

Biogeochemical implications for phosphorus cycling in sandy and muddy rhizosphere sediments of *Zostera marina* meadows (Denmark)

Marianne Holmer*, Cristiana Carta, Frede Ø. Andersen

Institute of Biology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark

ABSTRACT: To date, few studies have investigated the implications of sediment biogeochemical conditions on differences in sediment phosphorus (P) pools and P availability in sandy (low-P) and muddy (high-P) siliclastic seagrass sediments. To determine the role of seagrass activity on seasonal changes in sedimentary P, we investigated the solid-phase P pools in seagrass *Zostera marina* sediments in a Danish fjord. During the *Z. marina* growth season sediments were fractionated by sequential extractions into 3 (and once into 5) chemically defined groups: loosely adsorbed inorganic P, inorganic P bound to oxidized metals (primarily Fe-bound), P adsorbed to clay minerals, Al and humic acids, Ca-bound P, and refractory organic P. Fe-bound P accounted for about 30% of total P in the muddy sediments, whereas this pool was small in the sandy sediment (<5%). Here the Ca-bound pool was the most important (>80%). The Fe-bound P at the muddy site showed seasonal variations with lower pools during late summer, and it was always lower in the vegetated sediment, except during maximum biomass in July, where Fe-bound P was higher in the deep rhizosphere sediments. The seasonal variation was less at the sandy site, and there was little difference between unvegetated and vegetated sites, suggesting that the vegetation had a limited effect on the sedimentary P pools. However, the presence of vegetation increased the availability of P in the water column at both types of site, as P was released across the sediment–water interface at the vegetated sites compared with an uptake during most of the sampling period at the unvegetated sites, although the fluxes were low and could only account for a minor fraction (<10%) of the seasonal changes in the sedimentary P pools. Estimates of P incorporation in seagrass biomass showed that the seasonal variation in sedimentary P pools were more than sufficient to support the P demand. We hypothesize that the higher P contents in seagrass tissues and the larger biomass at the muddy site are due to high P availability from the redox-sensitive Fe-bound and organic P pools at this site, whereas P may be a limiting factor for growth at the sandy site, where the mobility of P is lower due to binding in the Ca fraction.

KEY WORDS: Phosphorus · *Zostera marina* · Sediment · Nutrient content · Seasonal variation

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INTRODUCTION

Sediments of aquatic ecosystems play a key role in phosphorus (P) cycling between dissolved- and solid-phase pools, and control long-term P diagenesis. The major biogeochemical processes that transform inorganic P from a dissolved to a solid state include anion adsorption, precipitation/co-precipitation with elements such as calcium, iron and aluminum, and uptake

by microbes and plants. In tropical and subtropical areas dominated by carbonate sediments, formation of calcium-phosphate complexes may effectively make P non-accessible for seagrasses, whereas P is considered to be more mobile and available in siliclastic sediments due to binding to the redox-sensitive Fe–P complexes (Jensen et al. 1995, 1998). The redox state and chemical composition of the sedimentary iron pools in siliclastic sediments can significantly influence the avail-

*Email: holmer@biology.sdu.dk

ability of phosphate in the porewater and the release of phosphate across the sediment–water interface (Jensen et al. 1995, Azzoni et al. 2001). When sediments are enriched with organic matter, e.g. due to eutrophication, this may stimulate bacterial metabolism and in particular sulphate reduction, which can be the dominant carbon-oxidation pathway in coastal marine sediments (Canfield 1993). In sediments vegetated with rooted macrophytes, the availability of porewater phosphate for plants is indirectly linked to rates of sulphide production and reoxidation, as the reduction of ferric iron or the binding of iron into iron sulphides causes a release of the adsorbed phosphate (Roden & Edmonds 1997). This mechanism can provide a feedback between sediments and the water column, with increased phosphate fluxes stimulating primary productivity in the water column, which in turn fuels microbial activity in the sediments, causing further decreases in the sediment redox status and hence greater phosphate availability. In addition, sulphide produced during sulphate reduction is toxic (Azzoni et al. 2001) and may negatively effect growth of seagrasses (Holmer & Laursen 2002). P availability may, however, not always be influenced by the redox state in siliclastic sediments. Because sandy sediments are typically dominated by carbonates and silicates and have low iron contents compared with clay-dominated sediments, redox reactions may be of little importance in controlling sediment P transformations. To date, few studies have investigated sedimentary P pools or identified the chemical processes important in the P cycle in temperate seagrass meadows growing on different substrates.

Seagrasses form highly productive communities on a variety of substrates. Thus, eelgrass *Zostera marina* is abundant in temperate regions, e.g. from the Baltic Sea to northern Spain, and has a wide distribution from coarse-grained sandy sediments near the intertidal limit to fine-grained muddy sediments at deep locations. Most seagrasses are capable of absorbing nutrients from both sediment and water-column sources, but it has been assumed that nutrient uptake by roots dominate over leaf uptake in estuaries where porewater nutrient concentrations are high relative to surface waters (Brix & Lyngby 1985, Hemminga 1998, Sfriso & Marcomini 1999, Touchette & Burkholder 2000). However, for plants growing in P-rich waters or sandy P-poor sediments, P uptake by leaves can be higher than by roots (McRoy & Barsdate 1970, Thursby & Harlin 1984, Gras et al. 2003).

While nitrogen is not considered a limiting factor for seagrass growth in the N-rich coastal waters of Denmark (Pedersen & Borum 1992, Nielsen et al. 2002), knowledge about P limitation in this area is rather limited, but it has been suggested that growth of *Zostera*

marina on coarse-grained low-P substrates may be P-limited in the same way as tropical and subtropical seagrasses growing on carbonate sediments (Alcovarro et al. 1997, Jensen et al. 1998, Gras et al. 2003) because of the strong binding of P in calcium fluorapatite (Jensen et al. 1998). There is no detailed information available on the seasonal changes in P forms, fluxes between sediment and water, and P availability in *Z. marina* meadows, but growth and biomass of *Z. marina* has shown positive responses to fertilization with P, indicating P limitation (Murray et al. 1992).

