



## Quality and reactivity of dissolved organic matter in a Mediterranean river across hydrological and spatial gradients



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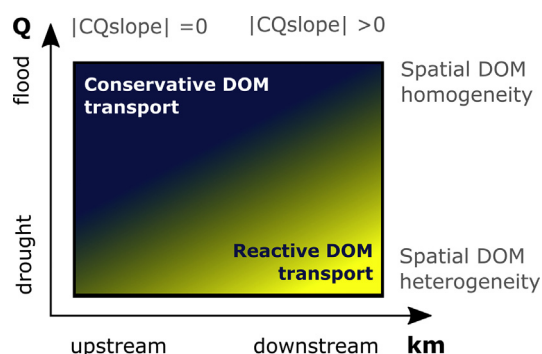
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### HIGHLIGHTS

- We measured DOM quality and reactivity along spatial and hydrological gradients.
- High flows favoured transport and spatial homogeneity of DOM properties.
- Low flows favoured in-stream reactivity and spatial heterogeneity of DOM properties.
- DOM vs discharge response evolved downstream from chemostatic to chemodynamic.
- DOM properties are sensitive to both hydrology and downstream distance.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Understanding DOM transport and reactivity in rivers is essential to having a complete picture of the global carbon cycle. In this study, we explore the effects of hydrological variability and downstream transport on dissolved organic matter (DOM) dynamics in a Mediterranean river. We sampled the main stem of the river Tordera from the source to the sea, over a range of fifteen hydrological conditions including extreme events (flood and drought). By exploring spatial and temporal gradients of DOM fluorescence properties, river hydrology was found to be a significant predictor of DOM spatial heterogeneity. An additional space-resolved mass balance analysis performed on four contrasting hydrological conditions revealed that this was due to a shift in the biogeochemical function of the river. Flood conditions caused a conservative transport of DOM, generating a homogeneous, humic-like spatial profile of DOM quality. Lower flows induced a non-conservative, reactive transport of DOM, which enhanced the spatial heterogeneity of DOM properties. Moreover, the downstream evolution of DOM chemostatic behaviour revealed that the role of hydrology in regulating DOM properties increased gradually downstream, indicating an organised inter-dependency between the spatial and the temporal dimensions. Overall, our findings reveal that riverine DOM dynamics is in constant change owing to varying hydrological conditions, and emphasize that in order to fully understand the role of rivers in the global carbon cycle, it is necessary to take into account the full range of hydrological variability, from floods to droughts.

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## 1. Introduction

Rivers have a fundamental role in the global carbon cycle, as they link terrestrial and marine systems by transporting carbon compounds from the land to the seas (Aufdenkampe et al., 2011; Schlesinger and Melack, 1981). Their importance is not limited to a relocation of carbon but, most importantly, it represents a reactive flux where about half of the carbon exported from the land is either outgassed as CO<sub>2</sub> to the atmosphere (at similar magnitudes to global terrestrial net production) or sequestered in sediments (at higher rates than those at the ocean floor) (Cole et al., 2007; Tranvik et al., 2009). Dissolved organic matter (DOM), a ubiquitous and complex mixture of organic compounds, is one of the major riverine carbon fluxes (Hope et al., 1994). Due to its high reactivity to a variety of ecosystem processes, such as bacterial respiration (del Giorgio, 2005), photochemical oxidation (Moran and Zepp, 1997) and primary production (Bertilsson and Jones, 2003), DOM is transformed, generated and retained during its riverine transit (Jaffe et al., 2008). Because of that, the DOM delivered to the sea can have not only different total loads, but also a different chemical composition compared with the DOM exported from the land. Due to the intrinsic longitudinal nature of river flows, DOM biogeochemical transformations occur along a downstream continuum, generating a spatial domain of DOM variability. On the other hand, hydrology is a time-dynamic physical variable which influences DOM biogeochemistry by regulating the land-stream connectivity and in-stream water residence times (Lake, 2000). Therefore, DOM dynamics has a complex variation influenced by both space and time. Most of the previous literature, though, has focussed on the significance of each dimension individually.

The role of space has been most influentially approached in the River Continuum Concept (RCC, Vannote et al., 1980). It theorized that DOM diversity would drastically decrease from the headwaters through higher order river reaches, due to a rapid depletion of labile moieties and a relative increase of the most recalcitrant compounds. The RCC framework has been expanded to take into account elements of spatial complexity such as the effects of dams (Ward and Stanford, 1983), tributary confluences (Benda et al., 2004; McClain et al., 2003; Rice et al., 2006) and floodplain interactions (Junk et al., 1989); and the idea of a downstream continuum evolved into a discontinuity of hierarchical geomorphological patches (Naiman et al., 1987; Poole, 2002; Thorp et al., 2006). However, despite this vast theoretical work, field evidence on longitudinal patterns of DOM properties remains scarce (Baker and Spencer, 2004; Harun et al., 2016; Slomberg et al., 2016), and only recently have there been attempts to empirically test the RCC predictions regarding DOM (Mosher et al., 2015). One of the core assumptions of the RCC was the system being in steady-state, thereby neglecting the effects from hydrological variability. This is despite the fact that rivers are continuously subject to floods and droughts as natural forms of disturbance (Naiman et al., 2008), which are expected to become more extreme due to anthropogenic climate forcing (Skoulikidis et al., 2017).

The role of hydrology on riverine DOM dynamics, has been mainly explored over the temporal domain, with space being reduced at individual or discreet sites within a catchment (for example, Fellman et al., 2009; Vidon et al., 2008). These sites are usually selected in headwater areas, where the stream-land connectivity is maximal. In these sites storm flows produce an immediate response on the quantity (Butturini et al., 2006; Evans and Davies, 1998), aromaticity (Fasching et al., 2015; Guarch-Ribot and Butturini, 2016), and biodegradability (Buffam et al., 2001) of the DOM flushed to the stream. However, the DOM-discharge relationship remains less explored at higher order river reaches, where DOM quality reflects not only the allochthonous inputs but also the in-stream transformations occurred upstream. According to that, different regions of the river continuum may respond differently to hydrological variation, suggesting a complex interaction between hydrology and space.

Previous studies performed in a Mediterranean river have demonstrated that contrasting hydrological conditions result in distinct spatial

patterns of DOM properties and biodegradability. Severe drought conditions caused drastic changes in the capacity of sediment microbial communities to degrade DOM substrates (Freixa et al., 2016), resulting in a longitudinal patchiness of DOM properties and retention rates (Butturini et al., 2016). This was particularly evident in a Mediterranean intermittent stream, when the hydrological fragmentation of the stream network in summer and the successive water flow reconvergence in autumn, strongly affected the planktonic microbial community structure within the same habitat (Fazi et al., 2013). The hydrological fragmentation altered the biogeochemical conditions with the depletion of oxidized solutes and caused changes in dissolved organic carbon characteristics (Vazquez et al., 2011). Floods were also found to profoundly change the biogeochemical function of the river by enhancing DOM transport and export (Butturini et al., 2016). Taken together, these studies provided evidence that longitudinal patterns of DOM sensitively respond to extreme hydrological events. However, our knowledge of how they change over a complete gradient of water flows – including gradual as well as extreme hydrological changes – still remains fragmentary.

