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The effect of nutrient additions on bacterial activity in seagrass (*Posidonia oceanica*) sediments

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Abstract

The influence of nutrient additions on benthic bacterial activity under seagrass meadows was tested by enriching five seagrass (*Posidonia oceanica*) meadows with nutrients over one year. We found a highly significant response of benthic bacterial activity to nutrient additions, which was reflected in greater (about two-fold) ammonification rates and, to a smaller extent, a significant tendency for a greater exoenzymatic activity. Nutrient additions significantly raised bacterial activity, without altering the seasonal changes in bacterial activity. As a result of the increased bacterial activity, the organic content of the sediments declined significantly, by about 33%, after one year of nutrient addition. Hence, nutrient additions to the seagrass meadows enhance seagrass production but also accelerate bacterial decomposition of seagrass carbon, thereby reducing the capacity of the sediments to store organic carbon. These results demonstrate that sediment nutrient availability limits bacterial activity in these *Posidonia oceanica* meadows, and identify bacteria as important nutrient consumers in these systems. © 1998 Elsevier Science B.V.

Keywords: Exoenzymatic activity; Ammonification rate; Nutrient availability; Seagrass meadows

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1. Introduction

The role of bacteria in aquatic ecosystems has been expanded from a dominant view of bacteria as decomposers responsible for nutrient remineralization, to one of them as consumers, as well as recyclers, of inorganic nutrients (Azam et al., 1983). The relative

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balance between these two roles appears to depend on the nutrient (C, N and P) balance of their organic substrates relative to the requirements for efficient bacterial growth (Goldman et al., 1987), which approaches the Redfield ratio (106 C:16 N:1 P; Thingstad, 1987). Efficient bacterial growth on organic substrates depleted of nitrogen and phosphorus relative to their requirements requires additional sources of nitrogen, usually taken up from the ammonium pool (Goldman et al., 1987), and phosphorus, often taken up as phosphate (e.g. Toolan et al., 1991; Coveney and Wetzel, 1992).

Bacterial demands for inorganic nutrients must be higher in benthic, compared to planktonic environments, because organic matter reaching the sediment is often characterized by high C:N and C:P ratios (Valiela, 1984). Marine macrophyte tissues, which represent the main source of organic carbon in many littoral areas, are often highly depleted of N and P relative to phytoplankton (Duarte, 1992). The detritus seagrass produce is, therefore, nutrient-depleted, resulting in very low decomposition rates (Enríquez et al., 1993). Yet, seagrass meadows are highly productive ecosystems, which are able to support a potentially high activity of benthic bacteria, if sufficient inorganic nutrients are available to the bacterial community. Marine angiosperms, which are often nutrient-limited (e.g. Short, 1987; Powell et al., 1989; Short et al., 1990; Duarte, 1990; Perez et al., 1991) also derive most of their nutrients from the sediment pool (e.g. Perez-Llorens and Niell, 1991). It is, therefore, likely that the availability of inorganic nutrients may limit bacterial activity under seagrass sediments in periods of active seagrass and bacterial growth, when the sediment's nutrient pool may be depleted (López et al., 1995).

We tested the influence of nutrient additions on benthic bacterial activity in five meadows of the seagrass *Posidonia oceanica* (L.) Delile, the dominant species in the Mediterranean, in the NE Spanish coast. Previous work demonstrated phosphorus to be depleted from the sediments of these meadows during periods of high activity of seagrass and bacteria (López et al., 1995), and demonstrated a tendency for bacterial activity to increase with increasing phosphorus concentration (López et al., 1995), strongly suggesting that this is a potentially limiting nutrient. We characterized benthic bacterial activity by measuring their exoenzymatic capacity, which reflects their potential for decomposition of organic matter (Hoppe, 1983; King, 1986; Meyer-Reil, 1987; Boon, 1989), and ammonification rates which, in addition to reflecting bacterial metabolism, have important influences on local nutrient cycles (López et al., 1995). We also tested for changes in the size of the sedimentary organic matter pool, to test if the increased bacterial activity expected in response to nutrient additions did indeed result in a more efficient recycling of the organic matter stored in the sediments.

