

Distribution of greenhouse gases, nitrite, and $\delta^{13}\text{C}$ of dissolved inorganic carbon in Lake Biwa: Implications for hypolimnetic metabolism

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Abstract. The vertical distribution of dissolved greenhouse gases (CH_4 , ΣCO_2 and N_2O), NO_2^- , and $\delta^{13}\text{C}$ of ΣCO_2 in Lake Biwa during a stagnant period was precisely determined. ΣCO_2 as well as NO_3^- was accumulated in the hypolimnion, whereas NO_2^- and CH_4 concentrations were generally higher in the epilimnion than in the hypolimnion. In August, NO_2^- and CH_4 were ephemerally accumulated at the thermocline. The concentration of CH_4 always exceeded equilibrium with respect to air/water exchange. N_2O was rather uniformly distributed in both time and space, and remained near equilibrium with respect to air/water exchange. All of these observations are similar to other stratified, oligotrophic lakes, in which the hypolimnia were well oxygenated. The $\delta^{13}\text{C}$ of ΣCO_2 became more negative with increasing depth, and showed a strong negative correlation with apparent oxygen utilization. From the data, the $\delta^{13}\text{C}$ value of organic matter decomposed into ΣCO_2 in the hypolimnion was calculated by isotope mass-balance, and found to be in a similar range to $\delta^{13}\text{C}$ of phytoplankton and benthic algae and distinctively higher than $\delta^{13}\text{C}$ of both terrestrial and sedimentary organic matters. This suggests that autochthonous organic matter was the major source of ΣCO_2 .

Introduction

Decomposition processes of organic matter in the tropholytic zone, i.e., the hypolimnion and sediment of lakes, are collectively referred to as hypolimnetic metabolism (Lehman 1988). Decomposition is also the major source of greenhouse gases like CO_2 , CH_4 , and N_2O dissolved in lake water. These three gases contribute to 80% of greenhouse effect (Rodhe 1990), and thus to understand mechanisms that control the emission of them from ecosystems is now of great environmental importance. The hypolimnetic metabolism includes both aerobic and anaerobic processes. The dynamics of the greenhouse gases in a lake is principally conditioned by the distribution of the

aerobic and anaerobic subsystems in the lake and whether the organic input is decomposed aerobically or anaerobically.

Decomposition of organic matter in freshwater systems has some distinctive characteristics that are not the case for terrestrial and oceanic systems. First, since freshwater systems have specific residence times for water, dissolved and suspended organic matter refractory enough not to be decomposed within the water retention time are flushed out intact. Therefore, properties of organic matter averaged over the total organic input to the system are not always equivalent to those of organic matter really decomposed in the system. A question of what part of the total organic input is actually decomposed in the system thus arises. The relative importance of autochthonous vs. allochthonous organic matter during decomposition is of special interest, because it is relevant to the question of the major causes of eutrophication, whether loadings of mineral nutrients or organic matters. Second, freshwater sediments easily become anoxic when organic input is high; however, unlike marine sediments, the role of sulfate reduction in anoxic sediment is relatively small due to low sulfate concentrations. Thus, a relatively large contribution of denitrification to anaerobic decomposition and the occurrence of methanogenesis at shallow layers in sediment or even in the water column are unique properties of freshwater systems, which may increase their importance as a potential source of N_2O and CH_4 .

In the hypolimnion of Lake Biwa (Table 1), oxic conditions prevail throughout the water column, and decomposition of organic matter predominantly proceeds through oxidative metabolism with CO_2 and NO_3^- as the end products, although a few percent of total mineralization proceeds through denitrification (Miyajima 1994). The oxic/anoxic interface, which is seemingly the active site of denitrification in this lake, usually exists at the sediment surface irrespective of water depth. Below the interface, methanogenesis presumably serves as the principal electron sink. CH_4 produced there diffuses into the oxic layer and is oxidized to CO_2 by methanotrophic bacteria. We address in this paper the mineralization of organic carbon by describing distribution of total dissolved inorganic carbon (ΣCO_2), CH_4 and stable carbon isotope ratio ($\delta^{13}\text{C}$) of ΣCO_2 . $\delta^{13}\text{C}$ values have been widely used to infer source of the carbon and food-web structure (Fry & Sherr 1988; Boschker et al. 1995). Since ΣCO_2 is virtually the sole sink of mineralized carbon in Lake Biwa, information on the $\delta^{13}\text{C}$ value of ΣCO_2 may be used to presume the origin of organic matter that is actually decomposed.

The oxidative mineralization of organic nitrogen into NO_3^- is divided into several steps. Organic nitrogen is first mineralized by heterotrophs into NH_4^+ , which is then oxidized into NO_2^- by ammonia oxidizers. A small fraction of NH_4^+ is also converted by the same organisms into N_2O which is not further

Table 1. The limnology of Lake Biwa.

Latitude	35°00'–35°30' N
Longitude	135°50'–136°15' E
Altitude (surface)	85 m
Area	674 km ²
Depth, maximum	104 m
Depth, mean	41 m
Seasonal stratification	April–January

metabolized under oxic conditions. NO_2^- produced is finally oxidized into NO_3^- by nitrite oxidizers. Denitrification is also divided into several steps, in which NO_2^- , NO and N_2O occur as intermediates. During aerobic and denitrifying mineralization, NO_2^- and N_2O are accumulated or consumed depending on the kinetic balance of the overall process (Kaplan & Wofsy 1985).

Studies on the distribution of NO_2^- , N_2O , and CH_4 in those lakes in which the hypolimnia are totally oxic are relatively rare, due to extremely low concentrations of these compounds. In this study, we applied special techniques for extracting them from lake waters, and precisely determined their concentration profiles. The results were compared with the cases in other lakes of similar setting, whereby possible common mechanisms underlying the observed profiles were discussed.

