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Key Points:
- The quantity and diversity of DOM displayed a bell-shaped pattern along the river continuum during nonstorm conditions
- In contrast, DOM showed a homogeneous longitudinal pattern during storm events
- We present a new framework for the dynamics of DOM in temperate river networks: the "Bending DOM Concept"

Supporting Information:
- Supporting Information S1
- Data Set S1
- Data Set S2

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Delineating the Continuum of Dissolved Organic Matter in Temperate River Networks

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Abstract The origin and reactivity of dissolved organic matter (DOM) have received attention for decades due to the key role DOM plays in global carbon cycling and the ecology of aquatic systems. However, DOM dynamics in river networks remain unresolved, hampered by the lack of data integrating the spatial and temporal dimensions inherent to riverine ecosystems. Here we examine the longitudinal patterns of dissolved organic carbon (DOC) concentration and DOM chemical diversity along a temperate river network under different hydrological conditions, encompassing small headwater streams to the river mouth and base flow to storm events. We show that, during nonstorm conditions, the concentration of DOC and the chemical diversity of DOM exhibit their maxima in the middle section of the network, depicting a bell-shaped pattern along the river continuum. In contrast, DOM shows a homogeneous longitudinal pattern during storm events, with highly concentrated and diverse DOM along the river network. We posit that these emerging patterns result from changes in the relative influence of catchment versus in-stream biogeochemical processes along the river continuum and that the degree of influence is modulated by river network hydrology. Based on these findings we put forward the "Bending DOM Concept," a new conceptual framework around which testable hypotheses on the spatiotemporal dynamics of DOM and the functioning of temperate river networks may be formulated.

1. Introduction

Dissolved organic matter (DOM) is a heterogeneous mixture of soluble organic compounds that constitutes the major form of organic matter in aquatic ecosystems (Birge & Juday, 1926; Schlesinger & Melack, 1981). In river networks, DOM is central to a multitude of disciplines from carbon cycling (Aufdenkampe et al., 2011) to food web dynamics (Pace et al., 2004) and ecosystem functioning (Prairie, 2008). Additionally, DOM is a water constituent of concern for drinking water supply (Chow et al., 2007) and is linked to the transport and reactivity of toxic substances (Aiken et al., 2011). Thus the source, processing, and fate of riverine DOM has traditionally been at the core of fluvial research (Findlay & Sinsabaugh, 2003), yet the spatiotemporal dynamics of DOM in river networks remain unresolved.

Few conceptual models have been put forward to understand riverine DOM dynamics. The river continuum concept (RCC) (Vannote et al., 1980), a mainstay in fluvial ecology for decades, sets a theoretical framework based on linear spatial changes in fluvial geomorphology to capture the origin and fate of organic matter in river networks. Although the RCC does not specifically address DOM dynamics, it does predict that DOM chemical diversity is maximal in headwater streams and that it decreases exponentially along the river continuum. The core concept underlying the RCC evolved into more complex models (McCluney et al., 2014;

Thorp et al., 2006) that extended previous efforts by including the role of floodplains (Junk et al., 1989) and autochthonous organic matter sources (Thorp & Delong, 1994). However, these subsequent models for river networks seldom addressed DOM dynamics in detail, and thus the RCC has remained the standard conceptual framework for riverine DOM studies over the last decades (e.g., Butturini et al., 2016; Coble et al., 2019; Kamjunke et al., 2019; Mosher et al., 2015; Oliver et al., 2016; Roebuck et al., 2020; Savio et al., 2015).

Our understanding of DOM sources, composition, and reactivity has greatly improved since the publication of the RCC with, first, the burgeoning use of optical characterization techniques (Fellman et al., 2010) and more recently the growth of ultrahigh resolution mass spectrometry as a tool for molecular-level analysis of DOM (Hutchins et al., 2017; Kellerman et al., 2018; Stubbins et al., 2010; Ward et al., 2013). Insights obtained through these and other analytical methods brought new DOM focused studies to the forefront of fluvial research, and explanations for the spatial or temporal dynamics of riverine DOM have lately been proposed. For example, Creed et al. (2015) observed a general tendency toward downstream homogenization of dissolved organic carbon (DOC) concentrations and a decrease in DOM aromaticity across thousands of streams in North America. These patterns led to a novel perspective of rivers as chemostats (Creed et al., 2015) and suggest a downstream shift in the controls on riverine DOM dynamics, from a dominance of hydrologic controls in headwaters to a greater relative influence of in-stream biogeochemical processes in larger rivers.

The Pulse-Shunt Concept (Raymond et al., 2016) adds a crucial temporal dimension to the RCC and the *chemostat* framework by discussing the role of network hydrological conditions in regulating the passive or active character of river networks in processing DOM. This concept postulates that during extreme discharge episodes (e.g., storm and snowmelt events) large amounts of DOM are transferred from soils to streams (*the pulse*) and exported conservatively to coastal systems due to extreme water velocities and reduced residence times (*the shunt*). In contrast, DOC that enters the river network at low to moderate discharge events spends longer times in transport and therefore has more opportunities to be processed before reaching the ocean. The role of hydrology and in-stream reactions in controlling the transport and transformation of riverine DOM was further developed in the River Network Saturation Concept (Wollheim et al., 2018).

These conceptual works have greatly advanced our knowledge of riverine DOM dynamics, but they have focused mostly either on space or time. Both dimensions have rarely been explicitly integrated into a more holistic framework founded on empirical observations. One of the main reasons for this is the lack of data sets that effectively capture the spatial and temporal variability of DOM along a single river network (e.g., Coble et al., 2019; Ejarque et al., 2017), as well as including larger rivers and data on DOM molecular composition. Therefore, despite the increasing availability of data, it remains unclear what are the patterns of DOM quantity and chemical diversity along the river continuum and how these vary across different flow conditions.

In this study, we aim to disentangle the inextricably connected spatial and temporal patterns of DOM quantity and chemical diversity along the river continuum. To do so, we analyzed a detailed data set of DOC concentrations and DOM chemical diversity and composition along the continuum of a medium-sized temperate river network, encompassing a wide range of stream sizes and hydrological conditions. DOC concentration was used as a proxy for DOM quantity, while electrospray ionization coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) and fluorescence spectroscopy were employed to assess DOM chemical diversity. Based on these data, we present a novel concept for explaining DOM spatiotemporal dynamics in temperate river networks. We then evaluate the representativity of our findings and the generality of the proposed concept by analyzing DOC concentration data from thousands of streams across the temperate biome.

