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The effects of manipulation of sedimentary iron and organic matter on sediment biogeochemistry and seagrasses in a subtropical carbonate environment

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Abstract The microbial metabolism of organic matter (OM) in seagrass beds can create sulfidic conditions detrimental to seagrass growth; iron (Fe) potentially has ameliorating effects through titration of the sulfides and the precipitation of iron-sulfide minerals into the sediment. In this study, the biogeochemical effects of Fe availability and its interplay with sulfur and OM on sulfide toxicity, phosphorous (P) availability, seagrass growth and community structure were tested. The availability of Fe and OM was manipulated in a 2 × 2 factorial experiment arranged in a Latin square, with four replicates per treatment. The treatments included the addition of Fe, the addition of OM, the addition of both Fe and OM as well as no addition. The experiment was conducted in an oligotrophic, iron-deficient seagrass bed. Fe had an 84.5% retention efficiency in the sediments with the concentration of Fe increasing in the seagrass leaves over the course of the experiment. Porewater chemistry was significantly altered with a dramatic decrease in sulfide levels in Fe addition plots while sulfide levels increased in the OM addition treatments. Phosphorus increased in seagrass leaves collected in the Fe addition plots. Decreased sulfide stress was evidenced by heavier δ^{34}S in leaves and rhizomes from plots to which Fe was added. The OM addition negatively affected seagrass growth but increased P availability; the reduced sulfide stress in Fe added plots resulted in elevated productivity. Fe availability may be an important determinant of the impact that OM has on seagrass vitality in carbonate sediments vegetated with seagrasses.

Keywords Florida Bay · Sediment geochemistry sulfate reduction · Sulfide stress · Nutrient limitation

Introduction

As a result of the world wide decline in seagrasses (Duarte 1999; Orth et al. 2006), increasing attention is being paid to these ecosystems to identify the factors that are contributing to the decline. Organic matter (OM) loading of the sediments of both anthropogenic and natural origin could be an important cause of decline (Delgado et al. 1999; Marba et al. 2002; Pergent et al. 1998), especially in carbonate sediments where levels of iron (Fe) are generally low compared to terrigenous systems (Duarte et al. 1995). Sources of

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anthropogenic OM to seagrass ecosystems include fish farming activities (Delgado et al. 1999) and sewage discharge from either terrestrial or marine activities (Duarte 1999; Marba et al. 2002). Natural sources include the release of OM from the seagrasses themselves, which can create a negative feedback on the health of seagrass beds (Carlson et al. 1994) with the deposition of detrital particles into the seagrass beds (Kamp-Nielsen et al. 2001).

Organic matter fuels microbial respiration and this activity quickly exhausts oxygen (O_2) in the sediments. Sulfate (SO_4^{2-}) is very abundant in oceanic environments and is widely used as an alternate electron acceptor in the absence of O_2. Sulfate reduction (SR) rates increase dramatically in the presence of OM, producing high levels of hydrogen sulfide (H_2S) along with reducing conditions in the sediment (Chambers et al. 2000; Terrados et al. 1999). The H_2S dissociates into free sulfide (S^{2-}) which is a potent phytotoxin that has been implicated in major seagrass die-off events in Florida Bay (Borum et al. 2005; Carlson et al. 1994; Koch and Erskine 2001). Experimental manipulations of S^{2-} in the sediment in both the laboratory and the field have resulted in reduced growth of seagrasses (Halun et al. 2002; Koch and Erskine 2001). SR may also benefit seagrasses, because nutrient regeneration through OM mineralization is an important mechanism for the supply of nutrients to seagrass ecosystems (Holmer et al. 2001; Holmer and Nielsen 1997).

The susceptibility of seagrasses to OM loading is likely to be highly influenced by the sedimentary geochemical milieu in which they occur. In particular, the concentration and oxidation state of dissolved iron may mediate the impact of OM loading. Available Fe has been shown to limit the growth of phytoplankton in the open ocean (Landry et al. 2000; Martin and Fitzwater 1988), of land angiosperms found on calcareous continental soils (Miller et al. 1984), and possibly of seagrasses in carbonate sediments (Duarte et al. 1995). In addition, availability and reactivity of Fe controls the interlinked biogeochemical cycles of sulfur and phosphorus, and is implicated in two buffering systems: the S^{2-}/iron mono-S^{2-}/pyrite and the iron hydroxide/phosphate/S^{2-} buffers (Azzoni et al. 2005). These buffering systems affect the growth and performance of primary producers (Chambers et al. 2001), since the Fe-S buffer system has the potential to bind free S^{2-}, making it non-toxic, and the FeOOH-PO_4^{3-} system has the potential to influence the availability of P (Chambers and Odum 1990), which can be the resource that limits plant growth in seagrass beds, especially in carbonate sediments (Short 1987). Anoxia and bacterial SR stimulate Fe^{3+} conversion to Fe^{2+} along with the release of Fe-bound P to the porewater (Azzoni et al. 2005). In anoxic carbonate sediment environments where Fe levels are low, much of the Fe is sequestered by sulfur (S) in the form of FeS (iron monosulfide) and FeS_2 (pyrite) and has no effect on P availability. Low P availability in such environments is then caused by the co-precipitation of P with $CaCO_3$ (Jensen et al. 1998; Short 1987).

A number of studies have investigated the effect Fe has on the removal of toxic S^{2-} and, therefore, on the growth of seagrasses growing on carbonate sediments (Chambers et al. 2001; Duarte et al. 1995; Holmer et al. 2005). It is also well established that an increase in OM loading to sediments will increase SRR and nutrient regeneration (Westrich and Berner 1984). However, experiments that simultaneously manipulate Fe and OM are called for in order to more fully understand the interplay between the geochemical cycles of sulfur, iron and phosphorus, and how such interplay affects the impacts of OM loading on seagrass ecosystems. In this study both OM and Fe concentrations were manipulated to address the effect each factor has individually as well as the potential synergistic effect of both factors in increasing nutrient regeneration and P availability while maintaining low S^{2-} levels in the sediment. Specifically, we hypothesized that OM loading would have a deleterious effect on the growth of the tropical seagrass *Thalassia testudinum*, but that Fe would be able to buffer against the negative effects of the addition of OM by the removal of toxic S^{2-} and the release of P to the porewater. Furthermore, we hypothesized that Fe loading into a Fe-poor carbonate environment would stimulate plant growth by both eliminating Fe limitation and increasing P availability to the seagrasses through the FeOOH-P buffer.

