UNIVERSITAT DE BARCELONA

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TESI DOCTORAL

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Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals

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Effects of flow intermittence on dissolved organic matter quality

Efectes de la intermitència del flux en la qualitat de la matèria orgànica dissolta

Memòria presentada per Verónica Granados Pérez per optar al títol de Doctora per la Universitat de Barcelona

Verónica Granados Pérez
Barcelona, Desembre de 2021

Vist-i-plau dels directors de la tesi:

Dr. Andrea Butturini
Professor agregat i tutor de la tesi
Universitat de Barcelona

Dr. Biel Obrador
Professor agregat
Universitat de Barcelona

A mi familia

y a todas y todos los que me han acompañado en el camino,

Ya estoy en el final de esta carretera

Tantas encrucijadas quedan detrás

Ya está en el aire girando mi moneda

Y que sea lo que

Sea

Todos los altibajos de la marea

Todos los sarampiones que ya pasé

Yo llevo mis experiencias como

bandera

Y que sea lo que

Sea

Lo que tenga que ser, que sea

Y lo que no, por algo será

Jorge Drexler

CONTENTS

ADVISORS' REPORT

Dr. Andrea Butturini and Dr. Biel Obrador, professors in the Department of Evolutionary Biology, Ecology and Environmental Sciences (Ecology section), at the University of Barcelona, as supervisors of the Doctoral Thesis presented by Verónica Granados Pérez entitled: Effects of flow intermittence on dissolved organic matter quality.

INFORM:

That the research studies developed by Verónica Granados Pérez for her Doctoral Thesis have been organized in three chapters, which corresponds to three scientific papers, two already published and another one under review.

The list of the published chapters, indicating the Journal Impact Factor according to SCI of ISI Web of Science:

Granados, V., Gutiérrez-Cánovas, C., Arias-Real, R., Obrador, B., Harjung, A., Butturini, A., 2020. The interruption of longitudinal hydrological connectivity causes delayed responses in dissolved organic matter. Sci. Total Environ. 713, 136619.

DOI: 10.1016/j.scitotenv.2020.136619

The impact factor of Science of the Total Environment in 2020 was 7.963. This journal is included in the category of Environmental Sciences and is reported in the First Quartile being in the 18th position of the 128 journals included.

Granados, V., Butturini, A., 2019. Dissolved organic matter variability along an impacted intermittent Mediterranean river. Limnetica 38, 555–573.

DOI: 10.23818/limn.38.32

The impact factor of Limnetica in 2019 was 1.431. This journal is included in the category of Aquatic Sciences and is reported in the Third Quartile being in the 144th position of the 228 journals included.

The manuscript under review is:

Granados, V., Gutiérrez-Cánovas, C., Arias-Real, R., Obrador, B., Butturini, A., 2022. Duration and frequency of drying shape dissolved organic matter composition during rewetting in intermittent streams. Sci. Total Environ.

The impact factor of Science of the Total Environment in 2020 was 7.963. This journal is included in the category of Environmental Sciences and is reported in the First Quartile being in the 18th position of the 128 journals included.

And CERTIFY:

That Verónica Granados Pérez has participated actively on the development of the research and the elaboration associated to each of the papers listed. In particular, her contribution included the following tasks:

- Participation in setting objectives and in the experimental design of each one of the chapters developed.
- Sampling design and field work.
- Sample processing and analyses in laboratory.
- Statistical data analyses and interpretation of results
- Writing, reviewing and editing processes of the manuscripts

Finally, we certify that the co-authors of the papers that conform this Doctoral Thesis will not use any of the manuscripts in any other Doctoral Thesis.

Barcelona, 24th December 2021

Andrea Butturini Biel Obrador

ABSTRACT

Water availability is the primary driver of dissolved organic matter (DOM) dynamics in intermittent rivers and ephemeral streams (IRES). Therefore, current research manifests the global influence of flow disruption on organic matter transport and processing, given the wide distribution of intermittent flow watercourses. However, there are still gaps of flow intermittence knowledge, forgetting the continuous and multidimensional nature of the drying disturbance.

In this context, in chapter three, we determine the influence of opposite flow conditions, drought and high flow on DOM properties and how the river continuum can cushion the impact of contrary hydrology on DOM and inorganic solutes. Further, in chapter four, we study the evolution of the dry period as a "film frame", evaluating the diminution of flow daily, especially after the flow disruption. Then, how the hydrological connectivity influences the response timing and shape of the biogeochemical variables analysed and if they are influenced by spatial position, water temperature and volume of the disconnected. Finally, in chapter five, from a multi catchment approach of IRES with a gradient of intermittence from permanent to ephemeral, we investigated how multiple drying components shape dissolved organic matter (DOM) and dissolved organic carbon (DOC).

Hence, in the results in chapter three, the DOM quality changed according to hydrology. DOM under high flow conditions was more terrigenous, humified, aromatic, and degraded, and the concentration gradually increased downriver. In contrast, DOM was less degraded and more aliphatic under drought conditions. However, the hydrological variability did not impact the DOM quality uniformly along the river continuum: DOM quality is more sensitive to hydrological changes in headwaters than in

downriver reaches. In chapter four, the drying period determined the disruption of the fluvial continuum with a recession of stream continuum at a rate of ~60m/d and the gradual formation of a patched system of isolated pools of different sizes. Our results showed that the time that had elapsed since isolated pool formation (CI-days) was an essential factor for understanding how drying shaped the biogeochemistry of the fluvial system. Overall, drying caused a high DOC concentration and an increase in the humic-like fluorescence signal. Additionally, solutes showed contrasting responses to hydrological disconnection. Thus, we identified three temporal responses on the variables studied: 1) some inorganic solutes reacted before the fragmentation (CI-days<0). Then, DOM optical qualitative parameters replied after the fragmentation (CI-days>0), and 3) BIX and HIX did not show significant differences throughout the dry period. Finally, in chapter five, the sampling was performed during post-drought season and the results showed changes in DOC were driven by annual drying duration, whereas multiple drying components better explained DOM quality. So, we found high DOC concentration and prevalence of terrestrial DOM sources when drying is more extended and more frequent. Furthermore, because of the time of sampling, the DOM quality of the stream reconnection described a catchment "washed" with a terrestrial DOM origin, and it is transported downriver quickly without being processed for the microbial community.

The findings of this thesis reveal the role of hydrology as a main driver of DOM dynamics. In particular, the flow disruption does not induce a single biogeochemical response but rather stimulates a set of solute-specific responses that generates a complex biogeochemical mosaic in a single fluvial unit. Furthermore, the necessity of considering multiple drying elements as annual or previous to predict organic matter dynamics on IRES and highlight these systems influence on the global carbon budget.

RESUM

La disponibilitat d'aigua és el principal motor de la dinàmica de la matèria orgànica dissolta (DOM) en rius intermitents i rieres efímeres (IRES). Per tant, la investigació actual manifesta la influència global de la interrupció del flux en el transport i processament de la matèria orgànica, donada l'àmplia distribució dels cursos d'aigua de flux intermitent. Tanmateix, encara hi ha llacunes en el coneixement de la intermitència de flux, oblidant la naturalesa contínua i multidimensional de la pertorbació de l'assecament.

En aquest context, al capítol tres, determinem la influència de dues condicions hidrològiques oposades, la sequera i el cabal elevat, sobre les propietats de la DOM i com el continu fluvial pot esmorteir l'impacte de la hidrologia sobre la DOM i els soluts inorgànics. A més, en el capítol quatre, s'estudia l'evolució del període sec com si fos un pel·lícula: "fotograma a fotograma" avaluant diàriament la disminució del cabal, especialment després de la interrupció del flux. S'estudia també com influeix la connectivitat hidrològica en el temps de resposta i la forma de diverses variables biogeoquímiques, i si les basses desconnectades estan influenciades per la seva localització espacial, la temperatura de l'aigua i el volum d'aquestes. Finalment, en el capítol cinc, des d'un enfocament de multiconca d'IRES amb un gradient d'intermitència de permanent a efímer, vam investigar com múltiples components d'assecament condicionen la qualitat i quantitat de la DOM que és transporta després duna sequera.

En els resultats del capítol tres, la qualitat del DOM va canviar en funció de la hidrologia. La DOM en condicions de cabal alt era més terrígena, humificada, aromàtica i degradada, i la concentració va augmentar gradualment riu avall. En canvi, la DOM estava menys degradada i amb més compostos alifàtics en condicions de sequera. Tanmateix, la variabilitat hidrològica no va afectar la qualitat de la DOM de manera uniforme al llarg

del continu fluvial: la qualitat és més sensible als canvis hidrològics en capçalera que no pas en trams riu avall. Al capítol quatre, el període d'assecament va determinar la interrupció del continu fluvial, amb una recessió a una velocitat de ~60 m/dia, així com la formació gradual d'un sistema fragmentat de piscines aïllades de diferents mides. Els resultats van mostrar que el temps que havia transcorregut des de la formació de les piscines aïllades (dies CI) és un factor essencial per entendre com l'assecament defineix la biogeoquímica del sistema fluvial. De forma general, l'assecament va provocar una alta concentració de DOC i un augment del component húmic. A més, els soluts van mostrar diferents respostes a la desconnexió hidrològica. Així, vam identificar tres respostes temporals sobre les variables estudiades: 1) alguns soluts inorgànics van reaccionar abans de la fragmentació (CI-dies<0). Els paràmetres qualitatius òptics de la DOM van respondre després de la fragmentació (CI-dies> 0), i 3) els índexs BIX i HIX no van mostrar respostes significatives. Finalment, al capítol cinc, el mostreig es va realitzar durant la temporada posterior a la sequera i els resultats van mostrar que els canvis en el carboni orgànic dissolt (DOC) estaven determinats per la durada anual de l'assecament, mentre que diversos components d'assecament explicaven millor la qualitat de la DOM. Així doncs, vam trobar una alta concentració de DOC i prevalença de fonts de DOM terrestres quan l'assecament és més estès i més freqüent. A més, a causa del moment del mostreig, la qualitat de la DOM va reflectir un "rentat de la conca", amb una DOM d'origen terrestre, i que es transporta riu avall ràpidament sense ser processat per a la comunitat microbiana.

Els resultats d'aquesta tesi revelen el paper de la hidrologia com a motor principal de la dinàmica de la DOM. En particular, la interrupció del flux no indueix una única resposta biogeoquímica sinó que estimula un conjunt de respostes específiques dels diferents soluts, la qual cosa genera un mosaic biogeoquímic complex en una única unitat fluvial. A més, es posa de manifest la necessitat de considerar múltiples elements d'assecament, com la duració i la freqüència anual o prèvia de l'assecament, per predir la dinàmica de la matèria orgànica en IRES.

GENERAL INTRODUCTION

1.GENERAL INTRODUCTION

Streams and rivers connect the terrestrial ecosystems to the marine/oceanic ones and, in a biogeochemical context, are a significant component of the carbon cycle at local, regional and global scales (Cole et al. 2007, Battin et al. 2008). The intermittent rivers and ephemeral streams (IRES) are characterized by a flow disruption throughout a determined period among these systems. The relevance of IRES is increasing worldwide as a consequence of climatic alteration, the increase of water demand and the land-use changes (Döll and Schmied 2012, Schneider et al. 2017). Flow cessation has severe implications on fluvial ecosystems structure and function, and during the last 20 years, its impact has been at the centre of intense research, especially in the Mediterranean region. Several studies focus on biogeochemistry, and more specifically, on organic matter. These studies highlight how the flow interruption and its successive recovery exert an essential control on the availability, composition, and processing of dissolved organic matter (DOM) flowing in running waters. DOM is the primary carbon and energetic source for heterotrophic microorganisms thriving in aquatic ecosystems. The present thesis aims to expand our knowledge about links between flow-intermittence and DOM, emphasising the importance of spatial-temporal components of drying phenomena along with fluvial networks.

1.1. INTERMITTENT STREAMS

IRES are running watercourses with periods of flow cessation. IRES include from ephemeral streams, totally dry for most of the time with unpredictable abrupt and short-term flow conditions, to almost permanent streams with seasonal short-term severe drought episodes. Within these two extremes exist a continuum of intermittence degrees, with most streams flowing from 20 to 80% of the year (Datry et al. 2017) Figure 1.1

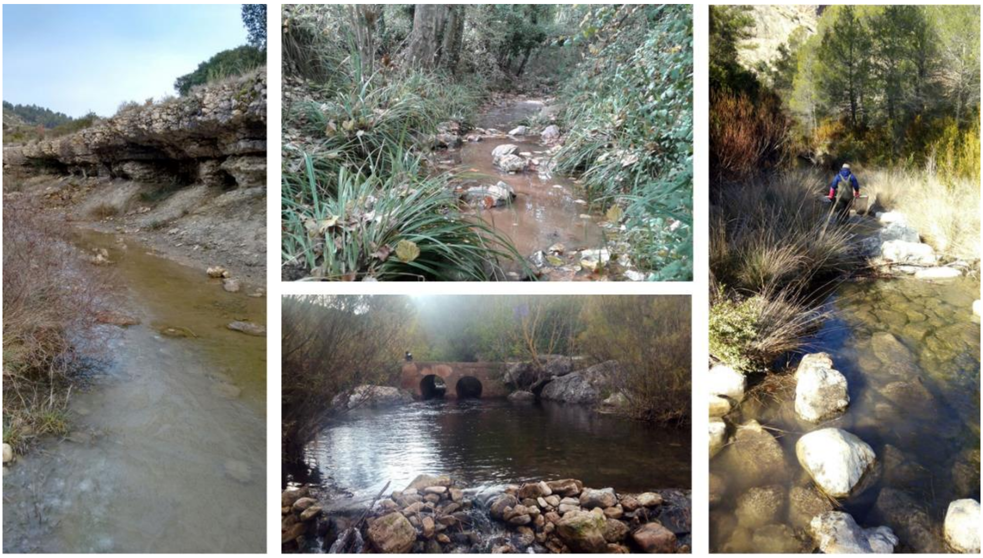


Figure 1.1. Photographs of some streams studied in this thesis with different intermittence gradients (NE Iberian Peninsula).

Worldwide, IRES comprise more than half of the global river network and are observed in all the terrestrial climatic areas (Datry et al. 2014), including arctic areas and alpine regions (Meyer and Wallace 2001). Nevertheless, IRES are highly relevant in arid, semiarid areas and Mediterranean regions.

Mediterranean IRES are characterized by seasonal high flow conditions mainly in fall and spring, a seasonal drought episode in summer, drying over an annual cycle, and great hydrological, sedimentary, and geomorphological diversity (Tockner et al. 2009, Bonada and Resh 2013, Skoulikidis et al. 2017). In the last 20 years, Mediterranean basins have been turning to dry up and exposing more extreme flooding and drying events (Butturini 2021), and the climate model projections predict an increase in the frequency of these events in the future (Pachauri et al. 2014). Furthermore, anthropogenic pressures as urban, industrial, touristic and agricultural development increase water demand (Skoulikidis et al. 2017). With this scenario, fluvial hydrology is altered: perennial rivers can shift to intermittent or vice versa if they are used as wastewater effluents (Luthy et al. 2015) or water releases from dams, industrial or a basin water transfer (Larned et al. 2010a, Steward et al. 2012, Datry et al. 2014). Irreversibly, the human-induced hydrological alterations affect the biodiversity and water quality of fluvial ecosystems (Sabater and Tockner 2010, López-Doval et al. 2013).

1.2. THE HYDROLOGICAL REGIME OF IRES

Hydrology has a strong influence on all freshwater ecosystems. Specifically, the research of flow cessation in IRES is essential because of its impact on aquatic organisms, their habitats, biogeochemical cycling and DOM composition (Leigh and Datry 2017). Each IRES has periods of zero-flow determined by regional and local factors influencing the hydrological regime’s magnitude, frequency, and duration (Costigan et al. 2016).

1.2.1. HYDROLOGICAL CONNECTIVITY

Flow cessation in IRES determines the rupture of the fluvial longitudinal continuum that links headwaters to downriver sections of a fluvial network, impacting biological, physical and chemical processes (Boulton et al. 2017). “Hydrological connectivity is the transport of matter, energy, and organisms through water and or using elements of the hydrological cycle” (Pringle 2001, Freeman et al. 2007). Hydrological connectivity has three spatial dimensions: longitudinal, lateral and vertical and one temporal dimension which interrelates between them (Ward 1989) Figure 1.2.1**.** Furthermore, hydrological connectivity is influenced by the following factors:

river geomorphology, for instance: channel morphology, riparian interface, and floodplain contour. The confluence of tributaries, meandering geometry, gravel bars and upwelling-downwelling zones or pools, and the size distribution of streambed sediments (Jaeger et al., 2017).

The flow regime relates to the quantity, timing and variability of flow, and is the most determinant factor for the hydrological connectivity (Sauquet et al., 2008). It includes magnitude, frequency, duration, timing, and rate of change of hydrological conditions. Magnitude is the discharge, the volumetric flow rate of water transported through a given cross-sectional area. Frequency is the number of times that repeats the same magnitude of flow along a specific time, for example, the annual number of zero-flow events. Duration is the time during which particular flow occur, for instance, the annual number of zero flow days (Arias-Real et al., 2020; Snelder et al., 2013). Timing is when a particular event is foreseeable, such as the autumn rainfall in the Mediterranean climate. Lastly, the rate of change is the velocity of flow changes from a magnitude (Leigh and Datry, 2017).

Other additional drivers can alter the hydrological connectivity, such as anthropogenic activities (e.g., land-use modification, flow regulation, water removal), and to meteorological conditions (e.g., timing and magnitude of precipitation and evaporation). Also, the biological activities (e.g., the algal accumulation in the sediment can affect the vertical hydrological connectivity or the tree fall leaves in the longitudinal hydrological connectivity have an effect on the biological comunity). The factors mentioned in this section are all interconnected (Boulton et al., 2017).

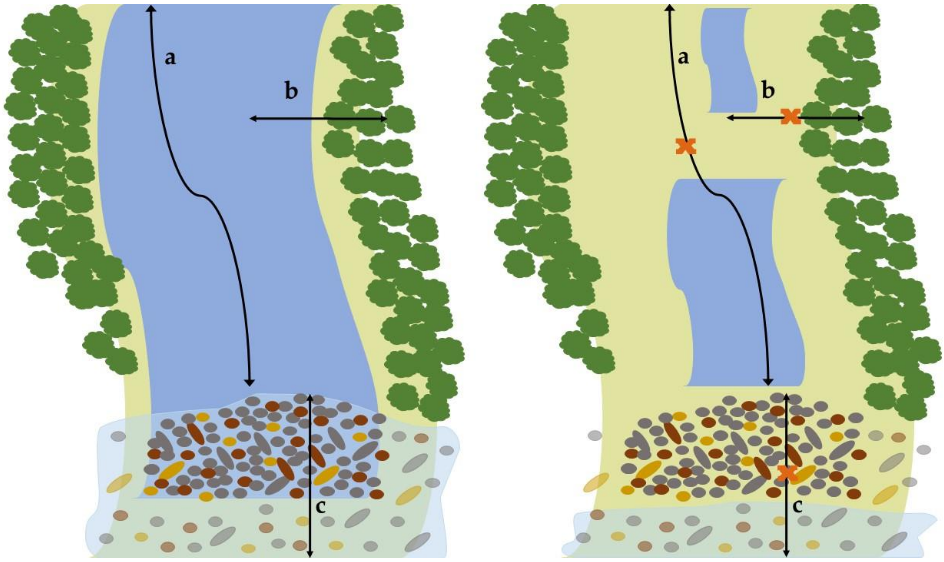


Figure 1.2.1. Three spatial dimensions of hydrological connectivity. Longitudinal (a), horizontal (b) and vertical (c) connectivity. Orange crosses indicate interruption of hydrological connectivity. This figure is modified from Chapter 2.3 in Datry et al., 2017 Book.

In IRES, the hydrological connectivity is interrupted in at least one or more of the three hydrological dimensions previously mentioned, creating spatial and temporal zero-flow dynamics that influence stream ecology. In particular, the interruption of lateral hydrological connectivity affects the displacements of organic carbon, nutrients, sediments and some aquatic biota between the floodplain and main stream channel (Amoros and Bornette 2002). The vertical hydrological connectivity originates principally from the exchange between the water surface and the subsurface (i.e., hyporheic zone) of the main stream channel or the inundated floodplain. So, the connectivity stops when surface flows finish, causing changes in inorganic and organic solutes and gas in the saturated hyporheic zone and increasing microbes productivity (Boulton et al. 2010, Harjung et al. 2019). Longitudinally, the flow cessation interrupts the downriver transport of sediments, solutes and organisms, and drives the fluvial ecosystem's fragmentation into a set of isolated and temporal water pools along the stream channel. The size and permanence of these pools strongly depend on water residence time (Figure 1.2.2), which in turn is modulated by the permeability of the substrate, the groundwater inputs and outputs, and the evaporation and precipitation balance (Fellman et al. 2011, Costigan et al. 2016). In short, the rupture of the hydrological connectivity of a stream channel promotes the formation of a heterogeneous mosaic of small and temporal aquatic habitats disconnected among them (see section 3.3).

Figure 1.2.2. Isolated pools at the end of the dry period in Fuirosos catchment (NE Iberian Peninsula).

1.2.2. CHARACTERIZATION OF FLOW REGIME IN IRES

A significant obstacle to investigating the IRES is characterising their hydrological regime because they are primarily ungauged and misrepresented in maps. Mainly, the intermittent headwater streams are disregarded because of their difficulties to obtain data with current techniques. In addition, their abundance and extension are typically underestimated because IRES does not have the same social-economic value as permanent rivers and are far from urban areas (Snelder et al. 2013, De Girolamo et al. 2015, Eng et al. 2016).

In this context, some feasible approximations to characterize the flow regime of IRES rely, for instance, the wet-dry mapping, remote sensors, field loggers hydrological metrics and modelling.

The wet-dry mapping is helpful to track the hydrological connectivity. For instance, it can precisely track in both time and space. The formation of isolated pools along the main reach (chapter four) or with trained citizen scientists could obtain more data of intermittent streams over large areas (Datry et al. 2016). Hence, the mapping is appropriate to describe the flow regime qualitatively, such as categorizing the aquatic state of IRES or phases of dry period or connected versus isolated (Taylor 1997, Bonada et al. 2006, Rupp et al. 2008, Gallart et al. 2012). Remote sensors are used as proxies of discharge and are suitable in large rivers, but they are not appropriate for small headwaters streams with high-density vegetation cover (Benstead and Leigh 2012).

More recently, some studies promoted the implementation of field data loggers to characterize the flow regime of IRES (Arias-Real et al., 2021). Water temperature loggers applied as flow surrogates advance our knowledge of intermittence properties and reduce the dependency on gauging stations. Furthermore, their easy installation in the riverbed and low cost allows for installing several sensors along the main stem, covering large spatial dimensions of the fluvial system even if they are in remote areas. However, field data loggers have some issues, and they are lost due to vandalism or large floods. Also, regular maintenance and downloading of the data are needed. Hence, water temperature data allow translation into hydrological metrics tracking the flow and the zero-flow situation with a temporal and spatial high resolution (Constantz et al. 2001, Gungle 2006). Therefore, hydrological metrics are indices or statistics computed from time series of discharge or temperature data. They are appropriated to characterize the intermittent flows, especially the drying elements of IRES, for instance, the number of zero-flow days, zero-flow period, percentage of zero-flow months, percentage of flow permanence and rate of zero-flow events in each season (Larned et al. 2010b, Stromberg et al. 2010, Snelder et al. 2013, Belmar et al. 2013, Reynolds et al. 2015, Paillex et al. 2020).

In addition, modelling can be applied to translate data into metrics. Two types of prediction models: the daily flow time series and, with this information, calculate the metrics, or the statistical models, where the flow metrics are estimated instantly (Carlisle et al. 2010). For instance, models can simulate flows under global change, switch the environmental conditions or modify the water extraction. Nonetheless, most models are developed for perennial rivers, obtaining the data flow from gauging stations (Larned et al. 2010b, Belmar et al. 2013). The difficulty appears for simulating the zero-flow conditions or even the low flow, overall the hydrological extremes such as floods or dry period. To approach some of these difficulties to modelize the intermittence, an example of modelling is the Soil Water Assessment Tool (SWAT) (Gassman et al. 2007) is a public domain program and the model most applied to catchment-scale hydrological transport, which has been helpful to model the flow intermittence in IRES (D'Ambrosio et al. 2017). It has recently been modelling drying dynamics from observations of flow state (i.e., flowing vs drying) in a continuous-time series that includes climate, hydrology, groundwater level, and basin information (Beaufort et al. 2019).

A second example was modelling the effect of flow intermittence of IRES during the dry period on DOM properties and inorganic solutes. The low flow data were registered until the flow disruption of the stream. Especially, getting the time data and volume of isolation pools is complex. Hence, temperature data loggers were set up covering the main stream. The temperature data collected was transformed into CI-days (i.e., in each sampling site: the number of days when the stream has flow, CI-days had a negative value until the flow disruption. On the contrary, when the stream was disrupted and transformed into isolated pools, CI-days had a positive value, the number of days isolated). Thus, to determine the effects of the variables, we tested the more appropriate model. For instance, if the variable had a non-linear response, it was modelled by GAMMS. On the other hand, if the variable had linear response was modelled by LM or GLMM, using the AIC criterion as statistical support. After that, we fitted the proper model of each variable, including CI-days, pool volume, temperature, and spatial location, as a fixed factor (chapter four).

The last example, in chapter five, was modelling the importance level of drying components on DOM quality with a multicatchment approach of IRES with a different gradient of intermittence, from permanent to ephemeral streams. As specified before, there are some difficulties to obtain zero-flow data, which is crucial in IRES systems. Hence, temperature data loggers were set up in 35 IRES along a hydrological year and sampled the streams after rewetting at the end of temperature loggers monitoring. The temperature data collected was transformed into drying components. It was defined as two main categories of drying components. 1) Annual drying elements that included: a) the annual drying duration. The annual number of days with zero-flow (ZFT, zero-flow total days) and b) the annual drying frequency. The annual number of zero-flow periods (ZFP, zero-flow periods). 2) Previous drying elements comprised: c) the number of zero-flow days in the last zero-flow period (ZFL, zero-flow last) and d) the number of days since the last rewetting event (RE, rewetting) (Arias-Real et al. 2021a). Then, with the two environmental factors altitude and water temperature determined and the four drying components, linear regression models and a multi-model inference approach were used.

I.3. IMPLICATIONS OF FLOW CESSATION ON DOM DYNAMICS AND INORGANIC SOLUTES

Organic matter (OM) constitutes the basic source of energy and carbon of aquatic food webs, and the dissolved fraction of OM, named DOM, plays a crucial role in the global carbon cycle, being the predominant form of organic carbon in freshwater ecosystems (Findlay and Sinsabaugh 2004). Furthermore, DOM exerts a key control on the ecosystem functioning and structure. In particular, IRES are affected by flow disruption along the hydrological year, and they are scarcely investigated. Flow cessation causes the diminution of the aquatic habitats with effects on the biotic structure (species richness and diversity) and ecosystem functioning (metabolism and biogeochemical processes).

I.3.1. THE ROLE OF LONGITUDINAL DOM TRANSPORT. AT CATCHMENT SCALE

DOM represents approximately 25 % of the total carbon transported downriver in the fluvial ecosystems (Meybeck 1982). Throughout the transport across the stream channel, DOM is susceptible to change in both quantity and quality (Jaffé et al. 2008). DOM is a complex mixture of thousands of organic molecules, and their composition reflects internal transformation processes (i.e., autochthonous or in-stream derived DOM) and external inputs (i.e., allochthonous or terrestrially-derived DOM) from terrestrial sources, from tributaries, groundwater and, when present, anthropogenic outlets from wastewater treatment plants, industries and urban settlements. Accordingly, the River Continuum Concept (RCC) (Vannote et al. 1980) theorizes about longitudinal DOM dynamics and anticipates variations in the DOM processing from upstream to downstream. RCC is an integrated model of fluvial functioning that describes rivers with physical and hydrological conditions that regulate the longitudinal distribution of biological communities. Thus, in agreement with the RCC model, expected downstream patters with a diminution of DOM heterogeneity, a reduction of the size of organic matter and, an increment of the contribution of recalcitrant molecules. The microbiota breaks down the labile compounds, and the recalcitrant fragment persists downriver (Amon and Benner 1996, Fellman et al. 2014). Creed et al. (2015) suggested a tendency towards a convergence/confluence of DOM concentration and composition along the river continuum, i.e., there will be high DOM variability and low DOM reactivity in headwaters and little DOM variability downriver. However, RCC is in question due to its simplicity and for not evaluating rivers' spatial variation. However, both Vannote and Creed theories do not consider the impact of natural and anthropogenic effluents. For instance, wastewater provides significant increases in protein-like fluorophores (Baker 2001, Saadi et al. 2006, Butturini and Ejarque 2013). The tributaries or groundwater inputs have water with different DOM compositions regarding the main stem and may accelerate or vice versa the biogeochemical processes (McClain et al. 2003).

The RCC (Vannote et al. 1980) and its adaptations to DOM longitudinal transport in rivers (Fellman et al. 2014, Wollheim et al. 2015, Creed et al. 2015) has recently been adapted to the spatial and temporal patterns of DOM quantity and chemical diversity (Casas-Ruiz et al. 2020a). Nonetheless, the extreme hydrological episodes, floods or drought that frequently occurred in IRES systems are still not considered (Butturini et al. 2016, Ejarque et al.

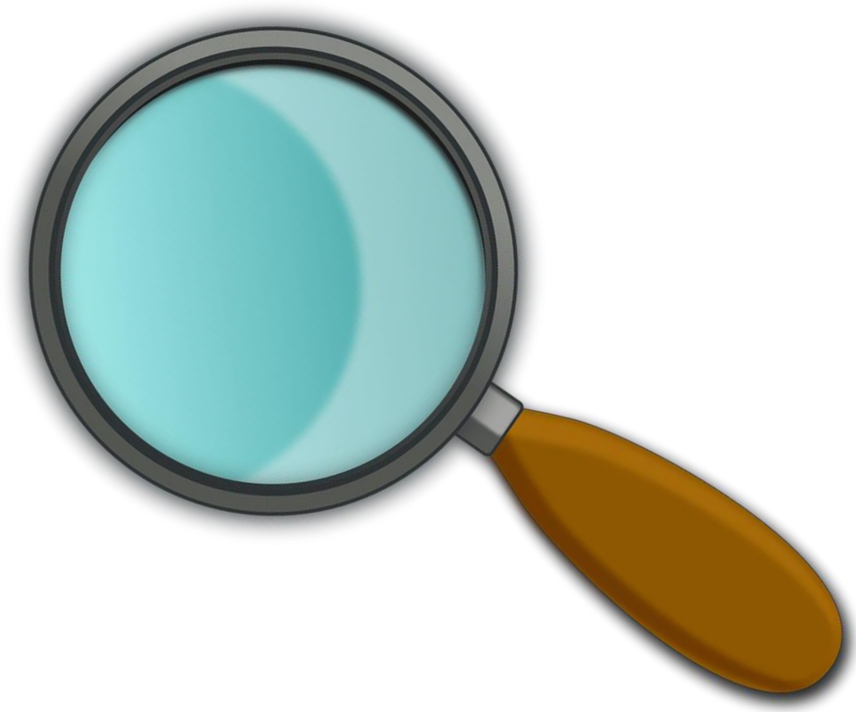
2017). So, during floods, the in-stream DOM processing is almost non-existent due to the short water residence times. Additionally, the terrestrially-derived DOM increase substantially as precipitation pulse this DOM from riparian soils to the river (Raymond et al. 2016), and DOM shows a homogeneous longitudinal pattern (Casas-Ruiz et al. 2020a). However, during droughts, water has typically a high residence time, which facilitates the in-stream microbial respiration, photochemical oxidation, primary production, and therefore the increase of the contribution of autochthonous DOM (Gao and Zepp 1998, Findlay and Sinsabaugh 2004, Maranger et al. 2005). Therefore, the extreme hydrological episodes will increase in time and duration due to climate change. These changes will condition DOM composition, food webs, trophic interactions and overall the ecosystem functioning of IRES.

1.3.2. THE ENTIRE FRAME OF THE DRY PERIOD ON THE DOM COMPOSITION. FROM BASE FLOW TO ISOLATED POOLS

The dry period is frequent in the arid and semiarid regions like the Mediterranean area. During this period, the flow disrupts, and the hydrological disconnection may be longitudinal, lateral or vertical dimension as described in section 1.2.1. Furthermore, the streams can have different longitudinal patterns of drying, for instance, downstream, headwaters or mid-reach drying (Lake 2003). Thus, hydrological transition from continuum to discontinuum is not equal along the stream, areas where the flow and connectivity are very heterogeneous, is both space and time. Hence, the streams may transform into fragmented pools or dry riverbeds, where the isolated pools can appear at different times during the dry period, persist from days to months and have disparate volumes (Boulton et al. 2017).

The mosaic habitats of established by drying acts as a refuge for aquatic invertebrates, fungi and bacteria, and they are seed banks for the aquatic plant (Larned et al. 2010a, Lake 2011, Datry et al. 2016). This mosaic of pools also influences organic matter availability and associated biogeochemical processes (Dahm et al. 2003, Gómez et al. 2009, Vázquez et al. 2015, Ejarque et al. 2017, Harjung et al. 2018, Bernal et al. 2018). Several studies revealed a prevalence of the autochthonous DOM origin during drying because of the water residence time increase and the decreasing of terrestrial inputs. Especially in compounds with a protein-like character when groundwater dominates the flow in base flow conditions (Fellman et al. 2011, Vazquez et al. 2011, von Schiller et al. 2015, Siebers et al. 2016, Casas-Ruiz et al. 2016). The DOM concentration does not change considerably along this period, but the DOM composition shifts to non-humic, less aromatic and low molecular weight compounds (Vazquez et al. 2011, von Schiller et al. 2015), suggesting in-stream microbial and algal processing (von Schiller et al. 2015). For instance, the streams with less canopy cover, few leaves inputs and high light availability have more autochthonous DOM from algal leachates (Dahm et al. 2003). However, other studies that focused on the evolution of drying in isolated pools showed the accumulation of terrestrial DOM with high aromaticity and relatively high molecular weight together with a gradual increase in DOM concentration (Harjung et al. 2018, 2019). In addition, the dry period progression develops anoxic isolated pools as consequence of high water temperature, high evaporation rates and solute concentration (Lillebø et al. 2007, Gómez et al. 2009, Fellman et al. 2011, von Schiller et al. 2015). Moreover high dissolved carbon dioxide concentration has been reported emission despite fluxes are low because of minor turbulence in the pools (Gómez-Gener et al. 2015). Anoxic habitats alters the growth and development of microbial and fungal communities. These physical, chemical and biological transformations disturb the organic matter processing (Fazi et al. 2013, Canhoto et al. 2016, Battin et al. 2016).

Most of previous studies provided static information or monitored small stream reaches. It is still missing a detailed description of the hydrological changes during this period covering the entire fluvial system and its effects on DOM composition and inorganic solutes.



RESEARCH OBJECTIVES

2.RESEARCH OBJECTIVES

This thesis studies how flow intermittence influences the biogeochemical dynamics from different perspectives. As opposed to IRES, this knowledge is more consolidated in permanent rivers. This thesis is structured in three chapters, each one corresponding to an independent publication.

2.1. OBJECTIVES OF CHAPTER 3

This chapter explores the divergence of DOC concentration, DOM qualitative properties and inorganic solutes between opposite hydrological conditions along a longitudinal continuum of an intermittent Mediterranean river 70 km-long.

Thus, the research questions are:

1) Do opposite hydrological conditions (high flow and drought) regulate the DOM descriptors?

2) Does the river continuum modulate the impact of hydrology on DOM descriptors and inorganic solutes, as predicted by Creed's hypothesis in permanent rivers?

In agreement with this approach, in headwaters, DOM has high variability and low reactivity. On the contrary, the downriver has low DOM variability. Thus, we expect a reduction in the contribution of aromatic substances and increases in small and aliphatic molecules downriver. In parallel, we expected smaller differences on DOM quantity and quality along the fluvial continuum.

2.2 OBJECTIVES OF CHAPTER 4

This chapter analyse the effect of DOC concentration, DOM composition, and inorganic solutes during the drying period, especially to study the fragmentation process covering the entire fluvial continuum with acute precision. After the fragmentation, the process is scantly researched when the stream transforms to isolated pools on different dates and water retention times. The research questions are:

1) How fast is the drying of a river continuum?

2) How the fluvial continuous is converted into a mosaic of fragmented isolated pools?

3) How does the hydrological fragmentation impact on DOM quantity and quality in isolated pools?

Our hypothesis is that the timing of fragmentation (CI-days) is the primary driver of the variability in DOC, DOM and inorganic solutes. So, we predict the biogeochemical changes that happen simultaneously to the moment of fragmentation of the stream continuum, increasing the DOC concentration and autochthonous DOM sources.

2.3 OBJECTIVES OF CHAPTER 5

This chapter has a broader perspective on the flow intermittence and focuses on how drying components impact DOM optical qualitative parameters. We apply a multi-catchment approach by sampling once thirty-five Mediterranean streams, which cover all flow intermittence gradients, during the post drought period during the rainy season. The research questions are:

2) How many drying components can be defined?

3) Which drying components impacts more significantly on post-drought DOM quantity and quality?

1) How affect the duration and frequency of drying on DOM quantity and quality?

Thus, we expect the recent duration of the latest drying or rewetting event to be more important than annual drying components for DOC and DOM quality. So, an increase of DOC concentration and a higher proportion of autochthonous DOM sources is expected as drying evolves.

CHAPTER 3

Dissolved organic matter variability along an impacted intermittent Mediterranean river

Dissolved organic matter variability along an impacted intermittent Mediterranean river

Verónica Granados [1] and Andrea Butturini [1]

[1] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona, Diagonal 643, 08028 Barcelona, Spain

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3.1.ABSTRACT

Hydrological variability is the key factor that modulates allochthonous inputs and in-river biotic processes and, thereby, the fate of dissolved organic matter (DOM) in rivers. However, little is known about how these factors, combined, change DOM quantity and quality along river courses. This study explored how DOM quantity (in terms of dissolved organic carbon – DOC) and quality (in terms of optical properties) varied along a Mediterranean river, under contrasting hydrological conditions: drought and high flow. The study was performed in the Matarranya river, a system severely affected by water abstraction for agricultural irrigation and inputs of untreated residual waters. DOM properties changed according to hydrology. DOM under high flow conditions was more terrigenous, humified, aromatic, and degraded and the concentration gradually increased downriver. In contrast, DOM was less degraded and more aliphatic under drought conditions. DOM spatial variability under drought and high flow conditions revealed that hydrology has a greater impact on DOM quality in headwaters than at downriver sites. Longitudinal changes in DOM were more evident under drought conditions. For instance, a longitudinal depletion of DOC, together with a decrease of the fresh DOM pool, was observed in a large fluvial segment (35 km long) that does not receive any notable anthropogenic inputs. In contrast, the contribution of the most aromatic and humified DOM pool was significantly higher downriver. This study confirms the role of hydrology as a main driver of DOM dynamics. Additionally, it shows that hydrological variability does not impact DOM properties uniformly along the river continuum. On the contrary, DOM properties are more sensitive to hydrological changes in headwaters than in downriver reaches.

3.2.INTRODUCTION

Dissolved organic matter (DOM) is a complex mixture of soluble organic compounds that vary in their reactivity and ecological role (Fellman et al., 2010) DOM includes complex, polymeric compounds (humic and fulvic substances) as well as simple, well-defined molecules (sugars, proteins, lipids, organic acids, phenols, alcohols, among others). The molecular weights of DOM compounds rang from less than 100 to over 300 000 Daltons (Hayase & Tsubota, 1985; Thurman, 1985). These compounds can originate from terrestrial or aquatic sources as well as from anthropogenic inputs, such as treated and untreated wastewater effluents (Leenheer, 2009). DOM plays an important role in aquatic food webs, because it supplies energy, carbon (Wetzel, 1992) and nitrogen (Keil & Kirchman, 1991) for the heterotrophic community. However, the extent to which the dissolved organic compounds are metabolized in freshwater ecosystems depends on their biochemical composition (Benner, 2003).

