

# Iron Additions Reduce Sulfate Reduction Rates and Improve Seagrass Growth on Organic-Enriched Carbonate Sediments

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## ABSTRACT

Here we demonstrate, through experimental iron additions to a Mediterranean seagrass meadow, that iron plays a pivotal role in seagrass systems on carbonate sediments, directly through its role as a limiting nutrient, and indirectly by stimulating phosphorus recycling through the activity of the enzyme alkaline phosphatase and by buffering the development of reduced conditions in sediments. Iron additions were performed throughout the active root zone (30 cm depth) to two *Posidonia oceanica* meadows, one on organic-enriched sediments and one on organic poor sediments (Reference). Seagrass growth, nutrient incorporation and sediment biogeochemical conditions were followed for four months. Iron additions had positive effects on seagrass growth (leaf production increased with 55%) and nutrient incorporation (increased 46–91%) in the organic-enriched site, increasing to

levels found at the Reference site. There was no effect of iron additions in the Reference seagrass meadow suggesting that iron was not the most important controlling factor at this site. The iron pools were about two times higher compared to the organic-enriched site. The main effect on the sediment biogeochemical conditions at the organic-enriched site was a suppression of sulfate reduction activity to the levels encountered at the Reference site ( $6.7 \text{ mmol m}^{-2}\text{d}^{-1}$  vs.  $4.7\text{--}5.9 \text{ mmol m}^{-2}\text{d}^{-1}$ ). This suggests that the sulfide stress on the seagrasses was removed and that the iron availability increased due to reduced precipitation of iron-sulfides and thus improving seagrass growth conditions in these organic-enriched sediments.

**Key words:** seagrass; carbonate sediments; sulfur cycling; iron addition; remediation.

## INTRODUCTION

Seagrasses form highly productive ecosystems that are declining world-wide (Marbá and others 1996; Short and Wyllie-Echeverria 1996, Hemming a and Duarte 2000; Duarte 2002). The declines have often

been attributed to reduced light conditions due to enhanced phytoplankton growth, epiphyte cover or macroalgae overgrowth (Nielsen and others 2002), but in some cases, such as for the seagrass *Posidonia oceanica* in the Mediterranean, declines have also been found in areas where light conditions are still excellent (Marbá and others 2002). *P. oceanica* often extends over carbonate-rich sediments characterized by low organic contents that favor oxidized conditions and thus seagrass growth. Organic

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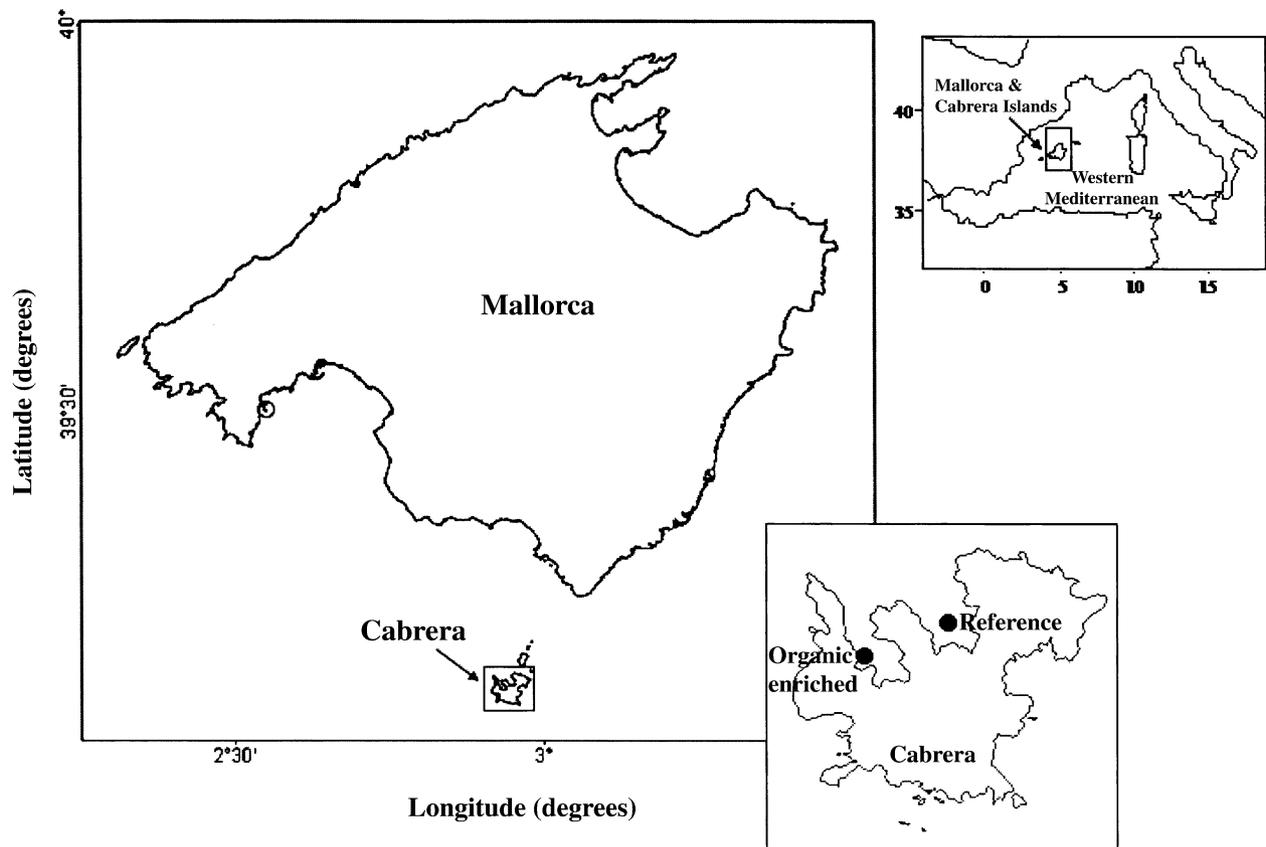