Materials and methods

Site description

The study took place in Florida Bay, a shallow (<2 m deep) oligotrophic lagoon which is colonized by seagrass beds dominated by *T. testudinum*. Florida Bay is bounded to the north by the Florida mainland, to the west by the Gulf of Mexico and to the east and south by the Pleistocene coral reef limestone formation of the Florida Keys (Fourqurean and Robblee 1999). Shallow mud banks restrict circulation of water through the bay dividing it into numerous semi-isolated basins. Experiments were performed on a shallow mud bank adjacent to the Bob Allen Keys (24°01.501′N, 80°0.911′W) in the central portion of the bay. This area is characterized by fine grained carbonate sediments covered with dense *T. testudinum* and low P availability in the porewater (Fourqurean et al. 1992b).

Experimental setup

The experiment consisted of four treatments with four replicates each: the addition of Fe (denoted as +Fe), the addition of OM (shown as +OM), the addition of both (+Fe + OM) or no addition (−Fe−OM) for a total of 16 experimental units, organized in a grid formation. This layout corresponds to a 2 × 2 factorial design, with two factors (Fe, OM) having two levels each (ambient, enhanced). In order to control for unforeseen spatial environmental gradients across the grid that may have confounded our results, we employed a Latin square design. A 12 m × 12 m area was parceled and divided in four rows and four columns. In each resulting cell a 0.25-m^2 PVC quadrat was established and a treatment was assigned to meet the restrictions imposed by a Latin square design (i.e., no treatment was repeated in any row or column). Treatments were applied to an area twice the size of the quadrats (0.5 m^2 with the 0.25 m^2 in the center) on a monthly basis to lessen edge effects around the area to be sampled.

Dissolved OM was added as an aqueous solution of sodium acetate, and Fe as an aqueous solution of ferrous chloride ($FeCl_2$). Acetate has been shown to be an effective stimulator of SR that strongly fractionates sulfur isotopes (Kleikemper et al. 2004). Ferrous iron (Fe^{2+}) is the cation involved in the precipitation of S^{2-} and release of P (Smolders et al. 1995). In order to provide a large but ecologically relevant OM dose to increase SR, the OM loading rate was equivalent to the maximum rate of productivity of seagrass leaves from Florida Bay (3.42 g C m^2 day^{-1}; Zieman et al. 1989). Similarly, sufficient Fe was added to titrate the S^{2-} produced by the maximum SRR measured in Florida Bay of 200 mM $year^{-1}$ (Ku et al. 1999). This yielded experimental loading rates of 140 g of sodium acetate m^{-2} $month^{-1}$ and 160 g of $FeCl_2$ m^{-2} $month^{-1}$. Interestingly, the loading rates of OM (to produce S) and Fe were stoichiometrically balanced for the formation of pyrite despite the independent methods used to determine them. Each plot was amended with the appropriate treatment by dissolving either substance in 500 ml of seawater and injecting it into the sediment up to a depth of ∼25 cm with ten 60 ml plastic syringes, ensuring the whole sediment volume was infused. Control plots were treated with sham injections of seawater alone. This procedure was performed once a month for 15 months starting in March 2005, and ending June 2006. Data and samples on seagrass and sediments were collected four times: September, 2005, December, 2005, April, 2006 and finally in June, 2006. Porewater was collected from each experimental unit for chemical analyses in June 2006.

Data collection

To measure the effects of OM and Fe additions on the sediment biogeochemical conditions, sediment and porewater samples were extracted. Sediment cores to a depth of 10 cm were collected at each plot using cut off 60 ml syringes as piston corers, kept cold and stored frozen until analysis. These cores were sectioned at 2-cm intervals; 1 ml was placed in a scintillation vial for determination of bulk sediment properties. The remainders of the core sections were used for Fe and P analysis. In this paper, we report sediment parameters integrated over the 10 cm depth of the cores.

Samples for bulk sediment properties were weighed wet and then oven dried at 80°C until constant weight. Bulk density and porosity were determined by loss of water. A dry oxidation, acid hydrolysis extraction followed by colorimetric analysis of phosphate was performed on dried ground samples for determination of total P concentrations (Fourqurean et al. 1992a). Total Fe was determined by a modification of the total P method by adding hydroxylamine–HCl to reduce any Fe^{3+} present to

Fe^{2+} and analyzing the extract for Fe^{2+} concentration colorimetrically (Lovley and Phillips 1987).

Porewater samples were collected with a stainless steel capillary probe inserted in the sediments to a depth of 10 cm and filtered through a GF/F filter. Porewater for S^{2-} analysis was immediately fixed with a 1% Zn acetate solution and S^{2-} concentrations determined with the methylene blue method (Cline 1969). The remainder of the porewater was analyzed for Fe, soluble reactive P (SRP), NH_4^+, SO_4^{2-} and Cl^-. Total Fe was determined colorimetrically by the same method described above (Lovley and Phillips 1987). The SRP and NH_4^+ were determined by standard colorimetry on an autoanalyzer (Alpkem 304) after first acidifying and sparging the sample with helium to drive off H_2S. Concentrations of Cl^- and SO_4^{2-} were determined after a 1:500 dilution using an ion chromatograph (Dionex 120). The ratio of SO_4^{2-} to Cl^- was calculated on a molar basis.

To test whether Fe and OM had any effect on the growth of the seagrass *T. testudinum*, plant responses were determined by changes in elemental and isotopic content, as well as biomass and growth measurements. Total P and Fe in the plants were measured using the same techniques as for sediment, after the leaves were gently scraped of epiphytes with a razor blade, dried to constant mass at 70°C and homogenized to a fine powder.