2. Materials and methods

Experimental nutrient addition was conducted in parallel to measurements of seagrass growth responses (Alcoverro et al., 1997), in five contrasting *Posidonia oceanica* meadows on the NE Spanish Coast (Mediterranean Sea). Three of the sites (Port Lligat, Giverola and shallow Medes, at depths of 5 m) were located towards the up-slope limit of the plant distribution and the two others (deep Medes and Blanes, at depths of 13 m) were closer to the deep limit (Alcoverro et al., 1997). Data on sediment characteristics, bacterial activity and its regulation, and plant growth and nutrient dynamics are reported elsewhere (Alcoverro et al., 1995; López et al., 1995; Alcoverro et al., 1997).

Two 1 m^2 experimental plots were established at each site, one used as a control and the other enriched at intervals of about 40–50 days over one year (i.e., nine sampling events, from October 1990 to November 1991). Sediments at the treatment plot were enriched by inserting 30 slow-release mixed fertilizer sticks, representing a loading of about 6.5 g of N and 0.89 g of P m^{-2} each time. Laboratory experiments on nutrient release from the fertilizer bars showed an average release rate of 2% of the nutrients added per day (Agawin, 1995). The release rate achieved exceeds calculated average daily seagrass requirements (Alcoverro et al., 1997) by at least ten-fold, and is similar to the loading rates received by heavily eutrophied coastal waters (Borum and Sand-Jensen, 1996). In addition, the N:P atomic ratio supplied was sixteen, close to the requirements for balanced bacterial (Thingstad, 1987) and seagrass growth (Duarte, 1990). Sediment samples, consisting of three replicated corers (5 cm internal diameter), pushed to a depth of about 5 cm into sediments, were collected from each plot at each sampling event prior to the addition of fertilizer sticks and these were kept refrigerated until processed (within 24 h). The sampling of sediments impacted 5% of the area of each of the experimental plots over the entire experiment. Once at the laboratory, we extracted sediment subsamples to measure nutrients (ammonium, nitrite, nitrate, phosphate) and organic matter and water contents, and to examine bacterial activity. Nutrient concentrations were measured in pore water extracted from the supernatant of centrifuged subsamples (3000 g, 10 min), which had been kept frozen until analyzed. Ammonium was measured as exchangeable ammonium, which was extracted by adding 20 ml of KCl (2 M) to the pore water (Alef and Kleiner, 1986). All nutrient analyses were performed on an autoanalyzer following standard methods (Grasshoff et al., 1983). The water content and the organic matter content of sediment were measured after desiccation (24 h) and ignition (2 h) at 105 and 450°C of the fresh and dried sediments, respectively.

Exoenzymatic bacterial activity was represented by the exoproteolytic (EPA) and exoglucosidase (EGA) activities, as described previously (López et al., 1995). The measurements were conducted on the supernatant (700 g for 5 min) of a slurry prepared by suspending 2–3 g of sediment subsample in 50 ml of filtered (0.2 μm Nuclepore filter) seawater, and stirred at 30°C for 1 h prior to the addition of appropriate substrates. These were L-leucil-β-naphthylamide (2 mM, Sigma N-8381) and 4-methylumbelliferyl-α-D-glucoside (20 μM, Sigma M-9766), which are surrogates of aminopeptidase and α-glucosidase activities, respectively.

Potential ammonification rates were measured by following the conversion of arginine to ammonium (Alef and Kleiner, 1986). For each replicate corer, duplicate slurries were prepared, consisting of 1–1.5 g wet weight of sediment subsample, 1 ml of seawater and 0.5 ml of an arginine solution (0.2% in sterile distilled water). One of each duplicate was frozen at −20°C and subsequently analyzed for ammonium concentration (see above), to be used as a blank, and the other was incubated overnight in darkness at 30°C in a shaking water bath prior to ammonium determination, to measured ammonium formation.