Figure 1. Map of location with experimental sites in the Mediterranean.

enrichment of carbonate sediments has been suggested to result in seagrass decline (Hemminga and Duarte 2000; Duarte 2002; Marbà and others 2002; Holmer and others 2003), by elevating the anaerobic microbial production of reduced sulfides, which is a phytotoxin (Goodman and others 1995; Erskine and Koch 2000; Holmer and Bondgaard 2001). Seagrasses growing on carbonate sediments may be particularly sensitive to organic enrichment (Hemminga and Duarte 2000), because carbonate sediments are often iron-deficient (Duarte and others 1995). Iron-deficient sediments have a limited capacity to bind sulfides (Berner 1984), and are therefore poorly buffered against organic enrichment, so that damage caused by organic enrichment to seagrasses is likely greater in these iron-deficient sediments. In addition, iron limitation has been suggested to affect benthic marine primary producers including seagrasses growing on biogenic carbonate sediments, where iron concentrations are typically low (Berner 1984; Duarte and others 1995; Chambers and others 2001). Organic enrichment may lower iron availability for plant uptake even further due to enhanced binding of sulfides to iron (Berner 1984).

The seagrass *P. oceanica* forms extensive meadows in the Mediterranean, particularly under oligotrophic conditions, where it may extend to depths of 35–40 m. It is, however, the slowest growing seagrass in the world (Hemminga and Duarte 2000), and small changes in growth rate or apical extension rate may be crucial for population dynamics and thus the distribution of this seagrass. There is evidence that *P. oceanica* meadows are declining throughout the Mediterranean, both in areas impacted by local human activity and in areas away from any source of disturbance (Marbà and others 1996). Conservation policies and the development of remediation techniques is needed to halt the decline and to restore seagrass habitat. On the basis of available evidence, we hypothesize that iron additions may reverse the decline of *P. oceanica* meadows due to organic enrichment by (1) interfering with the processes responsible for sulfide accumulation in the sediments, and (2) alleviating iron-limited plant growth.

Here we test the role of iron by experimental iron additions to declining Mediterranean seagrass meadows (*P. oceanica*) growing on carbonate sediments affected by organic loading. Iron additions

**Table 1.** Site Characteristics

	Organic-enriched	Reference
Seagrasses		
Shoot density (shoot m <sup>-2</sup> )	162 ± 15	288 ± 51
Below-ground biomass (g DW m <sup>-2</sup> )	1394.8 ± 114.4*	2029.5 ± 224.4
Sediments		
Particulate organic carbon (%DW)	2.56 ± 0.15**	0.47 ± 0.12
Particulate organic nitrogen (%DW)	0.245 ± 0.004**	0.032 ± 0.002
Total phosphorus (μmol (g DW) <sup>-1</sup> )	17.1 ± 1.1**	2.8 ± 0.2
C:N ratios (molar)	12.2	17.3

The shoot density is given as mean (± SE, n = 10) as reported in Holmer and others 2003. The below-ground biomass is given as mean (± SE, n = 8) for 0–30 cm and was sampled after 4 months.

The sediment particulate characteristics and total phosphorus content are given as mean (± SE, n = 3) for surface sediment (0–2 cm). The below-ground biomass was significantly lower (\*P = 0.02) and the nutrient contents significantly higher (\*\*P < 0.01) at the Organic-enriched site.

were also undertaken in a seagrass meadow receiving no organic inputs. Sediment conditions were examined by measuring sulfide production, sulfide pools and nutrient availability. We discuss the possible use of sediment iron additions as a remediation technique in declining seagrass meadows.