The purpose of the present study was to examine the forms and availability of sedimentary P in 2 eelgrass meadows with contrasting substrates (sandy vs. muddy sediment) during the maximum growth period (June to September). Biogeochemical parameters such as sediment organic content, redox profiles and pools of sulphides (free sulphides, iron monosulphides and pyrite) were also measured to assess their effects on P dynamics and in particular on the redox-sensitive pools of the sedimentary P. A sequential extraction of the sedimentary P pools was undertaken during the growth season with focus on the most mobile pools during 3 samplings (loosely adsorbed P and Fe-bound P) and a full sequential extraction was made at the end of the season (also including adsorbed P, Ca-bound P and organically bound P) in unvegetated and vegetated sediments. The sediment metabolic activity and flux of P across the sediment–water interface were studied in sediment incubations. The sedimentary P dynamics were related to the seasonal changes in biomass and nutrient content of the seagrasses.

MATERIALS AND METHODS

Study area and sampling procedures. Sediments and plants were collected during the maximum growth season between June and September in 2004 at a coastal site on the southwest coast of Fyn in Lillebælt, Denmark (55° 28.958' N, 9° 43.956' E). The stations were at shallow depths (~1 m) with eelgrass *Zostera marina* present in patches, and the sediments were either sandy or muddy, the sandy site being wind exposed. The water quality was assumed to be similar at the 2 sites, as the stations were located within 500 m of each other in a strait where tides reverse every 6 h. Sediment samples (3 replicates) from each station were collected manually using acrylic cores (inner diameter 8 cm, length 32 cm) in eelgrass meadows and in adjacent bare sediments. The cores were brought to the laboratory and stored at 15°C in a tank with aerated water from the sampling site. The water level in the tank was at least 2 cm above the upper edge of the

cores. Each core was fitted with a stirring magnet (60 rpm), and the tank water was aerated using several air-pumps. The cores were kept in darkness.

Plant measurements. Eelgrass density and above-ground biomass were determined within 3 randomly positioned rings (0.08 m^2) at each station. The shoots were harvested inside the rings and transported back to the laboratory, where the shoot density was quantified. The below-ground biomass was determined only in September by analysing 3 cores from the eelgrass meadows. The cores were sectioned in the intervals 0–1, 1–5, 5–10 and 10–15 cm and sieved. The plant material was then separated into root and rhizome. Dry weight (DW) of plant material was determined by drying to constant weight at 60°C . Before nutrient analysis, the eelgrass tissues were ground to a powder, and organic carbon (POC) and nitrogen (TN) were determined by elemental analysis on a Carbo Erba Elemental Analyser 1100EA. For total phosphorus (TP), approximately 0.06 to 0.08 g (triplicates for each sample) was ignited at 520°C for 2 h and subsequently boiled at 120°C for 1 h in 8 ml of 1 M HCl, after which dissolved inorganic phosphate (DIP) was measured spectrophotometrically using the molybdate blue method (Koroleff 1983).

Sediment flux measurements (O_2 and DIP). Sediment oxygen uptake (SOU) was determined by short-term incubations (3 to 4 h) of the collected sediments cores 1 to 2 d after sampling. An initial sample (T_0) was taken from the water phase inside each core while still submerged, and oxygen (O_2) was determined according to the Winkler method. A gas-tight lid was placed on top of the cores, while underwater, and the height of the water phase in cores was noted. At termination of the incubation, after 3 to 4 h, the final sample (T_1) was taken from each core, and the O_2 uptake was calculated from the decline in O_2 concentration. Fluxes of DIP were obtained similarly, except that the top of the sediment cores was kept above the water level in the tank, the water column was kept aerated, and the incubation was prolonged to about 24 h. The samples were GF/C filtered and stored frozen until analysis as described above.

Redox potential. After termination of the flux incubations, redox potential (E_h) profiles were measured with a Pt electrode using a calomel electrode as reference. E_h was recorded in the water column at 2 cm above the sediment and at 0.5, 3.0, 7.5 and 12.5 cm depth in the sediment.

Sequential extraction of P and Fe pools. After redox measurements were taken, the sediment cores were sliced in the intervals 0–1, 1–5, 5–10, and 10–15 cm in a glove bag in an N_2 atmosphere to prevent oxidation of iron and sulphides. Phosphorus was extracted according to Jensen & Thamdrup (1993), with a few

modifications. Approximately 1 g of wet sediment from each slice was subsampled and transferred to a 50 ml polyethylene centrifuge tube, where replicate samples from the 3 sediment cores from bare and vegetated sites were pooled, respectively. In Step 1, 1 M MgCl_2 (pH 8) was added to the sediment to extract loosely adsorbed P and porewater P (P_{MgCl_2}). In Step 2, sodium-bicarbonate-buffered dithionite solution (BD-reagent: 0.11 M NaHCO_3 + 0.11 M $\text{Na}_2\text{S}_2\text{O}_4$) was added to the sediment pellet to extract phosphate bound to reducible forms of iron and manganese (P_{BD}). Steps 1 and 2 were carried out in an N_2 atmosphere. In Step 3, the sediment was extracted with 0.1 M NaOH (P_{NaOH}). This step is supposed to extract phosphate adsorbed to clay minerals, Al oxides and humic acids. In Step 4, the sediment was shaken with 0.5 M HCl to extract calcium-bound P (P_{HCl}). In Step 5 the remaining sediment pellet was dried and combusted at 520°C for 6 h and subsequently boiled in 8 ml of 1 M HCl at 120°C for 1 h to extract the residual P. This pool represents the most refractory part of the organic P components (P_{RES}). The extracted inorganic phosphate was measured as described above for DIP. Only Steps 1, 2 and 5 were executed in June, July and August, whereas all the steps were completed in September to get a detailed description of all the pools once at each station. To determine the amount of non-reactive P (NRP) leached in Steps 1 to 3, the extracts were subjected to wet oxidation by heating the sample with persulphate for 30 min at 105°C in an autoclave, after which TP was measured. NRP, which is supposed mainly to consist of organically bound P, was calculated as the difference between the TP and DIP. As NRP contents were low (<1% of TP), only TP values are presented for the first 3 steps.

