

Role of dissolved organic matter in hypolimnetic mineralization of carbon and nitrogen in a large, monomictic lake

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Abstract

We tested the hypothesis that dissolved organic matter (DOM) is delivered to deep layers by convective mixing in winter, where it contributes to the mineralization of C, N, and P in the oxygenated hypolimnion of large (surface area 674 km², maximum depth 104 m), monomictic Lake Biwa. Basin-scale, seasonal measurements of DOM concentrations revealed that dissolved organic carbon (DOC) and nitrogen (DON) accumulated in the epilimnion during warm seasons and were redistributed into the deeper layer during winter overturn. Hypolimnetic DOC and DON decreased in concentration over the stratification period, indicating mineralization; the contributions of DOM to the total mineralization of C and N were 8% and 30%, respectively. Higher contribution of N relative to C suggests that the N-rich DOM was mineralized at depth. We failed to detect a significant contribution of dissolved organic phosphorus to P mineralization in the hypolimnion, which could be explained by substantial depletion in P relative to C and N of DOM; C:P and N:P ratios were 1,978 and 147, respectively. The data suggest that convective mixing in this monomictic basin delivers DOM to hypolimnetic depths, where it is mineralized during subsequent periods of stratification.

Hypolimnetic mineralization of organic carbon and nutrients in stratified waterbodies of large lakes represents an important biogeochemical process that affects the magnitude and spatiotemporal patterns of hypolimnetic oxygen depletion and the internal loading of N and P (Cornett and Rigler 1979; Wetzel 2001). Traditionally, studies have considered that this process is primarily linked to sedimentation and the subsequent mineralization of particulate organic matter (POM; Hutchinson 1938). Little is known as yet about the role of dissolved organic matter (DOM) in the regulation of hypolimnetic metabolism of large lakes, even though DOM often represents the largest organic matter pool in lake waters (Wetzel 2001; Biddanda and Cotner 2002). Recently, McManus et al. (2003) found that hypolimnetic oxygen depletion far exceeded the value that could be accounted for by sinking fluxes of particulate organic carbon (POC) in Lake Superior, suggesting that the oxidation of dissolved organic carbon (DOC) might explain the major fraction of hypolimnetic oxygen consumption. However, rigorous testing of this hypothesis has been hampered by a paucity of data on the dynamics and consumption of DOC in the hypolimnion of large lakes.

Coherent examinations of seasonal, horizontal, and depth-dependent variations of DOM in lake waters could provide useful information regarding the contribution of DOM to hypolimnetic metabolism. In marine environments, investi-

gators have examined seasonal variations of DOC profiles to estimate the vertical flux of DOC to the mesopelagic zone (Copin-Montégut and Avril 1993; Carlson et al. 1994). These studies have demonstrated that vertical mixing in winter introduces DOM into the mesopelagic zone where DOC is mineralized during the stratification period, suggesting that the DOC flux driven by this mechanism (the DOM pump) accounts for a significant fraction of the total downward flux of organic carbon (Copin-Montégut and Avril 1993; Carlson et al. 1994).

Studies have also suggested that the downward transport of DOC is coupled with the transport of dissolved organic nitrogen (DON) and phosphorus (DOP), a process regulated by the C:N:P ratio and the stoichiometry of the decomposition of DOM (Hopkinson and Vallino 2005). Because the C:N:P ratios of bulk DOM are typically high compared with those of POM, one would expect that the DOM pump more efficiently delivers C relative to N and P (Hopkinson and Vallino 2005), yet significant amounts of nutrients could be transported depending on the lability of DON and DOP. Whether or not the emerging paradigm just described regarding the DOM pump in marine systems applies to large, monomictic freshwater lakes has yet to be examined.

Our objective here was to examine, on the basis of extensive measurements of basin-scale, seasonal variations in DOM, whether DOC, DON, and DOP contribute to the mineralization of C, N, and P in the hypolimnion of Lake Biwa, a large (surface area 674 km²) tectonic lake located in the central part of Honshu Island, Japan (Horie 1984). Our study was conducted in the monomictic North basin (water volume 28.1 km³; water residence time 5.5 yr), which is deep (maximum depth 104 m), mesotrophic (annual mean levels of total phosphorus, chlorophyll *a*, and primary production are 0.2 μmol L⁻¹, 2 μg L⁻¹, and 104 mmol C m⁻² d⁻¹, respectively; Yoshimizu et al. 2001, Nishimura et al. 2005), and oxygenated (the annual minimum concentration of hypolimnetic dissolved oxygen (DO) concentration is ~4 mg L⁻¹),

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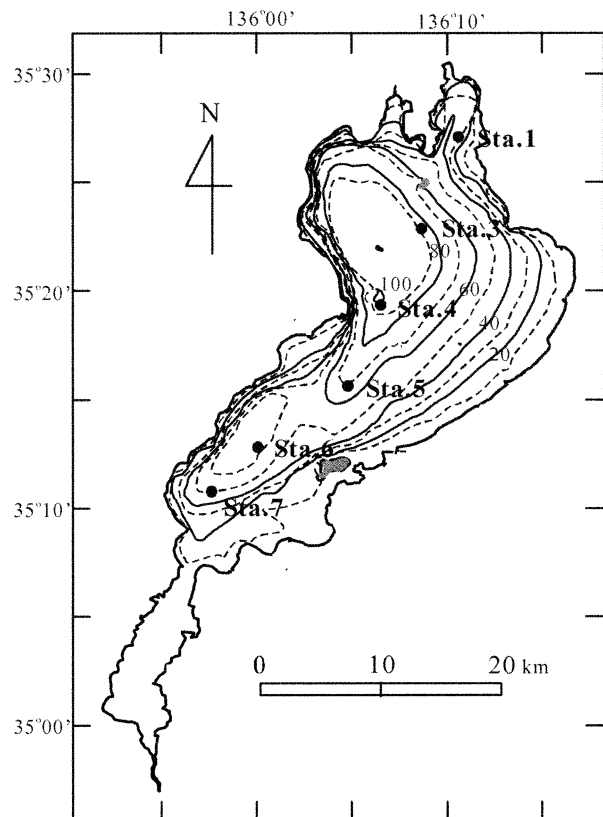


Fig. 1. Lake Biwa and sampling stations. Depth is shown in meters.

providing a useful test of the hypothesis that the DOM pump operates in large freshwater lakes.

Materials and methods

Thermal regime of the water column in the study area—Lake Biwa is a warm monomictic lake with surface water temperatures ranging from $7.8^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ in March to $27.6^{\circ}\text{C} \pm 2.3^{\circ}\text{C}$ in August (average \pm SD for the period between 1990 and 2000; <http://www.ecology.kyoto-u.ac.jp/biwako/teikan/index.htm>). Winter cooling combined with strong seasonal wind promotes vertical water mixing, allowing the whole water column to be included in the circulation between late January and March (overturn period). Surface water temperature starts to increase in April to establish thermal stratification (Horie 1984).

