

Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms

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1. Background

This report is one part of a larger study—the Wild Rice Sulfate Standard Study—coordinated by the Minnesota Pollution Control Agency (MPCA) on the effect of elevated sulfate concentrations on wild rice. Minnesota currently has a water quality standard of “10 mg/L sulfate - applicable to water used for production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels.” (Minn. R. 7050.0224, subpart 2). In 2010, the MPCA initiated a multi-year effort to clarify implementation of the state’s wild rice sulfate standard, which had recently come under increased questioning and contention. Based on a review of available studies and information, the MPCA determined that additional studies were needed to evaluate the effects of sulfate on wild rice before a revision to the numeric sulfate standard could be considered. In 2011 the Minnesota Legislature provided funding to gather this additional information in the Legacy Amendment Bill (Laws of Minnesota, 2011, First Special Session, ch. 6, Art. 2, Sec. 5(j)).

Wild rice is an important aquatic plant in parts of Minnesota, particularly northern Minnesota. It provides food for waterfowl, is also a very important cultural resource to many Minnesotans, and is economically important to those who harvest and market wild rice.

The goal of the overall Wild Rice Sulfate Standard Study is to enhance understanding of the effects of sulfate on wild rice and to inform a decision as to whether a revision of the wild rice sulfate standard is warranted. The Study consists of several research efforts that have been conducted by several groups of scientists at the University of Minnesota campuses in Duluth and the Twin Cities. The data collection phase of the study was completed in December 2013, and is documented in individual reports, along with associated data, from the researchers working on each component of the study.

The primary hypothesis driving the Study has been that if elevated sulfate has a negative effect on the growth of wild rice it is mediated through the formation of hydrogen sulfide in the rooting zone of wild rice, and that elevated iron would mitigate the toxicity of the sulfide by forming insoluble iron sulfide compounds.

The Study components include:

- Field study of wild rice habitats to investigate physical and chemical conditions correlated with the presence or absence of wild rice stands, including concentrations of sulfate in surface water and sulfide in the rooting zone.
- Controlled laboratory hydroponic experiments to determine the effect of elevated sulfate and sulfide on wild rice growth and development.
- Outdoor container experiments utilizing natural sediments to determine the response of wild rice to a range of sulfate concentrations in the surface water, and associated sulfide in the rooting zone.
- Collect and analyze rooting zone depth profiles of dissolved chemicals at wild rice outdoor experiments and field sites to characterize sulfate, sulfide, and iron in the rooting zone of wild rice.
- Sediment incubation study to explore the difference ambient temperature has on the rate that elevated sulfate concentrations in water enter underlying sediment, convert to sulfide, and later release sulfate back into the overlying water.

The MPCA will review the results from individual reports along with existing monitoring data, other relevant scientific studies, pertinent ecological, cultural and historical information, and the original basis for the wild rice sulfate standard to determine if a change to the current wild rice sulfate standard is warranted, and what that change might be. If change(s) are proposed, they would be adopted into Minnesota Rules via the administrative rulemaking process and subject to U.S. EPA approval before the changes could be implemented.

In the data reported here, high resolution (1.5 cm) pore water samples were collected from two situations: (1) Sulfate-amended wild rice microcosms throughout the summer of 2013 and analyzed for sulfate, sulfide, iron, pH and two trace metals (copper and zinc). Results were used to characterize the effect of amended sulfate on the rooting zone geochemistry and to define seasonal differences in the rooting zone geochemistry to which wild rice populations in the tanks were exposed. (2) Two field sites, in an attempt to characterize seasonal variability of porewater geochemistry in a field setting (Second Creek and Sandy Lake).

Sulfur participates in a host of chemical and biological reactions in anoxic sediment. The oxidized form of sulfur, sulfate, is present in most oxygenated surface waters and moves into reduced sediment pore fluids via diffusive or advective transport. Once sulfate reaches an area where oxygen and other more favorable electron acceptors have been consumed, biologically mediated reactions reduce sulfate (+VI oxidation state) to sulfide (-II oxidation state) or other intermediate forms. In addition to heterotrophic anaerobic metabolism, sulfide can also be produced via methane oxidation by anaerobic bacteria, which reduces sulfate to sulfide (Nauhaus et al. 2002). If transported to an area of more oxidizing conditions, sulfide can be re-oxidized to sulfate by oxygen or other electron acceptors, even without bacterial involvement. Though sulfate is relatively unreactive under oxidizing conditions, sulfide participates in a host of different reactions including acid-base, complexation, precipitation/dissolution, and volatilization. As a diprotic acid, freely dissolved sulfide exists in one of two primary forms at environmentally relevant pH values: hydrogen sulfide (H_2S) at low pH (below $pK_1 = 7.1$), predominantly as a dissolved gas, or as bisulfide (HS^-) above pH 7.1. Sulfide forms insoluble complexes with many metals including lead, copper, cadmium, iron, nickel, zinc, and manganese, in rough order of low to high solubility.

As a result of this complex geochemical setting in sediment pore fluids, a number of different chemical and biological reactions influence dissolved sulfide concentrations. The presence of more favorable electron acceptors can inhibit the production of sulfide by limiting the activity of sulfate reducing bacteria, which produce sulfide. Environments with pH significantly higher than 7 can shift the speciation of dissolved sulfide to bisulfide and limit the quantity of H_2S , a dissolved gas that may readily cross plant membranes (Armstrong and Armstrong 2005). Perhaps most importantly, the presence of dissolved, uncomplexed, or loosely bound ferrous iron in sediments can precipitate with dissolved sulfide due to the low solubility of ferrous-sulfide compounds (Lovley and Phillips, 1986).

If dissolved iron is present in excess, it can continue to keep dissolved sulfide concentrations low for some time even in the event of a consistent source of sulfide. It is important to note that ferrous iron in sediments exists in several forms, and that the dissolved fraction in porewaters typically makes up a relatively small amount of the total iron. In addition to the truly dissolved fraction, ferrous iron exists in forms loosely bound to the solid phase (organic complexes, bound to silicate or other mineral surfaces) and in more recalcitrant, insoluble forms (pyrite) (Rauret 1997). These solid phase pools of iron are often 10s to 100s of times more abundant than the quantity of iron dissolved in pore fluids. The dissolved fraction and many of the loosely bound fractions are available to consume sulfide introduced

to sediment, while recalcitrant forms do not participate in many reactions if they remain in strongly reduced conditions.

2. Methods

2.1. Experimental setting

Sulfate amendments to tank mesocosms (Appendix Figure A7) similar to those used by Walker et al. 2010 began in Summer 2011 and continued through 2012 and 2013 at five different amendment levels: control (~10 mg sulfate /L), low (50 mg sulfate / L), medium-low (100 mg sulfate / L), medium (150 mg sulfate / L), and high (300 mg sulfate / L). Water and sulfate levels in mesocosms were maintained by weekly sampling and appropriate additions of well water (~10 mg/L sulfate) and sodium sulfate stock solution to account for rain water dilution, evaporation, and flux of sulfate into sediment. During the fall of 2012, overlying water sulfate levels were not maintained actively after plant senescence in September and sulfate amendments did not begin again until June 2013.

During the summer of 2013, one end of each of the 30 mesocosm tanks was isolated from plant roots with a thin Plexiglas sheet. The Plexiglas sheet was placed approximately 17 cm from the end of each tank, isolating about 15% of the sediment in each tank. Plants were not allowed to develop in this zone of each mesocosm. The purpose of the plant-free zone was to assess the effect of wild rice roots on porewater. Macrophyte roots may oxidize sediment by releasing oxygen (Armstrong and Armstrong 2005) or reduce sediment by releasing dissolved organic compounds (e.g. Windham-Myers et al. 2013).

2.2. Sampling design

Mesocosms

During the summer of 2012, passive porewater equilibrators (peepers) were deployed to collect depth-profiles of samples approximately monthly in duplicate control, low (50 mg/L), medium (150 mg/L), and high (300 mg/L) mesocosms.

During the summer of 2013, peepers were again deployed monthly at the four sulfate treatment levels. Once a month from May to September, duplicate peepers were deployed within an individual tank at each treatment level. One peeper was inserted in the plant-free zone approximately 5 cm away from the Plexiglas sheet and the other peeper was located near the center of the tank where wild rice was allowed to grow. Sediment cores were extracted monthly during peeper retrieval in locations coincident with peepers, and sectioned into intervals consistent with peeper well spacing.

Field sites

In an attempt to characterize seasonal variability of porewater geochemistry in a field setting, peepers were also deployed at two field sites: one river site, Second Creek (47.52042 -92.1925), and one lake site, Sandy Lake (47.61872 -92.59314) during the summer of 2013. Both sites have sulfate concentrations in the overlying water elevated above regional background levels (150 – 700 mg/L, in contrast to regional background levels of less than 10 mg/L). Both sites have potential for groundwater flow due to coarse sediment and regional hydraulic gradients imposed by human alterations of the landscape. At each site, duplicate peepers were deployed and collected monthly: one in an area of coarser sediment and one in an area of finer, organic sediment. The solid phase was also characterized monthly at these field sites, but was limited spatially to an analysis of the homogenized top 10 cm of

sediment. The results from field samples collected with peepers are reported and described in Appendix B.

2.3. Sampling methods

Peepers similar to those described in Teasdale et al. (1995) were constructed of half-inch thick, 24 x 6 inch polycarbonate sheet plastic beveled at one end and milled to contain thirty-five 1-cm deep wells spaced 1.56 cm apart. A vinyl T-post was affixed to one side with machine screws to provide a hard surface for pounding while driving peepers into coarse river and lake sediments. Large diameter (~12 inch circular filter paper (0.45 μm polyethersulfone, Supor, Pall Life Sciences) and a protective nylon mesh (200 μm openings, Industrial Netting) was placed over the wells and secured in place using a face plate with openings corresponding to each well. Small stainless steel machine screws were used to seal the face plate tightly against the filter paper and avoid the movement of water bypassing filter material or between adjacent wells.

Peepers were assembled in the lab while submerged in distilled water to avoid any bubbles and immediately placed in an upright container filled with distilled water purged of oxygen by a continuous flow of nitrogen through a fine bubble diffuser. This nitrogen purge continued for at least 1 week until peeper deployment. Peepers were transported in deoxygenated water to field sites, inserted into sediment, and secured to the edge of mesocosms or to shore or a buoy at field sites. Following a 2-3 week deployment (US EPA, 2001), peepers were removed from the sediment, submerged in water purged of oxygen, and placed in a nitrogen-filled bag during the brief transport to a processing station. Mud was quickly removed from the filter paper surface and the peeper was placed into another nitrogen-filled bag for sample extraction (Koretsky et al. 2006). Beginning with the deepest well, samples were extracted from each peeper by puncturing the nitrogen-filled bag, nylon mesh, and filter paper with a hypodermic needle affixed to a polypropylene syringe barrel. The extracted sample was immediately injected into vials preloaded with reagents for iron and sulfide analysis. pH was immediately measured on another sample aliquot prior to acidification (0.5 % concentrated nitric acid) for metals preservation, and the final aliquot of sample - for anion analysis - was filtered through a cation exchange filter (Dionex, model #) to remove iron, acidified with 0.5 % concentrated hydrochloric acid, and purged with nitrogen (10 minutes) to remove dissolved sulfide. While all anion samples were acidified, only those with a noticeable colorimetric response for sulfide were purged with nitrogen. The entire extraction and preservation process for 12-16 wells in a single peeper typically took less than 45 minutes, during which the peeper was under a nitrogen atmosphere.

