

Deterioration of Sediment Quality in Seagrass Meadows (*Posidonia oceanica*) Invaded by Macroalgae (*Caulerpa* sp.)

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Abstract Species of the macroalgae *Caulerpa* sp. are increasingly being observed in meadows of the endemic Mediterranean seagrass *Posidonia oceanica*, and in particular *Caulerpa taxifolia*, has been considered as an invasive species leading to seagrass decline. Studies have so far failed to reveal the underlying mechanisms of the success of the macroalgae, and here, we examine how biogeochemical changes of the environment associated to indigenous (*Caulerpa prolifera*) and non-indigenous (*Caulerpa racemosa* and *C. taxifolia*) species affect the habitat of *P. oceanica*. Two of the species (*C. prolifera* and *C. racemosa*) affect the sediment biogeochemical conditions by increasing organic matter pools, microbial activity, and sulfide pools of the sediments, and limited effects were found for *C. taxifolia*. Biomass of the macroalgae contributed to the extent of impacts, and high sulfide invasion into the seagrasses and regression of the meadow were pronounced at the location with the highest *Caulerpa* biomass. This suggests that *Caulerpa* invasion contributes to seagrass decline probably because *Caulerpa* thrives better than the seagrasses in the modified environment.

Keywords Non-indigenous macroalgae · Seagrass decline · Sediment biogeochemistry · Sulfide pools · Mediterranean

Introduction

Increased nutrient availability in the coastal zones, caused by eutrophication, increased sedimentation, increased rainfall, etc., are almost always followed by loss of seagrass habitat, and the competitive advantage of macroalgae for nutrients is considered a paradigm for seagrass decline (Valiela et al. 1997). The increasing focus on non-indigenous species (NIS) has provided additional evidence of macroalgae as major threats to habitat diversity including loss of seagrass meadows (Jousson et al. 2000; Pedersen et al. 2005). The spread of the green macroalgae, *Caulerpa racemosa*, invading the Mediterranean Sea from the Red Sea, and *Caulerpa taxifolia*, originally from Australia and accidentally released into the Mediterranean Sea, are considered to represent detrimental invasive events in the Mediterranean Sea, as widely colonized areas have been found in the whole basin interfering with native communities (Verlaque et al. 2000, 2004).

Although *Caulerpa* sp. is considered a threat to *Posidonia oceanica* (Ceccherelli et al. 2000), there are only few direct manipulative studies of interactions between *Caulerpa* species and *P. oceanica* and these show mixed results. For instance, *C. racemosa* negatively affected seagrass growth, whereas *C. taxifolia* had no measurable impact (Ceccherelli and Campo 2002; Ceccherelli and Sechi 2002; Piazzini et al. 2005; Piazzini and Ceccherelli 2006), and the mechanisms leading to the displacement of *P. oceanica* under pressure from high abundance of *Caulerpa* species thus remain unclear. An alternative, yet unexplored, possibility is that *Caulerpa* species may displace *P. oceanica* through the modification of sediment properties to generate an adverse environment for growth. Other macroalgae NIS, such as *Sargassum muticum* and *Gracilaria vermiculophylla*, have been found to increase the nutrient cycling due to faster

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decomposition of the detritus compared to native species (Pedersen et al. 2005; Tyler and McGlathery 2006). Chisholm and Moulin (2003) demonstrated that *C. taxifolia* stimulates N_2 fixation due to release of photosynthetic metabolites through plant rhizoids, and these metabolites could also stimulate other microbial processes in the sediments, such as sulfate-reducing bacteria. Indeed, previous studies of sediment biogeochemical cycling in *Caulerpa prolifera* stands show significantly elevated sulfate reduction rates and increased pools of sulfide compared to those in adjacent seagrass meadows (Holmer et al. 2004). Hydrogen sulfide is highly toxic to plants, and threshold concentrations as low as 10 μ M in the porewaters have been associated with the decline of *P. oceanica* populations (Calleja et al. 2007). Increased sulfide production and possible sulfide intrusion into *P. oceanica* as a result of *Caulerpa* sp. abundance in the meadows may thus pose a threat to the growth and survival of the seagrass.

The aim of the present study was to examine the possible effects of ecosystem modifications due to abundance of the macroalgae *Caulerpa* sp. in seagrass *P. oceanica* meadows in the Mediterranean. Observations of *Caulerpa* sp. presence in *P. oceanica* meadows are increasing around the Balearic Islands (A. Grau, personal communication) where most meadows are currently declining (Marbà et al. 2005). We evaluate the hypothesis that the effects of three different *Caulerpa* species (two non-indigenous *C. racemosa* and *C. taxifolia* and one

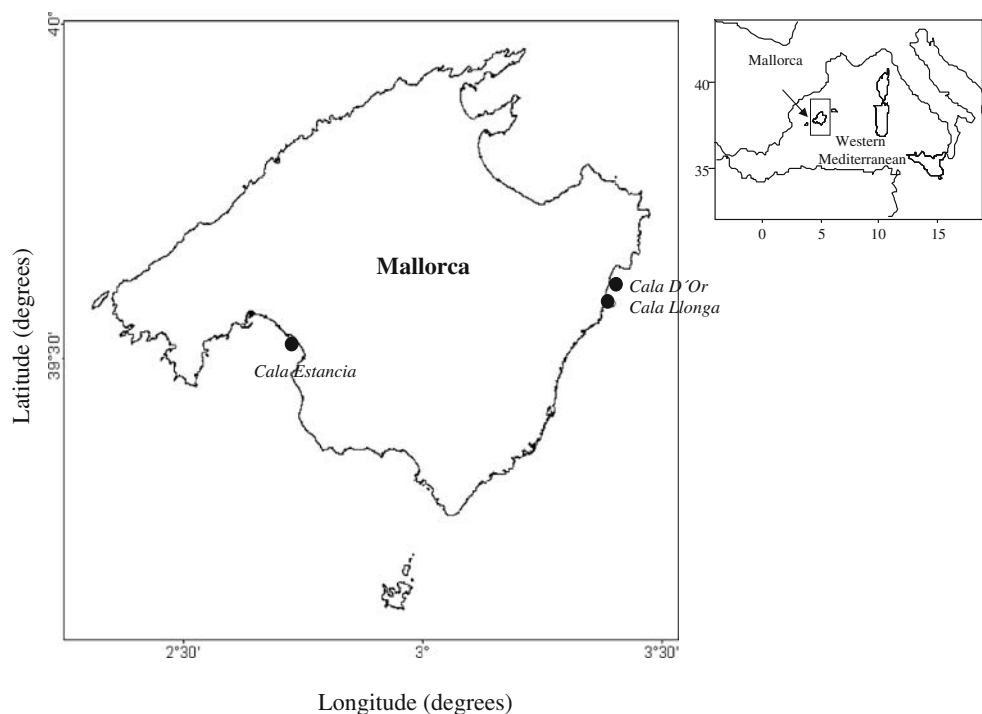
native *C. prolifera*) on *P. oceanica* meadows may be mediated by the development of adverse sediment biogeochemical conditions to support *P. oceanica* growth. We hypothesize that *Caulerpa* abundance in seagrass meadows increases sediment pools of organic matter, enhances microbial activity in the sediments, and increases sulfide exposure of *P. oceanica*.

