Control of Mercury Methylation in Wetlands Through Iron Addition

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Abstract

The San Francisco Bay-Delta System lost an estimated 85-95% of its historical tidal marshes to urban development, agriculture, and commercial salt production since the middle of the nineteenth century. Fortunately, there are many recent initiatives underway throughout the estuary to re-establish the important ecosystem functions and critical wildlife habitat that these tidal wetlands offer. However, there is a significant potential drawback to these restorations, as wetlands have been shown to play a major role in the production and export of methylmercury (MeHg), which is a potent neurotoxin that affects both humans and wildlife. While mercury pollution is a global problem, it is of special concern in the San Francisco Bay-Delta, where substantial additional inputs of inorganic mercury from historical mining activities have resulted in increased mercury levels in ecosystem. The potential exacerbation of MeHg health effects due to wetland restoration and construction is a serious concern that has been recognized in recent mercury regulations. However, restoration and management technologies have not yet been developed to control MeHg production and export from wetlands. The research described in this report tested the efficacy of one such potential control: the application of an iron sediment amendment to tidal wetland microcosms.

The conversion of inorganic mercury to MeHg is predominantly a biologically-driven process under typical wetland sediment conditions. The net production of MeHg is controlled by both bacterial activity and the bioavailability of inorganic mercury species to the microbial community. Under the reducing conditions typical of wetland sediments, dissolved mercury speciation and concentration is controlled by the presence of reduced sulfur, and it has been hypothesized that it is the uncharged dissolved Hg-S species that are readily available for methylation, as they are the species able to diffuse into bacterial cells. In this research, we tested the hypothesis that amending wetland sediments with iron will reduce net methylmercury production by decreasing dissolved porewater sulfide concentrations through the formation of insoluble iron-sulfur minerals, which correspondingly decreases the pool of mercury available to the methylating bacteria.

Two laboratory microcosm experiments were conducted using in-tact sediment cores collected from a tidal salt marsh in the San Francisco Bay estuary, where one experiment used sediments that had the vegetation removed and the second included live wetland plants. Microcosms in the devegetated experiment were split into four dosing groups (0, 180, 360, and 720 g- Fe/m^2) and were monitored for a period of 17 weeks. Shortly after iron addition, porewater S(-II) concentrations decreased significantly at all iron doses relative to the control, and net MeHg production and export to the overlying surface water decreased by over 90% at the highest iron doses. Despite some conversion of $FeS_{(s)}$ to pyrite, the effects persisted for at least 12 weeks. The inclusion of wetland vegetation substantially increased the amount of variation between triplicate cells, but general trends were similar to those found in the devegetated experiment.

This project was the first work to demonstrate that an iron sediment amendment has the potential to be an effective control of methylmercury production in tidal wetland sediments at the microcosm scale. These results have laid the groundwork for future studies to evaluate the efficacy of an iron

amendment at the field scale, which could demonstrate that this technique is a viable landscape-scale control on methylmercury production in restored and constructed tidal wetlands.

Introduction and Problem Statement

Wetland ecosystems provide many benefits to both society and the environment, as they provide habitat for threatened and endangered species, offer flood mitigation and aquifer recharge, improve water quality, and have aesthetic and heritage value. However, wetlands were historically treated as valueless wastelands, and they were often drained or filled in to make more 'useful' lands. This historical trend resulted in the loss of an estimated 53% of the wetland acreage in the continental US between the 1780's and 1980's, where California lost an estimated 91% of its wetlands, which was the highest percentage of any state [1]. However, near the end of the 20th century both government agencies and private landowners started to realize the many benefits that wetlands offer and began restoring and creating wetland habitat, which is evident in the increase of wetland acreage by 43,000 acres in the United States between 1986 and 1997 [2].

The San Francisco Bay-Delta estuary lost an estimated 85-95% of its historical tidal marshes to urban development, agriculture, and commercial salt production since the middle of the nineteenth century [3]. There are many current initiatives underway to re-establish important ecosystem functions and critical wildlife habitat throughout the estuary, with the South Bay Salt Pond Restoration Project being one of the largest and most well known, where it is slated to restore over 15,000 acres of tidally influenced salt marsh surrounding the southern portion of the San Francisco Bay [4]. This restored wetland acreage throughout the watershed will provide much needed habitat for a large number of migratory bird species that winter in the region or stop-over on the their migration along the Pacific Flyway, as well as viable habitat to resident federally listed endangered species like the salt marsh harvest mouse (*Reithrodontymys raviventris*) and California clapper rail (*Rallus longirostris obsoletus*). Additionally, the restoration can offer flood protection for the surrounding urban areas and increased local wildlife-oriented recreation opportunities.

A potential drawback to wetland restoration and construction, however, is the formation of monomethylmercury (MeHg) in the anoxic sediments, which is a potent neurotoxin that affects both humans and wildlife. While MeHg is typically found in sub-nanomolar levels in natural waters, even in those that are heavily impacted such as the San Francisco Bay-Delta estuary, it poses a significant health risk to high trophic status consumers since concentrations increase in each successive trophic level in the food web. This process, known as biomagnification, can result in MeHg concentrations that are up to 6 or 7 orders of magnitude higher in fish than in the surrounding water column [5, 6]. This is of special concern, as the primary exposure pathway for humans is through the consumption of fish, which is especially problematic in communities around the globe that rely on local fisheries as the primary source of protein in their diet. The dangers of mercury contamination are recognized as a public health hazard throughout the United States, where 80% of all fish consumption advisories issued by the EPA are due, at least in part, to elevated levels of MeHg [7]. Since

MeHg is a neurotoxin, the neurological development of fetuses and young children is especially susceptible to MeHg exposure, and chronic exposure in adults has been shown to cause impairment of the peripheral vision, speech, hearing, motility, and even coma and death [8]. MeHg also poses a significant threat for the reproductive success and survivability of piscivorous bird and mammal species [9], as well as benthic omnivores in tidal wetlands, such as the endangered California clapper rail. In fact, studies have shown that elevated mercury levels in failed California clapper rail eggs resulted in deformities, embryo hemorrhaging, and embryo malpositions [10], and chronic low-level dietary exposure to MeHg has been shown to alter the behavior of great egret juveniles [11].

A majority of the MeHg found in aquatic food webs can be traced back to anthropogenic emissions of inorganic mercury into the environment, such as those emitted by coal-fired power plants, metal mining and production facilities, and the chlor-alkali industry. While mercury pollution is a global problem, it is of special concern in the San Francisco Bay-Delta estuary in California, where there are substantial additional local inputs of inorganic mercury. Historical mining activities, including the use of mercury in hydraulic gold mining in the Sierra Nevada Mountains and several mercury mines in the Coast Range Mountains have increased the inorganic mercury loading through continual transport from tributaries and rivers. This continual flow from upstream sources, in addition to background deposition, has resulted in the elevated mercury concentrations found in the water [12], sediment [13], and biota [14] of the Bay-Delta.

In the aquatic environment, mercury is typically found in the Hg(II) oxidation state, and can be converted to MeHg via a process known as methylation. Under the typical anoxic conditions found in wetland sediments, the process is driven by microbial activity, where it is believed to be a passive metabolic process primarily mediated by sulfate-reducing bacteria [15, 16] and some iron-reducing bacteria [17, 18]. Because methylation is primarily a biological process, the production rate of MeHg is dependent on both the bacterial growth rates and on the bioavailability of the species of mercury present. In the presence of S(-II), which is typically present in the porewater of anoxic wetland sediments, the concentration of dissolved Hg(II) and its speciation is controlled by the presence of excess cinnabar ($HgS_{(s)}$). It has been hypothesized that only small, uncharged mercury complexes (such as HgS^0 and $Hg(HS)_2^0$) are capable of passively-diffusing into bacterial cells, and are therefore the only species bioavailable for methylation [19, 20]. Demethylation of MeHg also occurs in the aquatic environment both as a biotic control of MeHg toxicity [21] and as an abiotic photochemical process [22], and the balance of the competing methylation and demethylation rates yields the net MeHg production rate, which dictates the concentration of MeHg found in an aquatic ecosystem.

