

Mud-water fluxes of inorganic nitrogen and manganese in the pelagic region of Lake Biwa: seasonal dynamics and impact on the hypolimnetic metabolism

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With 9 figures and 5 tables in the text

Abstract

In the north basin of Lake Biwa (Japan), the oxic/anoxic interface is usually found at the sediment surface. Reduced manganese (Mn^{2+}) and nitrogen (NH_4^+) which were always accumulated in pore waters were detected little in the overlying lake water, which suggested the prevalence of Mn^{2+} - and NH_4^+ -oxidizing microbial activities at the sediment surface. This was proved by adding biological inhibitors such as azide to intact mud samples which induced rapid emission of Mn^{2+} and NH_4^+ from sediment to overlying water. Using data of emission or uptake rates by sediment of Mn^{2+} , NH_4^+ , NO_2^- and NO_3^- in the presence and absence of inhibitors, the rates of manganese oxidation, nitrification and denitrification were estimated by mass balance calculation over a one-year cycle. Both manganese oxidation and nitrification rates were found to decline towards the end of the stagnation period. This is presumably due to decrease in the oxygen availability at the sediment surface in this season, though the hypolimnetic oxygen concentration had never fell to zero. Concomitantly, denitrification rate was also lowered which resulted in enhancement of the regeneration of NH_4^+ from sediment to lake water. Of the whole oxygen consumption below the thermocline during the stagnation period, 9.5, 3.4 and 0.6% were ascribed to nitrification in the hypolimnetic water, nitrification at the sediment surface and manganese oxidation at the sediment surface, respectively. When comparing with the nitrogen economy of this lake found in literature, denitrification proved to have removed about half of nitrogen input to this lake.

Introduction

Transformations of inorganic nitrogen and manganese in aquatic environments are usually under control of various biological processes. Inorganic nitrogen species which usually exist as dissolved ions such as NH_4^+ , NO_2^- and NO_3^- are not only required for biosynthesis by primary producers, but some specific microorganisms also depend on them as electron donors for chemolithotrophy (nitrification) or as electron acceptors for anaerobic respira-

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Table 1. Equilibrium potentials ($p\epsilon^0(W)$) of some important redox couples for unit activities of oxidants and reductants at pH 7.0 and 25 °C (STUMM & MORGAN 1981).

Reaction	$p\epsilon^0(W)$
$O_2 + 4H^+ + 4e^- = 2H_2O$	+13.75
$2NO_3^- + 12H^+ + 10e^- = N_2 + 6H_2O$	+12.65
$\delta\text{-MnO}_2 + 4H^+ + 2e^- = Mn^{2+} + 2H_2O$	+7.8
$NO_3^- + 2H^+ + 2e^- = NO_2^- + H_2O$	+7.15
$NO_3^- + 10H^+ + 8e^- = NH_4^+ + 3H_2O$	+6.15
$NO_2^- + 8H^+ + 6e^- = NH_4^+ + 2H_2O$	+5.82
$\gamma\text{-MnOOH} + 3H^+ + e^- = Mn^{2+} + 2H_2O$	+4.3
$am\text{-FeOOH} + 3H^+ + e^- = Fe^{2+} + 2H_2O$	-3.9

tion (denitrification). By this reason, spacial and temporal distributions of inorganic nitrogens within a given basin often bring much information on the productivity and the trophic status as well as on the redox conditions of the basin (RIPL & FEIBICKE 1992). In contrast to nitrogen, manganese in reduced and oxidized states usually exists as free cation (Mn^{2+}) and insoluble oxide (MnO_2), respectively, which often results in anomalous concentration of this element near the oxic/anoxic interface (DAVISON 1985). Thus, manganese redox cycling may have a substantial contribution to the electron flow across the interface under natural conditions. The manganese redox processes have not been received much attention from ecologists as no higher organism has any interactions with them. The involvement of microbiological mediation, however, is evident in both manganese oxidation and reduction (NEALSON et al. 1988, LOVLEY 1991).

Environmentally important redox couples involving inorganic nitrogen or manganese have similar standard redox potentials to one another, all of which are lower than that of the oxygen couple (O_2/H_2O) and higher than that of the iron couple ($Fe(OH)_3/Fe^{2+}$, Table 1). This is why some important redox processes of nitrogen and manganese often occur sympatrically within the redox gradient found in natural aquatic systems (e.g. FROELICH et al. 1979). However, except for inorganic reduction of manganese oxide by nitrite reported for soils (BARTLETT 1981), evidences for direct coupling between redox reactions of inorganic nitrogen and manganese are relatively few under natural conditions. This is presumably related to the fact that, however the ambient redox potentials may be subject to the inorganic equilibria, net redox reactions proceed predominantly through the biological electron transfer processes. In the biological systems, the oxidation of reduced species such as NH_4^+ , NO_2^- and Mn^{2+} is principally coupled by chemolithotrophic bacteria with reduction of oxygen, whereas the reduction of oxidized species such as NO_2^- , NO_3^- and MnO_2 is usually coupled by anaerobic bacteria to oxidation of organics, hydrogen and sulfide. Actual activities of elementary redox processes involving nitrogen or manga-

nese, however, may be modified by one another through some indirect interactions such as competition for oxygen by nitrifiers and manganese oxidizers, and that for organic substrates by denitrifiers and manganese reducers.

In this study, the author evaluated the in-situ activities of nitrification, denitrification and manganese oxidation in the sediment of the north (main) basin of Lake Biwa, traced out their seasonal dynamics, and estimated their impacts on the whole-lake metabolism. Lake Biwa, the largest lake in Japan, is mesotrophic and the biological productivity in the pelagic region is largely limited by the phosphorus availability. Although there are a number of studies in this basin on the primary productivity, particulate material flux, decomposition and nutrient regeneration within the water column (e.g. MIYAJIMA 1992, and literature cited in it), metabolic processes within the sediment as well as the mud/water interactions have been received little attention as yet (ICHIKI & TIMPERLEY 1986 as an exception). Since the hypolimnetic water of this basin is not completely depleted of oxygen during the stagnation period, the oxic/anoxic interface usually appears just at or slightly deeper than the sediment surface. An oxidative mud layer characterized by the presence of manganese and iron oxides covers the sediment surface with a thickness of several millimeters over the major part of a year. This oxidative layer of the sediment is thought to be the most active locus for the redox transformation of both nitrogen and manganese in this basin. In the pelagic sediment of this basin, ambient temperature varies little throughout a year (8.0 ± 0.5 °C), so that the oxygen concentration is the only environmental factor that shows significant seasonality. Although oxygen is not depleted from the hypolimnetic water, the oxygen availability at the sediment surface, which is determined by the dynamic balance between the diffusion rate of oxygen from the overlying water and the oxygen consumption rate due to respiration of sedimentary biota, may vary drastically both in time and in space. As to the depositional input of organic matter to the sediment, MAEDA et al. (1987) reported that the flux increased both at the beginning of the stagnation period (May) and in the rainy season (July). Such an elevated loading of organic matter will intensify the competition for oxygen by heterotrophic and chemolithotrophic organisms residing at the sediment surface.

