Biogeochemistry (2012) 110:75–86
DOI 10.1007/s10533-011-9690-z

Influence of salinity on dimethyl sulfide and methanethiol formation in estuarine sediments and its side effect on nitrous oxide emissions

C. Magalhães · P. Salgado · R. P. Kiene · A. A. Bordalo

Received: 15 March 2011 / Accepted: 4 December 2011 / Published online: 21 December 2011

Abstract We investigated the regulatory effect of salinity on the production of dimethylsulfide (DMS) and methanethiol (MeSH) in estuarine sediments and the potential interactions with the nitrous oxide (N_2O) reductase step of the denitrification pathway. This was achieved by monitoring DMS, MeSH and N_2O accumulation in sediment slurries retrieved from a temperate estuary (Ave, NW Portugal). Treatments were performed with and without amendments of potential sulfur gas precursors, DMSP (0–50 μM) or methionine (0–500 μM) at different salinities (0, 15 and 30 ppt). Experimental increases of salinity inhibited DMS accumulation under both oxic and anoxic incubation conditions, and the pattern was observed whether DMSP or methionine was added or not, i.e. lower salinities stimulated DMS net production. In contrast, MeSH tended to accumulate to higher concentrations in higher salinity treatments (15 and 30 ppt). Our results also suggest that while salinity had a direct influence on N_2O accumulation, it also may modulated N_2O production through its regulatory effect on the formation of MeSH, a compound previously shown to inhibit N_2O reduction activity. Overall, our results suggest that changes in salinity may have an important regulatory role in net production of DMS, MeSH and N_2O and their potential emissions to the atmosphere.

Keywords DMS · Methanethiol · Nitrous oxide · Salinity effect · Estuarine sediments

C. Magalhães (✉) · P. Salgado · A. A. Bordalo
CIMAR/CIIMAR—Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas, no 289, 4050-123 Porto, Portugal
e-mail: cmag@icbas.up.pt

R. P. Kiene
Department of Marine Sciences, University of South Alabama, LSCB 25, Mobile, AL 36688, USA

R. P. Kiene
Dauphin Island Sea Lab, Dauphin Island, AL 36528, USA

A. A. Bordalo
Laboratory of Hydrobiology, Institute of Biomedical Sciences, University of Porto (ICBAS-UP), Largo Prof. Abel Salazar no 2, 4099-003 Porto, Portugal

Introduction

Organic sulfur compounds are widespread in aquatic environments. These compounds are released to the external environment by a variety of processes like the death of organisms, physiological stress and cell senescence (c.f. Kiene et al. 2000; Stefels 2000). Once released into the dissolved phase, organic sulfur compounds rapidly disappear because of microbial degradation (Kiene and Bates 1990; Kiene et al. 2000; Kiene and Linn 2000a, b). In marine waters, organic sulfur compounds can satisfy a substantial part of the sulfur and/or carbon demand for many marine prokaryotes (Kiene et al. 2000; Zubkov et al. 2002).

Dimethylsulfoniopropionate (DMSP) is an organic sulfur compound that has been studied extensively because of its role as a precursor of dimethylsulfide (DMS) and methanethiol (MeSH) (Kiene 1996; Schlessinger 1997; Kiene et al. 2000). DMS emissions from marine environments make an important contribution to the global biogenic-sulfur flux to the atmosphere with potential impact on global climate (Charlson et al. 1987; Andreae 1990; Visscher et al. 2003; Vallina and Simó 2007). In addition to the production from DMSP, DMS can arise from the methylation of MeSH, which can be originated from the degradation of methionine, or via methylation of hydrogen sulphide (Kiene and Capone 1988; Visscher et al. 1996, 2003; Lomans et al. 1997, 1999; Kiene et al. 2000).

DMSP is produced by many groups of micro and macroalgae (e.g. Reed 1983; Stefels 2000; Van Alstyne et al. 2003), and by some salt marsh grasses (e.g. *Spartina* sp.; Otte et al. 2004). The physiological function of DMSP in the DMSP producers is still not well understood, and several roles for this low molecular weight organic compound have been proposed (Kirst et al. 1991; Sunda et al. 2002; Van Alstyne and Houser 2003; Fredrickson and Strom 2009). In some algal species DMSP functions as a compatible solute, allowing the algae to cope with osmotic changes (Kirst 1989, 1996). The effects of salinity on DMSP biosynthesis and on intracellular DMSP concentrations have been studied (cf. Stefels 2000), and these findings indicated that hyper- and hypoosmotic shocks might show a variety of results depending on the species studied and the experimental conditions (Vairavamurthy et al. 1985; Dickson and Kirst 1986; Van Alstyne et al. 2003; Stefels et al. 1996). While intracellular DMSP content increased with salinity in several micro- and macroalgal species (Vairavamurthy et al. 1985; Edwards et al. 1988), in other species, DMSP was only slowly produced or remained unchanged (Van Alstyne et al. 2003; Stefels et al. 1996). The same is true for a decrease in salinity, where rapid DMSP release from cells was observed in some species of microalgae (Dickson and Kirst 1986), and in others intracellular DMSP concentrations did not change during hypoosmotic shocks (Stefels et al. 1996). There are, however, limited data available on the environmental controls of the biological pathways of DMSP degradation and DMS/MeSH formation in natural systems (Visscher et al. 2003; Niki et al. 2007). The few studies accessible indicate that DMS production is a function of salinity, temperature and dissolved oxygen in hypersaline microbial mats (Visscher et al. 2003), and that salinity affects water column DMSP and DMS dynamics in a coastal environment (Niki et al. 2007). In our study, we hypothesized that because the formation of these volatile organic sulfur compounds are mainly regulated by biological pathways, environmental factors may exert strong constraints on the emissions/distributions of these compounds. We therefore focused on the impact of salinity changes on DMS and MeSH net production in slurries prepared from sediments collected along the salinity gradient of the Ave River estuary (NW, Portugal). Our experiments included non-amended sediments and sediments that were amended with DMSP or methionine. We used methionine as a main precursor for the production of the methylated sulfur compound MeSH (Kiene and Visscher 1987). While MeSH is a major thiol formed during the anoxic degradation of DMSP though the demethylation/demethiolation pathway, in our locations, the MeSH accumulation from DMSP degradation was found to be of minor importance (Magalhães et al. 2011a, b). Our previous studies demonstrated that MeSH interfered with the nitrous oxide (N_2O) reductase step of denitrification, potentially leading to the accumulation of a potent greenhouse gas in the atmosphere (Magalhães et al. 2011a). Thus, in this study we also evaluated how salinity could modulate N_2O fluxes through its effect on MeSH net production.

