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# Characterization of variability of water and nutrient cycles in small floodplain water bodies using a geochemical multi-tracer

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Abstract. River floodplains contribute to river ecosystems by supporting high biological productivity and biodiversity. Within floodplains, semi-enclosed water bodies develop, among which those partially connected to the river are known as backwater (i.e., locally called Wando in Japanese). Although backwater serves as habitats for aquatic organisms, studies on the origin of spring water within backwater and the associated nutrient supplies remain limited. In this study, we investigated the origins and pathways of water and the internal nutrient dynamics (sources, concentrations and composition ratios) using multiple geochemical tracers—ion balance, chromophoric dissolved organic matter (CDOM), <sup>222</sup>Rn, stable hydrogen and oxygen isotope ratios in water ( $\delta^2$ H &  $\delta^{18}$ O–H<sub>2</sub>O), and stable nitrogen and oxygen isotope ratios in nitrate ( $\delta^{15}$ N &  $\delta^{18}$ O–NO<sub>3</sub>)—at three distinct backwaters sites within a 5-kilometer section of the middle reaches of an urban river (Tama River) in Tokyo. Each geochemical tracer exhibited significantly different values between the surrounding shallow groundwater and the main river along the backwaters, serving as an effective indicator for evaluating the contribution of both sources to the water supplied to the backwaters. The water sources differed not only among the three backwaters locations within a short river section (5 km) but also across seasons. River water exhibited relatively high phosphate concentrations (3.4–12.4 µmol L<sup>-1</sup>) and low dissolved silicate (DSi) concentrations (157–218 μmol L<sup>-1</sup>), whereas shallow groundwater exhibited lower phosphate (0.7–1.3 μmol L<sup>-1</sup>) and higher DSi concentrations (236–730 μmol L<sup>-1</sup>). While no significant difference in DIN concentration was observed between rivers and groundwater, the increase in  $\delta^{15}$ N &  $\delta^{18}$ O-NO<sub>3</sub> observed in one backwater site, coinciding with the decrease in nitrate concentration, suggested denitrification occurring in subsurface flow paths. As a result, in the backwaters, strongly influenced by the urban river, nutrient conditions reflected inputs from treated wastewater, leading to relatively stable N:P ratios in space and time. In contrast, the backwaters, which was primarily replenished by groundwater, showed pronounced seasonal fluctuations in N:P ratios due to variations in microbial activity, fertilizer inputs, and river inflow rates. Given the influence of nutrient environments on microbial communities and primary producers, the ecological functions of backwater can be better understood through intensive research focused on water-quality processes.

#### 1. Introduction

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30 Rivers form distinctive landforms and landscapes in their surrounding areas through processes such as erosion, transportation, and deposition. Among these, floodplains, which are areas where water overflows from rivers during heavy rainfall, function as transition zones connecting terrestrial and aquatic ecosystems (i.e., an aquatic/terrestrial transitional zone) (Junk et al., 1989). Floodplains have diverse aquatic ecosystems, including secondary channels, stagnant waters, ponds, and lakes isolated from the main channel (Amoros and Bornette, 2002; Galat et al., 1997; Ward et al., 2002). These areas are referred to as floodplain 35 water bodies (Nagayama et al., 2015), which function as a foundation for maintaining high biodiversity by creating diverse landscapes within rivers through spatiotemporal changes in physical and hydrological conditions in response to flood intensity and frequency (Amoros and Bornette, 2002; Junk et al., 1989; Keruzoré et al., 2013; Tockner et al., 2000; Ward et al., 2002). Floodplain water bodies are affected by overflow and underflow from the main river channel, as well as by groundwater discharge from surrounding highlands (Hancock, 2002). Since underflow seeps into sediments from riverbeds and banks and 40 reemerges through floodplains, the decomposition of organic matter in the underflow process induces changes in the aerobicanaerobic environment (Lewandowski and Nützmann, 2010), thereby influencing nutrient dynamics (Boulton et al., 1998; Brunke and Gonser, 1997; Findlay, 1995; Hendricks and White, 1988).

Organic matter decomposition in underground streams also leads to high concentrations of nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) at the spring point, which serve as hotspots for algal production (Coleman and Dahm, 1990; Grimm and Fisher, 1984; Valett et al., 1994). On the other hand, the anaerobic condition during the underflow process causes denitrification (Gooddy et al., 2014; Haycock and Burt, 1993) and is also known to function as a system for removing excessive nitrogen loads in water bodies.

Floodplains are considered to have high productivity in river ecosystems (Lewis et al., 2001; Sparks, 1995; Spink et al., 1998), and studies on nutrient dynamics and biological production have been conducted in large floodplain water bodies extending several kilometers in length. Bondar-Kunze et al. (2009) in their study of the Danube River, revealed that floodplain water bodies serve the function of supplying organic matter to the main river through primary production using nutrient inputs from the main river. In addition, the vast floodplain of the Mississippi River is reported to perform net primary production and exhibit autotrophic characteristics during certain seasons (Houser et al., 2015).

In Japan, river channel improvement has been actively carried out for a long time for flood control and water utilization. As a result, extensive natural floodplains are very rare in many rivers, and floodplains within channels restricted by embankments have formed (Nagayama et al., 2015). Such rivers form small floodplain water bodies (i.e., backwater) called Wando in Japanese that are several dozen to several hundred meters long, and many of these backwaters have springs (Figure 1). Previous studies have highlighted the ecological usefulness of river mainstems as evacuation sites during floods and as breeding grounds for juvenile fish (Denda et al., 2002, 2006; Dole-Olivier, 1998; Kimizuka, 1998; Minagawa et al., 2015; Nakajima et al., 2008). However, many other aspects, such as water quality and primary production, remain poorly understood. We hypothesized that, similar to large floodplains, the surrounding environment and water supply routes in backwater affect nutrient dynamics, which in turn may be related to phytoplankton communities and primary production in the floodplain.





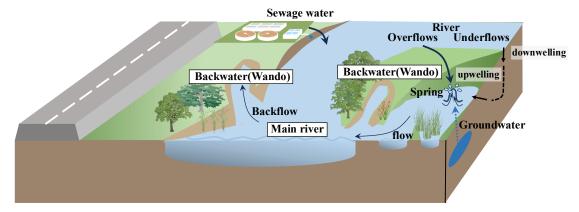


Figure 1 Floodplain of urban rivers in Japan. Floodplains restricted by embankments form floodplain water bodies other than the main stream due to the effects of sedimentation and erosion. backwaters are formed in accordance with the main stream being supplied water from river backwater, underflow/overflow water from upstream, and groundwater from the surrounding area.