In this study, we aimed at providing a combined space-time analysis of riverine DOM dynamics by exploring DOM quality and reactivity along a downstream gradient, and over a broad range of hydrological conditions. In particular, our integrated approach entailed to address fundamental research questions that remained largely unexplored in space or time approaches separately: i) how does hydrology influence the spatial variability of DOM quality and reactivity?, ii) how does space influence the temporal variability of DOM quality and reactivity?, iii) what is the inter-dependency between space and hydrology? In order to answer these questions, we measured DOM quantity and its optical properties along a Mediterranean river, from the headwaters to the river mouth; over 15 different hydrological conditions, which included a full spectrum of hydrological variation, from drought to flood conditions. Additionally, a space-resolved mass balance performed on four discreet hydrological conditions provided further insight on the in-stream reactivity of DOM associated with the observed spatial variability of DOM properties.

## 2. Materials and methods

### 2.1. Study site

The study was conducted in the catchment of the river Tordera (870 km<sup>2</sup>), which lies in the north-eastern part of the Iberian Peninsula (41.7°N, 2.5°E; Catalonia, Spain). From its source, the river flows along 60 km before discharging to the Mediterranean Sea. Due to orographic and geologic elements of the landscape, the river course has two main big bends (at about 20 and 40 km from the river source) which divide the river into three main sections with distinctive and well-defined biological and geomorphological characteristics. In the headwaters (0 to ≈20 km from source), the river flows through forested mountainsides, descending steeply from 1600 down to 150 m.a.s.l. In the middle part (from ≈20 to ≈40 km from the river source, between the two main bends), the river receives the most important tributaries in terms of drainage area and discharge contribution. These tributaries cover a range of water qualities, from pristine to moderately impacted, as some receive the waters from WWTP or industrial outlets. Finally, the last 20 km are characterised by a long alluvial plain, where the river adopts a curved and meandering trace, and has a high connection between the surface and subsurface water flows. In the whole catchment, forest land cover dominates (77%), especially in the upper parts. Agriculture (16%) and urban areas and industry (7%) also have an important presence, especially in the lowland (Caille et al., 2007). The hydrological regime of river Tordera is influenced by a Mediterranean climate. Accordingly, discharge exhibits extreme and contrasted events over an annual cycle, including stormflows and flood events in autumn and spring, and drought periods in summer. Floods are generally short in time (in

the order of hours to some few days), but intense in magnitude. Contrastingly, drought periods are prolonged in time. They naturally occur in summer, between the months of June and September.

## 2.2. Sampling strategy

Longitudinal samplings were conducted in order to characterise DOM quantity and quality along a river continuum, under a range of hydrological conditions. We sampled the main stem of river Tordera at a number of sites distributed all along the river course, from the headwaters to the river mouth (Fig. 1). In total, 15 longitudinal sampling campaigns were conducted between January 2009 and March 2013 (Table 1), representing a spectrum of hydrological conditions which covered a range of four orders of magnitude in the water flow (from  $156 \text{ m}^3 \text{ s}^{-1}$  during flood, to  $0.065 \text{ m}^3 \text{ s}^{-1}$  during drought, Fig. 2). The discharge at the gauging station at 45 km was used as a categorical variable defining the general hydrological conditions for the whole river length. Samples were analysed for DOM concentration and optical properties. The final data set included 189 records (15 sampling campaigns, 9 to 21 sites per campaign, Table 1).

Four of the longitudinal sampling campaigns (including the two most extreme events of flood and drought, plus two intermediate baseflow conditions, Table 1 and Fig. 2) were extended to perform a space-resolved mass balance. These mass balance samplings were aimed at determining whether the observed DOM longitudinal patterns were the result of hydrological or biogeochemical processes. That is, we wanted to identify to what extent longitudinal DOM patterns were shaped by a passive hydrological mixing of a series of tributaries successively introducing DOM with distinctive properties, or by a suite of in-stream biogeochemical processes which retained, generated and transformed DOM along the river length.

A snapshot mass balance approach (Grayson et al., 1997; Salvia et al., 1999) was performed in the middle part of the main stem, comprising the length between the two main bends (total length of 26 km, Fig. 1). This reach was chosen because it is the part of the main stem which receives the most important tributaries of the catchment, introducing a

**Table 1**  
Summary of the sampling campaigns performed.

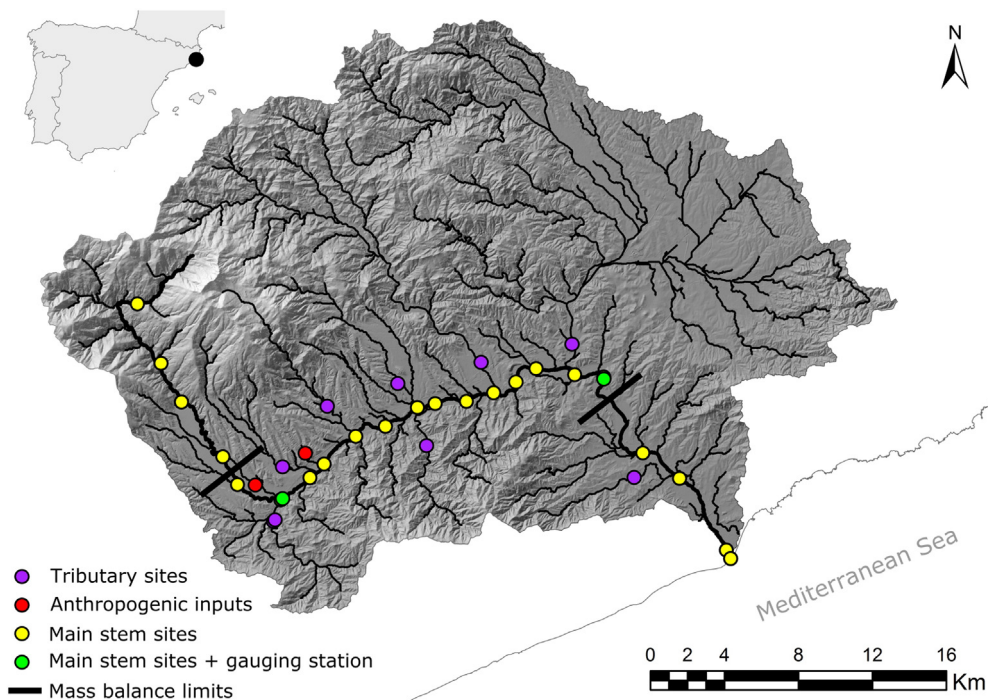
Date	Sampling type	Q 24 km <sup>a</sup> (m <sup>3</sup> s <sup>-1</sup> )	Q 45 km <sup>a</sup> (m <sup>3</sup> s <sup>-1</sup> )	n sites <sup>b</sup>
22/01/2009	Longitudinal	3.070	3.400	10
09/02/2009	Longitudinal	2.820	8.280	10
27/03/2009	Longitudinal	0.510	1.270	10
29/05/2009	Longitudinal	0.270	0.800	10
29/06/2009	Longitudinal	0.100	0.290	9
15/07/2009	Longitudinal	0.080	0.250	9
02/06/2010	Longitudinal	0.740	2.810	10
06/07/2010	Longitudinal	0.290	0.740	9
15/07/2010	Longitudinal	0.090	0.220	9
11/07/2011	Longitudinal	0.011	0.470	15
23/02/2012	Longitudinal	0.296	1.532	9
11/07/2012	Longitudinal + mass balance (drought)	0.002	0.065	19
26/04/2012	Longitudinal + mass balance (low baseflow)	0.203	1.900	21
22/05/2012	Longitudinal + mass balance (high baseflow)	0.514	3.104	20
06/03/2013	Longitudinal + mass balance (flood)	10.800	156.000	19

<sup>a</sup> Data provided by the Catalan Water Agency. In those occasions when their monitoring program was discontinued, data correspond to our own measurements.

