). However, current sampling methods do not facilitate direct measurement of geochemical solutions in contact with native microorganisms, making it difficult to evaluate specific processes and to assign representative reaction rates. In this article, we describe an in situ sampling technique, referred to as Native Organism Geochemical Experimentation Enclosures (NOGEEs), that allows for the direct measurement of water and sediments at the same spatial and temporal scale and use it to explore the kinetic controls on sulfate-reduction rates as landfill-leachate-contaminated groundwater discharges to a wetland.

Site Description

NOGEE experiments were conducted in a wetland-slough system at the Norman Landfill research site in Norman, Oklahoma (Figure 1A). This unlined landfill is located near the Canadian River in an alluvial aquifer system. A leachate plume containing elevated concentrations of dissolved organic carbon (DOC), chloride, ammonia, and methane developed in the aquifer beneath the landfill, resulting in a layered system with a series of interfaces between water masses (Cozzarelli et al. 2000; Scholl et al. 2005). Sulfate reduction, in particular, has been documented to be an important process in the leachate-contaminated groundwater (Tuttle et al. 2000; Ulrich et al. 2003; Scholl et al. 2006). Groundwater flow in this region is from the landfill toward the slough and the Canadian River (Scholl and Christenson 1998). Areas of ponding from a beaver dam in a shallow stream adjacent to the landfill mound have resulted in a wetland-slough system. Hydrologic conditions in the alluvial aquifer riparian zone have been shown to vary seasonally in response to variability in changing recharge conditions at the site (Figure 1B) (Scholl et al. 2005). Chemical and physical measurements indicate that attenuation of the leachate compounds from the landfill is driven by fresh contributions of electron acceptors from recharge waters (Figure 1B) (Cazull et al. 2006; Scholl et al. 2006; Kneeshaw et al. 2007).

NOGEE Design

NOGEEs were designed to accomplish four main tasks: (1) trap a native microbial population, (2) isolate the population from the surrounding environment in situ, (3) introduce a geochemical solution, and (4) measure the resulting geochemical concentrations. A single NOGEE consisted of a well-like apparatus made of nested 2.54 and 3.31-cm schedule 40 PVC pipe and machined PVC couplings. The lower 6.35 cm of the outer pipe was screened and a seal was fitted just above this interval that allowed movement of the inner pipe, creating a sealable chamber of approximately 60 mL volume. The inner pipe was plugged 8.13 cm up from the bottom but was connected to the surface by tubing (Figure 2). The screened interval was covered with a 5.0-μm polycarbonate membrane filter (Sterlitech Corporation, Kent, Washington). Additionally, this chamber enclosed a polycarbonate substrate for microbial colonization, housed in a perforated PVC tube (Figure 2). Preliminary studies for this research established that the polycarbonate substrate allowed for representative colonization of a native microbial community (Kneeshaw et al. 2008; Smith et al. 2008).

NOGEE experiments consisted of two phases: phase 1—colonization, and phase 2—experimentation. NOGEEs were designed so that during phase 1 (Figure 2) an internal PVC pipe was raised above the main chamber allowing passive diffusion of native pore water and microorganisms into the chamber. During phase 2 this pipe is lowered over the main chamber, passing through a seal at the base, effectively isolating the chamber area from the surrounding environment. Isolating the main chamber in situ allowed test solution to be introduced and samples to be collected through two tubing ports set at the bottom and top of the chamber.

NOGEE Experiments

NOGEE experiments were conducted at three different times between 2007 and 2008. An initial study, in May 2007, was conducted (1) to ensure the functionality of the NOGEE apparatus for conducting in situ rate studies, and (2) to evaluate how reaction rates change as a native microbial community responds to repeated geochemical

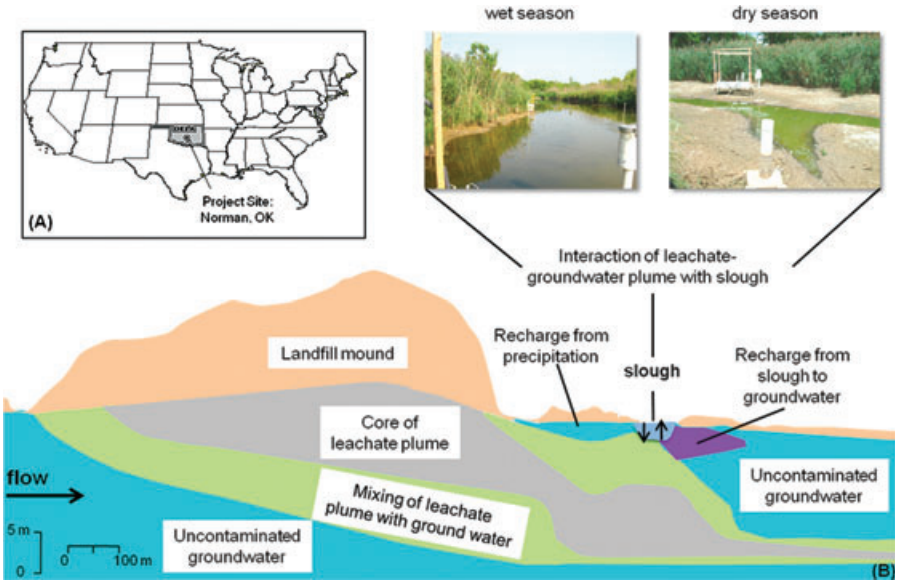

Figure 1. Site map (A) modified from U.S. Geological Survey Fact Sheet 040-03 (Christenson and Cozzarelli 2003) and conceptual model (B) of the transport and reaction zones at the Norman Landfill (Bjerg et al. 2003). Photographs show contrasting hydrologic conditions that perturb degradation processes in the wetland-slough system during different seasons.

perturbations (e.g., perturbations resulting from recharge processes). Four NOGEEs (two reactive, R1 and R2, and two controls, C1 and C2) were installed in the wetland sediments. A subsequent study was conducted to evaluate the effect of sulfate concentration on sulfate-reduction rates. These experiments were conducted in October 2007 and in February 2008. Eight NOGEEs (S1 to S6, reactive, and C1 and C2, controls) were installed in the wetland sediments in October 2007 and again in February 2008.

