Universitat de Girona

CONTROLS ON THE DYNAMICS OF RIVERINE DISSOLVED ORGANIC MATTER: INSIGHTS FROM A MEDITERRANEAN RIVER NETWORK

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Doctoral Thesis

Controls on the dynamics of riverine dissolved organic matter

Insights from a Mediterranean river network.

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The thesis contains one annex containing the supplementary material at the end of the document.

Hereby, Dr. Rafael Marcé of the Catalan Institute for Water Research and Dr. Pilar López from the University of Barcelona,

CERTIFY:

That the doctoral thesis entitled "Controls on the dynamics of riverine dissolved organic matter: Insights from a Mediterranean river network" submitted by Joan Pere Casas Ruiz has been completed under our supervision and meets the requirements to opt for the *International Doctor* mention.

In witness whereof and for such purposes as may arise, the following certification is signed:

Dr. Rafael Marcé   Dr. Pilar López

Girona, April 2017

A l'avi,

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LIST OF ADDITIONAL PUBLICATIONS

In addition to the papers included in this thesis, I have contributed to the following works:

- von Schiller, D, Marcé R, Obrador B, Gómez-Gener L, Casas-Ruiz JP, Acuña V, Koschorreck M (2014). Carbon dioxide emissions from dry watercourses. *Inland Waters* 4: 377–382.

- Abril M, Muñoz I, Casas-Ruiz JP, Gómez-Gener L, Barceló M, Oliva F and Menéndez M (2015). Effects of water flow regulation on ecosystem functioning in a Mediterranean river network assessed by wood decomposition. *Science of the Total Environment* 517:57–65.

- Gómez-Gener L, Obrador B, von Schiller D, Marcé R, Casas-Ruiz JP, Proia L, Acuña V, Catalán N, Muñoz I, Koschorreck M (2015). Hot spots for carbon emissions from Mediterranean fluvial networks during summer drought. *Biogeochemistry* 19:1-18.

- López P, López-Tarazón JA, Casas-Ruiz JP, Pompeo M, Ordoñez J, Muñoz I (2015). Sediment size distribution and composition in a reservoir affected by severe water level fluctuations. *Science of the Total Environment* 540:158–167.

- Gómez-Gener L, Obrador B, Marcé R, Acuña V, Catalán N, Casas-Ruiz JP, Sabater S, Muñoz I, von Schiller, D (2016). When Water Vanishes: Magnitude and Regulation of Carbon Dioxide Emissions from Dry Temporary Streams. *Ecosystems*, 19(4): 710-723.

- Proia L, von Schiller D, Gutierrez C, Casas-Ruiz JP, Gómez-Gener L, Marcé R, Obrador B, Acuña V, Sabater S (2016). Microbial carbon processing along a river discontinuum. *Freshwater Science* 35(4): 1133-1147.

- Gómez-Gener L, von Schiller D, Marcé R, Arroita M, Casas-Ruiz JP, Staehr PA, Acuña V, Sabater S, Obrador B (2016). Low contribution of internal metabolism to carbon dioxide emissions along lotic and lentic environments of a Mediterranean fluvial network. *al   al   a Biogeosciences* 121(12): 3030-3044.

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SUMMARY

Dissolved organic matter (DOM), composed of thousand of organic compounds, is a central component of global biogeochemical cycles. In river networks, DOM constitutes the major pool of organic carbon, and plays a key role as energy source, control on underwater light regimes, and modulator of toxic substances availability. The quantity and composition of riverine DOM depends on the combination of different sources as well as the in-stream processes DOM undergoes during transport. Yet not all DOM is processed or reacts in the same way. Depending on its chemical composition, some DOM fractions are more susceptible to in-stream processing than others; and even when chemical properties are similar, differences in environmental factors such as temperature, nutrients, irradiance, or water residence time may lead to different DOM fate. Detangling how the interplay between chemical composition and environmental factors modulate the processing of DOM remains challenging, but is crucial to understand river networks functioning and their role in global carbon cycling. Furthermore, the quantity and composition of riverine DOM highly vary with space and time, but the controls on the spatiotemporal dynamics of DOM in river networks are poorly understood.

In this thesis, we provide insights into the controls on riverine DOM processing, and into how in-stream reactions and changing DOM sources modulate DOM dynamics in river networks. To attain this, we evaluated the quantity and composition of DOM across a medium-sized river network (Fluvià River, North-East of the Iberian Peninsula) throughout a full hydrological year, covering from headwaters to the river mouth and from drought to storm conditions. Several techniques were employed to characterize DOM, including absorbance-fluorescence spectroscopy, size-exclusion chromatography, stable and radiocarbon isotopes, and ultrahigh resolution mass spectrometry. In addition, ex situ incubations were used to assess the biodegradability and biodegradation kinetics of bulk and different DOM size-fractions.

The results of this thesis are organized in four scientific papers that focus each on different aspects of riverine DOM dynamics. In paper I we focus on the biodegradation kinetics of different DOM size fractions under laboratory conditions. Using different discrete and continuous decay models, we identify a faster and predictable decay of the DOM fractions grouping the smallest and largest DOM compounds, while humic substances present an unpredictable, non-monotonic pattern during degradation. In paper II, we assess the sources of DOM and the DOM pool that is processed in different riverine environments during a drought episode, including isolated water parcels, running water reaches, and small weirs. Here we find that drought induces great spatial heterogeneities in the quantity and

composition of DOM resulting from varying sources at each of the environments. In paper III, we evaluate changes in DOC and DOM composition along several stream and river reaches to detangle the controls on DOM in-stream dynamics at the network scale. In this study, we find water residence time as the major constrain for DOM processing, and identify DOM properties and nutrient availability as the controls on the net balance between in-stream gains and losses of DOM. Finally, in paper IV we investigate patterns of DOM concentration and chemical diversity along river continua. Here, we identify an emerging global pattern of DOC with maxima in medium-sized rivers, and propose a conceptual framework to comprehend and predict the spatiotemporal dynamics of DOM in river networks.

The findings derived from this thesis open new windows into the controls of riverine DOM quantity and composition, and provide critical information for scaling up globally relevant processes such as the processing of terrestrial organic carbon in river networks and its export to the ocean.

RESUM

La matèria orgànica dissolta (DOM), constituïda per milers de compostos orgànics, és un component central dels cicles biogeoquímics globals. En xarxes fluvials, la DOM és el major reservori de carboni orgànic, i hi juga un paper clau com a font d'energia, control del règim lumínic de la columna d'aigua i modulador de la reactivitat i disponibilitat de substàncies tòxiques. La quantitat i composició de la DOM fluvial depèn de la combinació de diferents fonts així com del processos i transformacions que pateix durant el transport riu avall. Però no tota la DOM reacciona de la mateixa manera. En funció de la composició química, algunes fraccions de la DOM són més susceptibles a reaccionar que no pas altres; i fins i tot quan les propietats químiques són semblants, diferències en factors ambientals com la temperatura, els nutrients, la llum o el temps de residència de l'aigua poden donar lloc a diferents resultats. Entendre com la interacció entre la composició química i factors ambientals modulen el processament de la DOM és complex, però és vital per entendre el funcionament dels sistemes fluvials i el seu paper en el cicle global del carboni. A més, la quantitat i composició química de la DOM varia enormement amb l'espai i el temps, però el controls de les dinàmiques espacials i temporals de la DOM en xarxes fluvials estan encara per resoldre.

L'objectiu d'aquesta tesi és el d'entendre com diferents fonts i processos modulen les dinàmiques de la DOM en xarxes fluvials. Per tal d'assolir-ho, es va avaluar la concentració i composició de la DOM al llarg d'una xarxa fluvial (Riu Fluvià, Nord-Est de la península ibèrica) al llarg d'un any hidrològic, incloent des de rius de capçalera fins la desembocadura, i des d'episodis de tempesta fins a sequera. Es van utilitzar vàries tècniques per a la caracterització de la DOM, incloent-hi espectroscopia de absorbància i fluorescència, cromatografia per exclusió de mida, isòtops estables i radioactius i espectrometria de masses d'alta resolució. A més, es van dur a terme incubacions ex situ per tal d'analitzar la biodegradabilitat i cinètiques de degradació de la DOM i les seves diferents fraccions de mida.

Els resultats d'aquesta tesi estan organitzats en quatre articles científics, cadascun dels quals adreça diferents aspectes de les dinàmiques de la DOM fluvial. En un primer article s'investiguen les cinètiques de degradació de diferents fraccions de DOM en condicions de laboratori. Utilitzant diferents models discrets i continus de degradació, en aquest treball identifiquem un degradació ràpida i previsible de les fraccions que agrupen els compostos més grans i petits de la DOM, mentre que les substàncies húmiques presenten patrons de degradació no monotònics i imprevisibles. En el segon article s'investiguen les fonts de DOM i les fraccions que són processades en diferents ambients fluvials durant un episodi de sequera, des de basses aïllades i trams d'aigua corrent fins a petites preses. En aquest treball trobem que la sequera indueix una gran heterogeneïtat espacial en la quantitat i composició

de la DOM a conseqüència de fonts i processos contrastats en els diferents ambients. En el tercer article s'avaluen canvis de concentració i composició al llarg de diferents trams de riu per identificar els controls del processament de DOM a escala de conca. Aquí trobem que el temps de residència de l'aigua és el factor clau que determina que la DOM reaccioni o pel contrari es transporti passivament aigües avall. A més, les propietats de la DOM i la disponibilitat de nutrients s'erigeixen com els determinants del balanç net de generació i retenció de DOM en sistemes fluvials. Finalment, en un quart article s'investiguen patrons de concentració i diversitat química de DOM al llarg del continu fluvial. En aquest treball s'identifica un patró global emergent de concentració de DOM amb el màxim situat en rius de mida mitjana, i es proposa un marc conceptual per entendre i predir les dinàmiques espacials i temporals de la DOM en xarxes fluvials.

Les troballes derivades d'aquesta tesi representen un pas endavant en la difícil tasca d'entendre els controls de la concentració i composició de DOM fluvial, i ofereixen informació vital per a l'extrapolació de processos de rellevància global com el processament de carboni orgànic terrestre i la seva exportació cap a l'oceà.

RESUMEN

La materia orgánica disuelta (DOM), constituida por miles de compuestos orgánicos, es un componente central de los ciclos biogeoquímicos globales. En redes fluviales, la DOM es el mayor reservorio de carbono orgánico, y juega un papel clave como fuente de energía, control del régimen lumínico de la columna de agua y modulador de la reactividad y disponibilidad de sustancias tóxicas. La cantidad y composición de la DOM fluvial depende de la combinación de diferentes fuentes así como de los procesos y transformaciones que sufre durante su transporte río abajo. Pero no toda la DOM reacciona de la misma manera. En función de la composición química, algunas fracciones de la DOM son más susceptibles a reaccionar que otras; e incluso cuando las propiedades químicas son similares, diferencias en factores ambientales como la temperatura, los nutrientes, la luz o el tiempo de residencia del agua pueden dar lugar a diferentes resultados. Entender cómo la interacción entre la composición química y factores ambientales modulan el procesamiento de la DOM es complejo, pero es vital para entender el funcionamiento de los sistemas fluviales y su papel en el ciclo global del carbono. Además, la cantidad y composición química de la DOM varía enormemente con el espacio y el tiempo, pero los controles de las dinámicas espaciales y temporales de la DOM en redes fluviales están aún por resolver.

El objetivo de esta tesis es el de entender como diferentes fuentes y procesos modulan las dinámicas de la DOM en redes fluviales. Para lograrlo, se evaluó la concentración y composición de la DOM a lo largo de una red fluvial (Río Fluvià, Noreste de la Península Ibérica) a lo largo de un año hidrológico, incluyendo desde ríos de cabecera hasta la desembocadura, y desde episodios de tormenta hasta sequía. Se utilizaron varias técnicas para la caracterización de la DOM, incluyendo espectroscopía de absorbancia y fluorescencia, cromatografía por exclusión de tamaño, isótopos estables y radiactivos y espectrometría de masas de alta resolución. Además, se llevaron a cabo incubaciones ex situ para analizar la biodegradabilidad y cinéticas de degradación de la DOM y sus diferentes fracciones de tamaño.

Los resultados de esta tesis están organizados en cuatro artículos científicos, cada uno de los cuales se centra en diferentes aspectos de las dinámicas de la DOM fluvial. En un primer artículo se investigan las cinéticas de degradación de diferentes fracciones de DOM en condiciones de laboratorio. Utilizando diferentes modelos discretos y continuos de degradación, en este trabajo identificamos una degradación rápida y previsible de las fracciones que agrupan los compuestos más grandes y pequeños de la DOM, mientras que las sustancias húmicas presentan patrones de degradación no monotónicos e imprevisibles. En el segundo artículo se investigan las fuentes de DOM y las fracciones que son procesadas en diferentes ambientes fluviales durante un episodio de sequía, desde balsas aisladas y tramos de agua

corriente hasta pequeñas presas. En este trabajo encontramos que la sequía induce una gran heterogeneidad espacial en la cantidad y composición de la DOM, consecuencia de fuentes y procesos contrastados en los diferentes ambientes. En el tercer artículo se evalúan cambios de concentración y composición a lo largo de diferentes tramos de río para identificar los controles del procesamiento de DOM a escala de cuenca. Aquí encontramos que el tiempo de residencia del agua es el factor clave que determina que la DOM reaccione o por el contrario se transporte pasivamente aguas abajo. Además, las propiedades de la DOM y la disponibilidad de nutrientes se erigen como los determinantes del balance neto de generación y retención de DOM en sistemas fluviales. Finalmente, en un cuarto artículo se investigan patrones de concentración y diversidad química de DOM a lo largo del continuo fluvial. En este trabajo se identifica un patrón global emergente de concentración de DOM con el máximo situado en ríos de tamaño medio, y se propone un marco conceptual para entender y predecir las dinámicas espaciales y temporales de la DOM en redes fluviales.

Los hallazgos derivados de esta tesis representan un paso adelante en la difícil tarea de entender los controles de la concentración y composición de DOM fluvial, y ofrecen información vital para la extrapolación de procesos de relevancia global como el procesamiento de carbono orgánico terrestre y su exportación hacia el océano.

PREFACE

Dissolved organic matter (DOM) is nothing more than a mixture of organic compounds that water inherits from soils, leaves, braches, and whatever organic material it encounters while draining a landscape. If you come, like I do, from the Mediterranean region you may not be familiar with DOM. However, I bet you once ran into a small pond with a mysterious dark brown color, something that reminded you an over-steeped tea. That was DOM; and despite sometimes imperceptible, it is ubiquitous in all aquatic ecosystems on Earth, where it plays a fundamental role in many ecological processes. For instance, because of its color, DOM absorbs a share of the light that reaches the oceans, lakes and rivers surface, controlling how deep light penetrates into the water. I actually really like to introduce the role DOM plays in aquatic ecosystems with the following statement:

> *"W   a   t   t   a   a al   t   t   v l m b tt   a t   t,*
> *DOM is more akin to an equalizer whereby different components of aquatic ecosystems are filtered to different extent "*

This was written few years ago by Yves Prairie, a Canadian professor of Limnology[1] that summarized in a perspective paper the importance of understanding what DOM is and what it does. Perhaps you are aware of my devotion to sound engineering (or at least now you do), so I guess you can imagine how exciting my first encounter with Prairie's paper was. Anyway, what this statement intended to say is that DOM modulates many biogeochemical processes that take place in aquatic systems as well as many other aspects that are of critical human concern, for example the formation of carcinogenic products in drinking water and the availability of heavy metals in lakes and rivers. DOM has therefore gained attention in the last decades, and it has been investigated in all type of aquatic systems, from the ocean to alpine small streams, and even in urban artificial waters.

This work is about riverine DOM, that is, the DOM that is transported trough streams and rivers. River networks contain some of the most complex, dynamic and diverse ecosystems on Earth. One could actually say that riverine ecosystems are 'the hotspot of hotspots', as they harbor not only a disproportionately large biodiversity but also numerous biogeochemical reactions and ecological interactions. In this thesis, with the priceless help of my colleagues, I try to shed light into the complex functioning of river networks through a detailed analysis of riverine DOM. In particular, I investigate how different sources and biogeochemical processes control the spatial and temporal dynamics of DOM, with special emphasis on the factors that constrain its reactivity and fate.

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[1]The term "Limnology" derives from the ancient Greek word λίμνη (limne), meaning lake or pond, and is widely understood as the study of inland waters or non-marine aquatic ecosystems. This includes streams, rivers, lakes, ponds, estuaries, wetlands, ephemeral washes, and even dry areas of watercourses that recurrently desiccate.

1 INTRODUCTION

What is dissolved organic matter and why do we care about it

DOM can be defined as all organic matter that passes through a filter of specified pore size, usually ranging from 0.2 to 0.7 μm. This is an entirely operational definition, yet carries with it important consequences that clearly differentiate DOM from its counterpart, particulate organic matter. Essentially, DOM is smaller and travels with water, which may sound of little importance but is fundamental. On one hand, organic matter is the basis of heterotrophic metabolism, but only small compounds of about 500–1,000 Daltons are transported through the microbial cell membrane (Nikaido and Vaara 1985; Weiss et al 1991). DOM constitutes therefore the primary source of energy to microbial metabolism in riverine ecosystems. On the other hand, DOM is not subject to gravitational settling, and thus represents the major form of organic matter in transport in streams and rivers (Naiman 1982). But these are only two of the features that made riverine DOM a hot research topic in the last decades. DOM is involved in many biogeochemical processes that constitute the basis of stream and river ecosystem functioning: it regulates bacterial nutrient uptake (Bernhardt and Likens 2002), shapes the composition of microbial communities (Judd et al 2006), alters the bioavailability of toxic substances (Aiken et al 2011), and influences underwater light regimes (Kirk 1994) to name a few.

From a more biogeochemical perspective, DOM has received special attention in the last few years because of the central role it plays in the global carbon (C) cycle. DOM constitutes the major pool of organic C in streams and rivers (Fisher and Likens 1973; Volk et al 1997), and so its transport *per se* represents a relevant lateral flux of C from the continents to the ocean (Aufdenkampe et al 2011). However, streams and rivers do much more than just passively shunt terrestrial C downstream. Instead, they actively transform, store and outgas more than 75% of the C they

receive from terrestrial ecosystems (Cole et al 2007; Tranvik et al 2009; Battin et al 2009; Bastviken et al 2011; Raymond et al 2013; Borges et al 2015). And DOM plays a main role in this story. For instance, DOM is involved through its in-stream mineralization to the emission of significant amounts of carbon dioxide to the atmosphere (Duarte and Prairie 2005; Raymond et al 2013); and its flocculation and subsequent settling contributes to C burial in sediments (Cole et al 2007; Wachenfeldt and Tranvik 2008). Therefore, unraveling the sources of DOM and the factors that favor its processing in river networks has taken on a new urgency.

A primer on the characterization of dissolved organic matter

Because DOM is a complex amalgam of compounds, understanding DOM dynamics in river networks requires an accurate characterization of its properties. The amount of DOM in rivers is commonly measured as DOC via combustion or other oxidation techniques. Thus, the terms DOC and DOM are sometimes used interchangeably, yet DOC strictly refers to the carbon content of DOM[2]. In the case of composition, there are several analytical tools available to characterize DOM at different levels, of which a selection is presented below.

The burgeoning use of optical techniques, namely absorbance and fluorescence, has tremendously advanced our capability of detecting differences in DOM composition (Fellman et al 2010). Metrics like the DOC specific UV absorbance, the absorbance spectral slope, or the humification index give information on the aromaticity (i.e. the content of benzene or equivalent ring molecules) and apparent molecular size of DOM (Zsolnay et al 1999; Weishaar et al 2003; Helms et al 2008), and can be used, for instance, to assess DOM sources to aquatic systems (Huguet et al 2009; Catalán et al 2014). In addition, the combination of fluorescence data with multi-component deconvolution techniques has proven to be useful in tracking changes in DOM composition (Kothawala et al 2012; Mostovaya et al 2016). Optical techniques are often chosen as a tool to characterize DOM because of the relatively low cost of analysis and low demands on sample preparation.

DOM can also be described according to its apparent molecular weight distribution. Usual techniques to attain this include, but are not limited to, size exclusion chromatography (SEC; e.g., Kothawala et al 2006) and fractionation via ultrafiltration (e.g., Amon and Benner 1996). Both techniques separate DOM by the hydrodynamic volume of its constituents. SEC analysis often use UV-Vis absorbance

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[2] In this text, I will use DOM when referring broadly to the compartment of dissolved organic compounds, and DOC when discussing only its concentration in terms of C.

to detect chromatographic DOM fractions; nevertheless, differences in DOM specific absorptivity or the presence of optically invisible DOM may introduce bias (Pereira et al 2014). To overcome this, an organic C detector can be coupled to SEC, providing thus a more accurate depiction of the molecular-weight distribution of DOM (Huber et al 2011).

Stable and radiocarbon isotopes of DOC can also be used to characterize DOM, as the isotopic signature of DOC varies with source, age, and history of degradation. For example, terrestrial DOM derived from C3 and C4 plants have different isotopic composition due to different fractionation during photosynthesis (Marshall et al 2007), and can be easily discriminated by their ^{13}C signature. Radiocarbon (^{14}C) data provide useful information about the age of DOM, and can also be used to assess the sources of DOM to river networks. For instance, it has been successfully used to detect the signature of ancient permafrost (Spencer et al 2015) and atmospheric deposition of fossil fuel combustion byproducts in streams and rivers (Stubbins et al 2012). In addition, stable and radiocarbon isotopes provide a powerful tool to infer the pools of DOM that are incorporated into or metabolized by microbial communities (e.g., McCallister and Del Giorgio 2012; Guillemette et al 2015; Hotchkiss and Hall 2015)

More recently, the use of advanced molecular-level techniques has opened new windows into the dynamics of DOM in inland waters. Ultrahigh resolution mass spectrometry currently stands apart as the most powerful tool to characterize DOM, as it provides information on the thousands of compounds that constitute DOM mixtures. Due to the high mass accuracy (<1 ppm), using this tool we are able to precisely and unambiguously assign molecular formulas to each individual compound, although structural information cannot yet be obtained because isomers are not distinguished. Furthermore, when coupled to a soft ionization introduction system that disperses colloidal and loosely associated DOM into individual compounds, ultrahigh resolution mass spectrometry provides information on the actual molecular weight of DOM molecular constituents (as opposed to the apparent molecular-weight obtained through SEC and other filtration techniques). The use of molecular-level techniques has notably advanced our understanding of DOM degradation processes in aquatic systems (Stubbins et al 2010; Singer et al 2012), and has helped identifying the drivers of DOM variability across climatic regions (Jaffé et al 2012; Kellerman et al 2014). Nevertheless, despite the increasing availability of data at different levels, much work still remains to comprehend the dynamics of riverine DOM as well as the controls on its processing.

What we know and what is yet to be known

Research during the last three decades has tremendously advanced our knowledge of riverine DOM. We now know that DOM is made up of a complex mixture of organic compounds of varying origin, age, size and reactivity, and that it undergoes several in-stream processes during transport.

Depending on its origin, riverine DOM can be classified as either aquatic when produced within the river or terrestrial when imported from land. Aquatic sources include the in-stream production of DOM by algae, bacteria, fungi and macrophytes (Bertilsson and Jones 2003). In contrast, terrestrial DOM originates from the leaching of plants and organic soils of the catchment, and its transfer to streams is mostly regulated by the advective transport of surface runoff and subsurface water flows (Aitkenhead-Peterson et al 2003). The amount and composition of riverine DOM result from the mixing of these different sources in addition to biogeochemical processes that take place within river networks. Some examples of these processes are mineralization via microbial or photochemical degradation (Cory et al 2014; Fasching et al 2014), sedimentation out of the water column via flocculation (Sholkovitz 1976; Wachenfeldt and Tranvik 2008), or incorporation into higher trophic levels (Pace et al 2004; Fellman et al 2015). A mechanistic understanding of these processes is paramount to advance knowledge on the dynamics of riverine DOM, yet progress has been hampered by the high compositional complexity of DOM.

DOM comprises thousands of degradation by-products and freshly produced compounds with different properties (Koch and Dittmar 2006; Kellerman et al 2014; Raymond and Spencer 2015), and these compounds generate gradients of reactivity, from highly reactive to chemically stable. Thus, treating DOM as a single pool of molecules with shared properties may result into mismatches between mechanistic models and empirical observations. It is therefore imperative to detangle how in-stream processes affect the different compounds or fractions of DOM. But constraining DOM chemical composition might be not enough to predict the fate of DOM in river networks. Recent advances in soil science have led to the emerging view that organic matter persistence is controlled not only by its chemical composition but also by physical, chemical and biological factors from the surrounding environment (Schmidt et al 2011; Dungait et al 2012). This has helped explaining, for example, why thermodynamically unstable organic compounds can remain intact in deep soil profiles for decades, centuries or even millennia, and why these same compounds are degraded once transferred to streams and rivers (Marín-Spiotta et al 2014). Understanding how the interplay between DOM chemical composition and environmental factors modulate the processing of DOM in streams and rivers remains challenging, yet is central to constrain the role of river networks in C cycling.

River networks comprise longitudinal gradients of altitude, slope, riparian cover and catchment land use; and these gradients translate in turn into changes in hydraulics, light availability, nutrient loads, and connectivity with terrestrial ecosystems (Vannote et al 1980; Minshall et al 1985). Hence, the sources and processing of DOM may highly vary along a river continuum, and so may its amount and composition (Vannote et al 1980; Creed et al 2015). Likewise, temporal changes in hydrology can directly affect DOM in streams and rivers. For instance, since water residence time in the network controls the opportunity for DOM to react (Battin et al 2008; Weyhenmeyer et al 2012; Oldham et al 2013), the in-stream processing of DOM might be important during low flow periods while practically insignificant during storm events (Raymond et al 2016). Nevertheless, current knowledge on the spatiotemporal dynamics of DOM in river networks is mostly theoretical (Vannote et al 1980; Creed et al 2015; Raymond et al 2016), hampered by the lack of empirical datasets encompassing full river continua under different hydrological conditions. It is particularly unclear what are the sources and in-stream processes that shape riverine DOM during extreme events such as drought episodes and storms, despite these are recognized as hot moments of DOM processing and export to oceans, respectively (Raymond and Saiers 2010; Butturini et al 2016). In this sense working in Mediterranean networks appears ideal, as they are highly dynamic systems that experience a wide range of hydrological conditions, from floods to very low flows (Gasith and Resh 1999; Bernal et al 2013). Notably, during drought episodes, many river networks get intensely contracted due to lowering water tables, and even flow interruption may occur in some parts of the network if drought conditions last enough (Dahm et al 2003; Vazquez et al 2011). This phenomenon is more than usual in arid and semi-arid regions worldwide, but how drought episodes and particularly flow interruption affect DOM dynamics in river networks remain poorly understood.

