Title: Spatial and temporal variation in nutrients in water and riverbed sediments at the mouths of rivers that enter Lake Hachiro, a shallow eutrophic lake in Japan

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Abstract

We evaluated the spatial and temporal variations in dissolved inorganic nitrogen ($\text{DIN} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) and soluble reactive phosphorus (SRP) concentrations and the processes that control them in water and riverbed sediments at the mouths of five main rivers (MTN, BBM, IKW, TYK, and BFM) that enter Lake Hachiro, a shallow eutrophic lake in Japan. At the river mouths, we sampled water monthly and analyzed the nutrient concentrations and dissolved N_2O and CH_4 concentrations. We also sampled riverbed sediments to determine the SRP release potential, inorganic P fractions, and denitrification potential (dNp). Riverine SRP concentrations showed large spatial and temporal variations, with values increasing from summer to autumn in the three southernmost rivers (IKW, TYK, and BFM). High dissolved CH_4 concentrations were observed in association with high SRP concentrations, indicating that SRP was released from the sediments under hypoxic conditions. In contrast, NO_3^- concentrations did not differ among the rivers and decreased during summer, likely owing to denitrification, thereby decreasing DIN/SRP ratios to a level suitable for the formation of summer algal or cyanobacterial blooms in the three southernmost rivers. Sediment incubation demonstrated high SRP release rates and high dNp values in the sediments of these rivers due to their higher iron (Fe)-bound P and organic matter contents. The riverine SRP concentration was significantly correlated with dissolved CH_4 and sediment dNp in positive, and DIN/SRP in negative. In conclusion, differences in Fe-bound P and sediment organic matter content suggested to cause spatial and temporal variations in water quality, with high SRP release and denitrification under hypoxic summer conditions, resulting in lower DIN/SRP ratios that may trigger algal or cyanobacterial blooms in this shallow eutrophic lake.

1 **Keywords:** Phosphorus release, denitrification, N/P ratio, shallow eutrophic lake

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1. Introduction

Nitrogen (N) and phosphorus (P) are both required to support plant growth and are the key limiting nutrients in most aquatic and terrestrial ecosystems (Conley et al., 2009; Elser et al., 2007). Human activity has greatly altered N and P flows from river watersheds into aquatic ecosystems, thereby increasing primary production and causing widespread eutrophication (Howarth et al., 1996; Carpenter et al., 1998). Eutrophied lakes sustain damage from blooms of harmful organisms such as cyanobacteria, which can release toxins into the water (Paerl and Tucker, 1995).

Generally, sediments carried in flowing water play an important role in determining the effects of biogeochemical N and P cycles on the functioning of shallow lakes (e.g., Boström et al., 1988; Holmroos et al., 2012; Nizzoli et al., 2010; Søndergaard et al., 2003). Previous studies have focused on the release of P from the sediments under hypoxic conditions in shallow lakes and the dependence of this release on biological, chemical, and physical factors (Lukkari et al., 2008; Søndergaard et al., 2001). Iron (Fe)-bound P in these sediments is a primary factor that determines the release of P from sediments under hypoxic and anoxic condition (Lukkari et al., 2008). Hypoxic and anoxic conditions also affect N cycles by affecting processes such as denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in sediments and the water column (Burgin and Hamilton, 2007). These N and P dynamics will change the N/P ratio in the water, which will in turn affect interactions between algae or cyanobacteria and the nutrients they require for survival and growth (Correll, 1998; Hecky and Kilham, 1988; Niemistö et al., 2008). A suitable N/P ratio (Klausmeier et al., 2004; Smith, 1983) may create conditions suitable for the development of a harmful algal bloom in lake water. Thus, it is prudent to focus on both N and P dynamics unless

1 there is clear evidence or a strong reason to believe that only one of these nutrients is
2 important in a given ecosystem (Conley et al., 2009; Paerl et al., 2011). Unfortunately,
3 the biogeochemical responses of N and P in shallow-water ecosystems and their
4 relationships to eutrophication and hypoxia are less well understood than those in larger,
5 deeper estuaries and coastal marine ecosystems (Howarth et al., 2011).

