

A tale of pipes and reactors: Controls on the in-stream dynamics of dissolved organic matter in rivers

Joan P. Casas-Ruiz,^{1*} Núria Catalán,² Lluís Gómez-Gener,³ Daniel von Schiller,⁴ Biel Obrador,³ Dolly N. Kothawala,⁵ Pilar López,³ Sergi Sabater,^{1,6} Rafael Marcé¹

¹Catalan Institute for Water Research (ICRA), Girona, Spain

²Department of Ecology and Genetics/Limnology, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

³Department of Ecology, University of Barcelona, Barcelona, Spain

⁴Faculty of Science and Technology, Department of Plant Biology and Ecology, University of the Basque Country, Bilbao, Spain

⁵Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden

⁶Institute of Aquatic Ecology, University of Girona, Girona, Spain

Abstract

The potential for rivers to alter the flux of dissolved organic matter (DOM) from land to ocean is widely accepted. Yet anticipating when and where rivers behave as active reactors vs. passive pipes of DOM stands as a major knowledge gap in river biogeochemistry, resulting in uncertainties for global carbon models. Here, we investigate the controls on in-stream DOM dynamics by evaluating changes in DOM concentration and composition along several reaches of a medium-sized river network over one full hydrological year. Roughly half of the observations over time and space showed active reactor conditions and, among these, similar proportion of gains and losses was measured. High water residence times promoted the active over passive behavior of the reaches, while DOM properties and nitrate availability determined whether they supplied or removed DOM from the river. Among different DOM fractions, protein-like DOM both of terrestrial and aquatic origin seemed to drive bulk DOM patterns. Our study emphasizes the role of water residence time as a physical constraint for in-stream processes, and provides new insights into the key factors governing the net balance between in-stream gains and losses of DOM in rivers.

The riverine flux of dissolved organic matter (DOM) from terrestrial ecosystems to the ocean is a major component of the global carbon cycle (Battin et al. 2009; Ciais et al. 2013). Yet our ability to predict the fate of DOM relies on an accurate comprehension of the in-stream biogeochemical processes that supply and remove DOM from rivers. River networks have been identified as active conduits through which DOM can be not only transported but also produced,

buried in sediments, or mineralized and emitted to the atmosphere (Cole et al. 2007; Aufdenkampe et al. 2011; Raymond et al. 2013). Still, whereas relevant DOM processing has been reported in some bioassays and field studies (Mcdowell and Fisher 1976; Dawson et al. 2001; Guillemette and del Giorgio 2011), many others have found limited evidence of DOM alteration in rivers (Hanley et al. 2013; Kothawala et al. 2015; Wollheim et al. 2015). Therefore, unraveling the factors that promote in-stream processing of DOM (i.e., removal and/or generation) vs. its passive transport downstream is essential for a better understanding of the role of rivers in DOM cycling.

Among possible factors, the time DOM spends in the system has been usually invoked as a major constraint for in-stream DOM processing in small to medium-sized river networks (Temnerud et al. 2007; Ågren et al. 2014; Kothawala et al. 2015). In fact, from a strictly physical point of view, the ratio between reaction and transport timescales determine the opportunity for in-stream processes to occur (Oldham et al. 2013). At the same time, the chemical composition of DOM may influence its fate because reactivity can be highly variable among DOM fractions (Cory and Kaplan 2012).

*Correspondence: jpcasas@icra.cat

Additional Supporting Information may be found in the online version of this article.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Special Issue: Headwaters to Oceans: Ecological and Biogeochemical Contrasts Across the Aquatic Continuum

Edited by: Marguerite Xenopoulos, John A. Downing, M. Dileep Kumar, Susanne Menden-Deuer, and Maren Voss

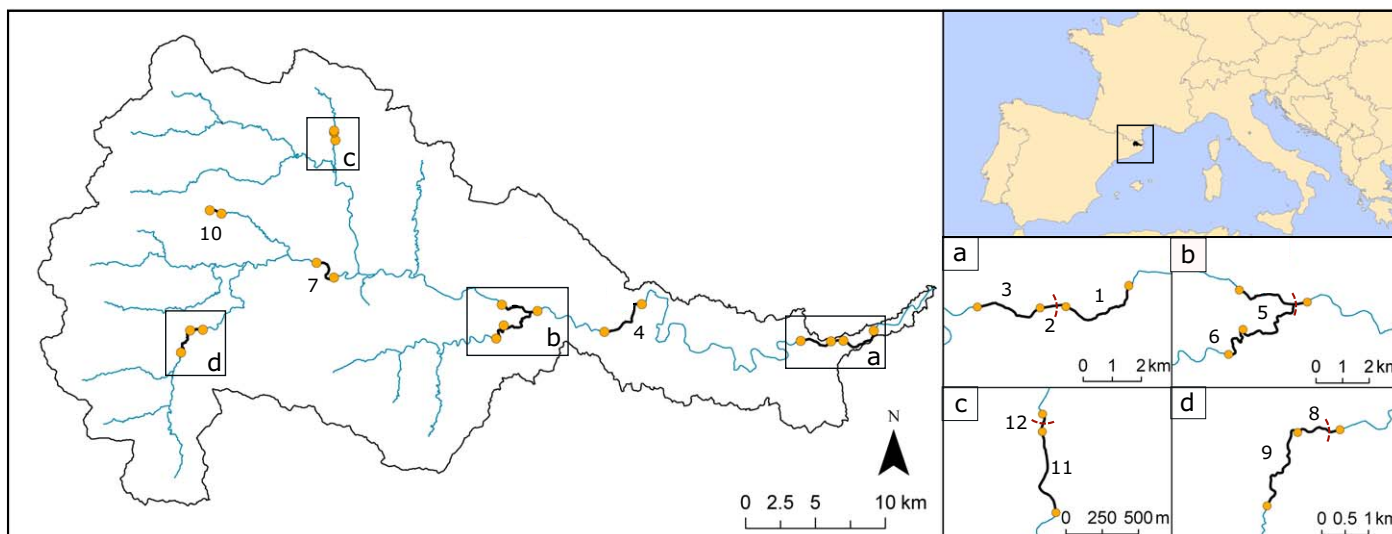


Fig. 1. Map of the Fluvià river catchment, with all studied river reaches indicated by heavy black lines. Yellow solid circles indicate the upper and lower ends of the reaches. The location coordinates of the study reaches are given in Supporting Information Table 1. The upper right panel shows the situation of the catchment in South-Western Europe. The lower right panel shows enlarged views of some of the study areas for visual clarity. Red dashed lines represent weirs. Note that reach 5 is a weir-delimited reach with two inflows.

