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Sedimentary anaerobic microbial biogeochemistry in the Gulf of Trieste, northern Adriatic Sea: Influences of bottom water oxygen depletion

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Abstract. Sediment samples from two locations in the Gulf of Trieste (northern Adriatic Sea) were collected during periods of maximum and minimum temperatures for two years. Both sites were rich in carbonate material and inhabited by a diverse benthic infaunal community. However, Site F exhibited a deeper dwelling faunal community, higher content of carbonate minerals, and larger grained sediments than at site MA, which was closer to shore. Depth profiles of sulfate reduction and potential rates of iron and manganese reduction were determined together with measurements of pore water and solid phase chemistry. Bottom waters at all sites were nearly saturated with oxygen for all of the dates sampled except for September 1993 when bottom waters at site F were less than 50% saturated. Sulfate reduction rates were as high as 400 nmol ml^{-1} day^{-1} during late summer and fall when temperatures were >20 °C, while rates during March (~ 8 °C) were <30 nmol ml^{-1} day^{-1}. Potential rates of iron reduction, as determined by the accumulation of both dissolved and acid-soluble reduced iron, were high in surficial sediments at each site except at site F when bottom waters were partially depleted in oxygen. In the latter instance, sulfate reduction overwhelmed metal reduction. Although the portion of metal reduction due directly to enzymatic use by bacteria was not determined, the potential rate data suggested that Fe and perhaps Mn reduction were significant components of anaerobic carbon degradation in these sediments during much of the year. Both sites appeared to support active metal-reducing bacterial communities. However, occasional depletion of oxygen in bottom waters appeared to cause a decrease in irrigation/reworking activity by infauna which depressed redox cycling of elements enhancing the importance of sulfate reduction. A shift from metal reduction to sulfate reduction potentially exacerbates toxic effects of oxygen depletion on fauna by increasing the accumulation of toxic sulfide.

Introduction

The microbially-mediated decomposition of organic material in sediments occurs at the expense of a variety of terminal electron acceptors, the choice of which depends primarily on thermodynamic and enzymatic controls (Froelich et al. 1979; Lovley et al. 1982; Lovley & Phillips 1987a). In near-shore marine sediments, oxygen (O_2) is usually consumed rapidly near the surface, below which decomposition occurs anaerobically. Considerable work has appeared on the role of sulfate (SO_4^{2-}) (Howarth & Teal 1979; Hines et al. 1982; Jørgensen 1982; Christensen 1989; Hines et al. 1989) and nitrate (NO_3^-) (Horrigan & Capone 1985; Jørgensen & Sørensen 1985; Christensen et al. 1987) reduction in marine sediments. However, it was demonstrated that oxidized iron (Fe) and manganese (Mn) can serve as significant terminal electron acceptors in anoxic sediments (Aller et al. 1986; Lovley 1987; Hines et al. 1991; Canfield et al. 1993; Aller 1994) and pure cultures of bacteria have been isolated that are capable of using these as electron acceptors during the oxidation of organic compounds (Nealson et al. 1989; Caccavo et al. 1992, 1994). Bacterial metal reduction is more difficult to quantify than S or N transformations in most instances, due to interferences from abiotic processes. Hence, the quantitative importance of the former is uncertain.

The role of Fe and Mn cycles in sediments is complicated further by the activities of benthic infauna. The irrigation of anoxic sediments during the pumping of overlying water into burrows and the mixing of surficial oxidized sediments into anoxic subsurface regions can drastically enhance the recycling of Fe and Mn, which tends to resupply oxidized metals for use by benthic microorganisms (Aller 1980; Hines 1991). An increased importance of metal reduction in sediments is beneficial to infauna since this pathway of organic matter decomposition can out compete SO_4^{2-} reduction preventing the possible accumulation of toxic hydrogen sulfide. The regeneration of Fe and Mn oxides provides a chemical sink for sulfide as well.

In shallow marine basin areas, water density stratification during the late summer can isolate bottom waters and sediments from well mixed and oxygenated surface waters, causing bottom water hypoxia or even anoxia in some instances. These seasonal events are often responsible for catastrophic loss of benthic, and sometimes environmentally important pelagic species (Tyson & Pearson 1991). The pathway by which sedimentary organic matter decomposes may also be affected greatly by seasonal and interannual variations in the extent of bottom water hypoxia. The isolation of the benthos during summer stratification, and the O_2 deprivation that follows, may prevent the redox recycling of material near the sediment-water interface. Without the introduction of sufficient O_2 to regenerate certain electron acceptors, it is conceivable that organic matter decomposition in summer is restricted to SO_4^{2-} reduction; further enhancing the demise of benthic macroorganisms.

The present study was undertaken to examine modes of decomposition in surficial sediments of the Gulf of Trieste in the northern Adriatic Sea. Waters overlying these sediments are periodically depleted in O_2 and occasionally become anoxic (Faganeli et al. 1991; Stachowitsch 1991), and massive mortalities of benthic fauna during these events have been recorded (Stachowitsch 1984). Our results presented here indicated that local depletion in bottom water O_2 content may alter the biogeochemistry of the sediments, influencing the pathways of organic matter decay, and potentially harming fauna.

Materials and methods

Sampling locations

The Gulf of Trieste is a 500 km^2, shallow (20–25 m) basin in the northernmost part of the Adriatic Sea (Figure 1). The gulf is bounded by Slovenia to the southeast and Italy to the north and west. The marine geology has been described elsewhere (Ogorelec et al. 1991). The gulf is partially isolated from the rest of the Adriatic by a shoal that runs northeast and southwest between Italy and Slovenia. Freshwater enters the system from several rivers, e.g., the Dragonia and Rizana streams in the southeast, and the Isonzo and Timavo Rivers in the northwest. The northwestern rivers are the main sources of freshwater. The salinity of gulf waters ranges between 33 and 38.5‰, and bottom water temperatures from 8 to 22 °C. A density gradient in late summer often results in bottom water hypoxia (Faganeli et al. 1991). The sedimentary detrital material to the east is derived from the erosion of flysch while the northern and western sediments of the gulf originate primarily from the Isonzo River which delivers carbonate material from the Julian Alps. The sediments are composed primarily of clay and silt sized material along the eastern shore which grade quickly into silty sands within a few km of the Slovene coast. The carbonate content increases from $<30\%$ to $>70\%$ along this same gradient. Sedimentation rates (^{210}Pb) are $\sim$1 mm yr^{-1} in the central part of the gulf to over 5 mm yr^{-1} near shore in the bays (Faganeli 1989; Ogorelec et al. 1991).

Two sedimentary sites were sampled from October, 1992 through March, 1994 (Figure 1). The March 1994 samples were used only for conducting certain laboratory experiments. The sampling dates represented periods of maximum (September, October) and minimum (March) bottom water temperatures. Site F is a transitional site between the southeastern flysch coast and much of the Gulf of Trieste. Sediments at F are silty sands (Ogorelec et al. 1991) that are high in biogenic carbonate and are inhabited by a surface population of brittle stars, sponges and tunicates. The sediments are bioturbated further by polychaetes (e.g., maldanids, *Lumbrinerius* sp., *Owenia* sp.) and bivalves (e.g., *Corbula* sp., *Nucula* sp.) (Avcin & Vriser 1983). Site MA is within the Bay of Piran and consists of clay and silt (Ogorelec et al. 1991). MA sediments appeared to be less bioturbated than site F (A. Avcin and B. Vriser, personal communication), and contained infauna that inhabited shallower depths as well, although infaunal densities were similar for the two sites (Avcin & Vriser 1983).