2. Materials and Methods

2.1. Study Site and Sampling Design

Water samples were collected from 22 sites across the Fluvià, a sixth order river network located in the North-East of the Iberian Peninsula (latitude 42°11′N, longitude 3°6′E; supporting information Figure S1). Fluvià's main stem is 97 km long, and its network drains a 990 km^2 catchment covered by mixed forests (79%), agricultural (18%), and urban (3%) areas. Catchment geology is mostly calcareous, with some areas (<15%) of siliceous materials. The 22 sampling sites encompass a wide range of stream orders (second to sixth) and subcatchment areas (2 to 960 km^2). The area draining into the smallest sampling sites is almost entirely dominated by mixed forests (>95%), whereas drainage areas of the medium-sized and large rivers sites have some contributions from agricultural fields (14–19%) and to lesser extent urban areas (1–4%) (Figures S1 and S2). Climate is typically Mediterranean, with air temperatures ranging from 6 to 26°C throughout the year, and precipitation mainly occurring in autumn and spring with an annual mean of 660 mm.

Samples were collected between 1 November 2012 and 31 December 2013. Discharge during the sampling period ranged from 0.1 to 50 $m^3 s^{-1}$, with a median and interquartile range of 1.4 and 0.9–2.8 $m^3 s^{-1}$, respectively (Figure 1a). Two major storm events took place in March and November 2013, with a peak discharge of 50 and 37 $m^3 s^{-1}$ at the river mouth, respectively. Additional storms events of lower magnitude occurred throughout the spring season. All sites were sampled 10 times at different hydrological conditions, from low to high flows, and covering the four seasons (Figure 1a). Additionally, we collected discrete water samples during the two major storm events of March and November 2013. During these extreme discharge events, most of the sampling sites became inaccessible due to road flooding. Therefore, storm event data were obtained only from a subset of sites (red symbols in Figure S1). Note also that storm sites were slightly different for the two storm events depending on site accessibility, particularly in headwater streams. DOC concentration and DOM diversity may change along the course of a storm event (e.g., hysteresis; Butturini et al., 2006; Wagner et al., 2019). Since we only took one sample per site during each storm event, these rapid changes in DOM properties are not captured by our sampling design, and thus within-storm dynamics cannot be assessed. We therefore interpret our storm data as a representation of the average DOC concentration and DOM diversity along the river continuum during storm events.

Stream discharge was measured at every site and sampling date (except storm events) using an acoustic Doppler velocity meter (Flow Tracker, YSI, USA) (Figure S3). Discharge at the river mouth was estimated at 10-min intervals for the whole study period using pressure sensors (Solinst levelogger and barologger 3001, Canada). Absolute pressure was corrected for atmospheric pressure and calibrated with the measured discharge.

2.2. Sample Collection and Analysis

Three replicate water samples for DOC concentration and FT-ICR MS analyses were filtered in situ through pre-combusted (450°C for 4 hr) 0.7 μm glass fiber filters and stored in acid washed 125 ml polyethylene bottles. Water samples for fluorescence analysis were collected in 11 ml pre-cleaned polypropylene tubes after filtering through pre-rinsed 0.2 μm nylon filters. Samples were transported to the laboratory in cool and dark conditions and kept in the fridge (4°C in the dark) until analysis. A representative subset (62 samples) were frozen and kept at −20°C for ultrahigh-resolution FT ICR-MS measurements.

DOC concentration was measured by high-temperature catalytic oxidation on a Shimadzu TOC-V-CSH analyzer (Shimadzu Corporation, Japan). Excitation-Emission matrices (EEMs) were obtained on a fluorescence spectrophotometer (F-7000, Hitachi, Japan) with a 1-cm quartz cuvette. Scans were collected over 3 nm increments for the excitation (248–449 nm) and the emission (250–550 nm) wavelengths. EEMs were blank subtracted, corrected for inner-filter effects and instrument specific biases, and normalized to the Raman peak area. Parallel Factor Analysis (PARAFAC) (Stedmon et al., 2003) was used to deconstruct EEMs into six independent fluorescence components (Figure S4; Casas-Ruiz et al., 2017) that were validated through visual inspection of the residuals and split-half analysis (Figure S5). All components have already been identified in previous models available in the OpenFluor database (Murphy et al., 2014).

For FT-ICR MS analysis, DOM was solid-phase extracted with 50 mg Bond Elut PPL cartridges (Agilent Technologies, Santa Clara, CA, USA) following methodology that is described in detail elsewhere (Dittmar et al., 2008). Briefly, each sample was acidified to pH 2 with HCl prior to loading onto the stationary phase. After loading, the cartridges were rinsed with three cartridge volumes (3 ml) of 0.01 N HCl and dried with N_2 prior to elution of DOM with methanol. A final concentration of 50 mg C L^{-1} in methanol was achieved for each extract by adjusting the sample volume passed through the cartridge based on DOC concentration. Eluates were stored at −20°C in acid washed and precombusted (550° for >5 h) amber glass vials until analysis. The order of analysis for the 62 extracts was randomized prior to data acquisition. Negative-ions produced by electrospray ionization (ESI) were directly infused into a custom-built 9.4 tesla

FT-ICR MS (National High Magnetic Field Laboratory, NHMFL; Tallahassee, FL, USA) (Blakney et al., 2011; Kaiser et al., 2011). Molecular formulae were assigned for all signals with magnitude greater than 6σ root mean square baseline noise with an in-house software (EnviroOrg©™, Corilo, 2015) developed at the National High Magnetic Field Laboratory following an internal "walking" calibration of each mass spectrum (Savory et al., 2011). Formulae with elemental combinations of $C_{1\text{-}45}H_{1\text{-}92}N_{0\text{-}4}O_{1\text{-}25}S_{0\text{-}2}$ were considered for assignment, and the mass error did not exceed 200 ppb. The average deviation of the number of assigned formulae for 10 extracts run in triplicate was ±7%. From the FT-ICR-MS data we assigned molecular formulae compound categories based on the stoichiometry of their molecular formula. The categories relevant to our study are delineated by the aromaticity index (AI; Koch & Dittmar, 2006, 2016) and H/C cutoffs (Šantl-Temkiv et al., 2013): aromatic compounds (AI > 0.50), highly unsaturated and phenolic compounds (AI ≤ 0.50 and H/C < 1.5), and aliphatic compounds (1.5 ≤ H/C ≤ 2.0).