In freshwater ecosystems, DOM represents approximately 25% of the total carbon transported downriver (Schlesinger & Melack, 1981; Meybeck, 1982) and its transport, reactivity and fate along fluvial courses is the product of internal (autochthonous) transformation processes and external (allochthonous) inputs from tributaries, occasional anthropogenic outlets (e.g., wastewater treatment plants, industries and urban settlements) and diffuse inputs from groundwater. The quantity and quality of allochthonous DOM inputs are strongly modulated by hydrology and, especially, the occurrence of extreme episodes such as droughts and floods (Buffam et al., 2001; Butturini et al., 2016).

Longitudinal DOM dynamics were theorized by the River Continuum Concept (RCC) (Vannote et al., 1980). According to this model, DOM heterogeneity and processing is expected to decrease from upstream to downstream, whereas the contribution of recalcitrant molecules is expected to increase (Fellman et al., 2014). Thus, larger and more reactive molecules would be more abundant in headwaters while smaller and more recalcitrant molecules would be more plentiful downriver (Amon & Benner, 1996). More recently, Creed et al. (2015) suggested a tendency towards a convergence/confluence of DOM concentration and composition along the river continuum. According to Creed's approach, high DOM variability and low DOM reactivity is expected in headwaters. In contrast, low DOM variability is expected downriver.

However, these scenarios ignore the impact of a succession of inputs from natural and anthropogenic tributaries (Fisher et al., 2004) and the occurrence of extreme hydrological episodes (Butturini et al., 2016). For instance, low in-stream DOM processing may be expected during floods due to low water residence times (Raymond et al., 2016). Therefore, terrestrially-derived molecules may reach lowland river sites. During drought, long water residence times may stimulate in-stream microbial respiration (Maranger et al., 2005), photochemical oxidation (Gao & Zepp, 1998) and primary production (Findlay & Sinsabaugh, 2003), thus increasing the amount of potentially labile compounds.

Predictions formulated by the RCC and successive extension and/or readjustments (Junk et al., 1989; Creed et al., 2015; Raymond et al., 2016) motivated the study of DOM longitudinal transport and fate along fluvial networks (Fellman et al., 2014; Kaushal et al., 2014; Creed et al., 2015; Wollheim et al., 2015; Butturini et al., 2016). Most of these studies were performed under baseflow conditions, whereas few studies have focused on drought and floods (Butturini et al., 2016; Ejarque et al., 2017). However, data obtained under droughts and floods are essential to understand the biogeochemical functioning of fluvial ecosystems. This is especially crucial in Mediterranean systems because a change in the frequency and magnitude of both hydrological extremes is expected in Mediterranean catchments (Vicente-Serrano et al., 2014; Barrera-Escoda & Llasat, 2015).

In this context, we aimed to analyse the quantitative and qualitative changes in DOM along a longitudinal continuum of an intermittent Mediterranean river, under two contrasting hydrological conditions: drought and high flows.

Our two research questions were:

- How do flow extremes determine DOM properties?
- How does the river continuum modulate the impact of hydrology on DOM properties?

According to Creed's hypothesis, we expected a reduction in the contribution of aromatic substances and concurrent increases in small and aliphatic molecules downriver. In parallel, differences in DOM quantity and quality between high and low flow conditions were expected to become smaller along the fluvial continuum. To achieve our aims, the main stem of a large intermittent river (70 km long) was sampled at high spatial frequency. DOM characterization included its quantification in terms of carbon (DOC) and qualitative DOM descriptors through spectroscopy techniques. Euclidean distance was also estimated to quantify the impact of hydrological conditions on DOM longitudinal patterns.

3.3.MATERIAL AND METHODS

3.3.1. STUDY AREA

This study was performed along the Matarranya river, the last important tributary joining the Ebro from the right-hand side of the river. It drains a catchment of 1738.45 km^2 from Els Ports de Tortosa-Beseit (1296 m a.s.l.) to the Ribarroja reservoir 100 km downstream (70 m a.s.l.). The basin is part of the geological Maestrazgo–Catalànides domain, which can be divided into two main areas: 1) Els Ports de Beseit, dominated by Mesozoic materials, in the headwaters; 2) The Ebro depression, dominated by Cenozoic (Tertiary and Quaternary) materials, in the middle and final sections of the Matarranya river. The geology is dominated by rocks of terrigenous origin (conglomerates, sandstones and marls), but limestones and evaporites (gypsum) outcrop along the entire route of the riverbed. The catchment is covered by brushwood (33%), forest (14%), urban areas (14%) and agriculture (33%), the latter especially in the middle and downriver reaches. The climate is relatively warm and arid. Downriver, air temperature and annual rainfall average 14-16 °C and 350 mm respectively. In contrast, in the headwaters air temperature and annual rainfall average 12-14 °C and 700-900 mm (El plan hidrológico del río Matarraña, 2008). The river shows a typical Mediterranean hydrology, characterized by moderate-high flow conditions in spring and autumn and a severe dry period in summer. The fluvial network contributes 156 hm^3 to the Ebro discharge. It comprises four main headwater tributaries (Matarranya, Ulldemó, Pena and Tastavins). Downriver the largest tributary is the Algars river. Fluvial hydrology is altered by human activities. There are two main reservoirs: the Pena reservoir (17 hm^3) and Vallcomuna (2.42 hm^3) as well as numerous small reservoirs in the catchment. Finally, a dense drainage system takes water from the river to irrigation systems during spring and summer. Since 1995, water from the Matarranya has been diverted into the Pena reservoir in winter (El plan hidrológico del río Matarraña, 2008). There are seven villages along the river. In the text they are named from “1” (headwaters) to “7” (downriver). The full names of the villages are given in the caption of Figure 3.3.1.1.

3.3.2. SAMPLING STRATEGY

The Matarranya river was sampled along its main stem and at the main natural and anthropogenic inflows to the main stem (Figure 3.3.1.1.). The main stem was sampled at 45 sites, from the headwaters (2 km from source; 40°48'57.87''N / 0°11'11.94''E) to its mouth (71 km from source; 41°12'40.13''N / 0°14'57.74''E). The surface water was sampled in the middle of the river channel. The distance between sampling sites averaged 1.6 ± 0.9 km.
Sampling sites were selected in order to take into account all main tributaries (at kilometres 3, 7.5, 14, 29), untreated wastewater inputs (at kilometres 2.5, 8.5, 46, 55.6) and irrigation return waters (at kilometres 38, 40, 48.8 and 62). Samples were collected during two opposite contrasting hydrological conditions. The first set of samples was collected in July 2015, under summer drought conditions. The second set was collected under high flow conditions in November 2015, after a severe high flood event.

3.3.3. CHEMICAL AND FIELD MEASUREMENTS

FIELD MEASUREMENTS

Electrical conductivity (EC, WTW 3310 set 1 conduct-meter) and water temperature were measured at each site. At the end of each sampling day, when samples were at the same temperature, DOM fluorescence in unfiltered samples was measured using two fluorescence sensors: a humic-

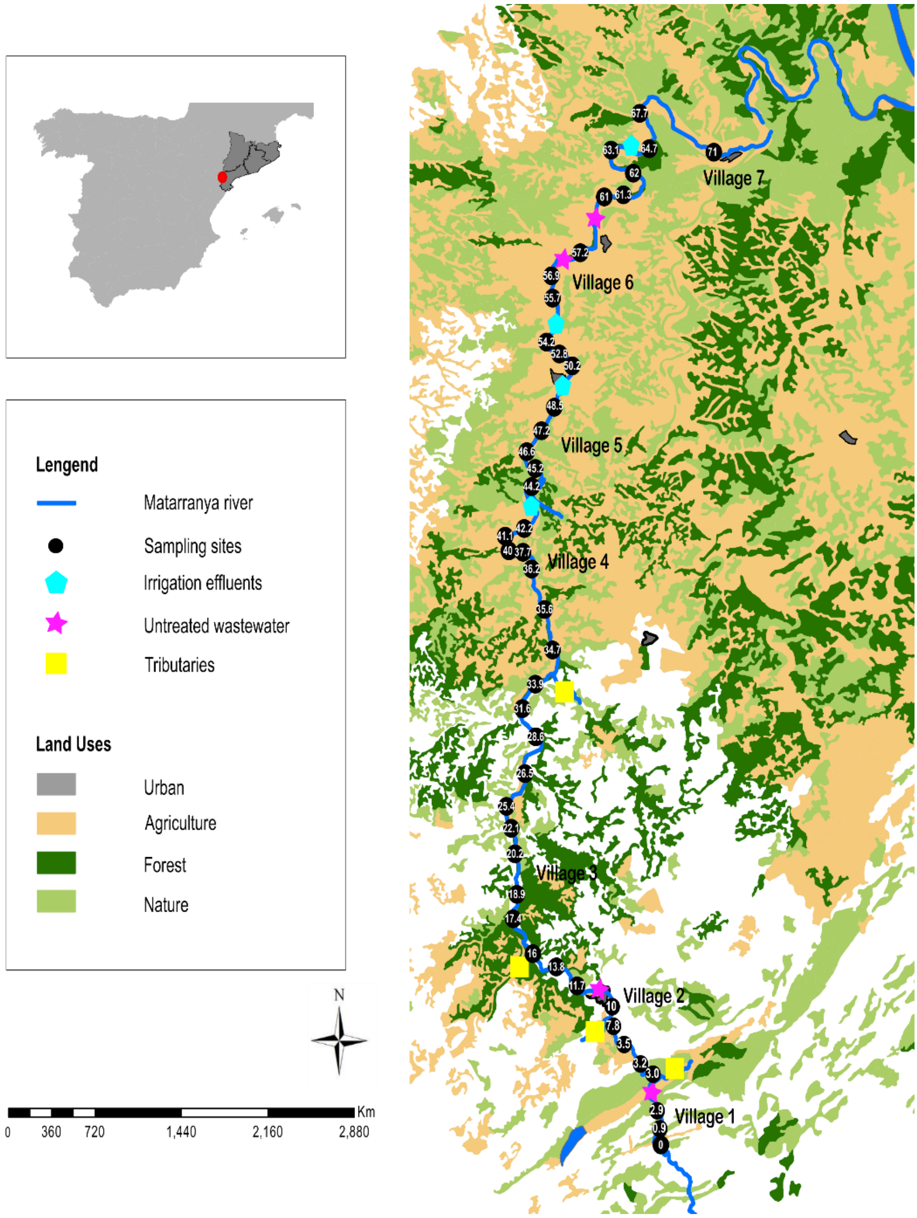

Figure 3.3.1.1. Matarranya river main stem and land uses. Black dots are sampling sites (numbers inside black dots indicate the distance in km from headwaters). Villages along the river are named with numbers (from 1 to 7). The villages are, beginning from the headwaters: Beseit, Vall-de-roures, La Torre del Comte, Massalió, Maella, Favara and Nonasp.

like sensor (TurnerDesigns Cyclops 7, Ex/Em, 325/470nm) and a tryptophan-like sensor (TurnerDesigns Cyclops 7, Ex/Em, 285/350nm).

CHEMICAL ANALYSES

For chemical analyses, all samples were filtered in the field using precombusted (450 °C) glass fibre filters (Whatman GF/F 0.7 µm pore size) and then 0.22 µm pore nylon filters. The filtered samples were placed in amber glass bottles previously washed with acid. We stored the samples on ice and in the dark, and immediately transported them to the laboratory where they were stored at 4 °C for later analysis. Chemical parameters were grouped as follows: inorganic solutes (named DIM) included: electrical conductivity, chloride, sulphate, $^{18}\Delta O$, ΔD, ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}); DOM descriptors (named DOM) included: DOC, HIX, FI, BIX, S_R, E_2:E_3, $SUVA_{254}$, $SUVA_{350}$, $Fluor_{Humic\text{-}like}$ and $Fluor_{Prot\text{-}like}$.

INORGANIC SOLUTES

Ammonium concentration was measured using the salicylate method (Reardon, 1969), and the soluble reactive phosphor (SRP) was measured using the molybdate method (Murphy & Riley, 1962). Inorganic anions (nitrate, chloride and sulphate) concentrations were analysed with an ion chromatograph Metrohm 761 Compact IC with the column Metrosep A Supp5 - 150/4.0.

To perform a better geochemical discrimination between the two opposite hydrological conditions stable water isotopes $^{18}\Delta O$ and ΔD were analysed (Laudon & Slaymaker, 1997). Stable isotopes analysis were performed at the Scientific and Technological Centre of the University of Barcelona. For $^{18}\Delta O$ the equilibrium was achieved with CO_2 and He and measurements were executed on a MAT 253 from Thermofisher. ΔD was measured by water pyrolysis analysis of the resulting H_2 separated by column chromatography on an EA-TC-IRMS-DELTA PLUS xp Thermofisher.

DOC CONCENTRATION AND OPTICAL PROPERTIES OF DOM

Samples for DOC analysis only were filtered by glass fiber filters (Whatman GF/F 0.7 μm pore size) and later, they were acidified with 10% HCl and refrigerated before analysis. DOC was determined by oxidative combustion and infrared analysis using a Shimadzu TOC Analyser VCSH coupled with a TN analyser unit. Qualitative optical properties of DOM were estimated in terms of absorbance and fluorescence. DOM absorbance spectra were measured using a UV-Visible spectrophotometer UV1700 Pharma Spec (Shimadzu) and a 1 cm quartz cell. Absorbance data were obtained in double beam mode with wavelength scanned from 200 to 800 nm and deionized water as the blank. Excitation-Emission Matrices (EEM) were generated by an RF-5301 PC spectrofluorimeter (Shimadzu). Spectra were measured using a 1 cm quartz cell. EMMs were measured over (Ex/Em) wavelengths of 240-420 nm and 280-690 nm and they were standardized based on the method of Goletz et al. (2011) using Mathematica (Wolfram Research) software. EEM data were corrected using the same method. The absorbance data for each sample respectively were used to correct the inner filter effects (Lakowicz, 2006). To correct wavelength-dependent inefficiencies of the detection system the following methods were used: Gardecki & Maroncelli's method (1998) for emission measurements and Lakowicz's method (2006) for excitation correction. Data were normalized with daily measurements of the area under the Raman peak using MilliQ water blanks (Lawaetz & Stedmon, 2009).

Seven qualitative descriptors of DOM were estimated in this study The four chromophoric indices were as follows: (a) Specific Ultra Violet Absorbance at 254 nm ($SUVA_{254}$) and (b) Specific Ultraviolet Absorbance at 350 nm

($SUVA_{350}$) as the absorption coefficient at 254 or 350 nm normalized by DOC concentration (Weishaar et al., 2003). Higher values are typically related to greater aromaticity (Hansen et al., 2016). (c) The ratio of absorbance at 250 nm to 365 nm (E_2:E_3), which provides information about DOM molecular size. Low E_2:E_3 values suggest a proportional increase in average DOM size (De Haan & De Boer, 1987). (d) Spectral slopes ratio (S_R), which also integrates shifts in the molecular size of DOM. This is a dimensionless parameter, estimated by calculating the ratio of the logarithmically transformed absorbance spectra slope at 275–295 nm ($S_{275–295}$) to that estimated at 350–400 nm ($S_{350–400}$). High S_R values indicate an increase in the proportion of the small DOM molecular fraction (Helms et al., 2008). Three fluorophoric indices were also estimated: (a) Humification index (HIX), which is the area under the emission spectra 435-480 nm divided by the peak area 310-345 nm from the spectra at an excitation wavelength of 254 nm. HIX indicates the extent of humification by quantifying the shift in the emission spectra toward longer wavelengths, due to lower H:C ratios. HIX values range from 0 to 1 with higher values indicating a greater degree of DOM humification (modified from Ohno, 2002). (b) Fluorescence index (FI), which is the ratio of emission intensities at 470 nm and 520 nm emitted at an excitation of 370 nm and provides information about DOM sources. High values suggest the prevalence of autochthonous DOM and low values the prevalence of allochthonous DOM (Cory & McKnight, 2005). (c) Biological index (BIX), which was calculated at an excitation of 310 nm as the ratio of the fluorescence intensity emitted at 380 nm. β fluorophore is the maximum of intensity and emitted at 430 nm, which corresponds with humic fraction. The β fluorophore is typical of recent autochthonous DOM release. Therefore, high BIX values (>1) suggest the presence of autochthonous and fresh DOM, whereas BIX values of 0.6–0.7 indicate low or zero autochthonous DOM production (Huguet et al., 2009).

3.3.4. HYDROLOGICAL MEASUREMENTS

During drought conditions, discharge was calculated at 19 sampling sites along the main reach. The velocity-area method (Di Baldassarre & Montanari, 2009) was used. A flowmeter (Global Water FP111 Flow, sensor range 0.1-6.1 m s^{-1}) was used to measure the mean water velocity. The river channel cross-section was divided into 0.2-0.5 m subsections (depending on the river width). The mean water velocity was estimated at 2/3 of the total depth. During high flow conditions, it was not feasible to estimate discharge in situ. Discharge values from the *Confederación hidrográfica del Ebro* were available for one headwaters site (40°49'27.82"N; 0°11'7.61"E) and one downstream site (41°11'42.42"N; 0°10'17.92"E). Values at these two sites were used to interpolate discharge along the entire longitudinal continuum.

3.3.5. STATISTICAL ANALYSES

The non-parametric Mann-Whitney-Wilcoxon test (Wilcoxon, 1945; Mann & Whitney, 1947) was used to test differences in the solute content between drought and high flow conditions. Correlations between variables were considered significant at the 5% level. Spatial and contrasting hydrological conditions (high flow and drought) variability were explored using principal components analysis (PCA). Two PCAs were run: the first one included only the DIM solutes (PCA_{DIM}). The second PCA integrated ten DOM descriptors (PCA_{DOM}). Clusters in the PCA analysis were identified using the optimization significant test silhouette (Rousseeuw, 1987). In order to quantify how alterations in DOM and DIM properties, induced by hydrology, changed downriver, we estimated the Euclidean distance (d_E, thereafter) between scores obtained under drought and high flow conditions at each sampling site, and plotted these distances with respect to the downriver distance. The Euclidean distance, d_E, describes the biogeochemical dissimilarity among samples collected at the same site but under the two contrasting hydrological conditions. Low d_E values indicate low biogeochemical dissimilarity (or high similarity) among hydrological conditions i.e., high chemostasis. High d_E values indicate the opposite (Figure 3.3.5.1).

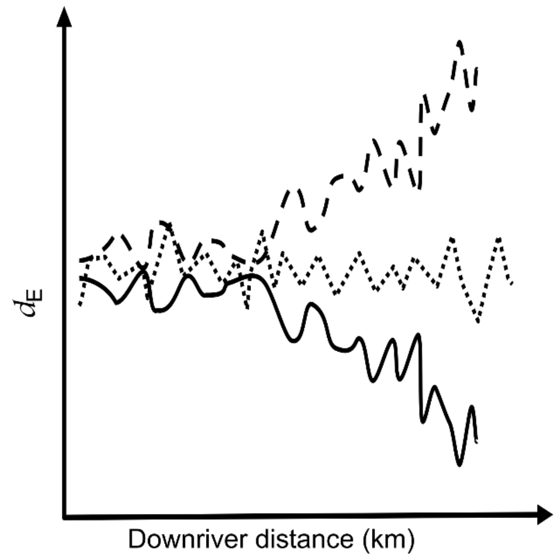

Figure 3.3.5.1. Three hypothetical trends in the dissimilarity index d_E with respect to the downriver distance. The dotted line describes the tendency when d_E is unrelated to the downriver distance. The dashed line describes the tendency when d_E increases downriver. The solid line describes the opposite situation, when d_E decreases downriver. Additional details are given in the statistical analyses section.

Two dissimilarity indices were estimated: one for inorganic solutes ($d_{E\text{-DIM}}$) and the other for DOM ($d_{E\text{-DOM}}$) parameters. Normality was tested using the Kolmogorov–Smirnov test (Massey, 1951). The null hypothesis was rejected at 5%. Mathematica (Wolfram Research) software was used for all statistical analyse

3.4.RESULTS

3.4.1. HYDROLOGY

Under drought conditions, discharge increased gradually from 0.055 to 0.55 $m^3 s^{-1}$ from the source to kilometre 12 (just after village 2). It then decreased to 0.4 $m^3 s^{-1}$ at kilometre 35. From this site, all water was taken for irrigation and runoff was nil, except in villages 5 and 6. From kilometre 40 to the last sampling site (kilometre 71) the fluvial continuum vanished, and water was confined to isolated and stagnant pools. The discharge profile was clearly different under high flow conditions. According to the information provided by the Confederación hidrográfica del Ebro, discharge increased from 0.61 $m^3 s^{-1}$ between the source and kilometre 58 to 12.5 $m^3 s^{-1}$ at the final downriver site (kilometre 71) (Figure 3.4.1.1).

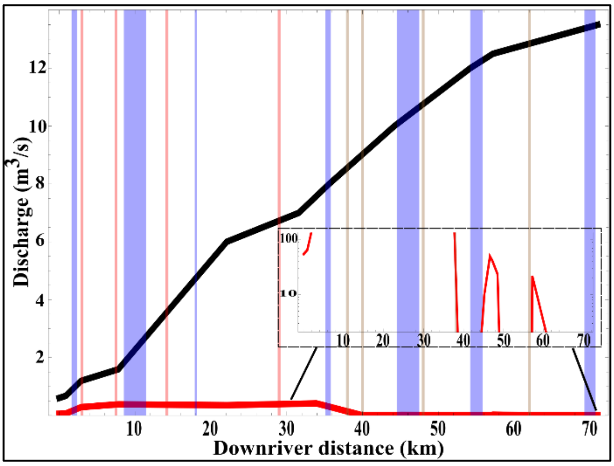

Figure 3.4.1.1. Longitudinal discharge profile along the river continuum during drought (red line) and high flow (thin black line) conditions. Purple vertical lines show the location of the seven villages next to the Matarranya river. The thickness of the lines is proportional to population size. Red vertical lines show the location of the main affluent. Brown vertical lines are the irrigation return flows. The inset shows the discharge at most downriver sites during drought. Discharge under high flow conditions (thin black line) was interpreted for illustrative purpose only, because discharge was only available for kilometre 0.9 and 58. Discharges were interpolated assuming that the discharge increased downriver according to a potential model.

3.4.2. INORGANIC SOLUTE CONCENTRATIONS. DROUGH VS HIGH FLOW

Under drought conditions, EC values ranged from 473 (headwaters) to 2060 µS cm^{-1} (kilometre 47) and were significantly higher than during high flow (U=1597, p<0.001; Figure 3.4.2.1a). The highest increases started at kilometre 40 and peaks occurred at kilometres 47, 56 and 62 (corresponding to

wastewater effluents from villages 5, 6, and irrigation return flows, respectively) (Figure 3.4.2.2a). During high flow,
conductivity increased slightly along the fluvial continuum from 451 (headwater) to 671 µS cm^{-1} (downriver). Abrupt increases were minor and occurred at kilometre 15 (after the confluence with the Tastavins affluent) and at kilometre 56 (village 6). Nitrate (NO_3^-) concentrations were significantly higher during drought conditions than during high flows (U=1485, p<0.001; **Figure 3.4.2.1b**) with values oscillating from 0.59 to 86 mg L^{-1}. Lower concentrations were typically found in the headwaters, but the concentration increased abruptly to 32 mg L^{-1} at kilometre 18 (village 3) (Figure 3.4.2.2b). Downstream, the NO_3^- concentration remain relatively stable until kilometre 45 and additional abrupt increases were detected downstream from the untreated wastewater inputs at village 5 (kilometre 47, 86.01 mg L^{-1}) and 6 (kilometre 57, 70.10 mg L^{-1}). Under high flows, the NO_3^- concentration increased from 1.34 (headwaters) to 12 mg L^{-1} (kilometre 57)

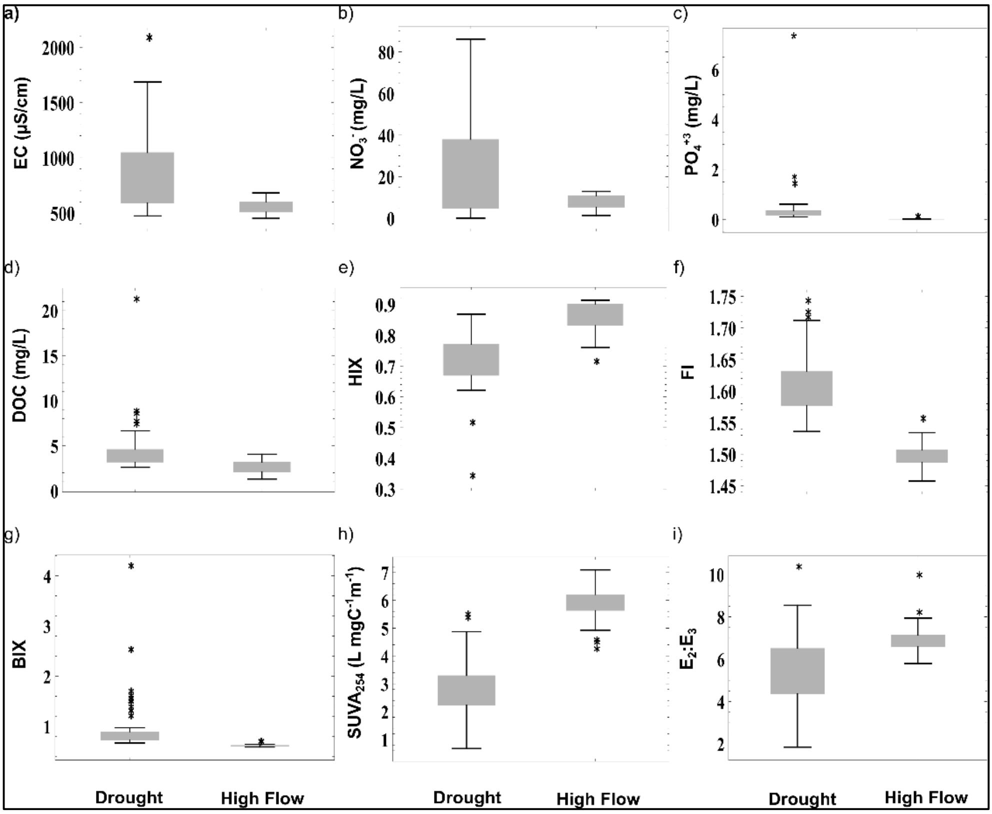

Figure 3.4.2.1 Box plots summarize the differences between drought (left box plot) and high flow (right box plot) conditions for the following variables: a) electrical conductivity (EC), **b)** nitrate (NO_3^-), **c)** phosphate (PO_4^{3+}), **d)** dissolved organic matter (DOC), **e)** humification index (HIX), **f)** fluorescence index (FI), **g)** biological index (BIX), **h)** specific ultra-violet absorbance at 254 nm ($SUVA_{254}$) and **i)** ratio of absorbance at wavelength 250nm:365nm (E_2:E_3). Asterisks symbolized the outliers of each variable.

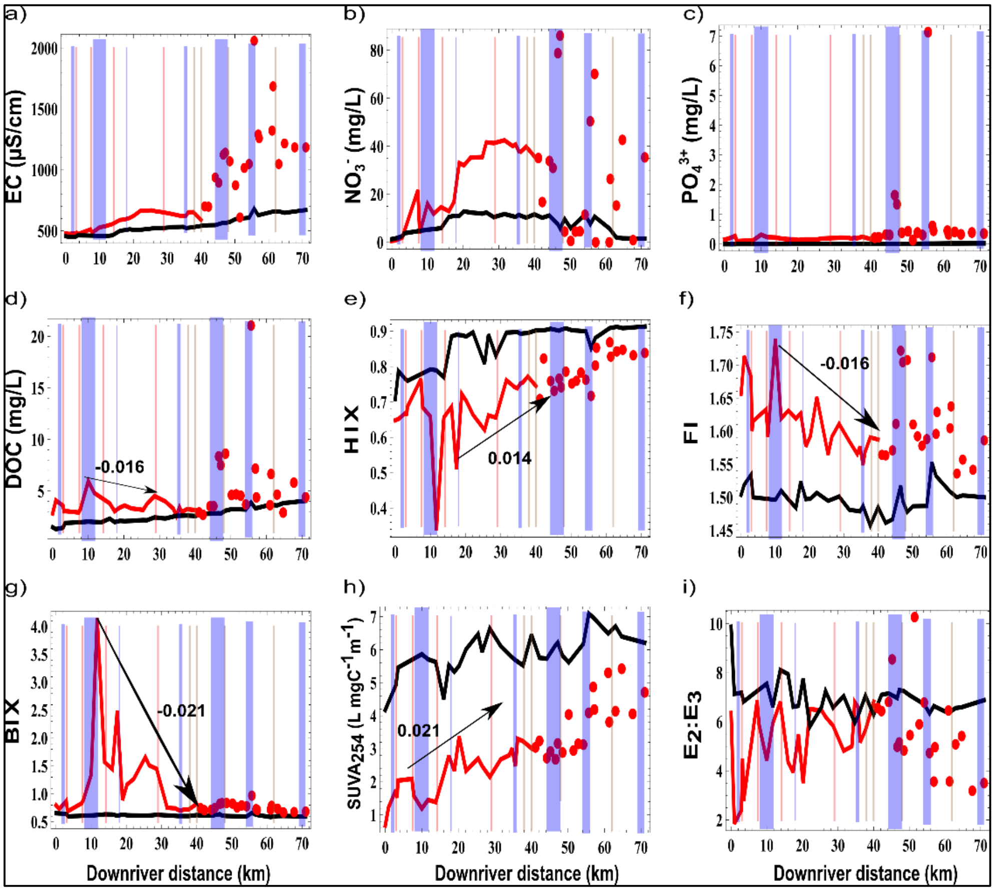

Figure 3.4.2.2. Longitudinal profiles, along the river continuum, of the same variables detailed in figure 4 during drought (red line and red dots) and high flow (black line) conditions. Red dots remark that, from kilometer 40 the water samples were collected from isolated pools. Vertical lines are the same as those in **figure 3.4.1.1**. Red arrows emphasize the longitudinal linear trend of some DOM parameters from kilometres 10 to 45. The values show the rescaled slope of these trends (statistical significance of all slopes are of $p<0.01$). See discussion for additional information.

then decreased to less than 2 mg L^{-1} up to kilometre 71. Phosphate (PO_4^{-3}) concentrations were significantly higher during drought (U=2025, $p<0.001$; Figure 3.4.2.1c). PO_4^{-3} concentrations ranged between 0.10 and 7.13 mg L^{-1} during drought (Figure 3.4.2.2c). The highest concentrations were recorded at kilometres 10, 47 and 56 just downstream from the untreated wastewaters at villages 2, 5 and 6, respectively. During high flow, phosphate concentrations were typically lower than the detection level (<0.005 mg L^{-1}) in headwaters and around 0.03 mg L^{-1} at downstream sites.

3.4.3. DOM CONCENTRATIONS AND PROPERTIES. DROUGH VS. HIGH FLOW

DOM was significantly higher under drought conditions than during high flow (in terms of DOC concentration, U=1747, $p<0.001$, Figure 3.4.2.1d). It was also less humified (in terms of HIX, U=150, $p<0.001$; Figure 3.4.2.1e), less aromatic (in terms of $SUVA_{254}$, U=18, $p<0.001$; Figure 3.4.2.1h), more autochthonous (in terms of FI, U=2022, $p<0.001$; Figure 3.4.2.1f), more fresh (in terms of BIX, U=20225, $p<0.001$; Figure 3.4.2.1g) and smaller in size (in terms of E_2:E_3, U=284, $p<0.05$, Figure 3.4.2.1i). DOC concentrations ranged between 2.60 and 21.05 mg L^{-1} during drought conditions, with no clear longitudinal trend (Figure 3.4.2.2d). However, the largest abrupt concentration increases coincided with untreated wastewater inputs (kilometres 10, 47 and 56) and an irrigation return flow (kilometre 49). Under high flow, DOC concentrations increased progressively downriver from 1.30 (kilometre 0.1) to 4.05 mg L^{-1} (kilometre 71). HIX values ranged between 0.34 and 0.87 during drought. The lowest values were found between kilometres 12 (village 2) and 17 (village 3) (Figure 3.4.2.2e). Downriver from these sites, the HIX values gradually increased up to 0.85. Under high flow, HIX values ranged between 0.76 and 0.91. The lowest values were detected in the headwaters (from kilometre 0 to 14). Thereafter they remained relatively constant at around 0.9. FI fluctuated between 1.54 and 1.74 and tended to decrease along the river continuum during drought (Figure 3.4.2.2f). The highest values were detected at kilometre 0.9 (headwater) and 10, 47 and 56 (coinciding with untreated wastewater inputs). On the other hand, FI did not show any longitudinal trend under high flow conditions. Peak FI values coincided with villages 1, 2, 3, 5 and 6. Under drought conditions, the highest BIX value was detected at kilometre 10 (coinciding with wastewater input from village 2, Figure 3.4.2.2g). Downstream, the BIX value decreased rapidly to less than 1. However, two additional peaks were detected at kilometres 17 and 26. During high flow, BIX values fluctuated between 0.56 and 0.66 along the main stem and did not show any longitudinal trend. $SUVA_{254}$ showed a tendency to increase downriver during both hydrological conditions. In contrast to other DOM descriptors, residual waters did not show any consistent change in $SUVA_{254}$. For instance, this index was slightly lower (or unchanged) at villages 2, 3 and 5, and only increased after village 6 during drought conditions (Figure 3.4.2.2h). The spectral slope index (S_R) showed larger fluctuations and a longitudinal pattern during drought conditions than during high flow although the differences in S_R values between the two hydrological periods were not statistically significant (U=1176, n.s.). Higher S_R values were detected in headwaters under drought conditions. Downstream S_R decreased rapidly until kilometre 27 and then gradually increased up to kilometre 68. On the other hand, the S_R values decreased slightly from 1.1 to 0.9 during high flow conditions. Under drought conditions the E_2:E_3 ratio showed marked oscillation between 2 and 10, with a minimum at kilometre 0.9 and a maximum at kilometre 52 (Figure 3.4.2.2i). Under high flow E_2:E_3 was relatively steady between 5 and 8 along the longitudinal axis, with the highest value at the headwater site (E_2:E_3 ~ 10).

3.4.4. PRINCIPAL COMPONENT ANALYSIS AND $D_{E\text{-}DIM}$ AND $D_{E\text{-}DOM}$ ALONG THE FLUVIAL CONTINUUM

Figure 3.4.4.1 shows the scores of the first and second components obtained from the PCA_{DIM} (the inorganic solutes). The two main components explained 59 and 18 % of the total variance respectively. PCA1 separated the data, under drought conditions, according to their location. Silhouette analysis identified three main clusters: Cluster 1 (negative PCA2 scores) integrated all data collected during high flow conditions. Cluster 2 (positive PCA2 scores) integrated data from headwaters and middle reaches (up to kilometre 45) under drought conditions. Cluster 3 (negative PCA1 scores) was the most heterogeneous and integrated mainly data from downstream sampling sites (from kilometre 46 to kilometre 71) under drought conditions. PCA of the DOM parameters explained 78% of total data set variance (Figure. 3.4.4.2). PCA1 axis (59% of total variance), separated the data set according to hydrology. High flow conditions were associated with negative PCA1 scores and drought conditions with positive PCA1 scores. The inset in figure 3.4.4.2. shows that PC1 scores decreased from nil to more negative values from headwaters to middle reaches and downstream reaches. PCA2 explained 19% of the total variance. This axis separated the data collected under drought conditions according to their location along the main stem: headwaters and middle reaches with positive scores and downstream with negative scores.

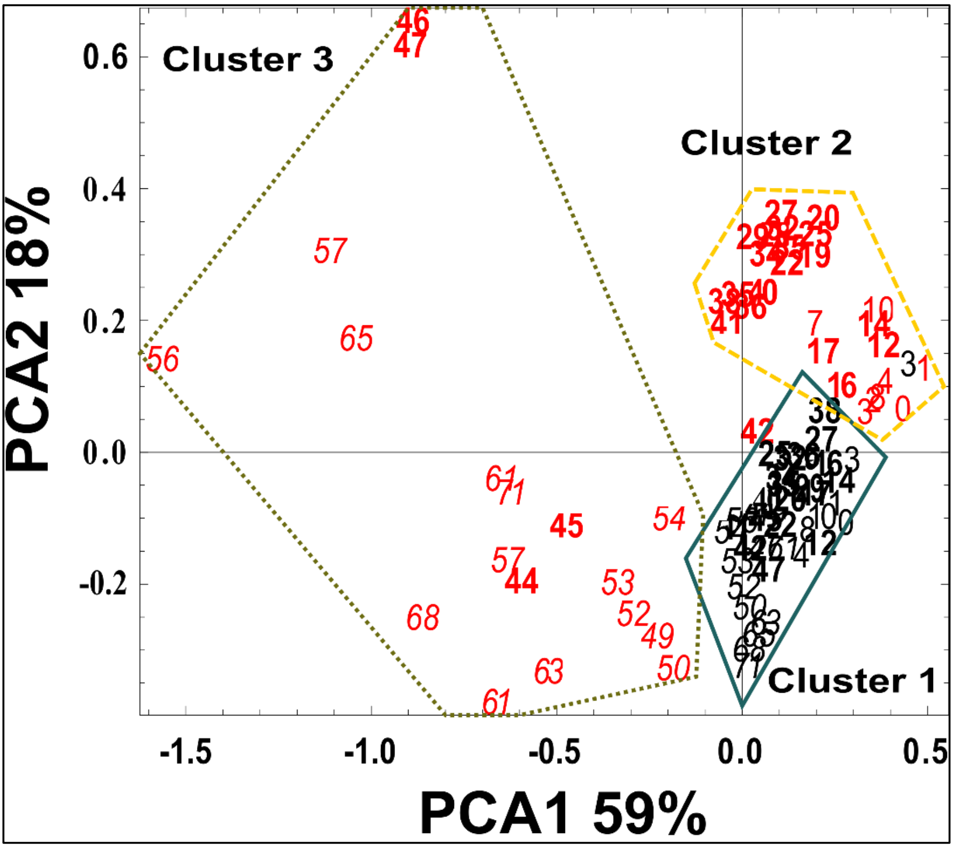

Figure 3.4.4.1. Principal component analysis of the inorganic solutes (PCAdim). Variables are: electrical conductivity, chloride, sulphate, $^{18}\Delta O$,ΔD, ammonium nitrate and phosphate. Red values are samples collected under drought conditions. Black values are samples collected under high flow conditions. Values indicate the distance (in kilometres) from the headwaters. Normal font show headwaters sites from 0 to 10 km, bold font show middle channel sites from 11 to 47 km, italic font show downstream sites from 48 to 71 km. Clusters were obtained using the Silhouette significance test.

