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Sulphate reduction in the root zone of the seagrass *Zostera noltii* on the intertidal flats of a coastal lagoon (Arcachon, France)

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ABSTRACT: Rates of sulphate reduction were determined in sediments from the eutrophic Bay of Arcachon, France. A comparison was made between sediment overgrown with the eelgrass *Zostera noltii* and unvegetated sediment. Rates of sulphate reduction were about twice as high in the root zone from 1 to 4 cm depth in the *Z. noltii* sediments (about 600 nmol cm^{-3} d^{-1}) as compared to the activity in the equivalent layer of the unvegetated sediment. The sulphate reduction in the root zone was not stimulated by light, which in other studies with other submersed macrophytes has been shown to promote sulphate reduction due to heightened excretion of organic molecules. Furthermore, in a field experiment, we were not able to detect any diurnal variation in the activity within the root zone. The stimulation of sulphate reduction by the presence of the *Z. noltii* was most likely due to degradation of leaf and root fragments which were found mixed into the sediment. Incubation times with radioactive sulphate had to be kept very short (preferably 15 min) to avoid a substantial reoxidation of sulphide and hence an underestimation of the rate of sulphate reduction.

KEY WORDS: Diurnal · Reoxidation · Root exudation · Excretion Oxygen · Photosynthesis · Regulation · Submersed macrophytes · Sulphate-reducing bacteria · Sulphide

INTRODUCTION

Seagrass meadows are among the most productive aquatic ecosystems and have been subject to intensive ecological investigations in the past (reviewed in Pollard et al. 1993). It has been suggested that the plants, by root excretion of organic compounds and oxygen, may have considerable impact on the activity and structure of the microbial community in the sediment (Smith et al. 1984, Moriarty et al. 1985, Moriarty et al. 1986, Caffrey & Kemp 1990, Pollard & Moriarty 1991), and several investigations have demonstrated a diurnal variation in the plant-microbial community interactions (Oremland & Taylor 1977, Caffrey & Kemp 1991, Koepfler et al. 1993), where sulphate reduction has also been found to vary through the day and night (Pollard & Moriarty 1991).

The loss of oxygen and organic compounds from the roots may have an influence on sulphate reduction. The presence of oxygen in the rhizosphere will enable aerobic bacteria to compete with sulphate reducers for organic substrates and consequently decrease rates of sulphate reduction. Oxygen can also oxidise reduced sulphur compounds, or it can oxidise ferrous iron and reduced manganese compounds which then subsequently can oxidise sulphide, and sulphate reduction measurements based on the accumulation rate of radiolabelled sulphide will then be more or less underestimated depending on the length of the incubation period (Jørgensen 1994, Moeslund et al. 1994). The organic exudates probably constitute a pool of easily degradable components. Blackburn et al. (1994) argued in favour of such a pool, as they observed a pronounced decrease in the accumulation of radiolabelled sulphide after an approximately 7 to 8 h incubation of oligotrophic sediment covered with the tropical submersed macrophyte *Halodule* sp.

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Sulphate reduction in seagrass beds has mainly been investigated in tropical or subtropical subtidal areas (Moriarty et al. 1985, Pollard & Moriarty 1991, Blackburn et al. 1994) and to our knowledge no studies have been made on the influence of the temperate seagrasses (in our study the intertidal *Zostera noltii*) on sulphate reduction.

The purpose of our investigation was to study in detail sulphate reduction in the root zone, focusing on the following questions:

(1) Are the measured sulphate reduction rates affected by the duration of the incubation due to a possible reoxidation of sulphide caused by oxygen-excreting roots (Sand-Jensen et al. 1982, Caffrey & Kemp 1991)?

(2) Will the sulphate reduction decrease in the dark, due to a decrease in availability of easily degradable organic matter when there is no photosynthesis (Smith et al. 1984, Moriarty et al. 1985, Pollard & Moriarty 1991)?

(3) Is the pattern of sulphate reduction rates obtained in laboratory experiments representative of *in situ* conditions where changes in temperature, oxygen tension, light, and tidal impact may be difficult to simulate?

