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Sulfur cycling and seagrass (*Posidonia oceanica*) status in carbonate sediments

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Abstract. Sulfur cycling was investigated in carbonate-rich and iron-poor sediments vegetated with *Posidonia oceanica* in oligotrophic Mediterranean around Mallorca Island, Spain, to quantify sulfate reduction and pools of sulfide in seagrass sediments. The oxygen penetration depth was low (< 4.5 mm) and sulfate reduction rates were relatively high (0.7–12 mmol $m^{-2}d^{-1}$). The total pools of reduced sulfides were remarkably low (< 5 mol S m^{-2}) indicating a fast turnover of reduced sulfides in these iron-poor sediments. The sulfate reduction rates were generally higher in vegetated compared to bare sediments possible due to enhanced sedimentation of sestonic material inside the seagrass meadows. The sulfate reduction rates were positively correlated with the seasonal variation in water temperature and negatively correlated with the shoot density indicating that the microbial activity was controlled by temperature and release of oxygen from the roots. The pools of reduced sulfides were low in these iron-poor sediments leading to high oxygen consumption for reoxidation. The sediments were highly anoxic as shown by relatively low oxygen penetration depths (< 4.5 mm) in these low organic sediments. The net shoot recruitment rate was negative in sediments enriched with organic matter, suggesting that organic matter enrichment may be an important factor for seagrass status in these iron-depleted carbonate sediments.

Introduction

Sulfur cycling is a major driver of the carbon cycling of coastal sediments, where it has been studied intensively (Skyring 1987; Thode-Andersen and Jørgensen 1989). Shallow coastal sediments often support dense seagrass cover, which may further impact the carbon cycling there. Seagrass activity has been shown to enhance sulfate reduction rates in vegetated sediments (Holmer and Nielsen 1997), which may induce a negative feedback on the plants, since sulfide is considered to be toxic to them (Fürtig et al. 1996; Erskine and Koch 2000; Holmer and Bondgaard 2001).

Studies on carbon cycling in shallow coastal sediments are, however, biased towards terrigenous sediments, and the biogenic carbonate sediments that cover a significant fraction of the world's coastal zone remain poorly studied (Berner 1984; Chambers et al. 2001). Carbonate sediments are characterized by low organic matter contents (Chambers et al. 2001), which favor oxidized conditions and promote seagrass growth. Organic enrichment of carbonate sediments, e.g., due to anthropogenic activity or eutrophication, greatly impacts their biogeochemical conditions. The particular sensitivity of carbonate sediments to organic enrichment derives from the fact that they are characteristically iron-poor, having a limited capacity to remove sulfides through the formation of iron-sulfide compounds such as pyrite (Berner 1984). It has therefore been suggested that the seagrasses growing on carbonate sediments may be particularly prone to sulfide toxicity (Hemminga and Duarte 2000). Indeed, iron addition experiments to vegetated carbonate sediments have been shown to enhance seagrass growth and affect their nutritional status (Duarte et al. 1995; Chambers et al. 2001).

Posidonia oceanica, forms extensive meadows, covering about 50,000 km^2 between 1 and 35 m isobaths in the Mediterranean (Béthoux and Chopin-Montégut 1986; Pergent et al. 1989), where it is one of the most productive ecosystems (Duarte and Chiscano 1999). *P. oceanica* forms extensive meadows on the nutrient-poor carbonate sediments that are present in some regions of the Mediterranean, such as the Balearic Islands (Canals and Ballesteros 1997). 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 (Marbá et al. 1996). For instance a tendency towards *P. oceanica* decline has been reported in the waters of the Cabrera Island National Park (Marbá et al. in press), where light penetration is still excellent (Ballesteros 2000). The observed net decline of *P. oceanica* populations in well illuminated carbonate sediments was attributable to reduced recruitment, rather than increased mortality (Marbá et al. in press). These sediments were enriched with organic matter, and suggest that recruitment of *P. oceanica* may be particularly sensitive to even moderate organic enrichment when growing on carbonate sediments. The possible link between this sensitivity and sulfur cycling remains, however, hypothetical due to the paucity of information on sulfur cycling in shallow carbonate sediments.

Here we examine the sulfur cycling in carbonate sediments of the Cabrera Island National Park and Mallorca Island (Balearic Islands, Western Mediterranean) encompassing a range of sediment organic matter contents and seagrass (*Posidonia oceanica*) cover. Our goal is to examine the rates and fate of sulfide production in relation to sediment characteristics and seagrass cover and to explore the possible relationship between sulfur cycling and seagrass status.

Methods

The study was conducted at Mallorca Island and the Cabrera Island National Park (39°9′ N, 2°56′ E) a protected marine area, where *Posidonia oceanica* meadows extend from near the surface down to below 35 m depth. *P. oceanica* forms thick mats of rhizome and root material that occupy a substantial fraction of the sediment volume (Hemminga and Duarte 2000). Six study sites were selected on the

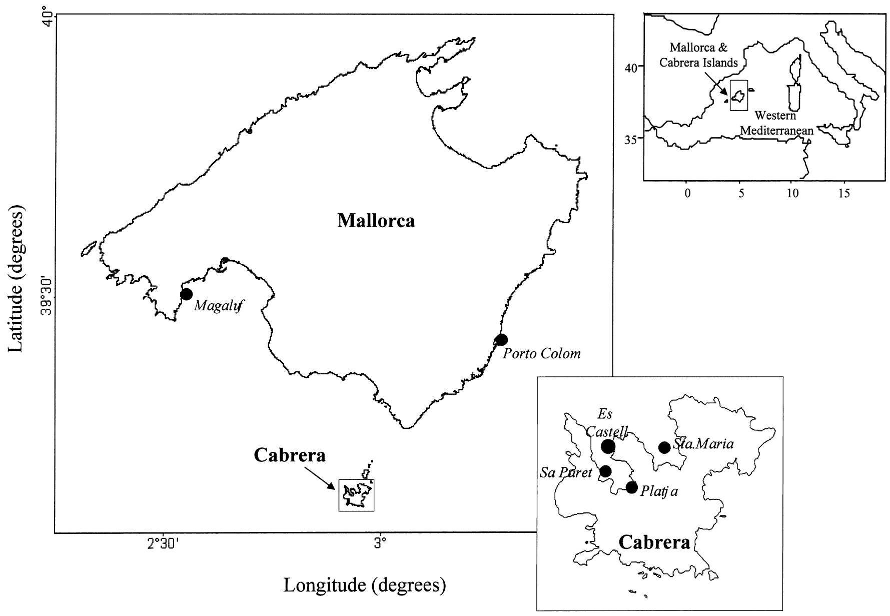

Figure 1. Map showing the sampling locations around Mallorca Island in the Mediterranean.