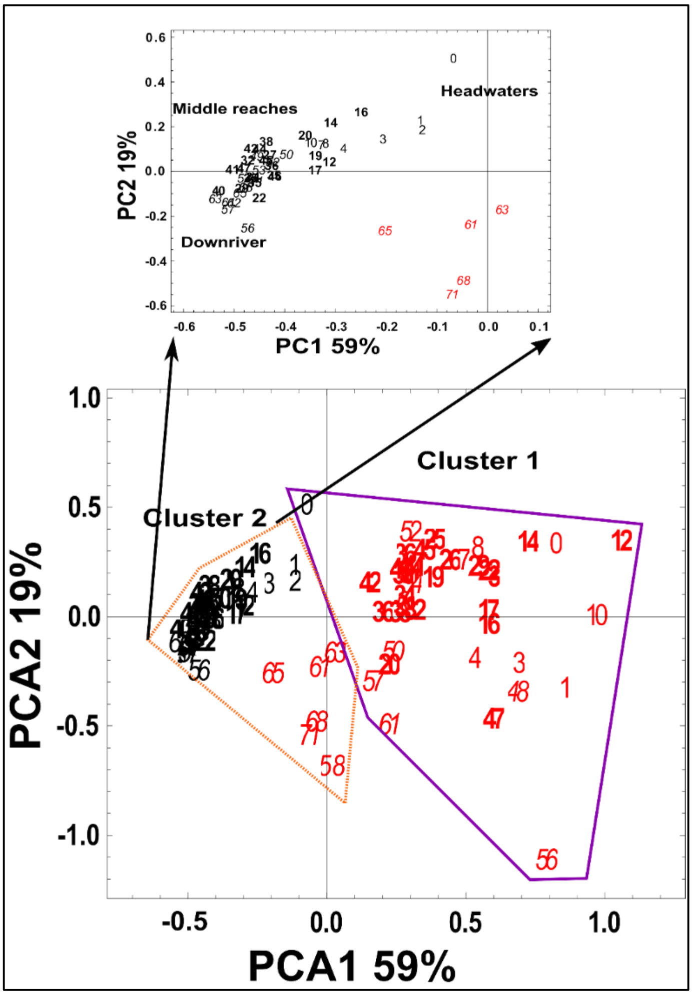

Figure 3.4.4.2. Principal component analysis of the dissolved organic matter parameters. Variables are: DOC, HIX, FI, BIX, S_R, E_2:E_3, $SUVA_{254}$, $SUVA_{350}$, $Fluor_{Humic\text{-}like}$ (FlHu) and $Fluor_{Prot\text{-}like}$ (FlPr). Red values are samples collected under drought conditions. Black values are samples collected under high flow conditions. Values indicate the distance (in kilometres) from the headwaters. Normal font show headwaters sites from 0 to 10 km, bold font show middle channel sites from 11 to 47 km, italic font show downstream sites from 48 to 71 km. Clusters were obtained using the Silhouette significance test. The inset expands the PCA plot for data collected during high flow conditions.

Data separation along the fluvial continuum was much more gradual during high flow conditions. Silhouette analysis revealed two clusters: Cluster 1 integrated samples collected from headwaters and middle reaches during the drought episode. Cluster 2 integrated samples collected during high flow and most of the downstream samples collected during drought.

Figure 3.4.4.3 presents the longitudinal trend of the dissimilarity indices obtained for the DIM and DOM parameters ($d_{E\text{-DIM}}$ and $d_{E\text{-DOM}}$ respectively) along the main stem of Matarranya river. A gradual and significant decrease in $d_{E\text{-DOM}}$ was detected along the river continuum (r^2=0.1, d.f.=43, p<0.05). This trend suggests that the qualitative properties of DOM tended to be unrelated to hydrological oscillations at the downriver sites. The trend is even more significant if the abrupt increase in dissimilarity that emerged downstream from the untreated wastewater inputs at village 2 (kilometre 10), village 5 (kilometre 46) and village 6 (kilometre 55) is removed (r^2=0.45, d.f.=43, p<0.001). On the other hand, the dissimilarity index of inorganic solutes ($d_{E\text{-DIM}}$) showed the opposite trend to the DOM trend and increased significantly downriver (r^2=0.32, d.f.=43, p<0.001). However, $d_{E\text{-DIM}}$ was low and relatively constant from the headwaters to kilometre 40 and the dissimilarity index increased abruptly downstream from the wastewater inputs.

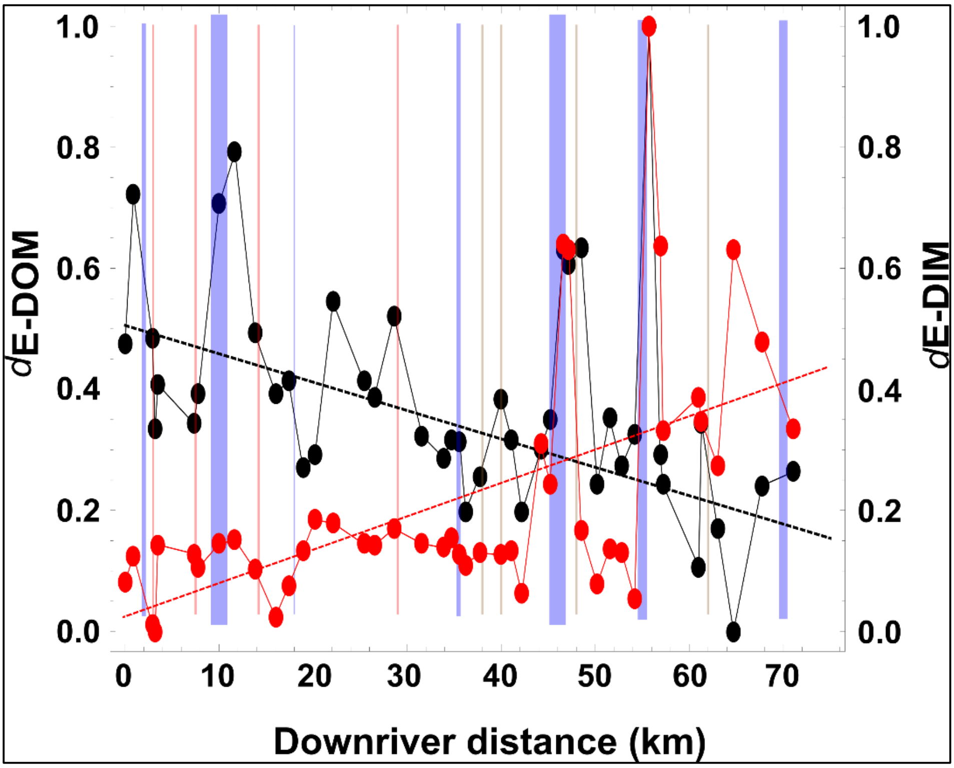

Figure 3.4.4.3. Longitudinal profile of the dissimilarity index of DIM ($d_{E\text{-}DIM}$, red disks) and DOM properties ($d_{E\text{-}DOM}$, black disks) along the river continuum. Vertical lines are the same as those in figure 3. The dashed black and red lines show the linear model (p<0.05) that relates dissimilarity of the $d_{E\text{-}DOM}$ or $d_{E\text{-}DIM}$ index to the downriver distance respectively.

3.5.DISCUSSION

This study clearly demonstrates that hydrological conditions strongly affect the concentration of inorganic solutes (i.e.,DIM) and quantity and properties of organic solutes (i.e.,DOM) that flow along a fluvial continuum. However, the significance of the impact depends on biogeochemistry composition (i.e, DIM or DOM) and, additionally the impact is not uniformly distributed throughout the fluvial continuum. The river continuum thus exerted some control on biogeochemical responses related to hydrological variability. This control was evident for DOM but not for DIM.

3.5.1. DROUGHT VS. HIGH FLOW CONDITIONS: DOM QUALITY

DOM properties were affected by hydrological conditions as follows: DOM was fresher and less degraded (i.e., high BIX), and more aliphatic and less humified (i.e., low $SUVA_{254}$ and HIX respectively) under drought conditions than under high flow conditions. This suggests that it is more autochthonous (i.e., high FI and low HIX) under drought conditions and more allochthonous (i.e., low FI and high HIX) under high flow conditions. This is consistent with other studies performed in human-altered Mediterranean rivers (Ejarque Gonzalez, 2014; Butturini et al., 2016). However, some caution is necessary regarding the term "autochthonous". This term means that the DOM fraction is a byproduct of in-stream processes. However, it is well known that DOM from (i.e., treated and untreated) wastewater produces a fluorescent signal associated with small protein-like substances (Baker, 2002; Saadi et al., 2006; Butturini & Ejarque, 2013). This fluorescence moiety modifies the excitation-emission florescence matrices, resulting in high FI and BIX values and low HIX values. Therefore, the high FI and BIX values detected under drought conditions do not necessarily indicate in-stream autochthonous DOM generation. On the contrary, the most relevant increases in BIX and FI were abrupt and coincided with villages 2, 5 and 6, revealing the severe impact of wastewater discharge from these villages. Therefore, it is important to take into account these anthropogenic inputs.

Overall, additional anthropogenic DOM inputs appeared to be minor between village 2 (i.e., kilometre 10) and village 5 (i.e., kilometre 45). Consequently, village 3 and 4 did not alter the DOM signal (i.e., although, nitrate increased sharply at village 4 under drought conditions). In addition, the two effluents that drain into the main stem (i.e., Tastavins and Calapatos) were almost completely dry during the summer sampling. Therefore, in this large 35-km-long fluvial segment, allochthonous (i.e., natural and anthropogenic) DOM inputs should be insignificant under drought conditions. Thus, longitudinal changes in qualitative DOM detected in this segment under drought conditions, might provide information about the magnitude of in-situ DOM processing. The results show that downriver from the suspected anthropogenic DOM inputs at village 2, DOM decreased slightly in terms of concentration (i.e., dDOC/dX= -0.016 km-1) and increased in terms of aromaticity (i.e., dSUVA/dX= 0.021 km-1) and humification (i.e., dHIX/dX= 0.014 km-1). These findings suggest the longitudinal accumulation of aromatic and large molecules, probably as a consequence of the degradation of labile aliphatic substances and the concurrent accumulation of more refractory aromatic humics. The gradual decrease in the FI index (i.e., dFI/dX= -0.016 km^{-1}) and the decrease in the BIX index (i.e., dBIX/dX= -0.021 km^{-1}) also suggest the degradation of labile substances. Remarkably, $SUVA_{254}$ increased at a constant rate throughout the entire fluvial stem from kilometre 10 to 71. This increase, although less pronounced, was also significant under high flow conditions. Overall, this positive trend contrasts with that observed in la Tordera (Ejarque et al., 2017) and that reported by Creed et al. (2015). They attributed the longitudinal decrease in aromatic substances to losses in allochthonous aromatic DOM and a parallel increase in autochthonous aliphatic DOM. Accordingly, this DOM processing route appeared to be inconsistent in the Matarranya river.

3.5.2. DOM DISSIMILARITY TREND ALONG THE RIVER CONTINUUM

The dissimilarity index of the DIM pool ($d_{E\text{-}DIM}$) increased significantly downriver. However, a more detailed analysis revealed that $d_{E\text{-}DIM}$ was relatively steady, and relatively low, from kilometre 0 to 42, indicating that the chemical variability of DIM solutes related to high flow and drought conditions was unrelated to river size. The $d_{E\text{-}DIM}$ descriptor increased abruptly as a consequence of the impact of wastewater inputs at villages 5 and 6 under drought conditions, when, from kilometre 43 to 71, the river was totally dry and most of the discharge was from these anthropogenic sources. The position of these abrupt increases in $d_{E\text{-}DIM}$ (at kilometre 46 and 56 respectively) reduce the significance of the $d_{E\text{-}DIM}$ vs. downriver distance relationship. Therefore, the longitudinal increase in $d_{E\text{-}DIM}$ seems to be unrelated to river size: if wastewater inputs were located in the headwaters the observed positive longitudinal trend in $d_{E\text{-}DIM}$ would probably vanish.

In contrast, the $d_{E\text{-}DOM}$ showed a clear and robust tendency to decrease downriver. According to the results, hydrological oscillations affect the DOM composition of headwaters more severely than at downriver sites. The negative trend is robust enough to dampen the noise (i.e., abrupt high $d_{E\text{-}DOM}$ values) caused by the impact of anthropogenic wastewater inputs under drought conditions. Overall, this negative trend is consistent with the hypothesis proposed by Creed et al. (2015). These authors suggested that the impact of hydrological variability should decrease downriver and that a river will tend towards chemostasis. This situation is thought to reflect the hydrological, biogeochemical and biological factors that control variations in DOM quantity and quality in time and space. Accordingly, high DOM oscillations are expected in headwaters, where hydrological conditions determine the drainage of different soils and ground water sections and stimulate the leaching of allocthonous DOM. The hyporheic and riparian interfaces (Butturini et al., 2003) are hydro-chemical compartments that can strength terrestrial-aquatic connections in headwaters and thus condition the mobilization and composition of DOM in these ecosystems (Vázquez et al., 2007, 2015). On the other hand, the strength of these connections should decrease downriver. DOM in downriver reaches reflects the mixing of DOM inputs from allochthonous (i.e., natural and/or anthropogenic tributaries and groundwater) and autochthonous sources. In fact, low river gradients, enhanced water residence times and instream autochthonous biogeochemical processes are reported to be more relevant in downriver reaches.

3.6.CONCLUSION

This study demonstrates the drastic impact of the hydrological regime on DOM properties. More significantly, it reveals that variations in DOM are more notable in headwaters than downriver reaches. This result is consistent with the chemostatic hypothesis formulated by Creed et al. (2015). DOM properties drastically changed with both hydrological regimes. Terrigenous, humified, aromatic and degraded DOM is flushed downriver under high flow conditions. Furthermore, these properties gradually become more predominant downriver. In contrast, DOM is less degraded, more aliphatic and less humic under drought conditions. These properties were mainly determined by putative inputs of wastewater rather than in-stream autochthonous processes. Under drought conditions, the fluvial segment between village 2 (i.e., kilometre 10) and village 5 (i.e., kilometre 43) did not receive relevant anthropogenic inputs. Therefore, this large segment is appropriate for exploring the magnitude of in-stream DOM retention/release processes under low flow conditions. The results demonstrated a depletion of DOC and a decrease in the fresh and poorly degraded DOM pool: on the contrary, the the most aromatic and humified DOM pool accumulated significantly downriver. These findings contrast with those reported in other studies (Creed et al., 2015; Ejarque et al., 2017).

3.7. Acknowledgements

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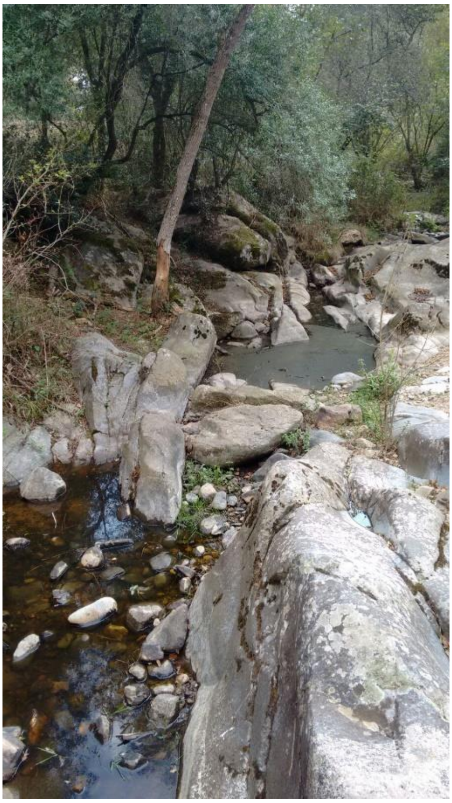

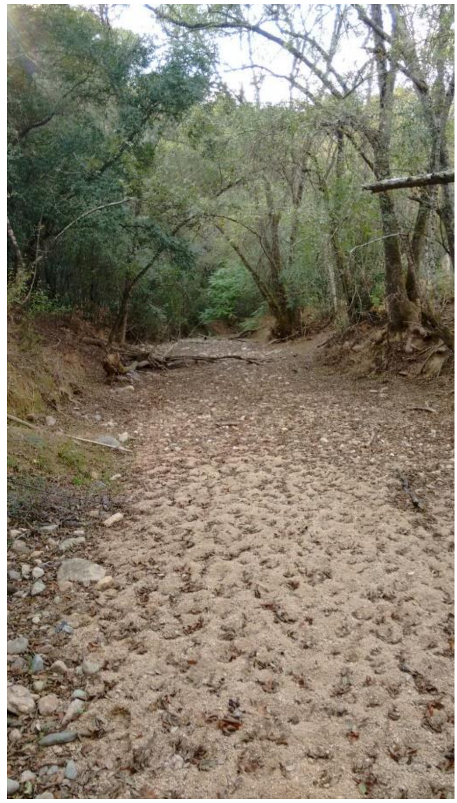

CHAPTER 4

The interruption of longitudinal hydrological connectivity causes delayed responses in dissolved organic matter

The interruption of longitudinal hydrological connectivity causes delayed responses in dissolved organic matter

Verónica Granados[1*], Cayetano Gutierrez-Cánovas[2,3], Rebeca Arias-Real[1], Biel Obrador[1], Astrid Harjung[4], Andrea Butturini[1].

[1] Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Barcelona, Spain.

[2] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal.

[3] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal.

[4] Department of Limnology and Bio-Oceanography, University of Vienna, Vienna, Austria.

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4.1.ABSTRACT

Hydrology is the main driver of dissolved organic matter (DOM) dynamics in intermittent rivers and ephemeral streams. However, it is still unclear how the timing and the spatial variation in flow connectivity affect the dynamics of DOM and inorganic solutes. This study focuses on the impact of flow cessation on the temporal and spatial heterogeneity of DOM quantity and quality along an intermittent stream. We monitored a headwater intermittent stream at high spatial and temporal frequencies during a summer drying episode and analysed dissolved organic carbon (DOC) and its spectroscopic properties, inorganic solutes and dissolved CO2. The drying period determined the disruption of the fluvial continuum with a recession of stream continuum at a rate of ~ 60 m/d and the gradual formation of a patched system of isolated pools of different sizes. Our results showed that the period of time that had elapsed since isolated pool formation (CI-days) was an essential factor for understanding how drying shaped the biogeochemistry of the fluvial system. Overall, drying caused a high DOC concentration and an increase in the humic-like fluorescence signal. Additionally, solutes showed contrasting responses to hydrological disconnection. Electrical conductivity, for instance, is a clear "sentinel" of the fragmentation process because it starts to increase before the hydrological disruption occurs. In contrast, DOC, most spectroscopic DOM descriptors and CO2 showed delayed responses of approximately 5-21 days after the formation of isolated pools. Furthermore, the spatial location and volume of each isolated pool seemed to exert a significant impact on most variables. In contrast, the temperature did not follow a clear pattern. These findings indicate that the fragmentation of longitudinal hydrological connectivity does not induce a single biogeochemical response but rather stimulates a set of solute-specific responses that generates a complex biogeochemical mosaic in a single fluvial unit.

4.2.INTRODUCTION

Freshwater ecosystems play a key role in global carbon cycling and organic matter processing (Sinsabaugh and Findlay 2003, Cole et al. 2007, Battin et al. 2008). Specifically, dissolved organic matter (DOM) is an essential energy source for microbial communities with crucial implications on aquatic food webs (Benner 2003). Hydrology is one of the main drivers of DOM dynamics (Fellman et al. 2009, Voss et al. 2015, Guarch-Ribot and Butturini 2016, Ejarque et al. 2017), particularly in intermittent rivers and ephemeral streams (IRES), which comprise more than half of the global river network (Datry et al. 2014). For example, in arid and semiarid regions such as the Mediterranean, most of the stream networks exhibit hydrological intermittence characterized by alternating dry and wet seasons (Vazquez et al. 2013). During the dry period, the surface flow disrupts hydrological connectivity, and some streams transform into a set of patched and fragmented water pools or into totally dry riverbeds. Thus, hydrological disconnection creates habitat mosaics with lotic and lentic habitats (Larned et al. 2010c, Datry et al. 2016), which affect organic matter and biogeochemistry dynamics (Dahm et al. 2003, Gómez et al. 2009, Vázquez et al. 2015, Harjung et al. 2018, Bernal et al. 2018).

Previous studies have explored how DOM changes after a dry period using static information (i.e., before and after drought comparisons) and monitoring one or a few sampling sites (Fellman et al. 2011, Vazquez et al. 2011, von Schiller et al. 2015, Casas-Ruiz et al. 2016, Harjung et al. 2018). As a result, the precise timing in which surface water DOM tracks hydrological changes in a continuum from flowing conditions to flow cessation is still unclear. A better understanding of the biogeochemical dynamics during the drying process will help quantify the role of hydrology in DOM variation and anticipate climate change effects caused by aridification and water abstraction.

During the dry period, it was revealed that most intermittent streams experienced autochthonous-originated DOM increases as a consequence of low terrestrial inputs (Casas-Ruiz et al. 2016). As drought advanced, DOM shifted to non-humic, less aromatic (Vazquez et al. 2011, von Schiller et al. 2015) and low molecular weight compounds, suggesting a major contribution of in-stream algal and microbial processes (von Schiller et al. 2015). Furthermore, as drought evolves, higher temperatures and evaporation enhance the respiration rates and the formation of anoxic habitats (Lillebø et al. 2007, Gómez et al. 2009, Fellman et al. 2011, von Schiller et al. 2015). These changes in environmental conditions influence the growth and development of microbial and fungal communities (Medeiros et al. 2009, Febria et al. 2015, Canhoto et al. 2016), which in turn can affect organic matter processing (Tzoraki et al. 2007, Fazi et al. 2013, Battin et al. 2016).

Most of these previous studies grounded their findings in studying small stream reaches. As a consequence, a comprehensive picture at the scale of the whole fluvial system is still missing. Additionally, the impact of the heterogeneity of habitats that are transitorily formed and destroyed during drying on biogeochemistry, and more specifically on DOM, has not been considered in most studies. Thus, it remains unclear whether the conclusions reported in these studies can be extrapolated to the entire system or, on the contrary, if they describe reach-specific patterns.

The objective of this study was to investigate how DOC, DOM and inorganic solutes change in space and time during a drying period, covering the entire fluvial continuum. Specifically, we investigated a) how hydrological connectivity influences the response timing and shape of the biogeochemical variables analysed and b) how these responses can be further influenced by spatial position along the stream and by local conditions (e.g., water temperature and volume of the disconnected pools). We hypothesized that the timing of fragmentation (CI-days) was an essential driver of changes in DOC, DOM and inorganic solutes. Therefore, we expected that these changes would coincide with the fragmentation of the fluvial continuum and the formation of the isolated pools. In addition, we hypothesized there would be an increase in the bulk DOC concentration and the contribution of autochthonous molecules.

4.3. MATERIAL AND METHODS

4.3.1. STUDY SITE

The Fuirosos River (NE Iberian Peninsula, 41° 42′ N; 2° 34′ E) is a third-order stream that drains a 15.2 km^2 in a forested granitic catchment, ranging 50–700 meters above sea level (m a.s.l.). It is a small representative river of the siliceous Mediterranean climate mountains (River type code, 2a) (Munné and Prat 2011). The catchment is mainly forested (coniferous, oak and deciduous vegetation), with minor agricultural use (<2%) (Figure 4.3.1.1). The climate is semiarid Mediterranean Csa, (Köppen climate classification) with a mean monthly air temperature ranging from 3°C in January to 24°C in August. Mean annual precipitation ranges 500-900 mm, being concentrated in autumn and spring with sporadic summer storms (Guarch-Ribot and Butturini 2016). Fuirosos is an intermittent stream with a dry period during summer and a clear hydrological seasonal pattern: a non-flowing dry period (July to September), a wet period (October-June) with basal discharge ranging between $5·10^{-3}$ to $3·10^{-2}$ m^3 s^{-1} (Vazquez et al. 2013, Guarch-Ribot and Butturini 2016). Rewetting from dry to wet phases is a typical abrupt transition triggered by the first severe autumnal rain episode (typically larger than 50 mm (Butturini et al. 2003). The main reach is 8 km long and the drainage density is approximately 0.6 km km^{-2} (Sala 2013). A reservoir (nominal capacity: 45,000 m^3) is located at 6 km from the river mouth. Stream morphology is prevalently step-pool in headwaters and, pool-riffle in downriver reaches. Streambed substrate is prevalently sandy-gravel and large cobbles. Unfractured granitic bedrock comprises approximately 10% of the total streambed surface.

4.3.2. SAMPLING STRATEGY

We selected 16 sampling sites along the main reach (8 km long), ranging in altitude from 941 m a.s.l. (41º40'02.2" N; 2º36'04.0" E) to 302 m a.s.l. (41º43'04.1" N; 2º34'21.3" E) (Annex I; Appendix A). The sampling started on 09/06/2016, 22 days after the last high flow episode, when the entire stream continuum was connected (base flow conditions) and finished on 02/09/2016, when the entire streambed was disconnected and the last remnant isolated pools were almost totally dry. During this period, we performed 10 sampling campaigns. Sampling sites are labelled with natural correlative numbers from downstream ("0") to headwaters ("15"). Sampling sites are clustered into two geomorphological classes with contrasting substrate permeability. The first group of sites (those at km 1, 1.7, 2.9, 4.2, 4.5, 4.8, 4.9, 7.4 and 8.1) are characterised by an impermeable granitic bedrock substrate where isolated pools can occur during the dry period. The second group of sites (those at km 1.5, 2.5, 5.1, 5.9, 6.2, outlet of the reservoir, and 6.9), are placed on permeable sand-cobbles-gravel substrates, where surface water rapidly vanished during the drought period (Figure 4.3.1.1). We performed a continuous assessment of the hydrological dynamic of drought, dividing it into four phases: pre-drought, contraction, fragmentation and dry.

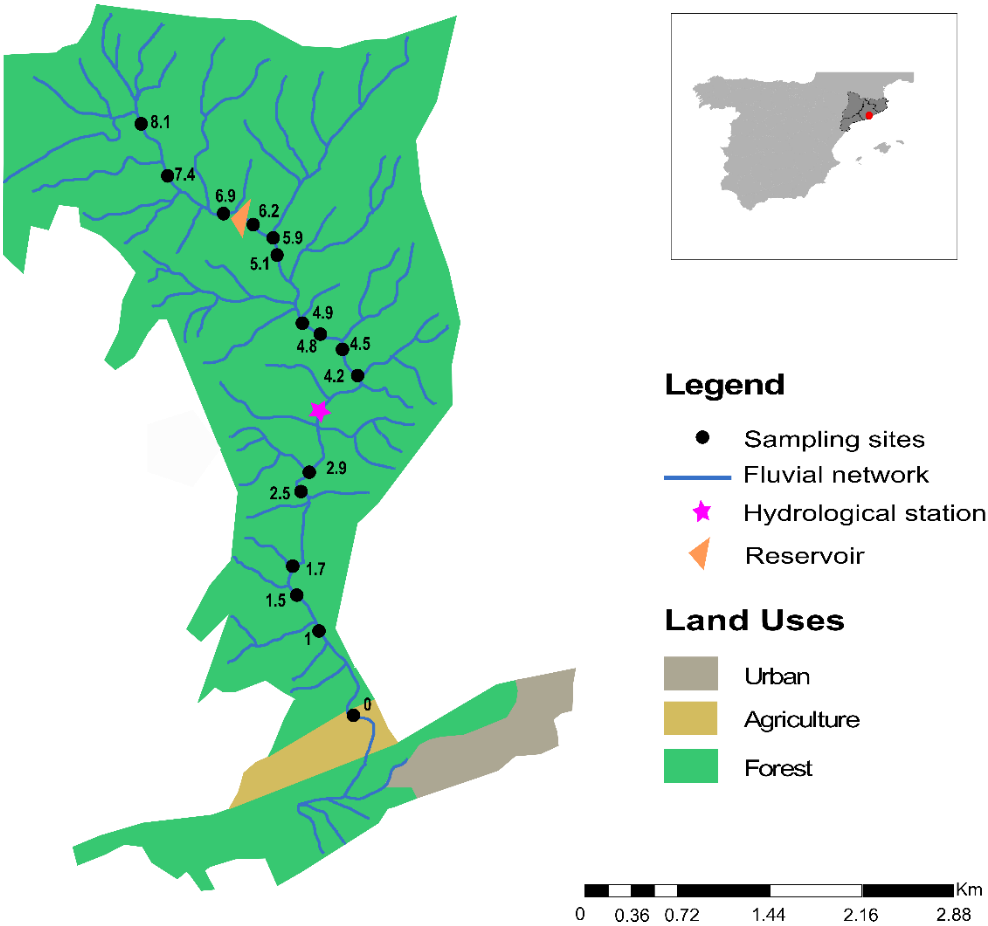

Figure 4.3.1.1. The study area map. Black dots are the sampling sites; numbers indicate the distance from downstream (km).

4.3.3. HYDROLOGICAL MONITORING

At each sampling site and date, stream discharge was estimated using the slug chloride addition method (Gordon et al. 2004). Additionally, water lever was continuously monitored at the sampling site "6" (hydrological station) with a water pressure transducer (Druck sensor PDCR/PTX 1830 or PTX 1730) connected to the automatic logger (Campbell CR10X). To determine the timing of flow cessation, we installed 15 temperature data loggers (SmartButton, ACR Systems) in the streambed along the fluvial continuum.

We assumed a dry streambed when data loggers recorded anomalously high daily temperature oscillations, and this assumption was validated after a visual inspection of each logger on each sampling date. At each sampling site, we estimated the time elapsed since the formation of isolated pools (defined as connected-isolated days, CI-days). CI-days can have positive or negative values. Negative CI-days indicate lotic conditions, i.e., the sampling site is still hydrologically connected with upstream reaches. In contrast, positive CI-days values indicate that the sampling site is hydrologically disconnected from upstream reaches, forming an isolated pool. When the CI-days value is equal to zero, this indicates the first day when the hydrological fragmentation of the river continuum occurred.

4.3.4. CHEMICAL ANALYSIS OF INORGANIC SOLUTES

At every sampling campaign and site, we measured electrical conductivity, temperature (EC, WTW 3310 set 1 conduct-metre), dissolved oxygen (YSI 20 Pro oxygen sensor) and dissolved carbon dioxide (CO_2) (GM70 Hand-held CO2 Metre, Vaisala) in the middle of the channel. We collected water samples for chemical analysis, which were filtered using pre-combusted (450°C) glass fibre filters (Whatman GF/F 0.7-µm pore size) followed by 0.22-µm pore nylon filters. The filtered samples were placed in amber glass bottles that had been previously pre-combusted. Samples were kept on ice in dark conditions and immediately transported to the laboratory, where they were stored at 4 °C until analyses (all the water samples were analysed after a few days of the sampling date). Once in the laboratory, we determined biogeochemical descriptors that included inorganic solutes (NH_4^+, SRP, NO_3^-, Cl^- and SO_4^{2-}), DOC and 10 qualitative descriptors of DOM composition based on spectroscopic properties (see next section). We measured NH_4^+ concentration using the salicylate method (Reardon 1969) and soluble reactive phosphorus (SRP) using the molybdate method (Murphy and Riley 1962). We analysed inorganic anion (NO_3^-, Cl^- and SO_4^{2-}) concentrations using an electrical conductivity detector waters (model 432) with a UV/V Kontron detector (model 332) and the column waters IC-Pak anions. To analyse DOC, we filtered the samples, acidified them with 10% HCl and refrigerated them before analysis on a Shimadzu TOC analyser VCSH with the oxidative combustion and infrared method (±0.5 μg L^{-1}). In addition, we added different standards with specific concentrations during the analysis to verify our results.

4.3.5. DOM SPECTROSCOPY

DOM spectroscopic properties were determined by DOM absorbance spectra using a UV-visible spectrophotometer UV1700 Pharma Spec (Shimadzu) and a 1 cm quartz cell. We obtained absorbance data in double-beam mode with wavelengths scanned from 200 to 800 nm and deionized water as the blank. We generated excitation-emission matrices (EEMs) with an RF-5301 PC spectrofluorimeter (Shimadzu). To determine spectra, we used a 1 cm quartz cell and we measured EMMs over (Ex/Em) wavelengths of 240-420 nm and 280-690 nm and they were standardized with the method of (Goletz et al. 2011) using Mathematica (Wolfram Research) software. We used the same method to correct the EEM data and the absorbance data for each sample to correct the inner filter effects (Lakowicz, 2006). We employed the following methods to correct the wavelength-dependent inefficiencies of the detection system: (Gardecki and Maroncelli 1998) for emission measurements and (Lakowicz 2006) for excitation correction. To normalize the data of each day of analysis with the spectro-fluorimeter, we applied daily measurements of the area under the Raman peak using MilliQ water blanks (Lawaetz and Stedmon 2009).

We evaluated seven qualitative descriptors of DOM composition: four chromophoric indices and three fluorophoric indices. The chromophoric indices were (a) specific ultra violet absorbance at 254 nm ($SUVA_{254}$), (b) specific ultraviolet absorbance at 350 nm ($SUVA_{350}$), (c) ratio of absorbance at 250 nm to 365 nm ($E_2:E_3$), and (d) spectral slope ratio for 275 – 295 nm ($S_{275-295}$). The $SUVA_{254}$ and $SUVA_{350}$ were determined as the absorption coefficient at 254 or 350 nm, respectively, normalized by DOC concentration (Weishaar et al. 2003). Higher values of $SUVA_{254}$ are associated with greater aromaticity (Hansen et al. 2016). The $E_2:E_3$ ratio provides information about DOM molecular size, where $E_2:E_3$ decreases with an increasing molecular weight (De Haan and De Boer 1987). $S_{275-295}$ also integrates variations in the molecular size of DOM, indicating a progressively greater proportion of the small DOM molecular fraction (Helms et al. 2008). $S_{275-295}$ is a unitless parameter, estimated by calculating the ratio of the logarithmically transformed absorbance spectra slope at 275–295 nm. The three fluorophoric indices considered were (a) the humification index (HIX), (b) the fluorescence index (FI) and (c) the biological index (BIX). The HIX is the area under the emission spectra at 435-480 nm divided by the peak area at 310-345 nm from the spectra at an excitation wavelength of 254 nm. The HIX indicates the extent of humification by quantifying the shift in the emission spectra towards longer wavelengths, due to lower H:C ratios. Higher HIX values indicate a greater degree of DOM humification (modified from (Ohno 2002)). The FI provides information about the DOM sources, where high values suggest the prevalence of autochthonous DOM and low values suggest the prevalence of allochthonous DOM (Cory and McKnight 2005a). This index is calculated as the ratio of emission intensities at 470 nm and 520 nm emitted at an excitation of 370 nm. Finally, the BIX was calculated at an excitation of 310 nm as the ratio of the fluorescence intensity emitted at 380 nm and 430 nm. Therefore, high BIX values (>1) suggest the presence of autochthonous and fresh DOM, while BIX values of 0.6–0.7 indicate low or zero autochthonous DOM production (Huguet et al. 2009b). Furthermore, we performed PARAFAC analysis (Bro 1997) using the drEEM toolbox (R. Murphy et al. 2013). First, Raman and Rayleigh scatters were interpolated and the EEMs were normalised to avoid high correlation among the components. Then the model was checked for outliers and four samples were excluded from the modelling procedure. The model was chosen from the best fit of the runs, applying a "nonnegativity" constraint. A three-component PARAFAC model with a core consistency of 44.6% was validated with split-half analysis (Annex I; Appendix B) as recommended in Murphy et al., 2013: all three are humic substances (humic-like or fulvic-like components) **(Table 4.3.5.1).** We expressed DOM fluorescence of the three PARAFAC components as maximum fluorescence intensity (Fmax) in raman units (R.U.). (i.e., in the results C1, C2, C3 are shown as humic component 1 (Humic Comp 1), humic component 2 (Humic Comp 2) and fulvic component 3 (Fulvic Comp).

4.3.6. STATISTICAL ANALYSES

To explore the response timing and shape of biogeochemical variables and the influence of spatial and local conditions, we quantified their responses to longitudinal hydrological connectivity (CI-days as the temporal predictor), spatial location (as the spatial predictor), volume (i.e., volume of the disconnected pool) and temperature (i.e., temperature of each disconnected pool at every sampling campaign). Given that response variables could show different shapes and dependence structures (repeated measures and temporal autocorrelation), we tested the appropriateness of different models and used AIC values to evaluate their statistical support (Zuur et al. 2009).

These models could render different statistical responses; however, their statistical support and reliability vary and can be evaluated by the comparison of AIC values (i.e., some models could misrepresent data properties). For example, in some cases, we detected non-linear responses that were more modelled more precisely by GAMMS. A linear response was modelled more precisely by linear models or GLMM when repeated measures were statistically relevant. Therefore, we fitted different models, including CI-days, pool volume, temperature (without significant effect on any variable) and spatial location, to source as predictors (fixed factors) by following a two-step strategy. First, following (Zuur et al. 2009), we assessed the response shape (linear vs. non-linear) and the necessity of including a temporal autocorrelation structure and a random intercept to account for repeated measures in the same site. To do this, for each response variable, we fitted a linear model (LM, generalized least squares, Nlme v.3.1 – 131.1) and generalized linear mixed-effects models (GLMMs) (Lme4 v.1.1-15) with a Gaussian error distribution and identity- or log- links. When scatterplots suggested a non-linear pattern and/or residuals showed a clear temporal dependence pattern, we also fitted generalized additive mixed models (GAMM) with an autoregressive integrated moving average (ARIMA) term. We then compared and ranked all these models using the Akaike information criterion (AIC) value and retained the model showing the lowest AIC value (Zuur et al. 2009). Second, using the most statistically supported model type (i.e., the lowest AIC) to select the best predictor structure for each response variable, we performed a stepwise backward selection based on the AIC values starting from a full model that included the three predictors. All model residuals were visually checked for normality and homoscedasticity of their distributions. The LMM, GLMM and GAMM models included sampling site as a random intercept to account for repeated measures at the same river location.

Table 4.3.5.1. Emission and excitation of the parallel factor analysis (PARAFAC) components. They are applied on 16 sampling sites in 10 sampling campaigns, 108 samples altogether. The references are taken from other PARAFAC models published in the online OpenFluor database ***("OpenFluor" in press)*** from other freshwater ecosystems with a similarity score of at least 0.9 ***(Murphy et al. 2014)***.

	Excitation	Emission	Components identified from previous studies	Description
C1	300	440	(Søndergaard et al. 2003) (C1 from Horsens river and Gribs lake) (Murphy et al. 2018) (C1 from tropical lake) (Garcia et al. 2018) (C4 from temperate river Patagonia) (Lapierre and del Giorgio 2014) (C1 from lakes, rivers, streams, wetlands)	Humic-like fluorophore related to microbial degradation in freshwater ecosystems
C2	<250	460	(Li et al. 2015) (C3 from Yangtze Estuary) (Asmala et al. 2018) (C3 from Roskilde Fjord) (Osburn et al. 2016) (C3 from estuaries) (Lambert et al. 2016) (C4 from tropical river) (Osburn et al. 2017) (C4 from tropical river) (Graeber et al. 2012) (C4 from Central European mixed forests)	Humic-like fluorophore, degraded, described as recalcitrant to microbial uptake
C3	270/370	470	(Wünsch et al. 2017) (Component 4 from Rio Negro, Neotropic) and (Component 2 from Lake Lillsjön, Artic) (Walker et al. 2013) (Component 2 from river warter, Artic)	Fulvic acid fluorophore. Terrestrial delivered

The LM and LMM were fitted using maximized loglikelihood (ML), while the GLMM and GAMM were fitted by maximizing the restricted log-likelihood (REML). Finally, to identify the inflection points in the temporal dynamics of each chemical descriptor (abrupt changes in each variable), we performed a nonparametric regression using conditional inference trees (Party v.1.2-4) (Hothorn et al. 2006). Before modelling, to reduce distribution skewness, we performed a log- or square root-transformation of some variables. We executed all statistical analyses in R 3.5.1 (R Core Team 2018). R code is provided in Annex I; Appendix C.

