Effect of dissolved oxygen on nitrogen and phosphorus fluxes from lake sediments and their thresholds based on incubation using a simple and stable dissolved oxygen control method

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Abstract

Laboratory incubation of intact sediments is a popular and useful method for obtaining nutrient fluxes from lake sediments to the overlying water. However, one of the main problems facing this method is that the lake sediment core itself consumes oxygen from the overlying water due to the decomposition of organic matter. In this study, addition of headspace with controlled oxygen concentration and circulation of overlying water to the traditional intact sediment incubation method allows for incubation with a stable dissolved oxygen (DO) concentration in the overlying water. In 24 columns incubated for 179–269 h, the mean DO concentration in the overlying water ranged from 0.35 to 9.95 mgO2 L−1. In most cases, stability was high, with the standard deviation of the fluctuation in DO concentration less than 0.1 mgO2 L−1. The DO concentration of the overlying water could be adjusted by changing the partial pressure of oxygen in the headspace. This method supported a detailed description of the relationship between DO concentration and nutrient fluxes from sediments. The soluble reactive phosphorus fluxes and first-order reaction constants of NO2− + NO3− fluxes increased rapidly when the DO concentration decreased below the thresholds of 1.5 and 3.0 mgO2 L−1, respectively, in Lake Biwa. The thresholds and precise relationships between DO concentration and nutrient fluxes provide useful information for predicting the effect of DO concentration decreases in the lake bottom on nutrient release from sediments. Our results indicate that this incubation method is a powerful tool for clarifying those relationships.

The biogeochemical cycles of nitrogen and phosphorus in lakes, as in other aquatic ecosystems, are strongly affected by the external loading rates, with internal processes such as loading from sediment also playing important roles (Forsberg 1989; Jensen and Andersen 1992). The lake water and sediment are generally large pools of nitrogen and phosphorus, and sediments can act as a source and sink of nitrogen and phosphorus for lake water. In particular, many lakes do not show decreases in phosphorus content despite large reductions in external loading (Marsden 1989; Sondergaard et al. 2003). Hence, lake water quality management and prediction require not only measurement of the external loads of nitrogen and phosphorus but also clarification of nitrogen and phosphorus exchange processes between lake water and sediment.

Nitrogen and phosphorus fluxes between sediment and the overlying water are strongly influenced by biological and chemical processes at the interface between the sediment and overlying water (Mortimer 1941, 1942; Gächter et al. 1988; Forsberg 1989; Li et al. 2018). The dissolved oxygen (DO) concentration affects the activity of the microbial community and redox reactions in surface sediments. Numerous studies have reported that soluble reactive phosphorus (SRP) release, ammonium (NH4+) release, and nitrate (NO3−) consumption rates in sediment are generally much greater under anoxic conditions than oxic conditions (Liikanen et al. 2002; Fisher et al. 2005; Haggard et al. 2005; Jiang et al. 2008; Small et al. 2014). These findings suggest that changes in the DO
concentration in lake bottom water affect cycling of nutrients such as nitrogen and phosphorus in the lake.

Recently, changes in stratification structures have been reported in lakes around the world due to global warming (North et al. 2014; Kraemer et al. 2015; Ficker et al. 2016; Rogora et al. 2018; Stetler et al. 2021). Furthermore, in some lakes, changes in thermal stratification have been found to contribute to hypoxia and changing nutrient dynamics at the lake bottom (North et al. 2014; Ficker et al. 2016; Rogora et al. 2018). Therefore, determining the effects of oxygen concentration on nitrogen and phosphorus fluxes between the sediment and overlying water is essential not only for determining the present exchange of nitrogen and phosphorus between lake water and sediment but also for elucidating the impacts of ongoing global warming on biogeochemical cycles in the lake.

Nutrient flux from lake bottom sediments to the overlying water is commonly measured in the laboratory by intact sediment core incubation. Intact sediment core incubation has the advantage of allowing measurement of fluxes under various conditions. However, the biggest problem with this method is that the lake sediment core itself consumes oxygen from the overlying water due to the decomposition of organic matter. This consumption does not allow the sediment to be incubated at a stable DO concentration. It is particularly difficult to incubate sediments at DO concentrations between oxic and anoxic. Therefore, many studies focusing on the effect of the oxygen concentration of lake water on nutrient fluxes from sediments are conducted using arrested controlled DO concentrations (such as oxic or anoxic) or large-scale equipment. However, nutrient fluxes at DO concentrations between oxic and anoxic are very important because seasonal or long-term changes between oxic and anoxic conditions at the bottom of most lakes do not occur instantaneously (North et al. 2014; Rogora et al. 2018; Yamada et al. 2021). Large-scale incubation experiments make it difficult to conduct multiple simultaneous incubations under different conditions. These major issues hamper clarification of the precise relationships and thresholds between DO concentration and nitrogen and phosphorus fluxes in various lakes.

A method to control the oxygen concentration easily and stably in a small-scale incubation would enable further elucidation of the relationships between the DO concentration and nutrient fluxes. This precise relationship, along with the spatiotemporal distribution of lake bottom DO concentrations in the field, will provide extremely important information about the nutrient dynamics of the lake when DO at the lake bottom decreases.

The first aim of this study is to develop a simple and compact method for incubating intact sediment cores while accurately and stably controlling the DO concentration of the overlying water. Specifically, intact sediment cores and lake water were incubated in a closed system using an acrylic column. A headspace with controlled oxygen concentration was created above the overlying water so that oxygen is supplied to the lake water through dissolution in equilibrium with the headspace, even if the lake bottom sediment consumes oxygen during incubation. Then, we conducted incubation experiments while continuously monitoring the DO concentration in the overlying water using a fiber-optic oxygen meter in a closed system. Then, we applied these methods to Lake Biwa to confirm the usefulness of our method. Lake Biwa, the largest monomictic lake in Japan, exhibits a change in the DO concentration of its bottom water due to changes in the stratification pattern resulting from global warming (Yoshimizu et al. 2010; Yamada et al. 2021).

