Influence of hydrology on dissolved organic matter quantity and properties in a temporal Mediterranean stream

Influència de la hidrologia sobre la quantitat i disponibilitat de la matèria orgànica dissolta en una riera temporal mediterrània

Eusebi Vazquez Garcia

TESI DOCTORAL

Departament d'Ecologia

Universitat de Barcelona

Programa de doctorat: Ecologia fonamental i aplicada

Bienni: 2002 - 2004

Influence of hydrology on dissolved organic matter quantity and properties in a temporal Mediterranean stream

Influència de la hidrologia sobre la quantitat i disponibilitat de la matèria orgànica dissolta en una riera temporal mediterrània

Memòria presentada per Eusebi Vazquez Garcia per optar al grau de doctor per la Universitat de Barcelona

Eusebi Vazquez Garcia

Barcelona, Juny 2013

Vist-i-plau dels directors de tesi

Dr. Andrea Butturini i Dr. Francesc Sabater

Professors del Departament d'Ecologia

Universitat de Barcelona

Als meus pares

A la Carol

The hours of folly are measur'd by the clock, but
of wisdom: no clock can measure.

What is now proved was once, only imagin'd.

Proverbs of Hell - The Marriage of Heaven and Hell

William Blake

Agraïments

No sé ben bé com he arribat fins aquí, ni que vol dir específicament "aquí" però al menys es pot dir que finalment la tesi s'ha acabat i és hora d'agrair a totes aquelles persones que d'una manera o altra m'han ajudat durant aquests anys. La llista és llarga i espero que si m'oblido d'algú no m'ho tingui en compte.

En primer lloc vull agrair a l'Andrea Butturini pel seu ajut i paciència tots els anys que ha durat això. Si voleu saber exactament quants, podeu preguntar-li quants anys té el seu fill gran. Estic molt agraït al Quico Sabater per haver-me obert les portes del departament i el seu recolzament.

Malgrat que una beca BRD em va permetre mantenir-me durant els primers anys, he d'agrair a Jordi Catalán el seu suport econòmic al contractar-me dins el projecte GRACCIE.

També tinc presents a la Susana Bernal que em va ensenyar el funcionament tant de les coses pròpies del departament com els diferents aparells com el CIA i el Technicon, i que va haver d'aguantar les interrupcions i preguntes de "el nou", i al Vicenç Acuña, qui amb la Susana i l'Andrea, em va guiar pels camins de Fuirosos.

Tirant una mica més enrera en el temps també he de donar les gràcies a la Gora i al Jesús per deixar-me compartir els seus mostrejos a la Tordera i el Gurri el meu darrer any de llicenciatura. Sense haver treballat amb ells potser no m'hagués interessat tant per aquest camp de recerca.

Els anys que he passat al departament d'ecologia sempre he estat ben acompanyat i han sigut molts els moments de riure , ja des de els temps del DEA amb coneguts com l'Ainhoa, el Biel, la Neus i l'Enric i els que van aterrar pel departament (Ernesto, Gerardo, Gonzalo, Blanca). Tot i que haver gaudit de la companyia de força gent del departament, vull agrair especialment a les persones amb qui he passat moltes estones agradables i que, possiblement, han hagut de patir més el meu mal humor (les confiances fan fàstic). Sense cap ordre en particular: la Lola, el Julio (ciclista, jugaire, "remeru", radical), la Marta (se't troba a faltar), la Bet (vols fer un cafè?), la Lídia, l'Esther (ànims!!), el Jaime, l'Alba, la Mary (o Mariángeles), el Dani ("he d'escriure un correu"). Ah, no em puc oblidar de les croquetes del Gonzalo, perdó, vull dir del Gonzalo ni de l'Isis i les seves rialles i no tornar a escriure els noms de l'Ainhoa, la Neus i el Biel. No he fet cap estada però hi ha qui ha vingut cap aquí: records a la Dijana. Tampoc m'oblido de la Gemma, el Carles i l'Oriol de la secció marítima, els Ecobill i les noves generacions del departament: Núria C. (màquina), Ada, Silvia, Max, Pau (vaig a pensar a la cambra fosca), Anna, Marc (allau!) i tots els altres. Un record per la Montse Bacardit, la Bel Turró i en

Lluís Camarero que van fer la meva "estança" a Vielha molt més agradable. Més agraïments a absents ja del departament: Luciano, Izaskun, Iraima, Xavi de Pedro, l'Isabel...