## MATERIALS AND METHODS

The experiment was located in Cabrera Island National Park (39° 9' N, 2° 56' E) a protected marine area close to Mallorca Island in the NW Mediterranean (Figure 1). *P. oceanica* meadows extend from near the surface down to below 35 m depth (mean light attenuation coefficient 0.063 m<sup>-1</sup>, Ballesteros and Zabala 1993), and form thick mats of rhizome and root material. The experiment was conducted in an area affected by organic loading from sewage inputs (Organic-enriched site). These inputs derive from sewage produced by the visitors on land (summer average 380 visitors day<sup>-1</sup>) and – in particular – from the visitors who reside on the average of 50 ships moored daily in the bay (about 200 persons day<sup>-1</sup>), which release their raw sewage directly to the bay waters, causing the decline of the seagrass meadows (Marbá and others 2002). The sediment organic content was elevated by a factor of 5.4 compared to an adjacent bay receiving no organic inputs (Marbá and others 2002), and the low C:N ratio and high total P content of the surface sediments (Table 1) suggest that sewage rather than seagrass detritus is the dominating source of organic matter. Seagrass detritus has a high C:N ratio typically greater than 20 and low P contents (Duarte 1992), whereas Oviatt and others (1987) characterized sewage dominated sedimentation by low C:N ratios and high P contents. The sediments at the Organic-enriched site were en-

riched with fine particles and had a lower carbonate content (88%) compared to the Reference site (94%) (see detailed description in Holmer and others 2003, where the Organic-enriched site is identical to vegetated Sa Paret and Reference to vegetated Sta. Maria). The water depth was 13–18 m and the shoot density ranged from 162 m<sup>-2</sup> at the Organic-enriched to 288 m<sup>-2</sup> at the Reference site, and the below-ground biomass was 31% lower at the Organic-enriched site (Table 1). *P. oceanica* populations are declining at the Organic-enriched site (Marbá and others 2002), where elevated sulfide production rates have been demonstrated (Holmer and others 2003).

In February 2001, eight 0.25-m<sup>2</sup> plots were established for iron enrichment at each site, and an additional eight plots served as controls. To each of the experimental plots, chelated Fe<sup>3+</sup> (Quelagro D, Infoagro), was injected into the sediments to a depth of 30 cm. Nine injections of 60 ml were done in each plot providing an approximate enrichment of 0.7 mol Fe m<sup>-2</sup>. The injections were repeated two months later. Control plots were injected with seawater. Seagrass growth and sediment biogeochemical conditions were followed for four months.

Ten replicate seagrass shoots in each plot were marked to estimate leaf growth in February and April using the leaf punching method (Short and Duarte 2001). The leaf elongation rate was converted into net production of leaf mass per shoot by multiplying leaf elongation rate by the specific leaf mass. The alkaline phosphatase activity (APA) was measured on three replicate fresh seagrass leaves (the youngest and second youngest leaves) from each plot within 48 hours after collection (Pérez and Romero 1993). The phosphorus and iron content of the leaf tissue produced during the experiment was determined by acid hydrolysis

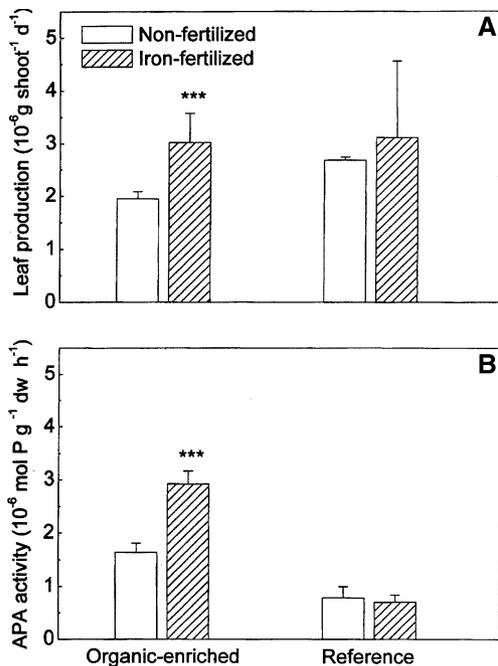


Figure 2. Leaf production (a) and alkaline phosphatase activity in leaf (b, APA) after iron additions. Each bar represents the mean of four experimental plots  $\pm$  SE. \*\*\* significant effect of iron addition at 0.001 level.

extraction of dried leaf tissue followed by colorimetric determination as described below. The nitrogen content was obtained by elemental analysis by use of Carlo Erba Elemental analyzer 1100EA. Nutrient incorporation was calculated as the product of the production and nutrient contents of new seagrass leaf tissue.

Three sediment cores were collected to a depth of 30 cm from each of the control and experimental plots 2 (April) and 4 months (June) after the iron injections for determination of sulfur cycling (i.d. 2.6 cm cores), porewater and solid phase characteristics (i.d. 9 cm cores) and below-ground biomass (i.d. 9 cm cores). Sulfate reduction rates (SRR) were determined by the core-injection technique (Jørgensen 1978). Two microliters of <sup>35</sup>S-sulfate (70 kBq) were injected with 1-cm intervals through predrilled silicone filled holes and the cores were incubated in darkness for 1–2 h. The incubation was terminated by sectioning the core into 3-cm intervals and fixing the sediment in 1 M zinc acetate (vol:vol). The samples were stored frozen until distillation according to the 1-step extraction scheme obtaining a total pool of reduced sulfides (TRS, Fossing and Jørgensen 1989). The concentrations of reduced sulfides were determined by spectrophotometry (Cline 1969).