The objectives of this study are comprehensively identifying the origin and pathways of spring water and associated nutrients (N, P, Si) in different backwater systems by measuring ion composition, <sup>222</sup>Rn concentration, stable hydrogen and oxygen isotope ratios (δ<sup>2</sup>H–H<sub>2</sub>O and δ<sup>18</sup>O–H<sub>2</sub>O) in water, and chromophoric dissolved organic matter (CDOM) concentration at the spring. Finally, we will discuss nutrient dynamics that influence ecological systems, such as phytoplankton production and communities.

# 2. Materials and methods

# 2.1. Study site

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The target river, the Tama River, flows through the Kanto region of Japan (river length: 138 km, watershed area: 1,240 km², average flow rate: 20.9 m³ s⁻¹) and mainly flows through urban areas of Tokyo. The population of the river basin exceeds 4 million, and approximately 34% of the basin is an urban area (Figure 2a). The Kanto region has a humid subtropical climate, with rainfall concentrated in the rainy summer seasons from June to September. River flow generally increases in these months and decreases from November to February (Figure 2c). The survey area is located in the middle reaches of the Tama River, where effluent from five sewage treatment plants flows into the river.



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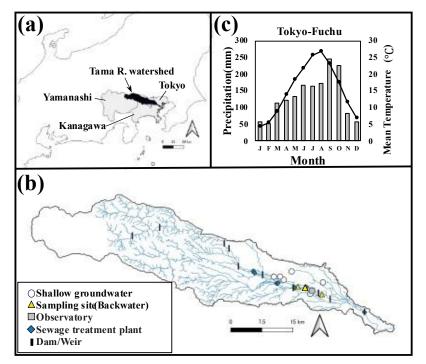


Figure 2 (a) The watershed of the target river, the Tama River. (b) The sampling locations (white circles and yellow triangles), public observatory (gray squares), sewage treatment plants (blue diamonds), and dams and weirs (black bars) in the Tama River. (c) The monthly average temperature (line graph) and precipitation (bar graph) from 1991 to 2020 at meteorological observation stations near the survey sites.

The Tama River forms river terraces from its middle to lower reaches. It was assumed that the numerous springs along the terraced cliffs would eventually flow into the Tama River and the floodplain as shallow groundwater. We selected three small backwaters (i.e., Backwater A, B, and C from upstream, Figures 2b, 3) within a 5 km section of the middle reaches of the Tama River. Backwater A is formed by a sandbar protruding from the riverbank. The location of springhead varies depending on the season, when main river water level was high in summer, the spring point and a small river about 100 m long appeared at upper end of the backwater (Figure 3a,e,d). The drainage channel from a factory is located approximately 600 m upstream of the backwater. During the survey period, the drainage flowed into the floodplain, but no inflow was observed flowing into Pond A or the main river as surface water. Backwater B was formed by a sandbar protruding from the riverbank, similar to Backwater A. A spring point was confirmed not only at the bottom of the upper end of the backwater but also from the riverbank at a higher elevation than the main water surface (Figure 3b,d). Since the dry channel trace continues upstream from Backwater C to the main river, it is possible that Backwater C was formed by a temporary stream during high water level. The headwaters of Backwater C form a small river approximately 50 meters long that flows into the backwater (Figure 3c).



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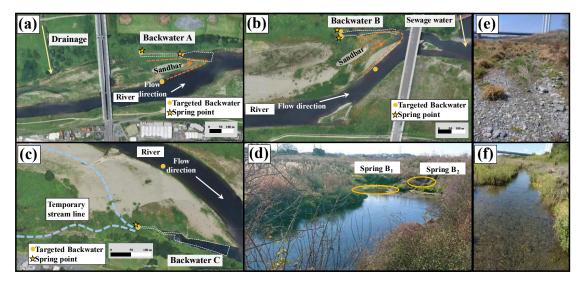


Figure 3 Overview and the sampling points at (a)Backwater A, (b)Backwater B and a sewage treatment plant located 50 meters downstream from Backwater B, and (c)Backwater C, respectively. The yellow circles indicate the survey locations, and those with the star marks indicate the spring outlets. (d) The location of two spring points at Backwater B. Backwater B<sub>1</sub> emerges from the bottom floor, while Backwater B<sub>2</sub> emerges from the terrace cliff. View upstream of Backwater A in (e) dry season and (f) rainy season. The backwater area extend upstream due to the rise in water level after April. Base maps in (a)–(c) were obtained from © Google Maps (© Google, Map data © Google, Maxar Technologies, 2025). Image was cropped and annotated with sampling sites.

Water samples (n=3) were collected at each of the backwaters and adjacent river mainstreams approximately once a month from February to November 2022. At each backwaters, water was collected directly from the spring if it was visible, otherwise from the vicinity of the upstream end. If more than one spring was found (e.g., on the bottom floor and at the terrace cliffs), water samples were collected at both locations to verify the contribution of different water sources. The water samples were immediately filtered through a 0.2  $\mu$ m cellulose acetate filter (CS020AS, ADVANTEC, Japan) for analysis of stable hydrogen and oxygen isotope ratios in water ( $\delta^2$ H–H<sub>2</sub>O,  $\delta^{18}$ O–H<sub>2</sub>O), and through a 0.45  $\mu$ m cellulose acetate filter (CS045AS, ADVANTEC, Japan) for analysis of nutrient, dissolved ions, and stable nitrogen and oxygen isotope ratios in nitrate ( $\delta^{15}$ N–NO<sub>3</sub>,  $\delta^{18}$ O–NO<sub>3</sub>), and then stored frozen or refrigerated until analysis. The water for <sup>222</sup>Rn analysis was collected in a 250 ml glass bottle, filling it to the brim without allowing any air bubbles to enter. To identify the water source of the spring water in the backwaters, spring water sampling was also conducted at six locations along the cliff lines around the survey site in November 2022. Rainwater was also collected from December 2021 to October 2022 within the grounds of Tokyo University of Agriculture and Technology (35.684657°N 139.483360°E). These samples were also processed in the same manner as above. Dissolved oxygen (DO), water temperature (Multi 3510 IDS, WTW, Germany), pH (HORIBA D-55, Horiba, Japan), and



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electrical conductivity (EC) (HORIBA ES-71, Horiba, Japan) were measured in the field. Only in November 2022, we measured the concentration of chromophoric dissolved organic matter (CDOM) using a CDOM sensor (Cyclops-7-Logger, Tuner, USA) by immersing the sensor in water collected in a bucket for 10 minutes. River flow and water quality data at the Ishihara (35.642695°N 139.527151°E) and Tama suidobashi (35.625582°N 139.569326°E) observatories were obtained from the public database of the Ministry of Land, Infrastructure, Transport and Tourism. We also used weather data from the Fuchu Observatory (35.684254°N 139.484085°E) published by the Japan Meteorological Agency.