For each experiment, NOGEEs were pushed in by hand so that the screened interval was in a shallow, reduced silty-clay layer within the wetland sediments. NOGEE chambers were then filled with deoxygenated Nanopure water and left to colonize for approximately 6 weeks, a sufficient time for colonization based on data from previous studies (Bengtsson 1989). After colonization, initial water samples were collected from the main chamber of all NOGEEs. The internal tube of the NOGEEs was then lowered to isolate the main chambers. Native water from the landfill-leachate-contaminated groundwater beneath the wetland sediments was used to make all test solutions

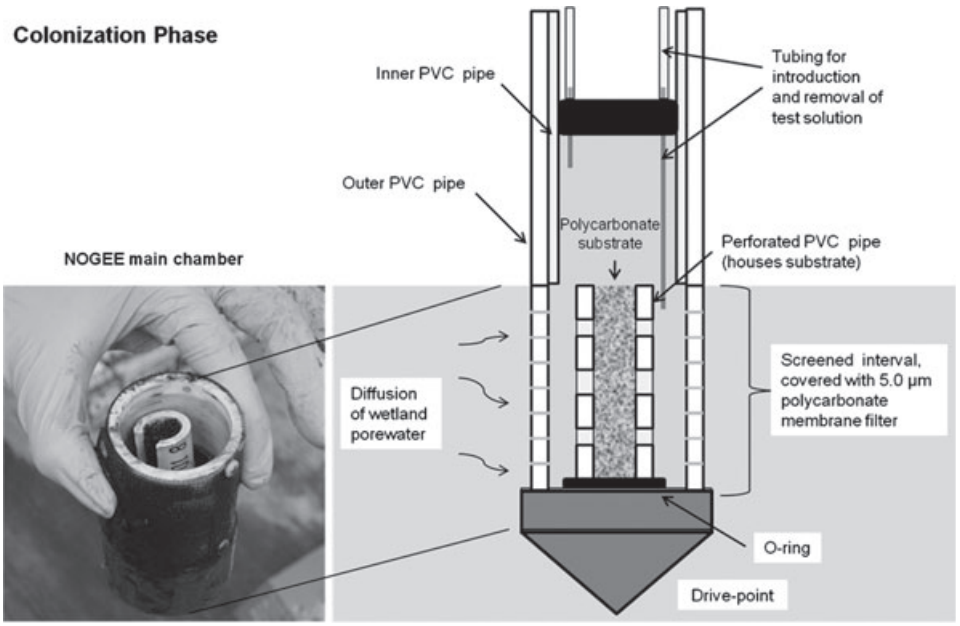

Figure 2. Photograph of NOGEE main chamber (left) and schematic cross section through NOGEE main chamber during colonization phase.

in an argon atmosphere to maintain anaerobic conditions. Test solutions for the initial experiment (May 2007) consisted of landfill-leachate-contaminated groundwater amended with sulfate (~100 mg/L SO_4^{2-}, prepared from Na_2SO_4, Acros Organics, Morris Plains, New Jersey) to serve as electron acceptor; lactate and acetate (~30 mg/L, prepared from $C_3H_6O_3$ and $NaCH_3CO_2$, respectively, Acros Organics) to serve as electron donor; and bromide (~100 mg/L Br^-, prepared from NaBr, Acros Organics) as a conservative tracer.

In the October 2007 and February 2008 experiments, three test solutions, A, B, and C, of approximately 10, 25, and 100 mg/L sulfate, respectively, were prepared in the same manner with the same electron donor and tracer concentrations described in the preceding section. NOGEEs S1 and S2 received test solution A. NOGEEs S3 and S4 received test solution B and NOGEEs S5 and S6 received test solution C. All control NOGEEs received test solution consisting of the landfill-leachate-contaminated groundwater and tracer without any addition of sulfate or electron donor.

Analytical Methods

Samples were syringe-filtered using Millex-HA 0.45-µm filters (Millipore, Bedford, Massachusetts) and analyzed for anions (Cl^-, Br^-, SO_4^{2-}, NO_3^-), ammonium, organic acids (acetate and lactate), iron (Fe^{2+}), sulfide (H_2S), DOC, alkalinity, and CH_4. Anion samples were preserved with formaldehyde and organic acid, and NH_4^+ samples were preserved by flash freezing; all were measured in the laboratory using a capillary electrophoresis system (Agilent Technologies, Wilmington, Delaware). Samples for Fe^{2+} and H_2S determination were preserved with trace-metal grade hydrochloric acid and zinc acetate, respectively; concentrations were determined photometrically in the field using a Spectronic#20D+ spectrophotometer (Thermo Spectronic, Rochester, New York). Cation samples were preserved with hydrochloric acid and analyzed by capillary electrophoresis (Agilent Technologies). Alkalinity samples were measured upon collection by acid titration and Gran plots for graphical determination (Stumm and Morgan 1996). Samples for DOC were filtered through a Whatman GD/X 0.20-µm syringe filter (Whatman, Piscataway, New Jersey) into a baked glass bottle, preserved with hydrochloric acid to a pH of less than 2, and analyzed using a Shimadzu TOC Vcsn analyzer (Shimadzu Corporation, Kyoto, Japan). Methane samples were collected following the method of Baedecker and Cozzarelli (1992) and analyzed using a 5890 Series II HP Gas Chromatograph split/splitless inlet FID (flame ionization detector) with a fused silica capillary column.