Tidal wetlands contain large areas of highly productive anoxic sediments that are ideal for MeHg production, and even though they can be net sinks for inorganic Hg(II), they are often found to be sources of MeHg [23-25]. Thus, the question of whether or not increased tidal wetland area from restoration activities will exacerbate existing mercury contamination problems must be seriously considered. While there are a variety of potential controls that could be evaluated for reducing MeHg concentrations in restored wetlands, the most promising

approach is to address the problem from the bottom-up – decreasing MeHg production in wetland sediments by limiting the bioavailability of inorganic Hg(II) to the methylating bacteria. We have proposed the utilization of an iron sediment amendment for this purpose. The presence of reduced iron in the sediment porewater decreases dissolved sulfide concentrations via the formation of $FeS_{(s)}$, and as sulfide concentrations decrease, the total pool of dissolved mercury complexes is correspondingly reduced, resulting in decreased concentrations bioavailable neutral mercury-sulfide complexes.

Research in both freshwater [26, 27] and estuarine [28, 29] environments has shown that the addition of ferrous iron to anoxic sediments can result in substantial reductions of porewater sulfide concentrations, and we are interested in its use for controlling Hg(II) bioavailability to microbial methylators in tidal marsh sediments. Previous studies by our research group have shown that the addition of 10^{-2} M ferrous iron to pure cultures of sulfate reducing bacteria in a closed anoxic system decreased net mercury methylation by around 75% relative to controls without changing microbial metabolic rates during the three day incubation [30]. A follow-up study was carried out using anoxic incubations of sediment slurries collected from five estuarine wetlands around the San Francisco Bay, which again found that the addition of Fe(II) reduced net MeHg production [31]. In this project, laboratory microcosm experiments were conducted to further assess the potential for using an iron sediment amendment as a landscape-scale control of methylmercury production in tidal wetlands.

Objectives

To reconcile the competing objectives of restoring aquatic habitats with the need to manage mercury-contaminated sediments, scientists need a better understanding of the factors controlling MeHg production and export from wetlands. Moreover, engineers need approaches for restoring wetlands in ways that maximize habitat potential without increasing MeHg concentrations in food webs. The objective of this research project was to develop new approaches for wetland construction and restoration that minimize MeHg production without compromising habitat potential. Specifically, we evaluated the use of an iron sediment amendment to control methylmercury production in tidal wetland microcosms. This project was designed to address the following research aims.

Aim 1. *Evaluate the effect of an iron amendment on net methylmercury production in a simulated tidal wetland environment.* The principle behind the iron amendment has been demonstrated in simplified laboratory incubations, but not under conditions encountered in the wetland environment. The microcosm experiments allowed for conditions such as simulated tidal flushing and exposure of sediments to the atmosphere to be included, and for the experiments to be run at timescales of up to 4 months.

Aim 2. *Evaluate the long term effect of iron addition on sulfur chemistry, and its linkage to net MeHg production.* Iron has a finite capacity to remove dissolved sulfide from porewater, so it is necessary to evaluate

the potential for long-term effectiveness of the amendment. The aging of iron-sulfide minerals potentially complicates the long-term efficacy as well, as the $FeS_{(s)}$ species initially formed will be converted less reactive forms over time. This may influence mercury bioavailability as well, since inorganic Hg is known to coprecipitate with authigenic pyrite [32], meaning that the production of pyrite from the amended iron may sequester inorganic mercury within the wetland sediments, resulting in long-term reduction in net MeHg production.

Aim 3. *Evaluate the effect of wetland vegetation on net methylmercury production in the presence of an iron amendment.* Wetland vegetation can have a profound effect on sediment biogeochemistry in salt marsh environments [33, 34] as plants are able to alter the biogeochemistry of the rhizosphere. Recent work has suggested that wetland vegetation may also play an important role in net MeHg production in salt marsh sediments [23, 35, 36]. It is important to consider the effect that a common tidal marsh plant species has on MeHg production in the presence of the iron amendment, since it is possible that wetland vegetation may increase MeHg production through the stimulation of microbial communities. Additionally, it is possible that vegetation may introduce oxygen into the sediments, which could affect the formation and cycling of reduced iron-sulfur minerals.

Procedure

In order to evaluate the use of an iron amendment under conditions that more realistically simulate an actual wetland environment, two separate microcosm studies were conducted. The use of microcosms allowed for the experiments to be carried out under controlled conditions for a period of weeks to months, while including factors that could influence mercury methylation such as a simulated tidal period, sediment exposure to the atmosphere and available for gas-exchange, and the presence of wetland vegetation. The first experiment was conducted using sediments that had the above-ground vegetation removed before the iron amendment, while the second set was conducted with live pickleweed plants (*Sarcocornia pacifica*).

Sampling Site and Microcosm Collection

Microcosm sediments were collected at Gambinini Marsh, a privately-owned tidal salt marsh near Petaluma, CA. This marsh experiences daily tidal fluctuations of estuarine water from the Petaluma River, which drains into San Pablo Bay in the San Francisco Bay estuary, and the high marsh plain is dominated by pickleweed. Historical mining practices have resulted in elevated mercury concentrations in the sediments of many tidal wetlands in the San Francisco Bay area, and it was unnecessary to add any additional mercury to the microcosms. This means that all measurements represent biogeochemical processes that occurred at in-situ mercury concentrations. Two separate sampling trips were taken to collect sediments for the microcosm experiments; sediments

were collected for the devegetated experiment in October 2007, and a return trip was conducted in September 2008 for the vegetated experiment.

The laboratory microcosms consisted of 38L (50cm L x 25cm W x 30cm H) acrylic aquariums (purchased from GlassCages.com). In-tact sediment cores were cut out of the high-marsh plain using hand shovels, and the block of sediment was extracted by hand. The cores were then shortened to a depth of around 15cm by removing sediments below that depth. The sized core was placed into the microcosm container and any areas that were not filled all the way to the acrylic walls were filled with additional sediment from the coring hole to avoid pooling of surface waters or short circuiting to the effluent port. Twelve microcosms were collected in this fashion from the same area of the marsh plain, and they were selected to have a similar density of vegetation based on appearance. Samples were then transported back to the laboratory and connected to the simulated tidal system.

Laboratory Microcosm Operation

Microcosms were kept in the lab under simulated light and tidal conditions (Fig. 1). Two 1000-W metal halide grow lamps were operated on a daily automated schedule of 15 hours of light, 9 hours of darkness. The microcosms were separated into two sets of six, and each group was placed under one grow lamp to provide uniform light coverage to each microcosm. Each microcosm was connected to an individual reservoir (14-L HDPE plastic bucket) that contained 5L of simulated estuarine water (salinity ~12 parts per thousand). The microcosms were operated on a weekly basis, where the water was sampled and then replaced by fresh simulated estuarine water on the first day of the week and was kept in the reservoir for the remainder of the week. The simulated estuarine water was made by mixing deionized water with Instant Ocean aquarium salts to the desired salinity, followed by the addition of half-strength Hoagland's Nutrient Solution reagents to support microbial and plant growth. Since evaporation of the reservoir water occurred during exposure to the grow lamps, additional deionized water was added to bring each reservoir back to approximately 5L in total volume during the middle of the week.