6 The nutrient and sediment loads transported into lakes by rivers depend on the
7 availability of nutrients in the watershed and their potential for movement to the lake
8 (Fraterrigo and Downing, 2008). Because the nutrients and sediments transported from
9 watersheds depend strongly on rate of loading and of sediment erosion into rivers
10 (Lukkari et al., 2008; Pacini and Gächter, 1999) and on watershed characteristics such
11 as hydrology, geology, soils, topography, and land use (Iglesias et al., 2011; Jiang et al.,
12 2010, 2012; Woli et al., 2004), they are expected to vary in space and in time in a lake.
13 Jin et al. (2006) reported spatial variation in different P fractions in sediments and a
14 relationship between these variations and the trophic status of different parts of Taihu
15 Lake. The sedimentation rate, organic matter content, and amount of sediment-sorbed P
16 transported by erosion were important determinants of the behavior of P in sediments
17 (Lukkari et al., 2008). Despite extensive research on the P dynamics in sediments, the
18 biogeochemical processes that affect N and P in the sediments and water where rivers
19 enter a lake are not yet well understood. To support efforts to improve lake water quality,
20 managers must have access to detailed information about the quality of transported
21 sediment and its spatial and temporal variations before they can predict the impact on a
22 lake's water quality and take measures to mitigate problems, particularly for shallow
23 eutrophic lakes.

24 Lake Hachiro in Japan's Akita Prefecture is an example of such a shallow

1 eutrophic lake. Currently, cyanobacterial blooms occur every summer (Kondo, 2010;
2 Okano et al., 2015), and managers consider it urgently necessary to improve the lake's
3 water quality (Kondo, 2010; Sasaki, 2010). Twenty significant rivers enter the lake, and
4 differences in the nutrients and sediments transported from each river's watershed will
5 influence the water quality where these rivers enter the lake. Unfortunately, there is little
6 or no information on these factors. To provide this information, we designed a study of
7 the biogeochemical processes that affect N and P transport in riverbed sediments and
8 their delivery into the lake at river mouths, which represent the interface between river
9 watershed and the lake. We hypothesized that these processes, that is, P release from
10 and denitrification in riverbed sediments would control the spatial and temporal
11 variations in water quality among the rivers that enter Lake Hachiro. To test this
12 hypothesis, we selected the five main rivers that enter the lake and evaluated the spatial
13 and temporal variations in dissolved inorganic nitrogen (DIN) and soluble reactive
14 phosphorus (SRP) concentrations and the processes that control them in water and
15 riverbed sediments.

2. Materials and methods

2.1. Site description

Lake Hachiro and its watershed are located in western Akita Prefecture, facing the Sea of Japan (Fig. 1). The entire watershed covers 894 km². Lake Hachiro had a surface area of 220 km² and was the second largest lake in Japan before the implementation of the Hachirogata National Land Reclamation Project, which was designed to improve self-sufficiency in food production (Ogata-mura Village Office, 2014; Sasaki, 2010). The project started in 1957 and finished in 1977 (Sasaki, 2010). As part of the project, Japan's largest polder, Ogata-mura Village, was constructed, leading to reclamation of nearly 80% of the lake's area and transforming the remaining lake water from brackish to fresh by controlling the lake water table by the floodgate (Fig. 1). Currently, Lake Hachiro is a shallow eutrophic lake, with a mean depth of 2.8 m, a maximum depth of 12 m, an area of 48.3 km², and a residence time of about 1 month. In 2007, it was designated as requiring conservation under the Law Concerning Special Measures for Conservation of Lake Water Quality in Japan. The lake serves as an important water resource for agriculture and aquaculture, and is also important as a tourist spot because of the beautiful scenery. Therefore, degradation of the lake's water quality is problematic for farmers, the fisheries industry, tourists, and residents.

Data for the region monitored by the Gojome meteorological recording station from 1981 to 2010 (Japan Meteorological Agency 2013) reveal that precipitation averages 1553 mm year⁻¹ and that the annual mean temperature is 10.8 °C. The lowest mean monthly temperature occurs in January (−0.9 °C) and the highest in August (24 °C). The average maximum snow depth during winter (December to February) is 48 cm. The elevation in the watershed ranges from −6.3 m a.s.l. inside the polder to 1037 m

a.s.l., and is generally higher in the southern part than in the northern part of the forested area of the watershed. There are mountains mainly in the east and the river flows downhill towards the west (Fig. 1). The agricultural land area (rice paddies and croplands) occupy 38% of the total watershed area. Rice paddies occupy about 85% of the agricultural land area in the watershed.

We established sample sites for determination of the water quality at the mouths of the lake's five main rivers (MTN, BBM, IKW, TYK, and BFM; Fig. 1). Table 1 summarizes the catchment area, mean water depth, and key sediment properties at the five river mouths. The catchment area ranges from 25.0 to 206.9 km². Mean river water depth ranges from 150 to 290 cm.