Water sampling—Bimonthly samplings were carried out between June 2001 and June 2002 at six transect stations deployed along the major axis of the lake (Fig. 1). Because of rough weather, Stas. 1 and 5 were not visited in December 2001. Additional samplings were carried out at selected stations (Stas. 4 and 6) until February 2003 with a bimonthly interval and in October 2003. Each sampling was completed within 2 or 3 days to obtain quasi-synoptic data. The hydrographic structure was determined with a CTD probe (SBE-911plus, Sea Bird Electronics Sealogger), and vertical sampling was conducted at predetermined depths. We used

5-liter Niskin-X bottles (General Oceanics Miami) for water sampling. The water samples near the bottom (1, 3, and 5 m above the bottom) were collected with the use of three sampling bottles sequentially attached to the wire at appropriate intervals. An echo sounder was used to help determine the position of the bottle for sampling at 1 m above the bottom. Actual sampling depths were ascertained with depth loggers attached to the sampling bottles. Before each survey, sampling bottles were cleaned by detergent followed by soaking with 0.6 mol L^{-1} hydrochloric acid and then vigorously rinsed with Milli-Q water (Milli-Q A10 Gradient, Millipore). Sample waters for the measurement of dissolved constituents, except for dissolved oxygen, were gravity filtered through GF/F glass fiber filters (Whatman, acid-rinsed and precombusted) with in-line filtration manifolds (Advantec Co.) directly connected to the outlet of the Niskin bottles.

For the measurement of DOC, the filtrate was dispensed into precombusted glass vials and sealed with acid-washed Teflon-faced silicone septa. These samples were acidified (2 mmol L^{-1} HCl) and stored in a refrigerator until analysis (principally within 100 h). For the measurement of other dissolved constituents, the filtrate was contained in acid-washed polyethylene bottles and stored at -20°C until analysis. During sampling, plastic gloves were worn and care was taken to minimize contamination. Contamination associated with sampling was examined by measuring DOC concentrations in Milli-Q water that had been in Niskin bottles and then filtered as just described. Results showed that DOC contamination was undetectable ($<1 \mu\text{mol L}^{-1}$). Water samples for the measurement of particulate constituents were filtered through acid-rinsed, precombusted GF/F glass fiber filters (Whatman), and the filters were stored frozen until analysis.

Chemical analyses—DOC concentration was determined by high-temperature catalytic oxidation with a TOC-5000A (Shimadzu) with autosampler injection after being purged with the CO_2 free carrier gas for 10 min at 105 mL min^{-1} . The high-sensitivity catalyst (Shimadzu) was conditioned by repeated injection (Benner and Strom 1993). Instrumental and Milli-Q water blanks were typically $2.1\text{--}3.2$ and $2.7\text{--}5.4 \mu\text{mol L}^{-1}$, respectively, accounting for $<10\%$ of the minimum DOC concentration in our water samples. Standards were made in the range $0\text{--}166 \mu\text{mol L}^{-1}$ with solutions of potassium hydrogen phthalate. The analytical precision (C.V.) of the replicate measurements ($n = 3\text{--}5$) was $<2\%$.

Nitrate, nitrite, and soluble reactive phosphorus (SRP) were measured spectrophotometrically with an autoanalyzer (AACS II, Bran + Luebbe) according to the manufacturer's manual. Ammonium was analyzed fluorometrically after the derivatization with orthophthaldialdehyde (Holmes et al. 1999). Total dissolved nitrogen (TDN) and phosphorus (TDP) were simultaneously measured by the wet oxidation method of Pujo-Pay and Raimbault (1994). Recovery of glycine after this oxidation was 95%. Analytical precision (C.V.) was 0.7% for TDN and 2.8% for TDP. Concentrations of dissolved organic nitrogen (DON) were estimated as differences between TDN and total DIN (nitrate + nitrite + ammonium), and those of DOP were estimated as differences between TDP and SRP. Concentrations of particulate car-

bon and nitrogen were measured with a CHN analyzer (PE-2400II, Perkin Elmer), whereas particulate phosphorus was determined as SRP after wet oxidation (Pujo-Pay and Raimbault 1994). Although these analyses do not discriminate inorganic and organic forms of particulate matter, we interpret these data as representing the concentrations of POC and particulate organic nitrogen (PON) and phosphorus (POP); we could ignore the possibility that calcium carbonate composes a large fraction of particulate carbon because particulate calcium concentrations are typically low in the area studied ($<1 \mu\text{mol L}^{-1}$; Mito et al. 2002). Dissolved oxygen concentration was determined by the Winkler method.

Results

Spatiotemporal variations and the whole-basin inventories of organic C, N, and P—About 600 samples obtained from 48 hydrocasts conducted between 2001 and 2003 were analyzed to characterize seasonal, horizontal, and depth-dependent variations of C, N, and P inventories over the whole north basin of Lake Biwa. Results revealed the maxima of DOC, DON, and DOP in the upper 15–20-m layer between June and October, suggesting net production of DOM in the

euphotic zone (typical examples shown in Figs. 2, 3). Concentrations of DOC (mean \pm SE, $110 \pm 9.2 \mu\text{mol L}^{-1}$, $n = 161$) and DON ($8.35 \pm 1.28 \mu\text{mol L}^{-1}$, $n = 161$) exceeded those of particulate counterparts (POC = $32.7 \pm 10.0 \mu\text{mol L}^{-1}$, $n = 161$; PON = $3.60 \pm 1.25 \mu\text{mol L}^{-1}$, $n = 161$), whereas POP ($0.130 \pm 0.044 \mu\text{mol L}^{-1}$, $n = 161$) generally exceeded DOP ($0.073 \pm 0.027 \mu\text{mol L}^{-1}$, $n = 155$) in the epilimnion. On occasion, we found a substantial accumulation of POC, PON, and POP near the bottom (Figs. 2, 3), indicating the development of a benthic nepheloid layer (defined in this study as a layer near the bottom in which concentration of POC exceeded $15 \mu\text{mol L}^{-1}$). Generally, we detected no significant increase in DOC, DON, and DOP within the benthic nepheloid layer.

