

## Reductive Dissolution of Manganese Coupled to Mineralization in Lake Water/Sediment Microcosms

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**Abstract** Organic substrates such as acetate, glucose and monomethylamine added to intact Lake Biwa sediment cores were mineralized with relatively constant rates. Concomitantly, reductive dissolution of manganese oxide which had initially accumulated on the sediment surface was observed. Iron was also reduced if initial accumulate of  $\text{MnO}_2$  was small. Excess nitrate inhibited reduction of Mn and Fe, but sulfate had no effect. These observations suggested sequential bacterial reduction of available electron acceptors from nitrate via Mn(IV) and Fe(III) to sulfate. The results are compared with the previous studies on  $\text{MnO}_2$  reduction in different environments such as paddy soils. Potential ecological significances of manganese redox cycling in lake sediments are discussed.

### INTRODUCTION

The oxidation-reduction cycle of manganese in aquatic environments is mediated principally by biological agents. In well-oxygenated waters or sediments, reduced manganese species such as  $\text{Mn}^{2+}$  and  $\text{MnCO}_3$  are oxidized rapidly by specific microorganisms into insoluble manganese oxide  $\text{MnO}_2$  (Nealson et al., 1988) which tends to deposit towards more reductive environment because of its large specific gravity. In reductive environments such as anoxic sediments, manganese oxide is readily reduced and solubilized into  $\text{Mn}^{2+}$  because the standard redox potential of  $\text{MnO}_2/\text{Mn}^{2+}$  is high enough, and  $\text{Mn}^{2+}$  thus produced diffuses freely towards the oxic/anoxic boundary. As a results, manganese in the hydrosphere is often concentrated anomalously at the oxic/anoxic interface. The manganese redox cycling near the interface may not only have a substantial contribution to the electron flow from sedimentary anoxia to overlying oxic domain, but also exert a critical influence upon the distribution of minor elements in lake and sea waters by the large adsorption capacity of manganese oxide precipitates (Sugiyama et al., 1992).

Lake Biwa is the largest, mesotrophic and monomictic lake in Japan (surface area, 674  $\text{km}^2$ ; max. depth, 104 m). In the major (north) basin of this lake, the lake water is stratified thermally from May to January. Although no hypolimnetic anoxia is formed at present, this basin is said to be in danger of seasonal oxygen depletion due to recent

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artificial eutrophication. Kawashima et al. (1988) found the manganese oxidation below the thermocline to be enhanced by some biological process(es) in the south (minor) basin of Lake Biwa. The present author reported later that this enhancement could be ascribed to the activity of a manganese-oxidizing bacterium *Metallogenium* sp. (Miyajima, 1992a). The same organism appears also in near-bottom turbid water of the north basin of this lake. Oxidation of  $Mn^{2+}$  at the sediment surface of this basin has also been confirmed to be bacterially catalyzed (Miyajima, unpublished data). In contrast to the oxidation process, reductive dissolution of manganese oxide has not been studied intensively in this lake. In his report on the survey at a dredged area where seasonal bottom anoxia emerges, the author suggested the possibility of dissolution of bacterially produced  $MnO_2$  caused by redox reaction with  $Fe^{2+}$ , based on a thermodynamic consideration of the redox equilibria (Miyajima, 1992b). In fact, both inorganic and biochemical processes are possible for the reduction of manganese in natural environments. Inorganic reduction includes reactions of  $MnO_2$  with hydrosulfide ( $HS^-$ ) and nitrite ( $NO_2^-$ ) as well as  $Fe^{2+}$ , whereas biochemical process includes respiratory electron transport from organic substrates to manganese oxide and the reduction by some organic metabolites produced by anaerobic bacteria. As it is not sure by what means solid oxides such as  $MnO_2$  are transported to the inside of the cellular membrane where terminal respiratory electron acceptors are usually reduced, it may be dangerous at present to refer to all the manganese reduction processes coupled to oxidation of organic substrate as "respiration" (Ghiorse, 1989). But there are increasing evidences that this type of the reduction of manganese as well as of iron is ubiquitous for suboxic freshwater and marine sediments. It seems possible that the biological reduction of  $MnO_2$  is by far more predominant than inorganic counterparts in the geochemical manganese cycling in the hydrosphere (see reviews of Lovley, 1991 and Nealson and Myers, 1992).

