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Characteristics and behavior of dissolved organic matter in the Kumaki River, Noto Peninsula, Japan

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Abstract

Dissolved organic matter (DOM) in river water was studied to understand the transport behavior of DOM in a small watershed with forest and paddy fields. Field experiments were conducted under normal flow conditions in the Kumaki River, which is located in the central part of the Noto Peninsula in Japan, during the period 2009–2010. The concentrations and structural properties of fulvic acid-like components, which are the major components of DOM, were determined using three-dimensional excitation–emission matrix spectroscopy and high-performance size-exclusion chromatography. The relative fluorescence intensity for fulvic acid-like components at an excitation wavelength of 305–335 nm and an emission wavelength of 425–440 nm increased from the upper forest area to the lower paddy field area and increased seasonally in this river system in the following order: winter, autumn, spring, summer. Fulvic acid-like components with a higher molecular weight were observed in the summer samples. These results suggest that higher precipitation and agricultural activity in the summer season increase the amount of fulvic acid-like components with higher molecular weight that are transported from the watershed into the river.

Keywords

DOM, Fulvic acid-like components, Excitation–emission matrix spectroscopy, High-performance size-exclusion chromatography (HPSEC)

Introduction

Dissolved organic matter (DOM) in natural waters is composed of a heterogeneous mixture of organic components with molecular weights ranging from less than 100 to over 300,000 Daltons (Da) (Thurman 1985; Kalbitz 2001; Mostofa et al. 2013). This DOM typically originates from three major sources: (i) allochthonous sources, such as terrestrial material from soils; (ii) autochthonous sources, such as surface water-derived matter of algal or phytoplankton origin; and (iii) synthetic organic substances of man-made or industrial origin. DOM is known to contribute significantly to material cycles in terrestrial and aquatic ecosystems (Dawson et al. 1981; Qualls et al. 1991; Kalbitz et al. 1999; Kalbitz 2001), particularly because of its complexation ability (especially for trace elements) and its pH buffering ability. DOM also plays an important role in the mobilization of organic pollutants in aquatic environments (Chiou et al. 1986; Fukushima and Tatsumi 1999; Sparks et al. 1996).

Numerous researchers have reported that the concentrations and chemical properties of DOM in soil and river waters depend on the land use patterns and vegetation types in the associated watersheds and the impacts of human activities (McDowell and Likens 1988; Qualls et al. 1991; Kalbitz 2001, Park et al. 2002; Chantigny 2003). Dissolved organic carbon (DOC) concentrations are generally higher in coniferous forests than deciduous forests (Qualls et al. 1991; Currie et al. 1996; Yano et al. 2000), and the abundance of high-molecular-weight DOM is typically greater in forest soil than in agricultural and paddy soil because the degradation of litter occurs in surface soils in forest areas (Cronan et al. 1999; Leinweber et al. 2001; Chantigny 2003). Agricultural activity leads to increases in the export of DOM from watersheds into river waters due to increases in the supply of drainage waters from paddy fields and upland fields (Lee et al. 2002). However, the DOC concentration flowing into a river is decreased by the growth of underbrush and inactive irrigation drainage after the abandonment of paddy fields. Moreover, the amount of high-molecular-weight DOM flowing into a river decreases with increasing the area of upland agricultural zone (Cronan et al. 1999).

To better understand the transport behavior of DOM in river systems, we focus on particular components of DOM in aquatic environments. Humic substances composed of humic and fulvic acid account for 40–80% of DOM in river water (Malcolm 1985; Mostofa et al. 2010). Thus, they play an important role in biogeochemical processes, such as buffering pH and aiding complexation of trace elements in aquatic environments (Matsunaga et al. 1998; Krachler et al. 2005; Nakagawa et al. 2008). Moreover, these substances exhibit various structural features because of differences in their source materials and formation environment (Malcolm 1985; Kalbitz 2001). Therefore, humic substances can be used as a parameter of DOM export, reflecting differences in watershed environments (Malcolm 1985; Coble 1996; Kalbitz et al. 1999). Many studies in various large rivers that include forest and agricultural areas in their watersheds have reported the transport of humic substances, especially fulvic acids, which constitute a major fraction of humic substances (Coble 1996; Cronan et al. 1999; McKnight et al. 2001; Baker 2005; Mostofa et al. 2010). Japanese river systems typically have small watersheds and high river bottom slopes and experience high precipitation. A few previous studies have reported the dynamics of humic substances in such small river systems incorporating forest and agricultural areas (e.g., Nagao et al. 2003; Mostofa et al. 2005; Sazawa et al. 2011). Typically, transport of humic substances and its seasonal variation have been shown to be controlled by precipitation, temperature, agricultural activities, and plant communities (Sazawa et al. 2011; Asakawa et al. 2007).

The present study aims to investigate spatial and seasonal variations in the concentrations and characteristics of humic substances in river water running through forests and paddy fields, corresponding to a typical Japanese river system. The river research was conducted under normal flow conditions in the Kumaki River, the watershed of which includes distributed forests and paddy fields, in the Noto Peninsula from June 2009 to July 2010. This area is suitable for investigation of the export of humic substances in river systems because it is a small watershed area with a simple land use pattern, as shown in Fig. 1. We focus on fulvic acid and determine its abundance through direct analytical methods using three-dimensional excitation–emission matrix (3-D EEM) spectroscopy and high-performance size-exclusion chromatography (HPSEC). Therefore, the present study expresses dissolved humic substances as fulvic acid-like components and discusses their dynamics and sources in the Kumaki River over a year.

Materials and methods

Study area

The Kumaki River system is located in the central part of the Noto Peninsula in Ishikawa Prefecture, Japan. The river system consists of the Kumaki River and two major tributaries (Fig. 1). The Kumaki River has a watershed area of 47.2 km^2 and a main channel length of 14.8 km (Ishikawa Prefecture 2008). The river runs through forest, farmland, and rural areas in its watershed and flows into the western part of Nanao Bay with a semi-closed coastal bay. Mean annual precipitation was 2183 mm at the Nishiyachi Station in 2009–2012 (Rivers Division of Ishikawa Prefecture 2012); this is higher than the average value (ca. 1700 mm) for Japan (Ministry of Land, Infrastructure, Transport and Tourism, Japan 2012). Many small rainfall events occur each year in this watershed and most of the annual precipitation is concentrated in the summer season (Rivers Division of Ishikawa Prefecture 2012). Here, we focus on the dynamics of fulvic acid-like components under normal flow conditions, when the water level is 0.08–0.32 m at the Kamo-Bashi Bridge in the downstream reaches of the river (Fig. 2 and Table 1) (Rivers Division of Ishikawa Prefecture 2012). For most observation dates, no rain events were recorded within the 5 h preceding the research; the sampling in February 2010 was the exception to this. In 2009–2012, the mean annual water level at the Kamo-Bashi Bridge was 0.07 m (Rivers Division of Ishikawa Prefecture 2012).

Around 93% of the upstream area is covered by forest. Paddy fields are distributed over 4% of the midstream area and 12% of the downstream area. The forest in the Kumaki River basin is composed primarily of a plantation forest and a secondary forest (Fig. 1). The dominant tree species are cedar (*Cryptomeria japonica*) and Cypress (*Chamaecyparis obtusa* and *Thujopsis dolabrata* var. *hondae*) and the dominant species in the secondary forest is Japanese oak (*Quercus serrata Thunb.*) (Ministry of the Environment of Japan 2001). The soil is classified as Haplic Red in the mountain ridge area, Terrace Yellow and Terrace Brown Forest soil in the mountain side area, and Strong Gley Lowland and Haplic Gray Lowland soil around the Kumaki River (National Institute for Agro-Environmental Sciences 2011).

Materials

River water samples were collected from surface water in the center of the river at three sites along the Kumaki River (Fig. 1) from June 2009 to July 2010 under normal flow conditions. These water samples were collected using a 5 l of bucket. The mean water level is 0.03 m in April–May (spring), 0.10 m in June–August (summer), 0.09 m in September–November (autumn), and 0.11 m in December–February (winter) (Rivers Division of Ishikawa Prefecture 2012). The seasonal fluctuation of the river water level is smaller than that of other rivers during April to August owing to agricultural activities. However, drainage water from paddy fields flows into the midstream and downstream areas, especially during the rice planting period in spring. Samples of drainage water from a paddy field were collected near the midstream site in August 2009 and April 2010. All water samples were filtered with Whatman GF/F glass fiber filters (pore size ca. 0.7 μm) after combustion at 450 °C for 6 h; then, the filtered samples were kept in a freezer at –30 °C until analysis. We focused on fulvic acid-like components because fulvic acids account for 60–80% of humic substances and have high fluorescence (Malcolm 1985; Mostofa et al. 2010). To identify the peak of fulvic acid-like components in the 3-D EEM spectra and the basic features of the size-exclusion chromatogram, humic and fulvic acids were isolated and purified from two river water samples from the Teshio and Tokachi Rivers, the watersheds of which are 47–70% forest and 14–27% agricultural area (Hokkaido Regional Development 2012).

