Aquatic Nitrogen Transformations at Low Oxygen Concentrations

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Nitrite and nitrous oxide are produced in both nitrification and denitrification processes of the nitrogen cycle and accumulate briefly in the metalimnion and hypolimnion of lakes during thermal stratification (15, 17, 24). Nitrification is thought to be responsible for nitrous oxide production in the euphotic zone of the world’s oceans (9, 28) and for the upper nitrite maximum (18, 19). Large accumulations of nitrite and nitrous oxide have been found under well-oxygenated conditions at depths between 50 and 60 m in Lake Vanda, Antarctica, which corresponded to a peak in nitrification activity (25).

Under conditions of low-oxygen tensions the mechanisms for the production of nitrite and nitrous oxide are ambiguous. Nitrite and nitrous oxide accumulate in nitrate-amended soils and in denitrifying bacterial cultures under low-oxygen conditions (1). Large accumulations of nitrite and nitrous oxide in the hypolimnion of two Japanese freshwater lakes were considered to be due to denitrification, and 0.14 g m⁻³ was considered to be the optimum oxygen concentration for nitrous oxide accumulation from denitrification (27). It has also been suggested that large concentrations of nitrite and nitrous oxide in an O₂-depleted hypolimnion of mesotrophic Lake St. George were due to denitrification (15). However, it has also been shown that cultures of Nitrosomonas sp., an ammonium-oxidizing bacterium, could oxidize ammonia to nitrite and produce nitrous oxide at oxygen concentrations as low as 0.1 g m⁻³ (10).

The present study reports on a large accumulation of nitrite and nitrous oxide throughout the hypolimnion of a deep mesotrophic New Zealand lake and examines the mechanisms which could be responsible for their accumulation.

MATERIALS AND METHODS

Lake Rotoiti. Lake Rotoiti is a deep (maximum depth of 126 m) mesotrophic lake situated in the Okataina volcanic center of the Taupo Volcanic Zone, on the North Island of New Zealand. The lake is warm and monomictic with stratification from October to May. The lake itself is divided into two arms. The western arm contains the outlet at the Kaituna River and also an input from eutrophic Lake Rotorua, through the Ohau Channel, which represents 73% of the average total annual input into Lake Rotoiti. The lake catchment is composed mainly of farmland and forest.

Sampling procedures. Temperature and dissolved-oxygen profiles were measured with a Yellow Springs Instruments dissolved-oxygen meter, and water samples were collected with a 6-liter Van Dorn sampler. Samples for nitrous oxide analysis were removed with a 50-ml plastic syringe containing 0.2 ml of a 30-g liter⁻¹ concentration of mercuric chloride to inhibit bacterial activity. The syringe tip was pushed into the end of a plastic tube on the outlet spigot of the sampler, and 50 ml of the sample was withdrawn slowly to avoid air bubbles and cavitation within the sample. The syringe was then sealed with a hypodermic needle pushed into a rubber bung. The nitrous oxide concentration was measured on the day of collection by gas chromatography (24).

Chemical analysis. Samples for chemical analysis were dispensed into 500-ml linear polyethylene bottles. Samples were filtered through washed Whatman GF/C glass fiber filters on the day of collection and stored frozen prior to analysis. Nitrate and nitrite were analyzed by the method of Downes (8), and ammonium was analyzed by a modification of the colorimetric technique of Crooke and Simpson (7).

Biochemical assays. Nitrification assay samples were dispensed into 130-ml borosilicate glass bottles covered in black polyvinyl chloride tape to exclude light. To prevent excessive aeration of the sample, the Van Dorn outlet tube was placed at the bottom of each bottle and the water sample was allowed to overflow the bottle before it was stopped. Triplicate control and test samples were taken at each depth. A 50-µl portion of 1.62 g of technical-grade nitrapyrin (Ivon Waterk Dow NZ, Ltd.) liter⁻¹, a nitrification inhibitor (2, 23), in acetone solvent, was added to each test sample to give a final concentration of 5 mg of inhibitor liter⁻¹, and a similar volume of solvent alone was added to the control bottles soon after collection. After 1 h, 1 ml of a sterile 25-µCi ml⁻¹ ¹⁵O-labeled sodium bicarbonate solution was added to all bottles and the samples were incubated for 4 h in the dark within ±2°C of lake temperatures. After incubation, 40-ml samples were filtered through 0.22-µm (pore size) Millipore membrane filters. The filters were then air dried, placed in liquid scintillation cocktail, and counted with a Beckman liquid scintillation counter. The decrease in bicarbonate uptake between controls and inhibited samples was considered a measure of nitrifier activity.