2.2. Water and sediment sampling

River water samples were collected once a month from May to December 2012 at the surface (from August to December in IKW, TYK, and BFM) and near the bottom (ca. 15 cm above the sediment). Because we lacked data from May to July for the surface water at three sites, we have not compared the surface water values and have instead focused on water near the river bottom. We measured water temperature, pH, and electrical conductivity (EC) using a pH/EC meter (D-54, Horiba, Kyoto, Japan) and measured the dissolved oxygen (DO) concentration using a DO meter (D-55, Horiba) on site. We could not measure DO in September and October because the sensor failed. At the same time as the water sampling, sediment samples (5 samples per river, each about 1 kg) were obtained from the riverbed of each sample site to a depth of about 10 cm using an Ekman-Birge grab sampler (S-15, Miyamoto Riken Ind. Co. Ltd., Osaka, Japan). Sediment samples were obtained in midstream, working from a bridge, and

passed through a 2-mm sieve at the site to remove coarse debris, and were combined into a single bulked sample for analysis.

2.3. Chemical analysis

2.3.1. Chemical analysis of the water and sediments

After sampling, the water and sediment samples were stored on ice until they could be transported to the laboratory, where they were then stored at 4 °C until analysis. The water samples were filtered through a 0.45-μm membrane filter. We measured the NO₃⁻ concentrations in the water using an ion chromatograph (DX-120, Dionex, Sunnyvale, CA, USA). The concentrations of NH₄⁺, NO₂⁻, and soluble reactive phosphorus (SRP) were determined using an autoanalyzer (QuAatro2-HR, BLTEC, Osaka, Japan). The fresh sediment was extracted twice with distilled water (soil:water, 1:2.5 for pH and 1:5 for EC) by shaking for 60 minutes, and pH and EC of in the extract were determined using pH/EC meter (LAQUA F-74BW, Horiba). Sediment total C and N were determined using a NC analyzer (NC-22F, Sumika Chemical Analysis Service, Ltd., Osaka, Japan) and these values were regarded as sediment organic C and N contents. Sediment total S content was determined by a direct combustion/infrared detection method (S632, LECO, MI, U.S.A.). For the determination of sediment total P contents, sediment samples were digested with a combination of HF-HNO₃-HClO₄ acids in Teflon beakers at 180 °C and total P concentration in the solutions were determined by ICP-OES (iCAP 6000, Thermo Fisher Scientific K.K., Yokohama, Japan).

2.3.2. Fractionation of sediment inorganic P

The sediment inorganic P was fractionated according to the method of Zhang et al. (2009). The fractionation procedure is based on the differential solubilities of the various forms of inorganic P in various extracts. In brief: 1 M ammonium chloride (NH_4Cl) is used first to remove the soluble and loosely bound P. Al-P complexes are separated from Fe-P complexes using 0.5 M NH_4F (pH8.2), then the Fe-P complexes are extracted using 0.1 M NaOH. Reductant-soluble P minerals are removed with sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$)–sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$)–sodium bicarbonate (CDB) extraction. Ca-P complexes are extracted with 0.25 M sulfuric acid (H_2SO_4), because they are insoluble in CDB. The SRP concentration was determined using the molybdenum blue method. We calculated the sediment inorganic P as sum of these five fractions.

2.4. Measurement of dissolved N_2O and CH_4

The concentration of dissolved N_2O (N_2O) and CH_4 (CH_4) in the stream water was measured by using a headspace technique based on the principle of vapor–liquid equilibrium (Minamikawa et al., 2011; Sawamoto et al., 2002). We collected 30 mL of stream water using a 60-mL syringe in triplicate, without degassing, and the rest of the syringe was filled with 30-mL of O_2 -free ultrapure N_2 gas obtained from a Tedlar bag. After 3 min of vigorous shaking by hand to produce equilibrium between the vapor and liquid phases, the headspace gas was transferred from the syringe into a 15-mL evacuated glass vial. The concentrations of N_2O and CH_4 were determined using a gas chromatograph (GC-14B, Shimadzu, Kyoto, Japan) with an electron-capture detector and a flame-ionization detector, respectively. The concentrations of N_2O and CH_4 in the stream water were calculated on the basis of Henry's law as a function of the water

temperature (Sawamoto et al., 2002).