## 2.2. Chemical analyses

The major nutrients including PO<sub>4</sub><sup>3-</sup>, NH<sub>4</sub><sup>+</sup> and dissolved silicate (DSi) were colorimetrically determined by the molybdenum blue method, the indophenol blue method, and the molybdenum yellow method, respectively, using a spectrophotometer (U-1500, HITACHI). Major anions (Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>), and cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>) were determined using an ion chromatography system (DIONEX ICS-1100 for cations and DIONEX DX-120 for anions, Dionex Corporation, USA). The bicarbonate (HCO<sub>3</sub><sup>-</sup>) concentration was calculated from the alkalinity using a titration method.

 $\delta^{15}$ N-NO<sub>3</sub> and  $\delta^{18}$ O-NO<sub>3</sub> were determined using denitrifying bacteria following Sigman et al. (2001) and Casciotti et al. (2002). In brief, NO<sub>3</sub> was converted into nitrous oxide (N<sub>2</sub>O) by denitrifying bacteria that lack N<sub>2</sub>O-reductase activity. The N<sub>2</sub>O was purified using cryogenic trapping (Precon System Finnigan MAT, Bremen, Germany), separated chromatographically (Finnigan GasBench Thermo Fisher Scientific, Germany), and analyzed using mass spectrometry (Finnigan Delta<sup>plus</sup>XP, Thermo Fisher Scientific, Germany). Standard materials USGS32, USGS34, and IAEA were used, and analytical precision for  $\delta^{15}$ N and  $\delta^{18}$ O was generally better than 0.2‰ and 0.6‰, respectively.  $\delta^{2}$ H-H<sub>2</sub>O and  $\delta^{18}$ O-H<sub>2</sub>O were analyzed using a cavity ring-down spectroscopy (L2410-I, Picarro, USA). The analytical accuracy of measurements was 0.1 ‰ for  $\delta^{18}$ O and 0.8 ‰ for  $\delta^{2}$ H based on replicate measurements of standard. The stable isotope ratios in water and NO<sub>3</sub><sup>-</sup> were expressed relative to Vienna standard ocean water (V-SMOW) and atmospheric nitrogen and were calculated as follows.

$$\delta_{sample}(\%_0) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

Where R = <sup>2</sup>H/<sup>1</sup>H, <sup>18</sup>O/<sup>16</sup>O or <sup>15</sup>N/<sup>14</sup>N. R<sub>sample</sub> and R<sub>standard</sub> indicate the isotope ratio (‰) of the sample and the standard material, respectively. The <sup>222</sup>Rn was measured using RAD-H<sub>2</sub>O system that is basically a portable 250 ml Rn-emanation bottle attached to an electronic radon detector (Durridge, RAD7, USA).

#### 2.3. Statistical analysis

Statistical analyses were performed using statistical software R ver4.4.1. Significant differences in measurement data at each location were determined using the Kruskal-Wallis test, followed by the Dunn test with the package software car, FSA, respectively, with p<0.05 considered significant. For comparisons between the two groups, the Wilcoxon rank sum test was used, and p<0.05 was considered significant. The correlation between each measurement data was calculated using Pearson's correlation coefficient with the psych package.





### 3. Results

### 3.1 EC and DO variation

The results of DO, EC, and water temperature for each backwaters and river are shown in Table 1. The water temperature in the backwater was generally cooler in summer and warmer in winter compared to that in the adjacent river. This temperature relationship has often been documented in groundwater and springs and adjacent river waters in temperate regions (e.g., Johnson et al. 2014). In February and March, during the dry winter season, no spring outlet was observed at the upstream of Backwater A, but after April, when precipitation increased, a spring outlet and a small river formed clearly. With this change in the water system, EC around the spring site increased from  $44.0 \pm 4.4$  mS m<sup>-1</sup> to  $66.8 \pm 5.5$  mS m<sup>-1</sup>, while DO decreased from  $11.2 \pm 0.9$  mg L<sup>-1</sup> to  $3.1 \pm 1.0$  mg L<sup>-1</sup>. On the other hand, Backwater B showed stable water quality throughout the observation period. In addition, the spring water from the terrace cliff (St. B<sub>2</sub> in Table 1) showed slightly lower EC and DO than that from the bottom floor (St. B<sub>1</sub> in Table 1), but no significant difference was found. In Backwater C, DO decreased clearly after April (i.e., from  $4.0 \pm 0.4$  mg L<sup>-1</sup> in February and March to  $0.85 \pm 0.54$  mg L<sup>-1</sup>), indicating an anaerobic environment.





Table 1 The maximum, minimum, average, and standard deviation values for electrical conductivity, pH, water temperature, and dissolved oxygen measured at each survey location from February to November 2022.