2.4. Analytical methods

Sulfate was measured on a Dionex ICS-1100 Ion Chromatograph (Thermo Scientific AS22 IonPac 4x250 mm anion exchange column) using the Chromelion software for peak integration. Sulfide and ferrous iron were quantified spectrophotometrically using the methylene blue method (Eaton et al. 2005a) as implemented with Hach sulfide reagents (Hach, 2010) and the phenanthroline method (Eaton et al. 2005b), respectively. The sulfide method quantifies all dissolved sulfide (both H_2S and HS^-), and is therefore not sensitive to pH. Sulfide was measured within an hour of collection on a field spectrophotometer and iron was stored on ice and transported to the lab for quantification. pH was measured with a Thermo Orion epoxy pH electrode with temperature correction calibrated in the field immediately prior to sample measurements. Zinc, copper, and total iron were quantified on an Elan 3000 ICP-MS using a customized standard from Inorganic Ventures. The detection and reporting limits for each analyte are summarized in Table 1. Reporting limit was calculated as the 99% confidence

interval based on the standard deviation of low-level samples or blanks. Detection limit is the 90% confidence interval.

AVS was quantified at the Minnesota Department of Health (MDH) as described in the MDH Environmental Health laboratory's SOP "Procedure for the Determination of Dissolved Sulfide in Water and Soil with Inline Distillation by Flow Injection Analysis Colorimetry" located in Appendix C of the Minnesota Wild Rice Sulfate Standard 2013 Field Survey - Quality Assurance Project Plan. Total sulfur was quantified at the National Lacustrine Core Facility (LacCore) using a coulometric titration method described in the laboratory's SOP, located in Appendix I of the 2013 Field Survey QAPP cited above. Extractable iron on the solid phase was quantified by the Science Museum of Minnesota - St. Croix Watershed Research Station with the Chemistry Department of Gustavus-Adolphus College by ICP-MS analysis (Agilent technologies 7700 Series ICP-MS)(EPA Method 6020A). These methods are detailed in Appendix F and G of the 2013 Field Survey QAPP.

3. Results

3.1. Vertical spatial gradients

Clear vertical spatial gradients were observed in all analytes quantified in mesocosm porewaters (Appendix Figure A1). For peepers placed in mesocosms, wells 1-3 were above the sediment-water interface and well 4 was the first sample collected from pore fluids. Sulfate was typically present at or near the target amendment level in wells 1-3 and less than 5 mg/L at all depths below well 6. One exception to this was in the high amendment tanks where sulfate was elevated in the deepest wells early in the summer. In the peepers placed in the middle of high sulfate amended tanks (Appendix Figure A1, panels a-d), a consistent reduction in porewater sulfate concentrations was observed between wells 4-10 as the summer progressed. This reduction in porewater sulfate is consistent with the biologically-driven conversion of sulfate to sulfide in anoxic sediments. The likely cause of sulfate penetration to deeper depths in some mesocosm porewaters is the movement of sulfate around the edges of the tanks when frozen sediment thawed early in the spring. Observations of a gap between the edge of sediment and the tank wall were made in several tanks during the peeper deployment in May 2013. This penetration of water into the bottoms of tanks could directly elevate sulfate concentrations in deep porewater in the case of amended tanks, or result in the oxidation of reduced sulfides at depth if oxidized water was transported to anoxic sediments at deeper depths. The consistent steep sulfate gradients observed in all amended mesocosms between wells 3-6 represent a driving force for net diffusional transport of sulfate into sediments. The maintenance of these gradients throughout the summer suggests a net consumption of sulfate from the porewaters at depths between 0-5 cm.

Observations of porewater sulfide concentrations were consistent with this biologically driven sulfate reduction in surficial sediments (Appendix Figure A2). Sulfide was consistently absent from the overlying water and highest in the first 1-2 wells below the sediment water interface. The peaks in porewater sulfide immediately below the sediment-water interface are indicative of net sulfide production at this depth and diffusional transport in both vertical directions. Oxygenated overlying water was likely responsible for rapid consumption of sulfide as it diffused upward. Lower sulfide concentrations at deeper sediment depths are likely a result of precipitation with dissolved iron and an absence of transport of sulfate from overlying water. Iron concentrations were highest in the deepest sediment pore fluids and lower near the sediment-water interface (Appendix Figure A3). In the porewaters of high and medium amendments, iron was depleted in the areas of elevated sulfide providing clear evidence for the consumption of sulfide from porewaters due to precipitation with ferrous iron. Oxidized overlying water also likely removed iron from porewaters near the sediment-

water interface in both unamended and amended mesocosms. The sustained gradients in iron represent a driving force for net transport of iron towards the surface water, in the opposite direction of sulfate. Though no external source of iron is present in mesocosms, a labile pool of ferrous iron on the solid phase makes a direct stoichiometric comparison of sulfate and iron measurements impossible.

The elimination of roots from one end of the mesocosms was expected to create further reducing conditions in sediment due to the removal of the potential oxidizing influence of rice roots on sediment geochemistry; however, the proximity of the rootless zone to the edge of the mesocosm may have created edge effects due to preferential transport of oxidized overlying water along the mesocosm tank walls. While differences among sulfate treatments were very clear, no consistent trend was apparent in porewater observations made in the same mesocosms in rooted and unrooted (edge) locations for any analytes (Appendix Table A1).

The presence of aquatic macrophyte roots can influence sediment sulfur processes in at least two important ways. The exudation of low molecular weight dissolved organic carbon from plant roots can provide energy for the heterotrophic metabolism of sediment microbes, including sulfate reducers (Holmer and Nielsen 1997). However, relative plant densities and the pool of organic carbon in the solid phase appears to greatly affect the rate of sulfate reduction and importance of root-derived organic carbon (Holmer et al. 1998, Boschker et al. 1999). The transport of oxygen to sediment surrounding plant roots and sediment-dwelling organism burrows has been shown to increase redox potentials and decrease concentrations of dissolved sulfide in sediment (Benoit et al. 2006, Schmidt et al. 2011). However, the flux of oxygen sufficient to oxidize sediment and avoid the buildup of sulfide likely depends on plant density, the organic content of sediment, and overall oxygen demand of the rooting zone (Holmer and Storkholm 2001).

3.2. Seasonal differences in porewater geochemistry

A summary of sulfate, sulfide, and iron in the porewaters of amended and control mesocosms is presented in Figure 1 and Appendix Table A1. Log scales are used for sulfate and sulfide due to very large differences among observations in different sulfate treatment levels. Values were averaged over either the top 10 cm (Figure 1, panels a-c) or the top 5 cm (Figure 1, panels d-f) to represent bulk concentrations in the rooting zone of rice plants. In the mesocosms, the sediment water interface was consistently between peeper wells 3 and 4 and for the purposes of this report, 0-5 cm averages are the average of wells 4-6 and 0-10 cm averages are the average of wells 4-10. Because clear differences were not present between edge (rootless zone) and mesocosm middle porewater chemistry, the average of two measurements made monthly in each tank were used in the summary. μM

Consistent with observations in field conditions (Koretsky et al. 2006), clear differences in average porewater sulfate, sulfide, and iron were apparent among treatments. Sulfate from the overlying water penetrates into the pore fluids of the amended mesocosms, resulting in increasing average porewater sulfate with amendment levels. Sulfide followed a similar trend with 0-10 cm average concentrations exceeding 50 μM in the high and medium amended porewater in July and August and below 10 μM in control and low amended porewater during all seasons. Porewater iron followed an inverse trend with control and low amended porewaters having similarly high average iron concentrations between 200 and 400 μM , while medium and high amended porewaters had iron concentrations less than 150 μM in all seasons. pH showed a pattern characteristic of aquatic sediments with a decrease near the sediment-water interface (relative to overlying water) due to acid-producing reactions in surficial sediments (Appendix Figure A4). Porewater pH was consistently between 6.75 and 7.25 in the 0-5 cm sediment interval and no strong trend in pH was observed either with season or sulfate amendment

level. This consistent, near neutral pH in surficial sediment of mesocosms means that the fraction of sulfide present in pore waters as the dissolved gas H₂S is between 40 and 70 % of total dissolved sulfide.

In the high and medium sulfate amended mesocosms, porewater concentration in the 0-5 cm interval were higher than in the 0-10 cm interval for sulfate and sulfide due to the sulfur source in the overlying water (Appendix Table A2). The only exception to this was sulfate in May, when the mesocosm amended with high sulfate experienced some preferential transport of sulfate or oxidation of sulfide in the bottom sediments. This trend of elevated sulfate and sulfide in the 0-5 cm interval was less pronounced but still consistently present in the mesocosms amended with low sulfate. In unamended tanks, surficial sediment was not consistently elevated due to relatively less sulfate transport from overlying water. Iron was lower in the 0-5 interval in all seasons and at all amendment levels.

Seasonal trends were most readily apparent in the medium and low amended mesocosm porewaters where sulfide increased and iron decreased throughout the summer 2013 season (Figure 1). These trends are consistent with the transport of sulfate from the overlying water, reduction to sulfide, and precipitation of iron sulfide solid phases as the summer progressed. Also consistent with increasing sulfate penetration from amended overlying water, a trend of increasing sulfate in surficial sediment was also seen in the medium and low amended mesocosm porewater. Timescales of diffusion can be estimated as $t_D = L^2 / D_{eff}$, where L is the distance and D_{eff} is the effective diffusion. With an effective diffusion of 8x10⁻⁶ cm² / sec, the approximate timescale for diffusion over a distance of 3-5 cm is 13 – 36 days, consistent with the observed seasonally increasing porewater sulfate.

3.3. Iron-sulfide equilibrium

The equilibrium of iron and sulfide is described by Equations 1 and 2.



$$K_{sp} = \frac{[Fe^{2+}]_{eq} [HS^-]_{eq}}{[H^+]_{eq}} \quad (2)$$

Although equation 2 describes the maximum concentration of iron and sulfide possible at chemical equilibrium, higher or lower concentrations are possible under conditions in which reactions or transport changes porewater concentrations more quickly than the processes of precipitation and dissolution.

The rate of precipitation of iron sulfide solid phases can be quantified in proportion to the oversaturation of porewaters with respect to iron and sulfide (Van Capellen and Wang 1996). Equation 3 describes the ion activity product (IAP), which is calculated using measured porewater concentrations. The IAP allows for comparison of measured concentrations with concentrations that would be present at equilibrium with a solid phase as described by Equation 2. Comparison of the IAP with the solubility product, K_{sp}, can be used to quantify the over or undersaturation of water chemistry with respect to a solid-phase mineral.

$$IAP = \frac{[Fe^{2+}] [HS^-]}{[H^+]} \quad (3)$$

Measured concentrations were used to calculate the IAP for the seasonal mesocosm porewaters as a means to quantify the potential for precipitation and accumulation of sulfide solid phases in the

sediment (Appendix Figure A5). Measurements of total dissolved sulfide were adjusted for pH to quantify the amount of bisulfide (HS^-) present for use in calculating the IAP. The steep vertical spatial gradients observed in depth-profiles (Appendix Figures A1-A3) showed that the 0-5 cm interval is the area in which iron and sulfur are undergoing the most rapid transformations. The average IAP over both the 0-5 cm and 0-10 cm interval is shown in Figure 2. Higher values (less negative) of the IAP represent conditions more saturated with respect to iron sulfide precipitation. The degree of oversaturation in mesocosm porewaters was clearly related to the sulfate amendment level. Seasonal trends in the 0-5 cm average IAP were present in the porewaters of the four amendment levels monitored, indicating increasing oversaturation of porewaters with iron and sulfide as the summer progressed.

Two common iron sulfide solid phases are *amorphous iron sulfide* and *mackinawite* having (log) solubility products of -2.7 to -3.0, and -3.5 to -4.2, respectively (Stumm and Morgan, 1996). Observations in the 0-5 cm interval of the control and low sulfate amended mesocosms show conditions at or near saturation with respect to amorphous iron sulfide. In the medium and high amended mesocosm porewaters, conditions were consistently oversaturated with respect to amorphous iron sulfide during all seasons. The control mesocosm had an ion activity product between the solubility product of amorphous iron sulfides and mackinawite during all seasons. This oversaturation of porewaters with iron and sulfide highlights the critical role of ferrous iron in controlling dissolved sulfide in porewaters and indicates that the precipitation of solid phase iron sulfides in surficial sediments represents a sink for removing dissolved iron and sulfide from sediment porewaters. It also provides an explanation for the consistent and sustained diffusional gradients of observed in porewaters for sulfate (Appendix Figure A1, from overlying water) and iron (Figure 1c, from deeper sediments).