In this sense, the measurement of DOM composition is essential to understand bulk DOM patterns across rivers (Jaffé et al. 2008; Wollheim et al. 2015). Several other extrinsic factors such as inorganic nutrient availability (Taylor and Townsend 2010; Wickland et al. 2012), microbial community composition (Arnosti 2003), ambient temperature (Raymond and Bauer 2000), irradiance (Stubbins et al. 2010), and chemical interactions with dissolved metals (Sharp et al. 2006) may also influence DOM processing. Therefore, both the distribution of residence times and the nature and kinetics of DOM reactions have been suggested as key factors determining when and where river networks are either passive conduits or active reactors of DOM (Battin et al. 2008; Raymond et al. 2016). However, in spite of some experimental and modeling studies focused on DOM processing (Dawson et al. 2001; Worrall et al. 2006; Temnerud et al. 2007; Tiwari et al. 2014; Palmer et al. 2016; Wollheim et al. 2015), the controls on the in-stream dynamics of DOM remain unclear, hampered by the complexity of tracing DOM changes under natural conditions.

In this study, we examine the role of in-stream processes on the net removal and generation of DOM, with a focus on the physical and biogeochemical factors that promote active DOM processing vs. passive transport. Using a reach-scale approach, we traced changes in DOM concentration and composition along several reaches of a medium-sized river network over one full hydrological year. Changes in DOM composition were assessed by optical spectroscopy and used to disentangle the potential role of different sources on bulk DOM dynamics. Our main hypothesis was that water residence time (RT) would be a key factor on

promoting active DOM processing over conservative transport, modulated by the composition and origin of DOM. Therefore, we predicted a positive relationship between RT and the magnitude of DOM changes along the reaches, and aquatic DOM sources to be more relevant for bulk dynamics than terrestrial DOM.

Methods

Study site

We conducted this study in the Fluvià River, located in the North-East of the Iberian Peninsula (Fig. 1). Fluvià's main stem is 97 km long, with a mean flow of $3.6 \text{ m}^3 \text{ s}^{-1}$ at the basin outlet (data from 2004 to 2014; Catalan Water Agency). Its river network drains a 990 km^2 catchment covered by mixed forests (79%), agricultural (18%), and urban (3%) areas. The catchment geology is mostly calcareous, with some areas (< 15%) of siliceous materials (Cartographic and Geological Institute of Catalonia 2006). Climate is typically Mediterranean, with air temperatures ranging from 6°C to 26°C over the year and precipitation mainly occurring in autumn and spring with an annual mean of 660 mm (data from 2004 to 2014; Catalan Water Agency).

A set of 12 linear reaches were defined across the river network, covering from headwaters to lowland reaches (Fig. 1). All study reaches were chosen as to avoid point sources or tributaries along the reach, and their length was defined as a compromise between having water residence time high enough to detect changes in DOM, and homogeneity of environmental conditions (i.e., canopy cover, morphology, and subcatchment land use). Due to the widespread abundance of small retention structures in

Mediterranean river networks (García-Ruiz et al. 2011), we purposely included four reaches (2, 5, 8 and 12 in Fig. 1 and Supporting Information Table 1) delimited by a small weir in their lower end. The presence of such weirs promoted an increase in residence time from an average of 8 h in weir-free reaches to an average of 29 h in weir-delimited reaches. A detailed morphometric and physicochemical description of the selected reaches is shown in Supporting Information Table 1.

Field and lab methods

Each reach was sampled 10 times during a hydrological year (from November 2012 to November 2013), which was characterized by a dry winter followed by rain storms in spring and a summer drought. Most of the samplings were done at base flow conditions, whereas two spring samplings (27 March 2013 and 01 May 2013) coincided with high flow periods. At each sampling, we collected stream water from the upper and lower end of the reaches. Three replicate water samples for dissolved organic carbon (DOC), chloride, and dissolved nutrient concentrations (i.e., nitrite, nitrate, and phosphate) were filtered in situ through pre-rinsed and pre-combusted 0.7- μm filters and placed into 125 mL polyethylene bottles. Water samples for DOM optical spectroscopy were collected in 11 mL polypropylene tubes after filtering through pre-rinsed 0.2- μm nylon filters. All plasticware was previously rinsed several times with filtered water to avoid contamination. Samples were transported to the lab in cool and dark conditions and kept in the fridge (4°C in the dark) until analysis. In addition, field probes were used to measure water temperature, conductivity, pH, and dissolved oxygen.

DOC concentration was analyzed within 1 d of sample collection by high-temperature catalytic oxidation on a Shimadzu TOC-V CSH analyzer (Shimadzu Corporation, Japan). Analytical precision for DOC (0.04 mg C L⁻¹) was determined from the standard deviation of identical samples reanalyzed. Dissolved chloride, nitrite, nitrate, and phosphate concentrations were analyzed by ionic chromatography (IC5000, DIONEX, U.S.A.) with a relative standard deviation at 1 ppm of 0.84%, 0.62%, 1.12%, and 1.18%, respectively.

Excitation-Emission matrices (EEMs) were obtained using a fluorescence spectrophotometer (F-7000, Hitachi, Japan) with a 1-cm quartz cuvette. EEMs were collected by measuring fluorescence intensity across the excitation range set from 248 nm to 449 nm and the emission range set from 250 nm to 550 nm, both at 3 nm increments. All EEMs were read in sample to reference signal ratio mode, and were corrected for instrument-specific biases after calibrating the instrument with Rhodamine B solution. EEMs were blank subtracted using the EEM of Milli-Q water measured every 10 samples. Fluorescence spectra were then corrected for inner filter effects using UV-visible absorbance spectra

measured on an Agilent 8453 spectrophotometer (Agilent Technologies, Germany). The integral of the water Raman scatter peak at excitation 350 nm was used for EEMs intensity calibration into Raman Units (RU; Lawaetz and Stedmon 2009).

Water residence time

We estimated the water residence time (RT) of the reaches at each sampling date by combining discharge measurements and river morphology data. We measured discharge at both ends of the reach with an acoustic Doppler velocity meter (Flow Tracker, YSI, U.S.A.). Then, the average water velocity along the entire reach was calculated using the hydraulic modeling software HecRas 2.2 (US Army Corps of Engineers), which was fed with the measured discharge and the cross-sectional morphology of the reach measured every ca. 100 m (data provided by the Catalan Water Agency). The RT of the reach at each sampling date was then calculated by dividing the reach length by the average water velocity along the reach. Since there were only few cross-sections available for the weir-delimited reaches, the RT in these systems was calculated by dividing the volume (calculated from digitized bathymetric maps) by the measured discharge.

