Dimethyl sulfoxide (DMSO) reduction potential in Mediterranean seagrass (*Posidonia oceanica*) sediments

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Abstract

Microbial activity was assayed in sediments under five nutrient-limited *Posidonia oceanica* meadows on the north-east coast of Spain by measuring potential DMSO reduction to dimethylsulfide (DMS) throughout an annual cycle. Nutrient enrichment of the sediments was used to examine the importance of nutrient availability for potential DMSO reduction. DMSO reduction was observed in all the sediments analysed. Values were higher under anaerobic conditions, and low in spring when seagrass uptake removed most of the interstitial sediment phosphorus. DMSO reduction was correlated with α-glucosidase activity suggesting a link with other microbial activities. Nutrient additions significantly increased DMSO reduction in the meadows studied. The extent of the response varied substantially over the year and was highest during November–December. Microbial DMSO reduction was coupled with nutrient cycles like other bacterial activities. This suggests a possibly important role of nutrient additions in bacterial activity in Mediterranean seagrass sediments.
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Keywords: Dimethyl sulfoxide reduction; Dimethylsulfide; Microbial activity; Sediments; Seagrass meadows

1. Introduction

Organosulfur compounds play a key role in sulfur cycling in marine environments (e.g. Kiene and Visscher, 1987). Dimethylsulfide (DMS) is the dominant volatile compound involved in the sulfur transfer from sea to the atmosphere (Andreae, 1986, 1990; Kelly and Smith, 1990). Marine DMS production appears to be closely associated with primary production, deriving mainly from dimethylsulfoniopropionate (DMSP) produced by phytoplankton (Keller et al., 1989, Malin et al., 1992), macroalgae (White, 1982; Karsten et al., 1990) and angiosperms (Dacey et al., 1987; Pakulski and Kiene, 1992). Bacteria can produce DMS during decomposition of organic matter containing sulfur (Andreae, 1985) or by using dimethylsulfoxide (DMSO). DMS can be used by phototrophic purple bacteria as an electron donor for CO_2 fixation (Zeyer et al., 1987) and by some chemoorganotrophs and chemolithotrophs for energy metabolism (De Bont et al., 1981; Suylen et al., 1986).

In addition, DMSO can be used as a terminal electron acceptor and subsequently reduced to DMS (Zinder and Brock, 1978a), which can occur under both aerobic and anaerobic conditions (Jonkers et al.,

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1996; Griebler and Slezak, 2001). Biological reduction of DMSO to DMS has been observed in several environments, including anoxic salt marsh sediments (Kiene and Capone, 1988), *Sphagnum* peats (Kiene and Hines, 1995), soils (Alef and Kleiner, 1989), seawater and freshwater environments (Griebler, 1996, 1997; Griebler and Slezak, 2001). DMSO is present in several marine environments at concentrations higher than those of DMS (Simó et al., 1997, 2000; Hatton et al., 1999). In natural environments DMSO is formed abiotically from photooxidation of DMS in surface waters and the atmosphere (Andreae, 1980; Brimblecome and Shooter, 1986) and from anthropogenic activities. The photochemical removal of DMS to DMSO appears to be particularly important (Brimblecome and Shooter, 1986; Kieber et al., 1996; Hatton, 2002a). Biological transformations such as degradation of marine phytoplankton (Andreae, 1980), and microbial oxidation of DMS (Zeyer et al., 1987) also contribute to DMSO formation. Marine phytoplankton can also produce DMSO directly (Simó et al., 1998) and dark production has also been observed (Simó et al., 2000). More recently, DMSO production within sedimenting material has been reported (Hatton, 2002b).

DMSO reduction has been used as a measure of microbial activity in soil (Alef and Kleiner, 1989) and recently in seawater and various freshwater habitats (Griebler, 1997; Griebler and Slezak, 2001). However, there is a paucity of knowledge on potential DMSO reduction rates in important marine ecosystems, such as seagrass meadows. Seagrass meadows are important components of the coastal ocean, and are important sites for sulfur cycling (Hemminga and Duarte, 2000). Moreover, seagrass promotes microbial activity in the underlying sediments (Hemminga and Duarte, 2000), resulting in active cycling of organic materials in these ecosystems. Yet, unlike other coastal angiosperms, examination of the potential DMS, DMSP and DMSO transformation in these ecosystems is still lacking.