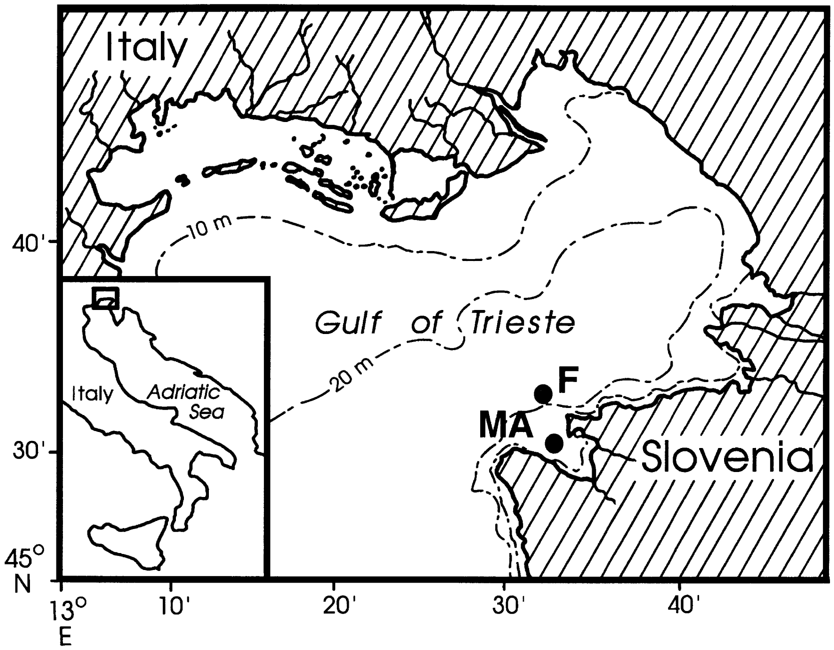

Figure 1. Sedimentary sampling locations in the Gulf of Trieste, northern Adriatic Sea.

Sample handling

Several 6 cm diameter sediment cores were collected by SCUBA at each of the sites on each date. After transport to the laboratory and removal of the overlying water by siphon, cores were placed into a N_2-filled glove bag. Individual horizontal sections from each core were removed, placed into glass jars, and homogenized. Subsamples were added to N_2-flushed serum vials for metal reduction measurements and 5 cc syringes for SO_4^{2-} reduction measurements. A portion of the sediment was placed in plastic bags and freeze-dried for determinations of metal content and CNSP analyses. Pore water was extracted from the remaining sediment using a nylon squeezer which was driven by N_2 pressure (Reeburgh 1967) and which contained a 0.45 μm final filter. Pore waters for nutrient analyses were frozen, waters for metal analyses were acidified to 1.0% with ultrapure HNO_3, waters for alkalinity, salinity, and SO_4^{2-} analyses were refrigerated, and waters for sulfide analyses were mixed with an equal volume of 6% zinc acetate to precipitate sulfides.

Activity measurements

Rates of SO_4^{2-} reduction were determined by injecting sediment subsamples in sealed syringes with $^{35}S\text{-}SO_4^{2-}$ and incubating overnight in the dark in a N_2-filled jar (Hines & Jones 1985; Hines et al. 1989). The reaction was stopped by freezing, and the incorporation of ^{35}S into reduced phases was determined by the chromium reduction method (Hines et al. 1989, 1996). Radioactivity in a portion of the ZnS trapped was determined by scintillation counting.

Potential rates of Fe reduction were determined in triplicate slurries in serum bottles (Lovley & Phillips 1986). Sediment samples (15 ml) were diluted with 20 ml of anoxic seawater and incubated at *in situ* temperatures in the dark under an N_2 atmosphere. After mixing, 0.1 ml samples were periodically removed (every 10–24 hr for 3–4 days) and mixed for one hour with 5.0 ml of 0.5 N HCl to dissolve Fe. A portion (0.1 ml) of this acidified slurry was added to 5.0 ml of a solution of 0.1% FerroZine in 50 mm Hepes (pH 7.0), mixed for at least 15 seconds, passed through a 0.4 μm Nuclepore filter, and Fe(II) determined as the A_{562} on a spectrophotometer. The accumulation of Fe(II) in slurries was used to estimate the rate at which slurries were reducing Fe. Since this rate was due to both chemical and microbial Fe reduction, it served as an estimate of Fe reduction potential. In some cases, we added $Na_2Mo_4^{2-}$ to inhibit SO_4^{2-} reduction to determine the portion of Fe that was reduced by sulfide. We also measured the quantity of total dissolved Fe in slurries using atomic absorption (AAS) and samples that were filtered and acidified with ultrapure HNO_3. In addition to measurements of reduced Fe, slurries were analyzed, at t_0, for reducible Fe oxide by mixing 0.1 ml of slurry with 5.0 ml of a solution of 0.25 M hydroxylamine hydrochloride and 0.25 M HCl followed by the determination of reduced Fe. Hydroxylamine reduces amorphous Fe oxides, and this pool, which is the difference between the hydroxylamine-treated subsamples and the HCL treated subsamples, is identical to the fraction designated “microbially-reducible Fe” by Lovley & Phillips (1987b).

The occurrence of Mn reduction was determined by measuring the accumulation of dissolved Mn in slurries over time. Samples were removed from slurries using syringes, immediately filtered (0.4 μm Nuclepore), acidified with HNO_3, and analyzed by AAS.

Chemical analyses

Water column temperature and oxygen profiles were determined using a CTD probe. Dissolved Fe and Mn in pore water samples were determined by AAS; sulfide colorimetrically (Cline 1969); SO_4^{2-} turbidimetrically (Tabatabai

1974), alkalinity by titration (Gieskes & Rogers 1973); NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-} colorimetrically (Grasshoff et al. 1983). Solid phase Fe and Mn were determined using AAS on sediment samples extracted with 5N HNO_3. Solid phase C, N, and S were determined using a Carlo Erba CHNS analyzer. Organic carbon was determined similarly on subsamples in which inorganic carbon had been removed by treatment with HCl. The $\partial^{13}C$ of sedimentary organic matter was determined using an Europa AA-20 Mass Spectrometer on dried sediment samples which were treated previously with 3M HCl to remove carbonate carbon (Faganeli et al. 1988). Total sedimentary P was determined colorimetrically after combustion of sediments at 450 °C, dissolution of material with 1M HCl, and centrifuging (Aspila et al. 1976). Organic P was determined as the difference between total P and inorganic P released by acid prior to combustion (Aspila et al. 1976).

Results

Preliminary experiments

Reduced Fe accumulated linearly for $\sim$3.0 days in sediments slurries from sites F and MA in March 1994 (Figure 2). Molybdate amendments had no effect on samples from the upper 3.0 cm at site F suggesting that Fe reduction was microbial. However, Fe(II) accumulation in samples from deeper than 3.0 cm was impeded when amended with molybdate indicating that a portion of the Fe was reduced by sulfide. In site MA sediments, molybdate decreased Fe reduction at all depths. However, molybdate was more effective at inhibiting Fe reduction in deeper sediments at MA as at F, suggesting that at least a portion of the Fe reduction in surficial sediments at MA was reduced microbially. We did not use molybdate treatments routinely, so the Fe reduction data indicate that bacterial Fe reduction was possible, but the exact mechanism was unknown.

Water column oxygen profiles

Measurements of water column temperature and oxygen concentrations were made on a monthly basis by the Marine Biological Station, Piran as part of a routine monitoring program in the Gulf of Trieste (and in Piran Bay), including sites F and MA. Oxygen was near saturation throughout the water column at all sites during October, 1992 and March 1993 (Figure 3). However oxygen levels were $<$50% saturated near the bottom at site F in September 1993.