2.5. Sediment incubation

2.5.1. Phosphorus release from the sediments

We used incubation to measure SRP release from the sediments. Samples of fresh, homogenized sediment (30 g) that were obtained in July were placed into ca. 550-mL glass bottles. A 400-mL aliquot of deionized water was added to the bottles, which were then closed with a butyl rubber septum and a crimped aluminum cover. Two stainless-steel needles (Terumo, 23 G $\times$ 32 mm, and GL Science, 19 G $\times$ 100 mm) were inserted through each septum and fitted with sterile three-way stopcocks. To achieve anoxic conditions, O₂-free ultrapure N₂ gas was bubbled through the solution for 30 min while simultaneously purging the headspace air. We also prepared bottles under oxic conditions by opening the stopcock to the air. The bottles were incubated in the dark for 30 days at 10 and 25 °C to study the effect of temperature in addition to oxic conditions. The bottles were prepared in triplicates for each treatment. The solution in the bottles was sampled (15 mL) at 1, 8, 15, and 30 days after the incubation started and was used for water analysis. SRP concentration was analyzed as described in section 2.3.1.

We also conducted an additional incubation to measure SRP and Fe released from the sediments. Sediment sample was obtained from the TYK river on July 2, 2013 and the fresh sample was incubated by the same method above. Fe concentration was analyzed by ICP-OES (iCAP 6000). SRP concentration was analyzed as described in section 2.3.1.

2.5.2. Denitrification measurements

The denitrification potential (dNp) of the sediment samples was measured to quantify the variation among sites in the quantity of electron donors available and the in situ activity of denitrifying organisms in the sediment. We defined dNp as the denitrification rate that occurred under anaerobic conditions with abundant NO_3^- at 25 °C, and measured it using the acetylene inhibition assay, which inhibits the final step in the conversion of N_2O gas into N_2 gas (Tiedje, 1994). All dNp assays were conducted within 2 days of sample collection. Samples of fresh, homogenized sediment (15 g) were placed into 150-mL glass bottles in triplicate. A 50-mL aliquot of solution containing nitrate (5 mg-N L^{-1} as KNO_3) was added to the bottles, which were then closed with a butyl rubber septum and a crimped aluminum cover. To achieve anoxic conditions, O_2 -free ultrapure N_2 gas was bubbled through the solution for 5 min, as described in section 2.5.1. Next, acetylene (C_2H_2) gas was added to the headspace to a final concentration of 10% v/v (at 10 kPa). Then we extracted the headspace gas with a gas-tight syringe at 6 h and calculated the denitrification rate assuming the linear portion of the curve for N_2O production as a function of time. N_2O was determined using a gas chromatograph, as described in section 2.4. Sediment dNp was calculated, and the sediment was oven-dried so that values ($\mu\text{g N kg}^{-1} \text{ h}^{-1}$) could be reported on a dry-weight basis (Hayakawa et al., 2012).

2.6. Statistical analysis

Statistical analyses were performed using the Excel Toukei 2010 software for Windows (Social Survey Research Information Co. Ltd., Tokyo, Japan). Because most the data were not normally distributed, correlations were calculated as Spearman's rank correlation coefficient. We used the Kruskal–Wallis test to identify significant

1 differences among rivers.

3. Results

3.1 Spatial and temporal variations in river water quality and sediment dNp

Water quality at the river mouths exhibited large spatial and temporal variations (Fig. 2). Water temperature increased to $>25\text{ }^{\circ}\text{C}$ during summer and decreased to $<10\text{ }^{\circ}\text{C}$ during autumn and winter at both depths in all rivers. DO near the river bottom decreased below 4 mg L^{-1} in summer (on 23 August) in all rivers, unlike in the surface water, and became higher in winter (Nov. and Dec.).

The SRP concentrations in water near the river bottom ranged from undetectable ($< 0.001\text{ mg P L}^{-1}$) to 0.046 mg P L^{-1} and increased from summer to autumn (Fig. 2(b)), especially in the three southernmost rivers (IKW, TYK, and BFM); in contrast, the SRP concentration in the two northernmost rivers (MTN and BBM) was low and showed little temporal variation. The SRP concentration in water near the river bottom differed significantly among the rivers (Fig. 3(b), $P < 0.01$). NO_3^- concentrations in the water also displayed large temporal variation, and tended to decrease during summer (Fig. 2(b)), but there was relatively small spatial variation among the rivers ($P = 0.20$, data not shown). NH_4^+ concentrations tended to increase during summer and autumn, but the spatial variation was not significant ($P = 0.26$, Fig. 2(b)). These patterns decreased the ratio of dissolved inorganic nitrogen to phosphate (DIN/SRP) during summer below the Redfield ratio (Redfield, 1958) of 16, especially at IKW and TYK (Fig. 2, 3(c), $P < 0.05$). NO_2^- concentrations in the water hardly detected during the study period ($< 0.01\text{ mg N L}^{-1}$, data not shown).

