Evidence of Aqueous Iron Sulfide Clusters in the Vadose Zone

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Iron and sulfur cycling is an important control on contaminant fate and transport. We present the effects of soil structure, specifically the presence of a soil layer, on linked biogeochemical and hydrological processes involving Fe and S cycling in the vadose zone using repacked soil columns. Synthetic rainwater was applied to a homogenized medium-grained sand column, a homogenized organic-rich loam column, and a sand-over-loam layered column. Water samples were voltammetrically analyzed for total sulfide, Fe$^{2+}$, and aqueous FeS clusters (FeS$_{aq}$) using a mercury drop electrode. Aqueous FeS clusters were observed in the loam and layered columns, with the greatest concentrations of FeS$_{aq}$ occurring at the sand–loam interface in the layered column. Redox potential (Eh) measurements showed rapid cycling of redox conditions at the interface (hours to days) suggesting that FeS$_{aq}$ formation was favored under conditions of disequilibrium. However, the relative stability and aqueous nature of FeS$_{aq}$ allowed for it to be transported, which has broader implications for chemical fate and transport in the vadose zone. The co-occurrence of FeS$_{aq}$ with iron-rich soil aggregates near the sand-loam interface in the layered column suggest that FeS$_{aq}$ may act as a precursor to the formation of aggregates that, in our column, subsequently caused an order of magnitude decrease in hydraulic conductivity. These findings suggest that FeS$_{aq}$ should be considered when predicting the transport of contaminants, particularly metals that may associate with FeS$_{aq}$ in systems with prominent iron and sulfur cycling.

Abbreviations: Eh, redox potential; FeS$_{aq}$, aqueous FeS clusters; PVC, polyvinyl chloride; TDR, time domain reflectometry.

Iron and sulfur cycling in the environment plays a major role in abiotic and biotic reactions, including redox reactions, precipitation, and sorption in soils. Metal-sulfide minerals are considered particularly important in toxic metal sequestration because of their low solubility and the ease with which toxic metals such as silver, cadmium, mercury, or lead are incorporated into the mineral structure and, thus, removed from the aqueous system. This low solubility is advantageous because it minimizes the transport and release of toxic metals into the environment. However, aqueous metal-sulfide species can also incorporate these toxic metals (Rozan et al., 2000), but in this case, the aqueous nature means that any associated (sorbed) or incorporated toxic metals become subject to transport rather than precipitation. As a result, these metals may be transported to uncontaminated or sensitive environments. These aqueous species can exist as “clusters,” defined as quantum-sized particles or complexes, which contain a discrete number of atoms small enough to behave as a dissolved species (Luther and Rickard, 2005), which, although often an intermediate, may persist for environmentally relevant periods of time even in oxic conditions (Rozan et al., 2000). Given the abundant nature of iron in the environment, the role of aqueous iron-sulfide clusters (FeS$_{aq}$) on chemical fate and transport in the vadose zone is of potential importance.

Numerous field and laboratory studies have used polarography, an electroanalytical method, to identify an aqueous iron-sulfide species (Davison et al., 1998; de Vitre et al., 1988; Theberge and Luther, 1997) in the environment. This species can form from the direct combination of Fe$^{2+}$ and S(-II) at low concentrations (Luther et al., 1996) or...
from dissolution of nanoparticulate mackinawite (Rickard, 2006; Wolthers et al., 2005). Conditions where formation of FeS\textsubscript{aq} begins to become favorable occur where pH is greater than 6 and Eh is less than \(-0.15\) V (see Rickard and Luther, 2007, for the complete Eh/pH diagram). The aqueous species form clusters that are polynuclear complexes of Fe and S. The presence of FeS\textsubscript{aq} not only has significance for overall Fe and S cycling, but also more broadly impacts contaminant fate and transport through processes such as increasing the transport of metals and removal of toxic H\textsubscript{2}S. Thus, documenting the presence of an aqueous FeS species has great implications for understanding and predicting contaminant fate and transport. These aqueous species have been observed in numerous environmental settings, but to our knowledge, have not been studied or documented in a partially saturated medium in the vadose zone and thus are not currently considered in current fate and transport models.

The first published account of an aqueous FeS species was voltammetrically measured in anoxic, hypolimnetic lake waters (Davison, 1977). Although the exact nature of the polarographic peak was unknown, the authors noted that this peak was “only observable when both ferrous iron and sulfide were present” and suggested the peak may be “a soluble, electroactive ferrous sulfide complex.” Since the first mention of this peak, many studies have worked to verify the identity of this substance, most commonly through titration of Fe\textsuperscript{2+} and S\textsuperscript{(-II)}. Regardless of the titrant used, either Fe\textsuperscript{2+} or S\textsuperscript{(-II)}, all results indicated the formation of an aqueous iron-sulfide cluster (Davison et al., 1998; de Vitre et al., 1988; Theberge and Luther, 1997).

The combination of Fe\textsuperscript{2+} and S\textsuperscript{(-II)} produces several iron-sulfide minerals including mackinawite (FeS\textsubscript{2}), greigite, (Fe\textsubscript{3}S\textsubscript{4}), and pyrite (FeS\textsubscript{2}). Iron-sulfide mineral formation occurs in a stepwise process whereby the most soluble polymorph (mackinawite, \(K_{sp} = 10^{-3.6}\)) precipitates first because nucleation of a more soluble phase is kinetically favored over that of a less soluble phase due to lower solid–liquid interfacial tension of the more soluble phase (Stumm and Morgan, 1996). This initial precipitate then acts as an intermediate that gives way to the formation of less soluble polymorphs (i.e., greigite, \(K_{sp} = 10^{-3.4}\); and pyrite, \(K_{sp} = 10^{-16.4}\)). The discovery of aqueous FeS clusters fits well with observations that more soluble products precipitate first. This suggests FeS\textsubscript{aq} may be the common intermediate from which other iron-sulfide minerals are formed.

