

RESEARCH ARTICLE

The Potential Role of Sediment Iron and Sulfur Speciation in Seagrass Meadow Loss and Recovery

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Oxidized iron (Fe) can reduce seagrass dieback when present in sufficient quantities in the sediment to fix sulfide as pyrite (FeS₂) or iron monosulfide (FeS). However, the oxidized Fe pool may become depleted over time as Fe is reduced and precipitated with sulfides. In this study, we estimated long-term variations in the speciation of solid forms of reduced and oxidized Fe along a eutrophication gradient in West Falmouth Harbor (WFH) (a temperate lagoon with substantial seagrass meadows) and conducted a 6-week microcosm study to assess the role of oxidized Fe in supporting seagrass recovery. We planted seagrass in sediments obtained from 2 WFH regions with differing Fe speciation. We found depletion of oxidized Fe over a decade following a seagrass dieback, even when the soluble sulfide levels decreased to concentrations unlikely to cause toxicity in seagrass. The continued absence of large concentrations of available oxidized Fe minerals in sediments, where most Fe was bound in FeS₂, could impede the recovery of seagrass in formerly vegetated regions. Seagrass grown in sediments with low Fe:S ratios exhibited an increased probability of survival after 4 weeks. Field and laboratory results indicated that even when the soluble sulfide levels decrease after seagrass dieback, sediments may not be able to support seagrass recovery due to the legacy effects of eutrophication on the sediment Fe pool. However, we observed signs of reoxidation in the Fe pool within a few years of seagrass dieback, including a decrease in the total sediment S concentration, which could help spur recolonization.

Introduction

Seagrass ecosystems have experienced considerable degradation and area loss in recent decades [1]; this loss is expected to increase in the coming years [2] partly due to increased coastal development and eutrophication [3]. A factor responsible for seagrass meadow mortality is the accumulation of soluble sulfides in the rooting zone [4,5]. Sulfides inhibit cellular respiration, and seagrass, as marine angiosperms, have root and rhizome structures that are often in direct contact with anoxic sediments [6]. However, even in regions of high sulfate reduction, soluble sulfides may not accumulate if they are precipitated with dissolved iron (Fe(II)) or by reacting with Fe minerals such as amorphous Fe-oxides, goethite, ferrihydrite, and lepidocrocite [7]. When soluble sulfide concentrations are sufficiently high, Fe monosulfide (FeS) readily precipitates. Pyrite (FeS₂) can also readily precipitate in microcrystalline forms, even at very low concentrations of soluble sulfides (conditions under which FeS does not precipitate) when there is a source of oxidizing power. In this case, some of the soluble sulfides are oxidized to produce elemental sulfur (S), which then readily

reacts with soluble sulfides to produce soluble polysulfides; these polysulfides can precipitate to form FeS₂ [8–11]. FeS can readily dissolve when the concentrations of soluble sulfides decrease, releasing Fe(II) and soluble sulfides. FeS₂ is many orders of magnitude less soluble than FeS, and it is more resistant to dissolution as the concentrations of soluble sulfides decrease [11–13]. Thus, FeS can be depleted from sediments via oxidation or dissolution, whereas FeS₂ is depleted only via dissolution. Because of the ability of Fe in decreasing soluble sulfide levels, several researchers have experimented with adding reactive Fe to seagrass meadow sediments to increase their probability of survival [14–16], especially in low-Fe carbonate sediments [7,17–19]. In some of these experiments, reduced mortality and improved seagrass health were observed after Fe injection [18,19]. In other experiments, no change was observed in some seagrass health parameters [7]. Clastic glacial sediments may contain high amounts of reactive Fe [20]; however, few studies on Fe addition have been conducted. The concentration of Fe-oxyhydroxides can be diminished over time if soluble sulfides continue to accumulate, leading to sediments with high concentrations of solid-phase reduced Fe, S, and soluble sulfides

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but with low amounts of reactive Fe [21,22]. This may occur in eutrophic conditions, where enhanced organic carbon (C) transport to sediment fuels increases sulfate reduction, leading to high-sulfide levels [22]. Seagrass ecosystems store high amounts of C in sediments per area [23]. Seagrass protection and recovery are considered highly important in actions against climate change and consequential for coastal health [24]. One reason that seagrass stores high levels of C is the preservation of organic matter (OM) in association with Fe-oxide minerals, preventing it from being respired [25]; thus, the preservation of Fe-oxides in sediments results in the preservation of organic C stocks and the prevention of sulfide buildup. Additionally, the presence of high amounts of Fe-oxides in sediments can slow down sulfate reduction by stimulating dissimilatory Fe reduction [18], which is a more thermodynamically favorable pathway than sulfate reduction [26].

In this paper, we present the results of a field study on Fe mineral composition in 3 areas of a nutrient-enriched lagoon with different histories of seagrass loss, porewater sulfide, and eutrophication. Additionally, by conducting an experiment on the lagoon microcosm, we investigated whether the addition of reactive oxidized Fe as FeOOH can increase the probability of seagrass survival in sediments obtained from Snug Harbor (SH) and Outer Harbor (OH).

Methods

Site description and history

We collected all seagrass and sediments from West Falmouth Harbor (WFH) (41°36'30"N, 70°38'33"W), which is a shallow lagoon adjoining Buzzards Bay on Cape Cod, MA (Fig. 1). WFH has experienced high nutrient loading since 2000 or so due to nitrate inputs from an aquifer contaminated by an

upstream wastewater treatment plant and diffuse sources in the watershed [27,28]. In this study, we focused on 3 basins within WFH: OH, Middle Harbor (MH), and SH. Most of the total nitrogen (N) load enters the harbor in the SH basin with an estimated load of 17 mmol of N m⁻² day⁻¹ [27]. However, inorganic N concentrations are low during the summertime because of rapid biological uptake; in general, N concentrations are high in SH, intermediate in MH, and low in OH (Hayn et al., in preparation). The outermost 2 basins (OH and MH) contain extensive seagrass meadows consisting of monospecific stands of *Zostera marina*; the innermost basin (SH) supported seagrass meadows until 2010 when a sudden dieback event led to the complete loss of seagrass [5]. The OH basin is characterized by sandy, low organic sediments, whereas MH and SH are characterized by organic fine-grained sediments, although OM has been decreased in SH since 2020 [5]. MH is further characterized by high soluble sulfide concentrations with values exceeding 2.0 mM being common in the seagrass rhizosphere, whereas SH is characterized by low-sulfide concentrations, generally below 0.5 mM [5]. The concentrations of soluble sulfides in OH are typically below 0.5 mM but spatially heterogeneous. Seagrass in MH typically has lower density, lower aboveground-belowground biomass, and more reduced tissue $\delta^{34}\text{S}$ than in OH; this indicates that the MH population is somewhat less healthy than the OH population [5]. Notably, before the seagrass loss in SH in 2010, soluble sulfide concentrations were very high, approaching 2.5 mM; however, they have decreased since then [5]. For further information on this site, see Table S1 [5,27].