Data analysis

In-stream processing of DOM

We used the river reach as the fundamental unit to evaluate in-stream DOM dynamics across the river network. The net change in DOC concentration (ΔDOC ; mg C L⁻¹) at each reach and sampling time was calculated as the difference between the down and upstream DOC concentrations. Hence, positive values indicate DOC net gain, whereas negative values indicate DOC net loss. This approach does not allow us to recognize the specific in-stream biotic and abiotic processes responsible for the observed net DOC changes. Thus, DOC gains include algal DOC production and release from particulate detritus, whereas DOC losses include biological and photochemical mineralization as well as flocculation and sedimentation processes.

A fundamental assumption in our calculations is that the study reaches were well-mixed systems in steady state during sampling, and that no major lateral inputs influenced the measured DOM variations. We used chloride as a conservative tracer to identify observations potentially influenced by unmeasured lateral inputs (e.g., groundwater, uncontrolled point sources). Observations showing chloride concentration variations higher than 5% between the up and downstream ends of a reach were considered likely to receive significant lateral inputs, and were therefore discarded for subsequent analyses. Following this criterion, we discarded 46 out of 120 observations. All data reported in descriptive tables and throughout the text refer to the dataset after removing these observations.

Given that the analytical precision of DOC measurements was 0.04 mg C L⁻¹ and that the error of a subtraction

propagates as the root of the sum of the squared error of the terms, any absolute variation in DOC below 0.06 mg C L^{-1} could not be considered different from zero. Therefore, observations with an absolute value of ΔDOC below 0.06 mg C L^{-1} are hereafter referred to as passive pipes, whereas those above it are referred to as reactors. Note however that we do not use the term passive pipe to refer to the total absence but to extremely low processing of DOM (it is indeed difficult to imagine any natural river reach without some DOM transformations, just those that are below the detection limit of the analytical approach, or that have equally balanced generation and degradation).

To calculate DOC net change rates ($\text{mg C m}^{-3} \text{ h}^{-1}$) we divided ΔDOC by the RT of the corresponding reach and sampling date. Same calculations were applied to the intensity of the six fluorescence components (see next section) in order to evaluate the in-stream gains and losses of different DOM fractions.

Optical indices and PARAFAC modeling

Parallel Factor Analysis (PARAFAC) was applied to decompose EEMs into underlying fluorescence components following the description by Stedmon and Bro (2008). The model included a total of 250 samples, 130 of which were collected at other locations within the same river network and included to increase the power of the analysis. Rayleigh scatter was replaced by a band of missing data prior to analysis. Potential model outliers were evaluated by examining residuals and leverage of each sample, and five samples were removed. The number of components best describing the dataset was initially assessed by checking randomness of residuals and visually examining the spectral shape of components. Then, the model was validated through split-half analysis and random initialization with 15 iterations. A six-component model was finally found to provide a robust description of the DOM fluorescence across our dataset. The position and spectral shape for the six components (referred as C1–C6 throughout the main text) are shown in Supporting Information Table 2 and Supporting Information Fig. 2, respectively. All PARAFAC steps were run in MATLAB 2012a (MathWorks, Massachusetts, U.S.A.) using the DOMFluor toolbox (Stedmon and Bro 2008). The modeled components were then compared to previous studies available in the OpenFluor database (Murphy et al. 2014).

Four humic-like (C1–C4) and two protein-like (C5 and C6) fluorescence components were identified across our dataset, with C2, C3, C4, and C5 being among the most commonly observed components in aquatic ecosystems (Murphy et al. 2014). C1 and C2 are located in the fluorescence region that usually define the ubiquitous humic-like Peaks C and A (Coble 1996), respectively, and are related to high molecular weight humic substances of terrestrial origin (Fellman et al. 2010). C4 is similar to Peak M, which have been previously associated to low molecular-weight, humic-like substances

both of terrestrial and aquatic origin. C3, the most red-shifted component in our study, has been previously related to terrestrial humic-like DOM in tropical and boreal systems (Stedmon and Markager 2005; Yamashita et al. 2010). Protein-like C5 and C6 spectra resemble those of tryptophan and phenylalanine free amino acids, respectively, and have been classified as originating from microbial DOM sources (Fellman et al. 2010).

In addition to PARAFAC modeling, we also calculated several optical indices that provide information about the origin of DOM as well as its aromaticity and degree of humification. The humification index (HIX; unitless) was calculated as the ratio between the peak area under the fluorescence emission spectra 435–480 nm and 300–345 nm, at an excitation wavelength of 254 nm (Zsolnay et al. 1999). HIX values increase with the extent of DOM humification. The biological index (BIX; unitless) was calculated by dividing the fluorescence intensity emitted at 380 nm by that at 430 nm for an excitation of 310 nm (Huguet et al. 2009), with higher BIX values corresponding to a higher autochthonous character and to the presence of freshly released DOM (Huguet et al. 2009; Wilson and Xenopoulos 2009). From the absorbance spectra, we calculated the specific UV absorbance at 254 nm (SUVA_{254} ; $\text{L mg C}^{-1} \text{ m}^{-1}$), an indicator of aromatic carbon content (Weishaar et al. 2003), by dividing sample absorbance at 254 nm by DOC concentration and cell length. Finally, we also calculated the slope of the absorbance spectra between 275 nm and 295 nm, which has been inversely related to DOM average molecular weight (Helms et al. 2008).

Statistics

In order to identify the factors determining when a river reach behaved either as a pipe or a reactor, a two-level categorical factor was regressed against biogeochemical and hydrological variables using stepwise multiple regression analysis on a binary response (referred as “binomial multiple regression” throughout the main text). Also, a stepwise multiple linear regression was used to identify which variables better explained ΔDOC . All hydrological, physicochemical and DOM compositional variables described above were included as explanatory variables in both analyses. Except for RT that relates to the whole reach, all variables used as explanatory referred to the upstream end as a representation of the reach initial conditions. Explanatory variables were log-transformed when necessary to avoid skewed distributions, and checked for colinearity before modeling. Since our dataset included multiple sampling sites and repeated measurements on the same reach, we first evaluated the full model performance before and after introducing spatial and temporal error terms using R packages nlme (Pinheiro et al. 2016), lme4 (Bates et al. 2015), and gstat (Pebesma 2004). Given there was no improvement in terms of Akaike Information Criterion (AIC) reduction, we kept the simplest

model (i.e., without error terms) for the subsequent stepwise procedure. Optimal models selection was done by backward elimination based on AIC. In the case of the multiple linear regression, normality of residuals and homocedasticity were checked by examining the residuals of the model. The level of significance was set to 0.05.

To further explore the role of RT in the dynamics of DOM processing, the rates of net change in DOC were compared with the water residence time of the reaches. As there is a common variable (RT) in the comparison, this relationship might embed a spurious mathematical component. Nevertheless, the dispersion of the common variable to its mean is fourfold smaller than the dispersion of the unique variable of the ratio, so relating these variables still provides valuable information (Kenney 1991). In any case, to keep in mind this potential artifact in our interpretations, we display a reference for this mathematical effect in figures.