Supporting studies have shown that FeS\textsubscript{aq} is an intermediate in pyrite formation (Rickard and Luther, 1997) and that pyrite formation was inhibited by FeS\textsubscript{aq} suppression (Rickard et al., 2001). The existence of an aqueous iron-sulfide phase is not currently included in traditionally accepted conceptual models of iron–sulfur dynamics in natural systems. However, the inclusion of FeS\textsubscript{aq} may lead to improved prediction of Fe and S distribution and reactivity, as well as a better understanding of heavy metal fate and transport in natural systems.

The calculation of equilibrium constants involving FeS\textsubscript{aq} formation depends on the stoichiometry of the clusters, which is currently unknown. In attempts to discover the stoichiometry of FeS\textsubscript{aq}, the structure of FeS\textsubscript{aq} has been studied, and although it has not been conclusively determined, several studies have successfully characterized aspects of its makeup. Rickard (1995) proposed that FeS\textsubscript{aq} was not a complex with a central atom to which other ligands were bound, but rather a molecular cluster that formed a multi-nuclear complex. These multinuclear complexes are called “clusters,” which we will also call FeS\textsubscript{aq} in this paper. Another study showed that these FeS\textsubscript{aq} clusters are arranged in a tetrahedral geometry (Theberge and Luther, 1997). These observations were confirmed by a study characterizing nanoparticulate (~2 nm) amorphous FeS (Wolthers et al., 2003), in which they determined that amorphous FeS was not truly amorphous but rather displayed a disordered tetragonal mackinawite structure. These molecular arrangements support that FeS\textsubscript{aq} clusters may easily transform into the more crystalline mackinawite and are an intermediate for more thermodynamically stable iron-sulfide minerals.

If FeS\textsubscript{aq} is an intermediate, then they should be readily observed in nature, and indeed, FeS\textsubscript{aq} has been documented in lakes (Buffe et al., 1988; de Vitre et al., 1988; Luther et al., 2003), river waters (Rozan et al., 2000), estuary sediments (Rickard et al., 1999), marine sediments (Luther et al., 1999; Luther et al., 1998), deep ocean hydrothermal vents (Luther et al., 2001), in flooded underground mines (Roesler et al., 2007), and even in sewage treatment effluent (Rozan et al., 2000). Thus, FeS\textsubscript{aq} clusters exist in many environments under various redox conditions, not unlike the wide range of redox conditions that have been observed in the vadose zone (Bekins et al., 2005; Oliver et al., 2003). As such, FeS\textsubscript{aq} clusters likely also exist in the vadose zone, although this has not yet been documented.

The complexities of the vadose zone provide a unique opportunity to explore how various redox states within the vadose zone impact Fe and S cycling, including the possible formation and transport of FeS\textsubscript{aq} clusters. One such complexity, common in many vadose zone environments, is physically distinct soil layers. Layers in soil systems are interesting from a chemical fate and transport perspective because the interface created between soil layers may be a “hot spot” of biogeochemical cycling, as these are locations where waters of differing redox state can interact (McClain et al., 2003; Baez-Cazull et al., 2007). In the vadose zone, layers may retard water flow and increase residence time for both water and chemicals (Nimmo et al., 2004; Hansen et al., 2011). This increased residence time may consequently lead to rapid consumption of dissolved oxygen and development of reducing conditions. Correspondingly, these soil interfaces have been shown to be populated by a greater number of microorganisms than in the soil matrix itself (Fredrickson et al., 1997; Madigan et al., 1997). Thus, due to their dynamic nature soil layers in the vadose zone are an ideal location to look for FeS\textsubscript{aq}.
The purpose of this paper is to present observations of FeS$_{aq}$ in unsaturated soil systems and the conditions in which these clusters were observed. In addition to presenting observations of FeS$_{aq}$, we discuss the potential implications of the presence of FeS$_{aq}$ on the linkages between iron–sulfur cycling and hydrologic flow in the vadose zone.

**Methods**

**Experimental Design**

Details of the experimental design are given in Hansen et al. (2011) and summarized briefly here. Two homogenous soil columns containing sand and loam respectively were characterized to evaluate geochemical transformations during fluid migration in a variably saturated system. Results from the homogeneous columns were then compared with a layered system (constructed of the same materials) to evaluate the effects of a soil layer interface. Both the sand and loam soils were collected near a closed municipal landfill in Norman, OK, USA (Breit et al., 2005; Kneeshaw et al., 2007)—the first, an alluvial medium-grained sand from the banks of the Canadian River and the second, an organic-rich loam from a wetland adjoining the capped landfill. The textural and chemical properties of the sand and loam soils are given in Hansen et al. (2011) in their Tables 1 through 3. Total organic carbon was measured by combustion in a high temperature (1800°C) furnace with oxygen using a Vario EL III elemental analyzer (Elementar).