Materials and Methods

Study Sites

We elected to test the hypothesis through a comparative analysis across adjacent (within 10 m) communities of *Caulerpa* and *Posidonia* and corresponding mixing of the two species. The study was conducted in July 2005 in Mallorca (Balearic Island, Spain, Fig. 1) in three subtidal study locations dominated by *P. oceanica* (L.) Delille, where each of the three *Caulerpa* species were present. These three study locations are Cala Llonga (39°22.030' N, 3°13.738' E) with *C. prolifera* (Forsskaal) Lamouroux at 3.5-m depth, Cala d'Or (39°22.164' N, 3°13.887' E) where *C. taxifolia* (Vahl.) C. Agardh was merged with *P. oceanica* meadows at a water depth of 5 m, and Cala Estancia (39°32.17' N, 2°42.57' E) at 2.5-m deep with *C. racemosa* (Forsskaal) J. Agardh. Observations between 2003 and 2005 in Cala Llonga reveal that a nearby meadow of *P. oceanica* was declining while the *C. prolifera* cover increased,

Fig. 1 Study sites around Mallorca Island where *Caulerpa prolifera* was found in Cala Llonga, *C. racemosa* in Cala Estancia, and *C. taxifolia* in Cala D'Or



whereas the sampled *P. oceanica* meadow in Cala d'Or was in steady state or slightly expanding and the *C. taxifolia* cover remained constant (data not shown). No such observations are available from Cala Estancia.

Sampling and Handling of Samples

At each location, three 10-m-long transects were established and separated in four sections ranging from 1 to 3 m wide each, representing a different community: bare sediment (Bare), *Caulerpa* bed (Cau), mixed meadow with *Posidonia* and *Caulerpa* (Mix), and *P. oceanica* meadow (PO). The density of shoots of *P. oceanica* was obtained using two 400-cm² quadrats, one on each side of the transect in the vegetated sections. For *Caulerpa* sp. biomass, SCUBA divers collected all plant material within the quadrat, whereas a subsample of ten *P. oceanica* shoots was collected from Mix and PO to minimize sampling impact on the seagrass meadows. An exception was at the Mix section at Cala Estancia site, where *C. racemosa* was present at the border and not inside of the *P. oceanica* meadow, and the biomass was obtained from the border. In addition, five *P. oceanica* plants (separated into leaves, roots, and rhizome of *Posidonia*) were collected for analysis of stable sulfur isotopic composition ($\delta^{34}\text{S}$). The plants for stable sulfur isotopic composition analyses were rinsed in freshwater to remove salts and freeze-dried immediately after collection.

The algal and seagrass material used to estimate plant biomass was dried at 60°C for 48 h. For *Caulerpa*, estimates of biomass were based on fronds, and for *P. oceanica*, aboveground biomass was calculated as the product of *P. oceanica* shoot density and shoot mass.

One sediment core in acrylic core liners (i.d.=2.6 cm) with pre-drilled silicone-filled holes for each centimeter was collected at each section in each transect for determination of sulfate reduction rates. One additional core (i.d.=4.3 cm) was collected in Bare, Cau, and PO sections in each transect for determination of sediment characteristics, including $\delta^{34}\text{S}$ isotopic composition. The depth where the sulfide front was located was determined by inserting silver sticks into each of the sediment cores and leaving them for 6 h. The front was detected where a black precipitate of Ag_2S was formed and the sulfide-free zone defined as the distance from the sediment surface to this mark. At each location, two vials with seawater were collected for determination of $\delta^{34}\text{S}$ of seawater sulfate.

After sampling, SCUBA divers deployed six sediment traps in Bare, Cau, and PO sections with two replicate traps as described by Gacia et al. (1999) for 48 h. Each sediment trap consisted of five cylinders of transparent glass of a diameter of 1.6 cm with an aspect ratio of 5 in a gimbaled frame.

Analysis

Plants

After freeze drying, the plant material was homogenized. Analysis of $\delta^{34}\text{S}$ was done by elemental analysis where 5 mg of dried plant tissue was added to 9 mg vanadium oxide, packed in tin capsules, and analyzed at Iso-analytical, UK.

SRR and Sulfur Pools

Sulfate reduction rates (SRR) were determined by the core injection technique (Jørgensen 1978) where 2 μl of ^{35}S -sulfate (70 kBq) was injected at 1-cm intervals through predrilled silicone-filled holes within 1 h of collection and incubated for 2 h in darkness at in situ temperature. The incubation was terminated by sectioning the cores into 2-cm intervals down to 10-cm and 5-cm intervals to 15 cm then fixed in 1 M zinc acetate and frozen immediately. Sulfate reduction rates were obtained by the two-step distillation method by Fossing and Jørgensen (1989), which separates sulfides into acid volatile (AVS: porewater H_2S and iron monosulfides) and chromium reducible sulfides (CRS: elemental sulfur and pyrite) pools. Radioactivity was counted on a Packard TriCarb 2000 scintillation counter and sulfide concentrations were determined spectrophotometrically according to Cline (1969).