Spearman's correlation analysis was used to assess relationships between continuous variable pairs. A Sequential Bonferroni correction was used to reduce the probability of Type I errors in multiple pairwise correlations (Quinn and Keough 2002). All statistical analyses were performed in R 3.1.3 (R Core Team 2015).

Results

DOC concentration ranged from 0.38 mg C L^{-1} to 4.64 mg C L^{-1} across all the reaches and sampling dates, with a mean and median value of 1.34 mg C L^{-1} and 1.23 mg C L^{-1} , respectively. SUVA_{254} , a compositional index positively related with aromaticity (Weishaar et al. 2003), varied across the river network and over time ($0.95 - 4.30 \text{ L mg C}^{-1} \text{ m}^{-1}$), indicating a highly variable and heterogeneous DOM composition in our dataset. This was also indicated by a wide range of HIX and BIX values ($0.3 - 10.4$ and $0.55 - 0.93$, respectively; Huguet et al. 2009). A negative correlation between SUVA_{254} and RT was found ($r_s = -0.28$, $p = 0.021$, $n = 74$). In contrast, BIX values increased with RT ($r_s = 0.31$, $p = 0.007$, $n = 74$), together indicating a tendency toward fresher and less aromatic DOM with higher RT.

The net change in DOC along the reaches was generally low, with ΔDOC values showing a median value of 0.00 mg C L^{-1} and an interquartile range of $-0.07 \text{ mg C L}^{-1}$ to 0.06 mg C L^{-1} (Fig. 2a). Of the whole dataset ($n = 74$), 51% of observations had an absolute ΔDOC above the threshold for considering DOC variations (0.06 mg C L^{-1} ; see Methods section) and, among these, 47% were positive (i.e., DOM net gain), and 53% were negative (i.e., DOM net loss; Fig. 2a and Supporting Information Fig. 1). No evident patterns in ΔDOC were observed across space or time (Supporting Information Figs. 2 and 3). However, there was slightly less DOM processing during the two high flow events of 27th March and 1st May, as indicated by very low dispersion of the ΔDOC values around zero (Supporting Information Fig. 3).

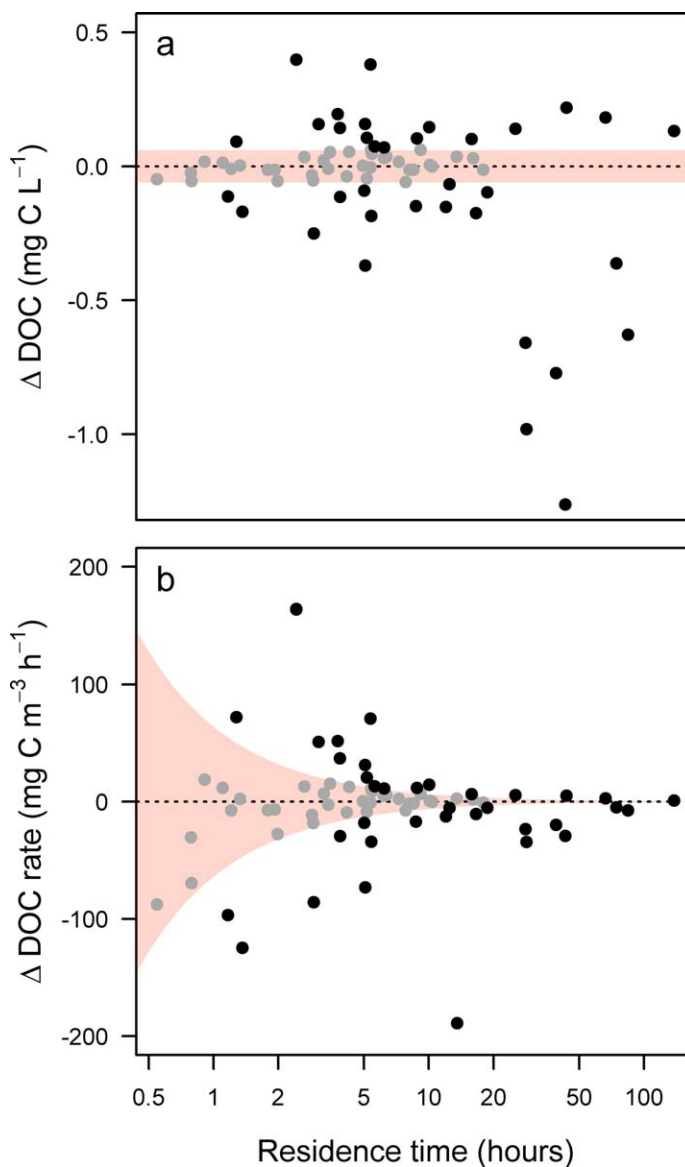


Fig. 2. In-stream dynamics of bulk DOM across the gradient of residence times measured in this study. **(a)** Relationship between the net change in dissolved organic carbon (ΔDOC) and the residence time of the reaches. **(b)** Relationship between the rate of ΔDOC and the water residence time of the reaches. In both panels positive values indicate net gain, while negative values indicate net loss. Red shaded area represents theoretical values of ΔDOC below the criterion for non-significance (0.06 mg C L^{-1} , See Methods section for details). Thus, data points inside this region (gray symbols) correspond to pipe observations, whereas black symbols correspond to reactors. All observations across space and time are included. Note that in **(a)**, point at coordinates (13.5, -2.56) was excluded for visual clarity.

In agreement with our prediction, a positive relationship was found between the absolute value of ΔDOC and RT across the whole dataset ($r_s = 0.39$, $p < 0.001$, $n = 74$). Accordingly, low RT observations were mostly identified as pipes, while the proportion of reactors increased with RT (Fig. 2).