Preceding the packing of the soils in the experimental columns, soils were air dried and then sieved, so that large-sized (>8 mm) organic matter (principally plant detritus and shells) could be discarded to obtain consistent soil-water properties. A piston compactor was used to pack soils into the columns in 3-cm increments to achieve a constant bulk density (1.5 Mg m$^{-3}$ for the sand and 1.1 Mg m$^{-3}$ for the loam) (Castiglione et al., 2003; Köhne and Mohanty, 2005). The measured saturated hydraulic conductivity values for the sand and loam materials were $1.06 \times 10^{-4}$ and $2.35 \times 10^{-5}$ m s$^{-1}$, respectively.

**Experimental Column Setup**

The soil columns were constructed from clear acrylic pipe (15 cm in diameter and 40 cm in height) (Fig. 1). At the bottom of the acrylic pipe, a mesh fabric made of nylon was glued to a densely perforated (one 0.19-cm-diam. hole per 1.16 cm$^2$) polyvinyl chloride (PVC) plate that was attached to the base of the column cylinder to allow water flow and prevent soil loss. A funnel-shaped cap that directed water into a single 1.9-cm outer diameter vinyl tube drained the column. Thus, the nylon mesh fabric at the bottom was open to the atmosphere via the vinyl tubing (Fig. 1). Glues and epoxys (hot melt adhesive, Adhesive Technologies, Inc. and Silvertip Gel Magic Adhesive, System Three) that did not leach organic acids such as acetate and formaldehyde after soaking in deionized water for 48 h were selected for column construction.

The upper surface of the column was open to the atmosphere, which allowed water to be introduced through a rainfall simulator made of a PVC reservoir and approximately 90 18-gauge needles (1 needle/1.96 cm$^2$). Water, delivered from a sealed Nalgene carboy, was delivered to the rainfall simulator through a digitally controlled peristaltic pump (Cole-Parmer). The temperature in the lab where experiments were conducted was at $22^\circ \pm 2^\circ$C.

Columns were equipped with collocated sets of measurement probes installed at selected depths to measure water content and collect water samples. In the sand and loam columns, sampling ports were located at depths of $-6, -16, -17,$ and $-27$ cm from the top of the column. In the layered column, ports were located at $-6, -11, -17, -19, -28,$ and $-36$ cm. A graphical representation of the sampling location depths in the columns are shown in Fig. 1 of Hansen et al. (2011). Time domain reflectometry (TDR) three-prong probes (8 cm long, 1.1-cm spacing between rods) were used to measure water content. Data from TDR probes were automatically collected using a TDR100 (Campbell Scientific) attached to a CR10X data logger (Campbell Scientific).

**Geochemical Sampling and Analysis**

Platinum electrodes, constructed after Wafer et al. (2004) and small suction lysimeters, used for aqueous geochemical sampling, were positioned with the collocated probe sets. Platinum electrode data were logged using a CR10X data logger (van Bochove et al., 2002). Data collected from these electrodes are reported as EMF values. Lysimeters were made from 6-mm-diameter ceramic cups (SDEC 220), aluminum tubing, and amber catchment vial connected to a vacuum. The ceramic cups were specifically designed to

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**Fig. 1.** Experimental setup of homogenous loam (left) and layered (right) columns.
prevent adsorption of cations to the ceramic material were selected for use in the experiment. To minimize physical and chemical artifacts during sampling, only 16 kPa of vacuum was applied to lysimeters for 5 min to recover a sufficient volume (7 mL) of water for geochemical analyses. Water samples were immediately divided for various analyses. The pH was measured, and then reduced species of S, Fe, and Fe\textsubscript{aq} were quantified voltammetrically using a hanging-drop mercury electrode (Metrohm). The electrode works by measuring current at a hanging-drop mercury electrode that is in contact with the water sample, while the voltage or potential between a reference and the hanging-drop mercury electrode is increased or decreased over time. At certain voltages, chemical species will be oxidized or reduced, which will be registered as a peak or a trough on a voltammogram. The height of the peak, which is proportional to concentration, is measured and then calibrated to a standard curve that was measured daily where possible. Concentrations of S(-II) and Fe\textsuperscript{2+} were measured by these means; however, because the stoichiometry of Fe\textsubscript{aq} is not known, only the currents and relative magnitudes of peaks are reported here. Samples were purged for 4 min with ultra-high-purity nitrogen gas before being measured with square-wave voltammetry. Purging time and rate were controlled via computer. This purging may cause a loss of H\textsubscript{2}S from solution. Therefore, it also may hasten a disassociation of Fe\textsubscript{aq}, creating more H\textsubscript{2}S (Theberge et al., 1997). Nevertheless, due to the variable pH of our soil solutions, the amount of H\textsubscript{2}S degassed during purging may have varied between samples and may have introduced some of the observed variation; still, we believe these effects to be minimal. The parameters used in the voltammetric analysis were: scanning range: 0 to −2100 mV; pulse height: 15 mV; step increment: 4 mV; frequency: 100 mHz; and scan rate: 80 mV s\textsuperscript{−1}. The balance of the collected Rainwater solutions were made with deionized water and reagent grade chemicals to emulate natural rainwater. The pH of the water was adjusted to approximately 5 with HCl to imitate the pH of natural rainwater. During some rainfall events, NO\textsubscript{3}\textsuperscript{−} and SO\textsubscript{4}\textsubscript{2−} were added (as sodium salts) to rainwater solutions to simulate the effects of polluted waters entering into the system. The concentrations of NO\textsubscript{3}\textsuperscript{−} and SO\textsubscript{4}\textsubscript{2−} ranged from 25 to 100 mg L\textsuperscript{−1}. Bromide and Cl\textsuperscript{−} were also added (as sodium salts) to rainwater solutions as a chemical tracer during some rainfall events. The dates in which NO\textsubscript{3}\textsuperscript{−}, SO\textsubscript{4}\textsubscript{2−}, Br\textsuperscript{−}, and Cl\textsuperscript{−} were added to rainwater are given in the next section.