Determination of Rates and Kinetic Parameters

For all experiments, any loss of tracer was noted and all other measured concentrations were corrected to account for dilution. After correction, zero-order rates were calculated using Equation 1 by determining the change in concentration of the initial reactant (c_{ri}) measured in the test solution minus the concentration of the final reactant (c_{rf}) collected at the end of each sampling event. For the October 2007 and February 2008 experiments, the Michaelis-Menten equation (Equation 2) Lineweaver-Burke plots (Equation 3) were used to estimate kinetic parameters (V_{max} and K_m) (Kaksonen et al. 2003).

$$k = \frac{c_{ri} - c_{rf}}{t} \tag{1}$$

$$v = \frac{V_{max} \cdot S}{K_m + S} \tag{2}$$

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{S} \tag{3}$$

Results and Discussion

Geochemical Response (May 2007 Experiment)

All five sampling time points for the two reactive NOGEEs in May 2007 revealed considerable decreases in sulfate concentration (60.1 to 91.9 mg/L) compared to changes in concentrations of tracer. Loss of tracer varied between 2% and 18%. Concentrations of sulfide increased (0.09 to 3.36 mg/L) in the final samples compared to concentrations in the initial test solution, and provided another indicator of microbial sulfate reduction. Concentrations of Fe^{2+} showed a decrease (11.33 to 18.71 mg/L) in final samples from initial test-solution concentrations. This decrease is attributed to removal due to reaction with the increased sulfide, likely as FeS minerals (Chapelle et al. 2009).

Initial test-solution concentrations of methane and DOC were somewhat variable between each test (1.4 to 4.5 mg/L CH_4 and 165 to 190 mg/L of C, respectively), which can be explained by variability in aquifer processes between pumping the well to make test solutions. In general, there was a slight decrease in methane concentrations (0.2 to 3.8 mg/L) from initial test-solution concentrations for each sampling event, which may be explained by methane oxidation coupled to microbial sulfate reduction (Grossman et al. 2002; van Breukelen and Griffioen 2004). All DOC results were within a range typical of reducing conditions (>150 mg/L of C). During the first two sampling events, DOC concentrations increased (18.7 to 59.4 mg/L) in both NOGEEs R1 and R2. DOC concentrations again increased during the last three sampling events in NOGEE R1 (20.4 to 57.2 mg/L) while, in contrast, decreases were observed in NOGEE R2 (5.1 to 15.4 mg/L). Decreases in DOC can be explained by utilization as electron donor coupled to sulfate reduction, whereas increases in DOC can be explained by the decomposition of particulate organic matter at a faster rate than DOC utilization as an electron donor. Thus, variability in DOC concentrations between the NOGEEs was likely due to differences in utilization rates. Little change was observed in other measured geochemical parameters (e.g., pH, alkalinity, Cl^-).

Geochemical analyses from control NOGEEs, C1 and C2, revealed little significant changes in concentrations

Table 1
May 2007 NOGEEs: Initial Geochemistry

NOGEE	H_2S (mg/L)	Fe^{2+} (mg/L)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	CH_4 (mg/L)	DOC (mg/L of Carbon)
R1	1.36	bdl	142.8	491.5	0.63	94.3
R2	2.28	bdl	47.3	471.5	0.66	99.3
C1	1.30	bdl	145.4	483.1	1.52	78.8
C2	bdl	bdl	68.2	444.1	NS	70.2

bdl = below detection limit.

of measured geochemical parameters (Fe^{2+}, H_2S, SO_4^{2-}, Cl^-, CH_4, DOC, pH, alkalinity) throughout the experiment. Initial geochemical samples from all NOGEEs (experimental and control) collected prior to experimentation indicated that chemical heterogeneity between each NOGEE location exists even in the small (<0.25 m) spatial scale (Table 1). Initial sulfate concentrations, for example, ranged from 47.3 to 145.4 mg/L. This suggests a possible similar heterogeneity in the initial microbial populations and further demonstrates the need to evaluate dynamic natural systems at this scale.

Comparison of Sulfate-Reduction Rates (May 2007 Experiment)

Sulfate-reduction rates were calculated for each sampling event using Equation 1. Comparison of sulfate-reduction rates for NOGEEs R1 and R2 for each of the five sampling events revealed rates (~0.8 to 1.9 mg/L/h in R1 and ~1.2 to 1.8 mg/L/h in R2) that were nearly identical (Table 2). Trends in rates were also nearly identical in both R1 and R2 with the slowest rates during the first sampling event (0.8 and 1.2 mg/L/h, respectively), followed by similar increases and decreases in rates, and then the fastest rates observed during the final sampling event (1.9 and 1.8 mg/L/h, respectively) (Table 2). The resulting rates were comparable within an order of magnitude to rates found in other studies evaluating in situ sulfate-reduction rates (Table 3) (Schroth et al. 2001; Kleikemper et al. 2002; McGuire 2002; Harris et al. 2005; Van Stempvoort et al. 2005; Kneeshaw et al. 2007). In addition, rates were comparable to those reported in Table 2 in McGuire et al. (2002), which provides a

Table 2
May 2007 NOGEEs: Sulfate-Reduction Rates

NOGEE	R1		R2	
Sampling Event	Time Exposed to Test Solution (h)	Sulfate-Reduction Rate (mg/L/h)	Time Exposed to Test Solution (h)	Sulfate-Reduction Rate (mg/L/h)
1	71.2	0.84	71.0	1.17
2	45.6	1.52	45.5	1.37
3	45.2	1.92	45.3	1.78
4	52.5	1.75	52.5	1.37
5	48.0	1.91	47.7	1.80

Table 3
Summary of In Situ Sulfate-Reduction Rate Measurements

Environment	Test	Order	Rate	Reference
LL-contaminated aquifer/wetland: Norman, OK	NOGEEs	Zero	0.2–46.1 (mg/L/d)	This study
PHC-contaminated aquifer (Canada)	Injection/ monitoring	Zero	4–6 (mg/L/d)	Van Stempvoort et al. (2005)
PHC-contaminated aquifer (Switzerland)	Push-pull	First	0.043–0.130 (d^{-1})	Schroth et al. (2001)
PHC-contaminated aquifer (Switzerland)	Push-pull	First	0.19–0.32 (d^{-1})	Kleikemper et al. (2002)
LL-contaminated aquifer/wetland: Norman, OK	Push-pull	First	3–14 (μmol (L sediment)$^{-1}$) d^{-1})	Harris et al. (2005)
PHC-contaminated aquifer (Canada)	Injection/ monitoring	First	0.003 and 0.01 (d^{-1})	Van Stempvoort et al. (2005)
LL-contaminated aquifer/wetland: Norman, OK	Push-pull	First	5.5–169.7 (d^{-1})	Kneeshaw et al. (2007)

LL = landfill leachate; PHC = petroleum hydrocarbon.

summary of sulfate-reduction rates determined from a number of field and laboratory methods.