Recent eutrophication of the north basin of Lake Biwa as illustrated by seasonal bloom of *Uroglena* (TEZUKA 1992) should have led to increasing organic loading to and accumulation in the hypolimnion and the sediment. In fact, the chemical oxygen demand (COD) level in the hypolimnetic water of the north basin of Lake Biwa has increased slowly during the last decade from 1.5 to 1.7 mg O₂ l⁻¹ (SONO & NOMURA 1991). Although the annual minimal oxygen concentration in the hypolimnion of the north basin of Lake Biwa showed no marked trend over the last two decades (TEZUKA 1992), an ephemeral emergence of anoxic water mass has been recognized recently near the deepest region of this basin (YOSHIOKA 1991). In addition to the increase in the

COD, the observed oxygen depletion is thought to be ascribed partially to recent trend of decreasing snowfall caused by global climatic warming, which is limiting the vernal inflow of cold and highly oxygenated snow-thaw water to the hypolimnion (KUMAGAI 1990). The decrease of snow water inflow elevated the average bottom temperature by ca. 2 degrees, whereby the oxygen consumption due to decomposition was activated. To examine such speculations and to predict responses of this lake to future climatic change and eutrophication, we need more information on the hypolimnetic and sedimentary biogeochemical processes and their general responses to expected environmental stresses. In this study, discussions are directed first to describing redox and stoichiometric structure of hypolimnetic nitrogen and manganese metabolisms, and then a stress is also laid on the seasonal change of their activities in relation to environmental oxygen availability.

Material and methods

The survey was carried out monthly from July 1991 to September 1992. Sediment core samples used in this study were all collected at a station (depth, 75 m) in the north basin of Lake Biwa (maximal depth, 104 m; see Fig. 1). In the bottom water of this sta-

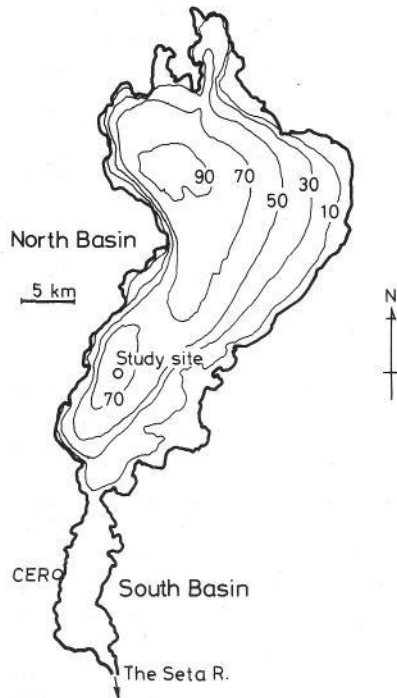


Fig. 1. Location of the sampling point (Study site) in Lake Biwa. Numbers in this map are depths in meters. CER, the Center for Ecological Research (our laboratory).

tion, water temperature was little variable throughout a year ($8.0 \pm 0.5^\circ\text{C}$) though oxygen saturation varied from near 100% at the beginning (May) down to 45% at the end (January) of the stagnation period. The sedimentation rate in the pelagic region of this basin is said to be in the order of mm per annum. MAEDA et al. (1987) reported that the flux of sedimenting organic matter to the sediment near this study site ranged from 20 to 70 $\text{mg-at.C m}^{-2} \text{day}^{-1}$ with maxima in May and July.

Water temperature was determined on the ship board with a thermistor thermometer. Oxygen concentration was determined by the Winkler method for water samples collected in a Van-Dorn sampler. Sediment core samples were collected with a gravity corer (\varnothing 5 cm; length 60 cm). Samples in which the surface layer was apparently disturbed were rejected. In some cases (Nov. 91 and Feb., May, Aug. 92), a core was sliced into 5 cm-long sections on ship board for subsequent extraction of pore waters by centrifugation (8000 g, 15 min at 8°C) in the laboratory. In one case (Oct. 91), two cores were cut into 2 to 5 cm sections, then dried and crushed for later analysis of the elemental composition (see Results). Other core samples collected were transported with ice water to the laboratory, where they were fixed vertically on a stand and incubated at in situ temperature in the dark. Each core usually consisted of a ca. 40 cm-long mud column overlain with a ca. 20 cm-deep water layer. At incubation, a head space (ca. 50 ml) was made at the top of each core to keep the overlying water oxygenated as it was under natural conditions.

Each incubation experiment was continued 12 days from the sampling, though data of the first 5 days were usually used for the flux estimation. During the incubation periods, subsamples of the overlying water were collected everyday: first, the overlying water was gently mixed by pipetting with much care taken not to disturb the sediment surface, then a 20 ml of subsample was withdrawn, and finally the same volume of filtered aged lake water (FALW) was added. FALW was prepared by filtering (Whatman GF/F) the lake water which was collected from 70 m layer at the study site and stored for about 3 months. The subsamples were analyzed later for concentrations of Mn^{2+} (BREWER & SPENCER 1977), NH_4^+ (BOWER & HOLM-HANSEN 1980), NO_3^- (SCHEINER 1974), and $\text{Si}(\text{OH})_4$ (molybdenum yellow). Obtained data of concentration changes were transformed into the amounts of the substance that were 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 (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 (μM) in the subsamples taken at days i and $i + 1$; C_a , concentration (μM) in the FLAW; V , volume (ml) of the water layer of the core; S , sediment surface area (cm^2) in the core.

In order to estimate the impact of microbiological activities upon the mud/water material fluxes, similar incubation experiments were done in the presence of specific or non-specific biological inhibitors. Single or duplicated cores per month were sacrificed to the inhibition experiment by adding a respiratory inhibitor sodium azide (NaN_3) to the overlying water (final concn. 2 mM) at the start of the incubation. NaN_3 at such a high concentration is thought to generally inhibit biological electron transport including both heterotrophic and chemolithotrophic ones. It does not interfere significantly any inorganic reactions involving ammonia or nitrate under near-neutral conditions. Although azide is known to coordinate to transition metal ions, inorganic chemical processes of manganese is not apparently affected by azide added to natural waters (ROSSON et al. 1985). Thus, any differences in the mud/water fluxes between the live and the NaN_3 -inhibited cores can be ascribed to microbiological activities though not specified.