Materials and procedures
Site description
Intact sediment cores and bottom water were collected at Sta. T1 (35°22.0′N, 136°06.0′E) in Lake Biwa (surface area: 670 km²; mean water depth: 41 m; maximum depth: 104 m), central Japan (Fig. 1). Sta. T1 is located in the northwestern part of the North Basin of Lake Biwa. The maximum depth of
the sampling area is approximately 90 m, and the pH of lake water varies from 6.9 to 7.8 among depths (Rasheduzzaman et al. 2018). The water temperature at the bottom of the lake changes little throughout the year, ranging between 7.5°C and 9.0°C.

The North Basin of Lake Biwa is a mesotrophic and monomictic lake. Mitamura et al. (2015) measured nutrient and chlorophyll (Chl) a concentrations at T1 at 14 depths monthly over 10 yr (2002–2011) and reported average concentrations of NH4+, NO3−, PO43−, and Chl a across all depths over the 10-yr period of 0.6 ± 0.3 (average ± standard deviation) μM, 14 ± 2 μM, 0.10 ± 0.05 μM, and 2.0 ± 1.1 μg L−1, respectively. Goto et al. (2017) measured the total organic carbon, total nitrogen, and total phosphorus concentrations of the 0–1.0 cm sediment layer at 65 points at depths greater than 40 m in the North Basin of Lake Biwa, reporting concentrations in dry sediments of 1.26–5.78% (average: 4.32%), 0.14–0.68% (average: 0.49%), and 0.023–0.090% (average: 0.056%), respectively.

In the North Basin of Lake Biwa, lake water generally circulates from January to March. DO is usually saturated in all layers during the circulation period and reaches its lowest levels around December, which is the end of the stratification period. The DO concentration is approximately 4–6 mgO2 L−1 near the bottom of the lake at the end of the stratification period (Mitamura et al. 2015). However, turnover of the lake did not occur during the winters of 2018–2019 and 2019–2020 (Shiga Prefecture 2021; Yamada et al. 2021). As a result, DO was generally depleted over a wide area of the lake bottom at the end of the stratification period in 2020 (Lake Biwa Environmental Research Institute 2021).

**Sediment core collection**

Sediment cores were collected on 10 September, 07 October, 02 November, 13 November, and 09 December 2020 and on 04 January 2021 from Sta. T1 in Lake Biwa. Sediment cores were collected using an intact core sampler (11 cm inner diameter and 50 cm length, HR-type core sampler 5172, Rigo, Japan), which enabled the collection of both a sediment core and the overlying water in undisturbed conditions. Sediment core samples for incubation experiments were collected immediately from the collected core using an acrylic column (8.4 cm inner diameter, 50 cm length). Water samples for sediment core incubation were collected from near the lake bottom using a Van Dorn water sampler (Van Dorn water sampler 5026, Rigo). The water temperature and DO concentration of bottom water were measured in situ using a multiple-parameter water quality sensor (AAQ175-ZF, JFE Advantech, Japan).

**Column incubation experiments**

The sediment core and overlying water were incubated in a system consisting of an acrylic pipe with a black rubber stopper and peristaltic pump (MP1000 and MP-3, EYELA, Japan; Fig. 2; Supplementary Fig. S1; Table 1). The joint between the black rubber stopper and acrylic column was covered with duct tape, and the joint between the butyl rubber stopper and acrylic column was covered with silicone sealant to seal the inside of the column more tightly. Incubation of the column was conducted in an incubator at the ambient water temperature of the lake bottom (∼9.0°C) in the dark. As four columns could be placed in the incubator, the incubation experiment was conducted using a set of four columns.

Before the start of incubation, the overlying water in the column was replaced with fresh lake bottom water adjusted to the required DO concentrations. Fresh lake bottom water was placed in a 2-liter polypropylene bottle, and He was bubbled through the end of a plastic tube inserted into the bottom of the bottle to reduce the DO of the fresh lake bottom water to the required value. The DO concentration was checked by constantly measuring it with a DO meter (FDO925 and Multi 3410, WTW, Germany). After the overlying water in the column had been drained from port 1, the column was filled with fresh lake bottom water using the siphon method to avoid disturbing the sediment (Supplementary Fig. S2). A three-way stopcock was attached to the end of a Tygon tube filled with fresh lake bottom water and placed in the column, and the other end was inserted into a 2-liter bottle filled with fresh lake bottom water. The three-way stopcock was adjusted...
to prevent the fresh lake bottom water from flowing too quickly; the flow rate was adjusted according to the difference in water level between the fresh lake bottom water in the 2-liter bottle and the three-way stopcock, to prevent disturbing the sediment in the column.

Then, the column was capped and sealed with a black rubber stopper, and a mixed gas of He and ambient air was injected through port 2 using a syringe, while the lake water was drained from port 1 to create headspace in the column. A mixture of He gas and air was prepared using a Tedlar bag. He gas flowed through the Tygon tube and a silicon tube connected to a He gas cylinder, on dipping the tip of the tube in water to prevent air from flowing back (Supplementary Fig. S3). He gas was collected from the tube with He gas flowing through it using a syringe with a needle. The He gas was mixed with ambient air collected using a syringe in a Tedlar bag.

The oxygen partial pressure of the headspace was calculated from the mixing ratio of He and ambient air, assuming that the oxygen concentration of air was 21%. The overlying water in the column was circulated using a peristaltic pump (flow rate, 400 mL h⁻¹). The DO concentration in the overlying water during the incubation experiment was controlled by adjusting the initial DO concentration of the overlying water and the ratio of He to air in the gas mixture used to create the headspace.

An O₂ spot sensor (OXSP50I, PyroScience, Germany) for measuring the DO concentration was attached inside the column, which allowed measurement of the DO concentration of the overlying water from outside the column using a fiber-optic oxygen meter (iSO2-4, PyroScience, Germany) and O₂ probe (SPFIB, PyroScience, Germany). Since we used a fiber-optic oxygen meter with a four-channel probe, the DO concentrations of only four spot sensors can be measured.