**Rainwater Solution**

Rainwater solutions were made with deionized water and reagent grade chemicals to emulate natural rainwater. The pH of the water was adjusted to approximately 5 with HCl to imitate the pH of natural rainwater. During some rainfall events, NO\textsubscript{3}\textsuperscript{−} and SO\textsubscript{4}\textsubscript{2−} were added (as sodium salts) to rainwater solutions to simulate the effects of polluted waters entering into the system. The concentrations of NO\textsubscript{3}\textsuperscript{−} and SO\textsubscript{4}\textsubscript{2−} ranged from 25 to 100 mg L\textsuperscript{−1}. Bromide and Cl\textsuperscript{−} were also added (as sodium salts) to rainwater solutions as a chemical tracer during some rainfall events. The dates in which NO\textsubscript{3}\textsuperscript{−}, SO\textsubscript{4}\textsubscript{2−}, Br\textsuperscript{−}, and Cl\textsuperscript{−} were added to rainwater are given in the next section.

**Experimental Period**

Experiments on the homogenous sand column were conducted before the set of experiments on the homogenous loam and layered column, which were conducted simultaneously. Before any of the experiments were started, columns were wetted up from the bottom to prevent any air pockets from being trapped in the sediments that would artificially alter water flow (Castiglione et al., 2003; Köhne and Mohanty, 2005).

Four rainfall events were applied to the homogenous sand column on sand column study Experimental Days 34, 35, 37, and 38. Twenty liters, or approximately six pore volumes of rainwater solution, with concentrations of 25 mg L\textsuperscript{−1} NO\textsubscript{3}\textsuperscript{−} and SO\textsubscript{4}\textsubscript{2−} and 50 mg L\textsuperscript{−1} Br\textsuperscript{−} were applied to the column during each rainfall.

The duration of the experiment on the loam and layered column was approximately 8 mo. Table 1 (adapted from Fig. 2 of Hansen et al., 2011) shows a graphical representation of the frequency, chemistry, and pumping rate of the rainwater applications. During the first month (Exp. Day 17–40) of the experiment, rainwater amended with NO\textsubscript{3}\textsuperscript{−}, SO\textsubscript{4}\textsubscript{2−}, Br\textsuperscript{−}, and Cl\textsuperscript{−} was used for the rainfall events. After this first month of the experiment, the column was exposed to a 5-mo dry period (where no rainfall was applied) that mimicked drought conditions in nature. This dry period was followed by two more months of rainfall (Exp. Day 119–239), during which time rainwater consisted of pH-adjusted deionized water, which simulated delivery of uncontaminated water to the system. It was during this post-drought time that the results presented in this study were collected.

**Analysis**

Sediment cores (3.8-cm diam., 40-cm length) taken from the soil columns were used for analyses. Cores were longitudinally split in two, and the halved sections were used for microbial enumeration analysis and imaging. Before the halved cores were imaged, the sediments were oven-dried (60°C) for 24 h. The temperature, at which the sediments were dried, was minimized in an attempt to reduce mineralogical alteration caused by drying. The dried sediment was saturated by matric and gravity-induced flow with a low viscosity Buehler epoxy. After the epoxy cured, the bonded sediments were cut, attached to a glass slide (1.3 × 3.8 cm), and polished. A Cameca microprobe equipped with an energy dispersive system was used to obtain back scattered electron and X-ray mapping images.

**Results and Discussion**

**Evidence of FeS\textsubscript{aq} Clusters**

In the three columns used in this study, FeS\textsubscript{aq} was observed in the homogenous loam and layered columns but not in the homogenous sand column. Similarly, reduced species, Fe\textsuperscript{2+} and S(-II), were observed in water samples from the homogeneous loam and layered columns but not in water samples from the sand column study (Hansen et al., 2011). The absence of reduced chemical species was likely due to a combination of short residence times of the water and lower organic carbon substrate for microbial growth (0.02% in the sand versus 1.50%). Figure 2 shows Eh and water content from the sand column for the period of 3 d (Exp. Days
The Eh values increased in response to rainfall events, but the data range is narrowly constrained from 0 to 80 mV. Water content values showed a response to rainfall as well, but also fall within a narrow range of 0.2 to 0.36 cm$^3$ cm$^{-3}$ (Fig. 2). In contrast, within the loam (Fig. 3) and layered (Fig. 4) columns, the Eh data show these columns to be more reducing overall, with the layered column exhibiting greatest variability in response to rainfall (Exp. Days 200–252). In addition, the layered column shows a wide distribution of Eh values with depth supporting the interpretation of enhanced geochemical cycling at the sand–loam interface (Fig. 4). Due to the lack of observations of reducing conditions and FeS$_{aq}$ in the sand column, any further discussion of these results will be limited, but are given here as baseline values for comparisons with the other two columns.