Diversity obtained by FT-ICR MS was defined as the total number of unique formulae assigned in each sample (i.e., richness). Diversity obtained by fluorescence was calculated based on the six PARAFAC components. In this case we did not use richness, because PARAFAC identifies the same number of recurring fluorescence components across samples. Instead, we calculated the evenness of the six PARAFAC components using an equation modified from the Pielou's evenness index (Pielou, 1966):

$$Evenness = -\frac{1}{lnN}\sum_{i=1}^{N} C_i \; lnC_i \tag{1}$$

where N is the number of components and Ci is the relative contribution of each component to the sum of all components' fluorescence intensity. Evenness may vary between 0 and 1 and reflects the relative contribution of the different components to bulk fluorescence, with a value of 1 corresponding to equal contribution (higher diversity).

2.3. Analysis of DOM Patterns Along the River Continuum

Longitudinal patterns were evaluated using the area of the upstream catchment associated to each site, as it provides a correlated, but continuous and more robust axis than stream order (Hughes & Omernik, 1983). Thus, sampling sites with small catchment areas correspond to headwater streams, and sites integrating large catchment areas correspond to larger rivers. Catchment areas for each site were calculated in ArcMap 10 (ArcGis, USA) using a digital elevation model with a 15-m resolution.

To statistically assess the longitudinal patterns of DOM we used generalized additive models (GAMs), as they do not assume a particular shape a priori. GAMs were fitted and visualized using packages *mgcv* (Wood, 2011) and *itsadug* (van Rij et al., 2017) in R 3.6.0 (R Core Team, 2019), with cubic regression spline as the smoothing function, and *catchment area* and *discharge condition* as smooth terms. *Discharge condition* was represented by the discharge measured at the river mouth at each sampling date, which provides an approximation of the network hydrological phase (e.g., base flow, high discharge, and storm event). The basis complexity (k) of the GAM models was set to 3 in order to only capture the overall patterns of DOM along the river continuum. A statistically significant effect of the smooth term *catchment area* on the response variable indicates that there exists a pattern along the river continuum (i.e., the pattern is not flat). A significant effect of *discharge condition* indicates that the response variable changes depending on the discharge condition (e.g., DOC concentration for a given site increases from low to high flow). A significant effect of the interaction between *catchment area* and *discharge condition* indicates that the overall shape of the pattern changes depending on the discharge conditions. All models were validated with plots of the distribution of residuals and of residuals against fitted values. A summary of model results from all GAMs is shown in the main text, while Tables 1 and S1 provide additional model information (F and p values and adjusted R^2). The level of significance was set to 0.05.

2.4. Patterns of DOC Across the Temperate Biome

To evaluate the representativeness of the patterns measured in the Fluvià data set we consulted the Global River Chemistry Database (GloRiCh) (Hartmann et al., 2014), a database that contains approximately 160,000 DOC measurements from 3,322 sites across different biomes. We first selected the sites within temperate regions by filtering all sites between latitudes 25 and 50 degrees in both hemispheres, leading to a total of 2,986 sites (Figure S6). We cannot ascertain the specific hydrological conditions under which these DOC

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Table 1
Analysis of the Spatiotemporal Patterns of Dissolved Organic Matter in the Fluvià River Network

Model	Catchment area		Discharge condition		Catchment area × Discharge condition		Sample size	Adjusted R^2
	F	*p*	*F*	*p*	*F*	*p*		
Dissolved organic carbon	23.58	<0.001	105.28	<0.001	3.27	0.017	229	0.62
Number of molecular formulas	7.78	0.001	11.11	0.002	1.032	0.281	61	0.35
PARAFAC evenness	16.66	<0.001	3.35	0.030	3.31	0.055	198	0.20

Note. Significance of the smoothing terms in the generalized additive models (GAMs) for dissolved organic carbon, the number of assigned formulas derived from FT-ICR-MS analysis, and the evenness of PARAFAC components. The term *discharge condition* is represented by the discharge measured at the river mouth during each sampling campaign.

measurements took place, although the database reflects mostly nonstorm conditions. The median number of DOC measurements per site in the database is five measurements (interquartile range from 2 to 18). We therefore used the median DOC concentration of each site for subsequent analyses. Data points with DOC concentrations above 100 mg C L^{-1} were considered as outliers and excluded from the analysis.

To assess the longitudinal pattern of DOC across temperate river networks we fitted a GAM model to the DOC data that has catchment area as smooth term but that also includes the mean annual precipitation, mean annual terrestrial primary productivity, and average slope of the catchment (data also available in GloRiCh). These variables were included in the GAM to account for climatic and environmental differences among catchments that may create different baselines of DOC and potentially mask the overall pattern along temperate river networks. Variables were first checked for collinearity based on pair-wise Spearman correlations. In addition, latitude and longitude coordinates were included as a Gaussian process smooth to account for spatial autocorrelation. The basis complexity k of the GAM models was set to 10, 15, 30, 30, and 100 for terrestrial productivity, precipitation, slope, catchment area, and latitude/longitude, respectively. We ensured that the choice of basis dimension (k) was sufficiently high using the "gam.check" function in R package mgcv. To confirm the resulting pattern, we additionally performed a random forest analysis with the same model structure as the GAM (excluding the Gaussian process smooth latitude/longitude, which was not statistically significant in the GAM analysis; $F = 0.435$, $p = 0.648$). The GAM and random forest models explained 52% and 62% of the variability in DOC across the temperate data set, respectively. When visualizing the predicted relationship between DOC and catchment area, values for all other predictors were set to their median value in the data set used to fit the GAM and random forest models.