If the rate of iron sulfide precipitation is proportional to the extent of oversaturation (Van Cappellen and Wang, 1996), the rates of iron sulfide precipitation can be compared among treatment levels.

$$r_{precip} = k_{precip} \frac{[Fe^{2+}][HS^-]}{[H^+][K_{sp}]} \quad (4)$$

Table 2 gives the ratio of the 0-5 cm average IAP in high mesocosm porewaters relative to the 0-5 cm average IAP in the porewaters of other mesocosms. These calculations suggest that iron sulfide precipitation is occurring 1-4 times, 3-12 times, and 30 to 40 times faster in the high mesocosms than in medium, low, and control mesocosms respectively.

3.4. Sulfur flux & accumulation

The net diffusional flux of sulfur from the overlying water into sediment mesocosms can be estimated using the observed concentration gradients near the sediment water interface. At 20 degrees celcius, the diffusion of sulfate in water adjusted for 95% porosity is $0.74 \text{ cm}^2/\text{day}$ ($8.6 \times 10^{-6} \text{ cm}^2/\text{sec}$) (Boudreau 1996). The observed difference in sulfate concentration between the wells located adjacent to the sediment-water interface were used to calculate the concentration gradient and estimate the net diffusive sulfate flux.

$$Flux_{SO_4^{2-}} = D_{eff} \frac{dC_{SO_4^{2-}}}{dx} \quad (5)$$

Figure 3 shows the net diffusive flux estimates for the four mesocosm treatment levels. Note the log scale due to large differences among observations. Table 3 gives the ratio of sulfate flux in high mesocosms relative to other mesocosms. Excluding the estimates which were affected by the intrusion

of sulfur into deeper sediments in the early summer, these calculations suggest that sulfate flux is occurring 2-4 times, 5-11 times, and 60 to 90 times faster in the high mesocosms than in medium, low, and control mesocosms respectively. These ranges are close to those independently estimated for iron-sulfide precipitation rates (Table 2).

Samples were collected in May and October 2013 to quantify the accumulation of sulfur in the solid phase by AVS and total sulfur. However, at the time of this report, total sulfur and AVS measurements were not available from the analytical labs to which they were sent. Flux estimates made from observed sulfate gradients and estimates of iron sulfide precipitation rates will be compared with the increases in sulfide observed in sediment solid phases when the data become available.

3.5. Sulfide's influence on zinc and copper in porewater

Elevated sulfide in the rooting zone of wild rice has the potential to limit the bioavailability of important trace elements including copper and zinc, which are both essential plant nutrients. Depth profiles of copper and zinc measurements made in the pore fluids of mesocosms during June, July, and August and are shown in Appendix Figure A5, and summarized in Figure 4 (0-5 cm averages) and Appendix Table A3. No clear depth-dependent trend associated with elevated sulfide in the 0-5 cm region of high and medium amended microcosms was apparent (Appendix Figure A5) in either copper or zinc observations. However, copper concentrations in pore water were higher in sulfate amended mesocosms during all seasons (Figure 4). Porewater zinc showed no trend with sulfate amendment level.

Figure 5 shows a log-log plot of pore water copper and zinc observations against sulfide observations made in the same peeper sample. Copper shows an increasing trend with increasing sulfide, while there is no trend in the zinc observations relative to sulfide. The significant scatter in the porewater metals measurements suggests that sulfide is not a major controlling factor for either zinc or copper in porewaters. Relative to the 2 to 3 order of magnitude differences observed in pore water sulfide among amendment levels, copper and zinc varied little in all pore fluid samples (about one order of magnitude). The reason for the trend of increasing copper with increasing sulfide was not ascertained with certainty, though previous research has shown that copper polysulfides can be important in defining copper solubility in the presence of covellite (copper sulfide, Shea and Helz, 1988).

4. Conclusions

The measurements made in the rooting zone of wild rice mesocosms provide a clear picture of the porewater geochemical environment in which rice plants grew during the 2013 season. Rice plants in the different treatments were exposed to a range of porewater sulfide concentrations in the top 10 cm of sediment, ranging from less than 1 μM on average in control mesocosms to over 50 μM on average for mesocosms amended with 300 mg/L sulfate in the overlying water. Additionally, measurements made provide a more detailed picture of the vertical structure of sediment chemistry present in wild rice rooting zones that cannot be captured by bulk sediment characterizations.

The observed vertical spatial gradients in the closed hydrologic tanks allow for the quantitative evaluation of transport and reaction rates related to iron sulfide precipitation. The significant oversaturation with respect to iron sulfide minerals observed in surficial porewaters of sulfate amended mesocosms highlights the critical role of iron in controlling sulfide in porewaters. In contrast to the hydrologically isolated mesocosms, groundwater or other sources could deliver iron to sediment in a field setting. While field systems are more difficult to analyze quantitatively, the results presented here

provide a clear indication that sulfate from the overlying water can be reduced to sulfide and accumulate in sediment pore fluids when iron is not present in excess quantities.

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Figures and Tables

Figure 1. Seasonal average porewater concentrations in control and amended mesocosm porewaters.

Figure 2. Seasonal average ion activity product in control and amended mesocosm porewaters.

Figure 3. Seasonal diffusional sulfate flux across the sediment-water interface in control and amended mesocosms.

Figure 4. Seasonal 0-5 cm average mesocosm porewater concentrations of (a) copper, and (b) zinc.

Figure 5. Scatter plot of porewater metals concentration vs. porewater dissolved sulfide.

Table 1. Methods, reporting units, and detection and reporting limits for chemical analyses.

Table 2. Ratio of 0-5 cm average IAP relative to porewaters in high amendment mesocosm.

Table 3. Ratio of sulfate flux across the sediment-water interface relative to that observed in high amendment mesocosm.

Table 1 Methods, reporting units, and detection and reporting limits for chemical analyses.

| Parameter Name | Units | Method | Detection Limit | Reporting Limit |
|----------------|----------------------|---|-----------------|-----------------|
| Iron (ferrous) | micromoles / liter | Phenanthroline spectrophotometry | 2.78 | 5.05 |
| Sulfide | micromoles / liter | Hach spectrophotometry (methylene blue) | 0.69 | 1.89 |
| pH | $-\log[H^+]$ (moles) | glass / epoxy pH electrode | | |
| Sulfate | milligrams / liter | Ion chromatography | 0.11 | 0.20 |
| Copper | micrograms / liter | Inductively coupled mass spectrometry | 0.23 | 0.42 |
| Zinc | micrograms / liter | Inductively coupled mass spectrometry | 1.49 | 2.71 |
| Iron-Total | micrograms / liter | Inductively coupled mass spectrometry | 394 | 715 |

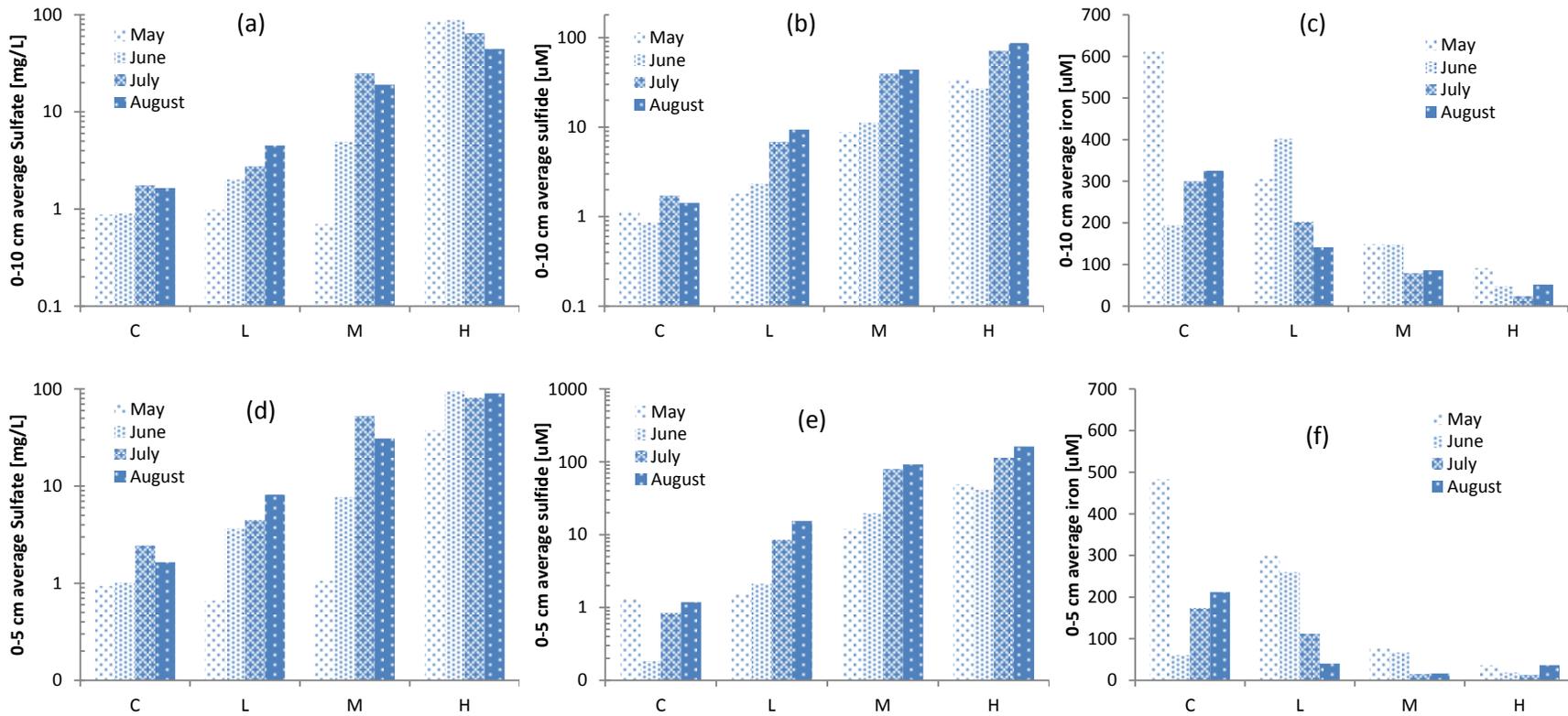


Figure 1 Seasonal average porewater concentrations in control and amended mesocosm porewaters. (a) 0-10 cm average sulfate, (b) 0-10 cm average sulfide, (c) 0-10 cm average iron, (d) 0-5 cm average sulfate, (e) 0-5 cm average sulfide, (f) 0-5 cm average iron. Values represent the average of measurements made in center and edge (rootless zone) of mesocosms.