In the loam and layered columns, FeS$_{aq}$ was manifest on voltammograms as a single or double peak at or centered around −1.2 V (vs. Ag/AgCl) (Luther et al., 2003) and are shown in Fig. 5. Complexities in cluster size and a lack of complete chemical characterization prevent determination of FeS$_{aq}$ concentration (Luther and Rickard, 2005). Instead current (A) from FeS$_{aq}$ peaks height are reported as a semi-quantitative representation of concentration. The largest peak heights for FeS$_{aq}$ measured during the experiment were observed at the soil-layer interface in the layered column. Furthermore, the maximum peak heights from the layered column were nearly double than those from the homogenous loam column.

Caution should be exercised when interpreting the FeS$_{aq}$ voltammetric signal in complex natural systems because other metal sulfide clusters (particularly copper-sulfide clusters) have displayed similar signals (Bura-Nakić et al., 2007). However, observations

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**Table 1. Experimental time table and conditions.**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Duration</th>
<th>Rainfall chemistry</th>
<th>Sampling schedule</th>
<th>Top boundary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wetting up</td>
<td>8 d</td>
<td>25 mg L$^{-1}$ NO$_3^-$ and SO$_4^{2-}$</td>
<td>On 8th day of saturation</td>
<td>Atmospheric</td>
</tr>
<tr>
<td>1st rain</td>
<td>12 h, rain every other 10 min</td>
<td>50 mg L$^{-1}$ NO$_3^-$ and SO$_4^{2-}$, 100 mg L$^{-1}$ Br$^-$, pH = 5.0</td>
<td>After 16 d after wetting up</td>
<td>Flux rate: 52 mL min$^{-1}$ (73.6 cm min$^{-1}$)</td>
</tr>
<tr>
<td>2nd rain</td>
<td>12 h continuously</td>
<td>25 mg L$^{-1}$ NO$_3^-$ and SO$_4^{2-}$, 50 mg L$^{-1}$ Cl$^-$, pH = 5.0</td>
<td>During rainfall</td>
<td>Flux rate: 52 mL min$^{-1}$ (73.6 cm min$^{-1}$)</td>
</tr>
<tr>
<td>3rd rain event</td>
<td>12 h continuously</td>
<td>100 mg L$^{-1}$ NO$_3^-$ and SO$_4^{2-}$, pH = 5.0</td>
<td>Before rainfall, 8 d after previous rainfall</td>
<td>Flux rate: 2.1 mL min$^{-1}$ (2.97 cm min$^{-1}$)</td>
</tr>
<tr>
<td>4th–9th rain events</td>
<td>6–18 h</td>
<td>NanopureWater, pH = 5.0</td>
<td>During rainfall</td>
<td>Flux rate: 0.8 mL min$^{-1}$ (1.13 cm min$^{-1}$)</td>
</tr>
</tbody>
</table>

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Fig. 2. (a) Water content and (b) redox potential (Eh) in the homogenous sand column over a 2-d period.
of $S(-II)$, $Fe^{2+}$ (Hansen et al., 2011), support the assumption that these peaks indeed represent $FeS_{aq}$. In addition, microprobe analyses also revealed an abundance of Fe and a lack of any other cluster-forming metals (e.g., copper). Titanium was present in appreciable amounts, but has not been shown to form an electroactive sulfide cluster.

In this study, at least 75% of $FeS_{aq}$ peaks were single peaked. Double-peaked signals were only detected in the homogenous (loam) column and are thought to represent either aged (hours) $FeS_{aq}$ (Bura-Nakić et al., 2007) or MnSH$^+$ (Luther et al., 1996). The voltammogram from the layered column exhibits a single $FeS_{aq}$ peak at $-1.2$ V (Fig. 5b) while the voltammogram from the loam column has a double $FeS_{aq}$ peak centered around $-1.2$ V, an $Fe^{2+}$ peak at $-1.5$ V, Mn(II) at $-1.7$ V, and an unknown peak at $-0.5$ V (Fig. 5a). The unknown peak at $-0.5$ V is likely a polysulfide complex (Luther et al., 2001). Assuming the system is in equilibrium, $S(-II)$ and $Fe^{2+}$ would be present in both the layered and loam columns where $FeS_{aq}$ is observed. However, both $Fe^{2+}$ and $S(-II)$ are notably absent from the layered column (Fig. 5b). This phenomenon is discussed in (Hansen et al., 2011), which showed enhanced Fe and S reduction within the layered system. This suggests that $S(-II)$ and $Fe^{2+}$ were produced but were removed by precipitation or sequestered by another mechanism before detection. In fact, $FeS_{aq}$ was only observed sporadically throughout the experiments, consistent with the interpretation that this phase may be an intermediate central to other iron–sulfur reactions.

**Timing of $FeS_{aq}$ Observation**

Aqueous FeS clusters were only observed in the last 2 mo of the 8-mo-long experiment. Figure 6 shows the $FeS_{aq}$ peak heights with time at selected sampling points from both columns as well as the occurrence of rainfall events. The difference between the early and later portions of the experiment was the frequency of rainfall events (days versus weeks). Results show that $FeS_{aq}$ was observed when the frequency of rainfall was much higher (i.e., in the second half of the experiment). Water sample chemistry (Hansen et al., 2011) indicates that the greater regularity of water allowed for higher microbial activity leading to different redox conditions (data not shown) than was observed in the first half of the experiment. Although increased frequency of rainfall maintained high water content values, the water content alone could not explain the formation of $FeS_{aq}$ as these clusters were observed at lower (~0.25 cm$^3$ cm$^{-3}$) and higher (~0.47 cm$^3$ cm$^{-3}$) water content levels. This demonstrates that $FeS_{aq}$ can form in unsaturated conditions and is not confined to saturated systems.