Materials and methods

We selected three sampling stations in the north basin of Lake Biwa: Stations A (90 m deep), B (73 m deep), and C (40 m deep), located in the north-west, central, and south-west regions of the basin, respectively. The survey was conducted twice (August 24 and September 16, 1993) at Station A, and three times (August 23, September 15 and November 26, 1993) at Stations B and C. The concentration of chlorophyll *a* in the euphotic zone was not so much different among the three stations ($2.2\text{--}5.5 \mu\text{g l}^{-1}$). For $\delta^{13}\text{C}$ of ΣCO_2 , additional samplings were done on October 8, 1993 and January 12 and March 11, 1994. Water samples were collected with a Van-Dorn sampler (3 l). For analyses of ΣCO_2 , $\delta^{13}\text{C}$ of ΣCO_2 , CH_4 and N_2O , sample water was packed in serum bottles (70 ml) with no air bubbles, 0.2 ml of saturated HgCl_2 solution was added as a preservative, and the samples were stored at room temperature until analyzed. Samples for oxygen analysis were packed in oxygen bottles (100 ml) and immediately fixed with MnCl_2 and alkaline-KI solutions. Samples for the other analyses were collected in polypropylene

bottles of appropriate volumes and transported to the laboratory on ice. Water temperature was determined *in situ* with a thermistor thermometer. pH was measured colorimetrically on board.

Chemical analyses were conducted as follows. Dissolved oxygen was determined by Winkler titration. NH_4^+ , NO_3^- , phosphate (PO_4^{3-}) and silicate ($\text{Si}(\text{OH})_4$) were determined by the methods of Bower & Holm-Hansen (1980), Scheiner (1974), Murphy & Riley (1962), and the molybdenum-yellow, respectively. NO_2^- was measured by the method of Wada & Hattori (1971a); briefly, NO_2^- in 1.0 l lake water was first reacted with sulfanilic acid and naphthylethylenediamine to form azo-dye, and the dye was once trapped on anion-exchange resin, then eluted with 15 ml of 60% (v:v) acetic acid, and quantified spectrophotometrically. A standard curve was prepared using the same concentration procedure. Analytical error is $\pm 5\%$. ΣCO_2 was determined with a dissolved-carbon analyzer (Shimadzu, TOC-500). $\delta^{13}\text{C}$ of ΣCO_2 was determined by headspace extraction in acid and GC/C/IRMS (Miyajima et al. 1995). $\delta^{13}\text{C}$ is defined as the relative deviation in isotope ratio r ($= {}^{13}\text{C}/{}^{12}\text{C}$) of a sample from the V-PDB standard:

$$\delta^{13}\text{C} = (r_{\text{sample}}/r_{\text{PDB}} - 1) \times 1000[\text{‰}].$$

The standard ${}^{13}\text{C}/{}^{12}\text{C}$ is 0.0112372. The analytical precision of this measurement is $\pm 0.3\text{‰}$ as $\delta^{13}\text{C}$. CH_4 was measured by headspace extraction; that is, a 5-ml portion of the sample water inside the serum bottle was replaced with helium to create a headspace, and after equilibrium between dissolved and headspace CH_4 was attained, the headspace CH_4 concentration was measured by FID-GC (Shimadzu, GC-14A). Dissolved CH_4 in original samples was calculated with an appropriate solubility constant. N_2O was determined by the method of Ueda et al. (1993); briefly, N_2O in 30 ml lake water was purged with helium and trapped on cold (0°C) molecular sieve 13X, then the trapped N_2O was eluted by heating (200°C) and introduced to an ECD-GC (Shimadzu, GC-8A). Analytical error in the CH_4 and N_2O analyses is $\pm 5\%$.

Results and discussion

Distribution of greenhouse gases and nitrite

Typical vertical profiles of some parameters in the water column at Station A are illustrated in Figure 1. It is convenient to divide the water column of Lake Biwa into four layers based on the distribution of O_2 : (i) *epilimnion*, in which O_2 (as well as water temperature) is uniformly distributed ($> 80\%$ O_2 saturation) due to mixing; (ii) *thermocline*, which is characterized by the oxygen