From the viewpoint of the carbon cycle, the iron reduction has sometimes been estimated to have considerable contribution to the mineralization of sedimentary organic matter in various aquatic environments (Lovley, 1987). Although studies on the contribution of manganese reduction are relatively fewer, some analyses of element profiles in sediments have implied significance of this process on sedimentary carbon cycles (e.g., Sørensen and Jørgensen, 1987). In the study reported below, the possibility of occurrence of biochemical manganese reduction was examined for intact sediment cores of Lake Biwa. For this aim, whether a supply of oxidized sediment with organic substrates induce reductive dissolution of manganese and iron was tested with the selectivity as electron acceptors compared for Mn and Fe oxides to nitrate and sulfate. Modes and potential geochemical significances of manganese redox transformation in lake sediments are discussed with a brief review over similar processes in different environments such as submerged soils extensively studied in Japan, which has been largely overlooked by recent reviewers of these subjects because of the linguistic barrier.

## MATERIALS AND METHODS

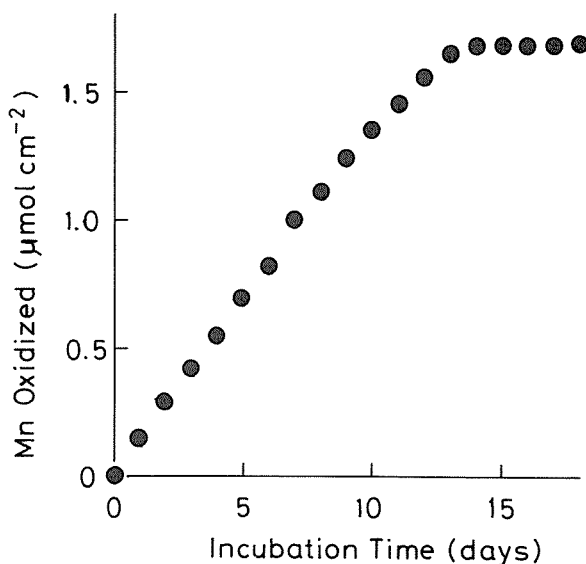
Sediment samples used in this study were collected at a station off Oumi-Maiko (depth, ca. 75 m), located in the south-western district of the north basin of Lake Biwa. Intact sediment cores were sampled with a gravity corer ( $\phi$  5.0 cm, 60 cm long). The obtained core samples usually contained a ca. 40 cm-deep mud layer overlain with a ca. 20

cm-deep water layer. These samples (called below “experimental systems”) were incubated at *in-situ* temperature (8°C) in dark with the water surface open to oxidative atmosphere (head space).

Newly collected samples often showed high biological activity at the sediment surface with significant amounts of  $\text{NH}_4^+$  and  $\text{Mn}^{2+}$  being emitted to the overlying water. During subsequent incubation for one month or more, the emission ceased gradually, and  $\text{Mn}^{2+}$  in the overlying water was eventually oxidized and accumulated as an  $\text{MnO}_2$  layer at the mud surface. After the  $\text{Mn}^{2+}$  concentration in the water layer fell down below detection limit (0.1  $\mu\text{M}$ ), further experiments were started. As the system of this state has a high manganese-oxidizing potential as revealed on adding  $\text{Mn}^{2+}$  to the overlying water (Fig. 1), reductive dissolution of the  $\text{MnO}_2$  layer does not occur spontaneously without further supply of electron donating substances.

The experiments were started by adding organic substrates with or without supplemental electron acceptors (see Results). Although the water surface of the experimental systems was always open to the head space, environment near the sediment surface should have become depleted of oxygen soon after the addition of organic substrates since the oxygen content of the water layer (ca. 400  $\mu\text{M}$  at saturation) was quite insufficient to oxidize the added substrates (2 mg-at.C  $\text{l}^{-1}$ ). The experiments were continued until the DOC concentration fell down below 10% of the initial concentration. The pH value in the water layer remained between 6.0 and 7.0 throughout the experimental period. During the experiments, subsamples were collected from the water layer everyday: the water layer was gently mixed by pipetting, subsample (20 ml) was withdrawn, and then 20 ml of filtered (Whatman GF/F) aged lake water was added to keep constant the bulk volume.

The subsamples were analyzed for concentrations of DOC (TOC analyzer, Shimadzu



**Fig. 1.** Oxidative precipitation of  $\text{Mn}^{2+}$  within a mud/water experimental system.  $\text{Mn}^{2+}$  was added in the water layer at the beginning of the incubation (final concn., 83  $\mu\text{M}$ ). Manganese was gradually accumulated as  $\text{MnO}_2$  during the incubation at the surface of mud.

