SOURCES OF DISSOLVED INORGANIC CARBON IN TWO SMALL STREAMS WITH DIFFERENT BEDROCK GEOLOGY: INSIGHTS FROM CARBON ISOTOPES

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ABSTRACT. Radiocarbon natural abundances ($\Delta^{14}C$) are being increasingly used to trace carbon cycling in stream ecosystems. To understand the ultimate sources of carbon, we determined the stable carbon isotope ratios ($\delta^{13}C$) and $\Delta^{14}C$ values of dissolved inorganic and organic carbon (DIC and DOC, respectively) and of particulate organic carbon (POC) in two small streams in central Japan, one of which flows over limestone bedrock (Seri) and the other does not (Fudoji). Investigations over four seasons revealed that the $\Delta^{14}C$ values of the DIC (from –238‰ to –174‰ for Seri and –23‰ to +10‰ for Fudoji) were less variable than those of the other carbon fractions (DOC: from –400‰ to –138‰ for Seri and –2‰ to +103‰ for Fudoji; POC: from –164‰ to –60‰ for Seri and –55‰ to +37‰ for Fudoji). Based on mass balance calculations using the $\delta^{13}C$ and $\Delta^{14}C$ values, the proportions of carbon in the DIC originated from (1) atmospheric CO_2 were 47% to 57% for Seri and 74% to 90% for Fudoji, (2) organic matter degradation were 29% to 35% for Seri and 4% to 21% for Fudoji, and (3) carbonate rock were 14% to 22% for Seri and 4% to 6% for Fudoji. We compared the results with previous studies that had been conducted in larger rivers and showed that in small streams, the dissolution of atmospheric CO_2 and weathering of carbonate rock are more important factors in the carbon cycling than the biological degradation of organic matter.

INTRODUCTION

In stream ecosystems, dissolved inorganic and organic carbon (DIC and DOC, respectively) and particulate organic carbon (POC) play important roles in biological production and carbon cycling (Raymond et al. 2004; Caraco et al. 2010; Zigah et al. 2014). In recent years, stable carbon isotope ratios ($\delta^{13}C$) and radiocarbon natural abundances ($\Delta^{14}C$) are being increasingly used to trace the sources, dynamics, and turnover of these carbon fractions, and large variations in the $\Delta^{14}C$ values have been reported from different regions (e.g. northeastern US: Raymond and Hopkinson 2003; Amazon: Mayorga et al. 2005; Alaska: Guo and Macdonald 2006; southern Asia: Martin et al. 2013). For example, Mayorga et al. (2005) reported that young (^{14}C-enriched) organic carbon is actively decomposed to DIC in the Amazon River, whereas McCallister and del Giorgio (2012) showed that a significant amount of aged (^{14}C-depleted) DOC is respired by bacteria and transferred into the DIC pool. However, the majority of previous studies were conducted in large rivers or lakes that had been considerably altered by human disturbance (Butman et al. 2015). The controls on the $\delta^{13}C$ and $\Delta^{14}C$ variations in DIC, DOC, and POC remain poorly understood in small streams in which the natural landscape is conserved (Palmer et al. 2001; Fellman et al. 2014).

The $\delta^{13}C$ values of DIC, DOC, and POC are potentially controlled by isotopic fractionation and the mixing proportions of end-members, such as atmospheric CO_2, organic matter (OM) degradation, and carbonate rock (Finlay 2003), whereas their $\Delta^{14}C$ values depend only on the mixing proportions of end-members (Raymond et al. 2004). One of the classical and plausible hypotheses is that the $\Delta^{14}C$ value of OM positively correlates with biological availability, as young OM includes more labile carbon fractions than old OM (Raymond and Bauer 2001; Mayorga et al. 2005). On the other hand, recent studies revealed that ^{14}C-depleted (relative to modern atmospheric CO_2) carbon also fuels carbon cycling (Marschner et al. 2008; Fellman et al. 2014), and is incorporated into aquatic animals (e.g. zooplankton and fish) (Caraco et al. 2010; Ascough et al. 2011; Keaveney and Reimer 2012; Fernandes et al. 2013; Ishikawa et al. 2014), suggesting that the relationship between the ^{14}C age and biological availability of OM in stream ecosystems is more complex than previously

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thought (Marín-Spiotta et al. 2014).

