Subarctic wintertime dissolved iron speciation driven by thermal constraints on Fe(II) oxidation, dissolved organic matter and stream reach

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Abstract

We studied the seasonal variations in Fe(II), Fe(III), humic-like dissolved organic matter (DOM), nitrate and nitrite (NO$_3$+NO$_2$), and silicate (Si(OH)$_4$) in river waters of three subarctic rivers flowing into Hakodate Bay in southwestern Hokkaido, Japan from May 2010 to February 2014. High Fe(II) concentrations were detected in winter at the sampling sites where the river bottom was comprised of sandy or silty sediment, primarily the lower and middle reaches of the rivers. Conversely, from early spring to late autumn Fe(II) levels were low or undetectable. We infer that soluble Fe(II) concentration in these subarctic river waters is driven by the balance between the influx of Fe(II) to the river and the Fe(II) oxidation rates that determines the dynamics in Fe(II) concentration in the river water. The Fe(II) may originate from reductive dissolution of Fe(III) in the river sediment or from Fe(II)-bearing groundwater. The latter seems to be the most likely source during winter time. The high Fe(II) concentrations during winter is predominantly attributed to the extremely slow oxidation rate of Fe(II) to Fe(III) at low water temperature rather than to an actual increase in the flux of reduced Fe(II). Nevertheless, we propose that the flux of reduced Fe(II) from river sediments and groundwater in lowland area of the catchment to overlying river waters might be the most important sources of iron in river waters. This provides an important insight into the role of river processes and the interaction between climate and river morphology in determining the inputs of iron to subarctic coastal marine waters.
1. INTRODUCTION

Iron is one of the most important micronutrients for phytoplankton growth in oceans and coastal waters. The sources for iron in these ecosystems are rivers, the atmosphere, shelf sediments, and submarine groundwater discharge. Many studies highlight the potential importance of sedimentary iron sources for coastal waters and even the open ocean (Croot and Hunter, 1998; Elrod et al., 2004; Johnson et al., 1999; Lam et al., 2006; Ussher et al., 2007; Hurst et al., 2010; Severmann et al., 2010). Recently, it has been reported that the release of Fe(II) from reducing sediments of the continental shelf is an important source of iron in coastal waters (Lohan and Bruland, 2008; Homoky et al., 2012a, b; Noffke et al., 2012; Hioki et al., 2014 and 2015; Chever et al., 2015).

Enrichment of iron in pore fluids and bottom waters is driven by the reductive dissolution of insoluble Fe(III) during the decomposition of organic matter in the sediment (Pakhomova et al., 2007; Noffke et al., 2012; Chever et al., 2015). However, our understanding of the biogeochemical and physical mechanisms that regulate iron, humic-like dissolved organic matter (DOM) and nutrients in river and lake waters is still limited although there are a lot of literature on the these mechanisms including redox processes and reductive dissolution of insoluble Fe(III) and nutrient dynamics in lakes, rivers, and wetlands (e.g., Shenker et al., 2005; Maassen and Balla, 2010).

Sediments can serve as sink or source with respect to iron and nutrients in the water column. The oxidation–reduction state (redox potential) of sediment is an important parameter affecting iron speciation and solubility (Stigliani, 1988). Anoxic conditions in sediment can promote a change from insoluble Fe(III) to soluble Fe(II), resulting in a flux of reduced Fe(II) from the river bottom sediment and groundwater to the water column. Conversely, oxidizing conditions in the water column oxidize soluble Fe(II) to insoluble Fe(III) by the formation of Fe(III) hydroxide (Baes and Mesmer, 1976; Millero et al., 1987; Millero and Sotolongo, 1989; Gonzalez-Davila et al., 2006;
In this study, we measured seasonal variations in river water of iron (Fe(II), dissolved Fe (D-Fe), and total Fe (T-Fe)), humic-like DOM measured as humic-like–fluorescence intensity (humic-F intensity), nitrate and nitrite (NO$_3$+NO$_2$), and silicate (Si(OH)$_4$) in three subarctic rivers in southern Hokkaido, Japan. The three rivers were in close proximity to one another but displayed some differences in catchment land use and riverbed composition (predominance of gravel-cobble-boulder, sand, or silt). Our goal was to identify factors controlling the seasonal variations in iron (primarily Fe(II) and T-Fe), humic-like DOM, NO$_3$+NO$_2$, and Si(OH)$_4$ in these subarctic river waters in relation to the redox conditions of the sediment–water interface.

2. MATERIALS AND METHODS

2.1. Study area and sample collection

The study area (Fig. 1) is located in southwestern Hokkaido, which is usually snow-covered from the middle of December to early March in the coastal lowlands and from early November to the middle of May in mountainous areas. Daily average air temperatures in winter (December–March) in the lowlands for 2010–2014 ranged from 0 to –6°C, with minimum temperatures being in the range of –10 to –20°C. For the same period, the daily average air temperatures during summer (June–September) were 15–24°C with maximum temperatures being in the range 26–33°C. Historical daily air temperature data were obtained from the daily climate normals in 2010–2014 for Hokuto, Hokkaido (Japan), close to the sampling stations, which were supplied by the Japan Meteorological Agency.

The rivers that were the subjects of the study were the Kunebetsu, the Oono, and the Moheji, all of which flow into Hakodate Bay in southwestern Hokkaido, Japan (Fig. 1, Table 1). The Kunebetsu River has a watershed of 123 km$^2$ and possesses several large
tributaries, which are probably influenced by the inflow of drainage from paddy fields.

The lower and middle reaches of the river pass through a large agricultural plain (vegetables and paddy fields). The Oono River has a watershed of 111 km$^2$ and no distinct tributaries, and although the lower and middle reaches pass through an agricultural plain (mainly vegetables fields), there is extremely less influence of drainage inflow. The Moheji River originates in a mountainous area and has the smallest watershed, 96 km$^2$, which comprises mostly steep hills covered in temperate deciduous forest with a small agricultural plain at the lower reach. The riverbed conditions at the lower-middle reaches of the Moheji River are remarkably different from those of the other two rivers (Table 1).

Sampling of the river water was conducted once or twice per month under low and normal river flow conditions from May 2010 to February 2014 at a sampling location in the lower reach (approximately 250–500 m far from the sea) of each of the three rivers. The sampling sites in the lower reach of the Kunebetsu, the Oono, and the Moheji Rivers are KR-1, OR-1, and MR-1, respectively (Fig. 1). Salinity of all river water samples, which were measured by a salt tester (Salt Testr 11, Eutech Instruments), was below 0.20 g/1000g. Therefore, the sampling locations were free of tidal and brackish water influences. An additional series of sampling was conducted, also once or twice per month in low and normal river flow, from May 2013 to February 2014 at locations in the lower (OR-1 and OR-2), middle (OR-3), and upper (OR-4 and OR-5) reaches of the Oono River (Fig. 1).

We collected surface water samples from the river center (maximum bottom depth: 1–2 m) from bridges using an acid-cleaned 5 L high-density polyethylene sampling bucket lowered to the water surface by rope. Approximately 10 mL of the sample was immediately (within 30 s of collection) transferred for on-site Fe(II) analysis into an acid-cleaned 15 mL polypropylene tube containing 200 µL of 0.01 mol ferrozine solution [0.514 g of ferrozine per 100 mL of Milli-Q water ($\rho = 18.2$ MΩ·cm)]. The
remaining water was used to fill acid-cleaned 1 L low-density polyethylene bottles for
laboratory analyses of iron (D-Fe and T-Fe), NO$_3$+NO$_2$, Si(OH)$_4$, and humic F-intensity.
Acid-cleaning the sampling bucket, bottles, and tubes was carried out by soaking for 3
days with 3 M hydrochloric acid and then washing with Milli-Q water.
The 1 L bottles containing water samples were immediately placed into an insulated
cooler box containing ice packs and promptly taken back to the laboratory and
immediately processed as follows: The water samples for D-Fe, NO$_3$+NO$_2$, Si(OH)$_4$,
and humic F-intensity were filtered through acid-cleaned 0.22 µm nitrocellulose
membrane filters (GSWP04700, Merck Millipore). The filtered samples (7–8 mL) for
NO$_3$+NO$_2$ and Si(OH)$_4$ were placed in 10 mL acrylic tubes and immediately frozen and
kept below –20°C in the dark until analyzed. The remaining filtered samples were used
for analyses of D-Fe and humic F-intensity. Unfiltered samples were used for T-Fe
analysis.