Pore waters and solid phase characteristics were obtained from similar depths. The sediment was centrifuged (1500 rpm for 5 min) in double centrifuging tubes equipped with a GF/F filter and flushed with N<sub>2</sub> to keep them anoxic. The pore water was sampled for analysis of sulfate (SO<sub>4</sub><sup>2-</sup>), sulfides (DS), ammonium (DIN), phosphate (DIP), and iron (Fe<sup>2+</sup> and Fe<sup>3+</sup>). Sulfate was determined by a Dionex autosuppressed ion chromatograph equipped with a conductivity detector. Sulfides were determined as described above, and ammonium was determined by the colorimetric method by Bower and Holm-Hansen (1980) and phosphate according to Koroleff (1983). Dissolved Fe<sup>2+</sup> was analyzed as described by Stookey (1970) and Sørensen (1982). The concentration of Fe<sup>3+</sup> was determined by subtraction after extraction in hydroxylamine according to Lovley and Phillips (1987). The particulate pools of Fe(II) and Fe(III) were extracted in 0.5 M HCl for 30 min and centrifuged from 5 min at 2000 rpm. The Fe(II) and Fe(III) was separated by use of hydroxylamine and analyzed as Fe<sup>2+</sup> as described above. Sediment density was obtained by weight of a known volume and the water content was obtained after drying overnight at 105°C. Porosity was calculated from sediment density and water content. The sediment particulate organic carbon (POC) and nitrogen (PON) were measured on dry sediment on three replicate surface samples with a Carlo Erba elemental analyser EA 1100A after removal of carbonates by acidification.

The cores for below-ground biomass were frozen, sectioned in 3 cm intervals down to 30 cm, rinsed free of sediment, and live roots were sorted out and dried at 60°C to obtain dry weight.

The effect of iron additions was analyzed using a one-way ANOVA. Because there was no significant difference in the effects of iron additions between the two sampling periods (April and June), the results are presented as a mean over the 4-month sampling period.

## RESULTS

Iron enrichment of the Organic-enriched sediments led to a 55% increase in leaf production (Figure 2), a 78% increase in the APA activity (Figure 2), and increased nutrient incorporation (46–91%, Figure 3). In contrast, no significant responses to iron additions were observed in Reference sediments (Figures 2 and 3). The leaf production was about 27% lower in the Organic-enriched control sediments compared to the Reference site, and the iron enrichment increased the production at the Or-

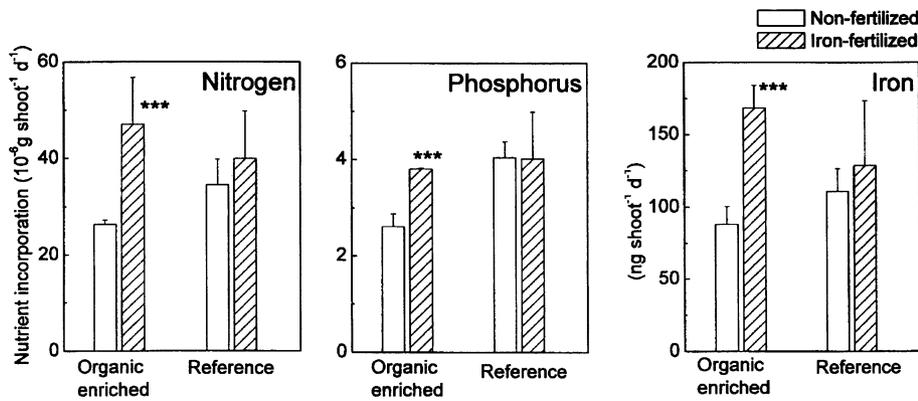


Figure 3. Nitrogen, phosphorus and iron incorporation in leaves in the experimental plots after iron additions. Each bar represents the mean of four experimental plots  $\pm$  SE. \*\*\* significant effect of iron addition at 0.001 level.