basis of a broader survey of plant status around Mallorca and Cabrera Islands (Figure 1). One site (Magalluf) in Mallorca was located in an exposed area, whereas the other site was in a rather sheltered bay (Porto Colom) with relatively high nutrient loading. Three sites (Platja, Es Castell, Sa Paret) at Cabrera Island were studied in a rather enclosed bay and one additional site (Sta. Maria) in a more exposed bay, which is environmentally protected from human activities with no access for the public (Marbá et al. in press). The study was conducted from fall of 2000 until June 2001, encompassing the reproductive season for *P. oceanica* (Buia and Mazzella 1991). Two sites (Sa Paret and Sta. Maria) were sampled 4 times during the year to examine the seasonal variation (October 2000, February, April and June 2001), whereas the other stations were sampled only once (October 2000 or June 2001). Sediments and seagrasses were sampled at water depths between 2 and 17 m, well above the 35 m depth limit of the plants. Light measurements at the deepest site (Sa Paret) showed that the light intensity was high throughout the year, 174 and 285 μmol m^{-2} s^{-1} in winter and summer, respectively.

Three sediment cores (i.d. = 4.3 cm) from each station were collected by SCUBA diving inside seagrass meadows and in adjacent bare sediments. The sediment cores were sectioned into 1-cm intervals (to 4 cm), in 2-cm intervals (4–10 cm) and in 5-cm intervals (10–15 cm). The sediment was centrifuged in double centrifuging tubes equipped with a GF/F filter and flushed with N_2 to keep them anoxic at 1500 rpm for 5 min. The pore water was sampled for analysis of sulfate (SO_4^{2-}), which was determined by a Dionex autosuppressed ion chromatograph equipped with a conductivity detector, and sulfides (DS), which were determined by colorimetric method of Cline (1969). 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 3 replicate surface samples (0–2 cm pooled) with a Carlo Erba elemental analyzer 1100EA by the method of Kristensen and Andersen (1987). The carbonate content was determined according to Smith and Atkinson (1984). The total phosphorus content (TP) was obtained from ignited sediment (3 replicate surface samples 0–2 cm pooled) after boiling in 1 M HC1 for 15 min. Particulate Fe(II) and Fe(III) were measured in vegetated sediments collected at Sta. Maria and Sa Paret by a modified version of the HC1 technique by Lovley and Phillips (1987), as described in Kristensen et al. (2000). Wet sediment was extracted in 0.5 M HC1 for 30 min and centrifuged for 5 min at 2000 rpm. The Fe(II) and Fe(III) were separated by use of hydroxylamine for reduction of Fe(III) and analyzed as Fe^{2+} as described by Stookey (1970) and Sørensen (1982).

Three sediment cores (i.d. = 2.6 cm) were collected, if possible without cutting of roots, to measure sulfate reduction rates (SRR) and total pools of reduced sulfides (TRS). The cores were within 1 hour of collection injected with 2 µl $^{35}SO_4$ (~ 70 kBq) at 1-cm intervals and incubated for 2 h in darkness at *in situ* temperature to obtain SRR (Jørgensen 1978). The incubation was terminated by sectioning the cores into intervals as described above and fixed in 1 M zinc acetate (equal volume zinc acetate to sediment) and frozen immediately. The rates were obtained by the one-step distillation method (Fossing and Jørgensen 1989). Radioactivity was counted on a Beckman LS-3801 or a Packard TriCarb 2000 scintillation counter. Pools of total reduced sulfides (TRS) were determined by the method of Cline (1969).

Oxygen penetration depth was measured in June 2001 at Sa Paret and Sta. Maria shortly after collection of cores (i.d. = 4.3 cm) to examine the oxygen dynamics in these sediments. The cores were collected without plants inside to avoid cutting of roots and rhizomes. The *Posidonia oceanica* rhizosphere is located relatively deep (> 10 cm, this study) in the sediments, and roots were thus not present in the upper layer (0–4 cm), and it is unlikely that the roots affected the surface oxygen penetration depth. Commercially available Clark-type microelectrodes (Unisense, Denmark) with a tip diameter < 10 µm, a 90% response < 1 s, and a stirring sensitivity of < 1% were used. The electrode signal was logged on a datalogger (PicoLog, England) connected to a picoammeter (Unisense, Denmark). The microelectrode was mounted on a micromanipulator, which allowed a spatial resolution of 10 µm.

In each studied meadow, two 20 m transects were lined and shoot density measured every second meter using 0.25 m^2 quadrats. Moreover, from each meadow, divers harvested between 100 and 200 *Posidonia oceanica* shoots, connected to horizontal rhizomes. Plant material was transported to the laboratory, where shoot age was estimated from the number of leaf scars on the shoots (Duarte et al. 1994). In April 2001 three replicated sediment plugs were collected using a steel corer (i.d. = 9.0 cm), and these were transferred underwater to PVC-tubes with a bottom stopper to preserve the rhizosphere structure. The sediment cores were frozen.

Later, the frozen cores were sectioned in 3 cm intervals down to 30 cm and rinsed free of sediment, and alive roots were sorted out and dried at 60 °C to obtain dry weight. The leaf material was dried and nutrient content (C, N, P) was determined on the two youngest leaves as described above for the sediments.

The status of the *Posidonia oceanica* populations sampled was derived from the age distribution of the shoots (Duarte et al. 1994). Shoot age (in years) was estimated as the total number of leaves produced during the life-span of a shoot (i.e., number of standing leaves plus number of leaf scars on the vertical rhizome supporting the shoot) divided by the average number of leaves produced by a shoot annually (Marbà et al unpubl. data). Shoot mortality rate (M, yr^{-1}) was derived from the exponential decline in the number of living shoots (N_0) with time (t, in years) as,

$$N_t = N_0 \times e^{-M \times t}$$

where N_0 is the number of shoots with age equal to the mode, and N_t is the number of shoots older than the modal age at time t. M was estimated using a semilogarithmic linear regression model, where constant mortality over shoot age classes and years was assumed. The annual gross shoot recruitment rate (R_{gross}, yr^{-1}) was calculated following Peterson and Fourqurean (2001) as,

$$R_{gross} = \frac{\sum_{j=1}^{k} e^{(\ln n_j + Mt_j)}}{N}$$

where n is the number of living shoots in the jth age class, t is the age of the jth age class, k is the oldest age class < 1 yr old, M is the per capita mortality rate, and N is the total number of living shoots in all age classes.