2.2. Determination of iron speciation
On-site Fe(II) concentrations were determined spectrophotometrically in the field
within 1 min of collection using the modified ferrozine method (Stookey, 1970; Kuma
et al., 1992 and 1995). The absorbance of the Fe(II)-ferrozine complex was measured at
a fixed wavelength of 560 nm with a 1 cm glass cuvette against Milli-Q water by a
compact photometer (model AP-1000M, APEL Co. Ltd., Saitama, Japan). The
absorbance was corrected by subtracting the absorbance of a pure river water sample,
i.e., without ferrozine water solution and reagent blank for ferrozine. The method
detection limit of Fe(II) is estimated to 0.2 µmol L$^{-1}$ with a 1 cm cuvette. The relative
standard deviation ($n = 5$) was 3.0% at the 2 µmol L$^{-1}$ level.
In the laboratory, D-Fe (filtered samples) and T-Fe (unfiltered samples)
concentrations were determined using the ferrozine method (Stookey, 1970). Briefly,
for both types of sample, 10–50 mL of sample was transferred to a 125 mL Erlenmeyer
flask and 1 mL of acid reagent solution was added. The acid reagent solution consisted of 514 mg of ferrozine, 10 g of hydroxylamine hydrochloride as a reducing reagent, and 50 mL of concentrated hydrochloric acid (about 12 mol L\(^{-1}\)) per 100 mL of solution (Stookey, 1970). The acidified solution was heated in a flask on a hot plate and held at the boiling point for 10 min. Consequently, the solution lost approximately 5 mL of water via the boiling. After the solution was cooled to room temperature, 1 mL of ammonium acetate buffer solution (40 g of ammonium acetate and 35 mL of concentrated ammonium hydroxide (about 14 mol L\(^{-1}\)) per 100 mL of solution (pH 5.5), Stookey, 1970) was added. The buffered solution was then quantitatively transferred to a 50 mL volumetric flask and the contents diluted to the mark with Milli-Q water. The absorbance was measured at 562 nm in 1 cm or 5 cm glass cuvettes by spectrophotometer (model U-2001, Hitachi Co. Ltd., Japan). The absorbance for D-Fe was corrected by subtracting those of the filtered water and reagent blank, and the absorbance of T-Fe was corrected by subtracting those of unfiltered river water and reagent blank. However, the acid reagent solution may be responsible for dissolving particles present in the unfiltered river water blank leading to reduced apparent absorption due to light scattering. The spectrophotometer was calibrated at Fe concentration in the range of 0–25 µmol L\(^{-1}\) using ferrous ammonium sulfate standard solutions prepared in acidified water. In addition, we subjected the 0.22 µm filtered water samples obtained in 2010–2012 to further filtering through 0.025 µm pore size nitrocellulose membrane filter (VSWP, Millipore) to determine the soluble Fe(III) (<0.025 µm fraction) concentrations. The data obtained are not shown in detail because the soluble Fe(III) values were extremely low—below the detection limit to ~0.1–0.3 µmol L\(^{-1}\), which is < ~3% of T-Fe(III) (T-Fe minus Fe(II)). Several studies have reported the limitations of the ferrozine method for determining Fe(II) in the presence of very high soluble Fe(III) (Anastacio et al., 2008; Gendel and Lahav, 2008). Soluble Fe(III) in solution can react with ferrozine, thereby interfering
with the coloration of the Fe(II)-ferrozine complex (Viollier et al., 2000). However, measuring dissolved Fe(II) in Fe(II)-Fe(III) mixture solution with low soluble Fe(III), such as river waters (pH of 6–8) in the present study, by ferrozine would not lead to the quantitative overestimation of Fe(II) concentrations (Viollier et al., 2000; Giokas et al., 2002). Reduction of the Fe(III)-ferrozine complex with time in the presence of humic substances could potentially lead to an artifact. However, the time required for complete complexation with free Fe(II) by ferrozine would be within 1 min, while the slow reduction of Fe(III) after ferrozine addition can take up to several minutes-hours. In our control experiments, ferrozine did not lead to overestimation of Fe(II) concentrations by measuring dissolved Fe(II) in Fe(II)-Fe(III) mixture water solution (pH of 7–8).

However, Fe(II) concentrations in river water samples from the lower reach site (KR-1) of the Kunebetsu River gradually increased with time (0 µmol L$^{-1}$ within 1 min to 0.1–0.4 µmol L$^{-1}$ after 1 hr) in the dark after ferrozine addition. Measurements were completed within 1 min in the present study; therefore too little Fe(III) reduction could have occurred after ferrozine addition to have caused overestimation of Fe(II) concentration. In addition, natural organic Fe(II) ligands may appear to be either too weak or too low in concentration to affect the recovery of Fe(II) in natural waters. In natural waters rich in DOM, however, Fe(II) may be present as a range of organic complexes or colloids that will equilibrate more slowly with ferrozine over time (Box, 1984) and the association of DOM with dissolved Fe may prevent the accurate measurement of D-Fe concentrations (Hopwood et al., 2014).

2.3. Experiments to quantify the in-situ oxidation rate of Fe(II) in river water

To determine the in-situ oxidation rate of Fe(II) at the in-situ water temperatures of winter, in the midday during early–mid winter of 2012/2013 (mid-November 2012–mid-January 2013), we measured oxidation of on-site Fe(II) in river water samples from the lower reach of the Kunebetsu River (KR-1, Fig. 1). Just after the
measurement of on-site Fe(II) concentration (0 min), approximately 10 mL samples were withdrawn from the sample vessel at 5, 10, 15, 20, 25, 30, 40, 45, and 60 min in the shadow and analyzed in exactly the same manner as for in-situ Fe(II). The water temperature of the sample vessel was regulated to within ±0.5°C of the in-situ water temperature throughout the 30–60 min of the oxidation experiment measurements. In addition, the photoreduction of Fe(III) was measured in river water samples (pH 7.6) with high Fe concentration that were collected from the lower reach of the Kunebetsu River (KR-1) during summer. The experiment was conducted under natural sunlight illumination at 5 and 10°C for 2–3 h in the same manner described in our previous studies (Kuma et al., 1992 and 1995). Because we did not detect any photo-reduced Fe(II) in this analytical method with nM level detection limit, the data are not shown.

2.4. Humic F-intensity, nitrite and nitrite, and silicate

Humic-like DOM in river water was quantified by measuring humic F-intensity. The humic F-intensity of the 0.22 µm-filtered river-water samples was measured in a 1 cm quartz cell by means of a fluorescence spectrophotometer (model F-2000, Hitachi Co. Ltd., Japan) at 320 nm excitation and 430 nm emission wavelengths and 10 nm bandwidths (Nagao et al., 2003). Relative fluorescence intensity (RFI) of the samples was expressed in terms of quinine sulfate units (10 QSU = 10 ppb quinine sulfate in 0.05 mol H2SO4, excitation 320 nm, emission 430 nm; Nagao et al., 2003). The monitoring wavelengths correspond to the position of the fluorescence maxima for fulvic acid, a major component of humic substances in river waters, and the humic F-intensity for fulvic acid at the concentration of 10 mg L⁻¹ was 80 QSU (Nagao et al., 2003).

The concentrations of NO₃⁻+NO₂⁻ and Si(OH)₄ were determined by means of an autoanalyzer (Technicon Corporation) using CSK standard solutions for nitrate and nitrite (Wako Pure Chemical Industries, Ltd., Japan), silicofluoride (Na₂SiF₆) standard
solution for silicate, and standard methods (Parsons et al., 1984).