Here we evaluate the potential microbial reduction of DMSO and its response to nutrient inputs in seagrass sediments from the north-east coast of Spain. Seagrass production has been shown to be nutrient-limited on the north-east coast of Spain (Perez et al., 1991; Alcoverro et al., 1997) where bacterial activity, as assessed by e.g. exoenzymatic activities, shows substantial seasonal variation and is also nutrient limited (López et al., 1995, 1998). We examined the seasonal pattern of potential DMSO reduction in five *Posidonia oceanica* meadows and tested, through in situ nutrient enrichment of the sediment, its response to experimental nutrient additions.

2. Materials and methods

The study was conducted in five *Posidonia oceanica* meadows located between -5 and -13 m depth along the north-east coast of Spain. Three of the meadows (Port Lligat, Giverola and shallow Medes) were located towards the upslope limit of the plant distribution and the other two (deep Medes and Blanes) were closer to the lower limit of occurrence (Alcoverro et al., 1997). The five meadows were representative of a wide range of plant cover (78 to 627 shoots m^{-2}) and plant production, which was highest in early summer and lowest in winter (Alcoverro et al., 1995). This region is characterised by steep slopes, sandy sediments, and oligotrophic unproductive waters that support sparse phytoplankton populations (Masó and Duarte, 1989). Detailed accounts of sediment characteristics, bacterial activity, plant growth and nutrient dynamics in these meadows are reported elsewhere (Alcoverro et al., 1995, 1997; López et al., 1995, 1998).

Two 1-m^2 experimental plots were established at each site, one was used as a control and the other enriched with nutrients at intervals of about $40–50$ days over one year (8 sampling events from October 1990 to November 1991). Sediments at the treated sites were enriched at each visit by inserting 30 slow-release fertiliser sticks, representing a loading of about 6.5 g N m^{-2} and 0.89 g P m^{-2} at each sampling event. Laboratory experiments showed an average release rate of 2% of the added nutrients per day (Agawin, 1995). This rate exceeds calculated average daily seagrass requirements (Alcoverro et al., 1997) at least 10 times and is similar to the loading rates found in heavily eutrophied coastal waters (Borum and Sand-Jensen, 1996). The input N: P atomic ratio ensures balanced bacterial (Thingstad, 1987) and seagrass growth (Duarte, 1990).

Sediment samples, consisting of three replicated corers each (5 cm internal diameter), were taken from

the 0–5 cm surface of the sediments in each plot at each sampling event prior to the addition of fertiliser sticks and kept refrigerated until processed (within 24 h). In the laboratory, the corer was extruded and the upper 2 cm of sediment were sliced off. Subsamples of this sediment fraction were used to determine nutrient concentrations, organic matter content and DMSO reduction. Nutrient concentrations were measured in pore water extracted from the supernatant of centrifuged subsamples (3000 g, 10 min) kept frozen until analysed. 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). Sediment water and organic matter contents were measured after desiccation (24 h at 105 °C) and heating (2 h at 450 °C) of the fresh and dried sediments, respectively.