Fe concentrations were determined in the BD, NaOH, HCl and RES extracts (Fe_{BD} , Fe_{NaOH} , Fe_{HCl} and Fe_{RES} , respectively) by spectrophotometric analysis according to Stookey (1970) and Sørensen (1982).

Reduced sulphides (AVS and CRS) pools. Immediately after sediment slicing for the P and Fe extractions, 10 ml of sediment from each slice were transferred into 50 ml pre-weighed centrifuge tubes and 10 ml of 1 M zinc acetate was added. The sediment–zinc-acetate mixture was stored frozen and subsequently distilled according to the method of Fossing & Jørgensen (1989). The distillation separates reduced sulphur compounds in the sediment into 2 pools, namely, acid volatile sulphides (AVS), composed mainly by iron monosulphide (FeS) plus free sulphides, and chromium reducible sulphides (CRS), composed primarily of pyrite (FeS_2) plus elemental sulphur. For AVS, 8 ml of 6 M HCl was added to the sediment distillation unit, and the distillation proceeded for 30 min. Zinc-acetate traps were changed, 16 ml of chromium

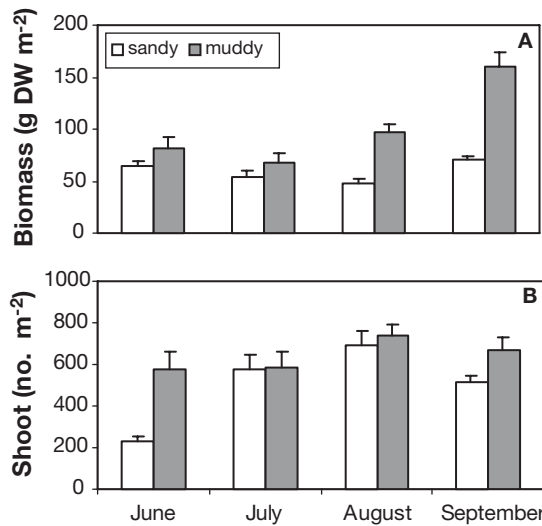


Fig. 1. *Zostera marina*. Seasonal variation in (A) above-ground biomass and (B) shoot density at the 2 sampling sites (n = 3, +SD)

solution was added and the mixture was distilled by boiling for 30 min to obtain the CRS. The sulphide concentrations were measured spectrophotometrically at 670 nm using the method of Cline (1969).

Density, water content and loss-on-ignition of the sediment. The density of the sediment was determined as the weight of a known volume. The water content was obtained by drying the sediment to constant weight at 105°C, and loss-on-ignition was obtained after combustion at 520°C for 6 h.

RESULTS

In the early summer the muddy site supported a dense eelgrass population, which remained constant in abundance but approximately doubled in biomass by September (Fig. 1). In contrast, at the sandy site the abundance increased in July and August, whereas the biomass remained almost constant throughout the sampling period (Fig. 1). The below-ground biomass was obtained in September, and for the muddy site 95% of the biomass was found in the surface layer (0 to 5 cm), whereas a smaller fraction (88%) was found in these layers in the sandy meadow (Fig. 2). The roots penetrated deeper at the muddy site and were present also in the 10 to 15 cm layer.

The TP content of the above-ground biomass showed distinct seasonal variation at the 2 sites: while the maximum TP content was found at the muddy site in July (0.404 ± 0.004% DW), the content continued to increase at the sandy site throughout (0.232 ± 0.005% DW; Fig. 3A). The TN content showed less seasonal variation and was not different between the 2 sites

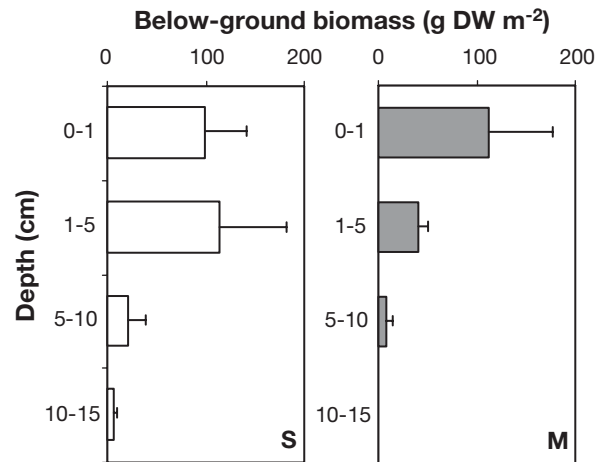


Fig. 2. *Zostera marina*. Depth profile of below-ground biomass in sandy (S) and muddy (M) sediments sampled in September (n = 3, +SD)

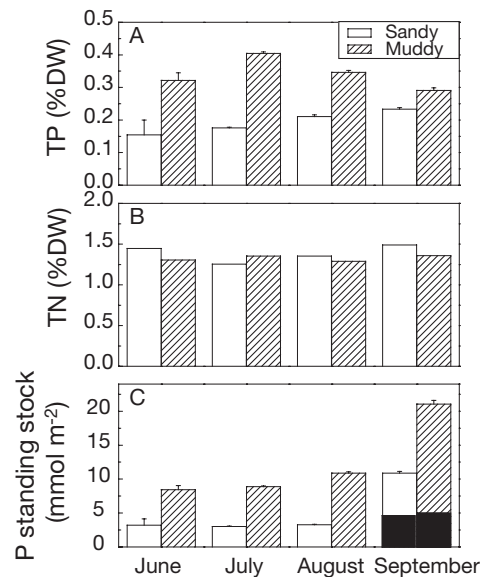


Fig. 3. *Zostera marina*. Seasonal variation in nutrient concentration and pools in biomass. (A) TP content in leaves (n = 3, +SD), (B) TN content in leaves (n = 1) and (C) standing stock of TP in leaves and (only for September) below-ground biomass (shown in black; mean, n = 3, +SD)