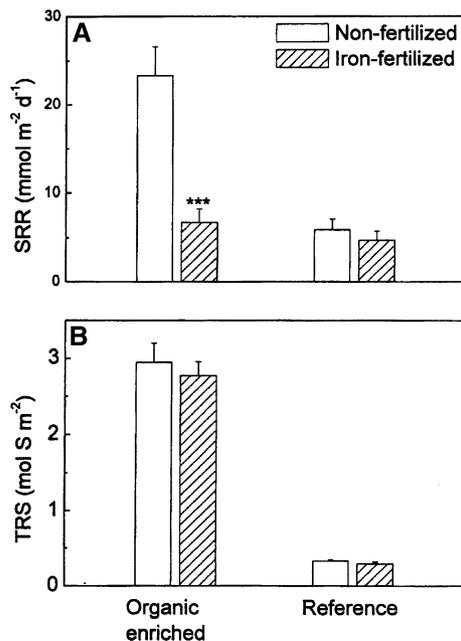


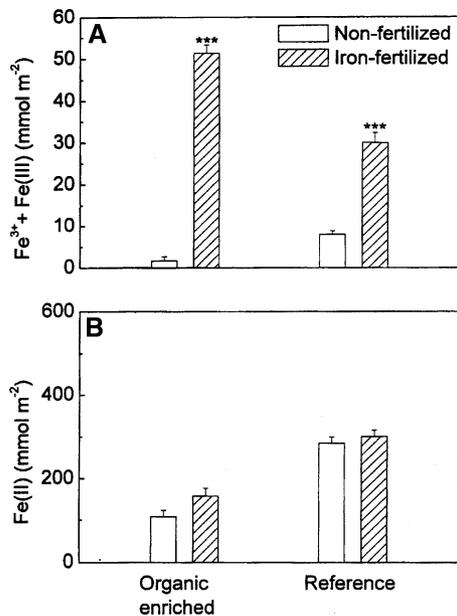
Figure 4. Depth integrated (0–30 cm) sulfate reduction rates (a) and total reduced sulfide pools (b) after iron additions. Each bar represents the mean of four experimental plots  $\pm$  SE. \*\*\* significant effect of iron addition at 0.001 level.

ganic-enriched site to the same level as at those observed in the Reference site. In contrast, the APA activity was higher in the Organic-enriched control sediments, and the iron enrichment increased this activity to a level three times higher compared to the Reference site. The nutrient content in the new leaf growth was not significantly different between seagrasses at the Organic-enriched and Reference sites (data not shown), whereas the nutrient incorporations were significantly lower in the Organic-enriched control sediments compared to the Reference site. The iron additions increased the nutrient incorporation at the Organic-enriched site to levels similar to the Reference site.

Iron addition reduced the sulfate reduction rate by 71% at the Organic-enriched site, however there was no effect at the Reference site (Figure 4). The sulfate reduction at the Organic-enriched site was four times higher than at the Reference site, but iron additions resulted in rates comparable to those at the Reference site. In contrast, there were no significant effects of iron additions on the total pools of reduced sulfides, which remained nine times higher at the Organic-enriched site compared to the Reference site.

The pools of reactive Fe(III) were very low, in particular at the Organic-enriched site with concentrations below  $100 \text{ nmol (gDW)}^{-1}$  and no significant trend with depth (data not shown). Also the less reactive Fe(II) showed low concentrations ( $<3500 \text{ nmol (gDW)}^{-1}$ ) with a tendency toward decreasing values with depth (data not shown). The depth accumulated pools of Fe(III) and Fe(II) were significantly higher at the Reference site ( $P < 0.001$ ), with pools about twice as high as at the Organic-enriched site. The iron additions increased the porewater pools of  $\text{Fe}^{3+}$  to very high concentrations (up to  $400 \mu\text{M}$ ), and the total pool of reactive iron ( $\text{Fe}^{3+} + \text{Fe(III)}$ ) was significantly enriched at both sites due to the addition of  $\text{Fe}^{3+}$  (Figure 5). Also the Fe(II) showed a tendency to increase by the iron additions at the Organic-enriched site (n.s.,  $P = 0.078$ ). No dissolved sulfides could be detected in the porewaters of the iron-fertilized sediments at the Organic-enriched site, whereas low concentrations ( $<13 \mu\text{M}$ ) were found in the control sediments at both sites (data not shown). The  $\text{Fe}^{2+}$  concentration was below detection ( $< 5 \mu\text{M}$ ) at both sites.

The nutrient availability in the porewaters was higher for ammonium at the Organic-enriched site, whereas the phosphate concentrations were almost similar at the two sites (Figure 6). The ammonium concentration showed a subsurface maximum at both sites in the 5–15 cm depth interval. Also



**Figure 5.** Depth integrated (0–30 cm) pools of oxidized iron (**a**,  $\text{Fe}^{3+} + \text{Fe(III)}$ ) and reduced iron (**b**,  $\text{Fe(II)}$ ) after iron additions. Each bar represents the mean of four experimental plots  $\pm$  SE. \*\*\* significant effect of iron addition at 0.001 level.

phosphate concentrations were higher in the surface layers. The iron additions did not have apparent effects on the nutrient availability in the sediments. The ammonium concentration increased slightly in response to iron additions at both sites, whereas there were no effects of iron additions on the phosphate concentrations.