4.4. RESULTS

4.4.1. HYDROLOGY

The drying process occurs gradually over period of 126 days (~4 months). The period started on 9/6/16, with the drying of the downriver main reach section (km 0 to 1; Figure 4.4.1.1), and finished on 13/10/16, after a severe rain episode of ~100 mm. Ten minor precipitation episodes occurred during this period (<10 L m^{-2}), but they did not have any impact on hydrology. Four different phases emerged during the drying process:

a) Pre-drought (days 0 to 15): water runoff rapidly disappeared from tributaries and the main reach contracted rapidly downstream (<1 km) and in the headwaters (>8 km). During this phase, the reservoir was almost full, and we did not observe hydrological fragmentation of the stream continuum.

b) Contraction (days 16 to 32): the reduction of the stream continuum accelerated and the stream bed was dry at the 1.5 and 2.5 km sampling locations, which resulted in a disruption of the stream continuum into three isolated reaches that were 5 km long (from 2.5 to 7.4 km). During this period, we observed the first isolated small volume (1-3 m^3) pools (at 1, 2.9, 7.4 and 8.1 km). These pools emerged in depressions of un-fractured granitic bedrock. The water volume of the reservoir decreased to ~50%.

c) Fragmentation (days 33 to 50): during this period, the length of the stream reaches with flowing water were reduced at a rate of approximately 60 m d^{-1}. As a result, running waters were constrained to a 1.7-km-long stream reach situated downstream of the reservoir outlet (km 6). During this period, we found five isolated pools, which was the highest number observed during the study period. Previously, four of the five isolated pools established at the beginning of the drought period dried up, while the other four new and larger isolated pools (15-30 m^3) emerged at 1.7, 4.2, 4.5 and 4.8 km.

d) Drought (days 51 to 125): 90% of the stream continuum was almost dry. A small volume of water persisted at the reservoir (<5,000 m^3) and in two pools (4.5 km and 7.4 km). The reservoir outlet (approximately 2 L s^{-1}) still fed a small stream reach (<0.5 km of longitude) of reduced discharge. This situation of extreme drought persisted until the rain events at the beginning of October, when rewetting took place (Figure 4.4.1.1).

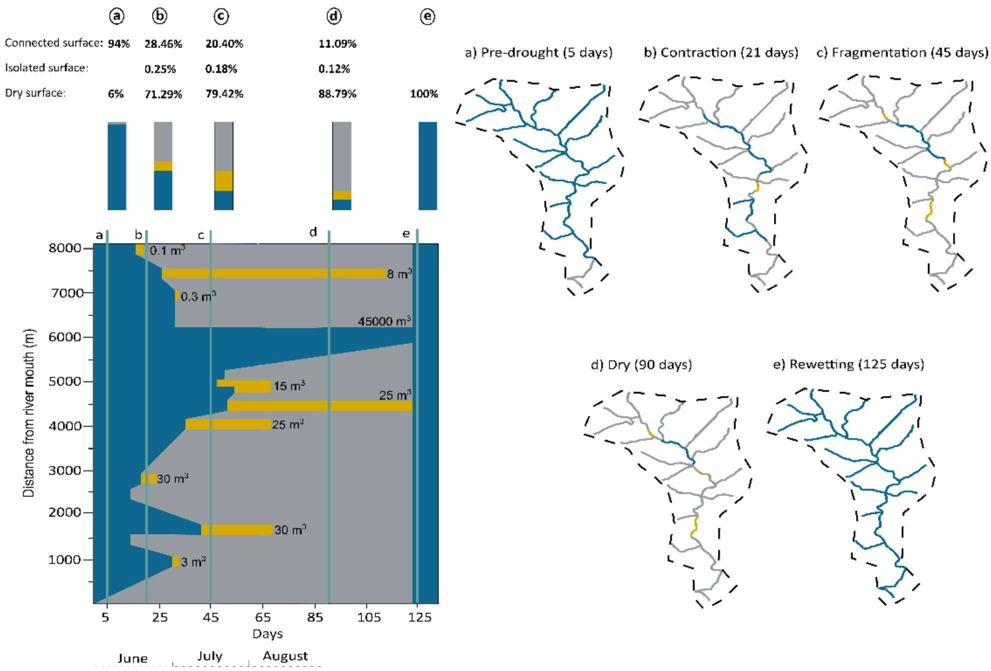

Figure 4.4.1.1. Hydrological dynamics during the drought across the Fuirosos catchment. Upper-left section: represents the percentage of connected surface (blue), isolated surface (yellow) and dry surface (grey) during the different phases of the dry period: a) pre-drought: 5 days after starting the study (9/06/16), b) contraction: 21 days, c) fragmentation: 45 days, d) dry: 90 days and e) rewetting: 125 days. The bottom-left plot illustrates the river length in the y-axis and days of the study in the x-axis, where a, b, c, d and e have the same information. Blue represents the connected sections. The numbers in the yellow bars indicate the volume of the isolated pools. The weir is represented with 45,000 m^3. The drawings on the right represent the stream network in the different phases described.

4.4.2. BIOGEOCHEMICAL RESPONSES

DOC, DOM optical parameters and inorganic solutes showed a wide range of variation over the course of the dry period (Annex I; Appendix D). As drying advanced, three different biogeochemical responses could be discerned (Figure 4.4.2.1).

The first type of pattern describes a positive relationship with CI-days. Electrical conductivity, DOC, $SUVA_{350}$, FI, NH_4^+, CO_2, $SUVA_{254}$ and the three humic PARAFAC components are shown (Figure 4.4.2.1). The most sensitive variable to fragmentation was electrical conductivity, which started to increase long before the formation of the first pools (CI-days= -47), and there was a successive acceleration at CI-days= 6. The second most responsive variable to drought was DOC, which started to increase at CI-days= 6 and exhibited an abrupt increase at CI-days= 21. Most of the other biogeochemical parameters showed a delayed abrupt response with respect to fragmentation, with CI-days oscillating from 5 to 21 days. The exception was CO_2, which responded almost at the onset of fragmentation (CI-days = -2) (Figure 4.4.2.2).

The second type of pattern showed a negative relationship with CI-days. This pattern was observed for E_2:E_3, $S_{275-295}$, O_2, NO_3^- and SO_4^{2-} (Fig. 3). The most responsive parameters to the dry period were NO_3^- (CI-days= -35) and SO_4^{2-} (CI-days= -13). However, E_2:E_3 and $S_{275-295}$ (CI-days= 6) and O_2 (CI-days= 7) showed a delayed response (Figure 4.4.2.2).

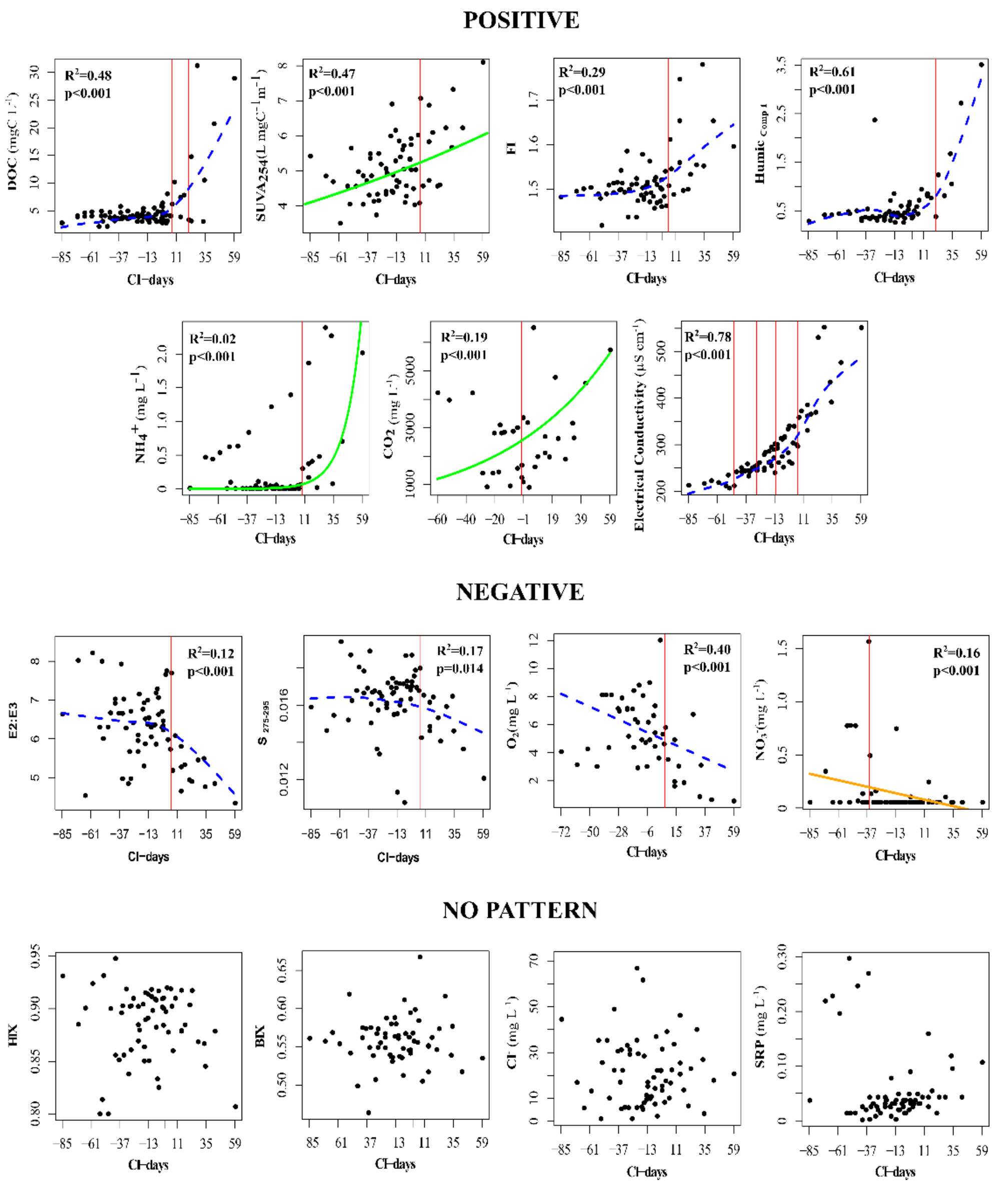

Figure 4.4.2.1. Changes in DOC, DOM optical parameters and inorganic solutes along CI-days. DOC, $SUVA_{254}$, FI, Humic $_{Comp\ 1}$, NH_4^+, CO_2, electrical conductivity, E_2:E_3, $S_{275-295}$, O_2, NO_3^-, BIX, HIX, Cl^-, SRP are on the y-axis. The CI-days on the x-axis are the connected-isolated days, where negative values are the days that the river is connected and the positive values the days that the river bed is disconnected. Plots show a positive, negative or no pattern with linear (LM, orange line; GLMM, green line) or non-linear (blue line) results. Vertical red lines explain the inflection points, which refer to the number of abrupt changes in each variable.

The third type showed a chemo-static response with respect to fragmentation. This response was detected for the BIX, HIX, Cl^- and SRP. Therefore, these variables were insensitive to fragmentation (Fig. 3). Focusing on those parameters significantly related to CI-days, the relationship was non-linear for DOM descriptors and linear for inorganic solutes (Table 4.4.2.1).

In addition to CI-days, water volume stored in pools emerged as a significant explanatory parameter for some DOM descriptors, such as $S_{275-295}$, BIX, PARAFAC humic components, SO_4^{2+}, SRP and O_2. Moreover, the spatial location was important in determining the changes in inorganic solutes such as Cl^-, NO_3^-, SRP, electrical conductivity, O_2, $SUVA_{350}$, and $S_{275-295}$. Interestingly, the temperature was unrelated to any of the biogeochemical changes (Table 4.4.2.1).

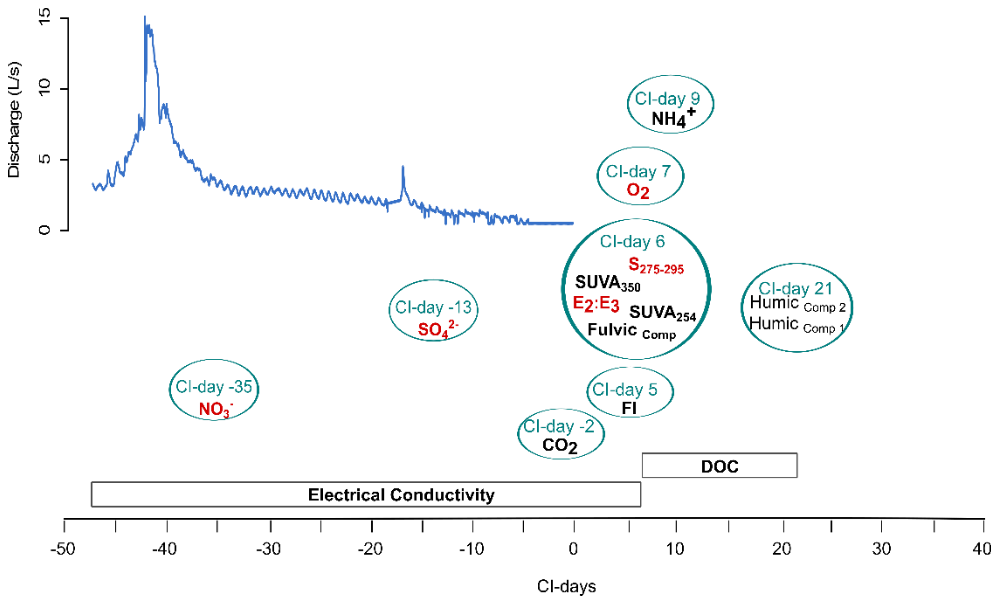

Figure 4.4.2.2. Timing of the response for each biogeochemical variable to CI-days. The inflection points were obtained using conditional inference trees. Horizontal white bars indicate the gradual response for electrical conductivity and DOM. The remaining biogeochemical variables that are circled show an abrupt response, and the CI-day starts their response. Additionally, black letters show a positive tendency over time, and red letters show a negative trend. The blue line in the upper panel illustrates the discharge at sampling site "6", a hydrological station located in the middle of the stream.

Table 4.4.2.1. Summary of the best fitting models for each response variable. P-values and regression coefficients are also shown for the predictors (CI-days, Volume and Spatial location) included in the best fitting model, along with explained variance and model type (LM, GLMM or GAMM). We did not show model coefficient for CI-days because in many cases we applied GAMM models, which did not provide such as information. Significant variables are highlighted in bold.

Variable	**CI-days**	**Volume**		**Spatial location**		**R^2**	**Model type**
	p-value	*Coefficient*	*p-value*	*Coefficient*	*p-value*		
DOC	**<0.001**					0.48	GAMM
$SUVA_{254}$	**<0.001**			0.0400	0.102	0.47	GLMM
$SUVA_{350}$	**<0.001**			0.0251	**0.048**	0.32	GAMM
$S_{275-295}$	**0.014**	0.0004	**<0.001**	-0.0002	**0.042**	0.17	GAMM
E2:E3	**<0.001**					0.12	GAMM
HIX	0.529					0	LM
FI	**<0.001**					0.29	GAMM
BIX	0.925	-0.0042	**0.040**			0.03	LM
Humic $_{Comp\ 1}$	**<0.001**	0.0416	**0.080**			0.61	GAMM
Humic $_{Comp\ 2}$	**<0.001**	0.0649	**0.033**			0.63	GAMM
Fulvic $_{Comp\ 1}$	**<0.001**	0.0296	**0.034**			0.54	GAMM
Cl^-	0.985			-0.4675	**0.004**	0.07	LM
NO_3^-	**<0.001**			0.0329	**0.029**	0.16	LM
SO_4^{2-}	**<0.001**	-0.2939	**0.009**			0.31	GLMM
SRP	0.660	0.5057	**<0.001**	0.2937	**0.008**	0.001	GLMM
NH_4^+	**<0.001**	1.3346	0.138	0.3019	0.560	0.02	GLMM
CO_2	**<0.001**	0.0236	0.265	0.0139	0.449	0.19	GLMM
Electrical conductivity	**<0.001**			0.3084	**0.089**	0.78	GAMM
O_2	**<0.001**	-0.8506	**<0.001**	-0.5574	**<0.001**	0.40	GAMM

4.5.DISCUSSION

Our results highlight that the timing of fragmentation (i.e., time since the formation of isolated pools, CI-days) is a key driver for most stream biogeochemical parameters. Most notably, our results revealed that the biogeochemical descriptors showed contrasting responses. While most DOM optical descriptors showed abrupt and delayed responses to fragmentation (CI-days>0), a few descriptors showed gradual responses or even anticipated fragmentation (CI-days<0). Additionally, the volume of pools was relevant in explaining changes in some DOM descriptors, while the spatial location of pools could also be important for most inorganic solutes.

4.5.1. TEMPORAL DYNAMICS OF HYDROLOGICAL FRAGMENTATION

Although longitudinal hydrological connectivity is recognized as a key driver of biogeochemical dynamics during dry periods, few studies have provided detailed temporal information on how drought episodes contract and fragment a fluvial network continuum (Stanley et al. 1997, Godsey and Kirchner 2014). The interruption of the hydrological connectivity is a fast-moving process in the Fuirosos catchment, showing a contraction of the stream continuum that began simultaneously at downstream and upstream reaches (Lake 2003). Furthermore, the estimation of the water retrocession rate (~ 60 m/d) confirms how abrupt the hydrological contraction is, determining that in a few hours, large fluvial reaches shift from wet to dry conditions. The contraction pattern detected in Fuirosos opposes to that reported for intermittent karst streams, where upstream areas were with permanent flow, middle reaches remained dry during the summer period and downstream areas developed several temporary springs (Meyer and Meyer 2000). The flow of these streams strongly depends on karstic groundwater inputs. In contrast, Fuirosos has a limited groundwater influence, and the drying pattern and location of isolated pools are mostly influenced by the location of permeable sediments and granitic bedrock outcrops. Overall, isolated pools with groundwater inputs have smooth biogeochemical responses, while without these inputs, concentrations increase more abruptly during the dry period (Dahm et al. 2003, Fellman et al. 2011, Siebers et al. 2016).

4.5.2. EFFECT OF THE EVOLUTION OF HYDROLOGICAL CONNECTIVITY ON DOM QUANTITY AND QUALITY AND INORGANIC SOLUTES

Considering CI-days as a temporal variable and also a descriptor of the hydrological state (connected or isolated), DOC concentration, most DOM optical parameters and inorganic solutes showed a significant effect on this predictor. In agreement with previous studies (Vazquez et al. 2011, Ejarque et al. 2017, Harjung et al. 2018), hydrological fragmentation accelerates the biogeochemical responses that occur during the dry period. Thus, DOC increased tenfold at the end of this period when the stream was almost dry, except in two pools and the remaining water of the reservoir. Additionally, aromaticity (in term of SUVA) and DOM molecular size (described as E2:E3 and $S_{275-295}$) increased with drying progression. Overall, the accumulation of DOM in the isolated pools reflects the impact of high evaporation, which is a consequence of the disconnection from upstream reaches and alluvial groundwater (Fellman et al. 2011, Siebers et al. 2016, Harjung et al. 2018). Moreover, in isolated pools, leaves from the riparian vegetation accumulated, causing an increase in the humic-like fluorescence signal and aromatic substances. This result coincides with that reported in other Mediterranean streams (Casas-Ruiz et al. 2016) as well as those rivers occurring in tropical (Yamashita et al. 2010), tundra (Balcarczyk et al. 2009) and temperate (Inamdar et al. 2012) systems. In addition, our results reflect a prevalence of humic substances and an absence of DOM production from algal metabolism, contradicting the results found in previous studies (Vazquez et al. 2011, von Schiller et al. 2015, Casas-Ruiz et al. 2016). These studies found a dominance of autochthonous DOM at the end of the dry period. However, we suggest that this divergence could be due to a deficient spatial and temporal frequency. Specifically, the accumulation of humic C1 and C2 would not have been captured by these studies (CI-days 21). These fluorescence components have been previously described as being especially recalcitrant to photodegradation or biodegradation. For example, humic C1 has proven to be more resistant to photodegradation then other components (Murphy et al. 2018). Moreover, a lack of inorganic nutrients with drying could explain a deficit in autochthonous production as observed in other intermittent streams (Martí et al. 1997, Skoulikidis and Amaxidis 2009).

The evolution of drying (CI-days) strongly modulates the availability of inorganic solutes. Electrical conductivity increased gradually during the dry period as did the DOC concentration and other inorganic components, suggesting a solute concentration of the system, especially in the isolated pools, at the end of this period. However, dissolved oxygen and CO_2 showed opposite trends, indicating high respiration activity at the bottom of the pools. Oxygen depletion in pools enhances the consumption of other electron acceptors (i.e., SO_4^{2-} and NO_3^-) (Kemp and Dodds 2002) along with the accumulation of NH_4^+ as a consequence of inhibited nitrification (Townsend 2002, Acuña et al. 2007, von Schiller et al. 2011). The emergence of these anoxic and reduced habitats with higher contents of salts, humic organic matter and ammonium may strongly limit the activity and abundance and alter the diversity and composition of prokaryotes (Medeiros et al. 2009, Fazi et al. 2013, Febria et al. 2015, Harjung et al. 2019) and fungi (Canhoto et al. 2016).

Although hydrological fragmentation had a strong impact on most of the studied biogeochemical variables, these variables did not respond simultaneously. We discerned three temporal patterns in response to fragmentation: 1) before fragmentation (CI-days<0), there were some inorganic solutes; 2) after fragmentation (CI-days>0), all were DOM optical parameters; and finally, 3) there were variables that did not show a significant change during the dry period, such as the BIX and HIX. Although some of these patterns were already found in previous studies that compared pre- and post-fragmentation periods (von Schiller et al. 2015, Harjung et al. 2018), this study provides new empirical evidence on how hydrological fragmentation shapes the dynamics of biogeochemical variables over time and space at a very detailed level. In particular, our main novelty was in detecting the precise moment when variables showed inflection points and in finding contrasting and delayed responses among biogeochemical descriptors.

4.5.3. EFFECT OF SPATIAL VARIABILITY AND ISOLATED POOL VOLUME ON DOM COMPOSITION AND INORGANIC SOLUTES

Although CI-days emerged as the most relevant driver of biogeochemical variation during the drying process, both spatial location and local pool conditions contributed to determining DOM and inorganic solute dynamics. The spatial location of isolated pools within the river continuum together with their size (water volume), had a substantial influence on some biogeochemical variables. Thus, the pools with greater volume had more LMW, humic DOM and anoxic environments. Regarding spatial pattern, DOM shifted from being aromatic and having high molecular weight (HMW) upstream to being less aromatic and having low molecular weight (LMW) downstream, which is consistent with empirical results from other studies (Ejarque et al. 2017). Although we had a longitudinal pattern for some DOM optical descriptors, this pattern fits better in studies performed at the regional scale, with high order watercourses (Creed et al. 2015). In addition, we found a link between solute concentration and water residence time, as was found in the following studies (Gómez et al. 2009, Fellman et al. 2011, Vazquez et al. 2011, von Schiller et al. 2011). Thus, we observed higher solute concentrations in the isolated pools after more days of disconnection (i.e., with a higher water residence time of isolated pools).

4.6.CONCLUSIONS

This study discerns different biogeochemical patterns in response to the hydrological fragmentation of a fluvial continuum. Our results demonstrate that the timing of fragmentation of the fluvial continuum is a key predictor of biogeochemical variability during the dry period and that most of the DOM descriptors showed a delayed and abrupt response just after fragmentation. The study of the biogeochemical process during the evolution of drying and the quantification of hydrological connectivity regarding DOM variability will enhance our understanding of the effects of climate change caused by aridification and water abstraction.

4.7. Acknowledgements

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CHAPTER 5

Multiple drying components shape dissolved organic matter composition in streams during post-drought conditions

Multiple drying components shape dissolved organic matter composition in streams during post-drought conditions

Verónica Granados[1*], Cayetano Gutierrez-Cánovas[2], Rebeca Arias-Real[1], Biel Obrador[1], Andrea Butturini[1].

[1] Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Barcelona, Spain.

[2] Biological Invasions group. Department of Integrative Ecology. Doñana Biological Station (EBD-CSIC). Av. Américo Vespucio, 26. Isla de la Cartuja. 41092 Seville, Spain.

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5.1.ABSTRACT

Water availability is a fundamental driver of biogeochemical processing on highly dynamic freshwater ecosystems. Recent evidence demonstrates that flow disruption can strongly influence biogeochemical cycles globally, given the wide distribution of watercourses exposed to episodic drying. However, the complexity of the drying process can give rise to different annual and antecedent hydrological conditions, but their effect on river biogeochemistry remains unclear. Here, using 35 streams over a long drying gradient (NE Iberian Peninsula), we investigated how characteristics of previous drought episodes affect the dissolved organic carbon (DOC) and dissolved organic matter quality (DOM). To do that, drought episodes are described by four drying components (annual drying duration and frequency, duration of the latest dry event, time since latest dry event) estimated from continuous measurements of water presence/absence during one year. Our results showed that DOC concentrations and contribution of humic-like compounds were positively associated with drying conditions. In addition, protein-like compounds decreased over the drying gradient. More specifically, changes in DOC were driven by annual drying duration, whereas multiple drying components better explained DOM quality. Finally, annual drying frequency and the duration of the latest dry event jointly explained more variance in DOM parameters than annual drying duration. These findings reveal the need to consider multiple drying components to better predict organic matter in highly dynamic streams.

5.2.INTRODUCTION

Fluvial ecosystems are hotspots of organic matter processing (McClain et al. 2003, Battin et al. 2008) because they receive large amounts of organic matter from terrestrial ecosystems flowing into the ocean. It is well established that hydrology has a key role in controlling biogeochemistry in flowing waters (Fasching et al. 2016, Guarch-Ribot and Butturini 2016, Baek et al. 2019) and recent evidence highlights the impact of flow intermittence on carbon cycle globally (Dewey et al. 2020). In this context, intermittent rivers and ephemeral streams (IRES) are receiving more attention due to their global distribution and expected expansion as consequence of global climate crisis (Datry et al. 2014, 2018, Shumilova et al. 2019, Blanchette et al. 2019). However, drying is a complex phenomenon that may entail different annual (drying duration and frequency) and previous components (duration of the latest dry event and rewetting) (Ejarque et al. 2017). Thus, understanding how these drying components influence organic matter dynamics is fundamental to better predict alterations in global carbon processing in response to environmental changes (Butturini 2021).

Previous studies have found that dry events and water availability strongly influence the concentration of dissolved organic carbon (DOC) and the composition of dissolved organic matter (DOM). For instance, drying reflects a reduction of hydrological connectivity with terrestrial ecosystems, which promotes the influence of autochthonous DOM sources, especially in protein-like character when groundwater dominates the flow during baseflow (Fellman et al. 2011, Vázquez et al. 2015, von Schiller et al. 2015, Siebers et al. 2016). Persistent drying conditions are also associated with a marked increase in DOC and the prevalence of the humic- signature (Harjung et al. 2018, 2019). However, DOM shows a more aromatic and humic-like character during rewetting, yet protein-like and microbial-

derived sources are discernible (Inamdar et al. 2011, Shumilova et al. 2019). The dry period and rewetting events are “hot moments” for DOM production, transformation and transport, and are considered key episodes to investigate the short time DOM quality in streams
(Vazquez et al. 2011, von Schiller et al. 2015, Casas-Ruiz et al. 2016, Harjung et al. 2018).

Nevertheless, recent research focuses on the intensity of drying—for example on the biogeochemistry of isolated pools in relation to its isolation time (e.g, chapter four), or on how drying duration and rewetting events in sediments lower DOC concentration and contribution of labile compounds after desiccation (Arce et al. 2021). Additionally, the importance to investigate the interactions between diverse nutrient cycles and the effects on surface-groundwater exchanges during the zero-flow. The biogeochemical processes in IRES are typically more influenced by temporal variations than spatial (Gómez-Gener et al. 2021). On the other hand, climate drivers (i.e., event variables as precipitation, discharge) determine the DOM quality, for instance, a heavy annual precipitation generated greater flux of DOM materials downstream on ephemeral streams. (Dewey et al., 2020).

Despite the progress in the field, previous studies have typically explored drying characteristics by focussing on qualitative descriptors (perennial vs intermittent), short-term assessments spanning weeks (pre and post-drying) or quantitative descriptions of annual drying duration (Larned et al. 2010b, Stromberg et al. 2010, Snelder et al. 2013, Belmar et al. 2013, Reynolds et al. 2015, Arias-Real et al. 2020). As a result, it is unclear how annual (drying frequency) or recent (duration of the latest drying or magnitude of rewetting events) can influence organic matter dynamics. According to the previous studies, we expect the recent duration of the latest drying or rewetting event to be more important than annual drying components for DOC and DOM quality. So, an increase of DOC concentration and a higher proportion of autochthonous DOM sources is expected as drying evolves.

To shed light on how droughts shape DOM quantity (in terms of DOC) and quality (in terms of DOM optical qualitative parameters) during post drought on IRES, our sampling strategy consisted on sampling one time during autumn under post-drought conditions 35 Mediterranean streams selected according to a flow intermittence gradient covering from permanent streams to ephemeral ones. We (1) characterise the annual drying elements (drying duration and frequency) and the previous drying element (duration of the last drying event, flowing time since the last rewetting). Next, (2) we investigated the effect and importance of annual and previous drying components on DOC and DOM quality while commanding other decisive environmental factors (water temperature and altitude) applying linear regression models and a multi-model inference approach.

5.3. MATERIAL AND METHODS

5.3.1. STUDY SITE AND SAMPLING STRATEGY

The study was conducted in 35 low-impacted streams over a hydrological gradient. These streams belong to nine different catchments in Catalonia (NE Iberian Peninsula) (AnnexII; Appendix A, Fig 1). Stream order ranges from two to five. Climate is typically Mediterranean with warm and dry summers, precipitation mainly occurring in autumn and spring with occasional summer storms. Forest land use predominates in most catchments, along with scrubland, grasslands and extensive agriculture (mainly olive groves and vineyards; more details are in (AnnexII; Appendix A, Table 1). All studied streams were sampled one time after rains of autumn 2017. In consequence, the collected samples integrated the drought and post-drought periods.

5.3.2. CHARACTERISATION OF DRYING COMPONENTS

To characterize the drying components is essential to determine the presence and absence of water in each stream during the study period. With this aim, temperature and water level were recorded from during the preceding year of the sampling date (from February 2016 to February 2017; see methodological details in Annual II; Appendix B). We calculated four drying components. First, the annual drying duration represents the number of days with zero-flow (ZFT, zero-flow total days). Second, the annual drying frequency was estimated as the annual number of zero-flow periods (ZFP, zero-flow periods). Third, the duration of the last dry event was calculated as the number of zero-flow days during the precedent dry event (ZFL, zero-flow last). Fourth, rewetting duration represents the number of days since the last dry event (RE, rewetting), during which water was continuously present (Fig 5.3.2.1) (Arias-Real et al. 2021a).

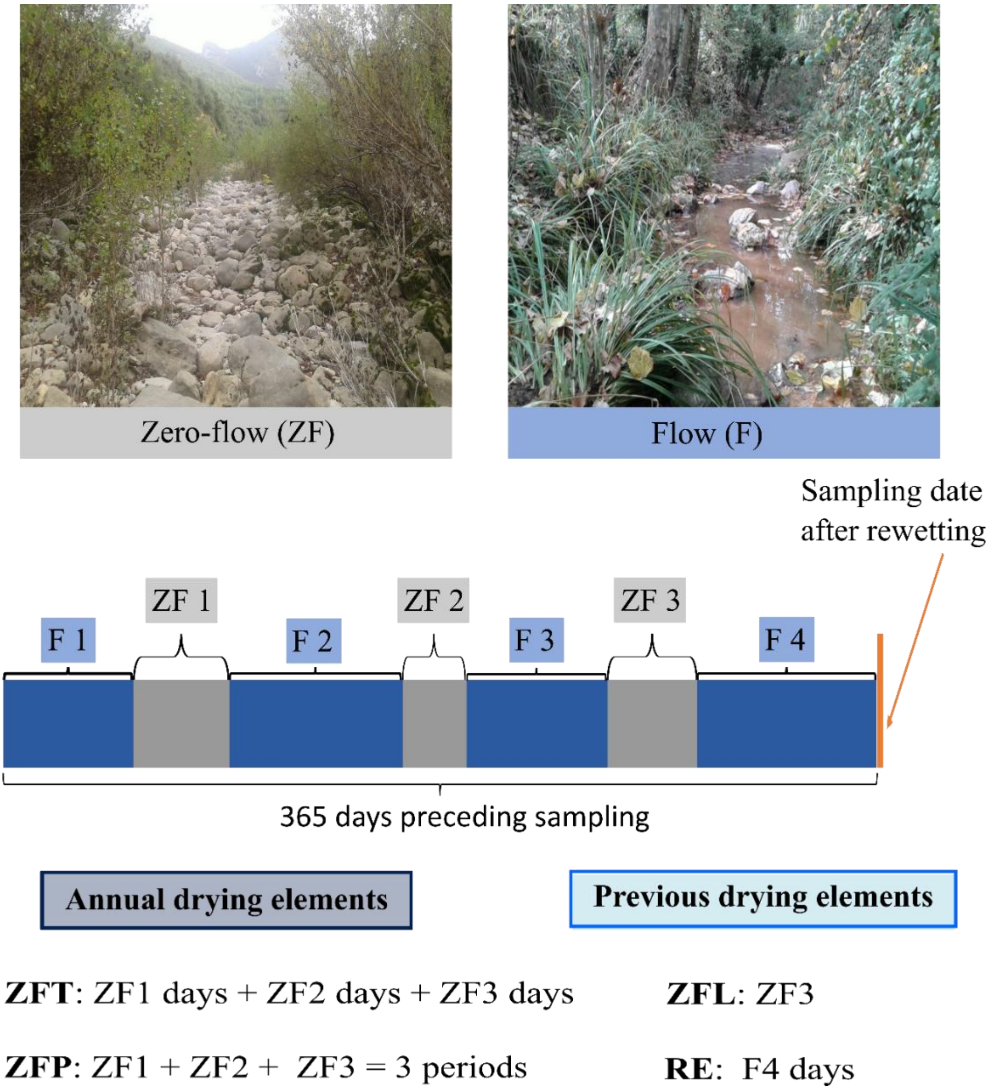

Figure 5.3.2.1. Visual description of how drying components were calculated. F corresponds to flowing conditions, ZF to zero-flow conditions. ZFT is the annual number of days with zero-flow, ZFP is the annual number of zero-flow periods, ZFL is the number of zero-flow days during the last drying period, and RE is the number of days since the last dry event.

5.3.3. LABORATORY ANALYSIS OF DOC AND DOM OPTICAL QUALITATIVE PARAMETERS

Water samples for chemical analysis were filtered using precombusted (450°C) glass fibre filters (Whatman GF/F 0.7 μm pore size) followed by 0.22 μm pore nylon filters. The filtered samples were placed in amber glass bottles previously washed with diluted acid. Samples were kept in ice on dark conditions and immediately transported to the laboratory, where they were stored at 4 °C until analyses.

Once in the lab, DOC concentration and DOM parameters (absorbance and fluorescence) were determined based on spectroscopic properties. Before analysing DOC, samples were acidified with 10% HCl before analysis on a Shimadzu TOC Analyser VCSH with oxidative combustion and an infrared detector. DOM absorbance spectra were determined using a UV-Visible spectrophotometer UV1700 Pharma Spec (Shimadzu) and a 1 cm quartz cell. The absorbance data were obtained in double-beam mode with wavelength scanned from 200 to 800 nm and deionised water as the blank. Excitation-Emission Matrices (EEM) were generated with an RF-5301 PC spectrofluorometer (Shimadzu). Spectra were obtained using a 1 cm quartz cell and over (Ex/Em) wavelengths of 240-420 nm and 280-690 nm. EEMs were standardised with the method of (Goletz et al. 2011) using Mathematica (Wolfram Research) software. We used the same previous method to correct each sample's EEM data and absorbance data to correct inner filter effects (Lakowicz 2006). We employed the following methods to correct wavelength-dependent inefficiencies of the detection system: the method of (Gardecki and Maroncelli 1998) for emission measurements and the method of (Lakowicz 2006) for excitation correction. To normalise the data of each day of analysis with the spectrofluorometer, we applied daily measurements of the area under the Raman peak using MilliQ water blanks (Lawaetz and Stedmon 2009).

DOM optical qualitative parameters included three chromophoric indices and three fluorophoric indices. The chromophoric indices were (a) Specific Ultra-Violet Absorbance at 254 nm (SUVA254), (b) the ratio of absorbance at 250 nm to 365 nm (E2:E3), and (c) the spectral Slopes Ratio (Sr). The three fluorophoric indices considered were (a) the Humification index (HIX), (b) the fluorescence index (FI) and (c) the Biological index (BIX). These optical parameters and their biogeochemical interpretation are described in **Table 5.3.3**.1

Table 5.3.3.1. Description of the DOM optical qualitative parameters used

Index	**Calculation**	**Interpretation**	**Reference**
$SUVA_{254}$ Specific Ultra-Violet Absorbance at 254 nm	The absorption coefficient at 254 is normalised by DOC concentration.	Higher values of $SUVA_{254}$ are typically related to greater aromaticity.	(Weishaar et al. 2003)
E_2:E_3	The ratio of absorbance at 250 nm to 365 nm.	The E_2:E_3 provides information about DOM molecular size. E_2:E_3 decreases with increasing molecular weight.	(De Haan and De Boer 1987).
Sr Slope ratio	Dimensionless parameter. The ratio of the logarithmically transformed absorbance spectra slope at 275–295 nm (S275–295) to that	The Sr also integrates shifts in the molecular size of DOM. Thus, high Sr values indicate an increase in the proportion of the small DOM molecular fraction.	(Helms et al. 2008)

	estimated at 350–400 nm (S350–400).		
HIX Humification index	The area under the emission spectra 435-480 nm divided by the peak area 310-345 nm from the spectra at an excitation wavelength of 254 nm.	HIX indicates the extent of humification by quantifying the shift in the emission spectra toward longer wavelengths due to lower H:C ratios. HIX values range from 0 to 1. Higher values indicate a greater degree of DOM humification.	(Ohno 2002)
FI Fluorescence index	The ratio of emission intensities at 470 nm and 520 nm emitted at an excitation of 370 nm.	The FI provides information about DOM sources. High values suggest the prevalence of autochthonous DOM, and low values the prevalence of allochthonous DOM.	(Cory and McKnight 2005b)
BIX Biological index	BIX was calculated at an excitation of 310 nm as the fluorescence intensity ratio emitted at 380 nm and 430 nm.	High BIX values (>1) suggest the presence of autochthonous and fresh DOM. Values of 0.6–0.7 indicate low or zero autochthonous DOM production.	(Huguet et al. 2009a)

5.3.4. DATA ANALYSIS

Using linear regression models and a multi-model inference approach (Burnham and Anderson 2002, Grueber et al. 2011), we estimated the effect and importance of drying components (ZFT, ZFP, ZFL and RE) on DOC and

DOM optical qualitative parameters (Table 5.3.4.1). To control for non-hydrological environmental factors, we also included altitude and water temperature as predictors in our models, as they are key drivers of biotic activity and organic matter dynamics (Ylla et al. 2012, Picazo et al. 2020). Next, we evaluated the explanatory capacity of 12 models considering all possible combinations of predictors, excluding those with high collinearity ($r \leq |0.70|$ or Variance Inflation Factor≤2). Thus, we avoided models including ZFT and ZFL simultaneously because they showed high collinearity (r= 0.78, see Annex II; Appendix C for more details). Afterwards, we ranked all 12 models using the values of the Akaike Information Criterion for small sample sizes (AICc) and retained those models with ΔAICc < 2 relatives to the model ranked first (Zuur et al. 2010). We also derived the explained variance (R^2) and Akaike weights to inform the explanatory power and relative likelihood of each model being the best model. We used the *MuMIM* R package to calculate AICc, rank models and estimate explained variance and Akaike weights. (Barton and Barton 2015). Furthermore, we applied a variance partitioning analysis to evaluate the contribution of drying components and environmental factors on DOC and DOM optical qualitative parameters (Hoffman and Schadt 2016). For all models, residuals plots were visually checked to verify model assumptions. Before analyses, to reduce distribution skewness, we performed a log-transformation for HIX, $SUVA_{254}$, DOC, Sr, a square-root transformation for E_2E_3, ZFT, ZFL, ZFP and fourth-root transformation for RE. Finally, we z-standardized (mean=0, SD=1) predictors to facilitate comparisons among model coefficients. All statistical analyses were executed in R 3.5.1 (R Core Team 2018).