Sediment Characteristics and Porewater

The top 0–2 cm of sediments were analyzed for particulate organic carbon (POC) and nitrogen (PON), whereas water content, density, and porewater were analyzed down to 15 cm. 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 and used for calculation of sulfate reduction rates. Sediment POC and PON contents were determined by elemental analysis according to Kristensen and Andersen (1987). Porewater was extracted by centrifugation of a sediment pellet in double centrifuging tubes (1,500 rpm, 5–10 min) and stored frozen until analysis. The porewater concentration of sulfate was determined by a Dionex autosuppressed ion chromatograph equipped with a conductivity detector (ICS-2500) and used for calculation of sulfate reduction rates.

$\delta^{34}\text{S}$ in Sediments and Seawater

Sedimentary sulfides for analysis of $\delta^{34}\text{S}$ were extracted as described for the sulfate reduction rates, except for a change in the traps which contained AgNO_3 . Ag_2S from the traps

Table 1 Results of two-way ANOVA tests of various parameters

	MS	df	F	P
Shoot density				
C species	191,769	2	3.76	0.054
Section	934,344	1	18.32	0.001*
C species × Section (Mix, PO)	38,811	2	0.76	0.489
Error	51,013	5		
Sedimentation				
C species	284.88	2	98.95	<0.0001*
Section	20.82	2	7.23	0.005*
C species × Section	49.15	4	17.07	<0.0001*
Error	2.88	18		
POC sedimentation				
C species	45,782	2	8.85	0.002*
Section	297,612	2	57.52	<0.0001*
C species × Section	7,064	4	1.37	0.285
Error	5,174	18		
PON sedimentation				
C species	3,126.67	2	63.09	<0.0001*
Section	645.06	2	13.02	<0.0001*
C species × Section	1,051.03	4	5.30	0.005*
Error	49.56	18		
S plant leaves				
C species	0.76	2	25.99	<0.0001*
Section	0.004	1	0.14	0.717
C species × Section (Mix, PO)	0.003	2	0.10	0.902
Error	0.029	5		
δ³⁴S rhizomes				
C species	6811	2	11.61	0.002*
Section	35.39	1	6.04	0.030*
C species × Section (Mix, PO)	3.20	2	0.55	0.592
Error	5.86	5		
SRR (log-transformed)				
C species	0.73	2	10.94	<0.0001*
Section	0.67	3	10.06	<0.0001*
C species × Section	0.13	6	1.93	0.120
Error	0.07	24		
TRS (log-transformed)				
C species	0.93	2	13.30	<0.0001*
Section	0.51	3	7.26	0.001*
C species × Section	0.14	6	1.98	0.110
Error	0.07	24		
Sulfide-free zone				
C species	23.34	2	6.75	0.005*
Section	47.47	3	13.72	<0.001*
C species × Section	15.50	6	4.48	0.004*
Error	3.46	24		

Caulerpa species (*C. prolifera*, *C. racemosa*, and *C. taxifolia*; C species) was the first factor, and section [Bare, Cau, Mix and PO] was the second factor

* $p < 0.05$

was filtered, and 0.3 g from both distillation steps was packed with 1 mg of vanadium oxide and analyzed for δ³⁴S at Iso-analytical, UK. Samples to determine the δ³⁴S of sulfate in seawater were prepared by centrifuging seawater (10 min, 1,500×g) followed by boiling of the supernatant

under acidic conditions (3 M HCl) and precipitating sulfate with BaCl₂ as BaSO₄. Isotopic analyses of BaSO₄ were made at Iso-analytical, UK.

δ³⁴S in Seagrasses

Seagrasses take up sulfur from three different sources: seawater sulfate through the leaves, porewater sulfate, and/or sulfide through the roots (Rennenberg et al. 1984). Due to the bacterial fractionation by sulfate-reducing bacteria, the sulfide produced in the sediments is isotopically lighter (lower δ³⁴S) than seawater and porewater sulfate (Kaplan et al. 1963). These differences allow quantification of sulfide invasion into the different parts of the seagrasses (e.g., Frederiksen et al. 2006). In order to estimate how much of the total sulfur in the plants was derived from sediment sulfide, the parameter F_{sulfide} was estimated using the following mixing equation:

$$F_{\text{sulfide}} = \frac{\delta^{34}\text{S}_{\text{tissue}} - \delta^{34}\text{S}_{\text{sulfate}}}{\delta^{34}\text{S}_{\text{sulfide}} - \delta^{34}\text{S}_{\text{sulfate}}} \times 100$$

where δ³⁴S_{tissue} is the value measured in the leaf, rhizome or root, δ³⁴S_{sulfate} is the value measured in the seawater, and δ³⁴S_{sulfide} is the value measured in the AVS pool which includes porewater sulfide potentially invading the seagrasses. The CRS pool was used for *C. taxifolia*, as it was not possible to obtain sufficient material for analysis from the AVS pool. This may have underestimated the F_{sulfide} with up to 3% based on lower δ³⁴S_{CRS} (−21.94‰) compared to δ³⁴S_{AVS} (average of *C. prolifera* and *C. racemosa* sites = −13.93‰).