The ratio between gross recruitment and mortality rates (R gross/M) was used to indicate whether populations were expanding (i.e., R gross/M > 1), declining (i.e., R gross/M < 1) or in steady state (i.e., R gross/M = 1, Duarte and Sand-Jensen (1990) and Duarte et al. (1994)). The use of this ratio assumes that recruitment and mortality rates have been maintained constant during the life span of the shoots (Fourqurean et al, accepted). In addition, if gross recruitment and mortality rates have been maintained similar during population life span, the ratio between gross recruitment and mortality rates equals the net population growth rate (r, Fourqurean et al, accepted).

The differences in the concentration of organic matter and TP and SRR between vegetated and bare sediments were tested with a one-way ANOVA followed by a Tukey's Post hoc analysis.

Results

The water temperature remained high (14 to 21 °C) during the study period (Table 1) and the salinity did not differ among the sites and was about constant (~ 38 PSU) throughout the sampling period. The sediments were coarse-grained carbonate sand (88–98% carbonates, Table 1), except at the deepest site, where the sediment was enriched with fine particles. The organic content was generally low (Table 1), and there were no consistent trends in POC and PON along the depth gradient, but the organic content was significantly higher (POC: 2.56% DW, PON: 0.45% DW) and the C:N ratio lowest (12.2) at the deeper site (Sa Paret). The total phosphorus content was low at most of the sites (TP < 3.0 μmol (g DW)$^{-1}$), except for the deepest site (17.1 μmol (g DW)$^{-1}$, Table 1), where the content was significantly higher in the vegetated sediments compared to the other sites. The total phosphorus contents were generally lower in the bare sediments although this was only significant at Platja and Sa Paret (Table 1).

Particulate Fe(II) and Fe(III) were extracted once and only at the Sta. Maria and Sa Paret, and were not significant different among stations ($p > 0.05$). The Fe(II) content was 1.4 ± 0.1 and 1.5 ± 0.3 μmol (g DW sed)$^{-1}$ [= 74 and 157 mmol Fe(II) m^{-2} (0–15 cm), respectively], and the Fe(III) 58 ± 32 and 29 ± 19 nmol (g DW sed)$^{-1}$ [6.3 and 1.4 mmol Fe(III) m^{-2} (0–15 cm), respectively] at Sta. Maria and Sa Paret, respectively.

The pore water concentrations of sulfate did not vary among sites and between vegetated and bare sediments (Table 1) and only very little with depth (data not shown). The concentrations ranged between 26.4 and 29.1 mM. The average sulfide concentrations in the pore waters were generally low (< 30 μM), but there was a large variation between sites and between vegetated and bare sediments. Highest concentrations were found at the vegetated sites Sa Paret, Sta. Maria and El Castell, whereas the concentrations were low in both vegetated and bare sediments of the Platja site.

There was a temporal variation in the sulfate reduction rates at Sta. Maria and Sa Paret (Figure 2). The sulfate reduction rates were up to 10 times higher in the surface layers at the deepest site with the organic enriched sediments compared to Sta. Maria, and the rates generally decreased with sediment depth. Similarly the total pools of reduced sulfides (TRS, Figure 2) were up to 9 times higher at Sa Paret, and the pools were generally increasing with depth, except for the sampling in October where high surface pools were encountered. The high sulfate reduction rates at Sa Paret were reflected in the depth integrated rates, which were between 1.4 and 3.6 times higher compared to Sta. Maria (Figure 3A). The temporal variation in the depth integrated rates showed a positive relationship with the water temperature at both sites, but only significantly at Sta. Maria (linear regression $R^2 = 0.95$, $p = 0.027$). Also the depth integrated TRS were much higher at Sa Paret compared to Sta. Maria (3.4–10.5 times higher) (Figure 3B). The relationship between the depth distribution of below-ground root biomass and sulfate reduction rates were studied in April and a significant negative linear correlation was found (Figure 4, $R^2 = 0.77$, $p = 0.002$).

Table 1. Site characteristics in 6 *Posidonia oceanica* meadows sampled in autumn 2000 and June 2001. The sediment particulate characteristics and total phosphorus content (TP) are given as mean (± SEM, n = 3) for the surface sediment (0–2 cm). Porewater concentrations are given as depth average (0–15 cm, n = 3). n.d., not determined

	Platja		Magalluf		Porto Colom		Es Castell		Sta. Maria		Sa Paret	
Water depth (m)	2		3		4		10		13		17	
Temperature (°C)	21		18		18		21		21		21	
Sediment particulates	Veg	Bare	Veg	Bare	Veg	~~n.d.~~ Bare	Veg	Bare	Veg	Bare	Veg	Bare
POC (%DW)	~~1.22~~ ± $0.10^{a,b}$	~~0.51~~ ± 0.02	~~0.38~~ ± 0.11	~~0.21~~ ± 0.01	~~0.71~~ ± 0.24		~~0.55~~ ± 0.02	~~0.47~~ ± 0.04	~~0.47~~ ± 0.12	~~0.47~~ ± 0.01	~~2.56~~ ± $0.15^{a,b,c}$	~~0.61~~ ± 0.02
PON (%DW)	0.068 ± $0.013^{a,b}$	0.019 ± 0.001	0.041 ± 0.012^{a}	0.017 ± 0.002	0.059 ± 0.012	n.d.	0.023 ± 0.002	0.021 ± 0.001	0.032 ± 0.002	0.033 ± 0.002	0.445 ± $0.004^{a,b,c}$	0.030 ± 0.001
C:N (molar)	21.0	32.0	10.9	14.0	14.1	n.d.	27.5	26.3	17.3	16.7	12.2	23.8
TP (μmol g^{-1}DW)	3.0 ± 0.3^{a}	2.2 ± 0.5	2.9 ± 0.6	2.4 ± 0.8	n.d.	n.d.	3.0 ± 0.5	1.9 ± 0.5	2.8 ± 0.2	n.d.	17.1 ± $1.1^{a,c}$	2.8 ± 0.4
Carbonates (%DW)	96	89	98	98	96	97	93	92	94	96	88	97
Porewater pools												
Sulfate (mM)	28.7	28.3	29.0	28.9	28.7	28.8	29.1	28.1	26.4	28.0	28.4	27.9
Sulfides (μM)	2.2	0.1	n.d.	n.d.	3.4	5.0	29.5	0.1	11.1	14.4	5.3	21.0