Table 5.3.4.1. Models used to evaluate the effect and importance of drying components and environmental factors on DOC and DOM parameters. Predictor labels: annual drying duration (ZFT), annual drying frequency (ZFP), duration of the latest dry event (ZFL) and rewetting days since the latest dry event (RE), altitude (Alt) and water temperature (Temp). Hypothesised drivers: E: environmental factors; A: annual drying components; P: previous drying components

Model	Predictors	Hypothesised drivers
0	Alt + Temp	E
1	Alt + Temp + ZFT	E+A
2	Alt + Temp + ZFP	E+A
3	Alt + Temp + ZFL	E+P
4	Alt + Temp + RE	E+P
5	Alt + Temp + ZFT + ZFP	E+A
6	Alt + Temp + ZFT + RE	E+A+P
7	Alt + Temp + ZFP + ZFL	E+A+P
8	Alt + Temp + ZFP + RE	E+A+P
9	Alt + Temp + ZFL + RE	E+P
10	Alt + Temp + ZFT + ZFP + RE	E+A+P
11	Alt + Temp + ZFP + ZFL + RE	E+A+P

5.4.RESULTS

DOC and DOM optical qualitative parameters showed high variability across the studied streams: DOC ranged from 0.7 to 14.6 mg C · L^{-1}, $SUVA_{254}$ varied from 1.83 to 8.59 L mg C^{-1} m^{-1}, HIX values fluctuated between 0.49 to 0.94, FI differed between 1.47 to 1.92, BIX varied from 0.48 to 0.81, E_2:E_3 ranged from 1.47 to 9.21 and Sr varied from 0.73 to 3.91. Furthermore, the drying components exhibited a wide range of variation, ranging from permanent-flow streams with 0 dry days to ephemeral streams with more than 324 dry days. Also, the dry periods ranged from 0 to 8. The duration of the latest dry period oscillated between 0 to 294 days, whereas rewetting duration since the last dry event ranged from 6 to 274 days (Fig 5.4.1 for more details, see Annex II; Appendix D)

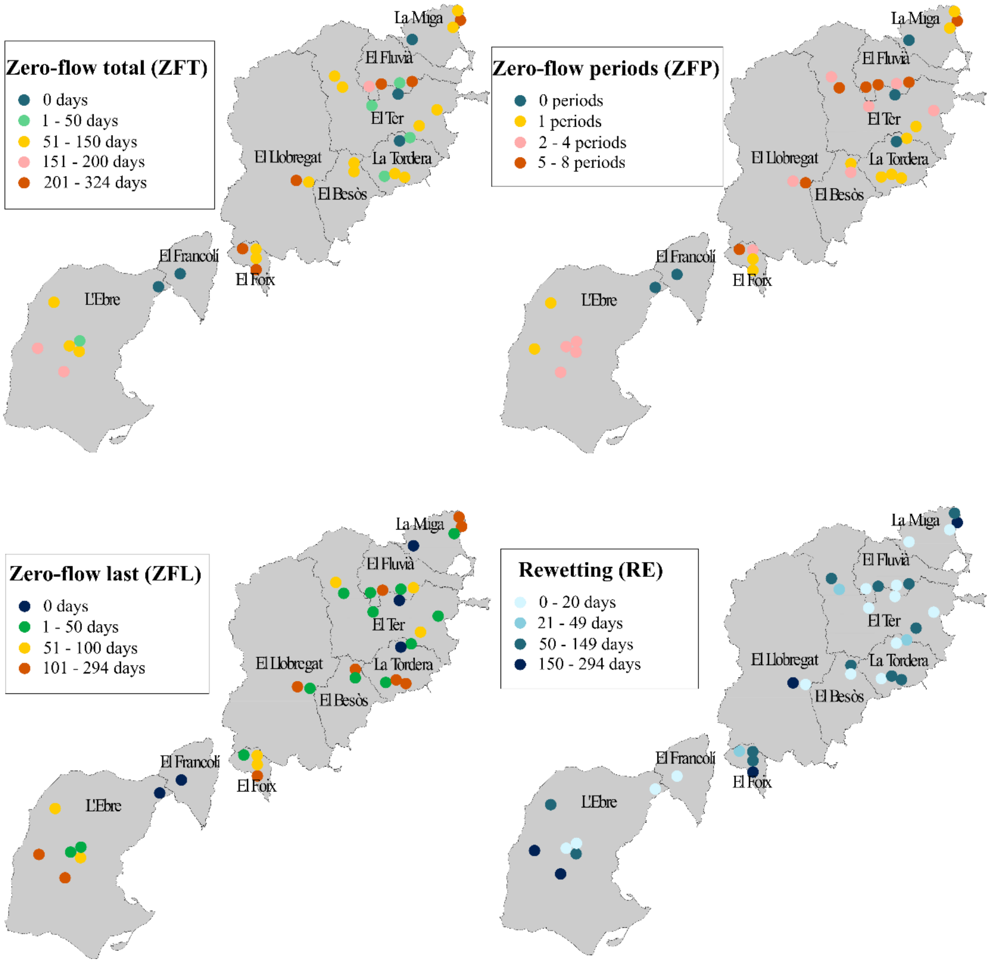

Figure 5.4.1. Range of variation of the drying components. Distributed as the annual drying duration (ZFT), annual drying frequency (ZFP), duration of the latest dry event (ZFL) and rewetting days since the latest dry event (RE) in 35 streams of the nine studied catchments in the Iberian Peninsula NE.

We identified two contrasting patterns in response to the annual (drying duration and frequency) and previous drying components (duration of the latest dry event, rewetting time since the latest dry event). The first group of variables, including DOC, HIX and E2:E3, tended to increase with drying conditions. DOC increased with both annual drying duration and frequency, whereas HIX and E2:E3 showed a positive response to annual frequency and the duration of the latest dry event. However, rewetting duration had positive (E2:E3), negative (HIX) or no effect (DOC) on these variables. On the contrary, the second group of variables, including $SUVA_{254}$, FI, BIX, and Sr, tended to decrease with drying conditions. All these variables decreased with annual drying duration but showed different strengths. $SUVA_{254}$ and Sr also decreased with annual drying frequency and the duration of the latest dry event. BIX and FI responded positively to rewetting duration, whereas Sr showed a negative response (Fig 5.4.2).

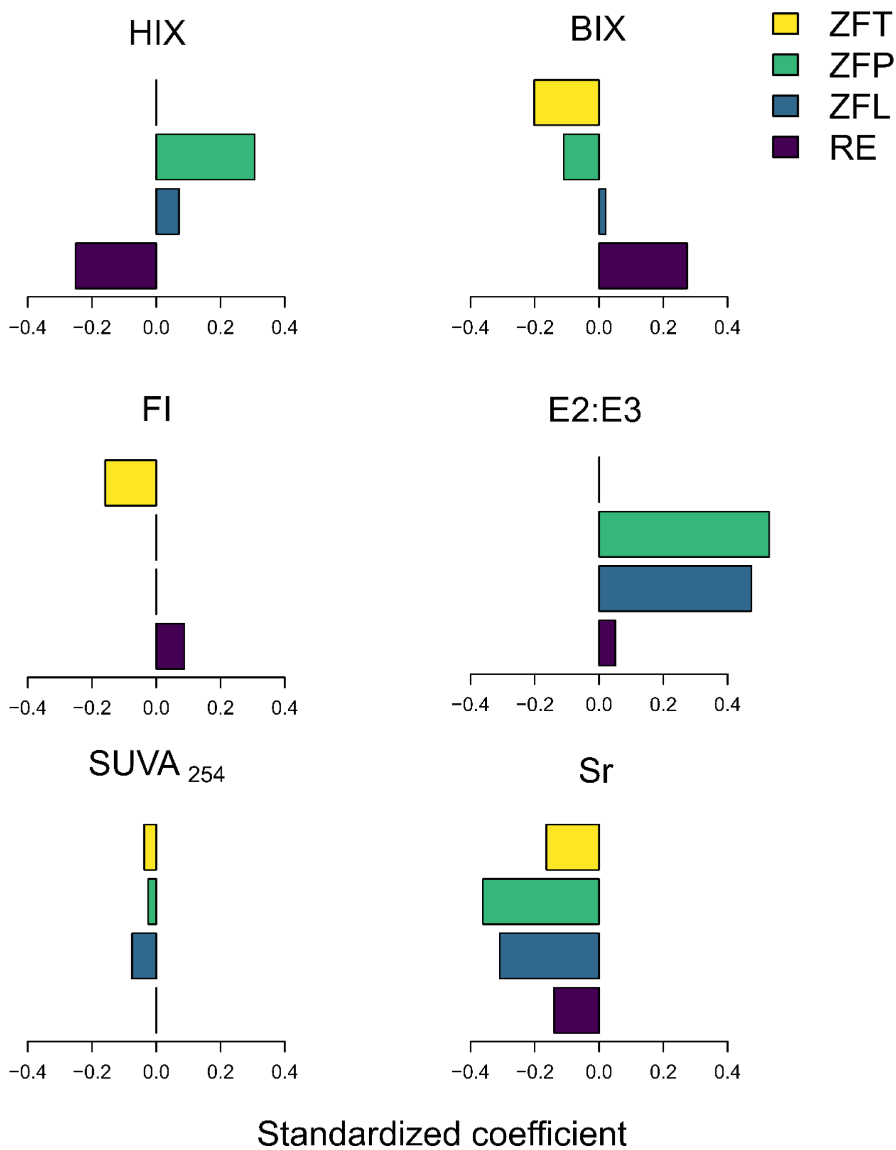

Fig 5.4.2. Weighted mean standardised coefficients of DOM optical qualitative parameters. The standardised coefficients represent the weighted mean values of retained models (ΔAICc ≤ 2). For more details, see Annex II; Appendix E. The annual drying duration (ZFT), annual drying frequency (ZFP), duration of the latest dry event (ZFL) and rewetting days since the latest dry event (RE).

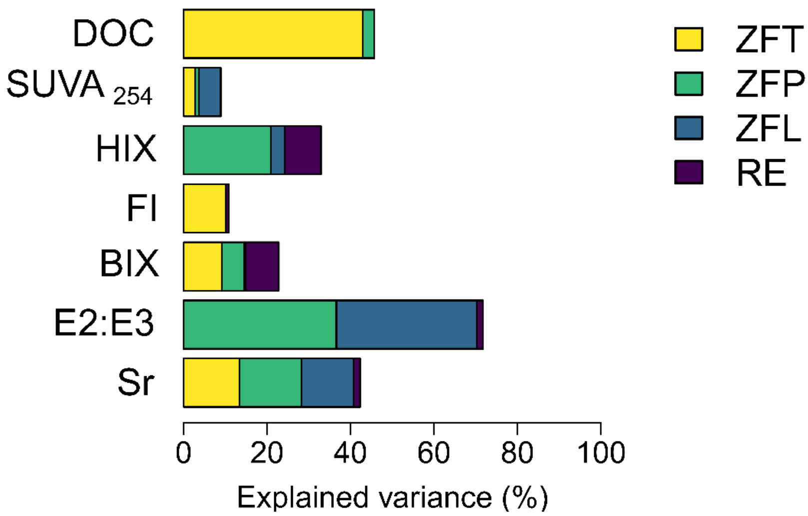

Fig 5.4.3. Mean explained variance of DOC concentration and DOM optical qualitative parameters by the four drying components (annual drying duration (ZFT), annual drying frequency (ZFP), duration of the latest dry event (ZFL) and rewetting days since the latest dry event (RE).

Annual drying duration was the best predictor of DOC changes (mean explained variance: 42.9%), whereas DOM parameters were explained by a combination of two or three drying components. Annual drying duration showed a limited capacity to predict variations in DOM parameters (mean explained variance of 5.9%) compared to annual drying frequency (mean explained variance: 13.1%) and the duration of the latest dry event (mean explained variance: 9.2%). Annual drying frequency was the best predictor of E2:E3 (mean explained variance: 36.5%), HIX (mean explained variance: 20.9%) and Sr (mean explained variance: 14.8%). The latest dry event's duration was also explanatory of E2:E3 (mean explained variance: 33.7%) and Sr (mean explained variance: 12.5%). Rewetting duration since the last dry event showed a certain explanatory capacity for BIX (mean explained variance: 8.7%) and HIX (mean explained variance: 7.9%). Temperature was generally relevant (mean explained variance: 7.2%, range: 1.3-17.9%), but altitude had a weak predictive capacity (mean explained variance: 1.1%, range: 0.0-3.2%). The temperature was particularly important for SUVA254 (mean explained variance: 17.9%) and FI (mean explained variance: 14.9%) (Fig 5.4.3 for more details, see Annex II; Table 1, Appendix E.

5.5.DISCUSSION

Our study demonstrates that the of organic matter quality are better explained by a combination of drying components than by a single descriptor integrating annual drying duration. Specifically, annual drying frequency, duration of the latest dry event and time since last dry event (i.e., rewetting time) jointly explained changes in molecular weight and aromatic-, humic- and protein-like compounds. We also found that DOC was better predicted by annual drying duration with a lower influence of other components. These findings suggest that the quantity and the quality of dissolved organic matter in streams respond differently to the temporal components of the drying process.

Our results align with previous studies finding that exposure to more prolonged drying conditions increases DOC concentrations (Vazquez et al. 2011, von Schiller et al. 2015, Casas-Ruiz et al. 2016, Harjung et al. 2018). This typical pattern is generally explained by the reduced capacity of microbes to process organic carbon during the dry phase. The limitation of the diffusion of the dissolved nutrients added to the transformation into anoxic and reduced habitats with higher contents of salts, humic organic matter and ammonium may have repercussions on the microbial activity and community structure (Romaní et al. 2017, Harjung et al. 2019, Arias-Real et al. 2021b). The main novelty of the present study was in revealing that the spatial patterns of organic matter quality are explained by a wider variety of drying components than previously thought. Thus, annual drying frequency, duration of the latest dry period and time since the latest dry period tracked changes in organic matter quality. We found that the intermittent stream's DOM quality was characterised by a higher proportion of lighter-weight molecules and aliphatic compounds and humic DOM sources (i.e., higher HIX). our results suggest that intermittent streams are dominated by terrestrial DOM sources, consequently, high allochthonous DOM quality. These DOM quality resembles that of soils,are also conditioned by the sampling time, after post-drought, in autumn coincidence of the leaf litterfall period and the proliferation of terrestrial vegetation in riparian zones, especially by the formation of roots (Inamdar et al. 2011, Catalán et al. 2013, von Schiller et al. 2015, Shumilova et al. 2019, Arce et al. 2019). Thus, our results revealed that prolonged and frequent dry periods increased the concentration of terrestrial DOM sources and compounds with low molecular weight as opposed to the autochthonous DOM sources found in the short-term studies (Vazquez et al. 2011, von Schiller et al. 2015, Casas-Ruiz et al. 2016). However, the lower aromaticity obtained by $SUVA_{254}$ results has a explained variance less than 10%, which could determine that drying components do not provide a solid explanation for the aromaticity in this study. Otherwise, reductions in protein-like compounds can be linked to the limited algal and microbial biomass production in IRES (Timoner et al. 2012, Gionchetta et al. 2019, Arias-Real et al. 2020, 2021b). Drying conditions reduce microbial biodiversity, inhibiting mechanisms driving biomass production in streambeds (Gionchetta et al. 2019, Arias-Real et al. 2020, 2021b). Nonetheless, flash storms can rapidly recover microbial activity (Amalfitano et al. 2008, Marxsen et al. 2010, Evans and Wallenstein 2014, Datry et al. 2014), resulting in solid peaks of organic matter processing and greenhouse gas emissions (Inamdar et al. 2011, Schiller et al. 2019, Shumilova et al. 2019). Our results showed that when rewetting last sufficiently, humic compounds can be processed locally or transported downstream (Vazquez et al. 2011, von Schiller et al. 2015, Harjung et al. 2018), and autochthonous production can recover. Interestingly, our study did not capture the impact of time since the last rewetting (i.e., RE) on DOC and DOM optical qualitative parameters. In small intermittent streams, rewetting is a flush and abrupt phase that causes a peak increase of allochthonous terrestrial DOC and a delayed microbial C processing peaks 2-3 days later of this event (Vazquez et al. 2011, von Schiller et al. 2015, Guarch-Ribot and Butturini 2016, Schiller et al. 2019, Shumilova et al. 2019). In most cases, the rewetting timing is almost unpredictable, and sampling the exact rewetting of 35 streams is a complex task. In our study, sampling never was performed until six days after the rewetting event. Consequently, we could not capture the biogeochemical signature of the abrupt rewetting accurately. However, the sampling strategy was accurate enough to describe the impact of drying.

In addition to drying components, water temperature contributed to track variations in DOM quality, whereas altitude was generally weakly non-explanatory. In our study, warming conditions were linked to more aromatic and less humified DOM, suggesting autochthonous origin. These findings match previous results, which have found that temperature controls organic matter processing by microorganisms at large scales (Schiller et al. 2019, Picazo et al. 2020, Arias-Real et al. 2021a).

Considering that drying conditions are expected to increase globally due to global change and water abstraction (Döll and Schmied 2012, Leigh and Datry 2017, Schneider et al. 2017), watercourses are expected to increase DOC concentrations and altered DOM properties. Accordingly, rivers might increase the amount of dissolved carbon to oceans due to reduced processed over drainage networks. In this vein, increasing drying conditions might turn IRES into more passive conduits than perennial flow watercourses (Cole et al. 2007, Casas-Ruiz et al. 2020b). Thus, our study can be helpful to understand better how future spatial changes in drying conditions can affect the global dynamics of dissolved carbon and improve global carbon budgets (Cole et al. 2007, Raymond et al. 2013).

5.6.CONCLUSIONS

Our comprehensive characterisation of the drying process of Mediterranean streams allowed us to evaluate the effect and importance of multiple drying components, finding that single indicators of drying (annual drying duration) do not fully capture the heterogeneity of DOM organic matter quality responses. Whereas annual drying duration was a good predictor of organic matter quantity, this study highlight on the importance to integrate multiple drying components to better predict variability of organic matter quality in highly dynamic streams.

5.7. Acknowledgements

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GENERAL DISCUSSION

6. GENERAL DISCUSSION

Flow is the primary driver of stream geomorphology and the dynamics of fluvial ecosystems. Thus, the hydrological regime is the descriptor of the flow structure and links with the biogeochemistry, habitats and biotic communities of the streams. However, climate change and human activities (e.g., pollution, river channelization, dams, land use and water abstraction for irrigation and to generate hydroelectric power) alter the patterns of the hydrological regime (Larned et al. 2010a, Zeiringer et al. 2018). Therefore, the distribution of IRES systems are increasing globally, and under a future scenario with a drier climate, the number of zero-flow days and their frequency will continue to rise (Döll and Schmied 2012, Jaeger et al. 2014, Schewe et al. 2014). In this context, it is necessary to investigate the hydrological regime in IRES. The characterization of flow intermittence is challenging, given the environmental and drying elements associated with flow disruption, among others mentioned in the introduction.

This thesis improves the knowledge of flow intermittence and its impact on the biogeochemistry of IRES through diverse perspectives: from the longitudinal quantitative and qualitative change of DOM, to the evaluation of the isolated pools formation during the dry period, to a higher scale approach to address all the drying components, the annual and the previous zero-flow elements in a multi catchment comparison.

6.1. THE RELEVANCE OF ESTIMATING FLOW INTERMITTENCE

The research on flow regime in IRES is essential to understand how the spatiotemporal variability of zero-flow exerts a direct or indirect impact on the habitat, organic matter processing and composition, nutrient transformation and community dynamics in these ecosystems (Leigh and Datry 2017, Rolls et al. 2018, Beaufort et al. 2019). Nonetheless, typifying the flow regime in IRES is difficult because most of them do not have gauging stations, or their spatial distribution is difficult to detect with the current mapping techniques. The maps are obtained with aerial photographs and satellite imagery, but none have enough resolution to detect small streams covered by vegetation (Meyer and Wallace 2001, Benstead and Leigh 2012, De Girolamo et al. 2015). Thus, to solve the limitations of flow regime characterization, most of the studies use qualitative indices; categorizing intermittent rivers (e.g., permanent, temporary-pools, temporary-dry and episodic), or phases (e.g., pre-drought, contraction, fragmentation, dry and rewetting), or conditions (connected vs disconnected)(Taylor 1997, Bonada et al. 2006, Rupp et al. 2008, Gallart et al. 2012, Harjung et al. 2018).

Furthermore, also have limitations to characterize the flow regime with quantitative predictors such as annual mean flow by applying hydrological models from gauging stations (Larned et al. 2010b, Stromberg et al. 2010, Snelder et al. 2013, Belmar et al. 2013, 2019, Reynolds et al. 2015). However, these approaches do not detect the variability imposed by flow disruption into IRES biotic and abiotic elements. Neither do they contemplate the variability in the frequency and duration of the drying or rewetting elements. In this context, autonomous temperature field loggers are suitable tools to describe flow regimes in IRES due to their ease of settling into the river bed, their simple interpretation and low cost (Constantz et al. 2001, Gungle 2006). As a result, high frequency temperature time series from the loggers can be used to track the flow or zero-flow status of IRES at high spatial and temporal resolution (Jansen, 2019; Paillex, 2019). The flow or zero-flow status can be transformed into hydrological metrics to evaluate the influence of flow regime, on any ecosystem structural or functional descriptor, either biotic or abiotic. This thesis highlights the advantages of estimating the flow intermittence in this way, as it allows more accurate identification of zero-flow characteristics and its effects on biogeochemical cycling. Thus, we determined the drying components in a multi catchment approach (chapter five) and the connected and isolated timing and duration of the stream (chapter four), in order to assess its effect on DOM composition.

6.2. EFFECT OF FLOW INTERMITTENCE ON DOM OPTICAL QUALITATIVE PARAMETERS

All chapters of this thesis show the influence of hydrological conditions on DOM optical qualitative parameters, and the concentration of the inorganic and organic solutes; the longitudinal research of the biogeochemistry transformations from headwaters to downstream. Chapter three shows the influence of antagonistic flow conditions, drought and high flow, on DOM properties and how the river continuum can cushion the impact of contrasting hydrology on DOM and dissolved inorganic matter properties. In chapter four, we address the effect of flow disruption on the biogeochemistry of isolated pools by studying the evolution of the dry period as a "film frame", monitoring the flow diminution at a daily scale. Finally, chapter five addresses, through a multi-catchment approach, the influence of the past drying components on DOM optical qualitative parameters.

6.2.1. LONGITUDINAL DIMENSION OF DOM OPTICAL QUALITATIVE PARAMETERS

The evaluation of DOM quality in contrasting flow conditions shows that during drought there is more autochthonous DOM production, whereas under high flow conditions allochthonous DOM sources prevail. These outcomes align with general patterns observed in human-altered Mediterranean rivers (Ejarque Gonzalez 2014, Butturini et al. 2016).

Although, the wastewater effluents (i.e., treated and untreated wastewater effluents) found along the main stem could favour the autochthonous signal. The studied network (Matarranya) showed a downstream diminution of DOC concentrations during drought. In addition, the DOM pool had more humic molecules and greater aromaticity, contrasting with other studies' DOM composition. (Creed et al. 2015, Ejarque et al. 2017). Thus, examining the detailed evolution of the dry period in chapter four, the remaining isolated pools had an allochthonous DOM origin (Harjung et al. 2018). Besides, chapter five shows that an allochthonous DOM origin coinciding with longer annual duration of drying. However, the drought episode in the Matarranya river had an in-stream autochthonous DOM production, likely because the flow interruption and the physicochemical water conditions obtained were not as extreme as the streams studied in chapter four and five.

The longitudinal analysis of the river continuum revealed that quantitative and qualitative DOM upstream were different between contrasting hydrological conditions (high flow and drought), but these differences in DOM vanishes downstream. Thus, the results agree with Creed's hypothesis "The river tends to chemostasis", which means that upstream DOM composition depends on hydrological conditions (Creed et al. 2015). This is explained by the terrestrial-aquatic connections that stimulate OM mobilization and promote the leaching of allochthonous DOM in headwaters. In contrast, the dissimilarity on DOM composition decrease downstream. Due to the higher water inputs from tributaries and groundwater and the longer water residence time, facilitated the in-stream biogeochemical processes, meaning the DOM autochthonous origin. Instead, dissolved inorganic matter concentrations contrast between the two hydrological conditions downriver, especially after the wastewater effluents during drought. Thus, dissolved inorganic matter denotes higher sensitivity to the anthropogenic inputs and the hydrological regime than DOM composition. However, in the latest study. However, a new concept was slightly dissimilar to our results and chemosthasis hypothesis for temperate river networks, which combine the spatial and temporal dynamics denominated "The Bending DOM Concept". Thus, during the base flow, the DOC concentration in the river continuum has a bell-shaped pattern, increasing the peak in the medium-sized streams, and the DOM quality was more variable, playing a key role, the aliphatic compounds. But, during the storms, longitudinal patterns of DOC concentration were higher, and DOM quality was less variable (Casas-Ruiz et al. 2020a).

6.2.2. TEMPORAL VARIABILITY OF DOM OPTICAL QUALITATIVE PARAMETS

We address the temporal variability of DOM composition caused by flow disruption from a broad approach, through multi catchment analysis (chapter five) and also from a detailed study of the dry period (chapter four), capturing when and where the flow is interrupted over the main steam as film frames of this “hot moment”.

During the dry period, the flow diminishes, and the fluvial continuum shrinks until its fragmentation, determining the biogeochemical processes of these intermittent systems. In chapter four, the hydrological connectivity cessation is fast. The Fuirosos fluvial network shifts from connected to disconnected in a few hours, with a rate of water receding of around sixty meters per day and a simultaneous regression pattern upstream and downstream (Lake 2003). This type of regression pattern is because the catchment has restricted groundwater inputs, as its sediments are impermeable with a granitic bedrock substrate. By contrast, karstic streams show groundwater entrances upstream (Meyer and Meyer, 2000).

As discussed in chapter three, the hydrological condition of drought has a strong influence on DOM composition and inorganic solutes. Nonetheless, little is known about how the hydrological connectivity affects the biogeochemistry of IRES. Hence, in chapter four, the drying progression increased DOC concentrations tenfold, also the DOM molecular size (i.e., E_2:E_3 and $S_{275-295}$). The increase of DOM concentration in isolated pools is a consequence of the high evaporation rates and the absence of groundwater inputs (Fellman et al. 2011, Siebers et al. 2016, Harjung et al. 2018). Furthermore, during drought leaves fall due to the water stress suffered by trees (Acuña et al. 2007). This accumulation of leaves in the isolated pools promotes an aromatic (i.e., high SUVA) and humic DOM signature. These results are in line with other studies in Mediterranean (Harjung et al. 2018), tropical (Yamashita et al. 2010), temperate (Inamdar et al. 2012) and tundra systems (Balcarczyk et al. 2009). However, the predominance of humic substances and allochthonous DOM sources contradict the DOM composition founded in previous studies at the same period (Vazquez et al. 2011, von Schiller et al. 2015, Casas-Ruiz et al. 2016) where they detected a dominance of autochthonous DOM derived from algal metabolism. These differences of DOM origin could relate to lower data resolution at both spatial and temporal scales. It is therefore needed to include high sampling sites along the catchment and more sampling dates to capture in detail the evolution of the dry period. When this high resolution strategy was applied in chapter five, we analyzed the annual duration and frequency of drying in several IRES with different intermittence gradients. We found that the ephemeral streams (long duration of zero-flow days over the annual drying), had a significant contribution of humic molecules and allochthonous DOM origin. It must be noted, though, that the terrestrial DOM pool might have a dominant origin also because the sampling was only performed in the rainy season. Regarding the inorganic solutes, they were dependent on the evolution of the dry period. Conductivity gradually increased along this period, indicating a solute concentration in the isolated pools and losing the surface water connection with the hyporheic zone and groundwater. Furthermore, isolated pools showed high respiration activity, raising the dissolved carbon dioxide in water and reducing the oxygen dissolved. Thus, the dissolved oxygen reduction favoured the consumption of SO_4^{2-} and NO_3^-, and NH_4^+ concentration constrained nitrification (Townsend 2002, Acuña et al. 2007, von Schiller et al. 2011). This reduced and anoxic habitat, with the accumulation of salts and organic matter not much biodegradable, inhibited the microbial activity and fungal communities (Medeiros et al. 2009, Febria et al. 2015, Canhoto et al. 2016, Harjung et al. 2019).

We expected that the timing of fragmentation, defined as zero CI-days, would have immediate consequences on the biogeochemistry of the stream. However, the fragmentation accelerated the biogeochemical responses but was not synchronized. Thus, we identified three temporal responses on the variables studied: 1) some inorganic solutes reacted before fragmentation (CI-days<0); 2) the response of some DOM optical qualitative parameters took place after the fragmentation (CI-days>0); and 3) some descriptors (e.g. BIX and HIX) did not respond to fragmentation throughout the dry period.

These findings match other DOM studies of pre-and post- fragmentation periods but with higher temporal and spatial precession of the dry period in the Fuirosos stream. (Harjung et al., 2018; von Schiller et al., 2015)

On the other hand, under a wider geographical perspective, we selected IRES with different zero-flow duration and frequencies, covering from the ephemeral to permanent streams, although in Mediterranean intermittent streams, the zero-flow duration and frequency are relatively predictable and seasonal. We evaluate the effect of the drying components on the DOC concentration and DOM quality responses. The drying components were the annual (i.e., drying duration and frequency) and previous drying components (i.e., duration of the last dry event, rewetting time since the last dry event). For instance, this study evidences that the quantity and quality of DOM responded differently to the drying components. DOC was better predicted by the long drying duration, aligned with the studies in intermittent Mediterranean streams (Vazquez et al. 2011, von Schiller et al. 2015, Casas-Ruiz et al. 2016, Harjung et al. 2019). However, the DOM quality was predicted by the annual drying frequency and the duration of the latest dry period. Notwithstanding, our results show that better knowledge of DOC concentration and DOM optical qualitative parameters is obtained if the annual drying duration and frequency (i.e., ZFT and ZFP) are included as explanatory variables of drying composition.

To sum up, the evolution of drying presented a humified character of DOM (i.e., higher HIX), a more significant proportion of lighter-weight molecules and aliphatic compounds which prevail the terrestrial DOM sources. Hence, the DOM composition becomes more similar to soils due to leaf litter inputs and the proliferation of terrestrial vegetation (Arce et al. 2019).

Rewetting is a fast event of stream reconnection. In the Mediterranean climate, it occurs as torrential rains between the end of the summer and the beginning of autumn. These flash floods cause an exacerbated increase of allochthonous C with high peaks of terrestrial matter accumulated during the dry period, and after two or three days of this episode, the C processing shifts to the highest peak of C microbial sources (Guarch-Ribot and Butturini, 2016; Schiller et al., 2019; Shumilova et al., 2019; Vazquez et al., 2011; von Schiller et al., 2015). However, in the multi-catchment study, rewetting did not have a significant influence because most samplings were performed six days after stream reconnection. This prevented us from capturing the fast transformation DOM composition and high peak of C. Furthermore, the previous flow duration (i.e., "rewetting") supported the same DOM composition, terrestrial DOM source and high aromaticity shown in other studies at lower spatio-temporal scales (Vazquez et al. 2011, von Schiller et al. 2015, Harjung et al. 2018). Also contributing to this DOM composition is the leaf fall period, which produces a humification of stream DOM (Inamdar et al. 2011, Catalán et al. 2013, von Schiller et al. 2015, Shumilova et al. 2019). To conclude, studying the rewetting requires precise knowledge of the timing of stream reconnection, with early and intensive sampling to capture the complex biogeochemical signal.

For instance, the increase in the temperature favours aromaticity and autochthonous sources, which is in line with other large-scale study (Picazo et al. 2020). Furthermore, a high-temperature benefits the organic matter processing by the stream microorganisms (Arias-Real et al., 2021; Picazo et al., 2020; Schiller et al., 2019).

In studies evaluating the short-term effects of drying, where the target is the previous drying phase (i.e., the dry period) (Vazquez et al. 2011, von Schiller et al. 2015, Casas-Ruiz et al. 2016, Harjung et al. 2019) some important information on the annual biogeochemical dynamics is missed. Hence, there are some discrepancies between the short-term research and the results of the fifth chapter, which also contemplates the annual drying duration and frequency in a multi-catchment study where the streams were sampled in the rainy season. The long duration and high frequency of zero-flow periods increase terrestrial origin of DOM (i.e., compounds with low molecular weight and lower aromaticity). This result contradicts patterns show in the short-term approaches, which found autochthonous DOM sources (Casas-Ruiz et al., 2016; Vazquez et al., 2011; von Schiller et al., 2015). However, investigations focalized at the end of the dry period (i.e., chapter four), the streamflow was disconnected and shifted to a cluster of isolated pools. The system was dominated by the allochthonous DOM sources, aromatic content, high molecular weight compounds, which announced difficulties of the biodegradation. In addition, the high water retention time promoted the accumulation of DOC concentration, and the physical-chemical of water pools switched it into an anoxic and reduced habitat, causing a diminution of the aquatic microbiota production and altering their diversity (Catalán et al. 2017, Harjung et al. 2018)

The flow regimes will get more significant duration and frequency of drying in the regions with IRES and in the areas that are predicted to expand by the flow intermittence due to climate change, water extraction, and land-use changes (IPCC, 2014; Edenhofer et al., 2014; Girolamo et al., 2017; Döll and Schmied 2012). Thus, the multi-catchment approach of chapter five indicates that more ephemeral streams have higher DOC concentration and it is from terrestrial origin. Accordingly, the global abundance of IRES (more than 70% worldwide, Fritz et al., 2013; Datry et al., 2014 a,b) and their expected increment will likely have a relevant impact on global carbon budgets (Cole et al., 2007). The drying components of IRES need to be studied, as well as their influence on DOC concentration and DOM composition, and how IRES are involved in the global carbon budget, from which they are currently excluded (Cole et al., 2007; Raymond et al., 2013).

6.3. TECHNICAL LIMITATIONS AND FUTURE RESEARCH

In the last few years, IRES research from different fields of knowledge has made a considerable leap by addressing how drying duration and frequency affect IRES ecosystems. This includes the changes on DOC concentration and DOM optical qualitative parameters (i.e., chapter five) to the effect on the invertebrate community response (Arias-Real et al., 2021), the biofilm biomass and metabolism (Colls et al. 2019) and the streambed microbial composition, density and diversity (Gionchetta et al. 2020). However, there is still much to research:

6.3.1. TECHNICAL GAPS

In the modelling context, it is needed to adopt hydrological models that contemplate the zero-flows and get a successful application in the prediction and knowledge of the intermittence of IRES. Significantly, the high spatial and temporal dissimilarity of IRES, besides the influence of lateral, vertical and longitudinal connectivity on the flow regime, make complex the flow intermittence modelling and find patterns between them. Furthermore, it is necessary to monitor a multi-year hydrological data to progress and obtain more accurate predictions. Unfortunately, neither is there much research that combines hydrological, biogeochemical, and ecological data. Nor enough data of flow monitoring when the stream is drying and keeps the pools or when the stream restarts the flow. Other difficulties are the scale limitations, the high-priced monitoring equipment and the significant variability of IRES types, for instance: in terms of geographic setting, geomorphology and hydrogeology, which are challenges for their study.

6.3.2. IRES REQUIRES SPECIFIC GUIDELINES AND WATER QUALITY STANDARDS

IRES have many physicochemical fluctuations, both spatially and temporally. In particular, in periods of flow disruption, significant biogeochemical responses occur. For instance, the dissolved organic matter and other solutes increase exponentially, and the oxygen concentration diminishes (chapter four). These characteristics imply understanding which factors make the intermittent ecosystems salubrious. However, unfortunately, these intrinsic biogeochemical and hydrological features of intermittent and ephemeral systems described are not included in the current guidelines and water quality standards adapted to the permanent streams.

6.3.3. GLOBAL MULTI-CATCHMENT STUDIES OF IRES OR WITH MORE EXTENDED AREAS ARE NEEDED

In addition to the difficulties obtaining data from IRES systems, primarily due to lack of global studies, there are geographically and climatic unequal in the research of these ecosystems. In the manner that, most of the studies are in the developed countries with the Mediterranean, temperate, semiarid and arid climates. Thus, it will likely be premature to extrapolate the biogeochemical processes developed in the Mediterranean IRES, which have more data. To the biogeochemical cycles of other climatic and geographic intermittence systems. Therefore, some research initiatives have been developed to counteract this imbalance, such as the IRBAS program "The 1000 Intermittent River project" http://1000_intermittent_rivers_project.irstea.fr (Datry et al., 2016). It was an international and collaborative network of researchers and institutions to share knowledge and co-operates in experiments of many IRES worldwide.

Furthermore, an example of the importance during the last years of the IRES research is the European cooperation H2020 COST Action. SMIRES (i.e., Science and Management of Intermittent Rivers and Ephemeral Streams) research: hydrology, ecology, biogeochemistry, ecohydrology, social sciences, and environmental economics of IRES. Thus, network with 200 researchers and 40 managers from different countries of Europe to resolve many of the knowledge gaps of IRES. Another example is the DryFlux initiative, recognizing that the dry aquatic systems also contribute to global

C cycling. This initiative aims to introduce research on ephemeral, intermittent and permanently drying inland waters to quantify the CO_2 emissions and estimate the losses of organic carbon from these systems worldwide https://www.ufz.de/dryflux/.

There is another network of scientific cooperation currently being developed in order to monitor the biodiversity of the Iberian Peninsula to get annual data from different ecology fields. The Global Iberian Observatory, with the aim of studying the impacts of global change on the Iberian rivers, and thus encourage cooperation between researchers, network with research companies and the administration, achieve citizen involvement, and raise awareness about the importance of biodiversity of our ecosystems.

CONCLUSIONS

This thesis contributes to the knowledge of key mechanisms of flow intermittence which affect the biogeochemistry of IRES. Essentially, it analyses different perspectives of IRES: in chapter three, the DOM quality behaviour along the longitudinal mainstream; in chapter four, the dry period of the stream and how it shifts DOC concentration and DOM optical qualitative parameters in the isolated pools; and in chapter five how drying components affect DOM under a multi-catchment approach. The following paragraphs summarize the conclusions of this thesis.

Chapter 3: DOM properties fluctuate more in headwaters than in downstream. Downriver tends to chemostasis.

1. Results from this study fitted the suggested chemostatic hypothesis. In this manner, the study of two opposite hydrological conditions, both under high flow and drought (i.e., low flow), reveal more variability in DOM properties in headwaters than downstream.
2. Under high flow conditions, DOM composition is from terrestrial sources, humic, degrades and rich in aromaticity moieties and, as solutes flow downriver, these DOM properties turn into more dominant.
3. Under drought conditions, DOM composition is less degraded, aliphatic and has little humic/aromatic. The wastewater inputs conditionate more the DOM composition than the in-stream autochthonous development. The results of DOM composition on the middle fluvial segment corroborate that it does not receive significant anthropogenic inputs and depicts this segment as suitable for exploring the in-stream DOM retention/release processes under drought conditions.
4. The results under drought conditions indicate a slightly DOC concentration decrease along the 35 km of the fluvial segment without anthropogenic inputs. DOM composition is less freshly and slightly

degraded, in contrast with other studies describing aromatic and humified DOM composition downriver.

Chapter 4: the DOM descriptors showed a delayed and abrupt response after the fragmentation of the fluvial continuum.

5. The dry period accelerates biogeochemical responses. In particular, the timing of fragmentation of the fluvial continuum (i.e., time since the formation of isolated pools, CI-days) is the crucial moment of biogeochemical variability.
6. The biogeochemical variables do not react in synchrony with the hydrological fragmentation. Three temporal patterns identify: 1) the inorganic solutes respond before the fragmentation (CI-days<0); 2) most DOM parameters respond after fragmentation (CI-days>0); and 3) some variables do not show any significant change during the dry period, such as the BIX and HIX. Essentially, most DOM descriptors show a delayed and quick response around five to twenty-one days after fragmentation.
7. The higher water residence time of isolated pools along drying increases DOC concentration tenfold. The aromaticity and DOM molecular size also increases, likely due to solute concentration of the system at the end of this period, especially in the isolated pools on unfractured granitic bedrock. Most inorganic components are also concentrated. However, dissolved oxygen and carbon dioxide show opposite trends, indicating high respiration activity at the bottom of the pools.
8. The spatial location and volume of each isolated pool significantly impacts the response of most variables.
9. The longitudinal hydrological fragmentation generates a complex and dynamic biogeochemical mosaic in a single fluvial network. In this framework, the study of DOM quality spatial variability during the

exacerbation of a drought provides a tool to quantify this biogeochemical uniqueness of IRES.