**pH Effects**

Another potential controlling factor on the formation of $FeS_{aq}$ may be pH. Davison et al. (1998) showed that the $FeS_{aq}$ signal increased with increasing pH, suggesting that concentration depends on pH. However, the actual stoichiometry would control the degree to which $FeS_{aq}$ formation is dependent on pH. Though the exact stoichiometry is unknown, in our experiments, the range of the pH of the soil pore waters in the layered column were higher...
(approximately 7–10) than in the loam column (approximately 7–8.5) (Fig. 7) and FeS\textsubscript{aq} was observed more frequently (and with larger peak heights indicating relatively higher concentrations) in the layered column. However, the pH ranges from the sediment pore waters observed in the experiment were limited, and a definitive correlation between the occurrence of FeS\textsubscript{aq} and pH conclusions cannot be drawn from these data.

**Distribution of FeS\textsubscript{aq}**

Aqueous FeS clusters were most frequently observed at the sand–loam interface in the layered column, as shown in Fig. 8, from Experimental Day 200 through 240. In addition to the near-constant presence of FeS\textsubscript{aq}, the largest peak current near the sand–loam interface was more than double than any other FeS\textsubscript{aq} measurements in the loam column. The frequent observation of larger-magnitude FeS\textsubscript{aq} Peaks near the sand–loam interface corresponds well with observations of enhanced biogeochemical cycling at this interface. With the exception of samples collected from the sand–loam interface, the behavior of FeS\textsubscript{aq} is transient and was rarely detected in the same location at consecutive sampling intervals. This transient behavior is consistent with observations that FeS\textsubscript{aq} is an intermediate in pyrite formation (Rickard and Luther, 1997) and highlights the importance of the soil layer in the formation of FeS\textsubscript{aq}. In addition, FeS\textsubscript{aq} was detected in the effluent water from the layered column only (Fig. 6 and 8), suggesting that this aqueous species was stable enough to be transported at least the length of the column.

**Redox Potential**

Ultimately, the presence of an interface between the sand and loam soils became a dominant factor that contributed to enhanced redox cycling and the formation of FeS\textsubscript{aq}. Within the layered column, the Eh data clearly show less fluctuation, in response to rainfall, at depth and enhanced geochemical cycling at the sand–loam interface (Fig. 4).

The Eh data from the two lowest probes at −28 and −36 cm in the layered column, show that reducing conditions (negative Eh values) were consistent throughout the experiment (below −200 mV for ~80% of the experiment). The data also show an increase of the Eh values by up to 80 mV each time a rainfall event was introduced. As the rainwater percolated through the soils, it transported dissolved O\textsubscript{2}, resulting in various oxidation reactions and an increased Eh. Oxidative conditions were short, with increased Eh values lasting several hours before the O\textsubscript{2} was depleted through either chemical oxidation of reduced minerals or biologic activity that consumed the dissolved O\textsubscript{2} causing the Eh to decrease to pre-rainfall levels.

However, the Eh data from the sampling location just below the sand–loam interface (−19 cm), where the greatest concentrations of FeS\textsubscript{aq} were observed (Fig. 4), show the greatest magnitude changes and provide the greatest insight on FeS\textsubscript{aq} development in soils. Similar to the data from the probes located lower in the column, the Eh values were negative before the introduction of rainwater, demonstrating that reducing conditions were prevalent. However, unlike in the lower column, the Eh response to rainfall is more variable. The greatest observed change in Eh was a change of nearly 200 mV in response to the rainfall on Experimental Day 239,
more than twice the change in Eh observed elsewhere. The amount of time that it took for the Eh to drop to the pre-rainfall values was much longer, on the order of days to a week. For example, after the rainfall event on Experimental Day 227, Eh values stabilized at approximately −80 mV after several days. On Experimental Day 234, the Eh dropped from −96 to −230 mV within 24 h. This pattern of stabilization after rainfall followed by rapid drop in Eh was repeated throughout the experiment.

Although Eh is not a definitive indicator of which terminal electron accepting process (TEAP) is active, the sharp drop in Eh supports that Fe(III) and SO₄²⁻ reduction became active within a short period of time after rainfall. The initiation of Fe(III) reduction and SO₄²⁻ reduction supplied the Fe²⁺ and S(-II) necessary to form Fe₃S₈. Measurements of the largest Fe₃S₈ current peaks at the sampling location immediately below the soil-layer interface (−19 cm) illustrate that the rapid redox cycling (Fig. 4) created favorable conditions for the formation of Fe₃S₈.

In contrast to the Eh data from the layered column, Eh data from the loam column, with the exception of the −6 cm sampling location, did not vary greatly through time (Fig. 3). Some minor fluctuation of Eh values were observed, associated with rainfall, as observed in the layered column, but the magnitude of Eh change was not nearly as great. The Eh data at −6 cm show that Eh dropped compared to the other sampling points in the loam column. There are two possible interpretations of the Eh data from this location: extended reducing conditions throughout the zone near the atmosphere interface (and around other sensors) or a more localized microenvironment. It is unlikely that such negative Eh values would be associated with an extended area so close to the top of the column where oxygen could more readily diffuse into the sediment, and we have no data to suggest that the extended reducing conditions exist. Further, redox microenvironments at this scale, even in lithologically homogeneous sediments, are quite common and well documented in natural and laboratory settings, so we believe this is the more likely explanation. This suggests that the sampling location at −6 cm was located in a microenvironment where the redox environment was limited to a localized area that was less than centimeter scale in size. The Fe₃S₈ observed in the loam column likely originated from microenvironments, such as this one located at −6 cm. It is possible the similar microenvironments also exist in the layered column, but based on the data, the overall contribution of these microenvironments would be small compared to the impact of the soil layer.

