Significance of Low-Oxygen Zone for Nitrogen Cycling in a Freshwater Lake: Production of N$_2$O by Simultaneous Denitrification and Nitrification

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Abstract

Denitrifying and nitrifying activities were determined to elucidate the mechanism of N$_2$O accumulation in the oxygen depleted zone of Lake Kizaki. Both activities were detected without any substrate enrichments in a zone of low-oxygen content (0.03–0.14 ml l$^{-1}$), where N$_2$O and NO$_2^-$ were accumulated. The determined nitrifying activity approximately coincided with the NH$_4^+$ utilization estimated from the NH$_4^+$ budget in this zone during the observation interval. On the basis of these activities and the change in N$_2$O concentration, it was suggested that the observed N$_2$O accumulation had dual origins of nitrification and denitrification and that these productions were in a dynamic equilibrium with further reduction of N$_2$O to N$_2$. The low-oxygen zone was found to be an active site for nitrogen cycling and was characterized by the presence of dissolved Mn and the absences of dissolved Fe and sulfide.

Key words: N$_2$O, denitrification, nitrification, low-oxygen zone

1. Introduction

N$_2$O is a by-product of nitrification and an obligate intermediate of denitrification (Ritchie and Nicholas, 1972; Payne, 1981). Since nitrification and denitrification dominate in oxic and anoxic environments, respectively, oxygen tension is thought to be of primary importance in regulating the distribution of N$_2$O in aquatic environments (Kaplan and Wofsy, 1985).

Often associated with oxygen-limited conditions, large accumulations of N$_2$O and NO$_2^-$ have been observed in several freshwater lakes (Knowles et al., 1981; Yoh et al., 1983; Yoh et al., 1988; Priscu et al., 1986; Downes, 1988). Yoh et al. (1988) reported that several $\mu$g atom N l$^{-1}$ of N$_2$O as well as NO$_3^-$ were invariably accumulated at layers of about 0.1 ml l$^{-1}$ oxygen concentration in both eutrophic Lake Fukami-ike and mesotrophic Lake Kizaki. Denitrification was considered to be responsible for these accumulations, since NO$_3^-$ concentration decreased with time at these layers. In fact, denitrifying activity was detected in such NO$_3^-$ decreasing layers (Terai et al., 1987; Terai, 1987).

However, a contribution of nitrification to N$_2$O production is also possible in oxygen-limited environments because nitrification can proceed with a high yield of N$_2$O at low oxygen tensions (Goreau et al., 1980). Downes (1988) observed NO$_2^-$ and N$_2$O accumulations in the oxygen depleted hypolimnion of a New Zealand lake and suggested that they were produced mainly by ammonium-oxidizing bacteria on the basis of measurements of the relative rates of nitrate and nitrite reduction. On the other hand, using marine sediment suspensions enriched with NO$_3^-$ and NH$_4^+$, N$_2$O was shown to be produced simultaneously by denitrification and by nitrification (Jorgensen et al., 1984).

The purpose of the present study is to elucidate the nitrogen metabolisms, with special reference to the mechanism of N$_2$O production, at the interface between oxic and anoxic...
zones and to describe other chemical conditions in detail. To evaluate the in situ processes of denitrification and nitrification, determinations of both activities were made without any substrate enrichments.

2. Materials and methods

2-1. Sampling
Water samples for chemical analysis (on 30 September and 29 October 1988) and for incubation experiments (on 29 September 1988) were collected at the deepest point in Lake Kizaki with a Van-Dorn sampler. For chemical analysis, they were filtered using Whatman GF/C filter in the field laboratory and kept frozen until analysis.

2-2. Chemical analysis
Dissolved oxygen was determined by the Winkler-method. NaN₃ was added to the KI solution to avoid the interference by nitrite. The concentrations of nitrate and nitrite were determined with a Technicon Autoanalyzer (Technicon Industrial Method, 1972). The concentrations of ammonium and sulfide were determined by the methods of Sagi (1966) and Clime (1969), respectively. The procedure for dissolved nitrous oxide determination was described previously (Yoh et al., 1988). Dissolved and particulate forms of manganese and iron were determined by the method described previously (Yagi and Shimodaira, 1986).