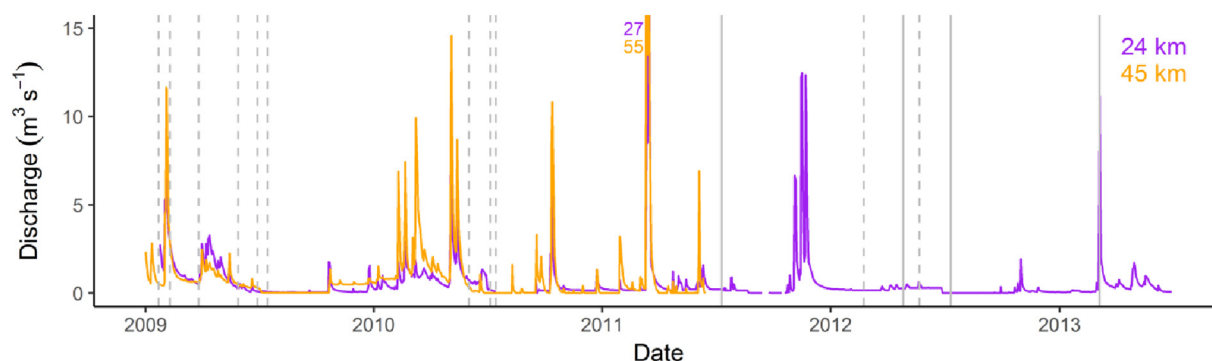
<sup>b</sup> Number of sites sampled along the main stem.

diversity of DOM sources and composition. Therefore, along these reaches both hydrological and biogeochemical processes may be controlling DOM spatial variation in an apparently indiscernible way. In addition, the water flow in this part of the river was relatively well constrained: there was no relevant surface water extraction or artificial flow regulation (e.g. dams).

The details of the sampling strategy we used for the snapshot mass balance are thoroughly explained in Butturini et al. (2016). In brief, it consisted on sampling all the direct water inflows (natural and anthropogenic) along the selected 26-km river reach, as exhaustively as possible, right before joining the main stem. Additionally, the main stem itself was sampled before every main junction, dividing the whole river reach into 13 segments, in each of which a mass balance could be calculated (Fig. 1).



**Fig. 1.** Catchment of river Tordera (Catalonia, North-East Spain), with the locations of the sampling sites. Tributaries and anthropogenic inputs were sampled only few meters before the confluence with the main stem. In this map, though, they have been represented further upstream for a clearer visualisation.



**Fig. 2.** Hydrograph during the study period, including the discharge measured at the gauging stations located at 24 and 45 km. Vertical lines indicate sampling dates (dashed lines correspond to longitudinal samplings, solid lines correspond to mass balance samplings). Discharge data provided by the Catalan Water Agency.

### 2.3. DOM mass balance calculations

We calculated the expected DOM fluxes ( $DOM_{exp}$ ) at the end of each river segment as the flow-weighted average of all observed DOM inputs. These included the inputs at the beginning of the river segment and those from any joining tributaries:

$$DOM_{exp} = \frac{Q_{upstr} \times DOM_{upstr} + Q_{trib} \times DOM_{trib}}{Q_{downstr}} \quad (1)$$

where  $Q$  refers to river discharge and  $DOM$  refers to either DOC concentration or DOM fluorophore intensity (see Section 2.4.1). Indices *upstr* and *downstr* refer to the beginning and end of the river segment, respectively; whereas *trib* refers to any joining tributaries. A preliminary water balance assessment revealed no groundwater sources.

Next,  $DOM_{exp}$  values were compared to their corresponding observed DOM fluxes ( $DOM_{obs}$ , Eq. (2)), in order to quantify the degree of DOM reactivity (%Reactivity, Eq. (3)) within each river segment.

$$DOM_{obs} = Q_{downstr} \times DOM_{downstr} \quad (2)$$

$$\%Reactivity = \frac{DOM_{obs} - DOM_{exp}}{DOM_{exp}} \times 100 \quad (3)$$

Positive values of %Reactivity indicate DOM generation, whereas negative values indicate retention. %Reactivity values near 0 indicate minimal in-stream transformation and, hence, a main hydrological control of DOM longitudinal variability. Mass fluxes were calculated for total DOC, as well as for individual fluorescence components.

### 2.4. Chemical and field measurements

#### 2.4.1. DOC concentration and DOM optical analysis

Water samples were filtered with precombusted GF/F filters and next with nylon 0.22  $\mu\text{m}$ -pore membranes. For DOC concentration analysis, an aliquot of 30 mL was acidified 3% v/v with HCl 2 M and stored refrigerated at 4 °C. For spectroscopic measurements, 30 mL aliquots were kept refrigerated in acid rinsed glass vials. All analyses were performed within a maximum of 5 days.

DOC content was determined by oxidative combustion and infrared analysis with a Shimadzu TOC Analyser VCSH. Absorbance spectra were measured using a UV-Visible spectrophotometer UV1700 Pharma Spec (Shimadzu). Data were collected in double beam mode with wavelength scanned from 200 to 800 nm and deionised water as the blank. The slit width was set to 1 nm. Excitation-Emission Matrices (EEM) were analysed using a Shimadzu RF-5301 PC spectrofluorometer equipped with a xenon lamp and a light-source compensation system (S/R mode). For every EEM, 21 synchronous scans were collected at 1-nm increments both in emission and in excitation. During each scan,

excitation was measured over a wavelength range of 230 nm <  $\lambda_{ex}$  < 410 nm. Initial emission wavelengths ranged from 310 nm to 530 nm, at intervals of 10 nm. The bandwidth used for both excitation and emission was 5 nm. Spectra were acquired using a 1-cm quartz cell.

Raw EEM data were corrected and normalised to allow for inter study comparison following the steps described by Goletz et al. (2011). An excitation correction function was determined using Rhodamine B as a quantum counter (Lakowicz, 2006), whereas for emission a correction file was obtained by comparing the reference spectra of quinine sulphate and tryptophan provided by the National Institute of Standards and Technology (NIST) according to the procedure described in Gardecki and Maroncelli (1998). Next, data were normalised by the area under the Raman peak of a deionised water sample at  $\{\lambda_{ex350}, \lambda_{em371-428}\}$  (Lawaetz and Stedmon, 2009). Inner filter effects were corrected by comparing absorbance measurements according to Lakowicz (2006), as described by Larsson et al. (2007). Finally, a blank EEM of deionised water, measured on the same day of analysis and having undergone the same correction and normalisation procedures, was subtracted from every EEM sample.