Ultimately, the presence of a sand–loam interface, where frequent redox cycling of sulfur and iron occurred, created conditions suitable for the consistent production of and largest peak heights (proxy for concentration) of Fe₃S₈. The formation of Fe₃S₈ at the interface implied that instead of precipitation of an insoluble mineral in this zone, percolating water would have transported Fe₃S₈ from the interface deeper into the soil column. The Fe₃S₈ was probably mineralized in the lower sections of the column as it was generally absent from the column effluent. However, Fig. 4 and 6 show Fe₃S₈ in the effluent and
demonstrate its ability to be transported away from the vadose zone. In this study, this distance could have been as great as 40 cm, but another study has shown Fe\textsubscript{aq} clusters that had been transported up to several kilometers (Rozan et al., 2000). This stability may not be so surprising given the observation that copper, even when complexed with EDTA, quickly formed sulfide clusters, indicating that Cu-sulfide clusters are more stable even than Cu-organic complexes. (Rozan et al., 2000). These results suggest that current conceptual models of iron-sulfide cycling may need to be adapted to include the possibility that Fe\textsuperscript{2+} and S(\text{-II}) may not rapidly precipitate as an insoluble mineral but may in fact be present in an aqueous phase subject to transport.

The Role of Fe\textsubscript{aq} on Linked Hydrological and Geochemical Processes

The presence of Fe\textsubscript{aq} in soils may influence soil structure which in turn may have a measured effect on the hydrologic properties of the vadose zone. The largest concentration of Fe\textsubscript{aq} (as evidenced by the largest peaks in electric current) was observed at −19 cm, immediately below the sand–loam interface in the layered column, and was accompanied by a greater two-dimensional density (aggregate area/matrix area) of soil aggregation (0.0640 cm\textsuperscript{2} cm\textsuperscript{-2}) than in the loam column (0.0195 cm\textsuperscript{2} cm\textsuperscript{-2}) at −10 cm (Hansen et al., 2011). The increased aggregate density at the sand–loam interface correlates with the observation of the greatest concentrations of Fe\textsubscript{aq} supports a relationship between the two. Further, we hypothesize that the Fe\textsubscript{aq} and aggregate formation are linked and their presence contributed to an observed decrease in hydraulic conductivity; in this case, the saturated hydraulic conductivity, in the layered column, decreased from 2.33 × 10\textsuperscript{-1} to 6.9 × 10\textsuperscript{-3} cm s\textsuperscript{-1}.

Aggregates are “secondary particles formed through the combination of mineral particles with organic and inorganic substances” (Bronick and Lal, 2005). Oades and Waters (1991) found that aggregates initially form as fragments of plant material that are encrusted by inorganic materials (in this case, metal bearing minerals) that protect them from rapid decomposition. It is this initial step, in which inorganic materials bind to the organic matter, where Fe\textsubscript{aq} may play a role in aggregate stabilization. Because Fe\textsubscript{aq} is reactive with organic matter (Grimes et al., 2001; Rickard et al., 2001), it would be the first inorganic material to begin to bind to the organic matter that would eventually become the core of the aggregate. The organically bound Fe\textsubscript{aq} would then facilitate precipitation of iron sulfide minerals around the organic matter. As the thickness of iron sulfide and other inorganic minerals increased, the inner organic material would become completely encrusted and the aggregate stabilized.

Microprobe analyses of soil aggregates from both columns were performed to determine their composition. Soil aggregates were largely composed of clays, organic material that ranged from microfossils to plant material, fine grained quartz, and various other minerals that included pyrite, ilmanite, iron-oxides, calcite, barite, anhydrite, and apatite. Aggregates near the sand–loam interface contained higher proportions of Fe than aggregates further away from the interface or in the loam column. This is consistent with observations of enhanced iron and sulfur cycling and Fe\textsubscript{aq} formation at the sand–loam interface, as shown in Fig. 8. An example of an aggregate near the sand–loam interface (−19 cm) from the layered column is shown in Fig. 9. The first image (a) is a back scattered electron image while the second (b) is a false-colored composite where the elements Fe, S, and Si are represented by red, gr...
green, and blue, respectively. Thus, red represents Fe oxide or Fe carbonate minerals, green corresponds to S, generally incorporated into organic material, blue represents quartz or feldspar; and yellow is iron-sulfide minerals (mostly pyrite). The presence of yellow (red + green = yellow) indicated that Fe and S were associated one with another in an iron-sulfur mineral. If any two elements are present at the same location, color additive mixing would produce secondary colors. Thus, the yellow is interpreted to be pyrite because of its thermodynamic stability and the abundance of pyrite framboids observed at higher magnification (data not shown).

The false-colored image shows the preferential accumulation of Fe within an aggregate, including the presence of iron oxides. Although Fe oxides played a role in aggregate formation, it does not nullify the role that FeS\textsubscript{aq} played as a bridge between the organic matter and inorganic material (such as Fe-oxides). In contrast, Fig. 10 shows an aggregate from the loam column at −30 cm. While there is some Fe (red color) present in the aggregate, its abundance is much lower than the amount of Fe in the aggregate shown in Fig. 9.

