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

Response1

(L8-L11 original text)

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.

(revised version)

Backwaters are semi-enclosed water bodies formed along rivers that serve as habitats for aquatic organisms (i.e., locally called Wando in Japanese). However, research the origin of the seepage water supplied to backwater and the associated nutrient supply remains limited.

(L22-L26 original text)

While no significant difference in DIN concentration was observed between rivers and groundwater, the increase in δ^{15}N & δ^{18}O–NO_3 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.

(revised version)

Although no significant difference in DIN concentrations was observed between river water and groundwater, the use of δ^{15}N & δ^{18}O–NO_3 revealed differences in the sources of NO_3 between urban rivers and groundwater, as well as the possibility of denitrification in the floodplain subsurface of some backwaters. Furthermore, it was suggested that differences in the geology and topography of the floodplains surrounding each backwater, which influence seasonal changes in river flow and microbial activity, may also lead to variations in N:P ratios among individual backwaters.

Response 2

(L284-286 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).

Response 3

(L25 original text)

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.

(revised version)

In contrast, the backwaters, which were primarily replenished by groundwater, showed pronounced seasonal fluctuations in N:P ratios due to variations in microbial activity, fertilizer inputs, and river inflow rates.

Response 4

(L27-L28 original text)

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.

(revised version)

This study, based on multiple geochemical tracer analyses, suggests that, in Japan’s steep-gradient urban rivers, hydrological connectivity and microbial activity can vary even within short river sections, and that backwaters exhibit spatially and temporally diverse nutrient environments.

Response 5

(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)

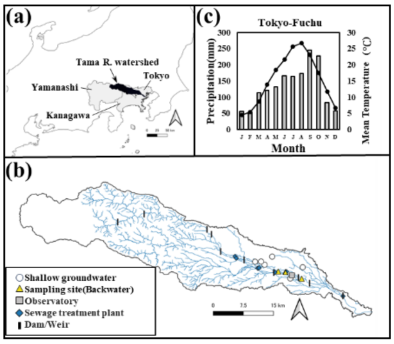
Rivers form distinctive landforms and landscapes in their surrounding areas through processes such as erosion, transport, and deposition. In particular, floodplains—where water overflowing from rivers flows during periods of high water caused by rainfall—function as transition zones connecting terrestrial 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).

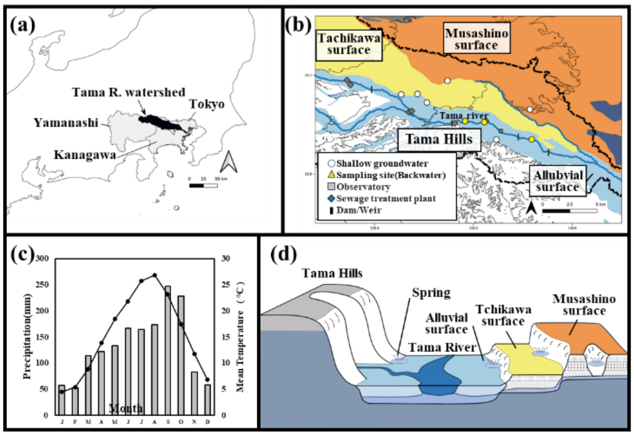
The hydrological conditions (e.g., river discharge and groundwater levels) and geology (e.g., grain size and organic matter content) surrounding floodplains are complex, leading to variations in the distribution of river underflow (Texier et al., 2024) and fluctuations in the mixing state of river water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





Original figure 2

revised version figure 2

Response 6

(L94-L95 original text)

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.

(revised version)

During the survey period, the drainage flowed into the floodplain, but no inflow was observed flowing into Backwater A or the main river as surface water.

Response 7

(L109-L110 original text)

Water samples (n=3) were collected at each of the backwaters and adjacent river mainstreams approximately once a month from February to November 2022.

(revised version)

Water samples were collected at each backwater and adjacent river mainstream approximately once a month from February to November 2022. At each site and sampling occasion, water was collected and subsampled into three separate bottles.

Response 8

(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.

Response 9

(L138-L139 original text)

Standard materials USGS32, USGS34, and IAEA were used, and analytical precision for δ^{15}N and δ^{18}O was generally better than 0.2‰ and 0.6‰, respectively.

(revised version)

Standard materials USGS32, USGS34, and IAEA-NO_3 were used, and analytical precision for δ^{15}N and δ^{18}O was generally better than 0.2‰ and 0.6‰, respectively.

Response10

(L235-L237 original text)

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.

(revised version)

In Backwater C, [DSi] were also higher than those in the adjacent rivers in most seasons, ranging from 189 to 252 μmol L^{-1}, but no statistically significant difference was observed between each river and backwater.

Response11

(L249-L250 Before)

The numbers shown on each symbol indicate the month of water sampling and its color indicate correspnding sampling site

(revised version)

The numbers shown on each symbol indicate the month of water sampling and its color indicate corresponding sampling site

Response12

(L268 original text)

(Dimova and Burnett, 2011; Ellins' et al., 1990).

(After)

(Dimova and Burnett, 2011; Ellins et al., 1990).

Response14

(L321-L322 original text)

In this study, $[NO_3^-]$ in groundwater ranged from 130 to 350 μmol L^{-1}, as high as in river water, but δ^{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.

(revised version)

In this study, $[NO_3^-]$ in groundwater ranged from 130 to 350 μmol 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., 12.7-18.1 ‰) affected by treated sewage.

Resoponse15

(L364 original text)

DIN and DSi concentrations of spring water in each backwaters, river water, and shallow groudwater plotted with dotted lines indicating typical compostion ratios.

(revised version)

DIN and DSi concentrations of spring water in each backwaters, river water, and shallow groundwater plotted with dotted lines indicating typical composition ratios.

Response16

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^2H & $\delta^{18}O–H_2O$, $\delta^{15}N$ & $\delta^{18}O–NO_3$) 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 $\delta^{15}N$ & $\delta^{18}O–NO_3$, 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.

Response17

(L395 original text)

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

(revised version)

The datasets generated and analyzed during this study are available in the Zenodo repository: https://doi.org/10.5281/zenodo.20115076.