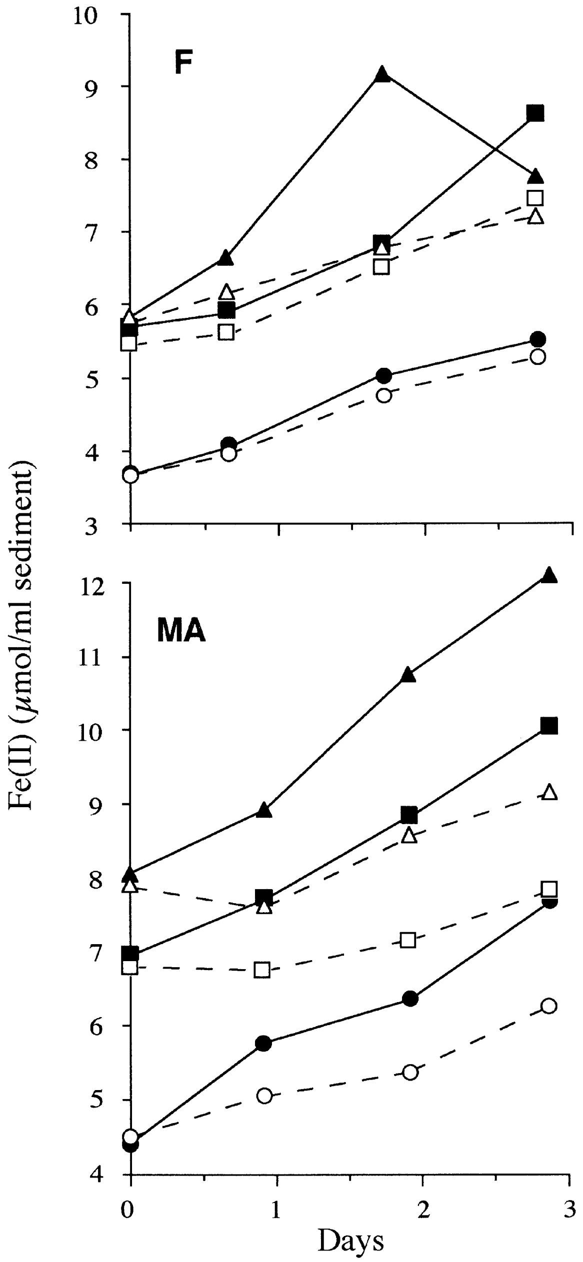

Figure 2. Reduced iron accumulation in sediment slurries from site F collected in March, 1994. Open symbols represent subsamples which were amended with sodium molybdate to inhibit sulfate reduction. (●) 0–1.5 cm; (■) 1.5–3.0 cm; (▲) 3–4.5 cm.

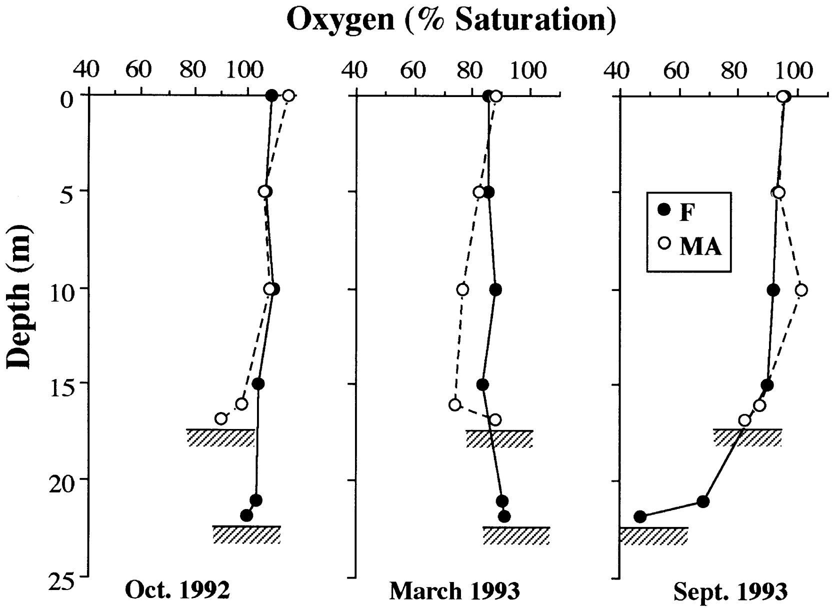

Figure 3. Depth profiles of dissolved oxygen saturation in the water column on different dates.

Activity measurements

Depth profiles of microbial activities varied greatly between sites and between sampling dates (Figure 4). Rates are expressed in terms of C oxidation, using accepted stoichiometries (Froelich et al. 1979), for comparative purposes even though metal reduction rates are simply rates of reduced Fe accumulation in slurries and do not necessarily represent C oxidation.

During October 1992, rates of SO_4^{2-} reduction at site MA were twice those at F in the upper 3.0 cm (Figure 4). However, site F exhibited subsurface maxima while rates at MA decreased with depth. The potential for Fe reduction was higher at site F and detected throughout the upper 8 cm. Potential Fe reduction was slow compared to SO_4^{2-} reduction at site MA.

SO_4^{2-} and potential Fe reduction in March 1993 were slower than in October (Figure 4). SO_4^{2-} reduction was <40 nmol ml^{-1} d^{-1} and displayed subsurface maxima indicative of the presence of other decomposition pathways. Potential Fe reduction rates were highest at site F in surficial sediments and rates at both sites were restricted to the upper 3–4.5 cm.

SO_4^{2-} reduction rates in September 1993 were rapid with a maximum in the upper 1.5 cm at both sites and a high subsurface maximum at MA (Figure 4). Potential Fe reduction rates at F were much slower than during the previous year (October), while rates at MA were quite high.

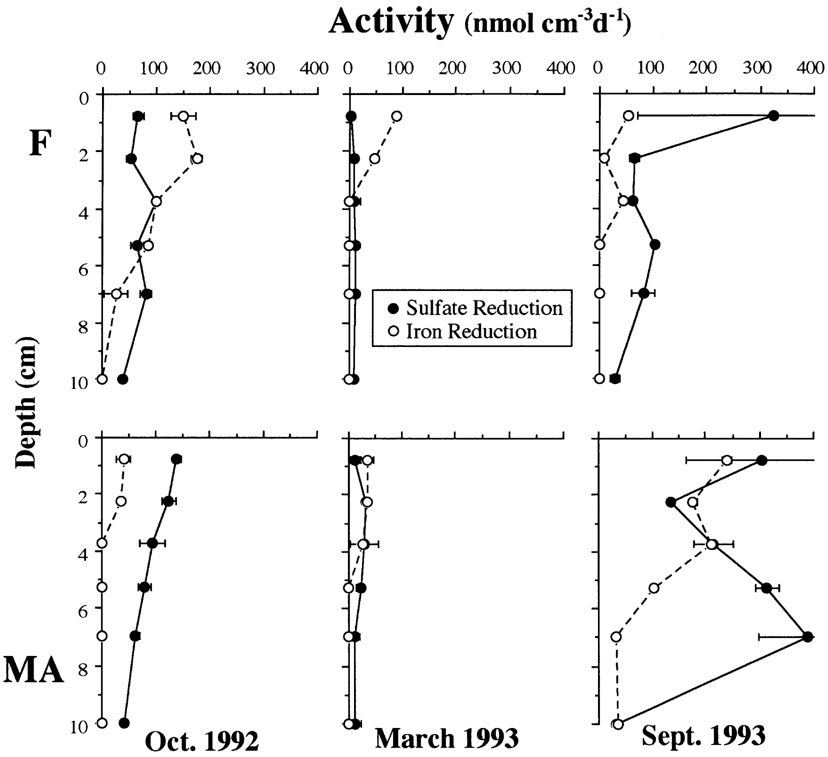

Figure 4. Sedimentary depth profiles of sulfate reduction and iron reduction rates. Both rates are presented as carbon oxidation equivalents using accepted stoichiometries (Froelich et al. 1979).