Ishikawa et al. (2012) tested the hypothesis that the weathering of carbonates is a source of ^{14}C-depleted carbon in stream ecosystems, and reported δ^{13}C and Δ^{14}C values of DIC and periphytic algae from a stream flowing over limestone bedrock. The results indicate that primary production transfers ^{14}C-depleted carbon from carbonate rock into stream food webs. However, the relative abundance of carbon originated from carbonate rock, dissolved atmospheric CO_2, and OM degradation in stream-water DIC has yet to be quantitatively assessed, which prevents comprehensive understanding of carbon cycling in stream ecosystems. To identify the ultimate sources of DIC, this study examines data from fieldwork conducted over four seasons in two small streams in central Japan. The two streams have contrasting geological characteristics: one stream flows over limestone bedrock (Seri) and the other does not (Fudoji). Samples of DIC, DOC, and POC were collected from both streams and their δ^{13}C and Δ^{14}C values were determined. A δ^{13}C and Δ^{14}C mixing model was used to partition DIC from each stream into three end-members (i.e. atmospheric CO_2, OM, and carbonate rock). We hypothesized that the dominant source of DIC is carbonate rock in Seri and atmospheric CO_2 in Fudo-ji. Finally, the results were compared with previous studies that had been conducted in larger rivers to test the hypothesis that atmospheric CO_2 is a more important source of DIC in smaller streams.

MATERIALS AND METHODS

Study Site

In June (summer), September (autumn), and December (winter) of 2009, and March–April (spring) of 2010, fieldwork was conducted in the upper part of the Seri River (watershed area = 30 km^2, 35°15′N, 136°20′E; hereafter Seri) and at the Fudoji Experimental Watershed in the upper part of the Daido River (watershed area = 6.0 km^2, 34°55′N, 136°00′E, hereafter Fudoji) in Shiga Prefecture, central Japan. The studied reaches of Seri and Fudoji flow over bedrock of Carboniferous–Permian limestone and Cretaceous granite, respectively. Several C$_3$ plant species dominate the terrestrial vegetation. For further details on the geography of Seri and Fudoji, see Ishikawa et al. (2012) and Asano et al. (2002), respectively. The stream width (m) was determined by a horizontal transect measured at a fixed site in each stream. The water velocity (m s^{-1}) and depth (m) were measured using a current meter (2310, Hiroi's System, San-ei, Japan) and a folding scale at every 20–50 cm along the transect. The stream discharge (L s^{-1}) was calculated as the water velocity multiplied by the water depth, integrated by the stream width. The water temperature (WT: °C), electric conductivity (EC: μS cm^{-1}), and pH were measured hourly between mid-morning and mid-afternoon using a YSI meter (Model 85 SCOOT, Yellow Springs Instruments, USA) (Table 1).

Table 1 Physicochemical characteristics of the study sites. WT: water temperature; EC: electric conductivity. The WT, EC, and pH are mean values of repeated measurements between mid-morning and mid-afternoon (ND: no data).

Stream	Season	Stream width (m)	Discharge (L s^{-1})	Depth (cm)	WT (°C)	EC (μS cm^{-1})	pH
Seri	Summer	11.5	644	25.5	15.9	132	8.8
	Autumn	10.5	402	23.3	16.0	145	8.0
	Winter	15.5	606	25.9	10.7	123	8.4
	Spring	20.5	2010	34.0	12.1	119	7.8
Fudoji	Summer	3.2	92	25.5	15.3	20	6.5
	Autumn	3.8	73	21.6	17.1	23	6.1
	Winter	4.6	119	28.7	6.1	16	6.2
	Spring	4.8	410	30.3	7.3	12	ND

Sample Processing

Three limestone cobbles were collected from Seri to determine the $\delta^{13}C$ value of the carbonates. Four replicates of DIC, DOC, and POC samples were collected from each site, every season. The water samples for DIC were collected following Ishikawa et al. (2012). In brief, the streamwater was collected in 250- or 500-mL glass bottles, carefully avoiding the introduction of atmospheric CO_2 into the bottles. The samples were injected with 1.5 or 3.0 mL of saturated $HgCl_2$ (final concentration: 1.6×10^{-3} mol L^{-1}) to inhibit microbial activity. Approximately 20 L of streamwater was collected in a tank and prefiltered using a 150-μm mesh to remove large particles. The water was then filtered through a glass fiber filter (GF/F: pore size 0.7 μm, Whatman, GE Healthcare Bio-Sciences, USA) that had been combusted at 450°C for 2 hr prior to the filtration. The material trapped on the filter was carefully rinsed with 0.1 mol L^{-1} HCl solution to remove carbonates and was dried in an oven at 60°C for 24 hr to obtain POC. The filtrates were collected in polycarbonate bottles (Nalgene, Thermo Fisher Scientific, USA) and were lyophilized using a freeze-drier (FDU-1200, EYELA, Japan). The precipitated powders were fumigated in a desiccator with a 12 mol L^{-1} HCl atmosphere to remove carbonates and were lyophilized again to obtain DOC. The DIC and DOC concentrations were measured using a high-temperature analyzer (TOC-5000A, Shimadzu, Japan) at the Center for Ecological Research (CER), Kyoto University, Japan.