Field study

In early July 2022, divers collected 5 replicate cores from the 3 basins mentioned above (15 cores in total; Table S1) using 6.4-cm-diameter acrylic tubes inserted into a sediment depth

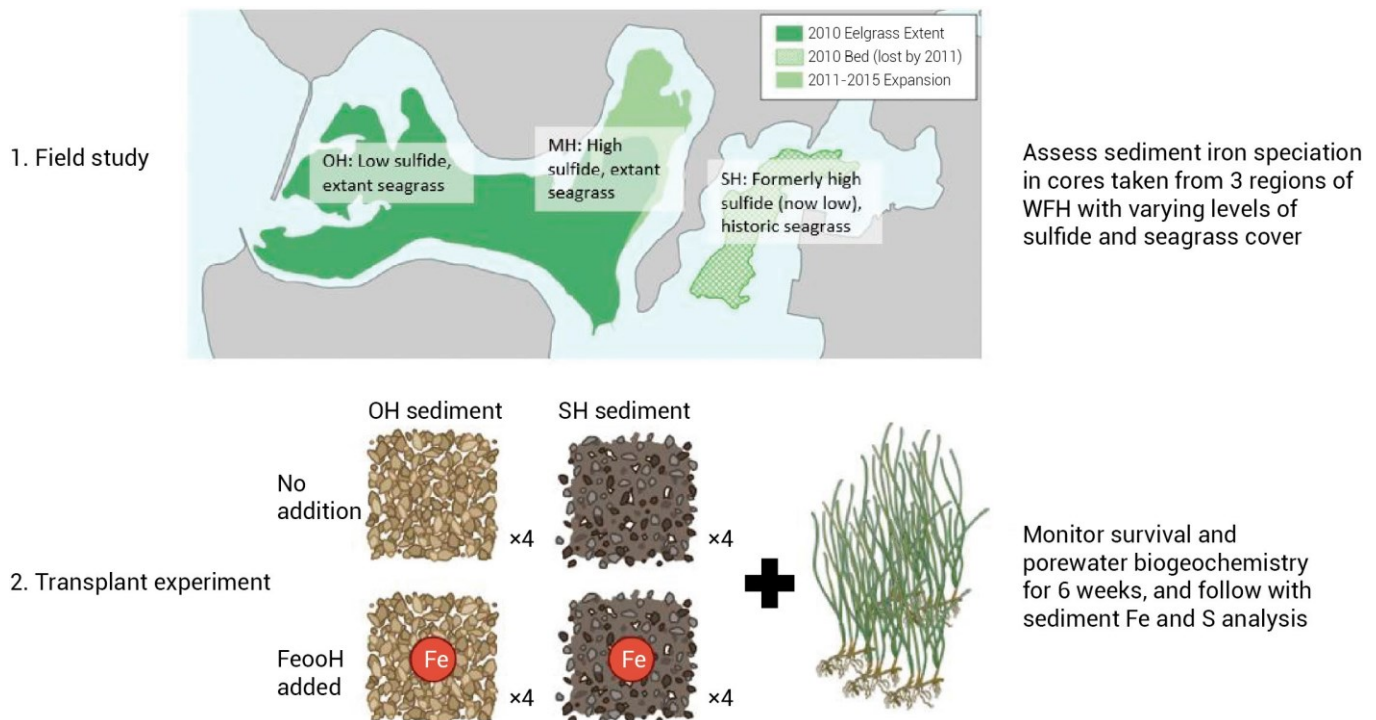


Fig. 1. Diagram showing the investigation process: (1) field study (described in the "Site description and history" and "Field study" sections); (2) experimental study (described in the "Fe amendment experiment on the WFH microcosm" section).

of ~12 cm. All cores were obtained within 1 m of each other. Care was taken to avoid seagrass in the 2 vegetated regions. When large rhizomes or blades were observed, the cores were resampled. The obtained cores were kept in an ice cooler in a dark space during their transportation to the Marine Biological Lab in Woods Hole, MA, ~12 km from the collection site. Of the 5 replicate cores, 3 were used for sediment solid-phase, one for porewater, and one for bulk density and porosity analyses. Within 3 h of collection, 4 of the 5 cores were placed in a glove bag for processing under an anoxic N₂ atmosphere. In 3 of the 5 cores, the top 8 cm of sediment was exuded and homogenized. From each homogenized top 8-cm sample, 8 to 10 g of wet sediment was weighed within the glove bag for acid-volatile sulfide (AVS) analysis, and 0.5 g was reserved for 0.5 M hydrochloric acid (HCl)-extractable Fe analysis. The remaining sediment was removed from the glove bag and dried at 100 °C for 48 h to be used in the subsequent analysis of the total S, total Fe, and OM concentrations. From the final core, we extracted a 5-ml porewater sample using a Rhizon porewater sipper and inserted 4 cm into the sediment to assess the porewater sulfide and Fe concentrations. The final core was extruded and sliced into 2-cm segments with an 8-cm depth. Each segment was placed in preweighed tins and weighed wet and dry to estimate the bulk density and porosity in the top 8 cm of the sediment. The analysis methods and relevant information are presented in Table 1.

We extracted AVS using passive diffusion [29]. Sediment samples were acidified using 9 M HCl in a deoxygenated apparatus with a 3% alkaline zinc acetate (ZnAc) trap. Sulfide that was volatilized using the acid solution was allowed to diffuse into the ZnAc trap for 24 h; then, it was measured using the methylene blue method [30]. The concentrations of amorphous Fe-oxides were estimated using a weak acid (0.5 M) [31]. The

samples were placed in Falcon tubes with 10 ml of 0.5 M trace-metal grade HCl, shaken overnight, and then centrifuged. The supernatant was assessed for total Fe concentration using flame atomic absorption spectroscopy (flame AA). Porewater samples were subsampled to perform 2 types of analysis: (a) the samples were fixed in 2% ZnAc and assessed for sulfide concentrations using the methylene blue method; (b) the porewater Fe concentration was measured using flame AA. Porewater Fe samples that fell below the detection limit of the flame AA were analyzed using the more sensitive ferrozine colorimetric method, which is described in detail in the “Fe amendment experiment on the WFH microcosm” section.

