

# Novel Insights into the Distribution of Reduced Sulfur Species in Prairie Pothole Wetland Pore Waters Provided by Bismuth Film Electrodes

Brandon C. McAdams,<sup>†</sup> Rachel M. Adams,<sup>†</sup> William A. Arnold,<sup>‡</sup> and Yu-Ping Chin<sup>\*,†</sup>

<sup>†</sup>School of Earth Sciences, The Ohio State University, 125 South Oval Mall, Columbus, Ohio 43210, United States <sup>‡</sup>Department of Civil, Environmental, and Geo-Engineering, University of Minnesota, 500 Pillsbury Drive Southeast, Minneapolis, Minnesota 55455, United States

**Supporting Information** 

**ABSTRACT:** Mercury/gold amalgam and bismuth film electrodes (BiFEs) were used to make the first centimeterscale measurements of redox species in benthic pore waters of prairie pothole wetlands across a hydrologic gradient. Sulfide in pore waters increased across this system from negligible sulfide in hydrologically up-gradient recharge wetlands to thousands of micromolar in down-gradient discharge wetlands. Field measurements of sulfides using the BiFE were tested against an established colorimetric assay. Sulfide measured with the BiFE agreed well with colorimetric measurements but is not subject to analytical artifacts associated with methods needed to extract the pore waters. Use of Hg/Au and BiFE electrodes should allow for rapid *in situ* detection of redox



active species, especially sulfide concentrations of >500  $\mu$ M, in pore waters over seasonal to decadal time scales. Such measurements are needed to understand important biogeochemical and environmental processes such as carbon cycling and contaminant attenuation tied to sulfur dynamics in these important ecosystems.

# INTRODUCTION

The prairie pothole region (PPR) spans more than 750,000 km<sup>2</sup> in central North America and is densely populated with wetlands and ephemeral lakes that form essential habitats in the many, often shallow, depressions of the landscape.<sup>1,2</sup> Little surface connectivity exists between individual water bodies,<sup>2</sup> but slow depression focused groundwater flow through pyrite and gypsum rich glacial until connects PPR wetlands.<sup>3</sup> The chemical weathering that occurs during groundwater flow creates a gradient from perched recharge wetlands that receive only rain and runoff to midgradient flow-through and down-gradient discharge wetlands that undergo an increase in ionic strength, predominantly sulfate (SO<sub>4</sub><sup>2-</sup>), along hydrologic flow paths.<sup>4,5</sup>

Sulfide concentrations as high as 2 mM have been observed in PPR wetland pore waters,<sup>6</sup> but the temporal dynamics of redox active species on seasonal and longer time scales in PPR wetlands remains mostly unresolved.<sup>7</sup> Previous pore water measurements have been made via centrifugation of bulk sediment<sup>6,8</sup> or via squeezing a sediment core, which expels water from an approximately 30–60 cm<sup>3</sup> section.<sup>6,7</sup> Both methods provide little information about fine-scale heterogeneity and therefore limit the observable processes governing the distribution of redox active species within these wetlands. An improved spatial and temporal knowledge of reactive redox species, especially high sulfide concentrations in PPR wetlands, is critical for understanding how these climatically dynamic systems<sup>9–11</sup> will respond in the future. Specifically, sulfur is important for anaerobic methane oxidation,<sup>12–17</sup> which may help regulate the release of methane from these wetlands that provide important carbon storage.<sup>18</sup> Sulfur also influences the fate of environmental contaminants such as mercury (Hg) and pesticides. Reduced sulfur species strongly bind with Hg and reduce its bioavailability,<sup>19–27</sup> and sulfate reduction can stimulate Hg methylation.<sup>28–30</sup> Reduced sulfur species are also highly reactive toward agricultural pesticides<sup>6,8</sup> and dissolved organic matter (DOM) in PPR porewaters.<sup>31</sup> Thus, reduced sulfur plays a critical role in pesticide attenuation in wetland systems.