Measurements of Stable Carbon Isotopic Compositions

The DIC samples in glass bottles were placed in a glass chamber that interfaced directly with a vacuum line. The samples were acidified to pH 2–3 with 30% H_3PO_4 and purged with ultra-high-purity He gas (Ishikawa et al. 2012). The evolved CO_2 gas was cryogenically purified in a vacuum line and collected in glass tubes that had been combusted at 450°C for 2 hr in advance. The $\delta^{13}C$ values were determined using a GV IsoPrime mass spectrometer (IsoPrime Ltd., UK) with a dual-inlet system for DIC in Fudoji (except for the summer sample) and with an automated carbonate preparation system (Sakai et al. 2009) for carbonates at the Japan Agency for Marine-Earth Science and Technology. The $\delta^{13}C$ values of the DIC in Fudoji for the summer sample were determined following Miyajima et al. (1995), using a gas chromatograph (GC) (HP 6890, Hewlett Packard, USA) connected to an isotope-ratio mass spectrometer (MAT252, Finnigan MAT, Germany) via an interface (GC combination III, Finnigan MAT) at CER. The POC concentrations and the $\delta^{13}C$ values of the DOC and POC were measured using a Flash EA1112 elemental analyzer (EA) connected to a Delta XP isotope-ratio mass spectrometer via a Conflo III interface (Thermo Fisher Scientific) at CER. The $\delta^{13}C$ values are reported relative to the international standard (Vienna Pee Dee Belemnite: VPDB). The data were corrected using internal standards (CERKU-01 DL-alanine: $\delta^{13}C_{VPDB} = -25.36‰$; CERKU-02 L-alanine: $\delta^{13}C_{VPDB} = -19.04‰$; CERKU-03 glycine: $\delta^{13}C_{VPDB} = -34.92‰$) that had been corrected relative to multiple international standards (Tayasu et al. 2011). The 1σ analytical precisions of the $\delta^{13}C$ measurements were better than 0.1‰, 1.8‰, and 0.3‰ for the IsoPrime, GC, and EA, respectively. The blank of combusted GF/F was assessed by the EA to correct $\Delta^{14}C$ values of POC (Table 4).

Radiocarbon Measurements

Samples for ^{14}C measurements were subjected to graphitization following Kitagawa et al. (1993) for samples containing >1 mg C and Yokoyama et al. (2010) for samples containing 0.1–0.6 mg C. Prior to graphitization, the DOC and POC samples were combusted with CuO in an evacuated quartz tube. The evolved CO_2 gas from all samples was cryogenically purified in a vacuum line and reduced to graphite under H_2 atmosphere using Fe as a catalyst. The ^{14}C concentrations of the samples containing >1 mg C were measured using an accelerator mass spectrometer (AMS) at the Rafter Radiocarbon Laboratory, GNS Science, New Zealand, and those of the samples containing 0.1 to

0.6 mg C were measured by AMS at the Atmosphere and Ocean Research Institute, University of Tokyo, Japan. The AMS results are reported as $\Delta^{14}C$ (‰) values (Stuiver and Polach 1977). The 1σ analytical precision of the $\Delta^{14}C$ measurements typically ranged from 2‰ to 15‰ (Tables 2–4).