Methods

Description of study area and sampling

In September 2010, intertidal sediments from four locations in the Ave River estuary were collected during low tide along the estuarine salinity gradient (Fig. 1). The Ave estuary is located in the NW coast of Portugal in a highly urbanized area. As such it is impacted by anthropogenic inputs including urban and industrial effluents (Guerreiro and Pereira 2002). Physical and chemical characteristics of this estuarine system have been presented previously (Gonçalves et al. 1992; Soares et al. 1999; Díez et al. 2005; Magalhães et al. 2011a, b). At each of the four sites, a total of twenty cores (3 cm diameter and 8 cm long)

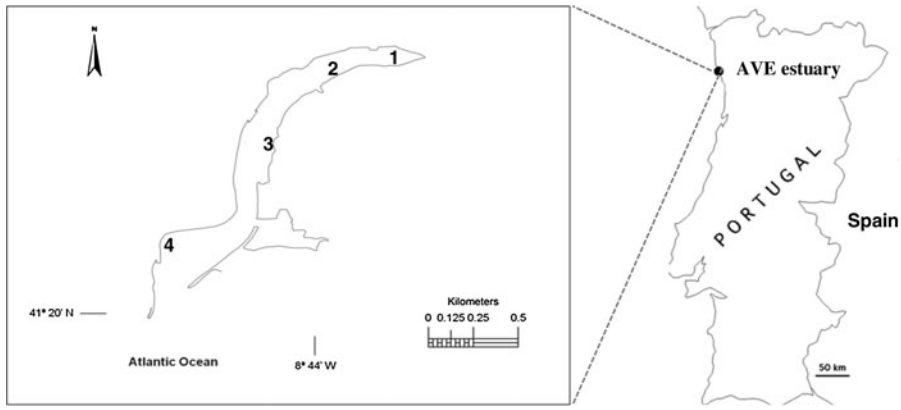

Fig. 1 Map of the Ave River estuary showing the location of sampling sites

were collected within 50 cm of each other, and all within approximately 3 m^2. The twenty cores collected from each site were volumetrically homogenized and used as a composite sample. At these same estuarine sites, 0.5 l of overlying water was collected in acid-cleaned polyethylene bottles. Additionally, 5 l of freshwater (0 ppt) was collected from the Ave River at a site upstream of Station 1. Samples were transported to the laboratory in the dark in refrigerated ice chests for later processing. In the laboratory, Ave River water (0 ppt) and overlying water from each site were immediately filtered (0.2 μm). A sub-sample of the filtrate was stored at −20°C for nutrient analysis; the remaining was stored in the refrigerator (4°C) for the slurry experiments. Sediment samples were stored at 4°C and processed within 2-4 days after collection according to the slurry experiments described below.

Slurry experiments

Sediment slurries were prepared by mixing 5 g of wet sediment with 10 ml of overlying water in 30 ml serum bottles as described in previous studies (Magalhães et al. 2011a, b). To test the effect of salinity changes on DMS, MeSH accumulation, slurries were made with water of different salinities (0, 15 and 30 ppt), prepared by adding artificial sea salts to Ave River freshwater according to the formula of Cavanaugh (1975). For each salinity treatment (0, 15 and 30 ppt), a set of slurries was incubated in the dark, under oxic (air headspace) and anoxic (purged with N_2 for 15 min) conditions. Within those treatments, sets were incubated with no amendments, or with either methionine (5, 50 and 500 μM) or DMSP additions (5 and 50 μM). Each treatment was run in triplicate and slurries were incubated for 4 h at constant temperature (20°C) with rotary shaking (70 rpm). N_2O, DMS, MeSH and H_2S accumulations in the slurries were monitored at time 0 h (incubation started after purging with N_2) and time 4 h of incubation by headspace analysis according to Magalhães et al. (2011a, b). Potential chemical production of N_2O, DMS and MeSH was evaluated at Site 1, in parallel experiments where biological activity was blocked by the addition of $ZnCl_2$ (2.5 M final concentration).