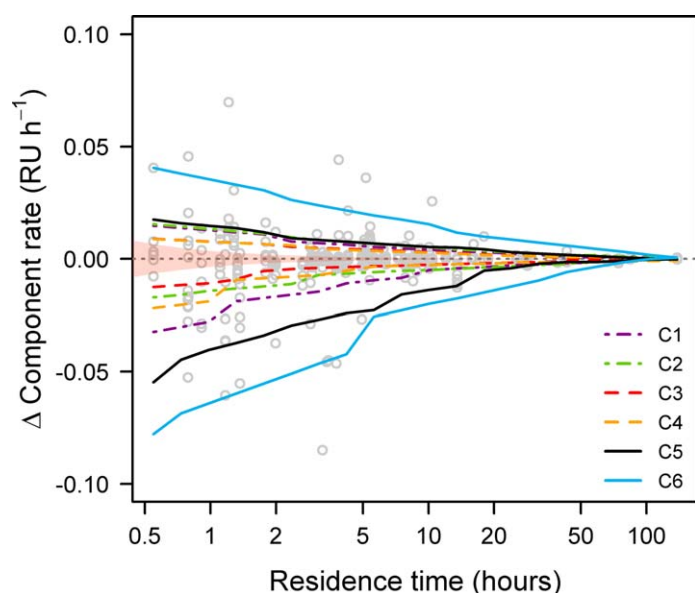


Fig. 3. Relationship between the rate of net change in fluorescence intensity (Δ Component rate) for each PARAFAC component (C1–C6) and the water residence time of the reach. Positive values indicate net gain; negative values indicate net loss. Color lines are the non-parametric 10th and 90th percentile regression for each component, as a representation of the distribution of the data. Note overlapping of some of the lines (e.g., 90th percentile line of C3 is hidden from view). Gray circles are the rates calculated for each component and included for reference. Red shaded area represents theoretical values of net change in fluorescence intensity below the criterion for non-significance (0.004 RU; based on repeated measures on the same sample). All observations across space and time are included.

Indeed, all observations with RT above ~ 18 h behaved as reactors. Δ DOC was also negatively correlated with inflowing DOC ($r_s = -0.60$, $p < 0.001$, $n = 74$). The rates of net DOC change ranged from $-189 \text{ mg C m}^{-3} \text{ h}^{-1}$ to $164 \text{ mg C m}^{-3} \text{ h}^{-1}$, with a median and interquartile range of -1 and -25 to $14 \text{ mg C m}^{-3} \text{ h}^{-1}$, respectively (Fig. 2b). In the case of reactors, net rates showed a non-linear trend towards zero along the RT axis, that is, the rates of net generation and net removal of DOM declined as RT increased (black symbols in Fig. 2b).

In contrast to bulk DOM, we found higher frequency of fluorescence losses than gains, with 64% of the observations showing a decrease in total fluorescence. Components C1, C3, and C4 showed almost identical dynamics, as indicated by the high correlation between their net fluorescence intensity changes (Supporting Information Fig. 4). Conversely, C2, C5 and particularly C6 showed lower correlation each with the rest of the components. The rates of removal and production of the fluorescence components showed a similar pattern than those of bulk DOM, approaching zero with increasing RT. However, there were conspicuous differences between the six components (Fig. 3). Humic-like components (C1–C4) behaved similarly and showed very small net

Table 1. Binomial multiple regression on the reactor vs. pipe behavior of the study reaches ($n = 74$). *Coefficient* indicates the increment in the log-odds-ratio corresponding to an increment of one unit for each explanatory variable. That is, positive coefficients indicate an increase in the odds of behaving as a reactor over a passive pipe.

Variable	Coefficient	Standard error	<i>z</i>	<i>p</i>
\log_{10} RT	2.157	0.702	3.074	0.0021
DOC	0.890	0.522	1.706	0.0879
Temp	0.089	0.052	1.728	0.0839

\log_{10} RT, logarithm of water residence time; DOC, dissolved organic carbon; Temp, water temperature.

Note- Variables shown are those included in the final model after the stepwise procedure based on the Akaike Information Criterion (see Methods for further details). Note that some of the included variables showed a slope that did not significantly differ from zero.

Table 2. Linear multiple regression on the net change in dissolved organic carbon (Δ DOC).

Variable	Coefficient	Standard error	<i>t</i>	<i>p</i>
\log_{10} RT	-0.133	0.035	-3.77	<0.001
DOC	-0.327	0.027	-12.08	<0.001
NO_3	0.075	0.012	6.26	<0.001
BIX	-0.804	0.238	-3.37	0.001

Intercept = 0.970 ± 0.190 ; $R^2 = 0.75$; *F*-statistic = 49.49; $p < 0.001$; $n = 74$.

\log_{10} RT, logarithm of the water residence time; DOC, dissolved organic carbon; NO_3 , nitrate; BIX, biological index.

Note- Shown are only the significant variables among those included in the final model after the stepwise procedure based on the Akaike Information Criterion (see Methods for further details).

rates of change along the RT axis, indicating limited in-stream loss and generation. In contrast, protein-like C5 and C6 appear to be the most reactive fractions, showing the highest in-stream retention rates. Only protein-like C6 showed marked in-stream generation (i.e., positive net change), suggesting an autochthonous origin.

The binomial multiple regression analysis indicated that high RT increased the probability for a reach to be a reactor over a pipe (Table 1). Then, the multiple linear regression revealed that RT, DOC, nitrate, and BIX were the variables most influencing the net change in DOC, explaining up to 75% of Δ DOC variance (Table 2; Fig. 4).

Discussion

The net in-stream variation of DOC along the study reaches was generally low, with almost half of the observations suggesting conservative DOM transport. Such small variations are in agreement with similar studies conducted in boreal (Temmerud et al. 2007) and temperate (Palmer et al. 2016) catchments, where in-stream variations in DOC

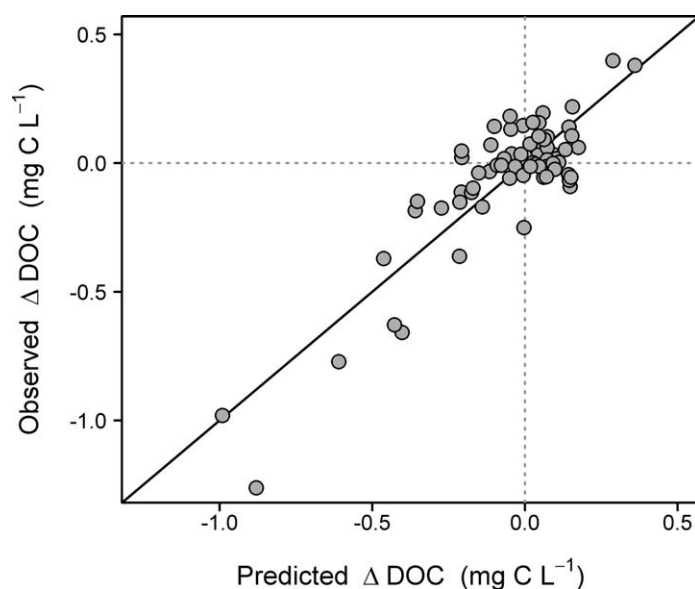


Fig. 4. Predicted relative to observed net change in dissolved organic carbon (ΔDOC). The 1 : 1 line is included for reference. The summary of the model is shown in Table 2.

were detected only at confluences due to sudden changes in water chemistry. Interestingly, only few studies have reported net gains of DOM across river networks, and those that have indicate low magnitude and frequency (Dawson et al. 2001; Temnerud et al. 2007; Palmer et al. 2016). Contrastingly, we found similar frequency for gains and losses, and the generation of DOM ($1 - 164 \text{ mg C m}^{-3} \text{ h}^{-1}$) was comparable to its removal (-189 to $-5 \text{ mg C m}^{-3} \text{ h}^{-1}$), suggesting in-stream losses might be compensated by gains at the catchment scale, at least at base flow conditions.