Potential DMSO reduction rates were assayed following the procedure of Alef and Kleiner (1989) modified for marine sediments. At the beginning of the study, experiments were conducted to assess the saturating concentration of DMSO, the linearity of DMSO reduction rates over time, and the optimal incubation time. The experiments were then conducted by adding DMSO at a final concentration of 2% vol/vol (280 mM) to sediment slurries, prepared by mixing a fresh sediment subsample of about 1.5–2.0 g with 0.5 ml of 0.2 μm (Millipore) filtered seawater, in triplicate 25 ml sterile flasks. Special care was taken to avoid the possible production of DMS by cut roots during preparation of the slurries (Howes et al., 1985). DMSO can be used as an

electron acceptor in respiratory processes, so that DMSO reduction rates may differ depending on the oxygen conditions in the sediments (Alef and Kleiner, 1989; Griebler, 1997). Accordingly, we determined potential DMSO reduction rates under both aerobic and anaerobic conditions, since sediment conditions range between these extremes from the sediment surface to the sediment interior. To ensure anaerobic conditions, sediment subsample flasks were encapsulated, bubbled for 10 min with He, and then tested by examining the resulting gas in the headspaces of the flasks. Sediment subsamples were incubated overnight at 30 °C with shaking. Controls were run using the same procedure with autoclaved sediment samples. A 100 μl subsample of the gas in the bottle headspace was run in a DANI HR gas chromatograph equipped with a flame ionisation detector. We used a 30 m long FSOT column, 0.25 μm film thickness (model DBFFAP, J. and W. Scientific), with He as the carrier gas. Chromatography was conducted with an injector temperature of 270 °C, detector temperature of 280 °C, and oven temperature of 60 °C isotherm, and gas flow of 2.2 ml/min for the carrier He, and N_2 make up 60 ml/min, H_2 at 60 ml/min, and air at 250 ml/min. DMS eluted, under this conditions, after 2.2 min, as confirmed with DMS (Merck) used for calibration, and the concentration was calculated using a Shimadzu CR-1A integrator.

Exoenzymatic bacterial activity was represented by α-exoglucosidase (EGA) activity as previously described (López et al., 1995). The measurements were conducted on the supernatant (700 g for 5 min) of a slurry prepared suspending 2–3 g of sediment subsample in 50 ml of filtered (0.2 μm Nuclepore filter)

Table 1
Annual average ($\pm$ SE) of sediment organic matter, nutrient concentration, exoglucosidase activity, and the overall percent variation in response to nutrient addition at the five meadows analysed ($-$ decline, $+$ increase)

Variable	Blanes	Giverola	Shallow Medes	Deep Medes	Port Lligat	Average of variation in response to fertilisation (%)
Organic matter (mg cm^{-3})	21.7 ± 2.08	25.9 ± 1.92	41.8 ± 2.36	47.3 ± 2.27	83.9 ± 6.63	-89
Dissolved inorganic nitrogen (μM)	283.5 ± 109.9	137.6 ± 57.3	188.4 ± 60.6	234.0 ± 62.4	334.7 ± 75.3	$+121$
Phosphate (μM)	2.64 ± 1.17	4.98 ± 1.87	20.26 ± 9.29	12.66 ± 3.90	16.13 ± 5.67	$+187$
α-Glucosidase activity (nmol cm^{-3} min^{-1})	0.068 ± 0.014	0.093 ± 0.022	0.096 ± 0.016	0.101 ± 0.016	0.126 ± 0.031	$+103$

seawater, and stirred at 30 °C for 1 h prior to addition of the 4-methylumbelliferyl-α-D-glucoside (20 μM, Sigma M-9766) as substrate. Fluorescence was measured with a Shimadzu RF-540 spectrofluorometer (excitation and emission wavelengths, 340 and 410 nm, respectively). All values measured were expressed as per cm^3 of sediment to avoid spurious correlations