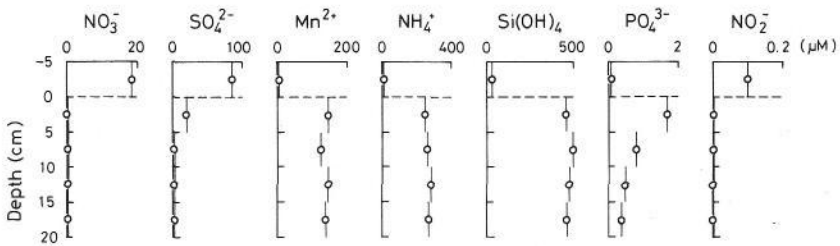


Fig. 2. Concentration profiles of some chemicals in the sediment pore water and the overlying lake water on August 1992. Dashed lines indicate the sediment-water interface. Sulfate concentration was determined by the ion chromatography (Yokogawa, IC-7000E), the others colorimetrically.

In some cases, more "specific" inhibitors such as chlorate, allyl thiourea and N-serve were tested (see Results for their dosages and effects). Subsamplings, chemical analyses and calculations in these inhibition experiments were performed after the methods already mentioned, although the analytical calibration was made with standard solutions which contained the inhibitor at the same concentration as the subsamples. For samples of chlorate-inhibition experiments, NO_2^- was determined by the Bendschneider-Robinson method. N-serve and allyl thiourea were found to give negative errors in the determination of ammonia, and chlorate interfered strongly the determination of nitrate.

Results and discussion

Redox structure of the mud/water interface

In the pelagic region of Lake Biwa, the redox gradient layer is usually formed near the mud/water interface as clearly seen when comparing concentrations of some redox-sensitive chemicals in sediment pore waters with those in overlying waters (Fig. 2). Oxidized species such as nitrate and sulfate which were abundant in the overlying water were nearly depleted in the pore waters, whereas reduced species such as ammonia and Mn^{2+} were present in well measurable concentrations only in the pore waters. The profiles of phosphate and silicate which are themselves redox-inactive seem to require other explanations such as steady-state mineralization and adsorption equilibria. As expected from their profiles, there should be oppositely directed diffusional fluxes at the mud/water interface of oxidized species (downward) and reduced ones (upward). To confirm this, mud/water fluxes of some solutes were estimated in intact core samples. The results (Fig. 3) showed that, if biological activity was stopped by sodium azide (2 mM), reduced species such as NH_4^+ and Mn^{2+} as well as Si(OH)_4 were leached rapidly out of sediment to overlying water in contrast to nitrate which was uptaken by sediment (data included in Fig. 7). In the control cores to which azide was not added, the effluxes of reduced species were significantly slower or sometimes absent. This indicated the prevalence of microbiological oxidizing activities upon NH_4^+ and Mn^{2+} at the sediment

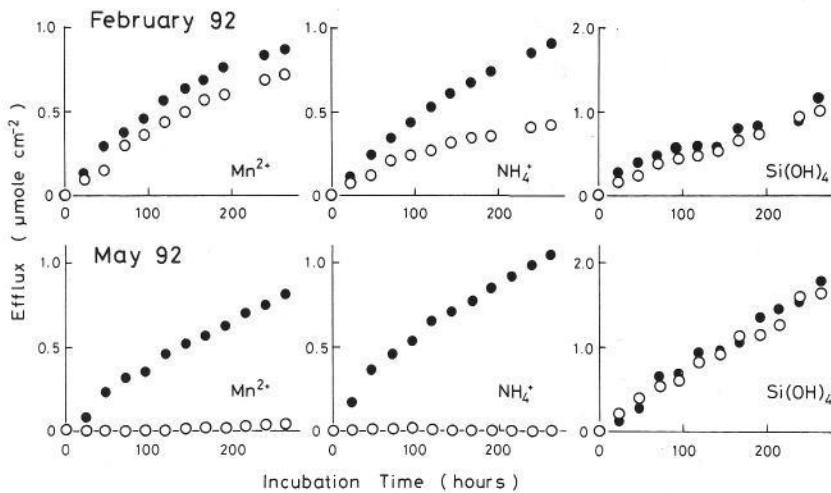


Fig. 3. Effluxes of Mn^{2+} , NH_4^+ and Si(OH)_4 from sediment to overlying water on February 1992 (top) and May 1992 (bottom). Incubation was started on the day of sample collection. Open circles, no inhibitor was added; solid circles, sodium azide (2 mM) was added to the overlying water.

surface. For Si(OH)_4 efflux, little difference was observed between inhibited and uninhibited cores. Phosphate leachage was never detected either in inhibited or control cores, presumably due to much lower phosphate concentration in pore waters.

The microbiological oxidizing activities expected from the above results should have resulted in net production of oxidized manganese and nitrogen at the sediment surface under natural conditions. Due to the extremely low solubility, oxidized manganese tends to accumulate as precipitate on the sediment surface. The profile of manganese in the bulk sediment (Fig. 4) can be explained by this effect though further speciation is needed for estimating real pool size of the sedimentary manganese oxide. If the environment of the sediment surface is sufficiently oxic, the oxidative precipitation of manganese at the surface and its reductive dissolution in underlying layers are thought to proceed at balanced rates under diffusion limitation, resulting in the manganese redox cycle to be closed within the vicinity of the oxic/anoxic boundary with pools of oxidized and reduced manganese remained above and below the boundary (DAVISON 1985). In contrast to manganese, nitrogen in the sediment is thought to be largely processed through a one-way redox transformation instead of a closed cycle. Nitrogen is originally supplied to the sediment as particulate organic nitrogen (PON), which is gradually mineralized by heterotrophic bacteria and macrobenthos into NH_4^+ and accumulated transitionally as free or adsorbed NH_4^+ in pore water. When NH_4^+ diffuses into the oxic do-

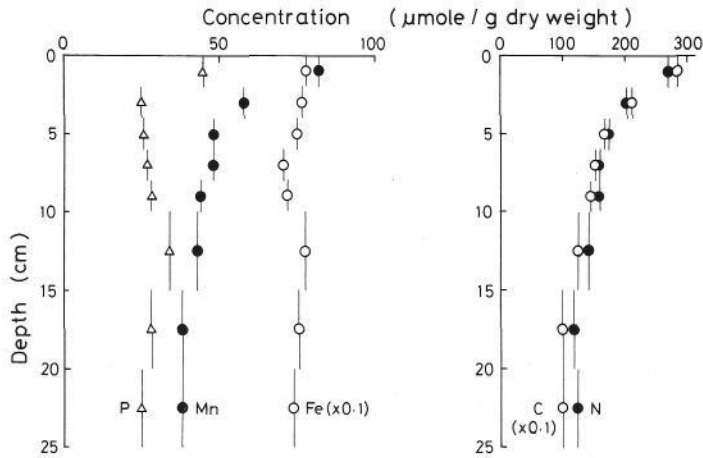


Fig. 4. Vertical profiles of acid-soluble phosphorus, manganese and iron (left) and of non-volatile carbon and nitrogen (right) in the bulk sediment cores (averages of two cores) collected on October 1991. P, Mn and Fe were leached from dry sediments by hot-acid treatment (1M HCl, 110°C, 10 min); C and N were determined by a CHN analyzer (Yanaco, MT-3).

main, it is oxidized by nitrifiers into NO_2^- and NO_3^- . Some oxidized nitrogen thus produced may diffuse out of sediment to overlying water, but the larger fraction seems to suffer subsequent denitrification within the sediment as discussed later. Thus, there are three pools of intermediates (PON, NH_4^+ and oxidized nitrogen) in the sediment, though the pool of oxidized nitrogen seemed negligible in size (Fig. 2). The vertical distribution of nitrogen and manganese in bulk sediment (Fig. 4) suggests that active mineralization and redox processes occur largely within upper several cm.

