PNIE PROCEEDINGS OF THE NATIONAL INSTITUTE OF ECOLOGY OF THE REPUBLIC OF KOREA

Original Articles

PNIE 2021;2(4):259-273
https://doi.org/10.22920/PNIE.2021.2.4.259
pISSN 2765-2203, eISSN 2765-2211

Stable Carbon Isotope Signature of Dissolved Inorganic Carbon (DIC) in Two Streams with Contrasting Watershed Environments: A Potential Indicator for Assessing Stream Ecosystem Health

Chulgoo Kim[1]*, Jong-Yun Choi[1], Byungwoong Choi[2], JunSeok Lee[1], Yonglak Jeon[1], Taewoo Yi[1]

[1] National Institute of Ecology, Seocheon, Korea
[2] National Institute of Environmental Research, Incheon, Korea

ABSTRACT

We conducted a study to investigate the characteristics of the carbon cycle of two streams (located in Shig a Prefecture, Japan), having similar size, namely, the Adokawa stream (length: 52 km, area: 305 km^2, watershed population: 8,000) and the Yasukawa stream (length: 62 km, area: 380 km^2, watershed population: 120,000), but with different degree of human activity. Samples were collected from these two streams at 14 (Adokawa stream) and 23 (Yasukawa stream) stations in the flowing direction. The dissolved inorganic carbon (DIC) concentration and the stable carbon isotope ratio of DIC (δ^{13}C-DIC) were measured in addition to the watershed features and the chemical variables of the stream water. The δ^{13}C-DIC (-9.50 ± 2.54‰), DIC concentration (249 ± 76 μM), and electric conductivity (52 ±13 μS/cm) in Adokawa stream showed small variations from upstream to downstream. However, the δ^{13}C-DIC (-8.68 ± 2.3‰) upstream of Yasukawa stream was similar to that of Adokawa stream and decreased downstream (-12.13 ± 0.43‰). DIC concentration (upstream: 272 ± 89 μM, downstream: 690 ± 37 μM) and electric conductivity (upstream: 69 ± 17 μS/cm, downstream: 193 ± 37 μS/cm) were higher downstream than upstream of Yasukawa stream. The DIC concentration of Yasukawa stream was significantly correlated with watershed environmental variables, such as, watershed population density (r = 0.8581, p<0.0001, n = 23), and forest area percentage of the watershed (r = -0.9188, p<0.0001, n = 23). δ^{13}C-DIC showed significant negative correlation with the DIC concentration (r = -0.7734, p<0.0001, n = 23), electric conductivity (r = -0.5396, p = 0.0079, n = 23), and watershed population density (r = -0.6836, p = 0.0003, n = 23). Our approach using a stable carbon isotope ratio suggests that DIC concentration and δ^{13}C-DIC could be used as indicators for monitoring the health of stream ecosystems with different watershed characteristics.

Keywords: Dissolved inorganic carbon, Stable carbon isotopes, Streams, Stream ecosystem health, Watershed

Introduction

Stream ecosystems are dependent on the characteristics and changes of the watershed environment. Studies have been conducted to determine the effects of changes in the watershed environment, especially the increase in human activity, on the river ecosystem (Clément *et al*., 2017; Li *et al*., 2018; Yirigui *et al*., 2019).

Received June 30, 2021; **Revised** October 7, 2021;
Accepted October 8, 2021

***Corresponding author:** Chulgoo Kim
e-mail ecorest@nie.re.kr
https://orcid.org/0000-0001-8669-0580

Several evaluation methods, namely, the Bellan's pollution Index (Bellan, 1967), the Saprobic Index (Zelinka & Marvan, 1961), the Shannon-Wiener Index (Shannon & Wiener, 1963), and the Index of Biotic Integrity (Clark *et al*., 2003) based on characteristics, such as, water quality measurement (dissolved oxygen (DO), chemical oxygen demand (COD), biological oxygen demand (BOD), pH, and electric conductivity (EC)) and biological community species composition, biomass, and production, are used to investigate the status of stream ecosystems. However, biological methodologies (e.g., diversity index and species number), as well as chemical and physical approaches, are insufficient for assessing the health of stream ecosystems (An *et al*., 1992; Karr, 1981).

Since the ecosystem metabolism is an integrated measure of the gross primary production and ecosystem respiration, the metabolic balance provides basic information for understanding the overall ecosystem status (Young & Matthaei, 2008). In a stream ecosystem, gross primary production uptakes CO_2 and releases O_2 , whereas ecosystem respiration is a reverse process in principle. CO_2 in water exists in various forms, such as, HCO_3^- , CO_3^{2-} , CO_2, and their compounds, depending on the pH condition, and they are collectively known as dissolved inorganic carbon (DIC). Therefore, in stream ecosystems, DIC is directly related to biological photosynthesis and organic matter degradation. However, the DIC concentration in stream water can also be changed by chemical weathering and degassing.

Information regarding which of these is the major mechanism is estimated by determining the correlation between the DIC concentration change and other accompanying variables. However, more direct information can be obtained using the stable carbon isotope ratio of DIC (δ^{13}C-DIC) (Nagata & Miyajima, 2008). For example, if photosynthesis, which is a biological process, is active in the water body, the DIC concentration will decrease and δ^{13}C-DIC will increase (Finlay, 2004; Herczeg, 1987; Hollander & KcKenzie, 1991; Quay *et al*., 1986; Stiller & Nissenbaum, 1999; Wang & Veizer, 2000). On the other hand, it is expected that the DIC concentration will increase and δ^{13}C-DIC will decrease in a respiration dominated waterbody (Atekwana & Krishnamurthy, 1998). These are explained by the isotope fractionation in the photosynthesis and respiration processes.

Rubisco enzyme fixes carbon in photosynthesis, adding CO_2 to a five-carbon compound to form a six-carbon sugar. The lighter carbon (^{12}C) reacts faster in this kinetic reaction with bond formation and the kinetic isotope effect is -29 per mill (Fry, 2006). Therefore, the kinetic isotope effect increases δ^{13}C value for DIC. However, the isotope fractionation by respiration is very insignificant (Nagata & Miyajima, 2008). Hence, δ^{13}C of organic substances used as a substrate for respiration is generally very low (-25~-30‰) and δ^{13}C of the decomposition product, DIC, has a low value.

Increase in human activity in the watershed affects streams in two ways, namely, nutrient pollution and organic pollution. In stream ecosystem dominated by nutrient pollution, active photosynthesis decreases the DIC concentration and increases the δ^{13}C-DIC. However, in stream ecosystem dominated by organic pollution, active respiration increases DIC concentration and decreases δ^{13}C-DIC.

Despite this theoretical establishment, variation patterns of the δ^{13}C-DIC values in actual streams are diverse and complex. Previous studies have shown that in some cases, δ^{13}C-DIC increased in the river flowing direction (Finlay, 2003; Telmer & Veizer, 1999) and decreased in some other cases (Aucuor *et al*., 1999), and these were mostly explained by the geological and hydrological characteristics of the watershed (Aucour *et al*., 1999; Das *et al*., 2005; Telmer & Veizer, 1999).

Furthermore, it has been shown that if artificial land use (agriculture, urban) at the watershed increased, the DIC concentration in the river would increase too (Barnes & Raymond, 2009). Such an increase might be due to the inflow of high concentrations of DIC in the watershed or might be due to the inflow of organic matter, which would accelerate organic decomposition in the river.

Therefore, in this study, we tested the suitability of DIC concentration and δ^{13}C-DIC values as indicators for monitoring the health of stream ecosystems by analyzing the watershed environment, water quality variables, and the DIC concentration and δ^{13}C-DIC values of the stream water of two streams, namely, the Yasukawa stream and the Adokawa stream, which have watershed environments with contrasting artificial impacts.

Materials and Methods

Watershed status

This study was conducted at the Adokawa stream and the Yasukawa stream, two river streams located in the same river system with contrasting watershed environments. Adokawa stream has a length of 52 km and a watershed area of 305 km^2 , and Yasukawa stream has a length of 62 km and a watershed area of 380 km^2 . The size of the two streams is roughly similar but the population size differs (8,000 and 120,000 people for the Adokawa stream and the Yasukawa stream, respectively).

The research stations for each stream are shown in Fig. 1. Field measurements and sampling were conducted at 14 stations for the Adokawa stream on May 28 and 29, 2004, and 23 stations for the Yasukawa stream on September 27 and 28, 2004. We referred to Kobayashi *et al*. (2009) for the geographic and demographic features.

Catchment area

Yasukawa stream was divided as upstream (St. Y1–Y7), midstream (St. Y9–Y11), and downstream (St. Y12-Y23) with catchment area ranges of 1.2–70 km^2, 119–152 km^2, 283–380 km^2, respectively. Adokawa stream was divided as upstream (St. A1–A6), midstream (St. A7–A9), and downstream (St. A10–A14) with catchment ranges of 0.3–66 km^2, 156–183 km^2, and 281–305 km^2, respectively (Table S1, Table S2).