Mass Balance Calculations

Using the *in situ* pH and water temperature (Table 1), the relative abundances of the aqueous CO_2 (CO_{2aq}), bicarbonate ions (HCO_3^-), and carbonate ions (CO_3^{2-}) in DIC were calculated according to the regression equations from the data in Plummer and Busenberg (1982). As the pH for the spring in Fudoji was not determined, the average of the pH values for other seasons in Fudoji (6.3) was applied to that for the spring. Assuming equilibrium with atmospheric CO_2, the $\delta^{13}C$ values of CO_{2aq}, HCO_3^-, and CO_3^{2-} were calculated according to the equations in Zhang et al. (1995). The $\delta^{13}C$ and $\Delta^{14}C$ values of the atmospheric CO_2 from 2009 to 2010 were set as –8.3‰ (data from Cape Kumukahi, Hawaii) (Keeling et al. 2005) and +45‰ (data from Vermunt, Austria) (Levin et al. 2010), respectively. The $\delta^{13}C$ value of the dissolved atmospheric CO_2 under equilibrium (hereafter $\delta^{13}C_{atm}$) was calculated using the relative abundances and $\delta^{13}C$ values of CO_{2aq}, HCO_3^-, and CO_3^{2-}, which ranged from 0‰ to +0.8‰ in Seri and from –6.3‰ to –4.1‰ in Fudoji. The $\Delta^{14}C$ value of atmospheric CO_2 (hereafter $\Delta^{14}C_{atm}$) does not change during the dissolution, as it is corrected for isotopic fractionation by $\delta^{13}C$ (Stuiver and Polach 1977).

Although there are a few studies comparing the biological availability of DOC and POC (e.g. Richardson et al. 2013), it depends on various factors including molecular structure and complexity (Marín-Spiotta et al. 2014), and parameters that cannot be easily defined. Raymond and Bauer (2001) suggested that DOC is the labile fraction whereas POC is the refractory fraction, based on their ^{13}C and ^{14}C compositions. In contrast, Ostapenia et al. (2009) showed that POC is derived primarily from algal production and is more labile than DOC. As no prior information about our system was available, we assumed that the rate of degradation of DOC and POC is equivalent. The $\delta^{13}C$ and $\Delta^{14}C$ values of DOC and POC were weighted by their concentrations to calculate the DIC fraction derived from organic matter degradation ($\delta^{13}C_{org}$ and $\Delta^{14}C_{org}$) as follows:

$$\delta^{13}C_{org} = \frac{[DOC]\,\delta^{13}C_{DOC} + [POC]\,\delta^{13}C_{POC}}{[DOC] + [POC]}$$

(1)

$$\Delta^{14}C_{org} = \frac{[DOC]\,\Delta^{14}C_{DOC} + [POC]\,\Delta^{14}C_{POC}}{[DOC] + [POC]}$$

(2)

Using $\delta^{13}C_{atm}$ (0‰ to +0.8‰ in Seri and –6.3‰ to –4.1‰ in Fudoji), $\delta^{13}C_{org}$, and the $\delta^{13}C$ value of carbonates collected from Seri ($\delta^{13}C_{carb}$ = +3.9 ± 0.3‰; mean ± SD, n = 3), the $\delta^{13}C_{DIC}$ value was derived as follows:

$$\delta^{13}C_{DIC} = f_{atm}\,\delta^{13}C_{atm} + f_{org}\,\delta^{13}C_{org} + f_{carb}\,\delta^{13}C_{carb}$$

(3)

where

$$f_{atm} + f_{org} + f_{carb} = 1$$

(4)

Using $\Delta^{14}C_{atm}$ (+45‰), $\Delta^{14}C_{org}$, and the $\Delta^{14}C$ value of the carbonate rock ($\Delta^{14}C_{carb}$ = –1000‰), with the additional assumption that the effect of the $\delta^{13}C$ correction on the $\Delta^{14}C$ value was negligible (Ishikawa et al. 2013), the $\Delta^{14}C_{DIC}$ value was derived as follows:

$$\Delta^{14}C_{DIC} = f_{atm}\,\Delta^{14}C_{atm} + f_{org}\,\Delta^{14}C_{org} + f_{carb}\,\Delta^{14}C_{carb}$$

(5)

RESULTS AND DISCUSSION

The mean DIC concentration in Seri (four-season mean ± SD: 19.7 ± 1.8 mg C L⁻¹) was higher than that in Fudoji (0.76 ± 0.35 mg C L⁻¹, Table 2). However, the mean DOC concentration was lower in Seri (0.19 ± 0.07 mg C L⁻¹) than that in Fudoji (1.25 ± 0.29 mg C L⁻¹, Table 3). Mean POC concentration was similar between the two streams (0.09 ± 0.05 mg C L⁻¹ for Seri; 0.10 ± 0.03 mg C L⁻¹ for Fudoji, Table 4). In Seri, the $\delta^{13}C_{DIC}$ (−7.2 ± 0.2‰) was less variable than the $\delta^{13}C_{DOC}$ (−24.2 ± 2.9‰) and $\delta^{13}C_{POC}$ (−25.0 ± 3.4‰). Both were highest in summer (Tables 3 and 4), and were higher than the typical $\delta^{13}C$ value of terrestrial C_3 plants (~ −28‰, Peterson and Fry 1987), suggesting that DOC and POC in Seri were mixtures of terrestrial- and aquatic-derived OM. In contrast, both the $\delta^{13}C_{DOC}$ (−28.4 ± 0.1‰) and $\delta^{13}C_{POC}$ (−27.9 ± 0.2‰) in Fudoji were less variable than those in Seri and were closer to the $\delta^{13}C$ value of terrestrial C_3 plants (Tables 3 and 4).