If it can be assumed that effective pools for diffusional fluxes of NH_4^+ and Mn^{2+} with respect to the oxic/anoxic boundary do not extend deeper than 5 (or 10) cm into the sediment and that their mud/water fluxes estimated in the azide-inhibited cores correspond to their actual fluxes through the oxic/anoxic boundary, then the effective pool sizes of NH_4^+ and Mn^{2+} as well as their turnover times can be calculated tentatively (Table 2). The turnover times similarly computed for the silicate pool were about twice as long as those for NH_4^+ and Mn^{2+} , probably reflecting some difference in the turnover mechanisms. There are pools in the sediment, too, for adsorbed NH_4^+ and Mn^{2+} , which are much larger than those for free ones. As the adsorbed fractions should have compensated the free ones with the latter decreasing during the incubation, the leaching rates of NH_4^+ and Mn^{2+} did not show much observable change over one calculated turnover period (Fig. 3). But the rates also seem to have decreased slightly towards the end of the incubations, presumably sug-

Table 2. Estimation for pool sizes and turnover times of free NH_4^+ , Mn^{2+} and $\text{Si}(\text{OH})_4$ in pore water of the sediment in the north basin of Lake Biwa (depth, 75 m).

	Species	Pool Size ¹ /mmol m ⁻²	Turnover Time ² /days
A	NH_4^+	7.7±1.8	8.1± 2.5
	Mn^{2+}	5.7±0.6	6.0± 1.5
	$\text{Si}(\text{OH})_4$	18.4±4.4	17.4± 5.5
B	NH_4^+	15.3±3.2	16.1± 4.2
	Mn^{2+}	9.9±1.7	10.5± 3.3
	$\text{Si}(\text{OH})_4$	36.3±9.7	34.4±11.6

* Estimates were made four times (Nov. 1991 and Feb., May, Aug. 1992). Mean values and standard deviations among the four estimates are presented here.

¹ Calculated from the pore water profiles. Pore water in the upper 5 cm (A) or 10 cm (B) layer of sediment only is assumed here to be the effective pool for the emission of each species across the oxic/anoxic boundary.

² Turnover times were calculated assuming that the emission rates of each species across the oxic/anoxic boundary were poised with the production rates in sediment.

gesting that the desorption rate rather than the diffusion rate became a predominant limiting factor as time went on. Accordingly, in later paragraphs, the author exclusively uses the estimates of mud-water fluxes which are calculated by linear regression only for the data of the first five days of the incubation experiments.

Effects of "specific" inhibitors in mud/water systems

In order to elucidate the roles for chemolithotrophic processes in the redox process of nitrogen at the sediment surface, effects of some "specific" inhibitors such as N-serve, chlorate (ClO_3^-) and allyl thiourea (ATU) were tested (OREMLAND & CAPONE 1988). In any case, inhibitor was added to the overlying water at the beginning of the experiments. N-serve and ATU are known to inhibit ammonia monooxygenase, an essential enzyme for ammonium oxidation, presumably by chelating the reaction-center Cu atoms. Therefore, both N-serve and ATU are expected to have similar effects to azide on the mud/water efflux of NH_4^+ . ATU, but not N-serve, does also inhibit nitrite oxidation. ClO_3^- is a competitive inhibitor for nitrite oxidation but does not affect by itself the ammonium oxidation (BELSER & MAYS 1980). Thus, on the addition of ClO_3^- instead of azide, NO_2^- instead of NH_4^+ is expected to be emitted to the overlying water. ClO_3^- and N-serve are said to inhibit denitrification at least partially.

N-serve was added as acetic solution to the final concentration of 20 ppm (HENRIKSEN 1980). Despite its popularity, N-serve was found to have a much poorer effect on NH_4^+ efflux than azide in the mud/water systems (data not shown). ICHIKI & TIMPERLEY (1986) carried out similar emission experi-

Table 3. Examples for rates of emission (or uptake) of ammonium, nitrite and nitrate by the sediment on the amendment of various "specific" inhibitors for chemolithotrophic nitrification.

Month	Inhibitors ¹	Emission rate ² /nmol cm ⁻² h ⁻¹		
		NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻
July 92	None	1.28±0.10	0.00	-0.67±0.08
	Chlorate	1.32±0.20	0.42±0.03	nd
	Azide	5.28±0.31	0.00	-0.09±0.14
Aug. 92	None	1.79±0.15	0.00	-0.36±0.05
	Chlorate	nd	0.13±0.01	nd
	ATU	3.34±0.27	0.00	-0.38±0.17
	Azide	3.59±0.21	0.00	-0.10±0.05
Sep. 92	None	1.90±0.10	nd	-0.51±0.08
	ATU	2.28±0.08	0.00	-0.74±0.09
	Azide	3.82±0.43	nd	-0.17±0.07

¹ Added by the following doses: sodium chlorate, 10 mM; sodium azide, 2 mM; allyl thiourea (ATU), 1 mM.

² Negative values mean uptake rates. nd., not determined.

ments and found slight increases in both NH₄⁺ emission and NO₃⁻ uptake by sediment caused by the addition of N-serve. Since the differences were very small, they concluded that the nitrification rate in Lake Biwa sediments was insignificant compared with NH₄⁺ production rate. Effect of ATU (added at 1 mM) was also not so clear as azide (Table 3). In one case (Aug. 92), the effluxes of NH₄⁺ in the presence of ATU and of azide were comparable, which seems to confirm the chemolithotrophic NH₄⁺ oxidation as the major factor mediating NH₄⁺ flux. However, ATU showed a significantly poorer effect than azide in another case (Sep. 92). These discrepancies between the NH₄⁺ consumption rates estimated by use of specific (N-serve and ATU) and non-specific (azide) inhibitors may be interpreted in two ways. First, the gross NH₄⁺ uptake is actually contributed not only by nitrification but also by heterotrophic NH₄⁺ assimilation. Since azide should have also inhibited both heterotrophic NH₄⁺ assimilation and production at the sediment surface, the difference in the NH₄⁺ emission rate between control and azide-inhibited systems must have exceeded the actual NH₄⁺ consumption rate due to nitrification if the heterotrophic NH₄⁺ assimilation had dominated over the NH₄⁺ production in the control system. This situation is, however, not feasible in Lake Biwa where both primary production and decomposition seem to proceed under phosphorus (not nitrogen) limitation. In fact, net NH₄⁺ production is always observed during aerobic dark decomposition of seston collected from Lake Biwa (TEZUKA 1986). Thus, the above-mentioned discrepancies can not be explained in this way. Second, it is likely that by some reason N-serve and ATU failed to inhibit completely the nitrification in the intact sediment