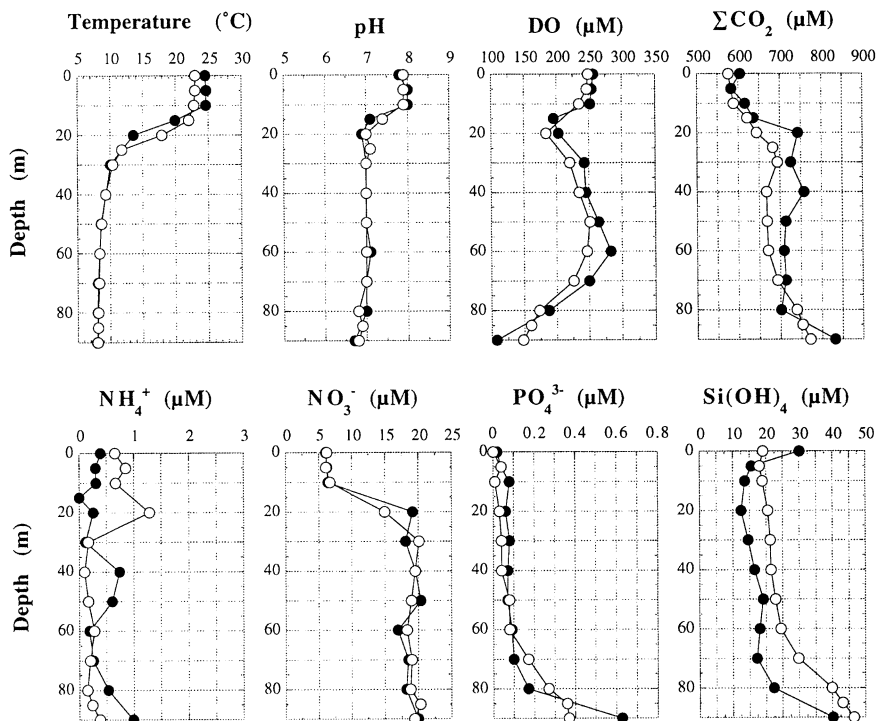


Figure 1. Vertical profiles of water temperature, pH, dissolved oxygen (DO), total dissolved inorganic carbon (ΣCO_2), ammonium (NH_4^+), nitrate (NO_3^-), dissolved inorganic phosphate (PO_4^{3-}), and monomeric silicate (Si(OH)_4) at Station A. Solid circles – 24 August 1993; open circles – 16 September 1993.

minimum (60–70% saturation); (iii) *upper hypolimnion*, in which dissolved oxygen is relatively high (65–80% saturation); (iv) *lower hypolimnion*, in which steep concentration gradient of O_2 appears towards the bottom (30–65% saturation). The O_2 minimum at the thermocline is thought to have been formed and maintained by respiration of plankton. The O_2 gradient observed in the lower hypolimnion is presumably due to O_2 consumption in the sediment. The concentration of ΣCO_2 increased with depth in the thermocline, indicating active mineralization of organic carbon in the hypolimnion. ΣCO_2 increased further with depth in the lower hypolimnion, reflecting mineralization within the sediment.

The concentration of NO_3^- increased from the epilimnion to the thermocline but did not increase further in the hypolimnion (Figure 1). Similar observations of NO_3^- profiles have been repeatedly reported by other researchers (Kawashima et al. 1976; Tezuka 1984; Miyajima 1992), and

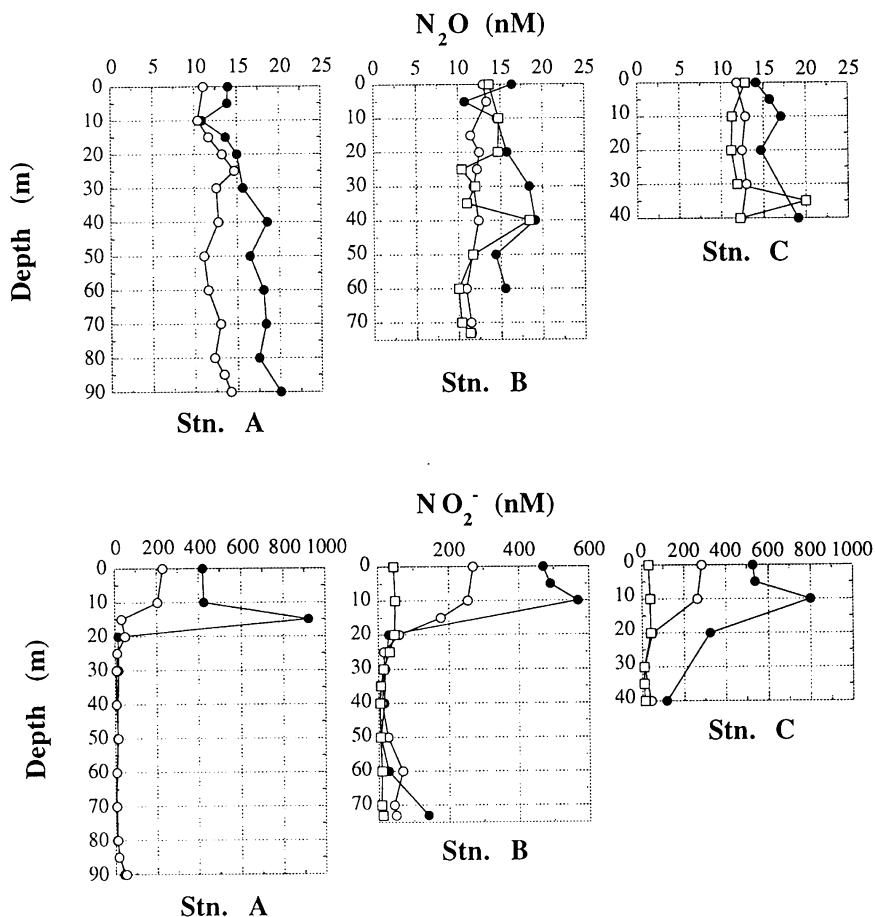


Figure 2. Vertical profiles of nitrous oxide (N_2O), nitrite (NO_2^-), methane (CH_4), and the stable carbon isotope ratio of dissolved inorganic carbon ($\Sigma\text{CO}_2 \delta^{13}\text{C}$) at three stations. Solid circles – 23–24 August 1993; open circles – 15–16 September 1993; open squares – 25 November 1993.

interpreted to suggest that net production of NO_3^- occurs rather uniformly in the hypolimnion but not in the sediment. In fact, although active nitrification occurs at the sediment/water interface, NO_3^- consumption by denitrification in the sediment offsets the production by nitrification (Miyajima 1994). In contrast, concentrations of PO_4^{3-} and $\text{Si}(\text{OH})_4$ did not increase in the thermocline but in the lower hypolimnion, which indicates that these nutrients were not regenerated in the water column but in the sediment. Tezuka (1990) ascribed the lack of PO_4^{3-} regeneration in the water column to characteristically low phosphorus contents in organic matters (mainly phytoplankton biomass) supplied to the hypolimnion of this lake. The regeneration of $\text{Si}(\text{OH})_4$ in