Yasukawa stream branches Y8' (47.5 km^2), Y15' (11.2 km^2), and Y18' (23.4 km^2) had catchment areas similar to the upstream catchment area size, and Y13' (117 km^2) had

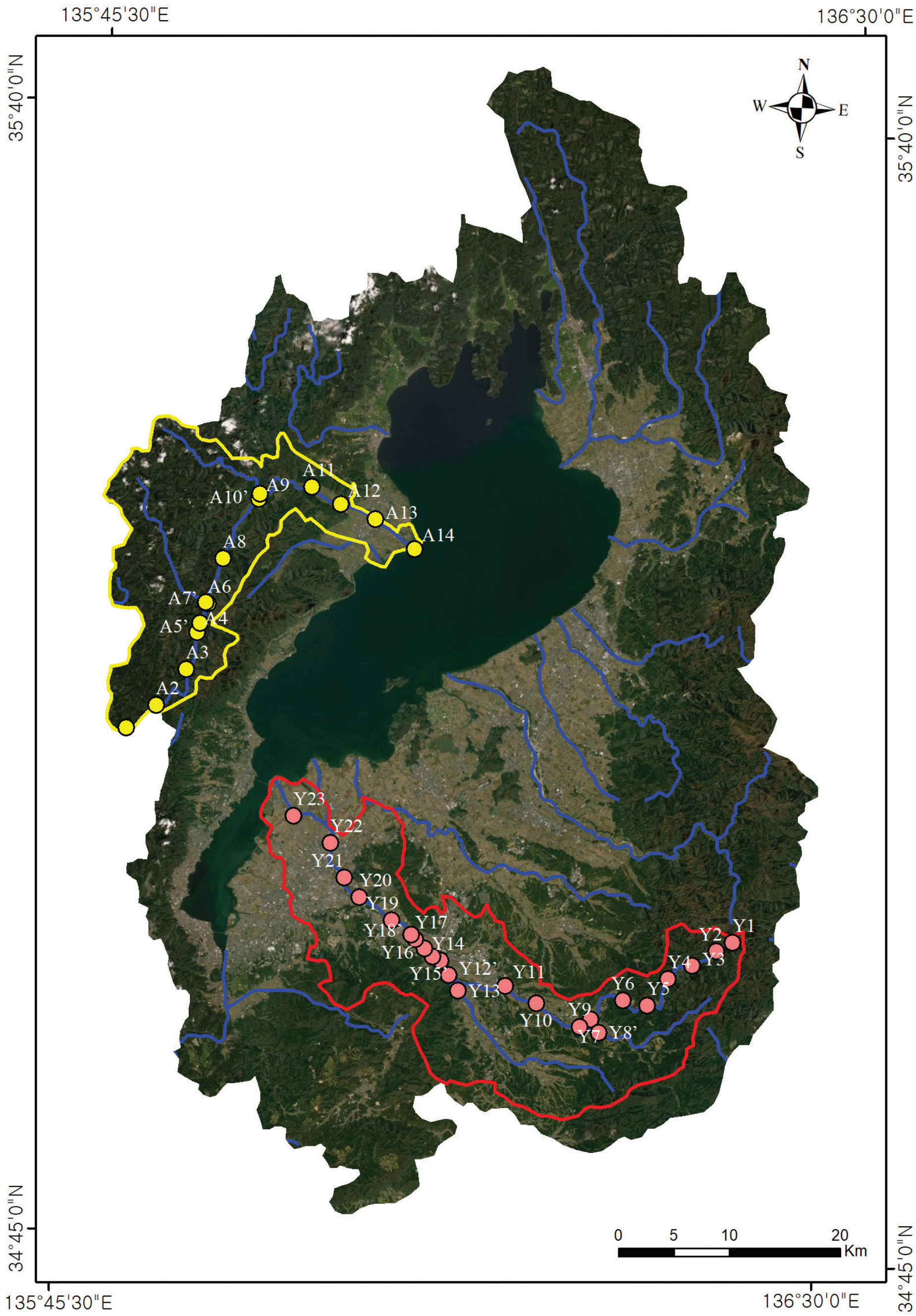

Fig. 1. Map of study sites in the Yasukawa stream and the Adokawa stream draining the Lake Biwa basin. Sampling stations are indicated by solid circles (•). Branches are indicated by a prime added to the site code. Background satellite image reproduced from ESRI.

a catchment area similar to that of the midstream.

Adokawa stream branches had a catchment area similar to that of the upstream.

Population density

The population density increased noticeably (0–321 people/km^2) at Yasukawa stream along with the increase in the flow distance, and three of the four branches, excluding Y8' (67 people/km^2), showed high population densities, similar to that of the downstream watershed (198–540 people/km^2) (Table S1, Table S2). On the other hand Adokawa stream, including the branches, showed a decreasing trend in the downstream direction (4–43 people/km^2) (Fig. 2).

Land use

The forest percentage along the Yasukawa stream watershed significantly decreased, following this order, upstream (90.9 ± 3.6%), midstream (78.6 ± 5.0%), and downstream (61.0 ± 1.8%) (Table 1, Fig. 3), but, the urban and rice field percentages increased (Table 1). Branches Y8' (80.8%) and Y15' (78.3 %) showed forest percentages similar to the upstream and midstream watershed, respectively. Y13' (54.0%) and Y18' (41.4%) showed the highest urban (Y13' = 6.6%, Y18' = 10.1%) and rice field (Y13' = 27.2%, Y18' = 27.5%) percentages among all stations (Table S1).

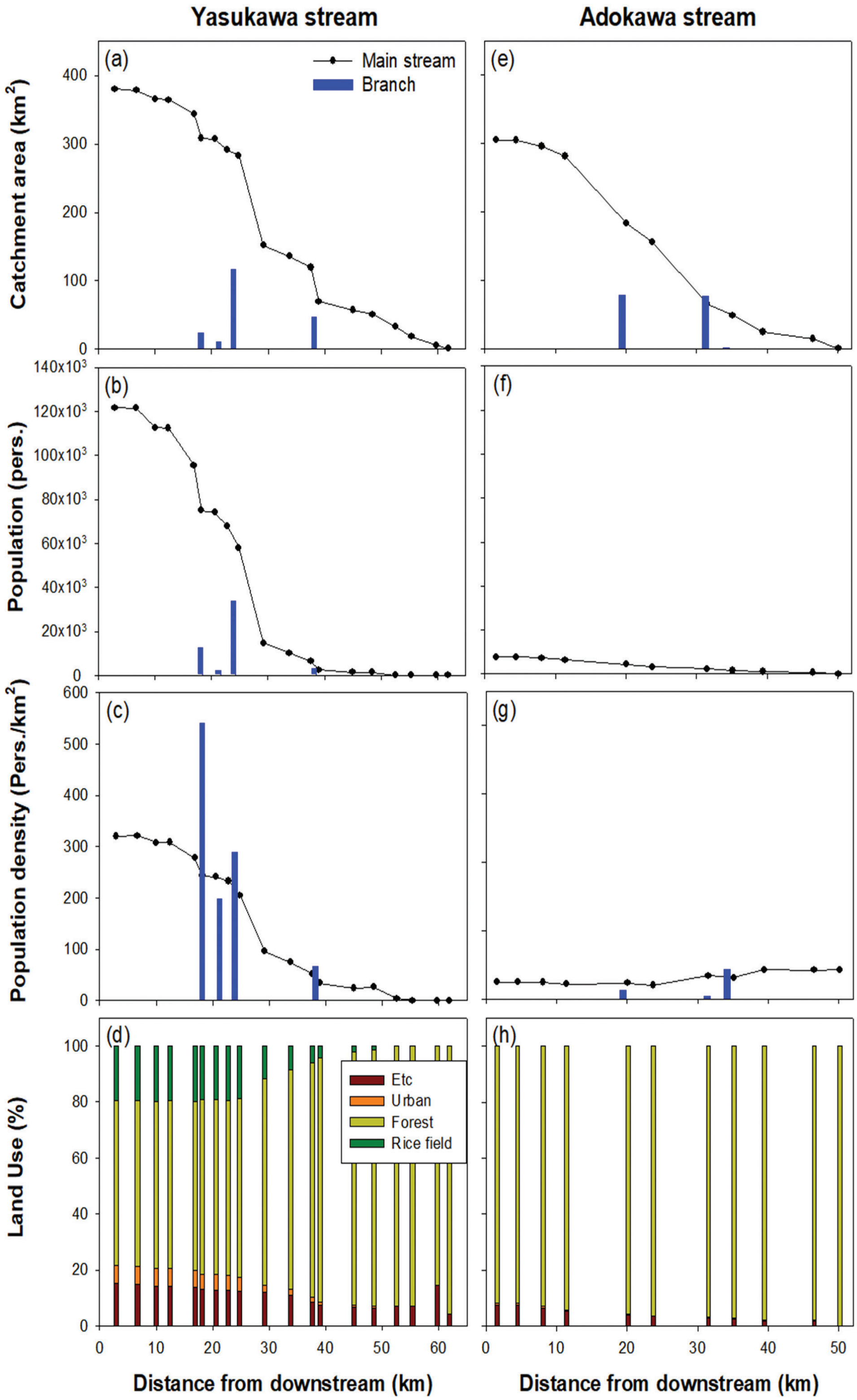

Fig. 2. Longitudinal changes of watershed features in the Adokawa and Yasukawa streams.