Our results indicate that RT was determinant on whether a reach behaved as a pipe or a reactor (Table 1), with high RT promoting active processing of DOM over passive transport downstream. This result confirms that RT exerts physical constraint on the opportunity for DOM to either react or be produced, which is further supported by the positive relationship between ΔDOC and RT. Interestingly, we found reactor conditions at very short RT (e.g., 2 h), and an upper boundary for pipe conditions at RT of ca. 18 h. This indicates that DOM dynamics can be influenced by in-stream processes acting at very short time scales (hours), which is consistent with daily variations in DOM concentration and composition reported elsewhere (Kaplan and Bott 1982; Spencer et al. 2007; Parker et al. 2010).

Since the distribution of RTs in a river network is tightly modulated by hydrology, in-stream DOM dynamics might be subject to strong temporal variability. In our case we did not find a marked temporal pattern, which is not surprising considering most samplings were done at base flow conditions. However, from our results one can expect that river networks will tend to behave as reactors during low flow periods, when

the network RT is high. On the contrary, even though our dataset does not cover much of high flows, our results point towards a passive role of river networks during high flow periods and flooding events. This would be indeed in agreement with the Pulse-Shunt Concept (Raymond et al. 2016), which identifies extreme hydrologic events as moments of low in-stream processing although of high relevance due to the export of large amounts of labile DOM to the ocean.

Besides RT, the multiple regression analysis indicated that DOC, BIX and nitrate concentration influenced ΔDOC (Table 2). The negative effect of DOC on ΔDOC indicates that high DOM concentrations enhanced its in-stream net loss. This is in agreement with DOM decaying according to first-order kinetics (Olson 1963), which would imply higher DOM degradation in reaches showing higher DOC. It is worth noting, however, that given the low range of DOC measured in our study (Supporting Information Table 1) we cannot extrapolate this result to other catchments with much higher DOC. For example, many studies in high DOC systems such as those influenced by wetlands have reported passive transport of DOM even though RT was high (e.g., Kothawala et al. 2015; RT up to 2 d), suggesting that other factors rather than DOC quantity and RT must play a role. Indeed, our results indicate that DOM composition and nutrient availability also influenced the net balance between gains and losses. We found a negative statistical effect of BIX on ΔDOC (Table 2), implying that the higher the BIX the higher the removal of DOM. Higher BIX values have been usually related to autochthonous DOM (Huguet et al. 2009; Wilson and Xenopoulos 2009), and recent advances on the molecular characterization of DOM have accordingly associated BIX with small-sized, highly unsaturated organic compounds derived from aquatic DOM sources (Kellerman et al. 2015). Therefore, the removal of DOM from the river was favored by the presence of in-stream produced and lowly altered DOM, which is supported by the fact that autochthonous component C6 showed the highest removal rates in terms of fluorescence intensity (Fig. 3). On the contrary, nitrate concentrations had a positive statistical effect on ΔDOC , promoting net DOM gains along the reaches. It is well known that nutrient availability favors gross primary production in rivers, with algal growth rates responding to nutrient concentration following a Monod kinetics (Borchardt 1996). Hence, we suggest that reaches receiving higher inputs of nitrate were more likely to show relevant production of photosynthates, becoming net exporters of highly reactive DOM to downstream reaches.

Interestingly, the net rates of DOM removal and generation approached zero as RT increased (Fig. 2b). In the case of DOM removal, a slowing down in the organic matter decay with increasing RT has already been observed along the land to ocean aquatic continuum (Catalán et al. 2016). At such a global scale, this pattern seems to result from a gradual loss of the most reactive components of DOM as it passes through inland

waters, suggesting that intrinsic DOM properties control decay rates (Catalán et al. 2016), and thus DOM persistence in aquatic systems (Kellerman et al. 2015). At the short timescale we focus, however, the significant correlations between RT and both BIX ($r_s = 0.31$, $p = 0.007$, $n = 74$) and SUVA₂₅₄ ($r_s = -0.28$, $p = 0.021$, $n = 74$) do not support a decrease in bio-reactivity as a plausible explanation for the observed trend. Therefore, given the symmetrical relationship between RT and the rates of DOM loss and generation (Fig. 2b), we hypothesize that this pattern is the result of catabolic and anabolic reactions balancing each other at high RT. If this holds true, we might expect a shift in the composition of DOM towards a more autochthonous character along those reaches with high RT, because DOM degradation should be compensated by aquatic DOM sources. Accordingly, using 18 h as an arbitrary threshold for DOM processing as RT increases, we observed a decrease in the most bioreactive terrestrial component (C5) coupled with an increase in autochthonous component C6 at high RT (Supporting Information Fig. 5). Therefore, whereas DOM processing at low RT may be constrained by time, higher RT would facilitate the co-occurrence of opposite reactions (e.g., primary production vs. heterotrophic respiration), resulting in lower net DOM variations than expected from single processes.

Results reported here provide insight into the role of rivers in the carbon cycle, as we identified the main factors determining in-stream DOM processing on its way from land to ocean. In particular, our finding that high RT increases the odds for river reaches to behave as reactors provides field-based evidence of the influence of hydrology on the biophysical opportunity for DOM to react (Battin et al. 2008; Raymond et al. 2016). However, our study builds up the binary concept of rivers as passive pipes vs. active reactors by including the alternation of DOM removal and generation conditions. Going a step further, we found that while RT determines the opportunity for in-stream reactions, DOM properties and nitrate availability control the net balance between removal and generation processes. We acknowledge that our small spatial representation precludes any direct extrapolation of our findings to regions other than Mediterranean climate latitudes. Nonetheless, results presented here may serve as a template for further understanding the complex role of in-stream reactions on the fate of riverine DOM. As a final corollary, our study stresses the relevance of in-stream DOM production on river carbon fluxes, which is often overlooked in current models despite it may support microbial respiration and energy flow across trophic levels in freshwaters (Thorp and DeLong 2002; Hotchkiss and Hall 2015).