between them (Bird and Duarte, 1989). The standard error of DMSO reduction measurements involving triplicate series of initial and incubated samples was calculated using bootstrap techniques (Efron and Tibshirani, 1986). Values were calculated using 100 bootstrap iterations. The meadows studied were specially selected to represent a range of conditions
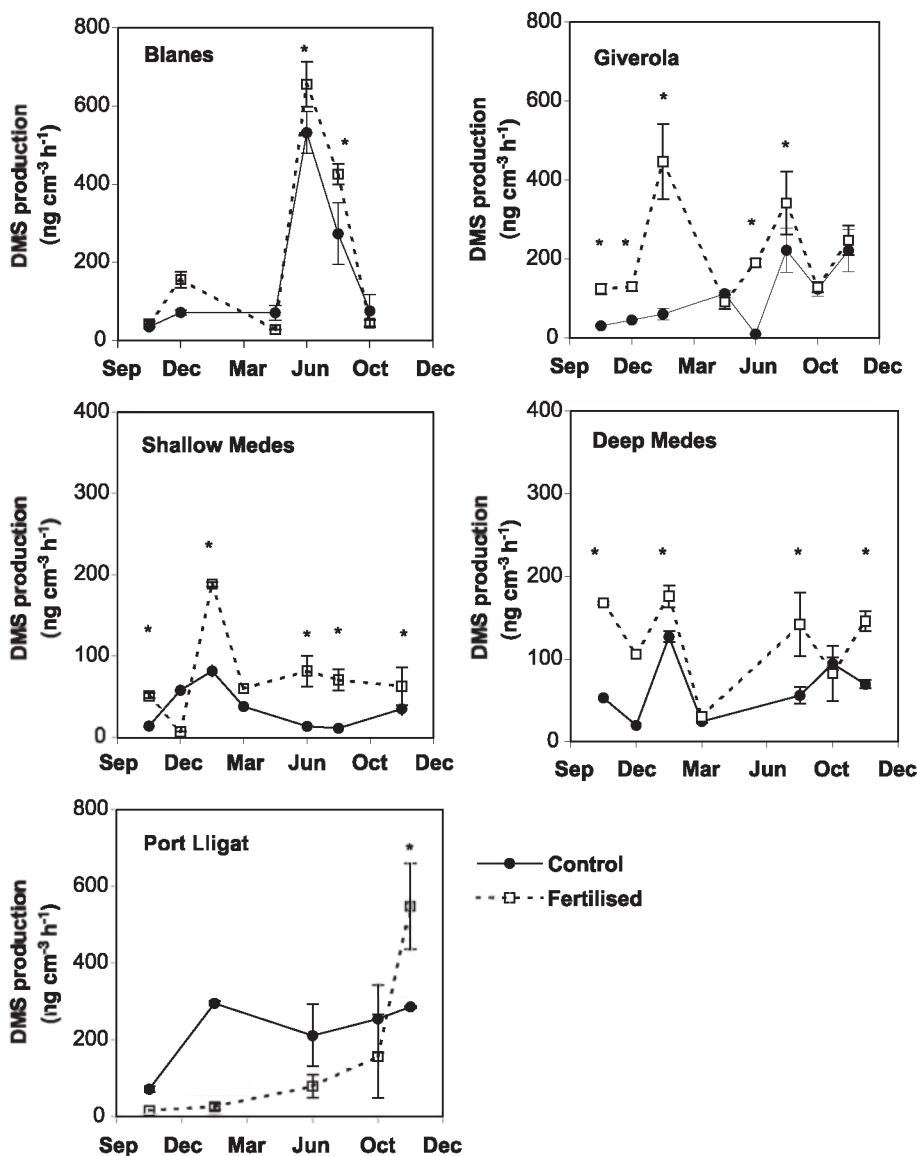

Fig. 1. Seasonal pattern of aerobic DMSO reduction in control and fertilised plots at the five sites studied. Values represent mean (± SE) for triplicate samples. Asterisks indicate significant differences (P < 0.05) between fertilised and control plots at each sampling event as determined by comparisons after rmANOVA.

encountered in this region. Yet, the experiments conducted in each meadow were independent of one another, and hence the five meadows analysed do not represent 'replicates'. Because consecutive observations in the control and fertilised plots within each meadow were not independent of one another, we used repeated-measures analysis of variance