Figure 1. Laboratory microcosm setup including acrylic microcosms, fluoropolymer connections and lines, automated grow-lamps and peristaltic pumps, and individual microcosm reservoirs.

A simulated tidal regime was provided via a system of automated multichannel peristaltic pumps (Fig. 2). Inlet and outlet ports were drilled in the acrylic microcosm before sample collection and were fitted with PTFE connectors for the FEP tubing used to provide the surface water supply. To minimize potential clogging of the effluent line due to sediment settling, water was brought in via the lower port, and sent out using a short length of FEP tubing oriented vertically from the top port such that its opening was just above the sediment surface.

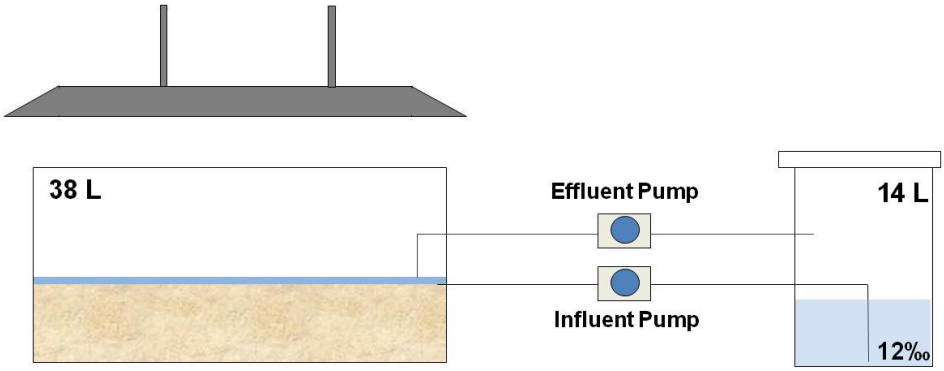

Figure 2. Schematic of laboratory microcosm setup. Each microcosm was connected to an individual reservoir, and a simulated daily tidal period was provided by automated peristaltic pumps.

The simulated tidal regime included two uniform high-tide events during each 24-hour period; one during the morning daylight hours and one during the dark overnight period. For these events, the inlet pump was turned on for 1 hour of incoming tide which gave 1-2cm of surface water over the microcosm

sediments. The surface water then was retained in the microcosm for 1 hour, before the effluent pump was turned on for 1 hour to drain the surface water. This resulted in the sediments being covered in water for a maximum of 6 hours per day with the remaining 18 hours open to exchange with the atmosphere. The microcosm system was checked daily for clogs, which occasionally occurred due to uptake of solid material into the effluent tubes. When this occurred, the clog was removed or that section of tubing was replaced, and the outgoing pump was turned on for a sufficient time to drain the remaining surface water. To reduce the growth of algae, aluminum foil was placed around the sediment filled portion of the microcosms, as well as over the inlet and outlet lines, and around the plastic reservoir buckets.

Iron Sediment Amendment

After collection in the field during October 2007, microcosms were allowed to equilibrate under laboratory conditions for a period of 4 months before the above ground vegetation was removed. The microcosms were randomly assigned to one of four treatment groups (n=3 tanks for each group): a Control dose (0 g-Fe/m^2), a Low dose (180 g-Fe/m^2), a Medium dose (360 g-Fe/m^2), and a High dose (720 g-Fe/m^2). These dosing levels were selected such that the dose applied to the medium group would approximately double the reduced iron already present in the sediments. The average concentration of acid-volatile sulfide in the microcosms was used as a proxy for reduced iron (ie, making the rough assumption that all AVS measured is in the form of $FeS_{(s)}$). These application rates were similar to the range found in the literature for the suppression of methane production in rice paddies [37] and phosphorus removal in treatment wetlands [38], and around an order of magnitude greater than the doses studied for reduction of sulfide toxicity in seagrass beds [28, 39].

The iron was amended as a subsurface injection of a ferrous iron-carbonate slurry using plastic 10-mL syringes and 16-gauge stainless steel needles at a depth of 2.5 cm. The slurry was created fresh for each microcosm just before injection from a mixture of de-aerated deionized water that had been buffered with Na_2CO_3/$NaHCO_3$ and the appropriate amount of $FeCl_2$*$4H_2O$ salts. A carbonate buffer was used to prevent a significant decrease in pH due to dissolution and hydrolysis of the iron salt, as well as to form an $FeCO_{3(s)}$ phase that is unstable in the presence of sulfide. For the control group, a suspension of $CaCO_{3(s)}$ was made and injected in the same fashion as for the iron-dose groups in order to control for any effects due to use of steel injection needles and sediment aeration.

Following the September 2008 sampling trip, microcosms collected for the vegetated experiment were allowed to equilibrate to the laboratory conditions for a period of 3 weeks before iron amendment. As in the first experiment, the 12 cells were randomly assigned into 4 different treatment groups: a control dose with the above-ground vegetation removed (Devegetated Control), and a Control, Low, and Medium iron dose with the vegetation present, where the amount of iron added in each dose group was the same as for the sediment experiment (0, 180, and 360 g-Fe/m^2 for the Control, Low, and Medium groups, respectively).

Sample collection and Analytical Methods

During the course of the experiment surface water and porewater samples were collected from each microcosm for the measurement of a variety of parameters at weekly or biweekly intervals. Surface water was collected from the reservoir following the morning high tide before reservoir water replacement (ie, exposure to the tidal regime for one week) by submerging the sample vessel under the water surface in the reservoir and filling to no headspace, and were analyzed for methylmercury, total mercury, sulfate, dissolved iron, total organic carbon, and pH. The total volume of surface water remaining in the reservoir was also measured to correct for differences in evaporation between microcosms, and surface water data are reported as normalized to the nominal 5-L reservoir volume concentration. Prior to the experiment, a permanent in-situ porewater sampler (10-cm Kijkelkamp Rhizon Soil Moisture Sampler) was installed in each microcosm at a depth of 3.5-cm, with an average pore size of 0.1 μm. On sample collection days, an average of 7-mL of porewater was collected in a 10-mL syringe from each sampler, allowing for the measurement of dissolved sulfide, sulfate, total dissolved iron, pH, and dissolved organic carbon.

Following the conclusion of the experiment, sediment cores were collected from each microcosm using acrylic tubes (6 cm i.d.), and were sectioned at 1-cm resolution to a depth of 10 cm. For the non-vegetated sediment experiment, sediment cores were taken between 8-11 weeks after the last surface water measurements, during which time the microcosms were continued under the standard operating conditions. Triplicate cores were collected from each microcosm and the depth layers from each of the three cores were combined into a single composite sample to account for spatial variability. Each composited section was homogenized and immediately analyzed via sequential extraction for acid-volatile sulfide (AVS) and chromium reducible sulfur (CRS) concentrations, and the remaining sediment samples were then frozen until analysis for total mercury and MeHg can be completed.

For the vegetated experiment, cores were collected for all microcosms during the week following the final water sampling date, and the in-tact cores were capped with rubber stoppers on both ends, wrapped in parafilm, and kept frozen until analysis between 8-10 weeks later. On the day of analysis, cores were thawed at room temperature in the dark for just enough time to allow them to be extruded from the acrylic coring tubes, and they were sectioned at 1-cm intervals using a stainless steel blade that was rinsed with dilute HCl between each layer. As in the sediment experiment, composite samples were made from each depth interval by mixing sediments from each of the three individual cores into one sample for analysis. After sectioning, samples to be preserved for future MeHg and total Hg analysis were kept frozen and placed into glass jars, and then moved to the freezer for storage. Subsamples of the composite were allowed to thaw enough to roughly homogenize, and then were immediately analyzed for the AVS-CRS extraction.