MATERIAL AND METHODS

Study area. The study was conducted in the Basin of Arcachon (44° 40' N, 1° 10' W) near Bordeaux, on the southwest coast of France. The total area of the coastal lagoon is 155 km^2, but only 40 km^2 are subtidal.

Sediment cores were sampled in May and September 1994 from sampling Stn B during the third and the fourth joint campaign of the CLEAN project (Coastal Lagoon Eutrophication and ANaerobic processes) sponsored by the European Union 'Environment' programme. The investigated site is located in the inner part of the Basin of Arcachon close to the shore and near the banks of a small channel draining the flat. In the following, the *Zostera noltii*-covered part of the station is referred to as Stn BZ, while the area free of *Z. noltii* is called Stn B.

At high tide, the water level at Stns B and BZ varied between 30 and 150 cm and the stations were above the water level for 10 to 12 h daily (the hydrobiological parameters of the lagoon are described in Robert et al. 1987). However, while the sediment at Stn B was directly exposed to air at low tide, the sediment at Stn BZ was covered by a 0.5 to 1 cm thick layer of water that was retained by the collapsed canopy. Apart from small areas along the draining channels, the sediment was densely covered by *Zostera noltii* with roots penetrating 3 to 4 cm into the sediment. Root and leaf biomasses in the area have been determined to be 70 to 100 g dry weight (dw) m^{-2} and 70 to 160 g dw m^{-2}, respectively (Auby 1991). The sediment surface of both sites was inhabited by large numbers of mud snails (*Hydrobia* sp.).

Sampling and incubation. ***Laboratory experiments:*** A series of sediment cores was collected in 1 patch with and in 1 patch without *Zostera noltii* at the start of Expts 1 and 2 (see below); samples for Expt 3 were collected twice, as light and dark cycle incubations were carried out separately due to the limited size of the incubation aquarium. Cores were taken at low tide with Plexiglas tubes (90 mm o.d., 87 mm i.d., 200 mm long). Care was taken not to push leaves into the sediment during sampling. The tubes contained 4 vertical rows of silicone-rubber filled injection ports separated by an angle of 90°. The vertical distance between the ports in each row was 10 mm. By this arrangement, we ensured a good distribution of the injected radiotracers.

After sampling, the cores were stored in dark containers held near the *in situ* temperature and quickly driven to the laboratory. Before incubation in an aquarium held at the *in situ* temperature (19°C), the sediment surface was pushed up so that it was flush with the upper edge of the Plexiglas tube. The sediment surface was covered by 10 cm of water during the incubation, allowing *Zostera noltii* leaves to move freely. The position of each core in the aquarium was random to avoid systematic errors. The aquarium was filled with sea water taken from in front of the marine station (31‰ salinity). Water in the aquarium was mixed by bubbling with air. Two 500 W halogen lamps served as light sources. The irradiance (400 to 700 nm range) at the sediment surface varied between 150 and 220 µE m^{-2} s^{-1} among the different cores, depending on their position in the aquarium. This light intensity was in good agreement with the intensity measured in the field around noon under an equivalent cover of water (see below).

Before starting the experiments, cores for experiments in the light were preincubated in the dark for 12 h and cores for dark experiments were preincubated in the light for 12 h. The following 3 laboratory experiments were conducted:

Expt 1. A possible effect of the duration of the illumination period on sulphate reduction rate (SRR) was studied by sampling 5 cores with and 3 without *Zostera noltii* for each incubation period (0.25, 1, 5, and 10 h).

Expt 2: The effect of duration of dark incubation on SRR was performed using 4 cores with and 2 without *Zostera noltii* for each incubation period (1, 2, 4, 8, 14, 22 and 48 h).

Expt 3: The diurnal variation in SRR was studied by making determinations at different time points during

an artificial 12 h light cycle and 12 h dark cycle. SRR measurements were made every 3 h by $^{35}SO_4^{2-}$ incubation of 5 cores with and 3 without *Zostera noltii* for half an hour. The first light incubation was made immediately after the light period was started, and similarly, for the first dark incubation was made immediately after the dark peroid was started.