Dried sediment samples were divided into 3 groups: total S samples, total Fe samples, and OM samples. The total S sample concentrations were assessed via combustion at 1,850 °C using a LECO S632 total S analyzer. Regarding the total Fe samples, 0.1 to 0.3 g of dried sediment was digested in a modified *aqua regia* solution in an unsealed 50-ml container. Initially, 5 ml of concentrated nitric acid (HNO₃) was added to each sample. Then, the samples were swirled and heated in a water bath at 70 °C for 1.5 h; next, 5 ml of concentrated HCl was added to each tube. The samples were heated for an additional 1.5 h, cooled, and then diluted using ultrapure distilled water; finally, they were filtered into a 100-ml volumetric flask, where they were brought to volume, and assessed for Fe concentrations using flame AA. The OM concentration was assessed using loss on ignition by placing the samples in preweighed tins; the samples were weighed before and after combustion at 500 °C in a muffle furnace for 4 h.

We calculated the concentrations of amorphous Fe-oxides similarly to the acid-soluble Fe described above minus the amount of Fe calculated from AVS. We assumed that AVS mainly contained FeS [11]. We estimated the concentrations of FeS₂-Fe as the remaining total Fe sediment that was not considered in the AVS or amorphous Fe-oxide analyses. We evaluated the contribution of crystalline Fe-oxides by comparing the molar Fe:S ratios calculated after subtracting Fe and S in FeS and amorphous Fe-oxides (see Results) from those in FeS₂ (0.5). Considering the high mineral content of these sediments, we assumed that the contribution of organic Fe to the total Fe pool was low or negligible [32]. Given the low organic content of the sediment, organic S was also unimportant.

The total S concentration in a subset of historic samples archived from previous WFH projects was assessed using a LECO S632 total S analyzer. These samples were collected in July 2005, 2007, 2013, and 2017 from the same sites by divers using 2-cm-diameter acrylic tubes inserted into a sediment depth of ~6 to 8 cm. The historic porewater sulfate and chloride concentrations investigated in this study were assessed using porewater samples by applying “peeper” techniques [5,33] and analyzed within 1 month of sampling using Dionex ion chromatography.

Fe amendment experiment on the WFH microcosm

In late July 2022, we collected 10 l of sediments from the OH and SH sites and kept the cores in oxygenated water for 3 days. We collected ~60 seagrass shoots with intact rhizomes from the OH site and kept them overnight in oxygenated water. The collected seagrass and sediments were transported in a cooler of oxygenated seawater to Cornell University (Ithaca, NY); the seagrass was allowed to acclimate for 10 days in a shallow tray containing an OH sediment in an aquarium environment and

Table 1. Methods used for the field, experimental, and historic sediment analyses. For the field analysis, information is provided on the number of replicate cores per sample and the specific core used.

Analyte	No. of cores (field)	Core no. (field)	Type of analysis
Total solid-phase Fe	3	1, 2, 3	Field and experimental
Total solid-phase S	3	1, 2, 3	Field, experimental, and historic
Acid-volatile S	3	1, 2, 3	Field
Acid-extractable Fe	3	1, 2, 3	Field
Porewater Fe	1	4	Field and experimental
Porewater sulfide	1	4	Field and experimental
Loss on ignition	1	5	Field and experimental
Bulk density	1	5	Field

then submersed in a 50%/50% ratio of seawater transported from WFH and artificial seawater (Instant Ocean). During the experiment, the plants were kept in filtered (TopFin PRO120 multistage canister filter) and oxygenated water under artificial growth lights for a 12-h light-dark cycle. We separated the remaining sediments into 16 1-l buckets, 8 of which contained SH sediments; the remaining 8 buckets contained OH sediments. Half of the sediment samples received a 1-ml 0.2-M Fe injection in the form of a Fe-oxyhydroxide slurry, which was created by mixing ~5 g of FeCl₃ with 100 ml of oxalic filtered seawater, achieving a total increase of ~200 µmol per sample. This process was based on previous studies on Fe addition, where beneficial effects on seagrass were reported [18,19]. The other half of the sediment samples received a 1-ml injection of filtered seawater. Control or treatment solutions were slowly injected directly into the sediments using tubing. A Rhizon sampler was inserted ~2 cm deep in each bucket, where it remained for the duration of the experiment. Then, seagrass of 4 to 5 plants per bucket was planted in the sediments and allowed to grow for 6 weeks. We counted the number of live and dead plants per bucket on a weekly basis. A plant was considered alive if it had any remaining green vegetation, and dead if it was completely brown or broken off at any point below the top of the meristem. We assessed seagrass health in terms of their overall survival rate throughout the entire 6-week experiment and after 1 month (referred to as "initial success" [34]). Once every 2 weeks, we sampled porewater to estimate the total porewater Fe and soluble sulfide concentrations. The soluble sulfides were fixed to 10% ZnAc and stored for ~2 months before the analysis using the methylene blue method described above. The total porewater Fe samples were immediately injected into a ferrozine solution, which was subsequently reduced using hydroxylamine hydrochloride, and then assessed spectrophotometrically [35,36].

At the end of the experiment, we sampled the surviving seagrass from the aboveground and belowground tissue biomass and leaf area. We collected all sediments from each bucket and dried them at 100 °C for 48 h. The sediments were digested in a modified *aqua regia* solution, as described previously, to estimate the total S and total Fe concentrations; then, they were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) (using a Perkin Elmer Optima 3300DV spectrometer) at SUNY ESF, Syracuse, New York. The total S concentration was assessed for a subset of samples using both a LECO S632 total S analyzer as well as modified *aqua regia* and ICP-OES for $R^2 = 0.99$ (Fig. S1). The C and N concentrations in the sediments were analyzed at the Cornell Stable Isotope Lab.

Statistical analysis

All statistical analyses were conducted using the R software. To assess the differences among the 3 groups in the field study, we used Tukey's multiple comparison test. To determine the correlations between variables, we used simple linear regression, where each basin (MH, SH, or OH) was incorporated as a block factor. To assess the differences in sediment conditions in the experiment, we used multiple *t* tests because the small sample size of each treatment group ($n = 4$ for sediments) violates the assumptions of hierarchical modeling. To assess the probability of seagrass survival in the aquarium environment under different sediment treatments, we used a Cox proportional hazards model in R [37]. All figures were created using the ggplot2 software [38] except for the Kaplan-Meier plot, which was created using the ggsurvfit software [39].