Total mercury in water was measured by BrCl oxidation, followed by reduction with $SnCl_2$, trapping on gold traps, thermal desorption, and cold vapor atomic fluorescence (CVAFS) detection [40]. Sediment samples were digested in concentrated hot acid (7:3 ratio of nitric to sulfuric), diluted to 100mL with 1% BrCl solution and analyzed following the same analytical process as for water

samples. Methylmercury in both water and sediments were measured by acidic chloride distillation [41, 42], aqueous phase ethylation, collection on Tenax traps, thermal desorption, GC separation, and detection by CVAFS [43]. Reduced sulfur speciation in sediments was conducted using a modified diffusion method for the sequential extraction of AVS and CRS [44]. Other chemical constituents were measured by standard methods [45], including graphite furnace atomic absorption spectroscopy for iron, ion chromatography for sulfate, and methylene blue colorimetric analysis for sulfide.

Results

Devegetated Sediment Microcosms

Samples were taken for 17 weeks following the iron amendment, following a weekly or biweekly measurement regime. The surface water was changed on the first day of every week, regardless of if samples were collected. We experienced a pump failure during Week 6, which resulted in a large flood tide in the microcosms, with water depth of around 6-8 cm and exposure to the permanently flooded conditions for up to 72 hours. Once the problem was discovered, the microcosms were drained back into their reservoirs, and the Week 6 samples were collected as scheduled. During the two week period until the pump system was replaced (Weeks 7 & 8), the microcosms were subjected to just one high tide per day during the morning hours. The Week 8 samples were collected from this low tidal frequency period, and the normal operating procedures were returned from Week 9 through the remainder of the experiment.

The simulated estuarine water provided on the first day of each week had an initial average sulfate concentration of 1400 mg/L. Figure 3 shows the concentration of sulfate remaining in the surface water at the end of the week, and the difference between the initial amount and the measured amount represents the net sulfate reduced by the microbial community. While there was some week to week variation in the surface water sulfate concentrations, the averages were typically similar between the groups during an individual week. This suggests that the iron addition did not significantly alter the net sulfate reduction rate.

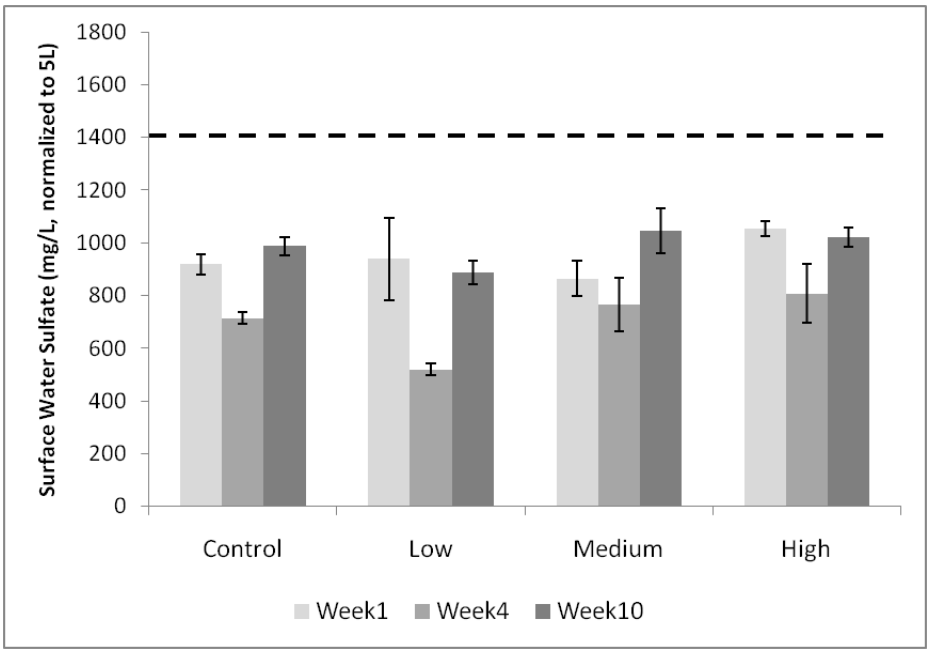

Figure 3. Concentration of sulfate remaining in microcosm reservoirs (normalized to 5L volume) after one week of tidal exposure. The difference between the initial concentration in the reservoir each week (dashed line) and the final concentration represents the net sulfate reduced within the microcosms. Data are shown as the average concentration of the three replicate microcosms ± one standard error of the mean (Avg±SE).

The porewater sulfide concentrations shown in Figure 4 supported the hypothesis that the addition of ferrous iron would reduce dissolved sulfide concentrations. The Low dose group showed decreases of greater than 70% for most weeks relative to the control group, and the Medium and High dose groups showed decreases of over 80-90% for most weeks. Additionally, the porewater sulfide concentrations were not high enough to account for the weekly losses of sulfate from the surface water, suggesting that there is another sink for the reduced sulfate, such as reduced sulfur minerals or volatilization of H_2S to the atmosphere.

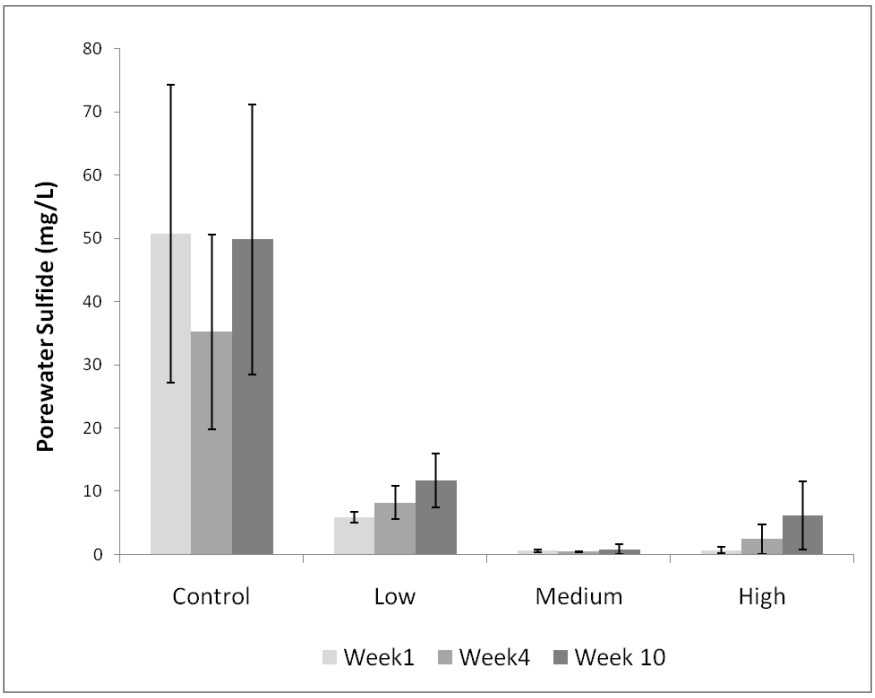

Figure 4. Porewater dissolved sulfide concentrations were decreased for all Fe-amendment doses relative to the Control group for the duration of the experiment, with the greatest effects evident in the Medium and High dose groups. Data shown as Avg±SE.