Table 2 Carbon concentration (mean ± SD, $n = 4$), $\delta^{13}C$ (mean ± SD, $n = 4$), and $\Delta^{14}C$ ($n = 1$) of DIC collected from Seri and Fudoji. For $\Delta^{14}C$, means of repeated measurements and 1σ analytical precisions are shown. The lab code YAUT indicates samples measured by AMS at the Atmosphere and Ocean Research Institute, the University of Tokyo.

Stream	Season	DIC concentration (mg C L⁻¹)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	AMS lab code
Seri	Summer	17.6 ± 0.73*	−6.9 ± 0.4*	−238 ± 4.1	†
	Autumn	21.9 ± 0.10	−7.4 ± 0.2*	−240 ± 2.4	†
	Winter	20.2 ± 0.14	−7.0 ± 0.3*	−215 ± 0.8	†
	Spring	19.3 ± 0.11	−7.3 ± 0.1*	−174 ± 1.7	†
Fudoji	Summer	0.47 ± 0.73*	−8.7 ± 2.0*	−6 ± 6.4	YAUT-012120
	Autumn	1.21 ± 0.04	−9.4 ± 0.01§	−16 ± 3.9	YAUT-005229-30
	Winter	0.85 ± 0.01	−6.4 ± 0.01§	−23 ± 5.0	YAUT-005209
	Spring	0.50 ± 0.01	−7.2 ± 0.01§	+10 ± 7.5	YAUT-005208

*Determined by GC/IRMS.
§Determined by dual-inlet IRMS.
†The $\delta^{13}C$ and $\Delta^{14}C$ values of DIC in Seri were reported by Ishikawa et al. (2012) as mean ± SD, $n = 4$.

Table 3 Carbon concentration (mean ± SD, $n = 4$), $\delta^{13}C$ (mean ± SD, $n = 4$), and $\Delta^{14}C$ ($n = 1$) of DOC collected from Seri and Fudoji.

		DOC			
Stream	Season	Concentration (mg C L⁻¹)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	AMS lab code
Seri	Summer	0.28 ± 0.03	−19.9 ± 3.65	−241 ± 9.5	YAUT-007415
	Autumn	0.18 ± 0.02	−25.6 ± 0.10	−211 ± 9.9	YAUT-007420
	Winter	0.15 ± 0.01	−25.1 ± 1.70	−400 ± 8.8	YAUT-007426
	Spring	0.13 ± 0.02	−26.3 ± 0.21	−138 ± 6.2	YAUT-012134
Fudoji	Summer	1.50 ± 0.07	−28.5 ± 0.11	+88 ± 6.5	YAUT-005217
	Autumn	1.48 ± 0.03	−28.4 ± 0.09	+70 ± 6.4	YAUT-005228
	Winter	1.12 ± 0.02	−28.4 ± 0.06	−2 ± 5.7	YAUT-005218
	Spring	0.91 ± 0.03	−28.3 ± 0.04	+103 ± 15.4	YAUT-005219

Table 4 Carbon concentration (mean ± SD, n = 4), $\delta^{13}C$ (mean ± SD, n = 4), and $\Delta^{14}C$ (n = 1) of POC collected from Seri and Fudoji. The lab code NZA indicates samples measured by AMS at the Rafter Radiocarbon Laboratory, GNS Science.

| Stream | Season | POC | | | AMS lab code |
		Concentration (mg C L^{-1})	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	
Seri	Summer	0.13 ± 0.10	−19.8 ± 0.9	−142 ± 10.5*	YAUT-007414
	Autumn	0.12 ± 0.09	−26.6 ± 0.4	−60 ± 2.1*	NZA-32354
	Winter	0.03 ± 0.01	−26.7 ± 1.1	−164 ± 9.9*	YAUT-007413
	Spring	0.08 ± 0.01	−26.9 ± 0.4	−70 ± 2.1*	NZA-32358
Fudoji	Summer	0.10 ± 0.03	−27.8 ± 0.1	+37 ± 2.2*	NZA-32350
	Autumn	0.14 ± 0.02	−28.1 ± 0.2	−7 ± 13.0*	YAUT-007421
	Winter	0.06 ± 0.01	−27.7 ± 0.3	−1 ± 12.7*	YAUT-007406
	Spring	0.10 ± 0.00	−27.9 ± 0.1	−55 ± 11.5*	YAUT-007407

*Data were corrected using the filter blank ($\Delta^{14}C$ = −1000‰, 0.6–3.7% of the total carbon in the samples) reported in this study.