Table 1. Summary of environmental variables in the Yasukawa and Adokawa streams. Variables are averaged for the Total (T), upstream (U), midstream (M), downstream (D), and branch (B) sites of each stream in order to examine downward trends. C.A.: catchment area (km^2); P.D.: population density (Peop./km^{-2}); EC: electric conductivity (μScm^{-1}); DIC: dissolved inorganic carbon in stream water (μM); $\delta^{13}C$-DIC: carbon stable isotope ratio of dissolved inorganic carbon (‰). C.A. and P.D. data from Kobayashi *et al*. (2009)

Stream name	Zone	Sites No.	C.A. (km^2)	P. D. (Peop. km^{-2})	Land cover (%) Forest	Land cover (%) Rice Field	Land cover (%) Urban	pH	EC (μScm^{-1})	DIC (μM)	$\delta^{13}C$-DIC (‰)
Yasu kawa	T	Y1~Y23	1.2-380	0-321	72.8±15.8	12.1±9.3	3.7±2.9	8.0±0.6	151±90	546±208	-10.80±2.03
	U	Y1~Y7	1.2-70	0-34	90.9±3.6	1.1±1.6	0.4±0.5	7.9±0.2	69±17	272±89	-8.68±2.3
	M	Y9~Y11	119-152	52-96	78.6±5.0	8.8±2.8	2.2±0.5	8.0±0.2	129±14	553±109	-10.95±1.21
	D	Y12~Y23	283-380	205-321	61.0±1.8	19.4±0.3	5.8±0.6	8.2±0.9	193±37	690±37	-12.13±0.43
	B	Y8',Y13',Y15',Y18	11-117	67-540	63.6±19.1	17.1±11.8	5.6±3.6	7.8±0.5	215±166	696±151	-11.42±1.39
Ado kawa	T	A1-A14	0.3-305	4-43	96.6±2.6	0.02±0.01	0.36±0.22	7.4±0.4	52±13	249±76	-9.50±2.54
	U	A1-A6	0.3-66	31-43	97.1±2.6	0.01±0.01	0.27±0.16	7.3±0.6	47±20	246±120	-10.47±3.77
	M	A8, A9	156-183	20-23	95.9±0.5	0.01±0.00	0.30±0.06	7.4±0.1	55±0.1	278±1	-9.31±0.13
	D	A11-A14	281-305	22-25	92.7±1.2	0.03±0.00	0.62±0.09	7.5±0.1	59±0.6	277±2	-9.60±0.14
	B	A5',A7',A10'	1.8-78	4-43	97.1±2.6	0.01±0.01	0.21±0.22	7.3±0.1	47±12	199±56	-7.87±2.68

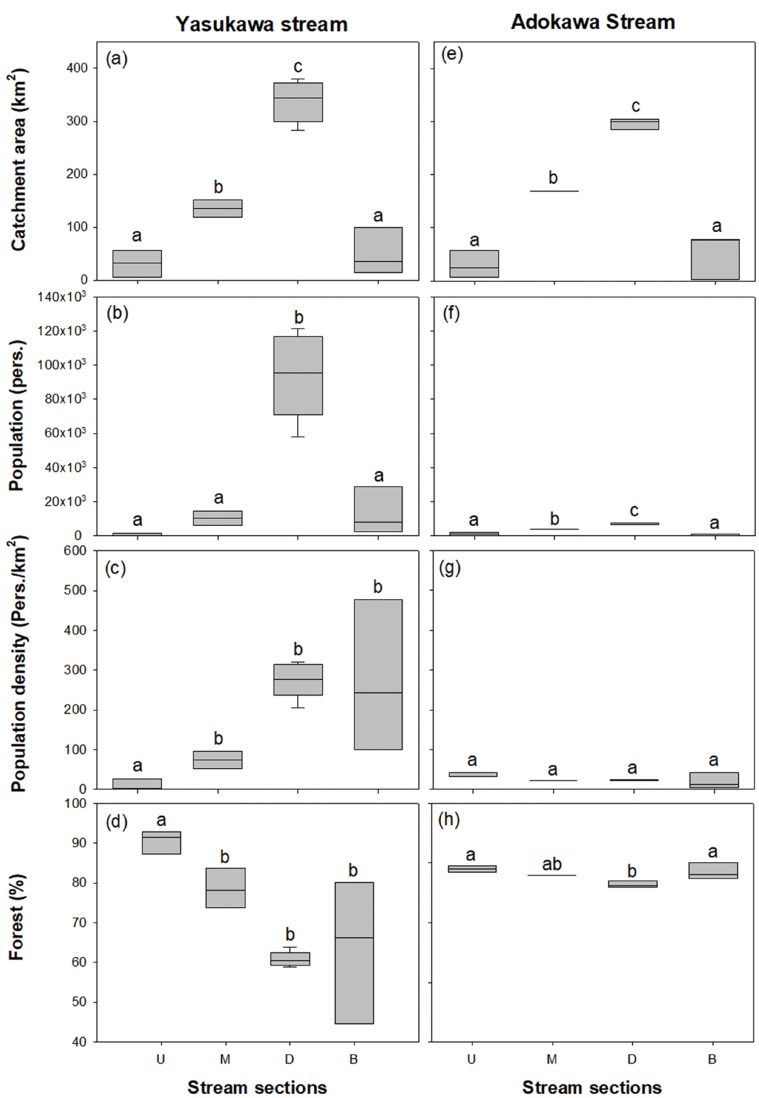

Fig. 3. Box plots of longitudinal changes of watershed features in the Adokawa and Yasukawa streams.

The forest percentage at the Adokawa stream watershed was highest (96.6 ± 2.6%), and the percentages rice field (0.02 ± 0.01%) and urban (0.36 ± 0.22%) activities were similar or less than those upstream of the Yasukawa stream (Table 1, Fig. 3).

On-site measurement of chemical variables

All chemical variables excluding the DIC were measured by Kobayashi *et al*. (2009). DIC concentrations were measured with a TOC-5000A total organic carbon analyzer (Shimadzu) (Kim *et al*., 2006; Maki *et al*., 2010).

The $\delta^{13}C$ values of DIC were determined using the headspace equilibration method, using an online system consisting of a gas chromatograph (GC-6890, Thermo Fis-

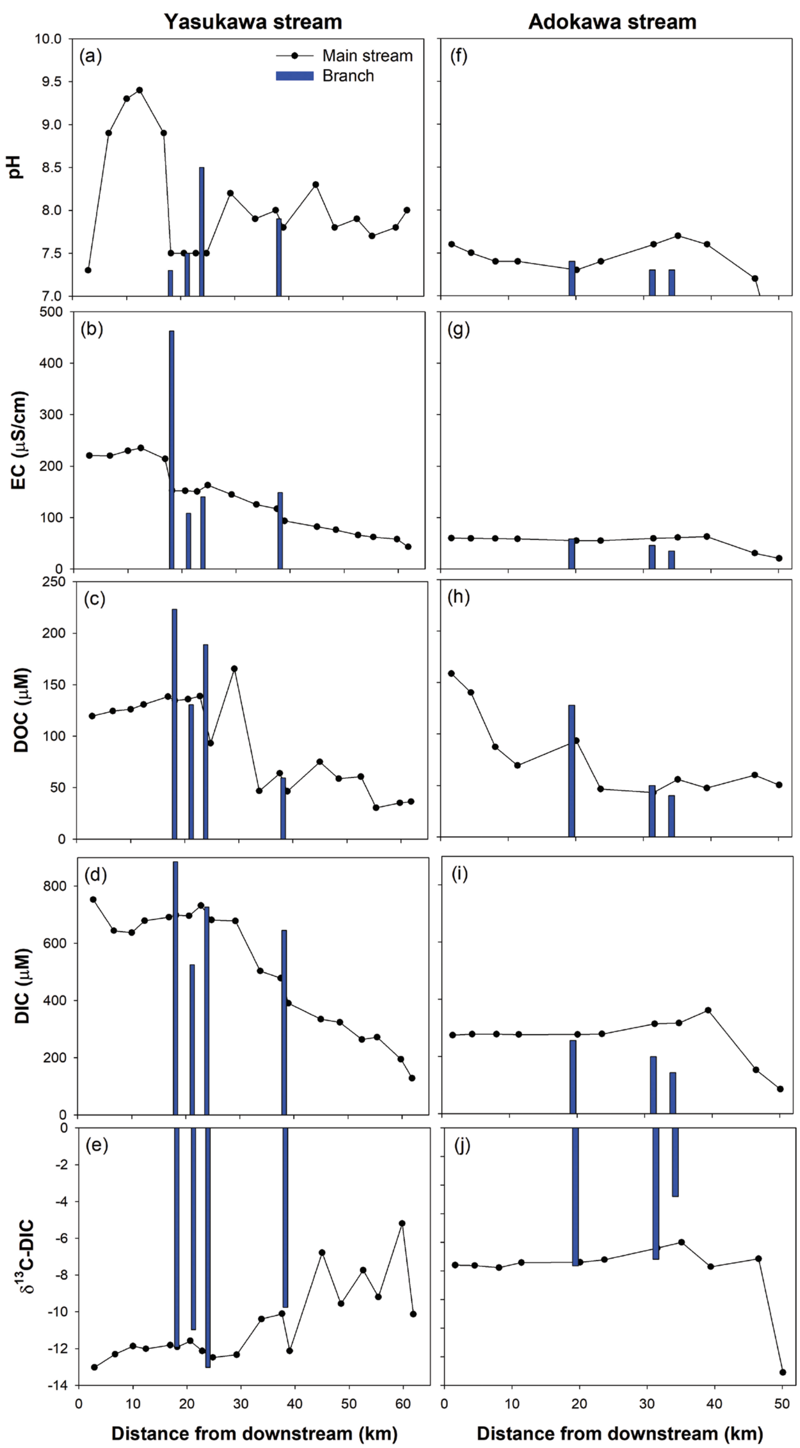

Fig. 4. Longitudinal changes of environmental variables in the Adokawa and Yasukawa streams.

her), combustion furnace (Combustion III, Thermo Fisher), and isotope ratio mass spectrometer (252, Finnigan MAT) (Maki *et al*., 2010; Miyajima *et al*., 1995).