Dissolved CH_4 concentrations in the water near the river bottom ranged from 7.19 to $220\text{ }\mu\text{g C L}^{-1}$, and increased during summer and autumn, especially in the three southernmost rivers (Fig. 4). It also differed significantly among the rivers ($P < 0.05$,

Fig. 3(e)). The dissolved N_2O concentration ranged from 0.25 to 1.11 $\mu\text{g N L}^{-1}$ and tended to decrease during summer, except in BFM, but the spatial differences were not significant ($P = 0.069$, Fig. 3(d)). The dNp in the sediments ranged from 0.23 to 90 $\mu\text{g N kg}^{-1} \text{ h}^{-1}$, and was consistently and significantly higher in the three southernmost rivers ($P < 0.01$, Fig. 3(f)).

3.2 Correlations among the water quality and sediment parameters

Table 2 summarizes the correlations among the water quality and sediment parameters for water near the bottom of the river. The SRP concentration was significantly correlated with DIN/SRP ($r = -0.846$, $P < 0.01$), CH_4 ($r = 0.370$, $P < 0.01$), and dNp ($r = 0.540$, $P < 0.01$). The NH_4^+ concentration was significantly correlated with CH_4 ($r = 0.349$, $P < 0.05$) and dNp ($r = 0.492$, $P < 0.01$). The NO_3^- concentration was significantly correlated with water temperature ($r = -0.498$, $P < 0.01$) and N_2O ($r = 0.639$, $P < 0.01$). DIN/SRP was negatively correlated with both CH_4 ($r = -0.326$, $P < 0.05$) and dNp ($r = -0.448$, $P < 0.01$).

3.3 Phosphorus release from sediments and inorganic P fractions in the sediments

The results of the incubation experiment clearly show that SRP was released under anoxic conditions at 25 °C in the sediments of the three southernmost rivers (Fig. 5(a)): the SRP concentration increased until 15 days after the incubation started and then decreased by 30 days. It increased slightly under anoxic conditions at 10 °C and oxic conditions at 25 °C (Fig. 5(b), (c)). Little or no SRP was released under oxic conditions at 10 °C (Fig. 5(d)). The MTN and BBM sediments, from the two northernmost rivers, released little to no SRP in any of the treatments.

1 The results of the fractionation of inorganic P in the sediments showed
2 differences in the relative amounts of the P fractions among the rivers (Fig. 6). Inorganic
3 P was higher in the BBM sediments and the sediments of the three southernmost rivers
4 (369 to 462 mg kg⁻¹) than in the MTN sediments (127 mg kg⁻¹). The main P fraction in
5 sediments from the three southernmost rivers and the MTN river was Fe-P, accounting
6 for 47% to 66% of the total. In contrast, Ca-P was most abundant in the BBM sediments
7 (64%). The median SRP concentration in the incubation water under anoxic conditions
8 at 25 °C was significantly positively correlated only with the Fe-P content in the
9 sediments ($r = 0.94$, $P < 0.05$, Fig. 7(a)). Furthermore, the results of the additional
10 incubation experiment in the TYK river clearly showed SRP was released along with Fe
11 especially under anoxic conditions at 25 °C (Fig. 8).

4. Discussion

The present study indirectly demonstrated the importance of the biogeochemical processes that occur in riverbed sediments and that control the spatial and temporal variations in DIN, SRP, and DIN/SRP in water at the mouths of the main rivers that enter Lake Hachiro (Fig. 2, 3, 4; Table 2).

4.1 Spatio-temporal variations of SRP and P release from sediments in Lake Hachiro

The large spatial and temporal variations in the SRP concentration in the water near the river bottom (Fig. 2, 3) are likely to have been caused by the release of Fe-bound P from the sediments due to reduction by the bottom water under anaerobic conditions. Previous studies have reported the importance of this release mechanism (e.g., Jensen and Anderson, 1992; Jin et al., 2006; Lukkari et al., 2008), although the exchange of P between the water and sediments is influenced by many factors, including biological (e.g., bacterial activity, mineralization processes, bioturbation), chemical (e.g., redox conditions, pH, the proportion of Fe-bound P), and physical factors (e.g., resuspension, sediment mixing) and their interactions (Søndergaard et al., 2001). If the pore water becomes anoxic due to aerobic microbial respiration in the sediments, the ferric ions are reduced to ferrous ions and binding with P weakens, allowing SRP to diffuse more freely. Lukkari et al. (2008) reported that Fe-bound P was the form of P in the sediments that changed most dynamically under anoxic conditions. The decrease of DO and increase of dissolved CH₄ in the bottom water indicated that the water near the river bottom and the pore water in the sediments became anoxic, especially in the three southernmost rivers (Fig. 2, 3(e)). The results of the incubation experiment (Fig. 5, 8) and the relative amounts of the P fractions (Fig. 6), and the

relationship between Fe-P and SRP (Fig. 7) suggested that anaerobic release of Fe-bound P from the sediments was enhanced by increasing water temperature and by anoxic conditions in the water. The amount of released SRP calculated by the maximum SRP concentration during the incubation accounted for 0.4-5.7% of Fe-P.