(Fig. 3B). The TP content of the below-ground tissues in September at the muddy site was up to 3 times higher (0.100 to 0.133% DW) than at the sandy site (0.059 to 0.075% DW), with no clear depth pattern at either site (Table 1). The TN content was also up to 3 times higher at the muddy site (0.82 to 0.94% DW) compared to the sandy site (0.30 to 0.68% DW, Table 1). The standing stock of P in above-ground biomass increased at both sites during the growth season and was almost 2 times higher in September than in June (5.3 and 15.0 mmol P m⁻² at the sandy and muddy

Table 1. Total P (TP) and total N (TN) concentrations in below-ground tissues at the sandy and muddy sites during sampling in September (average of 3 pooled cores). Molar concentrations are given in parentheses. -: no roots present

Depth interval (cm)	TP % DW ($\mu\text{mol g DW}^{-1}$)		TN % DW ($\mu\text{mol g DW}^{-1}$)	
	Sandy	Muddy	Sandy	Muddy
0–1	0.075 (24.2)	0.100 (32.3)	0.302 (215.7)	0.818 (584.3)
1–5	0.059 (19.0)	0.133 (42.9)	0.343 (245.0)	0.939 (670.7)
5–10	0.071 (22.9)	0.110 (35.5)	0.681 (486.4)	0.848 (605.7)
10–15	0.064 (20.6)	–	0.540 (385.7)	–

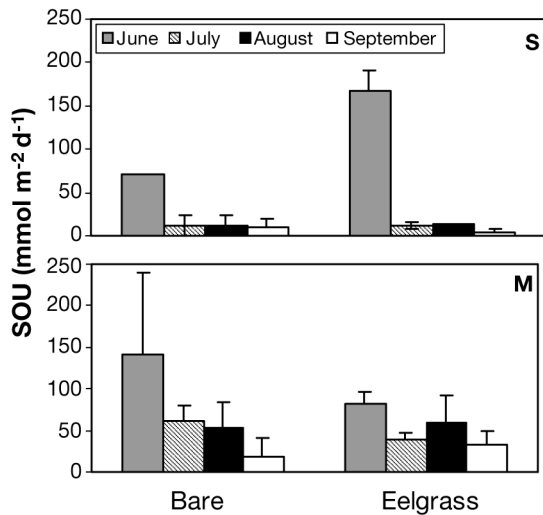


Fig. 4. Seasonal variation in sediment oxygen uptake (SOU) in vegetated (eelgrass *Zostera marina*) and bare sediment from sandy (S) and muddy (M) sites ($n = 3$, \pm SD)

sites, respectively; Fig. 3C). The below-ground pool accounted for 34 and 21% of the total pool at the sandy and muddy site, respectively.

In general, SOU was highest at the muddy site, except for a high rate in June at the vegetated sandy site. There was no significant difference (2-way ANOVA, $p > 0.05$) between the vegetated and bare muddy sites (Fig. 4). SOU was highest at the beginning of the growth season and decreased by 66% on average by September. For the sandy site, there was no significant difference between vegetated and bare sediments, except for September, where rates in the vegetated sediments were only 39% of the bare sediments (2-way ANOVA, $p = 0.002$; Fig. 4). As for the muddy site, rates decreased by up to 82% during the growth season.

DIP fluxes showed large seasonal variations; there were more distinct differences between vegetated and bare sediments, although these were not significant due to high heterogeneity (2-way ANOVA, $p > 0.05$). The fluxes were highest at the muddy site, and in the vegetated sediments changed from an uptake in June

to effluxes during the rest of the period (Fig. 5). In the bare sediments, only July showed an efflux. At the sandy site, the rates were generally lower compared with the muddy site (Fig. 5). The vegetated sediments showed an efflux during the sampling period, whereas DIP was taken up, except for a small efflux in August, in the bare sediments (Fig. 5).

Only very little sedimentary P (<11%) was obtained in the loosely adsorbed (P_{MgCl_2}) and Fe-bound pool (P_{BD}) at the sandy site (Table 2, Fig. 6). The pools were at a maximum in the surface layers and decreased with depth (Fig. 6). The depth-integrated pools (0–5 cm) show that there is a limited difference between vegetated and bare sediments (Table 2), but there was some seasonal variation: the P_{BD} fraction was at a maximum in June and September, and the P_{MgCl_2} fraction was at a maximum in June and July and at a minimum in August. The P_{RES} pool was 2 orders of magnitude higher than the P_{MgCl_2} and P_{BD} pools, and the full sequential extraction in September showed that the P_{HCl} pool was the most important fraction of P_{RES} (Fig. 6).

At the muddy site a significant fraction of the P was found in the P_{BD} pool (up to 39%; Table 2), and most of it was found in the surface layer (>50%; Fig. 6). The P_{BD} pool was generally higher in the bare sediment (Table 2). The P_{MgCl_2} pools were about 1 order of magnitude lower than the P_{BD} pool, and there was a minor