In addition, pH and water temperature were immediately measured using a handheld pH meter (model D21, Horiba Co. Ltd., Japan) and a thermometer after collecting river water samples in the 5 L sampling bucket.

3. RESULTS

3.1. Seasonal patterns in water temperature and discharge

The river water temperature in the lower reaches of the three rivers displayed similar patterns of seasonal variation (Fig. 2A). These temperatures were at their lowest (almost 0°C) in January–February and then gradually increased to peak at 18–23°C in August before commencing the decline towards the winter low (Fig. 2A).

The seasonal river discharge patterns of the three rivers were very similar (Fig. 2B). Monthly average water discharge of the Moheji River was two-fold to four-fold higher than that of both the Oono and Kunebetsu rivers (Table 1, Fig. 2B). All three rivers displayed seasonal patterns in discharge characterized by peak flows occurring due to snow melt from mid March to early May (spring flood period), tapering through to the fall, and occasional high discharges caused by rainfall during the summer through autumn period. From late fall, repeated snowing and melting in mountainous areas during mid November to mid December caused an increase in discharge until consistent snow cover and freezing occurred from mid December until early March (winter period), during which time discharge remained low.

The riverbed of the Moheji River comprises gravels, cobbles, boulders, and/or exposed bedrock throughout its entire length, whereas the riverbeds of the Kunebetsu River and Oono River are mainly composed of sandy and silty sediments throughout the lower and middle reaches (Table 1). Having the smallest watershed and highest water discharge, the Moheji River would have flows of remarkably higher energy, preventing
the deposition of fine inorganic and organic particles on the river bottom. The Moheji River in a large forested mountainous area with high precipitation slopes down steeply to the lower reach with large inflow of rainfall, groundwater and snow melt from many small valleys at the lower–upper reaches.

3.2. Seasonal variations in iron and humic F-intensity at the lower reach sites

The seasonal variations in Fe(II) at the lower reach sampling sites of the Oono River and Kunebetsu River displayed a very similar pattern over the period May 2010 to February 2014—high concentrations during winter and remarkably low or undetectable concentrations during spring–autumn (Fig. 2C). The higher Fe(II) concentrations (~10–17 µmol L⁻¹) tended to be strongly associated with low water temperature (0–4°C) during December–early March, whereas the extremely low or undetectable Fe(II) concentrations were associated with water temperatures higher than 7–8°C during spring to autumn (Figs 2A and 2C). During winter, Fe(II) concentrations were approximately two to three times higher in the Kunebetsu River than in the Oono River. The seasonal variations in D-Fe in the Oono River and Kunebetsu River displayed very similar results to those of Fe(II), with concentrations being high during winter, low during spring–autumn, and higher in the Kunebetsu River than in the Oono River (Figs 2C and 2D). Although there was no clear seasonal variation in T-Fe (Fig. 2E), seasonal variation in the Fe(II)/T-Fe ratio (as a percentage) in the Oono River and Kunebetsu River was evident, with remarkably high values of 20–60% during winter and extremely low values during summer, and similar in percentage and pattern between the two rivers (Fig. 2F). In contrast, Fe(II), D-Fe, and T-Fe concentrations in the Moheji River were extremely low or undetectable at all times (Figs 2C–F).

The seasonal variations in humic F-intensity displayed a pattern that was the reverse of that for Fe(II), D-Fe, and Fe(II)/T-Fe, that is, peak values in summer that were approximately two to three times higher than in autumn, winter, and spring (Fig. 2G).
The humic F-intensity values in the Kunebetsu River were about two to three times higher than those in the Oono River, whereas the Moheji River yielded consistently lower values than the Oono River, and which showed very little seasonal variation (Fig. 2G).

3.3. Seasonal variations in iron, humic F-intensity, and nutrients in the lower, middle, and upper reaches of the Oono River

The sampling of the Oono River from May 2013 to February 2014 was divided into five locations representing the lower (OR-1 and OR-2), middle (OR-3), and upper (OR-4 and OR-5) reaches of the river. The seasonal variation in Fe(II) was similar in pattern at the two lower and one middle reach sampling sites—high concentrations (~1–5 µmol L\(^{-1}\)) during winter and extremely low or undetectable concentrations during early spring–late autumn (Fig. 3A). The Fe(II) concentrations at the two lower reach sites tended to increase gradually from early November to late January with very similar concentrations and seasonal patterns. The winter values at the middle reach site (OR-3) were approximately half those at the lower reach sites (OR-1 and OR-2). The Fe(II) concentration values at the two upper reach sampling sites (OR-4 and OR-5) were extremely low or undetectable in all seasons (Fig. 3A).

The changes in D-Fe concentration from November to February were similar to those of Fe(II) during the same period (Figs 3B and 3C). During the warmer months (June–August), however, D-Fe and T-Fe concentrations were relatively high, either similar to or higher than those during winter, and behaved markedly different to the Fe(II) concentrations, which stayed constantly low during summer (Figs 3A–C). At the middle reach sampling site (OR-3), D-Fe during winter and T-Fe in all seasons were approximately half those at the lower reach sites (OR-1 and OR-2).

The Fe(II)/T-Fe ratio at the lower and middle reach sampling sites was high (20–50%) during winter and extremely low during summer with similar seasonal
patterns between the values of the Kunebetsu River and Oono River (Fig. 2F) or
between the values of the lower and middle reach samples of the Oono River (Fig. 3D).
The Fe(II)/T-Fe ratio values at the upper reach sites were extremely low throughout the
whole sampling period (Fig. 3D). Despite the remarkably different Fe(II) and T-Fe
levels between locations, the seasonal patterns in the Fe(II)/T-Fe ratio appear to be
spatially independent.

Humic F-intensity was approximately two to three times higher during early summer
(June–July) than in other seasons at all sampling sites of the Oono River (Fig. 3E).
During early summer, the order of humic F-intensity values was: lower reach (OR-1 =
OR-2) > middle reach (OR-3) > upper reach (OR-4 = OR-5). The humic F-intensity
values in the lower reaches were about two times those of the middle reach and four
times those of the upper reach.

The seasonal variations in NO$_3$+NO$_2$ in the samples from the lower reach (OR-1 and
OR-2) and middle reach (OR-3) sites of the Oono River displayed very similar
patterns—slightly elevated concentrations (~30–40 µmol L$^{-1}$) during the winter and low
concentrations (~20–25 µmol L$^{-1}$) during spring–autumn (Fig. 3F). However, in the
upper reach (OR-4 and OR-5), NO$_3$+NO$_2$ values were approximately half to one-third
(~15 µmol L$^{-1}$ during summer–winter and ~5–10 µmol L$^{-1}$ during spring–summer)
those in the lower and middle reaches (Fig. 3F). The order of the various sampling
locations with respect to the magnitude of NO$_3$+NO$_2$ concentrations in the three rivers
was: lower reach site (KR-1) of the Kunebetsu River > the lower reach sites (OR-1 =
OR-2) of the Oono River > the middle reach site (OR-3) of the Oono River > the upper
reach sites (OR-4 = OR-5) of the Oono River = the lower reach site (MR-1) of the
Moheji River.

Si(OH)$_4$ concentrations displayed similar seasonal patterns at all locations, with
concentrations being 5–7 times higher during the early summer–early winter period
(early June–early December) than during the middle winter–late spring period. The
order of the sampling sites with respect to the magnitude of Si(OH)$_4$ values was the
Kunebetsu River (KR-1) = the Oono River (OR-1–OR-5) > the Moheji River (MR-1)
(Fig. 3G). There were few or no differences among the lower, middle, and upper
reaches (OR-1–OR-5) of the Oono River with respect to seasonal Si(OH)$_4$
concentrations.