Sample preparation of fulvic acids (FA) isolated from river water

The water samples from the Teshio and Tokachi Rivers were acidified to pH 1.5

with HCl and pumped onto a column packed with Supelite DAX-8 resin (Thurman and Malcolm 1981). The humic substances adsorbed on the resin were eluted with 0.1 M NaOH and separated into fulvic acid at pH 1.5. The river fulvic acid was dissolved in a dilute NaOH solution and then adjusted using 0.01 M $NaClO_4$ solution at pH 8.0 to form fulvic acid with a concentration of 10 mg l^{-1}.

Analysis

The DOC concentration was measured according to a high-temperature combustion method (Peltzer and Brewer 1993). The water sample was acidified by 1 M HCl solution to remove carbonate and purged by N_2 gas for 1.5 min. Then, 150 µl of acidified and purged natural waters was injected into a Shimadzu TOC-V CSN total organic carbon analyzer. The injected samples were combusted at 680 °C and the resultant CO_2 was purified and measured with a Non-Dispersive Infra Red (NDIR) detector. Calibration curves were made using potassium phthalate as standard solution. All of the measurements were conducted at least in duplicate with a precision of ±3%. The detection limit was 50 µgC l^{-1}.

The 3-D EEM spectra of the water samples were measured with a Hitachi F-4500 fluorescence spectrophotometer with a 150 W ozone-free xenon lamp (Coble et al.

1993; Nagao et al. 1997). The spectra were recorded at excitation (Ex.) wavelengths from 250 to 500 nm and emission (Em.) wavelengths from 250 to 550 nm at 5 nm intervals. The scan speed was 2400 nm min^{-1}. The slit width was 5 and 10 nm for Ex. and Em. wavelengths, respectively. The photomultiplier voltage was set to 400 V. The relative fluorescence intensity (RFI) of the water samples was expressed in terms of quinine standard units (QSU), where 10 QSU corresponds to the fluorescence intensity of quinine sulfate (10 μg l^{-1} in 0.05 M sulfuric acid) at excitation and emission wavelengths of 345 nm and 455 nm, respectively.

The molecular size distribution of humic substances was measured using HPSEC (Nagao et al. 2003). Size-exclusion chromatography (SEC) column separation was performed with a stainless steel column (Hitachi GL-W530; 300 mm × 10.7 mm ID) packed with water-soluble polyacrylate gel resin. The water samples were injected into the SEC column at a carrier flow rate of 1 ml min^{-1}. The mobile phase was a 0.01 M Tris-HCl buffer solution containing 0.01 M NaCl and adjusted to pH 8.0. Fluorescence was monitored at excitation and emission wavelengths of 320 nm and 430 nm, respectively. The void volume (Vo) and total effective column volume ($Vo + Vi$) of the SEC column were 8.4 and 23.6 ml, respectively. The SEC column was calibrated with Blue Dextran (molecular weight (MW) of 2000000), polyethylene glycols (MW of 106, 194, 400, 1010, 1900, 22450, and 50000), and acetone (MW of 58). The peaks were detected by a refractive index detector. The relative standard deviation of the peak height was less than 9% for the natural waters in duplicate measurements. Lake Biwa fulvic acid purchased from the Japanese Humic Substances Society was measured in each analysis as a running standard to check reproducibility in peak retention volume, peak area, and peak height. Four peaks of the Lake Biwa fulvic acid were detected at retention volumes of 9.5 ml (Peak 1), 9.8 ml (Peak 2), 10.4 ml (Peak 3), and 11.9 ml (Peak 4). 10 mg l^{-1} solution of fulvic acid isolated from the water samples from the Teshio and Tokachi Rivers were also determined using 3-D EEM spectroscopy and HPSEC.

Parallel factor analysis (PARAFAC) modeling

PARAFAC statistically decomposes the complex mixture of DOM fluorophores into components without making any assumptions regarding their spectral shape or number (Stedmon et al. 2003). We used water samples collected from June 2009 to July 2011, including water samples collected during rainfall events, to obtain the necessary number of samples required to attain statistical significance for PARAFAC modeling. The water samples collected during rainfall events will be discussed in a future study.

The dataset for the PARAFAC modeling was composed of 76 river water samples, 5 drainage water samples from paddy fields, and 2 water samples from paddy fields. For PARAFAC modeling, excitation and emissions wavelengths of 240–450 and 300–500 nm, respectively, were used at 5 nm intervals. The analysis was conducted using MATLAB with the “N-way Toolbox for MATLAB” (Andersson and Bro 2000). Split-half analysis was used to validate the identified components (Stedmon et al. 2003; Cory and McKnight 2005).

Results

DOC concentration

DOC concentrations were found to be in the ranges 0.25–2.97 and 0.50–0.85 mgC l^{-1} in the Kumaki River water and drainage water, respectively (Table 2 and Fig. 3). In the river water, DOC concentrations were 0.25–1.13, 0.41–2.97, and 0.46–2.29 mgC l^{-1} in the upstream, midstream, and downstream areas, respectively. Thus, DOC concentrations generally increased from the upstream to the downstream region, except for the water samples from November 2009 and April 2010. The river water exhibited its highest DOC concentration in August 2009 and seasonal variation is apparent in the DOC concentration result.

3-D EEM spectra

Two fluorescent peaks in the 3-D EEM spectra were detected for the Kumaki River water samples (Table 3 and Fig. 4(a)–(d)), as follows: Ex./Em. of 305–340/425–440 nm for peak M and 320–340/445–465 nm for peak C. Three fluorescent components were identified by PARAFAC modeling using 83 EEM spectra of Kumaki River water samples and drainage water samples from paddy fields. The excitation and emission pairs of the main peak positions for the three components are shown in Table 4 and Fig. 5 together with the results of previous studies (Coble 1996; Yamashita et al. 2008; Mostofa et al. 2010). Component 1 indicates the occurrence of humic-like materials with two fluorophores at Ex./Em. of 310/410 nm of Peak M and 240/410 nm of Peak A1 (Fig. 5(a)). Component 2 corresponds to the occurrence of terrestrial humic-like materials with two fluorescence peaks at Ex./Em. of 245/480 nm of Peak A2 and 360/480 nm of Peak C (Fig. 5(b)). Component 3 corresponds to tryptophan-like materials with a fluorescent peak: Peak T at Ex./Em. of 275/315 nm (Fig. 5(c)). Fulvic acid peaks in the 3-D EEM spectra for the Teshio and Tokachi River water samples (Table 4 and Fig. 4(e) and (f)) were detected for Ex./Em. of 310/430–435 nm for peak M. These peak positions are similar to peaks M and C observed for the Kumaki River water samples and could correspond to terrestrial fulvic acid-like components originating from the decomposition of plant materials (Mostofa et al. 2013).

Figure 6 illustrates the variation in the RFI of fulvic acid-like components based on 3-D EEM spectra for the research period. The RFI values for peak M were in the range 4.0–24.4 QSU and appeared to exhibit seasonal variation, with high values in summer and low values in winter. Moreover, the RFI ranged from 4.6 to 9.5 for the upstream waters, 4.0 to 14.1 for the midstream waters, and 4.5 to 24.4 for the downstream waters of the watershed.

Size-exclusion chromatograms

Size-exclusion chromatograms of the water samples from the Kumaki River and drainage water obtained from a paddy field in August 2009 are shown in Fig. 7 together with two riverine fulvic acids. Four peaks were detected at retention volumes of 9.5 ml (Peak 1), 9.8 ml (Peak 2), 10.4 ml (Peak 3), and 11.9 ml (Peak 4). Four peaks were also detected in the size-exclusion chromatograms of the Teshio and Tokachi River water samples at the same retention volumes. Based on the calibration curve ($r^2 = 0.98$, $p < 0.04$) for the SEC column, the apparent molecular weights (MW) of peaks 1–4 were estimated to be >15.0, 12.4, 8.2, and 3.3 kDa, respectively. The higher MW fulvic acid-like components of peaks 1 and 2, which correspond to MW greater than 10 kDa and fluorescence at Em. wavelengths of 400–500 nm, are consistent with those of other river fulvic acids documented in previous studies (Dawson et al. 1981; Kalbitz 2001; Nagao et al. 2003; Asakawa et al. 2007). The peak height of peak 1 for fulvic acid-like components ranges from 268 to 4887 μV in the Kumaki River waters (Fig. 8(a)), with ranges of 375–2285, 317–2784, and 268–4887 μV in the upstream, midstream, and downstream areas, respectively. Moreover, ranges of 486–1308, 632–4887, 437–876, and 268–663 μV were found in spring, summer, autumn, and winter, respectively. Overall, the peak height remained almost constant from the upstream to downstream areas, except in June and August 2009 and April 2010, when peak height was found to increase downstream.

The peak height ratio of peaks 1 and 2 for the fulvic acid-like components, which is a simple indicator of the molecular size distribution, was found to range from 0.35 to 0.94 in the Kumaki River waters (Fig. 8(b)). This ratio was found to be 0.41–0.94, 0.35–0.85, and 0.34–0.87 in the upstream, midstream, and downstream areas, respectively, with ratios of 0.41–0.58, 0.45–0.94, 0.52–0.66, and 0.34–0.51 in spring, summer, autumn, and winter, respectively. Thus, this ratio exhibits a distribution similar to that of the peak height of peak 1.