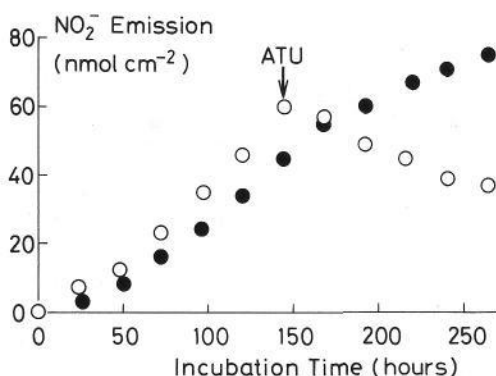


Fig. 5. Emission of nitrite from sediment on the addition of sodium chlorate (10 mM). Open circles, allyl thiourea (ATU, 1 mM) was added at 145 hours (arrow); solid circles, ATU was not added. Sample cores were collected on July 1992.

system used in this study. As for N-serve, BOON (1986) found that it exerted unexpectedly poor effect on the nitrification in seagrass-bed sediments presumably due to its poor diffusivity or inactivation in reductive environments prevailing in the sediments. Also, data of WOOD et al. (1981) shows that the inhibitory effect of ATU on nitrification is often far from being complete. In this study, after all, the difference in the NH_4^+ emission rate between the control and azide-inhibited systems was tentatively regarded as reflecting actual nitrification rate at the sediment surface.

In the presence of ClO_3^- , nitrite was emitted from the sediment surface (Table 3). This NO_2^- emission ceased on subsequent addition of ATU (Fig. 5), which suggested bacterial ammonium oxidation as the source of NO_2^- . As the NO_2^- emission was not observed in the control cores, the NO_2^- detected in the ClO_3^- -inhibited cores was thought to be what should have been consumed by nitrite oxidizers as soon as being produced in the absence of ClO_3^- . If so, the actual NO_2^- oxidation rate should have been largely balanced with the actual NH_4^+ oxidation rates. However, observed emission rates of NO_2^- were always significantly smaller than the differences in NH_4^+ efflux between the control and azide-inhibited cores which were regarded here as reflecting the actual NH_4^+ oxidation rates. This discrepancy may be ascribed to several reasons. First, NO_2^- produced at the sediment surface could diffuse both upward and downward, but the experiments detected only the fraction of NO_2^- that diffused upward to the overlying water. Thus, actual NO_2^- oxidation rate should have been about twice as high as the observed NO_2^- emission rate. Second, NO_2^- once produced might have been subsequently removed before escaping to overlying water, through either denitrification or inorganic redox reactions with indigenous manganese oxide (BARTLETT 1981) or ferrous iron (NELSON & BREMNER 1970). Third, if there were microsites where NO_2^- /

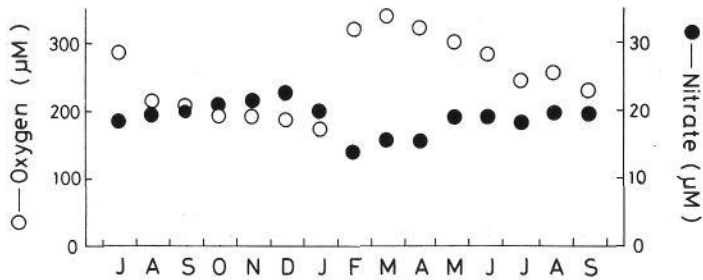


Fig. 6. Seasonal changes in the concentrations of oxygen and nitrate in the bottom lake water. Samples for oxygen determination were collected by a van-Dorn sampler and analyzed by the Winkler method. Ones for nitrate analyses were withdrawn from the overlying waters collected together with mud samples by a gravity corer.

ClO_3^- ratio is drastically higher than outside, NO_2^- -oxidizers residing within them would have survived with considerable NO_2^- -oxidizing activities. Finally, NH_4^+ oxidation itself might have been inhibited partially by chlorite (ClO_2^-) that is said to be produced through reduction of ClO_3^- by denitrifiers (HYNES & KNOWLES 1983). Therefore, these data of NO_2^- emission are probably considerable underestimates for the actual nitrite oxidation rates.

Seasonal changes in the mud/water fluxes

In the study site, temperature is largely invariable (around 8 °C) but oxygen content in the overlying lake water decreased gradually during the stagnation period (May to January; Fig. 6). The brown MnO_2 layer that usually covered the sediment surface tended to diminish towards the end of the stagnation period, which indicated that the ambient redox states near the sediment surface became more reductive. Such a trend in the redox states of sediment surface was also reflected in the mud-water fluxes of Mn^{2+} and NH_4^+ (Fig. 7). The effluxes of Mn^{2+} and NH_4^+ in the live cores (hatched bars) showed similar seasonal trends, with two minima in summer to early autumn of 1991 and in early summer of 1992 which indicate high activities of manganese- and ammonium-oxidizers in the early stagnation periods. In late stagnation periods of both years, relatively high effluxes of Mn^{2+} and NH_4^+ were observed in the live cores. These results suggested that bacterial Mn^{2+} - and NH_4^+ -oxidation activities were depressed towards the end of the stagnation period presumably due to decrease in the oxygen availability. The emission of $\text{Si}(\text{OH})_4$ in the live cores seemed to decline in the circulation period. Release of silicate from the mud is said to be controlled at least partially by the adsorption process onto ferric hydroxide (FUJINAGA & HORI 1982). It is thus possible that the formation of fresh $\text{Fe}(\text{OH})_3$ precipitates caused by increased oxygen supply in this period depressed the $\text{Si}(\text{OH})_4$ emission.