Voltammetry discriminates between different redox active species,<sup>32–34</sup> including some metal/ligand complexes.<sup>34–37</sup> Further, voltammetric electrodes make measurements possible at millimeter to centimeter depth intervals. Voltammetry has been widely used in benthic marine and estuarine environments

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to elucidate fine-scale biogeochemical processes<sup>38–41</sup> but has not been widely applied in lacustrine environments.<sup>42</sup> Mercury/ gold amalgam (Hg/Au) electrodes have been used for *in situ* detection of redox active substances in freshwater benthic environments that possess low levels of reduced sulfur (<500  $\mu$ M). In highly sulfidic environments (>500  $\mu$ M), however, sulfide adsorption interferes with the Hg/Au surface, rendering these electrodes ineffective.<sup>43</sup> Recently, the analytical potential of bismuth film electrodes (BiFEs) has been explored,<sup>44–46</sup> and BiFEs have quantified sulfide as high as 5500  $\mu$ M in the laboratory.<sup>43</sup> To date, BiFEs have not been used *in situ* to detect high levels of sulfide in benthic pore waters but may be useful for examining PPR wetland and other<sup>47–49</sup> highly sulfidic waters.

We used BiFEs to measure sulfide profiles in pore waters as a function of depth in sediment cores, mimicking an *in situ* application while allowing us to directly compare BiFEs against established colorimetric sulfide assays from the same pore fluids. The BiFE made possible measurements at 1 cm spatial resolution in the highly sulfidic benthic environments of some PPR wetlands. The profiles presented herein reveal heterogeneities in PPR wetland pore water redox processes, e.g., small-scale localized sulfide mineral formation,<sup>7</sup> in a manner that eluded previous measurements.<sup>6–8</sup>

## MATERIALS AND METHODS

Detailed descriptions of chemicals and reagents, working electrode fabrication and calibration, pore water squeezing, and methylene blue sulfide measurements and dissolved organic carbon (DOC) analyses are available in the Supporting Information.

Electrochemical scans were performed using a DLK-70 potientiostat from Analytical Instrument Systems (AIS, Ringoes, NJ) and a three-electrode arrangement composed of a Pt wire counter electrode, a saturated Ag/AgCl reference electrode, and a Hg/Au amalgam or BiFE working electrode.<sup>33,43</sup> Counter and reference electrodes were purchased from BASi (West Lafayette, IN) and used as received. Working electrodes housed in polyether-ether-ketone (PEEK) tubing were fabricated following described methods for Hg/Au amalgam<sup>39</sup> and BiFE<sup>43</sup> electrodes (details in the Supporting Information). Cathodic square wave (CSW) scans with the Hg/Au electrode were used to measure Mn(II) and Fe(II).<sup>33,35,39</sup> Sulfide was measured by cyclic voltammetry (CV) scans with the BiFE.<sup>43</sup> Calibrations performed in unfiltered pore water were similar (within the precision of the BiFE) to those in prepared buffered solutions (Figure S1). The working electrode was kept stationary in the sediment core during repeat measurement by locking it in place with a clamp attached to a ring stand (Figure S2).

Two pools of sulfide were measured. In the cathodic (negative sweeping) direction of both CV and square wave scans, free reduced sulfur ( $H_2S + HS^- + S^{2-}$  or  $\sum H_2S$ ) adsorbed to the electrode surface is stripped as the Hg or Bi is reduced and regenerated.<sup>43</sup> Speciation beyond  $\sum H_2S$  was not possible in our study because pH could not be measured concurrently in the pore water. In the anodic (positive sweeping) direction of CV scans, sulfide is plated onto the electrode surface. This process is strong enough to oxidize sulfide from both  $\sum H_2S$  and some iron sulfide and polysulfide complexes.<sup>43</sup> Because we are unable to resolve the sulfur speciation, the sulfide measured by the anodic sweep is termed acid volatile sulfide (AVS).<sup>50</sup>