In situ experiments: In order to compare data obtained in the laboratory with data collected in the field, the experiments described below were carried out at Stn BZ. In the morning (08:00 h) at low tide, 5 Plexiglas tubes (described above) were carefully pushed into the sediment. For all *in situ* incubations, $^{35}SO_4^{2-}$ was injected into each sediment core from above with a syringe equipped with a 10 cm long needle, and after 1 h of incubation the cores were withdrawn and the incubations were stopped. A second series of measurements was carried out in the middle of the day (14:00 h), when the seagrass meadow was covered by 50 cm of water. The water cover made direct injection into the sediment impossible, and cores were therefore withdrawn prior to injection and replaced in the sediment. A third series of measurements was made in the late afternoon (18:00 h) at low tide.

A simultaneous dark incubation was carried out by covering 2 m^2 of seagrass meadow with a thin light-impermeable aluminium sheet from the morning and until the end of the experiment. Incubations for sulphate reduction rates in the dark under the aluminium were started at 18:00 h using the same injection technique as described above. The aluminium sheet was only loosely fastened to the sediment to avoid an extreme change of physical and chemical conditions.

Sulphate reduction rates. Sulphate reduction rates were determined by the radiotracer method (Jørgensen 1977, Fossing & Jørgensen 1989). Carrier-free $^{35}SO_4^{2-}$ (15 µl; <0.1 nmol of $^{35}SO_4^{2-}$; 150 kBq; Isotope Laboratory, Risø, Denmark) diluted in sea water was injected through the side ports into the sediment core, giving an average activity of 10 kBq cm^{-3}

At the end of the incubation, the upper 2 cm of the cores were cut into 2 sections of 1 cm each, and the zone from 2 to 6 cm was sectioned into 2 slices of 2 cm each. The sediment was transferred to plastic bottles containing 5 or 10 ml of a 5% (wt/wt) Zn-acetate solution for 1 or 2 cm sections, respectively, to stop biological activity and to preserve sulphide. The plastic bottles were closed with a screw cap and shaken vigorously to mix the sediment with the Zn-acetate solution. The weight of the sediment slice was determined and a subsample for sulphate analysis was transferred to a plastic screw-capped centrifuge tube and stored in a freezer until further processing.

The formation of $H_2^{35}S$ was determined by the 1-step Cr^{2+} distillation method (Fossing & Jørgensen 1989). Sulphate reduction rates (SRR) were calculated from the following equation:

$$SRR = \frac{(SO_4^{2-}) \times (H_2{}^{35}S) \times 1.06}{((^{35}SO_4^{2-}) + (H_2{}^{35}S)) \times T}$$

where (SO_4^{2-}) = sulphate concentration; $(H_2{}^{35}S)$ = total counts of the radiolabelled sulphide; $(^{35}SO_4^{2-})$ = total counts in the radiolabelled sulphate; T = incubation time; and 1.06 is the S^{32}/S^{35} ratio (Sorokin 1962). SRR is expressed in nmol cm^{-3} d^{-1}.

Physical characteristics. Porosity and specific density of sediments were determined on 10 cm^3 triplicate samples from each 1 cm depth interval down to 10 cm. The porosity was determined from the weight loss by drying at 105°C for 12 h. Profiles of redox potential and pH in the sediment were obtained by carefully pushing a needle E_h electrode and a glass pH electrode (Radiometer), respectively, into the sediment, with readings on a portable voltmeter being taken for each 1 cm interval.

Chemical determination. ***Sulphate in pore water:*** Pore water was obtained by centrifugation of Zn-acetate-preserved sediment samples taken during the radiotracer experiment. Sulphate was determined by suppressed ion chromatography with a Sykam (Gilching, Germany) ion-chromatography system, consisting of a pump (model S1110), a column oven (model S4110), a conductivity detector (S3110) and an autosampler (model 851-AS; Jasco, Tokyo, Japan). The stainless-steel column (4 by 125 mm; LCA A09, Sykam) was kept at 60°C. The eluent contained Na_2CO_3 (7.5 mM), ethanol (5%, vol/vol), and 4-hydroxy-benzonitril (50 mg l^{-1}). The flow rate was 2 ml min^{-1} and the analysis time was 5 min.