Discussion

Variations in DOC concentration and RFI of fulvic acid-like components

The DOC concentrations observed in the present study are lower than or similar to those recorded for other Japanese river systems (Kawasaki et al. 2002: 1.5 mgC l^{-1}; Nagao et al. 2003: 1.1–2.1 mgC l^{-1}; Asakawa et al. 2007: 0.8–2.0 mgC l^{-1}; Sazawa et al. 2011: 0.5–1.2 mgC l^{-1}). The variation in DOC concentrations from upstream to downstream in the Kumaki River system is 0.25–2.97 mgC l^{-1}; this is higher than values reported previously (Nagao et al. 2003; Sazawa et al. 2011).

The distributions of RFI values of fulvic acid-like components and DOC concentrations in the Kumaki River are presented in Fig. 9. For the upstream waters, we found no correlation between DOC concentration and the RFI. A weak correlation ($r^2 = 0.47$, $p < 0.03$) is apparent for the midstream waters, except for the April sample. For the downstream waters, a positive correlation exists ($r^2 = 0.76$, $p < 0.0004$) for all samples. The ratio of the RFI value to the DOC concentration is 8.0 QSU (mgC $l^{-1})^{-1}$ for the midstream samples and 9.8 QSU (mgC $l^{-1})^{-1}$ for the downstream samples. This suggests that the concentrations of DOC and fulvic acid-like components exhibited similar variations in the midstream and downstream waters. Other studies in Japanese river systems have demonstrated a difference in RFI/DOC ratio between upstream and downstream water (Mostofa et al. 2005; Sazawa et al. 2011), which are typically associated with forest and agricultural areas, respectively. Thus, these results show that river water in agricultural areas typically exhibits a strong correlation between DOC concentration and RFI value, although no such correlation exists for river water in forest areas. In the present study, the Kumaki River system is a small watershed and has forest in its upstream areas and agricultural areas (such as paddy fields) along its midstream and downstream reaches. Therefore, the variation in correlation between DOC concentrations and RFI values found for the Kumaki River reflects differences in land use pattern between the upstream and midstream/downstream areas; such differences have been reported previously in several DOM studies (Cronan et al. 1999; Kalbitz 2001; Mostofa et al. 2005; Sazawa et al. 2011).

The distribution of RFI values of fulvic acid-like components and DOC concentrations for each season are illustrated in Fig. 10. The spring samples produced scattered plots or two correlation lines. The DOC concentrations in the upstream and midstream areas in April 2010 were higher than those in other months. Plowing and irrigation of the paddy field was undertaken in April before rice seedlings were transplanted, such that the majority of paddy water input to the river in its midstream reaches would have been associated with use of a paddy soil rotary in April 2010. In fact, the water level at the downstream observation point rose by 20 mm because of the input of drainage water from paddy fields in the spring season (Rivers Division of Ishikawa Prefecture 2012). The DOC concentration in the midstream area was higher than that in the downstream area in April 2010. However, the RFI values increased from upstream to downstream. Therefore, typical components released from paddy fields were relatively low-fluorescence organic materials. We found a positive correlation between RFI values and DOC concentrations in the summer (r^2 = 0.80, $p < 0.001$), autumn (r^2 = 0.64, $p < 0.01$), and winter samples (r^2 = 0.98, $p < 0.000005$). The ratio of RFI value to DOC concentration was found to be similar (9.2–10.6) in these three seasons, indicating that the proportion of fulvic acid-like components to DOM was almost constant from summer to winter.

The fluctuation of the river water level due to instantaneous variation is small, although antecedent precipitation of over 5 mm h^{-1} occurred in August and December of 2009 and in February and May of 2010 (Table 1). Plots of DOC and RFI values with maximum precipitation intensity within 24 h before sampling are shown in Fig. 11. We found a positive correlation between DOC and maximum precipitation intensity (r^2 = 0.84, $p < 0.00008$). Similarly, a positive correlation is evident for the RFI values and maximum precipitation intensity (r^2 = 0.79, $p < 0.0006$), except for the June sample, which was not associated with any precipitation. However, we found no correlation between DOC and RFI values and rainfall duration (not shown). These results indicate that the precipitation intensity within 24 h before sampling contributes to variations in the concentration of DOC and fulvic acid-like components in the Kumaki River.

Sources of fulvic acid-like components in the Kumaki River

A seasonal variation of MW is apparent for fulvic acid-like components in the Kumaki River (Fig. 8). The apparent variation of the river water level is small owing to agricultural activity (i.e., rice paddy cultivation) during April to August (Rivers Division of Ishikawa Prefecture 2012). However, the peak height ratios for the river water samples collected in summer were twice as high as those in spring and winter. This suggests that fulvic acid-like components exported from the watershed in summer are rich in higher molecular size fractions.

The average RFI values and peak height ratios of peaks 1 and 2 for fulvic acid-like components are shown in Fig. 12. The average RFI values were found to increase from upstream to downstream in the following order: winter = autumn < spring < summer. Conversely, the peak height ratio exhibits a different variation pattern. The ratio was found to be almost constant from upstream to downstream in autumn and winter, although the ratio in spring was higher in the midstream and downstream areas than the upstream area. In summer, the peak height ratios decreased slightly and the RFI values increased significantly from upstream to downstream. Asakawa et al. (2007) suggested that, in spring, autumn, and winter, humic substances with lower molecular size are derived from the deeper horizons of forest soil in a small forest watershed in Japan. The Kumaki River system exhibits lower RFI values and molecular weight of fulvic acid-like components in autumn and winter than summer. This is considered to be due to the contribution of fulvic acid-like components from basement flow in the deeper soil horizons. Conversely, the autumn samples show the presence of fulvic acid-like components with relatively high molecular weight. The difference in the RFI and peak height ratio may be delay in the transport of fulvic acid-like components from the surface soil to deeper soil horizons. In spring, the RFI and peak height ratio were higher than the winter season samples and increased from the upstream to downstream areas. The peak height ratio of drainage water from the paddy field was found to be 0.69, which is higher than that of river water in the midstream and downstream areas. The downstream fluorescent spectral form is consistent with that of drainage water from the paddy field (Fig. 4(c)–(d) and Table 3). Rice planting is performed in this area in April. Other studies conducted in forest and farmland catchments (Mostofa et al. 2005;

Sazawa et al. 2011) have shown that higher RFI values are observed not only at low water flow rates but also for high fluxes of drainage water from agricultural land (mainly paddy fields) located in downstream areas. Therefore, the variation observed in spring season indicates that a large contribution of fulvic acid-like components from the paddy fields occurred in the midstream and downstream areas in the Kumaki River. Cronan et al. (1999) have shown that the molecular size of DOM in river water is higher in forest areas than in agricultural areas. Moreover, Asakawa et al. (2007) demonstrated that humic substances in streams have higher molecular sizes in summer than that in autumn and winter and are typically derived from the surface of forest soil. Therefore, the peak height ratio for the Kumaki River samples in summer suggests that fulvic acid-like components with higher molecular size fractions are supplied from the forest soil located in the upstream area. The fulvic acid-like components are also supplied from the paddy fields located in the midstream and downstream reaches of the watershed. Summer is an active period for surface and subsurface flow from the upstream forest and the midstream and downstream paddy fields owing to the abundance of rainfall events, as shown in Fig. 2.

The results of a t-test conducted on each seasonal sample for comparison between seasons demonstrate that the seasonal variation of the peak height ratio is not significant for any of the watersheds. However, the summer season exhibits wide fluctuations in the peak height ratio for the fulvic acid-like components in the river water. The peak height ratio in June and August 2009 is about two times that in July 2010. Similar variations were found in the RFI values for fulvic acid-like components. These results suggest that the summer season in the Kumaki River system is particularly sensitive to the discharge of DOM and humic substances from the watershed.

Based on our results, we conclude that the Kumaki River watershed has three main sources of fulvic acid-like components that are transported into the river: 1) materials supplied by basement flow from deeper soil horizons; 2) materials derived from forests located in the upstream area; and 3) materials derived from paddy fields located in the midstream and downstream areas. Variations in the input of fulvic acid-like components from the watershed to the river depend on watershed conditions such as precipitation, land use type, and agricultural activity.

Conclusions

The export of fulvic acid-like components, which are the major components of DOM, was studied in the Kumaki River system under normal conditions in 2009–2010 to understand the factors controlling the transport behavior of fulvic acid-like components over a year. Field experiments were conducted at forest areas located in the upstream areas and paddy fields located in the midstream and downstream areas. The following results were obtained. Two fulvic acid-like component peaks were observed at excitation wavelengths of 305–335 nm and emission wavelengths of 425–440 nm in the 3-D EEM spectra of all water samples. Four peaks were detected in the size-exclusion chromatograms of all water samples, corresponding to the features shown in the chromatograms for the riverine fulvic acids. DOC concentration and relative fluorescent intensity (RFI) increased from the upstream to the downstream area. For the upstream waters, we found no correlation between DOC concentration and the RFI, but a positive correlation was observed for the midstream and downstream waters, except for the April sample. This suggests that the DOC concentration and fulvic acid-like components show similar variations in the midstream and downstream areas. The variation in RFI/DOC reflects differences in land use pattern. The RFI values increased in the following order: winter, autumn, spring, summer. The peak height ratio of peak 1/peak 2 (corresponding to MWs of 15.0 and 12.4 kDa) for the fulvic acid-like components was also found to be highest in the summer samples. The ratio was found to decrease broadly from upstream to downstream (upstream > midstream = downstream), although it increased from upstream to downstream in spring. These results suggest that, in summer, fulvic acid-like components with high molecular weight fractions are supplied from forest soil located in the upstream area and from the paddy fields located in the midstream and downstream reaches of the watershed. This can be attributed to the higher precipitation and more intense agricultural activity during this period.