Results

Field study

The bulk density and porosity of the samples obtained from the 3 sites were different. In the MH samples, the bulk density was 0.29 g cm⁻³, and the porosity was 77%. In the OH samples, the bulk density was 0.82 g cm⁻³, and the porosity was 32%. In the SH samples, the bulk density was 0.30 g cm⁻³, and the porosity was 60%. The concentrations of the MH porewater total soluble sulfides were the highest (920 µM), whereas those of the SH and OH sulfides were considerably lower (26 and 22 µM, respectively). The concentration of the MH porewater total Fe was below the detection limit in both the ferrozine and flame AA methods (<5 µM). The concentrations of the SH and OH porewater Fe were 0.7 and 15.1 µM, respectively. The average contents of OM in the MH, OH, and SH sediments were 12.2%, 7.1%, and 1.9%, respectively. Note that for each site, a single homogenized porewater sample obtained from the top 6 cm of each core was analyzed.

The Fe and S concentrations in the sample sediments obtained from each basin are presented in Table 2. The concentration of the total Fe in the MH sediments was the highest with an average value of 253 µmol per gram of dry sediment (the standard deviations are presented in Table 2). The average concentrations of the total Fe in the SH and OH sediments were 205 and 69 µmol per gram of dry sediment, respectively. The total Fe concentrations in the samples obtained from the 3 basins were significantly different (SH-MH: $P = 0.03$; SH-OH: $P < 0.01$; MH-OH: $P < 0.01$). The total S concentration followed the same pattern; it was the highest in the MH samples (average value: 307 µmol per gram of dry sediment), medium in the SH samples (251 µmol per gram of dry sediment), and lowest in the OH samples (67 µmol per gram of dry sediment); the differences were SH-MH: $P = 0.07$; SH-OH: $P < 0.01$; and MH-OH: $P < 0.01$. The total Fe and total S concentrations in the samples obtained from the 3 basins were closely correlated ($P < 0.01$, $R^2 = 0.98$). The average molar Fe:S ratio was the highest in the OH samples (1.1) and the lowest in the MH and SH (0.83 and 0.82, respectively); however, the differences were not significant ($P > 0.1$).

The average concentrations of the amorphous Fe-oxides in the MH, OH, and SH sediments were 19, 33, and 29 µmol per gram of dry sediment, respectively. The differences in the concentrations of the amorphous Fe-oxides among the basins were not significant because of the wide variability in concentrations among replicate cores (Fig. 2A, $P > 0.1$ for all). In the MH, OH, and SH sediments, the average contents of the amorphous Fe-oxides were 7.8%, 50.9%, and 14.8% of the total Fe pool, respectively (Fig. 2B); the differences were OH-MH: $P = 0.07$; OH-SH: $P = 0.1$; and SH-MH: $P = 0.9$. The highest concentration of AVSs, the majority of which was likely FeS, was 54 µmol of Fe per gram of dry sediment in MH, followed by 21 µmol of Fe per gram of dry sediment in SH, and 6 µmol of Fe per gram of dry sediment in OH; the differences were MH-OH: $P = 0.09$; MH-SH: $P > 0.1$; and SH-OH: $P > 0.1$ (Fig. S2). Again, wide variability among the replicate cores obtained from each basin was observed (Fig. 2), demonstrating the high spatial heterogeneity of Fe dynamics in these sediments. In the MH, SH, and OH samples, the average contents of the FeS-associated Fe were 21%, 10%, and 8% of the total Fe pool, respectively. FeS₂ was found at much higher levels in the SH and MH samples than in the OH samples; the average Fe concentrations were 125,

Table 2. Total Fe, total S, and different Fe mineral concentrations in the SH, OH, and MH sediments [mean value ± standard deviation (n)]

	Outer Harbor	Middle Harbor	Snug Harbor
Total Fe (μmol per gram of dry sediment)	69 ± 10 (3)	253 ± 25 (3)	205 ± 15 (3)
Total S (μmol per gram of dry sediment)	67 ± 23 (3)	307 ± 35 (3)	251 ± 6 (3)
S (%) by weight	0.2% ± 0.06 (3)	1.0% ± 0.09 (3)	0.8% ± 0.06 (3)
Molar Fe:S ratio	1.1 ± 0.3 (3)	0.83 ± 0.01 (3)	0.82 ± 0.06 (3)
AVS (Fe μmol per gram of dry sediment)	6 ± 3 (3)	54 ± 33 (3)	21 ± 21 (3)
Total Fe (%) in AVS	8% ± 6 (3)	21% ± 9 (3)	10% ± 8 (3)
FeS ₂ -Fe (Fe μmol per gram of dry sediment)	19 ± 19 (3)	125 ± 15 (3)	117 ± 18 (3)
Total Fe (%) in FeS ₂	27% ± 28 (3)	49% ± 1 (3)	56% ± 7 (3)
Amorphous Fe-oxides (Fe μmol per gram of dry sediment)	33 ± 12 (3)	19 ± 28 (3)	29 ± 23 (3)
Total Fe (%) in amorphous Fe-oxides	51% ± 27 (3)	8% ± 12 (3)	15% ± 12 (3)
Crystalline Fe-oxides (Fe μmol per gram of dry sediment)	11 ± 18 (3)	56 ± 20 (3)	39 ± 34 (3)
Total Fe (%) in crystalline Fe-oxides	14% ± 9 (3)	20% ± 16 (3)	19% ± 9 (3)
Porewater Fe (μM)	15.1 (1)	<5 (1)	<5 (1)
Porewater sulfide (μM)	22 (1)	26 (1)	920 (1)