(Winer, 1971) to test for differences in DMSO reduction between fertilised and control plots for each meadow. All the analyses used the basic model statement: treatment, seasonality, treatment $\times$ seasonality, with treatment representing the fixed variable and seasonality representing the repeated measure. Repeated-measured analysis of variance
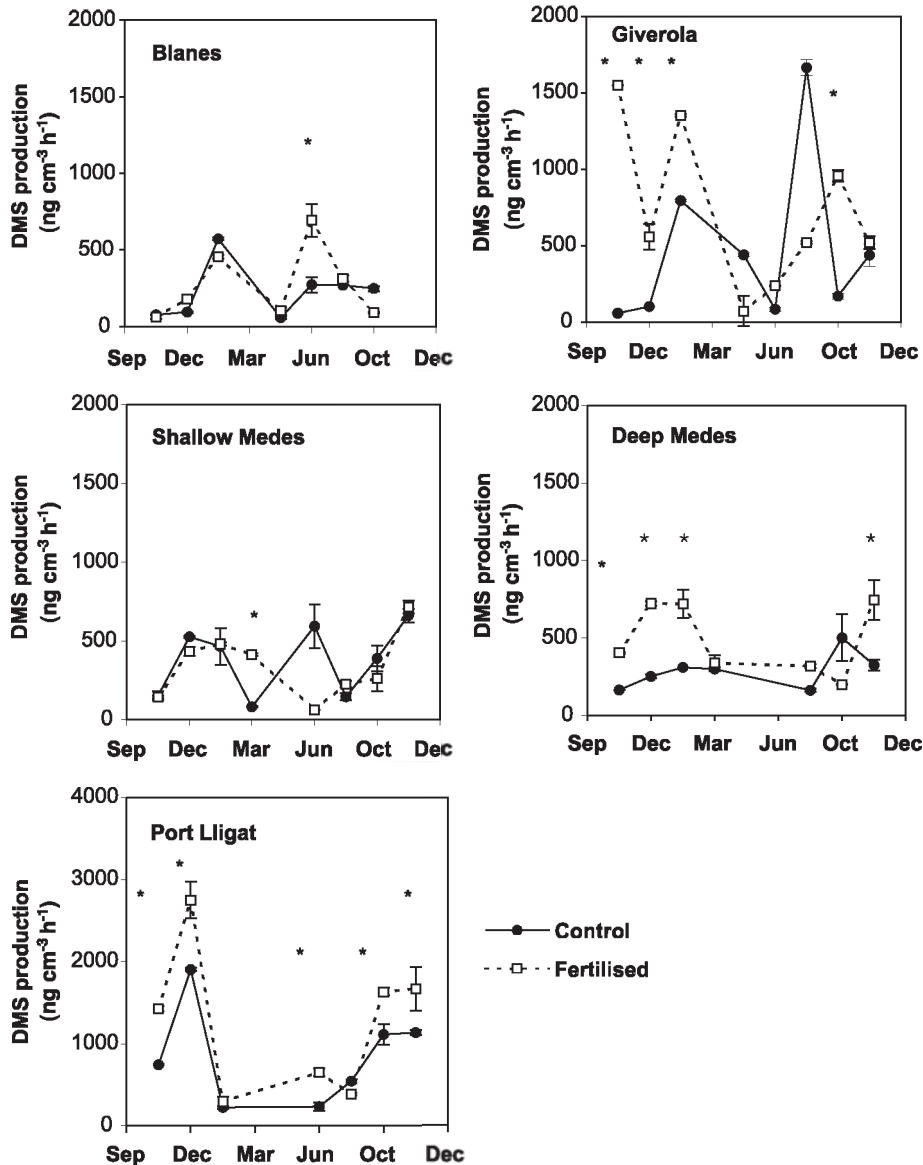

Fig. 2. Seasonal pattern of anaerobic DMSO reduction in control and fertilised plots at the five sites studied. Values represent mean ($\pm$ SE) for triplicate samples. Asterisks indicate significant differences (P < 0.05) between fertilised and control plots at each sampling event as determined by comparisons after rmANOVA.

(rmANOVA) was performed using BMDP statistical software (1993).