Mn reduction rates were determined from the accumulation of Mn in slurries for October 1992 and March 1993 samples only. A major portion of Mn that is liberated during Mn reduction in sediments is adsorbed as Mn^{2+} onto sedimentary Mn oxides (Murray 1974; Canfield et al. 1993). Since we did not empirically determine Mn^{2+} adsorption, Mn reduction rates were greatly underestimated. However, the Mn accumulation data were useful for determining where Mn reduction might be occurring. Mn reduction was restricted to the upper 1.5 and 3.0 cm at site F in October and March, respectively (Figure 5). Mn accumulation at site MA was more rapid than at F and was detected throughout the sediment core. A maximum Mn accumulation rate of $\sim$60 nmol ml^{-1} d^{-1} was detected in the upper cm at site MA in October.

Pore water chemistry

Dissolved sulfate displayed only slight decreases with depth at each of the sites despite the occurrence of sulfate reduction (data not shown). Dissolved

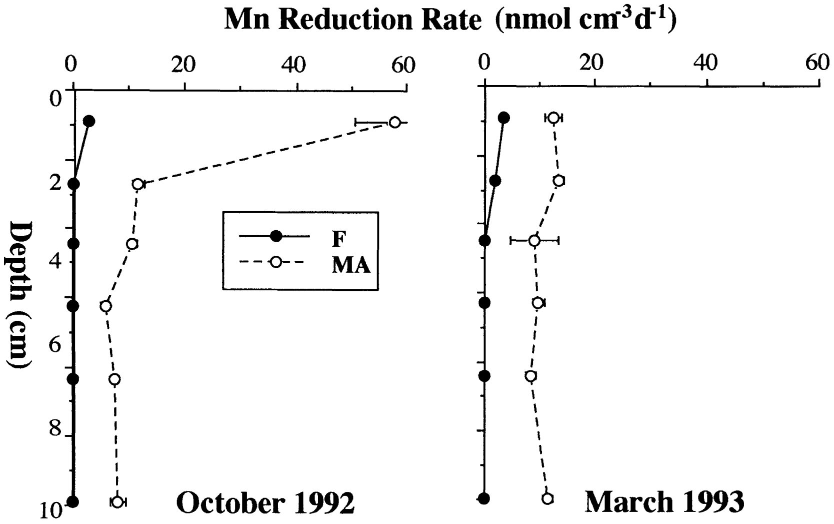

Figure 5. Sedimentary depth profiles of manganese reduction rates on two dates at sites F and MA. Rates, presented as carbon oxidation equivalents as in Figure 4, are from the accumulation of dissolved manganese in slurries and are not corrected for adsorption of Mn(II) by manganese oxides.

sulfide was <1.0 μM at both sites in October 1992 and in March 1993 (data not shown). However, in September 1993, sulfide reached 25 μM at site F and 8 μM at site MA.

Although the microbial rate profiles were quite different at sites F and MA (Figure 4), the dissolved metal profiles were relatively similar for the two sites (Figure 6). During warmer months, the highest Mn concentrations occurred near the surface with a subsurface Fe maximum (except for September 1993), suggesting that Mn reduction occurred near the surface and Fe reduction was situated within the 1–3 cm region. Some of the dissolved Mn may have resulted from the reduction of Mn by Fe^{2+} since these regions overlapped (Canfield et al. 1993). In winter, Mn concentrations were lower while dissolved Fe was usually higher.

Pore water nutrient data were acquired for October 1992 and March 1993 (Figure 7). Nitrate in 1992 was present in the upper few cm at site F and was depleted below 4.0 cm indicating that nitrate reduction was occurring in the upper few cm. Nitrate was virtually absent in MA sediments during that time. Examination of the carbon oxidation rates (Figure 4) indicated that nitrate at site F would have been used quickly during the slurry incubations, thus artificially favoring metal and SO_4^{2-} reduction. Nitrate was detected throughout the sediment column at both sites in March 1993. Ammonium concentrations at

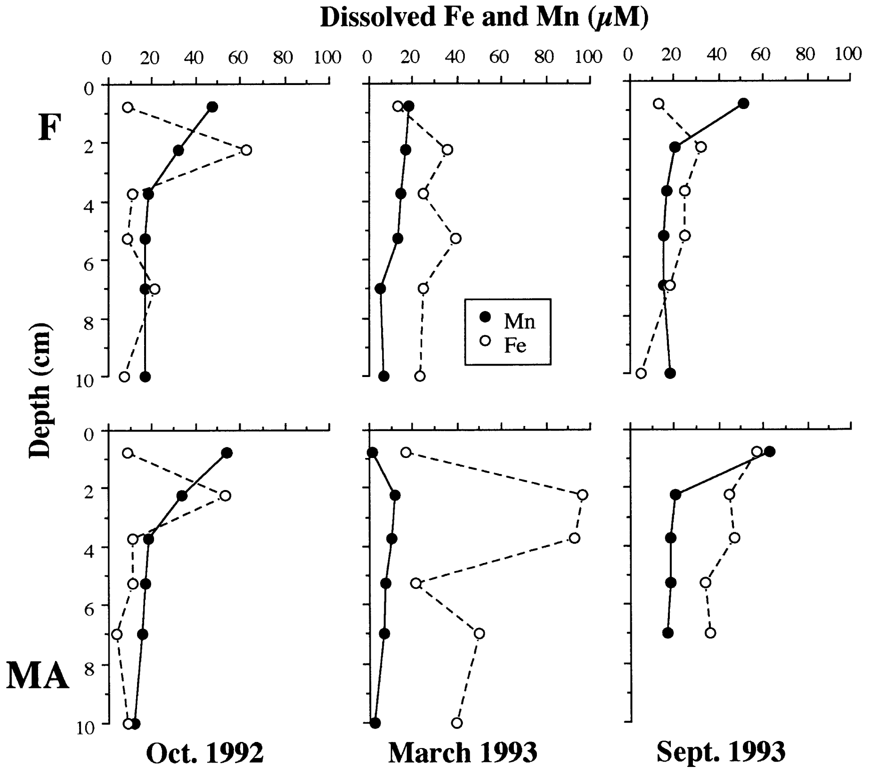

Figure 6. Sedimentary depth profiles of dissolved iron and manganese at sites F and MA on different dates.

site F in 1992 exhibited maxima as high as 375 μM, while at site MA, ammonium levels were relatively constant around 65 μM. Ammonium was high at both sites in March. Phosphate concentrations were low (<6.0 μM) throughout the sediments at both sites and during both sampling periods. Phosphate levels were low relative to ammonium, i.e., ammonium:phosphate ratios were higher (>20) than expected from the degradation of marine organic matter. Sediments at both F and MA contained significant quantities of carbonate material which may have caused a removal of phosphate from pore waters (Hines & Lyons 1982). Titration alkalinity concentrations were ~3.0 meq $l^{-1}$and essentially constant with depth at all sites (data not shown).