Gas and nutrient analysis

Volatile sulfur gases (DMS, MeSH and H_2S) were separated with a Mega-Bore silica plot gas chromatography column at 189°C and detected with a pulse flame photometric detector (P-FPD) as described in Magalhães et al. (2011a). DMS concentrations were determined using DMSP standards converted to DMS by sodium hydroxide (NaOH) treatment (Kiene and Service 1991) and H_2S concentrations were determined by using standard solutions of sodium sulphide (Na_2SO_4) converted to H_2S by hydrochloric acid (HCl) treatment. MeSH concentrations were estimated using the standard curve for DMS since the slopes of the standard curves of MeSH and DMS have been previously demonstrated to agree (Kiene 1996). The detection limit for both DMS and MeSH was 10 nM and for H_2S was 2 nM. Dissolved concentrations of DMS, MeSH and H_2S were calculated from measured headspace concentrations and empirical solubility coefficients, taking into account variations of salinity (Douabul and Riley 1979; Przyjazny et al. 1983). Nitrous oxide was quantified using a Varian gas chromatograph (CP-3800) equipped with an electron-capture detector (ECD) with two Hay Sep D columns according to Magalhães et al. (2005). N_2O

concentration was calculated using a standard curve generated from certified gas standards (N_2O in He, Scott Specialty Gas). Water column NH_4^+ and NO_2^- were quantified using methods described in Grasshoff et al. (1983). NO_3^- was assayed using an adaptation of the spongy cadmium reduction technique (Jones 1984), with the NO_2^- value subtracted from the total.

Sediment characteristics

DMSP concentrations were measured in triplicate sediment subcores (1 cc) from each sampling site, by cold alkali treatment (White 1982) and the amount of DMS released was measured by gas chromatography as described above. For sediment samples, it remains unclear whether DMS released with strong alkali represents exclusively DMSP or whether other potential precursors could contribute to DMS release (Kiene 1996). For simplicity, we refer to the alkali-labile DMS in sediments as DMSP. Chlorophyll *a* (Chl *a*) extraction from sediments was performed in triplicate subsamples with a mixed solution of acetone, methanol and water (45:45:10) according to Joye et al. (1996). Chl *a* concentration in the extracts was determined spectrophotometrically (Strickland and Parsons 1972). The percent organic matter content of sediment was determined by drying the sediment at 60°C to a constant weight, followed by ignition in a muffle furnace at 550°C for 4 h and reweighing.

Data analysis

Data analysis was performed at the 95% confidence level ($P < 0.05$). Data were tested for normality, using the Kolmogorov–Smirnov test and homoscedasticity, using the Leven's test (Zar 1996). In order to compare the means of each parameter between the different treatments, a one-way ANOVA Tukey's post hoc test analysis was performed (Zar 1996).

Results and discussion

DMS and MeSH production

In this study, DMSP concentrations were measurable at the four estuarine sites ranging between 2.6 ± 0.6 and 35.5 ± 6.7 μmol L^{-1} wet sediment. These values are within the range found in previous studies (Nedwell et al. 1994; Jonkers et al. 1998; Yoch 2002; Magalhães et al. 2011b). The concentrations of DMSP in the sediment correlated with sediment Chl *a* ($r = 0.97$, $p < 0.05$; data from Table 1), a pattern also observed in other marine systems and consistent with an algal source for DMSP (e.g. Reed 1983; Stefels 2000; Van Alstyne et al. 2003). Our results show higher rates of net DMS production at sites where higher DMSP concentration were measured (Table 1; Fig. 2), an observation that is likely due to the fact that DMSP is a major precursor of DMS (Kiene and Bates 1990). Steady-state concentrations and fluxes of DMS and MeSH in the sediment and surface waters depend on complex microbial formation and degradation processes (Kiene 1996; Lomans et al. 1997; Kiene et al. 2000), as well as on complex physicochemical interactions between the different natural elements (Mopper and Taylor 1986). In the sediment slurries from the Ave estuary, the production

Table 1 Physical and chemical characteristics of sediments and overlying water at the sampling sites in the Ave estuary

Dates	Sites code	Characteristics of overlying water				Characteristics of sediments				
		Salinity (ppt)	N inorganic (μM)			Chl *a* (mg g^{-1} wet sed)	DMSP (μmol L^{-1} wet sed)	OM (%)	Grain size (% dry weight)	
			NO_3^-	NH_4^+	NO_2^-				<63 μm	>2,000 μm
21 Sep 2010	1	2.1	160.3 ± 12.1	12.2 ± 0.7	6.0 ± 0.1	10.0 ± 2.1	16.0 ± 2.9	2.5 ± 0.2	8.3	15.5
21 Sep 2010	2	5.2	100.0 ± 3.1	12.5 ± 0.7	4.7 ± 0.0	7.1 ± 0.5	3.8 ± 0.5	2.3 ± 1.0	6.9	28.5
21 Sep 2010	3	12.4	77.4 ± 1.4	12.4 ± 0.8	3.7 ± 0.0	3.1 ± 0.3	2.6 ± 0.6	1.8 ± 0.2	3.2	20.6
21 Sep 2010	4	15.6	89.2 ± 4.0	8.1 ± 5.4	4.0 ± 0.1	18.9 ± 2.6	35.5 ± 6.7	1.2 ± 0.0	0.8	33.6