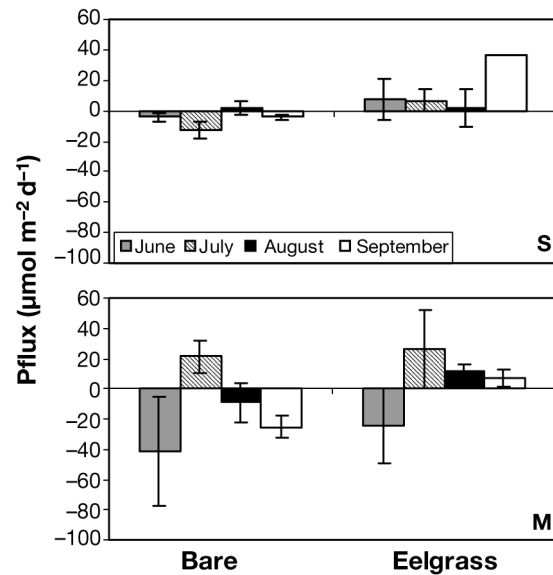


Fig. 5. P fluxes in vegetated (eelgrass) and bare sediment from muddy (M) and sandy (S) site during the sampling period. The standard deviation is not shown for the vegetated site in September due to a very high value ($\pm 81 \text{ mmol m}^{-2} \text{ d}^{-1}$; $n = 3$, \pm SD)

difference between vegetated and bare sediments (Table 2). The full sequential extraction showed that about 50% of the P in the vegetated sediments was bound in P_{RES} , whereas the P_{BD} fraction in the surface layer and the P_{HCl} fraction in the deepest layer contributed significant to the TP (50 and 59%, respectively) in the bare sediments (Fig. 6).

Table 2. Seasonal rhizosphere pools (0–5 cm sediment depth) of P and Fe from sequential extractions at the 2 study sites. P_{RES} for September were obtained by summation of P in Steps 3 to 5 of the sequential P-extraction procedure. The molar ratio between P_{BD} and Fe_{BD} is given. All pools are given in $mmol\ m^{-2}$ and represent 3 pooled cores

	P content			Fe content Fe_{BD}	$Fe_{BD}:P_{BD}$ (molar)
	P_{MgCl_2}	P_{BD}	P_{RES}		
Sandy bare					
Jun	12.0	9.1	276.2	71	7.8
Jul	10.5	5.8	298.8	51	8.8
Aug	5.4	6.8	278.1	76	11.1
Sep	7.8	28.5	269.0	84	3.0
Sandy vegetated					
Jun	10.4	12.0	289.9	62	5.1
Jul	10.4	4.3	261.8	43	10.2
Aug	5.3	10.6	319.9	84	7.9
Sep	8.8	13.0	259.0	73	5.6
Muddy bare					
Jun	8.0	248.3	622.0	6375	25.7
Jul	6.3	167.6	184.0	4369	26.1
Aug	9.7	160.0	633.9	3404	21.3
Sep	8.2	168.2	1050.4	4446	26.4
Muddy vegetated					
Jun	7.4	150.9	633.7	4977	33.0
Jul	6.0	168.0	274.5	4497	26.8
Aug	9.3	120.0	602.2	2370	19.8
Sep	9.2	81.8	755.7	2307	28.2

Only depth-integrated pools of Fe_{BD} are presented, and they show much higher values at the muddy site (2307 to 6375 $mmol\ m^{-2}$) than at the sandy site (43 to 84 $mmol\ m^{-2}$), corresponding to the low P_{BD} (Table 2). At both the sandy and muddy sites, pools were generally similar or lower in the vegetated sediments than in the bare sediments. The $Fe_{BD}:P_{BD}$ ratios were much lower at the sandy site (3 to 11) compared with the muddy site (15 to 24; Table 2). At the sandy site the ratios were at maximum in July and August, whereas the opposite pattern was found for the muddy site.

The rhizosphere pools of total reducible sulphides (TRS) were about 1 order of magnitude higher at the muddy site compared with the sandy site, and maximum pools were found in July (Table 3). The pools were generally higher at the vegetated muddy site

Table 3. Seasonal variation in rhizosphere pools (0–5 cm) of total reduced sulfide pools at the 2 study sites (TRS, $mmol\ S\ m^{-2}$), and the ratio between vegetated and bare sediments (Veg:bare) for the TRS, P_{BD} and Fe_{BD} pools in the rhizosphere sediment. Average values of 3 cores are given for TRS. –: not available

	TRS ($mmol\ S\ m^{-2}$)		TRS	Veg:Bare P_{BD}	Fe_{BD}
	Bare	Vegetated			
Sandy					
Jun	527	457	0.87	1.32	0.87
Jul	628	554	0.88	0.74	0.84
Aug	–	510	–	1.56	1.11
Sep	964	495	0.52	0.46	0.87
Muddy					
Jun	2439	4444	1.82	0.61	0.78
Jul	6865	9476	1.38	1.00	1.03
Aug	4211	3930	0.94	0.75	0.70
Sep	2331	2822	1.20	0.49	0.52

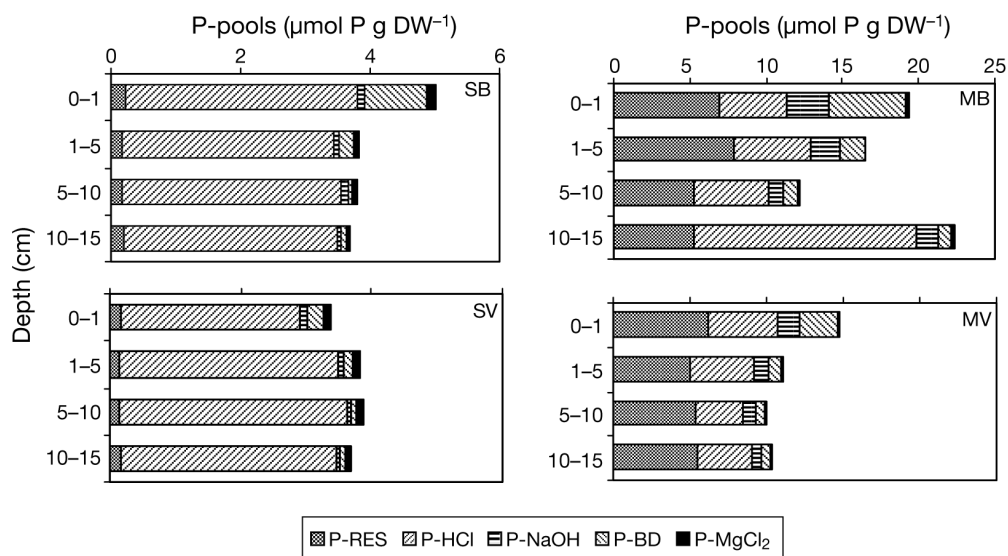


Fig. 6. P pools in September at vegetated (V) and bare (B) sediments from sandy (S) and muddy (M) sites

compared to the bare site (20 to 82 %), whereas the opposite pattern was found at the sandy site (12 to 48 % lower at the vegetated site). The AVS pools accounted for <10% of the TRS pool at both sites (data not shown).