To determine whether microbially-produced S^{2-} was being incorporated by the plants, the stable sulfur isotopic signatures ($\delta^{34}S$) of leaf and rhizome tissue were measured. Sulfide produced by bacterial SR, a process that fractionates sulfur stable isotopes very strongly, is isotopically lighter than the source sulfate (Kaplan and Rittenberg 1964). Because the stable sulfur isotopic signature of plant tissue is determined by the sulfur source, this fractionation provides an excellent tool to determine plant exposure to S^{2-}. With the removal of isotopically lighter S^{2-} via precipitation of iron monosulfides and pyrite formation, we expected plants to be enriched in the heavier isotope in iron addition plots. Conversely, it was expected that plants from plots with +OM would be isotopically lighter due to increased S^{2-} in the porewater, with subsequent uptake by the plants. Hence, we hypothesized that an increase in the $\delta^{34}S$ content of the seagrass would indicate an alleviation of S^{2-} exposure.

The $\delta^{34}S$ values of seagrass tissues were determined with a standard elemental analyzer isotope ratio mass spectrometer (EA-IRMS). The stable isotope composition was reported using standard δ values:

$$\delta^{34}S = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^{-3} (‰)$$

where R is the abundance ratio of the heavy to light isotopes of the element. These results are presented with respect to the international standards of Canyon Diablo Troilite (CDT). The reproducibility of the measurement is typically better than 0.2‰.

Net aboveground productivity of *T. testudinum* was measured using a modified leaf marking technique (Zieman et al. 1999). A 200-cm^2 quadrat was randomly placed in each plot. Within each quadrat all seagrass short shoots were punched by driving a hypodermic needle through the base of the leaves. Short shoots were harvested 10–14 days after and counted to estimate density and standing crop. *T. testudinum* leaves were counted, measured (length and width to nearest mm) and weighed to measure plant growth rate. Belowground biomass was obtained by coring the area selected for aboveground productivity after all the shoots were harvested. A rectangular stainless steel core was driven 10 cm into the sediment and all live roots and rhizomes were collected, cleaned of mud dried and weighed. For total Fe and isotopic determination, three rhizomes of approximately 5-cm each were taken from each plot and submerged in a 10% HCl solution for 5 min to dissolve external Fe-S deposits on the rhizomes and then thoroughly rinsed with deionized water (M.S. Frederiksen. Pers comm).

At the end of the experiment an Fe budget was constructed using bulk sediment parameters, seagrass density and biomass, and concentrations of Fe in the sediments, porewater and seagrass tissues to calculate the retention efficiency of the sediments and the efficacy of the method being used to deliver Fe to the system.

Statistical analysis

Data were tested for normality and equality of variances prior to analysis. Because of a marked seasonal pattern in the parameters measured, all data were analyzed using a two-way analysis of variance (ANOVA). At the conclusion of the experiment in June 2006, we evaluated the effects of FE and OM additions, the interaction of these additions, as well as the spatial position in the grid array (Latin square blocking factors) with ANOVAs. Tukey's post hoc tests were performed to evaluate differences among means in the four treatment groups: −Fe−OM (no addition),+Fe (iron addition only),+OM (addition of iron and OM), and +Fe + OM (addition of iron and OM). To track whether or not Fe availability to the seagrasses had increased over time, seagrass Fe content was analyzed using a two-way ANOVA with a repeated measures design. We did not use the repeated measures analysis for other response variables because the added complexity of the analyses did not improve our understanding of the effects of Fe and OM loading. Unless otherwise denoted, we defined statistical significance at the 95% confidence level. All analyses were performed on SAS 9.1.3 (SAS institute).

Results

Environmental conditions at the site varied throughout the year with winter minima temperature values of 16°C and summer maxima of 32°C. Salinity was highly variable from 18–41 ppt, with higher salinity in the winter dry season and lower salinity during the summer wet season. The calculated bulk density and porosity did not significantly differ from treatment to treatment (main effects: Fe and OM $p > 0.05$ for all cases) and the average bulk density and porosity of the sediments was 1.01 g cm^{-3} and 69%, respectively.

Sediment geochemistry

Our grid of experimental plots proved to be largely free from confounding spatial effects; rarely was there a significant effect of either row or column on any response variable. Consequently, we will not refer to the significance of these Latin square blocking factors.

Not surprisingly, Fe additions significantly increased the Fe content of the sediments (Fe main effect, Table 1) and this increase was almost threefold: 4,893 µg g^{-1} ± 1,362 S.E. in the +Fe treatment compared to 1,834 µg g^{-1} ± 39.98 and 1,744 µg g^{-1} ± 75.65 in the −Fe−OM and +OM treatments respectively (Fig. 1a). OM additions had no effect on the Fe content of the sediments, and there was no significant interaction between Fe and OM addition (Table 1). Neither Fe nor OM addition had any statistically significant effect on the phosphorus content of the sediments, nor was there a significant interaction between Fe and OM (Table 1, Fig. 1b).

The addition of Fe to the sediments caused a significant increase in dissolved Fe concentrations in the porewater; the addition of OM also had a significant effect on dissolved Fe concentrations in the porewater (Fe and OM main effects, Table 1; Fig. 2a). Porewater Fe concentration remained very low (generally less than 5 µM) in all treatments except for the +Fe treatment which had a concentration of 171.57 ± 26.46 µM. The effect of OM on porewater Fe concentration was a result of the strong interaction between Fe and OM addition; in the presence of added OM, added Fe did not increase the porewater Fe concentration. Porewater S^{2-} concentration was significantly influenced by the addition of both Fe and OM (Table 1, Fig. 2b). Measured S^{2-} concentrations ranged from 237 ± 107 µM in the +Fe treatment to 5,032 ± 1,307 µM in the +OM plots. The −Fe−OM and +Fe + OM treatments had values of 1,695 ± 711 µM and 1,604 ± 369 µM, respectively. Addition of OM tripled the S^{2-} concentrations in the porewater while Fe decreased by a factor of 8 the amount of dissolved S^{2-} present in the porewater (Fig. 2b). The concentration of Cl^- in the porewater averaged 0.61 ± 0.01 M, and did not vary among treatments, but SO_4^{2-} concentrations in the porewater were significantly affected by the addition of OM. In plots with added OM, SO_4^{2-} concentrations averaged 23.2 ± 0.2 mM compared to 29.8 ± 0.1 mM for plots with no OM addition. The SO_4^{2-}:Cl^- ratio can be used as a measure of the net effect of SR on sulfate concentrations in porewater. Normal Florida Bay surface water has a SO_4^{2-}:Cl^- of 0.051 (Rosenfeld 1979). All measured porewater SO_4^{2-}:Cl^- were lower than this surface water value (Fig. 2e), indicating a net loss of sulfate from the porewater. OM addition significantly lowered the SO_4^{2-}:Cl^- (Table 1, Fig. 2e), and Fe addition had no significant impact on SO_4^{2-}:Cl^-.