Chapter 5: the drying elements that describe two time-lengths have effects on DOC concentration and DOM composition.

10. The annual zero-flow duration and frequency, as well as the previous zero-flow duration, are needed to explain the organic matter dynamics in IRES.
11. The increment of the annual duration and frequency of drying, as well as the previous drying phase, increase DOC concentrations., DOM shows a higher proportion of lighter-weight molecules and aliphatic compounds added to a humified character, which makes DOM composition more similar to soils.
12. Under a multi-catchment perspective, rewetting does not have a significant influence on DOM, likely because the sampling as performed at a larger temporal scale that did not allow to properly capture the biogeochemical signal. Because the sampling was performed in the rainy season, the DOM quality was similar to the “rewetting period”, with terrestrial DOM source and high aromaticity, in agreement with shorter time-scale studies.
13. Temperature and altitude are less decisive than drying elements. For instance, the increase in the temperature favours aromaticity and autochthonous sources.
14. The annual duration and frequency of drying, bring more exhaustive knowledge on DOC and DOM dynamics than when only focusing on the previous drying phase.
15. The global expansion of flow intermittence suggests that in streams with a more considerable duration and frequency of drying, the DOM quality

will be from terrestrial sources, highlighting the need to integrate IRES in global carbon budgets.

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9. ANNEX I SUPPLEMENTARY INFORMATION CHAPTER 4

9.1.SUPPLEMENTARY INFORMATION CHAPTER 4

APPENDIX A

Table A.1. Characterization of the studied sampling sites in the Fuirosos catchment. Percentages of land use cover were referred to a Corine Land Cover 2006 data in a buffer area of 1 km around each sampling site.

Sampling sites	Distance	Altitude	Latitude	Longitude	Land use cover (%)		
	km	m a.s.l.	Decimal degrees	Decimal degrees	Forest	Urban	Agriculture
0	0	302	41° 43.068'N	2° 34.355'E	1.7	0	98.3
1	1	318	41° 42.615'N	2° 34.734'E	99.8	0	0.2
2	1.5	340	41° 42.416'N	2° 34.911'E	100	0	0
3	1.7	333	41° 42.293'N	2° 34.872'E	100	0	0
4	2.5	416	41° 41.948'N	2° 34.807'E	100	0	0
5	2.9	387	41° 41.790'N	2° 34.702'E	100	0	0
6	4.2	499	41° 41.324'N	2° 34.439'E	100	0	0
7	4.5	521	41° 41.189'N	2° 34.535'E	100	0	0
8	4.8	519	41° 41.167'N	2° 34.657'E	100	0	0
9	4.9	568	41° 41.100'N	2° 34.756'E	100	0	0
10	5.1	566	41° 40.737'N	2° 35.027'E	100	0	0
11	5.9	652	41° 40.679'N	2° 35.075'E	100	0	0
12	6.2	674	41° 40.612'N	2° 35.138'E	100	0	0
13	6.9	703	41° 40.524'N	2° 35.450'E	100	0	0
14	7.4	849	41° 40.316'N	2° 35.879'E	100	0	0
15	8.1	941	41° 40.044'N	2° 36.070'E	100	0	0

APPENDIX B

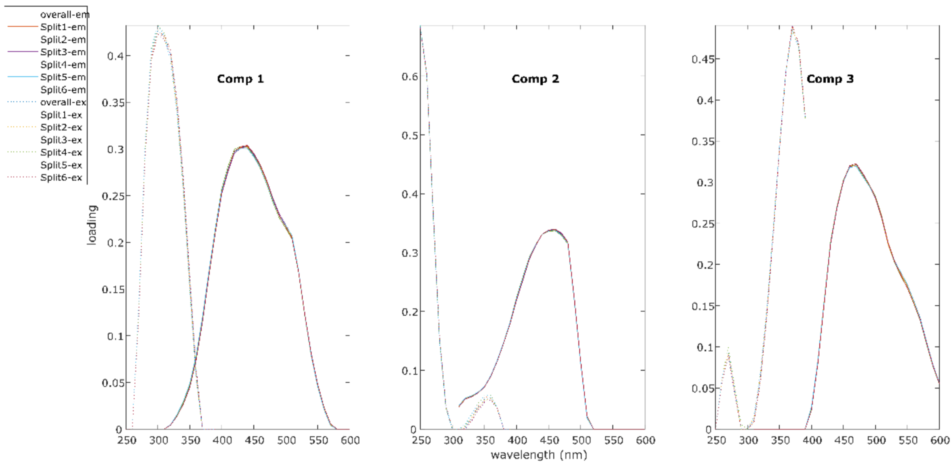

Fig B.1 Half split validation diagram of the 3 fluorescence components identified from the PARAFAC model for surface stream water samples (n = 108) at the 16 Fuirosos catchment during the dry period 2016.

APPENDIX C

R code Fuiros Model

Supplementary data to the chapter two can be found online at https://doi.org/10.1016/j.scitotenv.2020.136619.

APPENDIX D

Table D.1 Summary of biogeochemical variables. Minimum, mean, maximum and standard deviation.

	Mean	Sd	Min	Max
DOC (mg C · L^{-1})	5.03	4.45	2.099	31.18
SUVA $_{254}$ (L· mg C^{-1} · m^{-1})	4.98	0.84	3.403	8.00
SUVA $_{350}$ (L· mg C^{-1} · m^{-1})	1.12	0.30	0.604	2.40
$S_{275-295}$	0.02	0.002	0.011	0.02
E2:E3	6.30	0.93	4.337	8.48
HIX	0.89	0.03	0.800	0.95
FI	1.51	0.06	1.418	1.78
BIX	0.56	0.03	0.464	0.67
Humic $_{Comp\ 1}$	0.55	0.50	0.212	3.50
Humic $_{Comp\ 2}$	0.90	0.74	0.359	5.42
Fulvic $_{Comp}$	0.43	0.33	0.186	2.13
Cl^- (mg · L^{-1})	21.03	14.71	1	71.24
NO_3^- (mg · L^{-1})	0.15	0.23	0.050	1.56
SO_4^{2-} (mg · L^{-1})	5.52	6.01	1	25.50
SRP (mg · L^{-1})	0.05	0.06	0.001	0.30
NH_4^+ (mg · L^{-1})	0.19	0.48	0.001	2.39
CO_2 (mg · L^{-1})	2512	1533.68	850	6510
Electrical conductivity (μS · cm^{-1})	285.40	69.89	206	552
O_2 (mg · L^{-1})	5.71	2.35	0.540	12

10. ANNEX II SUPPLEMENTARY INFORMATION CHAPTER 5

10.1 SUPPLEMENTARY INFORMATION CHAPTER5

APPENDIX A

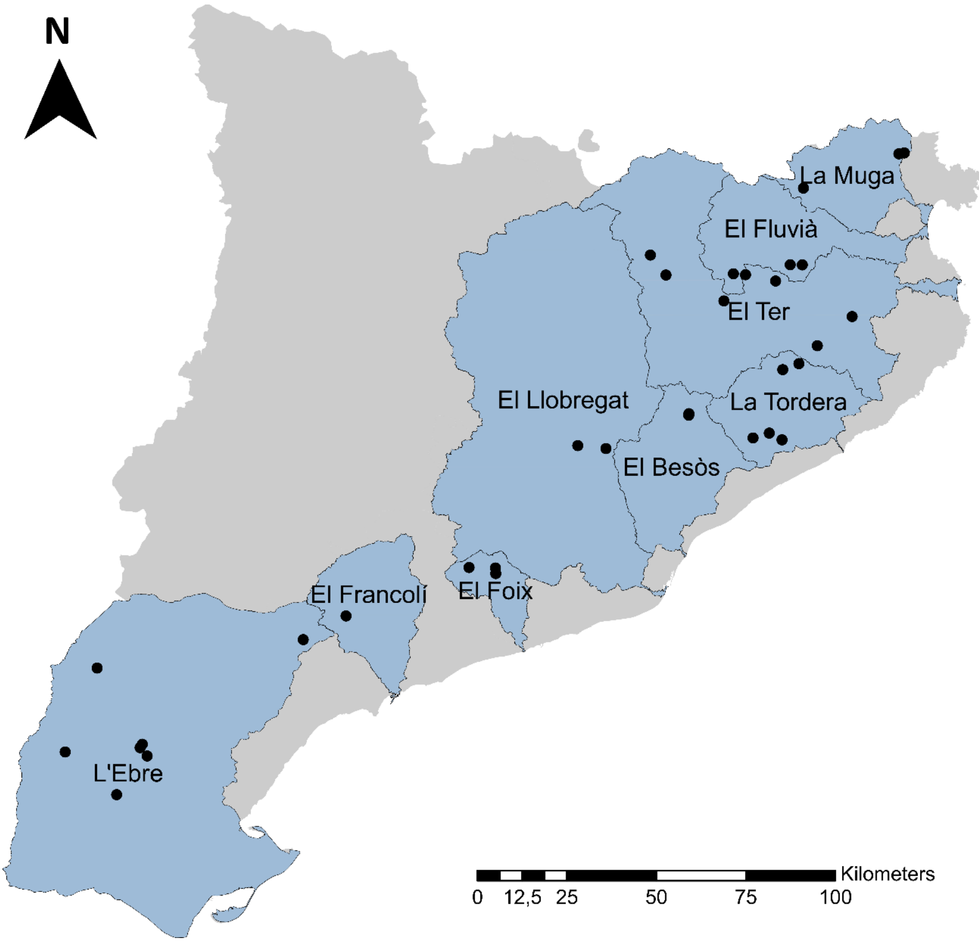

Fig 1. Map of the study area. The figure illustrates the streams distributed in 9 catchments of NE of the Iberian Peninsula: El Llobregat, El Besòs, El Foix, La Tordera, El Ter, El Francolí, El Fluvià, La Muga, L'Ebre.

Table 1. Geographic location and characterization of the studied sites. Percentages of land use cover were referred to a buffer area of 1 km around each sampling point. Agriculture land use is extensive agriculture; Forest land use include forest (broad-leaved forest, mixed forest and coniferous forest), scrubland and grasslands. Basins: Ll= El Llobregat, Be= El Besòs, Fo= El Foix, To= La Tordera, Te= El Ter, Fr= El Francolí, Fl= El Fluvià, Mu= La Muga, Eb= L'Ebre.

Site	Basin	Longitude	Latitude	Altitude	Time dry	Precipitation	Catchment area	Stream order	Land use cover (%)		
		Decimal degrees	Decimal degrees	m a.s.l.	%	mm	km^2		Urban	Agriculture	Forest
1	Ll	1°53'27.26"	41°42'25.71"	258	77.64	634.00	23.96	4	0	0	100
2	Ll	3° 3'6.24"	42°23'15.58"	325	86.10	732.00	23.96	3	2.19	0.94	96.87
3	Ll	1°59'4.98"	41°41'44.64"	468	37.46	692.00	8.68	2	0	0.57	99.43
4	Be	2°16'9.39"	41°46'14.72"	430	43.75	788.40	6.50	3	2.17	0.82	97.01
5	Be	2°16'6.24"	41°46'0.66"	540	25.00	748.40	19.35	4	2.14	0	97.86
6	Fo	1°30'20.34"	41°24'58.98"	620	63.76	568.50	1.65	2	0	9.28	90.72

7	Fo	1°35'36.65''	41°24'41.89''	390	51.71	574.00	1.89	3	0	96.44	3.56
8	Fo	1°35'36.94''	41°23'51.64''	330	27.96	590.07	26.87	4	0	100	0
9	To	2°38'54.46''	41°52'42.10''	170	13.72	908.25	12.44	3	0.79	3.27	95.94
10	To	2°35'35.04''	41°51'57.91''	655	0.00	874.60	49.06	5	0	0	100
11	To	2°28'43.62''	41°41'59.76''	156	6.56	908.25	12.44	4	0.65	1.42	97.93
12	To	2°32'2.87''	41°42'33.64''	110	51.73	864.60	49.06	3	0	0.59	99.41
13	To	2°34'31.31''	41°41'25.69''	290	39.85	874.00	49.06	4	11.56	10.30	78.14
14	Te	2°35'19.14''	42° 5'21.01''	385	0.00	1,013.00	13.41	2	0	0.11	99.89
15	Te	2°13'14.99''	42° 7'17.86''	530	28.19	963.00	13.32	2	0	0.24	99.76
16	Te	2°24'37.20''	42° 2'51.84''	920	6.69	1,063.00	3.71	2	0.03	0.45	99.52
17	Te	2°10'20.96''	42°10'26.33''	632	43.70	953.00	30.99	4	0	5.57	94.43
18	Te	2°50'15.69''	41°59'14.51''	81	29.80	815.00	7.71	4	30.78	12.68	56.54
19	Te	2°29'19.30''	42° 6'35.35''	526	71.05	963.42	13.32	3	0	0.58	99.42
20	Te	2°42'51.23''	41°55'12.78''	140	22.29	815.88	7.71	3	0	78.49	21.51
21	Fr	1° 5'16.00''	41°18'36.43''	605	0.00	469.82	28.93	4	0	14.13	85.87
22	Fl	2° 40'55.00''	42°07'30.80''	219	90.00	997.71	13.75	4	0	34.43	65.57

23	Fl	2°38'28.44''	42° 7'39.01''	266	3.28	997.00	13.78	3	0	1.23	98.77
24	Fl	1°35'36.94''	41°23'51.64''	484	81.67	1,026.25	15.72	4	0	1.14	49.34
25	Fl	2°26'53.16''	42° 6'50.94''	475	46.66	475.00	965.43	4	0.05	2.27	97.69
26	Mu	2°42'10.81''	42°19'1.93''	458	0.00	1,093.00	44.93	4	0	0	100
27	Mu	3° 3'6.24''	42°23'15.58''	100	51.01	841.83	13.53	3	0	99.49	0.51
28	Mu	3° 1'58.98''	42°23'10.92''	88	44.57	868.00	48.69	4	0	30.43	69.57
29	Eb	0° 8'7.56''	40°59'52.41''	415	58.08	638.00	116.46	3	0	98.99	1.01
30	Eb	0°18'02.8''	40°53'08.9''	390	53.29	540.00	1.727.00	4	0	0	100
31	Eb	0°56'29.8''	41°15'20.6''	214	0.00	595.00	627.30	5	0	0	100
32	Eb	0°15'6.35''	41°12'21.00''	170	27.01	540.00	1.035.67	4	0	30.49	69.51
33	Eb	0°24'24.60''	40°58'48.19''	85	26.72	709.00	29.17	3	0	1.75	98.25
34	Eb	0°23'4.08''	41° 0'5.81''	225	34.42	561.00	70.51	3	0	96.94	3.06
35	Eb	0°23'31.90''	41° 0'35.87''	487	4.74	427.00	34.99	3	0	95.28	4.72

We obtained land-use data from Corine Land Cover 2006 data in a buffer area of 1 km around each stream location, and we characterized as a percentage of agriculture, forest and urban land use. Climatic (Mean annual precipitation) and geomorphologic (stream order, catchment area and altitude) data were achieved from Servei Meteorològic de Catalunya (http://www.meteocat.es) and Agència Catalana de l'Aigua (ACA) public data (http://aca.gencat.cat), respectively.

APPENDIX B

Characterization of drying components

Temperature and water level were recorded during a one year (during the study period, from February 2016 to February 2017), at hourly intervals, with Solinst level loggers (Edge, model 3001, the precision of 0.05%) installed on the bottom of a riffle of the streambed in each stream. In addition, we installed Baraloggers (Solinst Barologger, the precision of 0.05%) in the riparian zone of each sampling site.

We determined the daily variation in the streambed temperature as the difference between each day maximum and minimum temperature and the highest daily change rate per hour. The recorded temperature was corrected for atmospheric pressure variations applying data from the Baraloggers. After temperature data were rectified, we smoothed the daily differences with fifth-order moving average and standardized each result with a fixed value per month. The fixed value was obtained from meteorological stations data closest to each sampling site (from Servei Meteorològic de Catalunya; http://www.meteocat.es), data from field observation and water level data from the Leveloggers. Moreover, we adjusted the possible similarity between the streambed water temperature and the air temperature employing the precipitation data from the meteorological stations, according to Arias-Real et al., (2021).

APPENDIX C

Table 1. Pearson correlation coefficient the drying components: zero-flow total days (ZFT), zero-flow period (ZFP), zero-flow last days (ZFL) and rewetting days (RE). Correlation coefficients r ≥ | 0.70 | are in bold.

	ZFT	**ZFP**	**ZFL**	**RE**	**Temp**
ZFP	0.58				
ZFL	**0.78**	0.10			
RE	-0.62	-0.45	-0.51		
Temp	-0.42	-0.18	-0.39	-0.25	
Alt	-0.13	0.01	-0.19	0.40	-0.23

APPENDIX D

Table 1. Median, mean, maximum and minimum of the drying components in the 35 streams selected: zero-flow total days (ZFT), zero-flow period (ZFP), zero-flow last days (ZFL) and rewetting days (RE).

	Annual drying elements		**Previous drying elements**	
	Zero-flow total (days) ZFT	**Zero-flow period (n) ZFP**	**Zero-flow last (days) ZFL**	**Rewetting (days) RE**
Median	95	2	41	51
Mean	109	2	70	81
Maximum	324	8	294	274
Minimum	0	0	0	6

APPENDIX E

Table 1. Ranking of linear models for DOC and DOM optical qualitative parameters. Only the most explanatory models are showed (ΔAICc ≤ 2) for each response variable. Predictor labels: annual drying duration (ZFT), annual drying frequency (ZFP), duration of the latest dry event (ZFL) and rewetting days since latest dry event (RE), altitude (Alt) and water temperature (Temp). R2: percentage of variance explained in each model. AICc: Akaike Information Criterion for small sample sizes, ΔAICc: AIC differences, and wi: model weight

Variables	ZFT	ZFP	ZFL	RE	Alt	Temp	R^2	AICc	ΔAICc	*Wi*
DOC	0.34	0.16			0.06	0.07	0.47	53.20	0.00	0.51
DOC	0.44				0.07	0.09	0.43	53.27	0.07	0.49
SUVA254					0.05	0.16	0.23	19.14	0.00	0.36
SUVA254			-0.08		0.03	0.13	0.27	19.52	0.38	0.30
SUVA254	-0.06				0.04	0.13	0.25	20.43	1.29	0.19
SUVA254		-0.05			0.05	0.15	0.25	20.77	1.63	0.16
HIX		0.18		-0.22	-0.12	-0.19	0.43	56.31	0.00	0.36
HIX		0.29	0.17		-0.14	-0.05	0.42	57.03	0.71	0.25
HIX				-0.32	-0.09	-0.24	0.38	57.18	0.87	0.23
HIX		0.29			-0.19	-0.13	0.37	57.84	1.53	0.17
FI	-0.03				0.00	0.03	0.27	-67.47	0.00	0.47
FI				0.03	0.00	0.05	0.26	-66.58	0.89	0.30
FI					0.01	0.04	0.19	-66.08	1.38	0.23

BIX				0.04	-0.01	0.01	0.23	-84.64	0.00	0.23
BIX	-0.04				-0.01	-0.02	0.23	-84.55	0.09	0.22
BIX		-0.02		0.03	-0.01	0.00	0.26	-83.82	0.83	0.15
BIX	-0.03	-0.02			0.00	-0.01	0.26	-83.47	1.18	0.13
BIX	-0.02			0.02	-0.01	0.00	0.24	-82.79	1.86	0.09
BIX			0.02	0.05	-0.01	0.02	0.24	-82.78	1.87	0.09
BIX		-0.03			0.00	0.00	0.19	-82.69	1.96	0.09
E2:E3		0.23	0.20		-0.05	-0.15	0.75	6.41	0.00	0.72
E2:E3		0.27	0.26	0.08	-0.07	-0.10	0.76	8.26	1.85	0.28
Sr		-0.30	-0.30	-0.21	0.10	-0.02	0.47	40.37	0.00	0.32
Sr		-0.20	-0.17		0.07	0.10	0.43	40.43	0.06	0.31
Sr	-0.24				0.06	0.09	0.38	41.11	0.75	0.22
Sr	-0.17	-0.11			0.08	0.10	0.40	42.03	1.66	0.14

11.ORIGINAL PUBLICATIONS

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Dissolved organic matter variability along an impacted intermittent Mediterranean river

Verónica Granados* and Andrea Butturini

Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona, Diagonal 643, 08028 Barcelona, Spain.

* Corresponding author: v.granados@ub.edu

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ABSTRACT

Dissolved organic matter variability along an impacted intermittent Mediterranean river

Hydrological variability is the key factor that modulates allochthonous inputs and in-river biotic processes and, thereby, the fate of dissolved organic matter (DOM) in rivers. However, little is known about how these factors, combined, change DOM quantity and quality along river courses.

This study explored how DOM quantity (in terms of dissolved organic carbon – DOC) and quality (in terms of optical properties) varied along a Mediterranean river, under contrasting hydrological conditions: drought and high flow. The study was performed in the Matarranya river, a system severely affected by water abstraction for agricultural irrigation and inputs of untreated residual waters.

DOM properties changed according to hydrology. DOM under high flow conditions was more terrigenous, humified, aromatic, and degraded and the concentration gradually increased downriver. In contrast, DOM was less degraded and more aliphatic under drought conditions. DOM spatial variability under drought and high flow conditions revealed that hydrology has a greater impact on DOM quality in headwaters than at downriver sites. Longitudinal changes in DOM were more evident under drought conditions. For instance, a longitudinal depletion of DOC, together with a decrease of the fresh DOM pool, was observed in a large fluvial segment (35 km long) that does not receive any notable anthropogenic inputs. In contrast, the contribution of the most aromatic and humified DOM pool was significantly higher downriver. This study confirms the role of hydrology as a main driver of DOM dynamics. Additionally, it shows that hydrological variability does not impact DOM properties uniformly along the river continuum. On the contrary, DOM properties are more sensitive to hydrological changes in headwaters than in downriver reaches.

Key words: Dissolved organic matter (DOM), river continuum concept (RCC), intermittent stream, hydrological variability, DOM dynamics, DOM optical properties, chemostasis

RESUMEN

Variabilidad de la materia orgánica disuelta a lo largo de un río mediterráneo impactado intermitentemente

La variabilidad hidrológica es el factor clave que modula las entradas alóctonas y los procesos bióticos en el río y, por lo tanto, el destino de la materia orgánica disuelta (MOD) en los ríos. Sin embargo, poco se sabe acerca de cómo estos factores combinados cambian la MOD y la calidad a lo largo de los cursos fluviales.

Este estudio explora cómo la cantidad de MOD (en términos de carbono orgánico disuelto - COD) y calidad (en términos de propiedades ópticas) varía a lo largo de un río mediterráneo, bajo condiciones hidrológicas opuestas: sequía (caudal muy bajo) y caudal alto. El estudio se realizó en el río Matarranya, un sistema severamente afectado por la extracción de agua para riego agrícola y aportes puntuales de aguas residuales no tratadas.

Las propiedades de la MOD cambiaron de acuerdo con la hidrología. La MOD bajo condiciones de caudal alto era más terrígena, humificada, aromática, degradada y la concentración aumentaba gradualmente aguas abajo. Por el contrario, la MOD estaba menos degradada y más alifática en condiciones de sequía. La variabilidad espacial de la MOD bajo condiciones de sequía y caudal alto reveló que la hidrología afecta la calidad de la MOD, siendo más severa en la cabecera que en el tramo *final del río. Los cambios longitudinales de la MOD fueron más evidentes en condiciones de sequía. Por ejemplo, en un segmento fluvial que no recibe ninguna aportación antropogénica notable (con 35 km de extensión), se observó una reducción de COD a largo del eje longitudinal, junto con una disminución de la MOD menos degradada. Por el contrario, la contribución de la MOD más aromática y humificada aumentó significativamente río abajo. Este estudio confirmó a la hidrología como impulsora principal de la dinámica de la MOD. Además, se evidencia que la variabilidad hidrológica no afecta a las propiedades de MOD uniformemente a lo largo del continuo fluvial. Por el contrario, las propiedades de MOD son más sensibles a los cambios hidrológicos en la cabecera que en curso bajo del río.*

Palabras clave: *Materia orgánica disuelta, concepto de río continuo, río intermitente, variabilidad hidrológica, dinámicas del MOD, propiedades ópticas del MOD, químio-homeostasis*

INTRODUCTION

Dissolved organic matter (DOM) is a complex mixture of soluble organic compounds that vary in their reactivity and ecological role (Fellman *et al.*, 2010) DOM includes complex, polymeric compounds (humic and fulvic substances) as well as simple, well-defined molecules (sugars, proteins, lipids, organic acids, phenols, alcohols, among others). The molecular weights of DOM compounds rang from less than 100 to over 300 000 Daltons (Hayase & Tsubota, 1985; Thurman, 1985). These compounds can originate from terrestrial or aquatic sources as well as from anthropogenic inputs, such as treated and untreated wastewater effluents (Leenheer, 2009). DOM plays an important role in aquatic food webs, because it supplies energy, carbon (Wetzel, 1992) and nitrogen (Keil & Kirchman, 1991) for the heterotrophic community. However, the extent to which the dissolved organic compounds are metabolized in freshwater ecosystems depends on their biochemical composition (Benner, 2003). In freshwater ecosystems, DOM represents approximately 25 % of the total carbon transported downriver (Schlesinger & Melack, 1981; Meybeck, 1982) and its transport, reactivity and fate along fluvial courses is the product of internal (autochthonous) transformation processes and external (allochthonous) inputs from tributaries, occasional anthropogenic outlets (e.g., wastewater treatment plants, industries and urban settlements) and diffuse inputs from groundwater. The quantity and quality of allochthonous DOM inputs are strongly modulated by hydrology and, especially, the occurrence of extreme episodes such as droughts and floods (Buffam *et al.*, 2001; Butturini *et al.*, 2016).

Longitudinal DOM dynamics were theorized by the River Continuum Concept (RCC) (Vannote *et al.*, 1980). According to this model, DOM heterogeneity and processing is expected to decrease from upstream to downstream, whereas the contribution of recalcitrant molecules is expected to increase (Fellman *et al.*, 2014). Thus, larger and more reactive molecules would be more abundant in headwaters while smaller and more recalcitrant molecules would be more plentiful downriver (Amon & Benner, 1996). More recently, Creed *et al.* (2015) suggested a tendency towards a convergence/confluence of DOM concentration and composition along the river continuum. According to Creed's approach, high DOM variability and low DOM reactivity is expected in headwaters. In contrast, low DOM variability is expected downriver.

However, these scenarios ignore the impact of a succession of inputs from natural and anthropogenic tributaries (Fisher *et al.*, 2004) and the occurrence of extreme hydrological episodes (Butturini *et al.*, 2016). For instance, low in-stream DOM processing may be expected during floods due to low water residence times (Raymond *et al.*, 2016). Therefore, terrestrially-derived molecules may reach lowland river sites. During drought, long water residence times may stimulate in-stream microbial respiration (Maranger *et al.*, 2005), photochemical oxidation (Gao & Zepp, 1998) and primary production (Findlay & Sinsabaugh, 2003), thus increasing the amount of potentially labile compounds.

Predictions formulated by the RCC and successive extension and/or readjustments (Junk *et al.*, 1989; Creed *et al.*, 2015; Raymond *et al.*, 2016) motivated the study of DOM longitudinal transport and fate along fluvial networks (Fell-

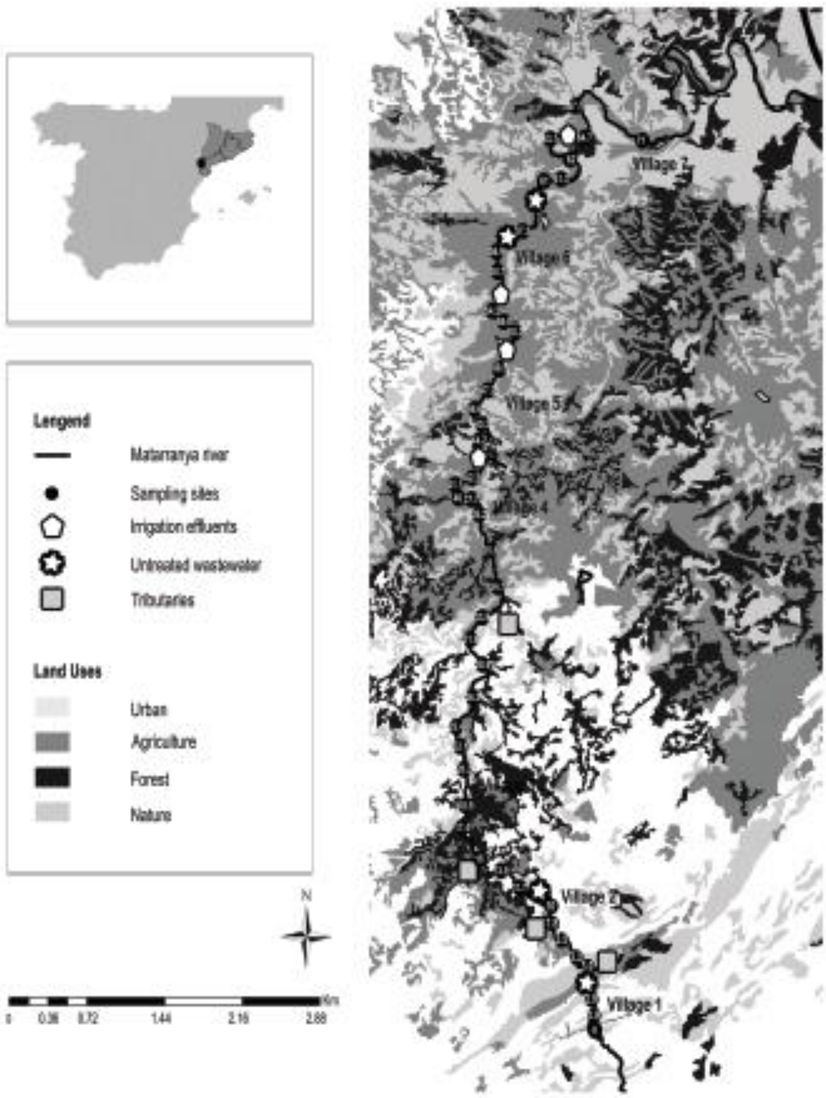

Figure 1. Matarranya river main stem and land uses. Black dots are sampling sites (numbers inside black dots indicate the distance in km from headwaters). Villages along the river are named with numbers (from 1 to 7). The villages are, beginning from the headwaters: Beseit, Vall-de-roures, La Torre del Comte, Massalió, Maella, Favara and Nonasp. *Eje principal del río Matarranya y usos del suelo. Los puntos de muestreo están indicados con círculos negros (los números en el interior de los círculos negros, muestran la distancia en km desde la cabecera). Los pueblos que se encuentran a lo largo del río, están indicados con números (del 1 al 7). Los pueblos son, desde la cabecera: Beseit, Vall-de-roures, La Torre del Comte, Massalió, Maella, Favara and Nonasp.*

man *et al.*, 2014; Kaushal *et al.*, 2014; Creed *et al.*, 2015; Wollheim *et al.*, 2015; Butturini *et al.*, 2016). Most of these studies were performed under baseflow conditions, whereas few studies have focused on drought and floods (Butturini *et al.*, 2016; Ejarque *et al.*, 2017). However, data obtained under droughts and floods are essential to understand the biogeochemical functioning of fluvial ecosystems. This is especially crucial in Mediterranean systems because a change in the frequency and magnitude of both hydrological extremes is expected in Mediterranean catchments (Vicente-Serrano *et al.*, 2014; Barrera-Escoda & Llasat, 2015).

In this context, we aimed to analyse the quantitative and qualitative changes in DOM along a longitudinal continuum of an intermittent Mediterranean river, under two contrasting hydrological conditions: drought and high flows. Our two research questions were: i) How do flow extremes determine DOM properties? ii) How does the river continuum modulate the impact of hydrology on DOM properties? According to Creed's hypothesis, we expected a reduction in the contribution of aromatic substances and concurrent increases in small and aliphatic molecules downriver. In parallel, differences in DOM quantity and quality between high and low flow conditions were expected to become smaller along the fluvial continuum. To achieve our aims, the main stem of a large intermittent river (70 km long) was sampled at high spatial frequency. DOM characterization included its quantification in terms of carbon (DOC) and qualitative DOM descriptors through spectroscopy techniques. Euclidean distance was also estimated to quantify the impact of hydrological conditions on DOM longitudinal patterns.

MATERIAL AND METHODS

Study area

This study was performed along the Matarranya river, the last important tributary joining the Ebro from the right-hand side of the river. It drains a catchment of 1738.45 km^2 from Els Ports de Tortosa-Beseit (1296 m a.s.l.) to the Ribarroja reservoir 100 km downstream (70 m a.s.l.). The basin is part of the geological Maestrazgo–Catalánides domain, which can be divided into two main areas: 1) Els Ports de Beseit, dominated by Mesozoic materials, in the headwaters; 2) The Ebro depression, dominated by Cenozoic (Tertiary and Quaternary) materials, in the middle and final sections of the Matarranya river. The geology is dominated by rocks of terrigenous origin (conglomerates, sandstones and marls), but limestones and evaporites (gypsum) outcrop along the entire route of the riverbed. The catchment is covered by brushwood (33 %), forest (14 %), urban areas (14 %) and agriculture (33 %), the latter especially in the middle and downriver reaches. The climate is relatively warm and arid. Downriver, air temperature and annual rainfall average 14-16 ºC and 350 mm respectively. In contrast, in the headwaters air temperature and annual rainfall average 12-14 ºC and 700-900 mm (El plan hidrológico del río Matarraña, 2008). The river shows a typical Mediterranean hydrology, characterized by moderate-high flow conditions in spring and autumn and a severe dry period in summer. The fluvial network contributes 156 hm^3 to the Ebro discharge. It comprises four main headwater tributaries (Matarranya, Ulldemó, Pena and Tastavins). Downriver the largest tributary is the Algars river. Fluvial hydrology is altered by human activities. There are two main reservoirs: the Pena reservoir (17 hm^3) and Vallcomuna (2.42 hm^3) as well as numerous small reservoirs in the catchment. Finally, a dense drainage system takes water from the river to irrigation systems during spring and summer. Since 1995, water from the Matarranya has been diverted into the Pena reservoir in winter (El plan hidrológico del río Matarraña, 2008). There are seven villages along the river. In the text they are named from "1" (headwaters) to "7" (downriver). The full names of the villages are given in the caption of Figure 1.

Sampling strategy

The Matarranya river was sampled along its main stem and at the main natural and anthropogenic inflows to the main stem (Fig. 1). The main stem was sampled at 45 sites, from the headwaters (2 km from source; 40° 48' 57.87" N / 0° 11' 11.94"

E) to its mouth (71 km from source; 41° 12' 40.13" N / 0° 14' 57.74" E). The surface water was sampled in the middle of the river channel. The distance between sampling sites averaged 1.6 ± 0.9 km. Sampling sites were selected in order to take into account all main tributaries (at kilometres 3, 7.5, 14, 29), untreated wastewater inputs (at kilometres 2.5, 8.5, 46, 55.6) and irrigation return waters (at kilometres 38, 40, 48.8 and 62). Samples were collected during two opposite contrasting hydrological conditions. The first set of samples was collected in July 2015, under summer drought conditions. The second set was collected under high flow conditions in November 2015, after a severe high flood event.

Chemical and field measurements

Field measurements

Electrical conductivity (EC, WTW 3310 set 1 conduct-meter) and water temperature were measured at each site. At the end of each sampling day, when samples were at the same temperature, DOM fluorescence in unfiltered samples was measured using two fluorescence sensors: a humic-like sensor (TurnerDesigns Cyclops 7, Ex/Em, 325/470nm) and a tryptophan-like sensor (TurnerDesigns Cyclops 7, Ex/Em, 285/350nm).

Chemical analyses

For chemical analyses, all samples were filtered in the field using precombusted (450 °C) glass fibre filters (Whatman GF/F 0.7 µm pore size) and then 0.22 µm pore nylon filters. The filtered samples were placed in amber glass bottles previously washed with acid. We stored the samples on ice and in the dark, and immediately transported them to the laboratory where they were stored at 4 °C for later analysis. Chemical parameters were grouped as follows: inorganic solutes (named DIM) included: electrical conductivity, chloride, sulphate, $^{18}\Delta O$, ΔD, ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}); DOM descriptors (named DOM) included: DOC, HIX, FI, BIX, Sr, E2:E3, $SUVA_{254}$, $SUVA_{350}$, $Fluor_{Humic-like}$ and $Fluor_{Prot-like}$.

Inorganic solutes

Ammonium concentration was measured using the salicylate method (Reardon, 1969), and the soluble reactive phosphor (SRP) was measured using the molybdate method (Murphy & Riley, 1962). Inorganic anions (nitrate, chloride and sulphate) concentrations were analysed with an ion chromatograph Metrohm 761 Compact IC with the column Metrosep A Supp5 - 150/4.0.

To perform a better geochemical discrimination between the two opposite hydrological conditions stable water isotopes $^{18}\Delta O$ and ΔD were analysed (Laudon & Slaymaker, 1997). Stable isotopes analysis were performed at the Scientific and Technological Centre of the University of Barcelona. For $^{18}\Delta O$ the equilibrium was achieved with CO_2 and He and measurements were executed on a MAT 253 from Thermofisher. ΔD was measured by water pyrolysis analysis of the resulting H_2 separated by column chromatography on an EA-TC-IRMS-DELTA PLUS xp Thermofisher.

DOC concentration and optical properties of DOM

Samples for DOC analysis only were filtered by glass fiber filters (Whatman GF/F 0.7 µm pore size) and later, they were acidified with 10 % HCl and refrigerated before analysis. DOC was determined by oxidative combustion and infrared analysis using a Shimadzu TOC Analyser VCSH coupled with a TN analyser unit. Qualitative optical properties of DOM were estimated in terms of absorbance and fluorescence. DOM absorbance spectra were measured using a UV-Visible spectrophotometer UV1700 Pharma Spec (Shimadzu) and a 1 cm quartz cell. Absorbance data were obtained in double beam mode with wavelength scanned from 200 to 800 nm and deionized water as the blank. Excitation-Emission Matrices (EEM) were generated by an RF-5301 PC spectrofluorimeter (Shimadzu). Spectra were measured using a 1 cm quartz cell. EMMs were measured over (Ex/Em) wavelengths of 240-420 nm and 280-690 nm and they were standardized based on the method of Goletz *et al.* (2011) using Mathematica (Wolfram

Research) software. EEM data were corrected using the same method. The absorbance data for each sample respectively were used to correct the inner filter effects (Lakowicz, 2006). To correct wavelength-dependent inefficiencies of the detection system the following methods were used: Gardecki & Maroncelli's method (1998) for emission measurements and Lakowicz's method (2006) for excitation correction. Data were normalized with daily measurements of the area under the Raman peak using MilliQ water blanks (Lawaetz & Stedmon, 2009).