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Table 1 Observed water level at downstream station and precipitation intensity and duration time within 24 h before sampling in the Kumaki River system from June 2009 to July 2010

Sample		Water level	Maximum precipitation intensity	Time before sampling	Total precipitation	Rainfall duration
		m	mm h^{-1}	h	mm	h
River water						
2009/6/18		0.13	0	0	0	0
2009/8/29	☆	0.13	15	12	29	5
2009/9/24		0.13	0	0	0	0
2009/10/16		0.13	0	0	0	0
2009/11/30		0.15	1	20	1	1
2009/12/28	☆	0.32	7	5	16	4
2010/1/27		0.13	0	0	0	0
2010/2/26	☆	0.08	6	0 *	9	3

2010/4/28		0.11	3	8	15	4
2010/5/26	☆	0.16	5	22 **	19	5
2010/7/1		0.11	0	0	0	0

* Rainfall during sampling period

** Rainfall of about 1 mm h^{-1} during sampling period

☆ Rainfall of over 5 mm h^{-1} within 24 h before sampling

Table 2 Water qualities and dissolved organic carbon (DOC) concentrations in the Kumaki River from June 2009 to July 2010

Sample	pH	DO (mg l^{-1})	EC (mS m^{-1})	TURB (NTU)	WT (°C)	DOC (mgC l^{-1})
River water						
2009/6/18						
Upstream	7.2	10.4	8.4	7.4	15.6	0.25 ± 0.01
Midstream	7.4	10.0	11.9	5.6	18.5	0.56 ± 0.02
Downstream	7.4	9.1	17.5	7.2	20.9	1.05 ± 0.00
2009/8/29						
Upstream	7.2	10.1	6.7	4.3	19.2	0.60 ± 0.00
Midstream	7.5	9.7	9.5	14.3	22.9	1.21 ± 0.00
Downstream	7.4	9.4	10.0	15.7	22.1	2.29 ± 0.02
2009/9/24						
Upstream	6.1	14.3	7.2	22.6	17.7	0.41 ± 0.00
Midstream	5.8	15.2	11.4	20.3	22.4	0.61 ± 0.01
Downstream	6.9	17.1	13.9	3.3	22.1	0.74 ± 0.01
2009/10/16						
Upstream	6.9	15.2	6.0	3.4	12.7	0.26 ± 0.00
Midstream	7.2	15.5	9.6	6.4	16.0	0.41 ± 0.01
Downstream	7.9	15.1	11.4	2.8	15.2	0.46 ± 0.01
2009/11/30						
Upstream	6.8	12.4	6.0	5.8	10.8	0.39 ± 0.01

Midstream	7.0	13.3	8.0	7.7	9.7	0.65 ± 0.01
Downstream	6.9	13.4	11.6	7.9	9.9	0.57 ± 0.01
2009/12/28						
Upstream	5.9	18.0	5.8	6.8	5.5	0.64 ± 0.00
Midstream	5.3	17.2	8.1	17.4	7.7	0.84 ± 0.01
Downstream	5.2	17.4	8.5	34.8	7.5	1.01 ± 0.01
2010/1/27						
Upstream	n.m.*	n.m.	n.m.	n.m.	n.m.	n.m.
Midstream	6.0	13.9	6.8	7.8	7.8	0.42 ± 0.00
Downstream	6.2	14.4	8.0	20.1	8.1	0.47 ± 0.01
2010/2/26						
Upstream	6.1	12.6	8.4	16.3	9.4	0.84 ± 0.01
Midstream	4.5	14.0	7.3	26.4	10.1	1.02 ± 0.00
Downstream	3.7	13.9	8.4	51.9	10.8	1.17 ± 0.01
2010/4/28						
Upstream	7.3	10.2	6.2	15.2	11.1	1.13 ± 0.23
Midstream	5.6	10.8	8.2	25.1	11.5	2.97 ± 0.05
Downstream	6.2	10.6	9.1	52.4	13.5	0.81 ± 0.08
2010/5/26						
Upstream	7.2	6.1	9.5	101.2	12.6	0.54 ± 0.01
Midstream	5.8	8.2	9.9	52.5	13.8	0.79 ± 0.01
Downstream	5.9	8.6	9.7	49.2	14.4	0.91 ± 0.00
2010/7/1						

Upstream	7.0	8.3	5.2	8.7	16.6	0.38 ± 0.00
Midstream	7.6	8.1	8.4	16.4	19.9	0.52 ± 0.01
Downstream	7.6	7.8	9.1	11.7	20.6	0.53 ± 0.00
Drainage water from paddy field						
2009/8/29	7.5	9.5	9.9	9.2	21.1	0.85 ± 0.01
2010/4/28	n.m.	n.m.	n.m.	n.m.	n.m.	0.50 ± 0.13

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* n.m. = river water samples not measured owing to snow cover

Table 3 Peak position and relative fluorescence intensity (RFI) of fulvic acid-like components in the Kumaki River from June 2009 to July 2010

Sample	Peak M		Peak C	
	Peak position (Ex./Em.)	RFI (QSU)	Peak position (Ex./Em.)	RFI (QSU)
Kumaki River water				
2009/6/18				
Upstream	310/435	8.0	335/455	7.5
Midstream	305/430	12.7	335/465	11.5
Downstream	335/430	20.3	340/460	17.7
2009/8/29				
Upstream	310/435	9.5	340/465	8.9
Midstream	305/435	14.1	330/465	13.0
Downstream	330/435	24.4	340/460	22.9
2009/9/24				
Upstream	310/430	7.2	340/465	6.9
Midstream	310/435	9.0	330/450	8.6
Downstream	310/435	11.2	335/460	10.4
2009/10/16				
Upstream	305/440	5.0	330/450	4.9
Midstream	310/430	6.3	340/460	5.8
Downstream	320/435	7.4	340/460	6.8
2009/11/30				
Upstream	310/440	4.8	335/460	4.4
Midstream	310/430	6.4	340/445	6.0
Downstream	325/430	8.4	335/460	7.5
2009/12/28				
Upstream	310/435	5.4	330/460	4.9
Midstream	320/435	7.8	335/460	7.4
Downstream	305/430	9.4	335/450	9.0
2010/1/27				
Upstream	n.m.*	n.m.	n.m.	n.m.

Midstream	325/435	4.0	340/465	3.3
Downstream	315/435	4.5	335/460	3.9
2010/2/26				
Upstream	310/430	7.1	325/455	6.4
Midstream	310/430	9.9	325/455	8.9
Downstream	310/435	11.6	330/460	10.4
2010/4/28				
Upstream	325/425	4.6	325/450	4.3
Midstream	310/430	8.4	330/460	7.7
Downstream	305/430	10.4	330/455	9.7
2010/5/26				
Upstream	310/435	7.0	320/445	6.6
Midstream	310/435	9.3	330/445	8.6
Downstream	310/430	11.6	330/450	10.5
2010/7/1				
Upstream	305/435	5.7	325/465	5.2
Midstream	310/430	6.7	330/455	6.1
Downstream	310/435	7.5	320/445	7.1
Drainage water from paddy field in the Kumaki River basin				
2009/8/29	340/435	12.4	340/460	11.8
2010/4/28	320/425	7.0	330/450	6.5

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* n.m. = river water samples not measured owing to snow cover

Table 4 Fluorescent peak position at excitation/emission (Ex./Em.) wavelength of dissolved humic substances

Samples	Peak C region			References
	Peak M Ex./Em. (nm)	Peak C Ex./Em. (nm)	Peak A Ex./Em. (nm)	
Yodo River FA	305/430	n.d. *	n.d.	Nagao et al. (2003)
Suwannee River FA	305/430	325/460	n.d.	
Ado River FA (extracted)	300–310/420–430	n.d.	260–270/430–440	Sugiyama et al. (2005)
Suwannee River FA (IHSS standard)	n.d.	333 ± 3/469 ± 6	n.d.	Mostofa et al. (2005)
Suwannnee River HA (IHSS standard)	n.d.	330 ± 13/484 ± 13	n.d.	
Bekanbeushi River FA	n.d.	345/460	n.d.	Kumegawa (2007)
Kumaki River **	310/410	360/480	240-245/410-480	This study
Teshio River FA	310/430	n.d.	n.d.	This study
Tokachi River FA				
Head water	310/430	n.d.	n.d.	
Upstream	310/435	n.d.	n.d.	
Downstream	310/435	n.d.	n.d.	

* n.d. = not detected

** This Kumaki River data is peak position by PARAFAC analysis.

Figure Captions

Fig. 1 Location maps of sampling sites (○), precipitation observatory station (△), and water level observatory station (□) in the Kumaki River system. The bold and thin black lines indicate the Kumaki River and its tributaries, respectively. The vegetation map of 2006 was produced by Asia Air Survey Co. Ltd.