The first sampling event resulted in the slowest sulfate-reduction rate, likely representing a transitional period in which the microbial population was adjusting to the introduction of new electron acceptors and donors. With subsequent exposure to sulfate-rich test solution, sulfate-reduction rates increased with similar magnitudes for the second and third sampling events in both NOGEEs R1 and R2. During the fourth sampling event, the sulfate-reduction rate decreased in both NOGEEs R1 and R2. The fifth and final sampling event revealed another increase in sulfate-reduction rates. The increase during the final sampling event occurred in NOGEEs R1 and R2 and resulted in rates that were nearly identical to those measured during sampling event 3, suggesting that a rate of approximately 1.8 to 1.9 mg/L/h might be an apparent V_{max} value for sulfate reduction for these conditions at this location.

Effect of Concentration on Sulfate-Reduction Rates

During the October 2007 and February 2008 NOGEE experiments, test solution was introduced and left in the main chamber for approximately 18 to 23 h before sample collection; this was repeated three times. All sampling events for the October 2007 NOGEEs (S1 to S6) revealed lower concentrations of sulfate (1.22 to 28.02 mg/L) than in the initial test solution, compared to changes in concentrations of tracer (bromide). Loss of tracer varied between 2% and 27%. It should be noted that for both experiments, there was some variability in initial test-solution concentrations of sulfate. All sampling events for the February 2008 NOGEEs (S1 to S6) showed less loss of sulfate compared to tracer. Loss of tracer varied between 2% and 16%. After correction, only very small changes in sulfate concentration were observed. It is hypothesized that this difference in rates may be related to differences in initial microbial consortia due to differences in seasonal geochemical conditions or to differences in seasonal temperatures between October 2007 (~24.7 °C average surface water temperature) and February 2008 (~4 °C average surface water temperature). Previous research has shown that microbial degradation rates decrease with decreasing temperature (Atlas and Bartha 1972; Atlas 1981; Leahy and Colwell 1990; Robador et al. 2009); however, as in situ temperature measurements were not determined this effect on enzymatic activity was not quantified.

Sulfide (H_2S) was not detected in any of the February 2007 NOGEE samples. Iron (Fe^{2+}) decreased (0.5 to 15.18 mg/L) from initial test-solution concentrations, with the exception of a few instances in which increases in iron were observed. In general, both October 2007 and February 2008 samples showed a slight decrease in methane concentrations (0.04 to 3.42 mg/L) from initial test-solution concentrations for each sampling event, but in some instances a slight increase (0.01 to 1.83 mg/L) in methane was observed. The decreases are again likely due to the oxidation of methane coupled to sulfate reduction, and the increases may have been due to methanogenesis occurring coincident with sulfate reduction. DOC concentrations in the test solutions for both NOGEE experiments were greater than 150 mg/L of C. In general, increases in DOC concentrations were observed during each sampling event for the October 2007 NOGEEs, again suggesting decomposition of particulate organic matter. These increases ranged from 2 to 70 mg/L of C and were significantly higher (>50% mg/L of C than test-solution concentrations) during the second sampling event for all October 2007 NOGEEs (S1 to S6). This trend suggests that decomposition of particulate organic matter was occurring but utilization of DOC as an electron donor was slower during this time. DOC concentrations for the February 2008 NOGEEs were more variable, with slight increases and decreases of a much smaller magnitude throughout all three sampling events. Little change was observed in other measured geochemical parameters.

Geochemical analyses from control NOGEEs, C1 and C2, during both experiments again revealed little change in concentrations of measured geochemical parameters throughout the experiment. Initial geochemical samples from all NOGEEs in the October 2007 and February 2008 experiments exhibited similar chemical heterogeneity between each NOGEE location, as in the initial May

Table 4
October 2007 NOGEEs: Initial Geochemistry

NOGEE	H_2S (mg/L)	Fe^{2+} (mg/L)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	pH	Alkalinity (mg/L $CaCO_3$)	CH_4 (mg/L)	DOC (mg/L of Carbon)
S1	bdl	2.08	19.46	298.83	7.02	520.03	2.16	71.79
S2	bdl	5.23	bdl	313.87	6.92	434.93	1.53	95.39
S3	bdl	4.93	bdl	337.73	7.02	406.57	1.86	64.31
S4	bdl	3.27	bdl	339.04	7.01	472.75	2.94	61.68
S5	bdl	0.93	26.59	332.14	7.2	520.03	2.98	68.62
S6	0.78	2.44	bdl	342.33	7.21	567.30	2.78	NS
C1	0.18	bdl	bdl	265.28	7.11	529.48	1.30	71.78
C2	bdl	1.35	19.50	283.37	7.14	510.57	1.75	89.80

bdl = below detection limit; NS = no sample.

Table 5
February 2008 NOGEEs: Initial Geochemistry

NOGEE	H_2S (mg/L)	Fe^{2+} (mg/L)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	pH	Alkalinity (mg/L $CaCO_3$)	CH_4 (mg/L)	DOC (mg/L of Carbon)
S1	bdl	4.93	16.95	292.68	7.12	567.30	3.17	82.05
S2	bdl	1.65	43.65	240.51	7.08	397.11	1.74	88.43
S3	bdl	1.31	37.73	211.18	7.04	359.29	3.61	76.51
S4	bdl	2.55	37.46	213.95	6.93	397.11	2.68	132.01
S5	bdl	3.87	35.86	204.75	6.94	378.20	NS	107.10
S6	bdl	2.22	24.16	222.19	6.96	302.56	NS	42.29
C1	bdl	7.72	39.90	390.08	7.20	756.40	3.67	95.72
C2	bdl	9.89	36.90	288.83	5.59	472.75	4.80	78.60

bdl = below detection limit.