2 AIMS AND OUTLINE

The work in this thesis aimed to provide new insights into the sources and processing of riverine DOM. More specifically I aimed to answer the following questions:

- What are the factors that promote in-stream processing versus passive transport of DOM in rivers? (Paper III)
- Is all DOM processed in the same way? (Papers I and III)
- How do extreme hydrologic events influence the sources and processing of DOM? (Papers II and IV)
- Can we predict the spatial and temporal dynamics of DOM in river networks? (Papers III and IV)

In paper I we tackle the question of whether all DOM is equally processed. We incubate water samples from different riverine environments and track the biodegradation kinetics of bulk DOM and different DOM size fractions with several decay models, from single-exponential to reactivity continuum models. In paper II, we look into the sources of DOM during drought episodes. To do so, we perform a detailed characterization of the DOM properties in different riverine environments that are typically found during drought, including isolated water parcels, running water reaches, and small weirs. In addition, we use stable and radiocarbon isotopes of DOC and dissolved inorganic C to identify the pools of DOM that are processed in each of the environments. In paper III, we evaluate changes in DOC and DOM composition along several stream and river reaches to detangle the factors that promote DOM in-stream processing at the network scale. In paper IV, we investigate patterns of DOC and DOM chemical diversity along a river continuum under different hydrological conditions, from low to storm flows. Additionally, we explore a global data set of DOC concentrations to test the commonality of the patterns we find, and build a novel conceptual framework for the spatiotemporal dynamics of DOM in river networks.

3 METHODS OVERVIEW

This section provides a brief summary of the study site characteristics and the materials and analytical methods used for the development of this thesis. The section is focused on DOM characterization methods, as it is a cross cutting theme in the four papers that constitute the core of the thesis. Detailed information of these and additional methods can be found in the respective papers.

Study site and hydrological context

The works included in this thesis were carried out during a two-year period in the Fluvià, a Mediterranean river network located in the North East of the Iberian Peninsula. Fluvià's main stem is 97 km long, with a mean flow of 3.6 m^3 s^{-1} at the basin outlet, and its river 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. Climate is typically Mediterranean, with air temperatures ranging from 6 to 26°C over the year and an annual mean precipitation of 660 mm. The Mediterranean climate is characterized by high seasonality and variability, with drought periods in summer and intense rainfall events in autumn and spring. The hydrological regime of Mediterranean river networks generally follows that of the rainfall pattern, and thus streams and rivers recurrently experience dry and wet periods throughout a hydrological year. Notably, during summer drought, scarce precipitation and high evapotranspiration rates in the catchment drastically reduce discharge in streams and rivers, which may even dry up in some areas of the network (Bernal et al 2013). On the contrary, intense precipitation events in autumn and spring can lead to extreme discharge events. In this sense, Mediterranean river networks make up a perfect scenario to test DOM dynamics under a range of hydrological conditions.

The four papers included in this thesis encompass different spatial and temporal scales, from the reach to the globe and from a single snapshot to a full hydrological year (Fig. 1). For papers I and II, we collected water samples in a single campaign from an upstream, medium-sized river reach during the summer drought of 2013 (Fig. 2 and 3). For paper III and IV, water samples were collected from several sites across the entire Fluvià river network, from headwaters to the river mouth (Fig. 2). In this case samplings were repeated ten times throughout a hydrological year in order to cover different hydrological conditions (Fig. 3). In addition, for paper IV we used a global database on river chemistry data (Hartmann et al 2014) to explore general longitudinal patterns of DOM.
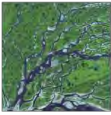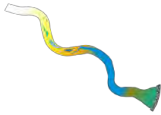
Fig. 1. Schematic overview of the spatial and temporal scales for which dissolved organic matter dynamics were addressed in each of the four papers comprising this thesis.
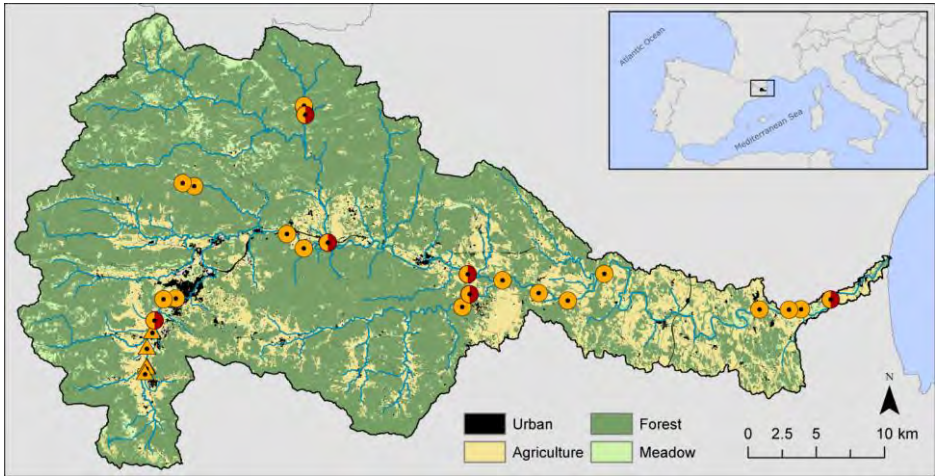
Fig. 2. Location of the sampling sites along the Fluvià River network (North-East of the Iberian Peninsula). Triangles indicate sampling sites for papers I and II, and circles correspond to papers III and IV. Symbols with a red half indicate sites that were sampled also during storm events for paper IV. The inset shows the location of the Fluvià watershed in South-Western Europe
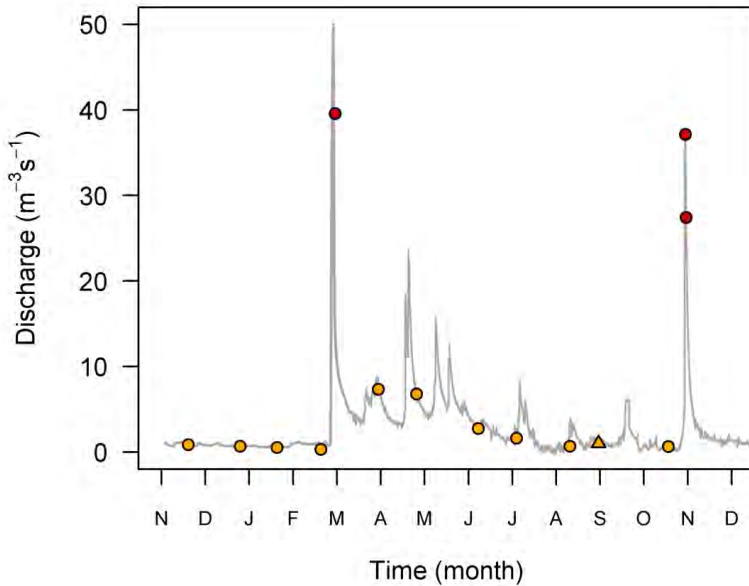
Fig. 3   ). Symbols indicate the sampling dates over the hydrograph (grey line) in the Fluvià river mouth. The triangle corresponds to the two studies carried out during a drought episode (papers I and II). Circles correspond to papers III and IV, with red symbols indicating samplings during storm conditions (paper IV). The shape and color of the symbols match those of the map (Fig. 1)

DOM characterization

DOC concentration was measured by high-temperature catalytic oxidation on a Shimadzu TOC-V CSH analyzer (Shimadzu Corporation, Japan). The following analytical methods were employed to characterize the optical properties and chemical composition of DOM.

Absorbance and fluorescence

Uv-vis absorbance spectra (190 to 800 nm) were measured on an Agilent 8453 diode array spectrophotometer (Agilent Technologies, Germany) using a 1-cm quartz cuvette. Excitation-Emission matrices (EEMs; Fig. 4a) were obtained using a fluorescence spectrophotometer (F-7000, Hitachi, Japan) with a 1-cm quartz cuvette. EEMs were collected by measuring fluorescence intensity across the excitation range set from 248 to 449 nm and the emission range set from 250 to 550 nm, both at 3 nm increments. All EEMs were read in sample to reference signal ratio mode, and were corrected for instrument-specific biases after calibrating the instrument with Rhodamine B solution. EEMs were blank subtracted, corrected for inner-filter effects, and normalized to the Raman peak area. Parallel Factor Analysis (PARAFAC) (Stedmon et al 2003) was used to decompose all EEMs (N=250) obtained from the Fluvià into six independent components that were validated through visual inspection of the residuals and split-half analysis.
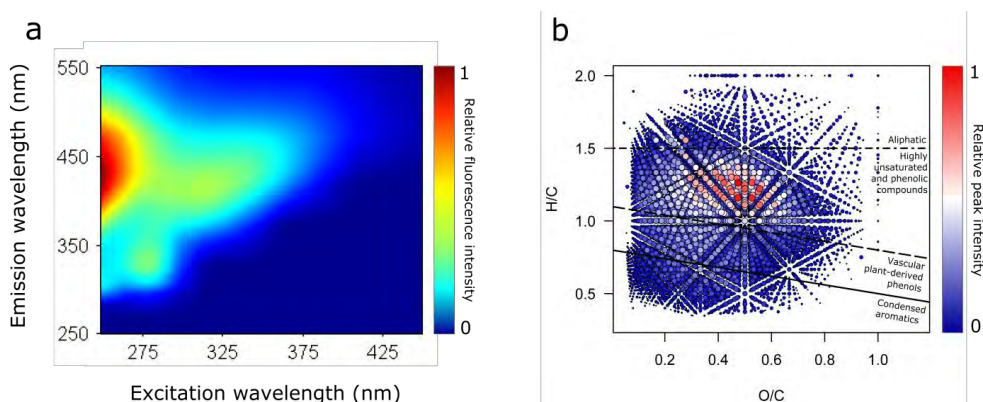
Fig. 4. Example of a fluorescence excitation-emission matrix (**a**) and a van Krevelen diagram (**b**) of riverine DOM. The categorical classification of compounds in panel **b** is based on Kellerman et al. (2014)

Size exclusion chromatography

SEC analysis was performed at the Leibnitz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany. In brief, DOC passed through a size-exclusion column packed with resin (Toyopearl HW 50S, volume of 250 x 20 mm). Phosphate buffer (0,029 mol L^{-1}, pH 6,5) was used as eluent in a flow rate of 1.1 ml min^{-1}. DOC was detected by infrared absorbance of CO_2 after UV oxidation at 185 nm in a cylindrical UV thin-film reactor (Graentzel-reactor). Fractions were identified by using fulvic and humic acid standards from the International Humic Substances Society (IHSS). In brief, the chromatograms were deconvoluted and apparent size fractions assigned based on the retention time and column affinity obtained for the IHSS standards. Fraction assignment was done by using the software FIFFIKUS (DOC-Labor Huber, Karlsruhe). Note that, in paper I, we refer to this analysis as LC-OCD, which stands for liquid chromatography organic carbon detection.

Stable and radiocarbon isotopes

In paper II we measured the ^{13}C and ^{14}C signature of DOM to constrain its origin. For analysis of ^{13}C, water samples of 10 mL volume were stabilized with 10 µL $HgCl_2$ and sent to the Colorado Plateau Laboratory (USA) for conventional isotopic ratio mass spectrometry. For ^{14}C analysis, water was filtered in the lab within 24 hours after collection (pre-combusted Whatman GF/F) and then enriched at 60 °C by evaporation with UHP nitrogen. Processed samples were measured by accelerator mass spectrometry at the Leibniz-Laboratory for Radiometric Dating and Isotope Research in Kiel, Germany.

Ultrahigh resolution mass spectrometry

Information at the molecular level of DOM was obtained through ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). This technique unambiguously assigns the molecular formula of each of the thousands of compounds that make up DOM mixtures. DOM was solid-phase extracted with 50-mg Bond Elut PPL cartridges (Agilent Technologies, Santa Clara, CA, USA) following the method that is described in detail in 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 0.01N HCl, and dried with N_2 gas prior to elution of the 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. Negative-ions produced by electrospray ionization (ESI) were directly infused into a custom-built 9.4 Tesla FT-ICR mass spectrometer

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(Tallahassee, FL, USA) (Blakney et al 2011; Kaiser et al 2011). Molecular formulas were assigned with an in-house software (EnviroOrg™, developed by Yuri E. Corilo) following an internal "walking" calibration of each mass spectrum (Savory et al 2011). The molecular composition of DOM is shown in van Krevelen diagrams (Kim et al 2003; Fig. 4b). The diagram is constructed using the elemental ratios H/C and O/C as the ordinate and abscissa, respectively. Since compound classes (e.g. lignin, carbohydrates, etc) have their own characteristic H/C and O/C ratios, different classes plot in different regions of the van Krevelen diagram, providing a visual representation of the molecular composition of DOM mixtures.

4 RESULTS & DISCUSSION

This section contains the four papers that constitute the main body of the thesis. The supplementary information for the papers is attached at the end of the thesis as an annex. Note that the labeling of the figures resets for each paper.

PAPER I

Biodegradation kinetics of dissolved organic matter
chromatographic fractions in an intermittent river
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RESEARCH ARTICLE

Biodegradation kinetics of dissolved organic matter chromatographic fractions in an intermittent river

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Abstract Controls on the degradation of dissolved organic matter (DOM) are complex but key to understand the role of freshwaters in the carbon cycle. Both the origin and previous degradation history have been suggested to determine DOM reactivity, but it is still a major challenge to understand the links between DOM composition and biodegradation kinetics. An appropriate context to study these links are intermittent rivers, as summer drought naturally diversifies DOM sources and sinks. Here we investigated the biodegradation kinetics of DOM in the main aquatic environments present in a temporary river. During dark incubations we traced the dynamics of bulk DOM and its main chromatographic fractions defined using LC-OCD: high molecular weight substances (HMWS), low molecular weight substances (LMWS), and humic substances and building blocks. Bulk DOM decay patterns were successfully fitted to the reactivity continuum (RC) biodegradation model. The RC parameters depicted running waters as the sites presenting a more reactive DOM, and temporary pools, enriched in leaf litter, as the ones with slowest DOM decay. The decay patterns of each DOM fraction were consistent throughout sites. LMWS and HMWS decayed in all cases and could be modeled using the RC model. Notably, the dynamics of LMWS controlled the bulk DOM kinetics. We discuss the mechanistic basis for the chromatographic fractions' kinetics during biodegradation and the implications that preconditioning and summer drought can have for DOM biodegradation in intermittent rivers.

1. Introduction

Organic matter degradation is a major component of the global carbon cycle [*Ciais et al.*, 2013]. Analyzing degradation data with process-based biogeochemical models is essential to quantify the turnover of organic matter in terrestrial [*Manzoni et al.*, 2012] and aquatic ecosystems [*Koehler et al.*, 2012; *Arndt et al.*, 2013]. Therefore, a number of models have been developed in order to describe the kinetics of organic matter decomposition in litter and soils [*Bosatta and Ågren*, 1995; *Koehler and Tranvik*, 2015], marine sediments [*Westrich and Berner*, 1984; *Middelburg*, 1989; *Boudreau and Ruddick*, 1991], and freshwater ecosystems [*Vähätalo et al.*, 2010; *Koehler et al.*, 2012]. Indeed, the decomposition of dissolved organic matter (DOM) in freshwaters has relevance at the global scale [*Cole et al.*, 2007; *Tranvik et al.*, 2009] and is known to be faster than in other Earth compartments [*Catalán et al.*, 2016]. Hence, for a comprehensive understanding of the carbon cycling in freshwater ecosystems, it is fundamental to unravel the kinetics of DOM biodegradation.

Classical models of organic matter biodegradation include discrete models based on a constant decay rate for the bulk organic matter (first-exponential decay models [*Berner*, 1964]) and multicomponent models that separate organic matter into a few groups with a distinctive decay rate [*Westrich and Berner*, 1984; *Canfield*, 1994]. Alternatively, continuum models provide a more realistic framework that assumes a continuous distribution of an infinite number of reactive types [*Boudreau and Ruddick*, 1991; *Bosatta and Ågren*, 1995; *Koehler et al.*, 2012]. However, although these models describe the behavior of DOM decay quite accurately, the lack of established links between biodegradation kinetics and the composition of DOM is a major shortcoming in our understanding of the carbon cycle in freshwaters [*Findlay and Sinsabaugh*, 2003]. Coupling compositional fractions with operational reactive types defined from the decay models is not straightforward.

DOM compositional fractions can be defined in terms of size or molecular weight [*Kothawala et al.*, 2006], optical properties [*Fellman et al.*, 2010], elemental composition [*Kellerman et al.*, 2014], or functional groups [*Helms et al.*, 2014], among others. Most of these techniques have been applied to assess the effects of

biodegradation on DOM composition, but usually the kinetics of compositional changes are not assessed [*Helms et al.*, 2008; *Paul et al.*, 2012]. Some studies using spectroscopic measurements have shown increases and decreases in DOM fluorescent fractions [*Guillemette and del Giorgio*, 2012; *Catalán et al.*, 2013a; *Sleighter et al.*, 2014], but assessing the contribution of different pools to bulk DOM decay remains essential to mechanistically describe the biodegradation process.

DOM compositional changes during biodegradation will be a function of the origin of DOM and the microbial community composition as well as of multiple physicochemical factors. These factors can influence both the composition and structure of the DOM (e.g., irradiation or pH) and also the biodegradation process itself (e.g., microbial function or temperature). However, the initial DOM composition has been shown to define DOM degradation patterns and subsequently the parametrization of the applied decay model. For example, significantly different parameters of the reactivity continuum model (RC) have been reported for DOM of clear and brown water lakes [*Koehler et al.*, 2012], and for the DOM of algal and terrestrial sources [*Guillemette et al.*, 2013]. Moreover, the character of the DOM sources is defined not only by its autochthonous or allochthonous origin but also by the previous processing they have undergone [*Catalán et al.*, 2013a]. Therefore, the spectrum of systems along the inland water continuum should constitute a complex and heterogeneous mosaic of DOM reactivities.

Interestingly, such heterogeneities in DOM sources and processing can be relevant not only at a regional but also at catchment and even reach scales. For instance, a distinguishing feature of temporary rivers (i.e., waterways that recurrently cease to flow at some points along their course) is a high level of spatial habitat heterogeneity, which has its maximum during dry periods [*Gasith and Resh*, 1999; *Steward et al.*, 2012]. In such periods, one may find a mixed sequence of running waters and isolated water pools over small distances, with each habitat presenting different sources of DOM and water residence times (and thus different degrees of degradation) [*Vazquez et al.*, 2011; *von Schiller et al.*, 2015]. In addition, since most temporary rivers are located in arid and semiarid regions, this heterogeneity is enhanced by a myriad of retention structures in response to high water supply demand [*Döll et al.*, 2009; *Zarfl et al.*, 2014]. Thus, during dry periods, temporary rivers turn into a very heterogeneous set of aquatic habitats, which may contain DOM with contrasting composition and degradation kinetics.

Here we aim to evaluate the biodegradation dynamics of DOM in the different aquatic environments present in a river undergoing summer drought (temporary pools, running water reaches and, a weir in its tail and dam). We analyzed the biodegradation patterns of bulk DOM and the compositional fractions in terms of size-exclusion chromatography by adjusting different decay models. We expect DOM decay kinetics to be different for each aquatic environment due to contrasting sources and degradation histories. Also, we aim to identify the chromatographic fraction driving the bulk DOM biodegradation patterns and to test if the reactivity continuum model (RC) is appropriate to capture the behavior of each independent fraction.

2. Materials and Methods

2.1. Study Site and Sampling

We collected water samples from different aquatic environments along the Fluvià river network (NE of the Iberian Peninsula) during September 2013. The Fluvià is a Mediterranean clear water river draining a predominantly calcareous watershed through mixed forests (79%), agricultural (18%), and urban (3%) areas. The Mediterranean climate has a dry and hot summer period during which the river flow gradually decreases, followed by flow cessation and the formation of isolated water pools. We selected different environments representative of the summer drought conditions linked to flow cessation, described to be diverse in terms of DOM sources and water residence time [*Casas-Ruiz et al.*, 2016]. Along a 7 km reach of the main stem and upstream to downstream, we sampled two isolated pools of less than 25 m^2 (A_Pool and B_Pool; Table 1 and supporting information Figure S3), two running water reaches (C_Run and D_Run), and finally two points in a small weir, the first one in the river mixing point (E_WTail) and the second near the dam (F_WDam). Isolated pools were 500 m away from each other and were located in shaded areas under riparian canopy, both with visible accumulation of tree leaves. Discharge in running water reaches was 0.122 m^3 s^{-1}, with a water velocity of 0.07 m s^{-1}, and a width and mean water depth of 5.1 and 0.33 m, respectively. The area, volume, and water residence time of the weir were 1.24 hm^2, 0.0174 hm^3, and 1.65 days, respectively. The water depth in

Table 1. Location and Physicochemical Properties of the Sampling Sites[a]

Site	Location		DOC(mg C L^{-1})	TDN(mg N L^{-1})	T(°C)	pH	Cond(µS cm^{-1})	DO(%)
	UTM x	UTM y						
A_Pool	454408	4663864	3.9 ± 0.1	1.5 ± 0.1	16.4	7.3	344	17
B_Pool	454536	4664300	4.2 ± 0.1	1.5 ± 0.1	16.5	7.2	336	3
C_Run	454549	4665842	1.3 ± 0.0	3.6 ± 0.1	15.1	7.7	724	81
D_Run	454919	4666891	1.5 ± 0.2	4.9 ± 0.1	16.0	7.7	610	113
E_WTail	456045	4669233	2.4 ± 0.2	2.9 ± 0.1	16.9	8.2	522	97
F_WDam	456464	4669278	2.2 ± 0.3	4.1 ± 0.4	16.0	8.2	506	78

[a]DOC, dissolved organic carbon; TDN, total dissolved nitrogen; T, water temperature; Cond, conductivity; and DO, dissolved oxygen saturation.

the weir was 0.6 m in E_WTail and 3.9 m in F_WDam. Samples were stored in the dark at 4°C until the experiment started within 24 h of water collection.

2.2. Experimental Setup

Water was filtered through 1.2 µm precombusted filters to remove larger particles (Whatman GF/C) and then through 0.2 µm filters (nylon membrane filters, Whatman, UK). All filter units were rinsed with Milli-Q water before use, and care was taken to avoid high pressure during the filtration process to prevent bursting of cells and unintended release of DOM. Samples were incubated in 60 mL precombusted glass vials with an inoculum (1.2 µm filtered water of each corresponding site) added in a 1:10 v/v proportion. No differences were found for initial bacterial abundance between the different sites (see section 2.3 and supporting information Figure S2). Samples were replicated 3 times and incubated in the dark at 20°C for 65 days. Different subsets of vials were prepared and sacrificed at each experimental time (0 h, 6 h, 18 h, 2 days, 4 days, 7 days, 14 days, 28 days, and 65 days). The difference in dissolved organic carbon (DOC) between the initial and the final time was considered the biodegradable DOM (BDOM).

2.3. DOM Properties and Bacterial Abundance

Prior to qualitative and quantitative DOM analysis, incubated samples were refiltered through 0.2 µm prerinsed filters to eliminate any newly formed bacterial biomass. Dissolved organic carbon (DOC) concentrations were determined in a Shimadzu TOC-VCS (Shimadzu Corporation, Japan) by high-temperature catalytic oxidation. The detection limit of the analysis procedure was 0.05 mg C L^{-1}. All DOC samples were acidified to pH 3.5 with 2 M HCl and preserved at 4°C in the dark until analysis.

The DOM size fractions were characterized by liquid chromatography-organic carbon detection (LC-OCD) following the procedure described in *Huber et al.* [2011]. In brief, LC-OCD is an automated size-exclusion chromatography system (SEC), coupled to a combination of UV and IR organic carbon detection [*Huber et al.*, 2011]. Filtered samples passed through a size-exclusion column packed with resin (Toyopearl HW 50S, volume of 250 × 20 mm). Phosphate buffer (0.029 M, pH 6.5) was used as eluent at a flow rate of 1.1 mL min^{-1}. DOC was detected by infrared absorbance of CO_2 after UV oxidation at 185 nm in a cylindrical UV thin-film reactor (Graentzel reactor). Fractions were identified by using fulvic and humic acid standards from the International Humic Substances Society [*Huber et al.*, 2011]. Analysis of the fractions was done by using the software FIFFIKUS (DOC-Labor Huber, Karlsruhe, Germany). LC-OCD allowed to differentiate three DOM fractions as in *Graeber et al.* [2015]: (i) high molecular weight substances (HMWS) of hydrophilic character (e.g., biopolymers like polysaccharides, proteins, or amino sugars), (ii) humic-like substances and building blocks (HS and BB), acids containing carboxyl and phenolate groups, and their breakdown products with a similar retention time as humic and fulvic substances standards, and (iii) low molecular weight acids and neutrals such as alcohols, ketones, sugars, or amino acids, combined as the low molecular weight substances (LMWS) fraction (more details in the supporting information Figure S4).

Bacterial abundance was measured following the procedure detailed in *Porter and Feig* [1980]. In brief, bacterial cells were counted in 20 fields per filter with epifluorescence microscopy (Nikon Eclipse 80i, Japan) after appropriate dilution (5–200) and staining (15 min) of samples with DAPI (4,6-diamidino-2-phenylindole; 2 µg mL^{-1}) and subsequent fixation on black polycarbonate filters (0.2 µm and 5 µm; Nuclepore, Whatman).

2.4. Modeling DOM Biodegradation

DOM biodegradation kinetics can be described using multiple models. Here we applied a reactivity continuum model, which considers each compound as a reactive type assumed to degrade following a first-order kinetics. Therefore, based on the probability distribution of reactivities, different groups of compounds or fractions degrading at specific decay rates (k) can be defined too. The initial distribution of reactivities most frequently chosen is the Gamma distribution [*Boudreau and Ruddick*, 1991; *Arndt et al.*, 2013], which captures very well the organic matter biodegradation dynamics observed in inland waters [*Koehler et al.*, 2012].

Following an approach as in *Koehler et al.* [2012], we used a Gamma distribution and modeled the relative DOC concentration (DOC_t/DOC_0) over time (t) as

$$\frac{DOC_t}{DOC_0} = \left(\frac{\alpha}{\alpha + t}\right)^\nu$$

where the parameter α (h) controls the lifetime of the most reactive compounds [*Arndt et al.*, 2013] or the apparent initial age of the bulk DOM [*Koehler et al.*, 2012], and ν (unitless) determines the shape of the distribution and is related with the prevalence of recalcitrant compounds. Therefore, high ν and low α values indicate dominance of labile compounds in the DOM, while low ν and high α values indicate a dominance of refractory compounds and low degradability [*Arndt et al.*, 2013]. Throughout the manuscript k (h^{-1}) states for the decay rate of DOM and from the fitted RC models, its decrease over the incubation time is calculated as $\nu/(\alpha + t)$ [*Koehler et al.*, 2012]. Model parameters were estimated using nonlinear regression package *nlme* in R. In order to test the RC model assumption, we fitted the data also to simple models usually used in OC decomposition modeling—a linear regression and a single-pool exponential model [*Olson*, 1963]—and calculated the Akaike weights of the three models, following the procedure described in *Koehler and Tranvik* [2015].

To determine the model best describing the patterns of change of each fraction from the LC-OCD, we followed a similar procedure but including also generalized additive models (GAM) in order to identify nonmonotonic trends not considered in the exponential-based models [*Manzoni and Porporato*, 2007]. We interpret the GAM fit as a null model, a flexible function not affected by prior assumptions based on the exponential decay behavior. Therefore, this fit will not allow to obtain a decay rate describing the biodegradation of a DOM fraction but might identify consistent temporal trends across the incubations. GAM models were fitted using *mgcv* Package in R [*R Core Team*, 2015] with cubic regression spline as the smoothing function. The degree of freedom was automatically selected with generalized cross validation in the GAMs.