All concentrations and rates measured were expressed per cm^3 of sediment to avoid spurious correlations (Bird and Duarte, 1989), except for nutrient concentrations, which were expressed per unit volume of interstitial water, to allow comparisons with published values. The standard error of measurements of bacterial activity was calculated using boot-strap techniques (Efron and Tibshirani, 1986) on triplicate series of initial and incubated samples. The meadows selected do not represent a random sample of the meadows present in the region, since they were selected to represent the range of conditions encountered there. Hence, the five meadows studied cannot be assumed to represent ‘‘replicates’’, and the experiments conducted at each meadow must be considered to be independent of one another. In addition, observations in the fertilized plots are not independent of one another, so we used repeated-measures analysis of variance (Winer, 1971) to test for differences in bacterial activities and organic matter content between fertilized and control plots for each meadow. All of the analyses used the basic model statement: treatment, seasonality, treatment × seasonality, with treatment representing the fixed variable and seasonality representing the repeated measure. Repeated-measures analysis of variance (rmANOVA) was performed using BMDP statistical software (1993). Bacterial response to nutrient enrichment could also be manifested by increasing any of the descriptors of bacterial activity at any one site and sampling date, which was tested by performing about 135 *t*-tests. The main goal of this study was not to test for the presence of enhanced bacterial activity at any particular meadow, but rather to test the hypothesis that the activity of benthic bacteria in NE Spanish seagrass meadows is nutrient-limited. Hence, we used meta-analysis to test the null hypothesis that the independent experiments conducted at each of the five meadows did not provide evidence of a general tendency towards increased bacterial activity in response to fertilization in NE Spanish *P. oceanica* meadows. Meta-analysis is well suited for this task, because it is designed to ‘‘summarize and analyze multiple independent studies’’ (Arnqvist and Wooster, 1995). The combined probability test, used to test the hypothesis for all five meadows in concert, followed the procedure described in Sokal and Rohlf (1969), based on the fact that the sum of the natural logarithms of the individual *t*-tests is distributed as a X^2 distribution.

3. Results and discussion

Nitrogen and phosphorus in fertilized sediment pore waters were often depleted down to control levels 40–50 days following additions, except for July–August and December (phosphate), and June and December (nitrogen), when uptake was apparently reduced (Fig. 1). Patchiness in nutrient concentrations within treatment plots was not a significant source of error and variation in this study, since the coefficient of variation of replicate cores collected from the same plot averaged 31%, both for control and treatment plots. The depletion of phosphorus concentrations down to the limit of detection at the onset of exponential plant growth in March (Alcoverro et al., 1995) was maintained despite nutrient additions, further indicating the strong phosphorus limitation of the benthic community (i.e. bacteria, seagrass) at that time. Nutrient additions to the sediments were, however, able to balance somewhat the sediments’ phosphorus deficiency, as reflected in the significant reduction in inorganic N:P ratios in the