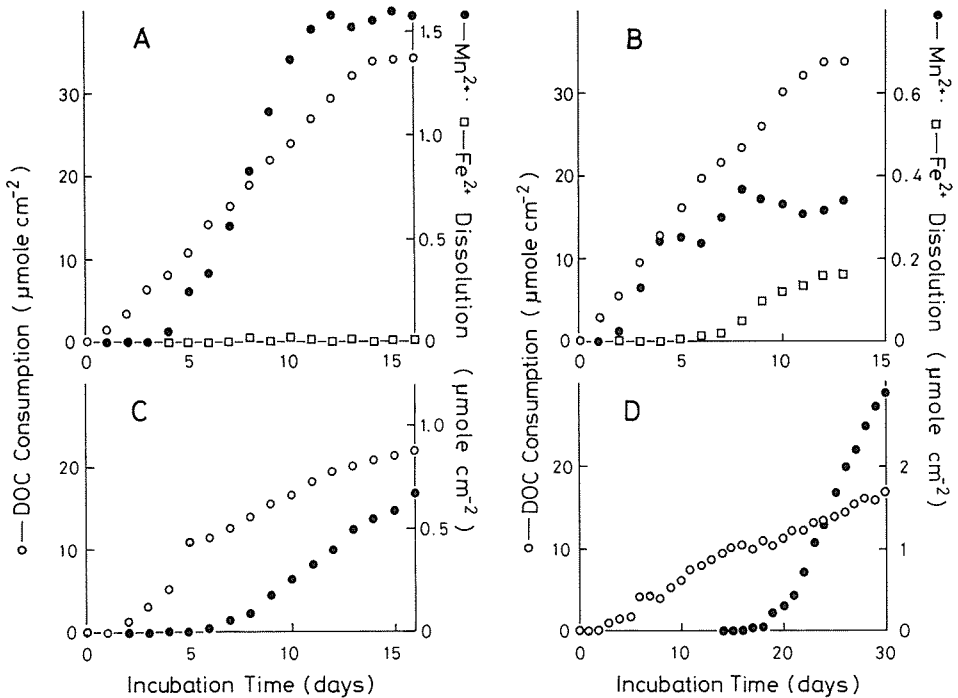
TOC-500),  $\text{Mn}^{2+}$  (formaldehyde, cf. Brewer and Spencer, 1971),  $\text{Fe}^{2+}$  (*o*-phenanthroline),  $\text{NO}_2^-$  (Bendschneider and Robinson's method),  $\text{NO}_3^-$  (sodium salicylate, cf. Scheiner, 1974), and  $\text{SO}_4^{2-}$  (ion chromatography, Yokogawa IC-7000E). The concentrations of the latter three ingredients were determined only if necessary. Obtained data of concentration changes were transformed into the amount of the substance that was emitted or uptaken by sediment, by the following formula:

$$A_i = (C_{i+1} \times V - C_i \times (V - 20) - C_a \times 20) / S$$

where  $A_i$ , the amount ( $\text{nmol cm}^{-2}$ ) emitted (if positive) or uptaken (if negative) by sediment between the sampling times of day  $i$  and of day  $i+1$ ;  $C_i$  and  $C_{i+1}$ , concentrations ( $\mu\text{M}$ ) in the subsamples taken at days  $i$  and  $i+1$ ;  $C_a$ , concentration ( $\mu\text{M}$ ) in the filtered aged lake water;  $V$ , volume (ml) of the water layer of the experimental system;  $S$ , sediment surface area ( $\text{cm}^2$ ) in the system.

## RESULTS

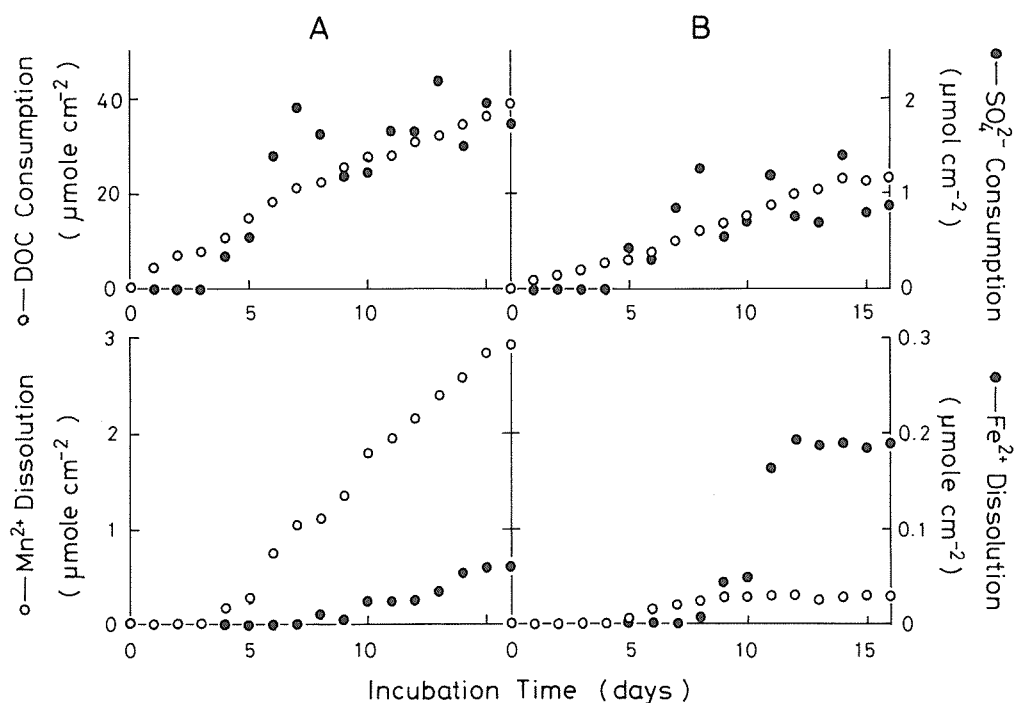
The first experiment examined the effect of organic addition to the experimental systems on the reductive dissolution of manganese and iron oxides. The experiment was started by adding organic substrates (acetate, glucose, or monomethylamine) to the water