The redox profiles generally showed that both bare and vegetated sediments were reduced in the deeper layers (>5 cm, Fig. 7). At the sandy site, the surface layers in the vegetated sediment had lower redox potential than for those in the bare sediment, whereas the opposite was found for the deeper layers (Fig. 7). In contrast, the muddy site had higher redox potentials in the surface layers, whereas the deeper layers were more negative in the vegetated sediment compared with the bare sediment. At the sandy site, the sediments were generally most reduced in August, whereas at the muddy site, the September sampling showed the lowest values.

The organic content was lowest at the sandy site (~0.5% DW), with no significant difference between

Table 4. Seasonal variation in depth-averaged sediment organic matter (OM) in bare and vegetated sediments at sandy and muddy site (%DW, n = 3, ±SD)

	Bare OM	Vegetated OM
Sandy		
Jun	0.41 ± 0.08	0.43 ± 0.05
Jul	0.44 ± 0.04	0.47 ± 0.04
Aug	0.56 ± 0.05	0.56 ± 0.09
Sep	0.52 ± 0.09	0.45 ± 0.07
Muddy		
Jun	2.37 ± 0.43	1.62 ± 1.18
Jul	1.86 ± 0.56	4.81 ± 1.86
Aug	1.69 ± 0.22	1.44 ± 0.36
Sep	1.48 ± 0.57	1.32 ± 0.28

the vegetated and bare sediments and no significant seasonal variation (Table 4). At the muddy site, a few high values in July, which may have been root detritus in the deeper layers, resulted in a high average content (4.8% DW), whereas the other samplings were in a range similar to that found for the bare site (1.5 to 2.4% DW). There was a decreasing trend during the sampling period, but it was not significant ($p > 0.05$).

DISCUSSION

General sediment biochemistry

The biogeochemical conditions of the rhizosphere sediments of the 2 eelgrass meadows differed considerably, in particular regarding the organic content, redox conditions, sulphide pools and the sedimentary forms of P. The organic content was, as expected, higher at the muddy site, but in contrast to other findings the presence of eelgrass did not increase the organic content significantly compared with the unvegetated sediments (Holmer et al. 2003b, Duarte et al. 2004). The lack of difference between the examined meadows may suggest that the meadows have been established recently (Pedersen et al. 1997, Pérez et al. 2001, Barrón et al. 2004), but both meadows are located at shallow depth and resuspension events may reposition the detritus pools and minimize the difference between bare and vegetated sediments (Frederiksen et al. 2004).

The modification of redox profiles due to presence of vegetation suggests that eelgrass is able to alter the biogeochemical conditions of the sediments. At the muddy site a positive anomaly was found in the surface layers and a negative anomaly in the deeper layers, whereas the sandy site

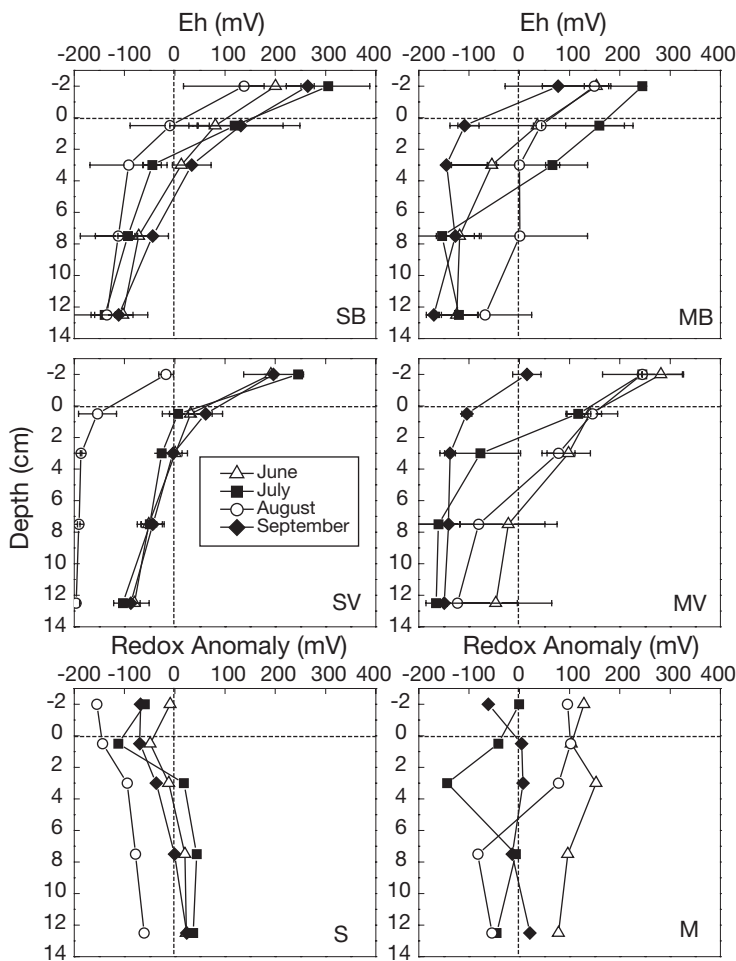


Fig. 7. Seasonal variation in depth profiles of sediment redox potential (E_h) in bare (B) and vegetated (V) sediment from sandy (S) and muddy (M) sediments (n = 3, ±SD). Redox anomaly [$E_h(V) - E_h(B)$] is shown in the lowest panel for sandy and muddy sediments