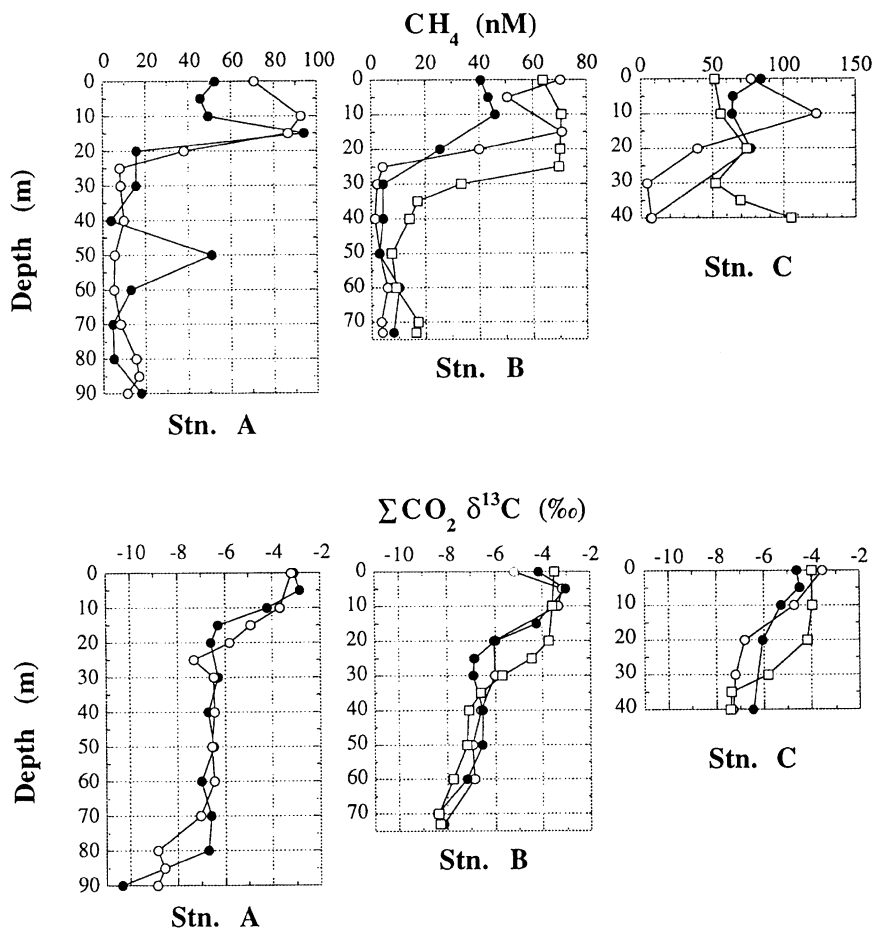


Figure 2. Continued.

the sediment might be partially due to the feeding activity of macrobenthos (Miyajima et al. 1995).

The nutrient dynamics described in the previous paragraphs illustrate the difference in the mode of mineralization between the hypolimnion and the sediment. One of the points of this study is to examine how hypolimnetic metabolism is reflected in the profiles of N_2O , NO_2^- , CH_4 , and $\delta^{13}\text{C}$ of ΣCO_2 . Three station profiles are shown in Figure 2. These data showed that concentration of N_2O did not vary vertically in any major way. Taking account of atmospheric concentration of N_2O (310 ppbv), the equilibrium concentration for air/water exchange at temperatures of the hypolimnion (8–10 °C, which also corresponds to surface water temperature during the

holomictic period) is 12–13 nM. Thus, the data indicates that accumulation of N_2O in the hypolimnion was generally small. The concentration of NO_2^- was relatively high in the epilimnion, and a concentration maximum was observed at the thermocline in August. NO_2^- was extremely low in the hypolimnion, though a small accumulation was observed in the lower hypolimnion at Station B.

The concentration ranges of N_2O and NO_2^- observed in this lake are similar to the cases of deep, oligotrophic basins, such as the Great Lakes (Lemon & Lemon 1981) and Lake Taupo (Vincent & Downes 1981), where O_2 is never depleted from the water column. In eutrophic lakes where the hypolimnion becomes anoxic, N_2O and NO_2^- are often accumulated substantially at the oxic/anoxic interface (Knowles et al. 1981). Although the interface usually appears at the sediment surface in Lake Biwa, no clear evidence was obtained for significant accumulation of these substances in the bottom waters. Studies in which the distribution of NO_2^- was precisely determined in oligotrophic lakes are rare. In the case of the North Pacific Ocean, a subsurface maximum of NO_2^- has been observed, and Wada & Hattori (1971b) ascribed its formation to NH_4^+ oxidation by nitrifiers. Although NO_2^- production due to NO_3^- reduction by primary producers was observed on addition of NO_3^- in the light, they excluded its contribution to the NO_2^- accumulation, because NO_3^- concentration *in situ* was much lower than NH_4^+ concentration. In the case of Lake Biwa, however, the reverse is the case; i.e., NO_3^- was the predominant species of inorganic nitrogen in the epilimnion (Figure 1). This suggests that NO_3^- reduction by primary producers was at least one of the major sources for the epilimnetic NO_2^- . The NO_2^- production by NH_4^+ oxidizers and lateral transport of NO_2^- produced in littoral region to the pelagic stations could also have been significant sources. The relative importance of these sources is to be evaluated in future studies.