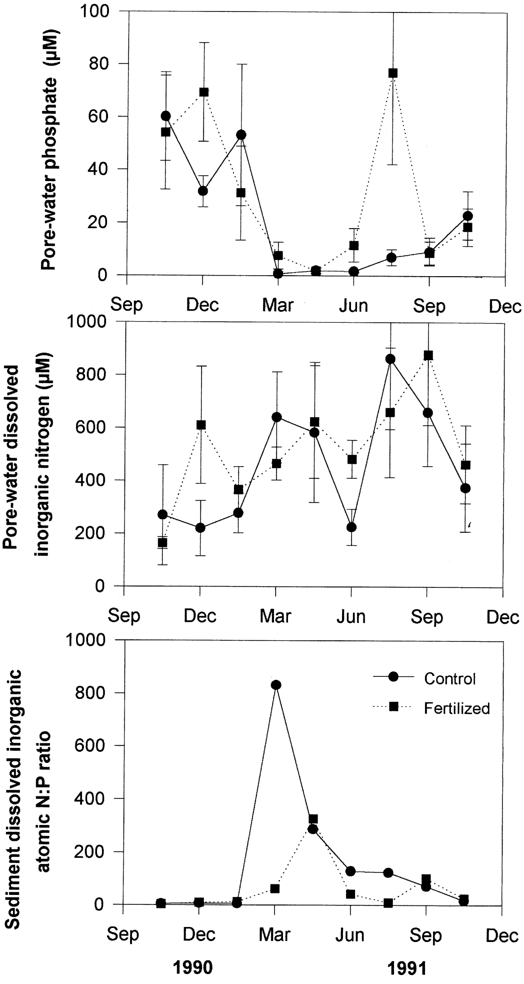

Fig. 1. Average seasonal time course of sediment pore water-dissolved inorganic nitrogen (DIN) and phosphate concentrations, and the corresponding atomic N:P ratio of fertilized and control plots. Error bars represent the variation (±1 SE) among meadows. The data shown represent porewater nutrient concentrations before each new application of fertilizer.

fertilized plots (Fig. 1). The sharp phosphorus depletion observed in spring, along with sustained high N:P ratios in fertilized plots, indicates that nutrient additions failed to saturate community demands at that time. Further elucidation of the fate of the added nutrients is, however, impossible with the data on hand. The time interval between measurements (40–50 days) is too long to provide a detailed description of the dynamics of the sedimentary nutrient pools, since it was designed to allow observation of both direct and indirect, through enhanced seagrass growth, effects of the added nutrients. Although nutrient additions did lead to increased seagrass growth and nutrient contents (Alcoverro et al., 1997), seagrasses are unlikely to be a significant sink for the added nutrients. The reason for this contention is that added nutrients exceeded seagrass requirements (cf. Alcoverro et al., 1997) by at least an order of magnitude. Some of the nutrients may have accumulated in living benthic biomass, and some, likely the bulk of the added nutrients, may have diffused out of the sediments.

All of the analyses tested the effect of treatment, as the fixed effect, seasonality, as the repeated measure, and the interaction between these two effects: Exoenzymatic activities showed the lowest responses, while EGA activity was significantly higher on fertilized than on control plots throughout the year in Deep Medes meadow (rmANOVA, $P = 0.0007$), EPA activity only showed a significant response to fertilization throughout the year in the Shallow Medes meadow (rmANOVA, $P = 0.0003$). Ammonification rates showed the higher responses throughout the year. Results were significant in meadows at Blanes (rmANOVA, $P = 0.0482$), Giverola (rmANOVA, $P = 0.0210$), Shallow Medes (rmANOVA, $P = 0.0044$) and Port Lligat (rmANOVA, $P = 0.0028$). No significant responses were observed at Deep Medes (rmANOVA, $P = 0.9106$). All of the activities showed strong seasonality (Table 1). The responses of EGA, EPA and ammonification (except at shallow Medes meadow) to fertilization varied seasonally, as revealed by a significant interaction term between nutrient addition and seasonality in the rmANOVA analysis (Table 1).

However, overall bacterial activity in the meadows examined increased significantly (combined probability test, X^2, $P < 0.001$) in response to nutrient additions over one year. We observed bacterial activity to be significantly greater, as reflected by simultaneous consideration of all three descriptors of bacterial activity, in enriched plots in half of the samples. This significant increase in nutrient enrichment was observed for all descriptors of bacterial activity (combined probability test, X^2, $P < 0.001$). The extent of the response varied substantially throughout the year and differed among bacterial activities (Fig. 2). Yet, the overall response to nutrient additions (i.e. the

Table 1
Summary of the rmANOVA (*F*-values) for exoglucosidase activity, exoproteolytic activity and the ammonification rate at the five meadows studied

Meadow	Blanes	Giverola	Shallow Medes	Deep Medes	Port Lligat
Exoglucosidase activity					
Treatment	13.01	2.57	0.00	87.02^{c}	2.18
Seasonality	78.45^{c}	20.87^{b}	51.88^{c}	45.11^{b}	41.71^{c}
Treatment × seasonality	3.09	38.50^{c}	65.68^{c}	11.31^{b}	63.86^{c}
Exoproteolytic activity					
Treatment	0.58	0.15	129.80^{c}	1.06	42.25
Seasonality	26.14^{b}	128.01^{c}	1176.11^{c}	1054.51^{c}	733.16^{c}
Treatment × seasonality	13.63^{b}	16.19^{b}	91.41^{c}	28.47^{b}	94.80^{c}
Ammonification rate					
Treatment	7.91^{a}	13.61^{a}	33.47^{b}	0.01	42.90^{b}
Seasonality	34.67^{c}	18.42^{c}	6.38^{a}	17.56^{b}	31.39^{c}
Treatment × seasonality	17.94^{c}	6.41^{a}	4.28	1.19	4.73^{a}