#### 2.4.2. Optical indices and EEM fluorescence components

Specific Ultra-Violet Absorbance (SUVA) – surrogate for DOM aromaticity – was calculated according to Weishaar et al., 2003, as the napierian absorption coefficient at 254 nm normalised by DOC concentration. The Humification Index (HIX) – indicator of humification degree – was calculated as the ratio between the area under  $\{\lambda_{ex254}, \lambda_{em435-480}\}$  and the area under  $\{\lambda_{ex254}, \lambda_{em330-345}\}$ , as described by Zsolnay et al., 1999. The Biological Index (BIX) – indicating an autotrophic origin of DOM – was calculated by dividing the fluorescence intensity at  $\{\lambda_{ex310}, \lambda_{em380}\}$  to that at  $\{\lambda_{ex310}, \lambda_{em430}\}$  (Huguet et al., 2009). Finally Fluorescence Index (FI) – indicator of the terrestrial vs microbial origin of DOM – was calculated as the fluorescence intensity at  $\{\lambda_{ex370}, \lambda_{em470}\}$  divided by that at  $\{\lambda_{ex370}, \lambda_{em520}\}$  (Cory et al., 2010; McKnight et al., 2001).

Individual fluorescence components were statistically resolved from the EEM data set using Self-Organising Maps (SOM), an unsupervised self-learning algorithm which treats EEM data holistically and can deal with nonlinear relationships (Bieroza et al., 2009; Cuss and Gueguen, 2016; Ejarque-Gonzalez and Butturini, 2014). A detailed explanation of the statistical procedure on this specific data set, as well as the characteristics of the resolved fluorophores, can be found in (Ejarque-Gonzalez and Butturini, 2014, 2015). In brief, SOM was applied on the Ex-Em pairs of the EEMs data set. Next, another SOM analysis was performed on the resulting correlation matrix of the component planes. After that, groups of highly correlated Ex-Em coordinates (interpreted as regions where a statistically independent fluorophore is fluorescing) were identified using hierarchical clustering analysis. Finally, the intensity of each fluorophore was quantified using Fluorescence Regional Integration (Chen et al., 2003). This analysis resolved 4

fluorescence components: C1 (in-stream generated, protein-like, similar to Coble's (1996) peak B), C2 (anthropogenically-related, protein-like, similar to Coble's (1996) peak T), C3 (humic-like, similar to Coble's (1996) peak A) and C4 (humic-like, similar to Coble's (1996) peak C). The resolved fluorescence components are further detailed in Fig. S1 (Supplementary material).

#### 2.4.3. Discharge measurements

During the study period, daily discharge on the main stem was monitored at two gauging stations located at 24 and 45 km by the Catalan Water Agency. Additionally, during the mass balance surveys and in the periods when the monitoring program of the Catalan Water Agency was interrupted, water discharge was measured at each sampling site. We used the velocity-area method (Di Baldassarre and Montanari, 2009), which consists on the summation of the product between the mean velocity and cross-sectional area of a series of sub-segments along a river transect. The mean velocity for every sub-segment was measured with a flow meter Global Water FP111, by sampling the point velocity at 4/10 of the total depth, in the middle of the segment width.

In flooding conditions, when the river flows were too high to take in-stream measurements with the flowmeter, river discharge was estimated using the Manning's equation (Manning, 1891) which estimates river discharge based on the cross-sectional area, hydraulic radius, river bed slope and the Manning's roughness coefficient. The latter was set to 0.05 following the reference tables in Chow (1959).

#### 2.5. Statistical analyses

Spatial and temporal variability of DOM properties was analysed on individual optical descriptors using boxplots, grouping the data by site (thereby representing temporal variability along space) and by hydrological condition (thereby representing spatial variability over a range of hydrological conditions). Further, DOM variability was explored multidimensionally using principal components analysis (PCA). We used a set of optical descriptors that covered a broad range of DOM optical properties: aromaticity (SUVA), humification degree (HIX), origin (BIX and FI), as well as humic- and protein-like composition (fluorescence components C1, C2, C3 and C4). Fluorescence components were expressed as relative intensities (percentage to the total fluorescence intensity) in order to emphasize qualitative over quantitative differences in DOM properties. For this same reason, DOC concentrations were not included in the analysis. As descriptors had different ranges of variation, they were previously mean-centered and scaled by their standard deviation. Analyses were performed with the Vegan package (Oksanen et al., 2016) for R (R Core Team, 2016).

The interaction between spatial and temporal variability was analysed by calculating DOM chemostasis along the river length. Chemostasis refers to the condition by which water solute concentrations do not respond to streamflow fluctuations and therefore, corresponds to the specific case in which the slope of the concentration-discharge (CQ) response approximates to 0 (Thompson et al., 2011). According to that, we computed CQ slopes as a measure of chemostasis using linear regression models (base R, R Core Team, 2016). CQ slopes were only calculated for sites which had 6 or more measurements available, including the two hydrological extremes (flood and drought). Discharge data were  $\log_{10}$ -transformed in order to minimise departures from normality of the model residuals. Slopes close to 0 indicate chemostasis whereas high values (in absolute terms) indicate a strong response of DOM properties to hydrological conditions.

### 3. Results

#### 3.1. Longitudinal patterns of DOM properties

DOM properties experienced an important variation in space (Fig. 3). DOC concentrations (Fig. 3a) increased from lowest values in

the headwaters (median ranging between 0.539 and 1.941 mg L<sup>-1</sup> up to site 19.2 km) up to 3.560 ± 2.593 mg L<sup>-1</sup> (median ± interquartile range) at site 28.1 km. Downstream, DOC remained at similar or slightly lower concentrations for the rest of the river length. Sites corresponding to the highest flood event presented differentiated pattern. Concentrations were higher than in the comparatively drier conditions and increased downstream gradually and continuously from 4.867 mg L<sup>-1</sup> in the headwaters to 10.819 mg L<sup>-1</sup> in the river mouth.

The individual fluorescence components presented a distinctive pattern compared with bulk DOC concentrations. The intensity of the protein-like component C1 (Fig. 3b) tended to increase downstream until km 38.5. In the final sites of the river, the temporal variability became much greater and blurred any longitudinal trends. The site at 42.2 km had significantly higher intensities compared with the sites immediately before and after. This site is located right after the tributary of Riera de Santa Coloma, which is the main tributary in the catchment. Contrastingly, the humic-like component C4 (Fig. 3c) followed the opposite pattern: it experienced a downstream decrease, achieving its minimum at 42.2 km – coinciding with the protein-like C1 peak. After that, C4 intensity increased moderately until the river mouth. Fluorescence components C2 and C3 followed similar patterns as C1 and C4, respectively (data not shown).