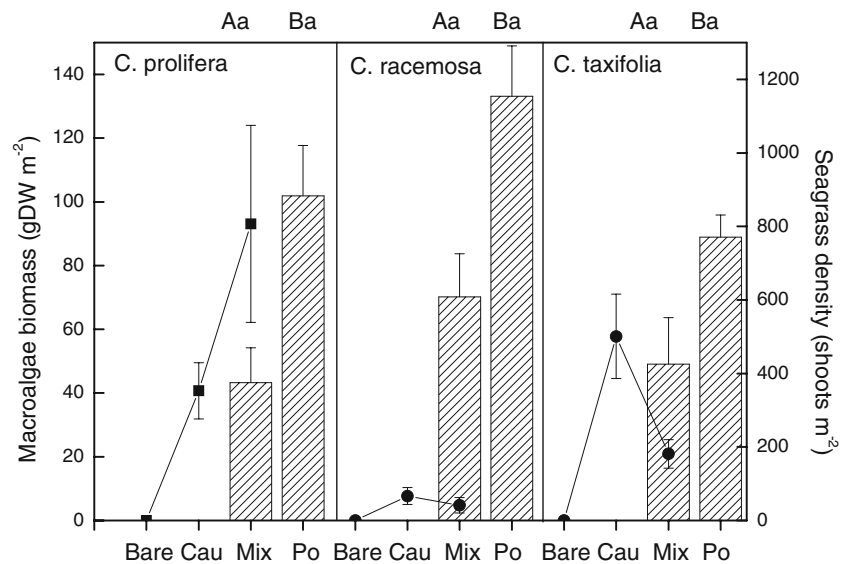
Sedimentation Rate

In the laboratory, the contents of one to three cylinders from the sediment traps were combined and collected on a combusted, pre-weighed Whatman GF/F filter (final replication 2 to 5). Dry weight of total sediment deposition was obtained after drying the filters at 60°C to constant weight. Sedimentation rates were estimated according to Blomqvist and Håkanson (1981) and Hargrave and Burns (1979) as described in detail in Gacia et al. (1999). The trap material was analyzed for nutrient contents (POC and PON) as described above for the sediments.

Statistical Analysis

A two-way analysis of variance (ANOVA) model (Systat 7.0) was used to test differences in sediment characteristics, sedimentation, and plant characteristics between sections and locations. Prior to analysis, data were tested for homogeneity, and normality assumption was verified by a Shapiro–Wilks test. The data were log-transformed if necessary. The ANOVA

Fig. 2 Average aboveground macroalgae biomass and seagrass shoot density at the three study locations ($n=3$, \pm SE). Bare unvegetated site, Cau *Caulerpa* section, Mix *Caulerpa* sp. and *P. oceanica* together, PO *P. oceanica* alone. Significant differences are given for *P. oceanica*. Small letters, significant ($p<0.05$) difference between locations (*C. prolifera* vs. *C. racemosa* vs. *C. taxifolia*). Capital letters, significant difference between Mix and PO



tests included tests for interactions and were followed by a Tukey post hoc test with a significance level of $p<0.05$.

Results

In the PO sections, no significant difference existed in *P. oceanica* shoot density among the three *Caulerpa* sp. locations (771 to 1,154 shoots per square meter; Table 1, Fig. 2). However, *P. oceanica* shoot density was significantly lower (45–58%) in the Mix sections that had both *Caulerpa* sp. and *P. oceanica* compared to the PO sections (Fig. 2). Similarly, the aboveground biomass of *P. oceanica* meadows in the PO section ranged between 874 and 1,974 g DW m⁻² and was threefold higher than that of Mix sections (data not shown). *Caulerpa* frond biomass

differed at the three locations, as *C. prolifera* attained the highest biomass (93 g DW m⁻²) compared to *C. taxifolia* (58 g DW m⁻²) and *C. racemosa* (8 g DW m⁻²; Fig. 2). At the *C. prolifera* location, the highest biomass was found in the Mix section, whereas the highest macroalgae biomass was observed in the Cau sections at the two other locations.

Sedimentation rates were not enhanced in the Cau section compared to the PO sections, but the sedimentation rates in Cau section for *C. racemosa* and *C. taxifolia* were higher compared to Bare (Tables 1 and 2, Figs. 3 and 4). The sedimentation varied significantly between locations, with highest rates at the *C. prolifera* location. Here, the Bare section had significantly higher sedimentation rate, and a high C/N ratio (18.8) compared to other sections indicating a relatively higher fraction of refractory material, which may be due to resuspension of sediments (Table 2).

Table 2 Sedimentation rates (average \pm SD, $n=3-5$), C/N ratio of the deposited material (average), and sediment C/N ratio (average) at the three locations

Parameter	Unit	Site	<i>Caulerpa prolifera</i>	<i>Caulerpa racemosa</i>	<i>Caulerpa taxifolia</i>
Sedimentation rate	grams DW per square meter per day	Bare	19.80 \pm 2.03 ^{a,b}	3.73 \pm 0.15 ^a	0.93 \pm 0.15 ^a
		Cau	9.51 \pm 1.69 ^{a,b}	4.96 \pm 0.30 ^a	2.10 \pm 0.11 ^a
		PO	8.68 \pm 1.17 ^{a,b}	5.54 \pm 0.33 ^a	2.32 \pm 0.09 ^a
Sedimentation C/N	M	Bare	18.8	8.2	12.5
		Cau	13.4	14.6	10.8
		PO	9.2	8.2	12.5
Sediment C/N	M	Bare	7.0	–	7.6
		Cau	21.7	27.1	16.3
		PO	9.3	21.0	30.3

Sedimentation and sediments were sampled at the bare section (Bare), in the *Caulerpa*-dominated section (Cau), and in the *Posidonia* meadow (PO). Sedimentation rates were tested for significant differences

^a Significant ($p<0.05$) difference between locations (*C. prolifera* vs. *C. racemosa* vs. *C. taxifolia*)

^b Significant ($p<0.05$) difference between sections. Data were log-transformed to meet statistical requirements