All samples from Seri (where limestone is present) were more ^{14}C-depleted than Fudoji (where limestone is absent) (Figures 1 and 2). The $\Delta^{14}C_{DIC}$ was less variable in both streams (−217 ± 31‰ for Seri; −9 ± 14‰ for Fudoji) than $\Delta^{14}C_{DOC}$ (−248 ± 110‰ for Seri; +65 ± 46‰ for Fudoji) and $\Delta^{14}C_{POC}$ (−109 ± 52‰ for Seri; −6 ± 38‰ for Fudoji). Except for the winter, the $\Delta^{14}C_{DOC}$ was higher than the $\Delta^{14}C_{POC}$ and $\Delta^{14}C_{atm}$ (+45‰) in Fudoji, suggesting that slightly older (i.e. bomb ^{14}C) OM from soil leaches into the streamwater (Raymond and Hopkinson 2003). In the Hudson River and Amazon River, DOC was more ^{14}C enriched than POC (Raymond and Bauer 2001), which is consistent with the results in Fudoji. On the other hand, the $\Delta^{14}C_{DOC}$ was lower than the $\Delta^{14}C_{POC}$ in Seri. The $\Delta^{14}C_{DOC}$ for the winter in Seri was the lowest (−400‰) in our data set. Fellman et al. (2014) reported a similar $\Delta^{14}C_{DOC}$ value (−452‰) from a spring-fed stream in Australia, suggesting that the groundwater is a source of ^{14}C-depleted DOC in Seri. The results indicate that both the DOC and POC are mixtures of OM originated from various sources with distinctive $\Delta^{14}C$.

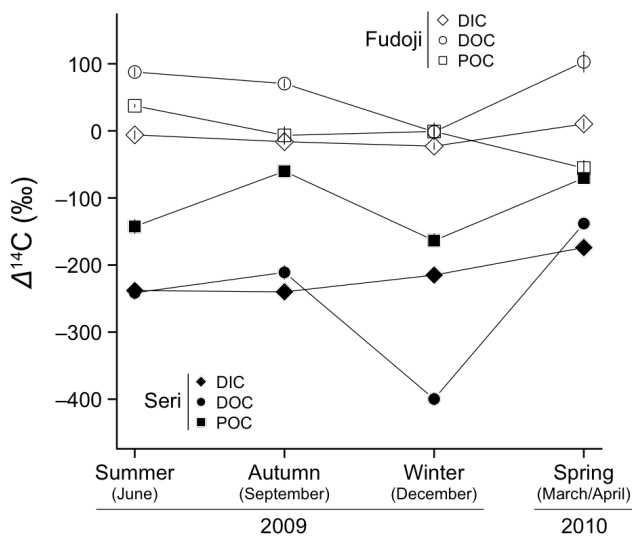Figure 1 Seasonal variations in $\Delta^{14}C$ values of DIC (diamonds), DOC (circles), and POC (squares) for Seri (filled symbols) and Fudoji (open symbols). Means of repeated measurements and 1σ analytical precisions are shown. The $\Delta^{14}C$ values of DIC in Seri are from Ishikawa et al. (2012).
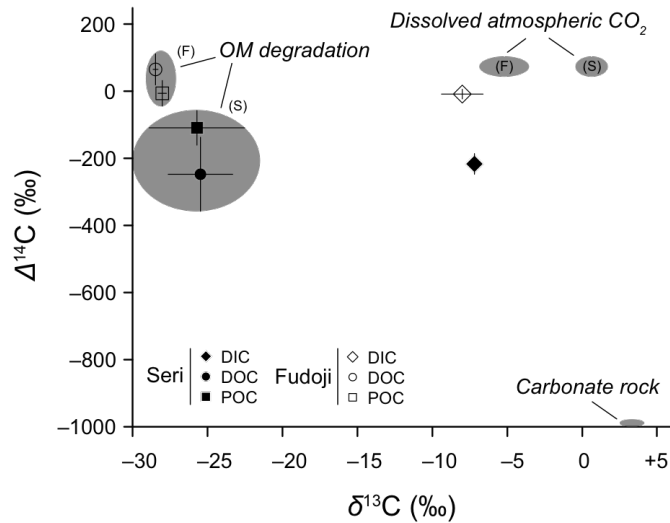
Figure 2 Biplot showing the four-season mean and SD of δ¹³C and Δ¹⁴C values of DIC, DOC, and POC for Seri and Fudoji. Potential DIC end-members are indicated as gray ovals, including: (1) dissolved atmospheric CO₂ (δ¹³C = 0‰ to +0.8‰ in Seri and –6.3‰ to –4.1‰ in Fudoji; Δ¹⁴C = +45‰ during 2009–2010); (2) organic matter (OM) degradation; and (3) carbonate rock (δ¹³C = +3.9 ± 0.3‰; Δ¹⁴C = –1000‰). (S) and (F) indicate Seri and Fudoji, respectively.

