We have listed below, both the original text and the revised version (in bold), which was updated based on reviewer's comments.

Response1

(L30-L74 original text)

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 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 reemerges through floodplains, the decomposition of organic matter in the underflow process induces changes in the aerobic-anaerobic 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_3^-) and ammonium (NH_4^+) 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.

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, ^{222}Rn concentration, stable hydrogen and oxygen isotope ratios (δ^2H & δ^{18}O–H_2O) 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.

(revised version)

Rivers form distinctive landforms and landscapes in their surrounding areas through processes such as erosion, transport, and deposition. In particular, floodplains—where water overflowing from rivers flows during periods of high water caused by rainfall—function as transition zones connecting terrestrial and aquatic ecosystems (Junk et al., 1989). In floodplains, hydrological changes such as flooding and seasonal fluctuations in flow result in the formation of diverse water bodies (floodplain water bodies). (Galat et al., 1997; Tockner & Stanford, 2002; Ward, 1989; Ward et al., 1999) and affect nutrient cycling (Bondar-Kunze et al., 2009) and primary production (Houser et al., 2015; Spink et al., 1998), and ecosystems (Amoros & Roux, 1988; Bornette & Amoros, 1991; Keruzoré et al., 2013). In particular, nutrient dynamics—which are closely linked to both productivity and algal communities—exhibit sink-source behavior within the complex interactions between floodplain waters and rivers (Gmitrowicz-Iwan et al., 2020; Pongsivapai et al., 2021; Zurbrügg et al., 2013).

The hydrological conditions (e.g., river discharge and groundwater levels) and geology (e.g., grain size and organic matter content) surrounding floodplains are complex, leading to variations in the distribution of river underflow (Texier et al., 2024) and fluctuations in the mixing state of river water and groundwater (Biehler et al., 2020). Furthermore, changes in the redox condition associated with the hydrological conditions influence the behavior of

phosphorus and nitrogen (e.g., phosphorus adsorption and desorption, nitrification and denitrification). Environmental tracers and stable isotope ratios have often been used in hydrological and water quality surveys in such floodplains (Gooddy et al., 2014; Verstraeten et al., 2002). A study of 100 floodplain water bodies scattered along the Rhine and Meuse rivers has shown that the composition of nutrients and ions in each backwater varies depending on the distance from the river and the location (inside or outside the levees) (Van Den Brink et al., 1993; Van Den Brink & Van Der Velde, 1991), as well as the composition of phytoplankton and small invertebrate communities (Brunke, 2002; Brunke et al., 2003; Van Den Brink et al., 1994). However, no studies have examined the relationship between nutrient dynamics and the origin (river water, groundwater, precipitation) and pathways (surface water, spring water) of water supplied to floodplain.

Since Japan is characterized by abundant rainfall, and approximately 70% of the country is mountainous, there are many rivers with steep gradients and fast-flowing currents. it has many rivers with steep gradients and fast-flowing currents (Oguchi et al., 2001) (Figure S1). In addition, residential areas and farmland are concentrated in the alluvial fans, plains, and plateaus of the middle reaches of rivers and downstream. Consequently, urban rivers have undergone channel modifications for flood control and water resource management (Nakamura & Oki, 2018; Takahashi & Uitto, 2004), resulting in many floodplains whose extent is restricted by levees (Nagayama et al., 2015). Within the floodplain, semi-enclosed water bodies are formed that are partially isolated from the river due to the development of sandbars (Kayaba et al., 1997). These are referred to as "Wando" in Japan (hereinafter referred to as "Backwater") (Figure 1). It has been reported that these floodplain water bodies contribute to the aquatic ecosystem by creating a physically stable environment (Denda et al., 2002, 2006; Kimizuka, 1998; Minagawa et al., 2015; Nakajima et al., 2008). In the middle reaches of urban rivers, which are dotted with backwaters, the quality of the water changes significantly over short distances due to agricultural runoff and effluent from wastewater treatment plants. On the other hand, permeable alluvial fans and abundant rainfall provide the plains with large quantities of groundwater, creating recharge sources with varying water qualities in the vicinity of Backwater. There has been no research on the contribution of such river water and groundwater to the origin of backwater.

We hypothesized that the complex contribution of abundant unconfined groundwater and fast-flowing urban rivers to floodplain waters leads to water-quality-forming mechanisms that differ from those of large continental rivers. Therefore, this study focused on the Tama River, which flows through the suburbs of Tokyo, Japan, and examined the hydrological connectivity between river water and surrounding groundwater by using multiple geochemical tracers in several floodplain water bodies We examined the impact of backwaters on river ecosystems by

investigating the dynamics of nutrients, including their sources and the supply processes influenced by biogeochemical processes, as well as the resulting concentrations and composition.