Organic matter loading caused a significant increase in the porewater concentrations of both

Table 1 The effects of Fe and OM additions on sediment, porewater and seagrass nutrient content and growth parameters, of the last sampling event (June, 2006), analyzed with a two-way ANOVA

Response variable	Source of variation		
	Fe	OM	Fe × OM
Sediment Fe	*	ns	ns
Sediment P	ns	ns	ns
Porewater sulfide	*	*	ns
Porewater Fe	*	*	*
Pore water NH_4^+	ns	**	*
Porewater SRP	ns	**	ns
Porewater ratio SO_4^{2-}:Cl^-	ns	*	ns
Thalassia testudinum %P	*	ns	*
Thalassia testudinum $\delta^{34}S$ (leaves)	**	ns	ns
Thalassia testudinum $\delta^{34}S$ (rhizomes)	*	ns	ns
Thalassia testudinum length	ns	**	ns
Thalassia testudinum width	**	*	*
Thalassia testudinum area	ns	*	ns
Thalassia testudinum density	**	ns	**
Thalassia testudinum standing crop	ns	ns	*
Thalassia testudinum areal productivity	**	ns	**
Thalassia testudinum leaf area productivity	**	ns	ns
Thalassia testudinum ss productivity	*	ns	ns
Thalassia testudinum leaf area productivity	*	ns	ns

*Designates $p < 0.05$, **Designates $p < 0.01$, *ns* designates $p > 0.05$

SRP and NH_4^+ (Fig. 2c, d and Table 1). SRP concentrations increased by approximately 0.3 μM with OM addition; Fe addition did not affect SRP concentrations in the porewater. OM addition caused a doubling of the dissolved NH_4^+ in the porewater. Adding Fe alone had no significant effect on NH_4^+ concentrations, but interestingly the addition of Fe with OM led to a decrease in NH_4^+ relative to Fe only additions (Fe × OM interaction in the ANOVA).

Seagrass elemental and isotopic content

Addition of Fe did not have an immediate impact on the Fe content of seagrass leaves in the experimental plots (Fig. 3). Over time, seagrass Fe content increased significantly in the Fe addition plots (Fe main effect, time main effect, time × Fe interaction, Table 2) from 102.3 ± 7.2 μg g^{-1} to a maximum of 599 ± 186.5 μg g^{-1} (Fig. 3). Seagrass leaf Fe content recorded 6 months after the beginning of Fe addition were still similar to the Fe limitation threshold of 100 μg g^{-1} (Duarte et al. 1995), but subsequently increased in the +Fe plots. Seagrass Fe content remained below the 100 μg g^{-1} threshold in all −Fe−OM and +OM treatments throughout the experiment with minimum values of 52.7 ± 5.0 μg g^{-1} and maximum of 86.9 ± 17.7 μg g^{-1}.

Addition of Fe had a modest but statistically significant effect on P concentration in seagrass leaves (Fe main effect, Table 1, Fig. 4a) but the effect was influenced by the level of OM (Fe × OM interaction, Table 1). *T. testudinum* P content ranged from 0.049% ± 0.002 in the −Fe−OM plots to 0.058 % ± 0.003 in the +Fe treatments with intermediate values for the +OM and +Fe + OM treatments. These values are all well bellow the P limitation threshold of 0.2% (Duarte 1990). Iron addition increased the $\delta^{34}S$ of both leaves and rhizomes of *T. testudinum* (Fig. 4b, Fe main effect, Table 1), but OM had no consistent effect on the $\delta^{34}S$ of leaves and rhizomes. However, there was a significant interaction of both factors (Fe × OM interaction, Table 1).

Seagrass vitality

Morphology of *T. testudinum* short shoots was affected by addition of both Fe and OM (Fe main effect, OM main effect, Table 1, Fig. 5a, b, c) and

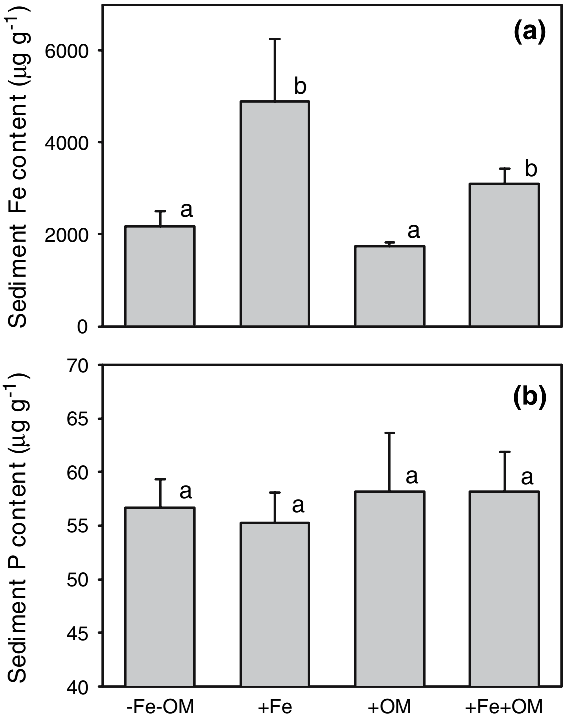

Fig. 1 Depth integrated sediment Fe (**a**) and P (**b**) content. *Bar* indicates S.E. ($n = 4$). *Bars* labeled with *same letter* were not significantly different (post hoc Tukey $p = 0.05$)

the interaction was only significant for leaf width (Fe × OM interaction). Leaves were shorter and narrower in the +OM treatments but wider in the +Fe treatments. As a result, +OM treatments had a smaller leaf area per shoot (5.5 ± 0.5 cm^2) compared to the rest of the treatments (OM main effect, Table 1, Fig. 5a, b, c).