3. Results

3.1. Sediment characteristics and bacterial activity

The sediments of the meadows were dominated by fine to coarse sand with organic contents ranging between 14 and 116 mg cm^{-3} and low nutrient

Table 2
Summary of the rmANOVA (F-values) testing for significant effects on DMSO-reduction rates at the five meadows studied

Aerobic conditions

Meadow	Blanes	Giverola	Shallow Medes	Deep Medes	Port Lligat
Treatment	8.46*	21.01**	54.18***	386.81***	1.23
Seasonality	71.99***	10.04***	27.87***	10.55***	6.75**
Treatment × Seasonality	2.44		6.00***	12.76***	3.83**	5.49**

Anaerobic conditions

Meadow	Blanes	Giverola	Shallow Medes	Deep Medes	Port Lligat
Treatment	2.62	32.53***	0.31	16.45**	8.07*
Seasonality	19.78**	34.74***	8.16***	2.46*	43.39***
Treatment × Seasonality	5.09**	41.48***	3.75**	3.18**	2.48*

Asterisks indicate: *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

concentrations, and supported active bacterial populations, ranging 2–30 fold in activity across meadows (Table 1). Sediments of the Port Lligat meadow had the highest bacterial activity and highest organic matter content (Table 1). Nitrogen and phosphorus concentrations in sediment pore waters were highly variable across the meadows (Table 1). Seasonal changes in sediment nitrogen and phosphorus resulted in unbalanced N: P ratios especially in spring, at the onset of exponential seagrass growth (López et al., 1995). Nutrient additions stimulated bacterial activity and enhanced nitrogen and phosphorus concentration (Table 1), leading to a decline in sediment organic matter content (López et al., 1998).

3.2. DMSO reduction

Microbial DMSO reduction was observed in all the sediments analysed, and the potential rates observed were higher under anaerobic conditions. Potential DMS production ranged between 10 and 532 ng cm^{-3} h^{-1} and between 56 and 1903 ng cm^{-3} h^{-1} under aerobic and anaerobic conditions, respectively. Sediments of the Port Lligat meadows showed on average the highest potential DMSO reduction rates under both aerobic and anaerobic conditions (Figs. 1 and 2).

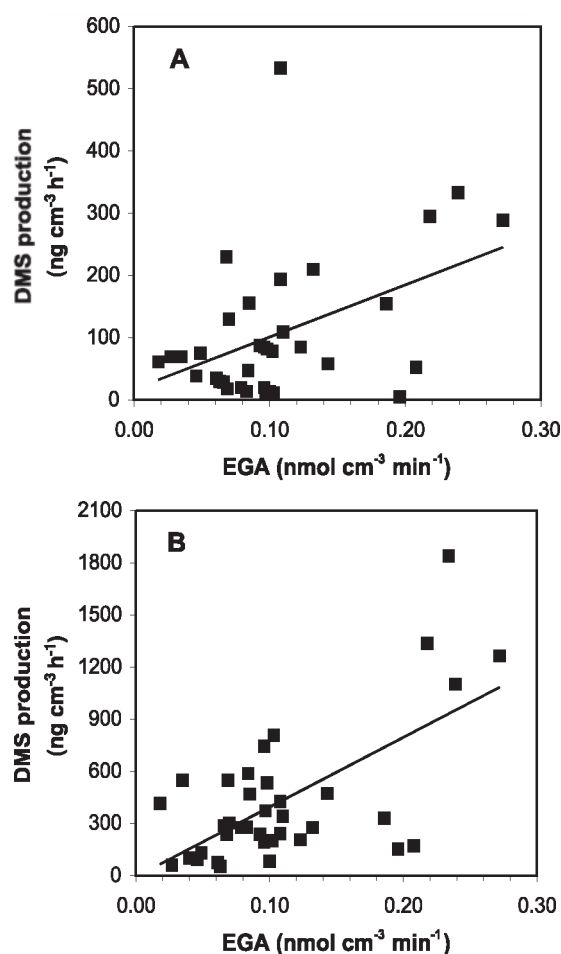The potential DMSO reduction showed an important temporal variation and a tendency to be lowest in