3. Results and Discussion

3.1. Spatiotemporal Dynamics of Riverine DOM

Our results show a clear differentiation between storm and nonstorm conditions in the quantity of DOM. Concentrations of DOC ranged from 0.3 to 6.5 mg C L^{-1} during nonstorm conditions, with a median value of 1.4 mg C L^{-1}, and were consistently higher during storm events (median = 5.5 mg C L^{-1}; range = 3.4–7.7 mg C L^{-1}; Figure 1b). The most striking feature of our data set is, however, the longitudinal patterns of DOC along the river network. During nonstorm conditions the concentration of DOC increased from headwaters to peak in medium-sized streams and then declined downstream while approaching the river mouth, depicting a bell-shaped pattern along the river continuum (Figure 1b). In contrast, patterns of DOC concentration were high and homogeneous during storm events. To validate these patterns, we fitted a GAM to the DOC data, using *catchment area* and *discharge condition* as smooth terms (the latter represented by discharge at the river mouth at any sampling date). The GAM model explained 62% of the DOC data variability across space and time, with both smooth terms and their interaction being statistically significant (Table 1). The results of the model support that there is indeed a bell-shaped pattern along the river continuum and that both DOC concentration and the shape of the pattern change with discharge conditions. The GAM model can also be used to visually illustrate the patterns of DOC across space and time. Gray lines in Figure 1b display the patterns predicted by the GAM model for three specific hydrological conditions that represent low and high discharge (fifth and 95th percentiles of the discharge measured at the river mouth, respectively) and a more extreme, storm event scenario (average discharge of our three storm samplings).

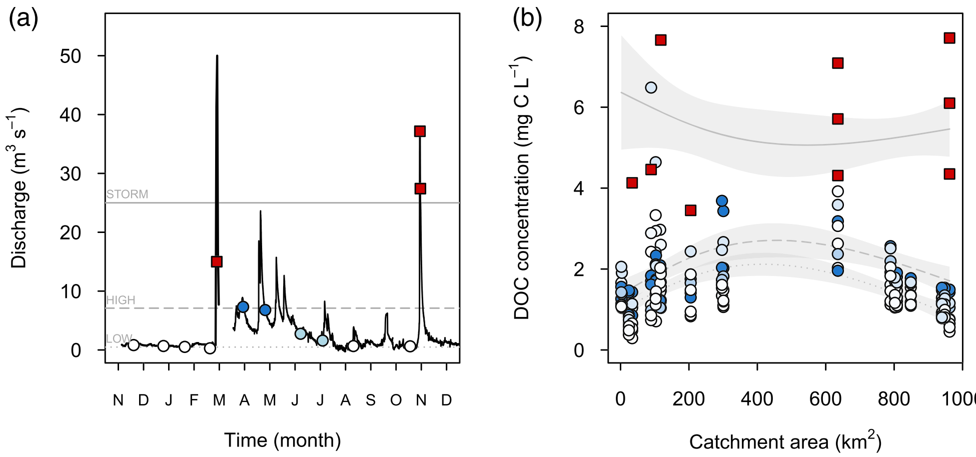

Figure 1. Hydrology and longitudinal patterns of dissolved organic carbon along the river continuum. (a) Hydrograph of the Fluvià River network measured at the river mouth during 2013. Circles show the sampling campaigns and are colored proportionally to discharge; red squares represent sampling during storm conditions. The dotted and dashed horizontal lines display the fifth and 95th percentiles of the annual discharge, representing low and high discharge conditions. The solid horizontal line displays the average discharge of the three storm samplings; (b) dissolved organic carbon (DOC) concentration along the river continuum. Gray lines display the patterns predicted by a generalized additive model for the three hydrological conditions (low, high, and storm) shown in panel (a). Gray envelopes display the 95% confidence interval of the model prediction.

The GAM predictions confirm that the bell-shaped pattern of DOC holds for most of the hydrological year, with the baseline of the pattern increasing from low to high discharge conditions. In contrast, during rare, extreme hydrological events, not only do DOC concentrations show a stark increase but also the pattern becomes flat across the river network (continuous gray line in Figure 1b).

The number of assigned formulae obtained by FT-ICR MS analysis varied from 6,900 to 20,200 during nonstorm conditions (median = 11,100) and was generally higher and less variable during storms (median = 14,500; range = 12,700–15,600; Figure 2a). The evenness of the PARAFAC components was also highly variable during nonstorm conditions (median = 0.92; range = 0.50–0.99), yet contrary to DOC and the number of molecular formulae, it was not higher during storms (Figure 2b). A lower evenness of the PARAFAC components during storms may be explained by the fact that our PARAFAC model includes a protein-like component (C6) that was negligible during storm events (Figure S7). Hence, even though there was a more balanced proportion of the rest of the fluorescence components during storms (higher diversity), the evenness index cannot mathematically reach higher values. This is a clear limitation of assessing DOM diversity through its fluorescence properties. As for the patterns in DOM diversity along the river continuum, both the number of assigned formulae and the evenness of the PARAFAC components also displayed a bell-shaped pattern during nonstorm conditions and a tendency to increase diversity with discharge conditions, which was again statistically validated using GAM models (Table 1). In the case of DOM diversity, the interaction between the smooth terms *catchment area* and *discharge condition* was not statistically significant for the number of assigned formulae yet marginally significant ($p = 0.055$) for the evenness of the PARAFAC components. Thus, while the patterns in DOM diversity seems to become flatter during the sampled storm events (red symbols in Figure 2), further research will be needed to conclude with confidence that the bell-shaped pattern of DOM diversity disappears completely under extreme discharge conditions.

To better understand the measured patterns of DOM quantity and diversity, we evaluated the spatiotemporal dynamics of the different molecular compound categories and fluorescence components of DOM. Figure 3 shows the patterns in relative abundance of aliphatic, aromatic, and highly unsaturated and phenolic compounds along the river network. Among the three compound categories, aliphatics is the one describing an analogous bell-shaped pattern to that of DOC and chemical diversity during nonstorm conditions, also in terms of number of aliphatic formulae (Figure S8). Thus, our molecular data suggest that aliphatic compounds play an important role for the patterns observed in bulk DOC concentration and DOM chemical diversity during nonstorm conditions. With the data available we cannot identify the ultimate origin of these