### Table 1. Average values and standard deviations in Experiment 2. The average values and standard deviations were calculated for the period indicated by the solid line (observed values) in Fig. 4.

| Run  | Column 1 DO (mgO₂ L⁻¹) | Column 2 DO (mgO₂ L⁻¹) | Column 3 DO (mgO₂ L⁻¹) | Column 4 DO (mgO₂ L⁻¹) | Temperature (°C) | Incubation time (h) | Sediment sampling day |
|------|------------------------|------------------------|------------------------|------------------------|------------------|---------------------|----------------------|
| Run 1 | 3.45                   | 3.47                   | 2.06                   | 2.37                   | 8.97             | 269                 | 07 Oct 2020          |
| Average | 0.07                   | 0.07                   | 0.10                   | 0.06                   |                  |                     |                      |
| Run 2 | 4.70                   | 6.54                   | 7.99                   | 9.95                   | 8.92             | 206                 | 02 Nov 2020          |
| Average | 0.10                   | 0.09                   | 0.17                   | 0.21                   |                  |                     |                      |
| Run 3 | 0.92                   | 1.23                   | 1.52                   | 2.59                   | 9.05             | 179                 | 13 Nov 2020          |
| Average | 0.06                   | 0.06                   | 0.09                   | 0.04                   |                  |                     |                      |
| Run 4 | 2.74                   | 5.86                   | 7.12                   | 9.16                   | 8.94             | 179                 | 13 Nov 2020          |
| Average | 0.19                   | 0.27                   | 0.10                   | 0.12                   |                  |                     |                      |
| Run 5 | 0.99                   |                       |                        |                        | 9.02             | 215                 | 09 Dec 2020          |
| Average | 0.06                   |                       |                        |                        |                  |                     |                      |
| Run 6 | 0.35                   | 0.44                   | 0.43                   | 0.40                   | 8.96             | 230                 | 04 Jan 2021          |
| Average | 0.04                   | 0.07                   | 0.08                   | 0.07                   |                  |                     |                      |
| Run 7 | 1.69                   | 2.93                   | 5.64                   |                        | 9.05             | 230                 | 04 Jan 2021          |
| Average | 0.08                   | 0.03                   | 0.06                   |                        |                  |                     |                      |

Osaka et al. Evaluating nutrient flux and its thresholds
simultaneously. Therefore, when we conducted incubation experiments using more than four spot sensors (four columns) simultaneously, the probes were connected to the spot sensors alternately, and the DO concentration was measured alternately. Specifically, the incubation experiments for Runs 3 and 4 in Experiment 2 (involving eight spot sensors), Experiments 1 and Run 5 in Experiment 2 (six spot sensors), and Runs 6 and 7 in Experiment 2 (seven spot sensors) described later were each conducted simultaneously. Therefore, it was not possible to measure the DO concentrations of all columns simultaneously in these experiments, and the DO concentrations were measured intermittently in some columns. By contrast, the DO concentrations were measured continuously in Runs 1, 2, and 5 in Experiment 2. We measured the DO concentration in the overlying water at 5-min intervals.

Each of the eight spot sensors had the same sensor code, and the spot sensors could be calibrated by entering that code into the DO monitoring software. Several other spot sensor calibration programs are available. In this study, we performed calibrations using both the above sensor codes, as well as two-point calibration with air-saturated water (bubbling with air) and anoxic water (bubbling sodium sulfite solution with He) using a column without sediment. However, there was only a small difference (within about ±0.2 mgO₂ L⁻¹) between the calibration using the sensor code and the two-point calibration. There was also little difference (±0.05 mgO₂ L⁻¹) when the probe of the fiber-optic oxygen meter was connected alternately to different spot sensors having the same sensor code to measure the DO concentrations alternately in the same air-saturated and anoxic water. Spot sensors with the same sensor code were used in all of the incubation experiments in this study. The fiber-optic oxygen meter has a port and probe specifically for measuring water temperature, which was also measured during the spot sensor calibration. In the experiment, the DO concentration was corrected by measuring the temperature of water in a 2-liter bottle placed in the incubator.

We conducted two incubation experiments with different settings for the following purposes:

**Experiment 1**: To clarify the effects of overlying water circulation and headspace creation in the column on the DO concentration during incubation, we incubated three columns. The first column had no headspace and no circulation, the second column had headspace and no circulation, and the third column had both headspace and circulation. In column 3, overlying water was collected using the same method as in Experiment 2 described below. We used the sediment core and lake bottom water sample collected on 09 December 2020 for this experiment.

**Experiment 2**: To clarify the effect of the DO concentration in the overlying water on nutrient flux from lake bottom sediment, column incubation was conducted with varying DO concentrations in the overlying water. We used the sediment core and lake bottom water samples collected on 07 October (Run 1), 02 November (Run 2), 13 November (Runs 3 and 4), 09 December (Run 5) 2020, and 04 January 2021 (Runs 6 and 7) at T1 for this experiment (Fig. 1; Table 1).

A 24-mL water sample was collected for chemical analysis from the lower port (port 1) using a plastic syringe, and 24 mL mixed gas containing He and ambient air at the same oxygen partial pressure as that of the head space was injected simultaneously into port 2. In column 3 in Experiment 1, 3 or 4 mL additional ambient air was injected into port 2. In the columns used for Runs 5 and 6 of Experiment 1, 1 or 2 mL additional ambient air was injected into port 2 at 160 h after the start of incubation (Run 5) or at 35 and 135 h after the start of incubation (Run 6) to maintain the initial DO concentration.