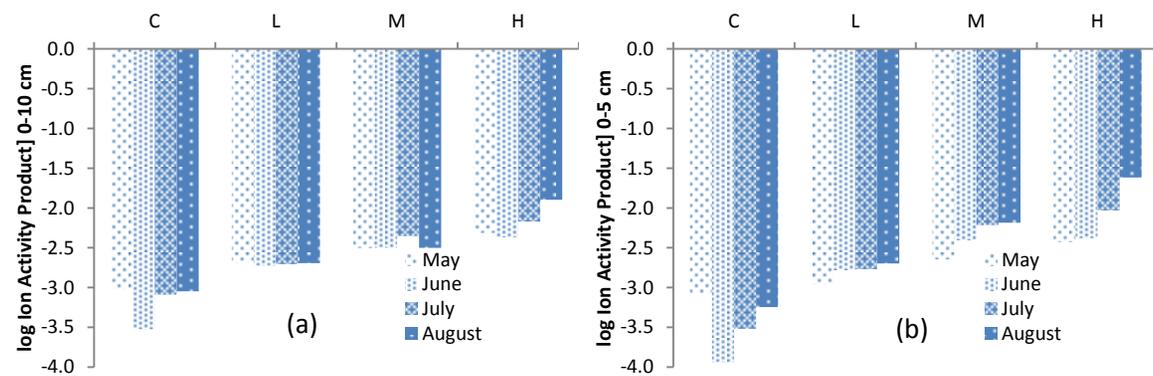


Figure 2 Seasonal average ion activity product in control and amended mesocosm porewaters. (a) 0-10 cm average IAP, (b) 0-5 cm average IAP.

Table 2 Ratio of 0-5 cm average IAP relative to porewaters in high amendment mesocosm.

| | H:C ratio | H:L ratio | H:M ratio |
|--------|-----------|-----------|-----------|
| May | 4.462 | 3.310 | 1.641 |
| June | 36.032 | 2.526 | 1.060 |
| July | 30.644 | 5.465 | 1.524 |
| August | 42.537 | 12.046 | 3.701 |

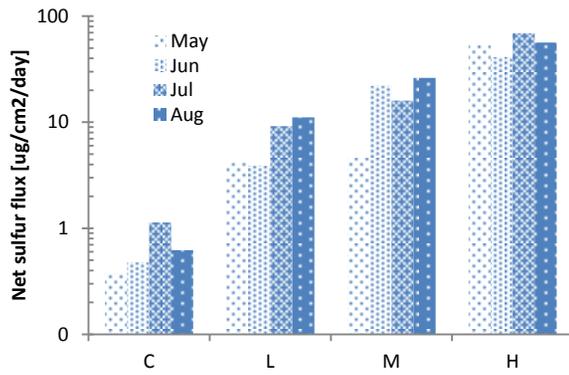


Figure 3 Seasonal diffusional sulfate flux across the sediment-water interface in control and amended mesocosms.

Table 3 Ratio of sulfate flux across the sediment-water interface relative to that observed in high amendment mesocosm.

| | H:C ratio | H:L ratio | H:M ratio |
|--------|-----------|-----------|-----------|
| May | 142.8 | 12.9 | 11.7 |
| June | 86.1 | 10.6 | 1.9 |
| July | 60.8 | 7.5 | 4.3 |
| August | 90.7 | 5.1 | 2.2 |

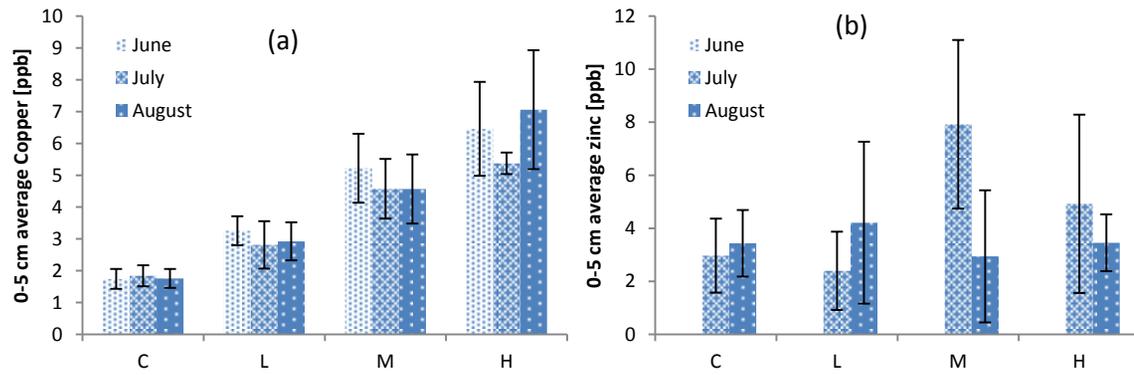


Figure 4 Seasonal 0-5 cm average mesocosm porewater concentrations of (a) copper, and (b) zinc.

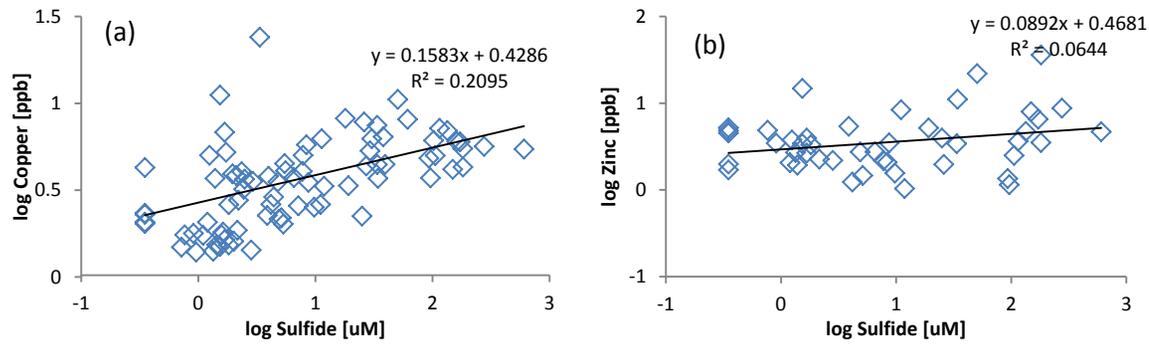


Figure 5 Scatter plot of porewater metals concentration vs. porewater dissolved sulfide. (a) log copper vs. log sulfide, (b) log zinc vs. log sulfide.

Appendix A. Depth profiles for chemical parameters in mesocosm porewater

Figure A1. Sulfate concentrations in wild rice mesocosm porewater.

Figure A2. Dissolved sulfide concentrations in wild rice mesocosm porewater.

Figure A3. Ferrous iron concentrations in wild rice mesocosm porewater.

Figure A4. pH in wild rice mesocosm porewater.

Figure A5. Ion activity product for iron sulfide in wild rice mesocosm porewater.

Figure A6. Dissolved zinc and copper concentrations in mesocosm porewater.

Fig. A7. Schematic of the stock tanks used as mesocosms.

Table A1. Comparison of porewater concentrations in 0-10 cm interval of rooted and unrooted zones of mesocosms.

Table A2. Seasonal average porewater concentrations in control and amended mesocosms.

Table A3. Zero to 5 cm average metals concentration in mesocosm porewater in June, July, and August 2013.

Figure A1 Sulfate concentrations in wild rice mesocosm porewater. Panels a-d: Center of 300 mg/L amended mesocosms in May, June, July, August, respectively; Panels e-h: Edge (rootless zone) of 300 mg/L amended mesocosms in May, June, July, August, respectively. Panels i-l: Center of 150 mg/L amended mesocosms in May, June, July, August, respectively; Panels m-p: Edge (rootless zone) of 150 mg/L amended mesocosms in May, June, July, August, respectively. Panels q-t: Center of 50 mg/L amended mesocosms in May, June, July, August, respectively; Panels u-x: Edge (rootless zone) of 150 mg/L amended mesocosms in May, June, July, August, respectively. Panels aa-ad: Center of control mesocosms in May, June, July, August, respectively; Panels ae-ah: Edge (rootless zone) of control mesocosms in May, June, July, August, respectively.

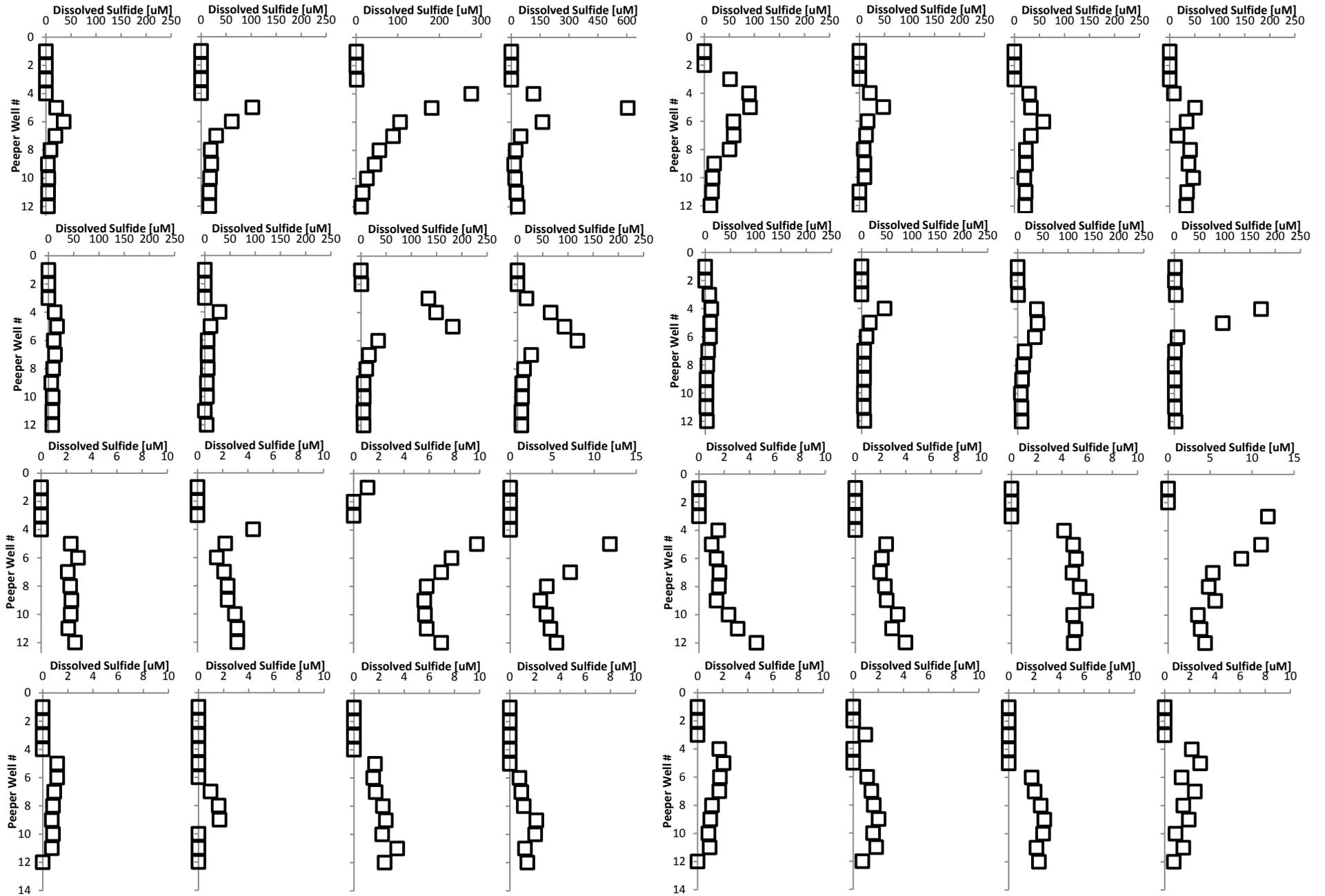


Figure A2 Dissolved sulfide concentrations in wild rice mesocosm porewater. Panels a-d: Center of 300 mg/L amended mesocosms in May, June, July, August, respectively; Panels e-h: Edge (rootless zone) of 300 mg/L amended mesocosms in May, June, July, August, respectively. Panels i-l: Center of 150 mg/L amended mesocosms in May, June, July, August, respectively; Panels m-p: Edge (rootless zone) of 150 mg/L amended mesocosms in May, June, July, August, respectively. Panels q-t: Center of 50 mg/L amended mesocosms in May, June, July, August, respectively; Panels u-x: Edge (rootless zone) of 150 mg/L amended mesocosms in May, June, July, August, respectively. Panels aa-ad: Center of control mesocosms in May, June, July, August, respectively; Panels ae-ah: Edge (rootless zone) of control mesocosms in May, June, July, August, respectively.