Isotope ratios were obtained relative to a high-purity CO_2 reference gas and were determined in standard δ notation as the difference in parts per thousand (‰) relative to international standards (Pee-Dee Belemnite):

$$\delta X = [(Rsample/Rstandard)-1] \times 10^3,$$

where, δ X is $\delta^{13}C$, and Rsample and Rstandard are $^{13}C/^{12}C$ ratios of the sample and the standard, respectively

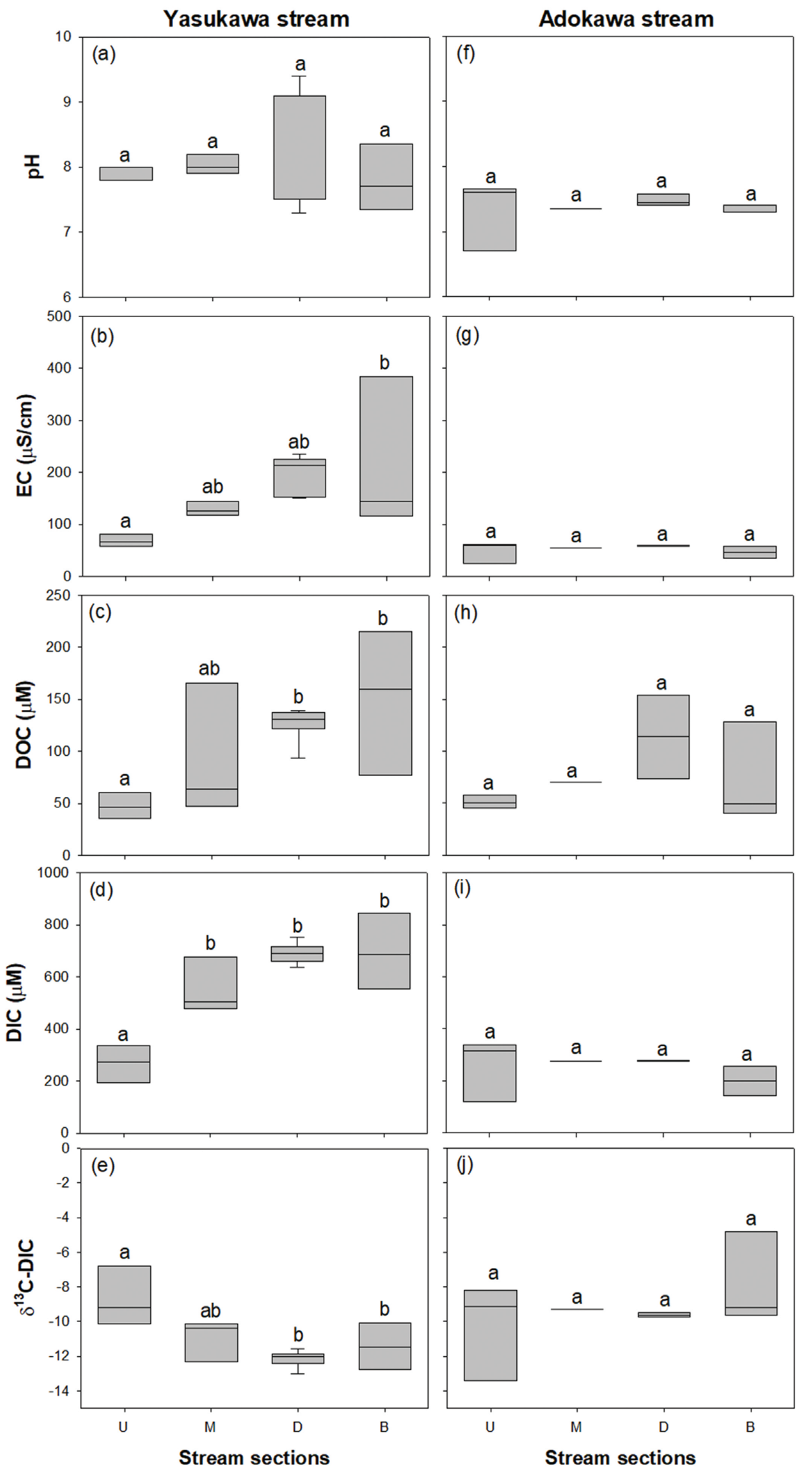

Fig. 5. Box plots of longitudinal changes of environmental variables in the Adokawa and Yasukawa streams.

(Maki *et al*., 2010; Miyajima *et al*., 1995).

Results

Chemical variables of stream water

pH

From the Yasukawa stream, it was observed that pH increased following this order: upstream (7.9 ± 0.2) < midstream (8.0 ± 0.2) < downstream (8.2 ± 0.9), but there was no significant difference (Fig. 4, Fig. 5, Table 1). In Adokawa stream, the difference in pH (7.4 ± 0.4) between stations was smaller than in Yasukawa stream (Fig. 4, Fig. 5, Table 1).

EC

EC is widely used as an indicator of the effects of human activities. EC in the Yasukawa stream significantly increased in the direction of upstream (69 ± 17 μS/cm), midstream (129 ± 14 μS/cm), and downstream (193 ± 37 μS/cm) (Fig. 4, Fig. 5, Table 1), whereas, in the Adokawa stream, low EC level (52 ± 13 μS/cm) was maintained, similar to that of Yasukawa stream upstream (Fig. 4, Fig. 5, Table 1).

DIC

The DIC concentration in Yasukawa stream significantly increased (Fig. 5) in the following direction: upstream (272 ± 89 μM) < midstream (553 ± 109 μM) < downstream (690 ± 37 μM). However, Y18' showed the highest value (885 μM).

In all sections of the Adokawa stream, a low DIC concentration (249 ± 76 μM) was recorded, similar to that of Yasukawa stream upstream (272 ± 89 μM).

δ^{13}C-DIC

In the Yasukawa stream, δ^{13}C-DIC significantly decreased in the following direction: upstream (-8.68 ± 2.3 ‰) > midstream (-10.95 ± 1.21 ‰) > downstream (-12.13 ± 0.43 ‰).

In all sections of Adokawa stream, low δ^{13}C-DIC level (-9.50 ± 2.54 ‰) was recorded, similar to that of Yasukawa stream upstream (-8.68 ± 2.3 ‰).

Correlation analysis

Catchment area vs. other variables

The catchment areas at each station of Yasukawa stream and Adokawa stream were positively correlated with the percentages of rice field and urban activities, and negatively correlated with the percentage of forest. When the mainstream data of Yasukawa stream only were analyzed, the population density, EC, and DIC were positively correlated with catchment area, and negatively correlated with δ^{13}C-DIC. Theses correlations increased further when Y1 was excluded (Table 2, Table S3).

In the case of the Adokawa stream, catchment area of the mainstream data was positively correlated with the percentage of rice field and negatively correlated with the percentage of forest at a significant level (Table 2, Table S4).

Table 2. Results of correlation analyses of the relationships between watershed features and environmental variables in the Yasukawa and Adokawa streams. Significantly correlated variables were collected (p<0.001). Abbreviations as in Table 1.

Total data				
	Yasukawa stream		Adokawa stream	
	positive correlation	negative correlation	positive correlation	negative correlation
C.A. (km^2)	Rice filed, Urban	Forest	Rice filed, Urban	Forest
P. D. (Peop.km^{-2})	Rice filed, Urban	Forest	-	-
EC (μScm^{-1})	P.D., Rice field, Urban, DIC	Forest	pH	-
DIC (μM)	P.D., Rice field, Urban, EC	Forest	pH, EC	-
δ^{13}C-DIC (‰)	Forest	P.D., Rice field, Urban, DIC	-	-

Mainstream data				
	Yasukawa stream		Adokawa stream	
	positive correlation	negative correlation	positive correlation	negative correlation
C.A. (km^2)	P.D., Rice filed, Urban, EC, DIC	Forest, δ^{13}C-DIC	Rice filed	Forest
P. D. (Peop.km^{-2})	C.A., Rice filed, Urban, EC, DIC	Forest, δ^{13}C-DIC	-	-
EC (μScm^{-1})	C.A., P.D., Rice field, Urban, DIC	Forest, δ^{13}C-DIC	pH, DIC	-
DIC (μM)	C.A., P.D., Rice field, Urban, EC	Forest, δ^{13}C-DIC	pH, EC	-
δ^{13}C-DIC (‰)	Forest	C.A., P.D., Rice field, Urban, EC, DIC	-	-

Population density vs. other variables

The population densities at each station of the Yasukawa stream were positively correlated with the percentage of rice field and urban activities, and negatively correlated with the percentage of forest. When the mainstream data of the Yasukawa stream only were analyzed, the catchment area, EC, and DIC were significantly correlated to the population density, and the correlations further increased when Y1 was excluded. In this case, in addition to the percentage of forest, δ^{13}C-DIC also became a variable with a negative correlation (Table 2, Table S3).