Seven qualitative descriptors of DOM were estimated in this study The four chromophoric indices were as follows: (a) Specific Ultra Violet Absorbance at 254 nm ($SUVA_{254}$) and (b) Specific Ultraviolet Absorbance at 350 nm ($SUVA_{350}$) as the absorption coefficient at 254 or 350 nm normalized by DOC concentration (Weishaar *et al.*, 2003). Higher values are typically related to greater aromaticity (Hansen *et al.*, 2016). (c) The ratio of absorbance at 250 nm to 365 nm (E2:E3), which provides information about DOM molecular size. Low E2:E3 values suggest a proportional increase in average DOM size (De Haan & De Boer, 1987). (d) Spectral slopes ratio (S_R), which also integrates shifts in the molecular size of DOM. This is a dimensionless parameter, estimated by calculating the ratio of the logarithmically transformed absorbance spectra slope at 275–295 nm (S275–295) to that estimated at 350–400 nm (S350–400). High S_R values indicate an increase in the proportion of the small DOM molecular fraction (Helms *et al.*, 2008). Three fluorophoric indices were also estimated: (a) Humification index (HIX), which is the area under the emission spectra 435-480 nm divided by the peak area 310-345 nm from the spectra at an excitation wavelength of 254 nm. HIX indicates the extent of humification by quantifying the shift in the emission spectra toward longer wavelengths, due to lower H:C ratios. HIX values range from 0 to 1 with higher values indicating a greater degree of DOM humification (modified from Ohno, 2002). (b) Fluorescence index (FI), which is the ratio of emission intensities at 470 nm and 520 nm emitted at an excitation of 370 nm and provides information about DOM sources. High values suggest the prevalence of autochthonous DOM and low values the prevalence of allochthonous DOM (Cory & McKnight, 2005). (c) Biological index (BIX), which was calculated at an excitation of 310 nm as the ratio of the fluorescence intensity emitted at 380 nm. β fluorophore is the maximum of intensity and emitted at 430 nm, which corresponds with humic fraction. The *β* fluorophore is typical of recent autochthonous DOM release. Therefore, high BIX values (> 1) suggest the presence of autochthonous and fresh DOM, whereas BIX values of 0.6–0.7 indicate low or zero autochthonous DOM production (Huguet *et al.*, 2009).

Hydrological measurements

During drought conditions, discharge was calculated at 19 sampling sites along the main reach. The velocity-area method (Di Baldassarre & Montanari, 2009) was used. A flowmeter (Global Water FP111 Flow, sensor range 0.1-6.1 m/s) was used to measure the mean water velocity. The river channel cross-section was divided into 0.2-0.5 m subsections (depending on the river width). The mean water velocity was estimated at 2/3 of the total depth. During high flow conditions, it was not feasible to estimate discharge in situ. Discharge values from the *Confederación hidrográfica del Ebro* were available for one headwaters site (40° 49' 27.82" N; 0° 11' 7.61" E) and one downstream site (41° 11' 42.42" N; 0° 10' 17.92" E). Values at these two sites were used to interpolate discharge along the entire longitudinal continuum.

Statistical analyses

The non-parametric Mann-Whitney-Wilcoxon test (Wilcoxon, 1945; Mann & Whitney, 1947) was used to test differences in the solute content between drought and high flow conditions. Correlations between variables were considered significant at the 5 % level. Spatial and contrasting hydrological conditions (high flow and drought) variability were explored using principal components analysis (PCA). Two PCAs were run: the first one included only the DIM solutes (PCA_{DIM}). The second PCA integrated

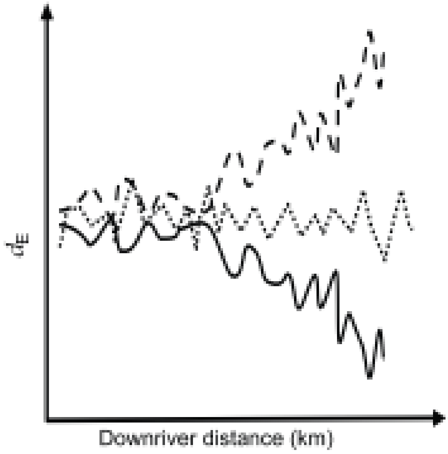

Figure 2. Three hypothetical trends in the dissimilarity index d_E with respect to the downriver distance. The dotted line describes the tendency when d_E is unrelated to the downriver distance. The dashed line describes the tendency when d_E increases downriver. The solid line describes the opposite situation, when d_E decreases downriver. Additional details are given in the statistical analyses section. *Tres tendencias hipotéticas del índice de disimilitud* d_E *respecto a la distancia aguas abajo. La línea de puntos describe la tendencia cuando* d_E *no está relacionada con la distancia aguas abajo. La línea discontinua muestra la tendencia cuando* d_E *aumenta río abajo. La línea continua describe la situación opuesta, cuando* d_E *disminuye aguas abajo. Para detalles adicionales, se encuentran en la sección de análisis estadísticos.*

ten DOM descriptors (PCA_{DOM}). Clusters in the PCA analysis were identified using the optimization significant test silhouette (Rousseeuw, 1987). In order to quantify how alterations in DOM and DIM properties, induced by hydrology, changed downriver, we estimated the Euclidean distance (d_E, thereafter) between scores obtained under drought and high flow conditions at each sampling site, and plotted these distances with respect to the downriver distance. The Euclidean distance, d_E, describes the biogeochemical dissimilarity among samples collected at the same site but under the two contrasting hydrological conditions. Low d_E values indicate low biogeochemical dissimilarity (or high similarity) among hydrological conditions i.e. high

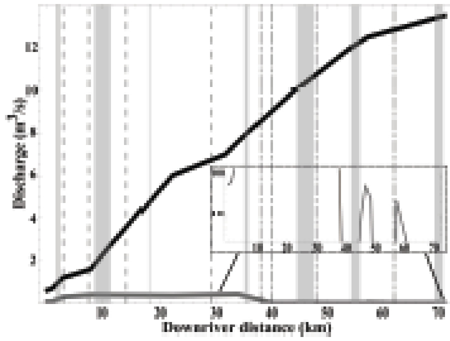

Figure 3. Longitudinal discharge profile along the river continuum during drought (horizontal grey line) and high flow (horizontal thin black line) conditions. Continuous vertical lines show the location of the seven villages next to the Matarranya river. The thickness of the lines is proportional to population size. Dashed vertical lines on the left show the location of the main affluent. Dashed vertical lines on the right are the irrigation return flows. The inset shows the discharge at most downriver sites during drought. Discharge under high flow conditions (thin black line) was interpreted for illustrative purpose only, because discharge was only available for kilometre 0.9 and 58. Discharges were interpolated assuming that the discharge increased downriver according to a potential model. *Perfil longitudinal del caudal a lo largo del continuo fluvial en condiciones de sequía (línea gris horizontal) y caudal alto (línea negra horizontal). Las líneas verticales continuas muestran la localización de los pueblos de alrededor del río Matarranya. El grosor de las líneas es proporcional al tamaño de la población. Las líneas discontinuas verticales de la izquierda, indican la localización de los afluentes principales. Las líneas discontinuas verticales de la derecha, indican la ubicación de los puntos de extracción de agua para el regadío. El recuadro muestra el caudal durante el periodo de sequía en la mayoría de puntos de muestreo aguas abajo. El caudal en condiciones de flujo alto (línea negra delgada) ha sido interpretado con una finalidad ilustrativa debido a que este solo está disponible del kilómetro 0.9 a 58. Los caudales fueron interpolados asumiendo que estos se incrementaron aguas abajo, se ha desarrollado a partir de un modelo potencial.*

chemostasis. High d_E values indicate the opposite (Fig. 2). Two dissimilarity indices were estimated: one for inorganic solutes ($d_{E\text{-}DIM}$) and the other for DOM ($d_{E\text{-}DOM}$) parameters. Normality was tested using the Kolmogorov–Smirnov test (Massey, 1951). The null hypothesis was rejected at 5 %. Mathematica (Wolfram Research) software was used for all statistical analyses.

RESULTS

Hydrology

Under drought conditions, discharge increased gradually from 0.055 to 0.55 m^3/s from the source to kilometre 12 (just after village 2). It then decreased to 0.4 m^3/s at kilometre 35. From this site, all water was taken for irrigation and runoff was nil, except in villages 5 and 6. From kilometre 40 to the last sampling site (kilometre 71) the fluvial continuum vanished, and water was confined to isolated and stagnant pools. The discharge profile was clearly different under high flow conditions. According to the information provided by the *Confederación hidrográfica del Ebro*, discharge increased from 0.61 m^3/s between the source and kilometre 58 to 12.5 m^3/s at the final downriver site (kilometre 71) (Fig. 3).

Inorganic solute concentrations. Drought vs. high flow

Under drought conditions, EC values ranged from 473 (headwaters) to 2060 µS/cm (kilometre 47) and were significantly higher than during high flow ($U = 1597$, $p < 0.001$; Fig. 4a). The highest increases started at kilometre 40 and

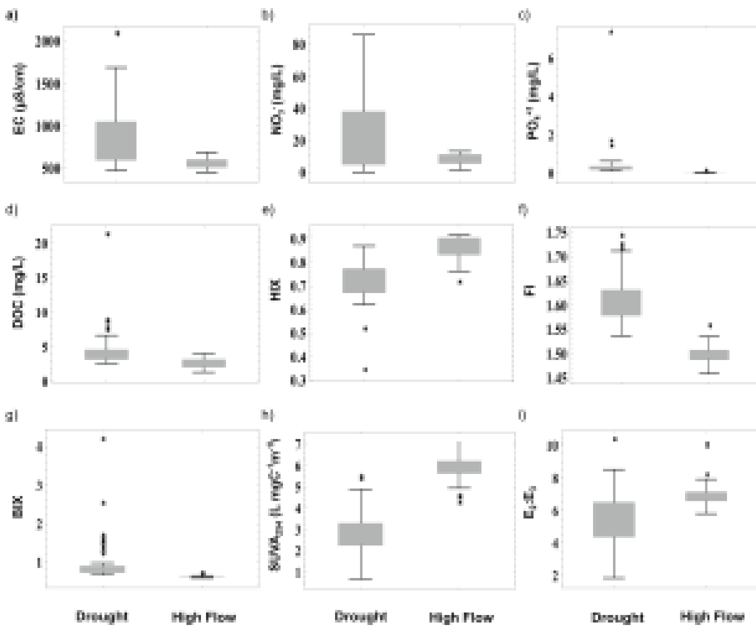

Figure 4. Box plots summarize the differences between drought (left box plot) and high flow (right box plot) conditions for the following variables: a) electrical conductivity (EC), b) nitrate (NO_3^-), c) phosphate ($PO4^{3+}$), d) dissolved organic carbon (DOC), e) humification index (HIX), f) fluorescence index (FI), g) biological index (BIX), h) specific ultra-violet absorbance at 254 nm ($SUVA_{254}$) and i) ratio of absorbance at wavelength 250nm:365nm (E_2:E_3). Asterisks symbolized the outliers of each variable. *Los diagramas de caja resumen las diferencia entre condiciones de sequía (diagrama de caja de la izquierda) y caudal alto (diagrama de caja de la derecha) para las siguientes variables: a) conductividad eléctrica (EC), b) nitrato (NO_3^-), c) fosfato ($PO4^{3+}$), d) carbono orgánico disuelto (DOC), e) índice de humificación (HIX), f) índice de fluorescencia (FI), g) índice biológico (BIX), h) absorbancia específica ultra-violeta a 254 nm ($SUVA_{254}$) y i) ratio de absorbancia en una longitud de onda de 250nm:365nm (E_2:E_3). Los asteriscos indican valores atípicos de cada variable.*

peaks occurred at kilometres 47, 56 and 62 (corresponding to wastewater effluents from villages 5, 6, and irrigation return flows, respectively) (Fig. 5a). During high flow, conductivity increased slightly along the fluvial continuum from 451 (headwater) to 671 µS/cm (downriver). Abrupt increases were minor and occurred at kilometre 15 (after the confluence with the Tastavins affluent) and at kilometre 56 (village 6). Nitrate (NO_3^-) concentrations were significantly higher during drought conditions than during high flows (U = 1485, $p < 0.001$; Fig. 4b) with values oscillating from 0.59 to 86 mg/L. Lower concentrations were typically found in the headwaters, but the concentration increased abruptly to 32 mg/L at kilometre 18 (village 3) (Fig. 5b). Downstream, the NO_3^- concentration remain relatively stable until kilometre 45 and additional abrupt increases were detected downstream from the untreated wastewater inputs at village 5 (kilometre 47, 86.01 mg/L) and 6 (kilometre 57, 70.10 mg/L). Under high flows, the NO_3^- concentration increased from 1.34 (headwaters) to 12 mg/L (kilometre 57) then decreased to less than 2 mg/L up to kilometre 71. Phosphate (PO_4^{-3}) concentrations were significantly higher during drought (U = 2025, $p < 0.001$; Fig. 4c). PO_4^{-3} concentrations ranged between 0.10 and 7.13 mg/L during drought (Fig. 5c). The highest concentrations were recorded at kilometres 10, 47 and 56 just downstream from the untreated wastewaters at villages 2, 5 and 6, respectively. During high flow, phosphate concentrations were typically lower than the detection level (< 0.005 mg/L) in headwaters and around 0.03 mg/L at downstream sites.

DOM concentrations and properties. Drought vs. high flow

DOM was significantly higher under drought conditions than during high flow (in terms of DOC concentration, U = 1747, $p < 0.001$, Fig. 4d). It was also less humified (in terms of HIX, U = 150, $p < 0.001$; Fig. 4e), less aromatic (in terms of $SUVA_{254}$, U = 18, $p < 0.001$; Fig. 4h), more autochthonous (in terms of FI, U = 2022, $p < 0.001$; Fig. 4f), more fresh (in terms of BIX, U = 20225, $p < 0.001$; Fig. 4g) and smaller in size (in terms of E2:E3, U = 284, $p < 0.05$, Fig. 4i). DOC concentrations ranged between 2.60 and 21.05 mg/L during drought conditions, with no clear longitudinal trend (Fig. 5d). However, the largest abrupt concentration increases coincided with untreated wastewater inputs (kilometres 10, 47 and 56) and an irrigation return flow (kilometre 49). Under high flow, DOC concentrations increased progressively downriver from 1.30 (kilometre 0.1) to 4.05 mg/L (kilometre 71). HIX values ranged between 0.34 and 0.87 during drought. The lowest values were found between kilometres 12 (village 2) and 17 (village 3) (Fig. 5e). Downriver from these sites, the HIX values gradually increased up to 0.85. Under high flow, HIX values ranged between 0.76 and 0.91. The lowest values were detected in the headwaters (from kilometre 0 to 14). Thereafter they remained relatively constant at around 0.9. FI fluctuated between 1.54 and 1.74 and tended to decrease along the river continuum during drought (Fig. 5f). The highest values were detected at kilometre 0.9 (headwater) and 10, 47 and 56 (coinciding with untreated wastewater inputs). On the other hand, FI did not show any longitudinal trend under high flow conditions. Peak FI values coincided with villages 1, 2, 3, 5 and 6. Under drought conditions, the highest BIX value was detected at kilometre 10 (coinciding with wastewater input from village 2, Fig. 5g). Downstream, the BIX value decreased rapidly to less than 1. However, two additional peaks were detected at kilometres 17 and 26. During high flow, BIX values fluctuated between 0.56 and 0.66 along the main stem and did not show any longitudinal trend. $SUVA_{254}$ showed a tendency to increase downriver during both hydrological conditions. In contrast to other DOM descriptors, residual waters did not show any consistent change in $SUVA_{254}$. For instance, this index was slightly lower (or unchanged) at villages 2, 3 and 5, and only increased after village 6 during drought conditions (Fig. 5h). The spectral slope index (Sr) showed larger fluctuations and a longitudinal pattern during drought conditions than during high flow although the differences in Sr values between the two hydrological periods were not statistically significant (U = 1176, n.s.). Higher Sr values were detected in headwaters

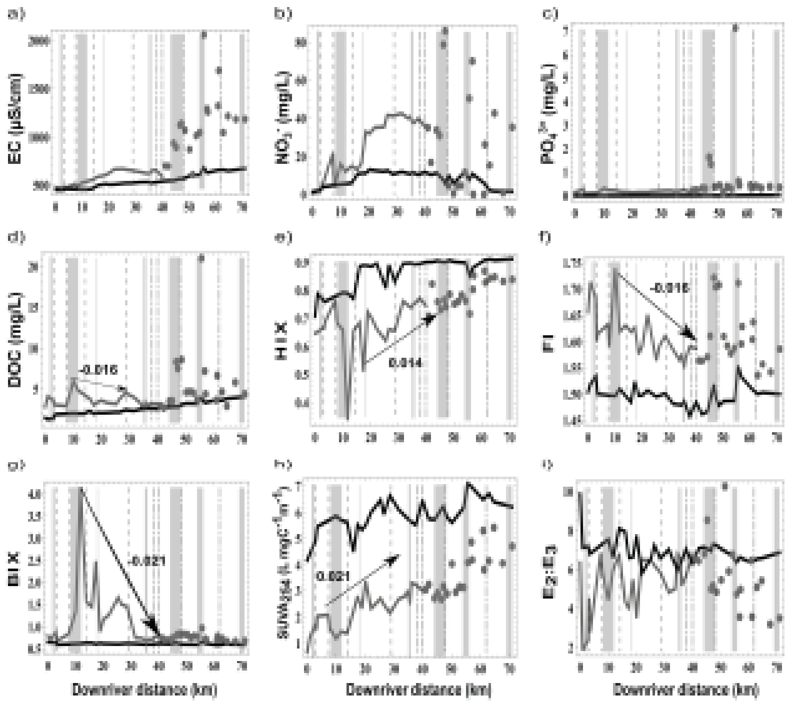

Figure 5. Longitudinal profiles, along the river continuum, of the same variables detailed in figure 4 during drought (horizontal grey line and grey dots) and high flow (horizontal black line) conditions. Grey dots remark that, from kilometer 40 the water samples were collected from isolated pools. Vertical lines are the same as those in figure 3. Arrows emphasize the longitudinal linear trend of some DOM parameters from kilometres 10 to 45. The values show the rescaled slope of these trends (statistical significance of all slopes are of $p < 0.01$). See discussion for additional information. *Perfiles longitudinales a lo largo del continuo fluvial, con las mismas variables detalladas en la figura 4 durante condiciones de sequía (línea gris horizontal y círculos grises) y condiciones de caudal alto (línea negra horizontal). Los círculos grises indican que a partir del kilómetro 40, las muestras de agua se han obtenido de las balsas aisladas. Líneas verticales son las mismas que en la figura 3. Las flechas enfatizan la tendencia lineal de algunos parámetros de la MOD desde el kilómetro 10 al 45. Los valores muestran la tendencia de las pendientes reescaladas (diferencias significativas de todas las pendientes con p < 0.01). Información adicional en la discusión.*

under drought conditions. Downstream Sr decreased rapidly until kilometre 27 and then gradually increased up to kilometre 68. On the other hand, the Sr values decreased slightly from 1.1 to 0.9 during high flow conditions. Under drought conditions the E_2:E_3 ratio showed marked oscillation between 2 and 10, with a minimum at kilometre 0.9 and a maximum at kilometre 52 (Fig. 5i). Under high flow E_2:E_3 was relatively steady between 5 and 8 along the longitudinal axis, with the highest value at the headwater site (E_2:E_3~10).

Principal component analysis and $d_{E\text{-}DIM}$ and $d_{E\text{-}DOM}$ along the fluvial continuum

Figure 6 shows the scores of the first and second components obtained from the PCA_{DIM} (the inorganic solutes). The two main components explained 59 and 18 % of the total variance

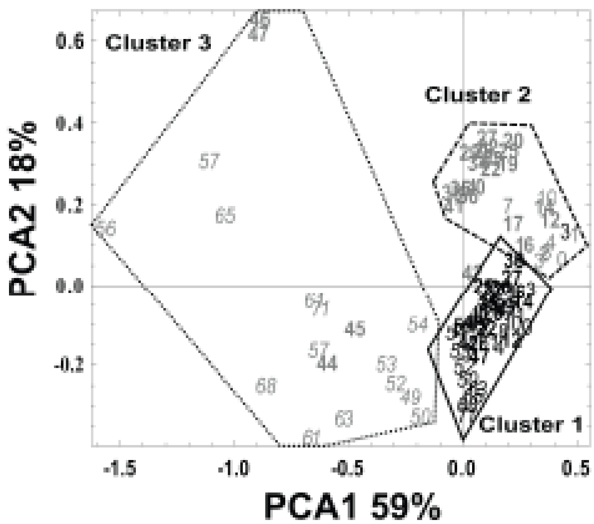

Figure 6. Principal component analysis of the inorganic solutes (PCA_{DIM}). Variables are: electrical conductivity, chloride, sulphate, $^{18}\Delta O$, ΔD, ammonium, nitrate and phosphate. Grey values are samples collected under drought conditions. Black values are samples collected under high flow conditions. Values indicate the distance (in kilometres) from the headwaters. Normal font show headwaters sites from 0 to 10 km. Bold font show middle channel sites from 11 to 47 km. Italic font show downstream sites from 48 to 71 km. Clusters were obtained using the Silhouette significance test. *Análisis de componente principal de solutos inorgánicos (PCA_{DIM}). Las variables son: conductividad eléctrica, cloruro, sulfato, $^{18}\Delta O$, ΔD, amonio, nitrato y fosfato. Valores en gris indican el resultado en condiciones de sequía y en negro, condiciones de caudal alto. Los números indican la distancia (en kilómetros) desde la cabecera del río. La fuente normal presenta los puntos de muestreo situados en la cabecera, desde 0 a 10 km. En negrita, muestra los ubicados en el cauce medio desde los 11 kilómetros a los 47. En cursiva, los puntos de muestreo aguas abajo desde los 48 kilómetros a los 71. Los grupos fueron obtenidos a partir de la prueba de significancia de silueta.*

respectively. PCA1 separated the data, under drought conditions, according to their location. Silhouette analysis identified three main clusters: Cluster #1 (negative PCA2 scores) integrated all data collected during high flow conditions. Cluster #2 (positive PCA2 scores) integrated data from headwaters and middle reaches (up to kilometre 45) under drought conditions. Cluster #3 (negative PCA1 scores) was the most heterogeneous and integrated mainly data from downstream sampling sites (from kilometre 46 to kilometre 71) under drought conditions.

PCA of the DOM parameters explained 78 % of total data set variance (Fig. 7). PCA1 axis (59 % of total variance), separated the data set according to hydrology. High flow conditions were associated with negative PCA1 scores and drought conditions with positive PCA1 scores. The inset in figure 7 shows that PC1 scores decreased from nil to more negative values from headwaters to middle reaches and downstream reaches. PCA2 explained 19 % of the total variance. This axis separated the data collected under drought conditions according to their location along the main stem: headwaters and middle reaches with positive scores and downstream with negative scores. Data separation along the fluvial continuum was much more gradual during high flow conditions. Silhouette analysis revealed two clusters: Cluster #1 integrated samples collected from headwaters and middle reaches during the drought episode. Cluster #2 integrated samples collected during high flow and most of the downstream samples collected during drought.

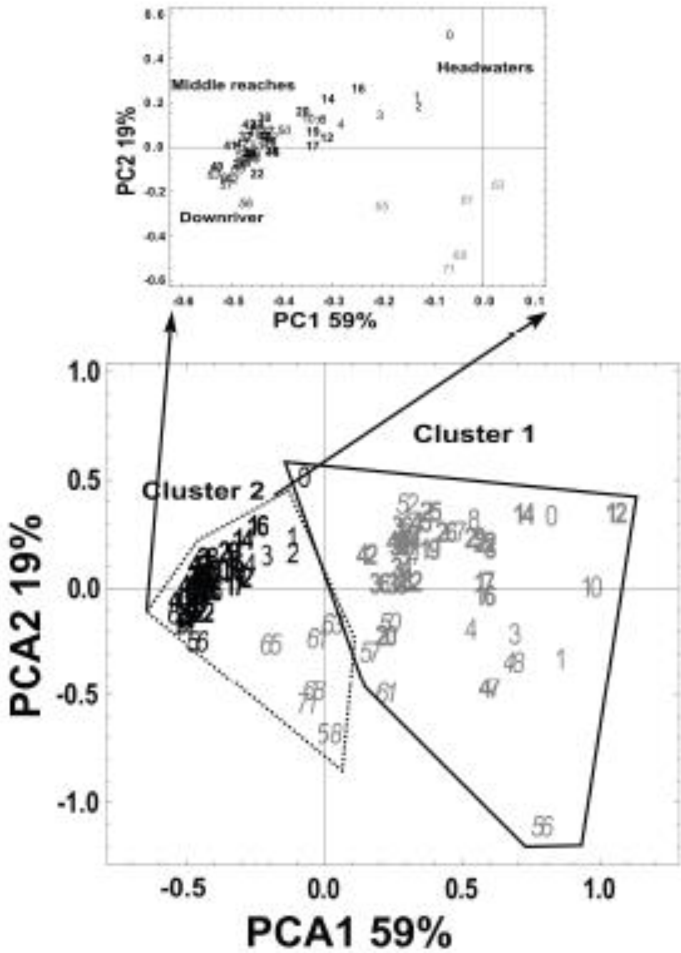

Figure 7. Principal component analysis of the dissolved organic matter parameters. Variables are: DOC, HIX, FI, BIX, Sr, E2:E3, $SUVA_{254}$, $SUVA_{350}$, $Fluor_{Humic\text{-}like}$ (FlHu) and $Fluor_{Prot\text{-}like}$ (FlPr). Grey values are samples collected under drought conditions. Black values are samples collected under high flow conditions. Values indicate the distance (in kilometres) from the headwaters. Normal font show headwaters sites from 0 to 10 km. Bold font show middle channel sites from 11 to 47 km. Italic font show downstream sites from 48 to 71 km. Clusters were obtained using the Silhouette significance test. The inset expands the PCA plot for data collected during high flow conditions. *Análisis de componente principal de los parámetros de la materia orgánica disuelta. Las variables son: DOC, HIX, FI, BIX, Sr, E2:E3, $SUVA_{254}$, $SUVA_{350}$, $Fluor_{Humic\text{-}like}$ (FlHu) and $Fluor_{Prot\text{-}like}$ (FlPr). Valores en gris indican el resultado en condiciones de sequía y en negro, condiciones de caudal alto. Los números indican la distancia (en kilómetros) desde la cabecera del río. La fuente normal presenta los puntos de muestreo situados en la cabecera, desde 0 a 10 km. En negrita, muestra los ubicados en el cauce medio desde los 11 kilómetros a los 47. En cursiva, los puntos de muestreo aguas abajo desde los 48 kilómetros a los 71. Los grupos fueron obtenidos a partir de la prueba de significancia de silueta. El recuadro amplía el gráfico de la PCA para los datos obtenidos en caudal alto.*

Figure 7 presents the longitudinal trend of the dissimilarity indices obtained for the DIM and DOM parameters (d_E-DIM and $d_{E\text{-}DOM}$ respectively) along the main stem of Matarranya river. A gradual and significant decrease in $d_{E\text{-}DOM}$ was detected along the river continuum ($r^2 = 0.1$, d.f. = 43, $p < 0.05$). This trend suggests that the qualitative properties of DOM tended to be unrelated to hydrological oscillations at the downriver sites. The trend is even more significant if the abrupt increase in dissimilarity that emerged downstream from the untreated wastewater inputs at village 2 (kilometre 10), village 5 (kilometre 46) and village 6 (kilometre 55) is removed ($r^2 = 0.45$, d.f. = 43, $p < 0.001$). On the other hand, the dissimilarity index of inorganic solutes (dE-DIM) showed the opposite trend to the DOM trend and increased significantly downriver ($r^2 = 0.32$, d.f. = 43, $p < 0.001$). However, dE-DIM was low and relatively constant from the headwaters to kilometre 40 and the dissimilarity index increased abruptly downstream from the wastewater inputs.

DISCUSSION

This study clearly demonstrates that hydrological conditions strongly affect the concentration of inorganic solutes (DIM) and quantity and properties of organic solutes (DOM) that flow along a fluvial continuum. However, the significance of the impact depends on biogeochemistry composition (i.e. DIM or DOM) and, additionally the impact is not uniformly distributed throughout the fluvial continuum. The river continuum thus exerted some control on biogeochemical responses related to hydrological variability. This control was evident for DOM but not for DIM.

Drought vs. high flow conditions: DOM quality

DOM properties were affected by hydrological conditions as follows: DOM was fresher and less degraded (high BIX), and more aliphatic and less humified (low SUVA254 and HIX respectively) under drought conditions than under high flow conditions. This suggests that it is more autochthonous (high FI and low HIX) under drought conditions and more allochthonous (low FI and high HIX) under high flow conditions. This is consistent with other studies performed in human-altered Mediterranean rivers (Ejarque Gonzalez, 2014; Butturini *et al.*, 2016). However, some caution is necessary regarding the term "autochthonous". This term means that the DOM fraction is a byproduct of in-stream processes. However, it is well known that DOM from (treated and untreated) wastewater produces a fluorescent signal associated with small protein-like substances (Baker, 2002; Saadi *et al.*, 2006; Butturini & Ejarque, 2013). This fluorescence moiety modifies the excitation-emission florescence matrices, resulting in high FI and BIX values and low HIX values. Therefore, the high FI and BIX values detected under drought conditions do not necessarily indicate in-stream autochthonous DOM generation. On the contrary, the most relevant increases in BIX and FI were abrupt and coincided with villages 2, 5 and 6, revealing the severe impact of wastewater discharge from these villages. Therefore, it is important to take into account these anthropogenic inputs.

Overall, additional anthropogenic DOM inputs appeared to be minor between village 2 (kilometre 10) and village 5 (kilometre 45). Consequently, village 3 and 4 did not alter the DOM signal (although, nitrate increased sharply at village 4 under drought conditions). In addition, the two effluents that drain into the main stem (Tastavins and Calapatos) were almost completely dry during the summer sampling. Therefore, in this large 35-km-long fluvial segment, allochthonous (natural and anthropogenic) DOM inputs should be insignificant under drought conditions. Thus, longitudinal changes in qualitative DOM detected in this segment under drought conditions, might provide information about the magnitude of in-situ DOM processing. The results show that downriver from the suspected anthropogenic DOM inputs at village 2, DOM decreased slightly in terms of concentration (i.e. $dDOC/dX = -0.016$ km^{-1}) and increased in terms of aromaticity (i.e. $dSUVA/dX = 0.021$ km^{-1}) and humification (i.e. $dHIX/dX = 0.014$ km^{-1}). These findings suggest the longitudinal accumulation of aromatic and large molecules,

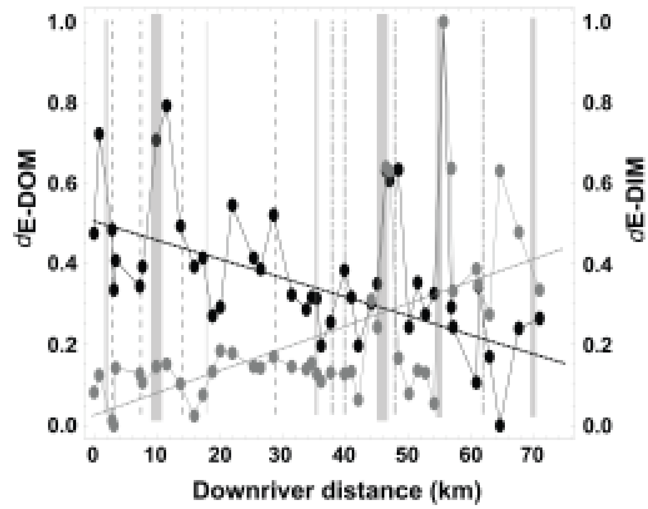

Figure 8. Longitudinal profile of the dissimilarity index of DIM ($d_{E\text{-}DIM}$, grey disks) and DOM properties ($d_{E\text{-}DOM}$, black disks) along the river continuum. Vertical lines are the same as those in figure 3. The dashed black and grey lines show the linear model ($p < 0.05$) that relates dissimilarity of the $d_{E\text{-}DOM}$ or $d_{E\text{-}DIM}$ index to the downriver distance respectively. *Perfil longitudinal del índice de disimilitud de la MID ($d_{E\text{-}DIM}$, discos grises) y de las propiedades de la MOD ($d_{E\text{-}DOM}$, discos negros) a lo largo del continuo fluvial. Las líneas verticales son las mismas que en la figura 3. Las líneas discontinuas horizontales negra y gris, indican un modelo lineal ($p < 0.05$) que muestra el índice de disimilitud $d_{E\text{-}DOM}$ o $d_{E\text{-}DIM}$ respecto a la distancia aguas abajo.*

probably as a consequence of the degradation of labile aliphatic substances and the concurrent accumulation of more refractory aromatic humics. The gradual decrease in the FI index (dFI/dX= -0.016 km^{-1}) and the decrease in the BIX index (dBIX/dX= -0.021 km^{-1}) also suggest the degradation of labile substances. Remarkably, $SUVA_{254}$ increased at a constant rate throughout the entire fluvial stem from kilometre 10 to 71. This increase, although less pronounced, was also significant under high flow conditions. Overall, this positive trend contrasts with that observed in la Tordera (Ejarque *et al.*, 2017) and that reported by Creed *et al.* (2015). They attributed the longitudinal decrease in aromatic substances to losses in allochthonous aromatic DOM and a parallel increase in autochthonous aliphatic DOM. Accordingly, this DOM processing route appeared to be inconsistent in the Matarranya river.

DOM dissimilarity trend along the river continuum

The dissimilarity index of the DIM pool ($d_{E\text{-}DIM}$) increased significantly downriver. However, a more detailed analysis revealed that $d_{E\text{-}DIM}$ was relatively steady, and relatively low, from kilometre 0 to 42, indicating that the chemical variability of DIM solutes related to high flow and drought conditions was unrelated to river size. The $d_{E\text{-}DIM}$ descriptor increased abruptly as a consequence of the impact of wastewater inputs at villages 5 and 6 under drought conditions, when, from kilometre 43 to 71, the river was totally dry and most of the discharge was from these anthropogenic sources. The position of these abrupt increases in $d_{E\text{-}DIM}$ (at kilometre 46 and 56 respectively) reduce the significance of the $d_{E\text{-}DIM}$ vs. downriver distance relationship. Therefore, the longitudinal increase in $d_{E\text{-}DIM}$

seems to be unrelated to river size: if wastewater inputs were located in the headwaters the observed positive longitudinal trend in $d_{\text{E-DIM}}$ would probably vanish.

In contrast, the $d_{\text{E-DOM}}$ showed a clear and robust tendency to decrease downriver. According to the results, hydrological oscillations affect the DOM composition of headwaters more severely than at downriver sites. The negative trend is robust enough to dampen the noise (i.e. abrupt high $d_{\text{E-DOM}}$ values) caused by the impact of anthropogenic wastewater inputs under drought conditions. Overall, this negative trend is consistent with the hypothesis proposed by Creed *et al.* (2015). These authors suggested that the impact of hydrological variability should decrease downriver and that a river will tend towards chemostasis. This situation is thought to reflect the hydrological, biogeochemical and biological factors that control variations in DOM quantity and quality in time and space. Accordingly, high DOM oscillations are expected in headwaters, where hydrological conditions determine the drainage of different soils and ground water sections and stimulate the leaching of allochthonous DOM. The hyporheic and riparian interfaces (Butturini *et al.*, 2003) are hydro-chemical compartments that can strength terrestrial-aquatic connections in headwaters and thus condition the mobilization and composition of DOM in these ecosystems (Vázquez *et al.*, 2007, 2015). On the other hand, the strength of these connections should decrease downriver. DOM in downriver reaches reflects the mixing of DOM inputs from allochthonous (natural and/or anthropogenic tributaries and groundwater) and autochthonous sources. In fact, low river gradients, enhanced water residence times and instream autochthonous biogeochemical processes are reported to be more relevant in downriver reaches.

CONCLUSION

This study demonstrates the drastic impact of the hydrological regime on DOM properties. More significantly, it reveals that variations in DOM are more notable in headwaters than downriver reaches. This result is consistent with the chemostatic hypothesis formulated by Creed *et al.* (2015). DOM properties drastically changed with both hydrological regimes. Terrigenous, humified, aromatic and degraded DOM is flushed downriver under high flow conditions. Furthermore, these properties gradually become more predominant downriver. In contrast, DOM is less degraded, more aliphatic and less humic under drought conditions. These properties were mainly determined by putative inputs of wastewater rather than in-stream autochthonous processes. Under drought conditions, the fluvial segment between village 2 (kilometre 10) and village 5 (kilometre 43) did not receive relevant anthropogenic inputs. Therefore, this large segment is appropriate for exploring the magnitude of in-stream DOM retention/release processes under low flow conditions. The results demonstrated a depletion of DOC and a decrease in the fresh and poorly degraded DOM pool: on the contrary, the the most aromatic and humified DOM pool accumulated significantly downriver. These findings contrast with those reported in other studies (Creed *et al.*, 2015; Ejarque *et al.*, 2017).

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The interruption of longitudinal hydrological connectivity causes delayed responses in dissolved organic matter

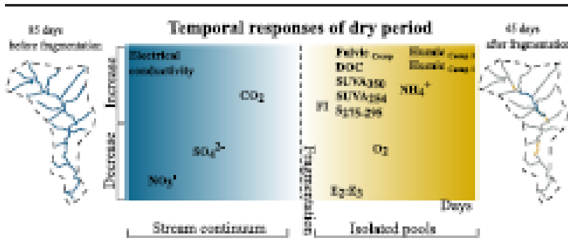
Verónica Granados [a,*], Cayetano Gutiérrez-Cánovas [b,c], Rebeca Arias-Real [a], Biel Obrador [a], Astrid Harjung [d], Andrea Butturini [a]

[a] Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Barcelona, Spain
[b] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal
[c] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal
[d] Department of Limnology and Bio-Oceanography, University of Vienna, Vienna, Austria

HIGHLIGHTS

- Timing of stream fragmentation is a key predictor of biogeochemical variability.
- Most of DOM parameters showed abrupt and delayed responses after fragmentation.
- Some inorganic solutes showed an anticipated response to fragmentation.
- Spatial location and pool volume were relevant in explaining some descriptors.

GRAPHICAL ABSTRACT



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ABSTRACT

Hydrology is the main driver of dissolved organic matter (DOM) dynamics in intermittent rivers and ephemeral streams. However, it is still unclear how the timing and the spatial variation in flow connectivity affect the dynamics of DOM and inorganic solutes. This study focuses on the impact of flow cessation on the temporal and spatial heterogeneity of DOM quantity and quality along an intermittent stream. We monitored a headwater intermittent stream at high spatial and temporal frequencies during a summer drying episode and analysed dissolved organic carbon (DOC) and its spectroscopic properties, inorganic solutes and dissolved CO_2. The drying period determined the disruption of the fluvial continuum with a recession of stream continuum at a rate of ~60 m/d and the gradual formation of a patched system of isolated pools of different sizes. Our results showed that the period of time that had elapsed since isolated pool formation (CI-days) was an essential factor for understanding how drying shaped the biogeochemistry of the fluvial system. Overall, drying caused a high DOC concentration and an increase in the humic-like fluorescence signal. Additionally, solutes showed contrasting responses to hydrological disconnection. Electrical conductivity, for instance, is a clear "sentinel" of the fragmentation process because it starts to increase before the hydrological disruption occurs. In contrast, DOC, most spectroscopic DOM descriptors and CO_2 showed delayed responses of approximately 5–21 days after the formation of isolated pools. Furthermore, the spatial location and volume of each isolated pool seemed to exert a significant impact on most variables. In contrast, the temperature did not follow a clear pattern. These findings indicate that the fragmentation of longitudinal hydrological connectivity does not induce a single biogeochemical response but rather

* Corresponding author.
E-mail address: v.granados@ub.edu (V. Granados).

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stimulates a set of solute-specific responses that generates a complex biogeochemical mosaic in a single fluvial unit.