In order to assess the relationship of the LC-OCD fractions with the decay rates, we performed linear regression analysis. Moreover, to check that these relationships were independent of the effect of DOM concentration, we performed a semipartial correlation analysis. To do so, we calculated the residuals of a linear regression between the natural logarithm of k and DOC_t/DOC_0 and related them to the LC-OCD fractions contribution. Regression analyses were validated by examining residuals and colinearity between explanatory variables. Variable values in the text are given as mean $\pm$ standard error unless stated otherwise. All the analyses were performed using R 3.2.2 [*R Core Team*, 2015].

3. Results

3.1. Site DOM Characterization

Initial DOM concentrations and quality differed between sites (Table 1 and Figure 1). The temporary pools showed the highest DOC concentrations ($4.21 \pm 0.06 \, mg \, C \, L^{-1}$), followed by the weir ($2.51 \pm 0.56 \, mg \, C \, L^{-1}$) and the running water sites ($1.34 \pm 0.01 \, mg \, C \, L^{-1}$).

The predominant chromatographic fractions in the initial samples were LMWS and HS and BB (Figure 1). The HMWS presented very low concentrations, being over the detection limit only in the pool sites (A_Pool: $0.31 \pm 0.04 \, mg \, C \, L^{-1}$ and B_Pool: $0.32 \pm 0.01 \, mg \, C \, L^{-1}$). Also, A_Pool and B_Pool presented higher concentrations of the HS and BB fraction than of the LMWS fraction, while an opposite composition was found for running water sites, with higher values of LMWS than of HS and BB. In the weir sites, LMWS were at higher concentrations than HS and BB, but the concentration of HS and BB was much lower in the dam (F_WDam) than in the tail (E_WTail).
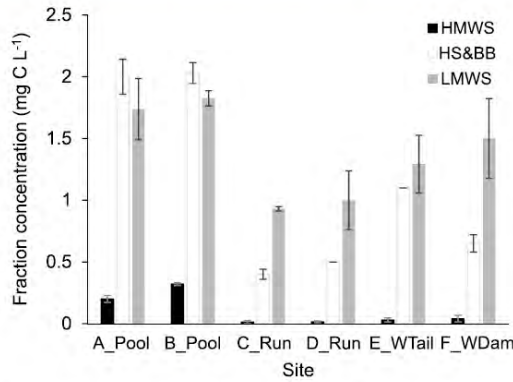
Figure 1. Initial composition of the DOM (mean ± SE) in the studied sites in terms of chromatographic fractions [*Huber et al.*, 2011]: high molecular weight substances (HMWS), low molecular weight substances (LMWS), and humic substances and building blocks (HS and BB). A_Pool and B_Pool refer to the two isolated pools, C_Run and D_Run to the running water reaches, E_WTail to the weir at its river mixing point, and F-WDam at its dam.

There was DOM consumption in all the incubations. The proportion of biodegraded DOM varied between 3 and 46%. The running waters showed the highest proportion of DOM biodegraded (42 ± 4% in C_Run and 41 ± 0% in D_Run), and lower values were found in the two pools (26 ± 1% in A_Pool and 32 ± 3% in B_Pool). In the weir, different amounts of DOM were consumed in the Tail (11 ± 8% E_WTail) and in the Dam (35 ± 5% F_WDam).

3.2. Bulk DOM Biodegradation Patterns

The different systems presented differentiated patterns in bulk DOM degradation, being similar for the two pools and the two running water reaches between them but not for the tail and dam of the weir (Figure 2).

The RC model properly described the degradation curves of the bulk DOM in all the sites (Figures 2 and 3 and Table 2). According to the model, the average lifetime of the most reactive compounds (a) was longer in the two pools than in the running waters or weir sites. Regarding parameter v, related with the relative preponderance of the most recalcitrant compounds, the weir sites presented lower values than the pools and the running waters. All the sites showed high initial decay coefficients (Figure 3a and Table 2), but they were higher in the running water sites and the two points within the weir than in pools. k declined slowly over time in pools, while sites C_Run and E_WTail presented a very strong initial decrease in decay rates followed by B_Run and F_WDam.
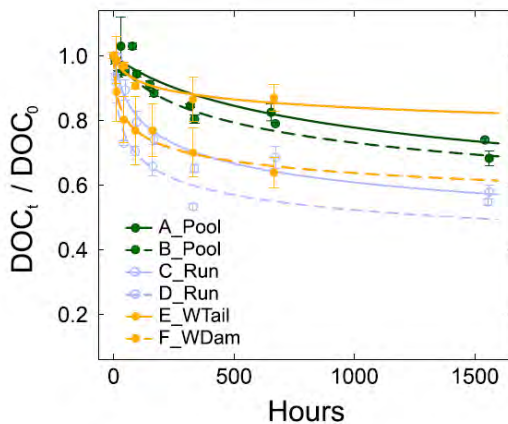
Figure 2. Time series of the relative decrease in dissolved organic carbon (DOC_t/DOC_0) during the incubations for the isolated pools A (A_Pool; green solid curve) and B (B_Pool; green dashed line), the running water reaches C_Run (blue solid curve) and D_Run (blue dashed line), and the weir at its tail (E_WTail; orange solid curve) and its dam (F_WDam; orange dashed curve). Mean and standard error values are shown. The lines show the values predicted by the reactivity continuum model.

At the end of the incubation time, the apparent first-order decay coefficients of all the sites started to converge, reaching an average value of 0.0004 h^{-1} after 1 year. The cumulative distribution functions (Figure 3b) show the probability of a DOM molecule of having a specific initial reactivity. Accordingly, around 20% of the DOM in the running water sites is likely to decay at $k = 0.01$ h^{-1} or faster, while this fraction represents around the 13% in weir sites and only around 3% in the two pools (Table 2). The fraction degrading more slowly ($k < 0.001$ h^{-1}) represents around 70–80% both in pool and weir sites, but only around 60% in running water sites.

3.3. Biodegradation Dynamics of the LC-OCD Fractions

The different fractions determined from LC-OCD presented very different patterns in time (Figures 4–6). LMWS and HMWS decreased, while the HS
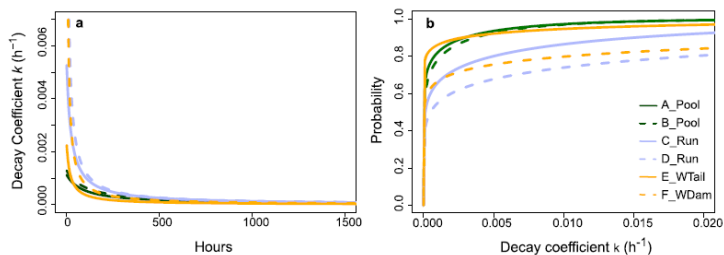
Figure 3. Output of the reactivity continuum model on bulk DOM. (a) Density functions of the models, showing the apparent first-order decay coefficient (k) over incubation time and (b) cumulative distribution function of initial reactivity for the isolated pools A_Pool (green solid curve) and B_Pool (green dashed line), the running water reached C_Run (blue solid curve) and D_Run (blue dashed line), and the weir at its tail (E_WTail; orange solid curve) and at its dam (F_WDam; orange dashed curve).

and BB fraction showed increases and decreases during the incubation time. These decreases reached up to the 60% of the initial LMWS (Figure 4) and up to the 80% of the HMWS (Figure 5). To gain insight into the fraction controlling the bulk DOM degradation pattern, we tested the relationship between the percentage of each fraction and the decay rates of the bulk DOM. Only the LMWS fraction showed a significant positive relationship with decay rates (Figure 7), and that relationship was independent of the bulk DOM concentration (Figure S1). We were unable to test this relationship for the HMWS, as this fraction was only available for two sites, and found no significant relationship with the HS and BB fraction.

The definition of the RC model as a continuous distribution of an infinite number of reactive types [*Boudreau and Ruddick*, 1991] implies that each compound conforming the DOM mixture decays following first-order kinetics. In agreement with that assumption, a reactivity continuum model could be fitted to the LMWS and HMWS fractions (supporting information Table S1). In both cases, in general, a better agreement was reached with the RC and GAM models than with the exponential or linear models (Figures 4 and 5; supporting information Tables S2 and S3) [*Wagenmakers and Farrell*, 2004]. The GAM model described slightly better than the RC decay of the LMWS fraction in sites A_Pool, D_Run, and E_WTail (Figure 4) and the HMWS fraction in site B_Pool (Figure 5). The parameters from the fitted RC models in these fractions are in accordance with the values obtained from the bulk DOM modeling (supporting information Table S1).

Regarding the HS and BB fraction, at the end of the incubation this fraction had decreased in A_Pool, B_Pool, and B_Run, while remained constant or increased in A_Run, E_WTail, and F_WDam (Figure 6). Throughout the incubation period, the HS and BB fraction showed increases and decreases and it was not possible to fit the gamma distribution of the RC model used. We further explored nonmonotonic trends using GAM models. We found significant trends (Figure 6); however, these trends were asynchronous and differentiated among sites, being impossible to find a common temporal pattern.

4. Discussion

4.1. Degradation Patterns of the Different DOM Fractions

The different DOM fractions presented consistent decay patterns across sites. Particularly, the LMWS fraction appeared to drive the biodegradation pattern of the bulk DOM in all the sites (Figure 7). LMWS are related to low molecular acids and low ion density molecules of amphiphilic or hydrophilic character, such as simple sugars or amino acids [*Huber et al.*, 2011]. Therefore, this fraction should be readily available for uptake into bacterial cells. Indeed, it presents a high bioavailability (up to 60% of the initial DOM), as previously found in studies using SEC during biodegradation [*Paul et al.*, 2012], and seems to be an important factor determining the decay rates of the bulk DOM (Figure 7). Moreover, the RC model, which assumes each compound decaying according to a simple exponential, was successfully fitted for LMWS in all the sites (Figure 4). The bacterial growth based on simple sugars and amino acids should translate in an exponential decrease of the concentration of each of these simple compounds [*Kaplan and Newbold*, 2003], in agreement with that model assumption.

Table 2. Estimated Parameters[a] of the Reactivity Continuum Models Fitted to the DOM Biodegradation Curves of the Different Sites

	A_Pool	B_Pool	C_Run	D_Run	E_WTail	F_WDam
α Average lifetime of the more reactive compounds (h)	89.5 ± 1.6	102.9 ± 1.4	24.1 ± 1.6	6.7 ± 1.95	19.8 ± 2.4	3.7 ± 0.6
ν Relative preponderance of the more recalcitrant compounds (unitless)	0.100 ± 0.012	0.132 ± 0.011	0.131 ± 0.012	0.133 ± 0.013	0.044 ± 0.014	0.080 ± 0.011
k Apparent initial first-order decay coefficient (h^{-1})	0.0011 ± 0.0007	0.0013 ± 0.0008	0.0054 ± 0.0007	0.0197 ± 0.0067	0.0224 ± 0.0057	0.0231 ± 0.0072
Reactivity class[b] 1 (%) $k > 0.01$	2.65	3.84	15.14	24.97	8.08	18.1
Reactivity class[b] 2 (%) $0.001 < k < 0.01$	15.37	18.66	20.41	15.61	10.85	16.17
Reactivity class[b] 3 (%) $k < 0.001$	81.99	77.5	64.45	59.42	81.07	65.72

[a]Model parameters reported with $\pm$ SE.
[b]Reactivity classes are defined as the proportion of initial DOM decaying with the defined k values [*Koehler et al.*, 2012].

The HMWS fraction, previously related with fresh organic materials [*Graeber et al.*, 2015], was also degraded to a large extent (up to 80%) and could be modeled with the RC model (Figure 5). The large molecular size of HMWS may indicate the presence of biopolymers, but it could also indicate the presence of supramolecular assemblies formed by several individual DOM compounds bonded through weak forces [*Piccolo et al.*, 2002; *Peuravuori and Pihlaja*, 2004; *Schaumann*, 2006; *Kellerman*, 2015]. In both cases, these processes would fit well with the multiexponential decay underlying the RC. Biopolymer breakage, for example, is mediated through exoenzymes, leading to an exponential decrease of a particular substrate [*Sinsabaugh and Follstad Shah*, 2012]. High molecular weight compounds have often been related with humic colored and highly aromatic materials, of dubious biodegradation potential [*Stedmon et al.*, 2007; *Helms et al.*, 2008]. Comparison between size fractions obtained throughout different methods is complex due to methodological biases [*Nebbioso and Piccolo*, 2013], but in the case of LC-OCD, the hydrophilic character and absence of response of this fraction in the UV detector points toward biopolymers [*Huber et al.*, 2011]. Therefore, the so called fast-decaying, short-term or labile fraction [*Guillemette and del Giorgio*, 2011; *Cory and Kaplan*, 2012] might in fact be the result of the biodegradation of both HMWS and LMWS representing, from aggregates to simple sugars, different stages in the disaggregation of biopolymers. This fast turnover related with biopolymers and monomers has been traditionally assessed during the study of the microbial loop in aquatic ecosystems [*Kirchman*, 2003; *Pomeroy et al.*, 2007] and envisaged as being the fraction of carbon with the fastest turnover. Naturally, some compounds within these fractions are likely to present a more persistent character, especially in the case of LMWS. Small chromatographic fractions have been also associated with carboxylic-rich alicyclic molecules that tend to be preserved in natural waters [*Amon and Benner*, 1996; *Kellerman*, 2015].

Less well known are the kinetics of the compounds comprised in the last fraction, the HS and BB. The humic fraction (HS) comprises the humic acids containing carboxyl and phenolate groups produced by biodegradation of dead organic matter (MW of approximately 1000 Da). The BB, molecular chains of polyphenolics and polyaromatic acids that have disaggregated, likely breakdown products from HS [*Huber et al.*, 2011]. It was impossible to fit this fraction to the RC, and it presented no clear temporal trends and a very poor fit to exponential or linear decays (Figure 6 and supporting information Table S4). Other studies on DOM biodegradation have reported simultaneous increases and decreases in different fluorophores, with increases mainly affecting humic-like regions of fluorescence, relating them with the simultaneous consumption and production of DOM during incubations [*Guillemette and del Giorgio*, 2012; *Catalán et al.*, 2013a]. Moreover, in contrast with the bulk DOM kinetics and the LMWS and HMWS fractions, we did not clear temporal pattern on the HS and BB biodegradation across sites. These results point toward the uncoupling between the decay kinetics applied to the bulk DOM, mainly dominated by easily decomposable compounds that will decay according to the RC model, and the kinetics of humic substances that are likely to follow complex nonmonotonic trends as exemplified here with the fitting to GAM models (Figure 6). We do not aim to present GAM models as the analytical solution to the transformation processes that humic DOM suffers during biodegradation. The use of complex nonmonotonic models might lead to impractical multiple parameterizations hard to apply, thereby dampening the understanding of the OM decomposition process [*Manzoni et al.*, 2012]. Our aim is instead to highlight the role of nonmonotonicity in the degradation of specific fractions of DOM, as has been shown for soil OM decomposition [*Neill and Gignoux*, 2006; *Manzoni*

Figure 4. Relative decrease in the LMWS fraction (mean ± SE) during the incubations in the isolated pools (a) A_Pool and (b) B_Pool, the running water reaches (c) C_Run and (d) D_Run, and the weir (e) E_WTail and (f) F_WDam. The curves represent the predicted loss of LMWS modeled by the reactivity continuum model (continuous line), a simple exponential model (dotted line), a linear model (dashed line), and a GAM model (dash-dotted line). When colored and present in the legend, the line shows the model presenting the best fit according to Akaike weights (see supporting information Table S2).

and Porporato, 2007]. In that sense, the use of GAM models could prove very useful to identify temporal patterns along incubations. Mainly in short-term experiments, organic carbon can present a fluctuating behavior due to strong biotic-substrate interactions [*Manzoni and Porporato*, 2007] related with the activity of oxidative exoenzymes, which can be especially relevant in the degradation of humic substances [*Arnosti*, 2003]. For example, further research and an increased number of samples might help elucidate if the initial increase followed by a decrease in the HS and BB fraction (Figures 6b–6d and 6f) is consistent. To conclude, in the timescales of most incubation studies of freshwater DOM, a fluctuating behavior leading to nonmonotonic trends in the biodegradation of humic fractions might be expected.

4.2. Composition Effects on Bulk DOM Degradation Patterns

The sites presented differentiated patterns of DOM biodegradation that were related with differences in the initial composition and site particularities. These site particularities include factors such as DOM sources and

Figure 5. Relative decrease in the HMWS fraction (mean ± SE) during the incubations in the isolated pools (a) A_Pool and (b) B_Pool. HMWS concentration was under the detection limit in the other sites (Figure 1). The curves represent the predicted loss of HMWS modeled by the reactivity continuum model (continuous line), a simple exponential model (dotted line), a linear model (dashed line), and a GAM model (dash-dotted line). When colored and present in the legend, the line shows the model presenting the best fit according to Akaike weights (see supporting information Table S3).

history of degradation, microbial communities, water isolation, pH, temperature, or availability of inorganic nutrients which might define the structure and biodegradation potential of the DOM in natural conditions [*del Giorgio and Davis*, 2003]. Therefore, here we consider the differences in the biodegradation patterns among systems as the result of divergent DOM sources and also of their preconditioning across environments. In general, the biodegradation of DOC from the six sites was extensive (up to 40% BDOM in the two running water reaches) and in the fast range of incubation studies of river DOM elsewhere [*Del Giorgio and Pace*, 2008; *Fellman et al.*, 2014]. A common trait of DOM in Mediterranean systems is its low color and aromaticity [*Vazquez et al.*, 2011; *Catalán et al.*, 2013b; *von Schiller et al.*, 2015], which leads to comparatively higher biodegradation potential than in systems dominated by colored, strongly humic substances like the boreal systems [*Berggren et al.*, 2010].

DOM from the two upstream pools presented the slowest decay; the lifetime of the most reactive compounds was long and the values of the decay coefficients declined slowly, indicating a broad spectrum of reactivities for these systems [*Koehler and Tranvik*, 2015]. However, despite showing the slowest bulk DOM decay, the pools included a fraction of HMWS that presented high biodegradation (Figure 5). The HMWS fraction, also called biopolymers [*Huber et al.*, 2011], is related with high molecular weight compounds (>10 kDa [*Rutlidge et al.*, 2015]), usually polymers as polysaccharides with some contribution of nitrogen-containing materials such as proteins or amino sugars [*Huber et al.*, 2011]. Structural polysaccharides are major components of leaf litter detritus [*Mansfield and Baerlocher*, 2005; *Engelhaupt and Bianchi*, 2001], which accumulates in the studied temporary pools [*Casas-Ruiz et al.*, 2016]. Leaf litter leaching has been reported to increase the presence of large, highly biodegradable molecules [*Romaní et al.*, 2006; *Cuss and Guéguen*, 2015]. Temporary pools not only have strong leaf litter inputs but also a longer water residence time than the running waters and the weir, which leads to an increase of in situ microbial activity and autochthonous DOM sources [*Fellman et al.*, 2011; *Vazquez et al.*, 2011]. Therefore, these large compounds in the pools were likely linked with fresh litter leaching and concomitant microbial activity. Accordingly, this HMWS fraction has been linked with fresh organic materials previously [*Graeber et al.*, 2015]. However, pool samples also present an important fraction of HS and BB. This fraction represents different acids resulting from the biodegradation of dead organic matter [*Rutlidge et al.*, 2015] and may be related to the presence of a very large reactivity class with very low decay rates (Figure 3b and Table 2). Accordingly, as sugars from fresh litter leachate are consumed, the humic fraction might become relatively more abundant [*Cleveland et al.*, 2004]. The accumulation of the old running water DOM and the byproducts of leaf litter decomposition [*Casas-Ruiz et al.*, 2016] shapes pools as spots where OM undergoes a more extensive degradation. As a result, slow-degrading compounds such as HS and BB accumulate in temporary pools, to be flushed down once the rain reconnects them to the main flow.

The DOM from the running water reaches presented a marked decay with high initial apparent k and a low percentage of compounds with slow decay (Reactivity Class 3; Table 2). Running waters presented also the highest relative content in LMWS. This fraction includes both low molecular acids and neutrals which might
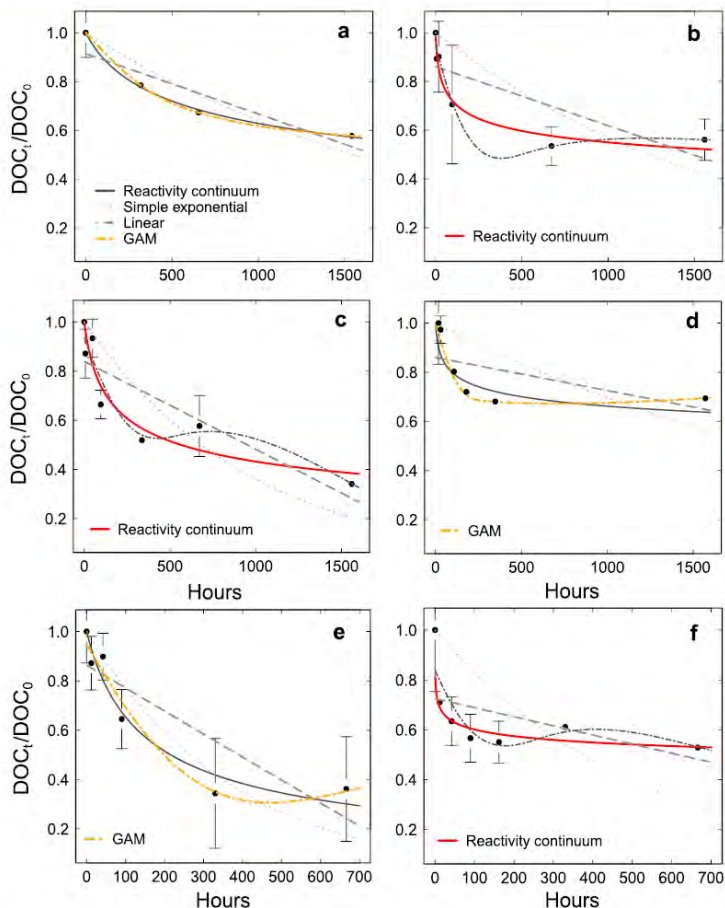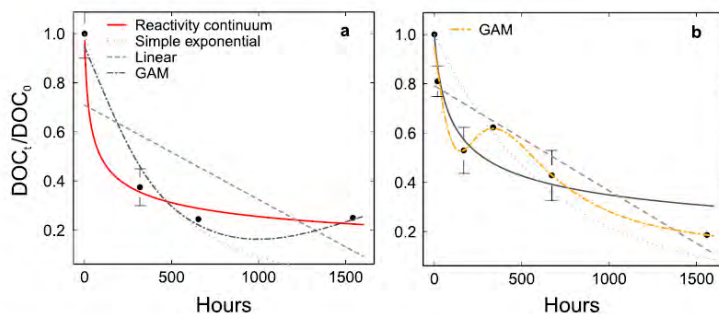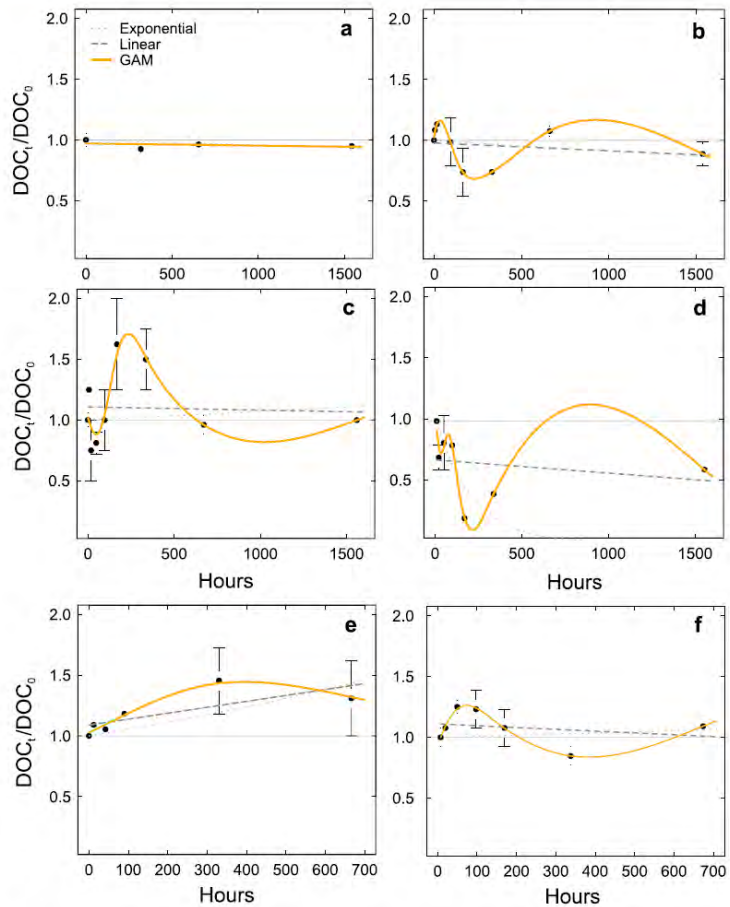
Figure 6. Relative decrease in the HS and BB fraction (mean ± SE) during the incubations in the isolated pools (a) A_Pool and (b) B_Pool, the running water reaches (c) C_Run and (d) D_Run, and the weir (e) E_WTail and (f) F_WDam. The curves represent the predicted loss of HS and BB modeled by a simple exponential model (dotted line), a linear model (dashed line), and a GAM model (dash-dotted line); the reactivity continuum model could not be fitted for this fraction. When colored and present in the legend, the lines show the model presenting a better fit according to Akaike weights (see supporting information Table S4). A grey continuous line at $y = 1$ (no change in DOC) is shown for clarity.

include aliphatic acids [*Kellerman*, 2015] and small humic and fulvic acids together with markedly biodegradable molecules like simple sugars or amino acids [*Her et al.*, 2002; *Huber et al.*, 2011]. Strong degradation of DOM from running waters has been reported previously [*Lapierre et al.*, 2013] and might be enhanced by important background concentrations of nutrients (total dissolved nitrogen (TDN); Table 1). Indeed, despite the central role of the LMWS on the decay rates of bulk DOM (Figure 7), the variability around this relationship (Figure S1) points towards factors other than composition that might also be influencing the degradation, such as nutrients or the microbial community. The low residence time of DOM in running waters with the consequent low exposure to previous degradation pathways might enable the presence of LMWS. Therefore, running water reaches might be mainly transporting less processed material [*Raymond et al.*, 2016]. Indeed, short water residence times correspond to a decrease in DOM processing, raising the instantaneous decay rates [*Catalán et al.*, 2016].
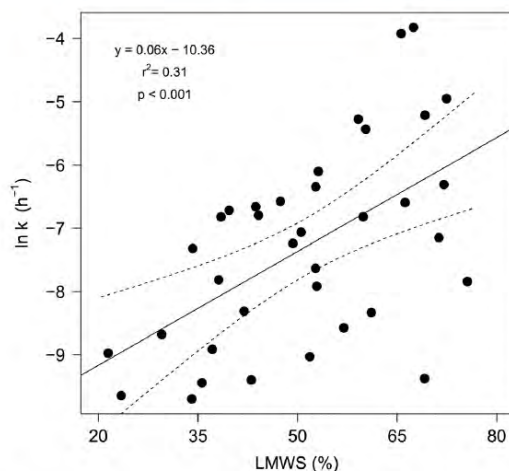
Figure 7. Relationship between the LMWS fraction and the modeled decay rates (k) at the corresponding incubation time in all the studied sites. Dotted lines show 95% confidence interval. An evaluation of the effect of DOC concentration on this relationship is presented in the supporting information Figure S1.