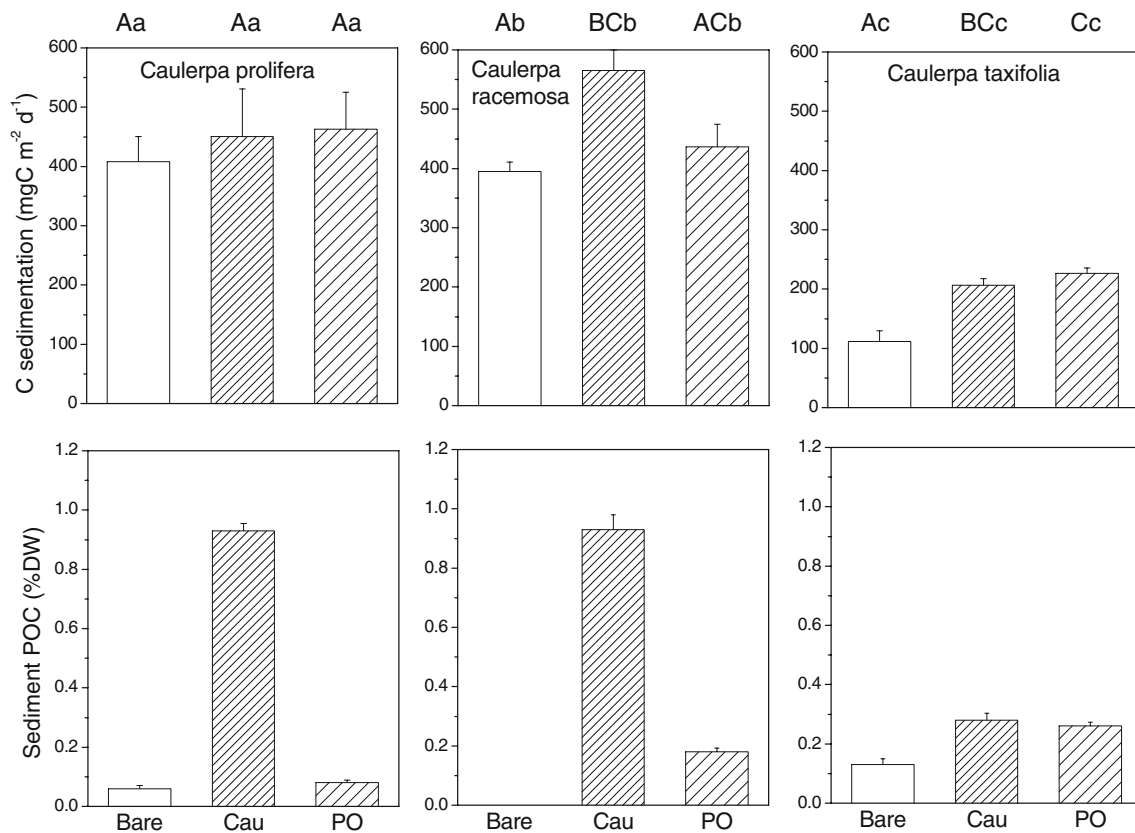


Fig. 3 Rates of sedimentation (*upper panel*) and sediment content of organic carbon (*lower panel*) at Bare, Cau, and PO sections ($n=3$, \pm SE). Significant differences are given for the sedimentation rate and not for the sediment POC due to lack of normality (Shapiro–

Wilk $p < 0.05$). *Small letters*, significant ($p < 0.05$) difference between locations (*C. prolifera* vs. *C. racemosa* vs. *C. taxifolia*). *Capital letters*, significant ($p < 0.05$) difference between Bare, Cau, or PO sections

Despite the lack of difference in rates of sedimentation between vegetation type, the sediment conditions varied considerably, with much higher POC (up to 16 times higher) and PON contents (up to five times higher) in the *Caulerpa*-dominated sections compared to the *P. oceanica* sections at all three locations (Figs. 3 and 4). The average sediment C/N ratios were generally high in Cau sections compared to Bare and PO sections, except at the *C. taxifolia* location where the C/N ratio was lower compared to the PO section (16.3 compared to 30.3, Table 2).

SRR were enhanced at the vegetated sections compared to Bare (Table 1, Fig. 5), and in particular presence of *Caulerpa prolifera*-enhanced SRR, which was about four times higher compared to the Bare section. The Bare sections generally reflected the sedimentation rates with highest rates at the *C. prolifera* location and lowest at *C. taxifolia*. Similarly, the pools of sulfides (TRS = AVS + CRS, where AVS < 1% of TRS) were higher in the vegetated sections compared to Bare and followed the SRR. The accumulation of TRS at the Bare sections reflected the sedimentation rates and sulfate reduction rates with highest accumulation at the *C. prolifera* location. On the other hand, the sulfide front was consistently closest to

the sediment surface in the Cau and Mix sections at all locations where sulfide was found already at 0.4- to 0.9-cm depth. At the *C. prolifera* location, no precipitate was found on the silver stick in the examined depth (=10 cm) in the PO section despite elevated TRS pools compared to the Bare and Cau sections.

The sulfate reduction rates were enhanced up to seven times in the Mix sections compared to the PO sections at two of the locations (*C. prolifera* and *C. racemosa*), whereas no significant differences between vegetation type were found at the location with *C. taxifolia* (Table 1). SRR were particularly high at the *C. prolifera* location where the highest *Caulerpa* biomass was found, and a positive correlation between *Caulerpa* biomass and SRR ($R^2 = 0.64$, $p < 0.001$) indicates that the abundance of macroalgae affects the sulfate reduction rates. Consistent with the high SRR, the TRS were high at the Mix sections at the *C. prolifera* and *C. racemosa* locations. Variability in sediment pools of sulfides was, however, not coupled to changes in *Caulerpa* biomass (regression analysis, $p > 0.05$).

The $\delta^{34}\text{S}$ of the seawater sulfate ranged between 19.6‰ and 20.5‰ (Table 3). The AVS pools were most depleted at the *C. prolifera* location (−16.5‰), whereas the $\delta^{34}\text{S}_{\text{CRS}}$