Fig. 3. Relationship between α-glucosidase activity (EGA) and DMS production for the overall data at the five meadows analysed. (A) DMS production in aerobic conditions ($y = 837.33x + 17.23$, $r = 0.44$, $n = 35$, $P < 0.01$). (B) DMS production in anaerobic conditions ($y = 4008.5x - 6.71$, $r = 0.64$, $n = 37$, $P < 0.01$).

spring (Figs. 1 and 2). Potential DMSO reduction rates were positively correlated with α-glucosidase activity under both aerobic and anaerobic conditions (P < 0.01, Fig. 3). Potential anaerobic DMSO reduction was positively correlated with the sediment organic matter content (Pearson correlation coefficient, P < 0.01).

3.3. Effect of nutrient additions on potential DMSO reduction

Nutrient additions significantly increased the potential aerobic DMSO reduction rates (except in the Port Lligat meadow), which also showed significant seasonality and fertilisation × time interactions (Table 2). The potential anaerobic reduction of DMSO was significantly positively affected by nutrient additions in 3 of the 5 meadows analysed, with a significant effect of seasonality and the fertilization × time interaction in all meadows (Table 2). The extent of the response to nutrient additions varied substantially over the year, as demonstrated by different significant effects of fertilisation in each meadow and each sampling event (Figs. 1 and 2). In general, the response was highest during November–December (Figs. 1 and 2).

4. Discussion

DMSO reduction rates have been shown to provide a sensitive and reliable estimate of microbial activity in freshwater environments, seawater, soils and sewage sludge samples (Alef and Kleiner, 1989; Sparling and Searle, 1993; Sklorz and Binert, 1994; Griebler, 1996, 1997; Griebler and Slezak, 2001). Here we show that the microbial capability to reduce DMSO to DMS is widespread in all the meadows analysed, which ranged broadly in sediment characteristics, organic matter content and plant productivity (López et al., 1995, 1998; Alcoverro et al., 1995, 1997). The analytical procedures to measure DMS have recently been improved (Griebler, 1997; Simó et al., 1998; Griebler and Slezak, 2001) relative to that we used in the present experiment (Alef and Kleiner, 1989), which may underestimate DMSO concentrations. Yet, the potential DMSO reduction rates in the seagrass meadows studied were high

relative to those reported in aquatic habitats (about 1.2 ng of DMS l^{-1} h^{-1} Griebler and Slezak, 2001) and similar to those found in freshwater sediments and soils (130–3000 ng of DMS g^{-1} h^{-1} Griebler, 1996, 1997; Alef and Kleiner, 1989). The potential DMSO reduction rates were on average highest in the Port Lligat meadow, which had reduced sediments with finer grain size and higher organic matter content than the other meadows (Alcoverro et al., 1995). An increase in DMSO reduction rates with decreasing grain size has also been reported for river sediments (Griebler, 1996).

The positive correlation between potential anaerobic DMSO reduction and organic matter content supports previous demonstrations of the importance of sediment organic matter in regulating bacterial activity (e.g., Meyer-Reil, 1987; Fabiano and Danovaro, 1998). The correlation between potential DMSO reduction and α-glucosidase activity observed in this work and previous records from aquatic habitats (Griebler, 1997; Griebler and Slezak, 2001) confirms that DMSO reduction is coupled with other microbial activities.

The concentration of DMSO in natural waters ranges broadly between 1 and 200 nM (Andreae, 1980; Kiene and Gerard, 1994). In western Mediterranean surface water, DMSO was the dominant dimethyl sulfur species in different location and seasons (Simó et al., 1997). Particulate matter, especially phytoplankton, is also an important source of DMSO in seawater (Simó et al., 1998; Lee and De Mora, 1999). Hence, the enhanced sedimentation rates reported within seagrass meadows (Gacia et al., 2002) suggest that DMSO reduction may occur naturally in the seagrass sediments examined, where sulfur cycling is intense (Hemminga and Duarte, 2000). However, the DMSO reduction rates reported here represent potential rates, which can be realised if sufficient DMSO is present in the environment. Although there are no reports on DMSO concentrations and reduction rates in seagrass meadows, DMSO is likely to be present there. Recent work in *Spartina alterniflora*-dominated salt marshes has demonstrated the presence of many DMSP degrading isolates able to reduce DMSO to DMS there (Ansede et al., 2001), and in addition the sedimenting material can be a source of DMSO (Hatton, 2002b) within the meadow.