Benthic sediment cores were collected from recharge (T9), flow-through (P7), and discharge (P8) wetlands at the U.S. Geological Survey Cottonwood Lakes Study Area near Jamestown, ND. Two cores were collected from wetland P8 at different locations: from within a vegetated mat (P8-VM) in the littoral zone and from an open water area (P8-OW). Push cores with polycarbonate liners that were ~30 cm long with a 6.5 cm diameter were used. Care was taken to ensure each core retained an inch or more of water above the sediment water interface. Collected cores were capped and kept on ice until they were measured. Cores from P7 and P8 were analyzed within 24 h of collection. The T9 core was analyzed 1 week after collection and was stored at 4 °C until it was analyzed.

Sediment cores are a compromise approach for mimicking *in* situ measurements because there was a lack of access to proper deployment infrastructures, e.g., landers and stable support craft, at the field site. The use of sediment cores (Figure S2) also allows *direct* comparison of BiFE-measured sulfide to *ex* situ measurements by colorimetric assays. After voltammetry was completed, P7 and P8 cores were squeezed to anoxically extract the pore waters at discrete depth intervals.<sup>6,8,51,52</sup> Extracted pore waters were analyzed for sulfide using the methylene blue method<sup>6–8,53</sup> to corroborate electrochemical measurements and for dissolved organic carbon (DOC) measurements using a Shimadzu TOC-V instrument (details in the Supporting Information).

## RESULTS AND DISCUSSION

While a  $\sum H_2S$  signal at -0.7 V was observed during CSW scans of the T9 core (Figure S3), the signal was below the reliable detection limit (<0.1  $\mu$ M) of the Hg/Au electrode.<sup>43</sup> Cathodic square wave scans in the T9 core also revealed a broad peak at -0.3 to -0.4 V (Figure S3) that possibly represents Fe(III)/DOM complexes (or, less likely, O<sub>2</sub>) present in the pore waters of this recharge wetland.<sup>39</sup> No other redox species were detected in the T9 core.

In P7, the flow-through wetland, CSW scans with the Hg/Au electrode did not detect oxygen and detected Mn(II) from 366  $\mu$ M at 1 cm decreasing with depth to 68.7  $\mu$ M at 10 cm, and Fe(II) was observed at only one depth [547  $\mu$ M at 1 cm (Figure 1)]. Fe(II) was below detection limits at depths greater than 2 cm, which could reflect its interaction with sulfides to form stable aqueous complexes or minerals, including mackinawite and pyrite. <sup>54–58</sup> At ≤2 cm,  $\Sigma$ H<sub>2</sub>S was above the detection threshold of the Hg/Au electrode. Thus, for  $\Sigma$ H<sub>2</sub>S and AVS only, we used the BiFE at deeper depth intervals within the P7 core.

 $\Sigma$ H<sub>2</sub>S increased initially with depth before being maintained at relatively constant levels followed by a decrease at depths below 9 cm [concentration range of 289–1350  $\mu$ M (Figure 1)]. AVS was ~300  $\mu$ M higher than  $\Sigma$ H<sub>2</sub>S in P7 and followed the same trend with depth (Figure 1). This relatively constant difference between  $\Sigma$ H<sub>2</sub>S and AVS in P7 may represent the existence of iron sulfides or S<sub>x</sub><sup>2-</sup> species.<sup>39,43</sup> The presence of iron sulfides could explain the loss of soluble Fe(II) from the pore fluids at this site.<sup>54,55,58</sup>

To further explore the spatial complexity of the geochemistry in these wetland sediments, we measured a second profile 2 cm horizontal from the original measurements within the same core. At 8 and 9 cm in this second sediment core profile,  $\sum H_2S$ was 300–400  $\mu$ M lower but AVS was 400–700  $\mu$ M higher than in the primary profile (Figure 1). An increase in AVS with a decrease in  $\sum H_2S$  may represent a zone of elevated sulfide