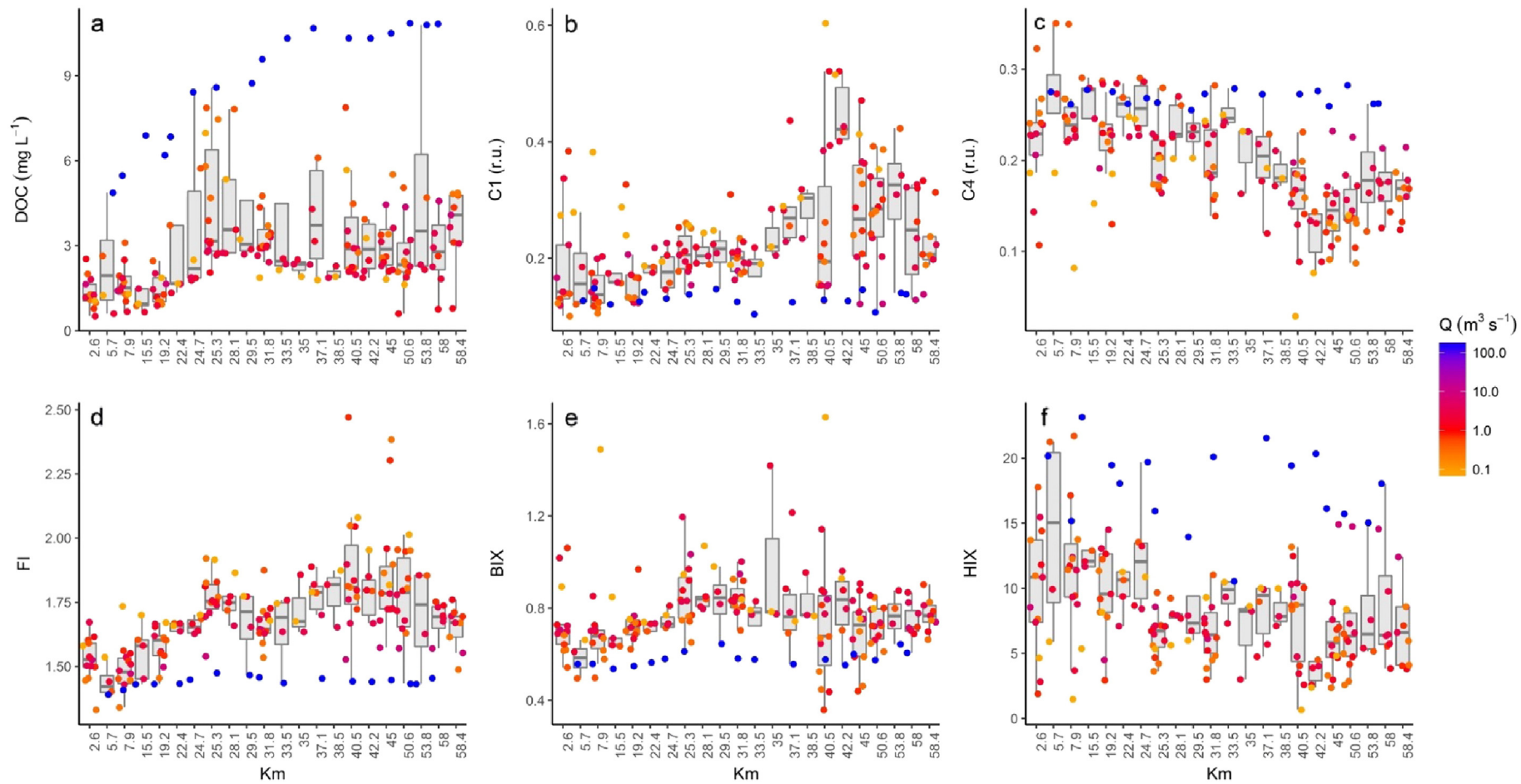
FI (Fig. 3d) overall tended to increase downstream. However, this increase was not continuous. There were two moderate peaks at 25.3 and 40.5 km, the latter being the main maximum at which some outlyingly high values were registered. After the second peak FI decreased moderately until the river mouth, although remaining at higher values compared with the headwaters. Similarly, BIX (Fig. 3e) tended to increase downstream, although only clearly until 29.5 km. After that, median BIX remained more stable until the river mouth; even though temporal variability became larger and the spatial trend less evident. HIX (Fig. 3f) longitudinal evolution was more consistent to the humic-like component C4, experiencing a decreasing trend along most of the river length, until site 42.2 km. The final sites remained median-stable around HIX = 6. Opposite to the rest of optical descriptors, the temporal variability of HIX in the headwaters was prominent.

#### 3.2. Spatial variability of DOM properties over a range of hydrological conditions

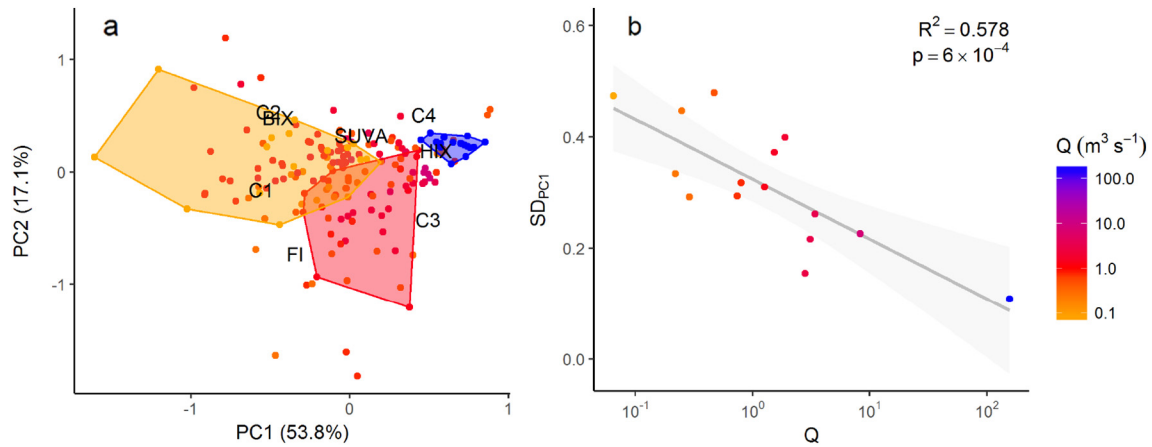
During the highest flood event, DOM properties at the headwaters remained essentially unchanged along the river length (Fig. S2, Supplementary material). This was evidenced by the flat evolutions of the fluorescence components C1 and C4, as well as FI and BIX, along space (Fig. 3), thereby exhibiting a remarkably low variability compared with the lower-flow conditions (Fig. S3, Supplementary material). HIX was the exception, as it exhibited a more random longitudinal pattern during these hydrological conditions. The opposite was observed for DOC concentration, and for HIX to some extent, for which spatial variability during these high-flow conditions was maximised.

Besides this extreme event, the variation of DOM properties in response to hydrology was not so clear when exploring each variable separately. The main features observable were reduced spatial variabilities at higher flows (especially remarkable for FI at  $Q \geq 1.53 \text{ m}^3 \text{ s}^{-1}$ ), increase in the humification degree (increasing HIX median at  $Q \geq 1.9 \text{ m}^3 \text{ s}^{-1}$ ) and decrease in the protein-like content and autochthonous origin (decreasing medians for C1, FI and BIX, at  $Q \geq 1.53 \text{ m}^3 \text{ s}^{-1}$ , Fig. S3 Supplementary material).