Fe increased while OM decreased shoot density in the plots (Fe main effect, OM main effect, Table 1, Fig. 5d). However, OM significantly changed the effect of Fe (Fe × OM interaction, Table 1) and the +Fe + OM plots had the greatest density with 1100.0 ± 112.5 shoots m^{-2}. Both Latin square blocking factors had significant effects on shoot density, indicating that there was a preexisting gradient in density across the experimental grid that was blocked out of the analysis. Seagrass standing crop was affected by OM and Fe additions, but in a more complex manner. The OM additions decreased the standing crop, but +Fe prevented OM from having a negative impact (Fe × OM interaction, Table 1). Iron addition increased the areal productivity and leaf area productivity significantly (Fe main effect, Table 1), and OM addition enhanced the positive effect of Fe addition (Fe × OM interaction, Table 1). The lowest values for areal productivity (mass of leaves produced per m^2 of seabed) and leaf area productivity (one-sided area of new leaves produced per m^2 of seabed) were found at the +OM plots (0.212 ± 0.043 g m^{-2} day^{-1} and 483.54 ± 84.04 cm^2 m^{-2} day^{-1}; Table 1).

On a per shoot basis, production of new plant material showed the same pattern with an increase in productivity in the Fe treatments and decrease in the OM treatments (Fe main effect, OM main effect, Table 1). However, the Fe by OM interaction was non significant (Fe × OM interaction, Table 1).

Over the course of the experiments, we added 335 g of soluble Fe to the porewater of each plot. At the end of the experiment, approximately 84.5% of the introduced Fe could be accounted for in the experimental plots. The largest part (281 g $plot^{-1}$) of the Fe was associated with particles in the sediment, and 2.4 g $plot^{-1}$ was found dissolved in porewater. Fe incorporated into seagrass tissues accounted for only 0.15 g $plot^{-1}$. The remaining 15.5% of the added Fe was unaccounted for.

Discussion

In the iron-poor, carbonate sediment environment of Florida Bay, increased OM loading in seagrass beds lead to increased S^{2-} and nutrient concentrations in sediment porewaters. Because of low Fe availability, this resulted in S^{2-} entry into seagrasses that led to decreased plant size, density and growth rate. The negative effects of increased S^{2-} concentrations overrode the stimulatory effects of increased nutrient availability that resulted from increased respiration. When Fe was added, the Fe prevented the accumulation of dissolved S^{2-} in the porewater, and led to enhanced plant size, density and growth rate. Our results suggest that low Fe environments like Florida Bay are more susceptible to OM loading than areas with higher Fe availability. We can conclude that the negative effects of high S^{2-} concentrations on seagrasses observed in Florida Bay (Borum et al. 2005; Carlson et al. 1994; Koch and Erskine 2001) and some other seagrass-dominated ecosystems (Calleja et al. 2007; Halun et al. 2002) can be partially attributed to very low Fe availability, which results

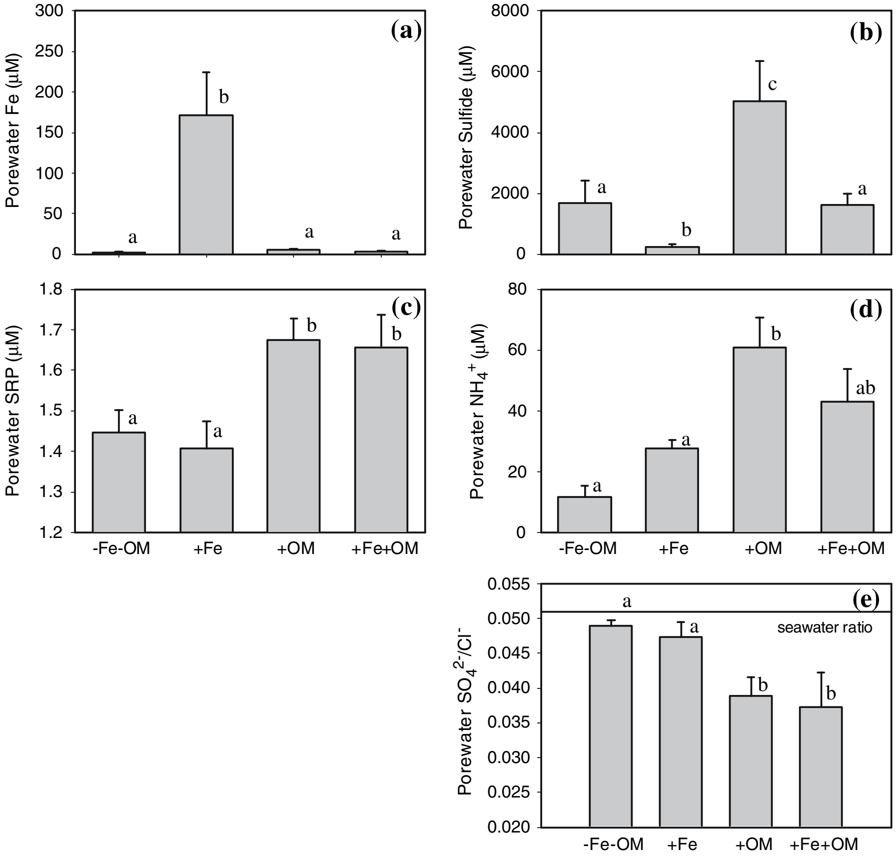

Fig. 2 Porewater concentrations of total dissolved Fe (**a**) S^{2-} (**b**) SRP (**c**) and NH_4^+ (**d**), and the ratio of porewater SO_4^{2-}:Cl^- (**e**) at all treatments. *Bar* indicates S.E. ($n = 4$). *Bars* labeled with *same letter* were not significantly different (post hoc Tukey $p = 0.05$)