Figure 1. Depth profile of the P7 core showing Fe(II), Mn(II),  $\sum H_2S$ , and AVS labeled according to the legend. Dashed lines and empty symbols indicate  $\sum H_2S$  and AVS measurements from the second exploratory profile taken 2 cm horizontally from the primary profile (solid lines). Error bars are one standard deviation from the mean of three or more measurements and are mostly within the size of the symbol.

reaction with Fe(II) such as pyritization that would remove more  $H_2S$  and form larger amounts of AVS than in the first profile. Spatially concentrated zones of pyritization have been observed by others in PPR wetland sediments,<sup>7</sup> but *ex situ* pore water measurements lack the resolution to elucidate this phenomenon.

Sulfide levels in cores from the discharge wetland P8 were similar to those observed in P7; thus, the BiFE was singularly employed in analyzing P8 pore waters.  $\Sigma H_2S$  in the P8-OW (open water) core generally increased with depth from a low of 164  $\mu$ M to a high of 1470  $\mu$ M at 4 cm (Figure 2a). The highest  $\Sigma H_2S$  in the P8-OW core was comparable to the highest  $\overline{\Sigma}$ H<sub>2</sub>S concentration observed in P7 (1350  $\mu$ M). Whereas  $\overline{\Sigma}H_2S$  in P7 eventually decreased with depth, sulfide did not follow this pattern in the P8-OW core. Similar to the primary P7 profile (Figure 1), AVS was 300-400  $\mu$ M greater than  $\Sigma H_2 S$  and followed the same pattern throughout the P8-OW core (Figure 2a), suggesting the presence of complexes such as iron sulfides and/or polysulfides. The presence of iron sulfides in P7 and P8 would indicate that iron continues to undergo biogeochemical removal processes along the hydrologic gradient from recharge to discharge in this system.

In an effort to independently corroborate our voltammetric measurements, sulfide profiles were obtained using the methylene blue method on squeezed pore waters from the identical P8 cores analyzed by voltammetry. The methylene blue method measured  $\Sigma H_2S$  values that were statistically similar or somewhat lower than  $\Sigma H_2S$  values measured using the BiFE [with a couple of exceptions in both cores (see Figure 2)]. Because the methylene blue measurements must be conducted *ex situ*, analytical artifacts such as hydrogen sulfide



**Figure 2.** Depth profiles from the (a) P8-OW and (b) P8-VM cores showing  $\sum H_2S$  and AVS measured with the BiFE and methylene blue (MB) measured sulfide. Note the different *x*-axis scales. Vertical error bars on methylene blue points indicate the vertical space squeezed from the core and integrated into that measurement. Horizontal error bars are one standard deviation from the mean of three or more measurements.

outgassing and oxidation through inadvertent contact with the atmosphere may occur. These potential artifacts are especially difficult to manage when extracting pore waters and transferring the samples from core to syringe and syringe to reaction vessel. Outgassing and oxidation may explain why some of our methylene blue-determined  $\Sigma H_2S$  values are lower than those measured by the BiFE. The intervals where methylene bluedetermined  $\Sigma H_2S$  concentrations were greater than the BiFE measurements could be attributed to mixing of higher- and lower-sulfide waters within the 30-60 cm<sup>3</sup> space of the squeezed core (as indicated by the vertical error bars in Figure 2). Thus, in addition to the analytical artifacts associated with measurements of  $\sum H_2S$  in sediments by methylene blue, i.e., extraction of pore fluids,<sup>7,8,47,59</sup> this approach lacks the much finer spatial resolution offered by the BiFE microelectrode. Regardless,  $\Sigma H_2S$  measurements by both methods were consistent and mostly similar throughout the cores.