Samples for measuring nitrous oxide production rates were collected as described above for samples collected for nitrous oxide concentration measurements. Samples (100 ml) were drawn into glass syringes and stored in the dark at hypolimnetic temperatures for 24 h before the assays were performed. Acetylene (50 ml) was added to each test syringe to inhibit the activity of nitrous oxide reductase (29), and the samples were shaken to dissolve the gas. Any excess gas (ca.
Nitrite and nitrous oxide appeared in the hypolimnion of Lake Rotoiti in late summer when dissolved-oxygen concentrations were between 0.5 and 1.0 g m⁻³ (Fig. 1). Hypolimnetic nitrite concentrations were between 90 and 120 mg of N m⁻³, and nitrous oxide concentrations ranged from 8 to 19 mg of N m⁻³. The highest nitrous oxide concentration was 25 mg of N m⁻³ at a depth of 30 m, the base of the metalimnion, on 11 April 1985 (Fig. 2). Hypolimnetic nitrate concentrations were always lower than the corresponding nitrite concentrations throughout this period. However, a nitrite peak was always present in the metalimnion, where nitrite was absent and oxygen levels were higher than in the hypolimnion (Fig. 2). A nitrite peak was still present at 25 m on 1 May 1985, a few weeks before turnover. Ammonium concentrations ranged from 5 to 15 mg of N m⁻³ in the epilimnion and increased sharply below 25 m (Fig. 2). The hypolimnetic ammonium concentrations were between 90 and 100 mg of N m⁻³ from 25 March to 3 April 1985 but then increased linearly at a net rate of 3 mg of N m⁻³ day⁻¹ (Fig. 1) until turnover.

Ammonium oxidizer activity as determined by the nitrapyrin assay was always much higher in the hypolimnion than in the epilimnion or metalimnion (Fig. 2), even when oxygen concentration was expelled, and the samples were left for at least 1 h to ensure complete inhibition of the nitrous oxide reductase enzyme. The assay was initiated by adding 1 ml of a solution of either nitrate or nitrite containing 5 mg of N m⁻³ into the syringes, followed by incubation in the dark at 12°C. Controls without acetylene were also run under similar conditions. Samples (5 ml) were removed periodically over a 24-h period with a 10-ml syringe, and the nitrous oxide concentration was measured by multiple equilibration and gas chromatography (24).

**RESULTS**

Nitrite and nitrous oxide appeared in the hypolimnion of Lake Rotoiti in late summer when dissolved-oxygen concentrations were between 0.5 and 1.0 g m⁻³ (Fig. 1). Hypolimnetic nitrite concentrations were between 90 and 120 mg of N m⁻³, and nitrous oxide concentrations ranged from 8 to 19 mg of N m⁻³. The highest nitrous oxide concentration was 25 mg of N m⁻³ at a depth of 30 m, the base of the metalimnion, on 11 April 1985 (Fig. 2). Hypolimnetic nitrate concentrations were always lower than the corresponding nitrite concentrations throughout this period. However, a nitrite peak was always present in the metalimnion, where nitrite was absent and oxygen levels were higher than in the hypolimnion (Fig. 2). A nitrite peak was still present at 25 m on 1 May 1985, a few weeks before turnover. Ammonium concentrations ranged from 5 to 15 mg of N m⁻³ in the epilimnion and increased sharply below 25 m (Fig. 2). The hypolimnetic ammonium concentrations were between 90 and 100 mg of N m⁻³ from 25 March to 3 April 1985 but then increased linearly at a net rate of 3 mg of N m⁻³ day⁻¹ (Fig. 1) until turnover.

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**FIG. 1.** Chemistry changes at 60 m in Lake Rotoiti hypolimnion between March and June 1985. (A) Ammonium (NH₄⁺) and nitrate (NO₃⁻) concentrations. (B) Nitrous oxide (N₂O) (10 times the actual values) and nitrite (NO₂⁻). (C) Oxygen (O₂) and ammonium oxidation potential (NH₄⁺ Ox.).

**FIG. 2.** Lake Rotoiti water chemistry on 11 April 1985. (A) Temperature (°C), oxygen (O₂), and ammonium oxidation potential (NH₄⁺ Ox.). (B) Nitrate (NO₃⁻), nitrite (NO₂⁻), ammonium (NH₄⁺), and nitrous oxide (N₂O) concentrations.
was below detectable levels in the hypolimnion, although nitrifier activity decreased steadily in the hypolimnion from the time that oxygen was undetectable until turnover (Fig. 1).

The denitrification assays performed on hypolimnetic water (Fig. 3) showed that nitrous oxide was produced from nitrite at four times the rate that it was produced from the equivalent concentration of nitrate, the opposite situation to that required to maintain high nitrite concentrations relative to nitrate concentrations by denitrification. The acetylene-inhibited assay also showed that all of the added nitrite and at least 80% of the added nitrate were reduced to nitrous oxide.

**DISCUSSION**

Nitrite is an intermediate, and nitrous oxide is a byproduct of nitrification, and both are intermediates in the denitrification pathway of the nitrogen cycle (14). However, they rarely accumulate in high concentrations. The oxidative and reductive pathways which lead to production and consumption of these nitrogen oxides are complex, with oxygen consumption being a key variable. In well-aerated aquatic systems, ammonium oxidation is thought to be the major source of N₂O supersaturation (9, 15, 17). Conversely, in situations where the oxygen concentration is below 0.2 g m⁻³, nitrite and nitrous oxide are used as terminal electron acceptors by denitrifying bacteria with dinitrogen as the final product. Under these circumstances, all forms of oxidized nitrogen tend to be absent (4, 6, 25, 26).