Table 6
October 2007 NOGEEs: Sulfate-Reduction Rates

	Sulfate-Reduction Rate (mg/L/h)					
Test Solution Received	Test Solution A (~10 mg/L SO_4^{2-})[1]		Test Solution B (~25 mg/L SO_4^{2-})		Test Solution C (~100 mg/L SO_4^{2-})	
NOGEE	S1	S2	S3	S4	S5	S6
1	1.138	1.136	0.584	0.793	0.519	0.998
2	0.399	0.057	0.307	0.586	1.035	1.200
3	0.524	0.530	0.593	1.367	1.015	1.448

[1]Actual test-solution concentration was 24.6 mg/L SO_4^{2-}.

2007 experiment (Tables 4 and 5). For example, initial pore-water sulfate concentrations, though on average lower than in May 2007, varied from below detection limit to 26.59 mg/L in the October 2007 NOGEEs and from 16.95 to 43.5 mg/L in the February 2008 NOGEEs.

Comparison of Sulfate-Reduction Rates (October 2007 and February 2008 Experiments)

Measureable sulfate reduction also occurred during each sampling event in October 2007 and in most sampling events in February 2008. In the October 2007 experiment, rates for each pair of NOGEEs that received identical test solutions were similar (positive correlation, mean $r = 0.83$) but rates varied by initial sulfate concentrations (Table 6). On average, sulfate-reduction rates were fastest in NOGEEs that received the highest concentrations of sulfate (91 to 115 mg/L SO_4^{2-}) and slowest in NOGEEs that received the lowest concentrations of sulfate (8.5 to 11 mg/L SO_4^{2-}). Sulfate-reduction rates for all sampling of the February 2008 NOGEEs (Table 7) were on average an order of magnitude slower than rates observed in October 2007. In four instances there were measureable rates of sulfate reduction. Rates for NOGEE pairs, S1 and S2 and S5 and S6, showed similar trends and were positively correlated ($r = 0.63$ and $r = 0.31$, respectively) whereas rates for S3

Table 7
February 2008 NOGEEs: Sulfate-Reduction Rates

	Sulfate-Reduction Rate (mg/L/h)					
Test Solution Received	Test Solution A (~10 mg/L SO_4^{2-})		Test Solution B (~25 mg/L SO_4^{2-})		Test Solution C (~100 mg/L SO_4^{2-})	
NOGEE	S1	S2	S3	S4	S5	S6
1	0.004	0.005	0.016	0.919	0.697	0.356
2	0.045	0.000	0.182	0.255	0.000	0.000
3	0.068	0.025	0.042	0.000	0.060	0.500

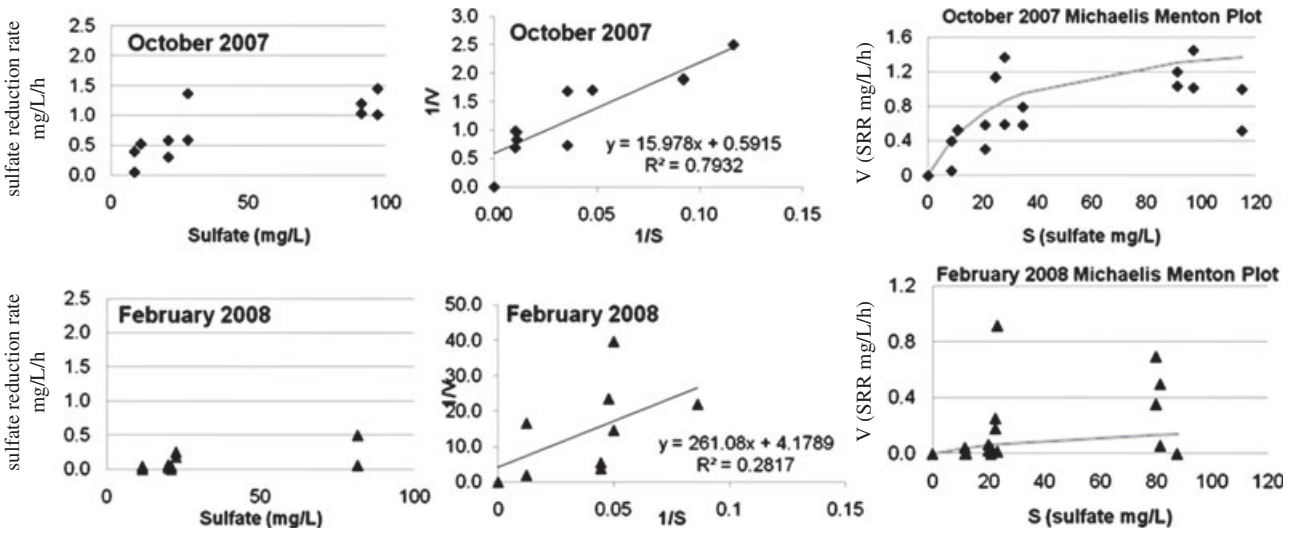

Figure 3. Plots of the measured sulfate-reduction rates against sulfate concentration, corresponding Lineweaver-Burk plots, and Michaelis-Menten curve fit of rate (mg/L/h) vs. substrate (mg/L) for October 2007 and February 2008 NOGEE experiments.

and S4 showed no correlation ($r = -0.39$). Additionally, sulfate concentration during February 2008 experiments appeared to have very little effect on the resulting rates.