Gràcies a tots amb qui he fet pràctiques de camp: Simone, Margarita, Elisabet, Núria de C., Josep Anton, Joan Armengol, Rafa, Marisol, Núria Bonada, Joan Gomà, Marta, Mikel, Emilia (gràcies pel recolzament!), José, Lourdes, Laura.

Per Fuirosos hi ha passat molta gent i amb l'Irene Ylla, l'Anna Romaní , els Stefano (Amalfitano i Fazi) i l'Emili hem col·laborat de forma estreta (i continuem), el resultats de la qual es poden llegir unes quantes pàgines més endins. Moltes gràcies per tot el que he aprés amb vosaltres i la vostra companyia. També he d'agrair especialment a l'Anna que m'ensenyes la tècnica d'ultrafiltració i què era allò del FI. Sense haver-hi treballat directament he compartit algunes estones a Fuirosos amb el Dani i el Miquel, i sense haver coincidit a Fuirosos, no puc deixar d'anomenar la tercera de la tríada, l'Alba i la seva nevera "blava" plena de mostres fins el capdamunt.

Gràcies a la Lola (un altre cop), la Santi, la Marta, el Vàngelis, el Lisandro, el Raül, la Maria, el Carlos, el Pablo, l'Eva, el Dani (vas al concierto?) per tots aquests anys. I un crit a la Patri, al Raül T, la Laia, al Sergio, al Guillem i la Núria (per on pareu?).

Estic especialment agraït també a l'Ariadna ("necessito voluntaris per un experiment sobre malària") per oferir-me la seva amistat tots aquests anys i les nostres trobades aperiòdiques per posar-nos al dia.

Gràcies a Paranoia desyerbar: Alan (es tan 2011...), Mari, Juan Carlos, Cristian, Alvaro, Mickey, Anna, Toni (Walk!). Sense els sopars de divendres (i tantes altres ocasions) i la vostra paciència res hagués sigut el mateix. Sou una medicina excel·lent pel mal humor i no hi hagut cap dia que al marxar cap a casa no m'hagi sentit millor. Als que van estar més d'un any pujant a l'Orient Express: Hermes (Minotauro!), Joan "Ridon" (the magician), David "Milos Perrier" Vendrell, Elio, Juan i la Carol. I a la resta que ens costa més de trobar-nos: Jota, Juanjo, Dani i Raül.

Gràcies per les converses estimulants i amenes (malauradament poc científiques) de la secció fanzinerosa: Alex Werden, Miguel Angel (trukulo) i Rubén (Aquilifer). Espero poder reemprendre alguns projectes que he deixat penjats ben aviat.

Moltes gràcies als "remerus" de Premià, sense poder remar amb vosaltres aquests darrers anys... no ho sé, no m'imagino com hagués sigut: Pau (veus com aquest cop anava de veritat?), Seve (remeros, remeritos i cagamandúrrias), Jordi (els macarrons els he fet jo), Marga (jo porto la cervesa), Xavi (fucking hell), Blanca (plou? Està plovent), Miquel Àngel (dimarts que ve vinc), Julio (again). Una salutació a les noves incorporacions.

Finalment, als meus pares i la meva germana per les raons més òbvies que es poden dir però que són de les més importants que es poden trobar i a la meva família, a les que han marxat, i als qui han arribat. Gràcies també a l'Aleydis, el Paco i la Montse.

I ara sí, donar les gràcies a la persona que ha hagut de patir el dia a dia amb mi durant tot aquest temps és quedar-se curt. Moltes gràcies Carol, per tot el recolzament incondicional i la comprensió, per tot aquest temps i el que ha de venir.