**Fig. 2.** Mud/water experimental systems were supplied with organic substrates and incubated at 8°C in dark. Amounts of dissolved organic carbon (DOC, open circles) removed from the water layer and of manganese (solid circles) and iron (squares) reduced and emitted to the water layer are plotted. Added substrates were: A and B, sodium acetate (1 mM); C, glucose (0.33 mM); D, monomethylamine hydrochloride (2 mM). Mud samples were collected on: A and B, Dec. 1991; C, May 1992; D, July 1992.

layer of the experimental systems at a concentration of 2 mg-at.C l<sup>-1</sup>. In any cases, the added substrates seemed to be consumed with relatively constant rates, though the area-based decrement rate of DOC significantly varied by the kind of substrate (Fig. 2). Reductive dissolution of manganese oxide that had been originally accumulated on the sediment surface occurred after an induction period of a few days (acetate and glucose) to a few weeks (methylamine). It was thought to have taken this period for the condition of the sediment surface to become anoxic enough for the manganese reduction to start. The final yield of reduced manganese varied from 0.4  $\mu\text{mol cm}^{-2}$  (Fig. 2-B) to 3.0  $\mu\text{mol cm}^{-2}$  (Fig. 2-D). The experimental design in which the water surface was exposed to oxidative head space makes it difficult to interpret such quantitative differences. But this variation seemed at least partially due to the difference in the initial amount of manganese oxide accumulated on the sediment surface. In the case where the manganese reduction yield was the smallest (Fig. 2-B), iron also began to be reduced as soon as the manganese reduction had reached plateau. This suggested that iron oxide was utilized as an alternative electron acceptor when manganese oxide was depleted, though whether the same microorganisms would have reduced iron oxide as did manganese oxide could not be determined.

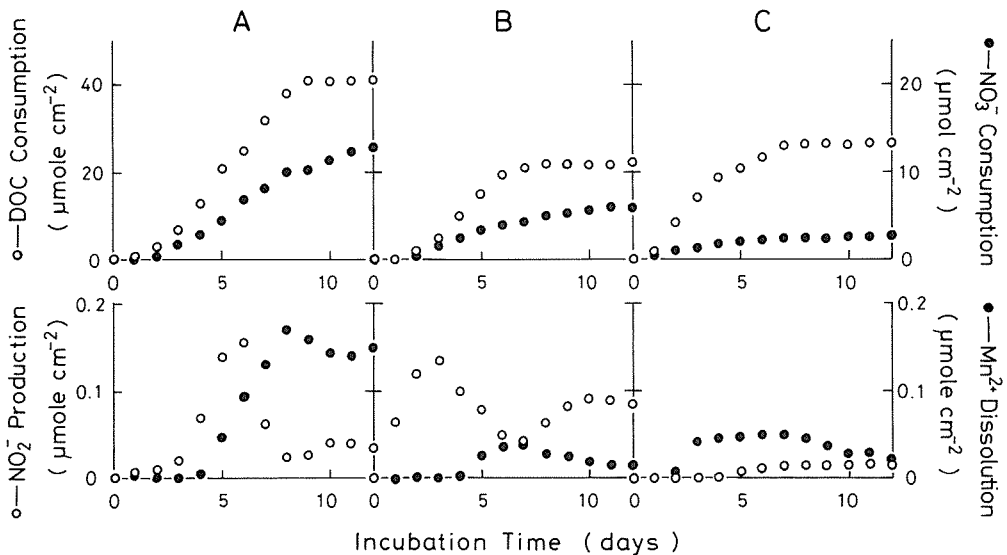
In the next experiment, organic substrate (acetate 1 mM) was added to the experimental systems in the presence of sulfate of sufficient concentration (1 mM) to