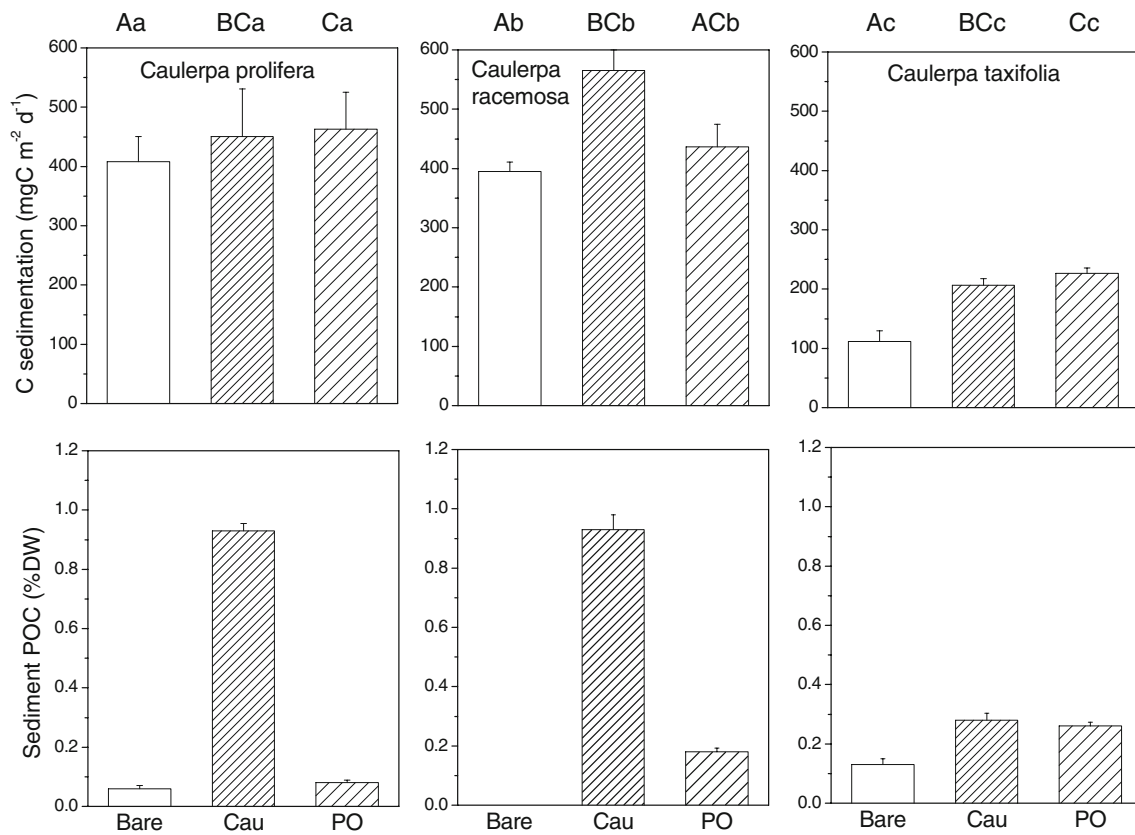


Fig. 4 Rates of sedimentation (*upper panel*) and sediment content of organic nitrogen (*lower panel*) at Bare, Cau, and PO sections ($n=3$, \pm SE). Significant differences are given for the sedimentation rate (log-transformed) and not for the sediment PON due to lack of

normality (Shapiro–Wilk $p<0.05$). *Small letters*, significant ($p<0.05$) difference between locations (*C. prolifera* vs. *C. racemosa* vs. *C. taxifolia*). *Capital letters*, significant ($p<0.05$) difference between Bare, Cau, or PO sections

pools ranged between -19.1‰ and -21.9‰ . The $\delta^{34}\text{S}$ of *P. oceanica* varied between the plant tissues, with the isotopic values in the roots and rhizomes more negative than those in the leaves (Table 4). The $\delta^{34}\text{S}$ of *P. oceanica* rhizomes at the *C. prolifera* location were significantly lower compared to the two other locations (Table 1), and there were significant differences between $\delta^{34}\text{S}$ at the Mix and the PO sections with higher values at the Mix section ($p=0.03$). F_{sulfide} followed the pattern observed for $\delta^{34}\text{S}$, although sedimentary sulfides were only detected in the leaves at PO section at the *C. prolifera* location (Table 4). The highest fraction of sulfides was found in the rhizomes and roots at the *C. prolifera* location where up to 40% of the total sulfur in the roots was derived from sedimentary sulfides. The contribution was up to 14% at the two other locations.

Discussion

The presence of *Caulerpa* sp. in *P. oceanica* meadows was coupled with a lower seagrass shoot density, suggesting either a negative effect of the macroalgae presence on the seagrass population dynamics or that the seagrasses were in

decline, allowing for colonization by the macroalgae. There was no significant difference between the shoot densities in the PO sections at the three locations, whereas the shoot density was 45–58% lower in sections with *Caulerpa* sp. present. Seagrass population dynamics, e.g., assessed through counts in permanent plots or changes in cover, have not been measured at the three locations, but observations between 2003 and 2005 suggest that the *P. oceanica* population in Cala d'Or with *C. taxifolia* present was in steady state or slightly expanding, whereas in Cala Llonga with *C. prolifera*, it was declining (decreased from 30% to 20% cover in 2 years; Terrados et al. 2009) along with an increase in *C. prolifera* cover (from 78% to 95%; Terrados et al. 2009). There are no observations for the *C. racemosa* location. The success of *C. prolifera* in Cala Llonga may thus be amplified by the modifications taking place in the seagrass meadow, similar to observations by Chisholm et al. (1997) for *C. taxifolia* in degrading *P. oceanica* meadows.

The presence of *C. prolifera*, and to some extent also *C. racemosa*, in *P. oceanica* meadows coincided with significant differences in sediment biogeochemical conditions, with enhanced sulfate reduction rates, shallower sulfide