Sediment: The content of organic carbon and total nitrogen was determined on dried and homogenised sediment samples after treatment with H_2SO_3 to remove inorganic carbon. The samples (40 to 50 mg) were processed in an HCN analyser (Carlo Erba Na 1500 nitrogen analyser; Strumentazione, Rodano, Milano, Italy).

The content of roots and leaves was determined in 1 cm sediment slices (60 cm^3). The sediment slices were washed on a sieve (mesh size 1 mm), and the retained material, which was a mixture of roots, leaves, and shells, was transferred to plastic cups and dried at 105°C for 12 h. Shells and larger sediment particles were removed, and the sample was washed with a solution of HCl to remove smaller particles of carbonates. The weight of the remaining organic part of the samples (= roots and leaves) was subsequently determined after drying for 48 h at 70°C.

RESULTS

Field observations

Sediment from Stns B and BZ consisted of silty material and was rich in detritus and organic matter. The sediment surface was inhabited by large numbers of mud snails. Except for a thin brown layer at the top (<0.5 cm), the sediment from Stn B was black throughout the core and released a strong odour of hydrogen sulphide. The surface was locally coated by small patches of purple sulphur and colourless sulphur bacteria. Sediment from Stn BZ, in contrast, was grey and did not smell of hydrogen sulphide. These observations also coincided with differences in redox potentials and pH profiles (Table 1). At Stn B, the redox potential decreased very rapidly with depth from +116 mV in the upper centimetre to <0 mV at 2 cm depth. A similar drop was seen in pH: 7.6 in the upper centimetre and 7.1 at 2 cm depth and below. In sediments from Stn BZ, both redox potential and pH were higher as compared to Stn B. The redox potential was about +200 mV in the upper centimetre and decreased to +100 mV at 7 cm depth. The pH values were uniformly 7.7 to 7.6 in all the analysed layers down to 7 cm depth (Table 1).

The upper centimetre at Stn BZ consisted mainly of loose silty material and detached leaves, and in the 1 to 4 cm layer, the root density was high. The content of organic matter that could be retained by a sieve with a mesh size of 1 mm (roots and leaves) was 10 to 20 fold higher in the upper 1 to 4 cm layer of *Zostera noltii*-covered sediment (Stn BZ) than in the same horizons of sediment cores from Stn B (Table 1). The highest amount was found at 4 cm depth at Stn BZ (25 mg dw cm^{-3}). In the 5 to 6 cm depth interval, the quantities of retained organic material were similar at both stations (6 to 10 mg dw cm^{-3}) and consisted mainly of leaf fragments. The organic content determined with an HCN analyser was about 1.2 mmol C cm^{-3} in the upper 6 cm of both sediments (Table 1), except for a peak in the 1 to 2 cm depth interval at Stn BZ (1.7 mmol C cm^{-3}). The C/N ratio was lowest in the top centimetre at both stations, ranging from 11.9 at Stn BZ to 10.2 at Stn B (Table 1). At Stn BZ, the C/N ratio increased to 15.2 in the root zone, and finally decreased to approximately 13.0 underneath the root layer. At Station B, the C/N ratio increased to 13.2 at 4 cm depth and remained constant below that horizon.