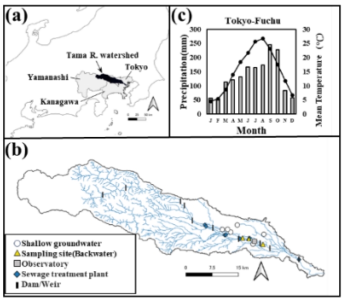
Response2

(L88-L89 original text)

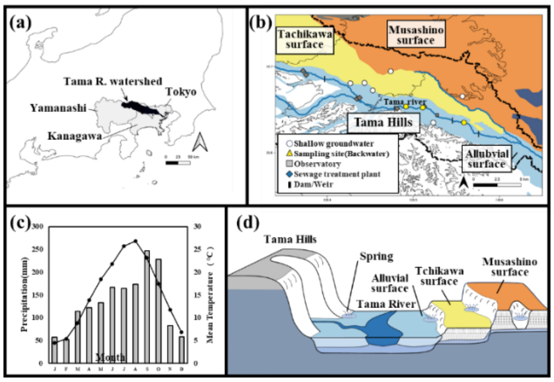
The Tama River forms river terraces from its middle to lower reaches. It was assumed that the numerous springs along the terraced cliffs would eventually flow into the Tama River and the floodplain as shallow groundwater.

(revised version)

The northern portion of the study area contains the central portion of the alluvial fan that was formed by the Tama River. The alluvial fan area is distinguished by a layer of loam on the surface, with a highly permeable gravel layer extending beneath it, resulting in an abundant supply of shallow groundwater. The shallow groundwater flows in a fan-shaped pattern from west to east, originating at the top of the alluvial fan (Shimano 1994, Yamanaka 2012). Groundwater levels in the area have been shown to flow southeastward and discharge into the Tama River (Hosono 2002, Shindo 1968). It has been established that these aquifers contribute to the replenishment of a deeper, confined aquifer. This assertion is supported by several lines of evidence, including groundwater contour lines (Shindo 1968), tritium (Shimada 1994a), and hydrogen and oxygen stable isotope ratios (Shimada 1994b). The Tama River's flow has the river terraces along the southern periphery of the alluvial fan. The intersection of the shallow groundwater's water table with the ground surface at the base of these terraces gives rise to numerous localized springs (Shimano 1994, Ohira 2003, Ueda 2000, Figure 2d). On the right bank of the Tama River, there is a hilly area that rises several dozen meters above the alluvial plain; like the alluvial fan on the left bank, its surface is covered by a layer of loam. The subsurface is comprised alternating layers of gravel, silt, and sand (Takano, 1994), and shallow groundwater flowing through highly permeable layers emerges at the boundary between the hilly terrain and the Tama River alluvial deposits.



Original figure 2



revised version figure 2

Response3

(L122-L124 original text)

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.

(revised version)

In November 2022, the concentration of colorimetric dissolved organic matter (CDOM) was measured using a CDOM sensor (Cyclops-7-Logger, Tuner, USA), which had a resolution of 0.5 ppb and a detection limit of 3.0 ppb based on factory calibration (Khamis et al., 2015), by submerging the logger in water collected in a bucket for 10 minutes.

(L284-L286 original text)

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.

(revised version)

The present study revealed discernible variations in CDOM concentrations among river water, shallow groundwater, and treated wastewater (Figure 6). The distinct concentration difference between river water and shallow groundwater remains consistent even when seasonal fluctuations in water volume occur due to rainfall (shallow groundwater: 5.5 ppb, 5.9 ppb; river water: 19.4 ppb, 57.2 ppb; in Ueba unpublished data, Tama River and surrounding shallow groundwater, comparing the rainy season and dry season, respectively).Additionally, dissolved organic matter (DOM) in urban rivers is regarded as being persistent due to the discharge of refractory DOM even after wastewater treatment, using microbial activity within circulation tanks (Dignac et al., 2000).

Response4

(L327-L337 original text)

Especially in Backwater C, δ^{15}N–NO_3 and δ^{18}O–NO_3 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.