The extremely low concentrations of N_2O and NO_2^- in the hypolimnion are somewhat surprising, when considering the active nitrification suggested by NO_3^- accumulation. N_2O yield in the NH_4^+ oxidation has been reported to be 0.2–10% relative to NO_2^- depending on oxygen tension (Goreau et al. 1980); at the O_2 concentration typical to the hypolimnion of Lake Biwa, N_2O accumulation of 0.2–0.5% vs. NO_2^- production would be expected. The low concentration of NO_2^- in the hypolimnion, on the other hand, indicates that the NH_4^+ oxidation was well coupled with the NO_2^- oxidation. Therefore, the NO_2^- production rate by NH_4^+ -oxidizers should be approximately equal to the NO_3^- accumulation rate. Miyajima (1992) reported that the NO_3^- accumulation rate in the hypolimnion at Station B was about $1.0 \mu\text{mol NO}_3^- \text{ l}^{-1} \text{ month}^{-1}$. Thus, N_2O production of 2–5 $\text{nmol l}^{-1} \text{ month}^{-1}$ is expected. As the water column is usually stratified after April, at least 10 nM of N_2O should

have been accumulated in excess of the equilibrium level by the survey time. This was not the case, however: from our data, the $\text{N}_2\text{O}/\text{NO}_2^-$ ratio during NH_4^+ oxidation must have been several times lower in Lake Biwa than in the experiments of Goreau et al. (1980). The low $\text{N}_2\text{O}/\text{NO}_2^-$ ratio might be due to a very low nitrification rate in this natural system as opposed to the incubation system of Goreau et al. (1980), because the ratio is presumably sensitive to kinetic conditions of the enzymes involved.

It has been observed in open ocean profiles that the concentration of both N_2O and NO_3^- is strongly correlated with the apparent oxygen utilization (AOU; Yoshinari 1976; Elkins et al. 1978). In the case of Lake Biwa, the vertical concentration profile of oxygen is strongly affected by sediment respiration. The stoichiometry between oxygen consumption and net nitrate production is different between the water-column and sedimentary processes, and as noted above, nitrate is absorbed by sediment from the overlying water. Consequently, the correlation between AOU and NO_3^- is weak in the hypolimnion of this lake. The lack of accumulation of N_2O in the hypolimnion suggests that the same mechanism also controls the behavior of N_2O . It is thus expected that the sediment functions as a sink of N_2O , supposedly through denitrification.

Like NO_2^- , the concentration of CH_4 was higher in the epilimnion and the thermocline than in the hypolimnion (Figure 2), although monthly variations were different between CH_4 and NO_2^- . Concentrations of the epilimnetic CH_4 were two orders of magnitudes higher than the equilibrium concentration with respect to exchange with the atmosphere. In addition, CH_4 was also accumulated in some cases in the lower hypolimnion. The mode of CH_4 distribution in this lake is very similar to the case of Lake Constance (Schmidt & Conrad 1993). Since CH_4 is produced only under strictly anaerobic conditions and readily oxidized by bacteria in oxic waters, accumulation of CH_4 in oxic waters should be a result of continuous supply of CH_4 from some adjacent anaerobic regions or anaerobic microsites within the oxic waters. Steep gradients of CH_4 observed in the thermocline and sometimes in the lower hypolimnion indicate active oxidation of CH_4 in these layers. The origin of CH_4 accumulated in the lower hypolimnion is thought to have been the anaerobic layer of the sediment. As the thickness of the oxic layer of sediment is small (usually a few millimeters), it is not surprising that a part of CH_4 produced in the anaerobic layer diffuses out of sediment without being oxidized. Likewise, at least a part of the CH_4 accumulated in the epilimnion is thought to have been produced in the anaerobic layer of littoral sediment and transported by lateral water flows to the pelagic stations, as pointed out by Schmidt & Conrad (1993). In fact, Yamada (unpublished data, 1995) observed that CH_4 concentration in some littoral waters was at least one

order of magnitude higher than in pelagic epilimnetic waters. It is known that high O_2 and low inorganic nitrogen concentrations, which are common in the epilimnion, antagonize the growth of methanotrophic bacteria (Rudd & Hamilton 1975; Rudd et al. 1976). This might be the reason that a relatively large amount of CH_4 was accumulated in this layer. Another possible source of the epilimnetic CH_4 is anaerobic microsites in the gut of zooplankton. To evaluate this possibility, we conducted a preliminary incubation experiment to demonstrate CH_4 emission from crustacean zooplankters in this lake, but the result was negative. Estimation of the turnover period of CH_4 is still needed, however, to elucidate relative strengths of these two possible sources of the epilimnetic CH_4 .

$\delta^{13}C$ of ΣCO_2 and the origin of hypolimnetic ΣCO_2

$\delta^{13}C$ of ΣCO_2 became more negative with increasing depth, and showed a similar pattern to those of the ΣCO_2 concentration; i.e., major gradients were observed in both the thermocline and the lower hypolimnion (Figure 2). In general, the $\delta^{13}C$ of ΣCO_2 is determined by both external and internal factors (McKenzie 1984). In the hypolimnion, ΣCO_2 contained in lake water at the onset of the stratification gives the background value of $\delta^{13}C$, and since then the value is gradually modified by the internal process, i.e., mineralization. Mineralization provides CO_2 of lower $\delta^{13}C$ than the background value, because the organic matter supplied to the hypolimnion is already depleted in $\delta^{13}C$ (−15 to −30‰). $\delta^{13}C$ of ΣCO_2 in the epilimnion is affected by exchange of CO_2 with the atmosphere and the photosynthetic CO_2 assimilation, both of which in effect raise the $\delta^{13}C$ of ΣCO_2 remaining in lake water. At equilibrium with respect to the exchange with atmospheric CO_2 ($\delta^{13}C = -7.8‰$), $\delta^{13}C$ of dissolved HCO_3^- will be about 0‰ (Mook et al. 1974). As a result, a steep gradient in $\delta^{13}C$ was developed in the thermocline. The further decrease in $\delta^{13}C$ in the lower hypolimnion, on the other hand, indicated accumulation of ^{13}C -depleted ΣCO_2 presumably produced by sediment respiration. The degree of decrease in $\delta^{13}C$ in the lower hypolimnion was apparently enhanced with increasing length of the water column (Figure 2). This depth dependency might have been resulted from difference in accumulation rate of organic matter to the sediment; i.e., deeper sediment might have received more organic matter due to concave basin morphology and produced more CO_2 by mineralization. Alternatively, at the shallower stations, lateral water flows driven by internal waves might have diluted the effect of sediment respiration on $\delta^{13}C$ in the overlying water.