## DISCUSSION

The additions of iron to organic-enriched sediments had a major impact on the sediment biogeochemical conditions with suppression of the sulfate reduction rates and increased pools of iron, whereas there was no effect of iron additions on the sulfate reduction rates at the Reference site. Sulfate reduction was most likely suppressed due to a shift in bacterial metabolism to microbial iron reduction. Oxidized iron is usually favored over sulfate as an electron acceptor in marine sediments due to more favorable growth kinetics (Thamdrup 2000), but is considered to be low in carbonate sediments due to low iron contents (Berner 1984). The addition of oxidized iron may have stimulated the microbial iron reduction and lowered the sulfide production and thus sulfide pressure on the seagrasses. There was no suppression of the sulfate reduction rates at the Reference site. Here sulfate reduction rates were generally much lower com-

pared to the organic-enriched site, probably controlled by oxidation of the sediments favoring oxidized metabolisms or by the availability of organic matter. The addition of iron may have stimulated the overall metabolism in the sediments due to enhanced availability of electron acceptors, but this was not tested in the current experiment. Microbial iron reduction in unvegetated sediments is often associated with an accumulation of  $\text{Fe}^{2+}$  in the porewaters (Thamdrup 2000), but this was not encountered in these sediments where the pools remained low throughout the examined depths at both locations. It is likely that  $\text{Fe}^{2+}$  was taken up by the seagrasses.

The iron pools increased in the iron-fertilized sediments suggesting that iron was available for plant uptake at both locations throughout the sampling period. The iron additions had only a marginal effect on the pools of precipitated sulfides, suggesting that iron cannot reoxidize the pools of sulfides already bound in the sediments. There was, however, a tendency toward lower pools at the organic-enriched site, where the sulfate reduction rates were suppressed. Only about 10% of sulfides produced during sulfate reduction are considered to be permanently buried in marine sediments and the rest is reoxidized (Thode-Andersen and Jørgensen 1989). The reoxidation may be even higher in vegetated sediments due to the release of oxygen from the roots, but remains to be examined in more detail (Pedersen and others 2004). It is therefore difficult to detect changes in the precipitation pattern over such relatively short periods of time, and a two year experiment with iron additions is now taking place at the same location to examine long term effects on the sulfur-iron dynamics.

The major changes in response to iron addition in sediment biogeochemical conditions at the organic-enriched site with reduced sulfate reduction and increased iron pools were associated with enhanced enzyme activity of alkaline phosphatase in seagrass leaves, increased nutrient incorporation and consequently increased seagrass growth at the iron amended organic-enriched site. In contrast, no significant response to iron additions on seagrass growth was observed in the Reference sediments. The cell-surface enzyme alkaline phosphatase is utilized by the seagrasses to recycle organic phosphorus compounds as an alternative or supplement to a direct uptake of inorganic phosphate (Cembella and others 1983), and may thus be very important for seagrasses growing on carbonate sediments, where the availability of inorganic phosphate is limited in the rhizosphere (Pérez and Romero