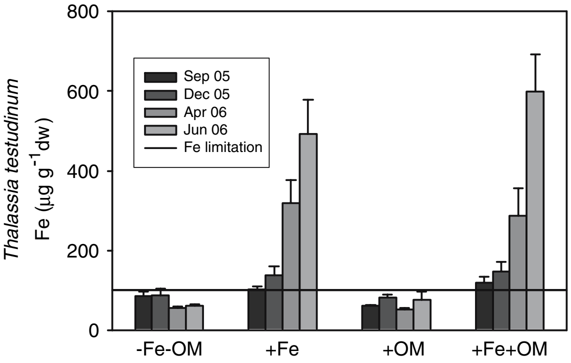

Fig. 3 Temporal variation in the Fe content of leaves of *Thalassia testudinum*, throughout the four sampling events. *Horizontal line* represents the threshold for Fe limitation in plants. *Bar* indicates S.E. ($n = 4$)

in poor capacity to remove from solution the sulfide produced by anaerobic respiration of OM.

The biogenic carbonate sediments in Florida Bay have relatively high levels of Fe in the sediments compared to other carbonate systems (Chambers et al. 2001; Duarte et al. 1995) and the regional spatial distribution of Fe is controlled by transport through the Everglades from a terrestrial source (Chambers et al. 2001). However, Florida Bay Fe concentrations are still low compared to coastal ecosystems underlain by terrigenous sediments (Chambers et al. 2000), and most of the Fe in Florida Bay sediments is already complexed with S in pyrite and iron monosulfides (Chambers et al. 2001). Because of this, the additional SR fueled by OM additions in our experiments produced S^{2-} that was not detoxified by Fe-S mineral formation and remained in the sediment porewater (Fig. 2b). Fe additions decreased the concentrations of S^{2-} in the porewater, and also apparently prevented the accumulation of isotopically depleted sulfur in seagrass plants (Fig. 4b). Because the S^{2-} produced by sulfate reducers is depleted in the heavy stable isotopes (Kaplan and Rittenberg 1964), the observed increase in δ^{34}S of seagrass tissues is consistent with a decrease in S^{2-} incorporation into seagrasses under Fe enrichment. It has been found that Fe addition can decrease SR in carbonate

Table 2 The effects of Fe and OM additions on seagrass Fe content analyzed with a two-way repeated measures ANOVA

Response variable	Source of variation	*p*-Value
Thalassia testudinum Fe (μg g^{-1})	Fe	**
	OM	ns
	Fe × OM	ns
	Time	**
	Time × Fe	**
	Time × OM	ns
	Time × Fe × OM	ns

Data were collected in September and December 2005 and April and June 2006

**Designates $p < 0.01$, *ns* designates $p > 0.05$

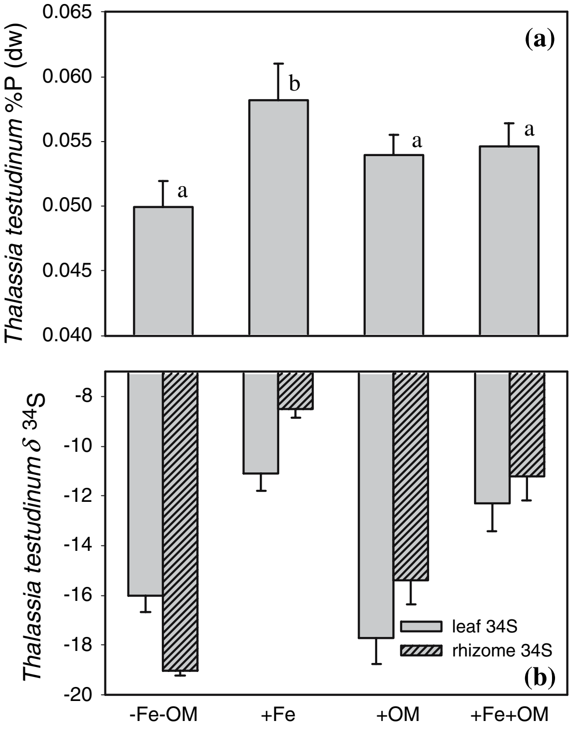

Fig. 4 Phosphorus content (**a**) and sulfur isotopic signature (**b**) of the seagrass *Thalassia testudinum*. *Error bar* indicates S.E. ($n = 4$). *Bars* labeled with *same letter* were not significantly different (post hoc Tukey $p = 0.05$)

sediments (Holmer et al. 2005), but our SO_4^{2-}:Cl^- data (Fig. 2e) suggest that the net effect of SR on the porewater was not affected by Fe addition, and argue that Fe additions decreased S^{2-} invasion and incorporation by titrating the S^{2-} into Fe-S minerals in our experiments.

Ambient levels of SR in Florida Bay seagrass beds produce sufficient S^{2-} to sequester a large amount of added Fe into Fe-S minerals. Ambient Fe content in seagrass leaves at our study site fell below the benchmark of 100 μg g^{-1} that is suggested to indicate Fe deficiency (Duarte et al. 1995; Miller et al. 1984). Even under the substantial Fe loading imposed in our experiments, there was no increase in the Fe content of seagrass leaves evident until fully 1 year after Fe loading began in March 2005; by June 2006, Fe content of seagrass leaves in +Fe and +Fe + OM plots were five to six times higher than the Fe limitation threshold (Fig. 3). By the end of our experiments, Fe-S mineral formation was apparently limited by S^{2-} availability in the +Fe plots, and substantial concentrations of dissolved Fe were present in the porewater (Fig. 2a). Interestingly, when SR was stimulated by OM addition, Fe addition had no effect on dissolved Fe concentrations in the porewater (Fig. 2a), likely because under these conditions Fe-S mineral formation was limited by S^{2-} availability. However, under combined Fe and OM loading, seagrasses could take up Fe and incorporate it into leaf tissue (Fig. 3) even though the porewater Fe concentrations remained low. This suggests that Fe-S mineral formation in sediments with high S^{2-} concentrations would not outcompete seagrass Fe uptake and be a primary cause of Fe limitation of seagrass growth in regions with relatively high Fe availability, but such competition could contribute to Fe limitation in low-Fe environments.