[a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$

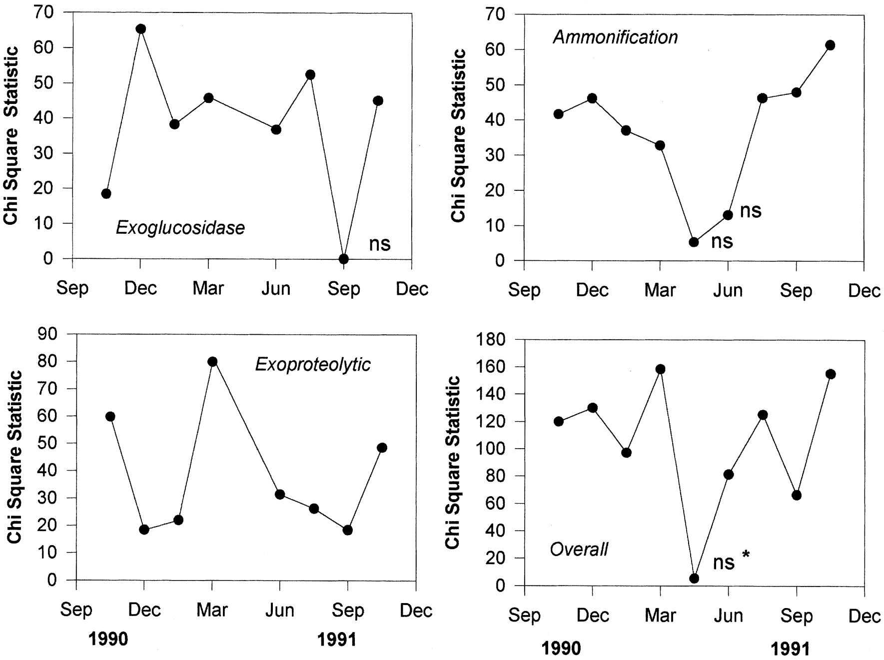

Fig. 2. Time course of the extent of the compound bacterial response to nutrient additions to sediments under five *Posidonia oceanica* meadows for individual descriptors of bacterial activity, or for a combination of all of the different estimates. Values on the y axis are the statistic $-2 \times \Sigma \ln P$, where P is the probability level of the individual t-tests (Sokal and Rohlf, 1969). Circles represent significant values (X^2 test, $P < 0.05$) and ns = non-significant values. *, This point only represents ammonification rates.

combined response of all three descriptors of bacterial activity) showed a maximum significance in March, when phosphorus was depleted in control sediments (Fig. 2). This pattern deviated from the fertilization enhancement of plant growth, which was highest in July–August (Alcoverro et al., 1997).

Despite the overall significant response of bacterial activity, the enhancement of EGA and EPA activity was, on average, small (Fig. 3). However, ammonification rates were substantially (two-fold on average, Fig. 3) enhanced by nutrient additions. These effects were greatest during summer and fall, reaching a five-fold increase in fertilized plots (Fig. 3). Bacterial activity in fertilized and control plots was, however, significantly ($P < 0.05$) correlated for all descriptors of bacterial activity, indicating that nutrient additions raised bacterial activity without altering the seasonal pattern.