Kinetic Parameters

Sulfate-reduction rates for the October 2007 and February 2008 NOGEE experiments exhibited Michaelis-Menten-like kinetics (Figure 3), despite scatter between the two data sets. In order to account for this, sulfate-reduction rates were plotted against sulfate concentration for each sampling event and then linearized using Lineweaver-Burk plots (Figure 3). The slope and the intercept of the best-fit line obtained from linear regressions of each data set were used to derive the Michaelis-Menton constant (K_m) and maximum sulfate-reduction rate (V_{max}). In order to get the most representative K_m and V_{max} values, rates for the first introduction of test solution and negligible rates were not included in the plots. This was done to eliminate data that was thought to represent an acclimation or lag phase. The V_{max} and K_m values obtained were then used to generate Michaelis-Menton curves (Figure 3). These figures illustrate two results: (1) sulfate-reduction rates were on average faster in October 2007 than in February 2008, and 92) the effect of sulfate concentration on rate was more apparent in October 2007 than in February 2008. This is evidenced by R^2 values from the Lineweaver-Burk plots that indicate that in October 2007, 79% of the variation in $1/V$ is due to the variation in $1/S$, while in February 2008, only 28% of the variation in rate could be attributed to sulfate concentration. The Michaelis-Menten constants (K_m) for October 2007 and February 2008 were 27.01 and 62.48 mg/L SO_4^{2-}, respectively (Figure 3). The V_{max} for sulfate reduction was significantly greater (1.69 mg/L/h) in October 2007 than in February 2008 (0.4 mg/L/h).

Conclusions

The rates reported in this study represent a range of sulfate-reduction rates likely to occur in a dynamic natural system prone to geochemical perturbations (i.e., in a "usual" state of disequilibrium). In general, the introduction of a native solution (landfill-leachate-contaminated groundwater) amended with sulfate and an electron donor resulted in measureable loss of sulfate. The sulfate-reduction rates determined for each set of NOGEE experiments (May 2007, October 2007, and February 2008) did, however, differ in response to changes in sulfate concentration. Results obtained from this new technique showed similarities in the rates and trends of the different experimental NOGEEs (Tables 2, 6, and 7). The resulting geochemical data suggests that the introduction of electron acceptors and donors elicits a relatively fast response in native microbial populations (<24 h). This is evidenced by notable changes in sulfate-reduction rates in response to the repeated introductions of test solution within each NOGEE experiment. The data presented here provide direct evidence on how disequilibrium conditions can affect reaction rates in natural systems. It is apparent from the experiments conducted that repeated geochemical perturbations, whether they are natural or anthropogenic, influence native microbial populations and impact the resulting geochemical data. It also seems apparent that differences in seasonal temperatures in near-surface systems may have a modulating effect on sulfate-reduction rates. As such, caution should be taken in interpreting geochemical results from (1) dynamic natural systems where environmental conditions are often changing and equilibrium states are rarely achieved, and (2) experiments repeatedly conducted within the same location (e.g., conducting push-pull tests repeatedly in the same location), which over time may produce inaccurate

rate data as the microbial community adapts to the repeated perturbation.

Geochemical results from the initial May 2007 NOGEE experiments, where repeated introductions of approximately 100 mg/L sulfate test solutions were introduced, revealed an apparent maximum sulfate-reduction rate of approximately 1.8 to 1.9 mg/L/h for this site (shallow wetland sediments) under the particular natural conditions during the time of the experiment. Geochemical results from the October 2007 and February 2008 NOGEE experiments were conducted to reveal sulfate concentration constraints on sulfate-reduction rates for this particular site. Results demonstrated that there is a concentration effect and that sulfate-reduction rates are faster at higher sulfate concentrations at this site. This concentration effect has implications for decisions regarding the utilization of zero-order vs. first-order rate equations. For example, it may be reasonable to use a first-order rate equation to determine sulfate-reduction rates for higher sulfate concentrations (e.g., >50 mg/L), as rates may be dependent on concentration above a certain threshold for a particular site. The effect of concentration may be further constrained by seasonal temperature differences, as sulfate-reduction rates were faster at the highest sulfate concentrations during October 2007, when average surface water temperatures were approximately 24.7 °C, than in February 2008, when average surface water temperatures approximately 4 °C. This effect may also be due in part to differences in native microbial populations in the wetland sediments due to different hydrologic conditions in October 2007 (dry) and February 2008 (wet). This suggests that the effects of hydrologic condition and temperature may also be important constraints on sulfate-reduction rates, especially in near-surface systems and when comparing rate data geographically. This study showed how in situ microcosms, like NOGEEs, can be used to evaluate the natural attenuation or bioremediation potential for a site. Future versions of NOGEEs could be configured to evaluate a wide variety of geochemical and microbiological parameters in both shallow and deeper groundwater environments.

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References

Adrian, N.R., J.A. Robinson, and J.M. Suflita. 1994. Spatial variability in biodegradation rates as evidenced by methane production from an aquifer. *Applied and Environmental Microbiology* 60, no. 10: 3632–3639.

Atlas, R.M. 1981. Microbial degradation of petroleum hydrocarbons: An environmental perspective. *Microbiology and Molecular Biology Reviews* 45, no. 1: 180–209.

Atlas, R.M., and R. Bartha. 1972. Biodegradation of petroleum in seawater at low temperatures. *Canadian Journal of Microbiology* 18, no. 12: 1851–1855.

Baedecker, M.J., and I.M. Cozzarelli. 1992. The determination and fate of unstable constituents of contaminated groundwater. In *Groundwater Contamination and Analysis at Hazardous Waste Sites*, ed. S. Lesage and R.E. Jackson, 425–461. New York: Marcel Dekker.

Baldwin, B.R., A.D. Peacock, M. Park, D.M. Ogles, J.D. Istok, J.P. McKinley, C.T. Resch, and D.C. White. 2008. Multilevel samplers as microcosms to assess microbial response to biostimulation. *Ground Water* 46, no. 2: 295–304.

Beeman, R.E., and J.M. Suflita. 1990. Environmental factors influencing methanogenesis in a shallow anoxic aquifer: A field and laboratory study. *Journal of Industrial Microbiology* 5, no. 1: 45–58.

Beeman, R.E., and J.M. Suflita. 1987. Microbial ecology of a shallow unconfined ground water aquifer polluted by municipal landfill leachate. *Microbial Ecology* 14, no. 1: 39–54.

Bekins, B.A., F.D. Hostettler, W.N. Herkelrath, G.N. Delin, E. Warren, and H.I. Essaid. 2005. Progression of methanogenic degradation of crude oil in the subsurface. *Environmental Geosciences* 12, no. 2: 139–152.