As expected, variations in concentrations of DOC and DON over depth were less pronounced in February (overturn period) than in other months. The concentrations of DOC and DON in February tended to be higher than the hypolimnetic concentrations during the stratification period (April–December; Fig. 3). This pattern is most clear by comparing two February (2001 and 2002) and three October (2001, 2002, and 2003) profiles obtained at Stas. 4 and 6 (Fig. 4). For both DOC and DON, the average hypolimnetic concentrations in October for three consecutive years were signif-

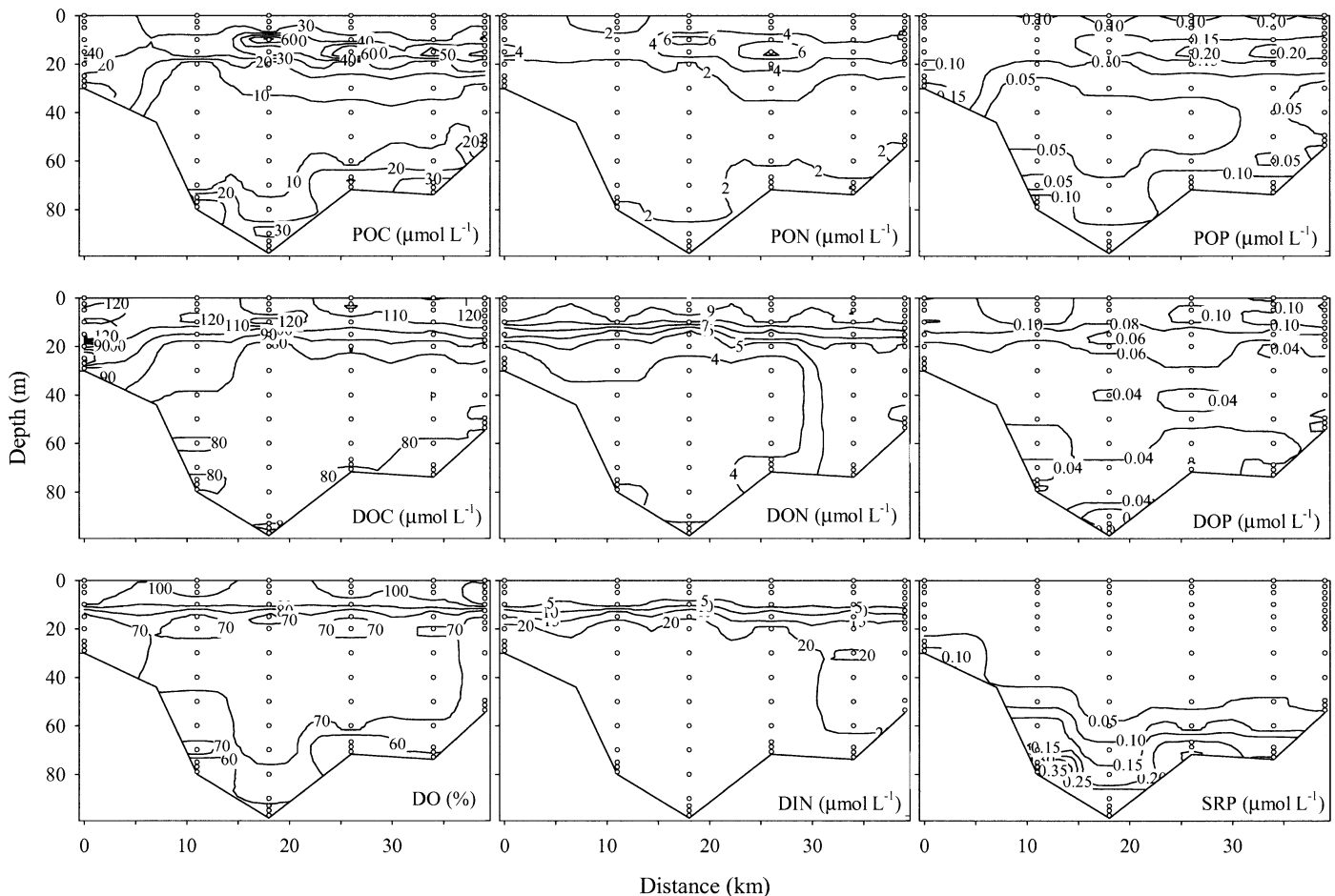


Fig. 2. Basin-scale distribution patterns of particulate and dissolved forms of C, N, and P; inorganic nutrients (SRP and DIN); and dissolved oxygen (DO) during the stratification period (August 2001). The x -axis indicates the distance (km) from Sta. 1.

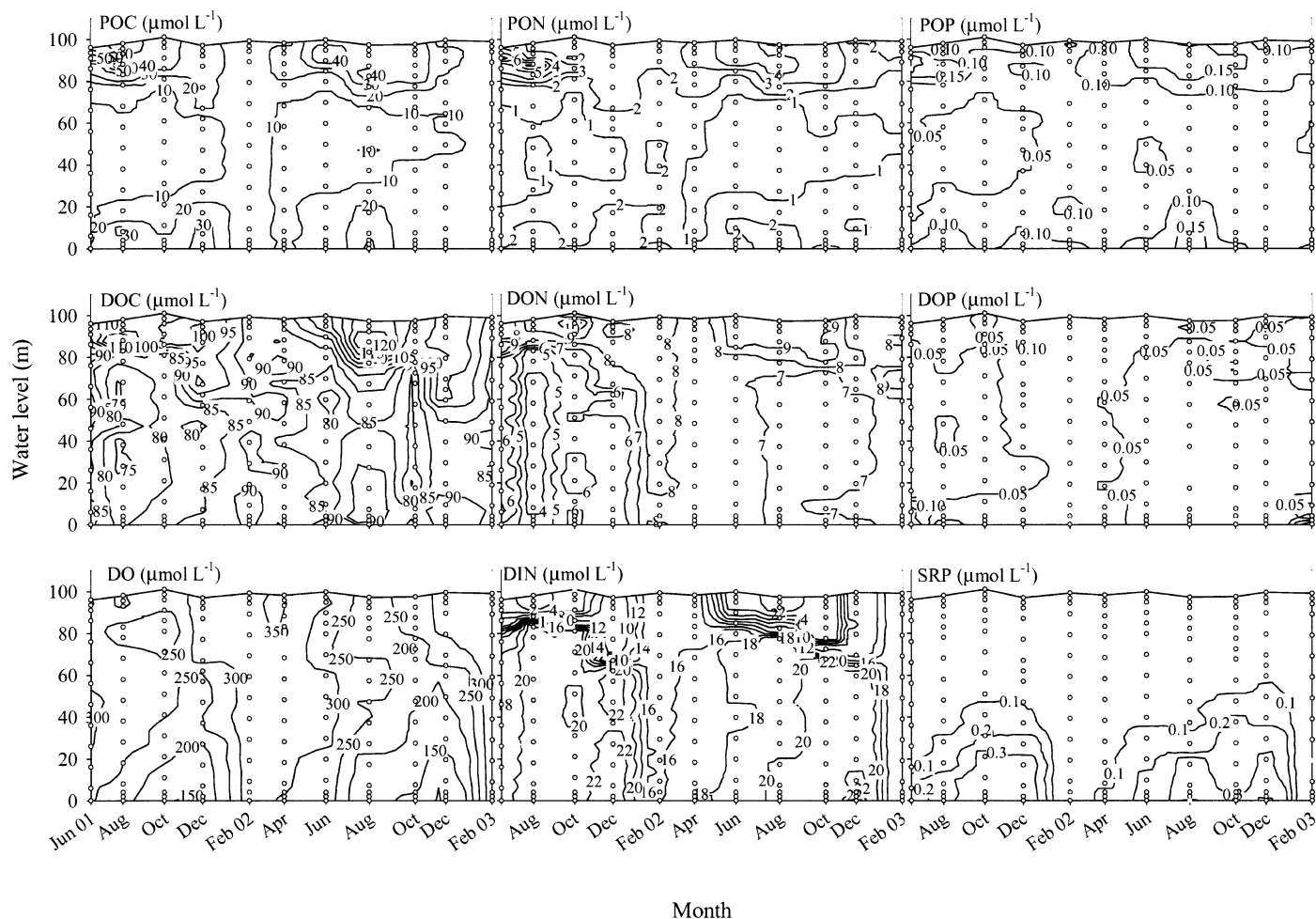


Fig. 3. Seasonal changes in vertical distributions of particulate and dissolved forms of C, N, and P; inorganic nutrients (SRP and DIN); and dissolved oxygen (DO) at Sta. 4.