However, when exploring the DOM response from a multivariate perspective, a clear response to hydrology was observed (Fig. 4). This was evidenced in the PCA ordination space (PC1 and PC2 accounting for 58.3% and 17.1% of the original variance, respectively) by grouping samples by hydrological condition. On the one hand, there was a displacement of the centroid from high PC1 scores (i.e. allochthonous, humic-like related region) during flood conditions to low PC1 scores (i.e. autochthonous, protein-like related region) during drought



**Fig. 3.** Longitudinal profiles of DOM properties. Boxplots represent hydrological variability at each site. Dot colours indicate the associated hydrological conditions in terms of river discharge ( $Q$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** PCA analysis on the longitudinal data set. a) The convex hulls of three selected contrasting hydrological conditions have been plotted to illustrate the increase in the dispersion area, and the displacement of the centroid, as flows decrease. b)  $SD_{PC1}$  of each of the convex hulls holds a significant correlation with log discharge ( $Q$ ). Shaded area corresponds to the 95% confidence band. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 4a); indicating a gradual shift in the average DOM properties. On the other hand, the dispersion of the samples within the PCA ordination space was inversely related to hydrological conditions. For instance, the samples collected specifically during the highest flood event ( $Q = 156 \text{ m}^3 \text{ s}^{-1}$ ) were highly clustered together and occupied only 1.4% of the total area. Contrastingly, the samples collected during the driest conditions ( $Q = 0.065 \text{ m}^3 \text{ s}^{-1}$ ) were highly dispersed and occupied 31.0% of the total area (Fig. 4a). According to that, we used the standard deviation (SD) on the PC1 as a measure of the degree of DOM heterogeneity for each of the 15 hydrological conditions and we found that  $SD(PC1)$  held a significant negative correlation with discharge (Fig. 4b;  $SD(PC1) = -0.046 \pm 0.010 \log(Q) + 0.325 \pm 0.019$ ,  $R^2 = 0.578$ ,  $p = 6 \cdot 10^{-4}$ ,  $F = 20.2$  on  $df = 13$ ).

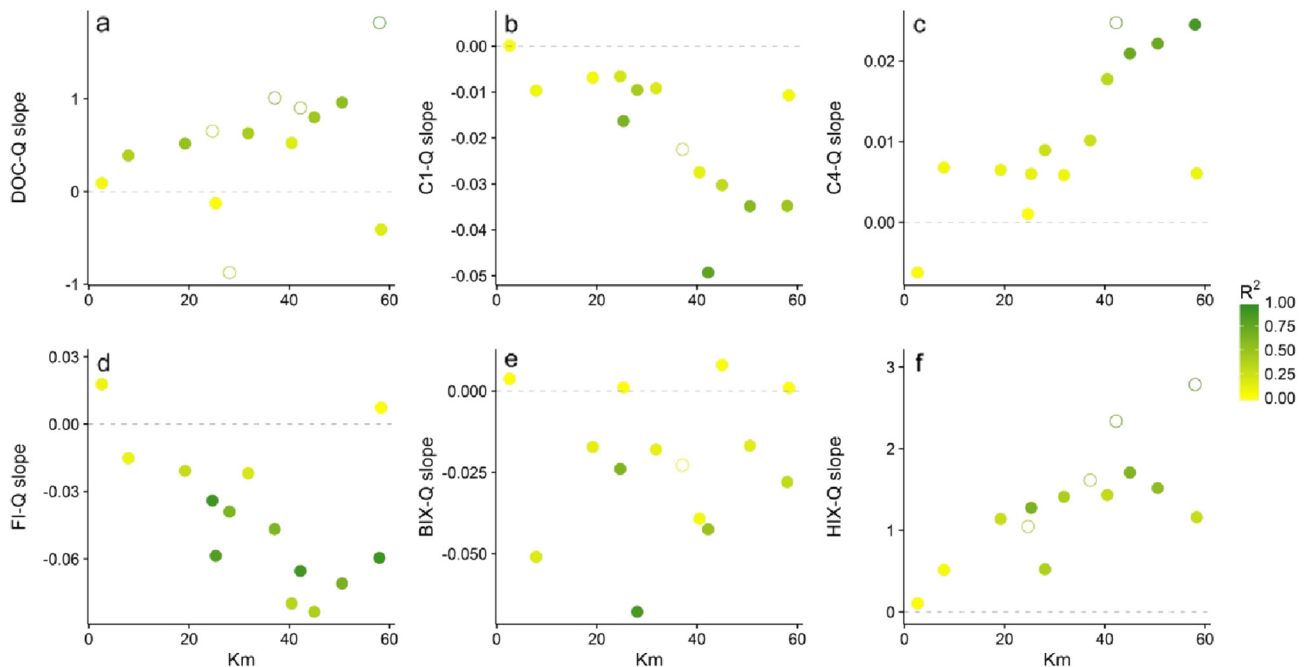
### 3.3. Downstream evolution of the DOM chemostatic response

A number of optical descriptors followed common longitudinal patterns on DOM chemostasis. Namely, FI, HIX, and the fluorescence

components C1 and C4, followed a gradual evolution from chemostasis in the headwaters, to flow-dependency at downstream sites (Fig. 5b–d, f). This was evidenced by an increase in the CQ slopes as well as by an increase in the  $R^2$  of the CQ linear regression along the river length, indicating that river discharge had an increasing explanatory role of DOM properties toward downstream distances. A similar behaviour was observed for total DOC concentration, which also evolved from chemostasis to chemodynamism over the downstream gradient (Fig. 5a). Contrastingly, the chemostatic response of BIX (Fig. 5e), SUVA and the fluorescence components C2 and C3 (data not shown) did not follow a systematic variation in space but rather an erratic downstream evolution.

### 3.4. Reactivity of DOM along the river continuum

The magnitude of in-stream DOM reactivity was very different at each of the four hydrological conditions. The lowest magnitudes were observed during flood conditions. Both generation and retention of DOC and fluorescence components was  $<20\%$  in all river segments,



**Fig. 5.** Downstream evolution of the DOM chemostatic response. Positive and negative values indicate, respectively, a positive and negative correlation between DOM properties and discharge. Values close to zero (dashed horizontal line) indicate chemostatic conditions. Solid and open circles indicate significant ( $p < 0.001$ ) and non-significant correlations, respectively.