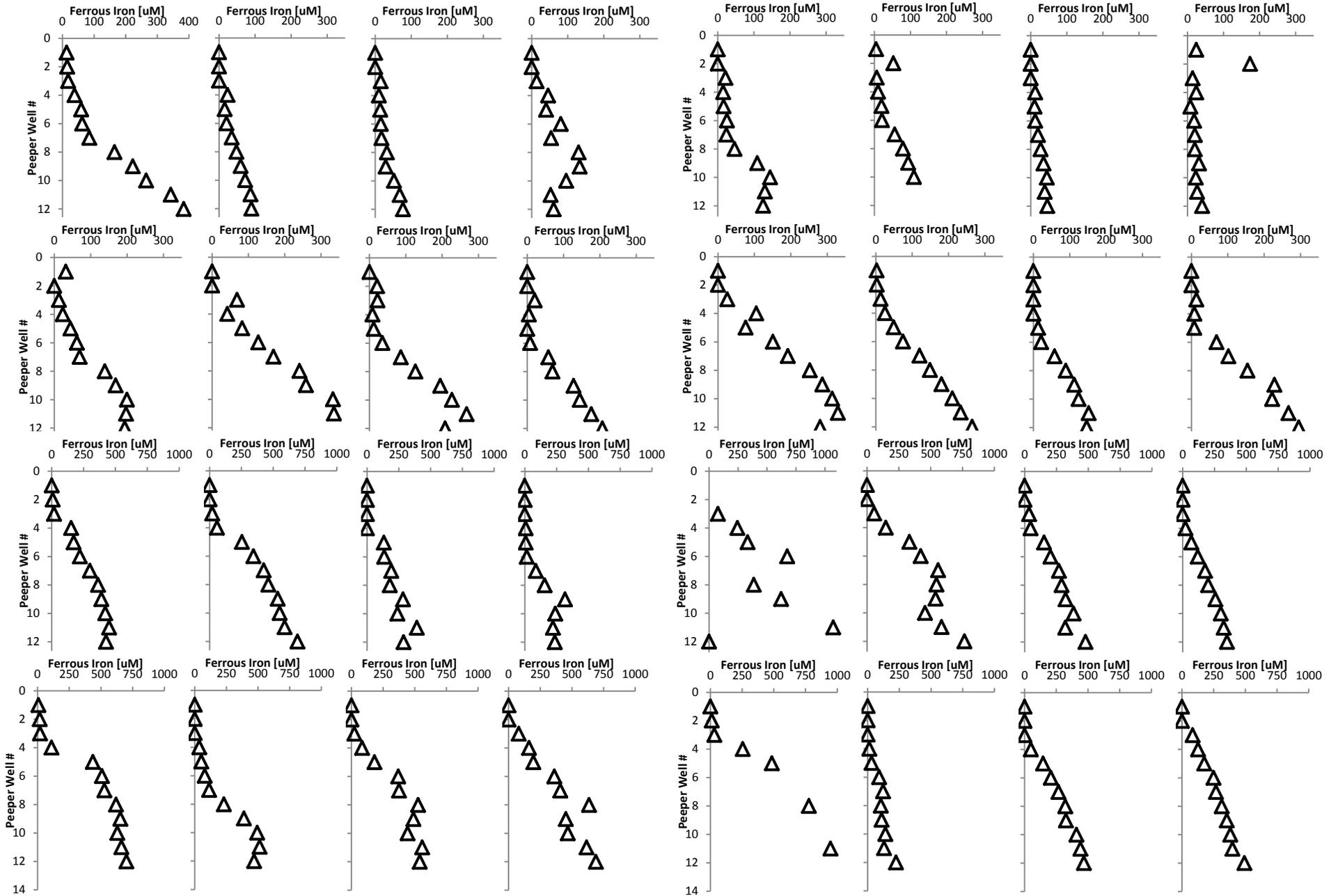


Figure A3 Ferrous iron concentrations in wild rice mesocosm porewater. Panels a-d: Center of 300 mg/L amended mesocosms in May, June, July, August, respectively; Panels e-h: Edge (rootless zone) of 300 mg/L amended mesocosms in May, June, July, August, respectively. Panels i-l: Center of 150 mg/L amended mesocosms in May, June, July, August, respectively; Panels m-p: Edge (rootless zone) of 150 mg/L amended mesocosms in May, June, July, August, respectively. Panels q-t: Center of 50 mg/L amended mesocosms in May, June, July, August, respectively; Panels u-x: Edge (rootless zone) of 150 mg/L amended mesocosms in May, June, July, August, respectively. Panels aa-ad: Center of control mesocosms in May, June, July, August, respectively; Panels ae-ah: Edge (rootless zone) of control mesocosms in May, June, July, August, respectively.

Table A1 Comparison of porewater concentrations in 0-10 cm interval of rooted and unrooted zones of mesocosms. Values are % difference from center porewater concentrations.

| | Sulfate | | | | Iron | | | | Sulfide | | | |
|--------|---------|-------|------|------|------|------|-----|------|---------|------|------|-----|
| | H | M | L | C | H | M | L | C | H | M | L | C |
| May | -85% | -27% | 43% | -53% | -58% | 95% | 12% | 46% | 314% | -38% | -25% | 85% |
| June | -40% | 179% | -30% | 7% | 41% | -35% | 14% | -5% | -50% | 40% | -18% | 83% |
| July | 44% | 107% | 74% | 27% | -5% | -40% | 43% | -30% | -72% | -63% | -42% | 0% |
| August | 31% | -100% | -65% | 4% | -75% | 92% | 37% | -30% | -76% | -17% | -4% | 88% |

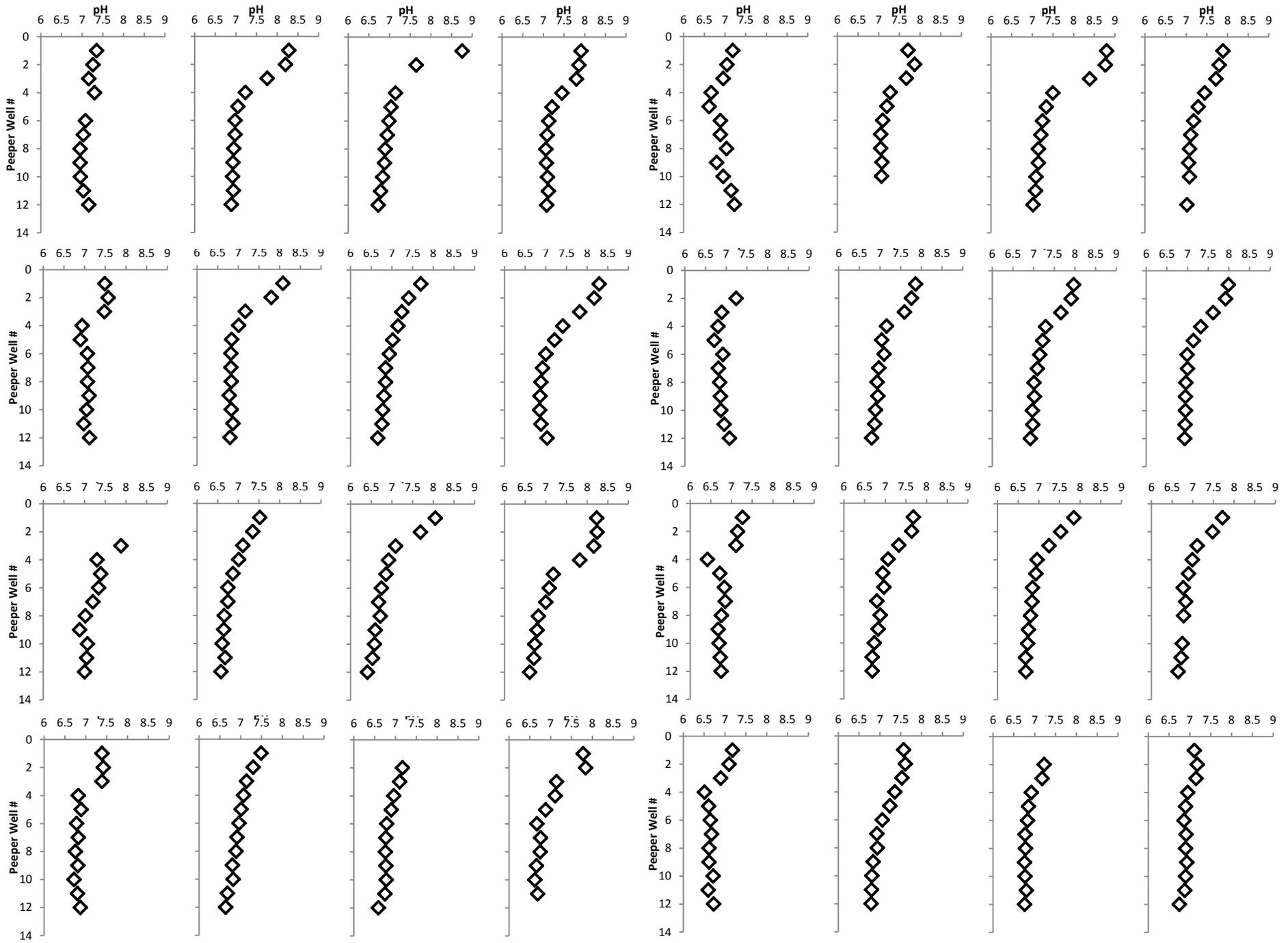


Figure A4 pH in wild rice mesocosm porewater. Panels a-d: Center of 300 mg/L amended mesocosms in May, June, July, August, respectively; Panels e-h: Edge (rootless zone) of 300 mg/L amended mesocosms in May, June, July, August, respectively. Panels i-l: Center of 150 mg/L amended mesocosms in May, June, July, August, respectively; Panels m-p: Edge (rootless zone) of 150 mg/L amended mesocosms in May, June, July, August, respectively. Panels q-t: Center of 50 mg/L amended mesocosms in May, June, July, August, respectively; Panels u-x: Edge (rootless zone) of 150 mg/L amended mesocosms in May, June, July, August, respectively. Panels aa-ad: Center of control mesocosms in May, June, July, August, respectively; Panels ae-ah: Edge (rootless zone) of control mesocosms in May, June, July, August, respectively.