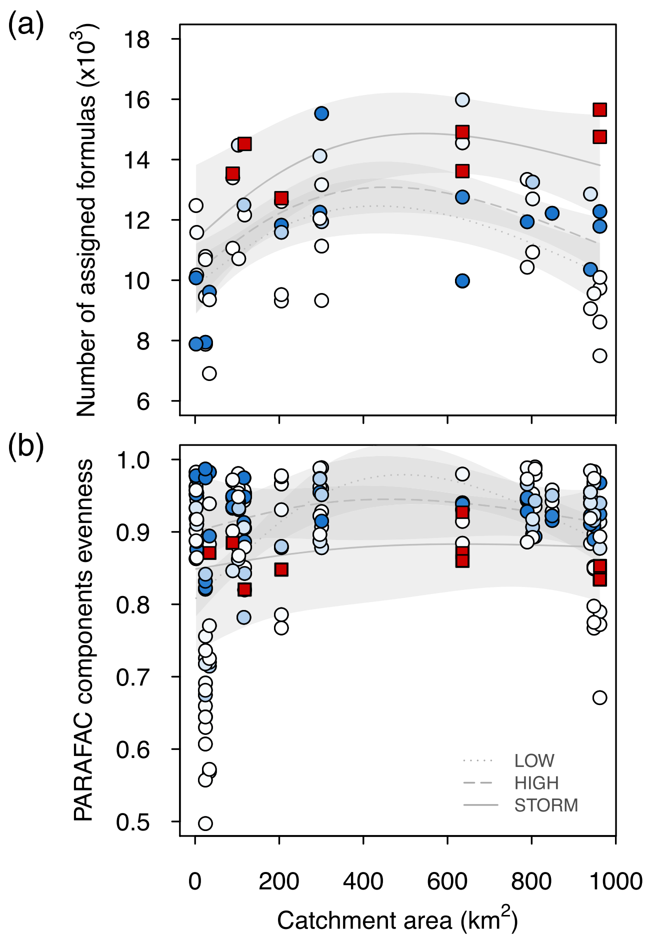

Figure 2. Chemical diversity of dissolved organic matter along the river continuum. (a) Number of assigned formulas derived from FT-ICR-MS analysis. For visual clarity, a point at coordinates (89.5, 20200) is not shown; (b) Pielou's evenness of the six fluorescence PARAFAC components. Circles and squares correspond to nonstorm and storm conditions, respectively. The color code reflects the hydrological condition of the river network and is the same as in Figure 1. Gray lines display the patterns predicted by a generalized additive model for low, high and storm discharge conditions (see Figure 1a and text for details). Gray envelopes display the 95% confidence interval of the models' prediction.

aliphatic compounds, yet recent work on the molecular composition of freshwater DOM has associated aliphatic materials with biodegradable, in situ produced DOM (Kellerman et al., 2014, 2018; Textor et al., 2018). An in situ origin of these aliphatic materials would be supported by two lines of evidence. First, we measured a similar bell-shaped pattern of PARAFAC components C4 and C5, which are typically associated with aquatic DOM sources (Fellman et al., 2010) (Figure S9). Second, Casas-Ruiz et al. (2017) measured significant and frequent net in-stream DOM production in the same river network, particularly in medium-sized streams. Furthermore, the Fluvià network encompasses an increase in agricultural land use from headwaters to medium-sized streams (Figure S2), which is often associated with increasing diffusive inputs of inorganic nutrients (Schindler, 2006) that stimulate autochthonous DOM sources in streams (Wagner et al., 2015; Wilson & Xenopoulos, 2009). Therefore, additions of in situ produced DOM in medium-sized streams and its degradation in larger rivers most likely accounted for an important share of the measured pattern of DOC concentration and DOM diversity in the studied river network during nonstorm conditions. Point source inputs derived from waste water treatment plant effluents or other anthropogenic activities can also introduce protein-like DOM into fluvial systems (Hosen et al., 2014; Parr et al., 2015). Thus, although urban land use is very low across the Fluvià River network, we cannot discard the possibility that urban point source inputs contributed to the increase in DOC concentration and DOM diversity in medium-sized streams.

In addition to aliphatics and protein-like DOM, we measured an analogous bell-shaped pattern for the PARAFAC component C1 (Figure S9), a humic-like component that is widespread in freshwaters but typically highest in streams draining wetlands and forested catchments (Fellman et al., 2010; Kothawala et al., 2015). Hence, our fluorescence data indicate that terrestrial DOM inputs and its degradation further downstream also contributed to the observed bell-shaped patterns of bulk DOC concentration and DOM diversity along the river continuum.

3.2. The Bending DOM Concept

On the basis of these findings, we propose a new conceptual framework that integrates both the spatial and temporal dynamics of DOM for temperate river networks: The *Bending DOM Concept* (Figure 4). During storm events, high stream water velocities and a strong connectivity with organic-rich catchment sources generate a high and uniform pattern of DOC concentration and DOM chemical diversity along the river continuum. In contrast, the pattern bends into a bell-shape with decreasing discharge conditions due to in-stream reactions and spatial changes in the source of DOM. We postulate that, along an average hydrological year, the patterns of DOC concentration and DOM chemical diversity along the river continuum bend and straighten as a function of network hydrological conditions.

Headwaters are strongly connected to the surrounding terrestrial ecosystems. Hence, even though they may harbor intense biogeochemical activity, DOM dynamics in small streams are typically controlled by continuous inputs of terrestrial DOM from the catchment (Creed et al., 2015; Hynes, 1975; Spencer et al., 2019). Under nonstorm conditions most stream flow in headwaters relies on groundwater leaching deeper soils (Freeze, 1972; Tiwari et al., 2014), which usually exhibits low DOC concentrations dominated by aged, plant-derived degraded compounds, and microbial metabolites (Barnes et al., 2018; Inamdar et al., 2011; Lambert et al., 2017; Shen et al., 2015). We hypothesize that this low-DOC groundwater signal is strong in