	Elec	etrical con	ductivity (	$(mS m^{-1})$	pH						Water temperature (°C)					
	n	Max	Min	Mean	SD	n	Max	Min	Mean	SD	n	Max	Min	Mean	SD	
Backwater A	9	72.2	40.9	61.7	10.6	9	9.3	7.3	8.0	0.5	9	23.1	11.8	18.7	3.3	
River A	9	58.6	23.0	38.6	10.2	9	9.2	7.7	8.4	0.4	9	28.2	13.3	20.7	4.2	
Backwater B <sub>1</sub>	9	44.3	36.1	40.7	2.7	9	8.0	7.0	7.5	0.4	9	24.0	18.0	20.3	1.8	
Backwater B <sub>2</sub>	7	42.5	37.0	40.2	1.8	7	8.0	7.0	7.5	0.3	7	22.2	17.9	20.0	1.6	
River B	9	48.5	23.9	38.7	6.7	9	9.0	7.6	8.2	0.5	9	28.5	16.3	22.1	3.6	
Backwater C	9	47.2	33.7	40.7	4.2	9	7.9	7.2	7.6	0.2	9	23.5	14.2	19.1	2.9	
River C	9	46.4	26.0	37.0	5.3	9	8.6	7.4	8.0	0.4	9	28.6	12.8	20.5	4.5	

	Dissolved oxygen (mg L <sup>-1</sup> )					Dissolved oxygen(%)				
	n	Max	Min	Mean	SD	Max	Min	Mean	SD	
Backwater A	8	11.9	2.4	5.1	3.7	113.5	26.7	53.9	34.6	
River A	8	11.1	8.6	9.9	0.9	141.2	100.1	112.9	13.9	
Backwater B <sub>1</sub>	8	8.4	5.3	6.2	1.0	91.2	59.5	69.8	9.8	
Backwater B <sub>2</sub>	6	6.2	4.6	5.3	0.6	71.4	52.1	59.5	6.6	
River B	8	12.6	8.3	10.9	1.4	158.6	98.9	128.5	22.2	
Backwater C	8	4.3	0.4	1.6	1.4	43.7	4.2	17.5	14.5	
River C	8	10.4	3.2	8.5	2.1	117.1	38.5	96.9	23.3	

# 3.2. 222Rn variation

The concentrations of <sup>222</sup>Rn in shallow groundwater (i.e., 700 ± 275 dpm L<sup>-1</sup>) were about one order of magnitude higher than those in river water (i.e., 85 ± 61 dpm L<sup>-1</sup>), as in previous reports from the Kanto region in Japan (Nakano-Ohta et al., 2005; Saito and Takata, 1994). The ranges of <sup>222</sup>Rn concentrations in Backwater A, B, and C were 204 dpm L<sup>-1</sup> to 617 dpm L<sup>-1</sup>, 317 dpm L<sup>-1</sup> to 589 dpm L<sup>-1</sup>, and 293 dpm L<sup>-1</sup> to 692 dpm L<sup>-1</sup>, respectively, clearly indicating higher concentrations than those in the rivers beside each backwaters. Rn concentrations in Backwater A and C increased from spring to summer and decreased in September when precipitation was heaviest, while in Backwater B they showed relatively constant activity (Table S1, Supplement).





# 3.3. $\delta^2$ H-H<sub>2</sub>O and $\delta^{18}$ O-H<sub>2</sub>O variation

The hydrogen and oxygen stable isotope ratios ( $\delta^2$ H and  $\delta^{18}$ O) of rainwater collected near the survey site ranged from -139% to -8.4% and -18.5% to -4.4%, respectively. The local meteoric water line (LMWL) was determined by simple regression analysis as  $\delta^2$ H =  $8.3 \times \delta^{18}$ O + 18 (Figure 4). The variation in  $\delta^2$ H and  $\delta^{18}$ O in rivers, backwaters, and shallow groundwater was confined to a narrow range (i.e.,  $\delta^2$ H: -59% to -47% and  $\delta^{18}$ O: -9.2% to -7.6%) compared to the variation in rainwater. The  $\delta^2$ H and  $\delta^{18}$ O values of the spring water of backwater and the adjacent river water sampled in the same month often had different values. However, in February, when no spring water was observed in Backwater A, the  $\delta^2$ H and  $\delta^{18}$ O values of the river and the backwater showed similar values (Table S2, Supplement).

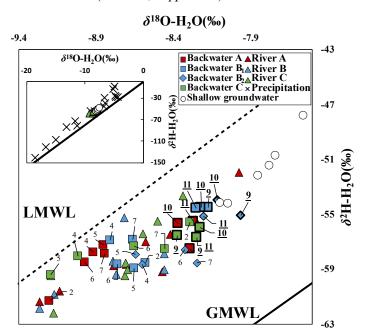


Figure 4 Overall diagram (upper left) of hydrogen and oxygen isotope ratios ( $\delta^2$ H,  $\delta^{18}$ O) in rivers, spring water at backwater, and shallow groundwater during the survey period and enlarged diagram. GMWL and LMWL represent the Global Meteoric Water Line ( $\delta^2$ H =  $8\delta^{18}$ O + 10) and the Local Meteoric Water Line ( $\delta^2$ H =  $8.3\delta^{18}$ O + 18), respectively. The numbers attached to each symbol indicate the month of sampling. The data obtained in autumn is indicated by symbols enclosed in thick lines and the month of sampling underlined.

#### 190 **3.4. Piper diagram**

The results of the Piper diagrams for each location are shown in Figure 5. The differences in ion balance between locations of the river were minor, while those differed from those of the backwaters spring and shallow groundwater. Shallow groundwater was characterized by a high proportion of Ca and Mg and was located on the left side of the graph. During the





summer period when water levels were high, Backwater A had high concentrations of Na and SO<sub>4</sub> and these values deviated from the linear line representing the mixture of the river and shallow groundwater. On the other hand, the water quality of Backwater B and C were plotted between the river and shallow groundwater, with Backwater B showing ion balance closer to that of shallow groundwater.

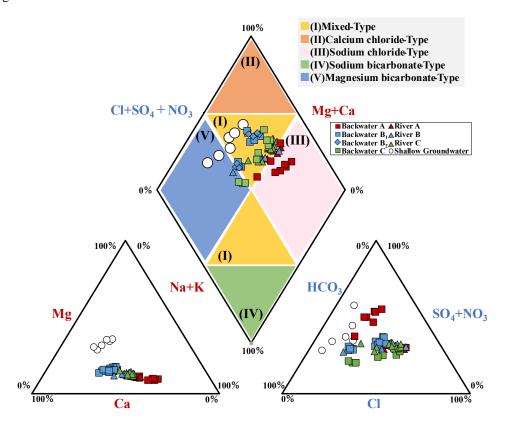


Figure 5 Characteristics of each water sample plotted on a Piper diagram based on the composition of cations (red color) and anions (blue color) in each water sample. Different water chemistry phases are indicated by different background colors.