The two weir sites presented intermediate reactivity although differentiated degradation patterns. The weir in its tail (i.e., closer to the running waters) showed a degradation pattern similar to the pools (Figures 3a and 3b), whereas that of the site close to the dam was similar to the river sites. Taking into account the lower biodegradation (BDOM) and higher proportion of HS and BB in the E_WTail site, its reactivity pattern might be related to higher influence of the sediments. Indeed, the tail of weirs and impoundments, where river water mixes with the lentic water body, is strongly influenced by the activity of the accumulated sediment [*Cardoso et al.*, 2013], especially during low flow periods [*Mosley*, 2015]. The F_WDam samples were taken close to the dam, where production in the water column is expected to increase. Therefore, sources of DOM will include in situ planktonic activity, and accordingly, increased biodegradation and LMWS were found. Small molecular weight compounds, such as aliphatic molecules with H:C ratio over 1 [*Kellerman*, 2015], have been related with aquatic production and found to increase with water residence time [*Köhler et al.*, 2013], which would explain the divergence between E_WTail and F_WDam.

The divergences in composition and decay kinetics between the aquatic environments generated during drought, with enhanced degradation in temporary pools and increased primary production in weirs, would slow down the catchment-scale DOM decay rates. The severity of this intermittency is expected to increase in regions predicted to suffer decreases in runoff such as the Mediterranean [*Larned et al.*, 2010; *Schewe et al.*, 2014] and, in agreement with our observations, dry conditions have been predicted to generate a decrease in the OC decay rates [*Catalán et al.*, 2016]. Intermittency is part of the idiosyncrasy of freshwater lotic ecosystems [*Steward et al.*, 2012], and its role should therefore be included in the assessment of the DOM cycling at catchment scales.

5. Conclusions

The patterns found in bulk DOM decay were linked to compositional differences between the DOM isolated from the aquatic environments found in a river during summer drought. The lowest decay rates of bulk DOM were found in the temporary pools, systems strongly influenced by leaf litter inputs and likely to process DOM to a larger extent than river sites. In the case of weirs, the higher decay rates and higher proportions of LMWS might be linked to DOM sources derived from autochthonous primary production. The modeling of each chromatographic fraction showed a consistent pattern across sites, with LMWS and HMWS decaying rapidly and fitting to the RC model. LMWS showed a positive relationship with decay rates of bulk DOM suggesting that this fraction controls the overall kinetics of bulk DOM degradation. The HS and BB fraction did not present consistent degradation dynamics across sites, highlighting the need to further investigate the kinetics of compounds within that fraction to confirm nonmonotonic patterns. The divergences in DOM composition and degradation kinetics between the different aquatic environments show that intermittency affects the processing of bulk and compositional fractions of DOM in rivers.

References

Amon, R. M. W., and R. Benner (1996), Bacterial utilization of different size classes of dissolved organic matter, *Limnol. Oceanogr.*, *41*(1), 41–51, doi:10.4319/lo.1996.41.1.0041.

Arndt, S., B. B. Jørgensen, D. E. LaRowe, J. J. Middelburg, R. D. Pancost, and P. Regnier (2013), Quantifying the degradation of organic matter in marine sediments: A review and synthesis, *Earth Sci. Rev.*, *123*, 53–86, doi:10.1016/j.earscirev.2013.02.008.

Arnosti, C. (2003), Microbial extracellular enzymes and their role in dissolved organic matter cycling, in *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*, edited by S. E. G. Findlay and R. L. Sinsabaugh, pp. 315–342, Elsevier Sci, San Diego, Calif.

Berggren, M., H. Laudon, M. Haei, L. Ström, and M. Jansson (2010), Efficient aquatic bacterial metabolism of dissolved low-molecular-weight compounds from terrestrial sources, *ISME J.*, *4*(3), 408–416, doi:10.1038/ismej.2009.120.

Berner, R. A. (1964), An idealized model of dissolved sulfate distribution in recent sediments, *Geochim. Cosmochim. Acta*, *28*(9), 1497–1503, doi:10.1016/0016-7037(64)90164-4.

Bosatta, E., and G. I. Ågren (1995), The power and reactive continuum models as particular cases of the q-theory of organic matter dynamics, *Geochim. Cosmochim. Acta*, *59*(18), 3833–3835, doi:10.1016/0016-7037(95)00287-A.

Boudreau, B. P., and B. R. Ruddick (1991), On a reactive continuum representation of organic matter diagenesis, *Am. J. Sci.*, *291*(5), 507–538, doi:10.2475/ajs.292.1.79.

Canfield, D. E. (1994), Factors influencing organic carbon preservation in marine sediments, *Chem. Geol.*, *114*(93), 315–329, doi:10.1016/0009-2541(94)90061-2.

Cardoso, S. J., L. O. Vidal, R. F. Mendonça, L. J. Tranvik, S. Sobek, and F. Fábio (2013), Spatial variation of sediment mineralization supports differential CO_2 emissions from a tropical hydroelectric reservoir, *Front. Microbiol.*, *4*(April), 1–8, doi:10.3389/fmicb.2013.00101.

Casas-Ruiz, J. P., J. Tittel, D. von Schiller, N. Catalán, B. Obrador, L. Gómez-Gener, E. Zwirnmann, S. Sabater, and R. Marcé (2016), Drought-induced discontinuities in the source and degradation of dissolved organic matter in a Mediterranean River, *Biogeochemistry*, *127*, 125–139, doi:10.1007/s10533-015-0173-5.

Catalán, N., B. Obrador, M. Felip, and J. L. Pretus (2013a), Higher reactivity of allochthonous vs. autochthonous DOC sources in a shallow lake, *Aquat. Sci.*, *75*(4), 581–593.

Catalán, N., B. Obrador, C. Alomar, and J. L. Pretus (2013b), Seasonality and landscape factors drive dissolved organic matter properties in Mediterranean ephemeral washes, *Biogeochemistry*, *112*(1-3), 261–274, doi:10.1007/s10533-012-9723-2.

Catalán, N., R. Marcé, D. N. Kothawala, and L. J. Tranvik (2016), Organic carbon decomposition rates controlled by water retention time across inland waters, *Nat. Geosci.*, *9*, 501–504, doi:10.1038/ngeo2720.

Ciais, P., et al. (2013), 2013: Carbon and other biogeochemical cycles, in *Climate Change 2013: The Physical Science Basis. Contribution Working Group I to the Fifth Assessment Report of the Intergovernmental Panel Climate Change*, pp. 465–570, Cambridge Univ. Press, Cambridge, U. K., and New York, doi:10.1017/CBO9781107415324.015.

Cleveland, C. C., J. C. Neff, A. R. Townsend, and E. Hood (2004), Composition, dynamics, and fate of leached dissolved organic matter in terrestrial ecosystems: Results from a decomposition experiment, *Ecosystems*, *7*(3), 275–285, doi:10.1007/s10021-003-0236-7.

Cole, J. J., et al. (2007), Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget, *Ecosystems*, *10*(1), 171–184, doi:10.1007/s10021-006-9013-8.

Cory, R. M., and L. A. Kaplan (2012), Biological lability of streamwater fluorescent dissolved organic matter, *Limnol. Oceanogr.*, *57*(5), 1347–1360, doi:10.4319/lo.2012.57.5.1347.

Cuss, C. and C. Guéguen (2015), Characterizing the labile fraction of dissolved organic matter in leaf leachates: Methods, indicators, structure, and complexity, in *SSSA Special Publication, Labile Organic Matter: Chemical Compositions, Function, and Significance in Soil and the Environment*, pp. 237–274, Soil Sci. Soc., America, doi:10.2136/sssaspecpub62.2014.0043.

del Giorgio, P. A., and J. Davis (2003), Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, in *Aquatic Ecosystems. Interactivity of Dissolved Organic Matter*, edited by S. E. G. Findlay and R. L. Sinsabaugh, pp. 400–425, Elsevier Sci, London, U. K.

del Giorgio, P. A., and M. L. Pace (2008), Relative independence of organic carbon transport and processing in a large temperate river: The Hudson River as both pipe and reactor, *Limnol. Oceanogr.*, *53*(1), 185–197, doi:10.4319/lo.2008.53.1.0185.

Döll, P., K. Fiedler, and J. Zhang (2009), Global-scale analysis of river flow alterations due to water withdrawals and reservoirs, *Hydrol. Earth Syst. Sci. Discuss.*, *6*(4), 4773–4812, doi:10.5194/hessd-6-4773-2009.

Engelhaupt, E., and T. S. Bianchi (2001), Sources and composition of high-molecular-weight dissolved organic carbon in a southern Louisiana tidal stream southern (Bayou Trepagnier), *Limnol. Oceanogr.*, *46*(4), 917–926, doi:10.4319/lo.2001.46.4.0917.

Fellman, J. B., E. Hood, and R. G. M. Spencer (2010), Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review, *Limnol. Oceanogr.*, *55*(6), 2452–2462, doi:10.4319/lo.2010.55.6.2452.

Fellman, J. B., S. Dogramaci, and G. Skrzypek (2011), Hydrologic control of dissolved organic matter biogeochemistry in pools of a subtropical dryland river, *Water Resour. Res.*, *47*, W06501, doi:10.1029/2010WR010275.

Fellman, J.B., R. G. M. Spencer, P. A. Raymond, N. E. Pettit, G. Skrzypek, P. J. Hernes, and P. F. Grierson (2014), Dissolved organic carbon biolability decreases along with its modernization in fluvial networks in an ancient landscape, *Ecology*, *95*(9), 2622–2632, doi:10.1890/13-1360.1.

Findlay, S. E. G., and R. L. Sinsabaugh (Eds.) (2003), *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*, Elsevier Sci, London, U. K.

Gasith, A., and V. H. Resh (1999), Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events, *Annu. Rev. Ecol. Syst.*, *30*, 51–81, doi:10.1146/annurev.ecolsys.30.1.51.

Graeber, D., et al. (2015), Global effects of agriculture on fluvial dissolved organic matter, *Sci. Rep.*, *5*, 16328, doi:10.1038/srep16328.

Guillemette, F., and P. A. del Giorgio (2011), Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems, *Limnol. Oceanogr.*, *56*(2), 734–748, doi:10.4319/lo.2011.56.2.0734.

Guillemette, F., and P. A. del Giorgio (2012), Simultaneous consumption and production of fluorescent dissolved organic matter by lake bacterioplankton, *Environ. Microbiol.*, *14*(6), 1432–1443, doi:10.1111/j.1462-2920.2012.02728.x.

Guillemette, F., S. L. McCallister, and P. A. del Giorgio (2013), Differentiating the degradation dynamics of algal and terrestrial carbon within complex natural dissolved organic carbon in temperate lakes, *J. Geophys. Res. Biogeosci.*, *118*, 963–973, doi:10.1002/jgrg.20077.

Helms, J. R., A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber, and K. Mopper (2008), Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter, *Limnol. Oceanogr.*, *53*(3), 955–969, doi:10.4319/lo.2008.53.3.0955.

Helms, J. R., J. Mao, A. Stubbins, K. Schmidt-Rohr, R. G. M. Spencer, P. J. Hernes, and K. Mopper (2014), Loss of optical and molecular indicators of terrigenous dissolved organic matter during long-term photobleaching, *Aquat. Sci.*, *76*(3), 353–373, doi:10.1007/s00027-014-0340-0.

Her, N., G. Amy, D. Foss, J. Cho, Y. Yoon, and P. Kosenka (2002), Optimisation of method for detecting and characterising NOM by HPLC-size exclusion chromatography with UV and on-line UV detection, *Environ. Sci. Technol.*, *36*(5), 1069–1076.

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Huber, S.a., A. Balz, M. Abert, and W. Pronk (2011), Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography–organic carbon detection–organic nitrogen detection (LC-OCD-OND), *Water Res.*, 45(2), 879–885, doi:10.1016/j.watres.2010.09.023.

Kaplan, L. A., and J. D. Newbold (2003), The role of monomers in stream ecosystem metabolism in aquatic ecosystems, in *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*, edited by S. E. G. Findlay and R. L. Sinsabaugh, Elsevier Sci, Avondale Pennsylvania.

Kellerman, A. M. (2015), Molecular-level dissolved organic matter dynamics in lakes: Constraints on reactivity and persistence, PhD thesis, Dep. of Limnol., Uppsala Univ., Uppsala, Sweden.

Kellerman, A. M., T. Dittmar, D. N. Kothawala, and L. J. Tranvik (2014), Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology, *Nat. Commun.*, 5, 3804, doi:10.1038/ncomms4804.

Kirchman, D. L. (2003), The contribution of monomers and other low-molecular weight compounds to the flux of dissolved organic material in aquatic ecosystems, in *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*, edited by S. E. G. Findlay and R. L. Sinsabaugh, pp. 218–237, Elsevier Sci., San Diego, Calif.

Koehler, B., and L. J. Tranvik (2015), Reactivity continuum modeling of leaf, root, and wood decomposition across biomes, *J. Geophys. Res. Biogeosci.*, 120, 1196–1214, doi:10.1002/2015JG002908.

Koehler, B., E. Von Wachenfeldt, D. N. Kothawala, and L. J. Tranvik (2012), Reactivity continuum of dissolved organic carbon decomposition in lake water, *J. Geophys. Res.*, 117, G01024, doi:10.1029/2011JG001793.

Köhler, S. J., D. N. Kothawala, M. N. Futter, O. Liungman, and L. J. Tranvik (2013), In-lake processes offset increased terrestrial inputs of dissolved organic carbon and color to lakes, *PLoS One*, 8(8), 1–12, doi:10.1371/journal.pone.0070598.

Kothawala, D. N., R. D. Evans, and P. J. Dillon (2006), Changes in the molecular weight distribution of dissolved organic carbon within a Precambrian shield stream, *Water Resour. Res.*, 42, W05401, doi:10.1029/2005WR004441.

Lapierre, J. F., F. Guillemette, M. Berggren, and P. A. del Giorgio (2013), Increases in terrestrially derived carbon stimulate organic carbon processing and CO2 emissions in boreal aquatic ecosystems, *Nat. Commun.*, 4, 2972, doi:10.1038/ncomms3972.

Larned, S. T., T. Datry, D. B. Arscott, and K. Tockner (2010), Emerging concepts in temporary-river ecology, *Freshwater Biol.*, 55(4), 717–738, doi:10.1111/j.1365-2427.2009.02322.x.

Mansfield, S. D., and M. O. Baerlocher (2005), Free amino acids, in *Methods to Study Litter Decomposition A Practical Guide*, edited by M. A. S. Graça, F. Bärlocher, and M. O. Gessner, pp. 25–31, Springer, Dordrecht, Netherlands.

Manzoni, S., and A. Porporato (2007), A theoretical analysis of nonlinearities and feedbacks in soil carbon and nitrogen cycles, *Soil Biol. Biochem.*, 39(7), 1542–1556, doi:10.1016/j.soilbio.2007.01.006.

Manzoni, S., G. Piñeiro, R. B. Jackson, E. G. Jobbágy, J. H. Kim, and A. Porporato (2012), Analytical models of soil and litter decomposition: Solutions for mass loss and time-dependent decay rates, *Soil Biol. Biochem.*, 50, 66–76, doi:10.1016/j.soilbio.2012.02.029.

Middelburg, J. J. (1989), A simple rate model for organic matter decomposition in marine sediments, *Geochim. Cosmochim. Acta*, 53(7), 1577–1581, doi:10.1016/0016-7037(89)90239-1.

Mosley, L. M. (2015), Drought impacts on the water quality of freshwater systems; Review and integration, *Earth Sci. Rev.*, 140, 203–214, doi:10.1016/j.earscirev.2014.11.010.

Nebbioso, A., and A. Piccolo (2013), Molecular characterization of dissolved organic matter (DOM): A critical review, *Anal. Bioanal. Chem.*, 405(1), 109–124, doi:10.1007/s00216-012-6363-2.

Neill, C., and J. Gignoux (2006), Soil organic matter decomposition driven by microbial growth: A simple model for a complex network of interactions, *Soil Biol. Biochem.*, 38(4), 803–811, doi:10.1016/j.soilbio.2005.07.007.

Olson, J. S. (1963), Energy storage and the balance of producers and decomposers in ecological systems, *Ecology*, 44(2), 322–331, doi:10.2307/1932179.

Paul, A., C. Dziallas, E. Zwirnmann, E. T. Gjessing, and H. P. Grossart (2012), UV irradiation of natural organic matter (NOM): Impact on organic carbon and bacteria, *Aquat. Sci.*, 74(3), 443–454, doi:10.1007/s00027-011-0239-y.

Peuravuori, J., and K. Pihlaja (2004), Preliminary study of lake dissolved organic matter in light of nanoscale supramolecular assembly, *Environ. Sci. Technol.*, 38(22), 5958–5967, doi:10.1021/es040041l.

Piccolo, A., P. Conte, E. Trivellone, B. Van Lagen, and P. Buurman (2002), Reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. Characterization of size-separates by Pyr-GC-MS and 1H-NMR spectroscopy, *Environ. Sci. Technol.*, 36(1), 76–84, doi:10.1021/es010981v.

Pomeroy, L., P. I. B. Williams, F. Azam, and J. Hobbie (2007), The microbial loop, *Oceanography*, 20(2), 28–33, doi:10.5670/oceanog.2007.45.

Porter, K. G., and Y. S. Feig (1980), The use of DAPI for identifying aquatic microflora, *Limnol. Oceanogr.*, 25(5), 943–948, doi:10.4319/lo.1980.25.5.0943.

R Core Team (2015), *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria. [Available at https://www.R-project.org/.]

Raymond, P. A., J. E. Saiers, and W. V. Sobczak (2016), Hydrological and biogeochemical controls on watershed dissolved organic matter transport: Pulse-shunt concept, *Ecology*, 97(1), 5–16, doi:10.1890/07-1861.1.

Romaní, A. M., E. Vázquez, and A. Butturini (2006), Microbial availability and size fractionation of dissolved organic carbon after drought in an intermittent stream: Biogeochemical link across the stream-riparian interface, *Microb. Ecol.*, 52(3), 501–512, doi:10.1007/s00248-006-9112-2.

Rutlidge, H., M. S. Andersen, A. Baker, K. J. Chinu, M. O. Cuthbert, C. N. Jex, C. E. Marjo, M. Markowska, and G. C. Rau (2015), Organic characterisation of cave drip water by LC-OCD and fluorescence analysis, *Geochim. Cosmochim. Acta*, 166, 15–28, doi:10.1016/j.gca.2015.05.042.

Schaumann, G. E. (2006), Soil organic matter beyond molecular structure Part I: Macromolecular and supramolecular characteristics, *J. Plant Nutr. Soil Sci.*, 169(2), 145–156, doi:10.1002/jpln.200521785.

Schewe, J., et al. (2014), Multimodel assessment of water scarcity under climate change, *Proc. Natl. Acad. Sci. U.S.A.*, 111(9), 3245–3250, doi:10.1073/pnas.1222460110.

Sinsabaugh, R. L., and J. J. Follstad Shah (2012), Ecoenzymatic stoichiometry and ecological theory, *Annu. Rev. Ecol. Evol. Syst.*, 43(1), 120,913,143,848,009, doi:10.1146/annurev-ecolsys-071112-124414.

Sleighter, R. L., R. M. Cory, L. A. Kaplan, H. A. N. Abdulla, and P. G. Hatcher (2014), A coupled geochemical and biogeochemical approach to characterize the bio-reactivity of dissolved organic matter from a headwater stream, *J. Geophys. Res. Biogeosci.*, 119, 1520–1537, doi:10.1002/2013JG002600.

Stedmon, C. A., S. Markager, L. J. Tranvik, L. Kronberg, T. Slätis, and W. Martinsen (2007), Photochemical production of ammonium and transformation of dissolved organic matter in the Baltic Sea, *Mar. Chem.*, 104(3–4), 227–240, doi:10.1016/j.marchem.2006.11.005.

Steward, A. L., D. Von Schiller, K. Tockner, J. C. Marshall, and S. E. Bunn (2012), When the river runs dry: Human and ecological values of dry riverbeds, *Front. Ecol. Environ.*, 10(4), 202–209, doi:10.1890/110136.

Tranvik, L. J., J. A. Downing, J. B. Cotner, S. A. Loiselle, R. G. Striegl, T. J. Ballatore, P. Dillon, K. Finlay, K. Fortino, and L. B. Knoll (2009), Lakes and reservoirs as regulators of carbon cycling and climate, *Limnol. Oceanogr.*, *54*(6_part_2), 2298–2314, doi:10.4319/lo.2009.54.6_part_2.2298.

Vähätalo, A. V., H. Aarnos, and S. Mäntyniemi (2010), Biodegradability continuum and biodegradation kinetics of natural organic matter described by the beta distribution, *Biogeochemistry*, *100*(1), 227–240, doi:10.1007/s10533-010-9419-4.

Vazquez, E., S. Amalfitano, S. Fazi, and A. Butturini (2011), Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions, *Biogeochemistry*, *102*(1), 59–72, doi:10.1007/s10533-010-9421-x.

von Schiller, D., D. Graeber, M. Ribot, X. Timoner, V. Acuña, E. Martí, S. Sabater, and K. Tockner (2015), Hydrological transitions drive dissolved organic matter quantity and composition in a temporary Mediterranean stream, *Biogeochemistry*, *123*, 1–18, doi:10.1007/s10533-015-0077-4.

Wagenmakers, E.-J., and S. Farrell (2004), AIC model selection using Akaike weights, *Psychon. Bull. Rev.*, *11*(1), 192–196, doi:10.3758/BF03206482.

Westrich, J. T., and R. A. Berner (1984), The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested, *Limnol. Oceanogr.*, *29*(2), 236–249, doi:10.4319/lo.1984.29.2.0236.

Zarfl, C., A. E. Lumsdon, J. Berlekamp, L. Tydecks, and K. Tockner (2014), A global boom in hydropower dam construction, *Aquat. Sci.*, *77*(1), 161–170, doi:10.1007/s00027-014-0377-0.

PAPER II

Drought-induced discontinuities in the source and
degradation of dissolved organic matter in a Mediterranean
river
Biogeochemistry, 2016

Abstract

The composition of dissolved organic matter (DOM) in rivers results from the different sources and in-stream transformations along the land to ocean aquatic continuum. Riverine DOM sources are highly dependent on the hydrological connection between the river channel and the surrounding terrestrial ecosystems, but how the lack of this connectivity (e.g., during drought episodes) affects the sources and biodegradation of DOM in rivers remains unclear. Here we identified the DOM sources as well as the different DOM pools that are respired along a Mediterranean river during drought by combining absorbance-fluorescence spectroscopy, size-exclusion chromatography, biodegradation assays, and stable and radiocarbon isotopes. DOM composition was highly heterogeneous along the river in response to different sources and in-stream processes in each distinct aquatic environment (i.e., isolated water pools, running waters, and impounded waters in weirs). The reduced hydrological connectivity with terrestrial ecosystems promoted the influence of autochthonous DOM sources. Still, tree leaves from overhanging canopies stood out as an important terrestrial DOM source, especially in sites where water residence time was high such as isolated pools and weirs. Degradation of leaf leachates was a relevant process in these sites, whereas autochthonous DOM and groundwater millennial DOM (>1300 year B.P.) seemed to be degraded in running waters. Overall, our results highlight that the drought-induced hydrological disconnection entails a great spatial heterogeneity in the sources of DOM, which at the same time determines the different DOM pools that are respired in each environment along the river.

Keywords

Mediterranean rivers; Drought; Dissolved organic matter; Degradation; Radiocarbon
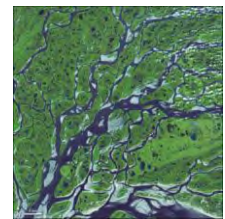
PAPER III

A tale of pipes and reactors: controls on the in-stream
dynamics of dissolved organic matter in rivers
Limnology and Oceanography, 2017

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

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Abstract

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

The riverine flux of dissolved organic matter (DOM) from terrestrial ecosystems to the ocean is a major component of the global carbon cycle (Battin et al. 2009; Ciais et al. 2013). Yet our ability to predict the fate of DOM relies on an accurate comprehension of the in-stream biogeochemical processes that supply and remove DOM from rivers. River networks have been identified as active conduits through which DOM can be not only transported but also produced, buried in sediments, or mineralized and emitted to the atmosphere (Cole et al. 2007; Aufdenkampe et al. 2011; Raymond et al. 2013). Still, whereas relevant DOM processing has been reported in some bioassays and field studies (Mcdowell and Fisher 1976; Dawson et al. 2001; Guillemette and del Giorgio 2011), many others have found limited evidence of DOM alteration in rivers (Hanley et al. 2013; Kothawala et al. 2015; Wollheim et al. 2015). Therefore, unraveling the factors that promote in-stream processing of DOM (i.e., removal and/or generation) vs. its passive transport downstream is essential for a better understanding of the role of rivers in DOM cycling.

Among possible factors, the time DOM spends in the system has been usually invoked as a major constraint for in-stream DOM processing in small to medium-sized river networks (Temnerud et al. 2007; Ågren et al. 2014; Kothawala et al. 2015). In fact, from a strictly physical point of view, the ratio between reaction and transport timescales determine the opportunity for in-stream processes to occur (Oldham et al. 2013). At the same time, the chemical composition of DOM may influence its fate because reactivity can be highly variable among DOM fractions (Cory and Kaplan 2012). In this sense, the measurement of DOM
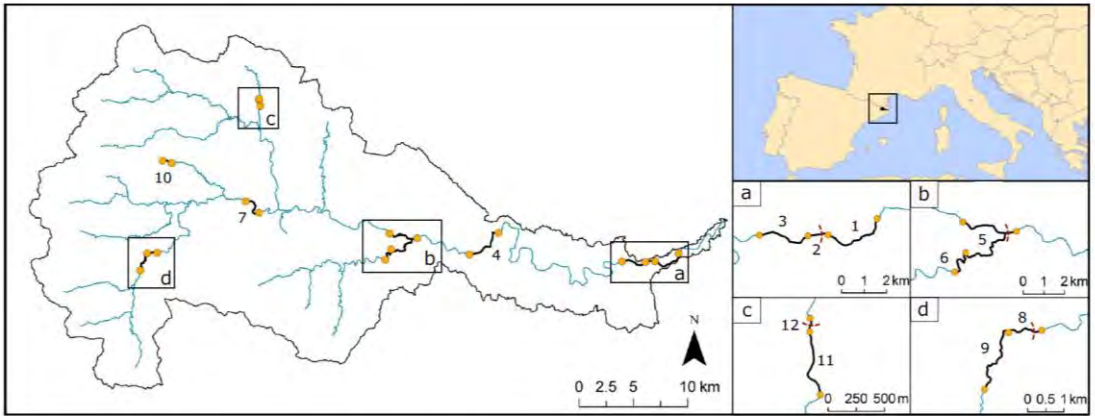
Fig. 1. Map of the Fluvià river catchment, with all studied river reaches indicated by heavy black lines. Yellow solid circles indicate the upper and lower ends of the reaches. The location coordinates of the study reaches are given in Supporting Information Table 1. The upper right panel shows the situation of the catchment in South-Western Europe. The lower right panel shows enlarged views of some of the study areas (**a–d**) for visual clarity. Red dashed lines represent weirs. Note that reach 5 is a weir-delimited reach with two inflows.