Iron exported in the surface water was similar and low for all treatment groups, with weekly averages ranging between 28-1500 µg/L (data not shown). There were differences in the porewater dissolved iron (Figure 5), where the highest-dose treatment groups had the correspondingly highest levels of dissolved Fe. There was a fairly constant initial decrease in porewater iron for both the Medium and High groups, which leveled off around Week 10 (following the pump failure flood event). Since export of iron in the surface water was low for all groups, these decreases suggest that there was a sink for a solid phase of iron within the microcosm. The most likely sink would be a reduced iron-sulfur mineral species, which was evident due to the formation of black sediment layers characteristic of Fe-S minerals in reduced sediments.

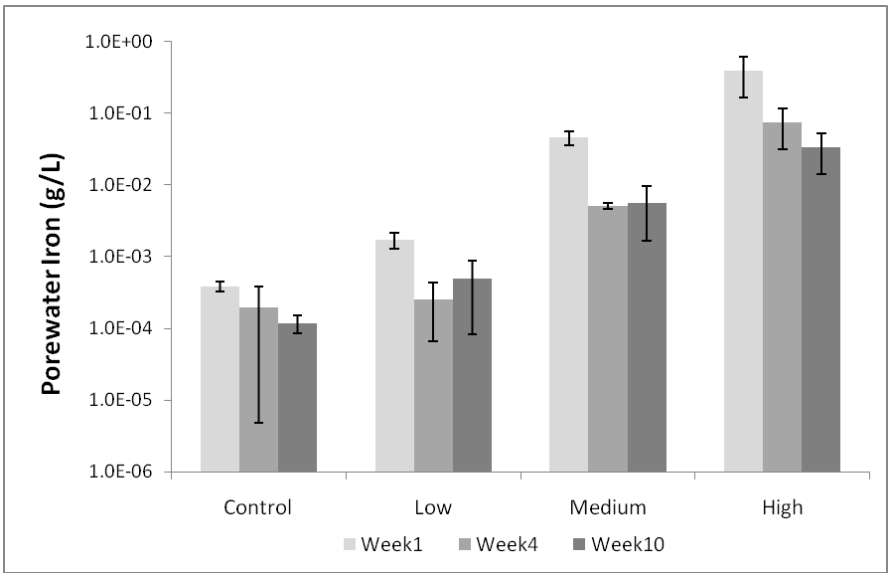

Figure 5. Dissolved iron concentrations in the porewater of the non-vegetated microcosms were initially stratified by amended iron dose. Decreases in the pool of dissolved iron suggest there was a solid-phase sink for iron within the sediments. Values shown as Avg±SE.

Total inorganic mercury in the surface water, defined as the difference between 5-L reservoir normalized concentrations of total mercury and methylmercury, was found to be similar between all treatment groups during most weeks, and had consistent average concentrations in the range of 4-10 ng/L (Fig. 6). This suggests that transfer of inorganic Hg from the sediment to the surface water was not affected by iron addition, and that differences seen in MeHg concentrations are not just due to a larger pool of total mercury in the surface water.

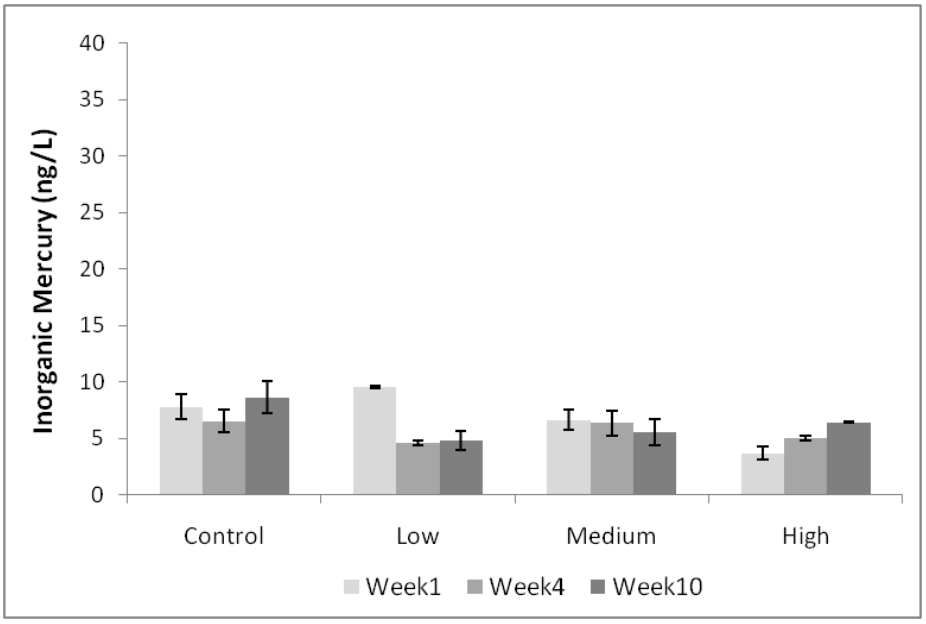

Figure 6. Total inorganic mercury (normalized to 5L reservoir volume) exported in the surface water was similar for all treatment groups in most weeks, and had a consistent range through the duration of the experiment. Values shown as Avg±SE.

Methylmercury concentrations in the surface water reservoirs showed clear differences between the amendment dose groups (Fig. 7). The Medium and High groups had the lowest averages and stayed low (averages less than 1 ng/L) during the entire 17-week observation period. The Low dose and Control groups had substantially higher concentrations, where the Medium group was found to have 12-94% decrease relative to the Control, and the High dose had a percent decrease of 40-94%. Additionally, concentrations for the Low and Control groups were much more variable around the time of the pump failure and return to normal operating conditions, with very large exports of MeHg following both the initial flooding event and the return to standard conditions during Week 10. After Week 12 of the experiment, the concentrations for the Low and Control groups dropped greatly, and were similar to the levels measured for the Medium and High groups. The reason for this decrease is not immediately clear. It is possible that the microbial community used up much of the labile organic carbon by this time and methylation rates slowed, although this is not represented in the net sulfate reduction observed. Additionally, it was possible that the pool of inorganic mercury that was most readily available for methylation within the sediments (ie, not strongly bound to recalcitrant substrates) was consumed by this time.

The Low dose group typically had an average concentration that exceeded that of the Control group, suggesting a possible increase in MeHg production due to the low level amendment. This increase is consistent with the results of the previous sediment slurry study [31], and warrants further attention in future experiments evaluating the iron amendment technique, as it would be problematic if it was found that a high-level Fe addition could eventually age into the conditions characteristic of the low-Fe amendment, resulting in increased MeHg concentrations. The reason for this increase is not immediately clear. It is possible that there was a small increase in the amount of sulfate reduction, as shown by slight differences in the average sulfate concentrations of the Low group during some weeks (Fig. 3). Alternatively, the low-level iron dose left an intermediate range of sulfide concentrations in the porewater (ie, 5 to 17 mg/L), which could have resulted in the shift of the predominant dissolved mercury-sulfide complex to one of the uncharged, bioavailable forms.