References

- Ågren, A. M., I. Buffam, D. M. Cooper, T. Tiwari, C. D. Evans, and H. Laudon. 2014. Can the heterogeneity in

stream dissolved organic carbon be explained by contributing landscape elements? *Biogeosciences* **11**: 1199–1213. doi:10.5194/bg-11-1199-2014

- Arnosti, C. 2003. Microbial extracellular enzymes and their role in dissolved organic matter cycling, p. 315–342. *In* S. Findlay and R. Sinsabaugh [eds.], *Aquatic ecosystems. Interactivity of dissolved organic matter*. Elsevier.
- Aufdenkampe, A. K., E. Mayorga, P. A. Raymond, J. M. Melack, S. C. Doney, S. R. Alin, R. E. Aalto, and K. Yoo. 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Front. Ecol. Environ.* **9**: 53–60. doi:10.1890/100014
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using {lme4}. *J. Stat. Softw.* **67**: 1–48. doi:10.18637/jss.v067.i01
- Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold, and F. Sabater. 2008. Biophysical controls on organic carbon fluxes in fluvial networks. *Nat. Geosci.* **1**: 95–100. doi:10.1038/ngeo101
- Battin, T. J., S. Luysaert, L. A. Kaplan, A. K. Aufdenkampe, A. Richter, and L. J. Tranvik. 2009. The boundless carbon cycle. *Nat. Geosci.* **2**: 598–600. doi:10.1038/ngeo618
- Borchardt, M. A. 1996. Nutrients, p. 183–227. *In* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe [eds.], *Algal ecology*. Academic Press.
- Catalán, N., R. Marcé, D. N. Kothawala, and L. J. Tranvik. 2016. Organic carbon decomposition rates controlled by water retention time across inland waters. *Nat. Geosci.* **9**: 501–504. doi:10.1038/ngeo101
- Ciais, P., Sabine, C., Bala, G., and others. 2013. Carbon and other biogeochemical cycles. *In*: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM), pp. 465–570. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Coble, P. G. 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Mar. Chem.* **51**: 325–346. doi:10.1016/0304-4203(95)00062-3
- Cole, J. J., and others 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* **10**: 172–185. doi:10.1007/s10021-006-9013-8
- Cory, R. M., and L. A. Kaplan. 2012. Biological lability of streamwater fluorescent dissolved organic matter. *Limnol. Oceanogr.* **57**: 1347–1360. doi:10.4319/lo.2012.57.5.1347
- Dawson, J. J. C., C. Bakewell, and M. F. Billett. 2001. Is in-stream processing an important control on spatial changes in carbon fluxes in headwater catchments? *Sci. Total Environ.* **265**: 153–167. doi:10.1016/S0048-9697(00)00656-2 doi:10.1016/S0048-9697(00)00656-2
- Fellman, J. B., E. Hood, and R. G. M. Spencer. 2010. Fluorescence spectroscopy opens new windows into dissolved organic

- matter dynamics in freshwater ecosystems: A review. *Limnol. Oceanogr.* **55**: 2452–2462. doi:10.4319/lo.2010.55.6.2452
- García-Ruiz, J. M., J. I. López-Moreno, S. M. Vicente-Serrano, T. Lasanta-Martínez, and S. Beguería. 2011. Mediterranean water resources in a global change scenario. *Earth Sci. Rev.* **105**: 121–139. doi:10.1016/j.earscirev.2011.01.006 doi:10.1016/j.earscirev.2011.01.006
- Guillemette, F., and P. A. del Giorgio. 2011. Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. *Limnol. Oceanogr.* **56**: 734–748. doi:10.4319/lo.2011.56.2.0734
- Hanley, K. W., W. M. Wollheim, J. Salisbury, T. Huntington, and G. Aiken. 2013. Controls on dissolved organic carbon quantity and chemical character in temperate rivers of North America. *Global Biogeochem. Cycles.* **27**: 492–504. doi:10.1002/gbc.20044
- Helms, J. R., A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber, and K. Mopper. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnol. Oceanogr.* **53**: 955–969. doi:10.4319/lo.2008.53.3.0955
- Hotchkiss, E. R., and R. O. J. Hall. 2015. Whole-stream ^{13}C tracer addition reveals distinct fates of newly fixed carbon. *Ecology* **96**: 403–416. doi:10.1890/14-0631.1
- Huguet, A., L. Vacher, S. Relexans, S. Saubusse, J. M. Froidefond, and E. Parlanti. 2009. Properties of fluorescent dissolved organic matter in the Gironde Estuary. *Org. Geochem.* **40**: 706–719. doi:10.1016/j.orggeochem.2009.03.002
- Jaffé, R., D. McKnight, N. Maie, R. Cory, W. H. McDowell, and J. Campbell. 2008. Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. *J. Geophys. Res. Biogeosci.* **113**: 1900–1908. doi:10.1029/2008JG000683
- Kaplan, L. A., and T. L. Bott. 1982. Diel fluctuations of DOC generated by algae in a piedmont stream. *Limnol. Oceanogr.* **27**: 1091–1100. doi:10.4319/lo.1982.27.6.1091
- Kellerman, A. M., D. N. Kothawala, T. Dittmar, and L. J. Tranvik. 2015. Persistence of dissolved organic matter in lakes related to its molecular characteristics. *Nat. Geosci.* **8**: 454–457. doi:10.1038/ngeo2440
- Kenney, B. C. 1991. Comments on “Some misconceptions about the spurious correlation problem in the ecological literature” by Y.T. Prairie and D.F. Bird. *Oecologia* **86**: 152.
- Kothawala, D. N., X. Ji, H. Laudon, A. M. Ågren, M. N. Futter, S. J. Köhler, and L. J. Tranvik. 2015. The relative influence of land cover, hydrology, and in-stream processing on the composition of dissolved organic matter in boreal streams. *J. Geophys. Res. Biogeosci.* **120**: 1491–1505. doi:10.1002/2015JG002946
- Lawaetz, A. J., and C. A. Stedmon. 2009. Fluorescence intensity calibration using the Raman scatter peak of water. *Appl. Spectrosc.* **63**: 936–940. doi:10.1366/000370209788964548
- McDowell, W. H., and S. G. Fisher. 1976. Autumnal processing of dissolved organic matter in a small woodland stream ecosystem. *Ecology* **57**: 561–569. doi:10.2307/1936440
- Murphy, K. R., C. A. Stedmon, P. Wenig, and R. Bro. 2014. OpenFluor- an online spectral library of auto-fluorescence by organic compounds in the environment. *Anal. Methods* **6**: 658–661. doi:10.1039/C3AY41935E
- Oldham, C. E., D. E. Farrow, and S. Peiffer. 2013. A generalized Damköhler number for classifying material processing in hydrological systems. *Hydrol. Earth Syst. Sci.* **17**: 1133–1148. doi:10.5194/hess-17-1133-2013
- Olson, J. S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. *Ecology* **44**: 322–331. doi:10.2307/1932179
- Palmer, S. M., and others. 2016. Sporadic hotspots for physico-chemical retention of aquatic organic carbon: From peatland headwater source to sea. *Aquat. Sci. Aquat. Sci.* **78**: 491–504. doi:10.1007/s00027-015-0448-x
- Parker, S. R., S. R. Poulson, M. G. Smith, C. L. Weyer, and K. M. Bates. 2010. Temporal variability in the concentration and stable carbon isotope composition of dissolved inorganic and organic carbon in two Montana, USA rivers. *Aquat. Geochem.* **16**: 61–84. doi:10.1007/s10498-009-9068-1
- Pebesma, E. J. 2004. Multivariable geostatistics in S: The gstat package. *Comput. Geosci.* **30**: 683–691. doi:10.1016/j.cageo.2004.03.012
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2016. {nlme}: Linear and Nonlinear Mixed Effects Models. R package version 3.1-128, <http://CRAN.R-project.org/package=nlme>.
- Quinn, G., and M. Keough. 2002. Experimental design and data analysis for biologists. Cambridge Univ. Press.
- R Core Team. 2015. R: A language and environment for statistical computing.
- Raymond, P. A., and J. E. Bauer. 2000. Bacterial consumption of DOC during transport through a temperate estuary. *Aquat. Microb. Ecol.* **22**: 1–12. doi:10.3354/ame022001
- Raymond, P. A., and others. 2013. Global carbon dioxide emissions from inland waters. *Nature* **503**: 355–359. doi:10.1038/nature12760
- Raymond, P. A., J. E. Saiers, and W. V. Sobczak. 2016. Hydrological and biogeochemical controls on watershed dissolved organic matter transport: Pulse-shunt concept. *Ecology* **97**: 5–16. doi:10.1890/14-1684.1
- Sharp, E. L., S. A. Parson, and B. Jefferson. 2006. Coagulation of NOM: Linking character to treatment. *Water Sci. Technol.* **53**: 67–76. doi:10.2166/wst.2006.209
- Spencer, R. G. M., B. A. Pellerin, B. A. Bergamaschi, B. D. Downing, T. E. C. Kraus, D. R. Smart, R. A. Dahlgren, and P. J. Hernes. 2007. Diurnal variability in riverine dissolved organic matter composition determined by in situ optical measurement in the San Joaquin River (California, USA). *Hydrol. Process.* **21**: 3181–3189. doi:10.1002/hyp.6887
- Stedmon, C. A., and S. Markager. 2005. Resolving the variability in dissolved organic matter fluorescence in a temperate