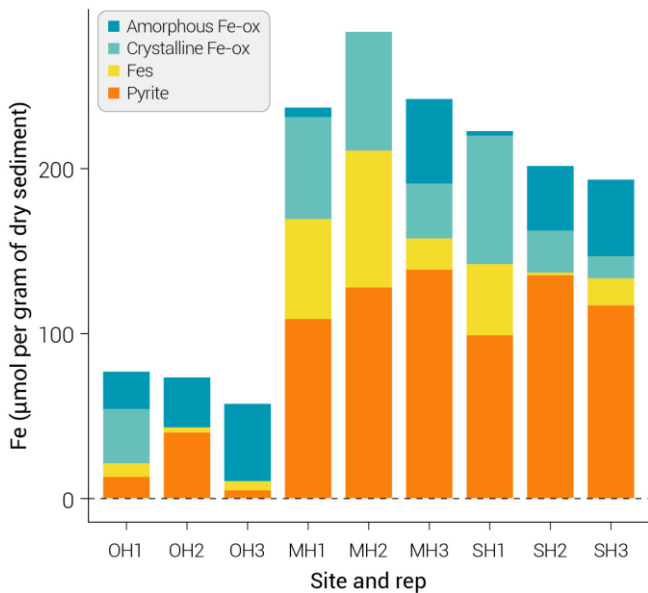


Fig. 2. Total Fe, amorphous Fe-oxides (Fe-ox, marked in teal), crystalline Fe-oxides (Fe-ox, marked in blue), FeS (marked in yellow), and pyrite (marked in orange) in the 3 regions of WFH, Outer Harbor (OH1, OH2, and OH3), Snug Harbor (SH1, SH2, and SH3), and Middle Harbor (MH1, M2, and MH3). The bars are scaled from the smallest to the largest according to the total Fe concentration; each bar represents one core.

117, and 19 μmol per gram of dry sediment in the MH, SH, and OH samples, respectively; the differences were OH-MH: $P < 0.01$; OH-SH: $P < 0.01$; and SH-MH, $P = 0.4$. In the MH, SH, and OH samples, the average contents of the FeS₂-Fe of the total Fe pool were 49%, 56%, and 27%, respectively. The concentrations of crystalline Fe-oxides did not significantly differ among the basins; the highest, medium, and lowest average concentrations were observed in the MH, SH, and OH samples,

respectively ($P > 0.1$ for all basin-level differences). The average contents of the crystalline Fe-oxides of the total Fe pool were comparable in the SH and MH samples (~20%) and ~14% in the OH samples (Table 2).

Historic decrease in S content in SH sediments

Historic sediments obtained from SH and assessed for total S content showed 2 separate patterns, depending on the seagrass presence or absence at the time of sampling (Fig. 3). The samples collected in 2005 were low on S content compared with current and other historic samples; they contained 0.7% of S by weight ($n = 6$) on average. The samples collected in 2007 showed a remarkable increase in S content; they exhibited the highest average S content among all samples used in the experiment 1.3% by weight ($n = 5$). Due to the seagrass loss in 2010, the average S content in the samples collected in 2013 had decreased considerably since 2007 (1.0% by weight, $n = 3$). This trend continued over the next 10 years; the average S content in the samples obtained in 2017 (0.86% by weight ($n = 3$)) was slightly lower than that in 2013; the average S content in the samples collected in 2022 was 0.78% by weight ($n = 6$).

WFH microcosm experiment

The seagrass survival rate per replicate core varied from 0% to 75% throughout the experiment, depending on the treatment and sediment origin. The initial success (survival rate after 4 weeks) varied from 0% to 100%. Of the 5 replicate cores with a 0% total survival rate throughout the experiment, 3 were obtained from the SH unamended sediment group and none from the OH + Fe sediment group. One replicate core of the untreated SH sediment was found to be heavily colonized by parchment worms (*Chaetopterus* spp.) with burrows directly adhered to seagrass roots. At least 6 distinct burrows were found; all were associated with different seagrass roots; their length varied between 3 and 14 cm. The sediment originating from SH was heavily colonized by *Chaetopterus* spp.;

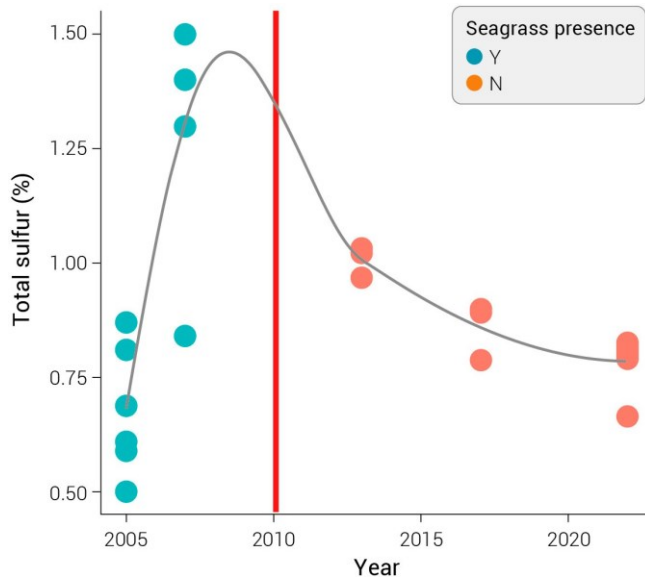


Fig. 3. Total solid-phase S content (expressed in percentage by weight) in the Snug Harbor sediments obtained from the same long-term monitoring site during 2005 to 2022. The total S content increased rapidly during 2005 to 2007, leading to a seagrass dieback event in the region in 2010 (red line); afterward, the total S content slowly decreased during 2013 to 2022. The gray line represents locally estimated scatterplot smoothing.

this sediment exhibited the highest total survival rate of 60% compared with all other samples obtained from the replicate cores; this rate is almost 3 times higher than the next closest rate exhibited by the replicate cores in the group. In all survival rate analyses and figures, this replicate core was omitted or included as a separate treatment class, labeled as “*Chaetopterus*”. On average, 56% of the seagrass planted in the OH + Fe sediment and 19% of the seagrass planted in the unamended OH sediment survived until the end of the experiment ($P < 0.05$). Similarly, 18% of the seagrass planted in the SH + Fe sediment and 8% of the seagrass planted in the unamended SH sediment survived until the end of the experiment ($P = 0.4$). Seventy-five percent of the seagrass planted in the OH + Fe sediment and 60% of the seagrass planted in the untreated OH sediment survived after week ($P = 0.2$). Fifty-three percent of the seagrass planted in the SH + Fe sediment and 17% of the seagrass planted in the corresponding untreated sediment survived after week ($P = 0.04$). The above results are presented in Fig. 4.

The aboveground and belowground biomass and the associated biomass ratio did not differ among treatment groups (Fig. S6). The concentrations of porewater soluble sulfides were low in all treatments in the WFH microcosm experiment; these concentrations exceeded 500 μM in the OH sediment samples before seagrass addition. After seagrass addition, the sulfide concentrations in both sediment classes and Fe treatments were consistently below 10 μM . The porewater Fe concentrations were similarly low; no differences between treatments were observed, regardless of the sediment origin or Fe addition.