Each collected water sample was immediately filtered through a cellulose acetate filter (0.45-µm pore size) and stored in a freezer in a polypropylene bottle. Water samples were collected at intervals of 1–4 d after the start of incubation. The initial amount of water in the columns ranged from 1394 to 1638 mL. For Experiment 2, we performed column incubations in Runs 1–7 (24 columns in total), changing the initial DO concentration of the overlying water and oxygen partial pressure in the headspace for each column.

The total amount of He used in all of these experiments was 1.5–2 m³.

**Chemical analysis**

Water samples were analyzed for NO₂⁻ + NO₃⁻, NH₄⁺, and SRP using a flow injection analyzer (OG-FI-300S for NO₂⁻ + NO₃⁻, OG-FI-3300NH for NH₄⁺, OG-FI-3300PO for SRP, Ogawa & Co.), which automatically performs spectrophotometric measurements. The dissolved inorganic nitrogen (DIN) concentration was calculated as the sum of NO₂⁻ + NO₃⁻ and NH₄⁺. In the sample used for total dissolved nitrogen (TDN) analysis, TDN was oxidized to NO₃⁻ through the addition of potassium persulfate and autoclaving, and NO₃⁻ was measured as TDN using a flow injection analyzer. The TDN concentration was measured only in Runs 3–7.

**Calculation of nutrient flux and first-order reaction constant**

The average nutrient flux was calculated for each column as

\[ J_{tn} = \left( \sum_{i=1}^{n} \left( t_{(i+1; t_i)} - t_{(i; t_i)} \right) \right) / t_i \]  

(1)

where \( J_{tn} \) is the average nutrient flux (mgN m⁻² d⁻¹ or mgP m⁻² d⁻¹) from the start of incubation to the \( n \)th water sampling time. \( t_i \) and \( t_{(i+1; t_i)} \) are the times (days) from the start of incubation to the \( i \)th water sampling time and to the sampling time immediately before the \( i \)th sampling time, respectively. \( t_{(i+1; t_i)} \) is the average nutrient flux (mgN m⁻² d⁻¹ or mgP m⁻² d⁻¹) from the sampling time immediately before the \( i \)th sampling time to the \( i \)th water sampling time, calculated as
\[
J(t_{i+1}; t_i) = \frac{(C_i - C_{(i-1)}) q_{(i-1)}}{t_i - t_{(i-1)}}
\]

where \(C_i\) and \(C_{(i-1)}\) are the nutrient concentrations (mgN L\(^{-1}\)) of the overlying water in the column at the \(i^{th}\) water sampling time and the sampling time immediately before the \(i^{th}\) sampling time, respectively. \(q_{(i-1)}\) is the water volume (L) of the overlying water in the column at the sampling time immediately before the \(i^{th}\) sampling time. \(A\) is the cross-sectional area of the inner column (m\(^2\)).

Assuming a first-order reaction, the rate constant for NO\(_3^-\) consumption \((k_1)\) is given by

\[
k_1 = \ln \left( \frac{C_0}{C_{n}} \right) / t_n
\]

**Assessment**

Effects of headspace and circulation on DO concentration stability in the overlying water

During Experiment 1, continuous DO concentration measurements using a fiber-optic oxygen meter allowed assessment of the effects of headspace and overlying water circulation on the stability of the DO concentration in the overlying water (Fig. 3). In column 3, in which headspace was created and the overlying water was circulated, incubation was possible while keeping the DO concentration of the overlying water constant. Additional air injection during the incubation also contributed to the stable DO concentration. The mean DO concentration was 4.90 mgO\(_2\) L\(^{-1}\), and the standard deviation was 0.07 mgO\(_2\) L\(^{-1}\) over 278 h of incubation. On the other hand, the DO concentration of the overlying water decreased during incubation in column 1, which had no circulation and no headspace, and in column 2, which had no circulation and had a headspace. Furthermore, in column 1, the DO concentration in the lake water differed depending on position in the column, with lower concentrations in the lower part of the lake water than in the upper part. In column 2, DO concentrations in the upper and lower portions of the lake water were almost identical. These findings demonstrate that the column incubation method with headspace and overlying water circulation proposed in this study can be used for incubations with stable DO levels for at least 10 d using simple tools.

As no headspace was present in column 1, and the incubation was conducted without supplying oxygen to the overlying water, the DO concentration in the overlying water may have decreased due to the decomposition of organic matter in the sediment. However, the rate of DO concentration decrease in the overlying water in column 1 was lower than expected. The sediment oxygen demand (SOD) calculated from the rate of decrease of the DO concentration in the overlying water in column 1 was 57.4 mgO\(_2\) m\(^{-2}\) d\(^{-1}\), while the SOD previously measured by Goto et al. (2017) at this study site ranged from 192 to 271 mgO\(_2\) m\(^{-2}\) d\(^{-1}\). In column 1, the difference in DO concentration.
concentration between the top and bottom of the overlying water indicates that the lake water was not mixed well (Fig. 3). As a result, the DO concentration immediately above the sediment decreased, and diffusion of DO from the overlying water into the sediment weakened, suggesting that SOD was lower than the calculated value. Several studies have confirmed the importance of the diffusive boundary layer in controlling SOD (Beutel 2003; Bryant et al. 2010; Bierlein et al. 2017) and noted that with relatively quiescent overlying water, the diffusive boundary layer becomes thicker, and the SOD decreases (Bryant et al. 2010). Our results are consistent with these findings.

In column 2, the DO in the overlying water decreased despite the creation of a headspace containing O₂ gas. This indicates that O₂ consumption by sediments exceeds the dissolution of O₂ from the headspace into the overlying water when a headspace is created alone. In other words, it is clear that creating a headspace containing O₂ and circulating the overlying water are both very important for maintaining a stable DO concentration in the overlying water during incubation. The fluctuation of the water surface due to circulation or passage of the overlying water through the headspace is probably essential for the dissolution of oxygen from the headspace into the water.