3.4. Oxidation rate of in-situ Fe(II) in river water

The most commonly verified rate law for oxidation of Fe(II) at near neutral pH over
the range of natural waters is that of Millero et al. (1987),

\[-d[\text{Fe(II)}]/dt = k[\text{OH}^-]^2p\text{O}_2[\text{Fe(II)}]\]  \hspace{1cm} (1)

where \(k\) is the rate constant with units mol$^{-2}$ atm$^{-1}$ min$^{-1}$, [OH$^-$] is the concentration of
hydroxyl ions, and [Fe(II)] is the concentration of total ferrous iron. At fixed pH and in
the presence of excess pO$_2$, equation (1) reduces to a pseudo first-order rate equation,

\[-d[\text{Fe(II)}]/[\text{Fe(II)}] = k_{\text{ox}} \, dt\]  \hspace{1cm} (2)

where \(k_{\text{ox}}\) is the pseudo-first-order rate constant and has units of inverse time.

The value of \(k_{\text{ox}}\) is strongly dependent upon pH and temperature. From our oxidation
rate experiment, we determined the oxidation rate constant of on-site Fe(II) in water
samples from the slope of the natural log of Fe(II) concentration against time using
least-squares linear regression from samples with in-situ water temperatures of 7.5, 4,
1.5, and 0.5°C (± 0.5°C) and pH in the range of 7.3–7.8. The oxidation rate constants
\((k_{\text{ox}})\) and half-lives \((T_{1/2})\) of Fe(II) obtained for each temperature are presented in Figure
4A. Low temperature resulted in an extremely low rate of Fe(II) oxidation, with \(T_{1/2} =
187\) min at near freezing temperature (0.5±0.5°C); an increase in temperature to just
1.5°C resulted in a reduction in $T_{1/2}$ to 62 min. Clearly, the oxidation rate was severely retarded as temperature approached 0°C.

The natural log of the $k_{ox}$ values from the rate equations at the four water temperatures were plotted against 1/T (T: absolute temperature in K; Fig. 4B). From the linear relationship between logarithmic $k_{ox}$ and 1/T and the Arrhenius equation (Brezonik, 1994), we can estimate the fairly rough values of $k_{ox}$ and $T_{1/2}$ at various water temperatures (for example, the values from 0 to 20°C in Fig. 4B). The estimated value (10.1 min$^{-1}$, Fig. 4B) of $k_{ox}$ in river water at 20°C in this study was roughly similar to those (approximately 1.3–5.6 min$^{-1}$) in water (20.5°C) at pH in the range of 7.5–8.0 in previous study (Stumm and Lee, 1961; Millero, 1985).

4. DISCUSSION

4.1. Sources of Fe(II) in river water

The detection of high Fe(II) concentrations during winter and low or undetectable concentrations during early spring–late autumn occurred only at the sampling sites where the river bottom comprises sandy and silty sediments—the lower reach sampling site (KR-1) of the Kunebetsu River and the lower and middle reach sites (OR-1–OR-3) of the Oono River (Figs 2A, 2C, and 3A, Table 1). In contrast, Fe(II) was low or undetected at all times of the year at sites where the riverbeds comprised gravels, cobbles, boulders, and/or exposed bedrock—the lower reach sampling site (MR-1) of the Moheji River and the upper reach sites (OR-4 and OR-5) of the Oono River (Figs 2A, 2C, and 3A, Table 1). The similarity in seasonal variations of Fe(II) and D-Fe in the Kunebetsu River and Oono River (higher Fe concentrations during winter than spring–autumn; Figs 2C, 2D, 3A, and 3B) is probably because of Fe(II) in D-Fe (the <0.22 µm fraction) during winter that was not oxidized to Fe(III) prior to filtration in the laboratory. D-Fe (the <0.22 µm fraction) in this study could still have potentially
contained Fe(II) that was not oxidized to Fe(III) until filtration in the laboratory.

Recent many studies have indicated that the release of Fe(II) from reducing sediments of the continental shelf might be an important source of iron in coastal waters (Pakhomova et al., 2007; Lohan and Bruland, 2008; Homoky et al., 2012a, b; Noffke et al., 2012; Chever et al., 2015). This may be the case in the sediments of lakes and rivers (Stigliani, 1988; Miao et al., 2006). Changes in sediment redox potential (Eh) and pH can cause changes in iron speciation and solubility, which can subsequently influence iron flux from river sediment to the water column. In anaerobic sediment, insoluble Fe(III) is reduced to water-soluble Fe(II) through microbial utilization of the oxygen contained in particulate Fe(III) oxides (Stigliani, 1988; Guo et al., 1997). In addition, it has been reported that microbial reduction of humic acids and subsequent chemical reduction of poorly soluble Fe(III) minerals by the reduced humic acids represents an important path of electron flow in anoxic natural environments such as freshwater sediments (Lovley and Blunt-Harris, 1999; Kappler et al., 2004).

In the present study, the higher Fe(II) concentrations during winter and the higher T-Fe concentrations in the Kunebetsu River than the Oono River (Figs 2C and 2E) may be due to the discharge of Fe(II)-bearing drainage water and groundwater to the surface water. The paddy fields in the lowlands of the catchments of the Kunebetsu River probably suggest reducing conditions in the soil/groundwater domain. In addition, the lack of Fe(II) and T-Fe in the river water of reaches with gravel and rock riverbeds indicates that the generation of soluble Fe(II) in the water column is probably driven by the reductive dissolution of insoluble Fe(III)—such as Fe(III) hydroxide and Fe(III) oxide—during the microbial decomposition of organic matter in reducing river sediments. However, the microbial reductive dissolution of insoluble Fe(III) in river sediments could be extremely slow at low temperature during winter. Therefore, it may also be due to the chemical composition of the groundwater discharging to the stream. Groundwater in lowland area where the subsurface is much more reactive due to the
presence of sedimentary organic matter is commonly more reduced and thus contains dissolved Fe(II) compared with groundwater in hilly area. The low river-water temperatures (below ~5°C) during winter in this subarctic environment leads to an apparent increase in the flux of reduced Fe(II) from particulate Fe in the riverine sediments and soil/groundwater in lowland area of the catchment, not necessarily because the flux has increased, but because the oxidation rate of the reduced Fe(II) back to Fe(III) is extremely slow at these low temperatures, thereby allowing a buildup of Fe(II) (Figs 2–4). This suggests that the release of reduced Fe(II) from riverbed sediments and groundwater to the water column might be the most important sources of iron in river waters.

Although photoreduction of Fe(III) is the dominant source of Fe(II) in acidic rivers (pH 2.3–3.1) and lakes (pH 4–5) (Collienne, 1983; Gammons et al., 2008), this mechanism was not the dominant source of Fe(II) in the pH 7–8 river waters of the present study as shown by the photoreduction experiment. This is consistent with previous findings that photoreduction of Fe(III) to Fe(II) is strongly dependent on pH, with high levels occurring at low pH and little to none at neutral and alkaline pH (Waite and Morel, 1984a, b).