icantly (Student's *t*-test, $p < 0.001$) lower than the average concentrations in February. The differences of hypolimnetic DOC and DON concentrations between February and October were $9.13 \mu\text{mol L}^{-1}$ (range Sta. 4–Sta. 6, 8.55–9.71) and $2.19 \mu\text{mol L}^{-1}$ (range Sta. 4–Sta. 6, 2.14–2.24), respectively. For DOP, the difference in concentrations between February and October profiles were not detected because of high variability from low concentrations ($<0.067 \mu\text{mol L}^{-1}$). The above seasonal differences in DOC and DON concentrations suggest that the DOM that was introduced into the deep-water columns in winter was degraded during the stratification period. The average rates of carbon and nitrogen mineralization between February and October were estimated to be $38.1 \text{ nmol L}^{-1} \text{ d}^{-1}$ (range 35.6–40.5) and $9.13 \text{ nmol L}^{-1} \text{ d}^{-1}$ (range 8.92–9.34), respectively (Table 1). However, on some occasions, the concentrations of DOC significantly increased by 4–5 $\mu\text{mol L}^{-1}$ in the hypolimnion, even when the water column was thermally stratified (October 2001 and August 2002 at all stations), suggesting significant inputs of DOC into the hypolimnion across the thermocline. Thus, our estimates of the mineralization rates of DOM, which assume no inputs of DOM into the deeper layer during the stratification period, are probably too low (see *Discussion*).

Between April and December, SRP tended to accumulate near the bottom, reflecting the depletion of dissolved oxygen (Fig. 3), suggesting the presence of diffusive flux of dissolved inorganic phosphorus from the sediments, the benthic nepheloid layer, or both. In contrast, DIN (mostly nitrate) was distributed homogeneously over depth (Fig. 3), suggesting that the oxidation of organic nitrogen occurred evenly throughout the hypolimnetic water column.

The data collected at Stas. 1, and 3–7 were used to estimate contributions of DOM to total organic matter (POM + DOM) in the water column of the entire north basin for each month (Fig. 5). The whole-basin data revealed that DOC accounted for a large (81–85%) and relatively constant ($\text{SD} = 1.5$) fraction of total organic carbon ($3.13\text{--}3.35 \times 10^9 \text{ mol}$) throughout the period of investigation. Similarly, DON represented a substantial (72–80%) and less variable ($\text{SD} = 2.7$) fraction of total organic nitrogen ($2.25\text{--}3.30 \times 10^8 \text{ mol}$). In contrast, relative contributions of DOP to total organic phosphorus ($3.37\text{--}4.76 \times 10^6 \text{ mol}$) were relatively low (25–53%) and variable ($\text{SD} = 9.5$) over the season.

Elemental compositions of DOM and POM—The elemental ratios of organic matter varied among pools (dissolved

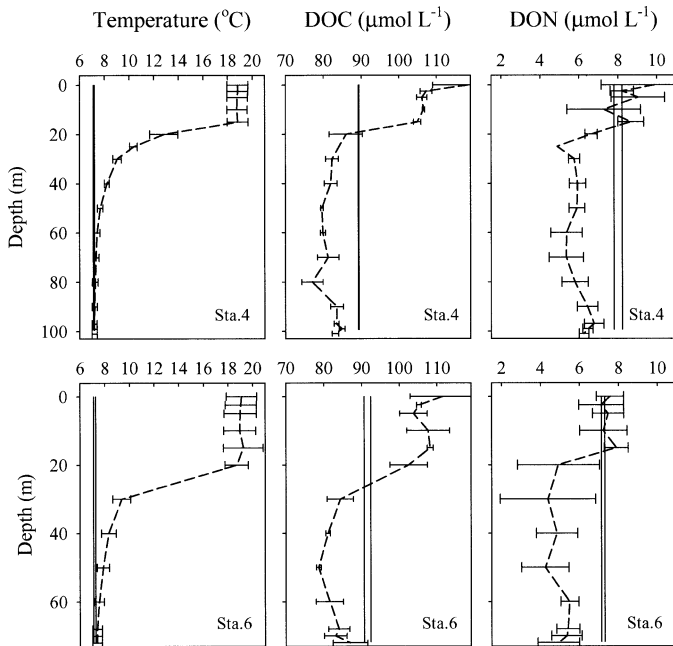


Fig. 4. Depth profiles of water temperature and DOC and DON concentrations at Sta. 4 (upper panel) and Sta. 6 (lower panel). Dashed lines represent composite profiles obtained with the October data collected during 3 consecutive years (2001, 2002, and 2003); errors are standard errors for individual depths ($n = 3$). Each of two vertical lines represents the value averaged over the whole water column in February of 2001 or 2002, although two lines are not separated for temperature and DOC at Sta. 4 because the average values varied little between two years. The February profile of each year consists of the data obtained at 13–15 sampling depths; coefficients of variation of the DOC and DON concentrations for each year were 1.6–3.8% and 2.6–7.7%, respectively.

vs. particulate), across layers (epilimnion vs. hypolimnion), and over season (overturn vs. stratification periods) (Fig. 6). The median values of C:N, C:P, and N:P ratios of POM in the epilimnion were 8.6, 247, and 29, respectively, which were significantly (Kruskal–Wallis ANOVA on ranks, $p < 0.005$) higher than those obtained in February (6.9, 144, and 21 for C:N, C:P, and N:P, respectively).

The POM collected in the hypolimnion had a significantly

($p < 0.001$) lower median value of C:P (168) and N:P ratios (18.5) compared with that collected in the epilimnion (C:P = 247, N:P = 29), indicating that POM was enriched with phosphorus with depth (Fig. 6). The particulate C:N ratio did not differ significantly ($p = 0.312$) between the epilimnion (8.6) and hypolimnion (8.9). Regardless of the layer and season, the C:N, C:P, and N:P ratios of DOM were much greater than those of POM: the overall C:N:P ratios averaged for the whole data set were 214:23:1 ($n = 603$) for POM and 1,978:147:1 ($n = 562$) for DOM. The median C:P (1,783) and N:P (129) ratios of DOM in the hypolimnion were significantly ($p < 0.05$) greater than those in the epilimnion (C:P = 1,385, N:P = 107). The C:N ratios of DOM were low (11.8) in February and high during the stratification period, with a tendency to increase with depth (12.9 and 13.5 in epi- and hypolimnion, respectively; Fig. 6).