**Stability and control of the DO concentration in incubations with various DO concentrations**

In Experiment 2, 24 columns were incubated at various initial DO concentrations and their DO concentrations were monitored (Fig. 4; Table 1). Although DO changed at the beginning of the incubation, DO equilibrium usually required a few hours (10 h at most). In the 24 columns incubated, the mean DO concentration in the overlying water during the incubation period ranged from 0.35 to 9.95 mgO₂ L⁻¹, and the standard deviation ranged from 0.03 to 0.27 mgO₂ L⁻¹. Of the 24 columns, 15 were incubated with standard deviations of less than 0.10 mgO₂ L⁻¹. These results show that our incubation method supports incubation with stable DO concentrations over a wide range of DO concentrations.

Figure 5 shows the relationship between the partial pressure of O₂ in the headspace and the average DO concentration in the overlying water during incubation. The DO concentration in the overlying water calculated using Henry’s law from the O₂ partial pressure of the headspace is also shown. In many incubation columns, the DO concentration in the overlying water was similar to the DO concentration calculated using Henry’s law from the partial pressure of O₂ in the headspace. These findings indicate that the DO concentration of the overlying water during incubation is affected by the DO concentration of the overlying water prior to headspace creation during the first few hours of incubation, and then it quickly shifts to being driven primarily by the partial pressure of oxygen in the headspace. In other words, we can easily control the DO concentration in the overlying water by changing the partial pressure of oxygen in the headspace.

The average DO concentration in the overlying water was slightly higher than the calculated value when the oxygen partial pressure in the headspace was less than 0.04 atm. Moreover, the average DO concentration in the overlying water was slightly lower than the calculated value when the oxygen partial pressure in the headspace was greater than 0.16 atm (Fig. 5). The possible reasons for this are as follows. Before the start of incubation, the overlying water was replaced with lake bottom water; this process is performed slowly to avoid disturbing the bottom sediment. Therefore, even if the oxygen partial pressure of the headspace is set to 0, oxygen is present in the overlying water. Therefore, in columns with low headspace oxygen partial pressure, the actual DO concentration will be slightly higher than the DO concentration calculated using Henry’s law. In Run 6, we replaced the lake water before incubation more quickly than in Runs 3 and 5, and injected ambient air during the incubation.
Fig. 6. Temporal variations in the (a) $\text{NH}_4^+$, (b) $\text{NO}_2^- + \text{NO}_3^-$, (c) DIN, (d) TDN, and (e) SRP concentrations during incubation.
Fig. 7. Relationship between the average DO concentration in the overlying water and (a) $\text{NH}_4^+$ flux, (b) $\text{NO}_2^- + \text{NO}_3^-$ flux, (c) $k_1$ of $\text{NO}_2^- + \text{NO}_3^-$, (d) DIN flux, (e) TDN flux, and (f) SRP flux. The data are from Tables 1 and 2.
In columns with a high DO concentration in the overlying water, organic matter decomposition in the sediments may be active, and thus oxygen consumption in the bottom sediments may be high. Several studies have shown that the rate of organic matter decomposition in water is higher in the presence of oxygen (Parr and Reuszer 1962; Moore et al. 1992). In Experiment 1, a small amount of gas with a higher O₂ partial pressure compared with the headspace was
added to the headspace during incubation, which improved stability against a decrease in the DO concentration. Particularly in incubations in which the DO concentration is high, this method can stabilize the DO concentration during incubation.

Temporal variation in nutrient concentrations during column incubation

The changes in the concentrations of nutrients in the overlying water of each column during Experiment 2 showed similar trends in terms of direction for each nutrient, especially for nitrogen compounds (Fig. 6). This tendency suggests that the proposed incubation method can maintain a stable environment for nutrient release and consumption from the sediment.

At all DO concentrations, NH$_4^+$, DIN, and TDN concentrations tended to increase, whereas those of NO$_2^- + NO_3^-$ tended to decrease. NH$_4^+$ and DIN concentrations tended to increase more slowly during the latter half than earlier half of the incubation. During the latter half of the incubation period, the differences in the concentrations of NH$_4^+$ and DIN sources between the sediment and overlying water decreased, suggesting that the diffusion and release rates of NH$_4^+$ and DIN decreased. Therefore, the fluxes of NH$_4^+$, DIN, and TDN were calculated using the concentrations 60–110 h after the start of incubation, and the flux of NO$_2^- + NO_3^-$ was calculated using the concentrations obtained at 133–206 h after the start of incubation. During incubation, SRP concentrations generally increased but sometimes showed irregular fluctuations near the end of the incubation period. While biological reactions are thought to be the main drivers of the fluctuations in nitrogen compound concentrations during incubation, fluctuations in phosphorus concentrations are strongly influenced by both biological and chemical reactions such as desorption from, dissolution in, and sorption onto sediment particles (Gächter et al. 1988; Hupfer and Lewandowski 2008). Fluxes of SRP were calculated using the concentrations measured at 60–110 h after the start of incubation, which is the same period used for NH$_4^+$, DIN, and TDN.

Relationships between nutrient fluxes from sediments and the DO concentration in the overlying water

Although each experiment was performed in an independent system, the change in flux with respect to DO concentration in the overlying water showed a uniform trend (Fig. 7). Under anoxic conditions, the fluxes of NH$_4^+$, DIN, TDN, and SRP and the negative flux of NO$_2^- + NO_3^-$ were larger than those under oxic conditions. In numerous studies comparing nutrient fluxes between oxic and anoxic environments, fluxes of NH$_4^+$ and SRP and the negative flux of NO$_2^- + NO_3^-$ increase under anoxic conditions (Moore et al. 1992; Liikanen et al. 2002; Haggard et al. 2005; Beutel et al. 2008), and the results of the present study are consistent with those findings.