4.2. Factors controlling seasonal variations in Fe(II) and Fe(III)

On-site Fe(II) concentrations showed a logarithmic decrease with increasing temperature at the lower reach sampling sites (KR-1 and OR-1) of the Kunebetsu River and Oono River (Fig. 5A) and at the lower and middle reach sites (OR-1–OR-3) of the Oono River (Fig. 5B). The logarithmic decrease in the Fe(II) oxidation rate constant \(k_{\text{ox}}\) against temperature (Fig. 4B) strongly suggests that the higher Fe(II) concentrations in river water during winter arise because of the slow oxidation rate of Fe(II) at low water temperature, and the low Fe(II) concentrations during summer are because of the remarkably faster oxidation rate of Fe(II) at higher water temperature
(Fig. 4). The rate that Fe(II) in the water column derived from reducing river sediments and Fe(II)-bearing groundwater oxidizes to insoluble Fe(III) hydroxide is governed by the temperature-dependent oxidation rate (Fig. 4). Recently, it has been reported that seasonal change in the temperature-dependent Fe(II) oxidation rate affects the dissolved Fe concentrations along the flow-path from anaerobic Fe(II) rich groundwater into surface water and the seasonal variation in in-stream reservoirs, resulting in the lower dissolved Fe concentrations at high temperature in summer compared to winter (van der Grift et al., 2014; Baken et al., 2015). Therefore, the Fe(II) concentration in river water reflects the balance between input and removal processes to and from the river water column. The Fe(II) concentration at the Oono River OR-1 sampling site was linearly related to the Fe(II) concentration measured on the same day at the Kunebetsu River site and at the other sites of the Oono River (OR-2–OR-5; Figs 6A and 6B). The correlation was especially strong for OR-2 and OR3 (Fig. 5B). The linearity of the relationships and different slopes suggest that the Fe(II) concentration in river waters is not only controlled by the spatially different supply rates of reduced Fe(II) from river sediment and Fe(II)-bearing groundwater to the water column—higher rate at lower reach sites—but also by the water temperature-dependent oxidative removal of Fe(II) from the water column. Moreover, despite remarkably different seasonal Fe(II) and T-Fe concentrations among the lower and middle reach sites of the Kunebetsu River and Oono River (Kunebetsu River (KR-1) > OR-1, OR-2 > OR-3; Figs 2C, 2E, 3A, and 3C), the seasonal Fe(II)/T-Fe ratios were spatially independent, with values of approximately 30–50% during winter at the Kunebetsu River lower reach site and the Oono River lower and middle reach sites (Figs 2F, 3D, and 7).

We estimated the seasonal variations in the release rate constant (a) of reduced Fe(II) from river sediment and groundwater to the river water column using the seasonal variation in the value of the temperature-dependent oxidation rate constant (b = kox) of Fe(II) to Fe(III) in river water and the observed seasonal variations in the Fe(II)/T-Fe
ratio ($R$, Figs 2F and 8) on the basis of three different assumed temperature-independent scavenging rate constants, $\gamma (\ln \gamma = -3, 0, \text{ and } +3)$, of T-Fe(III) corresponding to the removal of soluble, colloidal, and particulate Fe(III) from river water to the riverbed (Fig. A1, Appendix; Brezonik, 1994). The values of $b$ at in-situ water temperatures (Fig. 2A) were calculated from the linear relationship between logarithmic $k_{ox}$ and $1/T$ (Fig. 4B). In the present study, the soluble Fe(III) concentrations (<0.025 µm fraction) were negligibly low (below or near the detection limit) compared to T-Fe(III) (T-Fe minus Fe(II)) concentrations. The estimated seasonal patterns of $a$ at the lower reach sites of the Kunebetsu and Oono rivers revealed that $a$, similar to $b$, is strongly temperature-dependent, with high values during summer and low values during winter for all three values of $\ln \gamma$ (Fig. 8); however, the seasonal variations in reduced Fe(II) ($A$) in river sediment and groundwater are unknown (Fig. A1, Appendix). The estimated lower value of $a$ in winter compared to summer (Fig. 8) could suggest the slower microbial reductive dissolution of particulate Fe(III) in river sediments and soil/groundwater at low temperature during winter time. In addition, precipitation fell as snow during winter could diminish the discharge of Fe(II)-bearing groundwater into the river.

4.3. Relationship between T-Fe(III) and humic-like DOM

Fe(III) hydroxide solubility in natural waters is likely to be extremely low (below 0.1–1 nmol L$^{-1}$) at around pH 7–8 in the absence of organic ligand complexing (Stumm and Morgan, 1981; Kuma et al., 1996; Liu and Millero, 1999). Dissolved Fe (<0.2–0.45 µm size fraction) in rivers has been shown to consist almost entirely of colloids comprising a mix of Fe(III) hydroxide and organic compounds stabilized by dissolved organic matter (Krachler et al., 2012; Chekli et al., 2013; Fang et al., 2015). In the present study, therefore, a substantial amount of the T-Fe(III) in the river waters was in colloidal and particulate form rather than in soluble form. The colloidal and particulate
forms presumably included both Fe(III) hydroxide and Fe(III) organically bound to colloidal organic matter such as humic substances.

The seasonal variations in T-Fe (Figs 2E and 3C) and T-Fe(III) (data not shown) in the three rivers were relatively similar to those of humic F-intensity (Figs 2G and 3E), with levels being high during summer. The fact that humic F-intensity was higher at the lower reach sites of the Oono and Kunebetsu rivers than at the Moheji River, and at the lower reach sites than at the middle and upper reach sites of the Oono River (Figs 2G, 3E, and 9A) is attributed to the input of humic-like DOM from the river sediments to the water column and to the accumulation of humic-like DOM in the river water as it flowed downstream. Humic-like DOM is probably produced by the oxidation and remineralization of settling organic matter in the sandy and silty riverbed sediments, especially during summer. The plots of Fe(II) and T-Fe(III) against humic F-intensity at all sites revealed a significant (p<0.001) linear relationship between T-Fe(III) and humic F-intensity (Figs 9B and 9C) although DOM fluorescence in the present study might be more or less quenched by the presence of Fe. It has been reported that both Fe(II) and Fe(III) quenched DOM fluorescence with the varied degree of fluorescence quenching by the iron:DOC concentration ratio, DOM composition, and water samples (Poulin et al., 2014). This suggest that T-Fe(III) in these river waters is strongly associated with humic-like DOM (Fig. 9C), which may serve to stabilize the colloidal and particulate Fe(III) forms within the water column. Humic-like DOM in river water, which is supplied from river sediments in addition to downstream flow paths within soils/groundwater/wetland, complexes with Fe(III) as natural organic ligands, and this plays an important role in the formation of Fe(III)–organic colloids in river waters. Recently, it has been reported that land-derived humic substances may be of great importance as Fe(III) carriers in coastal waters, whereas autochthonously produced marine humic substances are of similar importance in the deep ocean (Tani et al., 2003; Kitayama et al., 2009; Laglera and van den Berg, 2009; Hioki et al., 2014). The
river-derived humic substances have functional groups that confer the ability to bind iron and keep it dissolved (Karlsson and Persson, 2010; Jones et al., 2011; Krachler et al., 2015). In boreal humic waters, iron is organically bound, particularly to high molecular weight organic colloids (Heikkinen, 1990 and 1994; Yan et al., 2013a, b). We speculate that humic-Fe(III) complexes are mainly produced during the rapid oxidation of Fe(II) close to the sediment–water interface following the large flux of soluble Fe(II) and humic substances from river sediments to the overlying water during summer (Figs 2C, 2E, 2G, 3A, 3C, 3E, 8, and 9C). Therefore, these results support the previous findings that humic substances play an important role in the supply of bioavailable Fe from rivers to coastal waters (Lofts et al., 2008; Krachler et al., 2010, 2015; Kritzberg et al., 2014) despite the large-scale removal of Fe from river water by precipitation and flocculation during estuarine mixing (Boyle et al., 1977; Mayer, 1982).

4.4. Nutrient sources and factors controlling their seasonal variation

The seasonal variation in NO$_3$+NO$_2$ concentrations showed summer values approximately two-thirds those of winter (Fig. 3F), probably because of biological uptake of NO$_3$+NO$_2$ during summer. In addition, NO$_3$+NO$_2$ concentrations were higher at the lower and middle reach sites (OR-1–OR-3) than at the upper reach sites (OR-4 and OR-5) of the Oono River; the highest seasonal concentrations were recorded at the Kunebetsu River site (Fig. 3F). The consistently high NO$_3$+NO$_2$ concentrations in the Kunebetsu River are probably due to the inflow of drainage from paddy fields. However, the differences in between the lower and middle reaches of the Oono River during summer were less for NO$_3$+NO$_2$ concentration than they were for humic F-intensity (Figs 9A and 10A), resulting from the biological uptake of NO$_3$+NO$_2$.