Laboratory experiments

Expt 1. Under illumination, there was a significant decrease in SRR as a function of duration of the incubation with $^{35}SO_4^{2-}$ in the 0 to 4 cm layer of the sediment from Stn B, whereas no effect could be seen in the 4 to 6 cm layer (Fig. 1). The most pronounced effect was observed in the upper centimetre, where the mean SRR decreased from 455 nmol cm^{-3} d^{-1} (0.25 h incubation in light) to 231 nmol cm^{-3} d^{-1} (1 h incubation) and finally to 87 nmol cm^{-3} d^{-1} (5 h incubation), i.e. a drop to 20% of the activity from the shortest to the longest incubation. ANOVA also indicated a highly significant reduction of the activity as a function of incubation time ($F_{3,8}$ = 34.07, $p < 0.0001$). In sediments from Stn BZ, the heterogeneity of the sediment due to the plants caused large standard errors, and although the 0.25 h incubations seemed to give higher values throughout the root zone than longer incubation times, this was not statistically significant except for the 0 to 1 cm layer (ANOVA, $F_{3,16}$ = 3.31, p = 0.047).

Expt 2. When cores were incubated with $^{35}SO_4^{2-}$ in the dark, the length of the incubation period generally did not have any significant effect on SRR in sediment from Stns B and BZ; thus, exhaustion of electron

Table 1. Sediment parameters determined in cores collected at Stns BZ and B

Depth (cm)	With *Zostera noltii*					Without *Zostera noltii*				
	Redox (mV)	pH	Leaves and roots (mg dw cm^{-3})[a]	Org. C (µmol cm^{-3})	C/N	Redox (mV)	pH	Leaves and roots (mg dw cm^{-3})[a]	Org. C (µmol cm^{-3})	C/N
Water column	230	7.8				129	7.9			
0–1	215	7.7	10.2 (1.7)	1166	11.9	116	7.6	0.8 (0.5)	1217	10.2
1–2	165	7.7	14.3 (14.8)	1730	15.3	83	7.3	0.5 (0.5)	895	11.4
2–3	156	7.7	16.2 (11.3)	1288	15.1	−18	7.1	0.6 (0.1)	1240	12.2
3–4	128	7.7	25.1 (12.3)	1016	13.9	−95	7.1	3.0 (2.5)	1278	12.5
4–5	109	7.7	15.8 (7.9)	1215	12.8	−109	7.1	6.2 (0.8)	1296	13.3
5–6	106	7.6	11.3 (6.2)	1091	12.9	−120	7.0	9.8 (4.0)	1560	13.2
6–7	100	7.7	6.7 (8.1)	828	12.9	−140	7.0	10.9 (1.7)	1537	13.2
7–8	125	7.7		880	13.5	−127	7.0		1264	12.3

[a]SD in parentheses

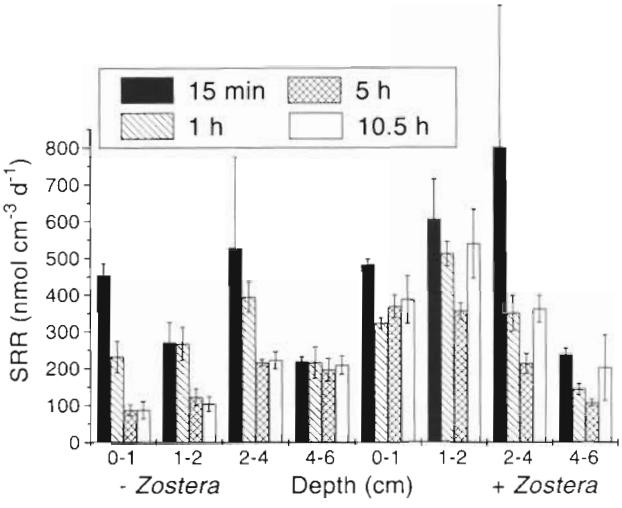

Fig. 1. Mean sulphate reduction rates (±SE) in sediment cores with (n = 5) and without (n = 3) *Zostera noltii*, incubated for 0.25, 1, 5, and 10.5 h in the light

donors excreted by the roots as a function of time after darkening did not seem to have an effect, even when the dark incubation was extended to 48 h (data not shown).