The maximum NH$_4^+$ and SRP fluxes during anoxic incubation in the present study were 17.99 mgN m$^{-2}$ d$^{-1}$ and 2.59 mgP m$^{-2}$ d$^{-1}$, respectively (Fig. 7a,f; Table 2). NH$_4^+$ and SRP fluxes from sediments under anoxic conditions have been reported to increase with increasing nutrient levels in the lake (Nürnberg 1988; Beutel 2006). The SRP fluxes in our study area were within the range of those in mesotrophic lakes (Nürnberg 1988), and the NH$_4^+$ fluxes were higher than in mesotrophic lakes, but lower than in eutrophic/hypereutrophic lake (Beutel 2006). Therefore, the NH$_4^+$ and SRP fluxes measured using the proposed method under anoxic conditions did not differ greatly from previously reported values.

When the DO concentration was relatively high, the NO$_2^- + NO_3^-$ flux was not affected by the initial NO$_2^- + NO_3^-$ concentration (Fig. 7b). However, when the DO concentration was low, the negative flux increased with increasing initial NO$_2^- + NO_3^-$ level at the same DO concentration. Therefore, when the relationship between the DO concentration and NO$_2^- + NO_3^-$ consumption rate was expressed as $k_i$, the negative value of $k_i$ decreased sharply when the DO concentration of the system was below $\sim 3.0$ mgO$_2$ L$^{-1}$, as the effect of the initial NO$_2^- + NO_3^-$ concentration disappeared (Fig. 7c). This finding indicates that the NO$_2^- + NO_3^-$ consumption potential increases rapidly when the DO concentration is below 3 mgO$_2$ L$^{-1}$ in our study area. Denitrification, which is considered to be the main driver of NO$_3^-$ flux and NO$_2^- + NO_3^-$ consumption in lake sediments under anoxic conditions, is affected by the NO$_3^-$ concentration (Mengis et al. 1997; Beutel et al. 2008; Kreiling et al. 2011). The results of the present study are similar to those of previous investigations. NH$_4^+$ flux from the sediment was always greater than the negative flux of NO$_2^- + NO_3^-$ at all DO concentrations. The DIN and TDN fluxes were positive at all DO concentrations (Fig. 7d,e).

As indicated by these results, nutrient fluxes from the sediments measured under oxic and anoxic conditions in this study are consistent with the trends reported in previous research.

Discussion

In this study, a simple method was established to incubate lake sediments while keeping the DO concentration of the overlying water stable for at least 10 d. Using this method, the DO concentration of the overlying water can be adjusted by simply changing the partial pressure of oxygen in the headspace. Moreover, the incubation can generally be performed with high stability, within 0.1 mgO$_2$ L$^{-1}$ standard deviation of the variation in DO concentration. This method allowed us to characterize in detail the relationship between nutrient fluxes from lake sediments and DO concentrations in the overlying water.
Incubation of lake sediments at various oxygen concentrations has been used in a number of studies to determine the effect of the lake DO concentration on nutrient fluxes from sediments (Nürnberg 1988; Haggard et al. 2005). These studies have provided very important information on nutrient dynamics when lake sediments are anoxic or oxic. However, most previous lake sediment incubation studies were roughly controlled under oxic or anoxic conditions, and few involved detailed measurements of nutrient fluxes in the range between oxic and anoxic conditions, which was the focus of this study. Therefore, the findings obtained using the method proposed in this study will enhance our understanding of the impact of hypoxia on nutrient cycling at the lake bottom. The importance of this research is that decreases in the DO concentration at lake bottoms occur over seasonal or long-term scales with gradual and continuous changes from oxic to anoxic (North et al. 2014; Rogora et al. 2018; Yamada et al. 2021). In other words, it is essential to understand not only oxic and anoxic conditions, but also the in-between states to fully clarify and evaluate the biogeochemistry related to hypoxia of the lake bottom. Specifically, information is needed on the absolute levels of nutrient fluxes between oxic and anoxic conditions, and whether nutrient fluxes increase or decrease gradually from oxic to anoxic conditions, or whether they increase or decrease rapidly around a certain threshold level. If such a threshold exists, determination of the threshold is important for interpretation of these processes in the environment.

The results of this study showed that the NH$_4^+$ flux increased relatively gradually as the DO concentration of overlying water decreased, SRP flux increased rapidly when the DO concentration decreased below 1.5 mgO$_2$ L$^{-1}$, and the $k_1$ negative value of NO$_2^−$ + NO$_3^−$ rapidly shifted to a more negative value when the DO concentration decreased below 3.0 mgO$_2$ L$^{-1}$ (Fig. 7). For nitrogen compounds, the $k_1$ negative value of NO$_2^−$ + NO$_3^−$ increased sharply when the DO concentration decreased below 3.0 mgO$_2$ L$^{-1}$. Due to the relatively large flux of NH$_4^+$, the amounts of DIN and TDN released from the sediment increased as the DO concentration decreased. In recent years, Lake Biwa has experienced some periods and areas in which the DO concentration at the bottom was below the threshold of 3.0 or 1.5 mgO$_2$ L$^{-1}$ due to the extensive stratification period (Lake Biwa Environmental Research Institute 2021; Yamada et al. 2021). These findings indicate that the internal loads of phosphorus and nitrogen in Lake Biwa have increased due to recent hypoxia at the lake bottom. If the precise relationship and threshold values of nutrient fluxes and DO concentrations can be determined in various environments, as demonstrated in this study, the spatiotemporal distribution of DO data can more accurately determine the area, duration, and magnitude of the increase in nutrient release associated with the decrease in DO concentration at the lake bottom. Such information will support better understanding and prediction of the impact of hypoxia on nutrient dynamics in lakes.

The relationships and thresholds of the DO concentration at the lake bottom and nutrient fluxes from sediments in various lakes should be clarified in the future, as nutrient fluxes from lake sediments under anoxic conditions have been reported to vary among lake environments (Nürnberg 1988; Beutel 2006; Hupfer and Lewandowski 2008). In particular, SRP release from lake sediments may be influenced not only by the nutrient status of the lake but also by the characteristics of the catchment, such as the presence of Al(OH)$_3$ and non-reducible Fe(III) minerals (Hupfer and Lewandowski 2008).