Figure 3 shows seasonal variations in $\delta^{13}C$ of ΣCO_2 at Station B from August 1993 through March 1994. $\delta^{13}C$ in the epilimnion irregularly changed, presumably due to temporal variations in photosynthetic activity and the river

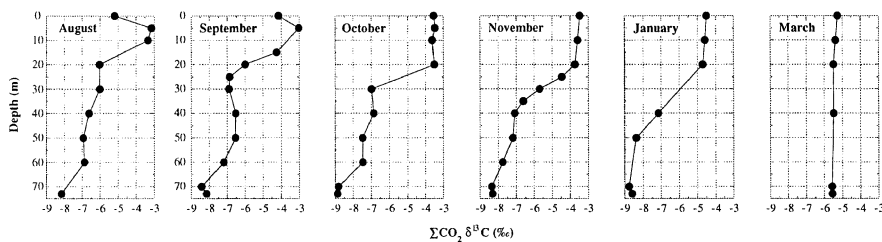


Figure 3. Seasonal changes in the vertical distribution of $\delta^{13}\text{C}$ of ΣCO_2 at Station B from August 1993 through March 1994.

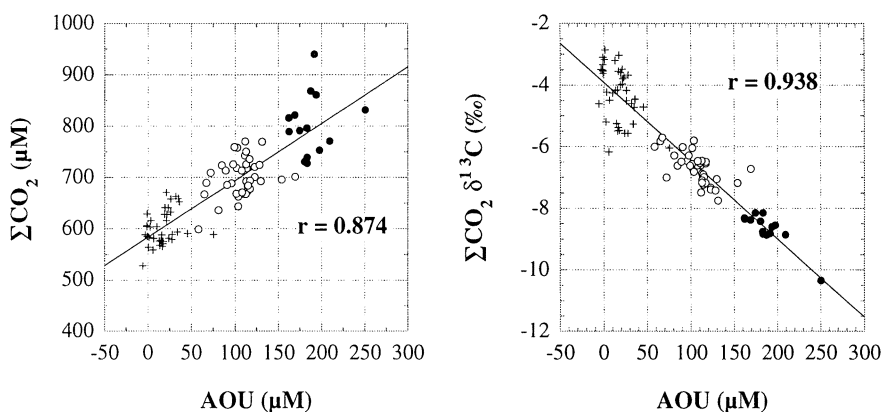


Figure 4. Linear regressions of the concentration (left) and the $\delta^{13}\text{C}$ (right) of ΣCO_2 against the apparent oxygen utilization (AOU). AOU is defined as the difference between the saturated oxygen concentration at the *in-situ* temperature and the observed oxygen concentration. Data from the three stations are pooled. Crosses – epilimnion and thermocline; open circles – upper hypolimnion; solid circles – lower hypolimnion.

water input. In contrast, $\delta^{13}\text{C}$ in the hypolimnion generally became more negative as time went on, especially at 30–60 m depth. This trend was not as clear in the deepest layers. Correspondingly, concentrations of O_2 and ΣCO_2 in the hypolimnion gradually decreased and increased, respectively (data not shown). Good correlations of ΣCO_2 and $\delta^{13}\text{C}$ of ΣCO_2 to the apparent oxygen utilization were observed (Figure 4). Notably, the correlation was stronger for the $\delta^{13}\text{C}$ of ΣCO_2 than for the ΣCO_2 concentration.

One of the aims of this study is to examine the possibility to elucidate, using $\delta^{13}\text{C}$ of accumulated ΣCO_2 , the origin of organic matter that is actually decomposed in the hypolimnion. To approach this problem, we tested two strategies. Firstly, we supposed that the $\delta^{13}\text{C}$ of organic matter mineralized

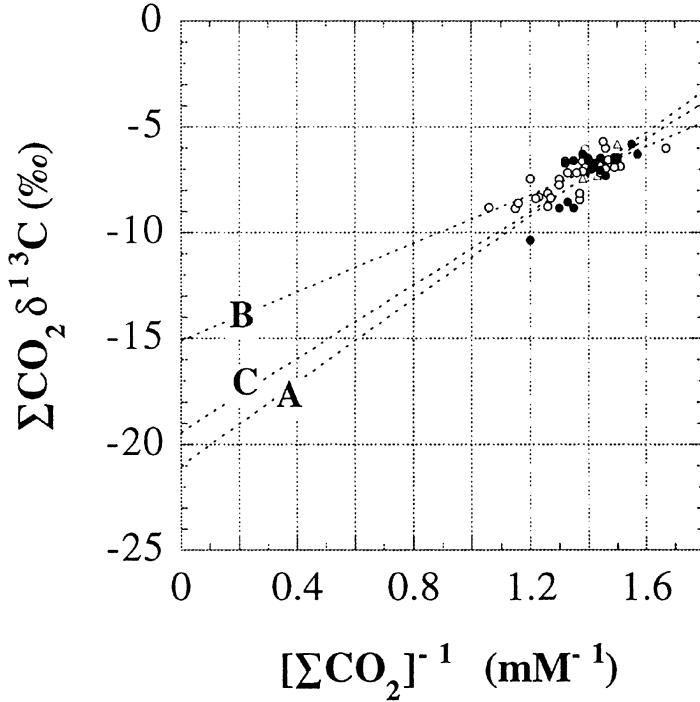


Figure 5. Linear regression between $\delta^{13}\text{C}$ of ΣCO_2 and the reverse of the concentration of ΣCO_2 . $r^2 = 0.595$ (Station A), 0.633 (Station B), 0.522 (Station C). Solid circles – Station A; open circles – Station B; open triangles – Station C.