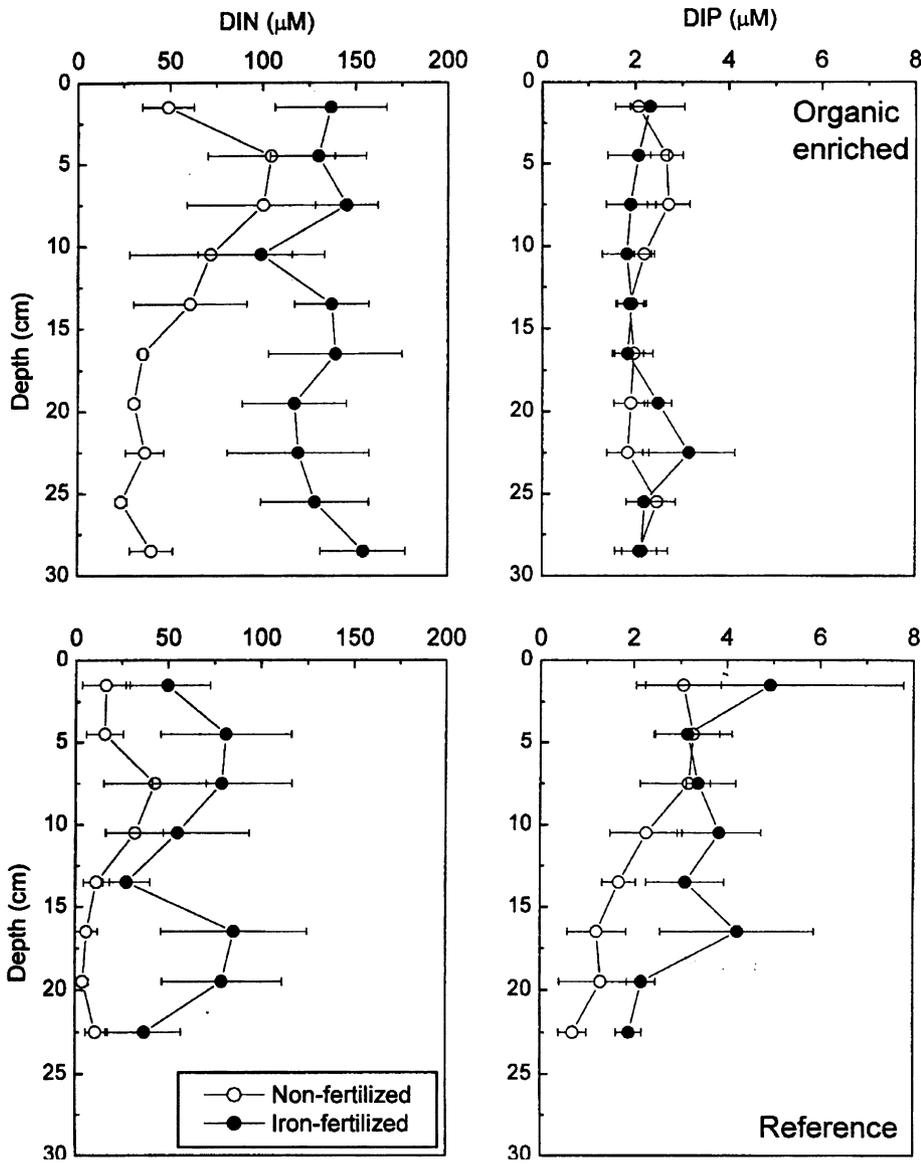


Figure 6. Porewater concentrations of ammonium (DIN, left panel) and phosphate (DIP, right panel) at the Organic-enriched and Reference site. Values represent mean of four experimental plots  $\pm$  SE.

Table 2. Nutrient Contents and Elemental Ratios in Seagrass Young Leaves at the Two Study Sites.

	Organic-enriched		Reference	
		+iron		+iron
Carbon (mg C (g DW) <sup>-1</sup> )	348.6 $\pm$ 11.6	347.75 $\pm$ 10.3	345.83 $\pm$ 7.6	337.91 $\pm$ 10.3
Nitrogen (mg N (g DW) <sup>-1</sup> )	13.58 $\pm$ 1.51	15.33 $\pm$ 3.40	12.98 $\pm$ 3.65	13.77 $\pm$ 0.94
Phosphorus (mg P (g DW) <sup>-1</sup> )	1.34 $\pm$ 0.18	1.28 $\pm$ 0.31	1.51 $\pm$ 0.41	1.33 $\pm$ 0.29
Iron ( $\mu$ g Fe (g DW) <sup>-1</sup> )	47.25 $\pm$ 11.37	52.75 $\pm$ 11.54	38.63 $\pm$ 10.41	38.88 $\pm$ 15.53
C:N ratios (weight)	25.6	22.7	26.7	24.5
C:P ratios (weight)	260.1	271.7	229.0	254.1

Values are average of samplings after 2 and 4 mo ( $\pm$  SE, n = 8). None of the treatments were significantly different ( $P > 0.05$ ).

1993). Alkaline phosphatase is, however, a major sink of iron in plants (Marschner 1986), and a low availability of iron may thus control the enzyme

activity. Iron deficiency has been suggested to control the nutrient uptake in seagrasses growing on carbonate sediments (Duarte and others 1995;

Chambers and others 2001), and our results indicate that this control may be exerted through the activity of plant enzymes, such as alkaline phosphatase. The concentrations of nutrients in seagrass leaves were below the critical levels found by Duarte (1990) and in particular the low phosphorus concentrations compared to findings in *P. oceanica* elsewhere in the Mediterranean (Alcoverro and others 2000) indicate strong nutrient limitation. Even though the phosphorus concentrations were high at the organic-enriched site, the pools in the porewaters were low suggesting that the plants took up the available inorganic phosphate. This site was characterized by a high alkaline phosphatase activity indicating that the activity of APA was stimulated by the presence of organic phosphorus, probably released from the sedimentary pools. Jensen and others (1998) also found higher pools of sedimentary P in anthropogenic influenced subtropical seagrass beds, where P was released as the sediments dissolved. They did not examine the underlying mechanisms behind the sediment dissolution, but Burdige and Zimmerman (2003) found increased carbonate dissolution as a result of increased mineralization activity stimulated by oxygen release from the roots. Additions of iron to the organic-enriched *P. oceanica* meadows increased the activity of APA and the uptake of phosphate was significantly enhanced in these plants suggesting that the APA was controlled by iron availability. There was no effect of iron additions on the APA activity at the Reference site. Here the activity was low probably limited by very low pools of phosphorus.

The iron concentrations in the seagrasses were below the critical levels for angiosperms ( $<100 \mu\text{g Fe (g DW)}^{-1}$ ) at both locations and in the low range of concentrations found in seagrasses growing in carbonate sediments (Duarte and others 1995). Nitrogen contents were also below the critical level found for seagrasses (Duarte 1992). Both nitrogen and iron incorporation increased in response to the iron additions at the organic-enriched site suggesting that enzymes responsible for nitrogen and iron uptake were also stimulated by the increased concentrations of iron. Most enzymes contain high amounts of iron and may be stimulated by higher iron availability, in particular enzymes involved in nitrogen fixation have been stimulated by Fe additions (Fu and Bell 2003; Igarashi and Seefeldt 2003). Seagrass meadows are generally characterized by high nitrogen fixation activity compared to unvegetated sediments, and the fixation is particularly high in rhizosphere sediments where diazotrophic bacteria benefit from organic substrates