In addition, the relationship between the DO concentration of overlying water and nutrient flux from sediments may vary seasonally for the following reasons. In this study, the relationship between DO concentration and NO$_2^−$ + NO$_3^−$ flux in lake water was affected by seasonal differences in the NO$_2^−$ + NO$_3^−$ concentration of lake bottom water (Fig. 7b). Previous studies have reported that nutrient fluxes from lake sediments vary with the amount of nutrients deposited from the lake surface layer (Burger et al. 2007) and with water temperature (Jensen and Andersen 1992; Jiang et al. 2008). Therefore, when investigating the relationship between DO concentration at the lake bottom and nutrient fluxes from sediments, it is desirable to perform sampling at multiple sites and during multiple seasons. This practical method, which is compact and simple and allows for simultaneous incubation at several different DO concentrations (up to eight levels in this study), will provide a powerful tool for clarifying the relationships between lake DO concentrations and nutrient fluxes from sediments in various lakes.

**Comments and recommendations**

With the addition of headspace and overlying water circulation to the traditional intact sediment incubation method, it is possible to conduct incubation experiments for several days with a stable DO concentration in the overlying water. Furthermore, because the incubation system is gas-tight, it can be used not only to measure nutrient and metal fluxes from sediments but also to determine the impact of the lake water DO concentration on greenhouse gas (CO$_2$, CH$_4$, N$_2$O, etc.) fluxes from the sediments and the rate of organic matter decomposition in the sediments (via measurement of CO$_2$).

In this study, DO concentrations were measured continuously using a fiber-optic oxygen meter. The same experiment conducted using a membrane or fluorescent-type DO meter would allow for DO measurements only at the beginning and end, but the experiments could be conducted at a lower total cost. In this paper, we confirmed that the DO concentration is constant during incubation using this method; therefore, if the DO concentration is similar at the beginning and end of the incubation, it is unlikely that the DO concentration varied significantly during the incubation period. Although we used He gas in this study, we believe that using N$_2$ gas instead of He will reduce the cost.
Although the experiments in this study were conducted with a constant circulation rate of overlying water, the circulation rate can be changed easily. Studies have suggested that the movement of water near the bottom of a lake affects the concentration gradient of materials in the boundary layer between the lake water and sediment, which in turn changes the diffusion rate of materials in the boundary layer (Bryant et al. 2010). This suggests that it may affect nutrient fluxes from sediments. The present method can be applied in future studies of the effects of the physical environment of the boundary layer between lake water and sediment on nutrient fluxes from sediments, by changing the circulation rate of the overlying water.

Data Availability Statement
The data used in this paper are available from the corresponding author.

References
Beutel, M. W. 2003. Hypolimnetic anoxia and sediment oxygen demand in California drinking water reservoirs. Lake Reserv. Manage. 19: 208–221. doi:10.1080/0743814039354086
Beutel, M. W. 2006. Inhibition of ammonia release from anoxic profundal sediments in lakes using hypolimnetic oxygenation. Ecol. Eng. 28: 271–279. doi:10.1016/j.ecoleng.2006.05.009
Beutel, M. W., N. R. Burley, and S. R. Dent. 2008. Nitrate uptake rate in anoxic profundal sediments from a eutrophic reservoir. Hydrobiologia 610: 297–306. doi:10.1007/s10750-008-9445-6
Bierlein, K. A., M. Rezvani, S. A. Socolofsky, L. D. Bryant, A. Wüest, and J. C. Little. 2017. Increased sediment oxygen flux in lakes and reservoirs: The impact of hypolimnetic oxygenation. Water Resour. Res. 53: 4876–4890. doi:10.1002/2016WR019850
Bryant, L. D., C. Lorrai, D. F. McGinnis, A. Brand, A. Wüest, and J. C. Little. 2010. Variable sediment oxygen uptake in response to dynamic forcing. Limnol. Oceanogr. 55: 950–964. doi:10.4319/lo.2010.55.2.0950
Burger, D. F., D. P. Hamilton, C. A. Pilditch, and M. M. Gibbs. 2007. Benthic nutrient fluxes in a eutrophic, polymeric lake. Hydrribiologia 584: 13–25. doi:10.1007/s10750-007-0582-0
Ficker, H., M. Luger, and H. Gassner. 2016. From dimictic to monomictic: Empirical evidence of thermal regime transitions in three deep alpine lakes in Austria induced by climate change. Freshw. Biol. 62: 1335–1345.
Fisher, M. M., K. R. Reddy, and R. Thomas James. 2005. Internal nutrient loads from sediments in a shallow, subtropical lake. Lake Reserv. Manag. 21: 338–349. doi:10.1080/07438140509354439
Forsberg, C. 1989. Importance of sediments in understanding nutrient cyclings in lakes. Hydrobiologia 176: 263–277. doi:10.1007/BF00026561
Gächter, R., J. S. Meyer, and A. Mares. 1988. Contribution of bacteria to release and fixation of phosphorus in lake sediments. Limnol. Oceanogr. 33: 1542–1558. doi:10.4319/lo.1988.33.6part2.1542
Goto, N., K. Fukuda, S. Omura, A. Yoshimura, and S. Ban. 2017. Sediment oxygen demand and bottom sediment environment in the northern part of the North Basin of Lake Biwa, Japan. Jpn. J. Limnol. 78: 169–178. doi:10.3739/rikusui.78.169
Haggard, B. E., P. A. Moore Jr., and P. B. Delaune. 2005. Phosphorus flux from bottom sediments in Lake Eucha, Oklahoma. J. Environ. Qual. 34: 724–728. doi:10.2134/jeq2005.0724
Hupfer, M., and J. Lewandowski. 2008. Oxygen controls the phosphorus release from lake sediments—a long-lasting paradigm in limnology. Int. Rev. Hydrobiol. 93: 415–432. doi:10.1002/iroh.200711054
Jensen, H. S., and F. Andersen. 1992. Importance of temperature, nitrate and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. Limnol. Oceanogr. 37: 577–589. doi:10.4319/lo.1992.37.3.0577
Jiang, X., X. C. Jin, Y. Yao, L. H. Li, and F. C. Wu. 2008. Effects of biological activity, light, temperature and oxygen on phosphorus release processes at the sediment and water interface of Taihu Lake, China. Water Res. 42: 2251–2259. doi:10.1016/j.watres.2007.12.003
Kraemer, B. M., O. Anneville, S. Chandra, et al. 2015. Morphometry and average temperature affect lake stratification responses to climate change. Geophys. Res. Lett. 42: 4981–4988. doi:10.1002/2015GL064097
Kreiling, R. M., W. B. Richardson, J. C. Cavanaugh, and L. A. Bartsch. 2011. Summer nitrate uptake and denitrification in an upper Mississippi River backwater lake: The role of rooted aquatic vegetation. Biogeochem 104: 309–324. doi:10.1007/s11053-010-9503-9
Lake Biwa Environmental Research Institute. 2021. The water chemistry of Lake Biwa. https://www.lberi.jp/learn/biwako/water
Li, J., Y. Zhang, and S. Katsev. 2018. Phosphorus recycling in deeply oxygenated sediments in Lake Superior controlled by organic matter mineralization. Limnol. Oceanogr. 63: 1372–1385. doi:10.1002/ino.10778
Liikanen, A., T. Murtoniemi, and H. Tanskanen. 2002. Effects of temperature and oxygen availability on greenhouse gas and nutrient dynamics in sediment of a eutrophic mid-oreal lake. Biogeochem 59: 269–286. doi:10.1023/A:1016015526712
Marsden, M. W. 1989. Lake restoration by reducing external phosphorus loading: The influence of sediment phosphorus release. Freshw. Biol. 21: 139–162. doi:10.1111/j.1365-2427.1989.tb01355.x
Mengis, M., R. Gächter, and B. Wehrli. 1997. Nitrogen elimination in two deep eutrophic lakes. Limnol. Oceanogr. 42: 1530–1543. doi:10.4319/lo.1997.42.7.1530
Mitamura, O., H. Azumi, M. Kihira, T. Akatsuka, K. Anbutsu, T. Ishikawa, and N. Goto. 2015. Ten-year variation of biogeochemical parameters in the north basin of Lake Biwa. Limnol. Study 2: 3–15.