Eusebi Vazquez Garcia

Premià de Mar - Barcelona

Primer dia d'estiu de l'any 2013.

Table of contents

GENERAL INTRODUCTION

Dissolved organic carbon (DOC) in fluvial systems

Organic matter, and more specifically the dissolved organic carbon pool, is a key source of energy in fluvial ecosystems (Fisher and Likens, 1973; McDowell and Fisher, 1976). The processing and transport of organic matter, from headwaters to the river mouth, greatly influences higher trophic levels, largely mediated by heterotrophic bacteria (Findlay et al., 1993). Furthermore, DOC contributes to pH regulation, metal binding, nutrient transport and light attenuation in the water column (Wetzel 2001; Kirk, 1994). The origin of DOC and changes affecting its quantity and properties present a significant impact on its chemical and biological reactivity, thus influencing system functioning (Kaiser et al., 2004; Porcal et al., 2009).

Under a physical perspective, organic matter is usually divided according to its size into three, operationally defined, pools: coarse particulate organic matter (CPOM; >1 mm) including leaves, branches and trunks; the fine particulate organic matter (FPOM; 1 mm - 0.7 μm); and dissolved organic matter (DOM; <0.7 μm, other criteria establish the cut-off at 0.45 μm).

Generally, DOM is usually estimated as dissolved organic carbon (DOC) since it comprises approximately 50% of total organic carbon in fluvial systems (Thurman, 1985) and more than 90% in marine environments (Wetzel 2001).

Origin of dissolved organic carbon

Considering DOC origin there are two main sources in aquatic systems: allochthonous organic matter, usually from pedogenic origin, composed of substances produced by the decomposition of higher plants by bacteria and fungi; and autochthonous organic matter formed in the aquatic system through the decomposition and excreta of plankton, algae and aquatic bacteria (Thurman, 1985; Wetzel 2001; Filella et al., 2009).

Dissolved organic matter in fluvial systems is the product of in stream processes (autotrophic activity, exudates and cell lysis), and the allochthonous inputs from the river catchment, being quantitatively higher than the autochthonous fraction. The most direct input into stream water is leaf litter leachate (McDowell and Fisher, 1976). In forested regions and, especially, in headwater areas, due to the proximity to the riparian forest and the low light irradiance, most streams are dominated by inputs of allochthonous organic matter (Fisher and Likens, 1973), usually greater than 1000 g $C \cdot m^{-2} \cdot year^{-1}$. In contrast, primary production is generally low, below 200 g $C \cdot m^{-2} \cdot year^{-1}$ (Fisher and Likens, 1973; Caraco and Cole, 2003; Mulholland, 1997; Sinsabaugh, 1997).

Additionally, other allochthonous organic matter inputs occur as a consequence of precipitation, surface runoff, and water movement through soils and ground water (Thurman, 1985; Qualls and Haines, 1992; Findlay 2001). Although DOC concentration in rain water is low, ranging from 0.8 to 3 mg·L^{-1} (Thurman 1985; Aitkenhead and Peterson, 2003), it becomes gradually DOC- enriched as it falls below the canopy line, leaching leaves and other surfaces, and the water drip from leaves and branches (throughfall) up to 10 mg·L^{-1} (Thurman, 1985).

Generally, DOC concentration in fluvial systems ranges from 0.5 to 50 mg·L^{-1}. It varies according to the size of the river, climate, vegetation within a river's basin, and the season of the year. Also, the presence of wetlands in the catchment increases the overall DOC concentration of the system, explaining an important part of its variability (Mulholland 2003). In small streams with discharges under 100 m^{3}·s^{-1} DOC concentration ranges between 1 and 4 mg·L^{-1} (Thurman 1985). In general, DOC concentration in ground water is lower than in throughfall and overland flow (Hinton et al., 1998).