Fig. 2 Hydrograph of the downstream area of the Kumaki River. Arrows indicate sampling dates. Precipitation and water level data were provided by the Integrated River Information System of Ishikawa Prefecture.

Fig. 3 DOC concentrations of river water in the upstream (●), midstream (▲), and downstream (□) areas of the Kumaki River

Fig. 4 3-D EEM spectra of river water from the (a) upstream, (b) midstream, and (c) downstream areas of the Kumaki River and from (d) drainage water from a paddy field in the midstream area in August 2009. Two spectra for fulvic acids of the (e) Teshio River and (f) Tokachi River are also shown in this figure. Arrows indicate peak positions of fulvic acid-like components. Contour intervals of all spectra are 1.0 QSU.

Fig. 5 3-D EEM spectra of the fluorescent components (a) 1, (b) 2, and (c) 3 of the Kumaki River samples, identified with the PARAFAC model

Fig. 6 RFI of fulvic acid-like components detected at peak M in 3-D EEM spectra of river water in the upstream (●), midstream (▲), and downstream (□) areas of the Kumaki River

Fig. 7 Size-exclusion chromatograms of river water from the (a) upstream, (b) midstream, and (c) downstream areas of the Kumaki River and (d) drainage water from a paddy field in the midstream area in August 2009. Two chromatograms for fulvic acids of the (e) Teshio River and (f) Tokachi River are also presented in this figure. The monitoring wavelength was Ex./Em.: 320/430 nm, corresponding to the fluorescent peak of riverine fulvic acid (Nagao et al. 2003; Asakawa et al. 2007).

Fig. 8 (a) Peak height of peak 1 and (b) peak height ratio of peaks 1 and 2 in the HPSEC chromatograms for fulvic acid-like components in the upstream (●), midstream (▲) and downstream (□) areas of the Kumaki River

Fig. 9 RFI values of fulvic acid-like components versus DOC concentrations in the (a) upstream, (b) midstream, and (c) downstream areas of the Kumaki River

Fig. 10 RFI values of fulvic acid-like components versus DOC concentrations in Kumaki River water in (a) spring, (b) summer, (c) autumn, and (d) winter during 2009–2010

Fig. 11 Plots of (a) DOC concentration versus maximum precipitation intensity and (b) RFI values of fulvic acid-like components versus maximum precipitation intensity within 24 h before sampling

Fig. 12 Average (a) RFI values and (b) peak height ratio of peaks 1 and 2 in the size-exclusion chromatograms for fulvic acid-like components in the Kumaki River. Error bars represent standard deviation (n = 2–3).

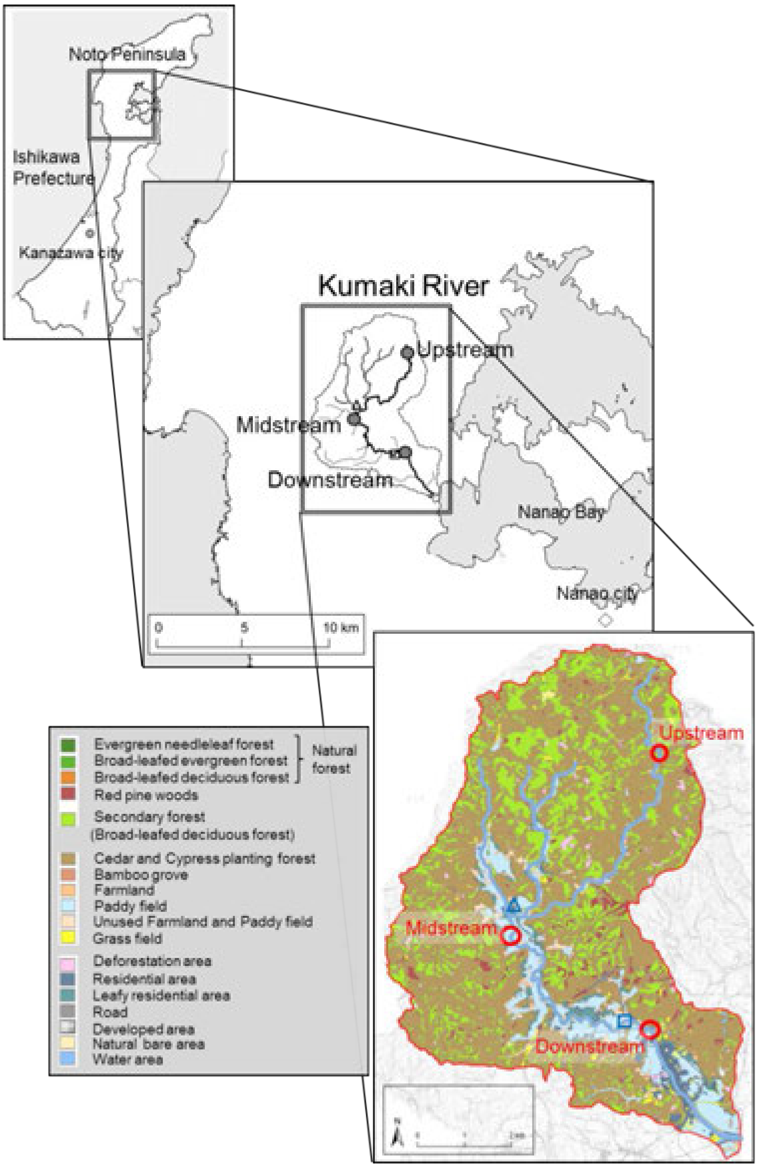


Fig. 1 Location maps of sampling sites (○), precipitation observatory station (△), and water level observatory station (□) in the Kumaki River system. The bold and thin black lines indicate the Kumaki River and its tributaries, respectively. The vegetation map of 2006 was produced by Asia Air Survey Co. Ltd.

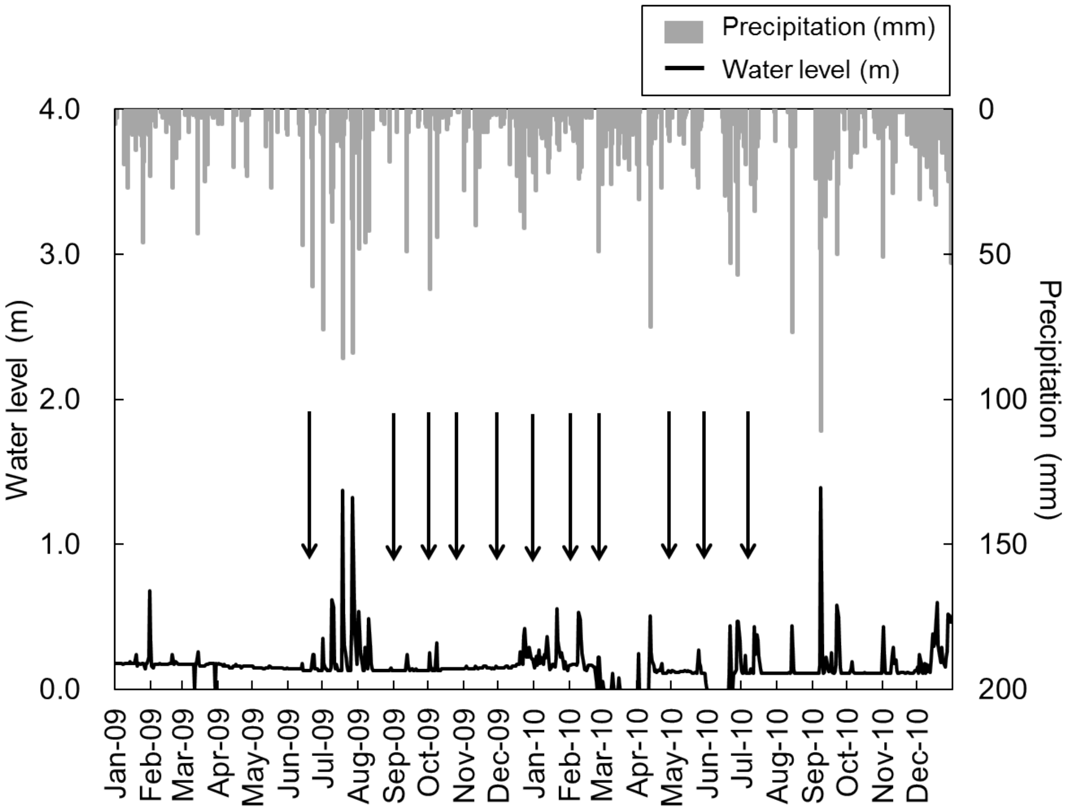


Fig. 2 Hydrograph of the downstream area of the Kumaki River. Arrows indicate sampling dates. Precipitation and water level data were provided by the Integrated River Information System of Ishikawa Prefecture.