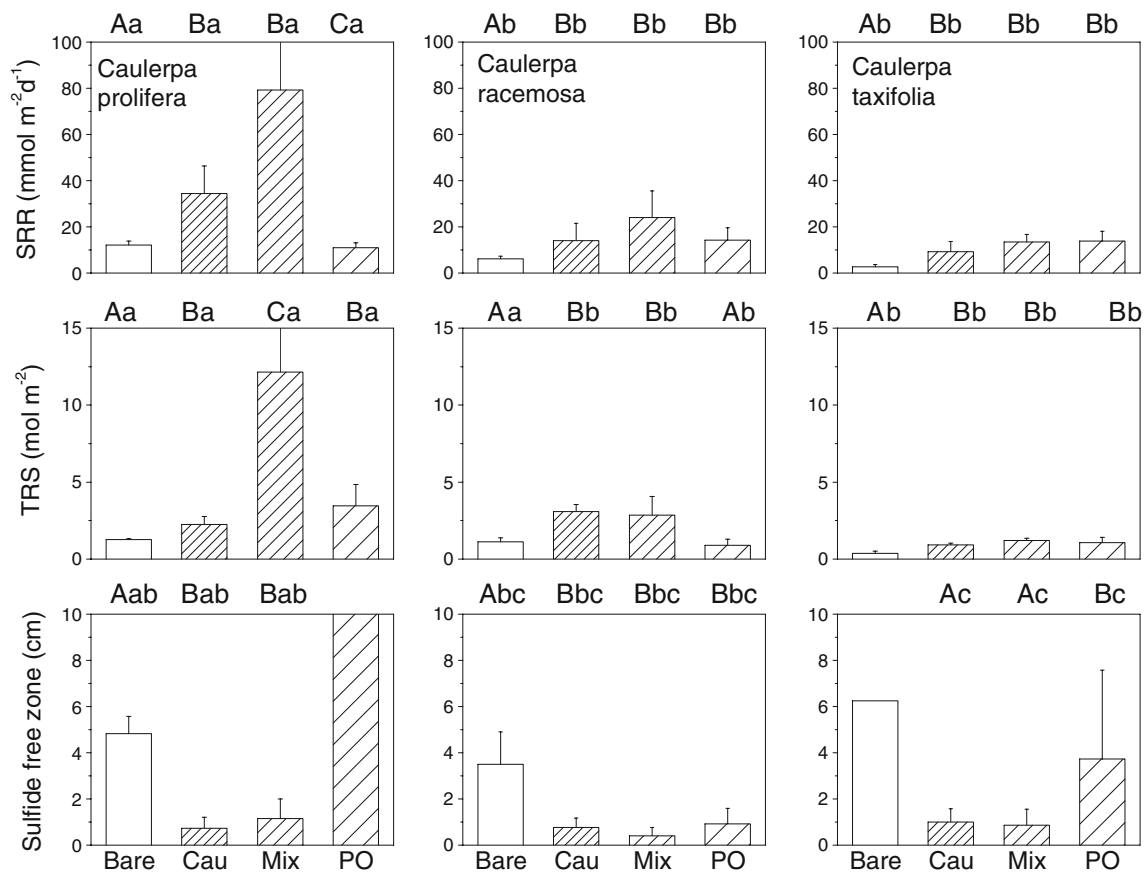


Fig. 5 Depth integrated sulfate reduction rates (SRR, 0–10 cm, $n=3$, \pm SE, upper), total pools of sulfide (TRS, mid), and the depth of the sulfide-free zone in the sediments (lower) at the three study locations. The sulfide front was >10 cm for *P. oceanica* section at the *C. prolifera* location. Significant differences are given for the sulfate

reduction rates (log-transformed), sulfide pools and for the sulfide front. Small letters, significant ($p<0.05$) difference between locations (*C. prolifera* vs. *C. racemosa* vs. *C. taxifolia*). Capital letters, significant ($p<0.05$) difference between Bare, Cau, Mix, or PO sections ($n=1$ for sulfide free zone at PO for *C. prolifera* and Bare at *C. taxifolia*)

front depth, and increased pools of sulfides in the sediments compared to seagrass sediments. Sulfate reduction rates in the mixed macroalgae–seagrass sections and in the *C. prolifera* stands were similar to observations from similar habitats underneath fish farm net cages in the Mediterranean (Holmer and Frederiksen 2007). Fish farm sediments receive extremely high organic loading compared to natural sediments, resulting in significantly enhanced sulfate reduction rates (Holmer and Frederiksen 2007). Similar

high sulfate reduction rates have been found in *C. prolifera* stands in a nearby bay (Holmer et al. 2004), suggesting that high abundance of *C. prolifera* stimulates the sulfate reduction rates in the sediments. The sulfate reduction rates were not as high at the *C. racemosa* location, probably due to a lower biomass, but a similar pattern with rates almost twice as high as in Mix compared to PO sections suggest a similar mechanism for this species. Our results suggest that the effect of *C. taxifolia* on sediment biogeochemistry is less, as the sulfate reduction rates were similar in Mix and PO sections despite relatively high macroalgae biomass. Chisholm et al. (1997); however, found major changes in sediment biogeochemistry in degrading *P. oceanica* meadows colonized by *C. taxifolia*, suggesting that the health of the *P. oceanica* meadow may play a role and that invaders are more successful in degraded environments as proposed by Didham et al. (2005).

Table 3 Seawater and sediment $\delta^{34}\text{S}$ values at the three locations from the *P. oceanica* section ($n=1-3$, \pm SE)

	<i>Caulerpa prolifera</i>	<i>Caulerpa racemosa</i>	<i>Caulerpa taxifolia</i>
$\delta^{34}\text{C}_{\text{SO}_4}$	19.57 \pm 0.14	20.49 \pm 0.30	n.a.
$\delta^{34}\text{S}_{\text{AVS}}$	-16.53 \pm 1.37	-11.33	b.d.
$\delta^{34}\text{S}_{\text{CRS}}$	-19.07 \pm 1.80	-21.32 \pm 3.40	-21.94 \pm 0.27

All values are given in ‰

n.a. not analyzed, b.d. below detection limit

Environmental differences at the three locations may also play a role for the observed differences. The sedimentation of organic matter was significantly higher at *C. racemosa* and *C. prolifera* sites, which may negatively

Table 4 Content of sulfur (S), $\delta^{34}\text{S}$ (‰), and F_{sulfide} (%) values in *P. oceanica* leaves, rhizomes, and roots for the sections where *Caulerpa* and *P. oceanica* occur together (Mix) and where *P. oceanica* grow alone (PO, average values \pm SD, $n=3$)

		<i>Caulerpa prolifera</i>	<i>Caulerpa racemosa</i>	<i>Caulerpa taxifolia</i>
S				
Leaves	Mix	0.79 \pm 0.16 ^a	1.18 \pm 0.23 ^a	1.54 \pm 0.10 ^a
	PO	0.88 \pm 0.10 ^a	1.16 \pm 0.10 ^a	1.55 \pm 0.21 ^a
Rhizomes	Mix	0.51 \pm 0.04	0.55 \pm 0.06	0.58 \pm 0.11
	PO	0.63 \pm 0.19	0.72 \pm 0.25	0.65 \pm 0.14
Roots	Mix	0.44 \pm 0.18	0.65 \pm 0.05	0.58 \pm 0.10
	PO	0.70 \pm 0.05	0.68 \pm 0.30	0.44 \pm 0.06
$\delta^{34}\text{S}$ (‰)				
Leaves	Mix	20.99 \pm 0.65	21.23 \pm 0.85	25.60 \pm 0.32
	PO	19.81 \pm 0.39	21.97 \pm 2.74	25.80 \pm 0.54
Rhizomes	Mix	12.45 \pm 2.95 ^{a,b}	18.64 \pm 0.36 ^{a,b}	19.49 \pm 1.27 ^{a,b}
	PO	10.69 \pm 3.13 ^{a,b}	16.45 \pm 2.88 ^{a,b}	15.02 \pm 2.58 ^{a,b}
Roots	Mix	10.21 \pm 8.85	21.69 \pm 2.01	18.42 \pm 5.56
	PO	5.94 \pm 2.90	15.83 \pm 4.57	19.08 \pm 1.76
F_{sulfide} (%)				
Leaves	Mix	0	0	0
	PO	0.7 \pm 0.9	0	0
Rhizomes	Mix	21.6 \pm 8.4	3.9 \pm 1.0	2.1 \pm 3.5
	PO	26.6 \pm 8.9	10.1 \pm 8.2	14.2 \pm 7.4
Roots	Mix	28.2 \pm 24.9	0.4 \pm 0.7	7.5 \pm 13.0
	PO	40.2 \pm 8.3	12.2 \pm 12.6	3.7 \pm 3.3