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

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

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

Methods

Study site

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

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

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

Field and lab methods

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

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

Excitation-Emission matrices (EEMs) were obtained using a fluorescence spectrophotometer (F-7000, Hitachi, Japan) with a 1-cm quartz cuvette. EEMs were collected by measuring fluorescence intensity across the excitation range set from 248 nm to 449 nm and the emission range set from 250 nm to 550 nm, both at 3 nm increments. All EEMs were read in sample to reference signal ratio mode, and were corrected for instrument-specific biases after calibrating the instrument with Rhodamine B solution. EEMs were blank subtracted using the EEM of Milli-Q water measured every 10 samples. Fluorescence spectra were then corrected for inner filter effects using UV-visible absorbance spectra measured on an Agilent 8453 spectrophotometer (Agilent Technologies, Germany). The integral of the water Raman scatter peak at excitation 350 nm was used for EEMs intensity calibration into Raman Units (RU; Lawaetz and Stedmon 2009).

Water residence time

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

Data analysis
In-stream processing of DOM

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

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

Given that the analytical precision of DOC measurements was 0.04 mg C L^{-1} and that the error of a subtraction

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

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

Optical indices and PARAFAC modeling

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

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

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

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

Statistics

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

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

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

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

Results

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

The net change in DOC along the reaches was generally low, with ΔDOC values showing a median value of 0.00 mg C L^{-1} and an interquartile range of −0.07 mg C L^{-1} to 0.06 mg C L^{-1} (Fig. 2a). Of the whole dataset ($n = 74$), 51% of observations had an absolute ΔDOC above the threshold for considering DOC variations (0.06 mg C L^{-1}; see Methods section) and, among these, 47% were positive (i.e., DOM net gain), and 53% were negative (i.e., DOM net loss; Fig. 2a and Supporting Information Fig. 1). No evident patterns in ΔDOC were observed across space or time (Supporting Information Figs. 2 and 3). However, there was slightly less DOM processing during the two high flow events of 27th March and 1st May, as indicated by very low dispersion of the ΔDOC values around zero (Supporting Information Fig. 3).
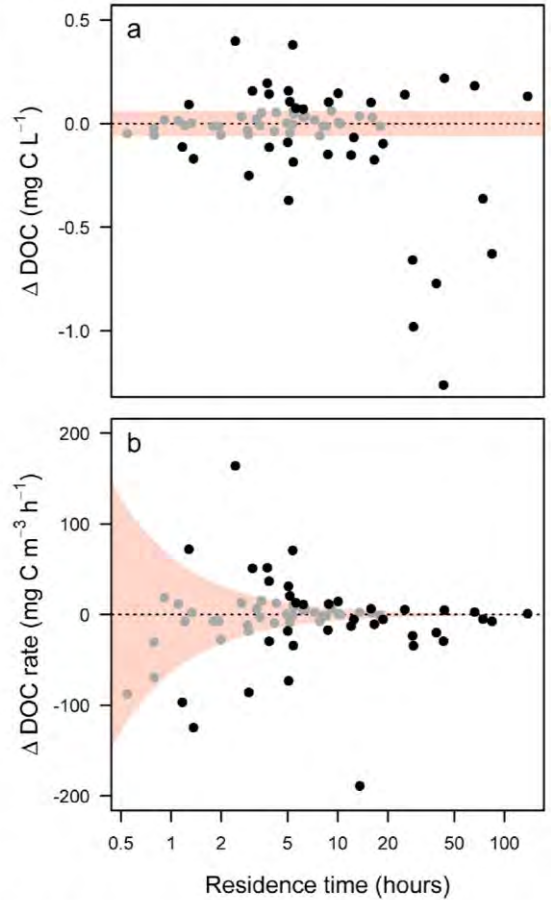
Fig. 2. In-stream dynamics of bulk DOM across the gradient of residence times measured in this study. (**a**) Relationship between the net change in dissolved organic carbon (ΔDOC) and the residence time of the reaches. (**b**) Relationship between the rate of ΔDOC and the water residence time of the reaches. In both panels positive values indicate net gain, while negative values indicate net loss. Red shaded area represents theoretical values of ΔDOC below the criterion for non-significance (0.06 mg C L^{-1}, See Methods section for details). Thus, data points inside this region (gray symbols) correspond to *pipe* observations, whereas black symbols correspond to *reactors*. All observations across space and time are included. Note that in (**a**), point at coordinates (13.5, −2.56) was excluded for visual clarity.

In agreement with our prediction, a positive relationship was found between the absolute value of ΔDOC and RT across the whole dataset ($r_s = 0.39$, $p < 0.001$, $n = 74$). Accordingly, low RT observations were mostly identified as *pipes*, while the proportion of *reactors* increased with RT (Fig. 2).
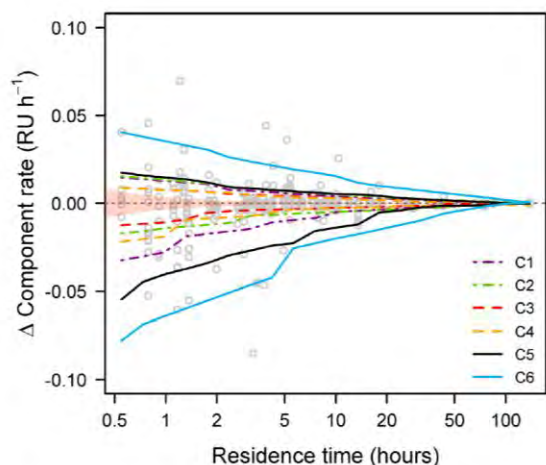
Fig. 3. Relationship between the rate of net change in fluorescence intensity (ΔComponent rate) for each PARAFAC component (C1–C6) and the water residence time of the reach. Positive values indicate net gain; negative values indicate net loss. Color lines are the non-parametric 10th and 90th percentile regression for each component, as a representation of the distribution of the data. Note overlapping of some of the lines (e.g., 90th percentile line of C3 is hidden from view). Gray circles are the rates calculated for each component and included for reference. Red shaded area represents theoretical values of net change in fluorescence intensity below the criterion for non-significance (0.004 RU; based on repeated measures on the same sample). All observations across space and time are included.

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

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

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

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

$\log_{10}RT$, logarithm of water residence time; DOC, dissolved organic carbon; Temp, water temperature.
Note- Variables shown are those included in the final model after the stepwise procedure based on the Akaike Information Criterion (see Methods for further details). Note that some of the included variables showed a slope that did not significantly differ from zero.

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

Variable	Coefficient	Standard error	t	p
$\log_{10}RT$	-0.133	0.035	-3.77	<0.001
DOC	-0.327	0.027	-12.08	<0.001
NO₃	0.075	0.012	6.26	<0.001
BIX	-0.804	0.238	-3.37	0.001

Intercept $= 0.970 \pm 0.190$; $R^2 = 0.75$; F-statistic $= 49.49$; $p < 0.001$; $n = 74$.
$\log_{10}RT$, logarithm of the water residence time; DOC, dissolved organic carbon; NO₃, nitrate; BIX, biological index.
Note- Shown are only the significant variables among those included in the final model after the stepwise procedure based on the Akaike Information Criterion (see Methods for further details).

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

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

Discussion

The net in-stream variation of DOC along the study reaches was generally low, with almost half of the observations suggesting conservative DOM transport. Such small variations are in agreement with similar studies conducted in boreal (Temnerud et al. 2007) and temperate (Palmer et al. 2016) catchments, where in-stream variations in DOC
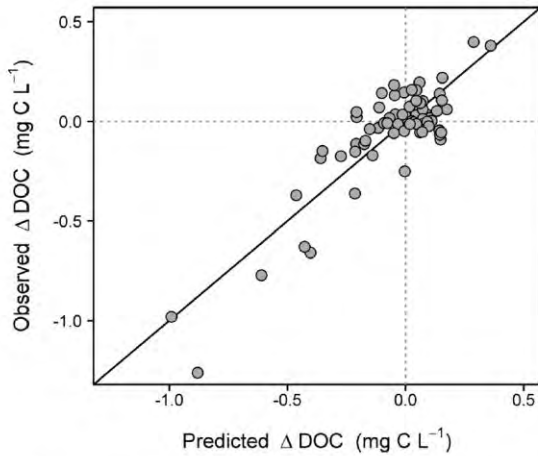
Fig. 4. Predicted relative to observed net change in dissolved organic carbon (ΔDOC). The 1 : 1 line is included for reference. The summary of the model is shown in Table 2.

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

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

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

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

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

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

waters, suggesting that intrinsic DOM properties control decay rates (Catalán et al. 2016), and thus DOM persistence in aquatic systems (Kellerman et al. 2015). At the short timescale we focus, however, the significant correlations between RT and both BIX ($r_s = 0.31$, $p = 0.007$, $n = 74$) and SUVA$_{254}$ ($r_s = -0.28$, $p = 0.021$, $n = 74$) do not support a decrease in bioreactivity as a plausible explanation for the observed trend. Therefore, given the symmetrical relationship between RT and the rates of DOM loss and generation (Fig. 2b), we hypothesize that this pattern is the result of catabolic and anabolic reactions balancing each other at high RT. If this holds true, we might expect a shift in the composition of DOM towards a more autochthonous character along those reaches with high RT, because DOM degradation should be compensated by aquatic DOM sources. Accordingly, using 18 h as an arbitrary threshold for DOM processing as RT increases, we observed a decrease in the most bioreactive terrestrial component (C5) coupled with an increase in autochthonous component C6 at high RT (Supporting Information Fig. 5). Therefore, whereas DOM processing at low RT may be constrained by time, higher RT would facilitate the co-occurrence of opposite reactions (e.g., primary production vs. heterotrophic respiration), resulting in lower net DOM variations than expected from single processes.

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

References

Ågren, A. M., I. Buffam, D. M. Cooper, T. Tiwari, C. D. Evans, and H. Laudon. 2014. Can the heterogeneity in stream dissolved organic carbon be explained by contributing landscape elements? Biogeosciences **11**: 1199–1213. doi:10.5194/bg-11-1199-2014

Arnosti, C. 2003. Microbial extracellular enzymes and their role in dissolved organic matter cycling, p. 315–342. *In* S. Findlay and R. Sinsabaugh [eds.], Aquatic ecosystems. Interactivity of dissolved organic matter. Elsevier.

Aufdenkampe, A. K., E. Mayorga, P. A. Raymond, J. M. Melack, S. C. Doney, S. R. Alin, R. E. Aalto, and K. Yoo. 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. Front. Ecol. Environ. **9**: 53–60. doi:10.1890/100014

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using {lme4}. J. Stat. Softw. **67**: 1–48. doi:10.18637/jss.v067.i01

Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold, and F. Sabater. 2008. Biophysical controls on organic carbon fluxes in fluvial networks. Nat. Geosci. **1**: 95–100. doi:10.1038/ngeo101

Battin, T. J., S. Luyssaert, L. A. Kaplan, A. K. Aufdenkampe, A. Richter, and L. J. Tranvik. 2009. The boundless carbon cycle. Nat. Geosci. **2**: 598–600. doi:10.1038/ngeo618

Borchardt, M. A. 1996. Nutrients, p. 183–227. *In* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe [eds.], Algal ecology. Academic Press.

Catalán, N., R. Marcé, D. N. Kothawala, and L. J. Tranvik. 2016. Organic carbon decomposition rates controlled by water retention time across inland waters. Nat. Geosci. **9**: 501–504. doi:10.1038/ngeo101

Ciais, P., Sabine, C., Bala, G., et al. 2013. Carbon and other biogeochemical cycles. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM), pp. 465–570. Cambridge University Press, Cambridge, United King- dom and New York, NY, USA.

Coble, P. G. 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Mar. Chem. **51**: 325–346. doi:10.1016/0304-4203(95)00062-3

Cole, J. J., and others 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems **10**: 172–185. doi:10.1007/s10021-006-9013-8

Cory, R. M., and L. A. Kaplan. 2012. Biological lability of streamwater fluorescent dissolved organic matter. Limnol. Oceanogr. **57**: 1347–1360. doi:10.4319/lo.2012.57.5.1347

Dawson, J. J. C., C. Bakewell, and M. F. Billett. 2001. Is in-stream processing an important control on spatial changes in carbon fluxes in headwater catchments? Sci. Total Environ. **265**: 153–167. doi:10.1016/S0048-9697(00)00656-2 doi:10.1016/S0048-9697(00)00656-2

Fellman, J. B., E. Hood, and R. G. M. Spencer. 2010. Fluorescence spectroscopy opens new windows into dissolved organic

matter dynamics in freshwater ecosystems: A review. Limnol. Oceanogr. **55**: 2452–2462. doi:10.4319/lo.2010.55.6.2452

García-Ruiz, J. M., J. I. López-Moreno, S. M. Vicente-Serrano, T. Lasanta–Martínez, and S. Beguería. 2011. Mediterranean water resources in a global change scenario. Earth Sci. Rev. **105**: 121–139. doi:10.1016/j.earscirev.2011.01.006 doi:10.1016/j.earscirev.2011.01.006

Guillemette, F., and P. A. del Giorgio. 2011. Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. Limnol. Oceanogr. **56**: 734–748. doi:10.4319/lo.2011.56.2.0734

Hanley, K. W., W. M. Wollheim, J. Salisbury, T. Huntington, and G. Aiken. 2013. Controls on dissolved organic carbon quantity and chemical character in temperate rivers of North America. Global Biogeochem. Cycles. **27**: 492–504. doi:10.1002/gbc.20044

Helms, J. R., A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber, and K. Mopper. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnol. Oceanogr. **53**: 955–969. doi:10.4319/lo.2008.53.3.0955

Hotchkiss, E. R., and R. O. J. Hall. 2015. Whole-stream 13C tracer addition reveals distinct fates of newly fixed carbon. Ecology **96**: 403–416. doi:10.1890/14-0631.1

Huguet, A., L. Vacher, S. Relexans, S. Saubusse, J. M. Froidefond, and E. Parlanti. 2009. Properties of fluorescent dissolved organic matter in the Gironde Estuary. Org. Geochem. **40**: 706–719. doi:10.1016/j.orggeochem.2009.03.002

Jaffé, R., D. McKnight, N. Maie, R. Cory, W. H. McDowell, and J. Campbell. 2008. Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. J. Geophys. Res. Biogeosci. **113**: 1900–1908. doi:10.1029/2008JG000683

Kaplan, L. A., and T. L. Bott. 1982. Diel fluctuations of DOC generated by algae in a piedmont stream. Limnol. Oceanogr. **27**: 1091–1100. doi:10.4319/lo.1982.27.6.1091

Kellerman, A. M., D. N. Kothawala, T. Dittmar, and L. J. Tranvik. 2015. Persistence of dissolved organic matter in lakes related to its molecular characteristics. Nat. Geosci. **8**: 454–457. doi:10.1038/ngeo2440

Kenney, B. C. 1991. Comments on "Some misconceptions about the spurious correlation problem in the ecological literature" by Y.T. Prairie and D.F. Bird. Oecologia **86**: 152.

Kothawala, D. N., X. Ji, H. Laudon, A. M. Ågren, M. N. Futter, S. J. Köhler, and L. J. Tranvik. 2015. The relative influence of land cover, hydrology, and in-stream processing on the composition of dissolved organic matter in boreal streams. J. Geophys. Res. Biogeosci. **120**: 1491–1505. doi:10.1002/2015JG002946

Lawaetz, A. J., and C. A. Stedmon. 2009. Fluorescence intensity calibration using the Raman scatter peak of water. Appl. Spectrosc. **63**: 936–940. doi:10.1366/000370209788964548

Mcdowell, W. H., and S. G. Fisher. 1976. Autumnal processing of dissolved organic matter in a small woodland stream ecosystem. Ecology **57**: 561–569. doi:10.2307/1936440

Murphy, K. R., C. A. Stedmon, P. Wenig, and R. Bro. 2014. OpenFluor- an online spectral library of auto-fluorescence by organic compounds in the environment. Anal. Methods **6**: 658–661. doi:10.1039/C3AY41935E

Oldham, C. E., D. E. Farrow, and S. Peiffer. 2013. A generalized Damköhler number for classifying material processing in hydrological systems. Hydrol. Earth Syst. Sci. **17**: 1133–1148. doi:10.5194/hess-17-1133-2013

Olson, J. S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology **44**: 322–331. doi:10.2307/1932179

Palmer, S. M., et al. 2016. Sporadic hotspots for physicochemical retention of aquatic organic carbon: From peatland headwater source to sea. Aquat. Sci. Aquat. Sci. **78**: 491–504. doi:10.1007/s00027-015-0448-x

Parker, S. R., S. R. Poulson, M. G. Smith, C. L. Weyer, and K. M. Bates. 2010. Temporal variability in the concentration and stable carbon isotope composition of dissolved inorganic and organic carbon in two Montana, USA rivers. Aquat. Geochem. **16**: 61–84. doi:10.1007/s10498-009-9068-1

Pebesma, E. J. 2004. Multivariable geostatistics in S: The gstat package. Comput. Geosci. **30**: 683–691. doi:10.1016/j.cageo.2004.03.012

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2016. {nlme}: Linear and Nonlinear Mixed Effects Models. R package version 3.1-128, http://CRAN.R-project.org/package=nlme.

Quinn, G., and M. Keough. 2002. Experimental design and data analysis for biologists. Cambridge Univ. Press.

R Core Team. 2015. R: A language and environment for statistical computing.

Raymond, P. A., and J. E. Bauer. 2000. Bacterial consumption of DOC during transport through a temperate estuary. Aquat. Microb. Ecol. **22**: 1–12. doi:10.3354/ame022001

Raymond, P. A., and others. 2013. Global carbon dioxide emissions from inland waters. Nature **503**: 355–359. doi:10.1038/nature12760

Raymond, P. A., J. E. Saiers, and W. V. Sobczak. 2016. Hydrological and biogeochemical controls on watershed dissolved organic matter transport: Pulse-shunt concept. Ecology **97**: 5–16. doi:10.1890/14-1684.1

Sharp, E. L., S. A. Parson, and B. Jefferson. 2006. Coagulation of NOM: Linking character to treatment. Water Sci. Technol. **53**: 67–76. doi:10.2166/wst.2006.209

Spencer, R. G. M., B. A. Pellerin, B. A. Bergamaschi, B. D. Downing, T. E. C. Kraus, D. R. Smart, R. A. Dahlgren, and P. J. Hernes. 2007. Diurnal variability in riverine dissolved organic matter composition determined by in situ optical measurement in the San Joaquin River (California, USA). Hydrol. Process. **21**: 3181–3189. doi:10.1002/hyp.6887

Stedmon, C. A., and S. Markager. 2005. Resolving the variability in dissolved organic matter fluorescence in a temperate

estuary and its catchment using PARAFAC analysis. Limnol. Oceanogr. **50**: 686–697. doi:10.4319/lo.2005.50.2.0686

Stedmon, C. A., and R. Bro. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: A tutorial. Limnol. Oceanogr. Methods **6**: 572–579. doi:10.4319/lom.2008.6.572

Stubbins, A., and others. 2010. Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. Limnol. Oceanogr. **55**: 1467–1477. doi:10.4319/lo.2010.55.4.1467

Taylor, P. G., and A. R. Townsend. 2010. Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. Nature **464**: 1178–1181. doi:10.1038/nature08985

Temnerud, J., J. Seibert, M. Jansson, and K. Bishop. 2007. Spatial variation in discharge and concentrations of organic carbon in a catchment network of boreal streams in northern Sweden. J. Hydrol. **342**: 72–87. doi:10.1016/j.jhydrol.2007.05.015 doi:10.1016/j.jhydrol.2007.05.015

Thorp, J. H., and M. D. Delong. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. Oikos **96**: 543–550. doi:10.1034/j.1600-0706.2002.960315.x

Tiwari, T., H. Laudon, K. Beven, and A. M. Ågren. 2014. Downstream changes in DOC: Inferring contributions in the face of model uncertainties. Water Resour. Res. **50**: 514–525. doi:10.1002/2013WR014275

Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ. Sci. Technol. **37**: 4702–4708. doi:10.1021/es030360x

Wickland, K. P., G. R. Aiken, K. Butler, M. M. Dornblaser, R. G. M. Spencer, and R. G. Striegl. 2012. Biodegradability of dissolved organic carbon in the Yukon River and its tributaries: Seasonality and importance of inorganic nitrogen. Global Biogeochem. Cycles **26**: GB0E03. doi:10.1029/2012GB004342

Wilson, H. F., and M. A. Xenopoulos. 2009. Effects of agricultural land use on the composition of fluvial dissolved organic matter. Nat. Geosci. **2**: 37–41. doi:10.1038/ngeo391

Wollheim, W. M., R. J. Stewart, G. R. Aiken, K. D. Butler, N. B. Morse, and J. Salisbury. 2015. Removal of terrestrial DOC in aquatic ecosystems of a temperate river network. Geophys. Res. Lett. **42**: 1–9. doi:10.1002/2015GL064647

Worrall, F., T. P. Burt, and J. Adamson. 2006. The rate of and controls upon DOC loss in a peat catchment. J. Hydrol. **321**: 311–325. doi:10.1016/j.jhydrol.2005.08.019 doi:10.1016/j.jhydrol.2005.08.019

Yamashita, Y., N. Maie, H. Briceño, and R. Jaffé. 2010. Optical characterization of dissolved organic matter in tropical rivers of the Guayana Shield, Venezuela. J. Geophys. Res. Biogeosci. **115**: G00F10. doi:10.1029/2009JG000987

Zsolnay, A., E. Baigar, M. Jimenez, B. Steinweg, and F. Saccomandi. 1999. Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. Chemosphere **38**: 45–50. doi:10.1016/S0045-6535(98)00166-0

Conflict of Interest

None declared.

PAPER IV

Delineating the continuum of dissolved organic matter in river networks

Manuscript

Delineating the continuum of dissolved organic matter in river networks

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Abstract

The origin and reactivity of dissolved organic matter (DOM) has 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 are still poorly understood, 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 river continuum under different hydrological conditions, encompassing small headwater streams to the river mouth, and base flow to storm events. We find a homogeneous longitudinal pattern of DOM during storm events, with highly concentrated and diverse DOM along the river network. In contrast, during non-storm conditions, the concentration of DOC and the chemical diversity of DOM exhibit their maxima in medium-sized streams, depicting a bell-shaped pattern along the river continuum. The analysis of a global dataset further shows that DOC concentration peaks in streams draining catchments of 300 600 km^2, indicating that longitudinal patterns of DOC depicting maxima in medium-sized streams are common in river networks worldwide. We posit that this emerging pattern results 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 river network functioning can be formulated.

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5 GENERAL DISCUSSION

In this thesis, we assessed the controls on the dynamics of riverine DOM quantity and composition in the framework of a Mediterranean catchment. Each of the papers examines different aspects of DOM dynamics, from biodegradation kinetics of different DOM fractions to longitudinal patterns of DOC and DOM chemical diversity along river continua. In this section I provide a broader discussion of the findings described in papers I to IV in a common context, and comment on additional relevant aspects that were not addressed in the papers.

A brief history of residence time: hydrologic control on the sources and processing of DOM

Results compiled in this thesis pose hydrology as the main variable controlling the sources and processing, and therefore the concentration and composition, of DOM in river networks.

The influence of hydrology on the quantity and composition of terrestrial DOM inputs is well documented. The properties of DOM transferred from soils to streams reflects those of bulk soil organic matter, and varies with soil depth and with hydrologic flowpath (Aitkenhead-Peterson et al 2003; Marín-Spiotta et al 2014). Top soil layers usually contain large amounts of organic matter dominated by young, plant-derived compounds such as lignin and polyphenols. In contrast, deeper soils have less organic content, and tend to accumulate older materials with greater contribution of microbially derived compounds (Kaiser and Kalbitz 2012). Hence, under low flow conditions, terrestrial DOM inputs resemble organic matter from older and deeper soils (Striegl et al 2005; Neff et al 2006; Spencer et al 2010), whereas at high and extreme discharge events, DOM exported from soils reflects surface organic-rich layers with high aromatic content (Raymond et al 2016 and references therein). This is in agreement with results from the Fluvià. For example, we measured very old radiocarbon ages in river DOC during low flows (>1,000 yr

BP, paper I), suggesting hydrological connection with deeper organic soil layers. In contrast, surface soils contributed most DOM to the river network during storm events, as indicated by the highest concentrations of terrestrial DOM throughout the hydrologic year (paper IV). At the same time hydrology modulates water residence times in the network, and therefore the contribution of in-stream DOM sources (paper III). Thus, the contribution of aquatic and terrestrial sources to bulk riverine DOM fluxes can highly vary with hydrological conditions. In our case, for instance, we observed that while large amounts of terrestrial, humic-like DOM are transferred to the Mediterranean Sea by the Fluvià during storms, protein-like DOM of aquatic origin dominates exports under low flow conditions (Fig 5).

The influence of hydrology on the processing of riverine DOM is much less known. Recent theoretical works have proposed hydrology as a key variable for DOM processing, as it modulates the time that molecules spend in a system, and thus the opportunity for their reaction (Battin et al 2008; Raymond et al 2016). In paper III, we empirically demonstrate that water residence time is a major constraint for DOM processing in river networks. Hence, it can be predicted that in-stream DOM transformations are relevant during low flow periods, and that during storm conditions river networks behave as passive pipes of DOM. But the role of residence time on controlling riverine DOM budgets can be much more complex. From our findings in paper III, one might expect that the higher the water residence time within a system the higher the in-stream retention and/or generation of DOM. And this may be valid for a single or a combination of similar processes, for instance heterotrophic respiration and/or photo-oxidation. It becomes complex, however, when opposite
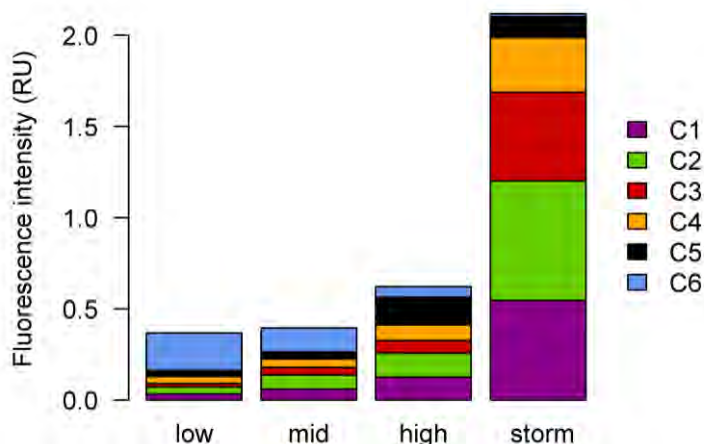

Fig. 5. Dissolved organic matter exported to the Mediterranean Sea by the Fluvià River under different hydrological conditions. Bars express the combined fluorescence of the six
) A A
C4 resemble terrestrial, humic-like materials; C5 is a protein-like component of terrestrial origin; C6 resembles protein-like compounds of aquatic origin

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processes co-occur. In such a case, if we assume that high residence times enhance the likelihood of opposite in-stream processes (e.g. catabolism vs. anabolism) to balance each other, a tendency to low net DOC change can be hypothesized with higher residence time (Fig. 6). In other words, at high residence times, gains may offset losses. Supporting this hypothesis, which I named the '*tempus compensabit* hypothesis', we observed a symmetrical decrease in the net rates of DOM production and degradation with increasing RT (Fig. 2b in page 81).