1. ^{a} Significant difference ($p < 0.01$) between vegetated and bare sediment
2. ^{b} Significant higher organic matter and TP content ($p < 0.01$) compared to other vegetated sites, except for Sa Paret
3. ^{c} Significant higher organic matter and TP content ($p < 0.01$) compared to other vegetated sites

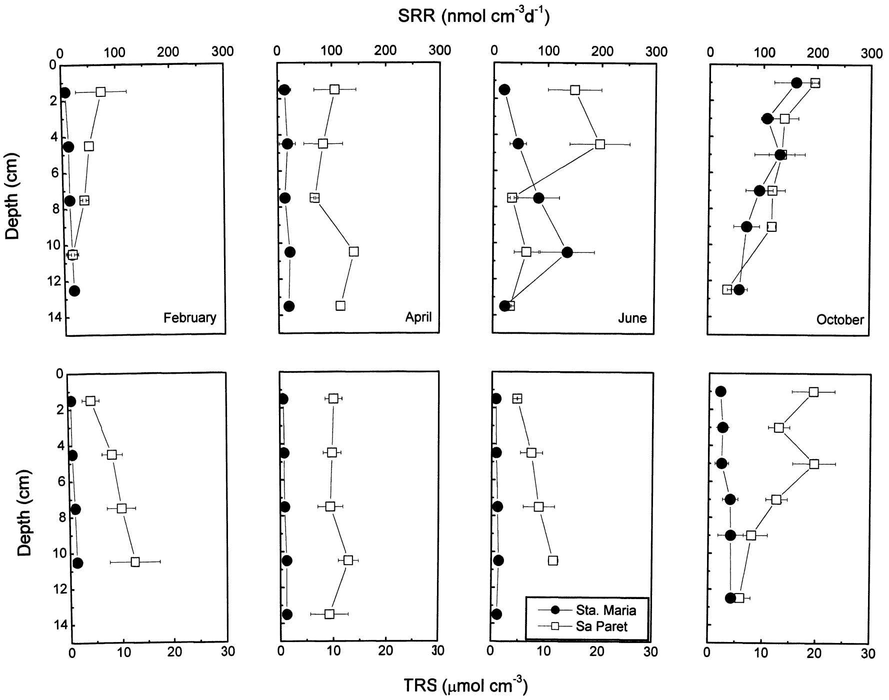

Figure 2. Seasonal variation in sulfate reduction (upper panel, SRR) and total pools of reduced sulfides (lower panel, TRS) in the *Posidonia oceanica* sediments at Sa Paret and Sta. Maria. Values are means (± SEM, n = 3).

Sulfate reduction rates were relatively high in vegetated compared to bare sediments, although this difference was only significant at Magalluf and Es Castell ($p = 0.01$, Figure 5A). An opposite tendency was found at the shallowest site (Platja, $p = 0.009$, Figure 5A). The variation in depth integrated SRR in the vegetated sediments between sites was high, and the rates at Sa Paret were 18 times higher compared to those at Magalluf, where the lowest rates were found. Also the TRS pools were generally higher in the vegetated sediments (significant at 3 sites, Figure 5B), and differed 20 fold between the site with the lowest (Magalluf) and that with the highest pool (Porto Colom). The oxygen penetration depth was 3 mm in Sa Paret and 4.5 mm in Sta. Maria sediments (Figure 6).

The shoot density of *Posidonia oceanica* decreased significantly along the depth gradient from 1019 shoots m^{-2} at the shallow site to 162 shoots m^{-2} at the deepest site (Table 2, linear regression $R^2 = 0.93$, $p = 0.03$). The shoot recruitment rate in the *P. oceanica* meadows showed a 3-fold variation, and was highest at the Porto Colom site (Table 2). In contrast, the recruitment rate was smaller at the other locations, and no recruitment was observed at Sa Paret (Table 2). There was less variability in the mortality rates, with the highest rate found in Porto Colom and the lowest in Sta. Maria. Three of the populations were expanding (positive net recruitment, Sta. Maria, Magalluf and Porto Colom), whereas the other three mead-

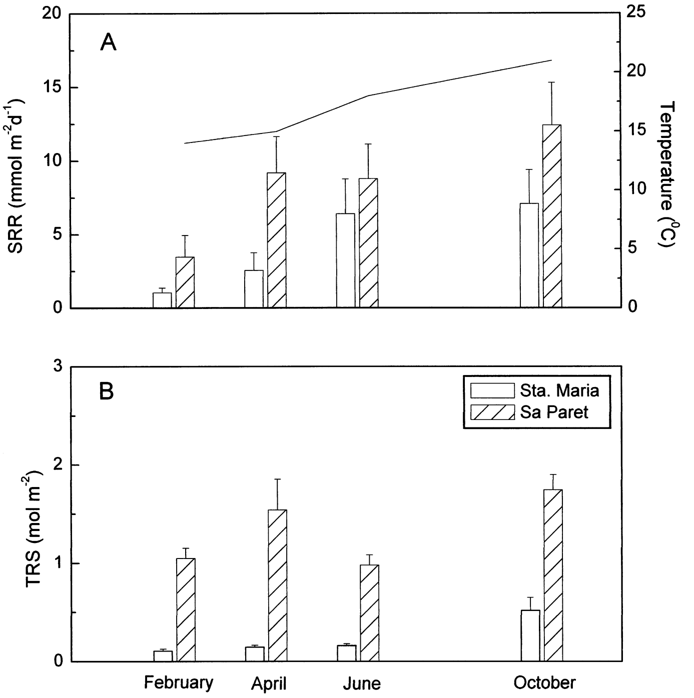

Figure 3. Depth integrated (0–15 cm) rates of sulfate reduction (A, SRR) and total pools of reduced sulfides (B, TRS) at Sa Paret and Sta. Maria. The seasonal variation in water temperature is shown in A. Values are means (± SEM, n = 3).

ows were in decline. The nutrient concentrations in the seagrass leaves were quite similar among the study sites (Table 2). Largest variation was found for the nitrogen content, which was relatively low at Sta. Maria and Sa Paret (1.17–1.28% DW) compared 1.70–1.73% DW at Platja and Es Castell. There were negative trends between shoot density and sulfate reduction rates (Figure 7A) and total pools of reduced sulfides (Figure 7B), but the trends were not significant (linear regression, $p > 0.05$). There was a significant negative relationship between the net shoot recruitment rate and the enhancement of reduced sulfur pools in vegetated sediments (Figure 8, linear regression $R^2 = 0.64$, $p = 0.050$). The expansion of the seagrass meadows thus ceased as the burial of sulfides increased.