**Fig. 3.** Experimental systems were supplied with sodium acetate (1 mM) and sodium sulfate (2 mM), and incubated at 8°C in dark. Amounts of DOC (open circles) and sulfate (solid ones) removed from water layer are plotted in upper panels, those of Mn (open circles) and Fe (solid ones) reduced and emitted to the water layer plotted in lower panels. The experiment was duplicated. Mud samples were collected on: A, May 1992; B, June 1992.

oxidize all the acetate by bacterial sulfate respiration. The results (Fig. 3) showed that, even in the presence of sulfate, temporal patterns of DOC consumption and reductive dissolution of manganese and iron oxides were essentially same as the cases without sulfate amendment (Fig. 2). Sulfate can penetrate the surficial manganese- and iron-oxide layers by diffusion into deeper sediment, and be reduced or merely accumulated there. Therefore, the apparent consumption of sulfate simultaneous with manganese reduction (Fig. 3) does not necessarily indicate sulfate respiration occurring under the same condition as the manganese reduction occurs. More likely, the finding that the presence of sulfate had no effect on the reductive dissolution of manganese and iron suggests that the manganese and iron respirations proceed under more oxic condition than the sulfate respiration does.

Next, a similar experiment was conducted with nitrate (2 mM) instead of sulfate as a supplemental electron acceptor. In this case (Fig. 4), manganese reduction seemed depressed severely with the yield of  $\text{Mn}^{2+}$  never exceeding  $0.2 \mu\text{mol cm}^{-2}$ , although the DOC consumption rate was significantly high compared to the cases without nitrate amendment (Figs. 2 and 3). Reduced iron was never detected in the water layer in this experiment. Where a small but unnegligible amount of manganese reduction was observed (Fig. 4-A), production of nitrite was also observed preceding the manganese reduction. It is possible that the manganese reduction in this case was caused by inorganic redox reaction of nitrite with manganese oxide (Bartlett, 1981). These results as a whole showed how the existence of a high concentration of nitrate enhanced, on one hand, mineralization of organic substrate by stimulating denitrifying activity but prevented, on the other, the redox potential of the sediment surface from lowering enough for the



**Fig. 4.** Experimental systems were supplied with sodium acetate (1 mM) and potassium nitrate (2 mM for A and B, 0.35 mM for C), and incubated at 8°C in dark. Amounts of DOC (open circles) and nitrate (solid ones) removed from water layer are plotted in upper panels, those of nitrite (open circles) and  $\text{Mn}^{2+}$  (solid ones) emitted to the water layer plotted in lower panels. Mud samples were collected on: A, Dec. 1991; B and C, Feb. 1992.

manganese or iron reduction to occur. Combining the results of Figs. 2-4, it is concluded that in the sedimentary redox sequence, various electron acceptors are consumed by bacterial respiration following the order that: (oxygen-) nitrate-manganese oxide-iron oxide-sulfate.

Since the organic substrates used here are not capable of directly reducing manganese oxide, it is evident that the observed reduction of manganese as well as iron oxides was caused in the course of bacterial degradation of the organic substrates. There are some ambiguities, however, in the results of this study as to whether the reduction of  $\text{MnO}_2$  was directly coupled with the oxidation of the added substrates. One possibility against the direct linkage is that the added organics were first utilized by iron- or sulfate-reducing bacteria to form  $\text{Fe}^{2+}$  or  $\text{HS}^-$ , which subsequently reduced  $\text{MnO}_2$  as abiotic reactions. Although the  $\text{Fe}^{2+}$  concentration in the water layer was below the detection limit in many experiments, it does not necessarily exclude this possibility because the oxidation of  $\text{Fe}^{2+}$  by  $\text{MnO}_2$  may be rapid enough to prevent the emission of  $\text{Fe}^{2+}$  (Myers and Nealson, 1988). This possibility may be discounted when considering that the iron and sulfate respirations bring less energetical benefits than the manganese respiration (see below). Another possibility is that the added substrates were first incorporated by aerobic bacteria residing in the water layer, the biomass of which was subsequently decomposed by manganese reducers after sedimentation. This is particularly plausible if a long induction period is observed before the manganese reduction commenced, as is the case in the experiment with methylamine (Fig. 2-D). Thus, further studies are needed with more elaborated designs to demonstrate the direct linkage between organic oxidation and manganese reduction as well as to elucidate the substrate spectrum for the bacterial manganese reduction.