Based on the estimates of the δ¹³C and Δ¹⁴C mixing model, the dissolution of atmospheric CO₂ contributed to 47–57% (Seri) and 74–90% (Fudoji) of the carbon in DIC (Figure 3). Although the water depth of the two streams was not greatly different, stream width and discharge were greater in Seri (watershed area = 30 km²) than Fudoji (6.0 km²) (Table 1), suggesting that the bubbling of water by air was a more important source of DIC in smaller streams. In Seri, the contribution of weathering from carbonate rock to DIC was 14–22%, whereas the contribution in Fudoji was 4–6%. These results indicate that the weathering of carbonate rock is an important source of DIC in streams where limestone is present. Furthermore, groundwater-derived OM with ¹⁴C-depleted carbon may also be an important source of DIC in Seri, whereas DIC in Fudoji scarcely had ¹⁴C-depleted carbon source, and was dominated by atmospheric CO₂ with minor contribution of soil-derived OM.
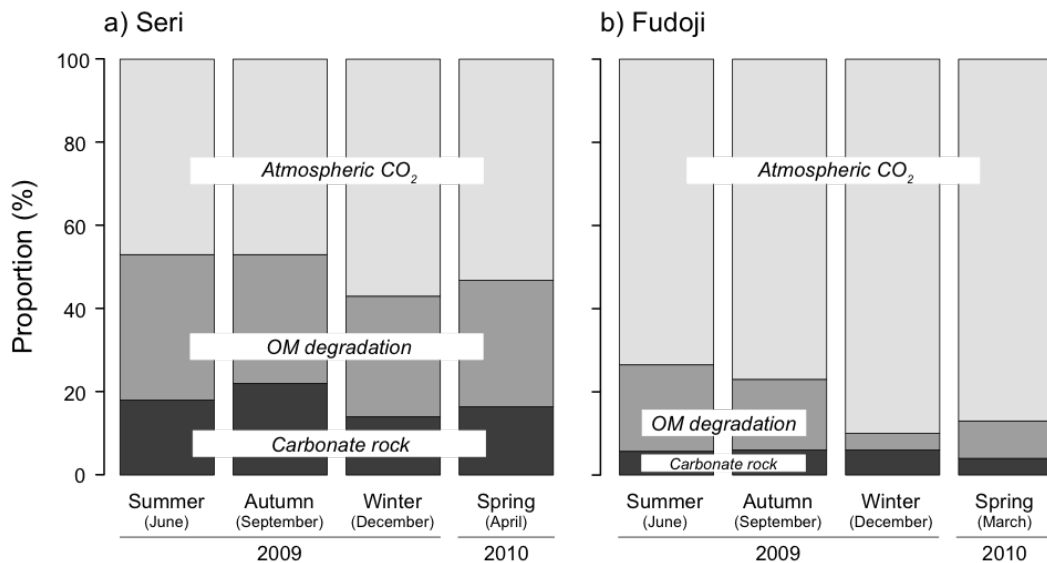

Figure 3 Proportions of carbon originated from atmospheric CO₂, organic matter (OM) degradation, and carbonate rock in DIC for (a) Seri and (b) Fudoji, as estimated using the δ¹³C and Δ¹⁴C mixing model.