Solid phase chemistry

The vertical distribution of hydoxylamine-reducible Fe was similar to the rates of Fe reduction at both sites in October 1992 and March 1993 (Figure

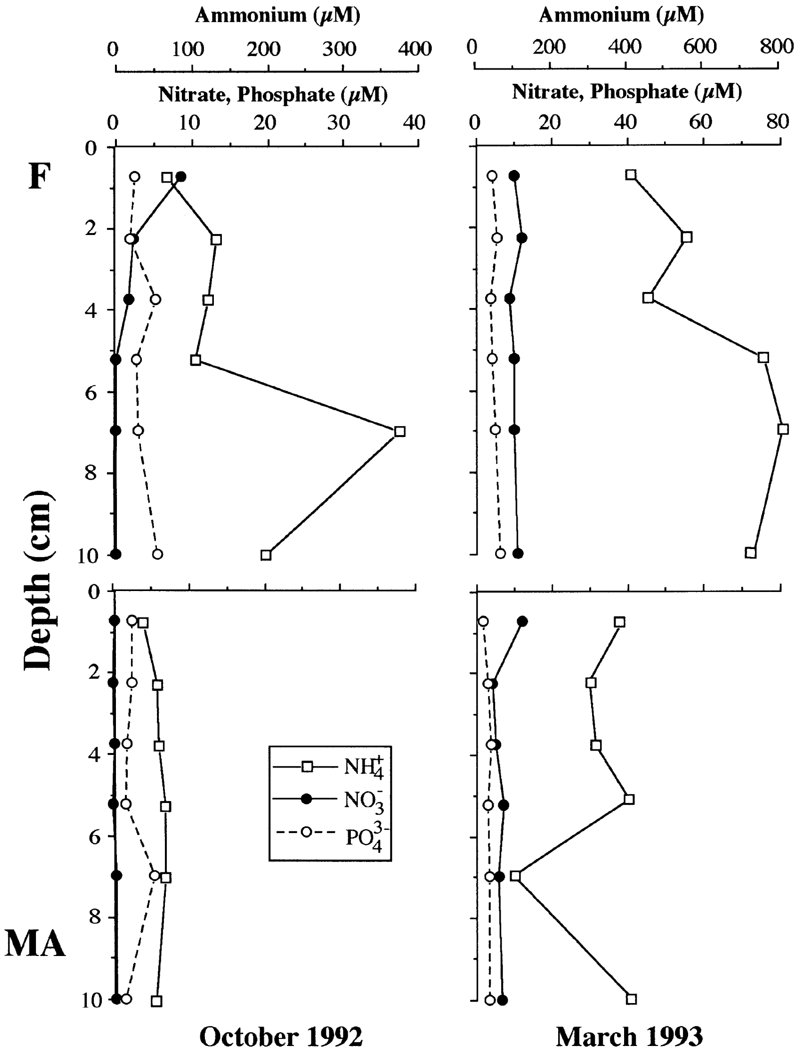

Figure 7. Sedimentary depth profiles of dissolved nutrients at sites F and MA on different dates.

8) suggesting that this Fe pool was preferentially utilized. This agrees with the findings of Lovley & Phillips (1987b) that microbial Fe reduction in sediments occurs at the expense of amorphous, easily-reducible Fe. In some instances, the quantity of hydoxylamine-reducible Fe was small enough that it was removed within less than two days and Fe accumulation ceased in those slurries prior to the end of the incubation. In those cases, Fe reduction was calculated from the increase in reduced Fe which occurred early.

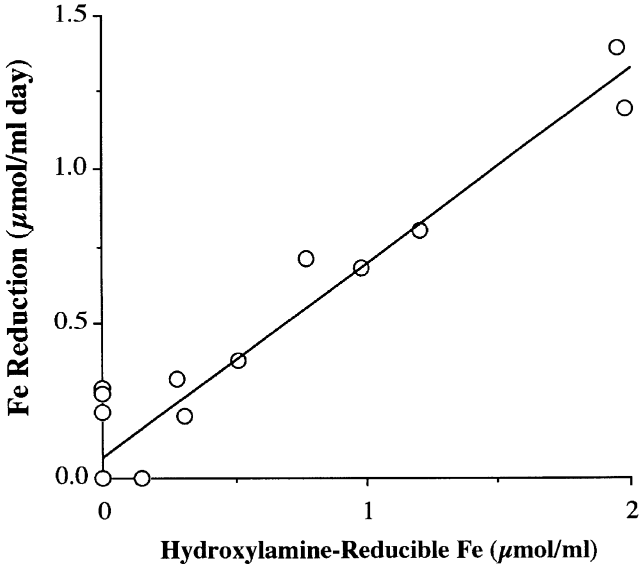

Figure 8. Iron reduction rates as a function of the concentration of hydroxylamine-reducible iron in sediments at sites F and MA in October, 1992 and March, 1993. Rates expressed as μmoles of iron reduced which differs from Figure 4 where rates are expressed as carbon equivalents.

∂^{13}C values of sedimentary organic matter were -22.5 ± 0.35‰ and -21.87 ± 0.29‰ for sites F and MA, respectively. These data indicated that the organic material deposited at both sites was primarily of marine origin.

Figure 9 displays average values for other solid phase chemicals in the sediments. Total sedimentary Fe and Mn concentrations were similar for the two sites at ~0.4 mmol g^{-1} and ~9.0 μmol g^{-1}, respectively. However, Fe levels at site MA were 20–40% higher than at F which reflects the fact that site MA is closer to shore and shallower. Site F was richer in carbonate material as seen by the higher total C concentrations. However, organic C levels were similar at the two sites. Total and reduced S was approximately two-fold higher at site MA than F. Site MA consisted of finer-grained material and was less subject to bioturbation than site F. Hence, reworking-mediated S oxidation was probably less active at MA. At both sites, surficial concentrations of reduced S exhibited temporal changes which are reflected in the larger variance in S data in the uppermost sediment layer. This variation was probably due to seasonal variation in bioturbation and to storm-induced resuspension of surface material. Solid phase N was higher at site MA than at F which most likely reflects the finer grained nature of site MA sediments. Although ammonium levels were fairly high at both sites, its contribution to the total N

Table 1. Estimates of percentage of carbon oxidation attributed to sulfate and iron reduction in sediments in the Gulf of Trieste (northern Adriatic Sea), and bottom water temperatures and oxygen concentrations on different dates. Rate values for the upper 3.0 cm only.

	%		Bottom water	
Sample	SO_4^{2-} reduction	Fe reduction	O_2 (% saturation)	Temp. (°C)
October 1992				
F	27	73	99.6	19.0
MA	78	22	89.7	18.2
March 1993				
F	8	92	91.2	7.8
MA	39	61	87.7	8.4
September 1993				
F	86	14	47.2	22.3
MA	51	49	82.3	22.3

was insignificant, indicating that the bulk of sedimentary N was organic. Like N, total and organic P concentrations were higher at MA than at F. Ratios of organic C to total N for both F and MA were 5–10, while N to organic P ratios were about 16. Both of these ratios are similar to the Redfield ratio which agrees with the stable isotope data indicating that organic material in these sediments is of marine origin.