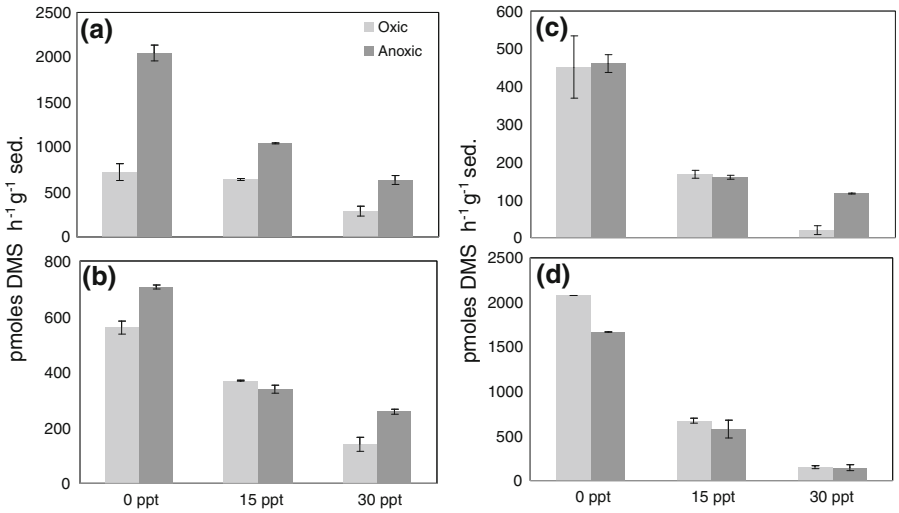

Fig. 2 Net DMS production rates in oxic and anoxic sediment slurries from sites 1 (**a**), 2 (**b**), 3 (**c**) and 4 (**d**) in the Ave estuary at three different salinities. For the salinity treatments, sediments from each site were slurried with either Ave River freshwater (0 ppt) or Ave River freshwater amended with different concentrations of sea salts to reach 15 and 30 ppt, respectively. The in situ salinities at each station are given in Table 1

of DMS always exceeded consumption, resulting in net DMS accumulation (Fig. 2). There were no significant differences in the magnitude of DMS accumulation between the incubations with and without oxygen (Fig. 2), contrary to what was observed in other investigations (Visscher et al. 2003). This finding suggests an important role of both aerobic and anaerobic metabolisms in DMS turnover by the communities inhabiting those sediments.

In contrast, MeSH accumulation was observed mainly in anoxic incubations, and only at the three stations furthest downstream (sites 2, 3 and 4). MeSH accumulations under oxic conditions were observed only at Station 4 and only at the two highest salinities

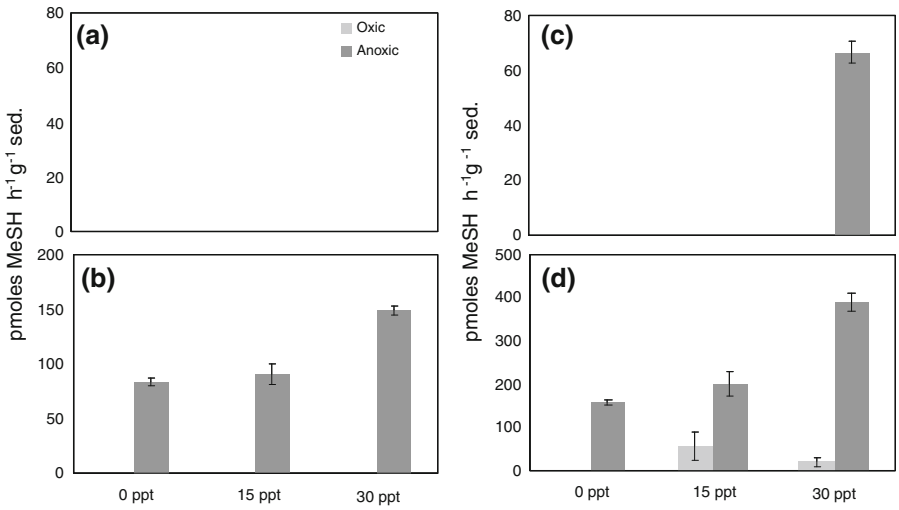

Fig. 3 Net MeSH production rates in oxic and anoxic sediment slurries from sites 1 (**a**), 2 (**b**), 3 (**c**) and 4 (**d**) in the Ave estuary at three different salinities. For the salinity treatments, sediments from each site were slurried with either Ave River freshwater (0 ppt) or Ave River freshwater amended with different concentrations of sea salts to reach 15 and 30 ppt, respectively. The in situ salinities at each station are given in Table 1. MeSH accumulation was not detected in any of the treatments in slurries from Station 1, the station with the lowest salinity

(15 and 30 ppt) (Fig. 3). In these cases, the MeSH accumulations under oxic conditions (from 19.5 ± 11.2 to 56.9 ± 32.8) were much less important than under anoxic conditions (from 157.3 ± 7.2 to 388.7 ± 19.9) (Fig. 3). MeSH net production was not observed at the uppermost station in the estuary, when incubations were performed with (air headspace) and without oxygen (purged with N_2) regardless the experimental salinity level used (Fig. 3). Actually, MeSH is likely to be subject to oxidation in the presence of O_2 (Suylen et al. 1987), but its oxidative metabolism is poorly understood. The absence of MeSH accumulation in oxic treatments has been suggested to be a result of rapid MeSH oxidation to dimethyl disulfide (DMDS) in the presence of oxygen (Visscher et al. 2003). Thus, it remains unclear whether lack of production or simply high rates of consumption were responsible for the minimal MeSH accumulations obtained. MeSH accumulation in anoxic treatments was not related to DMSP availability in the sediments (Table 1; Fig. 3) suggesting that other pathways of MeSH formation besides DMSP demethylation/demethiolation (Kiene 1996; Kiene et al. 2000) might be important. A multitude of MeSH production pathways may contribute to the MeSH fluxes in our anoxic incubations, including methylation of hydrogen sulfide (Mopper and Taylor 1986; Lomans et al. 1997, 1999), sulfide methylation by methoxylated aromatic compounds (Lomans et al. 2001) or by degradation of sulfur-containing amino acids (Lomans et al. 1997, 1999).