The addition of either Fe or OM to the system had no significant effect on P content of the sediments (Fig. 1b), but both OM and Fe addition significantly increased nutrient concentrations in sediment porewater (Fig. 2). OM additions stimulated SR, but the production of both SRP and NH_4^+ in plots that received OM additions suggests that added acetate led to a more general increase in the microbial breakdown of other organic compounds in addition to acetate, which contains no N or P. Along with remineralization of organic forms of nutrients, increased SR could also mobilize inorganic P in the sediments. In carbonate sediments, porewater SRP concentrations are reduced by the sorption of phosphate onto sediment particles (de Kanel and Morse 1978). Enhanced SR could also have increased SRP concentrations in the porewater by the dissolution of

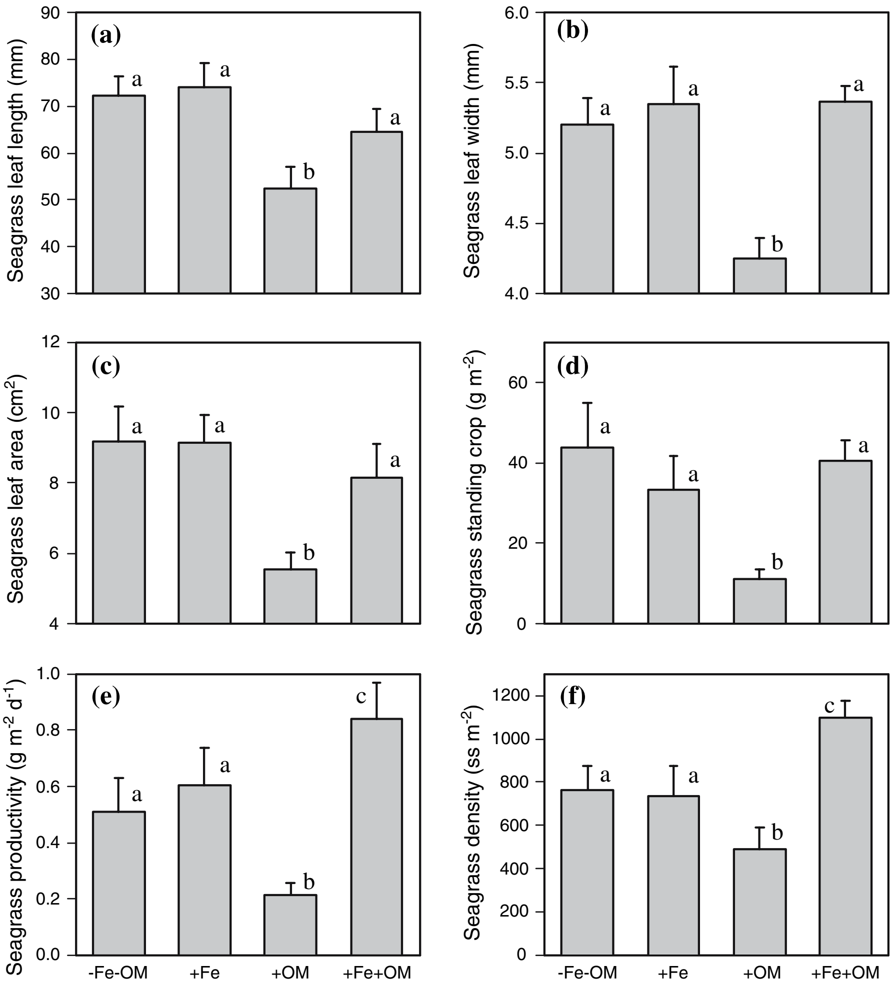

Fig. 5 *Thalassia testudinum* leaf length (**a**) leaf width (**b**) productivity leaf area (**c**), biomass (**d**), productivity (**e**) and density in short shoots per m^2 (**f**) measured by a modified leaf marking technique. *Bar* indicates S.E. ($n = 4$). *Bars* labeled with *same letter* were not significantly different (post hoc Tukey $p = 0.05$)

carbonate sediments, and concomitant release of sorbed P, that occurs in response to CO_2 production (Heijs and van Gemerden 2000; Jensen et al. 1998). The increased availability of nutrients stimulated by OM loading could have a positive impact on the size, density and growth rate of benthic primary producers at our site, at which the primary producers have been shown to be P-limited (Armitage et al. 2005; Fourqurean et al. 1992a). Our results are consistent with observations of seagrass communities under fish farm operations, where heavy organic loading of the sediments and high rates of nutrient remineralization have been recorded (Pergent et al. 1998).

Fe addition may further enhance the SRP pools by interacting with S^{2-}, because Fe^{3+} iron oxyhydroxides strongly sorb phosphates. The presence of iron oxyhydroxides in marsh soils trap P in sediments, and this P is made available to rooted plants when these oxyhydroxides are reduced (Chambers and Odum 1990). Similarly, O_2 exudation from the roots of aquatic macrophytes can cause a crust of oxidized iron oxyhydroxides to form around the roots that results in enhanced P availability (Hupfer and Dollan 2003). The P content of *T. testudinum* leaves increased when Fe was added to the sediments (Fig. 4a), indicating that Fe addition did increase the availability of P. In another iron addition experiment in a less oligotrophic region of Florida Bay approximately 15 km west of our current study, Fe additions increased the P pool in the sediments and in seagrass leaves of *Halodule wrightii*, but not in *T. testudinum* leaves (Chambers et al. 2001). Those authors argued that increased P caused by Fe addition was available for *H. wrightii* because of a shallower root system in closer contact with a layer of iron oxyhydroxide at the sediment-water interface, but not available for the *T. testudinum* with its deeper roots. In contrast, Fe addition in our experiments did not increase the total P pool in the sediments in the current study (Fig. 1b), perhaps because of the greater severity of P limitation at our site.