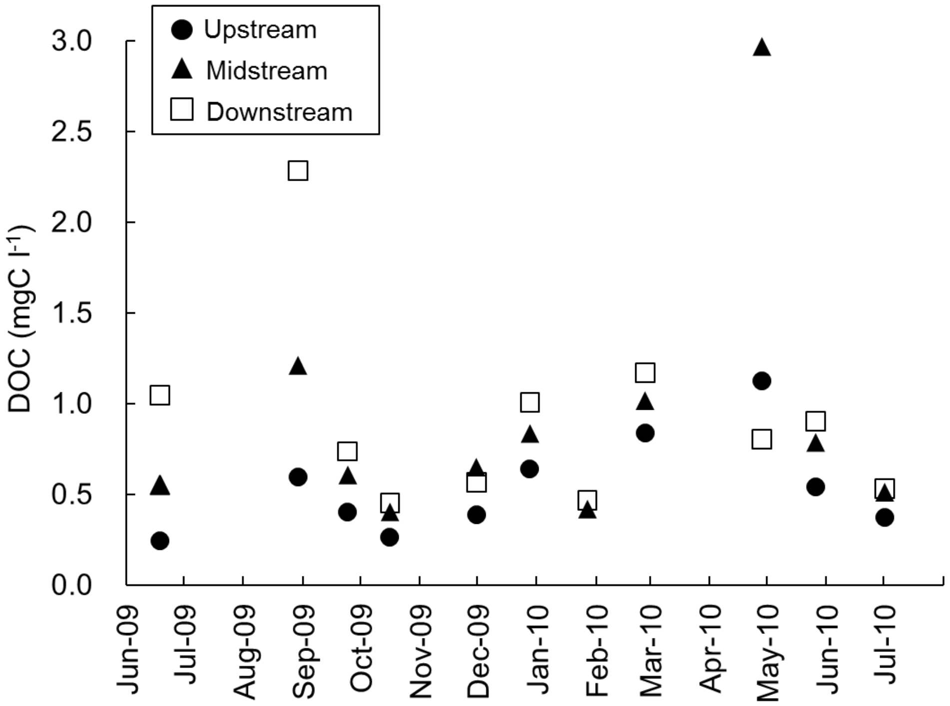


Fig. 3 DOC concentrations of river water in the upstream (●), midstream (▲), and downstream (□) areas of the Kumaki River

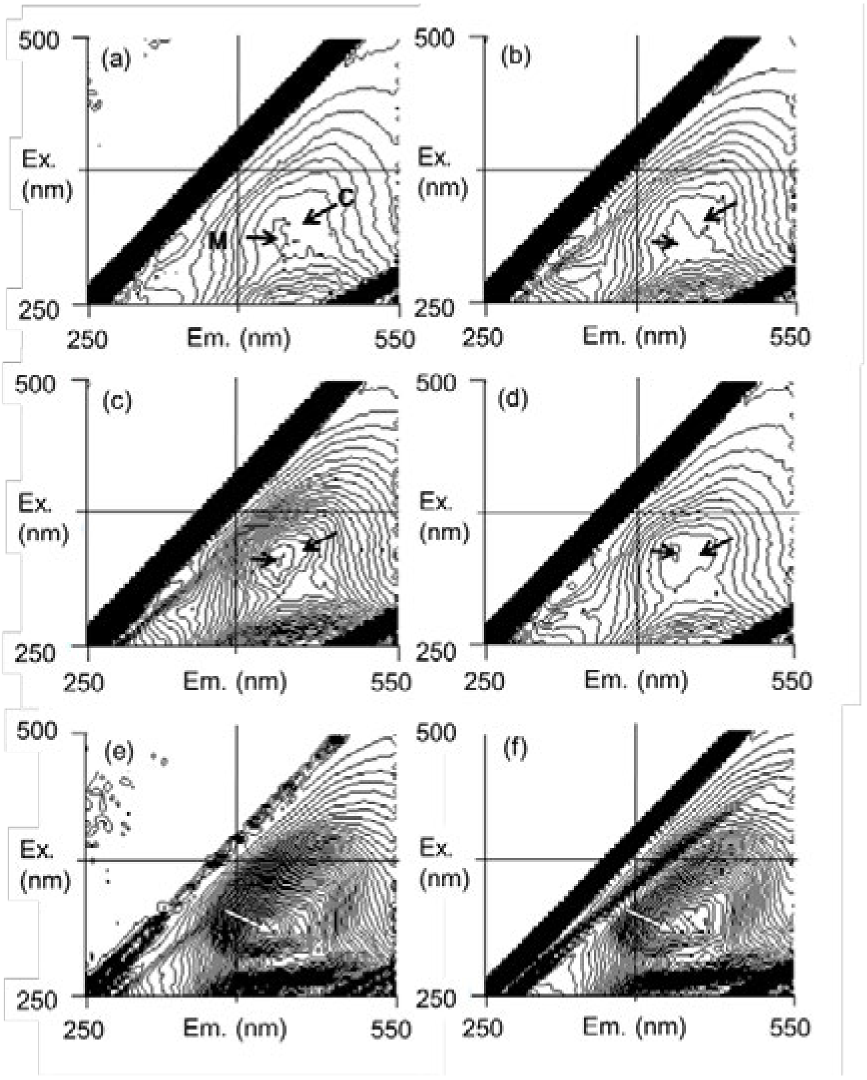


Fig. 4 3-D EEM spectra of river water from the (a) upstream, (b) midstream, and (c) downstream areas of the Kumaki River and from (d) drainage water from a paddy field in the midstream area in August 2009. Two spectra for fulvic acids of the (e) Teshio River and (f) Tokachi River are also shown in this figure. Arrows indicate peak positions of fulvic acid-like components. Contour intervals of all spectra are 1.0 QSU.

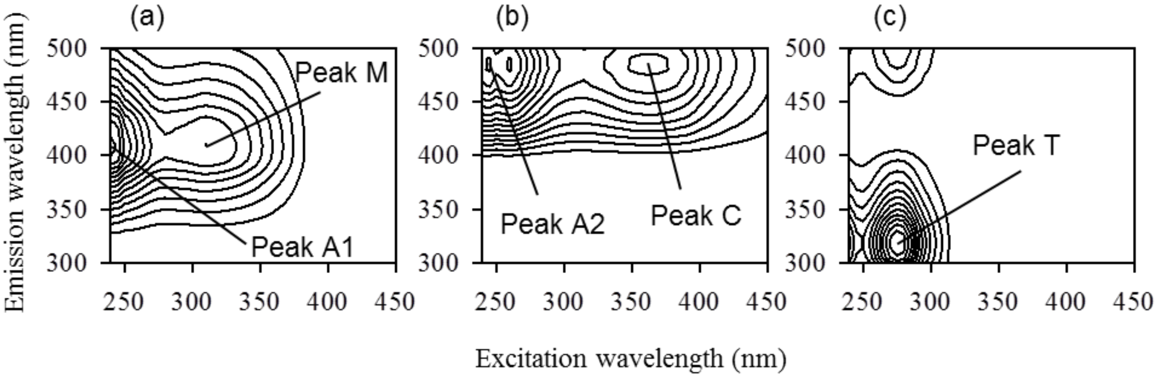


Fig. 5 3-D EEM spectra of the fluorescent components (a) 1, (b) 2, and (c) 3 of the Kumaki River samples, identified with the PARAFAC model

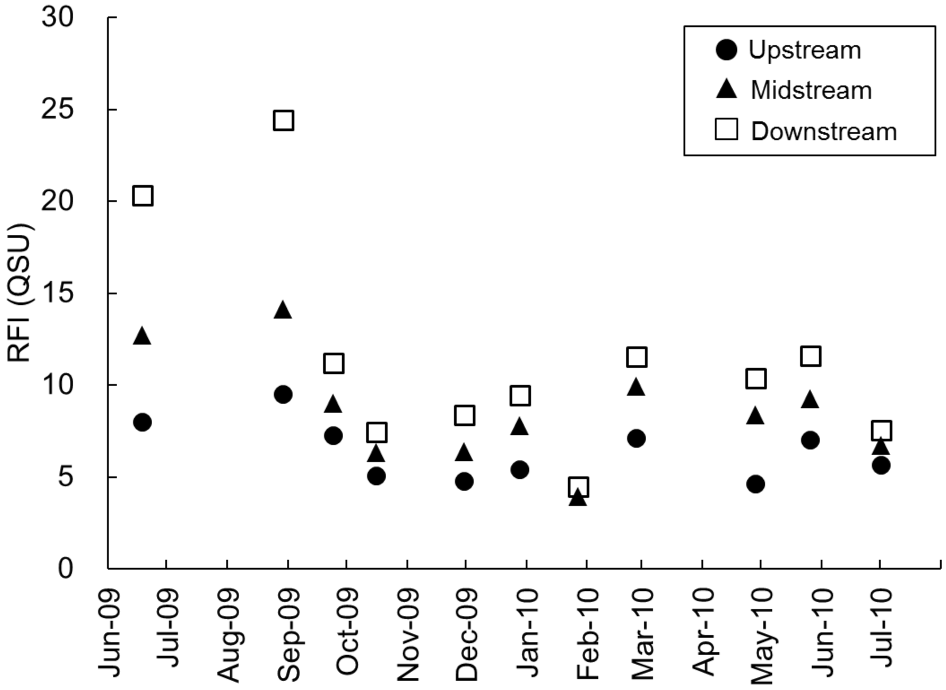
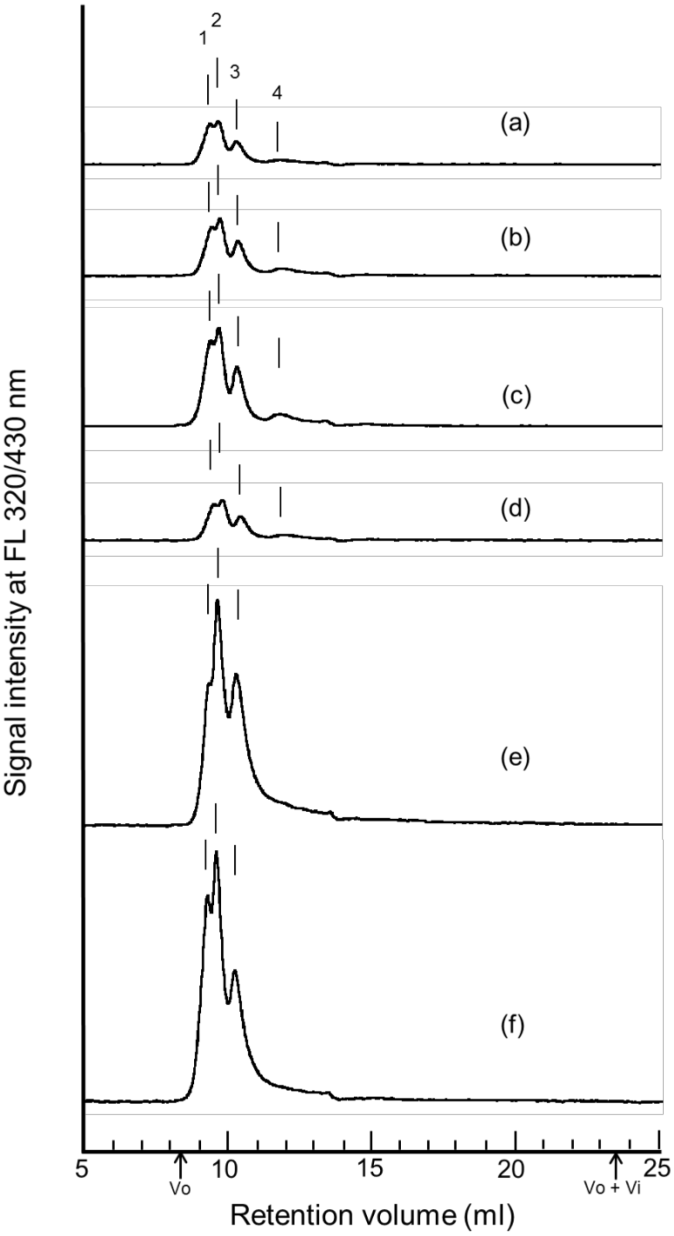