Bekins, B.A., E.M. Godsy, and E. Warren. 1999. Distribution of microbial physiologic types in an aquifer contaminated by crude oil. *Microbial Ecology* 37, no. 4: 263–275.

Bengtsson, G. 1989. Growth and metabolic flexibility in groundwater bacteria. *Microbial Ecology* 18, no. 3: 235–248.

Biggerstaff, J.P., M. Le Puil, B.L. Weidow, J. Leblanc-Gridley, E. Jennings, J. Busch-Harris, K.L. Sublette, D.C. White, and R.S. Alberte. 2007. A novel and in situ technique for the quantitative detection of MTBE and benzene degrading bacteria in contaminated matrices. *Journal of Microbiological Methods* 68, no. 2: 437–441.

Bjerg, P.L., H.J. Albrechtsen, P. Kjeldsen, T.H. Christensen, and I.M. Cozzarelli. 2003. The groundwater geochemistry of waste disposal facilities. In *Treatise on Geochemistry*, ed. B.S. Lollar, H.D. Holland and K.K. Turekian, 579–612. Oxford, U.K.: Elsevier.

Bjerg, P.L., K. Rugge, J. Cortsen, P.H. Nielsen, and T.H. Christensen. 1999. Degradation of aromatic and chlorinated aliphatic hydrocarbons in anaerobic part of the Grinsted landfill leachate plume: In situ microcosm and laboratory batch experiments. *Ground Water* 37, no. 1: 113–121.

Cazull, S.B., J.T. McGuire, I.M. Cozzarelli, A. Raymond, and L. Welsh. 2006. Centimeter-scale characterization of biogeochemical gradients at a wetland-aquifer interface using capillary electrophoresis. *Applied Geochemistry* 22: 2664–2683.

Chapelle, F.H., P.M. Bradley, D.J. Goode, C. Tiedeman, P.J. Lacombe, K. Kaiser, and R. Benner. 2009. Biochemical indicators for the bioavailability of organic carbon in ground water. *Ground Water* 47, no. 1: 108–121.

Chapelle, F.H., P.M. Bradley, D.R. Lovley, and D.A. Vroblesky. 1996. Measuring rates of biodegradation in a contaminated aquifer using field and laboratory methods. *Ground Water* 34, no. 4: 691–698.

Christensen, T.H., P. Kjeldsen, P.L. Berg, D.L. Jensen, J.B. Christensen, A. Baun, H.-J. Albrechtsen, and G. Heron. 2001. Biogeochemistry of landfill leachate plumes. *Applied Geochemistry* 16, no. 7–8: 659–718.

Christenson, S., and I.M. Cozzarelli. 2003. The Norman Landfill environmental research site: What happens to the waste in landfills? U.S. Geological Survey Fact Sheet FS-040-03.

Cirpka, O.A., E.O. Frind, and R. Helmig. 1999. Numerical simulation of biodegradation controlled by transverse mixing. *Journal of Contaminant Hydrology* 40, no. 2: 159–182.

Cozzarelli, I.M., B.A. Bekins, R.P. Eganhouse, E. Warren, and H.I. Essaid. 2010. In situ measurements of volatile hydrocarbon biodegradation rates in groundwater. *Journal of Contaminant Hydrology* 111, no. 1: 48–64.

Cozzarelli, I.M., J.M. Suflita, G.A. Ulrich, S.H. Harris, M.A. Scholl, J.L. Schlottmann, and S. Christenson. 2000. Geochemical and microbiological methods for evaluating anaerobic processes in an aquifer contaminated by landfill leachate. *Environmental Science and Technology* 34, no. 18: 4025–4033.

Cozzarelli, I.M., J.S. Herman, M.J. Baedecker, and J.M. Fischer. 1999. Geochemical heterogeneity of a gasoline-contaminated aquifer. *Journal of Contaminant Hydrology* 40, no. 3: 261–284.

Ekendahl, S., and K. Pedersen. 1994. Carbon transformations by attached bacterial populations in granitic groundwater from deep crystalline bed-rock of the Stripa research mine. *Microbiology* 140, no. 7: 1565–1573.

Godsy, E.M., E. Warren, I.M. Cozzarelli, B.A. Bekins, and R.P. Eganhouse. 1999. Determining BTEX biodegradation rates using in situ microcosms at the Bemidji site, Minnesota: Trials and tribulations. In *U.S. Geological Survey Toxic Substances Hydrology Program—Proceedings of the Technical Meeting Charleston South Carolina March 8–12, 1999*, vol. 3, Subsurface Contamination From Point Sources, Water-Resources Investigations Report 99-4018C.

Grossman, E.L., L.A. Cifuentes, and I.M. Cozzarelli. 2002. Anaerobic methane oxidation in a landfill-leachate plume. *Environmental Science and Technology* 36, no. 11: 2436–2442.

Harris, S.H., R.L. Smith, and J.M. Suflita. 2007. In situ hydrogen consumption kinetics as an indicator of subsurface microbial activity. *FEMS Microbiology Ecology* 60, no. 2: 220–228.

Harris, S.H., J.D. Istok, and J.M. Suflita. 2005. Changes in organic matter biodegradability influencing sulfate reduction in an aquifer contaminated by landfill leachate. *Microbial Ecology* 51, no. 4: 535–542.

Istok, J.D., M.D. Humphrey, M.H. Schroth, M.R. Hyman, and K.T. O'Reilly. 1997. Single-well "Push-pull" test for in situ determination of microbial activities. *Ground Water* 35, no. 4: 619–630.

Kaksonen, A., P. Franzmann, and J. Puhakka. 2003. Performance and ethanol oxidation kinetics of a sulfate-reducing fluidized-bed reactor treating acidic metal-containing wastewater. *Biodegradation* 14, no. 3: 207–217.

Kleikemper, J., M.H. Schroth, W.V. Sigler, M. Schmucki, S.M. Bernasconi, and J. Zeyer. 2002. Activity and diversity of sulfate-reducing bacteria in a petroleum hydrocarbon-contaminated aquifer. *Applied and Environmental Microbiology* 68, no. 4: 1516–1523.