## DISCUSSION

The lake water of the north basin of Lake Biwa always contains substantial dissolved oxygen so that the oxic/anoxic boundary is usually found at, or slightly below, the sediment surface. Thus,  $\text{Mn}^{2+}$  can be detected little in the lake water but is always present at 100 - 150  $\mu\text{M}$  in pore water. The sediment surface is usually covered with a brown oxidized layer of high  $\text{MnO}_2$  content (often several % as  $\text{MnO}_2$ ) which is visually distinct from underlying yellowish layer of ferric hydroxide. This structure apparently indicates the presence of oxidation-reduction cycling of manganese within the uppermost layer of sediment. Results of this study confirmed the existence at this layer of high potential activity of manganese reduction which was linked to mineralization of various organic substances. This conclusion conflicts apparently with the previous discussion of the mechanisms for manganese redox cycling at the redox gradient formed above the hypolimnetic anoxia (Miyajima, 1992b), where, based on the chemical equilibrium calculations,  $\text{MnO}_2$  reduction was ascribed to inorganic reaction with  $\text{Fe}^{2+}$ . Since both two studies proposed only potential but not necessarily predominant mechanisms responsible to the  $\text{MnO}_2$  reduction, it needs further studies to elucidate to what extents the respective processes actually function under natural conditions.

The observation that manganese reduction occurs between denitrification and iron reduction in the sedimentary redox sequence is consistent with the theoretical order of respiratory free energy outcome per given organic substrate, which decreases stepwise

when the terminal electron acceptor changes from oxygen via nitrate,  $\text{MnO}_2$ ,  $\text{Fe}(\text{OH})_3$ , sulfate to carbonate (Billen, 1982). It is thought that any electron acceptor, as a rule, can not be utilized until all the preceding electron acceptors are depleted enough. Observations of the sedimentary redox sequence similar to those in this study have been reported in different environments. In pelagic ocean sediments, the  $\text{MnO}_2$  layer similar to what is found at the surface of Lake Biwa sediment is formed at the sediment surface in some cases but more frequently in deeper subsurface sediment. Based on their analyses of pore water profiles, Froelich et al. (1979) concluded that denitrification and manganese reduction occurred at similar depths of oceanic sediment which were well below the aerobic regime but shallower than the zone of iron reduction. They could not discriminate the zones of denitrification and manganese reduction, presumably due to relatively low nitrate concentration in oceanic waters which might have failed to suppress the manganese reduction, or due to the coarse sampling interval which failed to detect finer-scaled vertical zonation. The vertical separation of zones responsible to nitrate and manganese reduction, both of which are several ten meters thick, is evident in the pycnocline of the Black Sea (Nealson et al., 1991). The manganese redox cycle in ocean may be of greater geochemical importance than in terrestrial environments because it is probably responsible to the formation of massive deposits of  $\text{MnO}_2$  on Pacific Ocean floor called manganese nodules.

Another example for the suboxic redox sequences can be quoted from the extensive studies on microbiological processes in submerged paddy soils in Japan. Since paddy soils usually contain much organic debris, they become strongly reductive states as soon as flooded, with Mn and Fe rapidly reduced and emitted into water layer. During prolonged incubation, decomposition activities in the soils tranquillize gradually until the surface of soils is covered with a thin layer of oxidized Fe and Mn. If excess nitrate or  $\text{MnO}_2$  is added on flooding, reduction of manganese and iron or iron alone, respectively, is significantly inhibited, which implies that nitrate and manganese reduction precedes manganese and iron reduction, respectively (reviewed by Takai and Kamura, 1966). The proliferation of nitrifiers are observed to follow the formation of the oxidized layer (Uehara and Takai, 1983), which should produce nitrate as a substrate for denitrification. In their later studies, the possibility of the direct coupling of bacterial respiration to the reduction of  $\text{MnO}_2$  was largely excluded, however. Kamura and Yoshida (1972) isolated many bacterial and yeast strains capable of  $\text{MnO}_2$  reduction from paddy soils, and demonstrated that the filtrates of the growth media spent for the incubation of mixed soil bacteria had a capacity to reduce  $\text{MnO}_2$ , which suggests the bacteria did not reduce  $\text{MnO}_2$  directly but only excreted some metabolite(s) capable of  $\text{MnO}_2$  reduction. Furthermore, Yoshida and Kamura (1975) found that the  $\text{MnO}_2$  reduction in paddy soils was often coupled also to the oxidation of  $\text{Fe}^{2+}$  which apparently originated in the bacterial iron reduction. After reviewing the results of these and other experiments, Yoshida (1975) concluded that 20 - 50% of the  $\text{MnO}_2$  reduction observed in submerged paddy soils was owed to the inorganic reaction with  $\text{Fe}^{2+}$ , with the remainder being ascribed to the action of some organic metabolites excreted during the bacterial growth. However, the excretion of reductive metabolites as well as the iron reduction in such soils seems to be confined within early periods of the flooding during which strongly reductive conditions prevail ephemerally with non-balanced degradation of native organic substances in paddy soils. Therefore, their interpretations for the  $\text{MnO}_2$  reduction can not be applied parallel to the phenomena



in natural lentic sediments such as found in Lake Biwa and open ocean, where the degradation of organic matters as well as various redox processes proceed nearly in the steady state.