Discussion

Our concurrent measurements of carbon, nitrogen, and phosphorus that reside in particulate and dissolved forms of organic matter, with coverage of seasonal, vertical, and horizontal extent, yielded a unique and extensive data set to examine characteristics of the organic matter field and potential roles of DOM in carbon and nutrient cycling. DOC concentrations close to those we found in Lake Biwa (73–138 $\mu\text{mol L}^{-1}$) have been reported in other large lakes, including Hovsgol (80–100 $\mu\text{mol L}^{-1}$, Hayakawa et al. 2003), Superior (110–120 $\mu\text{mol L}^{-1}$, Biddanda et al. 2001), and Constance (121–192 $\mu\text{mol L}^{-1}$, Weiss and Simon 1999), but our data are among the first that demonstrated the downward transport and subsequent mineralization of DOM in a large freshwater lake. We found that DOC and DON accumulate in the epilimnion during the stratification period to be redistributed into the deeper layer during the winter overturn period, followed by the mineralization at depth as indicated by significant decreases in concentrations in the hypolimnion over the stratification period. These results suggest that DOM plays a role in the hypolimnetic metabolism of carbon and nitrogen.

To examine to what extent DOM contributes to the total mineralization of carbon and nutrients, we compared the de-

Table 1. DOM and total mineralization rates of C and N, contributions of DOM to total mineralization, and C to N ratios of the mineralized organic matter in the hypolimnion of Lake Biwa. Values are the averages of the estimates derived for two sampling stations with the estimates in parentheses for individual stations including Stas. 4 (first value) and 6 (second).

	C	N	C:N
(A) DOM mineralization ($\text{nmol L}^{-1} \text{d}^{-1}$)*	38.1(35.6,40.5)	9.13(8.92,9.34)	4.2(4.0,4.3)
(B) Total mineralization ($\text{nmol L}^{-1} \text{d}^{-1}$)†	475(480,434)	30.1(29.6,30.7)	15.2(16.2,14.1)
% DOM (A/B×100)	8.4(7.4,9.3)	30.1(30.0,30.2)	

* DOM mineralization rates were estimated from the seasonal decline of DOC and DON concentrations between February (2002 and 2003) and October (2001, 2002, 2003) in the layer below 40 m. Average values obtained during consecutive years were used for this calculation (see Fig. 4). Because the depth of the thermocline was ~20–30 m in October, we assumed that the diffusive flux of DOM to the layer below 40 m was minimal.

† Total carbon and nitrogen mineralization rates were estimated from the seasonal decline and accumulation of dissolved O_2 and NO_3^- (or DIN), respectively, between February (2001 and 2002) and October (2001, 2002, 2003) in the layer below 40 m, which could include the benthic consumption of dissolved oxygen (see text for explanation).

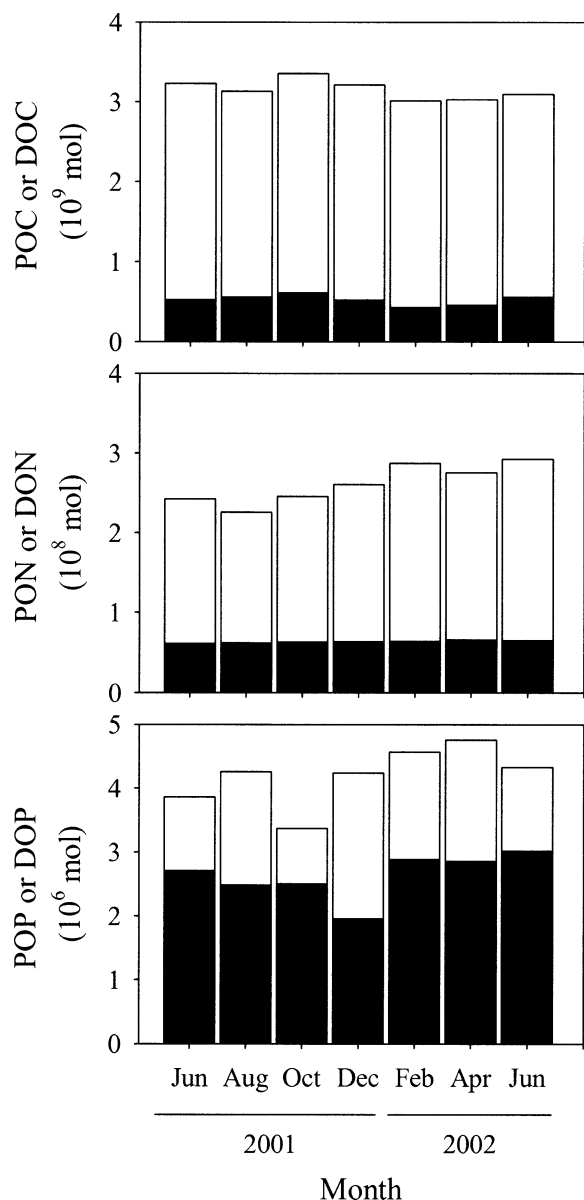


Fig. 5. Monthly inventories of whole-basin C, N, and P standing stock of particulate (filled bar) and dissolved forms (empty bar) of organic matter in the north basin of Lake Biwa. Concentrations of each constituent determined at a given depth (Z , m) were averaged for six sampling stations (except that the number of sampling stations was four in December 2001, see *Materials and Methods*) for each month. Assuming that this value represents the mean concentration in a Z -m depth horizon of the entire basin for each month, we estimated the total mass in the basin with the depth-volume curve of the basin.

crease of DOM in the hypolimnion during the stratification period with the total hypolimnetic mineralization of carbon and nitrogen. Total organic carbon mineralization was calculated as the sum of aerobic respiration ($\text{CH}_2\text{O} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O}$) and denitrification ($5\text{CH}_2\text{O} + 4\text{NO}_3^- + 4\text{H}^+ \rightarrow 5\text{CO}_2 + 2\text{N}_2 + 7\text{H}_2\text{O}$, Bédard and Knowles 1991). Aerobic respiration was estimated from the hypolimnetic depletion of O_2 with a subtraction of O_2 consumption because of ni-