Fig. 6 RFI of fulvic acid-like components detected at peak M in 3-D EEM spectra of river water in the upstream (●), midstream (▲), and downstream (□) areas of the Kumaki River



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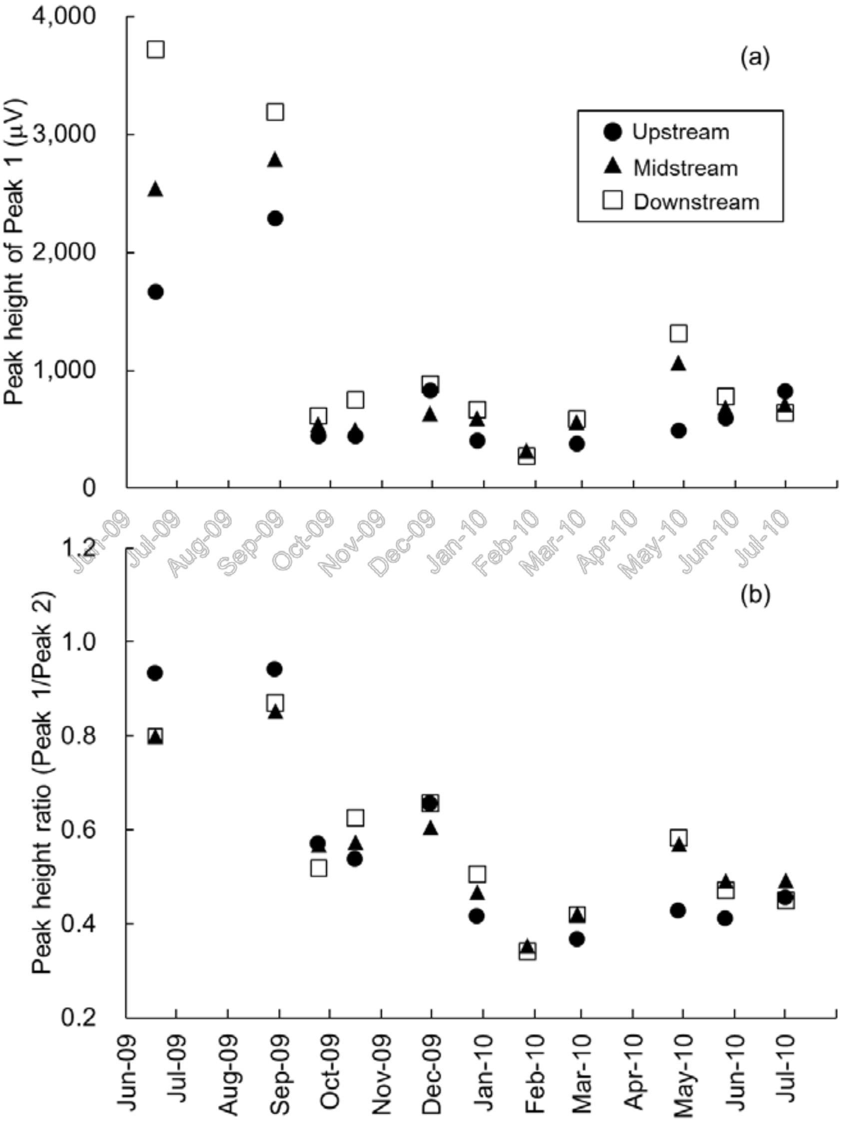

Fig. 8 (a) Peak height of peak 1 and (b) peak height ratio of peaks 1 and 2 in the HPSEC chromatograms for fulvic acid-like components in the upstream (●), midstream (▲) and downstream (□) areas of the Kumaki River

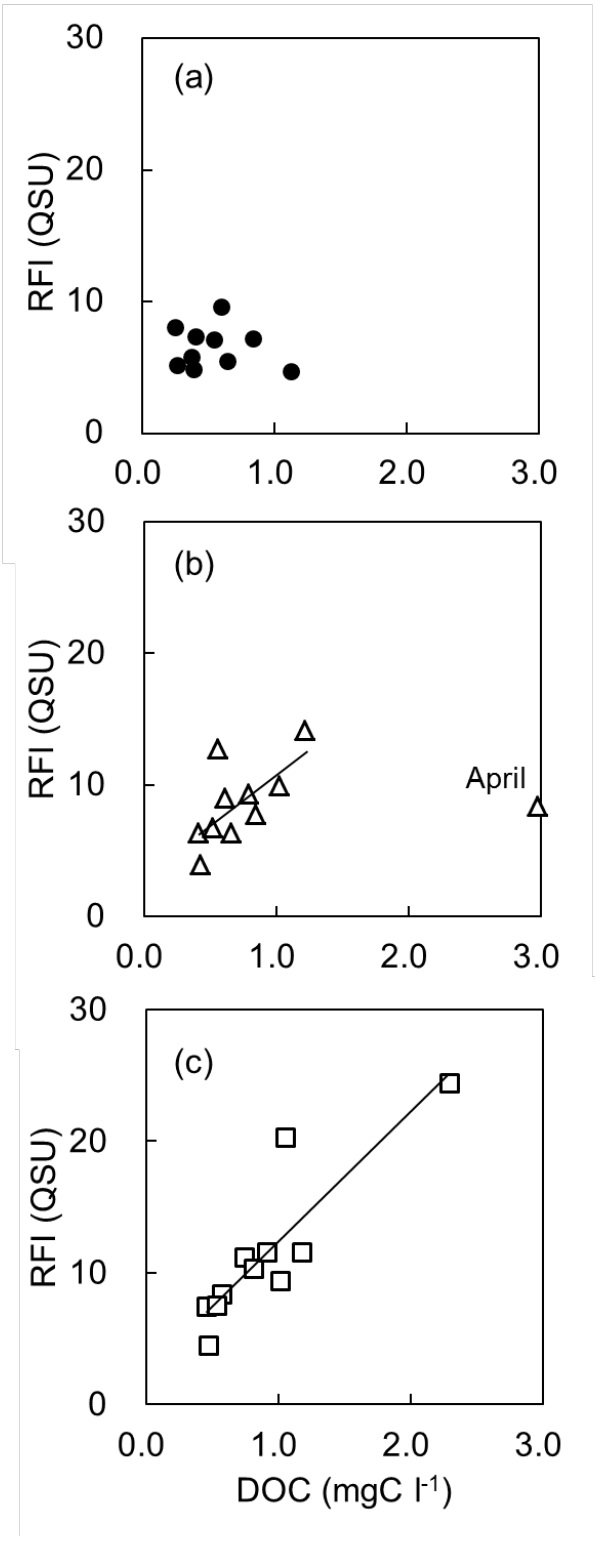

Fig. 9 RFI values of fulvic acid-like components versus DOC concentrations in the (a) upstream, (b) midstream, and (c) downstream areas of the Kumaki River