The OM content in the sediments at the end of the experiment depended on the sediment origin; the average OM content was higher in the SH sediments (6.9%) than in the OH sediments (1.0%). The average C contents in the OH and SH sediments were 0.4% and 3.0%, respectively ($P < 0.001$). This is a reverse trend compared with the trend observed in the field study, where OM in the OH sediments was higher than that in

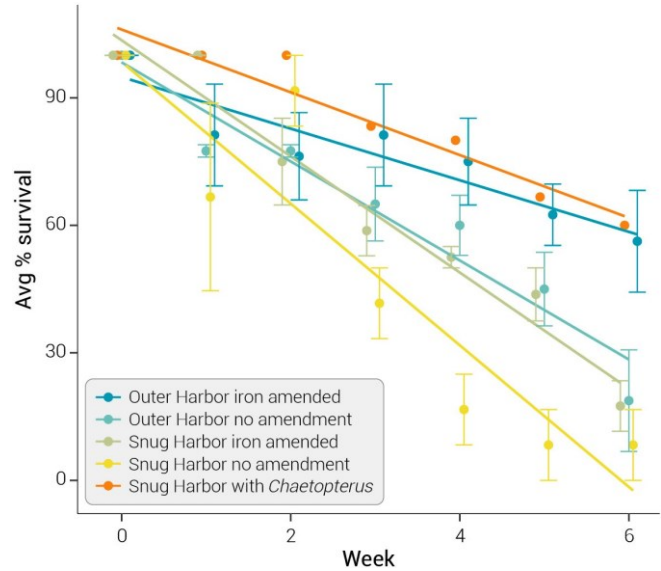


Fig. 4. Survival rate of each treatment group throughout the experiment. “Snug Harbor with *Chaetopterus*” represents a single replicate core. The bars represent the standard error.

the SH sediments. The average N contents in the OH and SH sediments were 0.05% and 0.3% by weight, respectively ($P < 0.001$). Fe addition resulted in a slight increase in the N and C contents in the OH sediments ($P = 0.07$ for both); however, there was no difference in the N and C contents in the SH sediments ($P > 0.1$); additionally, Fe addition did not affect OM in both OH and SH sediments ($P > 0.1$). The average C:N ratio was higher in the SH sediments (9.9) than that in the OH sediments (9.0) ($P < 0.001$).

The solid-phase Fe concentration at the end of the experiment was higher in the SH sediments (165.7 μmol per gram of dry sediment) than in the OH sediments (27.9 μmol per gram of dry sediment) ($P < 0.001$) and did not vary with Fe treatment ($P = 0.22$ for OH and $P = 0.17$ for SH). The solid-phase S concentration in the SH and OH sediments followed a similar pattern (200.6 and 26.5 μmol per gram of dry sediment, respectively) ($P < 0.001$) and did not vary in either sediment with Fe treatment ($P = 0.2$ for SH, $P = 0.28$ for OH). The molar Fe:S ratio in the OH sediments was higher than that in the SH sediments ($P < 0.001$); again, it did not vary with Fe treatment ($P > 0.1$ for both sediments), although the mean value in each of the Fe-addition sediments was higher than that in the untreated sediments (Fig. 5). The sediment within the *Chaetopterus* replicate core exhibited the highest concentrations of solid-phase Fe (192 μmol of Fe per gram of dry sediment) and solid-phase S (242 μmol of S per gram of dry sediment) among all sediments during the WFH microcosm experiment.

Discussion

Legacy effect of eutrophication and high sulfide

Our results indicate long-term alterations in sediment S for more than 10 years after a seagrass dieback event where soluble sulfides were a contributing factor, even after the soluble sulfide concentrations at that site (SH) had been considerably decreased [5]. In OH, the high content of total Fe in oxidized forms can reduce the soluble sulfide levels. Seagrass in OH typically exhibits lower sulfide intrusion and toxicity compared with that in

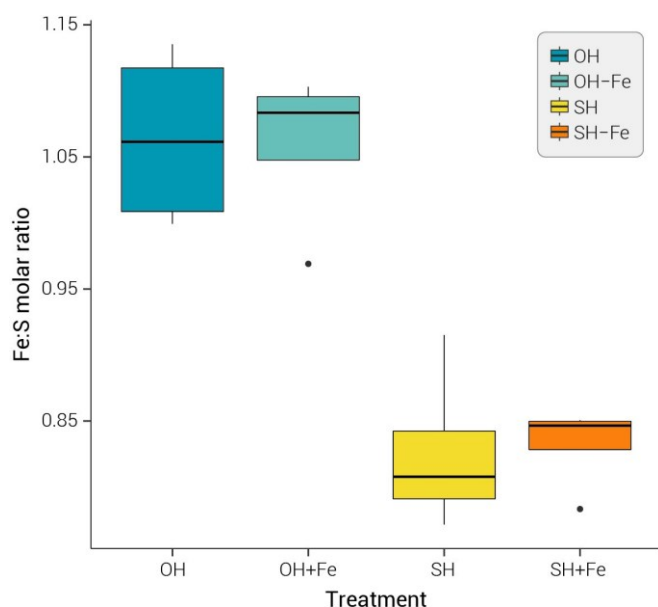


Fig. 5. Molar Fe:S ratio in the aquarium sediments at the end of the experiment. The difference between unamended and + Fe represents the integrated effect of treatment after 6 weeks of seagrass growth.

MH [5], where the Fe:S sediment ratio is below 1:1, indicating a low content of oxidized Fe.

The formation of FeS and FeS₂, both of which can be formed rapidly in regions of high sulfate reduction such as salt marshes and estuaries, depends on the environmental conditions such as concentrations of sulfide in sediments and redox conditions [8]. FeS₂ can precipitate, even when the S²⁻ activity is low; however, it requires a source of oxidizing power, such as oxidized Fe or oxygenation from seagrass roots and rhizomes, to produce elemental S. Conversely, FeS, which is a less oxidized mineral, is only formed where the S²⁻ activity is high [8]. When soluble sulfides decrease, as observed in SH in the period 2007 to 2019 [5] and in this study, FeS but not FeS₂ dissolves [8]. Seagrass typically creates regions of low pH in the rooting zone because of the exudation of organic acids [40,41] and increased oxygen levels via diffusion from roots [41,42]; this promotes the formation of FeS₂ [8,11].