Salinity effect on DMS and MeSH potential emissions

A consistent salinity effect on DMS net accumulations was observed in the salinity manipulation experiments for all sites (Fig. 2). Higher DMS production rates were observed in freshwater treatments (0 ppt), while higher salinity treatments resulted in the lowest rates of DMS accumulation (Fig. 2). This was the case regardless of the in situ salinity of the station where the sediment was collected (Table 1). The pattern of DMS accumulation in the different salinity treatments was found to be coherent in the oxic and anoxic treatments (Fig. 2). The role of salinity in regulating DMS flux to the atmosphere as been identified in previous research performed on microbial mats grown in hypersaline ponds with different salinities (Visscher et al. 2003). Higher DMS net accumulation was found in a lower salinity pond (90 ppt) compared with a higher salinity pond (115 ppt) (Visscher et al. 2003). While such high salinities were not tested in our study, our results are in agreement with these previous findings, suggesting that lower salinities stimulated DMS production rates. Salinity fluctuations have a direct impact on intracellular DMSP concentrations of micro and macroalgae inhabiting intertidal estuarine environments, where the osmotic function of DMSP has been described (Edwards et al. 1988). Intracellular concentrations of DMSP tend to decrease in response to a decrease in salinity and DMSP biosynthesis increased in higher salinities (Vairavamurthy et al. 1985; Dickson and Kirst 1986; Edwards et al. 1988). Thus, we hypothesize that the rapid release of DMSP from cells at lower salinities may increase the availability of DMSP to the DMSP-degrading microbial communities, resulting in high levels of DMS accumulation. Niki et al. (2007) also identified salinity as an important factor controlling the production of DMS in the water column of a coastal bay of Japan. In agreement with our results, it has been found that low-salinity shock leads to an increase in potential DMS production to the environment (Niki et al. 2007). However, low salinity can also increase algal DMSP lyase activity (c.f. Stefels 2000; Steinke et al. 2002), which can increase the relative contribution of algal DMS production compared to bacterial production of DMS. Our data on net potential DMS productivity do not allow us to discriminate between algal versus bacterial DMS production, but in light of the high Chl *a* levels found (Table 1), the algal role in DMS production may be relevant in Ave estuarine sediments and should be considered in future work.

DMSP additions from 0 to 50 μM at different salinity treatments (Fig. 4) showed a progressive increase in DMS accumulation with the same pattern of salinity effects observed for the unamended sediments (Figs. 2, 4); i.e. higher rates of DMS accumulation in the freshwater treatments. Interestingly, DMS accumulation in methionine treatments followed the same pattern of variation observed for the DMSP additions; lower DMS production was registered at the higher salinity (30 ppm) comparing to freshwater treatments (data not shown). These results together suggest that salinity alone influences the degradation rates of extracellular DMSP and methionine. The inverse relationship between DMS accumulation rate

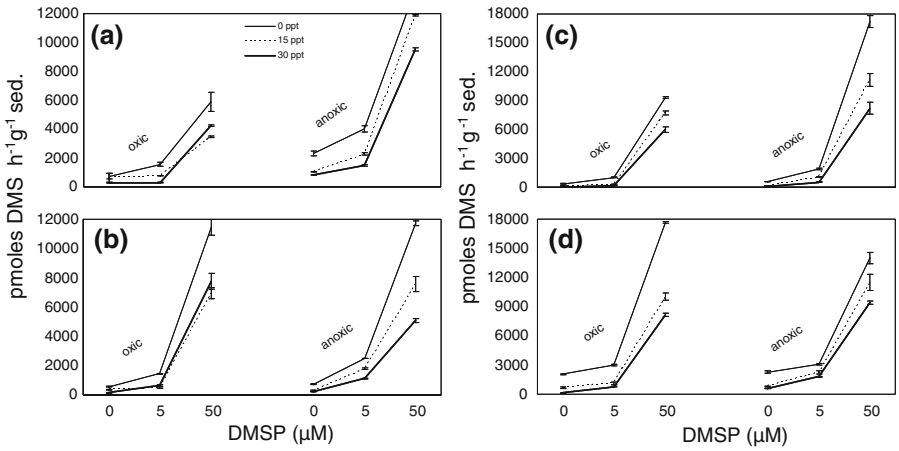

Fig. 4 The effects of DMSP additions and salinity treatments (0, 15 and 30 ppt) on net DMS accumulation rates in oxic and anoxic sediment slurries from sites 1 (**a**), 2 (**b**), 3 (**c**) and 4 (**d**) of the Ave estuary. For the salinity treatments, sediments from each site were slurried with either Ave River freshwater (0 ppt) or Ave River freshwater amended with different concentrations of sea salts to reach 15 and 30 ppt, respectively

and salinity can be attributed to either enhanced production of DMS from DMSP and methionine or suppressed decomposition of DMS at lower salinities.