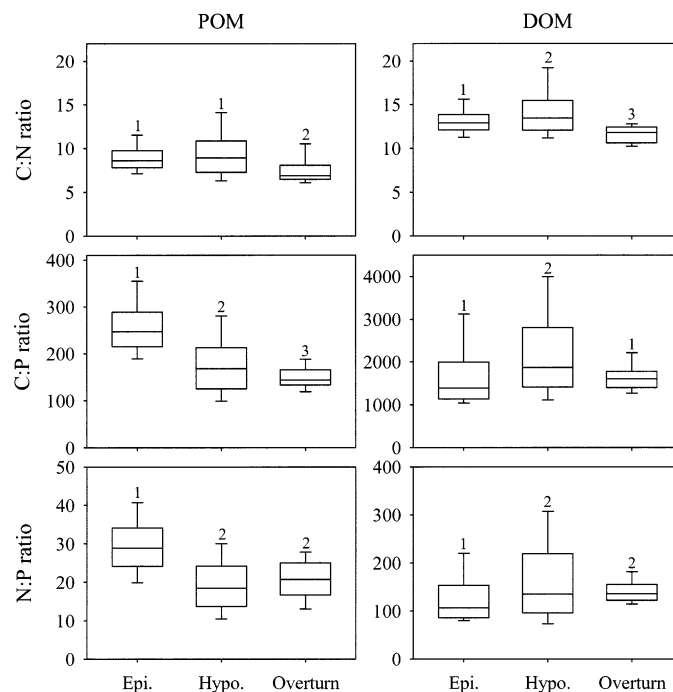


Fig. 6. C:N:P elemental ratios of particulate and dissolved pools of organic matter collected during the periods of stratification (April–December) and overturn (February). The data for the stratification period were separated into those collected in epilimnion (Epi.) and hypolimnion (Hypo.), which were defined as layers above and below the metalimnion (a layer in which vertical thermal gradient exceeded 1°C m^{-1}), respectively, except for April (without isothermal epilimnion), when Epi. and Hypo. represented the data obtained in layers above and below the 17-m depth horizon (compensation depth), respectively. The boundaries of each box indicate the 25th and 75th percentiles. Error bars above and below the box indicate the 10th and 90th percentiles. A line within the box marks the median. If ratios differ significantly (Kruskal–Wallis ANOVA on ranks, $p < 0.05$) among categories, different numerals are given. Number of samples is 179–188, 285–323, and 97 for Epi., Hypo., and overturn, respectively.

trification ($\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}$). Nitrification was estimated from the accumulation of nitrate in the hypolimnion after the correction for denitrification, which has been estimated to be 30% of nitrification in Lake Biwa (Miyajima 1994). The overall equation is (mol C mineralized) = (mol O_2 depleted) – ($2 \times$ mol nitrified N) + ($1.25 \times$ mol denitrified N) (Table 1). Our results indicated that the contributions of DOM to the total mineralization of carbon (35.6 – $40.5 \text{ nmol L}^{-1} \text{ d}^{-1}$) and nitrogen (8.92 – $9.34 \text{ nmol L}^{-1} \text{ d}^{-1}$) are 8.4% (range 7.4–9.3%) and 30.1% (30.0–30.2%), respectively (Table 1). The above results with volumetric comparisons generally agree with the results of areal comparisons on the basis of carbon and nitrogen fluxes previously reported in the literature (Maeda et al. 1987; Hama et al. 1990; Yoshimizu et al. 2001). Our estimates of the DOC and DON mineralization integrated over the deeper water column ($>40 \text{ m}$, 2.17 and $0.54 \text{ mmol m}^{-2} \text{ d}^{-1}$ for DOC and DON, respectively) represent 12% and 36% of the sinking fluxes of POC ($17.9 \text{ mmol m}^{-2} \text{ d}^{-1}$) and PON ($1.49 \text{ mmol m}^{-2} \text{ d}^{-1}$), respectively (Table 2). This flux of DOC accounts

Table 2. Comparisons of the areal hypolimnetic mineralization rates of DOM and POM settling fluxes (literature values) in the north basin of Lake Biwa.

	C	N
(A) Areal hypolimnetic mineralization of DOM (mmol m ⁻² d ⁻¹)	2.17*	0.54*
(B) POM settling flux (mmol m ⁻² d ⁻¹)	17.9(15.3,20.4)†	1.49(0.99,1.98)‡
A/B×100 (%)	12	36

* The areal fluxes were calculated for the water column (40–100 m) on the basis of the volumetric estimates of DOM mineralization rates of Sta. 4 obtained in the layer below 40 m (Table 1).

† Average and range (in parentheses) of the estimates of POC settling fluxes reported in the literature. The data include those of Maeda et al. (1987) (20.4 ± 15.8 mmol m⁻² d⁻¹, $n = 14$, between April and November 1984 and 1985, trap depth 30 m) and Yoshimizu et al. (2001) (15.3 ± 9.9 mmol m⁻² d⁻¹, $n = 11$, between July 1996 and August 1997, trap depth 30 m).

‡ Average and range (in parentheses) of the estimates of PON settling fluxes reported in the literature. The data include those of Maeda et al. (1987) (2.0 ± 1.2 mmol m⁻² d⁻¹, $n = 14$, between April and November of 1984 and 1985, trap depth 30 m) and Hama et al. (1990) (0.99 ± 0.49 mmol m⁻² d⁻¹, $n = 10$, between August 1985 and June 1986, trap depth 30 m).

for 2.1% of primary production (104 ± 61 mmol m⁻² d⁻¹, $n = 11$, between July 1996 and August 1997; Yoshimizu et al. 2001).

We consider that the above estimates of DOC and DON mineralization in the hypolimnion are conservative because DOM could be introduced into the hypolimnion even during the stratification period. Potential mechanisms that could drive this flux include diffusion, advection, release from sinking POM, and migration of zooplankton. In support of this hypothesis, we found, on occasion, seasonal peaks in hypolimnetic DOC concentrations during the stratification period (October 2001; August 2002), a phenomenon noted by an earlier study (Hori et al. 1998). The DOC introduced into the hypolimnion during the stratification period can contribute to hypolimnetic metabolism but is not accounted for in our calculations, which are based on the differences in concentrations between February and October. In addition to DOM fluxes across the thermocline, our estimates of DOC and DON mineralization rates could have errors associated with complexity in DOM delivery from multiple sources, including allochthonous DOM delivered via river inflow, ground water, shallow subsurface runoff, and aeolian depositions. Annual average DOC concentrations in the five main rivers flowing into the lake have been reported to be, on average, $111 \mu\text{mol L}^{-1}$ (range 48–212 $\mu\text{mol L}^{-1}$; Sugiyama et al. 2000), which is equivalent to or lower than the level of DOC in surface waters of the lake. The release of DOM from sediments to overlying water might also affect the spatial distribution of DOM in water columns, although we seldom observed an increase of DOM concentrations in the benthic nepheloid layer. We note that a potential sink, other than mineralization, of DOM includes flocculation and adsorption to settling particles of DOM. Inputs of clay mineral to a lake might enhance the scavenging of DOC in surface waters, although the equilibrium would be shifted toward desorption in hypolimnetic waters in which DOC concentra-

tions are low (Tietjen et al. 2005). Despite the above factors that potentially affect our interpretations of the spatiotemporal variations of DOM, the relative homogeneity over the basin (Fig. 2) and consistency over years (Fig. 4) of DOM profiles suggest that our calculations yielded reasonably accurate, albeit presumably conservative, estimates of hypolimnetic consumption of the DOM pool in Lake Biwa.