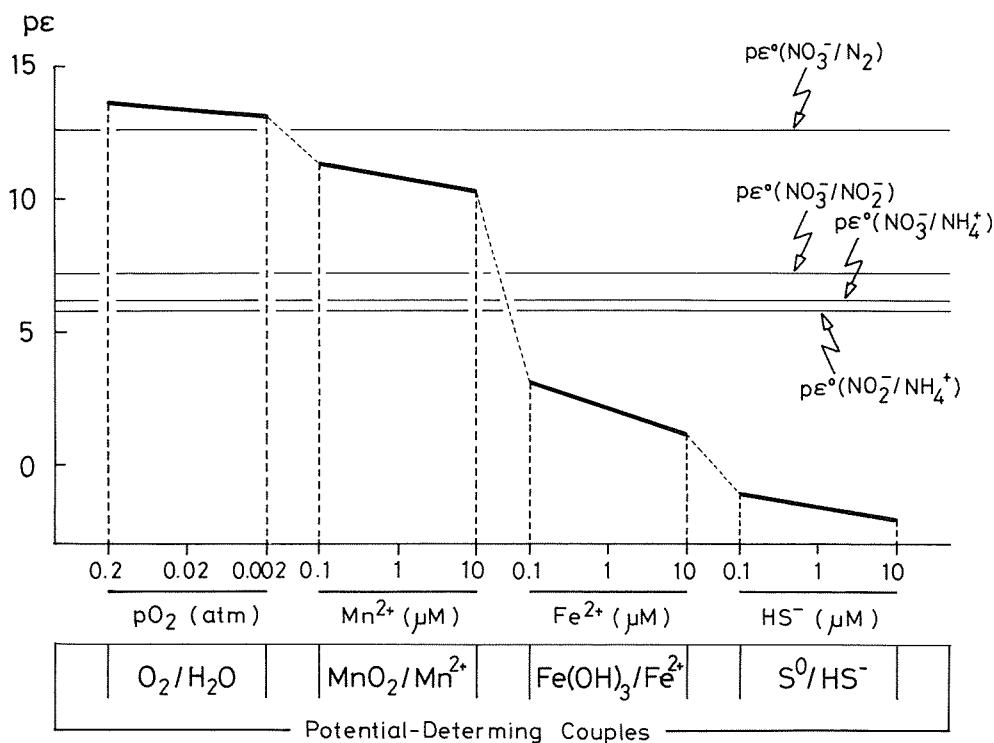
Ecological importances of the manganese redox cycle in natural lake sediments can be speculated in two ways. First, the cycle should function as an effective "electron shuttle" under suboxic conditions (Nealson and Myers, 1992) because of different mobility of manganese in oxic and anoxic environments (see INTRODUCTION). Since potential rates of reductive dissolution in the reduced layer as well as oxidative precipitation in the oxidative layer are high enough as revealed in this study, the cycle is thought usually turns over under the transport limitation. As the transport process depends on both diffusivity of  $Mn^{2+}$  and sedimentation or burial rate of  $MnO_2$ , the effectiveness of this cycle will increase when the oxic/anoxic interface appears in the water column or at the sediment surface rather than in subsurface sediment. The present author will report the actual contribution of manganese redox cycle to the hypolimnetic metabolism in Lake Biwa in another paper in preparation.

For manganese (or iron) reduction to be effectively coupled to the decomposition within the sediment, it may seem necessary that solid manganese (or iron) oxide be kept in touch with organic particles because the organic substrate is supplied to the sediment principally as particulate deposits. This difficulty, however, may be of less importance when hypothesizing metabolic consortia of fermentative and Mn- or Fe-reducing bacteria (Lovley and Phillips, 1988). Interstitial waters percolated from reduced paddy soils are known to contain some organic substances which are able to reduce both iron and manganese oxides (Matsumoto and Wada, 1972). Okazaki et al. (1976) reported that when submerged paddy soils of little Fe(III) contents was amended with debris of rice straws, interstitial waters became to contain much reductive (presumably organic) solutes, and that if the interstitial waters were introduced anaerobically to an ion-exchange resin loaded with  $Fe^{3+}$ , the  $Fe^{3+}$  was gradually reduced from the inlet downward. Unfortunately they did not determined whether the  $Fe^{3+}$  reduction was caused biologically or abiotically, but this observation seems to support the interconnection of decomposition and iron reduction via fermentative metabolites. Thus, future studies on the manganese reduction in natural sediments should be concentrated also on the elucidation of pathways of reducing power from particulate organic matter to manganese oxide.