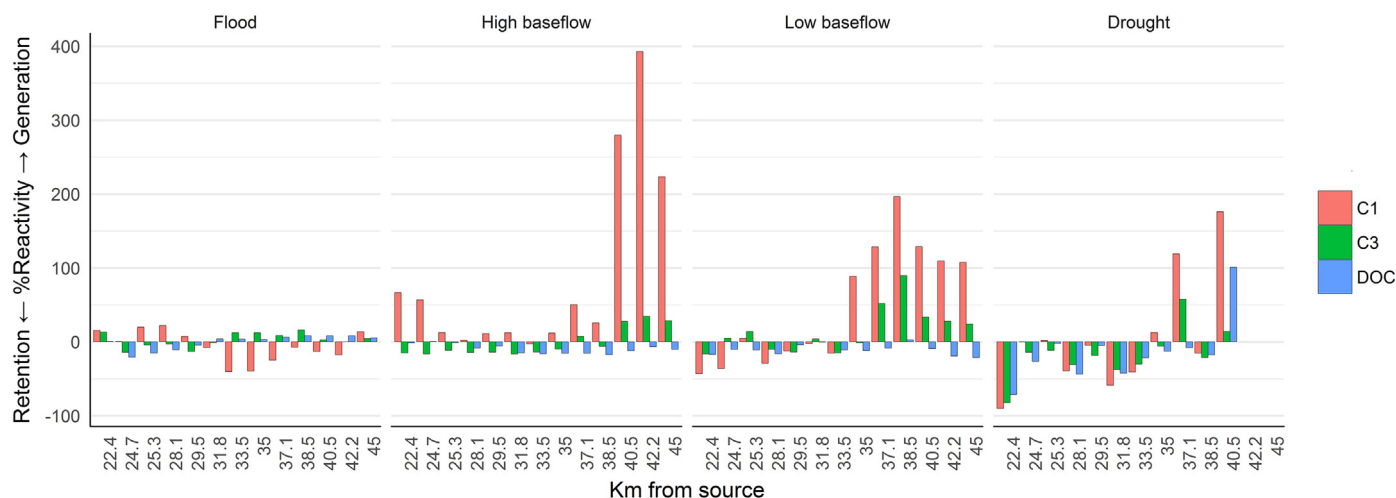


Fig. 6. Longitudinal profiles of DOM reactivity (%Reactivity) at four contrasting hydrological conditions. C2 and C4 exhibited similar patterns as C1 and C3, respectively.

except for three segments in the middle part of the river length which had a slightly higher retention of C1 (up to 40%, Fig. 6).

These low reactivity percentages were also found during high and low baseflow conditions, although only in the first 33 km of the river length. By contrast, in the lower parts of the river, there were very high rates of DOM generation, especially for the fluorescence component C1, which achieved generations between 200 and 400% at these sites. However, bulk DOC and the protein-like component C4 remained at low reactivity percentages (lower than 40%, as during flood conditions). From high to low baseflow, the region of DOM generation increased from 6.5 to 11.5 km. However, the generation percentages of C1 were more moderate than during high baseflow (between 75 and 200%). Also, in this case, the generation percentages of the humic-like DOM fraction (component C3) became more prominent, achieving generations up to 50–90%. Remarkably, despite these clear generation behaviour of both fluorescence fractions, the mass balance for bulk DOC revealed a predominantly conservative behaviour with a tendency to retention (retention percentages < 25%).

Finally, drought conditions created a mosaic of DOM biogeochemical behaviours, with an alternation of segments of retention, transport (i.e. very low %Reactivities) and generation. Retention predominated in the beginning and middle section of the river, for all DOM fractions. C1 generation at percentages > 100% still occurred at the downstream region of the river length, interestingly at two discontinuous sites. Fluorescence components C2 and C4 followed similar patterns as C1 and C3, respectively (data not shown).

## 4. Discussion

### 4.1. The spatial dimension: longitudinal patterns of DOM properties

The average spatial patterns that we found across hydrological conditions question some of the arguments of the RCC concerning the downstream succession of DOM quality. The RCC predicted a rapid depletion of labile DOM compounds at low-order sites, leaving a remaining pool of recalcitrant molecules flowing conservatively downstream (Vannote et al., 1980). In our study site, we accordingly observed a general pattern whereby a predominance of aromatic, humic-like compounds tended to decrease downstream. However, these aromatic signatures were gradually replaced downstream with protein-like moieties, typically considered more labile and readily available to microbial degradation than humic-like molecules (Cory and Kaplan, 2012). The reasoning behind the RCC prediction was that terrestrial sources of DOM are enhanced in headwater streams, representing a source of

fresh DOM, some components of which can be readily degraded by the riverine microorganisms. This idea has been supported by field evidence in our study site, where a higher utilisation of terrestrially-derived DOM by sediment bacteria was found in headwaters, in contrast to higher order sites (Freixa et al., 2016). However, our mass balance study revealed that, at lower reaches, there was not only a conservative transport of upstream subsidies of recalcitrant DOM. Further than that, there was an emergence of in-stream biogeochemical processes which transformed and replenished the pool of DOM molecules with autochthonously-derived compounds. Increased water residence times provide more chances for in-stream metabolism (Battin et al., 2008) and increased light availability may enhance autotrophic activity with respect to upstream canopy-shaded sites. Accordingly, we found that a conservative downstream transport of DOM shifted into an in-stream generation at downstream sites (starting at 35 km) under baseflow conditions. Most of the DOM production occurred in the form of protein-like fluorescence components, with rates between 100 and 400%, what is in line with common knowledge that protein-like substances are usually derived from microbial (Henderson et al., 2009) and autotrophic activity (Findlay and Sinsabaugh, 2003). But, most remarkably, there was also a production of humic-like components, at lower but significant rates of 25–90%. These findings add evidence to the recent revision of the traditional concept that humic-like substances are solely of terrestrial origin and that, on the contrary, microbial activity can be an important source of humic-like material (Jiao et al., 2010; Ogawa et al., 2001).

Parallel to the in-stream generation of specific fluorescence components, bulk DOC had a more conservative behaviour and experienced net retentions of only 10–20%. The production of fluorescence intensity of specific components parallel to total DOC decreases has already been observed (Asmala et al., 2014) and has been attributed to the capacity of microorganisms to transform uncoloured DOM forms into coloured ones (Guillemette and del Giorgio, 2012). Most importantly, this demonstrates that the measurement of total net DOC fluxes may underestimate the actual magnitude of in-stream reactivity, because they may not reflect simultaneous processes of retention and release that may quantitatively compensate each other. To our knowledge, previous DOC mass balance studies in streams focussed mainly in total DOC fluxes during stable flows (Eatherall et al., 2000; Moody et al., 2013; Worrall et al., 2006, 2007). Interestingly, these studies reported similar to slightly higher retention rates as those we observed in baseflow conditions. These results, added to the global budgets which depict inland waters as organic carbon sinks (Cole et al., 2007; Tranvik et al., 2009), contribute to conceptualise rivers as “filters”, which take terrestrial DOM, mineralise a fraction of it, leaving the rest to flow downstream.



However, our mass balance results for specific DOM fluorophores reveal a major potential for in-stream processes to actively shift DOM quality during its downstream transport, emphasizing the role of the river as a transformer.

#### 4.2. The temporal dimension: hydrological regulation of DOM spatial heterogeneity

Despite the average spatial patterns observed during baseflow, we found that the configuration of longitudinal gradients of DOM is highly sensitive to variations in discharge. The wide range of hydrological conditions included in this study allowed detecting a statistically significant correlation between hydrological conditions (as  $\log_{10}$  discharge) and the heterogeneity of DOM quality along a downstream gradient (as the standard deviation on the PC1 axis). Further analysis using a space-resolved mass balance approach (Fig. 6) revealed that the heterogeneity of DOM properties that emerged at decreasing flows could not be explained solely as the result of hydrological mixing of waters from different tributaries. Rather, it was the result of an increasing predominance of in-stream biogeochemical processes transforming DOM over hydrological transport. This systematic correlation between hydrological conditions and spatial heterogeneity of DOM reinforces the concept of hydrology as a fundamental physical variable that determines the opportunities for in-stream biogeochemical processing, a concept that so far has been explored theoretically (Battin et al., 2008) or semiquantitatively (Jantze et al., 2015).