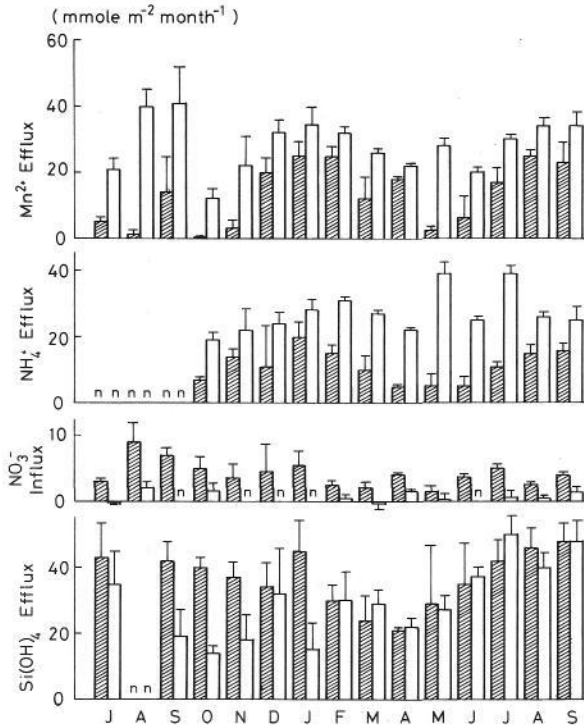


Fig. 7. Seasonal changes in the effluxes of Mn^{2+} , NH_4^+ and $Si(OH)_4$ from sediment and the influx of NO_3^- into sediment. Data of emission (illustrated in Fig. 3) for the first five days of incubation were regressed against the time, and the gradients and the standard errors were plotted as broad and narrow bars, respectively. Hatched bars, incubation without inhibitors ($n = 2$ to 4 for each month); hollow bars, with sodium azide (2 mM ; $n = 1$ or 2 for each month). 'n' means no data.

Figure 8 compares the manganese and ammonium oxidizing activities averaged for the early and late stagnation periods and for the circulation period. The Mn^{2+} and NH_4^+ oxidizing activities were estimated as the differences in the effluxes of Mn^{2+} and NH_4^+ , respectively, between the control and the azide-inhibited cores. Denitrification rates were also calculated as the sum of NH_4^+ oxidation rates and NO_3^- influx to sediment in the live cores. As expected, the NH_4^+ oxidation rate and the denitrification rate were significantly higher for the early stagnation than for the late one ($p > 0.95$, t -test). Similar trend was also found for the Mn^{2+} oxidation activity (insignificant statistically). As the oxygen saturation in overlying water had never fell below 40%, it may not appear natural to explain the observed declines in the Mn^{2+} and NH_4^+ oxidizing activities from oxygen availability. But the oxygen availability for Mn^{2+} and NH_4^+ oxidizing bacteria at the sediment surface is not only controlled by ambient oxygen concentration but affected also by competition

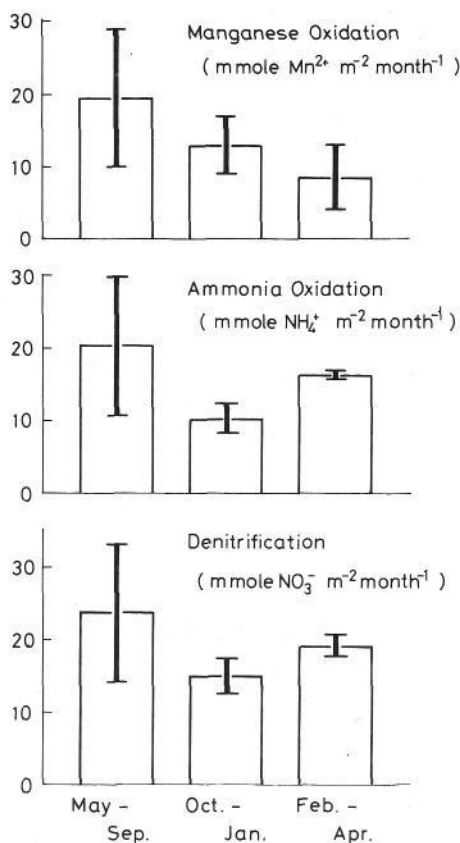


Fig. 8. Manganese oxidation, ammonium oxidation and denitrification activities at the sediment surface, averaged for the early stagnation period (May–September), the late stagnation period (October–January), and the circulation period (February–April). Statistically significant differences are detected by the *t*-test for: the early stagnation vs. circulation periods in the manganese oxidation ($p > 0.90$), the late stagnation vs. the other two periods in the ammonium oxidation and the denitrification ($p > 0.95$).

for oxygen with heterotrophic bacteria, with the latter being intensified when depositional load of organic substrates is high. The depositional organic flux to sediment is known to increase in the rainy season (July; MAEDA et al. 1987). Thus, it is probable that decomposition of fresh organic sediment deposited in this season decreased gradually the oxygen availability at the sediment surface which, in turn, lowered the activities of Mn²⁺ and NH₄⁺ oxidizers in the late stagnation period. The rates of Mn²⁺ and NH₄⁺ oxidation in the circulation period in which the oxygen concentration in the overlying lake water was the highest were significantly lower ($p > 0.90$) than respective rates for the early stagnation period (Fig. 8). This suggests that the Mn²⁺ and NH₄⁺ oxidation activities once lowered during the late stagnation period was not recovered at

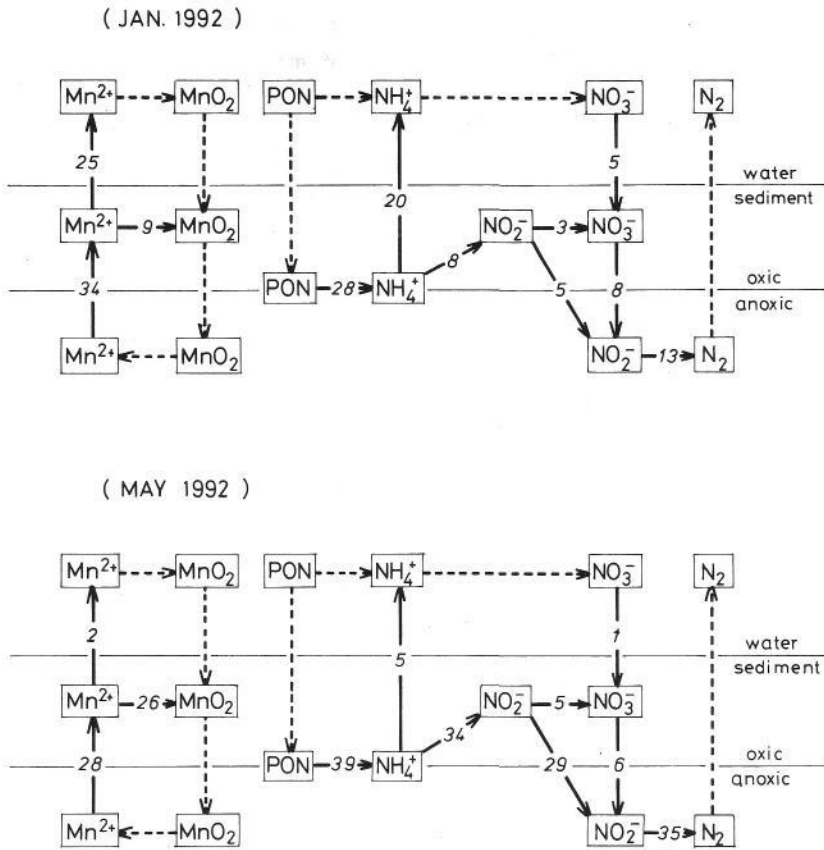


Fig. 9. The budgets for inorganic nitrogen and manganese in the vicinity of the redox boundary in the sediment on January and May 1992. Numbers are fluxes or reaction rates ($\text{mmol reactant m}^{-2}\text{month}^{-1}$) estimated directly or calculated in this study. Nitrite oxidation rates are estimated from the nitrite emission rates on the addition of chlorate, which should be underestimations (see text).

once even when oxygen concentration increased, presumably because of low growth rates of the relevant microorganisms under low temperature conditions.