The levels and seasonal variation in Si(OH)$_4$ were similar at each of the Oono River and Kunebetsu River sites, with elevated concentrations during early summer–early winter (early June–early December) and low concentrations during middle winter–late
spring (Fig. 3G). Notably, there were no differences in Si(OH)$_4$ levels between the lower, middle, and upper reach sites of the Oono River (OR-1–OR-5) even as levels varied with the season. This similarity between upstream and downstream sites was very different from the results for Fe, humic F-intensity, and NO$_3$+NO$_2$ (Figs 3, 5D, 9A, and 10). Si(OH)$_4$ in these river waters is likely to originate predominantly from the upper watershed rather than from river sediments in the lower and middle reaches as evidenced by the similar seasonal variations in Si(OH)$_4$ between rivers and the similar concentrations of Si(OH)$_4$ in all reaches of the Oono River (Figs 3G and 10B). The particulate Si fraction in soils and bedrock consists of crystalline minerals (e.g., quartz and other primary and secondary silicates) and amorphous Si (Cornelis et al., 2011). Dissolution of these minerals constitutes the most important source of Si(OH)$_4$ inputs to rivers, which is delivered via groundwater and surface and subsurface runoff (Ronchi et al., 2013). Base flow consists mainly of groundwater that has previously percolated through soil. Therefore, Si(OH)$_4$ concentrations in the river waters measured during base flow conditions in the present study may have been predominantly governed by the inflow of groundwater with high Si(OH)$_4$ concentrations in the upper reaches. The low Si(OH)$_4$ concentrations during winter and spring indicates that groundwater inflow to rivers was minimal from the middle of December to late May (Fig. 3G), probably because the groundwater may be lower during winter because of the reduced infiltration of water into the subsurface as precipitation fell as snow.

4.5. Main processes for the transfer of iron and humic substances into river

The main processes and a conceptual diagram describing iron redox transformations in river waters revealed by this study are shown in Fig. 11. This study shows that in subarctic rivers subject to near-freezing water temperatures in winter and warm temperatures in summer, soluble Fe(II) concentration in river water is driven by the reductive dissolution of insoluble particulate Fe(III) during the microbial decomposition
of organic matter in reducing river sediments and soil/groundwater. High Fe(II) concentrations in river water during winter are predominantly attributed to the slower oxidation rate of Fe(II) to Fe(III) at low water temperature, whereas low Fe(II) concentration during summer occurs because of the remarkably faster oxidation rate of Fe(II) at higher water temperature, despite the fact that the release rate of reduced Fe(II) from river sediments and groundwater is also expected to be higher during summer. When temperatures are high enough to support oxidation, the Fe(II) released from reducing river sediments and groundwater is removed from the water column by the oxidation to insoluble particulate Fe(III) oxyhydroxides that can be associated with DOC. Dissolved and particulate Fe(III) may complex with natural Fe(III)-binding organic ligands—such as humic substances—which are also released from river sediments and groundwater, also especially during summer. The result is that particulate Fe(III) is removed from the river water by precipitation and particle scavenging although colloidal and fine grained particulate Fe(III) can remain in the water column for long time especially under turbulent flow conditions.

5. CONCLUSION

Soluble Fe(II) concentration in river water reflects a balance between input processes at the sediment–water interface and removal processes within the water column, that is, the release of reduced Fe(II) from river sediment and groundwater to river water and the oxidative removal of reduced Fe(II) to insoluble particulate Fe(III) oxyhydroxides. In this study the flux of reduced Fe(II) from reducing river sediments and groundwater to the water column appeared to be the most important sources of both Fe(II) and Fe(III) in river water, as evidenced by the seasonal Fe fluctuations between the lower, middle, and upper reach sites of rivers. We suggest that the phenomenon with high Fe(II) concentration in river water during winter may apply in many subarctic and
arctic river environments where the riverbeds comprise reducing river sediments and the 
Fe(II)-bearing groundwater discharges into river.

APPENDIX A

We consider an Fe-based system comprising three compartments: reduced Fe(II) in 
river sediment (A), Fe(II) in river water (B), and mainly colloidal and particulate Fe(III) 
(C). A schematic representation of chemical flow for this system is shown in Fig. A1A. 
This system satisfies the following ordinary differential equations:

\[
\frac{dA}{dt} = -aA \quad \text{(A1)}
\]

\[
\frac{dB}{dt} = aA - bB \quad \text{(A2)}
\]

\[
\frac{dC}{dt} = bB - \gamma C \quad \text{(A3)}
\]

Thus, the complex chemical and physical processes for Fe are represented by three 
simple parameters: \(a\) is the release rate constant for \(A\), \(b\) is the oxidation rate constant 
for \(B\), and \(\gamma\) is the settlement or scavenging rate constant for \(C\).

We begin by examining the instantaneous input problem, and are interested primarily 
in the river-sediment source of Fe, that is, set \(A = A_0\) and \(B = C = 0\) at time \(t = 0\). The 
solution for each is then:

\[
A(t) = A_0 e^{-at} \quad \text{(A4)}
\]

\[
B(t) = \frac{aA_0}{b-a} \left( e^{-at} - e^{-bt} \right) \quad \text{(A5)}
\]
These yield the temporal variations shown in Fig. A1A. Next, the solution as a continuous input problem can be convolved with a source having an arbitrary time history. The convolution of $A$ is given as follows:

$$A_t = \int_0^t A(t-\tau)d\tau$$

$$= A_0 \frac{1 - e^{-at}}{a}$$ (A7)

On timescales that are long compared to the timescales of release and oxidation (presumably several hours), a steady state is obtained by letting $t \to \infty$ in the transit solution $A_t$, that is

$$A_\infty = A_0 a$$ (A8)

Similarly, solutions of $B_\infty$ and $C_\infty$ in the steady state are given as follows:

$$B_t = \int_0^t B(t-\tau)d\tau = \frac{a A_0}{b-a} \left\{ \frac{1 - e^{-at}}{a} - \frac{1 - e^{-bt}}{b} \right\}$$ (A9)

$$\therefore B_\infty = \frac{A_0}{b}$$ (A10)

$$C_t = \int_0^t C(t-\tau)d\tau = ab A_0 \left\{ \frac{1}{b-a} \left( \frac{1 - e^{-at}}{a} - \frac{1 - e^{-bt}}{b} \right) + \frac{1 - e^{-\gamma t}}{(\gamma-a)(\gamma-b)\gamma} \right\}$$ (A11)

$$\therefore C_\infty = \frac{A_0 \left( \gamma^2 - (a+b)\gamma + ab \right)}{(\gamma-a)(\gamma-b)\gamma}$$ (A12)

The observed Fe(II)/TFe ratio ($R$), which is spatially independent of any location, corresponds to our model ratio of $R = B_\infty/(B_\infty + C_\infty)$. The solutions of (A10) and (A12)
can be substitute into the following equation:

\[ R = \frac{\gamma(\gamma - a)(\gamma - b)}{\gamma(\gamma - a)(\gamma - b) + b^2\gamma(\gamma - (a + b)) + ab^2} \]  

(A13)

This can be transformed into a quadratic equation for the unknown parameter \( a \) as follows:

\[ D_1a^2 + D_2a + D_3 = 0 \]  

(A14)

where

\[ D_1 = R(\gamma^2 - b^2) - \gamma(\gamma - b) \]  

(A15)

\[ D_2 = (\gamma^2 - b^2)\{(1 - R) - Rb\} \]  

(A16)

\[ D_3 = (b - \gamma)\gamma b\{(1 - R) - Rb\} \]  

(A17)

Since \( a \) is always positive,

\[ a = \frac{-D_2 + \sqrt{D_2^2 - 4D_1D_3}}{2D_1} \]  

(A18)