In the SH sediments, the FeS₂ content is more than 50% of the total Fe pool (Fig. S4), even after soluble sulfides decreased to levels comparable with those in the OH sediments [5]. This persistent FeS₂ pool means that Fe-oxides remain lower in this basin than in OH, despite evidence of enhanced bioturbation and sediment oxidation; thus, the capacity of reactive Fe in the SH sediment pool to react with porewater dissolved sulfides is reduced. However, we do observe signs of recovery in SH; both crystalline and amorphous Fe-oxide contents are higher in SH than in the high-sulfide MH; additionally, the total sulfur content in the sediments decreased during the 10-year period after dieback (Fig. 3). When reactive solid-phase Fe is depleted in a region due to historic high-sulfide levels, the reintroduction of seagrasses may be difficult because the initial transplanting could elevate sulfide due to increased OM content trapped in seagrass sediments and root exudation, causing mortality in already stressed plants [43].

In the SH sediments, S increased rapidly during the period 2005 to 2007 (Fig. 3). During the same period, excess N entered the system via groundwater from upstream wastewater treatment,

resulting in increased eutrophication [27,28] and, consequently, high porewater sulfide levels; the S concentration in the SH sediments in 2007 approached 3.0 mM [5], which is nearly 3 times the concentration that causes adverse effects on the health of *Z. marina* [44,45]. Although no soluble sulfide measurements were conducted in 2005 at the SH site, the porewater sulfate-to-chloride ratios measured in 2005 indicate sulfate reduction in the sediments. Low SO₄²⁻:Cl⁻ values indicate a high degree of sulfate consumption and the presence of soluble sulfides via sulfate reduction. In SH in 2005, the average SO₄²⁻:Cl⁻ ratio was 0.048 in the top 12 cm of the sediments; this value is similar to the low-sulfide OH basin ratio of 0.05 measured in 2019 and virtually identical to the seawater SO₄²⁻:Cl⁻ ratio [5]. This indicates that soluble sulfide contents were not remarkably high in SH in 2005. By 2007, the average SO₄²⁻:Cl⁻ ratio decreased to 0.018 in the top 12 cm of the SH sediments; this value is similar to those in high-sulfide MH samples in 2019 [5]. This indicates a rapid increase in sulfide during the period 2005 to 2007, which could have led to the formation of FeS₂, resulting in a remarkable increase in the sediment total S during this 2-year period (Fig. 3).

After seagrass dieback in 2010, the total sediment S decreased considerably by 2013 and slowly afterward (Fig. 3). This initial decrease indicates the dissolution of FeS minerals in the sediments, whereas the slow decrease during 2013 to 2022 indicates a slow oxidation of FeS₂ minerals. Although this pattern indicates spatial heterogeneity of sediments within the study site, the peak before seagrass dieback is evident and represents the high S content measured in this study (Fig. 3). Bioturbation could affect the oxidation of FeS₂ and FeS by exposing them to highly oxic surface conditions [46]. In SH, the bioturbation caused by mantis shrimp (*Squilla empusa*) has been observed in recent years, which may have accelerated the oxidation of Fe-S minerals.

Impact of Fe addition on seagrass survival

In our experimental plantings, seagrass was 123% less likely to survive when placed in the SH sediment group (Cox proportional hazard survival analysis, $P < 0.01$), regardless of Fe treatment. Additionally, being in the Fe-amended group reduced the dieback risk by 68% ($P < 0.01$), regardless of the sediment class. Seagrass in the OH Fe-amended group was most likely to survive to the end of the experiment, whereas seagrass in the SH unamended group was most likely to die (Fig. 4 and Table S2). Interestingly, seagrass placed in the OH sediments with no Fe amendment and seagrass placed in the SH sediments with Fe amendment performed virtually the same throughout the experiment, indicating that small Fe amendments can improve recovery in the SH sediments. We found a strong linear relationship between sediment molar Fe:S and survival rate at week 4 (Fig. 6, $P = 0.009$), which could cause some of the differences in survival rates. The sediment molar Fe:S rate and the total survival rate exhibited a slight relationship at the end of the experiment ($P = 0.1$). The relationship could have been affected by the number of replicate cores with 0% survival rates at week 6 ($n = 5$) when sediments had become fully unvegetated during weeks 4 to 6; this probably affected the sediment biogeochemical conditions in these replicate cores.

These results indicate that the reactive oxidized Fe availability can assist seagrass in surviving transplanting. Of the 6 replicate cores, where the total survival rate at week 4 exceeded 50%, 5 were grown in sediments with a Fe:S ratio above 1,

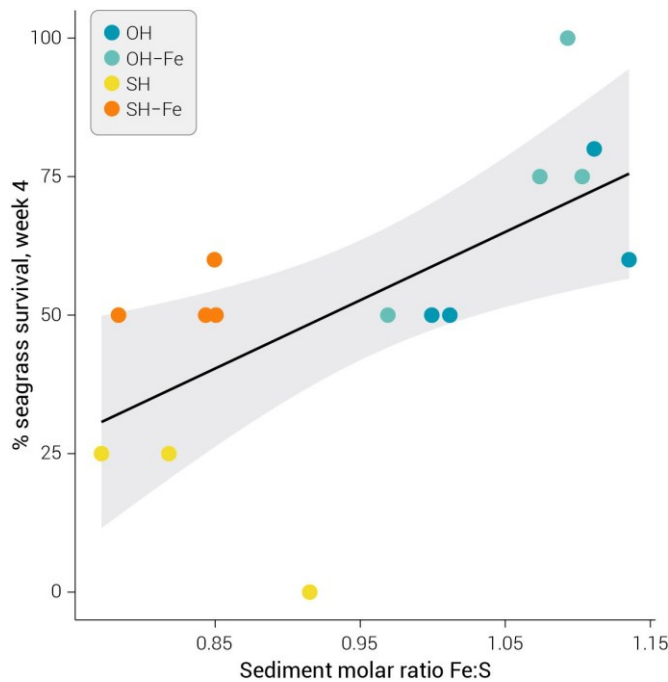


Fig. 6. Survival rate of seagrass after 1 month (4 weeks) in the aquarium vs. sediment molar Fe:S ratio ($P < 0.01$). The gray shading represents the 95% confidence interval, and the color points represent sediment origin (SH or OH) and treatment (Fe or no amendment).

implying available reactive Fe in the sediments. Additionally, the survival rate exceeded 50% in all except for one replicate core in the OH group, where field studies indicated considerable amounts of amorphous Fe-oxyhydroxides in the sediments. In this WFH region, the concentrations of soluble sulfides are typically low, and seagrass health parameters, including biomass allocation ($\delta^{34}\text{S}$) and light usage, indicate fair health [5]. However, OM has increased in OH in recent years, which can lead to increased sulfide concentrations; this can be detrimental to seagrass health in OH if this trend continues (7% on average in this study, as opposed to 4% in Haviland et al. [5]). In the SH sediments, the sulfide levels are similar to those in the OH sediments; however, seagrass could not recolonize this area, despite a robust population located nearby in the MH region. This may be partly due to the high Fe sulfurization because our results indicate that over 75% of the total sediment Fe is contained in FeS and FeS₂ in SH (Fig. 1).