Kneeshaw, T.A., J.T. McGuire, E.W. Smith, I.M. Cozzarelli, M.A. Voytek, and J.D. Kirshtein. 2008. A new approach for determining in situ microbial response to geochemical perturbations. *Geological Society of America Abstracts with Programs* 40, no. 6: 344.

Kneeshaw, T.A., J.T. McGuire, E.W. Smith, and I.M. Cozzarelli. 2007. Evaluation of sulfate reduction at experimentally induced mixing interfaces using small-scale push-pull tests in an aquifer-wetland system. *Applied Geochemistry* 22, no. 12: 2618–2629.

Leahy, J.G., and R.R. Colwell. 1990. Microbial degradation of hydrocarbons in the environment. *Microbiology and Molecular Biology Reviews* 54, no. 3: 305–315.

Mandelbaum, R.T., M.R. Shati, and D. Ronen. 1997. In situ microcosms in aquifer bioremediation studies. *FEMS Microbiology Reviews* 20, no. 3–4: 489–502.

Martino, D.P., E.L. Grossman, G.A. Ulrich, K.C. Burger, J.L. Schlichenmeyer, J.M. Suflita, and J.W. Ammerman. 1998. Microbial abundance and activity in a low-conductivity aquifer system in east-central Texas. *Microbial Ecology* 35, no. 3: 224–234.

McGuire, J.T. 2002. Quantifying redox reactions in an aquifer contaminated with waste fuel and chlorinated solvents. Ph.D. Diss., Michigan State University.

McGuire, J.T., D.T. Long, M.J. Klug, S.K. Haack, and D.W. Hyndman. 2002. Evaluating the behavior of oxygen, nitrate, and sulfate during recharge and quantifying reduction rates in a contaminated aquifer. *Environmental Science and Technology* 36, no. 12: 2693–2700.

Oya, S., and A.J. Valocchi. 1998. Transport and biodegradation of solutes in stratified aquifers under enhanced in situ bioremediation conditions. *Water Resources Research* 34, no. 12: 3323–3334.

Poindexter, J.S., K.P. Pujara, and J.T. Staley. 2000. In situ reproductive rate of freshwater caulobacter spp. *Applied and Environmental Microbiology* 66, no. 9: 4105–4111.

Robador, A., V. Brüchert, and B.B. Jørgensen. 2009. The impact of temperature change on the activity and community composition of sulfate-reducing bacteria in arctic versus temperate marine sediments. *Environmental Microbiology* 11, no. 7: 1692–1703.

Rogers, J.R., P.C. Bennett, and W.J. Choi. 1998. Feldspars as a source of nutrients for microorganisms. *American Mineralogist* 83, no. 11–12: 1532–1540.

Scholl, M.A., I.M. Cozzarelli, and S.C. Christenson. 2006. Recharge processes drive sulfate reduction in an alluvial aquifer contaminated with landfill leachate. *Journal of Contaminant Hydrology* 86, no. 3–4: 239–261.

Scholl, M.A., S.C. Christenson, I.M. Cozzarelli, D. Ferree, and J. Jaeschke. 2005. Recharge processes in an alluvial aquifer riparian zone, Norman Landfill, Norman, Oklahoma, 1998–2000. U.S. Geological Survey Scientific Investigations Report 2005-5238.

Scholl, M.A., and S.C. Christenson. 1998. Spatial variation in hydraulic conductivity determined by slug tests in the Canadian river alluvium near the Norman Landfill, Norman, Oklahoma. U.S. Geological Survey Water-Resources Investigations Report 97-4292, 28.

Schroth, M.H., J. Kleikemper, C. Bolliger, S.M. Bernasconi, and J. Zeyer. 2001. In situ assessment of microbial sulfate reduction in a petroleum-contaminated aquifer using push-pull test and stable sulfur isotope analyses. *Journal of Contaminant Hydrology* 51, no. 3–4: 179–195.

Smith, R.L. 1997. Determining the terminal electron accepting reaction in the saturated subsurface. In *Manual of Environmental Microbiology*, ed. C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenback and M.V. Walter, 577–585. Washington, DC: American Society of Microbiology.

Smith, E.W., M.A. Voytek, J.T. McGuire, I.M. Cozzarelli, T.A. Kneeshaw, and S.B. Cazull. 2008. A novel field apparatus

for conducting linked geochemical-microbiological experiments in shallow sediments. Eos Trans. AGU 89 (53). Fall Meeting Supplement, Abstract B13A-0433.

Stumm, W., and J.J. Morgan. 1996. *Aquatic Chemistry*, 1002. New York: John Wiley & Sons Inc.

Tuttle, M.L., G.N. Breit, I.M. Cozzarelli, and S.H. Harris. 2000. The impact of sediment-bound sulfate and ferric iron on degrading organic contaminants in a leachate plume at the Norman Landfill, Oklahoma. *Geological Society of America Abstracts with Programs* 32, no. 7: A127.

Ulrich, G.A., B.G. I.M. Cozzarelli, and J.M. Suflita. 2003. Sources of sulfate supporting anaerobic metabolism in a contaminated aquifer. *Environmental Science and Technology* 37, no. 6: 1093–1099.

van Breukelen, B.M., and J. Griffioen. 2004. Biogeochemical processes at the fringe of a landfill leachate pollution plume: Potential for dissolved organic carbon, Fe(ii), Mn(ii), NH_4, and CH_4 oxidation. *Journal of Contaminant Hydrology* 73, no. 1–4: 181–205.

Van Stempvoort, D., H. Maathuis, E. Jaworski, B. Mayer, and K. Rich. 2005. Oxidation of fugitive methane in ground water linked to bacterial sulfate reduction. *Ground Water* 43, no. 2: 187–199.

Weiss, J.V., and I.M. Cozzarelli. 2008. Biodegradation in contaminated aquifers: Incorporating microbial/molecular methods. *Ground Water* 46, no. 2: 305–322.

Wilson, J.T., J.F. McNabb, D.L. Balkwill, and W.C. Ghiorse. 1983. Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. *Ground Water* 21, no. 2: 134–142.