#### 3.5. CDOM

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CDOM concentrations showed a clear difference between river water (c.a. 70 ppb) and shallow groundwaters (c.a. 3 ppb) (Figure 6). Furthermore, CDOM concentrations in treated sewage water were as high as 200 ppb, indicating the possibility that the contribution of rivers, shallow groundwater, and treated sewage effluent to backwater can be determined by CDOM. The CDOM concentrations in each backwaters varied from site to site, with 34 ppb in Backwater A, 7.8 ppb in Backwater B, and 24 ppb in Backwater C.





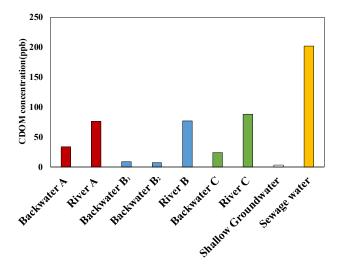


Figure 6 Bar graph showing CDOM concentrations at each survey location. All data are from November 2022.

## 3.6. Changes in the Nutrient Environment of the backwaters

NO<sub>3</sub><sup>-</sup> concentrations [NO<sub>3</sub><sup>-</sup>] in rivers were high, ranging from about 100 μmol L<sup>-1</sup> to 300 μmol L<sup>-1</sup> (Figure 7a, Tables S3-S5, Supplement). The highest concentrations were observed in winter (February and March). At Backwater A, [NO<sub>3</sub><sup>-</sup>] were as high as in the river during the winter (i.e., 298 μmol L<sup>-1</sup> in February), but lower than in the river (i.e., 75–122 μmol L<sup>-1</sup>) when the size of the backwater was expanding (i.e., April to November). In Backwater B, [NO<sub>3</sub><sup>-</sup>] fluctuated between about 120 μmol L<sup>-1</sup> and 230 μmol L<sup>-1</sup>, often lower than those in rivers. No apparent difference in [NO<sub>3</sub><sup>-</sup>] was observed between the spring water from the bottom floor and the terrace cliffs. Unlike the other backwater, Backwater C showed a significant decrease in [NO<sub>3</sub><sup>-</sup>] after early summer, reaching as low as 18 μmol L<sup>-1</sup> in November. The [NO<sub>3</sub><sup>-</sup>] in shallow groundwater ranged from about 130 μmol L<sup>-1</sup> to 350 μmol L<sup>-1</sup>, with significant differences among locations, and some locations had higher concentrations than rivers and backwater springs.





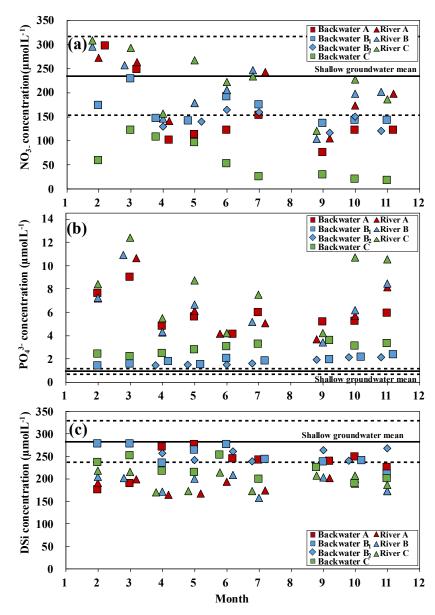


Figure 7 Seasonal variation in (a) NO<sub>3</sub><sup>-</sup>, (b) PO<sub>4</sub><sup>3-</sup>, and (c) DSi concentrations of backwater spring water and adjacent rivers. The solid line and broken lines in each graph indicate the average and standard deviation of shallow groundwater, respectively.

The PO<sub>4</sub><sup>3-</sup> concentration [PO<sub>4</sub><sup>3-</sup>] in rivers ranged from 3.4 μmol L<sup>-1</sup> to 12.4 μmol L<sup>-1</sup>, showing significant seasonal fluctuations (i.e., higher in February and March than the other months after April) consistent with the annual trend reported over the past 10 years in the public data for the Tama suidobashi, which is located downstream of the survey site (Table S7, Supplement).





 $[PO_4^{3-}]$  increased in winter and decreased from spring to summer (Figure 7b, Tables S3-S5, Supplement). Similarly, seasonal variation in  $[PO_4^{3-}]$  in Backwater A (i.e., 7.6 μmol  $L^{-1}$  in February, 9.0 μmol  $L^{-1}$  in March and 4.1 μmol  $L^{-1}$  to 5.9 μmol  $L^{-1}$  in the other months after April) was similar to that of rivers.

On the other hand, seasonal fluctuations in  $[PO_4^{3-}]$  were small in Backwater B and C, ranging from 1.4  $\mu$ mol  $L^{-1}$  to 2.3  $\mu$ mol  $L^{-1}$  and 2.2  $\mu$ mol  $L^{-1}$  to 3.6  $\mu$ mol  $L^{-1}$ , respectively. These values were slightly higher than  $[PO_4^{3-}]$  in the shallow groundwater, which averaged  $0.93 \pm 0.23 \ \mu$ mol  $L^{-1}$ .

During the survey period, DSi concentrations [DSi] in Backwater A and B were 175 to 276 μmol L<sup>-1</sup> and 209 to 276 μmol L<sup>-1</sup>, respectively, which were significantly higher than those in the adjacent rivers (Dunn test, p < 0.05, Figure 7c). In Backwater C, [DSi] were also higher than those in the adjacent rivers in most seasons, ranging from 14 to 40 μmol, but no statistically significant difference was observed. Seasonal fluctuations were small in both rivers and backwaters, but the trends in fluctuations were similar. Most samples in shallow groundwater showed [DSi] of approximately 200 to 300 μmol L<sup>-1</sup>, except one location with a distinctly high concentration of 730 μmol L<sup>-1</sup>.