The effect of temperature in regulating the release of P from sediments has been well documented (e.g., Böstrom et al., 1988; Jensen and Anderson, 1992). Under warm conditions, the organic P pool is mineralized by increased microbial activity. In our incubation experiment, more SRP was released at 25 °C than at 10 °C, but the impact was larger under anoxic than oxic conditions (Fig. 5). This suggests that the contribution of P release from the sediments during summer was larger from the Fe-bound P pool than from the organic P pool. Jensen and Anderson (1992) also reported a greater temperature effect with large proportions of Fe-bound P in the sediments. Organic P is also a possible fraction from which P was released (Bi et al., 2009; Katsaounos et al., 2007; Spears et al., 2007). Spears et al. (2007) reported that organic P would contribute to lower magnitude to long-term release of SRP during most of the year. The higher SRP concentrations during autumn and winter, and particularly in November (Fig. 2(b)), might indicate the release of P from organic P fractions especially in the three southernmost rivers that had high organic P contents in the sediments (Table 1). Although the inorganic P content in the sediments was high and within the same range in the three southernmost rivers, the SRP concentration was lower both *in situ* and *in vitro* in the BBM sediments (Fig. 2, 5), where the Ca-P fraction accounted for the highest proportion of inorganic P in the sediments (Fig. 6). This is likely because Ca-P mainly from apatite minerals is one of the most stable fractions, with slow SRP release (Lukkari et al., 2008), and releases its P only at a pH of 2 to 4

(Jin et al., 2006). SRP concentration in the MTN river was also low as same as that in the BBM river likely because of lower Fe-P and organic P contents in both the sandy sediments (Table 1, Fig. 2, 6, 7).

4.2 Effect of temperature and sediment qualities on N and P dynamics

Temperatures and the organic matter content in the sediments affect microbial activities under both oxic and anoxic conditions, and can affect the N and P dynamics in sediments and the water column. Under warmer conditions, DO decreased in the water near the river bottom (Fig. 2 (b)) as a result of aerobic decomposition of organic matter in the sediments, particularly in the three southernmost rivers, where the total C content was high in the sediments (Table 1). This probably resulted in P release from the organic and Fe-bound pools in the sediments, as described above. Under such anoxic conditions, organic matter can contribute as a carbon source to support denitrifying and methanogenic bacteria. The higher dNp in the sediments and the higher dissolved CH₄ concentration in sediments from the three southernmost rivers (Fig. 3, 4) during summer and autumn would result from the higher C contents in their sediments. The organic matter content of soils and sediments is a powerful predictor of *in situ* denitrification activity in riverbed sediments and riparian zones (Hayakawa et al., 2012; Inwood et al., 2005; Ullah and Faulkner, 2006). The dissolved CH₄ concentration at the all sample sites was extremely higher than that dissolved in distilled water equilibrated with air contains (0.031 µg C L⁻¹) (Fig. 4). Rice paddy is recognized as a dominant methane source (Kögel-Knabner et al., 2010) and is the main land use of agricultural land at each catchment in the present study, therefore, rice paddy would be a primary important source of the dissolved CH₄ in the river water as an external source, but in summer

when the effect of external loading from paddy became weak since irrigation water was stored in the paddy field, relative CH₄ source might be changed to sediments. A higher C content and a higher C/N ratio (Burgin and Hamilton, 2007; Nizzoli et al., 2010) and higher S content (Burgin and Hamilton, 2007) in sediments can enhance DNRA. The higher C and S contents, and higher C/N ratio in the riverbed sediments (Table 1) suggested that the higher potential DNRA in the three southernmost rivers might explain NH₄ source in the river water. We found significant positive correlations between the SRP concentration in the water near the river bottom and both the sediment dNp and the dissolved CH₄ concentration (Table 2). Zhang et al. (2015) reported that under warm and reducing conditions, microbes in the sediment showed increased expression of the genes encoding nitrate and nitrous oxide reductases to support denitrification, and increased levels of exopolyphosphatase, which supports polyphosphate degradation. Our results also suggest that SRP released from the sediments into pore water was coupled with increased activities of denitrifying and methanogenic bacteria in the sediments, resulting in higher dNp and a higher dissolved CH₄ concentration. However, it will be necessary to extend the measurements to clarify the interactions between SRP released from the sediments and microbial activities under anoxic conditions in the sediments, and their impacts on water quality.