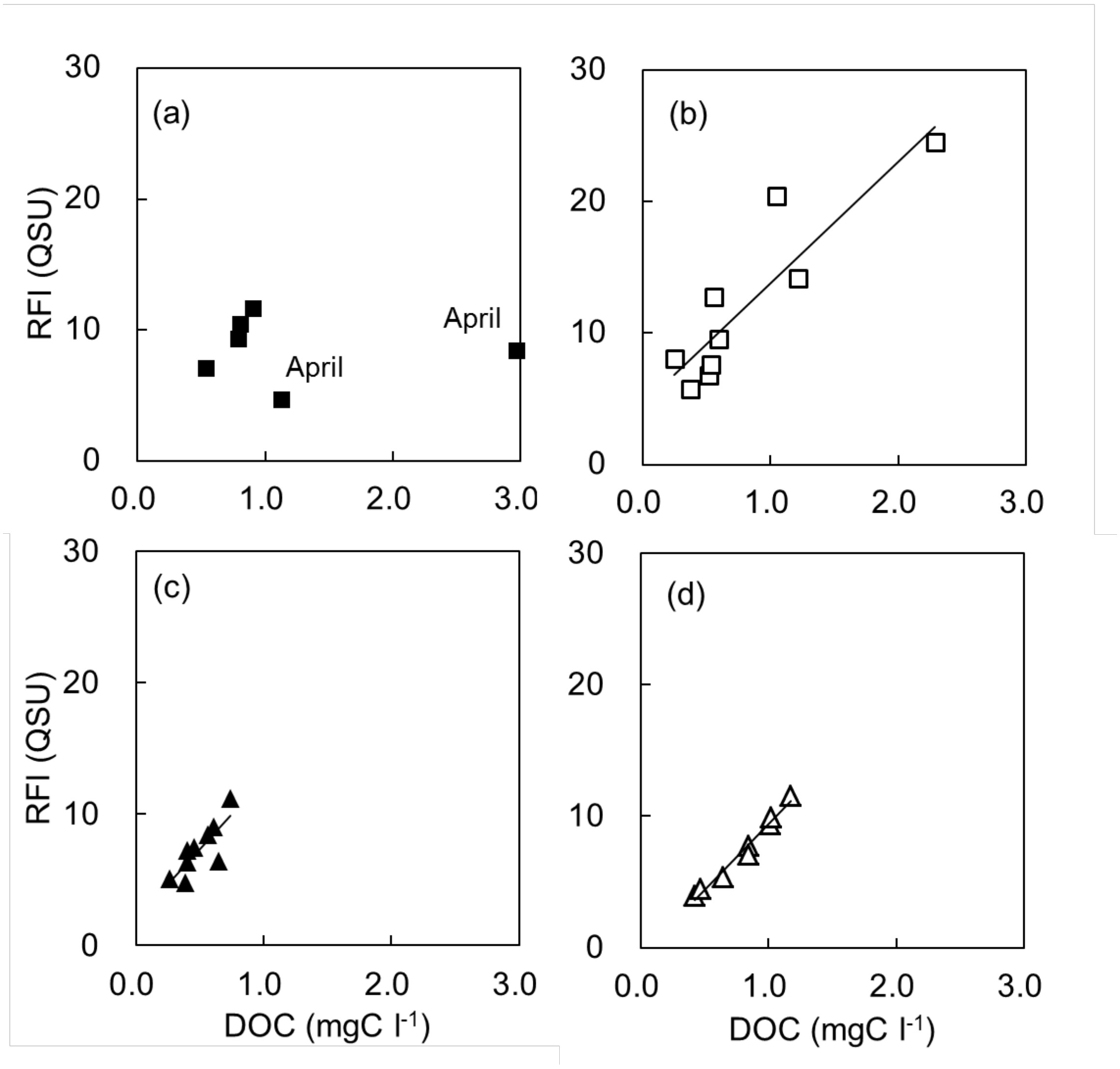

Fig. 10 RFI values of fulvic acid-like components versus DOC concentrations in Kumaki River water in (a) spring, (b) summer, (c) autumn, and (d) winter during 2009–2010

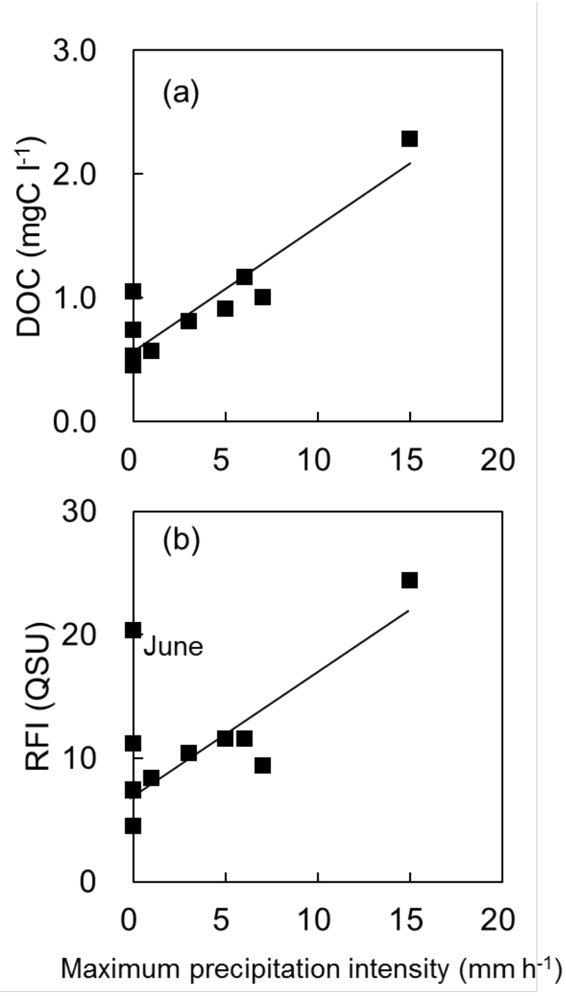

Fig. 11 Plots of (a) DOC concentration versus maximum precipitation intensity and (b) RFI values of fulvic acid-like components versus maximum precipitation intensity within 24 h before sampling

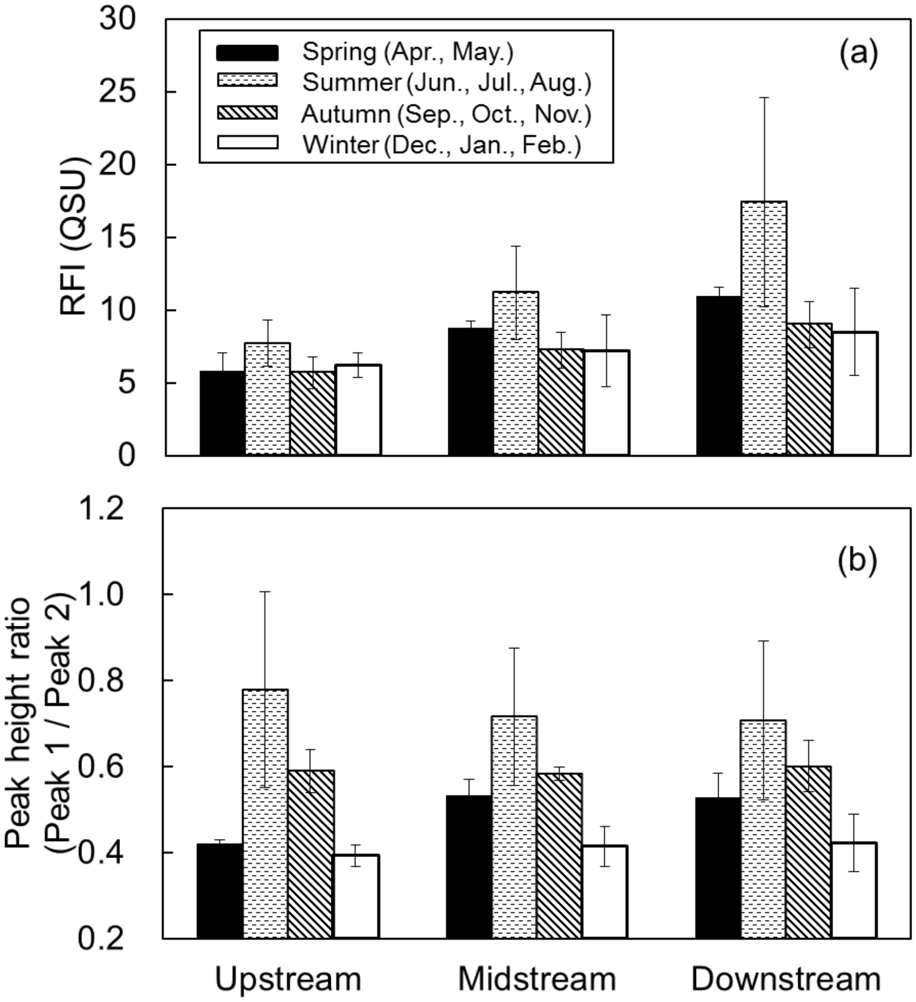

Fig. 12 Average (a) RFI values and (b) peak height ratio of peaks 1 and 2 in the size-exclusion chromatograms for fulvic acid-like components in the Kumaki River. Error bars represent standard deviation (*n* = 2–3).