It is interesting to note that the contribution of DON to total hypolimnetic mineralization (30%) far exceeded that of DOC (8%). This result apparently contradicts the expectation that DOM delivers C more efficiently than N because the average C:N ratio of DOM (13.5) is greater than that of POM (9.3) (Fig. 6) (cf. Hopkinson and Vallino 2005). A stoichiometric calculation suggests that the C:N ratio of DOM mineralized in the hypolimnion is 4.2 on average (Table 1), indicating the N-rich nature of the DOM that was mineralized in the hypolimnion. Thus, the fraction of DOM involved in the hypolimnetic DON mineralization appears to be distinctive, in terms of elemental composition, from other DOM components that are characterized by a high C:N ratio. Chemical structures and origins of these distinctive DOM components are unclear. Previous studies in marine systems have suggested that DOM can be classified into labile, semilabile, and refractory components according to its turnover time (Carlson and Ducklow 1995; Cherrier et al. 1996). Carlson et al. (1994) have postulated that semilabile DOM that turns over on an approximate timescale of months can mediate annual, convective transport of surface DOM down to the layer below the seasonal thermocline of the Atlantic. An important feature of the semilabile DOM is that, unlike labile DOM, it escapes from rapid (<days) degradation but is degraded slowly over seasons to allow the uncoupling in time and space of consumption from production of DOM. If this model applies to large freshwater lakes, the hypolimnetic mineralization of N that we found in Lake Biwa might be ascribed to the degradation of semilabile DOM produced in the surface layer. Alternatively, allochthonous DOM delivered into the deeper layer in winter might contribute to hypolimnetic N mineralization. Although river DOM generally exhibits a high C:N ratio, it still contains nitrogen that is available for microbial degradation (Stepanuskas et al. 2002).

We failed to detect clear patterns in the spatiotemporal dynamics of DOP primarily because of high variability associated with low DOP concentrations, which were near the detection limit of the analytical procedure used. The average C:P (1,978) and N:P ratios (147) of DOM that we derived are much (six- to ninefold) greater than the corresponding ratios of POM (C:P = 214, N:P = 23), indicating that DOM in Lake Biwa water is highly depleted in P relative to C and N. This P-poor nature of DOM probably reflects that bacterial communities are under severe limitation by P (Nishimura et al. 2005), leading to the substantial hydrolysis of DOP compounds and effective retention by microbes of P (Tezuka 1990). Consistent with this notion, we found that C:P and N:P ratios of DOM in the hypolimnion were higher than those in the epilimnion, whereas the reverse trend was evident for POM (Fig. 6), suggesting a conversion of dissolved to particulate (presumably biotic) phases of P in the hypolimnion. The above results suggest that, because of

the P-poor nature of the DOM pool and because of efficient microbial retention of P in the water column, DOM contributes little to hypolimnetic P mineralization in Lake Biwa, although this postulation does not exclude the possibility that DOP plays a pivotal role in rapid cycling of P in the microbial loop (Karl and Björkman 2002).

The extent and stoichiometry of DOM mineralization in the hypolimnion probably vary among freshwater lakes depending on nutrient regime and environmental setting. Conceivably, the role of allochthonous DOM in hypolimnetic carbon mineralization could be much greater in small lakes than in large, deep lakes, a notion consistent with the finding of Houser et al. (2003), who suggested that the contribution of DOC mineralization to total hypolimnetic mineralization can be as much as 100% in small humic lakes, with a general tendency of increase with increasing DOC concentration. Note that the lakes studied by Houser et al. (2003) are characterized by high concentrations of DOC (range 340–1,500 $\mu\text{mol L}^{-1}$), which are 3- to 14-fold higher than those in Lake Biwa. The relative importance of POC (sedimentation) and DOC (convective mixing) in organic carbon delivery might also depend on trophic status and community structure in surface waters. In oligotrophic waters, including open oceans, microbial food webs dominate in the euphotic zone, impeding the settling fluxes of POC (because of small cell size of primary producers; Legendre and Rassoulzadegan 1996) while promoting the production of DOM, including its semilabile components (Nagata 2000). Thus, one could hypothesize that the relative importance of DOC to POC in the delivery of organic carbon to the aphotic zone increases with increasing oligotrophy (cf. Biddanda et al. 2001). In support of this hypothesis, the data have suggested that the contribution of DOC to the total downward delivery (DOC + POC) can be high (>40%) in oligotrophic oceanic systems (e.g., Carlson et al. 1994) and in oligotrophic Lake Superior (McManus et al. 2003), whereas the corresponding value in mesotrophic Lake Biwa is moderate (8%), as suggested by this study. Concerning the stoichiometry of DOM mineralization, our finding that DOM contributes little to hypolimnetic P mineralization in Lake Biwa is consistent with a notion that P is preferentially mineralized relative to C, being intensively recycled in surface waters rather than delivered to deeper layers (Hopkinson and Vallino 2005). However, the high contribution of N relative to C in the hypolimnetic metabolism of Lake Biwa apparently contradicts the proposition that N is also preferentially mineralized relative to C in surface waters; hence, its contribution to the downward delivery is less significant than that of C (Hopkinson and Vallino 2005). It remains to be seen whether this pattern in C, N, and P mineralization in the hypolimnion applies to other large mesotrophic lakes. Also challenging for future studies is the evaluation of the sources and production and decomposition mechanisms of DOM in freshwater environments.

On the basis of the analysis of spatiotemporal variations of DOM, we found that the contributions of DOM to the hypolimnetic mineralization of carbon and nitrogen in Lake Biwa are significant, accounting for 8% and 30% of the total mineralization of carbon and nitrogen, respectively. Thus, winter turnover of the water column of a monomictic basin

should be considered a mechanism that delivers significant amounts of reduced carbon and nitrogen to the hypolimnion in the form of DOM, a process that has generally been overlooked in previous biogeochemical models of large lakes. Our data also suggested that the role of DOM in hypolimnetic mineralization of P appears to be insignificant, which might be explained in part by the elemental composition of DOM, which is characterized by the substantial depletion of P relative to C and N. The extent of DOM delivery and the relative importance of C, N, and P in total hypolimnetic metabolism probably depend on trophic status, nutrient regime, and hydraulics of the basins, a hypothesis that should be tested in future studies.