Water residence times can explain a shift from allochthonous to autochthonous sources of DOM. However, this cannot explain an increase in the overall diversity of fluorescence signatures detected along the river length. We argue that the underlying phenomenon is related to a weakening of the longitudinal connectivity as flows become extremely slow. Previous work in an intermittent Mediterranean stream provided evidence that flow interruption created isolated pools, each with independent chemical evolutions for DOM (Vazquez et al., 2011). Other studies also theorized about the main role of the longitudinal hydrological connectivity in determining downstream organic matter processing (Larned et al., 2011).

Our mass balance study, even though it included only four discrete hydrological conditions, illustrates how these two factors – water residence times and longitudinal connectivity – operate over gradually decreasing flows, determining the overall longitudinal patterns of DOM quality and reactivity. During flood, water residence times are extremely short and longitudinal connectivity is maximal, resulting in a conservative transport of terrestrially-derived DOM, leading to a homogeneous spatial profile of aromatic and humic-like DOM character. At baseflow conditions, water residence times become longer while still keeping a good longitudinal connectivity. These circumstances allow for a shift from allochthonous to autochthonous sources of DOM at lower reaches, as evidenced by a shift from humic-like to protein-like fluorescence properties, increased BIX and FI together with a decrease in HIX indices, and a shift from conservative transport to in-stream generation of DOM fluorophores. At lower baseflows, these patterns are enhanced so that the location of the DOM qualitative shift occurs further upstream, hence decreasing the length of influence of the terrestrial inputs from the headwaters. Interestingly, when flows decrease below a critical level, longitudinal connectivity is compromised so that each river reach becomes more independent of upstream DOM subsidies, thus maximising the heterogeneity of DOM fluorescence signatures over the longitudinal axis. This longitudinal disconnection during drought is also followed by biogeochemical functional changes, as the in-stream productivity of DOM (which dominated in baseflows) is replaced by a predominance of retention. Further research at higher temporal resolutions could shed light at the hydrological thresholds which trigger this emergence of spatial patchiness of DOM properties.

#### 4.3. Combined space-time effects: longitudinal patterns of DOM chemostasis

Given the relevance that both space and hydrology have in shaping DOM dynamics, a better understanding on how the two dimensions inter-relate with each other can be gained by analysing how DOM chemostatic behaviour (hydrological variability) varies as a function of downstream distance (spatial variability). In our analysis we found that a diversity of DOM properties, including DOC concentration, origin (FI), humification degree (HIX) and humic- and protein-like fluorescence composition (C1 and C4) had an increased chemodynamic response toward downstream distances. These results indicate that the DOM composition in the lower reaches of the river is more sensitive to changes in hydrology.

Our results contrast with previous works which reported increased chemostatic behaviours at downstream river reaches for inorganic solutes (Asano et al., 2009) and DOM (Creed et al., 2015). These studies argue that chemostasis at high order reaches is achieved as an effect of hydrological averaging of the diversity of concentrations received by upstream tributaries. We note here that such a mechanism could only be significantly operating under conditions of strong longitudinal connectivity, and therefore, this effect would not be meaningful during drought conditions. Further, Creed et al. (2015) also argue that an increased biogeochemical cycling of carbon at downstream sites would contribute to reduce temporal variability of DOM composition. However, our mass balance results provide evidence that the biogeochemical function of the river itself varies profoundly according to hydrological conditions, rather emphasizing the potential for in-stream processes to generate a diversity of fluorescence signatures. An explanation to the different results between this study and Creed et al. (2015) may lie in the fact that our study was focussed on an individual system, whereas Creed et al. (2015) explored patterns on a regional scale. Therefore, our results may be reflecting some factors that affect chemostasis, which may even out at the regional scale and blur the signal of individual river systems. These factors may include the effects of agricultural fertilisation (Thompson et al., 2011) or the distribution of organic-rich soils throughout the catchment (Herndon et al., 2015), both of which affect the temporal stability of DOM availability in soils and, therefore, the variability in the DOM flushing responses to storm events.

#### 4.4. Implications of hydrological variability and extreme events on DOM dynamics

In the last decade, during 50% of the days, the gauging station located at 24 km registered flows of  $<0.18 \text{ m}^3 \text{ s}^{-1}$ , flows which correspond, approximately, to the minimum environmental flows threshold established by the Catalan Government (which is of  $0.204 \text{ m}^3 \text{ s}^{-1}$  in the summer months, Boada et al., 2008). Due to the low levels of water discharge, these prolonged dry periods are responsible for only 7% of total water export. For the sake of this discussion, if we consider our drought sampling (performed at  $0.065 \text{ m}^3 \text{ s}^{-1}$ ) as representative of the DOC export during these dry periods, we can extrapolate that drought accounts only for 4% of the total DOC export. This illustrates the fact that over a long period, the longitudinal connectivity is extremely limited, so that the stream flow has minimal export influence on the receiving maritime ecosystems and, basically, the linking function of the river between the land and the ocean is practically inexistent. In this sense, droughts represent a discontinuity in the transport and linking function of the river. Contrastingly, drought maximised the role of the river as a reactor, hosting in-stream DOM transformations. This had profound consequences in the longitudinal patterns of DOM, as its overall quality and fluorescence signatures achieve a maximum heterogeneity. Due to the weak longitudinal connectivity associated with low flows, the quality of DOM in each stream reach became more independent of upstream subsidies, as processes like microbial

degradation, photochemical oxidation and autotrophic production of DOM became faster than transport. Our mass balance results highlight a predominance of DOM retention, what suggests a predominance of respiration processes.

On the other extreme, if we consider as stormflows those that were exceeded only 5% of the time ( $>4 \text{ m}^3 \text{ s}^{-1}$ ) over the last decade, then it turns out that stormflow events represent an export of 40% of the total water. Using DOC concentration data from our samplings performed at 3.4, 8.28 and  $156 \text{ m}^3 \text{ s}^{-1}$ , we obtain the rough estimate that flood events cause 47% of the total DOC export. This highlights the relevance that these events have despite their brevity in time: About half of the DOM that is relocated from the land to the sea via fluvial transport, is exported in the form of rapid pulses during storm events, undergoing minimal transformations, therefore being delivered to the seas with a pronounced humic-like and aromatic character.

## 5. Conclusions

In this study, we provided insights on the intricate relationship between space and hydrology in a fluvial continuum. The two dimensions of variation have to be necessarily considered in order to thoroughly understand the mechanisms that control DOM dynamics in rivers. The full gradient of hydrological conditions included in this study allowed expanding the knowledge provided by previous works which considered only some discreet hydrological states (i.e. [Butturini et al., 2016](#)). We found that hydrology determined the degree of spatial heterogeneity of DOM properties, as it could suppress (floods) or enhance (droughts) the effects of local spatial features. Heterogeneity emerged more predominantly at lower reaches as a result of in-stream processes generating protein-like components (baseflow) as well as respiring protein- and humic-like components (drought). Because of that, there was a spatial organisation of DOM temporal variability consisting in a gradual decrease of the DOM chemostatic response. This implies that DOM biogeochemistry in lower river reaches is likely more sensitive to hydrological variability than in headwater sites. Overall, our outcomes highlight the need to take into account the whole spectrum of hydrological variability of a specific system, including extreme hydrological events, as they may have profound consequences on the riverine biogeochemical function and carbon cycling.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.05.113>.

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