There are some interesting implications of the *tempus compensabit* hypothesis. On one hand, a null net balance in terms of DOC does not necessarily imply that a system does not process DOM. As long as RT is high enough, systems may reach a biogeochemical steady state whereby the net balance of DOM is buffered by the compensation between generation and removal processes. On the other hand, it is
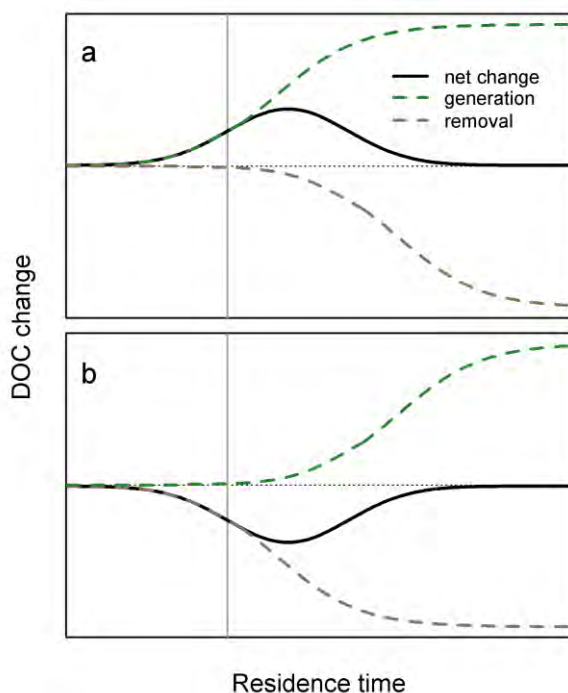
Fig. 6. Conceptual representation of the *tempus compensabit* hypothesis. **a** and **b** panels represent aquatic systems dominated by DOM generation and removal processes, respectively. The gray vertical line indicates a hypothetical residence time above which generation and removal processes co-occur. Below this threshold, only one type of process takes place in the system. According to the *tempus compensabit* hypothesis, the gross removal and generation of DOM increases with residence time until processes are limited by compositional and/or environmental factors (green dashed line in **a** and brown dashed line in **b**). Nevertheless, if the residence time of the system is high enough, generation and removal processes start balancing each other, buffering the net change of DOC (black lines). At residence times on the order of seconds and minutes, this phenomenon will be insignificant, or in any case unmeasureable with current analytical methods. However, beyond a hypothetical residence time threshold (gray vertical line), the balance between processes may become relevant for DOM and carbon budgets

expected a shift in DOM composition from terrestrial to aquatic character with increasing RT. In this regard, the evaluation of DOM compositional changes may provide useful information for discerning between actual passive pipes and reactors that simply are at a biogeochemical steady state. Indeed, in those reaches of the Fluvià network with high RT and apparently low rates of DOM processing, we observed a decrease in terrestrial humic-like fluorescence coupled to an increase of *in situ* produced, protein-like DOM (Fig. S5 in page 144). Notably, taking into account the potential compensation of DOM removal by in-stream additions of DOM might help explain current discrepancies in river network functioning. For instance, it might reconcile the high gross uptake rates of DOC calculated from whole stream reach studies (Mineau et al 2016 and references therein) with the low net removal rates observed at the network scale (Worrall et al 2006; Wollheim et al 2015).

I cannot assert how extendible may this hypothesis be to systems with much longer retention times such as lakes. However, it is true that, although lakes are hotspots of DOM degradation (Tranvik et al 2009), the net removal of DOC is usually lower than expected when compared with systems with much shorter residence times such as small streams (Mineau et al 2016; Catalán et al 2016). This is often indirectly attributed to differences in DOM reactivity (Catalán et al 2016), but the counterbalance of processes could be also involved. Indeed, Kothawala et al (2014) and Kellerman et al (2015) analyzed a survey of hundreds of boreal lakes to find that an aliphatic, protein-like fraction of DOM is ubiquitous in the water column of lakes, where it might be continuously recycled and partially offset terrestrial DOM degradation. I therefore suggest that the balance of catabolic and anabolic processes is significant for DOM and carbon budgets along the entire land-to-ocean continuum. Overlooking this phenomenon would lead to an underestimation of DOM decay rates, with potential implications for watershed biogeochemical modeling and predictions.

The counterbalance of biogeochemical processes in inland waters is more usual than one might think. For instance, the retention of nutrients in streams is often offset by release from the sediments through organic matter mineralization, desorption, and dissolution processes (von Schiller et al 2015). Hence, although we formulate this hypothesis from a perspective of carbocentric limnology[3], it may also be applicable to other biogeochemically active solutes.

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[3] "Carbocentric limnology" is a term coined by Jon Cole to informally refer to any research field involving C cycling in inland waters (see Prairie (2008))

On the inner and outer limits of DOM degradation

Why some DOM fractions or compounds react while some others do not is subject to intense debate, not only in river networks (Marín-Spiotta et al 2014) but also in soils, lakes, and the ocean (Schmidt et al 2011; Arrieta et al 2015; Kellerman et al 2015). In papers I and II, we find strong dependence of DOM reactivity on its composition. For instance, we show that DOM biodegradability increases with the share of low molecular-weight DOM compounds, and that these in turn control the biodegradation kinetics of bulk DOM. Furthermore, in paper III we find protein-like DOM to be more reactive along stream and river reaches than humic-like materials. These results support the classical paradigm of 'chemical' or 'intrinsic recalcitrance', whereby DOM persistence in the environment depends on the chemical composition of DOM (see Kleber (2010) for a detailed discussion on this concept). Nevertheless, our results indicate that other factors extrinsic to DOM composition are also at play. As mentioned above, in paper III we demonstrate that the time DOM spends in the system is determinant on its opportunity to react. Thus, chemically unstable DOM can be simply bypassed through river networks as long as water residence times are sufficiently low, for instance during extreme discharge events. On the contrary, it is only when residence times are high that DOM can react during transport. Other environmental factors such as water temperature and nutrient availability can also intervene on whether DOM is processed or not (paper III). In this sense, results from this thesis highlight that both chemical composition and environmental factors influence DOM reactions, and so none of them should be overlooked when addressing the controls on DOM persistence in river networks.

But these results are far from being conclusive. To the best of my knowledge, there is so far no standardized method to measure directly and independently the influence of intrinsic and extrinsic factors on DOM degradation. For instance, a traditional method to measure DOM intrinsic biodegradability is to incubate water samples in the dark. Nonetheless, the vast majority of studies do not consider that differences in water physicochemistry and bacterial inoculum used may interfere with the measurement, precluding comparison between systems and studies (e.g. papers I and II). I do not criticize the method *per se*, as it can be used to address other relevant questions (e.g. paper II; McCallister and Del Giorgio 2012; Guillemette et al 2015); however, traditional incubation methods do not provide conclusive information on the role of intrinsic DOM properties. In this sense, a critical line of future research is to develop standardized methods to address the influence of intrinsic and extrinsic factors on DOM degradation.

DOM maxima and where to find it

In paper IV we identified a longitudinal pattern of DOC and DOM chemical diversity along the Fluvià river continuum, with maxima in medium-sized streams; and the same pattern emerged upon analysis of thousands of globally distributed DOC measurements (Fig. 3 in page 94). On our attempt to unravel the factors that modulate the shape of the pattern, we hypothesized that catchment properties and the configuration of the dendritic network would be determinant. Certainly, the modeled maxima of DOC concentration and DOM chemical diversity in the Fluvià roughly coincides with the inflection point of the catchment area cumulative curve (Fig. 7). This curve shows the drainage area that the mainstem integrates as it flows downstream. Thus, it combines both the geomorphology of the catchment as well as the entrance of different tributaries along the river continuum. A logistic relationship between catchment area and stream length seems appropriate in the case of the Fluvià (Fig. 7), although we cannot assert whether this is common in other river networks. In fact, a power function with an exponent between 0 and 1 (the so-called "diminishing returns law" in economics) seems more likely from a conceptual point of view (Rosso et al 1991). Still, regardless of the appropriate specific function, it seems worthy to explore the catchment area cumulative curve as a potential tool to predict maxima of DOM amount and diversity for individual river networks.
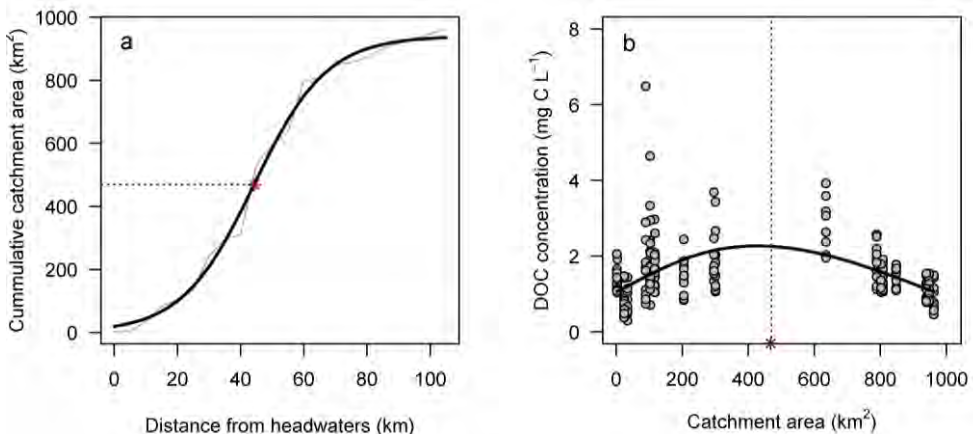
Fig. 7. Link between catchment geomorphology and dissolved organic carbon (DOC) in the Fluvià river network. **a**, cumulative catchment area integrated by the mainstem from headwaters to the river mouth (gray line, 5-km resolution). The black line shows the fit of a logistic function, with the inflection point indicated with a red asterisk. **b**, longitudinal patterns of DOC. The heavy line shows the fit of a generalized additive model. The dotted line corresponds with the inflection point in panel **a**.

Is it all in the middle?

We have already discussed the immediate repercussion of the emerging longitudinal patterns of DOM within paper IV, but because DOM intervenes in multiple ecological processes in aquatic systems (Prairie 2008), our concept may shed light on the patterns of other relevant ecological variables along river continua.

From a perspective of ecological theory, higher resource diversity induces specialization and increases species richness and functional diversity (Rosenfeld 2002; Clavel et al 2011). Thus, it can be hypothesized that elevated DOM chemical diversity translates into elevated biological diversity of those communities that rely on DOM as the main resource. This is the case, for instance, of riverine bacterial communities, and thus their dynamics could be tightly linked to those of DOM. Current knowledge suggests that bacterial taxonomic richness is maximal not in medium-sized rivers but in headwater streams (Ruiz-González et al 2015a; Savio et al 2015; Niño-García et al 2016); and this is consistent with the fact that riverine bacteria is dominated by taxa recruited from soils that enter networks through headwaters (Besemer et al 2012; Crump et al 2012; Ruiz-González et al 2015a). In this sense, it seems there is no direct coupling between DOM and bacterial community composition. However, recent findings demonstrate that the spatial distribution of bacterial functional traits in aquatic networks is strongly linked to the DOM pool to which bacteria are exposed, and that it cannot be reconstructed from patterns in community composition (Wilhelm et al 2015; Ruiz-González et al 2015b). In fact, it is intuitive to assume that not all taxa recruited from soils will remain active once entering streams. Hence, I predict that while community composition is more diverse in headwaters owing to the importation of soil communities, bacteria functional diversity follows the same pattern than their resource (DOM), with maxima in medium-sized streams (Fig 8). There is currently a paucity of detailed data on functional diversity along river continua, and so this prediction cannot be tested at the moment. Nevertheless, 16S rRNA sequencing is becoming more available as a tool to assess bacterial communities at the function level, and thus future studies should focus on investigating how functional diversity varies along river continua.

The taxonomic diversity of macroinvertebrate communities also exhibits maxima in medium-sized streams (Vannote et al 1980; Grubaugh et al 1996; Reyjol et al 2003; Tomanova et al 2007). In this case it is unlikely that a causal relationship between the dynamics of macroinvertebrates and DOM exists, because although some species can ingest DOM by piercing or sucking, most are adapted to utilize particulate organic matter as a resource (Wetzel 1983). However, an interesting corollary is that medium-sized streams might harbor the highest level of ecological information within river networks, as they contain the maximal diversity of DOM, potentially active bacteria, and macroinvertebrates.
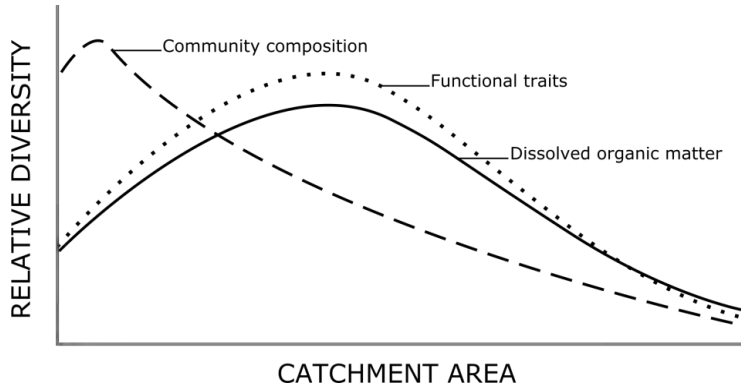
Fig. 8. Hypothetical distribution of bacterial compositional and functional diversity along with DOM chemical diversity through river continua. The aesthetics purposely resemble those of Fig. 2 in Vannote et al (1980).

6 CONCLUSIONS

The main conclusions of this thesis are as follows:

I.   Not all fractions of riverine DOM react the same way. DOM fractions encompassing the smallest and largest DOM compounds present fast and predictable degradation kinetics, while humic substances depict unpredictable, non-monotonic patterns. Tracking different fractions of DOM in laboratory and field studies is complex but necessary to improve our understanding of DOM dynamics in river networks.

II. The role of stream and river reaches as pipes or reactors of DOM can highly vary with space and time. River networks must be envisaged as complex sequences of ecosystems that switch between pipe and reactor conditions depending on network hydrology and water physicochemistry.

III. Water residence time is the main factor controlling the processing of DOM in river networks. DOC concentration and water temperature are also potential factors promoting DOM processing.

IV. The net balance between in-stream gains and losses of riverine DOM is controlled by the intrinsic properties of DOM and the availability of nutrients. Notably, the in-stream generation of DOM can be as much as important as its removal in terms of frequency and magnitude.

V.   The concentration and composition of DOM show great spatial heterogeneities during drought episodes. The hydrological disconnection from the catchment during drought seems to promote a relevant influence of autochthonous DOM sources. However, tree leaves from overhanging canopies stand out as an important source of DOM, especially in sites with high RT such as isolated water pools that favor the accumulation and decomposition of leaves.

VI.   During extreme discharge events large amounts of terrestrial DOM are transferred from soils to streams, which are then rapidly shunted towards the ocean without significant alteration. At such moments the quantity and chemical diversity of DOM are high and steady along the river continuum.

VII.   In contrast, a bell-shaped pattern with maxima in medium-sized streams arises with decreasing network discharge, probably due to the influence of DOM-poor groundwater in headwaters and in-stream degradation of DOM compounds in larger 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.

REFERENCES

Aiken GR, Hsu-Kim H, Ryan JN (2011) Influence of Dissolved Organic Matter on the Environmental Fate of Metals, Nanoparticles, and Colloids. Environ Sci Technol 45:3196–3201. doi: 10.1021/es103992s

Aitkenhead-Peterson JA, Mcdowell WH, Neff JC (2003) Sources, production, and regulation of allochthonous dissolved organic matter inputs to surface waters. In: Findlay S, Sinsabaugh R (eds) Aquat. Ecosyst. Interactivity dissolved Org. matter. Elsevier. Academic Press, pp 26–70

Amon RMW, Benner R (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnol Oceanogr 41:41–51.

Arrieta JM, Mayol E, Hansman RL, et al (2015) Dilution limits dissolved organic carbon utilization in the deep ocean. Science (80-). doi: 10.1126/science.1258955

Aufdenkampe AK, Mayorga E, Raymond PA, et al (2011) Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. Front Ecol Environ 9:53–60. doi: 10.1890/100014

Bastviken D, Tranvik LJ, Downing JA, et al (2011) Freshwater Methane Emissions Offset the Continental Carbon Sink. Science (80-) 331:50. doi: 10.1126/science.1196808

Battin TJ, Kaplan LA, Findlay S, et al (2008) Biophysical controls on organic carbon fluxes in fluvial networks. Nat Geosci 1:95–100. doi: 10.1038/ngeo101

Battin TJ, Luyssaert S, Kaplan LA, et al (2009) The boundless carbon cycle. Nat Geosci 2:598–600.

Bernal S, Schiller D, Sabater F, Martí E (2013) Hydrological extremes modulate nutrient dynamics in mediterranean climate streams across different spatial scales. Hydrobiologia 719:31–42. doi: 10.1007/s10750-012-1246-2

Bernhardt ES, Likens GE (2002) Dissolved Organic Carbon Enrichment Alters Nitrogen Dynamics in a Forest Stream. Ecology 83:1689–1700.

Bertilsson S, Jones JB (2003) Supply of dissolved organic matter to aquatic ecosystems: Autochthonous sources. In: Findlay S, Sinsabaugh R (eds) Aquat. Ecosyst. Interactivity dissolved Org. matter. Elsevier. Academic Press, pp 3–24

Besemer K, Peter H, Logue JB, et al (2012) Unraveling assembly of stream biofilm communities. ISME J 6:1459–68. doi: 10.1038/ismej.2011.205

Blakney GT, Hendrickson CL, Marshall AG (2011) Predator data station: A fast data acquisition system for advanced FT-ICR {MS} experiments. Int J Mass Spectrom 306:246–252. doi: http://dx.doi.org/10.1016/j.ijms.2011.03.009

Borges A V., Darchambeau F, Teodoru CR, et al (2015) Globally significant greenhouse-gas emissions from African inland waters. Nat Geosci. doi: 10.1038/ngeo2486

Butturini A, Guarch A, Romaní AM, et al (2016) Hydrological conditions control in situ DOM retention and release along a Mediterranean river. Water Res 99:33–45. doi: http://dx.doi.org/10.1016/j.watres.2016.04.036

Catalán N, Marcé R, Kothawala DN, Tranvik LJ (2016) Organic carbon decomposition rates controlled by water retention time across inland waters. Nat Geosci 9:501–504.

Catalán N, Obrador B, Pretus JL (2014) Ecosystem processes drive dissolved organic matter quality in a highly dynamic water body. Hydrobiologia 728:111–124.

Clavel J, Julliard R, Devictor V (2011) Worldwide decline of specialist species: Toward a global functional homogenization? Front Ecol Environ 9:222–228. doi: 10.1890/080216

Cole JJ, Prairie YT, Caraco NF, et al (2007) Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. Ecosystems 10:172–185. doi: 10.1007/s10021-006-9013-8

Cory RM, Ward CP, Crump BC, Kling GW (2014) Sunlight controls water column processing of carbon in arctic fresh waters. Science (80-) 345:925–928. doi: 10.1126/science.1253119

Creed IF, McKnight DM, Pellerin BA, et al (2015) The river as a chemostat: fresh perspectives on dissolved organic matter flowing down the river continuum. Can J Fish Aquat Sci 72:1272–1285. doi: 10.1139/cjfas-2014-0400

Crump BC, Amaral-Zettler LA, Kling GW (2012) Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. ISME J 6:1629–1639. doi: 10.1038/ismej.2012.9

Dahm CN, Baker MA, Moore DI, Thibault JR (2003) Coupled biogeochemical and hydrological responses of streams and rivers to drought. Freshw Biol 48:1219–1231. doi: 10.1046/j.1365-2427.2003.01082.x

Dittmar T, Koch B, Hertkorn N, Kattner G (2008) A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnol Oceanogr Methods 6:230–235. doi: 10.4319/lom.2008.6.230

Duarte CM, Prairie YT (2005) Prevalence of heterotrophy and atmospheric CO_2 emissions from aquatic ecosystems. Ecosystems 8:862–870. doi: 10.1007/s10021-005-0177-4

Dungait JAJ, Hopkins DW, Gregory AS, Whitmore AP (2012) Soil organic matter turnover is governed by accessibility not recalcitrance. Glob Chang Biol 18:1781–1796. doi: 10.1111/j.1365-2486.2012.02665.x

Fasching C, Behounek B, Singer G., Battin T. (2014) Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams. Sci Rep. doi: 10.1038/srep04981

Fellman JB, Hood E, Raymond PA, et al (2015) Evidence for the assimilation of ancient glacier organic carbon in a proglacial stream food web. Limnol Oceanogr 60:1118–1128. doi: 10.1002/lno.10088

Fellman JB, Hood E, Spencer RGM (2010) Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. Limnol Oceanogr 55:2452–2462. doi: 10.4319/lo.2010.55.6.2452

Fisher SG, Likens GE (1973) Energy Flow in Bear Brook , New Hampshire : An Integrative Approach to Stream Ecosystem Metabolism Author (s): Stuart G . Fisher and Gene E . Likens Reviewed work (s): Published by : Ecological Society of America Stable URL : http://www.jstor.org/stab. Ecol Monogr

43:421–439.

Gasith A., Resh VH. H (1999) Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. Annu Rev Ecol Syst 30:51–81. doi: 10.1146/annurev.ecolsys.30.1.51

Grubaugh JW, Wallace JB, Houston ES (1996) Longitudinal changes of macroinvertebrate communities along an Appalachian stream continuum. Can J Fish Aquat Sci 53:896–909. doi: 10.1139/f95-247

Guillemette F, McCallister SL, del Giorgio PA (2015) Selective consumption and metabolic allocation of terrestrial and algal carbon determine allochthony in lakes. ISME J 1–10. doi: 10.1038/ismej.2015.215

Hartmann J, Lauerwald R, Moosdorf N (2014) A Brief Overview of the {GLObal} {RIver} Chemistry Database, {GLORICH}. Procedia Earth Planet Sci 10:23–27. doi: http://dx.doi.org/10.1016/j.proeps.2014.08.005

Helms JR, Stubbins A, Ritchie JD, et al (2008) Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnol Oceanogr 53:955–969. doi: 10.4319/lo.2008.53.3.0955

Hotchkiss ER, Hall ROJ (2015) Whole-stream 13C tracer addition reveals distinct fates of newly fixed carbon. Ecology 96:403–416. doi: 10.1890/14-0631.1

Huber SA, Balz A, Abert M, Pronk W (2011) Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography--organic carbon detection--organic nitrogen detection (LC-OCD-OND). Water Res 45:879–85. doi: 10.1016/j.watres.2010.09.023

Huguet A, Vacher L, Relexans S, et al (2009) Properties of fluorescent dissolved organic matter in the Gironde Estuary. Org Geochem 40:706–719. doi: 10.1016/j.orggeochem.2009.03.002

Jaffé R, Yamashita Y, Maie N, et al (2012) Dissolved Organic Matter in Headwater Streams: Compositional Variability across Climatic Regions of North America. Geochim Cosmochim Acta 94:95–108. doi: 10.1016/j.gca.2012.06.031

Judd KE, Crump BC, Kling GW (2006) Variation in dissolved organic matter controls bacterial production and community composition. Ecology 87:2068–2079. doi: 10.1890/0012-9658(2006)87[2068:VIDOMC]2.0.CO;2

Kaiser K, Kalbitz K (2012) Cycling downwards – dissolved organic matter in soils. Soil Biol Biochem 52:29–32. doi: http://dx.doi.org/10.1016/j.soilbio.2012.04.002

Kaiser NK, Quinn JP, Blakney GT, et al (2011) A Novel 9.4 Tesla FTICR Mass Spectrometer with Improved Sensitivity, Mass Resolution, and Mass Range. J Am Soc Mass Spectrom 22:1343–1351. doi: 10.1007/s13361-011-0141-9

Kellerman AM, Dittmar T, Kothawala DN, Tranvik LJ (2014) Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. Nat Commun. doi: 10.1038/ncomms4804

Kellerman AM, Kothawala DN, Dittmar T, Tranvik LJ (2015) Persistence of dissolved organic matter in lakes related to its molecular characteristics. Nat Geosci 8:454–457.

Kim S, Kramer RW, Hatcher PG (2003) Graphical Method for Analysis of

Ultrahigh-Resolution Braodband mass spectra of Natural Organic Matter, the Van Krevelen diagram. Anal Chem 75:5336–5344. doi: 10.1021/ac034415p CCC:

Kirk JTO (1994) Light and photosynthesis in aquatic ecosystems. Cambridge university press

Kleber M (2010) What is recalcitrant soil organic matter? Environ Chem 7:320–332. doi: 10.1071/EN10006

Koch BP, Dittmar T (2006) From mass to structure: An aromaticity index for high-resolution mass data of natural organic matter. Rapid Commun Mass Spectrom 20:926–932. doi: 10.1002/rcm.2386

Kothawala DN, Evans RD, Dillon PJ (2006) Changes in the molecular weight distribution of dissolved organic carbon within a Precambrian shield stream. Water Resour Res 42:n/a--n/a. doi: 10.1029/2005WR004441

Kothawala DN, Stedmon CA, Müller RA, et al (2014) Controls of dissolved organic matter quality: Evidence from a large-scale boreal lake survey. Glob Chang Biol 20:1101–1114. doi: 10.1111/gcb.12488

Kothawala DN, von Wachenfeldt E, Koehler B, Tranvik LJ (2012) Selective loss and preservation of lake water dissolved organic matter fluorescence during long-term dark incubations. Sci Total Environ 433:238–246. doi: 10.1016/j.scitotenv.2012.06.029

Marín-Spiotta E, Gruley K., Crawford J, et al (2014) Paradigm shifts in soil organic matter research affect interpretations of aquatic carbon cycling: transcending disciplinary and ecosystem boundaries. Biogeochemistry 1–19. doi: 10.1007/s10533-013-9949-7

Marshall JD, Brooks JR, Lajtha K (2007) Sources of variation in the stable isotopic composition of plants. In: Michener R, Lajtha K (eds) Stable Isot. Ecol. Environ. Sci. Second Ed. Blackwell Publishing Ltd, pp 22–60

McCallister SL, del Giorgio PA (2012) Evidence for the respiration of ancient terrestrial organic C in northern temperate lakes and streams. Proc Natl Acad Sci U S A 109:16963–16968. doi: 10.1073/pnas.1207305109

Mineau MM, Wollheim WM, Buffam I, et al (2016) Dissolved organic carbon uptake in streams: A review and assessment of reach-scale measurements. J Geophys Res Biogeosciences n/a--n/a. doi: 10.1002/2015JG003204

Minshall GW, Cummins KW, Vannote RL (1985) Developments in Stream Ecosystem Theory. Can. J. Fish. Aquat. Sci.