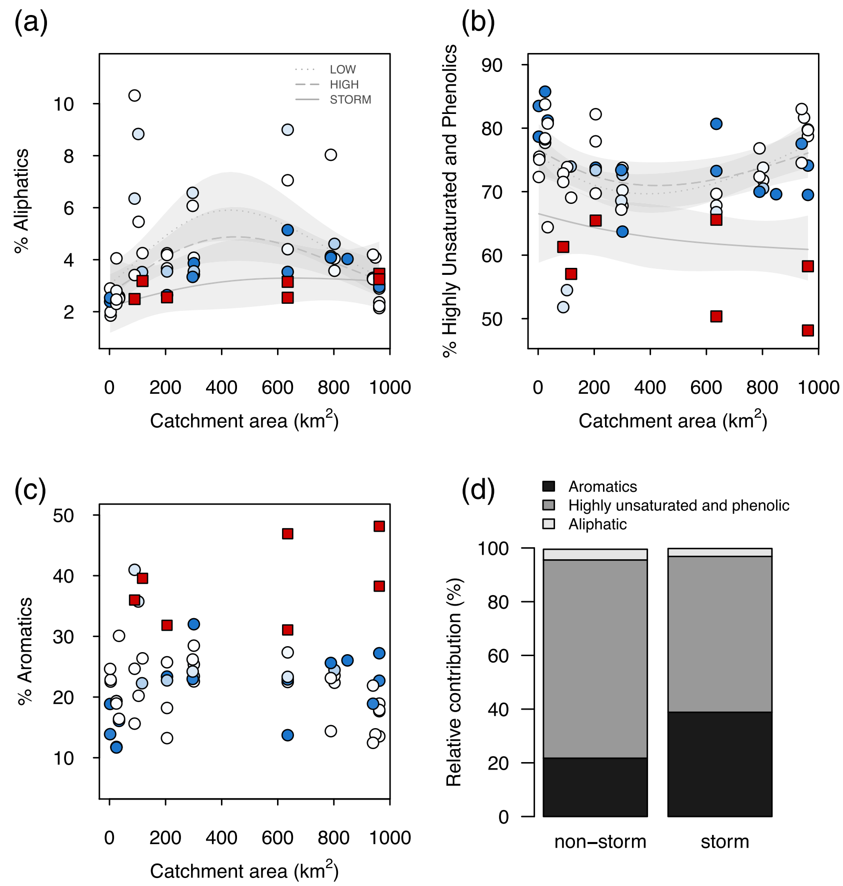

Figure 3. Spatiotemporal variation in the molecular composition of dissolved organic matter. Longitudinal patterns of the relative abundance of (a) aliphatic compounds; (b) highly unsaturated and phenolic compounds; (c) aromatic compounds. Squares and circles indicate storm and nonstorm conditions, respectively. The color code reflects the hydrological condition of the river network and is the same as in Figure 1. Gray lines in (a) and (b) display the patterns predicted by a generalized additive model for low, high, and storm discharge conditions (see Figure 1a and text for details). Model prediction is not shown in panel (c) because no statistically significant ($\alpha = 0.05$) pattern was found for the relative abundance of aromatic compounds. Panel (d) shows the mean contribution in terms of relative abundance of the three compound categories to bulk dissolved organic matter during storm and nonstorm conditions.

headwaters but that it is replaced downstream by the contribution of organic-rich terrestrial sources such as shallower soils (Kaiser & Kalbitz, 2012) and wetlands (Mulholland & Kuenzler, 1979) as well as agricultural and/or urban inputs in many catchments (Graeber et al., 2015; Meng et al., 2013), thereby increasing DOC concentration and DOM chemical diversity downstream (Figures 1b and 2). The confluence of several headwater streams may also contribute to an increase in DOM chemical diversity downstream. In fact, the headwater streams sampled in this study shared only 50% of their molecular formulae, indicating that each stream contributes its unique molecular signature as defined by the drained catchment. This is further substantiated by recent studies in North American headwater streams that identified general similarities in DOM molecular composition but also unique compounds that make each stream DOM pool distinct (Jaffé et al., 2012; Mosher et al., 2015). In addition to increasing DOM chemical diversity, previous studies have shown that the confluence of streams can also contribute to a homogenization of DOC concentrations downstream through hydrological mixing (Creed et al., 2015), a process that is particularly relevant in very small streams draining areas <10 km^2 (Coble et al., 2019; Hale & Godsey, 2019). Since aquatic primary production becomes more important as stream size increases (Battin et al., 2008; Vannote et al., 1980), algal-derived materials may also increase DOC concentration and DOM chemical diversity by the addition of new organic compounds. Algal DOM inputs may be particularly relevant in the presence of agriculture due to larger inputs of inorganic nutrients derived from the use of