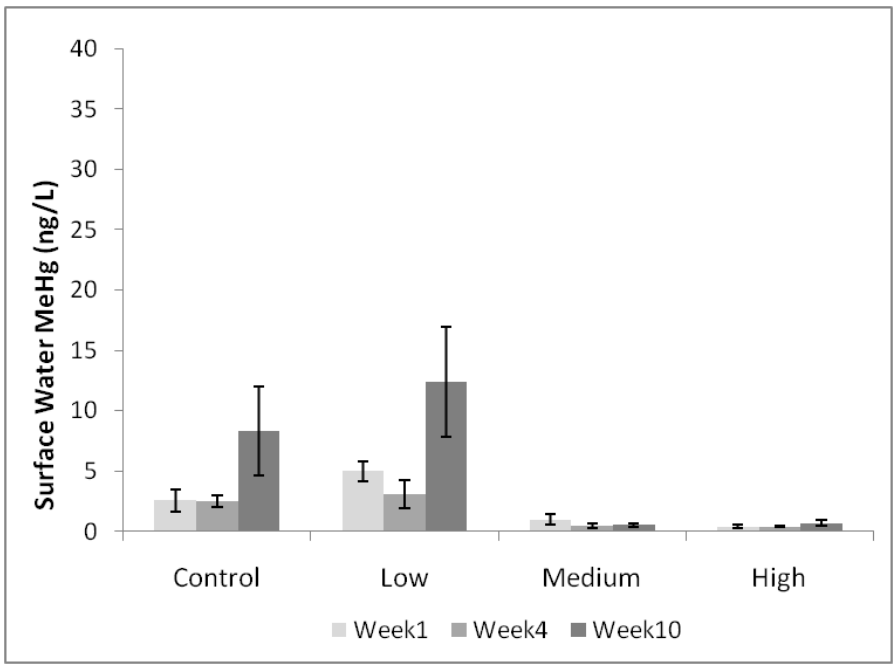

Figure 7. Methylmercury concentrations in the surface water, normalized to the 5-L reservoir volume. Concentrations for the Medium and High dose groups were substantially reduced relative to the Control group, and stayed consistently low throughout the duration of the experiment. Values shown as Avg±SE.

At the conclusion of the experimental period, sediment cores were extracted to evaluate the depth-dependent formation of reduced iron-sulfur minerals. The sequential extraction performed looked at two different experimental reduced sulfur fractions: acid-volatile sulfur (AVS) and chromium reducible sulfur (CRS). AVS is a measure of sulfide liberated from mineral phases that are soluble in 1N HCl, with metal monosulfides, like $FeS_{(s)}$, being the dominant form. The CRS measurement is able to extract reduced sulfur from minerals that are insoluble in 1N HCl, and when performed sequentially following AVS, the dominant phase measured for iron-sulfur minerals is pyrite ($FeS_{2(s)}$). Under the reducing conditions typically found in wetland sediments, it is believed that amorphous $FeS_{(s)}$ species will be formed initially and then converted into less reactive and more crystalline forms over time, like pyrite.

The depth profiles shown in Figure 8 include evidence of increased formation of AVS for the High dose group at the 2-3cm depth relative to the other groups. Additionally, all three treatment groups show elevated concentrations of CRS relative to the control group over the depth interval of 1-4cm. This suggests that over the duration of the experimental period, the amended iron enhanced the formation of pyrite within the microcosms. While pyrite formation is often thought to be a relatively slow process, it has been shown to form rapidly in estuarine marshes [46].

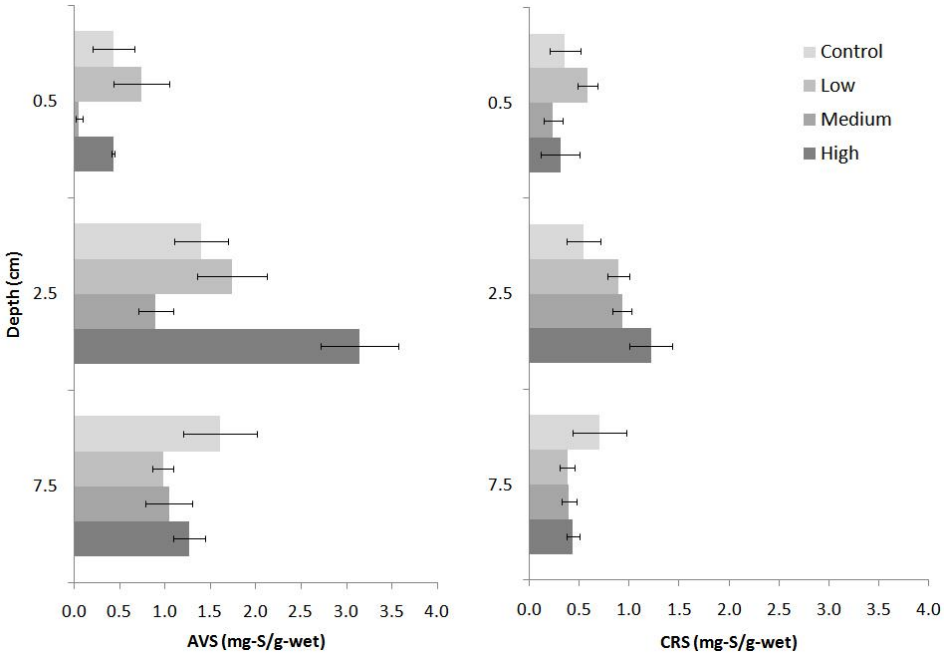

Figure 8. Depth profiles of reduced sulfur speciation for three segments from the top 10-cm of sediments from composite core samples. There is evidence of enhanced acid-volatile sulfide (AVS) production for the High dose group in the 2-3cm depth, as well as enhanced chromium reducible sulfur (CRS) formation for all iron amended groups over the depths of 1-4cm.Values shown as Avg±SE.

Vegetated Microcosms

Samples were collected from the vegetated microcosms for 8 weeks following the iron amendment. Around Week 5, plants in many of the microcosms had lost their vibrant green color and by the final sampling in Week 8, almost all plants were dormant.

Sulfate concentrations remaining in the surface water after one week of tidal exposure were similar to the range of values measured in the non-vegetated experiment. Again, concentrations were found to be similar between all groups from week to week (Fig. 9), which suggests that iron addition did not alter the net sulfate reduction rate in the microcosms. Additionally, since the devegetated control was similar to the other groups, it appears that the presence of live plants did not significantly alter net sulfate reduction.

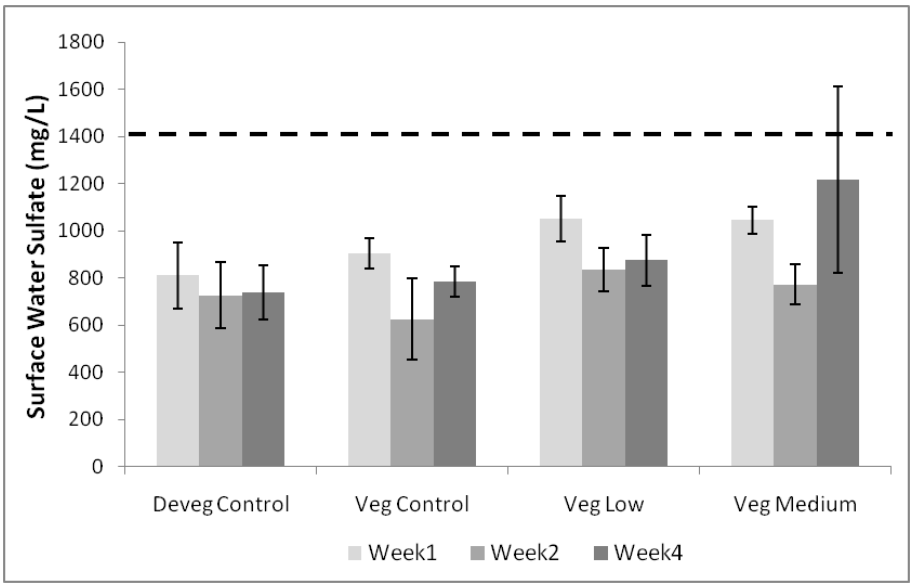

Figure 9. Surface water sulfate concentrations remaining after one week of exposure to the tidal regime (normalized to 5L reservoir volume) were similar between all treatment groups, and in the same typical range as for the devegetated experiment. The dashed line marks the initial sulfate concentration in the simulated estuarine water. Values shown as Avg±SE.