Discussion

The carbonate sediments studied had very low organic matter and phosphorus contents, except at Sa Paret and Platja, where the vegetated sediments were enriched compared to the adjacent bare sediments. Others have found enhanced sestonic particle trapping and retention under the canopies (Gacia et al. 1999; Gacia and Duarte 2001; Gacia et al. 2002), which may be the case at the examined sites as

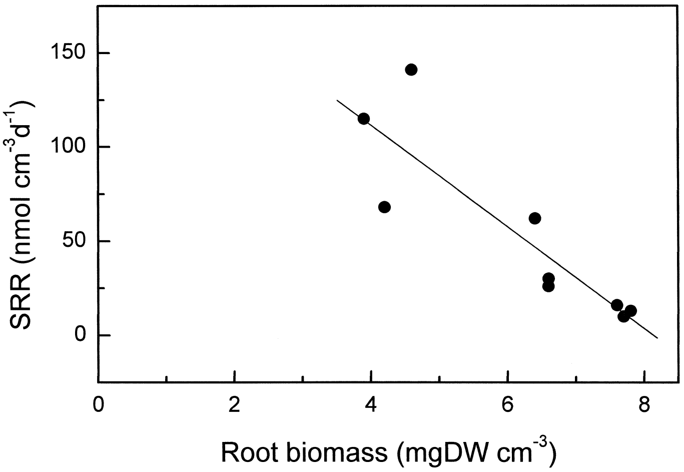

Figure 4. Relationship between depth distribution of sulfate reduction rates and root biomass at Sa Paret and Sta. Maria in April 2001. Linear regression $R^2 = 0.77$, $p = 0.002$.

well, since the C:N ratios of the surface sediments were much lower than those in the seagrass tissues. In particular, the relatively high organic matter in Sa Paret may be associated with high inputs of organic matter from sewage derived from a large number of visiting yachts in the Bay in summer (> 400 persons day^{-1}, Marbá et al. in press).

Sulfate reduction rates were comparable to those estimated for carbonate *Thalassia testudinum* sediments (5.5 mmol m^{-2}d^{-1}, Ku et al. (1999)) and tropical terrigenous seagrass sediments (Holmer et al. 2001), but lower than those found under temperate and eutrophic conditions in *Zostera marina* (Holmer and Nielsen 1997; Blaabjerg et al. 1998) and *Z. noltii* (Isaksen and Finster 1996) sediments. Sulfate reduction rates are primarily believed to be controlled by the supply of organic substrate to seagrass beds rather than sulfate, due to high sulfate concentrations in coastal sediments (Isaksen and Finster 1996; Boschker et al. 2000; Holmer et al. 2001). Sulfate concentrations were also high in all the examined sediments. The organic enrichment at Sa Paret resulted in increased rates of sulfate reduction in the surface layers, indicating that the organic matter was supplied from the water column rather than from the seagrasses. We found a negative relationship between the below-ground root biomass and the sulfate reduction rates suggesting that the rates were depressed in the presence of roots. Oxygen release from roots has not been documented in *Posidonia* sp., but has been found for other seagrasses (Pedersen et al. 1998; Connell et al. 1999), and is expected to occur from *P. oceanica* due to the large areal spaces observed inside the roots. This relationship was supported by the negative trend between shoot density and sulfate reduction rates observed at the study sites (Figure 7A) and between shoot density and total pools of reduced sulfides (Figure 7B). The negative trend with shoot density is opposite to findings in *Zostera marina* sediments, and suggests that the interactions between seagrasses and sediment biogeochemistry vary among species. *P. oceanica* is a large, slow-

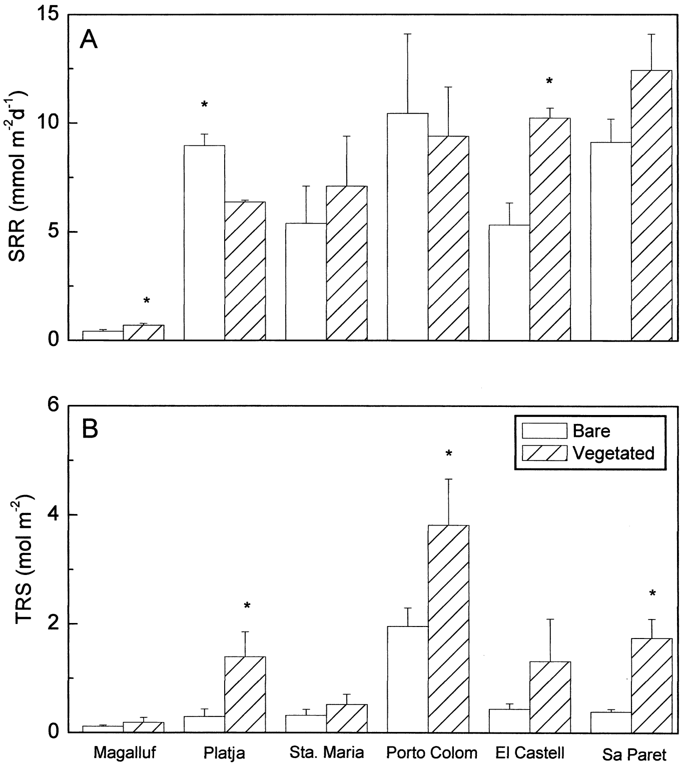

Figure 5. Depth integrated (0–15 cm) rates of sulfate reduction (A, SRR) and total pools of reduced sulfides (B, TRS) in bare and *Posidonia oceanica* sediments at the 6 study sites. Sites in Cabrera Islands were sampled in October 2000 at a water temperature of 21 °C and in Mallorca Island in June 2001 at a water temperature of 18 °C. Values are means (± SEM, n = 3). * significant difference ($p < 0.05$) between bare and vegetated sediment.

growing species with a large below-ground biomass and is primarily found in low organic sediments, and it is likely that *P. oceanica* is not able to withstand a reducing environment as *Z. marina* does. Release of dissolved organic matter has been found from some fast growing seagrasses as *Z. marina* and *T. hemprichii*, and is considered to stimulate the sulfate reduction activity in the rhizosphere sediments (Holmer et al. 2001). The slow growth of *P. oceanica* and the rigid roots suggest that an active excretion of organic components to rhizosphere sediments may be less plausible, but this has to be confirmed by further studies.