Expt 3. For the laboratory 12 h light-dark cycle experiment, the sediment cores were all incubated with $^{35}SO_4^{2-}$ for only half an hour to obtain the most realistic SRR. Statistical analysis (ANOVA) of the data showed SRR to be significantly affected by depth ($F_{3,283}$ = 23.8, p < 0.0001), station (± *Zostera*) ($F_{1,283}$ = 114.46, p < 0.0001), and day/night ($F_{1,283}$ = 6.36, p = 0.0122), whereas no effect of time during day or night could be detected ($F_{4,283}$ = 1.02, p = 0.3969) (Fig. 2). Hence, we pooled all data ignoring the time factor, so that only the effect of depth, station, and day/night on sulphate reduction is considered. It is notable that an increasing number of replicates (15 or 25 in Fig. 2 compared with the normal 3 or 5 replicates in Figs. 1 & 3) reduced the SE considerably. The rates measured in the surface layer and the root zone of the *Zostera noltii* bed (down to 4 cm) were about double the rates measured outside the vegetated area. Below the root zone, the SRR decreased and the mean rate for light and dark in the 4 to 6 cm depth interval was significantly lower than the rates determined in the overlying horizons (ca 27% of the mean rate in the 0 to 1 cm layer; Tukey *q*-test, p < 0.05). At this depth (4 to 6 cm), the mean rates from Stns BZ and B were comparable (188 and 172 nmol cm^{-3} d^{-1} for BZ and B, respectively). Except for the deepest layers, there was no significant difference between light and dark treatments, and there is no obvious explanation for our finding that the dark rates in the 4 to 6 cm layer were higher than the rates during illumination. The *in situ* incubation (see below) showed the opposite trend, and we think that the difference, although statistically significant (Stn BZ: Welch's approximate *t*-test, *t* = 3.6006, df = 31.1, p = 0.0011; Stn B: Welch's approximate *t*-test, *t* = 2.5691, df = 15.5, p = 0.0210), should not be considered real.

In situ experiments

The light intensity increased from approximately 100 µE m^{-2} s^{-1} at the start of the morning incubation (08:00 h) to about 500 µE m^{-2} s^{-1} at its end. From 10:00 to 11:30 h, the light intensity was high (700 to 1300 µE m^{-2} s^{-1}), but the shading effect of the incoming water during the tide decreased the light intensity reaching the *Zostera noltii* canopy drastically; it dropped from about 1000 µE m^{-2} s^{-1} before tidal water input to less than 100 µE m^{-2} s^{-1} under a water cover of 50 cm at the beginning of the mid-day incubation (14:00 h). During the second incubation period, the photon flux never exceeded 150 µE m^{-2} s^{-1}. The sediment temperature increased in the upper cm of the sediment from 20.5°C at 09:00 h to 23.5°C at 12:00 h and decreased again to 21.5°C at 18:00 h, and the temperature of the overlying water changed correspondingly. No temperature effect was caused by covering of the sediment with the light-impermeable aluminium sheet.

The rates of sulphate reduction in the upper 4 cm were about 30 to 40% lower than the corresponding data from the laboratory experiment (Fig. 2), but this can be explained by the use of a 1 h incubation period with radiotracer, which underestimates the SRR (Fig. 1). A much higher SRR was found in the upper 1 cm at noon (510 nmol cm^{-3} d^{-1}) as compared to the morning (268 nmol cm^{-3} d^{-1}) and afternoon (254 nmol cm^{-3} d^{-1}) values (Tukey *q*-test, p < 0.05), whereas no significant effect of time was observed in deeper layers

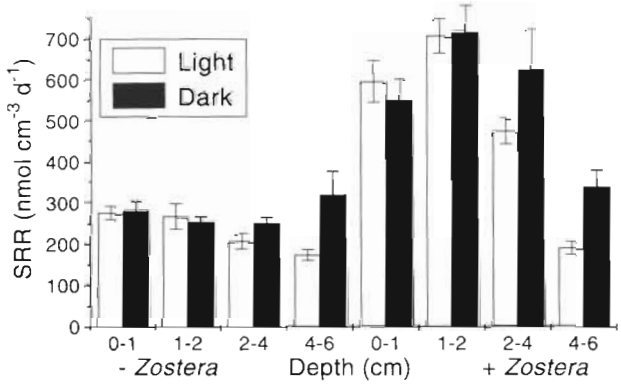

Fig. 2. Mean sulphate reduction rates (±SE) in sediment cores (n = 25) with and without (n = 15) *Zostera noltii*. For sulphate reduction rate determination, all samples were incubated for 0.5 h in the dark or light