(revised version)

In Backwater C in particular, dissolved oxygen (DO) and NO_3^- decreased significantly from spring to summer (Fig. 7), while $\delta^{15}N–NO_3$ and $\delta^{18}O–NO_3$ increased at a ratio of approximately 2:1 (Fig. 8). These findings are consistent with phenomena observed during denitrification and suggest that significant denitrification likely occurred (Kendall et al., 2008). The formation of anaerobic conditions and the activation of denitrification in floodplain aquifers have been reported in numerous studies (Bernard-Jannin et al., 2017; Obana et al., 2011). Denitrification in floodplains is influenced not only by a decrease in DO but also by other factors such as water quality parameters (organic matter content, water temperature, pH, etc.), groundwater level, soil particle size, and vegetation along groundwater flow paths (e.g., soil pH: Kaden et al., 2021; topography and soil particle size: Pinay et al., 2000; soil moisture: Pinay et al., 2007; groundwater level: MaCarty et al., 2006).Several studies suggest that in floodplains the presence of spatially localized areas with high denitrification capacity, along with the groundwater pathways and water level conditions required to access them, can significantly alter denitrification capacity (McCklain et al., 2003; Vidon and Hill, 2004; Kollogg et al., 2005). Even within the relatively small study area (within a 5 km radius), the fact that the degree of NO_3 concentration reduction varies from site to site compared to rivers and groundwater suggests that the depositional environments near each backwater differ significantly. Therefore, to evaluate denitrification capacity at each site and identify its determining factors, it is necessary to understand the floodplain subsurface in three dimensions from the perspectives of hydrology and sediment composition.

Response5

(L280-L283 original text)

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.

(revised version)

In Backwater A, spring water with high electrical conductivity (EC) and high concentrations of Na^+ and SO_4^{2-} was observed as river flow increased. Subsequent surveys revealed that in the

upstream section of Backwater A, effluent containing far higher concentrations of Na^+ and SO_4^{2-} (Na^+: approximately 11 mmol L^{-1}, SO_4^{2-}: approximately 7 mmol L^{-1} in Ueba unpublished data) and CDOM (167 ppb in Ueba unpublished data) were discharged into the floodplain. This suggests that seasonal increases in river flow caused the groundwater level in the floodplain to rise, supplying Backwater A with a mixture of discharge water and groundwater.

Supplementary Table：Water quality data for industrial discharges near backwater A

parameters	
Electrical conductivity (mS m^{-1})	205
CDOM (ppb)	167
Alkalinity (ppb)	364
Na^+ (µmol L^{-1})	10768
K^+ (µmol L^{-1})	373
Mg^{2+} (µmol L^{-1})	509
Ca^{2+} (µmol L^{-1})	2401
Cl^- (µmol L^{-1})	1989
SO_4^{2-} (µmol L^{-1})	7124
NO_3^- (µmol L^{-1})	54
NH_4^+ (µmol L^{-1})	2.0
PO_4^{3-} (µmol L^{-1})	0.77
DSi (µmol L^{-1})	570

Response6

(L22 original text)

While no significant difference in DIN concentration was

(revised version)

While no significant difference in DIN (NO_3^- + NH_4^+) concentration

(L131-L132 original text)

Major anions (Cl^-, NO_3^-, SO_4^{2-}) and cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) were determined using an ion chromatography system (DIONEX ICS-1100 for cations and DIONEX DX-120 for anions, Dionex Corporation, USA).

(revised version) Major anions (Cl^-, NO_3^-, SO_4^{2-}) and cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) were determined using an ion chromatography system (DIONEX ICS-1100 for cations and DIONEX DX-120 for anions, Dionex Corporation, USA). The sum of NO_3^- and NH_4^+ was defined as dissolved inorganic nitrogen (DIN) concentration.

Response7

We hypothesized that water quality in backwaters within the mid-reaches of urban rivers varies due to complex contributions from river water and groundwater, influenced by surrounding land use, geology, and topography. Therefore, in this study, we used multiple geochemical tracers (ion balance, CDOM, ^{222}Rn, δ^{2}H & δ^{18}O–H_2O, δ^{15}N & δ^{18}O–NO_3) to estimate the origin and pathways of water and to examine the relationship between nutrient dynamics of growth-limiting nutrients such as N, P, and Si. The results from each tracer suggested that backwaters consist of mixtures of urban river water and groundwater, with mixing ratios varying spatiotemporally, even within relatively short river sections. Phosphate and silicate concentrations in backwaters were influenced by inputs from urban rivers and shallow groundwater, which exhibited contrasting nutrient signatures (i.e., high phosphate and low silicate in urban rivers, and low phosphate and high silicate in groundwater). Although DIN concentrations were similar in urban rivers and shallow groundwater, analyses of δ^{15}N & δ^{18}O–NO_3, together with seasonal variations in NO_3^- concentrations and dissolved oxygen (DO), suggested the sources of NO_3^- in backwaters and its removal via denitrification in floodplain groundwater. As a result, the use of multiple geochemical tracers revealed that nutrient dynamics in backwaters are controlled by the mixing of urban river water and shallow groundwater, as well as microbial activity in the floodplain, thereby contributing to water-quality diversification within the river ecosystem.