Discussion

Measurements of rates of anaerobic decomposition in these northern Adriatic sediments yielded three general patterns. The two fall/late summer sampling dates displayed the most rapid rates and these were the warmest periods. However, site F was dominated by SO_4^{2-} reduction in 1993 and SO_4^{2-} and Fe reduction in 1992. Site MA behaved in an opposite manner to F in that SO_4^{2-} reduction dominated in 1992 while both rates were high in 1993. During March, SO_4^{2-} reduction rates decreased several fold at both sites, whereas Fe reduction rates did not decrease greatly. Therefore, metal reduction was a more significant proportion of the microbial activity during winter, yet in summer a site displayed either a dominance of SO_4^{2-} reduction or a co-occurrence of SO_4^{2-} reduction and metal reduction. Table 1 summarizes these findings by displaying the relative portion of anaerobic decomposition due to SO_4^{2-} reduction and Fe reduction. This analysis disregards the contribution of other modes of activity such as oxygen, nitrate, or Mn reduction, but is designed to depict how the samples varied temporally at the two sites.

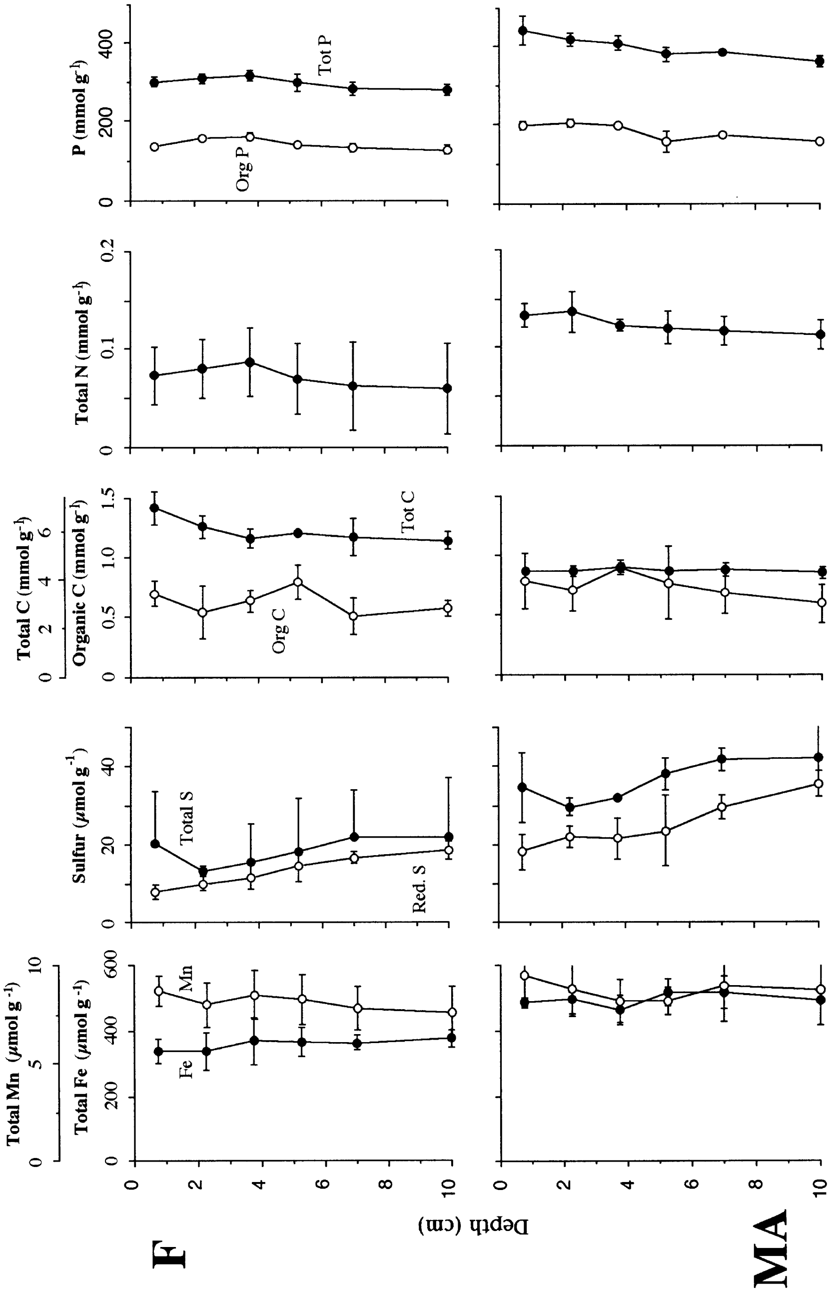

Figure 9. Sedimentary depth profiles of solid phase chemical constituents at sites F and MA on different dates.

Bertuzzi et al. (1996), using laboratory-based benthic chambers, determined O_2 consumption rates in Gulf of Trieste sediments to be $\sim$2.0 μmol m^{-2} d^{-1} in late summer, 1993. For a 20 cm sediment column, this is approximately 100 nmol ml^{-1} d^{-1} which is similar to rates of the other reduction processes measured in these sediments. Since O_2 consumption integrates respiration and the oxidation of reduced minerals in the sediments, we were unable to calculate the actual contribution of benthic O_2 respiration to carbon decomposition. However, considering only the rate of SO_4^{2-} reduction, during steady state conditions where reduced S compounds are not accumulating, the majority of O_2 consumption would occur via sulfide oxidation rather than by O_2 respiration.

Although the Fe reduction data are for potential rates only, the SO_4^{2-} reduction rate data alone provide evidence of the role of O_2 deprivation in controlling sedimentary decomposition pathways. At site F, SO_4^{2-} reduction maxima occurred several cm within the sediments in 1992 yet exhibited a surface, albeit variable, maximum in September 1993. The profile in 1992 was reminiscent of those reported during periods of bioturbation in other sediments in which metal dissolution was very rapid (Hines & Jones 1985). A near-surface maximum, like that noted at site F in September 1993, is indicative of the dominance of SO_4^{2-} reduction with only a minimal zone of metal reduction in the upper few mm (Canfield et al. 1993). Therefore, a change in the relative importance of SO_4^{2-} reduction and other pathways of decomposition was apparent. The data on potential Fe reduction rates support this conclusion.

Data for March indicated that slow winter rates of microbial activity resulted in a vertical zonation where a metal reduction (Fe reduction) zone occurred above the SO_4^{2-} reduction zone. This zonation was seen most clearly at site F where a 3.0 cm Fe reduction zone appeared (Figure 4). Macrofaunal activity in these sediments is probably minimal during winter, so it is expected that vertical zonation in sedimentary biogeochemical processes would be dictated by decomposition rates and the diffusion of oxidants. It appeared that site MA had faster rates of bacterial activity and a maximum rate of SO_4^{2-} reduction which was closer to the surface than at site F. However, in both cases it is not surprising that metal reduction would be a significant component of decomposition since slow microbial activity rates allow for the diffusion of O_2 into the sediments enhancing metal recycling.

The main difference between the 1992 and 1993 summer/fall data is that the relative importance of SO_4^{2-} and Fe reduction varied. SO_4^{2-} reduction dominated decomposition at site F in September 1993 when bottom waters were lower in O_2. Bottom water O_2 was abundant at site MA in 1993, and this site exhibited a significant Fe reduction component. Both sites have been shown to comprise a benthic macrofaunal community with the potential for bioturbation (Avcin & Vriser 1983), and the activity rate depth profiles exhibited subsurface maxima in some cases (Figure 4) which are reminiscent of bioturbation-influenced profiles (Hines & Jones 1985). The irrigation of burrows and the reworking of sediments during periods of high faunal activity increases the intrusion of oxidants into sediments which produces three dimensional redox gradients (Aller 1980, 1990) enhancing the recycling of redox-sensitive elements like Fe, Mn and S (Aller 1982; Hines et al. 1982; Hines & Jones 1985). Periods of active bioturbation should result in increased availability of Fe and Mn which will enhance rates of metal reduction. However, impairment of bioturbation by lower O_2 levels should favor bacterial SO_4^{2-} reduction since metal recycling is prevented. This phenomenon apparently occurred at site F in 1993, and rapid SO_4^{2-} reduction took place in surficial sediments.