released from the plants through root exudates (Welsh 2000). Additions of iron may thus stimulate nitrogen fixation and increase nitrogen availability to the seagrasses. The increased nutrient uptake was followed by increased leaf growth, consistent with previous iron additions for *Thalassia testudinum* on the Caribbean coast (Duarte and others 1995) and in Florida Bay (Chambers and others 2001). In addition to the improved sediment conditions with reduced sulfide production and sulfide concentrations, the increased availability of iron, may help improve seagrass growth in the organic enriched sediments, and the growth rates obtained were similar to the Reference site where the growth conditions were favorable for *P. oceanica* (Marbà and others 2002). The phosphorus and nitrogen content of the seagrass leaves, however, remained below the critical levels (Duarte 1990) indicating that the iron additions did not eliminate nutrient limitation.

The lack of effect at the Reference site on enzyme activity, nutrient uptake and seagrass growth suggests that iron was not the most important limiting factor there. Iron availability was generally higher and the utilization of iron in the sediments much less as indicated by lower rates of sulfate reduction and low particulate pools of reduced sulfides compared to the Organic-enriched site. Hence, sediment conditions were more favorable to support healthy *P. oceanica*.

There were several indications of impoverished growth conditions at the Organic-enriched site. The sediments were highly enriched with organic matter from increased organic inputs by visitors. The enriched sediments showed high sulfate reduction activity, four times higher compared to the Reference site. Sulfate reduction is an important mineralization process in organic-rich coastal sediments, but only few studies have been undertaken in carbonate sediments (for example, Ku and others 1999; Holmer and others 2003). Carbonate sediments tend to be organic poor and oxic mineralization processes are likely favored (King and others 1990), but a previous study at this site (Holmer and others 2003) showed low oxygen penetration depths ( $<5 \text{ mm}$ ). An organic enrichment may thus rapidly exhaust the aerobic respiration. Microbial iron reduction is not likely to be an important process in these sediments due to extremely low concentrations of oxidized iron (Thamdrup 2000). Sulfate concentrations remained high ( $>24 \text{ mM}$ ) to a depth of 30 cm suggesting that sulfate reduction was not limited by supply of sulfate. The sulfate reduction rates measured in the organic-enriched sediments were similar to those

in eutrophic coastal sediments (Moeslund and others 1994; Holmer 1999) whereas the rates at the Reference site were similar to findings in subtropical carbonate (Ku and others 1999) or tropical terrigenous (Holmer and others 2001) seagrass meadows. The high rates of sulfate reduction were reflected in a significant increase in the particulate pools of reduced sulfides. The accumulation of reduced sulfides was thus nine times higher compared to the Reference site, where pools were similar to seagrass meadows on carbonate sediments (Chambers and others 2001).

The precipitation of iron sulfides in carbonate sediments is generally considered to be iron limited (Berner 1984), and the pools of reactive iron were very low in the examined sediments, one to two orders of magnitude lower than found in a survey of carbonate sediments in Florida Bay, where the formation of iron sulfides was strongly iron limited (Chambers and others 2001). This suggests that the seagrasses at the Organic-enriched site experienced a significant sulfide stress enhanced by highly reduced conditions in the root zone. The pools of dissolved sulfides were, however, one to two orders of magnitude lower than the concentrations that affect growth and survival of the seagrass *T. testudinum* (Erskine and Koch 2000; Lee and Dunton 2000). Sulfide toxicity has not been examined for *P. oceanica*, and our results on reduced leaf growth and increased mortality at relatively low sulfide concentrations suggest this species to be more sensitive to sulfide or to the anoxic conditions invoked by large sulfide production.

The experimental results presented add to recent demonstrations of the importance of iron in controlling seagrass growth over carbonate sediments (Duarte and others 1995; Chambers and others 2001). Previous analyses, focused on *T. testudinum*, did not, however, examine the effects of iron on sulfate reduction rates nor its effects on enzyme activities. Hence, the results presented here extend our understanding on the mechanisms linking seagrass growth and health to iron supply. We demonstrated a pivotal role of iron in the control of key processes in the marine sediments spanning from direct limitation of plant growth in carbonate sediments, indirect control of phosphorus fluxes through iron-regulation of alkaline-phosphate activity and therefore phosphorus uptake by seagrasses, and the control, through the interaction between iron and sulfur cycling, of the benthic biogeochemical processes determining their suitability to support seagrass growth. Addition of iron to the active root zone in the *P. oceanica* sediments depressed the sulfate reduction rates and increased

the availability of reactive iron. Iron additions also increased the activity of the enzyme, which is responsible for uptake of phosphorus, but this stimulation was not sufficient to overcome the P limitation of seagrass growth at the site. The results presented support the contention that seagrass growing on carbonate sediments are highly vulnerable to organic inputs, and identify iron availability as a key component of the maintenance of healthy seagrass meadows in these areas.

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