The application of microelectrodes allows us to explore the heterogeneous nature of the sediments in different areas of the wetlands. For example, the greatest sulfide concentrations and changes with depth were seen in the P8-VM core [collected in a vegetated mat (Figure 2b)]. Areas of low sulfide discerned by the BiFE deeper in the core could be indicative of bioturbation,<sup>60–68</sup> the influence of littoral plants pumping oxygen into the rhizosphere,<sup>69–72</sup> and/or changes in the chemical properties of the pore fluids that can influence the metabolism of sulfate-reducing bacteria.<sup>73–75</sup> High DOC concentrations were also observed in P7 and P8 cores (see Figure S4), but no relationships were found between DOC and redox active substances within these wetland pore waters.

Acid volatile sulfide in the P8-VM core follows the pattern of  $\sum H_2S$ , but unlike the P7 and P8-OW cores, it was similar to or less than  $\sum H_2S$  (Figure 2b). Because AVS as defined previously should never be less than  $\sum H_2S$ , it is plausible that the upper detection limit of the BiFE microelectrode could be influenced

by matrix effects in the sediment pore waters. The most positive potential for the BiFE that does not compromise the electrode surface is -0.4 V.<sup>43</sup> At this potential, the anodic scan was unable to fully resolve the "in sediment" AVS signal in the same manner as the calibration scans (Figure S5). This may be due to differences in the AVS chemistry between our calibration standards [ $\Sigma$ H<sub>2</sub>S without other sulfide species (see the Supporting Information)] and the sediment pore fluids. Because detection of complexed sulfide in this manner first involves separating the sulfide from the complex,<sup>39,43</sup> broadening of the peak may result in fast scan rates<sup>39</sup> and cause it to be unresolved within the analytical potential window (from -1.6 to -0.4 V for the BiFE in our study). Slower scan rates may alleviate this problem but result in decreased sensitivity.<sup>43</sup>

The profiles measured as part of this study suggest that the chemical gradient observed in the surface waters of the PPR wetlands is also reflected in the benthic pore waters even though redox conditions differ, i.e., low to high reduced sulfur concentrations occurring along a hydrologic gradient from recharge to discharge wetlands. The fine spatial resolution also elucidated the influences of benthic processes such as bioturbation and rhizosphere processes on PPR wetland sulfur redox chemistry. Electrochemical measurements also support other findings that illustrate the importance of small-scale mineralization zones on sulfur regulation in these wetlands.7 Adaption of this approach for long-term deployment in wetlands should be possible in the future.<sup>39</sup> Such deployment will allow us to capture temporal changes in PPR wetland redox gradients associated with changing hydrologic conditions over seasonal and decadal time scales,<sup>9-11</sup> especially as these might occur with the future effects of global climate change in the region.

We have also reported the first direct field comparison of BiFE pore water down core sulfide measurements with methylene blue-determined sulfide for pore waters extracted from the same core. This comparison not only supported the efficacy of BiFE for future *in situ* measurements but also illustrated some of the limitations of *ex situ* approaches, e.g., outgassing and oxidation during sample handling and, perhaps more importantly, an inability to capture fine-scale heterogeneities in sulfur redox gradients. The BiFE is an attractive option for probing fine spatial variability and assessing temporal changes in high-sulfide redox environments globally and a useful tool for studying sulfur-influenced environmental and biogeochemical processes in sediments.

## ASSOCIATED CONTENT

#### **S** Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.6b00020.

A full list of chemicals and reagents used, the protocol for working electrode fabrication, calibration conditions, pore water extraction, and methylene blue and DOC analyses and figures that comprise plots of comparative calibrations in unfiltered pore water and prepared solutions, visual depictions of our down core method, cathodic square wave scans of the T9 core, sulfide and DOC in the P7 and P8 cores, and a calibration AVS signal compared to an in sediment AVS signal (PDF)

## AUTHOR INFORMATION

#### **Corresponding Author**

\*E-mail: chin.15@osu.edu. Phone: (614) 292-6953.

#### Notes

The authors declare no competing financial interest.

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