Patterns in the porewater sulfide data were less clear, since there was substantial variability among the triplicate microcosms (Fig. 10) which made it hard to draw definitive conclusions. On the average, groups amended with iron had lower sulfide concentrations than the Vegetated Control; for the Medium dose group, two of the three individual microcosms had values that stayed below 0.2 mg/L throughout the 8 week observation period, while a single microcosm (Tank 3) had a sulfide concentration that stayed around 30 mg/L throughout.

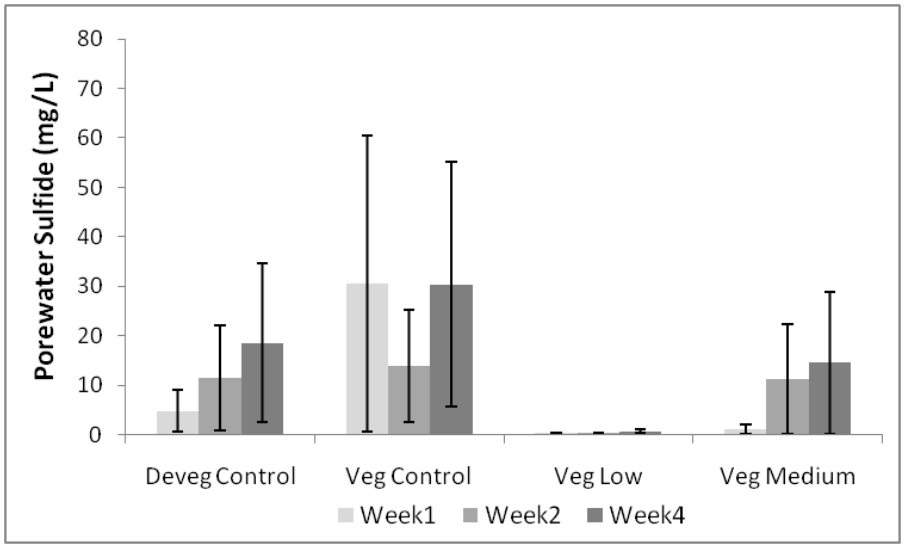

Figure 10. Porewater sulfide for the planted microcosms showed high variability within groups, however, the average concentrations demonstrated a reduction in dissolved sulfide in the porewater of the iron-amended groups. Values shown as Avg±SE.

There was substantial variation within dosing groups for the dissolved iron concentrations in the porewater as well (Fig. 11). On the average, the dosing groups were ordered in the expected fashion, where the highest doses had the highest levels of iron in the porewater. This was similar to what was found in the non-vegetated experiments, however, the decreases in porewater iron over the 8 week period were much smaller than the decreases found in the sediment experiment. The planted microcosms had iron concentrations that decreased by less than an order of magnitude, while the devegetated microcosms decreased by close to two orders of magnitude during their first 8 weeks. A possible explanation for this smaller decrease in dissolved iron for the planted experiment is that the picklweed roots may have helped to cycle the iron by continually oxidizing the recently formed pool of $FeS_{(s)}$.

For the Medium dose group, Tank 3 was again found to behave differently than the other two microcosms, where it had concentrations that were two orders of magnitude smaller than the others. While it is hard to infer what caused Tank 3 to be different than the other replicates within the Medium group, the relationship between porewater iron and sulfide makes intuitive sense. Since the porewater iron availability in Tank 3 was decreased to below $1.5x10^{-3}$ g/L levels by Week 2 (similar to the iron concentration found in both control groups), the sulfide concentrations were able to build up in the porewater since there was not enough iron present to precipitate it out as $FeS_{(s)}$.

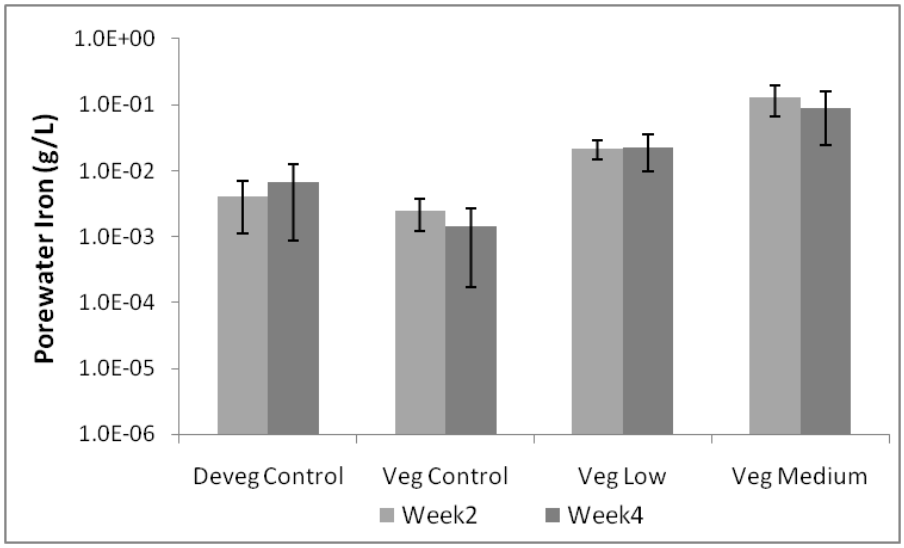

Figure 11. Porewater dissolved iron for planted microcosms showed higher average concentrations for the Medium and Low iron-dose groups, as expected. Decreases over time were found to be smaller than for the devegetated experiment.Values shown as Avg±SE.

Inorganic mercury concentrations were again found to be fairly similar between all groups, and with a consistent range of values over time (Fig. 12). This again suggests that the export of mercury from the sediments was not the main influence on methylmercury concentrations.

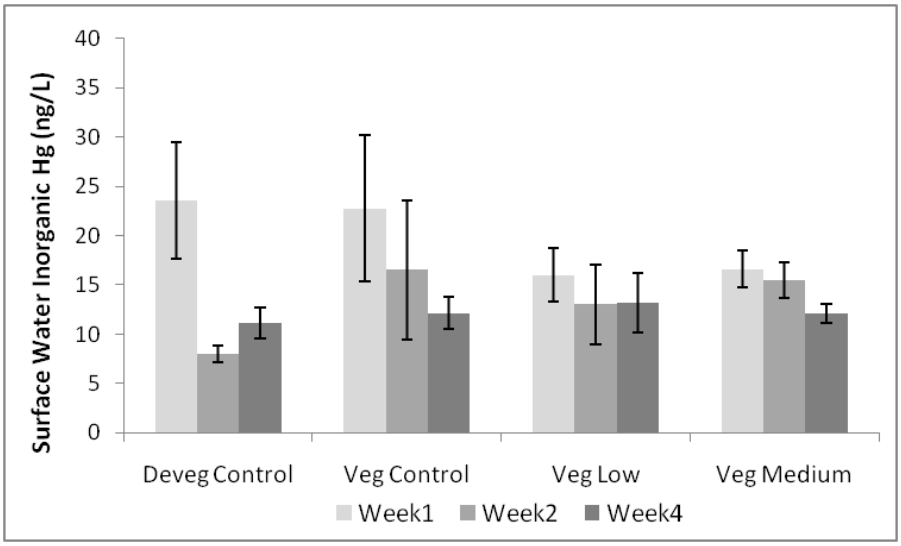

Figure 12. Inorganic mercury values (normalized to the 5-L reservoir volume) suggested similar export of mercury from the sediments was similar for all treatment groups. Values shown as Avg±SE.