Salinity also affected net MeSH production but in the opposite sense from that observed with DMS. MeSH accumulation was not detected at all Ave Estuarine sites, but when rates were measurable, higher values were registered for the higher salinity treatments (30 ppt) (Fig. 3). This reinforces the minor role of DMSP catabolism in the MeSH formation, already suggested by the observed absence of relationship between sediment DMSP content and rates of MeSH accumulation (Table 1; Fig. 2). In addition, rates of MeSH accumulation were higher for the highest salinities tested (15 and 30 ppt) in the anoxic treatments amended with methionine, except for the sediment from site 3 (Fig. 5a–d). These results are not in agreement with a previous study (Visscher et al. 2003), which found an increase in MeSH flux resulting from decreased salinity in hypersaline microbial mats. However, the similar regulatory effect of salinity on MeSH accumulation observed in slurries without and with added methionine (Figs. 3, 5a–d) suggests that sulfur containing amino acids can be potential precursors of MeSH in intertidal Ave estuary sediments. Other factors that promote MeSH accumulation in these sediments, such as higher sulfide concentrations and lower iron oxide availability (Mopper and Taylor 1986), cannot be excluded. Actually, in the higher salinity treatments, H_2S tended to accumulate to a greater extent than in the lower salinity treatments where lower magnitudes of MeSH accumulation were observed. These findings were consistent for all sampling sites (Fig. 6). Higher sulfide levels in more saline sediments would be expected due to the very high sulfate concentrations in seawater (Kiene and Capone 1988). In addition, the relationship observed between MeSH and H_2S accumulation rates at the different salinities tested ($R^2 = 0.9$, $n = 36$, $p < 0.001$; Fig. 6) provide evidences for an important role of H_2S methylation as a major mechanism of MeSH accumulation, already described as an important process of MeSH formation in freshwater sediments (Lomans et al. 1997).

Potential chemical production of MeSH and DMS

Potential chemical production of DMS and MeSH was evaluated in parallel experiments with DMSP and methionine additions, where biological activity was blocked by the addition of $ZnCl_2$ (Fig. 7). The inhibited slurries produced one to two orders of magnitude less DMS than non-inhibited slurries, and no signal of MeSH accumulation was ever observed in the $ZnCl_2$-treated slurries (Fig. 7). We checked whether $ZnCl_2$ interferes with DMS, MeSH and N_2O, and indeed results revealed that $ZnCl_2$ decreases DMS and MeSH concentrations in 13 and 35% respectively, while no binding effect was detected for N_2O. Although C_2H_2 bind to DMS and MeSH the high differences on DMS and MeSH accumulation rates observed between inhibited and non-inhibit

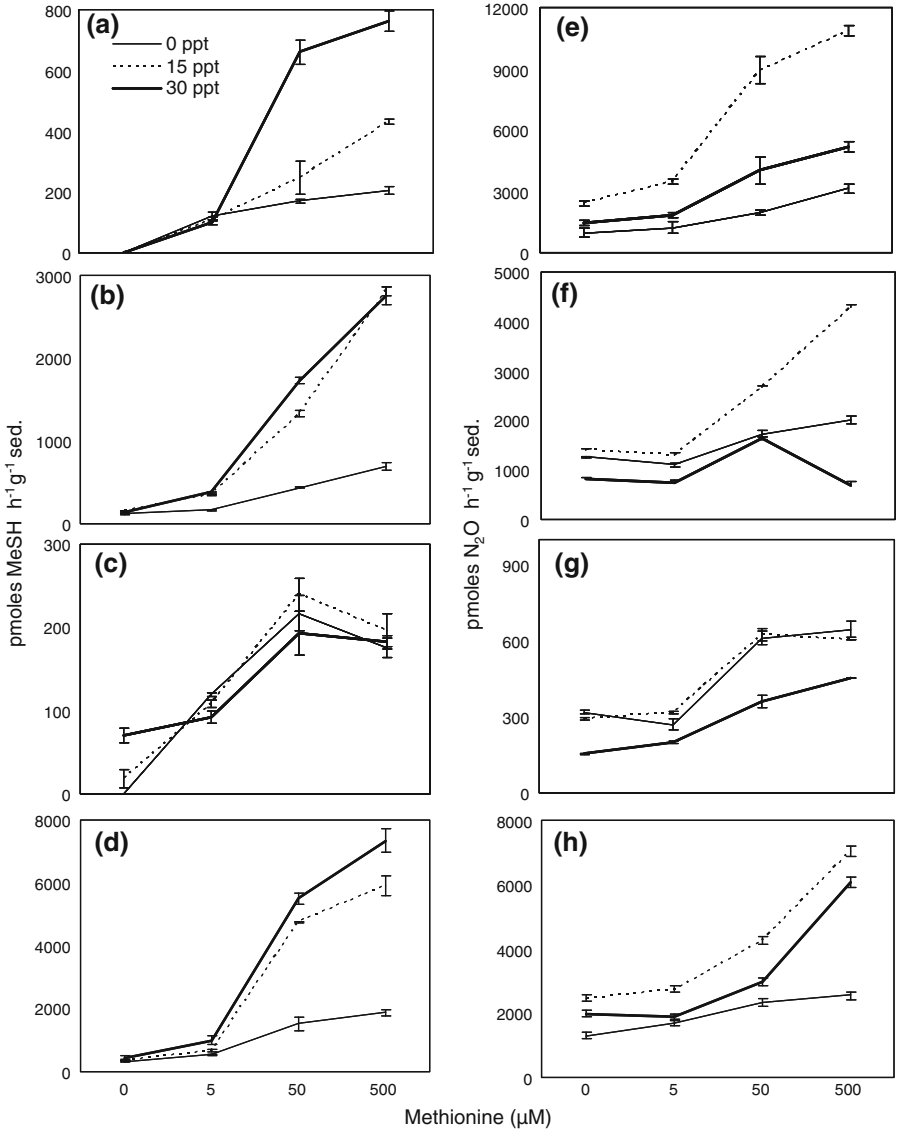

Fig. 5 The effects of methionine additions and salinity treatments (0, 15 and 30 ppt) on net MeSH and N_2O accumulation rates in anoxic sediment slurries from sites 1 (**a**, **e**), 2 (**b**, **f**), 3 (**c**, **g**) and 4 (**d**, **h**) of the Ave estuary. For the salinity treatments, sediments from each site were slurried with either Ave River freshwater (0 ppt) or Ave River freshwater amended with different concentrations of sea salts to reach 15 and 30 ppt, respectively

slurries (Fig. 7) still confirms the involvement of biological processes on the DMS and MeSH fluxes measured. In addition, different concentrations of artificial salt additions to freshwater may force pH to change and causing DMS and MeSH to react differently according to the salinity treatment. However, no differences in slurry pH resulted from the salinity treatments. This indicate a high buffering capacity of our estuarine sediments, which is in agreement with the absence of any significant differences in DMS and MeSH accumulation during the different salinity treatments in the $ZnCl_2$-treated slurries (Fig. 7).