Seasonal changes in sulfur cycling were examined for comparison at the most organic-enriched site, Sa Paret, and at the site with lowest organic content, Sta. Maria, and there was a large seasonal variation in the sulfate reduction rates at both sites. The seasonal changes reflected the variation in water temperature, but also the rates of sedimentation inside the seagrass beds are expected to increase with temperature due to enhanced pelagic primary production during summer (Gacia and Duarte 2001). The sulfate reduction rates were generally higher at the organic rich

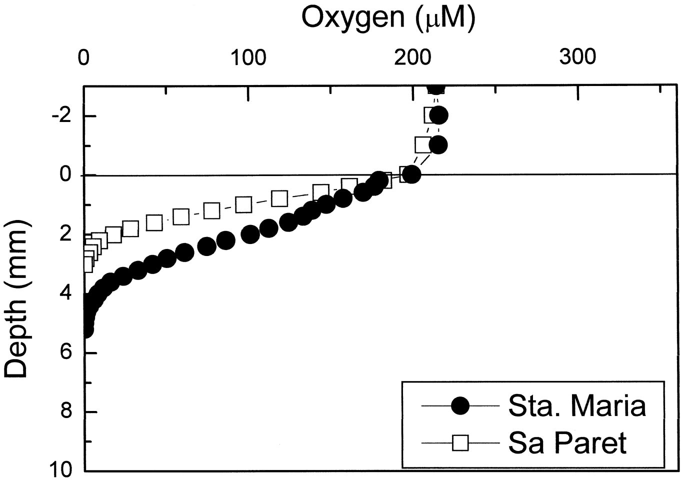

Figure 6. Oxygen profiles in *Posidonia oceanica* sediments at Sa Paret and Sta. Maria in June 2001. Values are means (n = 3).

Table 2. Seagrass characteristics in 6 *Posidonia oceanica* meadows sampled in autumn 2000 and June 2001. The shoot density is given as mean (± SEM, n = 10) and the mortality rate is given as mean (± SEM, n = 12–23), n.d., not determined

	Platja	Magalluf	Porto Colom	Es Castell	Sta. Maria	Sa Paret
Shoot density (shoot m^{-2})	1019 ± 60	607 ± 46	323 ± 54	342 ± 31	288 ± 51	162 ± 15
Recruitment rate (yr^{-1})	0.057	0.117	0.284	0.155	0.119	< 0.001
Mortality rate (yr^{-1})	0.331 ± 0.034	0.095 ± 0.031	0.160 ± 0.035	0.122 ± 0.022	0.043 ± 0.011	0.054 ± 0.015
Tissue C(%DW)	41.39	n.d	n.d.	41.40	40.95	39.99
Tissue N(%DW)	1.73	n.d	n.d.	1.70	1.28	1.17
Tissue P(%DW)	0.12	0.18	n.d.	0.16	0.21	0.11

site with the low shoot density and the temperature response was not as high as found at the low organic site. At Sa Paret the intensity of visitors increases during the spring and summer, which most likely enhances the sedimentation of fresh organic matter. This may stimulate the microbial activity in the sediments in excess of the temperature response.

Despite the production of sulfides in the vegetated sediments, the pools of reduced sulfides were low both in the pore waters and in the particulate pools compared to coastal sediments elsewhere (Moeslund et al. 1994; Holmer and Nielsen 1997; Lee and Dunton 2000). The pore water concentrations were also much lower than those measured in die-back events of *Thalassia testudinum* (Carlson et al. 1994). The particulate pools of reduced sulfides were similar to those reported in carbonate sediments vegetated with *T. testudinum* (Chambers et al. 2001), where

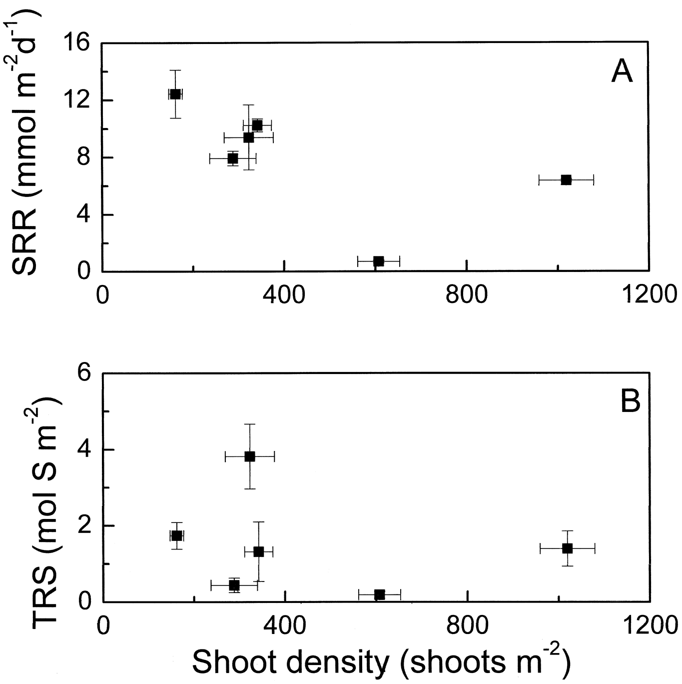

Figure 7. Depth integrated rates of sulfate reduction (A, SRR) and total pools of reduced sulfides (B, TRS) presented as function of the shoot density at the 6 study sites. Values are means (± SEM, n = 3).