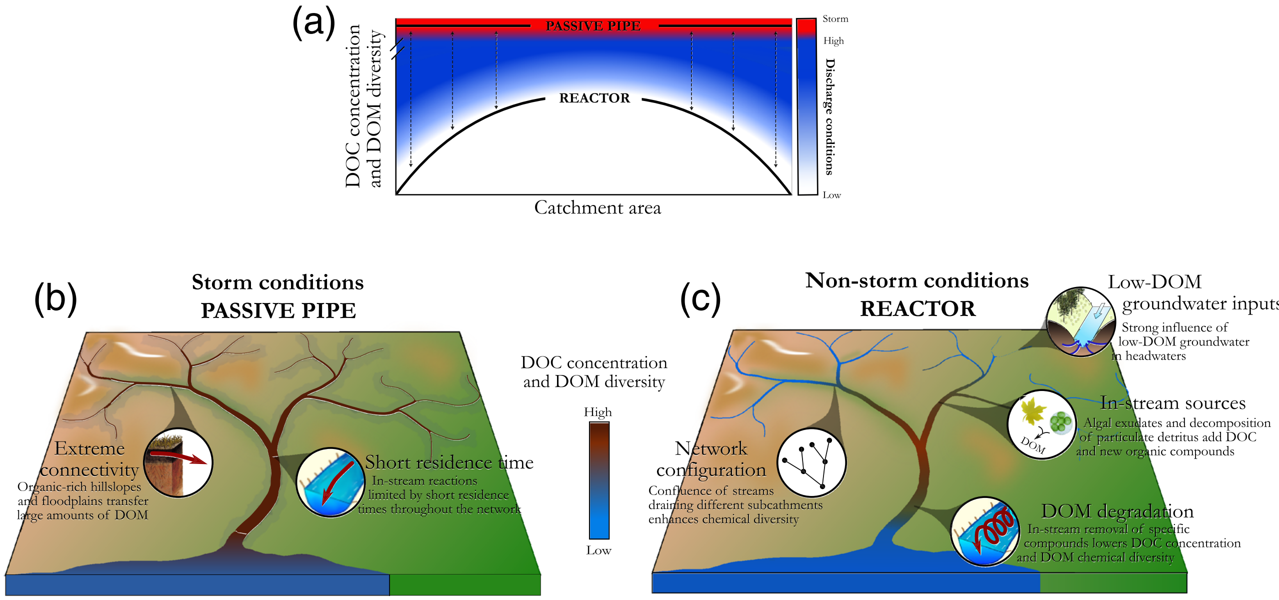

Figure 4. Conceptual representation of the Bending DOM Concept. (a) Longitudinal patterns of dissolved organic matter (DOM) in a theoretical river continuum as a function of network hydrological conditions. Panels (b) and (c) show potential processes that create the patterns during storm and nonstorm conditions, respectively. (b) During extreme discharge events large amounts of terrestrial DOM are transferred from soils to streams, which are then rapidly transported towards the ocean without significant alteration. At such moments fluvial networks behave as *passive pipes* where DOM concentration and chemical diversity are high and steady along the river continuum. (c) In contrast, the pattern bends into a bell-shape with maxima in medium-sized streams with decreasing network discharge due to the influence of DOM-poor groundwater in headwater streams and in-stream degradation of DOM compounds in large rivers. The confluence of tributaries, additions of algal-derived DOM, and decomposition of leaf and wood detritus also contributes to higher DOC and DOM diversity in medium-sized streams. The role of fluvial networks as *reactors* of DOM increases with decreasing discharge due to higher solute residence times in the network (Raymond et al., 2016).

fertilizers (Schindler, 2006) and pesticides (Hébert et al., 2019). In addition, the leaching and decomposition of terrestrial particulate organic matter such as leaves and wood that fall into streams can also contribute to the increase in DOC and likely of DOM diversity in medium-sized streams (McDowell & Fisher, 1976; Meyer et al., 1998). The relative importance of each of these processes to increasing concentration and diversity may vary among catchments and regions, especially if differences in land use and network configuration are important.

In larger rivers, the connectivity with terrestrial DOM sources decreases, and higher water residence times enhance the exposure of DOM to in-stream processes such as flocculation (Sholkovitz, 1976), photo-oxidation (Cory et al., 2014), and biological degradation (Guillemette & del Giorgio, 2011). Furthermore, algal production is often limited by depth and turbidity (Vannote et al., 1980). We therefore hypothesize that, under nonstorm conditions, the decrease in DOC concentration and DOM chemical diversity observed from medium-sized streams to the mouth of the river Fluvià (Figures 1b and 2) is promoted by the in-stream removal of specific compounds. In turn, the steepness of this decrease in DOM quantity and diversity will be regulated by the intensity of in-stream degradation processes. For instance, those large rivers where DOM degradation processes are hindered by environmental factors (e.g., low temperature and high turbidity) may only present a mild decrease or even a relatively constant DOC concentration and DOM diversity along the lower part of the river continuum. Our molecular composition data suggest that aliphatic compounds are the main DOM compounds that are lost in larger rivers, both in number of formulae and relative abundance (Figures 3 and S8). Nonetheless, other macromolecules that are traditionally thought to be chemically stable such as lignin have also been shown to be degraded upon entering river networks (Ward et al., 2013). Biological degradation of terrestrial compounds in large rivers may be additionally favored by previous phototransformation (Stubbins et al., 2010; Tranvik & Bertilsson, 2001) as well as by priming through additions of biolabile algal DOM (Bianchi, 2011; Guenet et al., 2014; Ward et al., 2016).

Our data set of the river Fluvià shows that the bell-shaped pattern of DOM quantity and diversity holds during most (>95%) of the hydrological year but that the baseline of the pattern raises with increasing discharge conditions (Table 1 and Figures 1b and 2). Such an increase in the pattern baseline was particularly evident for one of the humic-like components traditionally classified as of terrestrial origin (component C2), which showed an almost flat pattern yet a conspicuous increase in its relative abundance from low to high flows (Figure S9). In contrast, none of the materials previously related to aquatic DOM sources in the literature (aliphatics and protein-like DOM) increased their relative contribution with increasing discharge conditions (Figures 3 and Figure S9). Thus, changes in the source and magnitude of terrestrial DOM inputs are most likely behind the elevation of the pattern in DOM quantity and composition from low to high discharge conditions. This would be consistent with the recent findings by Zarnetske et al. (2018), who analyzed data on DOC concentrations and discharge across U.S. rivers to demonstrate that the delivery of DOM from the catchment to streams is transport limited. Hence, organic matter stocks in soils and wetlands may provide ample DOM to maintain delivery to rivers, but the actual transfer is limited by hydrological connectivity between terrestrial and aquatic ecosystems. We posit that, during nonstorm conditions, the bell-shaped patterns of DOC concentration and DOM diversity rise and fall as a function of the hydrological condition of the network due to changes in the connectivity with terrestrial DOM sources.

In contrast to the pattern observed during nonstorm conditions, we found that the concentration of DOC and the chemical diversity of DOM were high and homogeneous along the river continuum during storms events. Our molecular composition data show an apparent shift toward more aromatic and less processed materials with lower H/C and O/C ratios during storm events (Supplementary Figure S10), and this translates into an almost two-fold increase in the average relative abundance of aromatic compounds (Figure 3d). A high network homogeneity and enrichment in DOC and aromatics during and after storm peak discharge have been recently observed in a temperate forested watershed (Wagner et al., 2019) and are consistent with previous research showing that storm and flushing events maximize the connectivity between river networks and their catchment (Fasching et al., 2015; Laudon et al., 2011; Spencer et al., 2008), adding DOM from additional sources such as organic-rich surficial soils, hillslopes (Inamdar et al., 2011; Sanderman et al., 2009), and floodplains (Junk et al., 1989) that are often disconnected from streams. Previous studies have described hysteretic patterns of DOC concentrations and DOM composition during storms (Butturini et al., 2006; Vaughan et al., 2017; Wagner et al., 2019). Therefore, although not captured by our sampling design in the Fluvià River, the amount, composition, and diversity of DOM may change rapidly along the rising and falling limbs of storm peaks. Our results indicate that extreme discharge events transfer large amounts of aromatic, highly diverse DOM from the terrestrial landscape to streams. These materials, however, have limited opportunity to be processed within the river network due to extreme water velocities that reduce water residence times (Bernal et al., 2019; Butturini et al., 2016; Casas-Ruiz et al., 2017; Raymond et al., 2016; Wollheim et al., 2018).