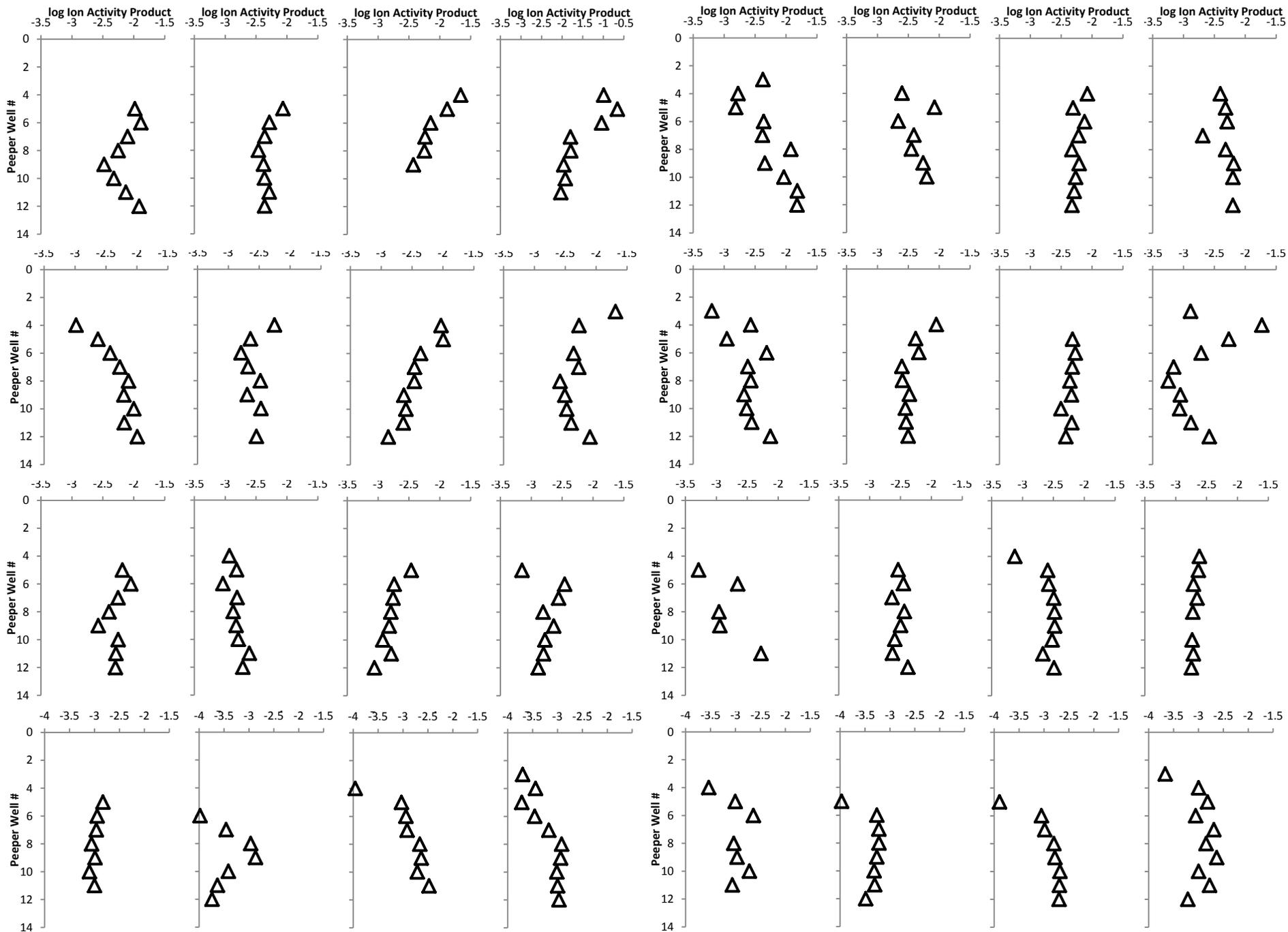


Figure A5 Ion activity product for iron sulfide in wild rice mesocosm porewater. Panels a-d: Center of 300 mg/L amended mesocosms in May, June, July, August, respectively; Panels e-h: Edge (rootless zone) of 300 mg/L amended mesocosms in May, June, July, August, respectively. Panels i-l: Center of 150 mg/L amended mesocosms in May, June, July, August, respectively; Panels m-p: Edge (rootless zone) of 150 mg/L amended mesocosms in May, June, July, August, respectively. Panels q-t: Center of 50 mg/L amended mesocosms in May, June, July, August, respectively; Panels u-x: Edge (rootless zone) of 150 mg/L amended mesocosms in May, June, July, August, respectively. Panels aa-ad: Center of control mesocosms in May, June, July, August, respectively; Panels ae-ah: Edge (rootless zone) of control mesocosms in May, June, July, August, respectively.

Table A2 Seasonal average porewater concentrations in control and amended mesocosms. Values represent the average of measurements made in center and edge (rootless zone) of mesocosms.

| | Sulfate [mg/L] | | | | | | | | Sulfide [μ M] | | | | | | | | Iron [μ M] | | | | | | | |
|------------|----------------|------|--------|------|------|-----|---------|-----|--------------------|-------|--------|------|------|------|---------|-----|-----------------|------|--------|------|-------|-------|---------|-------|
| | High | | Medium | | Low | | Control | | High | | Medium | | Low | | Control | | High | | Medium | | Low | | Control | |
| Depth [cm] | 0-10 | 0-5 | 0-10 | 0-5 | 0-10 | 0-5 | 0-10 | 0-5 | 0-10 | 0-5 | 0-10 | 0-5 | 0-10 | 0-5 | 0-10 | 0-5 | 0-10 | 0-5 | 0-10 | 0-5 | 0-10 | 0-5 | 0-10 | 0-5 |
| May | 85.1 | 37.6 | 0.7 | 1.1 | 1.0 | 0.7 | 0.9 | 0.9 | 34.1 | 49.1 | 8.8 | 12.0 | 1.8 | 1.5 | 1.1 | 1.3 | 91.3 | 36.1 | 148.8 | 77.0 | 305.6 | 299.5 | 611.7 | 482.9 |
| June | 88.5 | 94.5 | 4.9 | 7.7 | 2.0 | 3.7 | 0.9 | 1.0 | 27.0 | 41.4 | 11.3 | 19.7 | 2.3 | 2.1 | 0.9 | 0.2 | 47.7 | 18.7 | 147.3 | 66.9 | 402.4 | 259.8 | 194.4 | 60.5 |
| July | 64.7 | 81.0 | 25.0 | 52.7 | 2.7 | 4.5 | 1.8 | 2.4 | 71.4 | 114.0 | 39.5 | 79.4 | 6.9 | 8.5 | 1.7 | 0.8 | 24.0 | 12.7 | 79.2 | 15.0 | 202.3 | 112.0 | 300.0 | 173.2 |
| August | 44.4 | 90.0 | 19.1 | 31.0 | 4.5 | 8.2 | 1.6 | 1.6 | 87.1 | 162.4 | 44.0 | 92.0 | 9.4 | 15.4 | 1.4 | 1.2 | 51.8 | 36.2 | 86.0 | 16.3 | 141.2 | 40.2 | 325.3 | 211.9 |

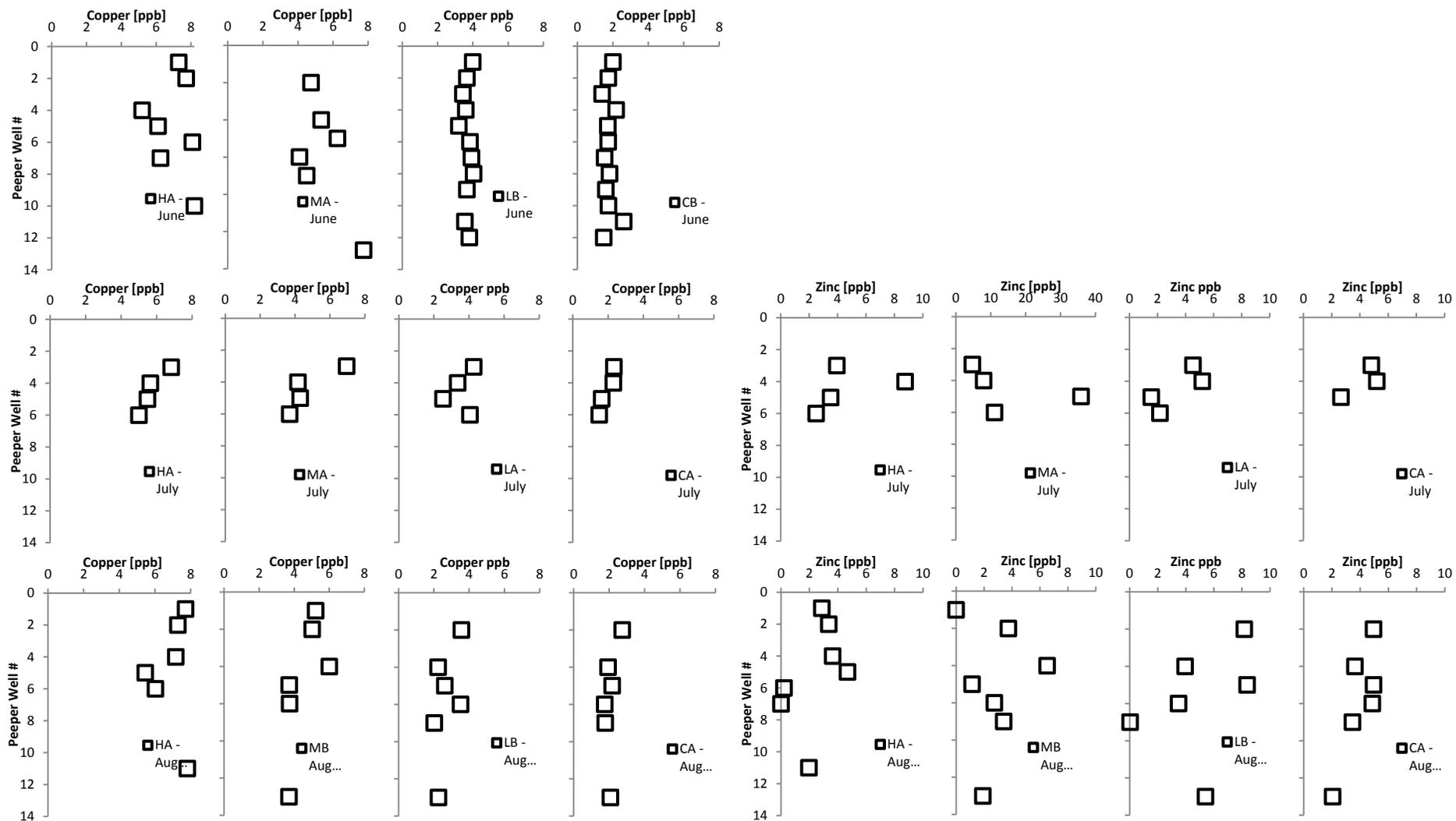


Figure A6 Dissolved zinc and copper concentrations in mesocosm porewater. Panels a-d: Copper in High, Medium, Low, Control amended tanks in June 2013. Panels e-h: Copper in High, Medium, Low, Control amended tanks in July 2013. Panels i-l: Zinc in High, Medium, Low, Control amended tanks in July 2013. Panels m-p: Copper in High, Medium, Low, Control amended tanks in August 2013. Panels q-t: Zinc in High, Medium, Low, Control amended tanks in August 2013.

Table A3 Zero to 5 cm average metals concentration in mesocosm porewater in June and August 2013.

| | Copper [ppb] | | | | Zinc [ppb] | | | |
|--------|--------------------|--------------------|--------------------|--------------------|-------------------|-------------------|-------------------|-------------------|
| | C | L | M | H | C | L | M | H |
| June | 1.7 (± 0.31) | 3.3 (± 0.45) | 5.2 (± 1.1) | 6.5 (± 1.5) | N/A ¹ | N/A ¹ | N/A ¹ | N/A ¹ |
| July | 1.8 (± 0.33) | 2.8 (± 0.75) | 4.6 (± 0.94) | 5.4 (± 0.34) | 3.0 (± 1.4) | 2.4 (± 1.5) | 7.9 (± 3.2) | 4.9 (± 3.4) |
| August | 1.8 (± 0.30) | 2.9 (± 0.60) | 4.6 (± 1.1) | 7.1 (± 1.9) | 3.4 (± 1.3) | 4.2 (± 3.1) | 2.9 (± 2.5) | 3.5 (± 1.1) |

¹ Zinc samples for June mesocosm pore water were compromised during analysis

Container design for 2013 sulfate-addition experiment in 100-gallon stock tanks (schematic)