showed the opposite pattern except for September, when the redox values were very low throughout the profile. Studies of other seagrass species have shown a positive anomaly throughout the root zone during the growth season (*Zostera noltii*, Isaksen & Finster 1996; *Thalassia testudinum*, Enríquez et al. 2001; *Cymodocea nodosa*, Marbà & Duarte 2001), whereas a seasonal study of *C. nodosa* documented negative values in the surface layers at the beginning and end of the growth season (Marbà & Duarte 2001). The positive anomaly at our study sites was coincident with the maximum below-ground biomass, and oxygen release from the roots may contribute to increased oxidation of the sediments compared with unvegetated sites (Pedersen et al. 2004). The negative anomaly in the sandy surface sediments could be due to the presence of labile organic matter, e.g. from microphytobenthos or seston settling in the seagrass meadow (Gacia et al. 2002) leading to increased microbial activity and consumption of oxygen.

The total pool of iron was about 20 times higher at the muddy site than at the sandy site, and this was clearly affecting the Fe_{BD} , the values for which were up to 70 times higher at the muddy site. Despite the high accumulation of reduced sulphides at the muddy site, the iron availability was apparently sufficient to allow a higher accumulation of oxidized iron compared with the sandy site. The presence of eelgrass decreased the pools of Fe_{BD} , in particular at the muddy site, which may be a mechanism by which P availability is increased for plant uptake. The P_{BD} was, however, still between 6 to 27 times higher at the muddy site than at the sandy site, and it is thus likely that very limited iron was available for binding of P and sulphide precipitation at the sandy site, which minimizes the pools of iron sulphides as well as the P_{BD} pools and may exert an anoxic and sulphide stress on the seagrasses (Terrados et al. 1999, Pedersen et al. 2004).

P dynamics in sedimentary pools

Based on the higher organic matter content and the high iron pools at the muddy site, it is expected that the P availability for plant uptake was higher there than at the sandy site. P_{BD} dominated the solid-phase P pools at the muddy site, whereas calcium-bound P (P_{HCl}) dominated at the sandy site, accounting for about 30 and 90% of the TP pool, respectively. The pools at the muddy site are within the lower range reported for coastal siliclastic sediments (Jensen et al. 1995), whereas the pools at the sandy site are similar to low-P carbonate sediments (Jensen et al. 1998, Koch et al. 2001). The most easily available P pools for mobilization and plant uptake in siliclastic and clay-dominated

sediments are considered to be the fractions extracted in the $MgCl_2$ and BD treatments (Jensen et al. 1995, 1998, McGlathery et al. 2001). The $MgCl_2$ extraction is considered to contain P loosely adsorbed to the sediments, and although this pool depends strongly on the adsorption sites and thus was expected to differ between the fine-grained muddy and coarse-grained sandy sediments (Jensen et al. 1995), the pool was quite similar in the 2 sediment types, with no major differences between bare and vegetated sediments, suggesting that neither the sediment type nor the activity of the plants affected this pool at the examined locations. Whereas there was no effect of season on the P_{MgCl_2} pools in the 2 sediment types, the P_{BD} pool was lower at the muddy site in the late growth season, coincident with low Fe_{BD} pools. Depletion of oxidized iron pools due to high sulphate reduction activity and low reoxidation capacity during late summer has been found for unvegetated sediments (Jensen et al. 1995, Holmer et al. 2003a), and this is consistent with the low redox potentials measured in September. The P_{BD} pools were generally lower in the vegetated sediment compared to the bare sediments, in particular at the rooted depths, which may reflect an uptake by the plants (McGlathery et al. 2001). At the sandy site the P_{BD} pools were lowest during July and August, when the pools of Fe_{BD} also were lower, suggesting that P was released due to reduction of oxidized iron. The low redox potentials measured in the surface layer of the sandy sediments further support reduction of iron oxides and release of P. A similar pattern with release of P from Fe oxyhydroxides during summer has been found in a *Ruppia cirrhosa* meadow (Azzoni et al. 2001).

The large difference in P and Fe pools in the BD fraction at the sandy and muddy sites resulted in a major difference in the $Fe_{BD}:P_{BD}$ ratios, with high ratios at the muddy site (16 to 24) and low ratios at the sandy site (3 to 11). The ratios at the muddy site are similar to findings in deep parts of the Skagerrak (~700 m; Jensen & Thamdrup 1993), where iron minerals are considered to be either less capable of adsorbing DIP or less saturated with DIP compared with more shallow locations with lower $Fe_{BD}:P_{BD}$ ratios. The P pools were lower at the muddy site compared with the Skagerrak, in particular in the vegetated sediments, which may be due to plant uptake. The low ratios and the seasonal variation with lower ratios during late summer at the sandy site were similar to observations in coastal surface sediment (water depth 16 m; Jensen et al. 1995). Such low ratios have been taken to indicate the limit for iron to bind P in freshwater sediments (Jensen et al. 1992), and it is thus likely that the low availability of oxidized iron is the limiting factor for binding of P in the BD fraction at the sandy site. Despite the large difference

in P_{BD} pools between the sandy and muddy sites, the depth-integrated pools of TP were at a maximum 2 times higher at the muddy site as a result of the relatively large pool of calcium-bound P at the sandy site. This indicates that the overall burial of P was not that different in the 2 types of sediment.