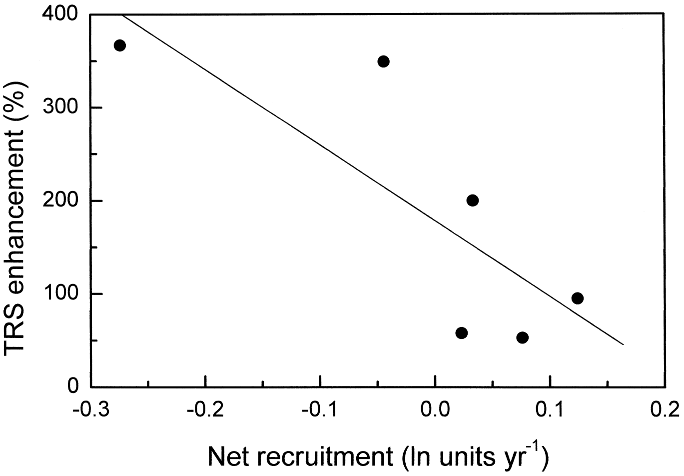

Figure 8. Relationship between net recruitment and the percentage enhancement of total pools of reduced sulfides in vegetated compared to bare sediments at the 6 study sites. Linear regression R^2 = 0.64, p = 0.050.

low concentrations have been considered to derive from internal oxidation by plant roots (Lee and Dunton 2000) and low iron concentrations. The iron pools were low in the examined sediments, comparable to levels found in carbonate sediments elsewhere (Jensen et al. 1998; Chambers et al. 2001), and possibly low enough as to render the plants iron limited (Duarte et al. 1995) and limit the precipitation of iron-sulfides, such as pyrite (Berner 1984). A link between iron supply and improved seagrass growth in carbonate sediments has already been demonstrated (Duarte et al. 1995). However, Duarte et al. (1995) attributed the response to iron limitation of seagrass growth, while Chambers et al. (2001) attributed the enhanced growth of seagrasses on carbonate sediments amended with oxidized iron to a depression of sulfide concentrations. Yet, no clear relationship between seagrass status and sulfide concentrations has emerged from that (Chambers et al. 2001) and other (e.g., Terrados et al. (1999) and references therein) studies, suggesting this link to be complex.

In this study the net shoot recruitment was negative in the two organic enriched seagrass meadows. The organic enrichment was associated with an increase in the pools of reduced sulfides compared to the adjacent bare sediments, and the vegetated sediments were thus more reduced. The negative correlation between the net shoot recruitment and the enhancement of sulfur pools at all the examined sites (Figure 8) suggests that there may be a more general connection between sulfur cycling and the expansion of *Posidonia oceanica* seagrass meadows. Stimulation of the production of reduced sulfides by even moderate increase in organic inputs may exceed the limited capacity for pyrite formation in carbonate sediments, thereby leading to the development of persistent anoxic conditions in the sediments and seagrass decline in iron-depleted carbonate sediments. Anoxic conditions in the examined sediments were confirmed by low oxygen penetration depths (< 4.5 mm). Seagrass meadows on iron-depleted carbonate sediments may, therefore, be much more sensitive to organic inputs than those on terrigenous, iron-rich sediments (Hemminga and Duarte 2000).

This study is the first attempt to examine the sulfur cycling in *Posidonia oceanica* carbonate-rich Mediterranean sediments, and shows that sulfur cycling is important despite the low organic matter contents in these sediments. The study shows clear evidence of negative effects of organic enrichment on the long-term performance of *P. oceanica* in the iron-depleted carbonate sediments in the Mediterranean. However, this and previous studies concur to show the link between seagrass status and sediment biogeochemical processes to be complex (e.g., Chambers et al. 2001, Holmer et al. 2001). There is, therefore, a need to experimentally test the feedback relationships between sulfate reduction rates, iron supply and seagrass performance.

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References

Ballesteros E. 2000. Flora marina. In: Esfagnos E.L. (ed.), Parque Nacional del Archipiélago de Cabrera. Gráficas Varona, Talavera de la Reina, Spain, pp. 175–188.

Berner R.A. 1984. Sedimentary pyrite formation: An update. Geochim. Cosmochim. Acta. 48: 605–615.

Béthoux J.P. and Chopin-Montégut G. 1986. Biological fixation of atmospheric nitrogen in the Mediterranean sea. Limnol. Oceanogr. 31: 1353–1358.

Blaabjerg V., Mouritsen K.N. and Finster K. 1998. Diel cycles of sulphate reduction rates in sediments of a *Zostera marina* bed (Denmark). Aquat. Microb. Ecol. 15: 97–102.

Boschker H.T.S., Wielemaker A., Schaub B. and Holmer M. 2000. Limited coupling of macrophyte production and bacterial carbon cycling in the sediments of *Zostera* meadows. Marine Ecology Progress Series 203: 181–189.

Buia M.C. and Mazzella L. 1991. Reproductive phenology of the Mediterranean seagrasses *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa* (Ucria) Aschers., and *Zostera noltii* Hornem. Aquat. Bot. 40: 343–362.

Canals M. and Ballesteros E. 1997. Production of carbonate particles by phytobenthic communities on the Mallorca-Menorca shelf, northwestern Mediterranean Sea. Deep-Sea Res. Part II-Top. Stud. Oceanogr. 44: 611–629.

Carlson P.R., Yarbro L. and Barber T. 1994. Relationships of sediment sulfide to mortality of *Thalassia testudinum* in Florida Bay. Bull. Mar. Sci. 54: 733–746.

Chambers R.M., Fourqurean J.W., Macko S.A. and Hoppenot R. 2001. Biogeochemical effects of iron availability on primary producers in a shallow marine carbonate environment. Limnol. Oceanogr. 46: 1278–1286.

Cline J.D. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14: 454–458.

Connell E.L., Colmer T.D. and Walker D.I. 1999. Radial oxygen loss from intact roots of *Halophila ovalis* as a function of distance behind the root tip and shoot illumination. Aquat. Bot. 63: 21–228.

Duarte C.M. and Chiscano C.L. 1999. Seagrass biomass and production: A reassessment. Aquat. Bot. 65: 159–174.

Duarte C.M., Marbà N., Agawin N., Cebrián J., Enríquez S., Fortes M.D. et al. 1994. Reconstruction of seagrass dynamics: age determinations and associated tools for the seagrass ecologist. Mar. Ecol. Prog. Ser. 107: 195–209.

Duarte C.M., Merino M. and Gallegos M. 1995. Evidence of iron deficiency in seagrasses growing above carbonate sediments. Limnol. Oceanogr. 40: 1153–1158.

Duarte C.M. and Sand-Jensen K. 1990. Seagrass colonization: biomass development and shoot demography in *Cymodocea nodosa* patches. Mar. Ecol. Prog. Ser. 67: 97–103.