2-3. Denitrifying Activity
Denitrifying activity was determined by the acetylene blockage technique (Yoshinari and Knowles, 1976). Lake water was incubated in a 100-ml vial with or without 5 ml of C₂H₂-saturated distilled water under dark at 7°C for 2 days. After incubation, 0.4 ml of saturated HgCl₂ solution was added and kept cold until analysis. At the laboratory, after equilibration of the sample water with He in a 30-ml glass syringe at a constant temperature, the gas phase was injected with a gas-tight syringe into a gas chromatograph equipped with electron capture detector (Yanaco G 180 EN). Dissolved N₂O concentration was calculated by the solubility formula of Weiss and Price (1980). The denitrifying activity was determined as the difference in N₂O concentration before and after incubation with C₂H₂.

2-4. Nitrifying activity
For the measurement of nitrifying activity, nitrapyrin (Dow Chemical Co., Ltd.; commercial formulation, "N-serve 24 E") and C₂H₂ were used as the specific inhibitors for autotrophic nitrification at the final concentrations of 10 ppm (Yoshoka et al., 1985) and 10 Pa (Hynes and Knowles, 1978), respectively. Lake water filled in a ground-glass stoppered bottle (ca. 250 ml volume) was incubated with or without the inhibitor at 7°C under dark for two days. After incubation, it was filtered through a Whatman GF/C filter and kept frozen until chemical analysis. The activity of nitrification was determined by the difference in NH₄⁺ concentration with and without the inhibitor after incubation. The analysis of NH₄⁺ was made in triplicate.

3. Results

3-1. Vertical distributions of inorganic nitrogen species and oxygen
On 30 September (Fig. 1), the oxygen concentration in the hypolimnion had been largely diminished; only 2.47 ml l⁻¹ in the uppermost hypolimnion. A depletion of oxygen, below 20 m depth, caused drastic changes in all the inorganic nitrogen species. Oxygen was present in trace amount between 20 m and 25 m depths but absent below 25 m. Exactly corresponding to this low-oxygen zone, a decrease to complete exhaustion of NO₃⁻ concentration was observed, together with N₂O and NO₂⁻ accumulations and an increase of NH₄⁺ concentration. All nitrogen oxides (NO₃⁻, NO₂⁻ and N₂O) were under their detection limits in the anoxic zone below 25 m depth, showing complete denitrification. The downward increase of NH₄⁺ concentration, which had a consistent gradient in the low-oxygen zone, turned sharper at the boundary from low-oxygen to anoxia. Accumulations of N₂O and NO₂⁻, in the mid course of the downward NO₃⁻ decrease, amounted to 0.22 and 2.34 µg atom N l⁻¹ at their maxima, respectively, having their ratio of 0.094.
On 29 October (Fig. 2), although oxygen was further consumed in the upper hypolimnion, the distributions of all nitrogen species were quite similar to those on 30 September. NO$_3^-$ concentration showed little change during a month throughout the low-oxygen zone, which was located in the same depth range as September. Maximum amounts of N$_2$O and NO$_2^-$ were 0.17 and 1.77 μg atom N l$^{-1}$, respectively, similar to a month earlier. In the low-oxygen zone, each pool of inorganic nitrogen species was thus found to be almost constant during a month interval.

These observations show that the distributions of all nitrogen species were definitely related to oxygen concentration. It is thus possible to describe their distributions in terms of oxygen concentration as follows: (1) in the well-oxygenated zone, down to 20 m, almost all inorganic nitrogen was comprised of NO$_3^-$; (2) in the low-oxygen zone, between 20 m and 25 m with oxygen concentration ranging from 0.14 to 0.03 ml l$^{-1}$, all nitrogen species showed drastic changes with the accumulations of N$_2$O and NO$_2^-$ whose amount comprised 11.5 and 6.4% of total inorganic nitrogen in September and October, respectively; and (3) in the anoxic zone, below 25 m, nitrogen oxides (NO$_3^-$, NO$_2^-$ and N$_2$O) disappeared completely and NH$_4^+$ was the only component of inorganic nitrogen.