In the case of the Adokawa stream, population densities were not significantly correlated to the other variables (Table 2, Table S4).

EC vs. other variables

The EC at each station of the Yasukawa stream were positively correlated with the population density, DIC, percentages of rice field and urban activities, and negatively correlated with the percentage of forest. When the mainstream data of the Yasukawa stream only were analyzed, the catchment area was significantly correlated to the EC, and the correlations increased further when Y1 was excluded. In this case, in addition to the forest percentage, δ^{13}C-DIC also became a variable with a negative correlation (Table 2, Table S3).

In the case of the Adokawa stream, only pH was significantly positively correlated with EC (Table 2, Table S4).

DIC concentration vs. other variables

The DIC at each station of the Yasukawa stream was positively correlated with the population density, EC, the percentage of rice field and urban activities, and negatively correlated with the percentage of forest. When the mainstream data of the Yasukawa stream only were analyzed, the catchment area have a significant correlation with DIC, and the correlations increased further when Y1 was excluded. In this case, in addition to forest percentage, δ^{13}C-DIC also became a variable with a negative correlation (Table 2, Table S3).

In the case of Adokawa stream, pH and EC were significantly positively correlated with EC (Table 2, Table S4).

δ^{13}C-DIC vs. other variables

The δ^{13}C-DIC at each station of the Yasukawa stream was positively correlated with the forest percentage, and negatively correlated with the population density, DIC, the percentage of rice field and urban activities. When the mainstream data of the Yasukawa stream only were analyzed, the catchment area also became a variable with a negative correlation (Table 2, Table S3).

In the case of the Adokawa stream, δ^{13}C-DIC was not significantly correlated to other variables (Table 2, Table S4).

Discussion

The DIC concentration and isotope ratio in the flow-direction of a stream can be altered by different mechanisms, such as, air-water CO_2 exchange, kinetic isotope effect of CO_2 dissolution, isotope conversion and equilibrium with CO_2 , CO_2 consumption by photosynthesis, and CO_2 generation by respiration (Nagata & Miyajima, 2008).

In the present study, the DIC concentration between Y1 and Y2 increased, and δ^{13}C-DIC sharply increased because of the CO_2 evasion mechanism of CO_2 over-saturation due to high CO_2 in the soil. The δ^{13}C of dissolved CO_2 was ≥ 8 per mill lower compared to the co -existing HCO_3^- . Therefore, the δ^{13}C of the residual DIC increased due to the degassing of dissolved CO_2 (Nagata & Miyajima, 2008). This phenomenon was also confirmed in the change between A1 and A2, the uppermost stations of the Adokawa stream.

On the other hand, the change in the DIC concentration was insignificant between Y3 (271.4 μM) and Y4 (263.7 μM), and between Y5 (324.2 μM) and Y6 (334.7 μM), but, δ^{13}C-DIC increased. This is likely because of the isotope fractionation caused by the active photosynthesis between these stations. Kobayashi *et al*. (2009) reported that the concentration of chlorophyll a in epilithon at these stations increased more rapidly at Y4 (0.792 μg cm^{-2}) and Y6 (2.366 μg cm^{-2}) compared to Y3 (0.263 μg cm^{-2}) and Y5 (2.573 μg cm^{-2}), respectively, implying a relatively more active photosynthesis.

However, while the overall DIC concentration increased in the river flowing direction in the Yasukawa stream, δ^{13}C-DIC showed an inversely proportional decreasing trend (Fig. 2, Fig. 6). Furthermore, the DIC concentration was significantly positively correlated with the BOD and DOC concentrations (Fig. 7) due to active respiration of living organisms in the stream water. The δ^{13}C of CO_2 generated by respiration was almost identical to the δ^{13}C-DIC of carbon that constituted organic matter, which became food, or decaying organic matter, and generally had a value between -25 and -30 per mill (Clark & Fritz, 1997; Nagata & Miyajima, 2008).

Therefore, as shown by the high level of correlations with the population density, land use, EC, BOD, and DOC in the watershed, the increase in human activity in the watershed led to an increase in the supply of organic matter to the stream. δ^{13}C-DIC demonstrated a possibility that the DIC concentration could have increased because of actively decomposing them.

However, as shown by Kobayashi *et al*. (2009) in a study

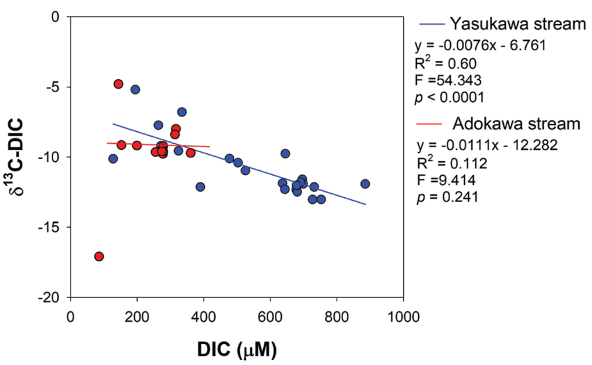

Fig. 6. Relationship between the DIC concentration and carbon stable isotope ratio of DIC (δ^{13}C-DIC).

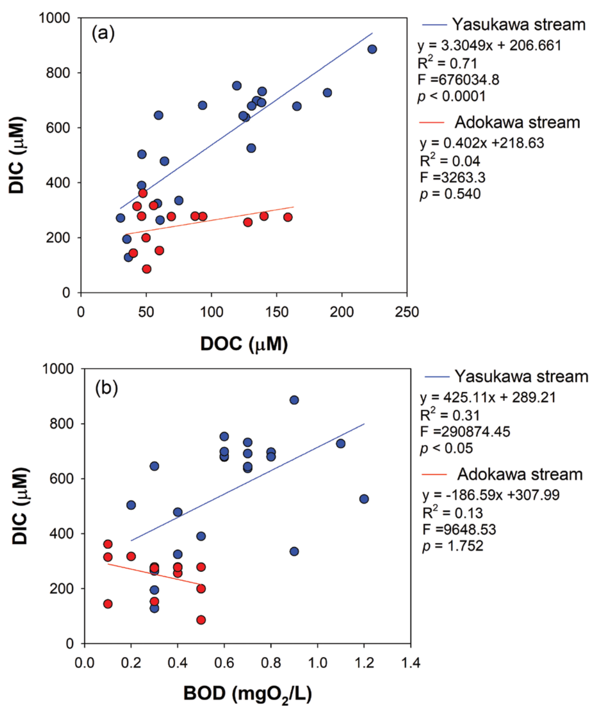

Fig. 7. Relationships between the organic matter (DOC, BOD) and DIC concentration.

which investigated nutrient pollution (nitrogen and phosphorus inventories) and organic pollution (DOC, BOD) by comparing the upstream and downstream of the Yasukawa stream, there was no clear evidence which proved that organic matter used in respiration inside the stream was derived from other sources due to the organic pollution or that the organic matter produced was related to nutrient pollution. Nevertheless, since the DIC consumed during the internal production process by the nutrient pollution and the DIC produced by the decomposition of autochthonous organic matter theoretically offset each other, the net increase in the DIC would be due to the decomposition of allochthonous organic matter in addition to autochthonous organic matter.

However, in the Adokawa stream, there was almost no change in the DIC concentration and δ^{13}C-DIC in the river flowing direction. Furthermore, the DIC concentration showed no significant correlation with the BOD and DOC concentrations (Fig. 7). The Adokawa stream showed very little changes in population density, land use, and EC in the flowing direction of the stream, which might explain the negligible effects of human activity in the watershed. Consequently, there was almost no change in the DIC concentration and δ^{13}C-DIC (Fig. 6).

It is interesting to note that DOC and BOD concentrations showed a significant positive correlation in the Yasukawa stream (r^2 =0.60, p<0.0001, n=23), whereas in the Adokawa stream, this was not the case (r^2 =0.04, p<0.50, n=14) (Fig. 8).

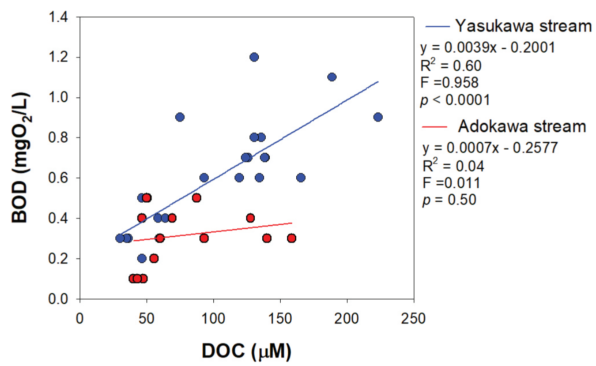

Fig. 8. Relationship between the DOC and BOD concentration.