4.3 Effect of watershed characteristics and the lake's structure on sediment qualities

Watershed characteristics and the lake's structure would control the spatial distribution of sediment inputs and quality, including the P fractions and C content in water and sediments at the mouths of the rivers. The high Ca-P content in the sediments of the BBM river (Fig. 6) might reflect its watershed geology. In the middle reaches of

the BBM river, there is a tributary with a high calcium concentration in the river water and riverbed sediments, likely because of the presence of calcareous sedimentary rock (Hayakawa et al., unpublished data), which would contribute to the higher Ca-P content in the sediments. A high sedimentation rate is a dominant factor for burial of P in sediments (Lukkari et al., 2008). A large allochthonous particulate P was transported during a single rain event to a lake and the particulate P content depended on particle size of suspended solids and increased with decreasing particle size (Pacini and Gächter, 1999). The MTN river, which had the lowest P and C contents in the sediments (Table 1, Fig. 6), is frequently in flood, and the available P and C provided from the watershed might have been buried in coarse sediments with lower P and C contents.

The lake's structure may also control sediment quality by restricting water movement. In Lake Hachiro, water movement to the center of the original lake and the mouths of the three southernmost rivers was restricted by the land reclamation and the floodgate that is only one water outlet in the lake (Sasaki, 2010; Fig. 1). The restriction would have contributed to organic matter accumulation in the sediments, which would easily create hypoxic conditions in the water near the river bottom, resulting in higher SRP concentration and lower DIN/SRP ratio in the three southernmost rivers (Fig. 2(b)). The trophic status in the river water will also affect the balance among the different P fractions in the sediments. Previous studies reported that in lakes with a more eutrophic status, the dominant P fraction in the sediments was Fe-bound P (Kaiserli et al., 2002; Ribeiro et al., 2008).

4.4 Biogeochemical N and P cycles and algal boom in Lake Hachiro

Biogeochemical N and P cycles between the sediments and the water can

change the N/P ratio in the water column, and this will affect the likelihood of an algal or cyanobacterial bloom in the water. The atomic ratios of nutrients in algal cells and in the water provide useful clues to the interactions between algae and nutrients (Hecky and Kilham, 1988). For N and P, the ratio is known as the Redfield ratio, for which $N/P \approx 16$ (Redfield, 1958). However, Klausmeier et al. (2004) noted that the optimal ratios ranged from 8.2 to 45.0, depending on the ecological conditions. Our results show that increased SRP caused a decreasing DIN/SRP ratio in the water column, and that DIN/SRP approached the range reported by Klausmeier et al. (2004) during summer and autumn, especially in the three southernmost rivers (Fig. 2(b), Table 2). In Lake Hachiro, blooms of cyanobacteria occur every summer (Kondo, 2010; Okano et al., 2015). Such blooms are favored high temperature ($25\text{ }^{\circ}\text{C}$), Okano et al., 2015) and under high nutrient loading rates, particularly if the availability of N is limited relative to the availability of P because of the ability of cyanobacteria to fix N_2 (e.g., Paerl and Tucker, 1995; Moisander et al., 2003). Our results suggest that the potential for cyanobacterial blooms would be higher near the mouths of the three southernmost rivers, which had higher concentrations of Fe-bound P (Fig. 6) and sediments rich in organic matter (Table 1).

5. Conclusions

The present study demonstrated the importance of the biogeochemical interactions between N and P in sediments at the mouths of the main rivers that enter Lake Hachiro. These interactions help to explain the spatial and temporal variations in river water quality. The dominance by Fe-bound P and the presence of sediments rich in organic matter in some rivers resulted in spatial and temporal variations in river water

quality as a result of higher SRP release and denitrification rates under anoxic conditions during summer, resulting in a lower DIN/SRP ratio in the river water and may trigger algal or cyanobacterial blooms in the lake. Especially in a shallow eutrophic lake such as Lake Hachiro, the watershed characteristics and lake structure will also control sediment quality and redox conditions, both of which affect the spatial and temporal variations in river and lake water quality. The present study revealed higher priorities in the three southernmost rivers for managers to improve water quality. Weaknesses of the present study include a lack of data on microbial activity in the sediments, and insufficient details and analysis of watershed characteristics (e.g., geology, hydrology, topography, and land use etc.). Future research should investigate the effects of these factors.

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- 3

Figure captions

Figure 1 Location of the Lake Hachiro watershed, in Japan's Akita Prefecture, and the land-use distribution and locations of the sampling sites in the five main rivers that enter the lake.