Salinity effect on N_2O production during MeSH accumulation

In a previous study we demonstrated that MeSH interferes with the N_2O reductase step of denitrification (Magalhães et al. 2011b) and leading to the accumulation of N_2O and its potential emission to the

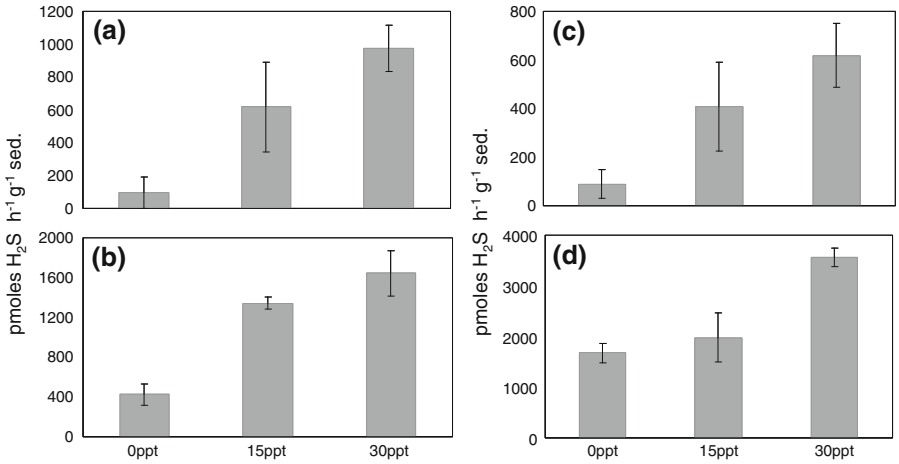

Fig. 6 Mean values of net H_2S accumulation rates in methionine treatments at different salinities from sites 1 (**a**), 2 (**b**), 3 (**c**) and 4 (**d**) of the Ave estuary. For the salinity treatments, sediments from each site were slurried with either Ave River freshwater (0 ppt) or Ave River freshwater amended with different concentrations of sea salts to reach 15 and 30 ppt, respectively

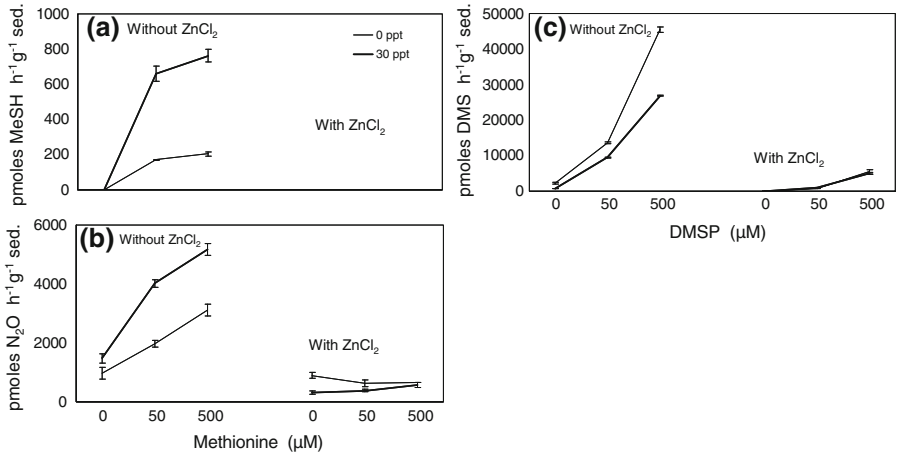

Fig. 7 Net MeSH (**a**), N_2O (**b**) and DMS (**c**) accumulation rates at site 1 anoxic sediment slurries treated without and with 2.5 M ZnCl (final concentration) at 0 and 30 ppt, amended with different Methionine and DMSP concentrations. For the salinity treatments, sediments of site 1 were slurried with either Ave River freshwater (0 ppt) or Ave River freshwater amended with different concentrations of sea salts to reach 30 ppt

atmosphere. In the present study, we hypothesized that salinity may modulate N_2O fluxes through its regulatory effect on the magnitude of MeSH accumulation. For sediment slurries incubated under anoxic conditions N_2O accumulated to a greater extent in the 15 ppt salinity treatment compared to the freshwater (0 ppt) treatment (Fig. 5e–h). Since MeSH accumulated to a much greater extent from methionine additions at higher salinities (Fig. 5), these findings are consistent with MeSH playing a role in the salinity effect on N_2O accumulation (Magalhães et al. 2011b). The interactions between salinity, MeSH and N_2O accumulation may be complex, as, we found that N_2O accumulation rates were lower at 30 ppt compared to those at 15 ppt (Fig. 5). We attribute these results to the direct effect of the higher salinity (30 ppt) in reducing potential N_2O net accumulation from non-amended slurries compared to 15 ppt slurries (Fig. 8). This was particularly evident at site 3 where no differences were observed in MeSH fluxes for different salinities tested, and lower N_2O accumulation rates were registered at the higher salinity treatment (Fig. 5g). N_2O accumulations measured in anoxic incubations (Fig. 5e–h) were likely to be a function of the rates of