The bacterial response to nutrient additions was partially direct and partially attributable to indirect effects that were dependent on plant production. While separation of these effects is not possible within the current experimental design, there was evidence that the increased bacterial activity, particularly the ammonification rate, in response to nutrient additions, increased with increasing seagrass production (Fig. 4, $P < 0.05$). The cumulative effects of the enhanced bacterial activity in response to nutrient enrichment is best represented by the progressive decline in the pool of organic

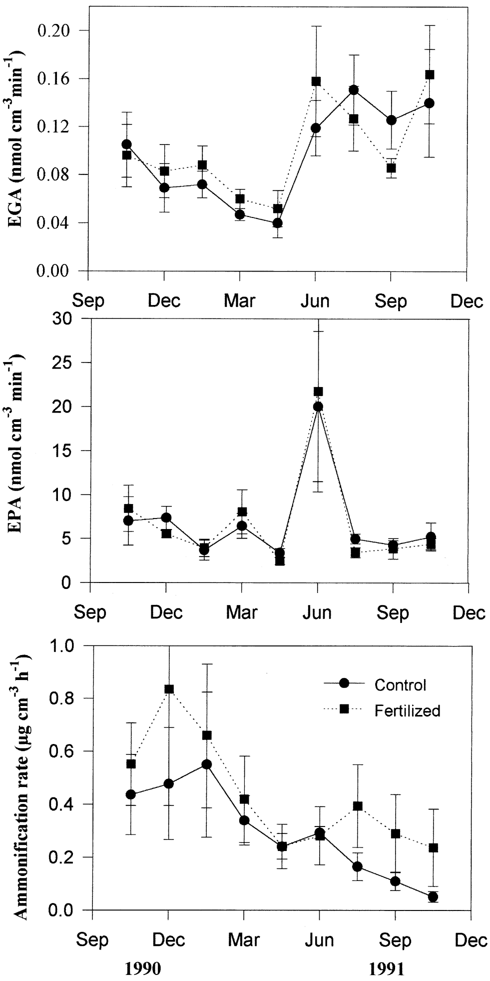

Fig. 3. Average seasonal time course of exoglucosidase activity (EGA), exoproteolytic activity (EPA) and the ammonification rate in fertilized and control plots in the seagrass sediments studied. Error bars represent the variation (±1 SE) among meadows.

matter in the sediments receiving nutrients (Fig. 5). A decrease in the organic matter content in fertilized compared to control plots was significant throughout the year in meadows at Blanes (rmANOVA, $P = 0.01$), Giverola (rmANOVA, $P = 0.0058$) and Deep Medes (rmANOVA, $P = 0.048$). No significant responses were observed at Shallow Medes and Port Lligat meadows (rmANOVA, $P = 0.266$ and 0.1166, respectively). The organic matter content at Blanes and Port Lligat meadows showed strong seasonal responses (Table 2). The response to fertilization varied seasonally at Blanes and Giverola meadows, as revealed by a significant interaction term between the addition of nutrients and seasonality in the rmANOVA analysis (Table 2).

The decrease in organic matter content is quite substantial (an average reduction of 33% in the large organic matter pool stored in the fertilized relative to an average

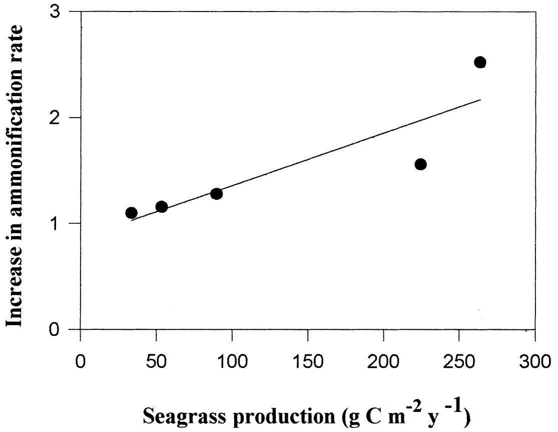

Fig. 4. Relationship between the average increase in ammonification rate (as the ratio of fertilized to control values) upon nutrient additions and the production of the five *Posidonia oceanica* meadows studied.

reduction of 14% in the control sediments), is significant (combined probability test, X^2, $P < 0.001$), despite the enhanced seagrass production in enriched plots (cf. Alcoverro et al., 1997). The decline in the organic matter of sediment indicates that nutrient addition increased the efficiency of bacterial processing of seagrass detritus. As a result, nutrient enrichment leads to a reduced capacity of the sediments to store carbon, despite greater carbon inputs derived from enhanced seagrass production.