Although the nitrification rate at the sediment surface was always positive, nitrate was always uptaken by sediment from the overlying water (Fig. 7). Thus, the sediment functions as a sink for nitrate produced in the hypolimnion and at the sediment surface. This clearly suggests the presence of an effective coupling of nitrification and denitrification within the sediment. As the nitrate influx from the overlying water was usually low compared to the estimated nitrification rate within the sediment, the denitrification in the sediment was principally limited by the latter. Since the nitrification rate within

the sediment seemed to be limited by the oxygen availability, the fraction of the denitrified nitrogen to the nitrogen emitted as ammonia to the overlying water should have also changed with the oxygen availability. According to the calculation based on the data of Fig. 7, about 90% of ammonia that had been supplied to the surficial oxidized sediment could be eventually denitrified within the sediment when the nitrification rate was high (May 1992, see Fig. 9). Similarly, 93% of Mn^{2+} diffused out of the reduced layer should have been oxidized before escaping to the overlying water. In contrast, when the nitrification rate was low (January 1992), more than 70% of Mn^{2+} and NH_4^+ were calculated to have been emitted to the overlying water without undergoing oxidation (Fig. 9). Thus, the oxygen limitation in the sedimentary system leads to increasing regeneration efficiency of nitrogen.

The effective removal of nitrate produced within the aerobic sediment due to the coupling of nitrification and denitrification is a common observation in oxidative estuarine sediments (e.g. JENKINS & KEMP 1984, KEMP et al. 1990). Without an extraordinarily high denitrification potential supposed, such an effective coupling seems difficult to be explained by the classical bilayer model which assumes an anaerobic layer for denitrification overlain with a distinct aerobic layer for nitrification (JAHNKE et al. 1982). Some researchers have recently proposed a new model to explain such observations, in which denitrification proceeds within anaerobic microsites dispersed in the oxidized layer (SEITZINGER 1988, KEMP et al. 1990). KEMP et al. (1990) stated that the presence of the oxidized layer which enabled the coupling of nitrification and denitrification should lower the regeneration efficiency of nitrogen to the overlying water column and thus depress the eutrophication.

Role of sedimentary metabolism in the whole lake system

The manganese oxidation as well as the nitrification at the sediment surface uses oxygen which is stored in the hypolimnion. Oxygen in the hypolimnion is also consumed through the aerobic mineralization of organic matter and by nitrification in the hypolimnetic water column. In Table 4, magnitudes of nitrification, denitrification and manganese oxidation at the sediment surface which were estimated in this study are compared with those of overall oxygen consumption and hypolimnetic nitrification estimated at the same site in 1988 (MIYAJIMA 1992). The ratio of oxygen consumption due to hypolimnetic plus sedimentary nitrification (here, 12.9%) can be related to both the C:N ratio of organic matter subject to mineralization and the ratio of mineralization due to denitrification (here, 2.7% on the electron basis). Reversely, if we can assume that all the nitrogen once mineralized be eventually nitrified (and in part denitrified further) within the basin, the average C:N ratio of mineralized organic matter can be calculated from the overall oxygen consump-

Table 4. Hypolimnetic and sedimentary nitrification and manganese oxidation as compared with the hypolimnetic oxygen consumption during the stagnation period.

1. Biological activities below the thermocline (20 m), estimated during the stagnation period (June to October) of 1988 (cited from MIYAJIMA 1992).	
Oxygen consumption	935 mmol m ⁻² month ⁻¹
Nitrate net production	40.5 mmol m ⁻² month ⁻¹
2. Biological activities at the sediment surface averaged over the same period as above but in 1991 and 1992 (estimated in this study).	
Ammonium oxidation	16.1 mmol m ⁻² month ⁻¹
Denitrification	20.0 mmol m ⁻² month ⁻¹
Manganese oxidation	11.7 mmol m ⁻² month ⁻¹
(Overall nitrification ¹)	60.5 mmol m ⁻² month ⁻¹)
3. Contributions of hypolimnetic and sedimentary biological activities to the hypolimnetic oxygen loss during the same period ² .	
Nitrification in the hypolimnetic water column	9.5 %
Nitrification at the sediment surface	3.4 %
(Nitrification as a whole)	12.9 %)
Manganese oxidation at the sediment surface	0.6 %
4. Ratio of the denitrification at the sediment to the hypolimnetic oxygen loss (on the electron-equivalent basis) ³ .	
	2.7 %

¹ Calculated as net nitrate production in the hypolimnion (1988) plus denitrification in the sediment (1991–92).

² Nitrification and manganese oxidation are assumed to always transfer 8 and 2 electrons to oxygen per atom of nitrogen and manganese oxidized, respectively.

³ Each nitrogen atom is assumed to always receive 5 electrons during the denitrification.

tion rate (say $R_{-O}/\text{mol O}_2 \text{ m}^{-2} \text{ month}^{-1}$), the overall nitrification rate ($R_N/\text{mol N m}^{-2} \text{ month}^{-1}$), and the denitrification rate ($R_D/\text{mol N m}^{-2} \text{ month}^{-1}$) as follows:

$$C/N = (R_{-O} - 2R_N + 1.25R_D)/R_N$$

where the chemical form of organic carbon subject to mineralization is assumed as $(\text{CH}_2\text{O})_n$. According to the data of Table 4, the C:N ratio is calculated to be about 14, which is slightly higher than the average C:N ratios of suspended or sedimentary organic matters found in this lake (ranging 8–13). This difference can be ascribed to several factors. First, the organic matters subject to mineralization include also dissolved organic matter which may have somewhat higher C:N ratio than the suspended and sedimentary ones. Second, the C:N ratio of the sedimentary organic matter tends to decrease with increasing depth (Fig. 4), which implies that the heterotrophic organisms in sediment mineralize selectively organic carbon concentrating organic nitrogen in their biomass. These two factors should result in actually higher C:N ratio in the mineralized organic matter than those found in the suspended and

sedimentary ones. However, although it is out of the scope of this study, the effective coupling of nitrification and denitrification as discussed in the preceding section may occur also at anaerobic microsites within particles suspended in the water column (KOIKE & YOSHINARI, in press). If so, the actual C:N ratio of decomposed organic matter must be lower than that calculated above because both R_N and R_D should have been underestimated equally.