A change in the relative importance of SO_4^{2-} reduction and other decomposition pathways can profoundly affect the activity and survival of benthic fauna. It has been shown that bottom water hypoxia and anoxia can decimate benthic communities including those in the northern Adriatic Sea (Justić et al. 1987; Stachowitsch 1991). However, an increase in the portion of decomposition that occurs via SO_4^{2-} reduction will add to this problem through increased production of toxic sulfide. Bioturbation activity that delivers sufficient oxidants to sediments for the maintenance of decomposition pathways other than SO_4^{2-} reduction will enhance faunal survival, including other anaerobic pathways. Once the occurrence of low O_2 has curtailed bioturbation to the point where nitrogen, Mn, and Fe oxides are no longer available, then sulfide production will increase and free sulfide will eventually accumulate exacerbating a further decrease in faunal activity. The O_2 levels noted in the bottom waters at site F in September 1993 were $\sim$2.0 ml l^{-1} which is sufficient to adversely affect benthic fauna (Tyson & Pearson 1991).

Although we report Fe reduction data in terms of organic carbon utilized, it was not possible for several reasons to determine the exact portion of Fe reduction due to microbial utilization as an electron acceptor. First, any NO_3^- that was present in the samples when collected would have been utilized quickly during incubation making it impossible to determine the role of NO_3^- reduction in the decomposition of organic matter. NO_3^- was present in surficial pore waters at site F in 1992 (Figure 7) and at both F and MA in March 1993. As mentioned previously, the contribution of NO_3^- reduction to decomposition in the slurries may have been small since the isolation of the samples in serum bottles would result in the rapid use of NO_3^- forcing metabolism towards other modes such as metal and SO_4^{2-} reduction. Second, it is possible that mixing of sediments during slurry preparation generated some amorphous Fe oxides which would enhance Fe reduction during incubation. In most instances we noted a close relationship between the depth distribution of hydroxylamine-reducible Fe and Fe reduction (Figure 8) which was similar to that reported by Lovley & Phillips (1986) in the Potomac River. We made every effort to maintain anoxia during sample collection and processing to minimize any reoxidation of reduced Fe. It is possible that mixing of surficial sediments with those from just below the surface caused some Fe oxidation and production of new hydroxylamine-reducible Fe. However, for samples where Fe reduction and hydroxylamine-reducible Fe were noted throughout the upper $\sim$6 cm, such as at site F in October 1992 (data not shown), it is likely that oxidants capable of oxidizing Fe at depth were present in situ, underscoring the role of bioturbation in maintaining a supply of oxidants at depth. In addition, SO_4^{2-} reduction rates were determined on samples that were not slurried, and the vertical distribution of these rates agreed with the Fe reduction profiles, in most instances, in that subsurface minima in SO_4^{2-} reduction corresponded with vertically significant rates of potential Fe reduction. Finally, interpretation of data was hampered by our inability to collect sufficiently narrow depth sections with the coring equipment at hand.

Despite the limitations of the Fe reduction rate data, the use of acid-soluble Fe, as opposed to simply dissolved Fe, provides a more direct measure of Fe reduction. Canfield et al. (1993) used the accumulation of dissolved Fe to determine the depth intervals over which Fe reduction was occurring. They then used the stoichiometry of ammonium and CO_2 production to calculate metal reduction rates indirectly. Although our approach, which mimics that of Lovely & Phillips (1986), provides a more direct estimate of Fe reduction, it has limited application because of the large background of reduced Fe in solid and dissolved phases in most sediments. In many instances it will not be possible to detect an increase in Fe(II) over a very large acid-soluble Fe pool associated with AVS in sediments. In general, these types of measurements are difficult to make in marine sediments that are rich in S. Low S environments like freshwater systems are much more amenable to examining the relative role of microbial metal reduction in carbon cycling.

Pore water profiles were not as good a measure of the effects of hypoxia on sediments as were the activity rate data. In some instances, pore water profiles of Fe and Mn were similar for two sites while the rate profiles were very different (Figures 4–6). This difference may simply represent the timing of the change in bacterial activity. If a system recently moved toward SO_4^{2-} reduction from Fe reduction, there might not have been sufficient time for pore water chemistry to respond completely. In addition, an increase in SO_4^{2-} reduction should enhance Fe dissolution for a period due to the reduction of

Fe by sulfide. This would maintain a dissolved Fe pool until easily reducible Fe is titrated.

Without Mn(II) adsorption data we were unable to calculate the true rate of Mn reduction. To provide a crude estimate of Mn reduction we multiplied the dissolved Mn accumulation data (Figure 5) by the range of Mn adsorption rates reported by Canfield et al. (1993). These calculations provided rate data that were similar in magnitude to those reported previously (Canfield et al. 1993), suggesting that Mn reduction may have been a significant process in the northern Adriatic sediments. However, since the rates were not determined directly like the Fe reduction rates, we did not consider them in our treatment of the effects of oxygen deprivation on sedimentary processes.

Conclusions

1. Interpretation of metal reduction data was difficult due to the: 1) inability to collect sediment core sections which were less than 1.0 cm thick; 2) fact that increases in reduced Fe were not large compared to the ambient pool of reduced Fe; 3) inability to adequately separate sulfide-mediated metal reduction from enzymatic reduction; 4) presence of a surficial NO_3^- reduction zone and the potential depletion of NO_3^- early during slurry incubations; 5) potential for the production of easily reduced Fe during slurry preparation, and; 6) lack of knowledge regarding the relative importance of the adsorption of reduced Mn by Mn oxides. Pore water data apparently were not good indicators of the effects of hypoxia on benthic microbial activity.
2. During winter when temperatures and microbial activity were lower, increased penetration of oxygen allowed for a predominance of metal reduction in sediments.
3. During warm periods when microbial activity (and presumably bioturbation) was active and bottom water O_2 was abundant, the activities of infauna maintained a subsurface cycling of redox-sensitive elements which enhanced the importance of metal reduction.
4. During warm periods when bottom waters became depleted in O_2, like what occurred at site F in September 1993, decreased subsurface cycling of metals and sulfur allowed for SO_4^{2-} reduction to dominate anaerobic decomposition. An increase in SO_4^{2-} reduction relative to metal reduction and cycling during periods of O_2 depletion increases sulfide concentrations which further impedes the activity of infauna.
5. Sedimentary SO_4^{2-} and metal reduction rates appeared to be similar in magnitude to O_2 consumption rates reported previously for these sediments.