No significant differences were found in the surface water MeHg concentrations between the treatment groups (Fig. 13). In the initial three weeks following the amendment, there was some ordering of the averages, where the treated groups were lower than the Vegetated Control. However, the averages for the Vegetated Control were increased by very high concentrations of MeHg from a single microcosm during each week. For example, in Week 1 Tank 5 had a normalized surface water MeHg concentration of 50 ng/L, while Tanks 12 and 10 had values of 5.6 and 0.2 ng/L, respectively. Then in Week 2, Tank 12 had a concentration of 48 ng/L while Tanks 5 and 10 had values of 2 and 0.8 ng/L, respectively. This heterogeneity in the exported MeHg resulted in very large standard errors, and made it challenging to evaluate if there were any differences between the control and planted groups. By Week 4 of the experiment, all of the microcosms were exporting a similar amount of MeHg, and continued to do so until the conclusion of the experiment. This is similar to the behavior found in the sediment microcosms, where all of the dosing groups were approaching a low value by Week 12.

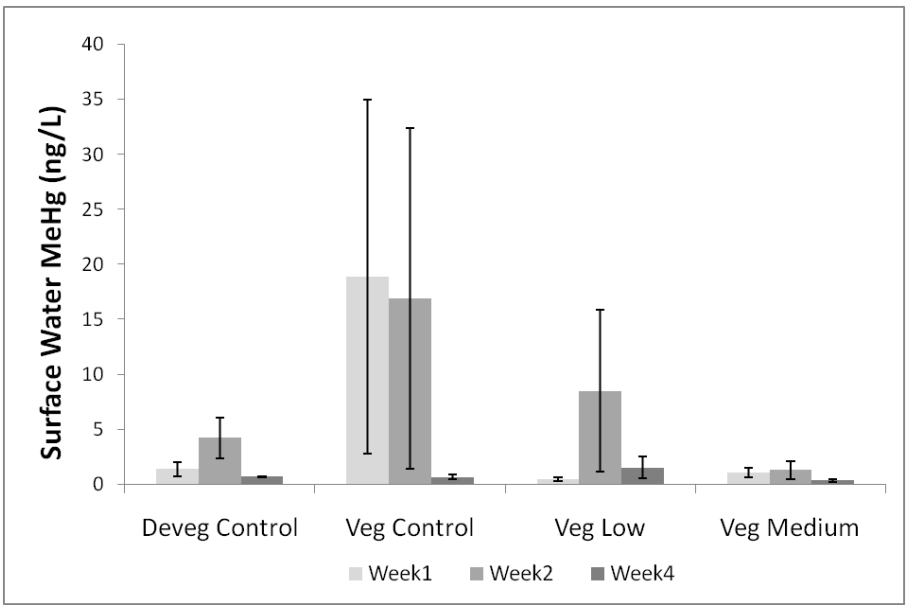

Figure 13. Surface water methylmercury values (normalized to the 5-L reservoir volume) did not show substantial differences between the treatment groups. Around the fourth week of the experiment, the microcosms were all exporting similarly low concentrations of MeHg. Values shown as Avg±SE.

In contrast to the non-vegetated experiment, small differences in the average AVS over the 2-5cm depth range were found in the planted microcosms, while no difference was found in CRS (Fig. 14). There are a few potential reasons of why this would be the case. The first is that the experiment was run for less than half of the time of the non-vegetated sediment study, so the potentially slow formation of pyrite (CRS fraction) may not have had sufficient time to form. It is also possible that redox cycling due to oxygen inputs from the plant roots could have played a role by keeping the pool of AVS in a constant flux, and not allowing the formed minerals enough time to react and form pyrite.

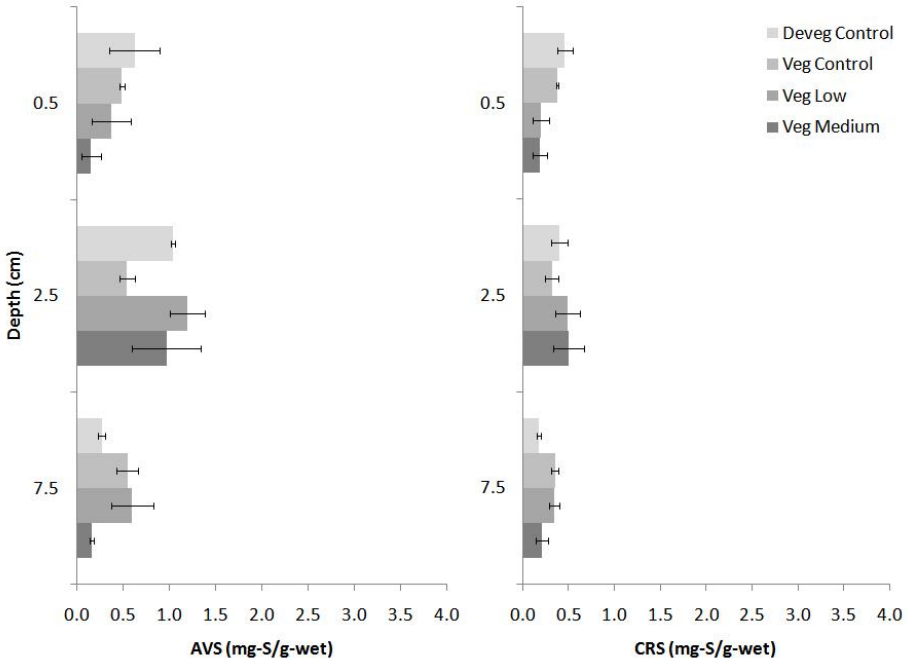

Figure 14. Depth profiles of reduced sulfur speciation for three segments of the top 10-cm of sediments from composite core samples. There is evidence of enhanced AVS production in the iron treatment groups for the 2-4 cm depth, and CRS values were similar between all groups at all depths.Values shown as Avg±SE.

Overall, the variability in the planted microcosms made it to draw many conclusions of if there was an effect of iron addition on the export of MeHg from the microcosms. By including plants in the system, we inherently made the biogeochemistry much more complicated, which may account for the increased variability. Additionally, the laboratory equilibration time before samples were collected was reduced from months for the devegetated sediment experiment to weeks for the planted experiment, which may have left a stronger signal of the initial heterogeneity found in an actual wetland environment.

Conclusions

There is a clear need to develop techniques for restoring and constructing tidal wetlands that will reduce the amount of methylmercury produced and exported from the sediments without sacrificing habitat quality. This project demonstrated the efficacy of a sediment iron amendment in laboratory microcosms using tidal marsh sediments. The non-vegetated sediment experiment showed significant decreases in methylmercury export of over 80% relative to the unamended control. Additionally, there was evidence of enhanced pyrite formation in the iron-amended groups, which could have implications for the long-term effectiveness of the iron amendment due to the potential for inorganic mercury to be contained within this fairly unreactive mineral matrix. The more complex case of including live wetland vegetation was evaluated as well, but the microcosms exhibited considerable variation within the treatment groups that made it hard to draw conclusions on the effectiveness of the iron amendment. However, the expected patterns of decreased porewater sulfide

concentrations and enhanced iron-sulfur mineral formation were found within this complex case, which suggests that the iron amendment may still be effective under different experimental conditions.

Before an iron-amendment management scheme could be designed for wetland restoration projects, further research is needed to demonstrate the efficacy of this approach under actual conditions encountered in the field, as well as over longer time periods. Additionally, future work should address the effect of varied tidal regimes and different wetland plant species, as well as the potential for unintended ecosystem consequences due to the introduction of excess iron into the sediments. However, the microcosm experiments described in this report, in addition to the previous work in pure cultures and sediment slurries, consistently indicate that this technique has the potential to be an effective method of controlling methylmercury production within restored tidal wetland sediments.

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