The stable isotope ratios of NO<sub>3</sub><sup>-</sup> (δ<sup>15</sup>N<sub>NO3</sub> and δ<sup>18</sup>O<sub>NO3</sub>) in rivers changed between 12.7% and 18.1% for δ<sup>15</sup>N<sub>NO3</sub> and between -2.1% and 1.4% for δ<sup>18</sup>O<sub>NO3</sub> (Figure 8). Backwater A and B showed δ<sup>15</sup>N<sub>NO3</sub> and δ<sup>18</sup>O<sub>NO3</sub> values similar to those of rivers in spring (April and May). On the other hand, in autumn (October and November), δ<sup>15</sup>N<sub>NO3</sub> and δ<sup>18</sup>O<sub>NO3</sub> in Backwater A showed an increasing trend, while δ<sup>15</sup>N<sub>NO3</sub> in Backwater B showed a decreasing trend. At Backwater C, δ<sup>15</sup>N<sub>NO3</sub> and δ<sup>18</sup>O<sub>NO3</sub> consistently showed more enriched values (i.e., 20.1‰–35.7‰ for δ<sup>15</sup>N, 6.2‰–15.9‰ for δ<sup>18</sup>O) compared with those in rivers. Shallow groundwater showed lower δ<sup>15</sup>N<sub>NO3</sub> and δ<sup>18</sup>O<sub>NO3</sub> values than those in rivers and backwater spring water, ranging from 1.7‰ to 6.9‰ for δ<sup>15</sup>N and from -1.5‰ to 1.7‰ for δ<sup>18</sup>O.

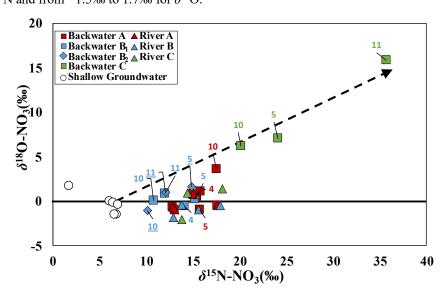


Figure 8 Distribution of stable nitrogen and oxygen isotope ratios in nitrate ( $\delta^{15}$ N-NO<sub>3</sub> and  $\delta^{18}$ O-NO<sub>3</sub>) of various water samples. The numbers shown on each symbol indicate the month of water sampling and its color indicate





corresponding sampling site (the numbers for Spring  $B_2$  are underlined to distinguish them from Spring  $B_1$ ). The broken arrow indicates the direction in which  $\delta^{15}N$  and  $\delta^{18}O$  typically change at a ratio of 2:1 when nitrate in groundwater around the survey site undergoes denitrification.

## 4. Discussion

#### 4.1. Estimation of Water Origins and Supply Routes Using a Multi-Tracer

Previous studies on large floodplain water bodies have focused on secondary channels within floodplains (Bondar-Kunze et al., 2009; Tockner et al., 1999), floodplain water bodies maintained by backflow from slow-flowing rivers (Houser et al., 2015), and isolated water formed in floodplains (ponds, floodplain lakes) (Forshay and Stanley, 2005). These studies demonstrate that the nutrients supplied by inflowing water and the nutrients released from sediments influence the nutrient dynamics in floodplain water bodies.

Many floodplains are mixed zones of river water and groundwater (Boulton et al., 2010; Hancock, 2002), and the sedimentary environment and redox conditions in the water bodies are heterogeneous (Appling et al., 2014; Gallardo, 2003; Lewandowski and Nützmann, 2010). Therefore, when considering nutrient dynamics in water bodies such as floodplains, it is essential to thoroughly examine the origin and pathways of water.

## 4.2. Estimated Origin of backwater Water

In this study, the mixing ratio of river underflow and shallow groundwater supplied to the backwater spring water changed over time and space. Among the multiple tracers used in this study, <sup>222</sup>Rn was effective in estimating the origin of spring water from backwater, as it exhibited higher concentrations in shallow groundwater than in rivers, consistent with previous studies (Dimova and Burnett, 2011; Ellins' et al., 1990). The <sup>222</sup>Rn concentration in the spring water of the backwaters remained at a high level from 204 dpm L<sup>-1</sup> to 660 dpm L<sup>-1</sup>, indicating that all the backwaters were affected by shallow groundwater.

The load of <sup>222</sup>Rn during the river underflow process in floodplains varies depending on the geology and residence time (Biehler et al., 2020; Schaper et al., 2022). It has been reported that it takes about 3 weeks for <sup>222</sup>Rn to reach an equilibrium with minerals that generate Rn. However, the residence time of the underflow can be relatively short in our study area, because the survey area consists of gravelly sediments, and the distances between the river and backwaters are short. Therefore, it is considered that the influence of <sup>222</sup>Rn loading during the underflow process is negligible.

The influence of shallow groundwater was also observed in the ion compositions. The Piper diagram (Figure 5) shows that through the study period, the ion composition of Backwater B and C was intermediate between shallow groundwater and river water, respectively, with the composition of Backwater B being more similar to that of groundwater. Therefore, it was suggested that both Backwater B and C are formed by the mixing of river water and shallow groundwater, with Backwater B in particular being strongly influenced by shallow groundwater. On the other hand, the ion composition of Backwater A was



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characterized by high concentrations of Na and was slightly out of the mix of river and groundwater. In Backwater A, the inflow of factory effluent was observed upstream of the floodplain, and springs with high EC were observed as river flow increased. This suggests that the rising groundwater level in the floodplain due to increased river flow caused wastewater and groundwater to mix, forming the spring water.

In this study, CDOM concentrations showed clear differences in rivers, shallow groundwater, and treated sewage water (Figure 6). Dissolved organic matter (DOM) in urban rivers is suggested to be persistent (Dignac et al., 2000) because sewage treatment plants release refractory DOM after sewage treatment using microbial activity in the circulation tanks. Therefore, the relatively low CDOM concentrations observed in the spring water at backwaters are thought to reflect dilution by shallow groundwater with a lower concentration of CDOM. Furthermore, CDOM concentrations can not only be used as an indicator of shallow groundwater inflow in the backwater, but also have the potential to allow a numerical estimation of the mixing ratio between river water and shallow groundwater. The CDOM concentrations in both Backwater B and C were lower than those in the river, but in addition, Backwater B, which was inferred from the Piper diagram to be more strongly influenced by shallow groundwater, showed CDOM concentrations closer to those of shallow groundwater than Backwater C.