3-2. Vertical distributions of manganese, iron and sulfide

Distributions of dissolved and particulate forms of Mn and Fe, and S$^{2-}$ were determined on 29 October to characterize the low-oxygen condition in Lake Kizaki. In the upper hypolimnion, concentrations of dissolved Mn and Fe were lower than their particulate concentrations, and S$^{2-}$ was absent (Fig. 3). The increase of dissolved Mn concentration was found to start at 21 m depth, accompanied by a decrease in particulate Mn concentration. An increase of dissolved Fe concentration and a decrease of particulate Fe concentration start-
ed simultaneously at 24 m depth, about 3 m deeper than the case of Mn. $S^{2-}$ also appeared below 24 m. All the gradients in profiles of dissolved Mn, dissolved Fe and $S^{2-}$ turned sharper at 25 m. This depth corresponded to the boundary between the low-oxygen zone and anoxic zone. The changes in these gradients suggest sinks for these species above this level and therefore corroborate the presence of trace oxygen in the low-oxygen zone. An appearance of dissolved Mn corresponded to the initiation of the low-oxygen zone. The low-oxygen zone thus contained an appreciable amount of dissolved Mn but little dissolved Fe and $S^{2-}$.

### 3-3. Denitrifying activity and nitrifying activity

Denitrifying activity and nitrifying activity were measured without any supplement of substrates at several layers in the deeper hypolimnion on 30 September.

The denitrifying activity was found in the 21 and 22 m layers (Fig. 4a). In other layers, however, the activity was almost or completely absent. These results show that denitrification took place only in the low-oxygen zone. The absences of the activity indicate that denitrification was arrested owing to the high oxygen concentrations (0.90 and 0.70 ml$\cdot$1$^{-1}$) in the upper layers (19 and 20 m) and the exhaustions of electron acceptors in the lower layers (24 and 28 m). The profile of the activity showed a striking resemblance to that of $N_2O$ and $NO_2^-$ concentrations having their maxima at 22 m depth (Fig. 1), suggesting the contribution of denitrification to these accumulations. The maximum activity of 0.14 $\mu$g atom N$\cdot$1$^{-1}$$\cdot$day$^{-1}$ at 22 m depth was comparable to or lower than the previous study in this lake (Terai et al., 1987; Terai, 1987).

The profiles of nitrifying activity determined with nitrapyrin or acetylene are shown in Figure 4b. The nitrifying activities were found in the 21, 22 and 24 m layers but not in the 19, and 20 m layers (Fig. 4b), showing that nitrification was also active in the low-oxygen zone. Because of the large background concentration...
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Fig. 3. Vertical distributions of dissolved and particulate manganese (○, ●), dissolved and particulate iron ([□], ■), and sulfide (△) in the hypolimnion of Lake Kizaki on 29 October 1988.

Fig. 4. Vertical distributions of denitrifying activity (a) and nitrifying activity (b) in the deeper hypolimnion of Lake Kizaki on 30 September 1988. Both activities were determined without any supplement of substrates. Nitrifying activity were determined with nitrapyrin (10 ppm) or C₂H₂ (10 Pa) as a inhibitor.
of NH$_4^+$ (47 μg atom N·1$^{-1}$), the activity can not be determined at 28 m depth. Although the vertical trends of activity were similar, lower values were obtained in the low-oxygen zone with C$_2$H$_2$ (0.18-0.35 μg atom N·1$^{-1}$·day$^{-1}$) than with nitrapyrin (0.41-0.46 μg atom N·1$^{-1}$·day$^{-1}$). This difference may have arisen either from less effective inhibition with C$_2$H$_2$ or from enhanced NH$_4^+$ regeneration by nitrapyrin. The restriction of the activity in the low-oxygen zone suggests that nitrification would be promoted by the abundant NH$_4^+$ in this zone. The intensity of the activity in the low-oxygen zone was 2-3 fold larger than the denitrifying activity (Fig. 4a) and as high as that in the active nitrification in this lake (Takahashi et al., 1982; Yoshioka et al., 1985).

4. Discussion

The decreasing profile of NO$_3^-$ concentration with increasing depth in the low-oxygen zone suggests the occurrence of denitrification. In such a NO$_3^-$-decreasing zone in Lake Kizaki, not only a successive decrease of NO$_3^-$ concentration with time (YouI et al., 1988) but a denitrifying activity (Terai et al., 1987; Terai, 1987) has been observed. Due to diffusion and NO$_3^-$ reduction, the NO$_3^-$ concentration should have decreased with time. The fact that the NO$_3^-$ concentration was nevertheless almost constant during a month (Figs. 1 and 2), therefore, suggests the occurrence of nitrification in this zone.