The concentration of DOC was higher than that of POC in both streams (Tables 3 and 4), indicating that contribution of DOC degradation to DIC was greater than that of POC, if we assume that the degradation rates of DOC and POC are equivalent. The OM degradation contributed to 29–35% (Seri) and 4–21% (Fudoji) of the carbon in DIC. Proportion of OM degradation for the winter in Fudoji was the lowest (4%), probably due to low water temperature (Table 1) and low biological activity. Our results were compared with two previous studies that reported $\delta^{13}C$ and $\Delta^{14}C$ values of DIC, DOC, and POC from US rivers (Hudson, Parker, and York) (Raymond et al. 2004) and the Amazon River (Mayorga et al. 2005). The same mixing model that was used in our analysis was applied to the data sets of Raymond et al. (2004) and Mayorga et al. (2005) to calculate proportions of carbon originated from atmospheric CO_2 and OM degradation in DIC. Figure 4 shows that the contribution of atmospheric CO_2 to DIC decreases with stream/river size (i.e. watershed area and discharge), whereas the contribution of OM degradation increases. This result indicates that the dissolution of atmospheric CO_2 is a more important driver of carbon cycling in small streams than the biological degradation of organic matter. Contribution of atmospheric CO_2 to DIC in Seri was slightly lower than that in US rivers (Figures 4a and 4c) because the weathering of carbonate rock is also important in Seri.
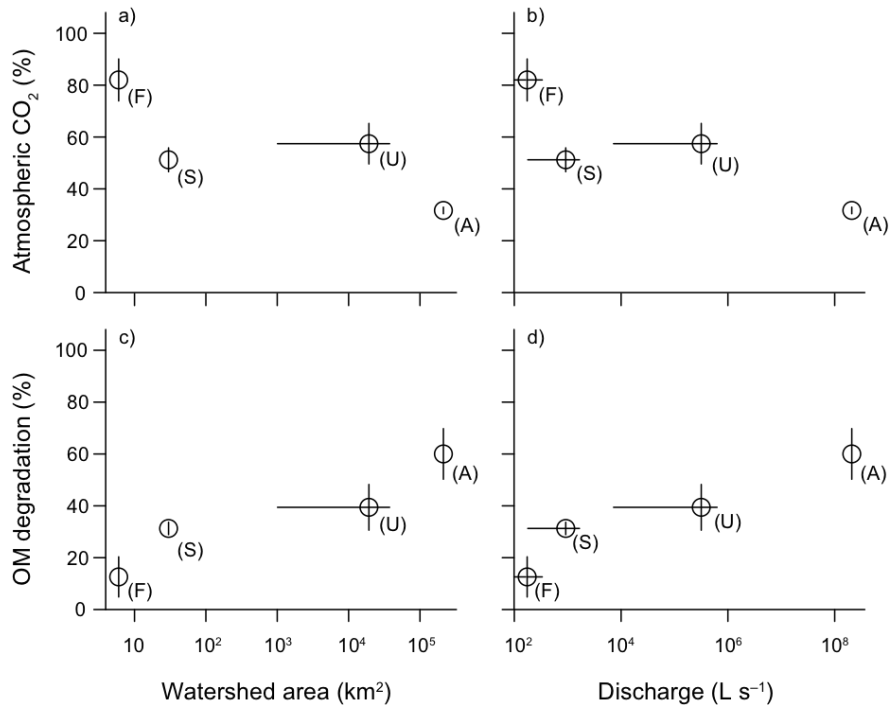

Figure 4 Biplot showing the proportions of carbon originated from (a, b) atmospheric CO_2 and (c, d) OM degradation in DIC versus (a, c) watershed area (km^2) and (b, d) discharge ($L\,s^{-1}$) (mean and SD) as estimated using the $\delta^{13}C$ and $\Delta^{14}C$ mixing model. (S), (F), (U), (A) indicate Seri ($n = 4$), Fudoji ($n = 4$), US rivers (Hudson, Parker, and York; $n = 9$) (Raymond et al. 2004), and the Amazon River ($n = 4$) (Mayorga et al. 2005), respectively.

Although there are limitations and uncertainties in our analyses associated with several assumptions (e.g. the degradation rates of DOC and POC are equivalent), ^{13}C and ^{14}C compositions are useful for constraining the sources of DIC in streams. This is partly because the $\Delta^{14}C$ value can be used to distinguish between dissolved atmospheric CO_2 under equilibrium and the weathering of carbonate

rock, both of which have similar $\delta^{13}C$ values in most cases. The approach presented in this study will be extensively used in future studies that aim to generalize the source of DIC by comparing multiple streams and rivers of different regions.

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