References

Aller RC (1980) Diagenetic processes near the sediment-water interface of Long Island Sound, II. Fe and Mn. In: Saltzman B (Ed) Estuarine Physics and Chemistry Studies in Long Island Sound (pp 351–415). Academic

Aller RC (1982) The effects of macrobenthos on chemical properties of marine sediment and the overlying water. In: McCall PL & Tevesz MJS (Eds) Animal-Sediment Relations (pp 53–102)

Aller RC (1990) Bioturbation and manganese cycling in hemipelagic sediments. Phil. trans. Roy. Soc. London 331: 51–68

Aller RC (1994) The sedimentary Mn cycle in Long-Island sound – its role as intermediate oxidant and the influence of bioturbation, O_2, and $C_{(org)}$ flux on diagenetic reaction balances. J. Mar. Res. 52: 259–295

Aller RC, Mackin JE & Cox RT, Jr (1986) Diagenesis of Fe and S in Amazon inner shelf muds: Apparent dominance of Fe reduction and implications for the genesis of ironstones. Continental Shelf Res. 6: 263–289

Aspila KI, Agemian H & Chau ASY (1976) A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. Analyst 101: 187–197

Avcin A & Vriser B (1983) The northern Istrian soft bottom communities: The example of Piran Bay (north Adriatic). Bioloski Vestnik 31: 129–160

Bertuzzi A, Faganeli J & Brambati A (1996) Annual variation of benthic nutrient fluxes in shallow coastal waters (Gulf of Trieste, northern Adriatic Sea). P.S.Z.N. I: Mar. Ecol. 17: 261–278

Caccavo F, Blakemore RP & Lovley DR (1992) A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay estuary, New Hampshire. Appl. Environ. Microbiol. 58: 3211–3216

Caccavo F, Lonergan DJ, Lovley DR, Davis M, Stolz JF & Mcinerney MJ (1994) Geobacter sulfurreducens sp nov, a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. Appl. Environ. Microbiol. 60: 3752–3759

Canfield DE, Thamdrup B & Hansen JW (1993) The anaerobic degradation of organic matter in Danish coastal sediments: Iron reduction, manganese reduction, and sulfate reduction. Geochim. Cosmochim. Acta 57: 3867–3883

Christensen JP (1989) Sulfate reduction and carbon oxidation rates in continental shelf sediments, an examination of offshelf carbon transport. Cont. Shelf Res. 9: 223–246

Christensen JP, Murray JW, Devol AH & Codispoti LA (1987) Denitrification in continental shelf sediments has major impacts on the oceanic nitrogen budget. Global Biogeochem. Cycles 1: 97–117

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14: 454–458.

Faganeli J (1989) Sedimentation of particulate nitrogen and amino acids in shallow coastal waters (Gulf of Trieste, Northern Adriatic). Mar. Chem. 26: 67–80

Faganeli J, Malej A, Pezdic J & Malacic V (1988) C:N:P ratios and stable C isotope ratios as indicators of sources of organic matter in the Gulf of Trieste. Ocean. Acta 11: 377–382

Faganeli J, Pezdic J, Ogorelec B, Herndl GJ & Dolenec T (1991) The role of sedimentary biogeochemistry in the formation of hypoxia in shallow coastal waters (Gulf of Trieste, northern Adriatic). In: Tyson RV & Pearson TH (Eds) Modern and Ancient Continental Shelf Anoxia (pp 107–117). The Geological Society, Pub. No. 58, London

Froelich PN, Klinkhammer GP, Bender ML, Luedtke NA, Heath GR, Cullen D, Dauphin P, Hammond D, Hartman B & Maynard V (1979) Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: Suboxic diagenesis. Geochim. Cosmochim. Acta 43: 1075–1090

Gieskes JM & Rogers WC (1973) Alkalinity determination in interstitial waters of marine sediments. J. Sed. Petrol. 43: 272–277

Grasshoff K, Ehrhardt M & Kremling K (1983) Methods of Seawater Analysis. Verlag Chemie, Weinheim

Hines ME (1991) The role of certain infauna and vascular plants in the mediation of redox reactions in marine sediments. In: Berthelin J (Ed) Diversity of Environmental Biogeochemistry (pp 275–286). Elsevier, Amsterdam

Hines ME, Bazylinski DA, Tugel JB & Lyons WB (1991) Anaerobic microbial biogeochemistry in sediments from two basins in the Gulf of Maine: Evidence for iron and manganese reduction. Est. Coastal Shelf Sci. 33: 313–324

Hines ME, Devereux R & Visscher PT (1996) Sulfur cycling. In: Newell S & Christian R (Eds) Manual of Environmental Microbiology (pp 324–333). American Society for Microbiology, Washington, DC

Hines ME & Jones GE (1985) Microbial biogeochemistry in the sediments of Great Bay, New Hampshire. Est. Coast. Shelf Sci. 20: 729–742

Hines ME, Knollmeyer SL & Tugel JB (1989) Sulfate reduction and other sedimentary biogeochemistry in a northern New England salt marsh. Limnol. Oceanogr. 34: 578–590

Hines ME & Lyons WB (1982) Biogeochemistry of nearshore Bermuda sediments. I. Sulfate reduction rates and nutrient generation. Mar. Ecol. Prog. Ser. 8: 87–94

Hines ME, Orem WH, Lyons WB & Jones GE (1982) Microbial activity and bioturbation-induced oscillations in pore water chemistry of estuarine sediments in spring. Nature 299: 433–435

Horrigan SG & Capone DG (1985) Rates of nitrification and nitrate reduction in nearshore marine sediments under varying environmental conditions. Mar. Chem. 16: 317–327

Howarth RW & Teal JM (1979) Sulfate reduction in a New England salt marsh. Limnol. Oceanogr. 24: 999–1013

Jørgensen BB (1982) Mineralization of organic matter in the sea bed – the role of sulphate reduction. Nature 296: 643–645

Jørgensen BB & Sørensen J (1985) Seasonal cycles of O_2, NO_3 and SO_4 reduction in estuarine sediments: The significance of an NO_3 reduction maximum in spring. Mar. Ecol. Prog. Ser. 24: 65–74

Justic´ D, Legovic T & Rottini-Sandrini L (1987) Trends in oxygen content 1911-1984 and occurnece of benthic mortality in the northern Adriatic Sea. Estuarine, Coastal and Shelf Science 25: 434–445

Lovley DR (1987) Organic matter remineralization with the reduction of ferric iron: A review. Geomicro. J. 5: 375–399

Lovley DR, Dwyer DF & Klug MJ (1982) Kinetic analysis of the competition between sulfate reducers and methanogens for hydrogen in sediments. Appl. Environ. Microbiol. 43: 1373–1379

Lovley DR & Phillips EJP (1986) Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac River. Appl. Environ. Microbiol. 52: 751–757

Lovley DR & Phillips EJP (1987a) Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Appl. Environ. Microbiol. 53: 2636–2641

Lovley DR & Phillips EJP (1987b) Rapid assay for microbially reducible ferric iron in aquatic sediments. Appl. Environ. Microbiol. 53: 1536–1540

Murray JW (1974) The surface chemistry of hydrous manganese oxide. J. Colloid Interface Sci. 46: 357–371

Nealson KH, Myers CR & Wimpee BB (1991) Isolation and identification of manganese-reducing bacteria and estimates of microbial Mn(IV)-reducing potential in the Black Sea. Deep-Sea Res Pt A-Oceanog Res 38: S907–S920

Ogorelec B, Misic M & Faganeli J (1991) Marine geology of the Gulf of Trieste (northern Adriatic): Sedimentological aspects. Mar. Geol. 99: 79–92

Reeburgh WS (1967) An improved interstitial water sampler. Limnol. Oceanogr. 12: 163–165

Stachowitsch M (1984) Mass mortality in the Gulf of Trieste: the course of community destruction. Marine Ecology 5: 243–264

Stachowitsch M (1991) Anoxia in the Northern Adriatic Sea; rapid death, slow recovery. In: Tyson RV & Pearson TH (Eds) Modern and Ancient Continental Shelf Anoxia (pp 119–129). Geological Soc. Spec. Publ. No. 58, London

Tabatabai MA (1974) A rapid method for determination of sulfate in water samples. Environ. Letters 7: 237–243

Tyson RV & Pearson TH (1991) Modern and ancient continental shelf anoxia: An overview. In: Tyson RV & Pearson TH (Eds) Modern and Ancient Continental Shelf Anoxia (pp 1–24). The Geological Society, London