The results presented support our previous suggestion that sediment nutrient availability limits bacterial activity in *Posidonia oceanica* meadows (López et al., 1995) and extend to these benthic systems the finding that bacteria are important nutrient consumers (Azam et al., 1983). The widespread occurrence of nutrient limitation in seagrass ecosystems, along with the low nutrient content of the detritus they produce, suggests that the interaction between seagrasses and benthic bacteria, and their possible competition for sediment nutrients, deserves closer attention.

Our results also show that increased nutrient loading to seagrass meadows may reduce their capacity to store organic matter, an important function of these ecosystems (Duarte and Cebrián, 1996), despite increasing the production of the seagrasses (Alcoverro et al., 1997). Our findings also showed that bacteria responded to increased sediment nutrients more than primary producers did (Alcoverro et al., 1997). The slow growth of *Posidonia oceanica* allows a remarkably efficient internal nutrient economy, which explains its capacity to reach the high primary production needed to support the abundant fauna and active sediment bacteria in the oligotrophic environments it inhabits. In contrast, bacteria must rely on those nutrients that are available, both organic and inorganic, in their environment to support their requirements.

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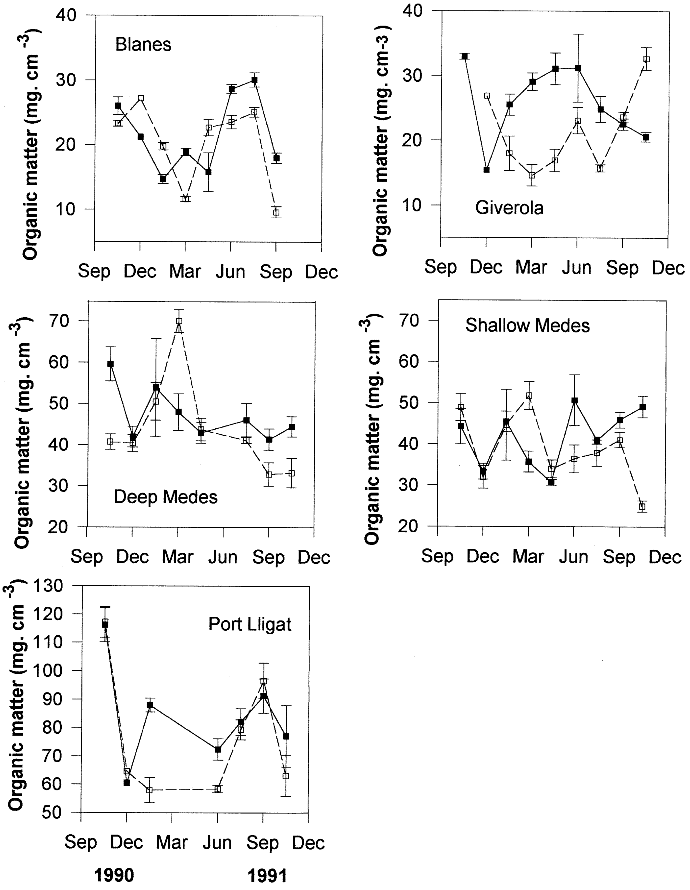

Fig. 5. Seasonal changes in organic matter content in control (solid line) and fertilized (broken line) plots at the five sites studied. Error bars represent the variation (±1 SE) among three replicated corers.

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Table 2
Summary of the rmANOVA (*F*-values) for organic matter content at the five meadows studied

Meadow	Blanes	Giverola	Shallow Medes	Deep Medes	Port Lligat
Treatment	20.13^b	28.80^b	1.66	7.94^a	3.98
Seasonality	44.99^c	3.14	2.87	4.40	33.34^c
Treatment × seasonality	13.22^b	10.64^b	3.50	3.16	3.79

^{a} $P < 0.05$, ^{b} $P < 0.01$ and ^{c} $P < 0.001$.

field, respectively. We would also like to thank Dr. Ana S. Haedo, for her advice on statistics, and two anonymous reviewers, who provided useful criticism.

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