Except for biological processes, the NH$_4^+$ budget in the low-oxygen zone (20-25 m depth) could be accounted for both by transport through diffusion and by NH$_4^+$ release due to organic matter decomposition within this zone. Using Fick’s first law, the influx of NH$_4^+$ from the lower zone is estimated to be 0.98 mg atom N·m$^{-2}$·day$^{-1}$, given the concentration gradient at the boundary layer of 7.5 μg atom N·1$^{-1}$·m$^{-1}$ (taken from the concentration gradient between 25-26 m on both observations) and the vertical eddy diffusion coefficient of 0.13 m$^2$·day$^{-1}$ (Takahashi and Sajo, 1981). In a similar way, the efflux of NH$_4^+$ to the well-oxygenated zone is estimated to be 0.22 mg atom N·m$^{-2}$·day$^{-1}$, employing the averaged NH$_4^+$ concentration gradient in the upper low-oxygen zone on both dates. Thus, the net flux into the low-oxygen zone is 0.76 mg atom N·m$^{-2}$·day$^{-1}$, or 0.15 μg atom N·1$^{-1}$·day$^{-1}$ on the average within this zone (20-25 m). On the other hand, on the basis of the organic matter composition in this lake (Takahashi and Sajo, 1981), the input of NH$_4^+$ through regeneration is calculated to be 0.05 μg atom N·1$^{-1}$·day$^{-1}$, assuming that the amount of decomposition within the low-oxygen zone is the same as that in the above oxic layers of 19 and 20 m, where about 0.5 ml·l$^{-1}$ of oxygen was consumed during the 29-day interval. As a whole, the NH$_4^+$ input into this zone was approximately 0.20 μg atom N·1$^{-1}$·day$^{-1}$. The actual NH$_4^+$ increase during the observation interval, however, averaged only 0.03 μg atom N·1$^{-1}$·day$^{-1}$ in this zone. Therefore, it is presumed that NH$_4^+$ would have been removed at a rate of 0.17 μg atom N·1$^{-1}$·day$^{-1}$, due to nitrification. This rate is comparable to the obtained nitrifying activities (Fig. 4b), corroborating the results of determination.

It has been demonstrated that nitrifiers can grow and oxidize their substrates even under oxygen-limited condition (Carlucci and McNally, 1969; Kaplan, 1983). Macfarlane and Herbert (1984) observed that at an oxygen concentration of 0.1 mg·l$^{-1}$ (0.07 ml·l$^{-1}$) NO$_2^-$ production was still 45% of that under fully aerobic conditions with chemostat cultures of Nitrosomonas N3. Laudelout et al. (1976) showed that ammonium oxidizers have higher affinity to oxygen than nitrite oxidizers. Hynes and Knowles (1984) actually observed NO$_2^-$ accumulation in an oxygen-depleted mixed culture of an NH$_4^+$ oxidizer and a NO$_2^-$ oxidizer, where NH$_4^+$ oxidation took place but NO$_2^-$ oxidation did not. On the other hand, Goreau et al. (1980) demonstrated that the N$_2$O yield relative to NO$_2^-$ production by NH$_4^+$ oxidizing bacteria increased from 0.3% to 10% as the oxygen level decreased. Jørgensen et al. (1984) also reported a maximum ratio of N$_2$O production relative to NH$_4^+$ oxidation of more than 20% with oxygen-limited marine sediment suspensions. These experimental results sug-
gest that the nitrification in the low-oxygen zone in Lake Kizaki would cause accumulations of both NO$_2^-$ and N$_2$O. Taking 0.4 $\mu$g atom N·1$^{-1}$·day$^{-1}$ as a nitrification rate (Fig. 4b) and a yield ratio of N$_2$O to NO$_2^-$ of 10% (GOREAU et al., 1980), it is estimated that N$_2$O would be produced at a rate of 0.04 $\mu$g atom N·1$^{-1}$·day$^{-1}$ in this zone.