From this solution, we can estimate the seasonal variation in \( a \) by assuming the appropriate order of \( \gamma \) and by substituting observed values of \( R \) and \( b \). The solution of \( a \) or \( R \) (eqs A13 and A15) is of interest when we examine the idealized limit state. In the limit state, \( \gamma < b, a \rightarrow b, \) and \( R \rightarrow \gamma/b \rightarrow 0 \). Note that this limit with \( R \rightarrow 0 \) may be close to the observed state in summer. In the inverse limit, \( \gamma \gg b, a \rightarrow \gamma, \) and

\[ R \rightarrow \gamma^3/(\gamma^3 + b^2 + b^3) \rightarrow 1 \]. A transition to the inverse limit, with \( R \sim 0.5 \), will occur with the onset of winter (Fig. A2).
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Van der Grift B., Rozemeijer J. C., Griffioen J. and van der Velde Y. (2014) Iron


Figure captions

Fig. 1. Location of the three rivers flowing into Hakodate Bay in southwestern Hokkaido, Japan used to study iron speciation in river water. The sampling sites were located in the lower reaches of the Kunebetsu, Oono, and Moheji rivers (KR-1, OR-1, and MR-1 for each lower reach sampling site, respectively) and also in the middle and upper reaches of the Oono River. The Oono River sampling sites are given labels to distinguish their locations: OR-1 and OR-2 for the sites in the lower reach, OR-3 for the site in the middle reach and OR-4 and OR-5 for the sites in the upper reach.
Fig. 2. Seasonal variations in river water parameters measured at the lower reach sampling sites (KR-1, OR-1, and MR-1) of the Kunebetsu, Oono, and Moheji rivers during May 2010–February 2014: (A) water temperature, (B) water discharge, (C) Fe(II), (D) dissolved Fe (D-Fe), (E) total Fe (T-Fe), (F) Fe(II)/T-Fe ratio, and (G) humic-like fluorescence intensity (humic F-intensity). Historical daily water discharge data of the three rivers for 2010–2012 were obtained from the Hokkaido Government (the data for 2013 and 2014 were not available for use in this study).

Fig. 3. Seasonal variations in river water parameters measured at the lower reach sampling sites (KR-1 and OR-1) of the Kunebetsu and Oono rivers and at the lower (OR-1 and OR-2), middle (OR-3), and upper (OR-4 and OR-5) reach sampling sites of the Oono River during May 2013–February 2014: (A) water temperature and Fe(II), (B) D-Fe, (C) T-Fe, (D) Fe(II)/T-Fe ratio, (E) humic F-intensity, (F) NO$_3$+NO$_2$, and (G) Si(OH)$_4$.

Fig. 4. A pseudo first-order rate of oxidation equation was applied to in-situ Fe(II) measurements from river-water samples collected with pH 7.3–7.8 and in-situ water temperatures of 7.5, 4, 1.5, and 0.5°C (± 0.5°C) to determine (A) estimated oxidation rate constants ($k_{ox}$) and half-lives ($T_{1/2}$) of in-situ Fe(II) at different in-situ water temperatures, and (B) the estimated $k_{ox}$ and $T_{1/2}$ values at water temperatures of 0, 5, 10, 15, and 20°C from the linear relationship between logarithmic $k_{ox}$ and 1/T (where T is absolute temperature in K) taken from the relationships shown in panel (A).

Fig. 5. In-situ Fe(II) concentrations as a function of in-situ water temperature at (A) the lower reach sampling sites (KR-1, OR-1, and MR-1) of the Kunebetsu, Oono, and Moheji rivers and (B) the lower (OR-1 and OR-2), middle (OR-3), and upper (OR-4
and OR-5) reach sampling sites of the Oono River, showing the logarithmic decrease in Fe(II) concentration with increasing temperature at the lower and middle reach sampling sites where the river bottom comprises sandy and silty sediments.

Fig. 6. The relationship between concurrently sampled in-situ Fe(II) concentration at the Oono River (OR-1) sampling site versus that at (A) the downstream sampling site of the Kunebetsu River (KR-1) and (B) each of the other sampling sites of the Oono River (OR-2–OR-5) showing linear relationships with different slopes that indicate that the concentrations were very similar at the Kunebetsu (KR-1) and OR-1 sites, but compared to OR-1, the concentrations progressively declined moving to the middle and upper reaches.

Fig. 7. Fe(II)/T-Fe ratio (%) against in-situ water temperature at (A) the lower reach sites of the Kunebetsu River (KR-1) and the Oono River (OR-1) during May 2010–February 2014 and (B) the lower and middle reach sites of the Oono River during May 2013–February 2014, showing the spatially independent relationship.

Fig. 8. Estimated seasonal variations of the release rate constant ($a$) of reduced Fe(II) from river sediment and groundwater to river water from the seasonal variations in temperature-dependent oxidation rate constant ($b = k_{ox}$) of Fe(II) in river water (Fig. 4B) and the observed seasonal variations in Fe(II)/T-Fe ratio ($R$, Fig. 2F) on the basis of three temperature-independent scavenging rate constants ($\gamma \ln \gamma = -3, 0, \text{and } +3$) at the lower reach sites of (A) the Kunebetsu River (KR-1) and (B) the Oono River (OR-1).

Fig. 9. (A) Humic F-intensity values at the Oono River sites OR-2–OR-5 against those at OR-1 from samples taken on concurrent dates, with linear relationships indicating
that as humic F-intensity increased, the values at upstream sites became progressively lower than at OR-1 (May 2013 to February 2014, Fig. 3E). (B) In-situ Fe(II) concentrations and (C) T-Fe(III) (T-Fe minus Fe(II)) concentrations against humic F-intensity values at all sites of each of the three rivers (May 2010 to February 2014, Figs 2C–G).

Fig. 10. Values measured at Oono River sites OR-2–OR-5 against those at OR-1 from samples taken on concurrent dates for (A) NO$_3$+NO$_2$ concentrations and (B) Si(OH)$_4$ concentrations (May 2013 to February 2014, Figs 3F and 3G).

Fig. 11. Conceptual diagram showing the main processes during summer and winter for the transfer of iron and humic substances between river sediment and the water column. The diagram shows the flux of reduced Fe(II) and humic substances from the reducing river sediments and Fe(II)-bearing groundwater in lowland area of the catchment to the water column, the oxidation of released Fe(II) to Fe(III), the complexing of dissolved and particulate Fe(III) with Fe(III)-binding ligands such as humic substances, and the precipitation, resuspension and deposition of the particulate Fe(III). The thickness of the arrows and the size of the label typeface show relatively the magnitude of the flux and the concentrations of the various forms.

Appendix A figure captions

Fig. A1. (A) Diagrammatic representation of the two-step first-order sequence ($A \rightarrow B \rightarrow C$) for the iron transformation system in and between river sediment and groundwater and the water column, showing the flux of reduced Fe(II) from bottom sediment and groundwater to the river water ($A \rightarrow B$), the oxidation of reduced Fe(II)
to Fe(III) in the water \((B \rightarrow C)\), and the scavenging of colloidal and particulate Fe(III) from the river water; (B) the change in concentration of \(A\), \(B\), and \(C\) with time \((t)\) in first-order reactions described by equations A4, A5, and A6, respectively.