- estuary and its catchment using PARAFAC analysis. *Limnol. Oceanogr.* **50**: 686–697. doi:10.4319/lo.2005.50.2.0686
- Stedmon, C. A., and R. Bro. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: A tutorial. *Limnol. Oceanogr. Methods* **6**: 572–579. doi:10.4319/lom.2008.6.572
- Stubbins, A., and others. 2010. Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. *Limnol. Oceanogr.* **55**: 1467–1477. doi:10.4319/lo.2010.55.4.1467
- Taylor, P. G., and A. R. Townsend. 2010. Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. *Nature* **464**: 1178–1181. doi:10.1038/nature08985
- Temnerud, J., J. Seibert, M. Jansson, and K. Bishop. 2007. Spatial variation in discharge and concentrations of organic carbon in a catchment network of boreal streams in northern Sweden. *J. Hydrol.* **342**: 72–87. doi:10.1016/j.jhydrol.2007.05.015 doi:10.1016/j.jhydrol.2007.05.015
- Thorp, J. H., and M. D. Delong. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. *Oikos* **96**: 543–550. doi:10.1034/j.1600-0706.2002.960315.x
- Tiwari, T., H. Laudon, K. Beven, and A. M. Ågren. 2014. Downstream changes in DOC: Inferring contributions in the face of model uncertainties. *Water Resour. Res.* **50**: 514–525. doi:10.1002/2013WR014275
- Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* **37**: 4702–4708. doi:10.1021/es030360x
- Wickland, K. P., G. R. Aiken, K. Butler, M. M. Dornblaser, R. G. M. Spencer, and R. G. Striegl. 2012. Biodegradability of dissolved organic carbon in the Yukon River and its tributaries: Seasonality and importance of inorganic nitrogen. *Global Biogeochem. Cycles* **26**: GBOE03. doi:10.1029/2012GB004342
- Wilson, H. F., and M. A. Xenopoulos. 2009. Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nat. Geosci.* **2**: 37–41. doi:10.1038/ngeo391
- Wollheim, W. M., R. J. Stewart, G. R. Aiken, K. D. Butler, N. B. Morse, and J. Salisbury. 2015. Removal of terrestrial DOC in aquatic ecosystems of a temperate river network. *Geophys. Res. Lett.* **42**: 1–9. doi:10.1002/2015GL064647
- Worrall, F., T. P. Burt, and J. Adamson. 2006. The rate of and controls upon DOC loss in a peat catchment. *J. Hydrol.* **321**: 311–325. doi:10.1016/j.jhydrol.2005.08.019 doi:10.1016/j.jhydrol.2005.08.019
- Yamashita, Y., N. Maie, H. Briceño, and R. Jaffé. 2010. Optical characterization of dissolved organic matter in tropical rivers of the Guayana Shield, Venezuela. *J. Geophys. Res. Biogeosci.* **115**: G00F10. doi:10.1029/2009JG000987
- Zsolnay, A., E. Baigar, M. Jimenez, B. Steinweg, and F. Saccomandi. 1999. Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. *Chemosphere* **38**: 45–50. doi:10.1016/S0045-6535(98)00166-0

Acknowledgments

We thank the Catalan Water Agency for providing river morphology data. The authors are also thankful to Carmen Gutiérrez, Meritxell Abril, and Lorenzo Proia for lab and field assistance. This research was funded by the Spanish Ministry of Economy and Competitiveness through the projects REMEDIATION (CGL2014-57215-C4-2-R) and FUNSTREAM (CGL2014-58760-C3-3-R). J. P. Casas-Ruiz and Ll. Gómez-Gener were supported by FPI predoctoral grants (BES-2012-059655 and BES-2012-059743), and N. Catalán held a Wenner-Gren foundation stipend (2014–2016; Sweden). This work profited from the research network NETLAKE COST Action (ES1201), supported by COST (European Cooperation in Science and Technology). Authors also acknowledge the support from the Economy and Knowledge Department of the Catalan Government through Consolidated Research Group (2014 SGR 291)—Catalan Institute for Water Research. All data used in this study is available upon request from the authors.

Conflict of Interest

None declared.

Submitted 16 June 2016

Revised 06 September 2016; 11 October 2016

Accepted 13 October 2016

Associate editor: Marguerite Xenopoulos