In the case of the nitrification at the sediment surface, the substrate ammonium is mainly supplied by diffusion from underlying anoxic layers. In contrast, the nitrification in the water column utilizes principally the ammonium which is produced by aerobic mineralization within the water column. Therefore, the ratio of the magnitude of nitrification within the hypolimnetic water column to that of the overall nitrification should reflect roughly the ratio of aerobic mineralization to the overall mineralization below the thermocline. According to the data of Table 4, about three-fourths (9.5%:12.9%) of the mineralization seems to proceed aerobically below the thermocline. Similarly, since the manganese oxidation exclusively utilizes Mn^{2+} supplied from underlying anoxic layers, the magnitude of manganese oxidation should be roughly poised with that of manganese reduction in anoxic layers which is presumably coupled to anaerobic mineralization (NEALSON & MYERS 1992). The oxygen consumption due to the manganese oxidation amounted to only 0.6% of the overall oxygen consumption (Table 4). These results altogether show that the majority of the decomposition process below the thermocline proceeds under aerobic conditions in Lake Biwa. This is presumably due to the vast capacity of the hypolimnion of this lake which has well compensated increasing organic loading due to recent eutrophication.

Table 4 shows that nitrate which was produced by nitrification at the expense of 12.9% of the overall oxygen consumption served as an electron acceptor for mineralization through the denitrification only by a small ratio (2.7%) compared to oxygen. Nevertheless, the denitrification may function as the major outlet in the nitrogen economy in this lake. As seen in Table 4, about one-third (20.0 vs. 60.5 $mmol\ m^{-2}\ month^{-1}$) of nitrogen nitrified below the thermocline was further denitrified at the sediment surface. The remainder of nitrate should be either transported to the euphotic zone and reutilized by primary producers or removed from the lake through the outlet river(s). According to previous studies of the nitrogen economy in Lake Biwa (reviewed by YOSHIOKA 1991; Table 5), 3543 to 4471 tons Na^{-1} was supplied to this lake by precipitation and through rivers and groundwaters, only 40 to 47% of which was flushed out from the outlet (the Seta River). The remainder is thought to be either trapped within the sediment, harvested by fishery, or denitrified. On the other hand, if the denitrification rate estimated in this study can be applied over the whole bottom area of this lake (674 km^2), annual denitrification within the whole basin is calculated to be about 2230 tons Na^{-1} .

Table 5. The nitrogen economy in Lake Biwa and the denitrification.

Literature ¹	KUNIMATSU (1981)	INOUE & MORISAWA (1981)
1. Nitrogen economy in Lake Biwa (unit: ton N a ⁻¹).		
Inflow by:		
Rain	1058	846
Ground waters	377	1004
Rivers	3036	1693
(Total)	4471	3543
Outflow ² by:		
The Seta River	2384 (53 %)	2125 (60 %)
Other outlets	2087 (47 %)	1418 (40 %)
2. Denitrification rate in the sediment estimated in this study ³ .		
2230 ± 180 ton N a ⁻¹ .		

¹ Cited from the review of YOSHIOKA (1991).

² The Seta River is the only outlet river from Lake Biwa. "Other outlets" here include nitrogen removals due to burial and immobilization within the sediment, harvest of fishery, denitrification, etc. Numbers in parentheses mean the ratios of respective outflows to the total inflow.

³ Estimates done at this study site (depth, 75 m) are summed up from Oct. 1991 to Sept. 1992, and adopted as a representative denitrification rate for the whole bottom area of Lake Biwa. This assumption is of course not very correct because denitrification rate may have a large regional variation due to, e.g., differences in temperature and organic loading.

Being estimated more than ten years later, this value is well comparable to the difference reported between the inflow and the outflow of nitrogen in this lake (Table 5), which suggests that the nitrogen economy of this lake is largely determined by denitrification. The extents to which denitrification serves as the outlet in the nitrogen economy are reviewed by SEITZINGER (1988) for various lakes and estuaries. According to him, in most lakes 20 to 50 % of nitrogen input is removed by denitrification and 0 to 20 % by burial in the sediment. Thus, the contribution of denitrification to nitrogen removal in Lake Biwa (perhaps 50 % or more) is one of the highest cases ever studied in the world.

Such a high contribution of the denitrification should be supported by the effective coupling of nitrification and denitrification at the sediment surface. As discussed in the preceding section, seasonal decrease in the oxygen availability at the sediment surface seemed to cause destruction of aerobic microbial community including nitrifiers and enhance the regeneration of ammonium from sediment to overlying water. Similarly, depletion of hypolimnetic oxygen caused by recent artificial eutrophication seems to have led to decoupling of the nitrogen metabolism, resulting in an observable change in the nitrogen/phosphorus balance in this lake. SONO & NOMURA (1991) reported that the concentrations of total nitrogen (TN) as well as COD in the hypolimnion of the north basin of Lake Biwa was gradually increasing during the last decade

with the ratio of TN to total phosphorus (TP) concentration raised from ca. 25 to ca. 35 as atomic ratio. Since the concentration of chloride in Lake Biwa has shown a similar trend of gradual increase for the last decade and this increase seems to be ascribed mainly to the internal condensation of chloride due to the recent trend of decreasing annual precipitation rather than any changes in external loading, the authors deduced that the observed increase in TN as well as COD levels had been also resulted solely from the internal concentration effect. The factors responsible to such a long-term change in TN and COD are, however, somewhat complicated because the behavior of these ingredients is not so conservative as chloride. Since the majority of TN in the hypolimnetic water is NO_3^- -N, the increase in the TN/TP ratio implies gradual increase of regeneration rate of nitrogen relative to phosphorus in the hypolimnion. If the N:P ratio of external loading has not so much changed, it is possible that the increasing nitrogen regeneration was at least partially resulted from prolonged prevalence of seasonal reductive condition over the sediment surface. In estuarine environments where the primary productivity is often limited by the nitrogen supply, increasing nitrogen regeneration due to decoupling of the nitrogen redox transformation is thought to act as a positive feedback effect for the eutrophication (KEMP et al. 1990). Since the primary productivity in lacustrine environments is, in contrast, usually limited by the phosphorus availability (SCHINDLER 1977), the increase in the nitrogen regeneration efficiency may not necessarily lead directly to changes in the productivity. However, the prevalence of reductive condition that is thought to have affected nitrogen transformation is also known to cause the reductive dissolution of sedimentary Fe(III)-PO_4^{3-} complex, which may result in the emission of biologically available phosphorus into the water column. Thus, further studies on the effects of changing redox condition upon the whole lake metabolism must be done with special reference to the behavior of phosphorus within the lake.

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