Figure 2 Temporal dynamics of precipitation and water quality parameters in (a) the surface water and (b) water near the river bottom at the five sampling sites. DO, dissolved oxygen content; DIN/SRP, ratio of dissolved inorganic nitrogen to phosphate. In the graphs of DIN/SRP, the horizontal line represents the Redfield ratio (= 16).

Figure 3 Box plots of (a) $\text{NO}_3^- \text{-N} + \text{NO}_2^- \text{-N} + \text{NH}_4^+ \text{-N}$ (DIN), (b) soluble reactive phosphate (SRP), (c) the DIN/SRP atomic ratio, (d) dissolved N_2O (N_2O), (e) dissolved CH_4 (CH_4) in river water, and (f) sediment denitrification potential (dNp) during May to December in 2012. Maximum and minimum values indicate extremes; horizontal lines represent the median, and bars represent the 25% and 75% percentiles.

Figure 4 Temporal dynamics of dissolved N_2O (N_2O) and dissolved CH_4 (CH_4) in the water near the river bottom, and of sediment denitrification potential (dNp), at the five sampling sites.

Figure 5 The results of the incubation experiment (P released as phosphate) with the sediments from the five sampling sites. (a) Anoxic conditions at 25 °C, (b) anoxic

conditions at 10 °C, (c) oxic conditions at 25 °C, and (d) oxic conditions at 10 °C.

Figure 6 The amounts and distribution of the inorganic P fractions in the riverbed sediments at the five sampling sites.

Figure 7 The relationships between median SRP concentration under anoxic conditions at 25 °C and (a) Fe-P, (b) Al-P, (c) Soluble-P, and (d) Ca-P in the sediment in the incubation experiment.

Figure 8 The results of the incubation experiment with the sediment from the TYK. (a) SRP concentration, (b) Fe concentration.

Table 1 Catchment size and water depth for the five main rivers that enter Lake Hachiro, and key properties of the sediments at the river mouths.

Parameter	MTN	BBM	IKW	TYK	BFM
<i>Catchment</i>					
Catchment area, km ²	151	207	53	25	36
Mean water depth, cm	290	230	240	150	160
<i>Sediment (July 2012)</i>					
Particle size	sandy	sandy	silty	silty	clayey
pH	6.27	6.49	5.93	5.84	6.34
EC, mS m ⁻¹	2.1	1.9	2.4	3.2	9.0
T-C, g kg ⁻¹	1.24	1.64	15.8	15.6	36.1
T-N, g kg ⁻¹	0.16	0.16	1.18	1.20	3.01
T-P, g kg ⁻¹	0.21	0.49	0.71	0.67	1.24
Inorg-P, g kg ⁻¹	0.13	0.41	0.46	0.37	0.46
Org-P*, g kg ⁻¹	0.08	0.08	0.25	0.31	0.77
T-S, g kg ⁻¹	0.04	0.10	0.53	0.66	1.24
C/N	7.8	10.0	13.4	13.0	12.0

*Org-P was calculated by the difference between T-P and Inorg-P

1 Table 2 Spearman's rank correlation coefficients for the relationships between river water quality parameters, dissolved gas concentrations
2 (N₂O, CH₄), and the sediment denitrification potential (dNp) at the mouths of the five main rivers that enter Lake Hachiro ($n = 40$).
3

	Water temp.	SRP	NH ₄ -N	NO ₃ -N	DIN/SRP	CH ₄	N ₂ O	dNp
<i>River water quality (water near the river bottom)</i>								
Water temp.	—	−0.043	0.234	−0.498 **	−0.232	0.059	−0.484 **	0.070
SRP		—	0.280	0.232	−0.846 **	0.370 *	0.138	0.540 **
NH ₄ -N			—	−0.075	−0.250	0.349 *	0.161	0.492 **
NO ₃ -N				—	0.249	0.067	0.639 **	0.117
DIN/SRP					—	−0.326 *	0.258	−0.448 **
<i>Dissolved gas (water near the river bottom)</i>								
CH ₄						—	0.144	0.475 **
N ₂ O							—	0.294
<i>Sediment</i>								
dNp								—

4 Significance levels: *, $P < 0.05$; **, $P < 0.01$
5 DIN/SRP, the ratio of dissolved inorganic nitrogen to phosphate.

Figure

Fig. 1 hayakawa_catena

Fig. 3 hayakawa_catena

Fig. 4 hayakawa_catena

Fig. 5 hayakawa_catena

Fig. 6 hayakawa_catena

Fig. 7 hayakawa_catena

Fig. 8 hayakawa_catena