at each station was time-independent. $\delta^{13}\text{C}$ of ΣCO_2 in the hypolimnion will be given as:

$$\delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{bg}} \cdot [\Sigma\text{CO}_2]_{\text{bg}} + \delta^{13}\text{C}_{\text{m}} \cdot ([\Sigma\text{CO}_2] - [\Sigma\text{CO}_2]_{\text{bg}})) / [\Sigma\text{CO}_2],$$

where $\delta^{13}\text{C}_{\text{bg}}$ and $\delta^{13}\text{C}_{\text{m}}$ are the background $\delta^{13}\text{C}$ (i.e., $\delta^{13}\text{C}$ of ΣCO_2 at the onset of stratification) and $\delta^{13}\text{C}$ of mineralized carbon, respectively, and $[\Sigma\text{CO}_2]$ and $[\Sigma\text{CO}_2]_{\text{bg}}$ are ΣCO_2 concentrations at the sampling time and the onset of the stratification, respectively. In this assumption, $\delta^{13}\text{C}$ of ΣCO_2 will linearly depend on the reverse of the ΣCO_2 concentration. In Figure 5, we plotted $\delta^{13}\text{C}$ against $[\Sigma\text{CO}_2]^{-1}$. The $\delta^{13}\text{C}$ of CO_2 derived from mineralization ($\delta^{13}\text{C}_{\text{m}}$) is then given as intersections between the regression lines and the ordinate. This value was calculated to be -21.0 , -15.1 and -19.5‰ for Stations A, B and C, respectively. However, the correlation between $\delta^{13}\text{C}$ of ΣCO_2 and $[\Sigma\text{CO}_2]^{-1}$ was not so strong, which might indicate that $\delta^{13}\text{C}_{\text{m}}$ was not time-independent.

Table 2. $\delta^{13}\text{C}_m$ ($\delta^{13}\text{C}$ of the organic carbon mineralized in the hypolimnion) calculated by an isotope mass-balance model (see text).

Site	$\delta^{13}\text{C}_m$ (‰, \pm s.d.)	Sample number
Station A, upper hypolimnion	-12.4 ± 2.3	15
Station A, lower hypolimnion	-20.0 ± 1.2	4
Station B, upper hypolimnion	-13.1 ± 3.0	20
Station B, lower hypolimnion	-16.3 ± 1.7	10
Station C	-14.0 ± 2.8	7

Secondly, using the above equation, we can calculate the $\delta^{13}\text{C}_m$ value independently from each datum of $\delta^{13}\text{C}$ of ΣCO_2 , provided $\delta^{13}\text{C}_{\text{bg}}$ and $[\Sigma\text{CO}_2]_{\text{bg}}$ are known. This estimate for $\delta^{13}\text{C}_m$ represents the average $\delta^{13}\text{C}$ of organic matter mineralized between the onset of the stratification and the sampling time. Here, we tentatively assumed that concentration and $\delta^{13}\text{C}$ of ΣCO_2 in the holomictic period (March 1994 in Figure 3) represented the background values $[\text{CO}_2]_{\text{bg}}$ and $\delta^{13}\text{C}_{\text{bg}}$. The $\delta^{13}\text{C}_m$ thus estimated is shown in Table 2. Most estimates for the upper and lower hypolimnia are found in the ranges between -7 and -17‰ and between -15 and -20‰ , respectively. In reality, ΣCO_2 in a given layer of the water column is gradually mixed by diffusion with ΣCO_2 in the adjacent layers, and this process also modifies $\delta^{13}\text{C}$ of ΣCO_2 , especially where the vertical gradient of $\delta^{13}\text{C}$ is steep. At some depths just below the thermocline, $\delta^{13}\text{C}$ of ΣCO_2 would increase by mixing with ΣCO_2 of a higher $\delta^{13}\text{C}$ diffusing from the epilimnion. Thus, it should be borne in mind that the relatively high estimates for $\delta^{13}\text{C}_m$ for the upper hypolimnia in Table 2 would have been partially due to the effect of mixing with epilimnetic ΣCO_2 .

It is interesting to compare the estimated $\delta^{13}\text{C}_m$ with $\delta^{13}\text{C}$ of possible sources of the hypolimnetic ΣCO_2 , e.g., plankton biomass, benthic algae in littoral zone, suspended organic detritus, sediment organic matter and organic matter of terrestrial origin (Fry & Sherr 1988). Measured $\delta^{13}\text{C}$ values of particulate organic matter and sediment are listed in Table 3. Particulate matters of the size fractions $40\text{--}70$ and $70\text{--}150\text{ }\mu\text{m}$ consisted mainly of phytoplankton ($>90\%$ in volume), and their $\delta^{13}\text{C}$, as well as $\delta^{13}\text{C}$ of littoral epilithic algae (-16.5‰), was similar to $\delta^{13}\text{C}_m$ calculated above. $\delta^{13}\text{C}$ of the size fraction $2.7\text{--}20\text{ }\mu\text{m}$, which consisted mainly of detritus, and $\delta^{13}\text{C}$ of sediment organic matter deviated negatively from the estimated $\delta^{13}\text{C}_m$ values. $\delta^{13}\text{C}$ of terrestrial organic matter (typically -27 to -30‰) is far more negative. $\delta^{13}\text{C}$ of the $20\text{--}40\text{ }\mu\text{m}$ fraction, which consisted mainly small cyanobacteria and detritus (up to 90 and 40% in volume, respectively), was intermediate between those of phytoplankton and sediment. These data strongly suggest that ΣCO_2 accu-

Table 3. $\delta^{13}\text{C}$ of particulate organic carbon collected from the epilimnion and sediment of the north basin of Lake Biwa during the stratification periods (April–January) of 1993 and 1994 (Yamada, unpublished data).