Even though the differences between vegetated and bare sediments were small for SOU, suggesting small differences in organic-matter mineralisation rates, higher P fluxes in the vegetated sediments indicated higher P availability. Dissolved inorganic phosphate was released from the muddy vegetated sediments from July to September, whereas efflux only was observed from the bare sediments in July, and at the sandy site there was a P efflux from the vegetated sediments during the entire sampling period and only in August for the bare sediments. There are only few observations of P fluxes in seagrass sediments, but for *Thalassia testudinum* a release of DIP was found for an anthropogenically affected site, probably due to higher mineralisation of organic-matter inputs, whereas the pristine sites showed an uptake of DIP (Jensen et al. 1998). However, the measured release in our study was low compared with the large changes in the sedimentary pools of P and could at maximum account for 10% of the observed changes in the surface sedimentary pools between samplings.

Implications of sedimentary P pools for P dynamics in eelgrass tissues

In contrast to the quite similar nitrogen content in the leaves, the P content was between 26 and 233% higher at the muddy site than at the sandy site, which is similar to observations by Short (1987), where eelgrass accumulated higher P pools in muddy compared with sandy substrates. There were quite different patterns of seasonal variation, with continuously increasing P concentrations at the sandy site, whereas the P maximum was reached in July at the muddy site and had decreased 31% by September. The muddy site was characterized by the highest efflux of P, in particular in July, which may explain the higher P content in the leaves in this period. However, most P uptake for seagrasses is believed to occur through the roots (McRoy et al. 1972), although recent studies of *Thalassia testudinum* growing on carbonate sediments have shown a larger uptake by leaves due to the strong binding of P to the sedimentary pools (Gras et al. 2003). There were no signs of P-limited growth at the muddy site, as the P concentrations in the leaf tissues were above the critical limits for growth (Duarte 1990). In contrast, the P content was below the critical limit at the sandy site, in particular at the beginning of the growth season,

suggesting P limitation in this sediment, where a significantly part of the P is bound in the calcium fraction. An additional indication of enhanced P availability for root uptake at the muddy site was the up to 2 times higher P content in the below-ground tissues compared with the sandy site. Also, the N content of the below-ground tissues was higher, up to 3 times, suggesting that mineralisation of organic matter may contribute nutrients to the plants. However, the evaluation of nutrient limitation on seagrass growth based solely on nutrient content should be done with caution, as both P and N can be translocated in the plants for growth optimisation (Pedersen & Borum 1992, Alcoverro et al. 2000, Gras et al. 2003).

To our knowledge there are no estimates of the P requirements of *Zostera marina* for growth, whereas rates of 30 to 100 $\mu\text{mol P m}^{-2} \text{d}^{-1}$ have been estimated for *Thalassia testudinum* (Patriquin 1972, Fourquenan et al. 1992). We did not measure primary productivity, but estimates can be made by assuming an annual production of $\sim 818 \text{ g DW m}^{-2} \text{yr}^{-1}$ by *Z. marina* based on extensive studies of meadows located under similar conditions (from Olesen & Sand-Jensen 1994). The average leaf P concentration was 60 $\mu\text{mol P g DW}^{-1}$ and 110 $\mu\text{mol P g DW}^{-1}$ for sandy and muddy sediments, respectively, and the annual P requirements are thus 49 and 90 $\text{mmol P m}^{-2} \text{yr}^{-1}$, respectively. The below-ground biomass was only estimated in September, and, assuming a production of 130 $\text{g DW m}^{-2} \text{yr}^{-1}$ (*Z. noltii*, from Duarte et al. 1998), the P requirements can be estimated to be 3 and 5 $\text{mmol P m}^{-2} \text{yr}^{-1}$ at the sandy and muddy sites, respectively. The total P requirements are then 52 and 95 $\text{mmol P m}^{-2} \text{yr}^{-1}$, and, assuming that most of the uptake occurs during the growth season examined here, the release of P across the sediment–water interface can only support a fraction of this amount (average rate 5 $\mu\text{mol m}^{-2} \text{d}^{-1}$, max. 2%). The water-column concentration of DIP was low during the growth period ($< 1 \mu\text{M}$, data not shown), and the water column should be fully mixed all the time to be able to support the estimated plant requirements. This suggests that the sedimentary pools play the most important role for P uptake, and the observed seasonal changes in the loosely adsorbed P_{MgCl_2} and P_{BD} pools were almost sufficient to support plant uptake at the muddy site, as the surface sedimentary pools were reduced by 86 mmol m^{-2} from July to September. However, at the sandy site the changes in sedimentary P_{MgCl_2} and P_{BD} pools were much lower and could not support plant growth, and this may explain the low P contents in plant tissues and the lower biomass of the meadow. Similarly, Jensen et al. (1998) found that the water-column pools of DIP were too low to support seagrass P requirements for *T. testudinum*, and a release of P from the particulate P pools of the sediment was

necessary to renew the porewater DIP for direct uptake by the roots or for P release across the sediment–water interface. They found that the sedimentary pools could only support long-term seagrass growth if these were available for plant uptake, and the same conclusion can be made for this study, where the total sedimentary P pools exceeded by several times the amount required by the plants, and even the easily available pools, such as the P_{MgCl_2} and P_{BD} pools, were 6 to 100 times higher than the requirements at the muddy site. However, P limitation may occur if the release of P from the particulate sedimentary pools is too slow to support maximum growth rates. This may be the case at the sandy site with high pools of calcium-bound P.

In conclusion, the large pools of easily available P at the muddy site, which become available during the growth season through increased mineralisation of organic matter due to increasing temperatures and reduction of the rhizosphere sediments, thereby releasing P from the redox-sensitive pools, provide P for plant uptake, and no P limitation was observed. In contrast, the availability of P was lower at the sandy site, as P was bound in the calcium fraction, which is more resistant to seasonal changes in sediment biogeochemical conditions.

Acknowledgements. We thank C. Kronborg for technical assistance during laboratory work, and T. Rützou and B. B. Knudsen for their help during field work. C.C. was supported by a grant from the Government of Sardinia, and M.H. and F.Ø.A. by a grant from the National Science Foundation (grant no. 21-02-0463, 272-05-0408).

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Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany

Submitted: November 3, 2005; Accepted: January 27, 2006
Proofs received from author(s): July 31, 2006