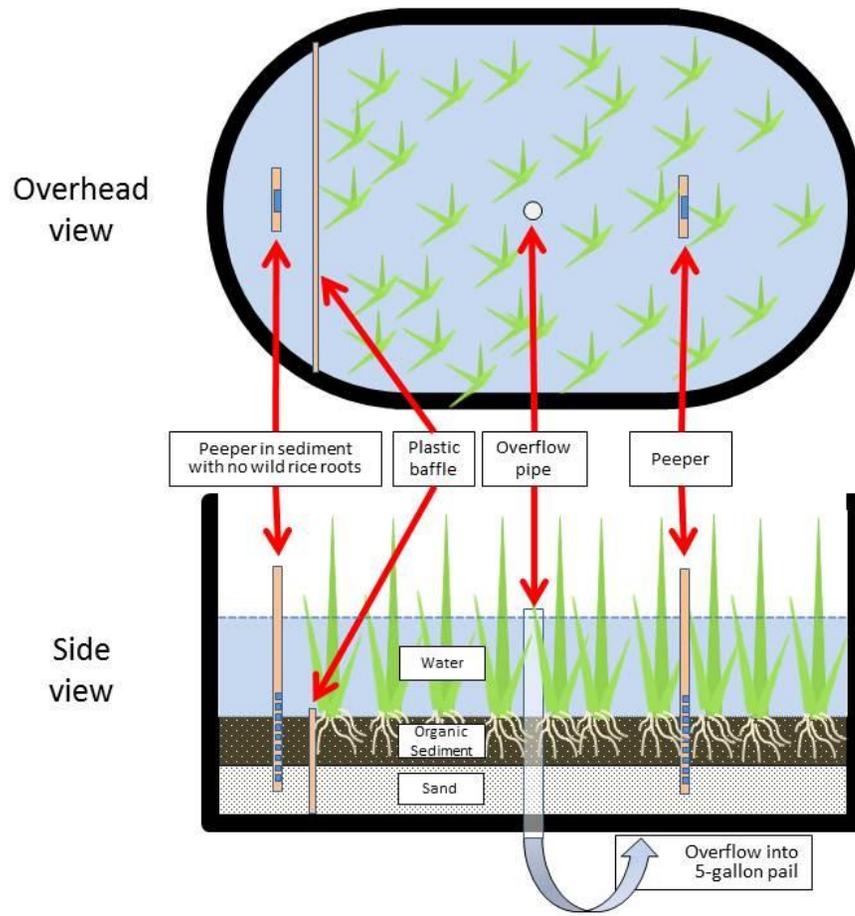


Figure A7 Schematic of the stock tanks used as mesocosms.

APPENDIX B. Observations of porewater chemistry at field sites

Figure B1. Porewater observations at Sandy Lake field site: sandy location.

Figure B2. Porewater observations at Sandy Lake field site: muddy location.

Figure B3. Porewater observations at Second Creek field site: sandy location.

Figure B4. Porewater observations at Second Creek field site: muddy location.

B.1 Observations of porewater chemistry at field sites

Porewater chemistry observations made at the two field sites, Sandy Lake and Second Creek, in Summer 2013 are shown in Figure B1 to B4. Two sets of replicate peepers—four peepers—were deployed at each field site once per month. Two peepers, spaced approximately 1 meter apart, were placed in a sandy location at each site, and two additional peepers, also spaced approximately 1 meter apart, were placed in a more muddy, organic location. Figure B1 and B2 show the porewater concentrations observed in Sandy Lake at sandy and muddy locations, respectively. Figure B3 and B4 show the porewater concentrations observed in Second Creek at sandy and muddy locations, respectively.

In general, sulfate depth profiles followed a pattern consistent with biologically driven sulfate reduction in anoxic surficial sediments: uniform overlying water concentration (typically wells 1-3) and rapid depletion in sediments. It is important to note that the peeper well number is used to represent the depths of each peeper well for field observations. Field peepers were routinely inserted at depths that allowed for quantification of sediment porewater chemistry to depths of greater than 25-30 cm, but these depths are not reflected on the plots in Figures B1 – B4. Therefore, the relative depths of measurements should be interpreted with caution until actual depths can be associated with each observation.

Sulfate in the overlying water was uniformly between 100 and 150 mg/L at Sandy Lake. In Second Creek, overlying water sulfate was near 300 mg/L in June and July, but increased to over 700 mg/L in August and September. Sulfate was almost always depleted to below 10 mg / L by 5 cm depth, except at the sandy location at Sandy Lake, where sulfate was routinely above 50 mg/L at deeper depths (except during July). Sulfide concentrations in sediment pore fluids were almost always less than 10 μM (compared with 1000 – 7000 μM sulfate in the overlying water), and were often below the method reporting limit of 0.7 μM . The steep gradients of sulfate, sustained throughout the summer, and a lack of buildup in porewater sulfide indicate a consistent removal mechanism for sulfur in sediments. Iron concentrations at field sites were frequently in excess of 200 – 500 μM and precipitation of sulfide as iron sulfides provide a likely explanation for the low dissolved sulfide concentrations.

In general, sulfide concentrations were higher and iron concentrations lower in the sandy locations at each field site. The lack of organic carbon may have resulted in less iron in pore fluids which allowed sulfide produced to remain in pore fluids at slightly higher levels than those at highly organic locations where high iron concentrations kept sulfide very low. The significant variability observed in peepers placed only 1 m apart indicates small-scale heterogeneity in the field sites, in contrast to the very consistent geochemical observations in the hydrologically isolated sulfate-amended mesocosm experiments. Despite having similar to or greater overlying water sulfate than the mesocosms, the ion activity product for iron sulfide in field sites were less consistent in their oversaturation with respect to iron sulfide than amended mesocosm pore waters.

Though steep gradients in sulfate were observed near the sediment-water interface at field sites, the potential for groundwater interactions cannot be ruled out at field sites. Diffusive sulfate flux estimates in mesocosms ranged from 0.5 to 50 $\mu\text{g}/\text{cm}^2/\text{day}$. Assuming an iron concentration of 250 $\mu\text{mol}/\text{L}$ in groundwater, an advective groundwater flow of 0.02 – 2 cm/day would be necessary to keep pace with diffusional sulfur flux from overlying water.

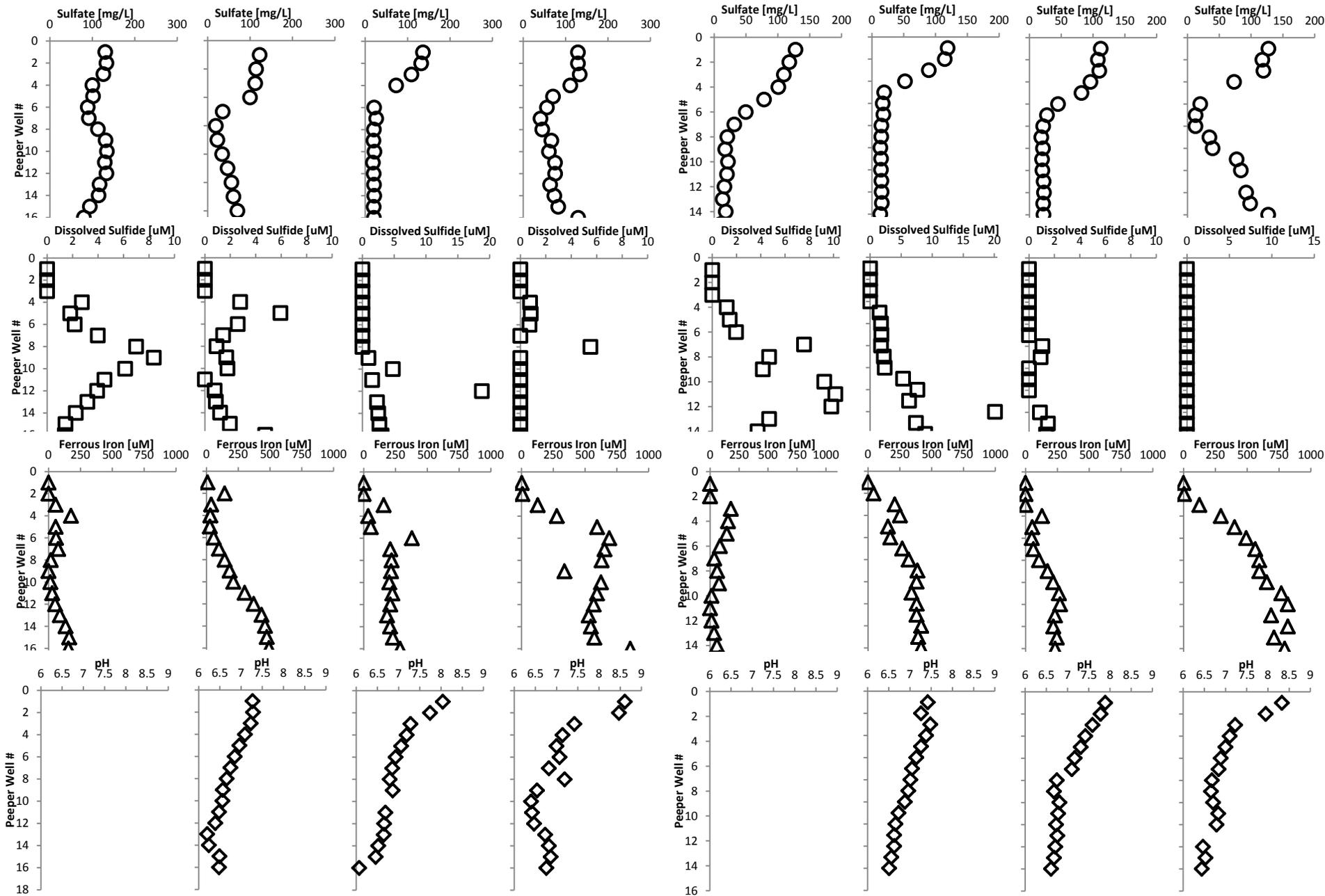


Figure B1 Porewater observations at Sandy Lake field site: sandy location. Panels a-d: Sulfate in sandy location during May, June, July, August, respectively; Panels e-h: Sulfate in replicate sandy location during May, June, July, August, respectively;. Panels i-l: Sulfide in sandy location during May, June, July, August, respectively; Panels m-p: Sulfide in replicate sandy location during May, June, July, August, respectively; Panels q-t: Iron in sandy location during May, June, July, August, respectively; Panels u-x: Iron in replicate sandy location during May, June, July, August, respectively; Panels aa-ad: pH in sandy location during May, June, July, August, respectively; Panels ae-ah: pH in replicate sandy location during May, June, July, August, respectively.