In addition, in the Yasukawa stream, both BOD (r^2=0.31, p<0.05, n=23) and DOC (r^2=0.71, p<0.0001, n=23) showed a significant positive correlation with DIC, whereas in the Adokawa stream, this was not the case (BOD: r^2=0.13, p<1.752, n=14; DOC: r^2=0.04, p<0.54, n=14) (Fig. 7).

This is because labile organic matter contributed to the increase of organic matter in the Yasukawa stream, which was significantly affected by human activities, but in the Adokawa stream, where the percentage of forest was significant, refractory organic matter contributed to the increase in DOC.

In stream ecosystems, the impacts of the watershed might change quantitatively and qualitatively in the flowing direction of the stream. Nevertheless, the metabolism is in equilibrium in the ecosystems that stably accommodate environmental changes of watersheds. In other words, gross primary production and ecosystem respiration are in equilibrium at the ecosystem level, and as shown in the case of the Adokawa stream, the variation range of DIC concentration and $\delta^{13}C$-DIC remained stable. On the other hand, as shown by the large changes in the DIC concentration and $\delta^{13}C$-DIC in the river flowing direction of the Yasukawa stream, the environmental changes of watersheds exceeded the accommodating capacity of the river ecosystem.

Therefore, this study suggests the possibility of using DIC concentration and $\delta^{13}C$-DIC as indicators for monitoring whether ecosystems with different watershed characteristics can accommodate the effects of artificial changes.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgments

This research was supported by a grant from the 5th National Ecosystem Survey of National Institute of Ecology (NIE), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIE-A-2021-01).

References

An, K.G., Hong, Y.P., Kim, J.K., and Choi, S.S. (1992). Studies on zonation and community Analysis of freshwater fish in Kum-River. *Korean Journal of Limnology*, 25, 99-112.

Atekwana, E.A., and Krishnamurthy, R.V. (1998). Seasonal variations of dissolved inorganic carbon and $\delta^{13}C$ of surface waters: application of a modified gas evolution technique. *Journal of Hydrology*, 205, 265-278. doi:10.1016/S0022-1694(98)00080-8

Aucour, A.M., Sheppard, S.M., Guyomar, O., and Wattelet, J. (1999). Use of 13C to trace origin and cycling of inorganic carbon in the Rhone river system. *Chemical Geology*, 159, 87-105. doi:10.1016/S00092541(99)00035-2

Barnes, R.T., and Raymond, P.A. (2009). The contribution of agricultural and urban activities to inorganic carbon fluxes within temperate watersheds. *Chemical Geology*, 266, 318-327. doi:10.1016/j.chemgeo.2009.06.018

Bellan, G. (1967). Pollution et peuplements benthiques sur substrat meuble dans la région partie. Le Secteur de Cortiu. R*evue Internatianle Océanographie Médicale*, 6, 53-87.

Clark, I.D., and Fritz, P. (1997). *Environmental isotopes in hydrogeology*. Boca Raton, FL: CRC Press.

Clarke, R.T., Wright, J.F., and Furse, M.T. (2003). RIVPACS models for predicting the expected macroinvertebrate fauna and assessing the ecological quality of rivers. *Ecological Modeling*, 160, 219-233. doi:10.1016/S0304-3800(02)00255-7

Clément, F., Ruiz, J., Rodríguez, M.A., Blais, D., and Campeau, S. (2017). Landscape diversity and forest edge density regulate stream water quality in agricultural catchments. *Ecological Indicators,* 72, 627–639. doi:10.1016/j.ecolind.2016.09.001

Das, A., Krishnaswami, S., and Bhattacharya, S.K. (2005). Carbon isotope ration of dissolved inorganic carbon (DIC) in rivers draining the Deccan Traps, India: Sources of DIC and their magnitudes. *Earth and Planetary Science Letters*, 236, 419-429. doi:10.1016/j.epsl.2005.05.009

Finlay, J.C. (2003). Controls of stream water dissolved inorganic carbon dynamics in a forested watershed. *Biogeochemistry*, 62, 231-252. doi:10.1023/A:1021183023963

Finlay, J.C. (2004). Patterns and controls of lotic algal stable carbon isotope ratios. *Limnology and Oceanorgraphy*, 49, 850-861. doi:10.4319/lo.2004.49.3.0850

Fry, B. (2006). *Stable isotope ecology*. New York: Springer. doi:10.1007/0-387-33745-8

Herczeg, A.I. (1987). A stable carbon isotope study of dissolved inorganic carbon cycling in a softwater lake. *Biogeochemistry*, 4, 231-263. doi:10.1007/BF02187369

Hollander, D.J., and McKenzie, J.A. (1991). CO_2 control on carbon-isotope fractionation during aqueous photosynthesis-a plaeo-pCO2 barometer. *Geology*, 19, 929-932. doi:10.1130/0091-7613(1991)019<0929:CCOCIF>2.3.CO;2

Karr, J.R. (1981). Assessment of biotic integrity using fish communities. *Fisheries*, 6, 21-27. doi:10.1577/15488446(1981)006<0021:AOBIUF>2.0.CO;2

Kim, C., Nishimura, Y., and Nagata, T. (2006). Role of dissolved organic matter in hypolimnetic mineralization of carbon and nitrogen in a large, monomictic lake. *Limnology and Oceanography*, 51, 70-78. doi:10.2307/4499561

Kobayashi, Y., Kim, C., Yoshimizu, C., Kohzu, A., Tayasu, I., and Nagata, T. (2009). Longitudinal changes in bacterial community composition in river epilithic biofilms: influence of nutrients and organi c matter. *Aquatic Microbial Ecology*, 54, 135-152. doi:10.3354/ame01258

Li, K., Chi, G., Wang, L., Xie, Y., Wang, X., and Fan, Z. (2018). Identifying the critical riparian buffer zone with the strongest linkage between landscape characteristics and surface water quality. *Ecological Indicators*, 93, 741–752. doi:10.1016/j.ecolind.2018.05.030

Maki, K., Kim, C., Yoshimizu, C., Tayasu, I., Miyajima, T., and Nagata, T. (2010). Autochthonous origin of semi-labile dissolved organic carbon in a large monomictic lake (Lake Biwa): carbon stable isotopic evidence. *Limnology*, 11, 143-153. doi:10.1007/s10201-009-0299-z

Miyajima, T., Yamada, Y., Handa, Y.T., Yoshii, K., Koitabashi, T., and Wada, E. (1995). Determining the stable isotope ratio of total dissolved inorganic carbon in lake water by GC/IRMS. *Limnology and Oceanography*, 40, 994-1000. doi:10.4319/lo.1995.40.5.0994

Nagata, T., and Miyajima, T. (2008). *Stable isotopes in environmental assessment of watersheds-Progress towards an integrated approach*. Kyoto: Kyoto University Press.

Quay, P.D., Emerson, S.R., Quay, B.M., and Devol, A.H. (1986). The carbon cycle for Lake Washington- A stable isotope study. *Limnology and Oceanography*, 31, 596-611. doi:10.4319/lo.1986.31.3.0596

Shannon, C.E., and Wiener, W. (1963). T*he Mathematical Theory of Communication*. Chicago: University of Illinois Press.

Stiller, M., and Nissenbaum, A. (1999). A stable carbon isotope study of dissolved inorganic carbon in hardwater Lake Kinneret (Sea of Galilee). *South African Journal of Science*, 95, 166-170.

Telmer, K., and Veizer, J. (1999). Carbon flux, pCO_2 and substrate weathering in a large northern river basin, Canada: carbon isotope perspectives. *Chemical Geology,* 159, 61-86. doi:10.1016/S0009-2541(99)00034-0

Wang, X.F., and Veizer, J. (2000). Respiration-photosynthesis balance of terrestrial aquatic eco systems, Ottawa area, Canada. *Geochimica et Cosmochimica Acta*, 64, 3775-3786. doi:10.1016/S0016-7037(00)00477-4

Yirigui, Y., Lee, S., Nejadhashemi, A.P., Herman, M.R., and Lee, J. (2019). Relationships between riparian forest fragmentation and biological indicators of streams. *Sustainability*, 11, 2870. doi:10.3390/su11102870

Young, R.G., Matthaei, C.D., and Townsend, C.R. (2008). Organic matter breakdown and ecosystem metabolism: Functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society*, 27, 605-625. doi:10.1899/07-121.1

Zelinka, M., and Marvan, P. (1961). Zur Präzisierung der biologischen Klassifikation der Reiheit fliessender Gewässer. *Archiv für Hydrobiologie*, 57, 389-407.

Supplementary Table S1. Geographic and demographic features and environmental variables at the sampling sites of the Yasukawa stream. Dis.: distance from mouth (km); Alt.: altitude (m); C.A.: catchment area (km^2); P.D.: population density (Peop. km^{-2}); EC: electric conductivity (μScm^{-1}); BOD: biological oxygen demand in stream water (mgL^{-1}); DOC: dissolved organic carbon in stream water (μM); DIC: dissolved inorganic carbon in stream water (μM); $\delta^{13}C$-DIC: carbon stable isotope ratio of dissolved inorganic carbon (‰). Watershed features, BOD, and DOC data from Kobayashi *et al*. (2009).