Second, the  $MnO_2$  layer which appears in the redox gradient may play a role as a redox buffer. This function should be emphasized here as previous reviews on the manganese cycle do not devote much in this point. The redox potential of sediment varies with depth depending on redox couple the most prevalent in each layer. When the oxidized side of a redox couple is solid as is the case for  $MnO_2$ ,  $Fe(OH)_3$  and  $S^0$ , often formed at a particular depth in the sediment is a conspicuous deposit of the oxidized species in which the redox potential is determined by the very redox couple. Figure 5 shows how the redox potential ( $p_e$ ) in the sediment should be determined by manganese, iron and sulfur redox couples with the reduced sides of the couples being at near-natural concentrations. When rotated 90°, this figure also shows schematically the vertical separation of characteristic layers and the corresponding change in  $p_e$  found in the uppermost layers of natural sediments. It seems of particular importance that when manganese functions as the redox-determinant element, sympatric occurrence of nitrification and denitrification is theoretically possible, because the equilibrium redox potentials of  $NO_3^-/NH_4^+$  and  $NO_3^-/N_2$  half-cells are lower

and higher, respectively, than the ambient potential imposed by the manganese equilibrium (Fig. 5). If the ambient potential is controlled by oxygen or iron redox couples, denitrification or nitrification, respectively, must be excluded. Thus, the presence of the  $\text{MnO}_2$  layer may support effective transformation of inorganic nitrogen by coupling nitrification to denitrification.

The importance of the layers of oxidized metals for nitrification is supported by some studies on paddy soil processes. As already noted, the proliferation of nitrifiers usually follows the appearance of the oxidized layer at the surface of submerged soils. In their studies on the manganese oxidation in paddy soils, Kamura et al. (1977) observed that nitrification proceeded rapidly only after the completion of manganese oxidation. This phenomenon may be explained by competition for oxygen between manganese oxidizers and nitrifiers and/or an inhibitory effect of  $\text{Mn}^{2+}$  on the nitrification (Oka and Wada, 1992). But it is also possible that the presence of  $\text{MnO}_2$  itself enhanced the nitrification by stabilizing redox conditions of the habitat of nitrifiers. Takai and Uehara (1973, 1976) reported, on the other hand, that in many cases, little nitrate and nitrite were accumulated even after the proliferation of nitrifiers. Since the denitrifiers did not always proliferate



**Fig. 5.** Values of redox potential as determined by particular redox couples which are abundant in lake sediments. Potentials are calculated by the Nernst's equations, and plotted as a function of the concentration of dissolved species. Equilibrium potentials (denoted as  $pe^0$ ) for  $\text{NO}_3^-/\text{N}_2$  couple and for  $\text{NO}_3^-/\text{NO}_2^-$ ,  $\text{NO}_3^-/\text{NH}_4^+$  and  $\text{NO}_2^-/\text{NH}_4^+$  couples at pH 7 with unit activities of the oxidized and reduced species (Stumm and Morgan, 1981) is also shown as a theoretical upper boundary for denitrification and as theoretical lower boundaries for nitrite oxidation, overall nitrification and ammonium oxidation, respectively.

parallel to the nitrifiers, the authors ascribed the rapid removal of oxidized nitrogen partially to inorganic oxidation of  $\text{Fe}^{2+}$  by nitrite (Nelson and Bremner, 1970). This reaction, if actually occurs, should aid the formation and maintenance of the oxidized layer itself.

Similar examples of rapid nitrate removal are found in studies of coastal marine sediments where the regeneration efficiency of nitrate may limit the primary productivity in overlying water column. Denitrification activity in these environments often seems to be limited by nitrate availability which is determined by nitrification within the sediments (Seitzinger, 1988). Jenkins and Kemp (1984) reported that sometimes more than 99% of nitrate produced by nitrifiers in well-oxygenated surficial sediments had been removed by denitrification before escaping to the overlying water. Since the denitrification in sediments often functions as a dominant outlet in the nitrogen economy of estuaries as well as of lakes (Seitzinger, 1988), it deserves extensive efforts to elucidate by what means such a tight coupling of nitrogen metabolism can be maintained. These researchers commented that such a tight coupling was difficult to be explained by the classical bilayer model which assumed a distinct anaerobic layer for denitrification overlain with an aerobic layer for nitrification. Rather, denitrification in such environments seems more presumably to occur within anaerobic microsites in the aerobic surface layer, where high diffusional flux of nitrate caused by steep microgradient may support the tight coupling of nitrification to denitrification (Seitzinger, 1988). Thus, microbial communities in oxidized sediments may be much more highly structured in space than previously thought, though knowledge about such microstructures is little more than what is speculated. The  $\text{MnO}_2$  layer where the manganese redox couple determines the ambient redox potential is one of the candidate sites that harbor structured microcommunities including the tight coupling of nitrification and denitrification. Further studies on spacial interactions among microbes capable of processing different elements such as nitrogen and manganese may offer an interesting approach in sedimentary microbial ecology.

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