Considering the morphologic characteristics of the basin and climatic factors (i.e. antecedent moisture conditions), another relevant input of DOC into stream water might soil water fluxes. In higher soil horizons DOC concentration and availability is higher: in the A horizon fresh and semi decomposed plant material accumulates. In the B horizon organic matter is older and possibly has been transported from the superior horizons while processes of complexation with iron and aluminium facilitate the adsorption (and immobilization of DOC) by clays. The C horizon contains little organic matter. Concentrations in soil solution vary depending on the biome. For instance in grasslands, annual DOC concentration averages 7 ± 4 mg·L^{-1}, while in coniferous forests 36 ± 23 mg·L^{-1} (Aitkenhead and Peterson, 2003).

Spatial variations in DOC concentration along the fluvial system are lower in magnitude than the alterations caused by hydrological changes (Mulholland, 2003). Furthermore, these hydrology alteration events might prompt changes in the spatial and temporal patterns of DOC sources at the watershed scale resulting in remarkable changes in concentration. For instance, during precipitation events, the flushing of DOC from near stream riparian soils causes an increase of DOC concentration in stream water (Hinton et al., 1998; Hornberger et al., 1994; Butturini and Sabater, 2001). Furthermore, the shift among water flowpaths, from deep to shallow soils, allows the mobilization of an accumulated DOC pool in surface soils. On the other hand, the delay in the connection between hillslope and riparian zone, depending on antecedent soil moisture conditions, may result in differential DOC mobilization due to the hydrologic demands in upslope soils (McGlynn and McDonell, 2003).

Transport of dissolved organic carbon

From a historical perspective, fluvial systems have been considered as a link between terrestrial and marine systems (Schlesinger and Melack, 1981; Schlesinger 1999). Under this conceptual framework, DOC from allochthonous origin is considered refractory, and thus a solute transported downstream without much biological interaction within the fluvial system. In recent times this view has been challenged and different studies point out that fluvial systems not only remineralize carbon at a local scale but also are a net source of CO_2 towards the atmosphere (Richey 2002, Mayorga 2005, Cole 2007). The estimated organic carbon flux from

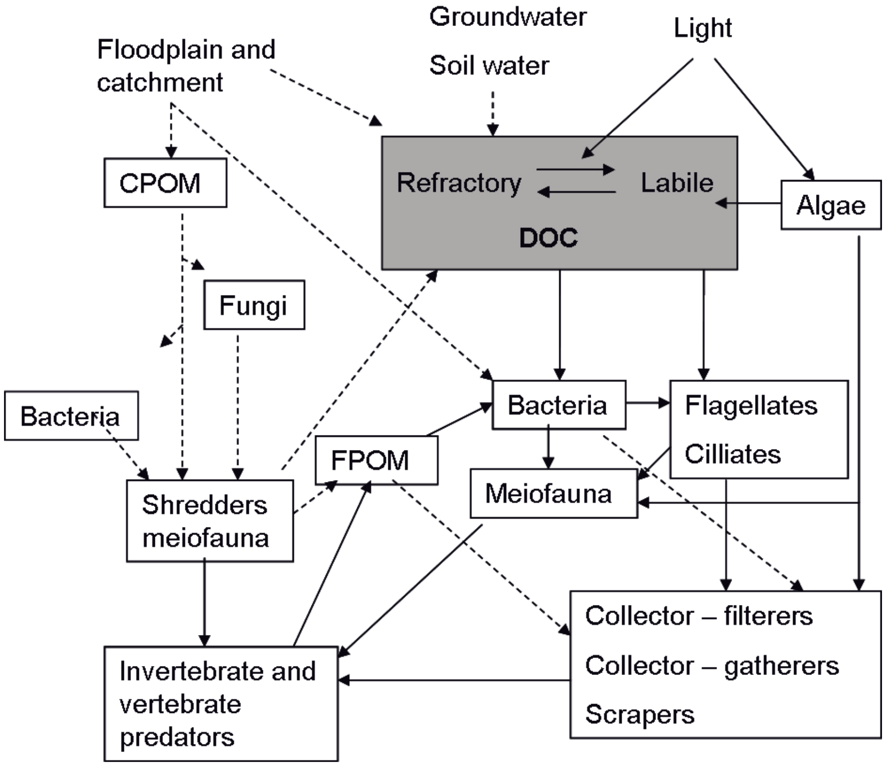


Figure I.1. The microbial loop in flowing waters (from Meyer, 1994). Lines indicate the pathways of organic carbon. Dotted lines indicate flows that are not present in planktonic systems.