Mostovaya A, Koehler B, Guillemette F, et al (2016) Effects of compositional changes on reactivity continuum and decomposition kinetics of lake dissolved organic matter. J Geophys Res Biogeosciences 121:1733–1746. doi: 10.1002/2016JG003359

Naiman RJ (1982) Characteristics of Sediment and Organic Carbon Export from Pristine Boreal Forest Watersheds. Can J Fish Aquat Sci 39:1699–1718. doi: 10.1139/f82-226

Neff JC, Finlay JC, Zimov S a., et al (2006) Seasonal changes in the age and structure of dissolved organic carbon in Siberian rivers and streams. Geophys Res Lett 33:L23401. doi: 10.1029/2006GL028222

Nikaido H, Vaara M (1985) Molecular basis of bacterial outer membrane permeability. Microbiol Rev 49:1.

Niño-García JP, Ruiz-González C, del Giorgio PA (2016) Interactions between hydrology and water chemistry shape bacterioplankton biogeography across boreal freshwater networks. ISME J 1–12. doi: 10.1038/ismej.2015.226

Oldham CE, Farrow DE, Peiffer S (2013) A generalized Damköhler number for classifying material processing in hydrological systems. Hydrol Earth Syst Sci 17:1133–1148. doi: 10.5194/hess-17-1133-2013

Pace ML, Cole JJ, Carpenter SR, et al (2004) Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature 427:240–243.

Pereira R, Isabella Bovolo C, Spencer RGM, et al (2014) Mobilization of optically invisible dissolved organic matter in response to rainstorm events in a tropical forest headwater river. Geophys Res Lett 41:1202–1208. doi: 10.1002/2013GL058658

Prairie YT (2008) Carbocentric limnology: Looking back, looking forward. Can J Fish Aquat Sci 65:543–548. doi: 10.1139/F08-011

Raymond PA, Hartmann J, Lauerwald R, et al (2013) Global carbon dioxide emissions from inland waters. Nature 503:355–359. doi: 10.1038/nature12760

Raymond PA, Saiers JE (2010) Event controlled DOC export from forested watersheds. Biogeochemistry 100:197–209. doi: 10.1007/s10533-010-9416-7

Raymond PA, Saiers JE, Sobczak W V (2016) Hydrological and biogeochemical controls on watershed dissolved organic matter transport: pulse-shunt concept. Ecology 97:5–16. doi: 10.1890/14-1684.1

Raymond PA, Spencer RGM (2015) Riverine DOM. Biogeochem Mar Dissolved Org Matter 509–533. doi: 10.1016/B978-0-12-405940-5.00011-X

Reyjol Y, Compin A, A-Ibarra A, Lim P (2003) Longitudinal diversity patterns in streams: comparing invertebrates and fish communities. Arch Fur Hydrobiol 157:525–533. doi: 10.1127/0003-9136/2003/0157-0525

Rosenfeld JS (2002) Functional redundancy in ecology and conservation. Oikos 98:156–162. doi: 10.1034/j.1600-0706.2002.980116.x

Rosso R, Bacchi B, La Barbera P (1991) Fractal relation of mainstream length to catchment area in river networks. Water Resour Res 27:381–387. doi: 10.1029/90WR02404

Ruiz-González C, Niño-García JP, del Giorgio PA (2015a) Terrestrial origin of bacterial communities in complex boreal freshwater networks. Ecol Lett 18:1198–1206. doi: 10.1111/ele.12499

Ruiz-González C, Niño-García JP, Lapierre JF, del Giorgio PA (2015b) The quality of organic matter shapes the functional biogeography of bacterioplankton across boreal freshwater ecosystems. Glob Ecol Biogeogr 24:1487–1498. doi: 10.1111/geb.12356

Savio D, Sinclair L, Ijaz UZ, et al (2015) Bacterial diversity along a 2600 km river continuum. Environ Microbiol 17:4994–5007. doi: 10.1111/1462-2920.12886

Savory JJ, Kaiser NK, McKenna AM, et al (2011) Parts-Per-Billion Fourier Transform Ion Cyclotron Resonance Mass Measurement Accuracy with a " al king" Calibration Equation. Anal Chem 83:1732–1736. doi:

10.1021/ac102943z

Schmidt MWI, Torn MS, Abiven S, et al (2011) Persistence of soil organic matter as an ecosystem property. Nature 478:49–56. doi: 10.1038/nature10386

Sholkovitz ER (1976) Flocculation of dissolved organic and inorganic matter during the mixing of river water and seawater. Geochim Cosmochim Acta 40:831–845. doi: 10.1016/0016-7037(76)90035-1

Singer GA. GA, Fasching C. C, Wilhelm LL., et al (2012) Biogeochemically diverse organic matter in Alpine glaciers and its downstream fate. Nat Geosci 5:710–714. doi: 10.1038/ngeo1581

Spencer RGM, Hernes PJ, Ruf R, et al (2010) Temporal controls on dissolved organic matter and lignin biogeochemistry in a pristine tropical river, Democratic Republic of Congo. J Geophys Res Biogeosciences 115:n/a--n/a. doi: 10.1029/2009JG001180

Spencer RGM, Mann PJ, Dittmar T, et al (2015) Detecting the signature of permafrost thaw in Arctic rivers. Geophys Res Lett 42:2830–2835. doi: 10.1002/2015GL063498

Stedmon CA, Markager S, Bro R (2003) Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Mar Chem 82:239–254.

Striegl RG, Aiken GR, Dornblaser MM, et al (2005) A decrease in discharge-normalized DOC export by the Yukon River during summer through autumn. Geophys Res Lett 32:n/a-n/a. doi: 10.1029/2005GL024413

Stubbins A., Hood E., Raymond PA., et al (2012) Anthropogenic aerosols as a source of ancient dissolved organic matter in glaciers. Nat Geosci 5:198–201. doi: 10.1038/ngeo1403

Stubbins A, Spencer RGM, Chen H, et al (2010) Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. Limnol Oceanogr 55:1467–1477. doi: 10.4319/lo.2010.55.4.1467

Tomanova S, Tedesco P a., Campero M, et al (2007) Longitudinal and altitudinal changes of macroinvertebrate functional feeding groups in neotropical streams: a test of the River Continuum Concept. Fundam Appl Limnol / Arch für Hydrobiol 170:233–241. doi: 10.1127/1863-9135/2007/0170-0233

Tranvik L, Downing J, Cotner J (2009) Lakes and reservoirs as regulators of carbon cycling and climate. Limnol … 54:2298–2314.

Vannote RL, Minshall GW, Cummins KW, et al (1980) The river continuum concept. Can J Fish Aquat Sci 37:130–137.

Vazquez E., Amalfitano S., Fazi S., Butturini A. (2011) Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions. Biogeochemistry 102:59–72. doi: 10.1007/s10533-010-9421-x

Volk CJ, Volk CB, Kaplan LA (1997) Chemical composition of biodegradable dissolved organic matter in streamwater. Limnol Oceanogr 42:39–44.

von Schiller D, Bernal S, Sabater F, Martí E (2015) A round-trip ticket: the importance of release processes for in-stream nutrient spiraling. Freshw Sci

34:20–30. doi: 10.1086/679015.

Wachenfeldt E, Tranvik LJ (2008) Sedimentation in Boreal Lakes—The Role of Flocculation of Allochthonous Dissolved Organic Matter in the Water Column. Ecosystems 11:803–814. doi: 10.1007/s10021-008-9162-z

Weishaar JL, Aiken GR, Bergamaschi BA, et al (2003) Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ Sci Technol 37:4702–4708. doi: 10.1021/es030360x

Weiss MS, Abele U, Weckesser J, et al (1991) Molecular architecture and electrostatic properties of a bacterial porin. Science (80-) 254:1627–1630. doi: 10.1126/science.1721242

Wetzel RG (1983) Limnology, second edition. Saunders

Weyhenmeyer GA, Fröberg M, Karltun E, et al (2012) Selective decay of terrestrial organic carbon during transport from land to sea. Glob Chang Biol 18:349–355. doi: 10.1111/j.1365-2486.2011.02544.x

Wilhelm L, Besemer K, Fragner L, et al (2015) Altitudinal patterns of diversity and functional traits of metabolically active microorganisms in stream biofilms. ISME J 9:2454–2464. doi: 10.1038/ismej.2015.56

Wollheim WM, Stewart RJ, Aiken GR, et al (2015) Removal of terrestrial DOC in aquatic ecosystems of a temperate river network. Geophys Res Lett 42:1–9. doi: 10.1002/2015GL064647

Worrall F, Burt TP, Adamson J (2006) The rate of and controls upon DOC loss in a peat catchment. J Hydrol 321:311–325. doi: http://dx.doi.org/10.1016/j.jhydrol.2005.08.019

Zsolnay A, Baigar E, Jimenez M, et al (1999) Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. Chemosphere 38:45–50. doi: 10.1016/S0045-6535(98)00166-0

ANNEX

Biodegradation kinetics of dissolved organic matter chromatographic fractions, a case study in an intermittent river

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Introduction
The tables compile the results for the Akaike information criteria analysis for the different models fitted to each of the chromatographic fractions.
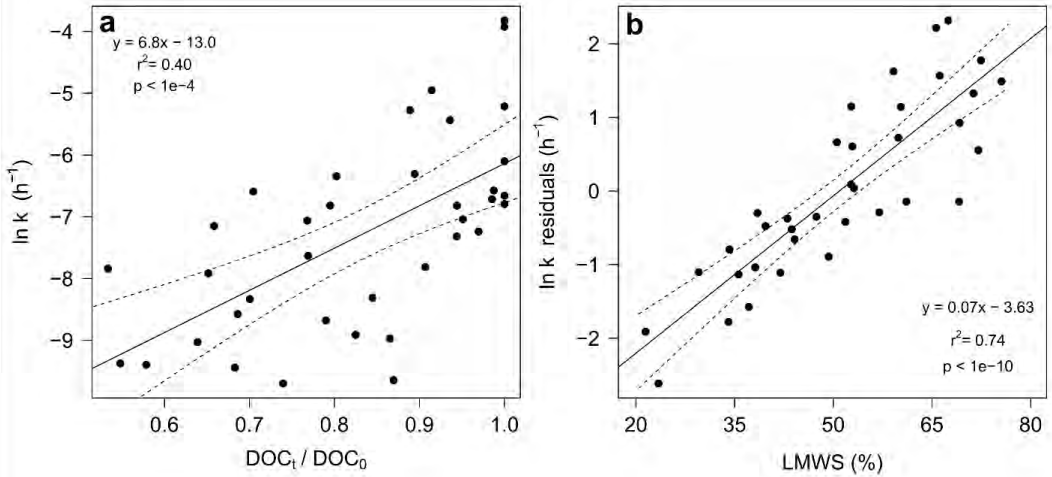
127

Figure S1. Relationship between the apparent decay coefficient (k) of the bulk DOM and the proportion of initial DOM (a). In (b), the residuals of that relationship are related to the low molecular weight fraction of DOM (LMWS) confirming the independence of the relationship between LMWS and k (Fig. 7 main text) from the DOC concentration.

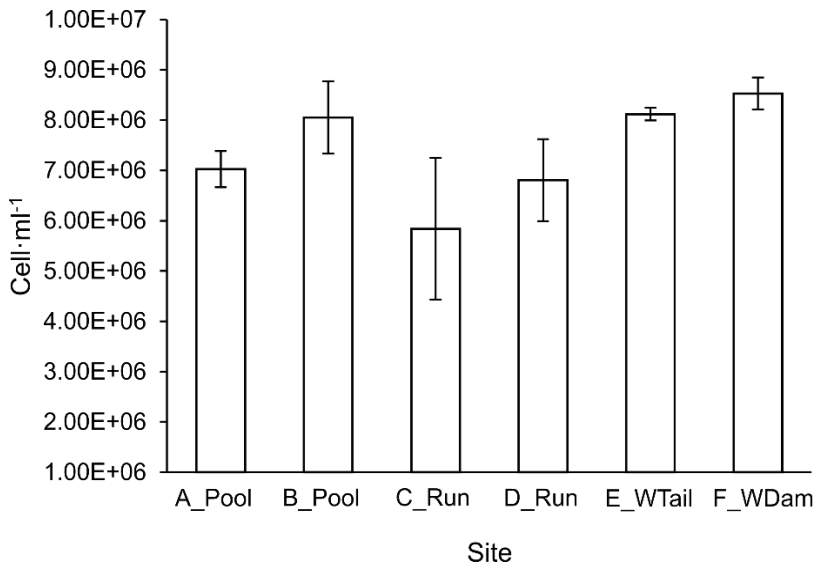**Figure S2.** Bacterial abundance (Cell ml^{-1}) at incubation time = 0 for each of the sites. Although Kruskall-Wallis test indicated differences between sites (X^2 = 13.82, p= 0.02), comparison by group indicated differences only between sites C_Run and F_WDam , z = 54 =) , w
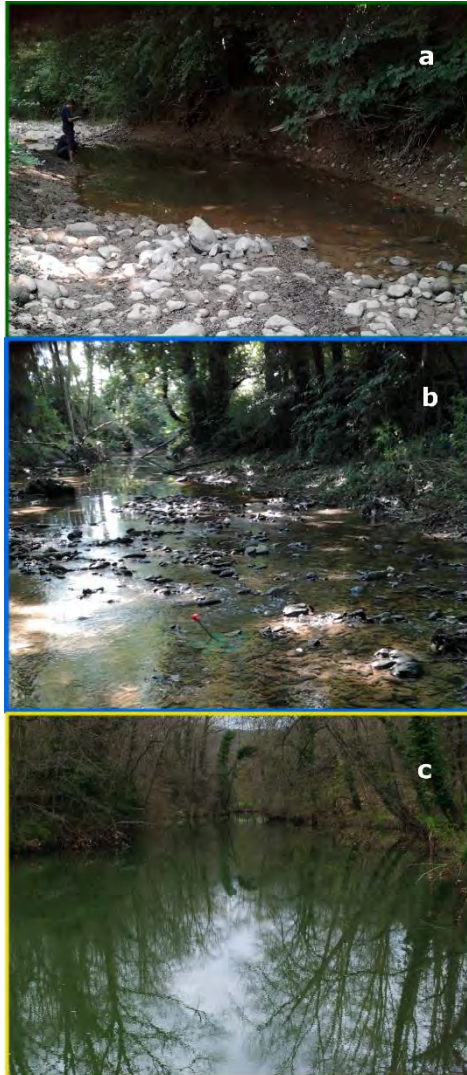
Figure S3. Pictures of some of the study sites a) A_Pool, b) C_Run and c) F_WDam (picture taken later that year)
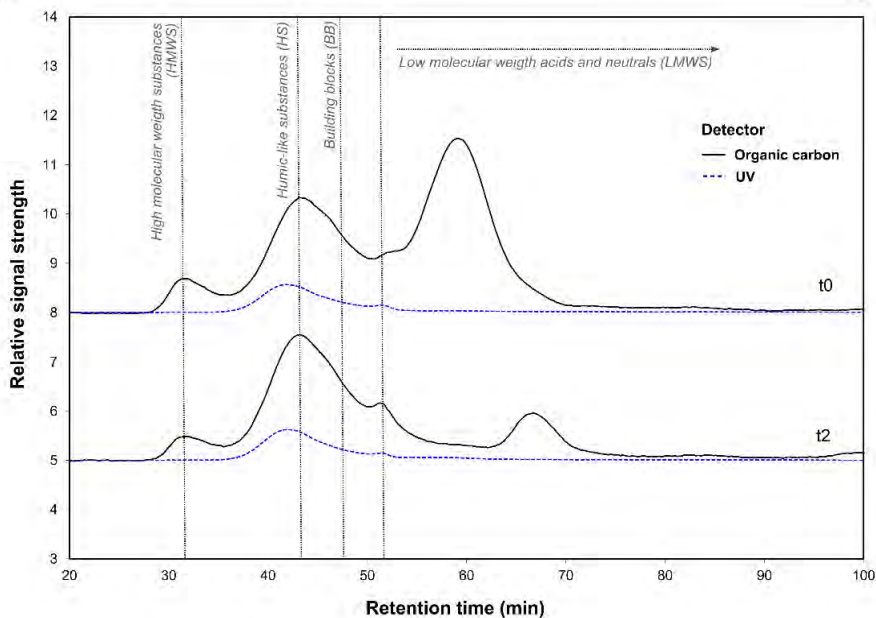
Figure S4. Chromatogram of size-exclusion chromatography (LC-OCD) for two samples from one of the pools at time 0 and 2 of the incubations. The distribution of the DOM fractions is shown. Humic-like substances (HS) and building blocks (BB) were integrated together as the HS&BB fraction. Similarly, low molecular weight acids and neutrals were integrated jointly as the low molecular weight substances (LMWS) fraction (*see* Graeber et al. 2015 for a similar approach).

Table S1. Parameters of the fitted RC models to the LMWS and HMWS fractions of each site.

Table S1

Parameter	Fraction	A_Pool	B_Pool	C_Run	D_Run	E_WTail	F_WDam
α Average lifetime of the more reactive compounds (h)	LMWS	58.29 ± 1.57	6.59 ± 1.91	47.18 ± 2.85	12.08 ± 3.37	113.39 ± 2.38	3.08 ± 3.59
	HMWS	117.47 ± 4.43	32.14 ± 3.94	NA	NA	NA	NA
ν Relative preponderance of the more recalcitrant compounds (unitless)	LMWS	0.156 ± 0.013	0.119 ± 0.012	0.271 ± 0.015	0.094 ± 0.015	0.626 ± 0.017	0.133 ± 0.014
	HMWS	0.652 ± 0.020	0.303 ± 0.017	NA	NA	NA	NA
Apparent first initial decay coefficient k (h^{-1})	LMWS	0.0027 ± 0.0008	0.0180 ± 0.0063	0.0057 ± 0.0005	0.0078 ± 0.0004	0.0055 ± 0.0007	0.0432 ± 0.0040
	HMWS	0.0056 ± 0.0005	0.0094 ± 0.0042	NA	NA	NA	NA

Values indicate modelled values ± SE

Table S2. Results of the AIC analysis for the 4 models fitted to the LMWS fraction of DOM in the six studied sites. AIC indicate the values of the Akaike Information Criterion; Δ_i =[AIC_i – min (AIC)] and w_i (AIC) are the rounded Akaike weigths [*Wagenmakers and Farrell*, 2004 and references therein], that can be interpreted as conditional probabilities for each model. Thus, the model showing the larger weight can be consider as the most adequate model.

Table S2							
Model		**A_Pool**	**B_Pool**	**C_Run.**	**D_Run**	**E_WTail**	**F_WDam**
Reactivity continuum	AIC	-22	-18	-10	-15	-10	-24
	Δ_i	-56	0	0	-35	-1	0
	w_i (AIC)	0.00	0.6399	0.5669	0.0000	0.4100	0.9998
Exponential	AIC	-6	-2	-4	-2	-6	3
	Δ_i	-72	-15	-6	-49	-6	-26
	w_i (AIC)	0.0000	0.0003	0.0230	0.0000	0.0349	0.0000
Linear	AIC	-4	-3	-4	-4	-1	-3
	Δ_i	-74	-14	-7	-47	-10	-21
	w_i (AIC)	0.0000	0.0005	0.0214	0.0000	0.0044	0.0000
GAM	AIC	-78	-17	-9	-50	-11	-7
	Δ_i	0	-1	-1	0	0	-17
	w_i (AIC)	1.0000	0.3694	0.3887	1.0000	0.5507	0.0002

Note- the number of parameter estimated for the Reactivity continuum is 2, for the Exponential and linear models 1, and for the GAM models, the smooth parameters were between 4 and 8.

Table S3. Results of the AIC analysis for the 4 models fitted to the HMWS fraction of DOM in the two studied pools. AIC indicate the values of the Akaike Information Criterion; Δ_i =[AIC_i – min (AIC)] and w_i (AIC) are the rounded Akaike weigths [*Wagenmakers and Farrell*, 2004 and references therein], that can be interpreted as conditional probabilities for each model. Thus, the model showing the larger weight can be consider as the most adequate model.

Table S3			
Model		**A_Pool**	**B_Pool**
Reactivity continuum	AIC	-12	-7
	Δ_i	0	8
	w_i (AIC)	0.9722	0.0206
Exponential	AIC	-1	-4
	Δ_i	10	11
	w_i (AIC)	0.0062	0.0043
Linear	AIC	5	-2
	Δ_i	17	12
	w_i (AIC)	0.0002	0.0022
GAM	AIC	-4	-14
	Δ_i	8	0
	w_i (AIC)	0.0213	0.9729

Note- the number of parameter estimated for the Reactivity continuum is 2, for the Exponential and linear models 1, and for the GAM models, the smooth parameters were between 4 and 8.

Table S4. Results of the AIC analysis for the models fitted to the HS & BB fraction of DOM in the six studied sites. AIC indicate the values of the Akaike Information Criterion; Δ_i =[AIC$_i$ – min (AIC)] and w_i (AIC) are the rounded Akaike weigths [*Wagenmakers and Farrell*, 2004 and references therein], that can be interpreted as conditional probabilities for each model. Thus, the model showing the larger weight can be consider as the most adequate model.

Table S4

Model		A_Pool	B_Pool	C_Run	D_Run	E_WTail	F_WDam
	AIC	-12	-5	8	6	-3	-3
Exponential	Δ_i	0	33	7	16	14	30
	w_i (AIC)	0.3437	0.0000	0.0237	0.0004	0.0009	0.0000
	AIC	-12	-3	9	6	-4	-4
Linear	Δ_i	0	35	9	16	13	29
	w_i (AIC)	0.3282	0.0000	0.0135	0.0003	0.0015	0.0000
	AIC	-12	-38	0	-10	-17	-32
GAM	Δ_i	0	0	0	0	0	0
	w_i (AIC)	0.3282	1.0000	0.9628	0.9993	0.9975	1.0000

Note- the number of parameter estimated for the Reactivity continuum is 2, for the Exponential and linear models 1, and for the GAM models, the smooth parameters were between 4 and 8.

SUPPLEMENTARY INFORMATION

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

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Supplementary Table 1. Morphological and physicochemical description of the 12 sampled reaches. Values reported are means and ranges (in brackets) corresponding to the ten sampling dates.

Reach	UTM coordinates		Length (m)	mean width (m)	mean depth (m)	Residence time (hours)	DOC (mg C/L)	NO$_3^-$ (mg N/L)	PO$_4^{3-}$ (mg P/L)	Temp (ºC)	pH	DO (mg O/L)
	X	Y										
1	504859	4669395	3220	44 (38-54)	0.28 (0.21-0.38)	3 (2-4)	1.29 (1.1-1.49)	1.66 (0.78-2.74)	0.007 (0.002-0.017)	20.8 (14.2-25.2)	8.2 (8.1-8.3)	11.8 (11.4-12.5)
2[†]	502689	4668683	900	41 (41-41)	0.74 (0.74-0.74)	7 (1-16)	1.11 (0.81-1.53)	1.85 (0.82-2.73)	0.008 (0.002-0.02)	16 (8.8-26.9)	8.2 (8.1-8.4)	12.1 (9.4-14.5)
3	501798	4668642	2450	33 (27-44)	0.31 (0.23-0.48)	4 (2-6)	1.06 (0.79-1.54)	1.8 (0.86-2.75)	0.009 (0.002-0.026)	16.4 (9.7-24.6)	8 (7.9-8.3)	9.8 (8.6-12.2)
4	488258	4671294	3810	26 (23-32)	0.11 (0.05-0.25)	5 (3-7)	1.27 (1.06-1.81)	1.72 (0.72-2.28)	0.028 (0.003-0.062)	15.7 (8.3-24.9)	8.1 (7.9-8.3)	10.5 (8.2-13.1)
5[†]	480787	4670809	1640	66 (66-66)	1.36 (1.36-1.36)	42 (5-84)	2.56 (1.57-3.49)	1.97 (1.04-2.62)	0.086 (0.034-0.159)	14.3 (6.1-24.2)	8.1 (8-8.5)	9.9 (7.2-12.4)
6	478372	4669780	1720	7 (6-9)	0.16 (0.1-0.27)	8 (1-19)	1.73 (1.05-2.97)	2.00 (1.16-2.77)	0.016 (0.002-0.043)	12.5 (3.8-20.7)	8.3 (8-8.5)	10.9 (8.2-14.1)
7	466208	4673166	2360	13 (9-18)	0.15 (0.07-0.23)	5 (1-8)	1.79 (1.54-2.04)	2.73 (2.29-3.18)	0.188 (0.128-0.247)	15.9 (13.6-18.2)	8.4 (8-8.8)	10.0 (10-10)
8[†]	456822	4669472	770	16 (16-16)	1.41 (1.41-1.41)	46 (3-138)	1.89 (1.12-4.64)	4.37 (2.19-5.77)	0.033 (0.019-0.09)	13.1 (5.4-17.8)	8.3 (8.1-8.5)	10.0 (8.5-12.5)
9	455915	4669416	2150	8 (6-11)	0.2 (0.09-0.38)	9 (1-25)	1.11 (0.74-1.62)	4.65 (2.24-5.85)	0.023 (0.015-0.031)	11.8 (6.8-15.7)	7.9 (7.6-8.2)	8.6 (7.7-10)
10	458166	4677743	660	2 (2-2)	0.1 (0.08-0.12)	5 (1-9)	1.68 (1.43-2.06)	0.01 (0-0.02)	0.004 (0.003-0.004)	16.2 (12-19.6)	8.4 (8.3-8.5)	9.3 (8.5-10.3)
11	466312	4682995	340	4 (2-5)	0.15 (0.09-0.34)	4 (1-6)	0.54 (0.38-0.85)	0.01 (0-0.06)	0.003 (<LOQ-0.004)	9.1 (5.2-13.8)	8.4 (8.2-8.6)	11.1 (10.3-11.7)
12[†]	466218	4683553	80	10 (10-10)	1.14 (1.14-1.14)	7 (1-16)	0.69 (0.38-1.47)	0.02 (0-0.06)	0.003 (<LOQ-0.004)	11.4 (6-19.2)	8.4 (8.3-8.8)	10.6 (9.2-11.7)

DOC, dissolved organic carbon; Temp, water temperature; DO, dissolved oxygen; LOQ, limit of quantification

[†]Reaches delimited by a small weir at their lower end (see Methods section)

Supplementary Table 2. Peak position for the six fluorescence components (C1– C6) identified by Parallel Factor Analysis.