In our study, Fe addition was small relative to the total amount of Fe in the sediment and did not significantly alter the Fe mineral pool after 6 weeks of seagrass growth in either sediment type. Additionally, no change in porewater Fe or soluble sulfides with Fe injection was observed, in contrast to studies indicating sediment biogeochemical alterations with Fe addition [18,19,47], albeit on sediments with much lower natural total Fe levels than those in WFH. Considering the estimated total Fe in each sample and bulk density, our Fe addition represents an increase in total Fe of only 0.3%–0.4% in either sediment and a 0.8% increase in oxidized Fe in OH and 2.3% in SH (see the Supplementary Materials). The small amount of oxidized Fe injection immediately before the introduction of seagrass may have kept sulfide levels low before our first porewater sample in the critical early transplanting phase where seagrass was acclimating to its environment [48]; this could have increased the survival rate we observed in the plants obtained from the

Fe-amended sediments (Table 2 and Fig. 4). Interestingly, we observed an increase in the SH sediment OM at the end of the experiment compared with the field conditions; this is possibly due to seagrass root exudation into the SH sediments. The reason we observed this increase in the SH and not in the OH sediments may be due to the prior presence of seagrass in the OH sediments, which may contain a microbial community that breaks down the specific mixture or root exudates released from *Z. marina* [49]. Additionally, seagrass addition in the SH sediments could cause a higher increase in OM than that in the OH sediments because seagrass in aquaria was planted at a much higher density than that in field conditions (SH density = 0 shoots per m²). In contrast, seagrass in the OH sediments was planted at a density similar to that in the field (density = 236 shoots per m² in the field study [5] compared with ~300 shoots per m² in the aquarium), albeit with less depth and, thus, less sediment volume in the experimental conditions compared with the field conditions. An additional cause could be the OM loss in all the sediments used in the experiment during placement in the tanks, which may have settled out within the SH sediments.

Impact of *Chaetopterus* worms

The replicate core that was heavily colonized by *Chaetopterus* spp. “parchment worms” exhibited remarkably different sediment Fe conditions than any other core (the concentrations of solid-phase Fe and S were the highest in the bulk sediment), and it was the only core with a measurable concentration (12 μM) of the total porewater Fe at the end of the experiment. The sediment C and N contents were insignificant, but the seagrass survival rate was much higher in this replicate core than in any other SH core. Recent research conducted in Long Island, just south of our study site, showed that *Chaetopterus* burrows host cable bacteria, which are members of the deltaproteobacterial Desulfobulbaceae family [50]; this is a group of filamentous bacteria with location-specific cells that perform multiple metabolic pathways across the oxic-anoxic interface including sulfide oxidation [51,52]. Cable bacteria are increasingly found in the seagrass rhizosphere and seem to affect seagrass tolerance to sulfide [53,54]. These bacteria can enhance dissolution of FeS and precipitation of Fe-oxides in sediments [55] and in the lining of *Chaetopterus* tubes [50], leading to a further reduction in seagrass mortality. In this study, the direct association of seagrass with *Chaetopterus* tubes adhered to seagrass roots is a novel observation. Although the objective of our study was not the assessment of the impact of *Chaetopterus* and cable bacteria on seagrass meadow sediment biogeochemistry, and our analysis was limited to a sample of only one sediment replicate core containing the organisms, the apparent impact of *Chaetopterus* on seagrass survival and solid-phase Fe and S is interesting and requires further investigation.

Ecological significance

Our study showed that even after soluble sulfide levels have fallen to values suitable for seagrass health, as in SH, sediments may not yet be suitable to support seagrass if the oxidized Fe pool remains saturated with Fe-S minerals. Even in sediments with relatively high total Fe content, seagrass is more likely to survive transplanting in sediments amended with small amounts of oxidized Fe. Notably, the majority of seagrass recovery projects have failed due to the stressful conditions after planting [56]. Recent research advances have improved seagrass recovery [57]. This study contributes to observations that Fe addition

increases seagrass survival under stressful transplanting conditions [18,19]; however, additional field studies are required to support this claim.

Conclusion

The results of our study showed an increase in total solid-phase S after long-term eutrophication in SH sediments, resulting in seagrass loss when the total solid-phase S content was at its highest (Fig. 3). Seagrass loss occurred when most of the Fe content was kept as FeS and FeS₂. After seagrass loss, we observed a slow recovery in the solid-phase S pool, where the total S levels in SH in 2022 were lower than those in high-sulfide MH (where seagrass was extant) and the Fe:S ratios were higher (Table 1). However, SH sediments and the low-sulfide OH basin did not support seagrass, although small injections of Fe improved seagrass survivability (Fig. 4). Both the field and experimental studies indicated a high seagrass survival rate for high Fe:S ratios. *Chaetopterus* worms may play an interesting role in seagrass survival under stressful organic-rich sediment conditions, possibly because of their mutualism with cable bacteria [50]; however, our study was limited to a single but fortuitous replicate core, and further investigation is required. Our results indicated that even after the porewater soluble sulfide levels decrease following a seagrass dieback event, sediments may still not be able to support successful seagrass recovery for a certain period due to the legacy effects of eutrophication on the Fe mineral pool by reducing the availability of reactive oxidized Fe. The timescale required for this pool to naturally recover is unclear; however, our field observations indicated a period over a decade.

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Competing interests: The authors declare that they have no competing interests.

Data Availability

Data will be made available by request immediately and will be uploaded to the Cornell eCommons database after publication.

Supplementary Materials

Figs. S1 to S6
Tables S1 and S2

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