Moore, P. A., K. R. Reddy, and D. A. Graetz. 1992. Water quality-nutrient transformations in sediments as influenced by oxygen supply. J. Environ. Qual. 21: 387–393. doi: 10.2134/jeq1992.00472425002100030014x

Mortimer, C. H. 1941. The exchange of dissolved substances between mud and water in lakes, I. J. Ecol. 29: 280–239. doi: 10.2307/2256395

Mortimer, C. H. 1942. The exchange of dissolved substances between mud and water, II. J. Ecol. 30: 147–201. doi: 10.2307/2256691

North, R. P., R. L. North, D. M. Livingstone, O. Köster, and R. Kipfer. 2014. Long-term changes in hypoxia and soluble reactive phosphorus in the hypolimnion of a large temperate lake: Consequences of a climate regime shift. Glob. Chang. Biol. 20: 811–823. doi: 10.1111/gcb.12371

Nürnberg, G. K. 1988. Prediction of phosphorus release rates from total and reductant-soluble phosphorus in anoxic lake sediments. Can. J. Fish. Aquat. Sci. 45: 453–462. doi: 10.1139/f88-054

Parr, J. F., and H. W. Reuszer. 1962. Organic matter decomposition as influenced by oxygen level and flow rate of gases in the constant aeration method1. Soil Sci. Soc. Am. J. 26: 552. doi: 10.2136/sssaj1962.03615995002600060012x

Rasheduzzaman, M., M. Kawaguchi, H. Obata, and M. Maruo. 2018. Determination of dissolved and particulate thiols in Lake Biwa water and extracted fulvic acids by solid phase extraction followed by HPLC with fluorescence detection. Limnology 19: 299–309. doi: 10.1007/s10201-018-0547-1

Rogora, M., F. Buzzi, C. Dresti, and others. 2018. Climatic effects on vertical mixing and deep-water oxygen content in the subalpine lakes in Italy. Hydrobiologia 824: 33–50. 10.1007/s10750-018-3623-y.

Shiga Prefecture. 2021. Lake Biwa water quality survey results- Today’s Lake Biwa. https://www.pref.shiga.lg.jp/ippan/kan kyoshizen/biwako/300014.html

Small, G. E., J. B. Cotner, J. C. Finlay, R. A. Stark, and R. W. Sterner. 2014. Nitrogen transformations at the sediment-water interface across redox gradients in the Laurentian Great Lakes. Hydrobiologia 731: 95–108. doi: 10.1007/s10750-013-1569-7

Søndergaard, M., J. P. Jensen, and E. Jeppesen. 2003. Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia 506: 135–145. doi: 10.1023/B:HYDR.000008611.12704.dd

Stetler, J. T., S. Girdner, J. Mack, L. A. Winslow, T. H. Leach, and K. C. Rose. 2021. Atmospheric stilling and warming air temperatures drive long-term changes in lake stratification in a large oligotrophic lake. Limnol. Oceanogr. 66: 954–964. doi: 10.1002/lo.11654

Yamada, K., H. Yamamoto, S. Hichiri, T. Okamoto, and K. Hayakawa. 2021. First observation of incomplete vertical circulation in Lake Biwa. Limnology 22: 179–185. doi: 10.1007/s10201-021-00653-3

Yoshimizu, C., K. Yoshiyama, I. Tayasu, T. Koitabashi, and T. Nagata. 2010. Vulnerability of a large monomictic lake (Lake Biwa) to warm winter event. Limnology 11: 233–239. doi: 10.1007/s10201-009-0307-3

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