	Excitation wavelength (nm)	Emission wavelength (nm)
C1	<250, 338	427
C2	<250	427
C3	<250, 377	487
C4	<250, 308	385
C5	278	325
C6	<250	304

	15-Nov-12	13-Dec-12	21-Jan-13	25-Feb-13	27-Mar-13	01-May-13	17-Jun-13	15-Jul-13	23-Sep-13	08-Nov-13
1					-0.06		-0.05	-0.11		
2		0.15	0.00	0.10	0.02	0.01	-0.25	0.14		-0.01
3		0.00	0.11	-0.04	-0.01	-0.01	0.16	-0.01	0.07	0.00
4		0.05	0.03		-0.03		0.16		0.05	0.02
5	-0.66	NA	-0.36	-0.63		-0.19	-0.18	-0.98	-1.26	-0.77
6	-0.37	0.04	-0.01	0.01	-0.17	0.00	0.01	0.02	-0.10	-0.15
7						-0.01			-0.06	
8		0.13				0.03	-0.07	-2.56	0.22	0.18
9		0.14				0.09	0.40		0.38	
10						-0.11	-0.04	-0.15		
11	0.20	0.06	0.04	-0.09						-0.05
12	0.03	-0.01	0.06	0.01	-0.02	-0.05	0.05	0.07		0.10

Supplementary Figure 1. Spatial and temporal variation of the net change in dissolved organic carbon (DOC). The colour code refers to the behaviour of the reaches: blue for *reactors* with net gain, red for *reactors* with net loss, and grey for *pipes*. Empty cells correspond to observations likely influenced by unmeasured lateral inputs (i.e., point sources and groundwater) and excluded from the dataset (See Methods section for details).
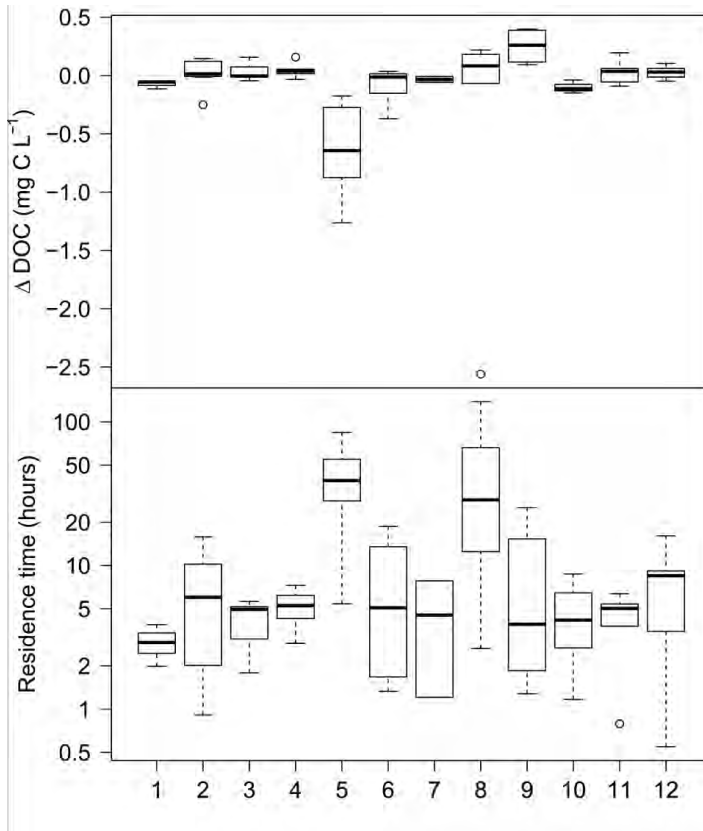
Supplementary Figure 2. Variation of the net change in dissolved organic carbon (ΔOC) (upper panel) and the residence time of the reaches (lower panel) across sampling sites. Sites are sorted from the river mouth (1) to headwaters (12). See Figure 1 for details on the distribution of the sites across the river network. Box plots display the 10th, 25th, 50th, 75th, and 90th percentiles, and individual outliers. The gray dashed line in the upper panel represents ΔDOC=0.
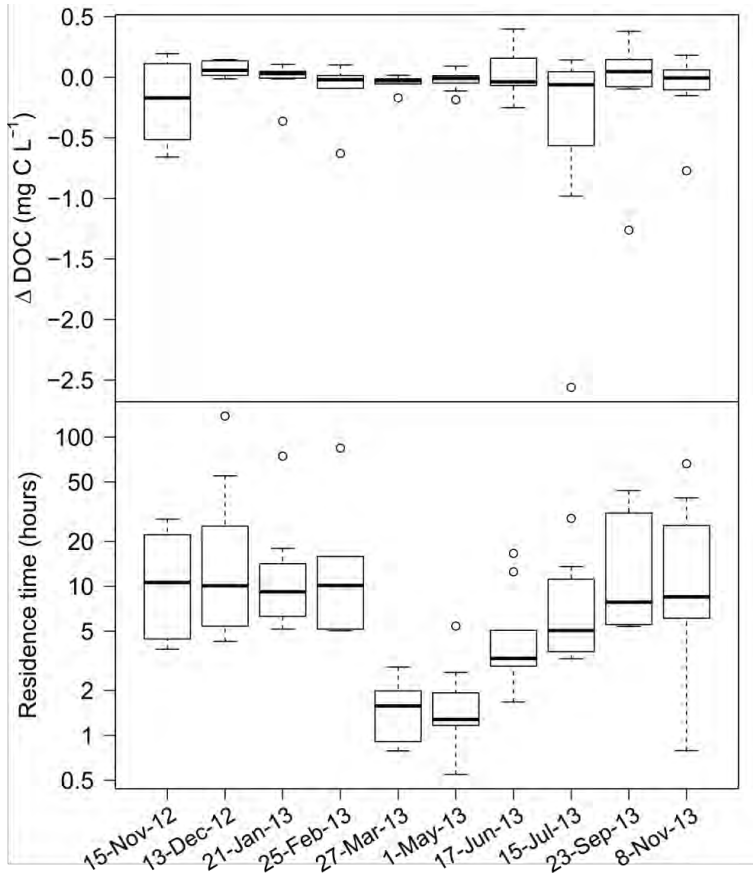
Supplementary Figure 3. Temporal variation of the net change in dissolved organic carbon (DOC) (upper panel) and the residence time of the reaches (lower panel). Box plots display the 10th, 25th, 50th, 75th, and 90th percentiles, and individual outliers. The gray dashed line in the upper panel represents DOC=0.
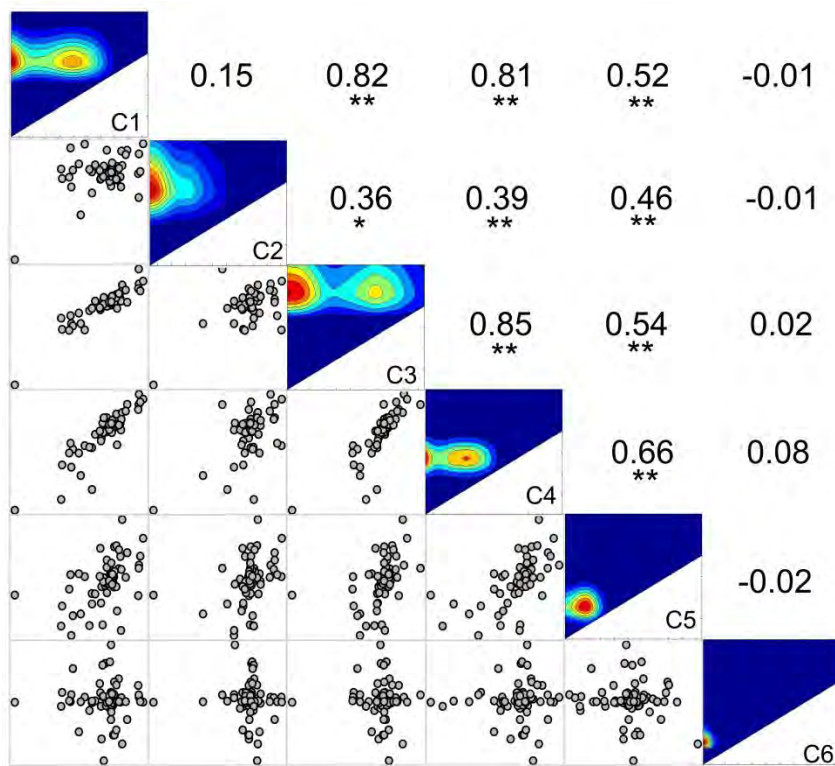
Supplementary Figure 4. Paired relationships between the net variation in fluorescence intensity along the reaches for the six components (C1 C6) identified by PARAFAC. Cells inside the matrix diagonal show the fluorescence spectra of each component. Values above the diagonal are Spearman correlation coefficients. Positive values mean coupling between components, that is, they both either increase or decrease along the reach. Negative values mean opposite dynamics. Asterisk shows statistical significance (* $p<0.01$; ** $p<0.001$).
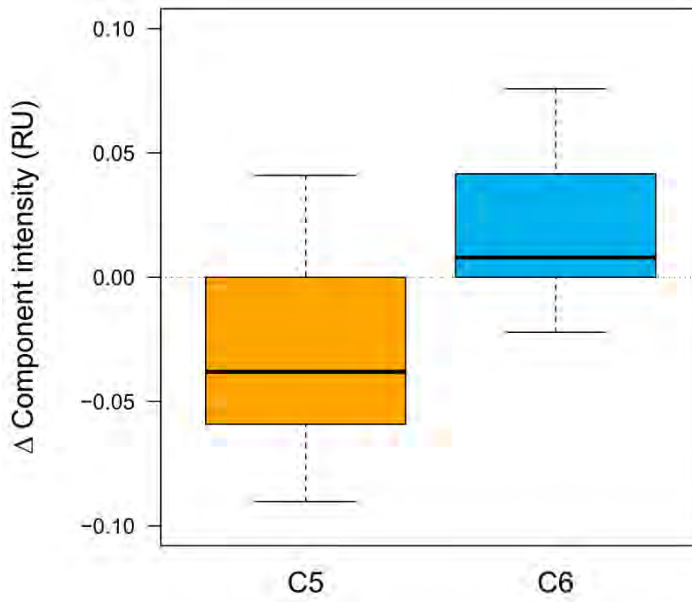
Supplementary Figure 5. Net change in fluorescence intensity of components 5 and 6 for *reactor* observations with a residence time above 18 hours (Mann-Whitney test, *p*<0.001).

Supporting Information

Delineating the continuum of dissolved organic matter in river networks

Short Title: Delineating the continuum of DOM in river networks

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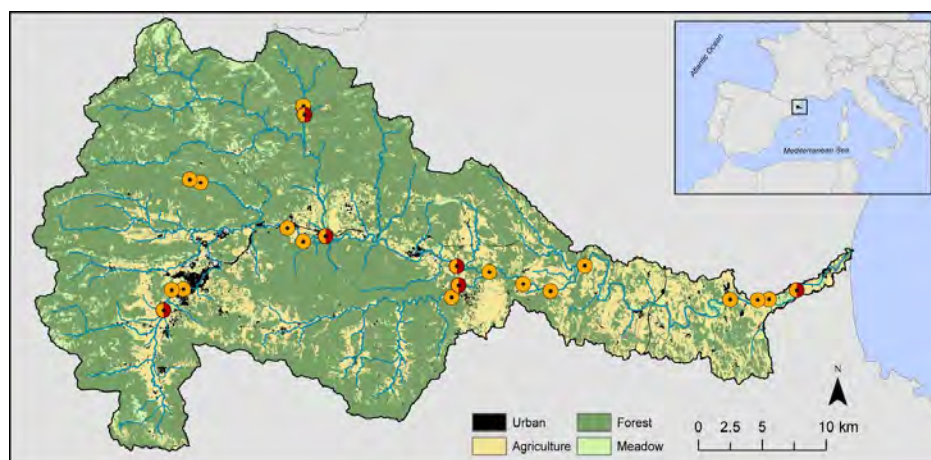
Fig. S1. Distribution of the study sites across the Fluvià river network (North-East of the Iberian Peninsula). All sites were sampled ten times over a hydrological year. Symbols containing a red half indicate sites that were sampled also during storm events. The inset at the upper-right corner shows the location of the catchment in South Western Europe.
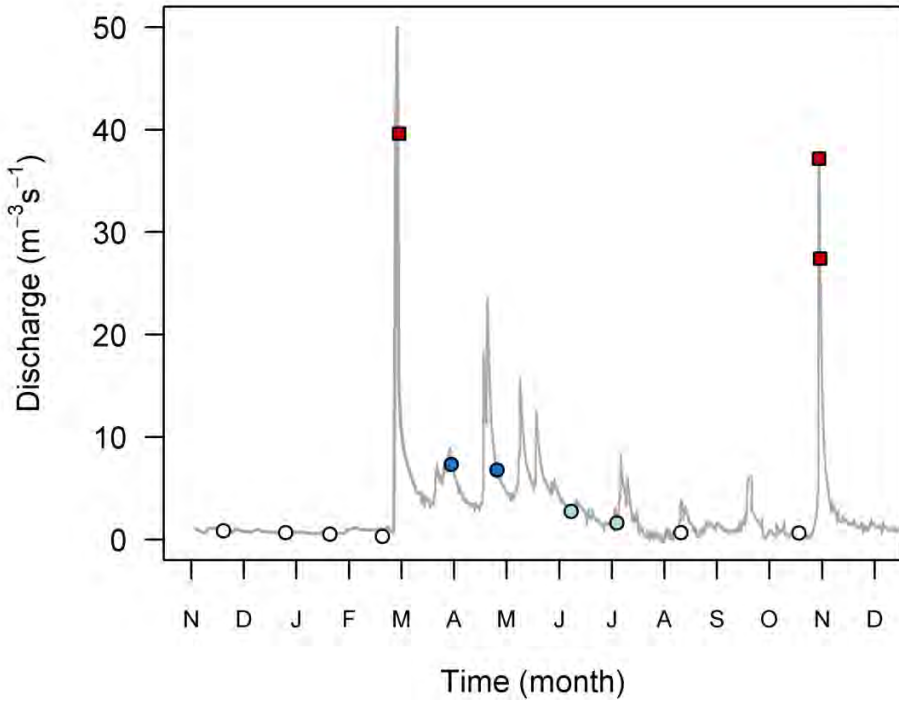
Fig. S2. Hydrological context of the samplings. Symbols indicate the sampling dates over the hydrograph (grey line) in the Fluvià river mouth during the study period (2012-2013). Color code same as Fig. 1.
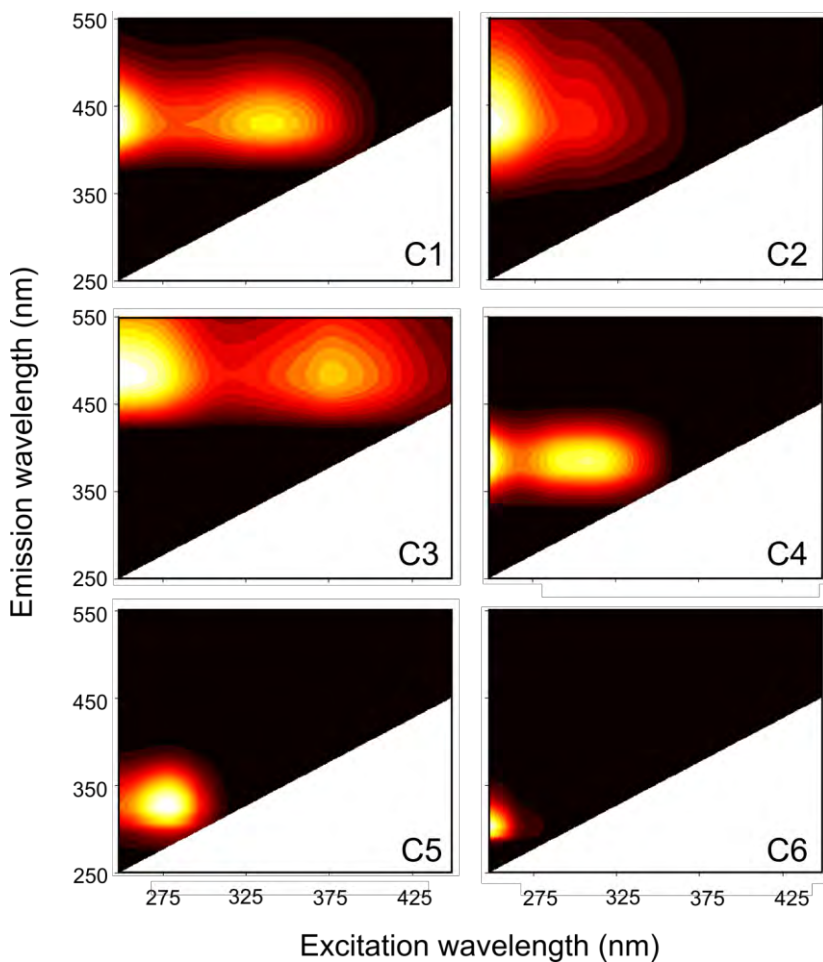
146

Fig. S3. Fluorescence spectra of the components identified using parallel factor analysis (PARAFAC). For a detailed description of the fluorescence components, the reader is referred to Casas-Ruiz et al (2017, in press, doi:10.1002/lno.10471)
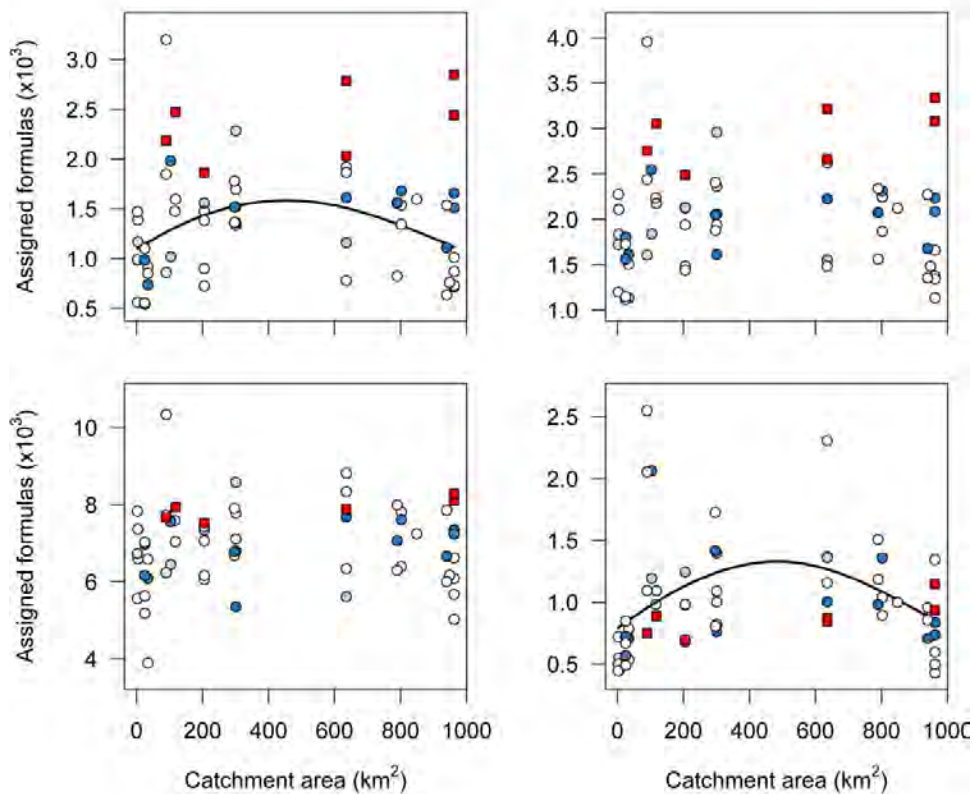
Figure S4: Longitudinal patterns of the number of assigned formulas for each of the molecular compound categories. a) condensed aromatics, b) polypenols, c) highly unsaturated and phenolic compounds, and d) aliphatic compounds. Black lines show statistically significant (α=0.05) GAM models for the non-storm conditions dataset. No significant patterns were found during storm events. The color code is the same as in Fig. 1.
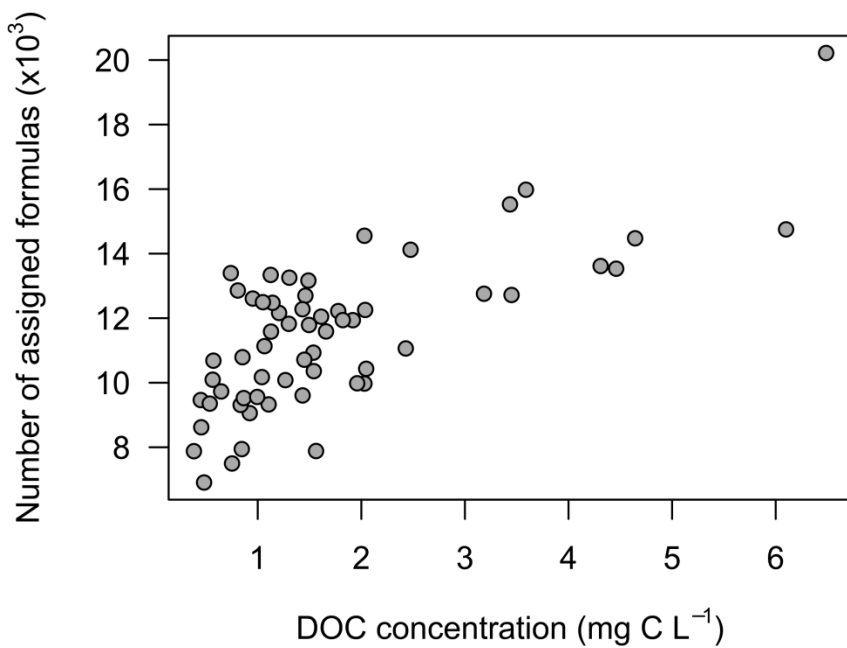
Fig. S5. Relationship between the concentration of dissolved organic carbon (DOC) and the number of assigned formulas from FT-ICR-MS analysis (*Spearman rho*=0.69, *p<0.0001, N*=62).

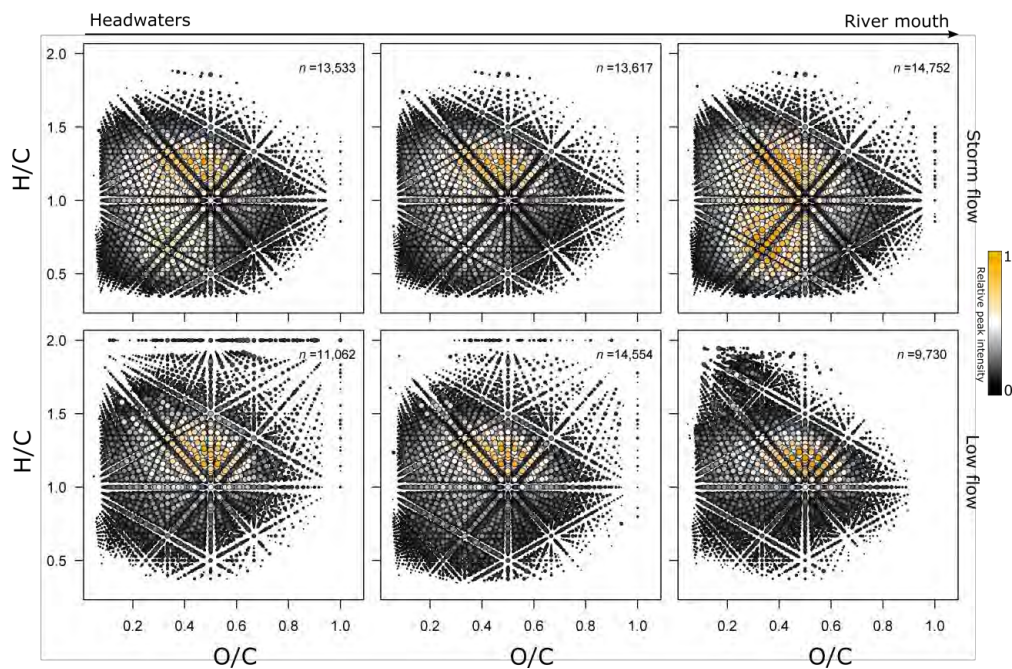**Fig. S6.** Patterns of DOM molecular composition along the Fluvià river network. The figure shows selected Van Krevelen diagrams of DOM from headwater streams to the river mouth under low and storm flow conditions. The color and size of the symbol indicates the relative peak intensity of molecular formulas for each sample. The total number of assigned formulas is shown in the upper-right corner of the diagrams.
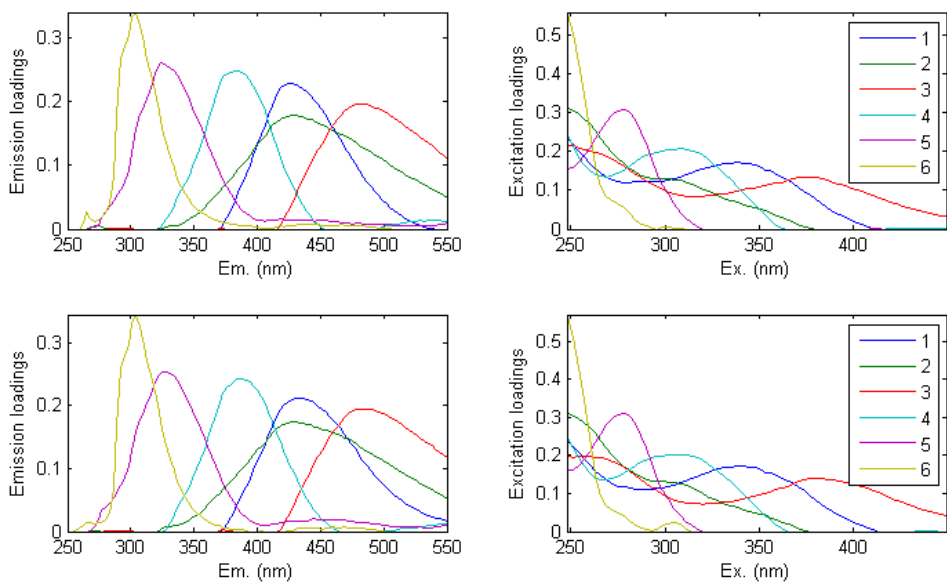
150

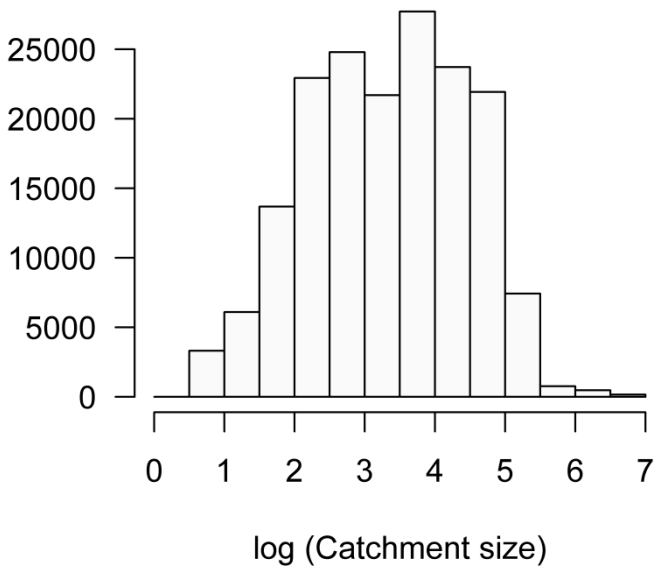**Fig. S7.** Split half validation of the six-component PARAFAC model

Fig. S8: Frequency hystogram of catchment area for all DOCmeasurments available in GloRiCh.