Erskine J.M. and Koch M.S. 2000. Sulfide effects on *Thalassia testudinum* carbon balance and adenylate energy charge. Aq. Bot. 67: 275–285.

Fossing H. and Jørgensen B.B. 1989. Measurement of bacterial sulfate reduction in sediments: Evaulation of a single-step chromium reduction method. Biogeochem. 8: 205–222.

Fürtig K., Rüegsegger A., Brunold C. and Brändle R. 1996. Sulphide utilization and injuries in hypoxic roots and rhizomes of common reed (*Phragmites australis*). Folia Geobot Phytotax 31: 143–151.

Gacia E., Duarte C.M. and Granata T. 1999. An approach to the measurement of particle flux and sediment retention within seagrass (*Posidonia oceanica*) meadows. Aquat. Bot. 65: 255–268.

Gacia E. and Duarte C.M. 2001. Elucidating sediment retention by seagrasses: sediment deposition and resuspension in a Mediterranean (*Posidonia oceanica*) meadow. Estuar. Coast. Shelf Sci. 52: 505–514.

Gacia E., Duarte C.M. and Middelburg J.J. 2002. Carbon and nutrient deposition in a Mediterranean seagrass (*Posidonia oceanica*) meadow. Limnol. Ocenogr. 47: 23–32.

Fourqurean J.W., Duarte C.M. and Marbà N. Elucidating seagrass population dynamics: Theory, constraints and practice. Limnol. Oceanogr., (accepted).

Hemminga M. and Duarte C.M. 2000. Seagrass Ecology. Cambridge University Press, Cambridge, 298 pp.

Holmer M. and Bondgaard E.J. 2001. Photosynthetic and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. Aquat. Bot. 70: 29–38.

Holmer M. and Nielsen S.L. 1997. Sediment sulfur dynamics related to biomass-density patterns in *Zostera marina* (eelgrass) beds. Marine Ecology Progress Series 146: 163–171.

Holmer M., Andersen F.Ø., Nielsen S.L. and Boschker H.T.S. 2001. The importance of sulfate reduction based mineralization for nutrient regeneration in tropical seagrass sediments. Aquat. Bot. 71: 1–17.

Isaksen M.F. and Finster K. 1996. Sulphate reduction in the root zone of the seagrass *Zostera noltii* on the intertidal flats of a coastal lagoon (Arachon, France). Mar. Ecol. Prog. Ser. 137: 187–194.

Jensen H.S., McGlathery K.J., Marino R. and Howarth R.W. 1998. Forms and availability of sediment phosphorus in carbonate sand of Bermuda seagrass beds. Limnol. Oceanogr. 43: 799–810.

Jørgensen B.B. 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. Geomicrob. J. 1: 11–27.

Kristensen E. and Andersen F.Ø. 1987. Determination of organic carbon in marine sediments: a comparison of two CHN-analyzer methods. J. Exp. Mar. Biol. Ecol. 109: 15–23.

Kristensen E., Andersen F.Ø., Holmboe N. and Holmer M. 2000. Carbon and nitrogen mineralization in sediments of the Bangrong mangrove area, Phuket, Thailand. Aquat. Microb. Ecol. 22: 199–213.

Ku T.C.W., Walter L.M., Coleman M.L., Blake R.E. and Martin A.M. 1999. Coupling between sulfur recycling and syndepositional carbonate dissolution: Evidence from oxygen and sulfur isotope compositions of pore water sulfate, South Florida platform, USA. Geochim. Cosmochim. Acta. 63: 2529–2546.

Lee K.-S. and Dunton K.H. 2000. Diurnal changes in pore water sulfide concentrations in the seagrass *Thalassia testudinum* beds: the effects of seagrasses on sulfide dynamics. J. Exp. Mar. Biol. Ecol. 255: 201–214.

Lovley D.R. and Phillips E.J.P. 1987. Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Appl. Environ. Microbiol. 53: 2636–2641.

Marbá N., Duarte C.M., Cebrián J., Enríquez S., Gallegos M.E., Olesen B. et al. 1996. Growth and population dynamics of *Posidonia oceanica* on the Spanish Mediterranean coast: elucidating seagrass decline. Mar. Ecol. Prog. Ser. 137: 203–213.

Marbà N., Duarte C.M., Holmer M., Martínez R., Basterretxea G., Orfila A. et al. 2002. Effectiveness of protection on seagrass (*Posidonia oceanica*) populations in Cabrera National Park (Spain). Environmental Conservation 29: 509–518.

Moeslund L., Thamdrup B. and Jørgensen B.B. 1994. Sulfur and iron cycling in a coastal sediment: Radiotracer studies and seasonal dynamics. Biogeochem. 27: 129–152.

Pedersen O., Borum J., Duarte C.M. and Fortes M.D. 1998. Oxygen dynamics in the rhizosphere of *Cymodocea rotundata*. Mar. Ecol. Prog. Ser. 169: 283–288.

Peterson J.B. and Fourqurean J.W. 2001. Large-scale patterns in seagrass (*Thalassia testudinum*) demographics in south Florida. Limnol. Oceanogr. 46: 1077–1099.

Pergent G., Boudouresque C.F., Lrouzet A. and Meinesz A. 1989. Cyclic changes along *Posidonia oceanica* rhizomes (lepidochronology)- present state and perspectives Marine Ecology-publicazion della stazione. Zoologica di Napoli 10: 221–230.

Skyring G.W. 1987. Sulfate reduction in coastal ecosystems. Geomicrobial. J. 5: 295–374.

Smith S.V. and Atkinson M.J. 1984. Phosphorus limitation of net production in a confined aquatic ecosystem. Nature 307: 626–627.

Stookey L.L. 1970. Ferrozine – a new spectrophotometric reagent for iron. Anal. Chem. 42: 779–781.

Sørensen J. 1982. Reduction of ferric iron in anaerobic, marine sediment and interaction with reduction of nitrate and sulfate. Appl. Env. Microbiol. 43: 319–324.

Terrados J., Duarte C.M., Kamp-Nielsen L., Borum J., Agawin N.S.R., Fortes M.D. et al. 1999. Are seagrass growth and survival affected by reducing conditions in the sediment? Aquat. Bot. 65: 175–197.

Thode-Andersen S. and Jørgensen B.B. 1989. Sulfate reduction and the formation of ^{35}S-labeled FeS, FeS_2 and S^0 in coastal marine sediments. Limnol. Oceanogr. 34: 793–806.