rivers to the oceans is 0.4-0.9 Pg C·year^{-1} (Schlesinger and Melack, 1981; Aitkenhead and McDowell, 2000). But while recent studies report similar fluxes from rivers to sea, the estimated terrestrial to fluvial carbon flux is 1.9 PgC·year^{-1} and, consequently, a flux towards the atmosphere ranging from 0.8 to 1.55 Pg C·year^{-1} (Cole et al., 2007, Battin et al., 2008). This CO_2 is produced by respiration processes either in the hyporheic zone or within the stream or river (Cole et al., 2007). On the other hand, further studies propose that photomineralization processes might be also a relevant source of CO_2 (Farjalla et al., 2009).

In the last decades, an increase in DOC concentration and export has been detected in temperate and boreal aquatic systems (especially peatlands) (Freeman 2004 & co). Although regional comparison is hindered by the estimation methods of long term studies in each area (a topic examined in Eimers et al., 2008), different studies have proposed different mechanisms but a definitive model or explanation is still to be found: temperature increase due to climate warming (Moore et al., 1998, Freeman et al., 2001, Worral et al., 2004), increase in atmospheric CO_2 (Freeman et al., 2004), nitrogen deposition (Sinsabaugh et al., 2004; Findlay et al., 2005), decrease in sulphate deposition (Evans et al., 2006; Monteith et al., 2007), changes in hydrology (including drought periods) (Worrall and Burt, 2008; Clark et al., 2005; Jager et al., 2008). While there is not a definitive explanation, in studies focused on the combined effects of some of the suggested hypothesis, discharge and hydrologic regime seems to take a prevalent weight (Pastor 2003, Eimers 2008). Thus, the alteration of hydrological regime, either by anthropogenic activity or climate change, should be carefully considered since DOC concentration, composition and processing might be seriously affected.

DOC in stream processing

While a fraction of DOC can be retained rapidly by abiotic processes (i.e. sorption in soils and sediments), DOC removal from fluvial systems is mainly caused by biotic processes (Findlay and Sobczak, 1996) either in surface, hyporheic or ground water. Although it is a slower process it accounts for the majority of DOC removal (Tank et al., 2010).

Overall, the incorporation of allochthonous DOC pool into the aquatic trophic web is mediated by microbial communities (and fungal activity as well). This alternative energetic pathway is known as microbial loop, although it was first observed in bacteria from marine water column (Azam et al., 1983), is also present in lotic systems although with a few particularities presented in Meyer (1994): the microbial loop gains relevance due to the higher inputs of allochthonous organic matter; second, planktonic and benthic microbial loops are closely linked since most of microbial communities are benthic; finally, there are fewer trophic transfers between the microbial and fungal communities and the top consumers. Furthermore, another alternative pathway was described by Kerner et al. (2003) which considers the abiotic formation of DOC macroparticles that can be consumed by protozoa, bypassing the microbial compartment.

1.2. Dissolved organic matter composition and lability

Although the incorporation of DOC into stream food web is mainly mediated by microbial communities, it is a complex mix of substances and not all the inputs can be assimilated by bacteria. Therefore, while quantification is relatively reliable and fast, the characterization of the substances hiding behind the general denomination of DOC is still an unattainable objective. Due to the high number of compounds included as DOC, it is an arduous task (if ever attainable) to identify and characterize every single substance. Under this limitation, different methods have been applied in order to offer a wide variety of approaches to its characterization (McDonald et al., 2004; Abbt-Braun, 2004).