Sites	Watershed features					Land use (%)				Environmental variables					
	Position	Dis. (km)	Alt. (m)	C.A. (km^2)	P.D. (Peop.km^{-2})	Etc	Urban	Forest	Rice field	pH	EC (μScm^{-1})	BOD (mgL^{-1})	DOC (μM)	DIC (μM)	$\delta^{13}C$-DIC(‰)
Y1	35°00.525’N, 136°24.462’E	61.9	710	1	0	4.1	-	95.9	-	8.0	43.19	0.3	36.4	128.0	- 10.13
Y2	35°00.083’N, 136°23.522’E	59.8	480	6	0	14.7	-	85.3	-	7.8	57.96	0.3	35.2	194.5	- 5.20
Y3	34°59.332’N, 136°22.145’E	55.4	400	18	0	7.1	-	92.9	-	7.7	62.34	0.3	30.3	271.4	- 9.20
Y4	34°58.617’N, 136°20.752’E	52.6	360	33	3.6	7.1	0.1	92.8	0.0	7.9	66.04	0.3	60.6	263.7	- 7.73
Y5	34°57.293’N, 136°19.592’E	48.5	300	51	26.6	6.3	0.9	91.4	1.5	7.8	76.05	0.4	58.6	324.2	- 9.56
Y6	34°57.500’N, 136°18.143’E	45	260	57	23.7	6.7	0.8	90.5	2.1	8.3	82.07	0.9	75	334.7	- 6.79
Y7	34°56.487’N, 136°16.278’E	39	220	70	34.4	7.3	1.1	87.4	4.2	7.8	93.60	0.5	46.4	389.9	- 12.13
Y8’	34°55.872’N, 136°16.780’E	38.2	220	47.5	66.5	9.1	2.1	80.8	8.0	7.9	148.83	0.3	59.5	645.2	- 9.77
Y9	34°56.112’N, 136°15.635’E	37.6	215	119	52.4	8.5	1.7	83.8	6.1	8.0	117.20	0.4	64	477.8	- 10.11
Y10	34°57.153’N, 136°13.020’E	33.8	200	136	74.5	10.9	2.3	78.2	8.6	7.9	125.33	0.2	46.7	503.2	- 10.40
Y11	34°57.922’N, 136°11.132’E	29.2	170	152	96.4	11.9	2.7	73.8	11.7	8.2	144.57	0.6	165.5	678.0	- 12.34
Y12	34°58.345’N, 136°07.777’E	24.8	140	283	204.9	12.6	4.8	63.8	18.8	7.5	163.13	0.6	93.2	681.2	- 12.48
Y13’	34°57.587’N, 136°08.353’E	23.9	140	117	289.3	12.3	6.6	54.0	27.2	8.5	140.10	1.1	188.9	727.1	- 13.03
Y14	34°59.012’N, 136°07.253’E	22.8	140	291	232.7	12.8	5.3	62.4	19.5	7.5	150.53	0.7	138.9	732.0	- 12.14
Y15’	34°59.190’N, 136°06.770’E	21.2	140	11	197.8	12.4	3.4	78.3	5.8	7.5	108.43	1.2	130.5	525.4	- 10.97
Y16	34°53.492’N, 136°06.473’E	20.6	140	307	241.5	12.9	5.4	62.6	19.1	7.5	152.23	0.8	135.9	696.3	- 11.59
Y17	35°00.113’N, 136°05.753’E	18.2	130	308	243.2	13.0	5.4	62.4	19.2	7.5	152.40	0.6	134.6	698.4	- 11.92
Y18’	35°00.353’N, 136°05.603’E	18.1	130	23	540.2	21.0	10.1	41.4	27.5	7.3	462.63	0.9	223.2	885.4	- 11.92
Y19	35°00.870’N, 136°04.217’E	16.9	130	344	277.7	13.9	6.0	60.4	19.7	8.9	213.93	0.7	138.4	691.3	- 11.81
Y20	35°01.667’N, 136°01.933’E	12.4	110	364	308.2	14.1	6.3	59.9	19.7	9.4	235.23	0.8	130.8	679.2	- 12.02
Y21	35°02.662’N, 136°01.093’E	10	105	366	308	14.3	6.3	59.6	19.7	9.3	229.93	0.7	126.1	637.3	- 11.87
Y22	35°04.483’N, 136°00.382’E	6.7	95	378	321.1	14.9	6.5	59.1	19.5	8.9	220.13	0.7	124.3	643.8	- 12.31
Y23	35°06.083’N, 135°53.387’E	2.9	87	380	319.9	15.3	6.5	58.8	19.4	7.3	220.33	0.6	119.5	752.8	- 13.03

Supplementary Table S2. Geographic and demographic features and environmental variables at sampling sites of the Adokawa stream. Abbreviations as in Table S1. Watershed features, BOD, and DOC data from Kobayashi *et al*. (2009).

Sites	Watershed features					Land use (%)				Environmental variables					
	Position	Dis. (km)	Alt. (m)	C.A. (km^2)	P.D. (Peop.km^{-2})	Etc	Urban	Forest	Rice field	pH	EC (μScm^{-1})	BOD (mgL^{-1})	DOC (μM)	DIC (μM)	$\delta^{13}C$-DIC(‰)
A1	35°09.600’N, 135°47.950’E	50.1	645	0.274	42.8	-	-	100.0	-	6.2	20.8	0.5	50.4	85.7	- 17.10
A2	35°10.788’N, 135°49.669’E	46.5	555	14.39	42.2	1.75	0.29	97.9	0.01	7.2	30.8	0.3	60.1	152.7	- 9.15
A3	35°12.582’N, 135°51.330’E	39.4	405	24.48	42.7	1.83	0.26	97.9	0.01	7.6	63	0.1	47.4	361.4	- 9.71
A4	35°14.427’N, 135°51.885’E	35.1	315	48.5	31.3	2.44	0.43	97.1	0.01	7.7	61.3	0.2	55.7	316.6	- 7.99
A 5’	35°14.875’N, 135°52.015’E	34.2	310	1.78	42.8	0.00	-	100.0	-	7.3	35.2	0.1	40.1	143.6	- 4.79
A6	35°15.858’N, 135°52.448’E	31.5	280	65.74	34.3	2.80	0.37	96.8	0.01	7.6	59.4	0.1	43	314.0	- 8.40
A 7’	35°15.892’N, 135°52.287’E	31.3	280	76.06	3.9	3.55	0.18	96.3	0.02	7.3	45.9	0.5	49.9	199.2	- 9.18
A8	35°18.070’N, 135°53.213’E	23.7	195	156.1	20.1	3.48	0.26	96.2	0.01	7.4	55.2	0.4	46.5	278.2	- 9.21
A9	35°21.065’N, 135°55.147’E	20.1	170	183.3	23.4	4.09	0.35	95.5	0.02	7.3	55.1	0.3	93.3	276.9	- 9.40
A10’	35°21.312’N, 135°55.212’E	19.4	165	77.81	13.1	4.65	0.44	94.9	0.02	7.4	58.6	0.4	127.9	255.6	- 9.64
A11	35°21.773’N, 135°58.257’E	11.4	120	281.1	22.3	5.23	0.48	94.3	0.02	7.4	58.7	0.4	69.3	276.7	- 9.42
A12	35°20.988’N, 136°00.040’E	8.1	105	296	24.7	6.53	0.65	92.8	0.03	7.4	59.3	0.5	87.4	278.0	- 9.77
A13	35°20.353’N, 136°02.132’E	4.5	90	304.5	25.2	7.39	0.66	91.9	0.03	7.5	59.6	0.3	140.3	278.1	- 9.62
A14	35°19.553’N, 135°03.798’E	1.6	85	305.1	25.3	7.57	0.68	91.7	0.03	7.6	60.1	0.3	158.6	274.1	- 9.59

Supplementary Table S3. Results of correlation analyses between watershed features and environmental variables in the Yasukawa stream. Bold type indicates $p<0.001$.