Significant differences are given for S in leaves and $\delta^{34}\text{S}$ in rhizomes, as these were the only data set showing normality (Shapiro–Wilk $p>0.05$)

^aSignificant ($p<0.005$) difference between locations (*C. prolifera* vs. *C. racemosa* vs. *C. taxifolia*)

^bSignificant difference between sections (Mix vs. PO)

affect *P. oceanica*, as the rates (~1 g DW OM per square meter per day, estimated from POC sedimentation considering POC=50% of OM) were close to the threshold value (1.5 g DW OM per square meter per day) for net population decline found by Diaz-Almela et al. (2008). In particular, sedimentation of allochthonous material may contribute to the enhanced microbial activity (Holmer et al. 2004). Sedimentation of nutrients was up to two times higher at the vegetated compared to the bare sections, but there were no significant differences between the *Caulerpa*-dominated and the PO sections. Regrettably, sedimentation was not measured at the Mix sections where it may differ from the PO sections due to the smaller seagrass shoots (Marbà et al., unpublished) with openings in the less dense meadow. The higher rates of sedimentation in the vegetated sections were reflected in higher sediment organic contents, although the organic enrichment did not strictly follow the sedimentation rates. *Caulerpa*-dominated sections showed much higher organic enrichment than PO sections, suggesting that *Caulerpa* detritus contributed directly to the

sediment organic pools and indirectly through enhanced trapping of organic matter (Piazzi et al. 2007). The enrichment was quite substantial at the *C. prolifera* and *C. racemosa* locations where the sediment organic carbon content was up to 11 times higher than found in the PO section. An important effect of *Caulerpa* presence may thus be an organic enrichment of the sediments, leading to the observed enhanced microbial activity in the sediments (Chisholm et al. 1997).

The significant and major decline in $\delta^{34}\text{S}$ values in roots ($\delta^{34}\text{S}$ decline=8–13‰) and rhizomes ($\delta^{34}\text{S}$ decline=6–7‰) of *P. oceanica* at the *C. prolifera* location suggests a high intrusion of sedimentary sulfides into the belowground parts (up to 40%). Depleted $\delta^{34}\text{S}$ values in *P. oceanica* belowground tissues have been found in sediments enriched by organic matter from phytoplanktonic inputs (decline 6–8‰; Marbà et al. 2007) and from fish farm facilities (decline 6–13‰; Frederiksen et al. 2007), where the sulfide exposure of the plants is enhanced due to high rates of sulfate reduction fuelled by high sedimentation of organic matter. There was, however, higher sulfide invasion in the PO sections compared to the Mix section, which is puzzling but may be due to a number of reasons: (1) The $\delta^{34}\text{S}$ values show large heterogeneity, indicating that the intrusion of sulfide is highly variable probably controlled by individual shoot characteristics, which could be addressed by analyzing a larger number of replicates. (2) *P. oceanica* shoots are long lived (years) and sulfide intrusion could have occurred earlier. (3) *P. oceanica* shoots are connected through rhizomes (Marbà et al. 2002) and sulfides may be translocated between shoots growing in the Mix and PO sections, as the distance between them was small (<1–2 m). Data on shoot size showed smaller shoots (17–55%) at the Mix sections probably as a result of decreased meristematic activity compared to the PO sections (Garcias-Bonet et al. 2008). Reduced growth upon sulfide exposure has been found for a number of different seagrasses, e.g., *Halophila ovalis* (Kilminster et al. 2008), *Zostera marina* (Goodman et al. 1995; Holmer and Bondgaard 2001), *Thalassia testudinum* (Koch et al. 2007), including *P. oceanica* (Marbà et al. 2007; Pérez et al. 2007). The observations of the seagrass population dynamics at the *C. prolifera* (declining) and *C. taxifolia* (steady state/expanding) locations are consistent with the generally negative effects on *P. oceanica* with *C. prolifera* presence and limited effects with *C. taxifolia*.

In conclusion, the presence of *Caulerpa prolifera* and *C. racemosa* in *P. oceanica* meadows was coincident with deteriorated sediment quality. Sediment organic matter pools were enhanced in *Caulerpa* sp. beds, whereby sediments became reduced almost to the sediment surface, and sulfate reduction rates and sulfide production were stimulated similar to findings in organic-enriched sedi-

ments. Sulfate reduction rates and sulfide pools were further enhanced in the *P. oceanica* meadows with co-occurrence of *Caulerpa* sp. $\delta^{34}\text{S}$ values in the seagrasses were depleted and a high fraction of sedimentary sulfides were found accumulating in the seagrass rhizomes and roots, suggesting a high invasion of sulfides into the plants. Low shoot density and small shoots in the Mix sections suggest that altered sediment biogeochemistry could eventually lead to seagrass decline being enhanced in modified environments. *Caulerpa* sp. seems to benefit from the deterioration of the sediments with high biomass in organic-enriched sediments. These results suggest that stimulation of high abundance of *Caulerpa* sp., in particular of *C. prolifera* and *C. racemosa*, could be displacing seagrass meadows through the modifications of sediment conditions to turn these adverse to support seagrass growth. This is a powerful mechanism for interactions between rapid colonizers and native seagrass species, in particular in habitats where the native species already are stressed by other factors.

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