The spatiotemporal patterns in DOC concentration and DOM chemical diversity found in the Fluvià River network appear robust and provide a testing ground for future research to unravel the processes that create the emerging bell-shaped pattern as well as the factors that modulate its form. We predict that, in the case of DOC concentration, the form of the pattern will depend mostly on the catchment's organic matter stock and its transfer efficiency to streams, as well as on the position of distinct functional units along river continua. For example, the location of wetlands will simultaneously affect the height and skewness of longitudinal patterns by adding large amounts of aromatic DOM to the river network (Mulholland & Kuenzler, 1979). In the presence of lowland wetlands, DOC maxima could even be found close to the river mouth. With respect to DOM chemical diversity, we foresee the assortment of DOM sources in the catchment (height) and the dendritic configuration of the river network (skewness) as the main shape modulators. Other potentially important functional units include lakes, reservoirs and floodplains (McCluney et al., 2014) as well as inputs of sewage and other anthropogenic DOM sources including agricultural products (Spencer et al., 2019; Wagner et al., 2015; Williams et al., 2016). In addition, factors controlling in-stream processes such as microbial community composition, temperature, nutrient availability, suspended sediments, and level of irradiance will play a critical role in shaping DOM, especially in large rivers and therefore in modulating the declining limb of the bell-shaped pattern.

3.3. Patterns of DOM Across Temperate River Networks

We have developed the Bending DOM Concept based on the structure and functioning of the Fluvià River, which is characterized by the particular bell-shaped pattern of DOM concentration and diversity along the

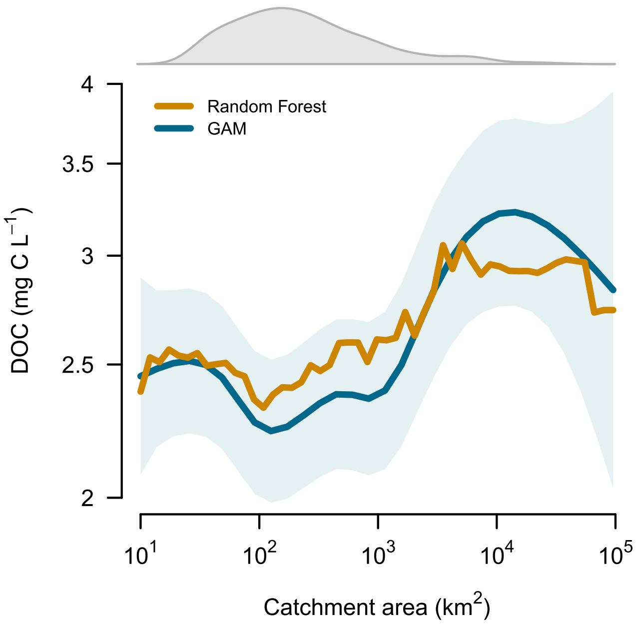

Figure 5. Relationship between dissolved organic carbon concentration (DOC) and catchment area across temperate streams and rivers. Partial dependence of dissolved organic carbon (DOC) on catchment area estimated through general additive modeling (GAM) and random forest analysis (see section 2 and Tables S2 and S3 for results). Lines represent the overall pattern of DOC along the temperate river continuum once the effects of different terrestrial net primary production, mean annual precipitation, and slope of the catchment are accounted for. Values for all predictors other than catchment area were set to their median value in the dataset used to fit the GAM and random forest models. The envelope corresponds to the 95% confidence interval of the GAM analysis. The catchment area data distribution is shown on top of the main panel.

river continuum. Yet this does not necessarily mean that the patterns measured in our system are entirely representative of temperate river networks worldwide. Unfortunately, whereas a wealth of DOC concentration data with global coverage is available in the literature, there is a relative paucity of data on DOM molecular composition. Yet in our data set the number of assigned formulae was positively correlated with DOC concentration (Spearman rho = 0.69, $p < 0.0001$), a correlation that has been also found in boreal lakes and Himalayan fluvial networks (Hemingway et al., 2019; Kellerman et al., 2014) and that together suggests a strong link between the amount and diversity of DOM along the aquatic continuum. We therefore evaluated the commonality of the bell-shaped pattern of DOM quantity and diversity by analyzing data on riverine DOC concentration available from the GloRiCh database (Hartmann et al., 2014), which covers a large fraction of the temperate biome and encompasses a range of catchment areas spanning several orders of magnitude. Sites available in GloRiCh are geographically distributed across wide climatic, topographic, and biogeochemical gradients, which create different baselines of river DOC concentration across regions that may mask the overall pattern. Thus, in this case we used a GAM analysis that has catchment area as smooth term but that also includes terrestrial primary productivity, mean annual precipitation, and average slope of the catchment to account for these climatic and environmental effects on DOC concentration (see section 2 for details on the analysis and Table S2 for model results). The GAM analysis shows that, while there is no clear pattern in small streams and rivers (catchment area <100 km^2), there is a generalized increase in DOC concentration toward medium-sized temperate rivers (Figure 5). In larger rivers, however, it is not clear that DOC concentrations decrease downstream as we observed in the Fluvià River. In fact, the wide 95% confidence interval of the GAM at large catchment areas indicates that multiple shapes are possible. Thus, whereas some river networks may present a full bell-shaped pattern, in other cases DOM degradation in large rivers may be too low or be offset by additional DOM inputs (e.g., from organic-rich and lowland wetlands), keeping DOC concentration relatively constant or even increasing along the lower part of the river continuum.

Patterns of DOC concentration and DOM diversity in regions other than the temperate zone might also differ from the ones we present here. As an example, small headwater streams in the boreal biome are strongly influenced by organic-rich riparian zones and wetlands (Ledesma et al., 2017; Schiff et al., 1998), and thus DOC concentration maxima in boreal river networks will likely be shifted toward headwaters (Tiwari et al., 2017). In this regard, a critical direction for future work is to test the Bending DOM Concept in other temperate networks but also to constrain the concept in other regions such as the arctic, boreal, and tropical zones, where additional elements and processes such as permafrost or a high connectivity with floodplains may play an important role. We also anticipate that highly complex river networks such as those subject to severe flow intermittence or influenced by glaciers may deviate from our predictions and will require particular attention.

Data Availability Statement

The two data sets of the Fluvià River used to generate the results have been deposited in Figshare Digital Repository (https://figshare.com/projects/Bending_DOM_concept/80270).

Conflict of Interest

The authors declare no competing interests.

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