On the other hand, the rate of N$_2$O production in the presence of C$_2$H$_2$ (5kPa) was 0.11 (21 m) and 0.14 $\mu$g atom N·1$^{-1}$·day$^{-1}$ (22 m) (Fig. 4a). When the process of denitrification is described as, NO $\rightarrow$ N$_2$O $\rightarrow$ N$_2$, with respective reaction rates of $k_1$ and $k_2$, the denitrifying activity corresponds to $k_1$ (TERAI et al., 1987). Thus, these rates represent the gross production rate of N$_2$O, implying that N$_2$O was supplied to the in situ N$_2$O pool with possible concurrent removal by the further reduction to N$_2$.

Although the net rate of N$_2$O production is unknown, it seems likely that some N$_2$O would be released into water during denitrification, as suggested in the previous study in this lake (YOH et al., 1983; YOH et al., 1988). The fact that the vertical profile of the denitrifying activity was quite similar to that of N$_2$O concentration (Figs. 1 and 4a) corroborates the contribution of denitrification to the N$_2$O accumulation.

Thus, the N$_2$O accumulations observed in the low-oxygen zone are considered to have dual origins of nitrification and denitrification. This conclusion is somewhat different from the results in a New Zealand lake (DOWNES, 1988), in which N$_2$O at low oxygen concentrations was suggested to be mainly produced by nitrifying bacteria.

The fact that the amount of N$_2$O accumulation was almost constant during a month (Figs. 1 and 2) despite the N$_2$O production indicates the presence of N$_2$O sinks. In a similar way as for the estimation of NH$_4^+$ flux, the N$_2$O loss due to upward and downward diffusion is estimated to average 0.005 $\mu$g atom N·1$^{-1}$·day$^{-1}$ in the low-oxygen zone. This rate appears to be insignificant compared to the N$_2$O production. Hence, it is suggested that most of the produced N$_2$O was removed by further reduction to N$_2$ within the low-oxygen zone by denitrifiers. If the N$_2$O production is almost balanced with its reduction, it is conceivable that more N$_2$O may be reduced than produced during denitrification. This seems to be especially the case at the 24 m layer; although N$_2$O production by nitrification was expected to be large in this zone, the denitrifying activity (gross N$_2$O production) was low, suggesting an active N$_2$O reduction (Fig. 4a,b). It should be noted that the rates of production and consumption were much larger than the observed change in the N$_2$O pool. Judging from the pool sizes of N$_2$O (Figs. 1 and 2), the average retention time of N$_2$O was only one or two days. This fact indicates that the N$_2$O accumulation was governed by a dynamic equilibrium between the production and the consumption.

In the present study, the observed N$_2$O concentrations at the maxima were ca. 0.2 $\mu$g atom N·1$^{-1}$ on both occasions, which are the lowest level ever recorded for this lake (YOH et al., 1983; YOH et al., 1988). The denitrifying activities were also at a low level compared with the previous determinations (TERAI et al., 1987; TERAI, 1987), and this is supported by little decrease of NO$_3^-$ concentration with time (Figs. 1 and 2). Terai (1987) found a correlation between denitrifying activities and N$_2$O concentrations in this lake. The present data sets of N$_2$O concentration and denitrifying activity also fit in with this relationship.

The large N$_2$O accumulations observed in other freshwater lakes (KNOWLES et al., 1981; PRISCU et al., 1986) appear to be associated with the rapid decreases in NO$_3^-$ concentration. It is thus suggested that the amount of N$_2$O accumulation may be regulated by the intensity of denitrification.

Basically, nitrification and denitrification are assumed to take place in the presence and absence of oxygen, respectively. Knowledge on the nitrogen metabolisms in the interface between the two extreme environments is limited. In the present study, it was found that there was a zone of low-oxygen content with an appreciable depth interval between oxic and anoxic zones. The fact that dissolved Mn and
dissolved Fe (and sulfide) concentrations started to increase with concomitant decreases in particulate Mn and Fe concentrations at the upper and lower boundaries of the low-oxygen zone, respectively (Fig. 3), indicates that this zone had a typical redox condition as the intermediate environment. In this zone, both denitrification and nitrification were active and \( \text{N}_2\text{O} \) as well as \( \text{NO}_2^- \) were accumulated. It is thus suggested that this zone was a site of active nitrogen cycling, where denitrification and nitrification were closely coupled in situ via \( \text{N}_2\text{O} \) and \( \text{NO}_2^- \).

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