From a hydrologic perspective, the sand–loam interface acted as a barrier to flow as water flowed from the sand (higher hydraulic conductivity) into the loam (lower hydraulic conductivity) and FeS\textsubscript{aq} likely played a key role in further reducing flow rate through this barrier. As the sand–loam interface is the location of the largest current peaks of FeS\textsubscript{aq} and FeS\textsubscript{aq} has been shown to be an intermediate in the formation of pyrite, iron-sulfide minerals may have precipitated near hydrologic barriers due to an accumulation of FeS\textsubscript{aq}. Likewise, this is also the location where soil aggregates were most densely accumulated, and FeS\textsubscript{aq} can act to form and stabilize soil aggregates. Ultimately, both precipitation of Fe–S minerals and aggregate formation act to decrease the hydraulic conductivity of the sediment.

### Implications of FeS\textsubscript{aq} in Contaminant Fate and Transport

While not enough is known about FeS\textsubscript{aq} clusters to draw a mechanistic conclusion about the exact nature of the relationship between FeS\textsubscript{aq} and formation of soil aggregates, the observation of FeS\textsubscript{aq} in the vadose zone is significant and has implications for contaminant fate and transport. In particular, the presence of a sand–loam interface caused some unique biogeochemical and hydrologic conditions to form. From a geochemical perspective, the observation of the largest FeS\textsubscript{aq} peaks at the soil layer was accompanied by large shifts in redox potential. This association suggests that FeS\textsubscript{aq} is not only an indicator of systems that are frequently in redox disequilibrium, but their formation is favored in these conditions. Certainly, observations of FeS\textsubscript{aq} in environments such as tidal flats (Taillefert et al., 2007), estuaries (Rickard et al., 1999), and deep ocean hydrothermal vents (Luther et al., 2001), where redox conditions are out of equilibrium, are consistent with observations from this study.

Aqueous FeS clusters have been shown to be stable in the absence of nanoparticulate mackinawite (Rickard, 2006) and O\textsubscript{2}, which allows for their transport from their source (Luther et al., 2003), as was observed in this study. Iron (II), often thought to be immediately precipitated in sulfide-rich areas, may be instead transported via FeS\textsubscript{aq} clusters through such regions. Toxic metals may be substituted into FeS\textsubscript{aq} clusters (Rozan et al., 2000), thus changing the flow and transport patterns, which could alter human health and ecologic risk.

Currently, and to our knowledge, the existence of an aqueous iron-sulfide species has not been integrated into current conceptual models of flow and transport. However, as this study has shown, there may be many roles that these clusters play in the environment.

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**Fig. 10.** (a) Backscattered electron image (1 × 1 mm) of a soil aggregate from (−30 cm) in the loam soil column. (b) Red–green–blue false color composite of the same soil aggregate where red represents iron, green is sulfur, yellow is iron-sulfur minerals, and blue is silicon.
They may be indicators of redox disequilibrium or enhanced biogeochemical cycling, predictors of decreased hydraulic conductivity, or may help to predict the fate and transport toxic metals and contaminants more accurately not only in the vadose zone, but also in other adjacent environments such as aquifers of fluvial systems. As our knowledge of FeS\textsubscript{aq} increases, revising contaminant fate and transport models to include FeS\textsubscript{aq} may prove vital to increase the accuracy of and benefits derived from these models.

**Conclusions**

This paper documents evidence for FeS\textsubscript{aq} in a vadose zone system. The FeS\textsubscript{aq} peaks were centered at $-1.2$ V (vs. Ag/AgCl) and both double and single peaks were observed. FeS\textsubscript{aq} was observed in both a homogenous loam column and a layered sand-over-loam column but not in the homogeneous sand column. However, the greatest FeS\textsubscript{aq} peak heights (semiquantitative proxy for concentration) were detected near the sand–loam interface, which is consistent with observations of enhanced biogeochemical cycling occurring at soil boundaries.

The sand–loam interface was also a zone of frequent and large-magnitude fluctuations in Eh, suggesting this was an active zone of biogeochemical cycling. Thus, FeS\textsubscript{aq} in unsaturated sediments may serve as an indicator of dynamic systems that are frequently out of equilibrium. This study also demonstrated the ability of the FeS\textsubscript{aq} to be transported through and out of the vadose zone. This observation is especially pertinent when coupled with results from Rozan et al. (2000), who discovered that toxic metals such as silver, cadmium, mercury, or lead can be incorporated in FeS\textsubscript{aq} clusters and may subsequently transport beyond the vadose zone. Thus, the incorporation of an aqueous iron-sulfide species into current conceptual models should be considered to account for complexities not presently taken into account especially in contaminant fate and transport.

Observations of FeS\textsubscript{aq} also have implications for hydrologic fluxes in the vadose zone. Aqueous FeS clusters were also observed in association with soil aggregates containing high amounts of Fe. Because FeS\textsubscript{aq} has been shown to be reactive with organics (Grimes et al., 2001; Rickard et al., 2001), it is likely that FeS\textsubscript{aq} was attracted to the organic constituents within soil aggregates and further cemented and stabilized these aggregates. The nature of the relationship between FeS\textsubscript{aq} and soil aggregates is unclear, but FeS\textsubscript{aq} may affect the formation of soil aggregates that may ultimately change the hydraulic properties of the soil. This suggests that the presence of FeS\textsubscript{aq} impacts not only biogeochemical cycling, but also the physical and flow properties of sediments, which has broader implications for the fate and transport of all chemical constituents in the system.

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**References**


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