Like other bacterial activities (López et al., 1995), the potential DMSO reduction showed considerable seasonal variation, being low in spring, when phosphate was removed from the sediment by seagrass uptake (López et al., 1995; Alcoverro et al., 1995). This is consistent with the reduced bacterial activity, as reduced exoproteolytic and exoglucosidase activities and ammonification rates, in these meadows following phosphorus depletion (López et al., 1995). Microbial transformations are believed to be the main loss mechanisms of DMSO in marine environments through its consumption or reduction to DMS (Lee et al., 1999). Our findings indicate that these losses occur through processes that take place under both aerobic and anaerobic conditions. A possible slow chemical reduction of DMSO to DMS at high H_2S concentrations has been reported (Zinder and Brock, 1978b). There are no data on H_2S concentrations in the seagrass meadows studied, but the predominance of anaerobic conditions that could deliver high H_2S concentrations was limited to a single site (Port Lligat). Although DMSO may not be the main DMS precursor in marine sediments, our findings demonstrate the potential capacity of DMSO reduction in microorganisms inhabiting these seagrass meadows.

The evidence of a relationship between nutrient dynamics and potential DMSO reduction rates was tested using experimental nutrient additions. The results support the hypothesis that increased nutrient inputs lead to DMSO reduction in NE Spanish seagrass meadows and substantiate previous results showing a positive response of other bacterial activities to experimental nutrient inputs (López et al., 1998). The increased bacterial activity, as reflected in increased potential DMSO reduction rates and increased exoenzymatic activities and ammonification rates resulting from increased nutrient inputs to these meadows (López et al., 1998), suggests that nutrient additions may increase the efficiency of bacterial processing of seagrass detritus, leading to the significant decrease in the sediment organic matter content (López et al., 1998), in spite of greater organic matter inputs derived from enhanced seagrass production with nutrient enrichment (Alcoverro et al., 1997; López et al., 1998). These results provide further evidence of the importance of nutrient supply as the main factor controlling biogeochemical processes in seagrass ecosystems, and suggest a possibly important role of nutrient additions in bacterial activity in Mediterranean seagrass sediments.

Efficient bacterial growth depends on the quality of the organic substrates available, and is highest whenever the relative nitrogen and phosphorus concentration in the organic matter approaches the Redfield ratio (106C: 16N: 1P, Thingstad, 1987). Bacteria in aquatic ecosystems can be consumers or recyclers of inorganic nutrients (Azam et al., 1983) dependent on the balance among C, N, and P in the organic matter used. Bacterial activity in seagrass sediments is expected to be influenced both by the production of the overlying macrophytes, which produce organic matter depleted in N and P (Alcoverro et al., 1997; Hemminga and Duarte, 2000), and the supply of nitrogen and phosphorus to satisfy bacterial nutritional requirements (López et al., 1995, 1998). The positive correlation between the potential anaerobic DMSO reduction rate and organic matter in the sediments explained the differences in potential DMSO reduction rate among the meadows examined. However, the enhancement of the potential DMSO reduction by nutrient inputs suggests that bacterial activity was nutrient limited within these meadows. Hence, the results presented here provide evidence that bacterial activity, as described by potential DMSO reduction rates in these meadows, depends on the supply of organic matter. However, the responses to experimental nutrient additions also reveal that the low quality of the organic matter available within *Posidonia oceanica* meadows leads to nutrient limitation of bacterial activity in these meadows.

Acknowledgements

anonymous reviewers for useful criticism. N.I.L. is a career investigator from CONICET.

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