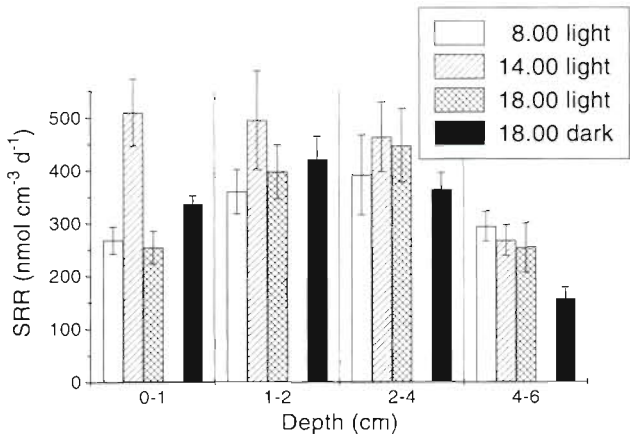

Fig. 3. *In situ* sulphate reduction rates (±SE, n = 5) in sediment with *Zostera noltii*. All samples were incubated for 1 h at different times of the day. The dark incubation was carried out in sediment covered by a light-impermeable aluminium sheet

(Fig. 3). The stimulation in the upper layer may have been due to a decreasing photosynthetic activity of the benthic microalgae community, lowering the penetration depth of oxygen and thereby lowering the reoxidation of reduced inorganic sulfur compounds (Moeslund et al. 1994 and Fig. 1 in this paper). Additionally, the 3°C increase in temperature could have been responsible for approximately 20 to 30% of the stimulation (Isaksen & Jørgensen 1994, Isaksen & Jørgensen 1996). For all the layers down to 4 cm, the dark values were similar to the mean of the light values. In the 4 to 6 cm layer, the rate in the dark was only 60% of those found in the light, which is exactly the opposite of the laboratory experiment results.

DISCUSSION

The presence of *Zostera noltii* obviously had a stimulating effect on the rates of sulphate reduction (Figs. 1, 2 & 3). The rates in the 0 to 4 cm layer with the roots were about twice as high as in sediments free of plant cover, whereas no significant difference could be found between sediments from Stn B and BZ below the root zone. The shape of the SRR profile (Stn BZ) is in good agreement with the distribution pattern of the sulphate reducer community, which has highest population densities (6×10^7 cm^{-3}) in the 1.0 to 1.5 cm depth interval (B. Schaub & H. van Gemerden pers. comm.).

Budgets for heterogeneous environments such as vegetated sediments should only be made with a large number of replicates. In this study we have analysed more replicates than in any previous study that we know of, but even so there were limitations concerning the statistical evaluation of data obtained in some of our experiments.

Reoxidation of hydrogen sulphide

In Expt 1 (Fig. 1) we investigated the influence of incubation time with radiotracer on the measured SRR. There was a significant decline of the measured SRR with time in the unvegetated sediment down to 4 cm depth, and although a similar trend was observed in the vegetated sediment, the heterogeneity in the analysed cores was so large that we do not have a statistically firm reason to argue that the same drop in activity occurred here. The drop in activity with incubation time must have been due to a reoxidation of sulphide by oxidised inorganic compounds such as $Fe(OH)_3$ and MnO_2, and in the vegetated sediments possibly also by oxygen excreted from the roots (Iizumi et al. 1980, Sand-Jensen et al. 1982, Smith et al. 1984, Caffrey & Kemp 1991). As the vegetated sediments did not have any sulphide smell and were found by redox potential analysis to be more oxidised than the unvegetated sediments, the reoxidation mechanism must have been at least as efficient as in the unvegetated sediments, but this was blurred by the heterogeneity. Below 4 cm depth, there seemed to be only little reoxidation at both Stn B and BZ.