The d-value (Dansgaard, 1964), which is known to preserve the evaporation process during raincloud formation over the sea, generally shows values < 10 % in summer and > 20 % in winter in Japan, located in the monsoon climate zone (Waseda and Nakai, 1983). In this survey, d-values of rainwater showed a similar trend (i.e., 7.3% to 13% in rainy season, 13% to 30% in dry season) (Table S2, Supplement). On the other hand, the d-values for rivers, shallow groundwater, and backwaters ranged from 7.5% to 14.5% regardless of season, showing values close to those during summer precipitation. Consequently, this indicates that summer rainfall is the primary recharge source for shallow groundwater. Furthermore, in rivers, dams in the upper reaches store rainfall during the rainy season in summer to ensure domestic water supplies. This water is then discharged into the Tama River either directly or indirectly via domestic water systems throughout the year, likely contributing to the pronounced influence of summer rainfall. In fact, in the survey period, 43% of the annual precipitation fell during the summer months (July, August, and September). Hydrogen and oxygen stable isotopes ( $\delta^2$ H and  $\delta^{18}$ O) in water showed altitude effects even in Tama river middle reaches, due to the higher elevation of its whole catchment area (i.e., 1021 m on average) (Inamura and Yasuhara, 2003). On the other hand, shallow groundwater in the plateau generally has relatively heavier  $\delta^2$ H and  $\delta^{18}$ O values than the adjacent rivers. This is because shallow groundwater is recharged by rain that falls in relatively lowland areas (i.e., about 8–50 m) compared to the Tama river whole catchment area, as also suggested by Shimada et al. (1994).

In this study,  $\delta^2$ H and  $\delta^{18}$ O values at the backwater spring outlet showed a trend toward heavier values after July to September (Figure 4). This change is consistent with the fact that most of the annual precipitation falls in summer and shallow groundwater is mainly replenished by summer rainfall, as discussed in terms of the d-value above. In our study sites, summer precipitation increased the supply of shallow groundwater, indicating that shallow groundwater became the dominant source of spring water in the backwaters.



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## 4.3. Nutrients Dynamics in the backwaters

In floodplain water bodies, nutrients are generally supplied by overflow water and underflow water from the main river (e.g., overflow during floods: Heiler et al., 1995; Knowlton and Jones, 1997; underflow: Tockner et al. 1999). Furthermore, it has been clarified that the effect of nutrient dilution occurs due to the inflow of groundwater, where nutrients are removed by denitrification and adsorption on minerals Bornette and Amoros, 1991; Carbiener et al., 1990. However, recent studies have often reported that groundwater can act as a source of nitrate and phosphate, influenced by surrounding land use and contamination (e.g., NO<sub>3</sub><sup>-</sup>: Arauzo et al., 2011; PO<sub>4</sub><sup>3-</sup>: Holman et al., 2010; Lewandowski et al., 2015), as well as by geology (PO<sub>4</sub><sup>3-</sup>: Huang et al., 2020). Therefore, when considering the nutrient dynamics of backwater, it is necessary to take into account the effects of nutrients transported by shallow groundwater.

In this study,  $[NO_3^-]$  in groundwater ranged from 130 to 350  $\mu$ mol  $L^{-1}$ , as high as in river water, but  $\delta^{15}N$  in  $NO_3$  ( $\delta^{15}N_{NO3}$ ) in groundwater (i.e., 1.7–6.9 ‰) was lower than that in river water (i.e., 15–20‰, Kumazawa, 1999) affected by treated sewage.

Therefore,  $NO_3^-$  in groundwater may be more influenced by chemical fertilizers applied to fields rather than leaking sewage pipes. As  $^{222}$ Rn and CDOM were used as indicators to clearly distinguish the contribution of shallow groundwater and river water to backwater (see above),  $\delta^{15}N_{NO3}$  could also be used to analyze the source of nitrate observed in backwater. On the other hand, in the spring water of Backwater A and C,  $NO_3^-$  concentrations decreased as DO decreased during the summer months. Especially in Backwater C,  $\delta^{15}N_{NO3}$  and  $\delta^{18}O_{NO3}$  increased in a ratio of approximately 2:1 (Figure 8), which is a typical phenomenon observed in the  $NO_3$ -isotopes when denitrification occurs under anaerobic conditions (Kendall et al., 2008). The formation of anaerobic conditions and the activation of denitrification in the floodplain subsurface have been reported in many studies (Bernard-Jannin et al., 2017; Obana et al., 2011). Denitrification in floodplains is not only caused by a decrease in dissolved oxygen (DO) but is also influenced by water quality parameters (such as organic matter content, water temperature, pH), groundwater level, soil particle size, and vegetation along the underflow path, among other factors (e.g., soil pH: Kaden et al., 2021; landscape and soil particle-size: Pinay et al., 2000; soil moisture: Pinay et al., 2007). The survey area is relatively narrow (i.e., within 5 km distance), and considering that there is little variation in the water quality of river water and shallow groundwater, the denitrification capacity of each backwaters may be influenced by localized transport and deposition processes in the meandering sections of the river and the resulting characteristic floodplain components such as grain size, vegetation, and organic matter content.

Due to the urbanization around the Tama River, we predicted that shallow groundwater would be contaminated with  $PO_4^{3-}$  in the same way as  $NO_3^-$ , but in fact,  $PO_4^{3-}$  concentration  $[PO_4^{3-}]$  in shallow groundwater in the middle reaches was only about  $1-2 \mu mol L^{-1}$  higher than  $[PO_4^{3-}]$  in upstream water in Tokyo (Wakamatsu et al., 2006). The reason for the discrepancy between  $[NO_3^-]$  and  $[PO_4^{3-}]$  in shallow groundwater may be attributed to volcanic ash-derived sediments (i.e., Kanto Loam layer) with high  $PO_4^{3-}$  adsorption capacity (Abdelwaheb et al., 2019; Uchida et al., 2022). As a result, the  $[PO_4^{3-}]$  in backwater reflects the influence of supply from river water (i.e., c.a. 6.8  $\mu mol L^{-1}$ ) and dilution by shallow groundwater (i.e., c.a. 0.9  $\mu mol L^{-1}$ ).