Size fraction (μm)	$\delta^{13}\text{C}$ (‰, \pm s.d.)	Sample number
70–150	-17.7 ± 3.7	13
40–70	-19.8 ± 3.4	15
20–40	-22.2 ± 2.7	16
2.7–20	-23.9 ± 2.0	13
Sediment	-24.2 ± 1.5	3

culated in the hypolimnion was principally derived from decomposition of phytoplanktonic and/or benthic algae, i.e., autochthonously produced organic matters.

The $\delta^{13}\text{C}_m$ estimated for bottom layers at Station A was deviated negatively from the typical $\delta^{13}\text{C}$ of phytoplankton, which might be an indication of contribution from sediment or detrital organic matter. An additional factor that affects the bottom-layer $\delta^{13}\text{C}_m$ is methanogenesis in anaerobic sediments (Oana & Deevey 1960). CO_2 -reducing methanogenesis proceeds with a large isotope fractionation (Oremland 1988), with isotopically heavier CO_2 being left behind. CH_4 produced is isotopically lighter than average sediment organic matter. If this light CH_4 diffuses to oxic hypolimnion and is oxidized into CO_2 , $\delta^{13}\text{C}$ of hypolimnetic ΣCO_2 becomes more negative. Although the heavy CO_2 left in anaerobic sediment gradually diffuses to the oxic hypolimnion and partially offsets the effect of CH_4 oxidation on $\delta^{13}\text{C}$ of ΣCO_2 , difference in $\delta^{13}\text{C}$ remains in ΣCO_2 between the oxic and methanogenic layers, as far as both the production and the oxidation of CH_4 continue at a measurable rate. The negatively deviated bottom-layer $\delta^{13}\text{C}_m$ might thus be indicative of CH_4 oxidation. However, the relative importance of the CH_4 oxidation would be small, as the concentration of CH_4 in the hypolimnion was 4–5 orders of magnitude lower than that of ΣCO_2 (Figures 1, 2).

The predominance of the autochthonous organic matter as substrate for the hypolimnetic metabolism may be explained by the following two factors. First, the input of autochthonous organic matter to the hypolimnion might be absolutely greater than that from allochthonous organic matter. Although the input of terrestrial organic matter to the lake would be large, most of it is presumably mineralized in the littoral regions. Second, the terrestrial organic matter that is transported to the hypolimnion without being mineralized in the littoral regions would be resistant against mineralization as compared to fresh autochthonous organic matter (Hedges et al. 1988). Thus, the former would be

eventually flushed out by water exchange or permanently incorporated in the sediment without substantial mineralization. The $\delta^{13}\text{C}$ of sediment organic matter that was significantly lighter than plankton (Table 3) suggests selective preservation of terrestrial organic matter in the sediment (Meyers 1994).

Summary

In Lake Biwa, CH_4 was accumulated in the epilimnion up to two orders of magnitudes higher than the equilibrium concentration, which suggests that CH_4 emission to the atmosphere was substantial. CH_4 was presumably produced in littoral sediment and transported to pelagic regions by lateral flows. The relative depletion of inorganic nitrogen and the abundance of O_2 in the epilimnion, both of which suppress the growth of CH_4 -oxidizing bacteria, might enhance the longevity of CH_4 . In contrast, CH_4 seemed to be effectively oxidized in the hypolimnion. On the other hand, the concentration of N_2O was always near the equilibrium level for air/water exchange. The relatively small accumulation of N_2O as compared to NO_3^- in the hypolimnion might be due to the high O_2 concentration and the relatively slow nitrification rate, which could decrease the N_2O yield during NH_4^+ oxidation. Both the over-saturated CH_4 and near-equilibrium N_2O concentrations in the epilimnion seem common features to deep, oligotrophic lakes with well-oxygenated hypolimnia; underlying mechanisms which maintain such distributions may well be common. Unlike the cases of CH_4 and N_2O , studies in which the concentration of NO_2^- has been precisely determined in oligotrophic lakes are rare. This study provided an example that NO_2^- was accumulated in the epilimnion. We tentatively concluded that this accumulation was the result of NO_3^- reduction by primary producers.

The $\delta^{13}\text{C}$ of ΣCO_2 was well correlated to the apparent oxygen utilization, indicating that $\delta^{13}\text{C}$ of organic matter actually mineralized in the hypolimnion was fairly constant. $\delta^{13}\text{C}$ of the mineralized carbon, which was estimated using isotope mass balance, suggested that the major organic substrate for the hypolimnetic metabolism was of autochthonous origin. This further implies that the trophic status of Lake Biwa will be highly sensitive to changes in nutrient loading which readily activates autochthonous primary production, rather than changes in organic matter loading. This point makes a major difference from dystrophic lakes, where the input of organic matter principally controls the lake metabolism. In addition, for controlling CH_4 emissions, protection of littoral sediments from eutrophication will be effective, as far as they are the major source of the epilimnetic CH_4 .

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