The surface sediments in Arcachon were very rich in oxidised iron minerals (L. Stal pers. comm.), but as oxidised iron occurs as highly insoluble compounds, and as oxygen penetrates only to very shallow depths by diffusion through the regular sediment surface, reoxidation can only be mediated either by local oxygen intrusion due to faunal or plant activity, or by more widespread resuspension of sediments (especially non-vegetated sediments) during high winds (Revsbech et al. 1980). The high iron content is ecologically very important as it serves as a redox buffer in periods when the oxygen supply to the sediment cannot match the need for reoxidation, especially of sulphide, but for our measurements the high reoxidation rates of sulphide represent a significant methodological problem. We know that the rates measured after 15 min incubations were higher than after longer incubations, but we do not know how much higher the rates would have been if we could have totally avoided reoxidation. To obtain the data shown in Fig. 2, we incubated the cores for only half an hour with radiotracer, but that is the shortest incubation time that allowed us to process the large series of cores. During the field experiment, where injections had to be made vertically, we even had to use 1 h incubations.

Diurnal cycle of sulphate reduction

From the data presented in Figs. 2 & 3, no impact of plant photosynthetic activity can be seen on the SRR in the root zone. The rates in the light and in the dark were very similar. This is in contrast to studies on tropical seagrass beds in Jamaica and northern Australia, where a stimulation was found during illumination (Moriarty et al. 1985, Pollard & Moriarty 1991, Blackburn et al. 1994). It has been proposed (Blackburn et al. 1994) that the stimulation by light was due to excretion of photosynthates, and this conclusion was supported by the finding that in a meadow of *Halodule beaudetti* SRR decreased to very low levels after 5 to 10 h in the dark. In oligotrophic tropical carbonate sediments, such exudates may constitute a much larger fraction of the total amount of electron donors available for the bacterial population than was the case in the eutrophic Arcachon sediments. It is also possible that *Zostera noltii* excrete much less organic carbon than *H. beaudetti*, or that the exudates are more heavily degradable. Excretion of organic matter from the roots is well documented for a number of marine macrophytes (Penhale & Smith 1977, Wetzel & Penhale 1979, Moriarty et al. 1986), but although we incubated sediment cores for up to 48 h in darkness, no decrease in SRR could be detected (data not shown).

The large difference in SRR between sediments from Stns B and BZ (e.g. Fig. 2) indicated that there was more degradable organic matter available in the upper 4 cm of sediment at Stn BZ. Interestingly, the content of organic carbon present as determined by HCN analysis was approximately the same at both stations (about 1.2 mmol cm^{-3}; Table 1), whereas the content of larger pieces of organic material such as roots and partly degraded leaves was very different. Fresh and easily degradable material may give rise to higher activity of heterotrophic fermentative bacteria and thereby to a higher production of fermentation products such as hydrogen and volatile fatty acids, which are the most important substrates for sulphate-reducing bacteria (Sørensen et al. 1981, Widdel 1988). The higher rates measured at Stn BZ were therefore most likely due to the quality rather than quantity of the organic carbon present.

The data from the laboratory experiments were supported well by the *in situ* experiments. The field experiment served as an extra control and also to elucidate whether very high light intensities had an effect. There was no effect of light regime on SRR in the root horizon from 1 to 4 cm depth.

CONCLUSIONS

The rate of sulphate reduction in the sediment was highly stimulated by the presence of *Zostera noltii* throughout the upper 0 to 4 cm layer, which was heavily infiltrated by roots. No difference between the rate of sulphate reduction in vegetated and non-vegetated sediment could be detected below the root zone. There was no difference between the rates of sulphate reduction in the root zone during illumination and darkness, and direct utilization by sulphate-reducing bacteria of root exudates originating from photosynthetic activity may therefore be less important than low-molecular-weight organic molecules produced by microbial hydrolysis of macromolecules. The results stress the importance of keeping the incubation time short when measuring sulphate reduction in relatively oxidised sediments, as 15 min incubations with radiotracer gave up to 6 times higher rates of sulphate reduction than incubation for several hours.

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