References

- BÉDARD, C., AND R. KNOWLES. 1991. Hypolimnetic O_2 consumption, denitrification, and methanogenesis in a thermally stratified lake. *Can. J. Fish. Aquat. Sci.* **48**: 1048–1054.
- BENNER, R., AND M. STROM. 1993. A critical evaluation of the analytical blank associated with DOC measurements by high temperature catalytic oxidation. *Mar. Chem.* **41**: 153–160.
- BIDDANDA, B., AND J. COTNER. 2002. Love handles in aquatic ecosystems: The role of dissolved organic carbon drawdown, re-suspended sediments, and terrigenous inputs in the carbon balance of Lake Michigan. *Ecosystems* **5**: 431–445.
- , M. OGDahl, AND J. COTNER. 2001. Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. *Limnol. Oceanogr.* **46**: 730–739.
- CARLSON, C. A., AND H. W. DUCKLOW. 1995. Dissolved organic carbon in the upper ocean of the central equatorial Pacific Ocean, 1992: Daily and finescale vertical variations. *Deep-Sea Res. II* **42**: 639–656.
- , AND A. F. MICHAELS. 1994. Annual flux of dissolved organic carbon from the euphotic zone in the north-western Sargasso Sea. *Nature* **371**: 405–408.
- CHERRIER, J., J. E. BAUER, AND E. R. M. DRUFFEL. 1996. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters. *Mar. Ecol. Prog. Ser.* **139**: 267–279.
- COPIN-MONTÉGUT, G., AND B. AVRIL. 1993. Vertical distribution and temporal variation of dissolved organic carbon in the north-western Mediterranean Sea. *Deep-Sea Res. I* **40**: 1963–1972.
- CORNETT, R. J., AND F. H. RIGLER. 1979. Hypolimnetic oxygen deficits: Their prediction and interpretation. *Science* **205**: 580–581.
- HAMA, T., K. MATSUNAGA, N. HANDA, AND M. TAKAHASHI. 1990. Nitrogen budget in the euphotic zone of Lake Biwa from spring to summer, 1986. *J. Plankton Res.* **12**: 125–131.
- HAYAKAWA, K., T. SEKINO, T. YOSHIOKA, M. MARUO, AND M. KUMAGAI. 2003. Dissolved organic carbon and fluorescence in Lake Hovsgol: Factors reducing humic content of the lake water. *Limnology* **4**: 25–33.
- HOLMES, R. M., A. AMINOT, R. KÉROUEL, B. A. HOOKER, AND B. J. PETERSON. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish. Aquat. Sci.* **56**: 1801–1808.
- HOPKINSON, C. S., AND J. J. VALLINO. 2005. Efficient export of carbon to the deep ocean through dissolved organic matter. *Nature* **433**: 142–145.
- HORI, T., Y. SUGIYAMA, AND M. SUGIYAMA. 1998. Chemical and physicochemical characteristics of dissolved organic carbon

- circulating in harmonic Lake Biwa, Japan. *Jpn. J. Limnol.* **59**: 39–52.
- HORIE, S. 1984. *Lake Biwa*. Dr W, Junk Publishers.
- HOUSER, J. N., D. L. BADE, J. J. COLE, AND M. L. PACE. 2003. The dual influences of dissolved organic carbon on hypolimnetic metabolism: Organic substrate and photosynthetic reduction. *Biogeochemistry* **64**: 247–269.
- HUTCHINSON, G. E. 1938. On the relation between the oxygen deficit and the productivity and typology of lakes. *Int. Rev. Gesamten Hydrobiol.* **36**: 336–355.
- KARL, D. M., AND K. M. BJÖRKMANN. 2002. Dynamics of DOP, p. 250–366. *In* D. A. Hansell and C. A. Carlson [eds.], *Biogeochemistry of marine dissolved organic matter*. Elsevier.
- LEGENDRE, L., AND F. RASSOULZADEGAN. 1996. Food web mediated export of biogenic carbon in oceans: Hydrodynamic control. *Mar. Ecol. Prog. Ser.* **145**: 179–193.
- MAEDA, H., O. MITAMURA, T. KODAMA, M. EGUCHI, H. KITADA, AND A. KAWAI. 1987. Sedimentation, decomposition and liberation of substances in the north basin of Lake Biwa. *Lake Biwa Research Institute Report* 86-A05.
- MCMANUS, J., E. A. HEINEN, AND M. M. BAEHR. 2003. Hypolimnetic oxidation rates in Lake Superior: Role of dissolved organic material on the lake's carbon budget. *Limnol. Oceanogr.* **48**: 1624–1632.
- MITO, S., M. KAWASHIMA, AND Y. SOHRIN. 2002. Characterization of suspended solids in Lake Biwa by measuring their elemental composition of Al, Si, P, S, K, Ca, Ti, Mn, and Fe. *Limnology* **3**: 11–19.
- MIYAJIMA, T. 1994. Mud-water fluxes of inorganic nitrogen and manganese in the pelagic region of Lake Biwa: Seasonal dynamics and impact on the hypolimnetic metabolism. *Arch. Hydrobiol.* **130**: 303–324.
- NAGATA, T. 2000. Production mechanisms of dissolved organic matter, p. 121–152. *In* D. L. Kirchman [ed.], *Microbial ecology of the oceans*. John Wiley.
- NISHIMURA, Y., C. KIM, AND T. NAGATA. 2005. Vertical and seasonal variations of bacterioplankton subgroups with different nucleic acid contents: Possible regulation by phosphorus. *Appl. Environ. Microbiol.* **71**: 5828–5836.
- PUJO-PAY, M., AND P. RAIMBAULT. 1994. Improvement of the wet oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. *Mar. Ecol. Prog. Ser.* **105**: 203–207.
- STEPANAUSKAS, R., N. O. G. JØRGENSEN, O. R. EIGAARD, A. VIKAS, L. J. TRANVIK, AND L. LEONARDSON. 2002. Summer inputs of riverine nutrients to the Baltic Sea: Bioavailability and eutrophication relevance. *Ecol. Monogr.* **72**: 579–597.
- SUGIYAMA, Y., M. SUGIYAMA, AND T. HORI. 2000. Environmental chemistry of rivers and lakes, part V: A comparative study of the chemical and physicochemical characteristics of organic carbon dissolved in river and lake waters. *Limnology* **1**: 171–176.
- TEZUKA, Y. 1990. Bacterial regeneration of ammonium and phosphate as affected by the carbon:nitrogen:phosphorus ratio of organic substrates. *Microb. Ecol.* **19**: 227–238.
- TIETJEN, T., A. V. VÄHÄTALO, AND R. G. WETZEL. 2005. Effects of clay mineral turbidity on dissolved organic carbon and bacterial production. *Aquat. Sci.* **67**: 51–60.
- WEISS, M., AND M. SIMON. 1999. Consumption of labile dissolved organic matter by limnetic bacterioplankton: The relative significance of amino acids and carbohydrates. *Aquat. Microb. Ecol.* **17**: 1–12.
- WETZEL, R. G. 2001. *Limnology: Lake and river ecosystems*, 3rd ed. Academic Press.
- YOSHIMIZU, C., T. YOSHIDA, M. NAKANISHI, AND J. URABE. 2001. Effects of zooplankton on the sinking flux of organic carbon in Lake Biwa. *Limnology* **2**: 37–43.

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