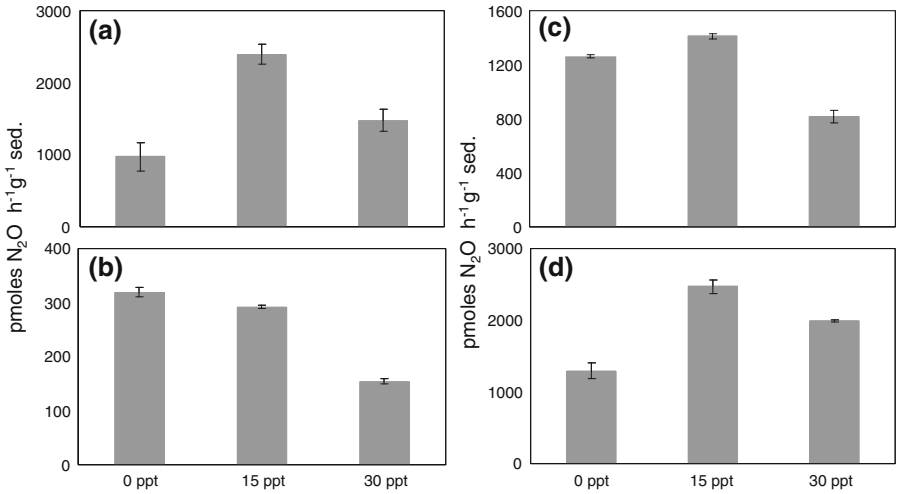

Fig. 8 Net N_2O production rates in anoxic sediment slurries from sites 1 (**a**), 2 (**b**), 3 (**c**) and 4 (**d**) in the Ave estuary at three different salinities. For the salinity treatments, sediments from each site were slurried with either Ave River freshwater (0 ppt) or Ave River freshwater amended with different concentrations of sea salts to reach 15 and 30 ppt, respectively. The in situ salinities at each station are given in Table 1

denitrification in our sediments. Previous studies evaluating the regulatory effect of salinity on denitrification pathway have found that tolerance to salinity may differ within denitrifier communities from different coastal and estuarine systems (Seitzinger et al. 1991; Rysgaard et al. 1999; Magalhães et al. 2005; Fear et al. 2005). For example Rysgaard et al. (1999) showed that denitrification decreased with increasing salinities in estuarine sediments where in situ salinities ranged from 3 to 13 ppt. However, Magalhães et al. (2005) reported that denitrification rates were independent of salinity in estuarine sediments exposed to a wide range of salinities (0–35 ppt). Other studies performed in estuarine sediments (Kana et al. 1998; Fear et al. 2005) indicated that denitrifying communities may adapt to the range of salinities inherent to a given ecosystem and that salinity becomes a driving force in selecting halotolerant or halophilic denitrifier communities.

MeSH is typically present at nanomolar to low micromolar concentrations in sediment pore waters (Mopper and Taylor 1986; Kiene 1991). In our experiments, N_2O started to accumulate at MeSH concentrations between 0.1 and 6.7 μM depending on the different sampling sites. This is in agreement with our previous study where we provide evidences that MeSH concentrations typically found in marine sediments can be sufficient to cause inhibition of the nitrous oxide step of denitrification (Magalhães et al. 2011a). In the present study, the magnitude of N_2O accumulation depended on the MeSH concentration, and generally lower MeSH concentrations yielded high N_2O accumulations. Thus, MeSH:N_2O ratios were always lower than one, while values vary according with the sites and the different salinity treatments; site 1 showed the lowest mean values of MeSH:N_2O (0.09 ± 0.05), while the other three sites presented higher MeSH:N_2O values (0.52 ± 0.21, 0.29 ± 0.12 and 0.59 ± 0.25, respectively for sites 2, 3 and 4). This variability could be attributed to the different sensitivities of the nitrous oxide enzyme from the different denitrifier communities to MeSH.

Conclusions

In this study we identified salinity as an important environmental factor regulating natural DMS and MeSH net accumulations in a temperate estuarine system. Our results indicated salinity changes affected the magnitudes of DMS and MeSH accumulations in an opposite pattern; while DMS production rates were stimulated in freshwater treatments (0 ppt), higher salinity treatments (15 and 30 ppt) resulted in higher rates of MeSH accumulation. These findings revealed that different communities/processes are implicated in the formation of these volatile sulfur compounds. In addition, salinity was suggested to be an important

factor modulating the previously identified inhibitory relationship between MeSH and nitrous oxide reductase enzyme activity in estuarine sediments (Magalhães et al. 2011a). Thus, salinity fluctuations that are typical of estuarine systems could play an important role in regulating DMS, MeSH and N_2O emissions to the atmosphere, with potential effects on the global climate balance (Dickinson and Cicerone 1986; Charlson et al. 1987; Visscher et al. 2003; Kiene et al. 2000; Vallina and Simó 2007).

Acknowledgments We thank T. Hollibaugh and W. Wiebe for their helpful comments on the results presented on this manuscript. This study was funded by the Portuguese Science and Technology Foundation (FCT) through PosDoc fellowships to C. Magalhães (SFRH/BPD/14929/2004, SFRH/BPD/76989/2011) and a grant to C. Magalhães (PTDC/MAR/098914/2008). Funding for participation of R. Kiene was provided by the NSF through grant OCE 0928968.

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