1. Introduction

Freshwater ecosystems play a key role in global carbon cycling and organic matter processing (Battin et al., 2008; Cole et al., 2007; Sinsabaugh and Findlay, 2003). Specifically, dissolved organic matter (DOM) is an essential energy source for microbial communities with crucial implications on aquatic food webs (Benner, 2003). Hydrology is one of the main drivers of DOM dynamics (Ejarque et al., 2017; Fellman et al., 2009; Guarch-Ribot and Butturini, 2016; Voss et al., 2015), particularly in intermittent rivers and ephemeral streams (IRES), which comprise more than half of the global river network (Datry et al., 2014). For example, in arid and semiarid regions such as the Mediterranean, most of the stream networks exhibit hydrological intermittence characterized by alternating dry and wet seasons (Vazquez et al., 2013). During the dry period, the surface flow disrupts hydrological connectivity, and some streams transform into a set of patched and fragmented water pools or into totally dry riverbeds. Thus, hydrological disconnection creates habitat mosaics with lotic and lentic habitats (Datry et al., 2016; Larned et al., 2010), which affect organic matter and biogeochemistry dynamics (Bernal et al., 2018; Dahm et al., 2003; Gómez et al., 2009; Harjung et al., 2018; Vázquez et al., 2015).

Previous studies have explored how DOM changes after a dry period using static information (i.e., before and after drought comparisons) and monitoring one or a few sampling sites (Casas-Ruiz et al., 2016; Fellman et al., 2011; Harjung et al., 2018; Vazquez et al., 2011; von Schiller et al., 2015). As a result, the precise timing in which surface water DOM tracks hydrological changes in a continuum from flowing conditions to flow cessation is still unclear. A better understanding of the biogeochemical dynamics during the drying process will help quantify the role of hydrology in DOM variation and anticipate climate change effects caused by aridification and water abstraction.

During the dry period, it was revealed that most intermittent streams experienced autochthonous-originated DOM increases as a consequence of low terrestrial inputs (Casas-Ruiz et al., 2016). As drought advanced, DOM shifted to non-humic, less aromatic (Vazquez et al., 2011; von Schiller et al., 2015) and low molecular weight compounds, suggesting a major contribution of in-stream algal and microbial processes (von Schiller et al., 2015). Furthermore, as drought evolves, higher temperatures and evaporation enhance the respiration rates and the formation of anoxic habitats (Fellman et al., 2011; Gómez et al., 2009; Lillebø et al., 2007; von Schiller et al., 2015). These changes in environmental conditions influence the growth and development of microbial and fungal communities (Canhoto et al., 2016; Febria et al., 2015; Medeiros et al., 2009), which in turn can affect organic matter processing (Battin et al., 2016; Fazi et al., 2013; Tzoraki et al., 2007).

Most of these previous studies grounded their findings in studying small stream reaches. As a consequence, a comprehensive picture at the scale of the whole fluvial system is still missing. Additionally, the impact of the heterogeneity of habitats that are transitorily formed and destroyed during drying on biogeochemistry, and more specifically on DOM, has not been considered in most studies. Thus, it remains unclear whether the conclusions reported in these studies can be extrapolated to the entire system or, on the contrary, if they describe reach-specific patterns.

The objective of this study was to investigate how DOC, DOM and inorganic solutes change in space and time during a drying period, covering the entire fluvial continuum. Specifically, we investigated a) how hydrological connectivity influences the response timing and shape of the biogeochemical variables analysed and b) how these responses can be further influenced by spatial position along the stream and by local conditions (e.g., water temperature and volume of the disconnected pools). We hypothesized that the timing of fragmentation (Cl-days) was an essential driver of changes in DOC, DOM and inorganic solutes. Therefore, we expected that these changes would coincide with the fragmentation of the fluvial continuum and the formation of the isolated pools. In addition, we hypothesized there would be an increase in the bulk DOC concentration and the contribution of autochthonous molecules.

2. Materials and methods

2.1. Study site

The Fuirosos River (NE Iberian Peninsula, 41° 42′ N; 2° 34′ E) is a third-order stream that drains a 15.2 km^2 in a forested granitic catchment, ranging 50–700 m above sea level (m.a.s.l.). It is a small representative river of the siliceous Mediterranean climate mountains (River type code, 2a) (Munné and Prat, 2011). The catchment is mainly forested (coniferous, oak and deciduous vegetation), with minor agricultural use (<2%) (Fig. 1). The climate is semiarid Mediterranean Csa, (Köppen climate classification) with a mean monthly air temperature ranging from 3 °C in January to 24 °C in August. Mean annual precipitation ranges 500–900 mm, being concentrated in autumn and spring with sporadic summer storms (Guarch-Ribot and Butturini, 2016). Fuirosos is an intermittent stream with a dry period during summer and a clear hydrological seasonal pattern: a non-flowing dry period (July to September), a wet period (October–June) with basal discharge ranging between $5 \cdot 10^{-3}$ and $3 \cdot 10^{-2}$ $m^3\ s^{-1}$ (Guarch-Ribot and Butturini, 2016; Vazquez et al., 2013). Rewetting from dry to wet phases is a typical abrupt transition triggered by the first severe autumnal rain episode (typically larger than 50 mm (Butturini et al., 2003)). The main reach is 8 km long and the drainage density is approximately 0.6 km km^{-2} (Sala, 2013). A reservoir (nominal capacity: 45,000 m^3) is located at 6 km from the river mouth. Stream morphology is prevalently step-pool in headwaters and, pool-riffle in downriver reaches. Streambed substrate is prevalently sandy-gravel and large cobbles. Unfractured granitic bedrock comprises approximately 10% of the total streambed surface.

2.2. Sampling strategy

We selected 16 sampling sites along the main reach (8 km long), ranging in altitude from 941 m a.s.l. (41°40′02.2″ N; 2°36′04.0″ E) to 302 m a.s.l. (41°43′04.1″ N; 2°34′21.3″ E) (Appendix A). The sampling started on 09/06/2016, 22 days after the last high flow episode, when the entire stream continuum was connected (base flow conditions) and finished on 02/09/2016, when the entire streambed was disconnected and the last remnant isolated pools were almost totally dry. During this period, we performed 10 sampling campaigns. Sampling sites are labelled with natural correlative numbers from downstream ("0") to headwaters ("15"). Sampling sites are clustered into two geomorphological classes with contrasting substrate permeability. The first group of sites (those at km 1, 1.7, 2.9, 4.2, 4.5, 4.8, 4.9, 7.4 and 8.1) are characterized by an impermeable granitic bedrock substrate where isolated pools can occur during the dry period. The second group of sites (those at km 1.5, 2.5, 5.1, 5.9, 6.2, outlet of the reservoir, and 6.9), are placed on permeable sand-cobbles-gravel substrates, where surface water rapidly vanished during the drought period (Fig. 1). We

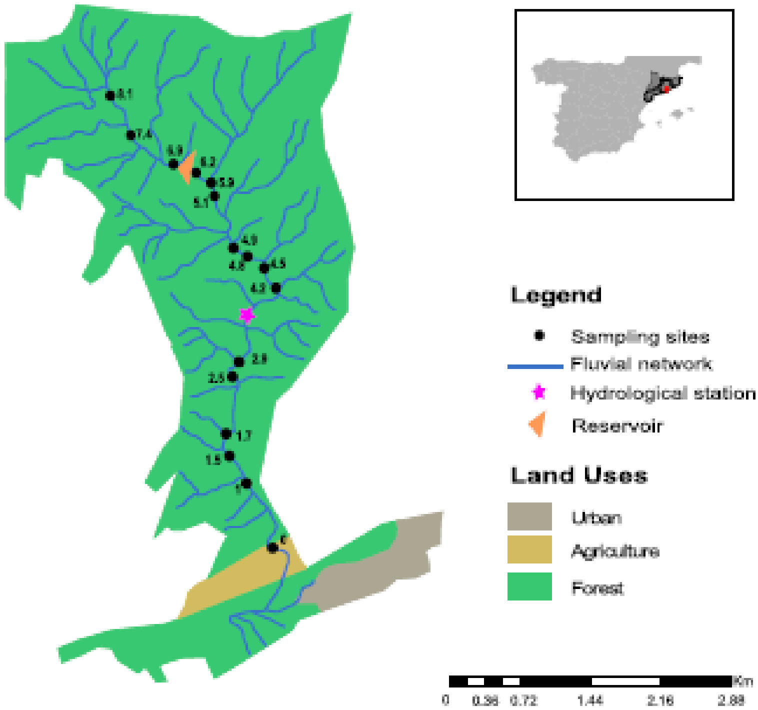

Fig. 1. The study area map. Black dots are the sampling sites, numbers indicate the distance from downstream (km).

performed a continuous assessment of the hydrological dynamic of drought, dividing it into four phases: pre-drought, contraction, fragmentation and dry.

2.3. Hydrological monitoring

At each sampling site and date, stream discharge was estimated using the slug chloride addition method (Gordon et al., 2004). Additionally, water level was continuously monitored at the sampling site "6" (hydrological station) with a water pressure transducer (Druck sensor PDCR/PTX 1830 or PTX 1730) connected to the automatic logger (Campbell CR10X). To determine the timing of flow cessation, we installed 15 temperature data loggers (SmartButton, ACR Systems) in the streambed along the fluvial continuum. We assumed a dry streambed when data loggers recorded anomalously high daily temperature oscillations, and this assumption was validated after a visual inspection of each logger on each sampling date. At each sampling site, we estimated the time elapsed since the formation of isolated pools (defined as connected-isolated days, CI-days). CI-days can have positive or negative values. Negative CI-days indicate lotic conditions, i.e., the sampling site is still hydrologically connected with upstream reaches. In contrast, positive CI-days values indicate that the sampling site is hydrologically disconnected from upstream reaches, forming an isolated pool. When the CI-days value is equal to zero, this indicates the first day when the hydrological fragmentation of the river continuum occurred.

2.4. Chemical analysis of inorganic solutes

At every sampling campaign and site, we measured electrical conductivity, temperature (EC, WTW 3310 set 1 conduct-meter), dissolved oxygen (YSI 20 Pro oxygen sensor) and dissolved carbon dioxide (CO_2) (GM70 Hand-held CO2 Meter, Vaisala) in the middle of the channel. We collected water samples for chemical analysis, which were filtered using pre-combusted (450 °C) glass fibre filters (Whatman GF/F 0.7-μm pore size) followed by 0.22-μm pore nylon filters. The filtered samples were placed in amber glass bottles that had been previously pre-combusted. Samples were kept on ice in dark conditions and immediately transported to the laboratory, where they were stored at 4 °C until analyses (all the water samples were analysed after a few days of the sampling date). Once in the laboratory, we determined biogeochemical descriptors that included inorganic solutes (NH_4^+, SRP, NO_3^-, Cl^- and SO_4^{2-}), DOC and 10 qualitative descriptors of DOM composition based on spectroscopic properties (see next section). We measured NH_4^+ concentration using the salicylate method (Reardon, 1969) and soluble reactive phosphorus (SRP) using the molybdate method (Murphy and Riley, 1962). We analysed inorganic anion (NO_3^-, Cl^- and SO_4^{2-})

concentrations using an electrical conductivity detector waters (model 432) with a UV/V Kontron detector (model 332) and the column waters IC-Pak anions. To analyse DOC, we filtered the samples, acidified them with 10% HCl and refrigerated them before analysis on a Shimadzu TOCanalyser VCSH with the oxidative combustion and infrared method (± 0.5 µg L^{-1}). In addition, we added different standards with specific concentrations during the analysis to verify our results.

2.5. DOM spectroscopy

DOM spectroscopic properties were determined by DOM absorbance spectra using a UV–visible spectrophotometer UV1700 Pharma Spec (Shimadzu) and a 1 cm quartz cell. We obtained absorbance data in double-beam mode with wavelengths scanned from 200 to 800 nm and deionized water as the blank. We generated excitation–emission matrices (EEMs) with an RF-5301 PC spectrofluorimeter (Shimadzu). To determine spectra, we used a 1 cm quartz cell and we measured EMMs over (Ex/Em) wavelengths of 240–420 nm and 280–690 nm and they were standardized with the method of (Goletz et al., 2011) using Mathematica (Wolfram Research) software. We used the same method to correct the EEM data and the absorbance data for each sample to correct the inner filter effects (Lakowicz, 2006). We employed the following methods to correct the wavelength-dependent inefficiencies of the detection system: (Gardecki and Maroncelli, 1998) for emission measurements and (Lakowicz, 2006) for excitation correction. To normalize the data of each day of analysis with the spectro-fluorimeter, we applied daily measurements of the area under the Raman peak using MilliQ water blanks (Lawaetz and Stedmon, 2009).

We evaluated seven qualitative descriptors of DOM composition: four chromophoric indices and three fluorophoric indices. The chromophoric indices were (a) specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), (b) specific ultraviolet absorbance at 350 nm ($SUVA_{350}$), (c) ratio of absorbance at 250 nm to 365 nm (E_2:E_3), and (d) spectral slope ratio for 275–295 nm ($S_{275-295}$). The $SUVA_{254}$ and $SUVA_{350}$ were determined as the absorption coefficient at 254 or 350 nm, respectively, normalized by DOC concentration (Weishaar et al., 2003). Higher values of $SUVA_{254}$ are associated with greater aromaticity (Hansen et al., 2016). The E_2:E_3 ratio provides information about DOM molecular size, where E_2:E_3 decreases with an increasing molecular weight (De Haan and De Boer, 1987). $S_{275-295}$ also integrates variations in the molecular size of DOM, indicating a progressively greater proportion of the small DOM molecular fraction (Helms et al., 2008). $S_{275-295}$ is a unitless parameter, estimated by calculating the ratio of the logarithmically transformed absorbance spectra slope at 275–295 nm. The three fluorophoric indices considered were (a) the humification index (HIX), (b) the fluorescence index (FI) and (c) the biological index (BIX). The HIX is the area under the emission spectra at 435–480 nm divided by the peak area at 310–345 nm from the spectra at an excitation wavelength of 254 nm. The HIX indicates the extent of humification by quantifying the shift in the emission spectra towards longer wavelengths, due to lower H:C ratios. Higher HIX values indicate a greater degree of DOM humification (modified from (Ohno, 2002)). The FI provides information about the DOM sources, where high values suggest the prevalence of autochthonous DOM and low values suggest the prevalence of allochthonous DOM (Cory and McKnight, 2005). This index is calculated as the ratio of emission intensities at 470 nm and 520 nm emitted at an excitation of 370 nm. Finally, the BIX was calculated at an excitation of 310 nm as the ratio of the fluorescence intensity emitted at 380 nm and 430 nm. Therefore, high BIX values (>1) suggest the presence of autochthonous and fresh DOM, while BIX values of 0.6–0.7 indicate low or zero autochthonous DOM production (Huguet et al., 2009). Furthermore, we performed PARAFAC analysis (Bro, 1997) using the drEEM toolbox (R. Murphy et al., 2013). First, Raman and Rayleigh scatters were interpolated and the EEMs were normalized to avoid high correlation among the components. Then the model was checked for outliers and four samples were excluded from the modelling procedure. The model was chosen from the best fit of the runs, applying a "nonnegativity" constraint. A three-component PARAFAC model with a core consistency of 44.6% was validated with split-half analysis (Appendix B) as recommended in Murphy et al., 2013: all three are humic substances (humic-like or fulvic-like components) (Table 1). We expressed DOM fluorescence of the three PARAFAC components as maximum fluorescence intensity (F_{max}) in raman units (R.U.). (i.e., in the results C1, C2, C3 are shown as humic component 1 ($Humic_{Comp\ 1}$), humic component 2 ($Humic_{Comp\ 2}$) and fulvic component 3 ($Fulvic_{Comp}$).

2.6. Statistical analyses

To explore the response timing and shape of biogeochemical variables and the influence of spatial and local conditions, we quantified their responses to longitudinal hydrological connectivity (CI-days as the temporal predictor), spatial location (as the spatial predictor), volume (i.e., volume of the disconnected pool) and temperature (i.e., temperature of each disconnected pool at every sampling campaign). Given that response variables could show different shapes and dependence structures (repeated measures and temporal autocorrelation), we tested the appropriateness of different models and used AIC values to evaluate their statistical support (Zuur et al., 2009). These models could render different statistical responses; however, their statistical support and reliability vary and can be evaluated by the comparison of AIC values (i.e., some models could misrepresent data properties). For example, in some cases, we detected non-linear responses that were modelled more precisely by GAMMS. A linear response was modelled more precisely by linear models or GLMM when repeated measures were statistically relevant. Therefore, we fitted different models, including CI-days, pool volume, temperature (without significant effect on any variable) and spatial location, to source as predictors (fixed factors) by following a two-step strategy. First, following (Zuur et al., 2009), we assessed the response shape (linear vs. non-linear) and the necessity of including a temporal autocorrelation structure and a random intercept to account for repeated measures in the same site. To do this, for each response variable, we fitted a linear model (LM, generalized least squares, Nlme v.3.1–131.1) and generalized linear mixed-effects models (GLMMs) (Lme4 v.1.1–15) with a Gaussian error distribution and identity- or log- links. When scatterplots suggested a non-linear pattern and/or residuals showed a clear temporal dependence pattern, we also fitted generalized additive mixed models (GAMM) with an autoregressive integrated moving average (ARIMA) term. We then compared and ranked all these models using the Akaike information criterion (AIC) value and retained the model showing the lowest AIC value (Zuur et al., 2009). Second, using the most statistically supported model type (i.e., the lowest AIC) to select the best predictor structure for each response variable, we performed a stepwise backward selection based on the AIC values starting from a full model that included the three predictors. All model residuals were visually checked for normality and homoscedasticity of their distributions. The LMM, GLMM and GAMM models included sampling site as a random intercept to account for repeated measures at the same river location. The LM and LMM were fitted using maximized log-likelihood (ML), while the GLMM and GAMM were fitted by maximizing the restricted log-likelihood (REML). Finally, to identify the inflection points in the temporal dynamics of each chemical descriptor (abrupt changes in each variable), we performed a non-parametric regression using conditional inference trees (Party v.1.2–4) (Hothorn et al., 2006). Before modelling, to reduce distribution skewness, we performed a log- or square root-transformation of some variables. We executed all statistical analyses in R 3.5.1 (R Core Team 2018). R code is provided in Appendix C.

Table 1
Emission and excitation of the parallel factor analysis (PARAFAC) components. They are applied on 16 sampling sites in 10 sampling campaigns, 108 samples altogether. The references are taken from other PARAFAC models published in the online OpenFluor database (http://openfluor.org; December 2019) from other freshwater ecosystems with a similarity score of at least 0.9 (Murphy et al., 2014).

	Excitation	Emission	Components identified from previous studies	Description
C1	300	440	C1 from Horsens river and Gribs lake (Sandergaard et al., 2003). C1 from tropical lake (Murphy et al., 2018). C1 from temperate river Patagonia (Garcia et al., 2018). C1 from lakes, rivers, streams, wetlands (Lapierre and del Giorgio, 2014).	Humic-like fluorophore related to microbial degradation in freshwater ecosystems
C2	<250	460	C1 from Yangtze Estuary (Li et al., 2015). C3 from Roskilde Fjord (Asmala et al., 2018). C3 from estuaries (Osburn et al., 2016). C4 from tropical river (Lambert et al., 2016) and (Osburn et al., 2017). C4 from Central European mixed forests (Graeber et al., 2012).	Humic-like fluorophore, degraded, described as recalcitrant to microbial uptake
C3	270/370	470	Component 4 from Rio Negro, Neotropic and component 2 from Lake Lillsjön, Artic (Wünsch et al., 2017). Component 2 from river water, Artic (Walker et al., 2013).	Fulvic acid fluorophore. Terrestrial delivered

3. Results

3.1. Hydrology

The drying process occurs gradually over period of 126 days (~4 months). The period started on 9/6/16, with the drying of the downriver main reach section (km 0 to 1; Fig. 2), and finished on 13/10/16, after a severe rain episode of ~100 mm. Ten minor precipitation episodes occurred during this period (<10 L m^{-2}), but they did not have any impact on hydrology. Four different phases emerged during the drying process:

a) Pre-drought (days 0 to 15): water runoff rapidly disappeared from tributaries and the main reach contracted rapidly downstream (<1 km) and in the headwaters (>8 km). During this phase, the reservoir was almost full, and we did not observe hydrological fragmentation of the stream continuum.
b) Contraction (days 16 to 32): the reduction of the stream continuum accelerated and the stream bed was dry at the 1.5 and 2.5 km sampling locations, which resulted in a disruption of the stream continuum into three isolated reaches that were 5 km long (from 2.5 to 7.4 km). During this period, we observed the first isolated small volume (1–3 m^3) pools (at 1, 2.9, 7.4 and 8.1 km). These pools emerged

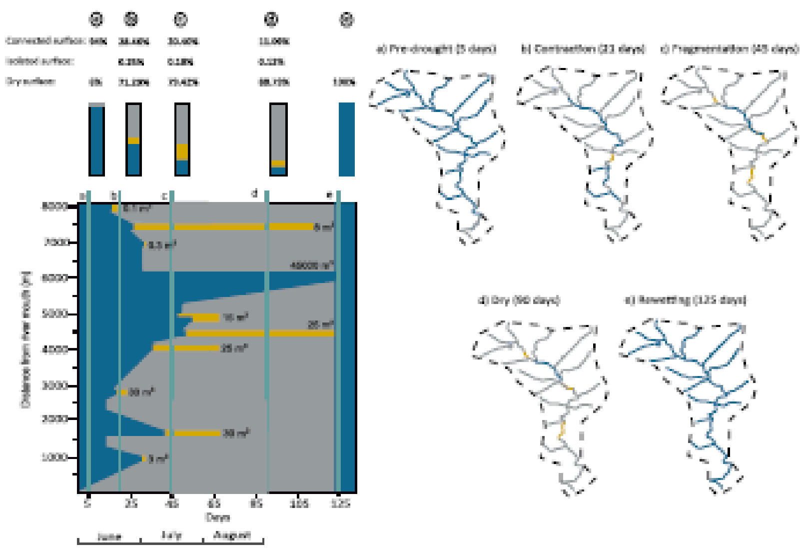

Fig. 2. Hydrological dynamics during the drought across the Fuirosos catchment. Upper-left section: represents the percentage of connected surface (blue), isolated surface (yellow) and dry surface (grey) during the different phases of the dry period: a) pre-drought: 5 days after starting the study (9/06/16), b) contraction: 21 days, c) fragmentation: 45 days, d) dry: 90 days and e) rewetting: 125 days. The bottom-left plot illustrates the river length in the y-axis and days of the study in the x-axis, whereas, b, c, d and e have the same information. Blue represents the connected sections. The numbers in the yellow bars indicate the volume of the isolated pools. The weir is represented with 45,000 m^3. The drawings on the right represent the stream network in the different phases described. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in depressions of un-fractured granitic bedrock. The water volume of the reservoir decreased to ~50%.

c) Fragmentation (days 33 to 50): during this period, the length of the stream reaches with flowing water were reduced at a rate of approximately 60 m d^{-1}. As a result, running waters were constrained to a 1.7-km-long stream reach situated downstream of the reservoir outlet (km 6). During this period, we found five isolated pools, which was the highest number observed during the study period. Previously, four of the five isolated pools established at the beginning of the drought period dried up, while the other four new and larger

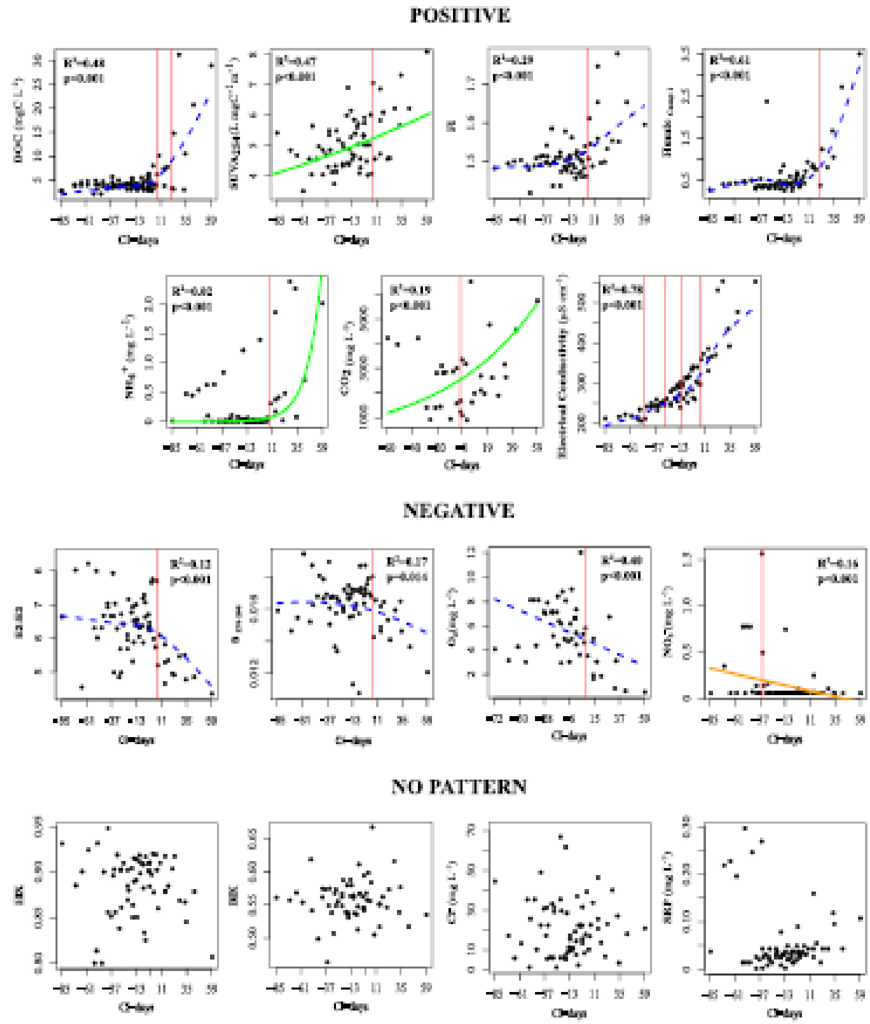

Fig. 3. Changes in DOC, DOM optical parameters and inorganic solutes along CI-days. DOC, $SUVA_{254}$, FI, $Humic_{Comp.1}$, NH_4^+, CO_2, electrical conductivity, E_2:E_3, $S_{[illegible]}$, O_2, NO_3^-, BIX, HIX, Cl^-, SRP are on the y-axis. The CI-days on the x-axis are the connected-isolated days, where negative values are the days that the river is connected and the positive values the days that the river bed is disconnected. Plots show a positive, negative or no pattern with linear (LM, orange line; GLMM, green line) or non-linear (blue line) results. Vertical red lines explain the inflection points, which refer to the number of abrupt changes in each variable. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

isolated pools (15–30 m^2) emerged at 1.7, 4.2, 4.5 and 4.8 km.

d) Drought (days 51 to 125): 90% of the stream continuum was almost dry. A small volume of water persisted at the reservoir (<5000 m^3) and in two pools (4.5 km and 7.4 km). The reservoir outlet (approximately 2 L s^{-1}) still fed a small stream reach (<0.5 km of longitude) of reduced discharge. This situation of extreme drought persisted until the rain events at the beginning of October, when rewetting took place (Fig. 2).

3.2. Biogeochemical responses

DOC, DOM optical parameters and inorganic solutes showed a wide range of variation over the course of the dry period (Appendix D). As drying advanced, three different biogeochemical responses could be discerned (Fig. 3).

The first type of pattern describes a positive relationship with CI-days. Electrical conductivity, DOC, $SUVA_{350}$, FI, NH_4^+, CO_2, $SUVA_{254}$ and the three humic PARAFAC components are shown (Fig. 3). The most sensitive variable to fragmentation was electrical conductivity, which started to increase long before the formation of the first pools (CI-days = −47), and there was a successive acceleration at CI-days = 6. The second most responsive variable to drought was DOC, which started to increase at CI-days = 6 and exhibited an abrupt increase at CI-days = 21. Most of the other biogeochemical parameters showed a delayed abrupt response with respect to fragmentation, with CI-days oscillating from 5 to 21 days. The exception was CO_2, which responded almost at the onset of fragmentation (CI-days = −2) (Fig. 4).

The second type of pattern showed a negative relationship with CI-days. This pattern was observed for E_2:E_3, $S_{275-295}$, O_2, NO_3^- and SO_4^{2-} (Fig. 3). The most responsive parameters to the dry period were NO_3^- (CI-days = −35) and SO_4^{2-} (CI-days = −13). However, E_2:E_3 and $S_{275-295}$ (CI-days = 6) and O_2 (CI-days = 7) showed a delayed response (Fig. 4).

The third type showed a chemo-static response with respect to fragmentation. This response was detected for the BIX, HIX, Cl^- and SRP. Therefore, these variables were insensitive to fragmentation (Fig. 3). Focusing on those parameters significantly related to CI-days, the relationship was non-linear for DOM descriptors and linear for inorganic solutes (Table 2).

In addition to CI-days, water volume stored in pools emerged as a significant explanatory parameter for some DOM descriptors, such as $S_{275-295}$, BIX, PARAFAC humic components, SO_4^{2-}, SRP and O_2. Moreover, the spatial location was important in determining the changes in inorganic solutes such as Cl^-, NO_3^-, SRP, electrical conductivity, O_2, $SUVA_{350}$ and $S_{275-295}$. Interestingly, the temperature was unrelated to any of the biogeochemical changes (Table 2).

4. Discussion

Our results highlight that the timing of fragmentation (i.e., time since the formation of isolated pools, CI-days) is a key driver for most stream biogeochemical parameters. Most notably, our results revealed that the biogeochemical descriptors showed contrasting responses. While most DOM optical descriptors showed abrupt and delayed responses to fragmentation (CI-days>0), a few descriptors showed gradual responses or even anticipated fragmentation (CI-days<0). Additionally, the volume of pools was relevant in explaining changes in some DOM descriptors, while the spatial location of pools could also be important for most inorganic solutes.

4.1. Temporal dynamics of hydrological fragmentation

Although longitudinal hydrological connectivity is recognized as a key driver of biogeochemical dynamics during dry periods, few studies have provided detailed temporal information on how drought episodes contract and fragment a fluvial network continuum (Godsey and Kirchner, 2014; Stanley et al., 1997). The interruption of the hydrological connectivity is a fast-moving process in the Fuirosos catchment, showing a contraction of the stream continuum that began simultaneously at downstream and upstream reaches (Lake, 2003). Furthermore, the estimation of the water retrocession rate (~60 m/d) confirms how abrupt the hydrological contraction is, determining that in a few hours, large fluvial reaches shift from wet to dry conditions. The contraction pattern detected in Fuirosos opposes to that reported for intermittent karst streams, where upstream areas were with permanent flow, middle reaches remained dry during the summer period and downstream areas developed several temporary springs (Meyer and Meyer, 2000). The flow of these streams strongly depends on karstic groundwater inputs. In contrast, Fuirosos has a limited groundwater influence, and the drying pattern and location of isolated pools are mostly influenced by the location of permeable sediments and granitic bedrock outcrops. Overall, isolated pools with groundwater inputs have smooth

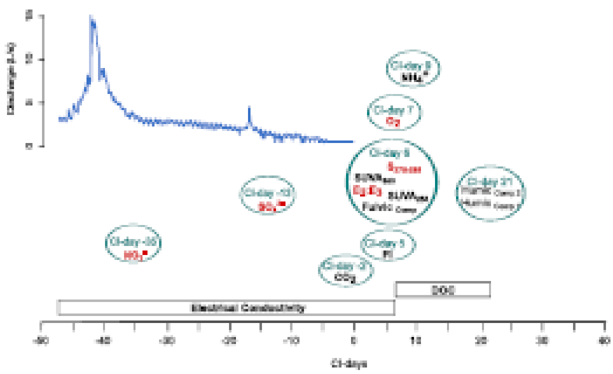

Fig. 4. Timing of the response for each biogeochemical variable to CI-days. The inflection points were obtained using conditional inference trees. Horizontal white bars indicate the gradual response for electrical conductivity and DOM. The remaining biogeochemical variables that are circled show an abrupt response, and the CI-days are their response. Additionally, black letters show a positive tendency over time, and red letters show a negative trend. The blue line in the upper panel illustrates the discharge at sampling site "6", a hydrological station located in the middle of the stream. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Summary of the best fitting models for each response variable. p-Values and regression coefficients are also shown for the predictors (CI-days, Volume and Spatial location) included in the best fitting model, along with explained variance and model type (LM, GLMM or GAMM). We did not show model coefficient for CI-days because in many cases we applied GAMM models, which did not provide such as information. Significant variables are highlighted in bold.

Variable	CI-days	Volume		Spatial location		R^2	Model type
	p-Value	Coefficient	p-Value	Coefficient	p-Value		
DOC	<0.001					0.48	GAMM
$SUVA_{254}$	<0.001			0.0400	0.102	0.47	GLMM
$SUVA_{350}$	<0.001			0.0251	0.048	0.22	GAMM
$S_{275-295}$	0.016	0.0004	<0.001	−0.0002	0.042	0.17	GAMM
E2:E3	<0.001					0.12	GAMM
HIX	0.529					0	LM
FI	<0.001					0.29	GAMM
BIX	0.925	−0.0042	0.040			0.03	LM
$Humic_{Comp\ 1}$	<0.001	0.0416	0.010			0.61	GAMM
$Humic_{Comp\ 2}$	<0.001	0.0649	0.033			0.63	GAMM
$Fulvic_{Comp\ 1}$	<0.001	0.0296	0.034			0.54	GAMM
Cl^-	0.865			−0.4675	0.004	0.07	LM
NO_3^-	<0.001			0.0329	0.029	0.16	LM
SO_4^{2-}	<0.001	−0.2939	0.009			0.21	GLMM
SRP	0.650	0.5057	<0.001	0.2987	0.008	0.001	GLMM
NH_4^+	<0.001	1.2346	0.138	0.2019	0.590	0.02	GLMM
CO_2	<0.001	0.0236	0.265	0.0129	0.449	0.19	GLMM
Electrical conductivity	<0.001			0.3094	0.009	0.78	GAMM
O_2	<0.001	−0.8506	<0.001	−0.5574	<0.001	0.40	GAMM

biogeochemical responses, while without these inputs, concentrations increase more abruptly during the dry period (Dahm et al., 2003; Fellman et al., 2011; Siebers et al., 2016).

4.2. *Effect of the evolution of hydrological connectivity on DOM quantity and quality and inorganic solutes*

Considering CI-days as a temporal variable and also a descriptor of the hydrological state (connected or isolated), DOC concentration, most DOM optical parameters and inorganic solutes showed a significant effect on this predictor. In agreement with previous studies (Ejarque et al., 2017; Harjung et al., 2018; Vazquez et al., 2011), hydrological fragmentation accelerates the biogeochemical responses that occur during the dry period. Thus, DOC increased tenfold at the end of this period when the stream was almost dry, except in two pools and the remaining water of the reservoir. Additionally, aromaticity (in term of SUVA) and DOM molecular size (described as E2:E3 and $S_{275-295}$) increased with drying progression. Overall, the accumulation of DOM in the isolated pools reflects the impact of high evaporation, which is a consequence of the disconnection from upstream reaches and alluvial groundwater (Fellman et al., 2011; Harjung et al., 2018; Siebers et al., 2016). Moreover, in isolated pools, leaves from the riparian vegetation accumulated, causing an increase in the humic-like fluorescence signal and aromatic substances. This result coincides with that reported in other Mediterranean streams (Casas-Ruiz et al., 2016) as well as those rivers occurring in tropical (Yamashita et al., 2010), tundra (Balcarczyk et al., 2009) and temperate (Inamdar et al., 2012) systems. In addition, our results reflect a prevalence of humic substances and an absence of DOM production from algal metabolism, contradicting the results found in previous studies (Casas-Ruiz et al., 2016; Vazquez et al., 2011; von Schiller et al., 2015). These studies found a dominance of autochthonous DOM at the end of the dry period. However, we suggest that this divergence could be due to a deficient spatial and temporal frequency. Specifically, the accumulation of humic C1 and C2 would not have been captured by these studies (CI-days 21). These fluorescence components have been previously described as being especially recalcitrant to photodegradation or biodegradation. For example, humic C1 has proven to be more resistant to photodegradation than other components (Murphy et al., 2018). Moreover, a lack of inorganic nutrients with drying could explain a deficit in autochthonous production as observed in other intermittent streams (Martí et al., 1997; Skoulikidis and Amaxidis, 2009).

The evolution of drying (CI-days) strongly modulates the availability of inorganic solutes. Electrical conductivity increased gradually during the dry period as did the DOC concentration and other inorganic components, suggesting a solute concentration of the system, especially in the isolated pools, at the end of this period. However, dissolved oxygen and CO_2 showed opposite trends, indicating high respiration activity at the bottom of the pools. Oxygen depletion in pools enhances the consumption of other electron acceptors (i.e., SO_4^{2-} and NO_3^-) (Kemp and Dodds, 2002) along with the accumulation of NH_4^+ as a consequence of inhibited nitrification (Acuña et al., 2007; Townsend, 2002; von Schiller et al., 2011). The emergence of these anoxic and reduced habitats with higher contents of salts, humic organic matter and ammonium may strongly limit the activity and abundance and alter the diversity and composition of prokaryotes (Fazi et al., 2013; Febria et al., 2015; Harjung et al., 2019; Medeiros et al., 2009) and fungi (Canhoto et al., 2016).

Although hydrological fragmentation had a strong impact on most of the studied biogeochemical variables, these variables did not respond simultaneously. We discerned three temporal patterns in response to fragmentation: 1) before fragmentation (CI-days<0), there were some inorganic solutes; 2) after fragmentation (CI-days>0), all were DOM optical parameters; and finally, 3) there were variables that did not show a significant change during the dry period, such as the BIX and HIX. Although some of these patterns were already found in previous studies that compared pre- and post-fragmentation periods (Harjung et al., 2018; von Schiller et al., 2015), this study provides new empirical evidence on how hydrological fragmentation shapes the dynamics of biogeochemical variables over time and space at a very detailed level. In particular, our main novelty was in detecting the precise moment when variables showed inflection points and in finding contrasting and delayed responses among biogeochemical descriptors.

4.3. *Effect of spatial variability and isolated pool volume on DOM composition and inorganic solutes*

Although CI-days emerged as the most relevant driver of biogeochemical variation during the drying process, both spatial location and local pool conditions contributed to determining DOM and inorganic solute dynamics. The spatial location of isolated pools within the river continuum together with their size (water volume), had a substantial influence on some biogeochemical variables. Thus, the pools with greater volume had more LMW, humic DOM and anoxic environments. Regarding spatial pattern, DOM shifted from being aromatic and having high molecular weight (HMW) upstream to being less aromatic and having low molecular weight (LMW) downstream, which is consistent with empirical results from other studies (Ejarque et al., 2017; Granados and Butturini, 2019). Although we had a longitudinal pattern for some DOM optical descriptors, this pattern fits better in studies performed at the regional scale, with high order watercourses (Creed et al., 2015). In addition, we found a link between solute concentration and water residence time, as was found in the following studies (Fellman et al., 2011; Gómez et al., 2009; Vazquez et al., 2011; von Schiller et al., 2011). Thus, we observed higher solute concentrations in the isolated pools after more days of disconnection (i.e., with a higher water residence time of isolated pools).

5. Conclusions

This study discerns different biogeochemical patterns in response to the hydrological fragmentation of a fluvial continuum. Our results demonstrate that the timing of fragmentation of the fluvial continuum is a key predictor of biogeochemical variability during the dry period and that most of the DOM descriptors showed a delayed and abrupt response just after fragmentation. The study of the biogeochemical process during the evolution of drying and the quantification of hydrological connectivity regarding DOM variability will enhance our understanding of the effects of climate change caused by aridification and water abstraction.

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CRediT authorship contribution statement

Verónica Granados: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing. **Cayetano Gutiérrez-Cánovas:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - review & editing. **Rebeca Arias-Real:** Investigation, Methodology, Validation, Visualization, Writing - review & editing. **Biel Obrador:** Writing - review & editing. **Astrid Harjung:** Methodology, Validation, Writing - review & editing. **Andrea Butturini:** Conceptualization, Data curation, Investigation, Methodology, Validation, Visualization, Writing - review & editing.

Declaration of competing interest

All authors agree with the content of the manuscript and approve of its submission to Science of the Total Environment. The authors declare no conflict of interest.

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