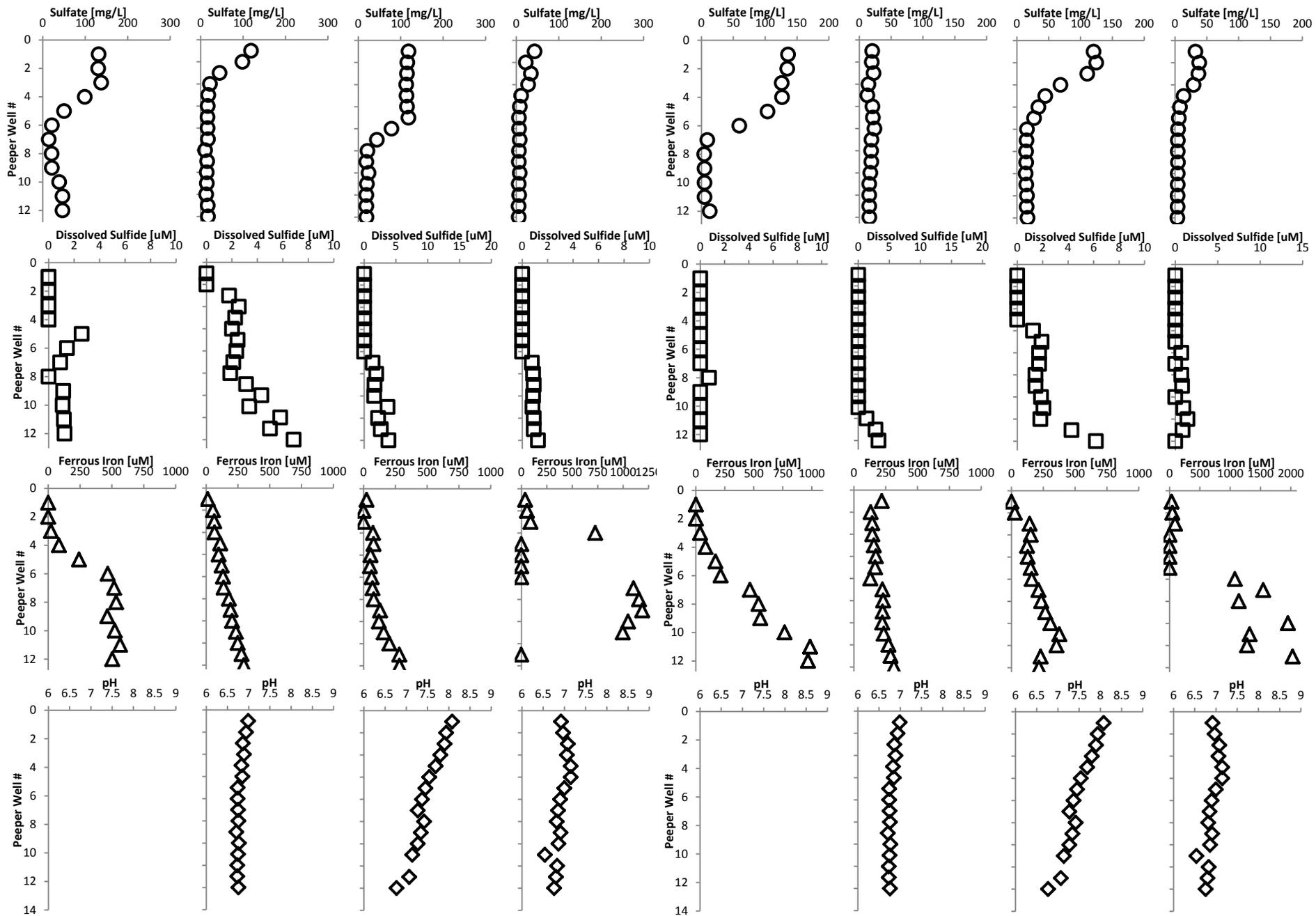


Figure B2 Porewater observations at Sandy Lake field site: muddy location. Panels a-d: Sulfate in muddy location during May, June, July, August, respectively; Panels e-h: Sulfate in replicate muddy location during May, June, July, August, respectively;. Panels i-l: Sulfide in muddy location during May, June, July, August, respectively; Panels m-p: Sulfide in replicate muddy location during May, June, July, August, respectively; Panels q-t: Iron in muddy location during May, June, July, August, respectively; Panels u-x: Iron in replicate muddy location during May, June, July, August, respectively; Panels aa-ad: pH in muddy location during May, June, July, August, respectively; Panels ae-ah: pH in replicate muddy location during May, June, July, August, respectively.

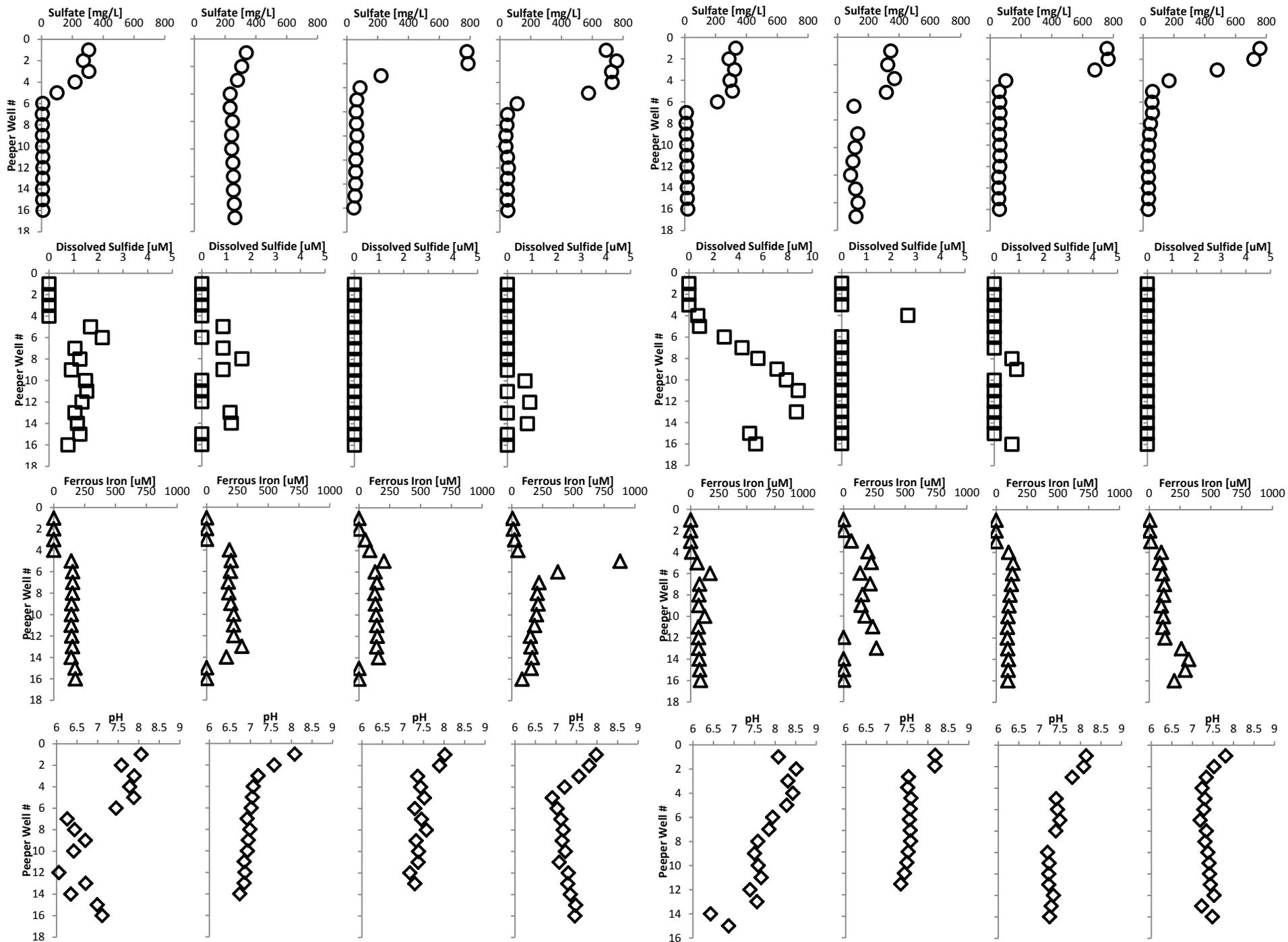


Figure B3 Porewater observations at Second Creek field site: sandy location. Panels a-d: Sulfate in sandy location during May, June, July, August, respectively; Panels e-h: Sulfate in replicate sandy location during May, June, July, August, respectively;. Panels i-l: Sulfide in sandy location during May, June, July, August, respectively; Panels m-p: Sulfide in replicate sandy location during May, June, July, August, respectively; Panels q-t: Iron in sandy location during May, June, July, August, respectively; Panels u-x: Iron in replicate sandy location during May, June, July, August, respectively; Panels aa-ad: pH in sandy location during May, June, July, August, respectively; Panels ae-ah: pH in replicate sandy location during May, June, July, August, respectively.

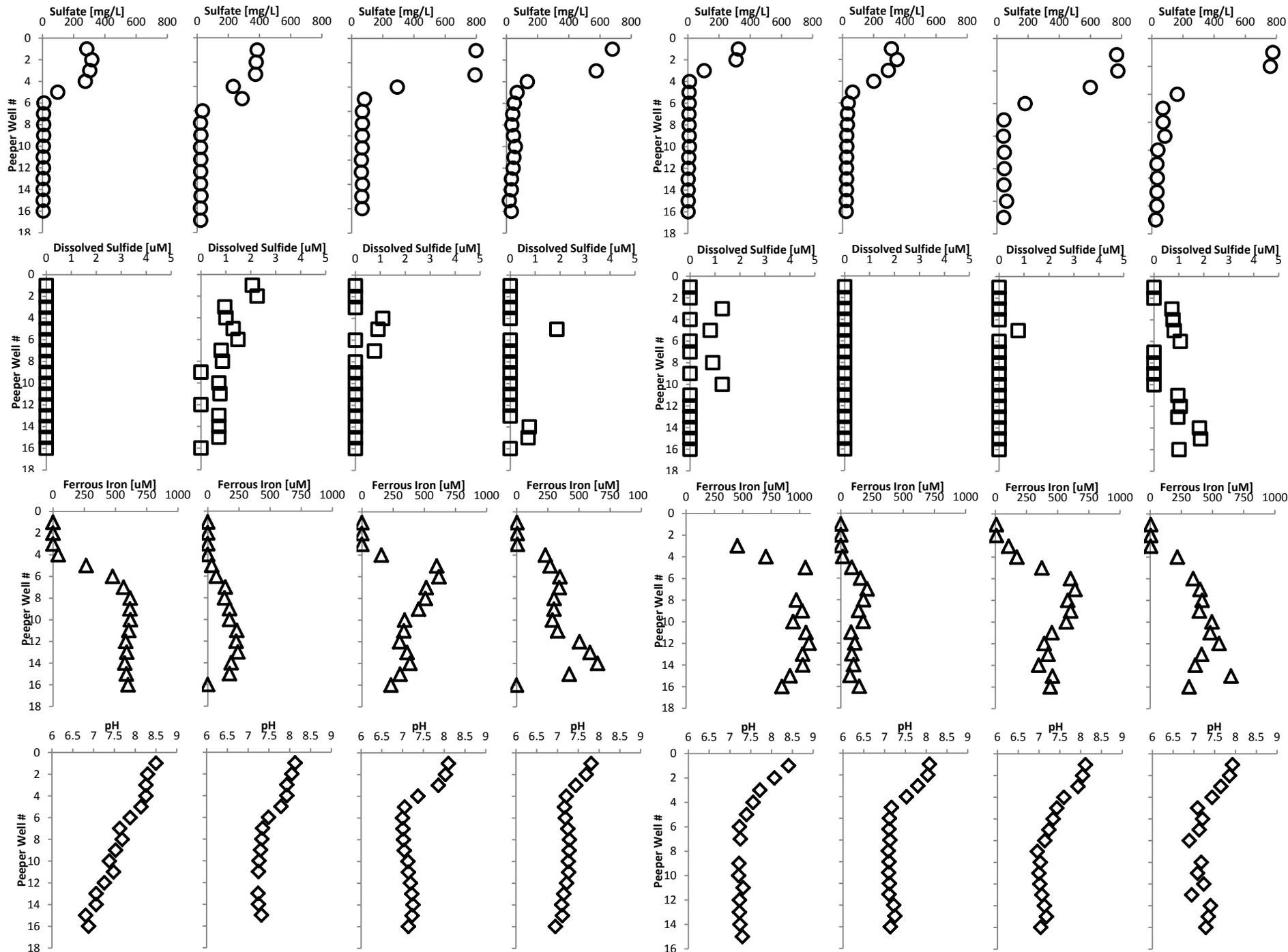


Figure B4 Porewater observations at Second Creek field site: muddy location. Panels a-d: Sulfate in muddy location during May, June, July, August, respectively; Panels e-h: Sulfate in replicate muddy location during May, June, July, August, respectively;. Panels i-l: Sulfide in muddy location during May, June, July, August, respectively; Panels m-p: Sulfide in replicate muddy location during May, June, July, August, respectively; Panels q-t: Iron in muddy location during May, June, July, August, respectively; Panels u-x: Iron in replicate muddy location during May, June, July, August, respectively; Panels aa-ad: pH in muddy location during May, June, July, August, respectively; Panels ae-ah: pH in replicate muddy location during May, June, July, August, respectively.