Between these two extremes of oxygenation is a region of partial oxygen depletion to concentrations between 1 and 0.2 g m⁻³ at which both nitrification and denitrification are active, and it is within this region of oxygen concentration that nitrite and nitrous oxide accumulated in Lake Rotoiti.

Although hypolimnetic denitrification has been demonstrated for this lake (21; this study), it is difficult to see how the high hypolimnetic nitrite concentrations would be maintained entirely by this process given the low hypolimnetic nitrate concentrations present during this period. The highest rate of nitrate denitrification reported by Priscu et al. (21) for Lake Rotoiti was about one-third the rate of nitrate reduction found in the present study. The denitrification assays reported here show that nitrate was reduced at a rate four times faster than nitrite (Fig. 2). Even in the control assays (Fig. 2A), when nitrous oxide reductase was not inhibited, the initial rate of nitrous oxide accumulation from nitrite was over three times faster than the initial rate of nitrous oxide production from nitrate with acetylene inhibition. This suggests that if nitrate reduction was the sole source of nitrite in the hypolimnion, then the reduction of nitrate to nitrite would be the rate-limiting step in the production of nitrous oxide and that nitrite would not have accumulated in the hypolimnion. Betlach and Tiedje (1) demonstrated with bacterial cultures and a kinetic model of denitrification that if the rate of nitrite reduction was five times greater than the rate of nitrate reduction little nitrite accumulated. The assays reported here also indicate that an additional mechanism, not linked to the denitrification of nitrate, was mainly responsible for the production of both nitrite and nitrous oxide.

An alternative process for the production of nitrite and nitrous oxide is nitrification. The nitrapyrin assays indicated high nitrification potentials in the hypolimnion with as much as 90% of the dark bicarbonate uptake being inhibited by nitrapyrin. Nitrification proceeds more rapidly at low oxygen concentrations (3), with ammonium oxidizers having a greater affinity for oxygen (Kₘ, 0.51 mg of O₂ liter⁻¹) than nitrite oxidizers (Kₘ, 1.98 mg of O₂ liter⁻¹) (16). This could lead to an accumulation of nitrite under the low hypolimnetic oxygen concentrations. Gower et al. (10) have also shown that the amount of nitrous oxide produced by ammonium oxidizers increases from 0.3% of nitrite produced under well-aerated conditions to nearly 10% under low-oxygen tensions, and up to 25% nitrous oxide has been reported from nitrification in marine sediments at low oxygen levels (13). The results presented here show that when nitrite was present in the hypolimnion of Lake Rotoiti, nitrous oxide ranged from 8 to 16% of the nitrite concentrations. Hypolimnetic ammonium levels also increased rapidly after nitrite levels dropped, suggesting that oxidation limited ammonium concentrations while oxygen was present (Fig. 1). Nitrifier bicarbonate assimilation rates also remained high even after oxygen was undetectable in the hypolimnion, with assimilation rates only dropping after nitrous oxide had disappeared (Fig. 1). The eventual loss of nitrite and nitrous oxide may be due to both the cessation of ammonium oxidation or higher rates of denitrification relative to the nitrification rate as oxygen levels drop below 0.2 mg m⁻³.

Hooper (11) and Richie and Nicholas (22) have suggested that nitifying bacteria produce nitrite reductase and can reduce nitrite to nitrous oxide under aerobic and anaerobic conditions. Under low-oxygen conditions *Nitrosomonas eu-
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VOL. 54, by high nitrifier oxygen concentrations. These observations in combination with the experimental data present the first evidence of the dual role of nitrifying bacteria in the metabolism of nitrous oxide in aquatic environments and are consistent with the following sequence of bacterial processes, with oxygen controlling which process was dominant and hence which products accumulated. (i) At oxygen concentrations >1.0 g m⁻³, the process was as follows: \( \text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^- \). This process was predominant in the metalimnion, where diffusion of ammonium and nitrite from the hypolimnion supplied the substrates for the nitrifiers. A very small amount of nitrous oxide would be produced as a by-product of ammonium oxidation to nitrite under these conditions (9). (ii) At oxygen concentrations between 1.0 and 0.2 g m⁻³, the process was as follows: \( \text{NH}_4^+ \rightarrow \text{NO}_2^- \). Low oxygen levels inhibited nitrite oxidation. This was coupled with the process \( \text{NO}_2^- \rightarrow \text{NO}_3^- \), nitrifier denitrification. At those oxygen levels, nitrite and oxygen competed as terminal electron acceptors. (iii) At oxygen concentrations <0.2 g m⁻³, the process was as follows: \( \text{NO}_2^- \rightarrow \text{N}_2 \rightarrow \text{N}_2 \), complete denitrification to dinitrogen. It should be stressed that these are considered the dominant processes, and it is likely that several of these processes were operating simultaneously to some degree while oxygen was present, possibly in microzones within the water column.

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**LITERATURE CITED**


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