In addition to the P-trapping of iron oxyhydroxides, other mechanisms may explain why Fe additions increased the P content of *T. testudinum* (Fig. 4a) and the similar response found earlier for *H. wrightii* (Chambers et al. 2001). In seagrass rhizospheres, the activity of alkaline phosphatase, an enzyme involved in the uptake and recycling of P within the plant, is stimulated by Fe addition, possibly enhancing the ability of the plants to uptake P (Holmer et al. 2005).

Sulfide intrusion into aquatic rooted macrophytes does occur under anaerobic conditions in the sediments (Holmer et al. 2006). This intrusion can be inferred by the elemental S content of the plants since they can store sulfur as S^0 (Holmer et al. 2005), and although it provides a good proxy of invasion, little information on the nature of the sulfur entering the plants is obtained. As reactions go to completion, the difference between the $\delta^{34}S$ of S^{2-} and the source sulfate diminishes and this has been used to determine the source of sulfur entering the plants (Carlson and Forrest 1982). More recently, research ties evidence of sulfur uptake by seagrasses to the amount of OM present in the sediments (Frederiksen et al. 2006). Our results suggest the addition of Fe protected the seagrasses from S^{2-} entry, since $\delta^{34}S$ was significantly less negative in leaves and rhizomes from plants from plots amended with Fe (+Fe, +Fe + OM) (Fig. 4b). The OM interacted with Fe (Fe × OM interaction, Table 1) which indicated that Fe was less capable of buffering S^{2-} intrusion under high OM inputs and increased SR rates.

Morphometric features of the leaves revealed that short shoots became smaller with addition of OM, but had no change in size when Fe was added (Table 2, Fig. 5a, b, c). Most productivity parameters were positively affected by the presence of Fe and negatively affected by the presence of OM (Fig. 5d, e, f), and in some instances (density 5f, areal productivity 5e) the Fe by OM interaction led to even greater productivity. This not only further reinforces evidence of S^{2-} stress to the plants and a contrasting positive effect of Fe on the growth of this seagrass, but highlights the complex effects of the interaction between Fe and OM.

Because of the reactivity of both dissolved Fe^{2+} to form Fe-S minerals and dissolved Fe^{3+} to form insoluble iron oxyhydroxides, the iron added to the plots was largely retained (84.5%) in the plots, mostly as part of the solid-phase sediment. The remaining 15.5% of the Fe injected may have been lost by burial below our sampling zone via mixing, to the water column in dissolved form, incorporated into unmeasured pools like macroalgae, or lost as particulate matter generated by processes like leaf sloughing and herbivory. The high retention of Fe in carbonate sediments is comparable to 82% P retention efficiencies in carbonate environments limited by P (Ferdie and Fourqurean 2004). In contrast, the retention of added N in seagrass beds is comparatively very low (<10%) (Ferdie and Fourqurean 2004; Stapel et al. 2001). Differences in retention efficiency between relatively immobile Fe and P compared to N suggest that any addition of Fe and P is likely to influence primary production and geochemistry for a much longer time than additions of N, which is relatively quickly lost from such systems by denitrification.

This study demonstrates that Fe and OM additions can significantly alter the biogeochemical conditions of vegetated sediments and these conditions can have dramatic consequences for seagrass growth. Enhanced growth and productivity of *T. testudinum* in carbonate environments is controlled by the availability of Fe and the subsequent ability of the plants to increase their P intake, but also through the alleviation of the toxic effects of S^{2-} and a decrease in the S^{2-} stress inflicted to the plants. However, the magnitude and direction of these effects depend on the interaction of both elements. Only about 15% of the S^{2-} produced through anaerobic respiration is permanently buried in the sediment (Thamdrup et al. 1994) and the rest is gradually reoxidized back to sulfur. Because of the lack of titrating agents that can reduce the levels of S^{2-} in the sediment, marine macrophytes are then likely to be more susceptible to the negative effects of increased deposition of OM in carbonate sediments. The negative effect of OM on the growth of

T. testudinum was relatively small, and after a year of substantial OM loading, no mass mortality was observed. With normally high background levels of S^{2-} in the porewaters, seagrasses must have adapted mechanisms to withstand such conditions. Potential mechanisms include exudation of O_2 through the roots and extracellular reoxidation of S^{2-} (Koch and Erskine 2001) and intracellular reoxidation and storage as elemental sulfur S^0 (Holmer et al. 2005). It has been hypothesized that increased S^{2-} levels (Carlson et al. 1994) was a major contributor to the yet unexplained onset and spread of several seagrass die-off events that have occurred in the Bay since 1987 (Robblee et al. 1991). In light of this and other research (Borum et al. 2005; Chambers et al. 2001; Koch and Erskine 2001) it is likely that OM from already decaying seagrass material fuels SR and the production of S^{2-}, triggering a negative feedback and spreading the die-off patches. The onset of the die-off, however, can not be attributed solely to the build-up of OM in the sediments and other factors (e.g., hypersalinity, elevated temperatures, Koch et al. 2007) must interact in concert to produce a negative O_2 balance in the root zone of the seagrasses followed by S^{2-} invasion (Borum et al. 2005). Similarly, the decline of seagrasses under fish farming operations may continue after the activities have been foregone (Delgado et al. 1999) due to organic loading and elevated S^{2-} production, but the initial decline must start by a combination of factors like eutrophication of the water column with subsequent increased turbidity, shading and epiphytization of the leaves.

Fe loading could be used as a tool to ameliorate the negative effects of OM and increase P availability to seagrasses growing in carbonate sediments. This has already been proposed as a bioremediation technique in places with considerable amounts of anthropogenically derived organic loading (Holmer et al. 2005), although further research would need to be done to assess the implications of this actions in the long term.

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