Fig. A2. Estimated seasonal variation in the release rate constant \((a)\) of reduced Fe(II) from the river sediment and the groundwater to the river water by assuming the appropriate order of magnitude of the scavenging rate constant \((\gamma)\) of colloidal and particulate Fe(III) from river water and by substituting the observed Fe(II)/T-Fe ratio \((R)\) and the oxidation rate constant \((b)\) of reduced Fe(II) in river water.
Table 1 Characteristics of three rivers

<table>
<thead>
<tr>
<th>No</th>
<th>River</th>
<th>Watershed (km²)</th>
<th>Water discharge (2011) monthly average</th>
<th>Water discharge (2012) monthly average</th>
<th>Water temperature (°C)</th>
<th>pH</th>
<th>Riverbed condition at lower–upper reaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kunebetsu</td>
<td>122.9</td>
<td>4~15</td>
<td>6~19</td>
<td>0~23</td>
<td>7.02~8.36</td>
<td>silt+sand</td>
</tr>
<tr>
<td>2</td>
<td>Oono</td>
<td>111.1</td>
<td>7~19</td>
<td>4~29</td>
<td>0~21</td>
<td>7.17~8.28</td>
<td>silt+sand</td>
</tr>
<tr>
<td>3</td>
<td>Moheji</td>
<td>96.3</td>
<td>35~77</td>
<td>14~71</td>
<td>0~19</td>
<td>7.08~8.15</td>
<td>gravel+cobble</td>
</tr>
</tbody>
</table>
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Fig. 3. Seasonal variations in river water parameters measured at the lower reach sampling sites (KR-1 and OR-1) of the Kunebetsu and Oono rivers and at the lower (OR-1 and OR-2), middle (OR-3), and upper (OR-4 and OR-5) reach sampling sites of the Oono River during May 2013–February 2014: (A) water temperature and Fe(II), (B) D-Fe, (C) T-Fe, (D) Fe(II)/T-Fe ratio, (E) humic F-intensity, (F) NO$_3$+NO$_2$, and (G) Si(OH)$_4$. 
Fe(II) (ln mole L$^{-1}$)

Time (min)

$k_{\text{ox}} = 0.0037 \text{ min}^{-1}$
($T_{1/2} = 187 \text{ min}$)

$k_{\text{ox}} = 0.0743 \text{ min}^{-1}$
($T_{1/2} = 9.3 \text{ min}$)

$k_{\text{ox}} = 0.0354 \text{ min}^{-1}$
($T_{1/2} = 19.6 \text{ min}$)

$k_{\text{ox}} = 0.0111 \text{ min}^{-1}$
($T_{1/2} = 62 \text{ min}$)

$Y = -30.87X + 107.62$ ($R=0.954$, $n=4$)

$0^\circ \text{C: } k_{\text{ox}}=0.00454 \text{ min}^{-1}, T_{1/2}=151 \text{ min}$

$5^\circ \text{C: } k_{\text{ox}}=0.0346 \text{ min}^{-1}, T_{1/2}=19.8 \text{ min}$

$10^\circ \text{C: } k_{\text{ox}}=0.246 \text{ min}^{-1}, T_{1/2}=2.9 \text{ min}$

$15^\circ \text{C: } k_{\text{ox}}=1.63 \text{ min}^{-1}, T_{1/2}=0.42 \text{ min}$

$20^\circ \text{C: } k_{\text{ox}}=10.1 \text{ min}^{-1}, T_{1/2}=0.068 \text{ min}$

Fig. 4 A pseudo first-order rate of oxidation equation was applied to in-situ Fe(II) measurements from river-water samples collected with pH 7.3–7.8 and in-situ water temperatures of 7.5, 4, 1.5, and 0.5°C to determine (A) estimated oxidation rate constants ($k_{\text{ox}}$) and half-lives ($T_{1/2}$) of in-situ Fe(II) at different in-situ water temperatures, and (B) the estimated $k_{\text{ox}}$ and $T_{1/2}$ values at water temperatures of 0, 5, 10, 15, and 20°C from the linear relationship between logarithmic $k_{\text{ox}}$ and $1/T$ (where $T$ is absolute temperature in K) taken from the relationships shown in panel (A).
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In-situ Fe(II) concentrations as a function of in-situ water temperature at (A) the lower reach sampling sites (KR-1, OR-1, and MR-1) of the Kunebetsu, Oono, and Moheji rivers and (B) the lower (OR-1 and OR-2), middle (OR-3), and upper (OR-4 and OR-5) reach sampling sites of the Oono River, showing the logarithmic decrease in Fe(II) concentration with increasing temperature at the lower and middle reach sampling sites where the river bottom comprises sandy and silty sediments.
Fe(II) (µmol L$^{-1}$) at Kunebetsu River (KR-1)

\[ Y = 1.722X + 0.375 \quad (R = 0.873, n = 52) \]

Fe(II) (µmol L$^{-1}$) at OR-2~5 at Oono River (OR-1)

\[ Y = 0.896X + 0.112 \quad (R = 0.977, n = 18) \]

\[ Y = 0.345X + 0.151 \quad (R = 0.971, n = 18) \]

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Fig. 6. The relationship between concurrently sampled in-situ Fe(II) concentration at the Oono River (OR-1) sampling site versus at (A) the downstream sampling site of the Kunebetsu River (KR-1) and (B) each of the other sampling sites of the Oono River (OR-2~OR-5) showing linear relationships with different slopes that indicate that the concentrations were very similar at the Kunebetsu (KR-1) and OR-1 sites, but compared to OR-1, the concentrations progressively declined moving to the middle and upper reaches.
Fig. 7. Fe(II)/T-Fe ratio (%) against in-situ water temperature at (A) the lower reach sites of the Kunebetsu River (KR-1) and the Oono River (OR-1) during May 2010–February 2014 and (B) the lower and middle reach sites of the Oono River during May 2013–February 2014, showing the spatially independent relationship.

\[ Y = -2.265X + 39.83 \quad (R = 0.894, n = 54) \]
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Fig. 8. Estimated seasonal variations of the release rate constant (a) of reduced Fe(II) from river sediment and groundwater to river water from the seasonal variations in temperature-dependent oxidation rate constant (b = kox) of Fe(II) in river water (Fig. 4B) and the observed seasonal variations in Fe(II)/T-Fe ratio (R, Fig. 2F) on the basis of three temperature-independent scavenging rate constants (γ: ln γ = -3, 0, and +3) at the lower reach sites of (A) the Kunebetsu River (KR-1) and (B) the Oono River (OR-1).
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(A) Humic F-intensity values at the Oono River sites OR-2-OR-5 against those at OR-1 from samples taken on concurrent dates, with linear relationships indicating that as humic F-intensity increased, the values at upstream sites became progressively lower than at OR-1 (May 2013 to February 2014, Fig. 3E). (B) In-situ Fe(II) concentrations and (C) T-Fe(III) (T-Fe minus Fe(II)) concentrations against humic F-intensity values at all sites of each of the three rivers (May 2010 to February 2014, Figs 2C–G).
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Fig. 10. Values measured at Oono River sites OR-2–OR-5 against those at OR-1 from samples taken on concurrent dates for (A) NO$_3$+NO$_2$ concentrations and (B) Si(OH)$_4$ concentrations (May 2013 to February 2014, Figs 3F and 5G).
Fig. 11. Conceptual diagram showing the main processes during summer and winter for the transfer of iron and humic substances between river sediment and the water column. The diagram shows the flux of reduced Fe(II) and humic substances from the reducing river sediments and Fe(II)-bearing groundwater in the lowland area of the catchment to the water column, the oxidation of released Fe(II) to Fe(III), the complexing of dissolved and particulate Fe(III) with Fe(III)-binding ligands such as humic substances, and the precipitation, resuspension and deposition of the particulate Fe(III). The thickness of the arrows and the size of the label typeface show relatively the magnitude of the flux and the concentrations of the various forms.
Fig. A1. (A) Diagrammatic representation of the two-step first-order sequence ($A \rightarrow B \rightarrow C$) for the iron transformation system in and between river sediment and groundwater and the water column, showing the flux of reduced Fe(II) from bottom sediment and groundwater to the river water ($A \rightarrow B$), the oxidation of reduced Fe(II) to Fe(III) in the water ($B \rightarrow C$), and the scavenging of colloidal and particulate Fe(III) from the river water; (B) the change in concentration of $A$, $B$, and $C$ with time ($t$) in first-order reactions described by equations A4, A5, and A6, respectively.
Fig. A2. Estimated seasonal variation in the release rate constant ($\alpha$) of reduced Fe(II) from the river sediment and the groundwater to the river water by assuming the appropriate order of magnitude of the scavenging rate constant ($\gamma$) of colloidal and particulate Fe(III) from river water and by substituting the observed Fe(II)/T-Fe ratio ($R$) and the oxidation rate constant ($\theta$) of reduced Fe(II) in river