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This is consistent with the relative order of [PO<sub>4</sub><sup>3-</sup>] in each backwaters (i.e., Backwater A: 5.9 µmol L<sup>-1</sup> > Backwater C: 2.9 µmol L<sup>-1</sup> > Backwater B: 1.8 µmol L<sup>-1</sup> on average) corresponded to the relative CDOM concentrations (i.e., Backwater A: 34 ppb > Backwater C: 24 ppb > Backwater B: 7.8 ppb), which serve as an indicator of river water mixing intensity. Additionally, even in the spring water at Backwater C, where anaerobic conditions had formed, there were no significant increases in [PO<sub>4</sub><sup>3-</sup>], although the release of PO<sub>4</sub><sup>3-</sup> and Fe<sup>2+</sup> from iron-bound phosphorus has often been reported in anaerobic environments (Carlyle and Hill, 2001). These results suggest that the PO<sub>4</sub><sup>3-</sup> load in the floodplain groundwater is highly limited, and the backwater is likely always to exhibit consistently lower [PO<sub>4</sub><sup>3-</sup>] than rivers.

The concentration of DSi [DSi] in backwaters (i.e.,  $175-277 \mu mol L^{-1}$ ) was higher than that in rivers in almost all seasons (i.e.,  $157-218 \mu mol L^{-1}$ ), with an average of 54  $\mu mol L^{-1}$  higher (Figure 7c). This suggests the possibility of inflow of groundwater with higher [DSi] (i.e.,  $231-730 \mu mol L^{-1}$ ) affected by geological factors (Asano et al., 2003), and the supply of shallow groundwater to the backwaters may form an ecosystem with higher [DSi] that is different from those of rivers throughout the year.

# 4.4 The Environment of the backwater from the Perspective of Spring Water

From a stoichiometric perspective in relation to aquatic phytoplankton dynamics, the backwater studied here may exhibit significant differences in phytoplankton dynamics due to seasonal and backwater-specific variations in nutrient concentrations and compositions (Filstrup et al., 2016). Therefore, the nutrient concentrations and composition of the water samples obtained in this study were plotted (Figure 9a,b) and discussed with reference to the Redfield ratio (C:Si:N:P = 106:15:16:1), which has commonly been used in ocean studies (Brzezinski, 1985; Redfield, 1934).



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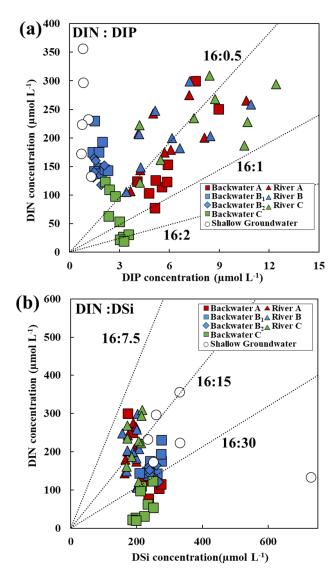


Figure 9 (a) DIN and PO<sub>4</sub><sup>3-</sup> concentrations and (b) DIN and DSi concentrations of spring water in each backwaters, river water, and shallow groudwater plotted with dotted lines indicating typical compostion ratios. The numbers shown on each symbol indicate the month of water sampling and its color indicate corresponding sampling site (the numbers for Spring  $B_2$  are underlined to distinguish them from Spring  $B_1$ ).

The N:P ratio of nutrients was greater than 16:1 in rivers and spring water at Backwater A and B, especially in Backwater B, ranging from 60 to as high as 150. On the other hand, in Backwater C, the N:P ratio decreased significantly from approximately 56:1 to 6:1 seasonally due to the reduction of NO<sub>3</sub><sup>-</sup> through denitrification. In backwaters such as Backwater B and C, where the contribution from shallow groundwater recharge is significant, [PO<sub>4</sub><sup>3-</sup>] remains consistently low, while



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[NO<sub>3</sub>] fluctuates due to seasonal changes in microbial activity, fertilization, and river influence. Consequently, the N:P ratio of nutrients in spring water at backwater may vary significantly depending on the season. On the other hand, in Backwater A, where CDOM concentrations were high, suggesting much greater contribution from river water, the N:P ratio of nutrients tended to be relatively stable. This is likely because urban rivers are influenced by treated wastewater-derived nutrients with a relatively stable N:P ratio throughout the year. Since treated wastewater is mixed with the river base flow containing low concentrations of nutrients, the nutrient concentrations in the river fluctuate seasonally depending on the mixing ratio, keeping the N:P ratio almost stable. Overall, backwaters are recharged by shallow groundwater, river water, and drainage water with specific nutrient characteristics. This leads to fluctuations in N and P concentrations and/or the variation in the N:P ratio, which may result in the formation of diverse phytoplankton communities (Kovalenko et al., 2017; Roozen et al., 2008; Unrein, 2002). The DSi concentration [DSi] in the Tama River was around 340 µmol L<sup>-1</sup> prior to 1950, slightly higher than the average (316 μmol L<sup>-1</sup>) for other major rivers in Japan (Kobayashi, 1961). Additionally, the average [DSi] in the Tama River decreased to about 300 μmol L<sup>-1</sup> around 2005 (Inoue and Akagi, 2006), and has further decreased to about 190 μmol L<sup>-1</sup> in 2022 (this study). Structures such as dams and weirs that disrupt the continuity of rivers cause DSi to be taken up by phytoplankton and precipitate as particulate silicon, thereby inhibiting the transport of DSi from upstream (Humborg et al., 1997; Maavara et al., 2020; Wei et al., 2015; Yang et al., 2018). The construction of multiple weirs on the Tama River may also be hindering DSi transport and causing low concentrations in the main river channel. In addition, [DSi] in rivers and spring areas also decrease significantly due to the direct supply of rainwater with lower [DSi] (i.e., below the detection limit). The [DSi] in shallow groundwater is at least 30 µmol L<sup>-1</sup> higher than that of rivers, and the DIN:DSi of spring water influenced by groundwater was clearly different from that of rivers, being up to 16:30 or higher (Figure 9b). Many studies have reported that DSi is one of the factors limiting diatom proliferation in freshwater ecosystems (Egge and Aksnes, 1992; Egge and Jacobsen, 1997; Justić et al., 1995; Turner et al., 1998). In backwater, the inflow of shallow groundwater may create an environment favorable to diatoms compared to rivers, potentially leading to changes in the composition of phytoplankton communities.

### Data availability

395 The datasets used in this study are available from the corresponding author upon reasonable request.

#### **Author contributions**

R.U. designed the study with guidance from Y.U. R.U. conducted the fieldwork, performed the data analysis, and wrote the manuscript. Y.U. supervised the research and revised the manuscript.





# **Competing interests**

400 The authors declare that they have no competing interests.

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