Total data

			Population density (Peop.km^{-2})	Land use (%) Forest	Rice field	Urban	pH	EC (μScm^{-1})	DIC (μM)	δ^{13}C-DIC (‰)
Catchment area (km^2)		r	0.5917	**-0.6632**	**0.6913**	**0.6465**	0.3818	0.4164	0.6352	-0.6206
		p	0.0029	**0.0006**	**0.0003**	**0.0009**	0.0722	0.0481	0.0011	0.0016
Population density (Peop.km^{-2})		r		**-0.9619**	**0.925**	**0.9906**	0.1454	**0.9048**	**0.8581**	**-0.6836**
		p		**p<0.0001**	**p<0.0001**	**p<0.0001**	0.508	**p<0.0001**	**p<0.0001**	**0.0003**
Land use (%)	Forest	r			**-0.982**	**-0.9845**	-0.1562	**-0.8513**	**-0.9188**	**0.7093**
		p			**p<0.0001**	**p<0.0001**	0.4767	**p<0.0001**	**p<0.0001**	**0.0002**
	Rice field	r				**0.9655**	0.1874	**0.7893**	**0.9238**	**-0.7705**
		p				**p<0.0001**	0.3918	**p<0.0001**	**p<0.0001**	**p<0.0001**
	Urban	r					0.163	**0.8861**	**0.9062**	**-0.7303**
		p					0.4575	**p<0.0001**	**p<0.0001**	**0.0001**
pH		r						0.1189	0.0519	-0.1001
		p						0.5888	0.8139	-0.6495
EC (μScm^{-1})		r							**0.7892**	-0.5396
		p							**p<0.0001**	0.0079
DIC (μM)		r								**-0.7734**
		p								**p<0.0001**

Main stream data

			Population density (Peop.km^{-2})	Land use (%) Forest	Rice field	Urban	pH	EC (μScm^{-1})	DIC (μM)	δ^{13}C-DIC (‰)
Catchment area (km^2)		r	**0.9944**	**-0.9796**	**0.9815**	**0.9984**	0.3285	**0.9618**	**0.9179**	-0.7497
		p	**p<0.0001**	**p<0.0001**	**p<0.0001**	**p<0.0001**	0.1697	**p<0.0001**	**p<0.0001**	**0.0002**
Population density (Peop.km^{-2})		r	1	**-0.9704**	**0.9665**	**0.9942**	0.3543	**0.9555**	**0.8818**	**-0.7267**
		p		**p<0.0001**	**p<0.0001**	**p<0.0001**	0.1367	**p<0.0001**	**p<0.0001**	**0.0004**
Land use (%)	Forest	r		1	**-0.988**	**-0.9835**	-0.269	**-0.935**	**-0.9359**	**0.7185**
		p			**p<0.0001**	**p<0.0001**	0.2654	**p<0.0001**	**p<0.0001**	**0.0005**
	Rice field	r			1	**0.9866**	0.2481	**0.924**	**0.9544**	**-0.7842**
		p				**p<0.0001**	0.3057	**p<0.0001**	**p<0.0001**	**0.0001**
	Urban	r				1	0.3205	**0.9556**	**0.9217**	**-0.7579**
		p					0.181	**p<0.0001**	**p<0.0001**	**0.0002**
pH		r					1	0.4993	0.1407	-0.1043
		p						0.0295	0.5657	0.671
EC (μScm^{-1})		r						1	**0.8803**	**-0.7283**
		p							**p<0.0001**	**0.0004**
DIC (μM)		r							1	**-0.8018**
		p								**p<0.0001**

Main stream data, head water data was excluded (Y1)

			Population density (Peop.km^{-2})	Land use (%) Forest	Rice field	Urban	pH	EC (μScm^{-1})	DIC (μM)	δ^{13}C-DIC (‰)
Catchment area (km^2)		r	**0.9951**	**-0.978**	**0.9796**	**0.9984**	0.3383	**0.9579**	**0.9156**	**-0.7714**
		p	**p<0.0001**	**p<0.0001**	**p<0.0001**	**p<0.0001**	0.1697	**p<0.0001**	**p<0.0001**	**0.0002**
Population density (Peop.km^{-2})		r	1	**-0.9715**	**0.9641**	**0.9941**	0.3614	**0.9547**	**0.8838**	**-0.7394**
		p		**p<0.0001**	**p<0.0001**	**p<0.0001**	0.1406	**p<0.0001**	**p<0.0001**	**0.0005**
Land use (%)	Forest	r		1	**-0.9881**	**-0.9835**	-0.2787	**-0.9256**	**-0.9296**	**0.7471**
		p			**p<0.0001**	**p<0.0001**	0.2628	**p<0.0001**	**p<0.0001**	**0.0004**
	Rice field	r			1	**0.9852**	0.2529	**0.9161**	**0.9608**	**-0.8055**
		p				**p<0.0001**	0.3114	**p<0.0001**	**p<0.0001**	**0.0001**
	Urban	r				1	0.3284	**0.952**	**0.9237**	**-0.7768**
		p					0.1834	**p<0.0001**	**p<0.0001**	**0.0001**
pH		r					1	0.5248	0.1453	-0.103
		p						0.0253	0.5651	0.6842
EC (μScm^{-1})		r						1	**0.8633**	**-0.7571**
		p							**p<0.0001**	**0.0003**
DIC (μM)		r							1	**-0.8692**
		p								**p<0.0001**

Supplementary Table S4. Results of Pearson's correlation analyses between watershed features and environmental variables in the Adokawa stream. Bold type indicates $p<0.0001$.

Total data

			Population density ($Peop.km^{-2}$)	Land use (%)			pH	EC (μScm^{-1})	DIC (μM)	$\delta^{13}C$-DIC (‰)
				Forest	Rice field	Urban				
Catchment area (km^2)		r	-0.4516	**-0.9225**	**0.864**	**0.8236**	0.3294	0.5511	0.3955	0.026
		p	0.105	**p<0.0001**	**0.0001**	**0.0003**	0.2502	0.0411	0.1616	0.9298
Population density ($Peop.km^{-2}$)		r	1	0.578	-0.5648	-0.3614	-0.2631	-0.4221	-0.2298	-0.0921
		p		0.0304	0.0353	0.2043	0.3634	0.1327	0.4294	0.7543
Land use (%)	Forest	r		1	**-0.9678**	**-0.9324**	-0.4811	-0.6703	-0.499	-0.078
		p			**p<0.0001**	**p<0.0001**	0.0816	0.0087	0.0693	0.7911
	Rice field	r			1	**0.8959**	0.4428	0.5864	0.423	0.0612
		p				**p<0.0001**	0.1129	0.0275	0.1318	0.8354
	Urban	r				1	0.5832	0.7275	0.6085	0.1278
		p					0.0286	0.0032	0.0209	0.6632
pH		r					1	**0.8392**	**0.8148**	0.7663
		p						**0.0002**	**0.0004**	0.0014
EC (μScm^{-1})		r						1	**0.9577**	0.3783
		p							**p<0.0001**	0.1823
DIC (μM)		r							1	0.3349
		p								0.2418

Main stream data

			Population density ($Peop.km^{-2}$)	Land use (%)			pH	EC (μScm^{-1})	DIC (μM)	$\delta^{13}C$-DIC (‰)
				Forest	Rice field	Urban				
Catchment area (km^2)		r	-0.8284	**-0.9574**	**0.9102**	0.8326	0.3427	0.5156	0.2848	0.2671
		p	0.0016	**p<0.0001**	**0.0001**	0.0015	0.3022	0.1045	0.396	0.4273
Population density ($Peop.km^{-2}$)		r	1	0.7481	-0.6368	-0.6119	-0.4049	-0.5741	-0.3871	-0.4151
		p		0.0081	0.0351	0.0454	0.2167	0.0647	0.2395	0.2043
Land use (%)	Forest	r		1	**-0.9627**	**-0.9421**	-0.5144	-0.606	-0.3918	-0.4191
		p			**p<0.0001**	**p<0.0001**	0.1054	0.0481	0.2334	0.1995
	Rice field	r			1	**0.913**	0.4773	0.5077	0.3099	0.4041
		p				**0.0001**	0.1376	0.1109	0.3536	0.2177
	Urban	r				1	0.6613	0.669	0.4845	0.5701
		p					0.0267	0.0244	0.131	0.0671
pH		r					1	**0.8925**	**0.8901**	**0.9404**
		p						**0.0002**	**0.0002**	**p<0.0001**
EC (μScm^{-1})		r						1	**0.9638**	0.7489
		p							**p<0.0001**	0.008
DIC (μM)		r							1	0.7548
		p								0.0072

Main stream data, headwater data (A1) excluded

			Population density ($Peop.km^{-2}$)	Land use (%)			pH	EC (μScm^{-1})	DIC (μM)	$\delta^{13}C$-DIC (‰)
				Forest	Rice field	Urban				
Catchment area (km^2)		r	-0.792	**-0.9604**	**0.9021**	0.8203	-0.0846	0.3575	-0.029	-0.5737
		p	0.0063	**p<0.0001**	**0.0004**	0.0037	0.8162	0.3105	0.9367	0.0829
Population density ($Peop.km^{-2}$)		r	1	0.6663	-0.5138	-0.459	0.1017	-0.3775	-0.0536	0.2069
		p		0.0354	0.1287	0.1821	0.7799	0.2822	0.8831	0.5662
Land use (%)	Forest	r		1	**-0.9474**	**-0.9243**	-0.0545	-0.3605	0.0248	0.5216
		p			**p<0.0001**	**0.0001**	0.8812	0.3061	0.9458	0.122
	Rice field	r			1	**0.8747**	-0.1129	0.158	-0.1961	-0.6809
		p				**0.0009**	0.7562	0.6628	0.5872	0.0302
	Urban	r				1	0.2291	0.3567	-0.0047	-0.3085
		p					0.5243	0.3117	0.9898	0.3858
pH		r					1	0.7504	0.774	0.3976
		p						0.0124	0.0086	0.2552
EC (μScm^{-1})		r						1	**0.913**	-0.021
		p							**0.0002**	0.954
DIC (μM)		r							1	0.1046
		p								0.7737