Usually, DOC (DOM, NOM) is compositionally divided into operationally defined by the chemical fractionation methods resulting in humic and non-humic pools. In natural stream water, the non-humic pool comprises lipids (carboxylic acids) (5%), free amino acids (5%), carbohydrates (10%) and hydrophilic acids (30%). The remaining 50% of DOC would include humic substances that can be divided into two different pools: humic acids (10%), that are soluble in water at any pH, and fulvic acids (40%), that become insoluble in water at pH lower than 2 (Thurman, 1985). Humin would be a third pool that comprises substances that are not soluble in water, but are found in soils rather than in aquatic systems. However the contribution of humic-like substances is variable and changes over time, mainly due to hydrological processes, and it can constitute up to 75% - 80% of DOC in surface waters (Volk et al., 1997) and ground waters (Kaplan and Newbold, 2003). The characterization of the non-humic fraction is usually done through the application of standard laboratory procedures (for instance, Ylla et al., 2010). On the other hand, due to their complexity, chemical reactivity and significant contribution to the DOC pool, humic substances have received a lot of attention and have become a focus in DOC characterization.

Structurally, humic substances are associated to other substances as amino acids, peptides, aliphatic substances, metal ions, clays and oxides of iron and aluminium. Thus, these substances bound to a humic core may alter the in situ biogeochemical properties and role in the aquatic system, in contrast with its properties when isolated.

An approach to DOC characterization is the fractionation according to molecular weight. The tangential ultra filtration is a widely used method to separate a DOC sample into subsamples of different molecular weight ranges (McDonald et al, 2004), although the comparison between studies might be hindered by the applied MW cut-offs, since usually are established arbitrarily. The fraction below 1 KDa is often referred as the low molecular weight fraction (LMW) while values over 1 KDa are usually referred as high molecular weight (HMW). Selecting other MW cut-offs allow further division of the DOC pool, thus considering ranges like medium (MMW), between 1 and 10 KDa, and high molecular weight (HMW), higher than 10 KDa or sometimes higher than 100 KDa (Amon and Benner 1996, Guéguen et al., 2006). Combining the chemical and physical fractionations, fulvic acids present low molecular weight (from 0.5 to 2 KDa), while that of humic acids are slightly higher (2 to 5 KDa). On the other hand, as humic substances present bindings with other compounds, the MW of the complex does not correspond to a single group of substances, even less to a single one.

Further DOC characterization is offered by spectroscopic techniques. Within these set of techniques UV/vis and fluorescence spectroscopy are currently widely applied since they are fast, reliable and relatively economic methods. Nonetheless there are some caveats on its use worth considering: as pointed by Filella (2009), the measured properties are only related to a DOC fraction, mainly that of humic substances. This fact added to the possibility of different substances having similar optic properties prevent obtaining quantitative data from these techniques (Filella et al., 2010). Furthermore, inter-sample comparison might be hindered because of this. In any case, spectroscopy can offer information on DOC characteristics such as humic content and origin, structure. Thus, for a limnologist DOC characterization must be carried under the premise that the accuracy is very limited and the application of different techniques offer a relatively wide window from which to study the role of DOC in the aquatic system.

Considering the DOC role in aquatic ecosystems and its bacterial uptake, a common approach to its study is the measure of its bioavailable fraction (BDOC).

One of the most common methods is to estimate the percentage of DOC incorporated by local (i.e. from the study site) microbial communities. Other methods include the estimation of bacterial respiration or bacterial growth (Meyer et al., 1994; Del Giorgio and Davies, 2003; Cammack 2004).

DOC bioavailability is affected by numerous factors including temperature, light, nutrient concentration, the composition of the bacterial community, the DOC composition and its chemical properties, and the exposure time (del Giorgio and Davis, 2003). The determination of DOC bioavailability is debated due to its complexity, especially that of humic substances, (McDonald et al., 2004) and the comparison between studies is difficult because the different methods that can be applied to measure it might provide different results (McDowell et al., 2006). In fact, BDOC content can vary between 1% and 75% in fluvial systems (Sun et al., 1997).

A recurrent topic regarding the changes in DOC bioavailability is the effect of light irradiance since photodegradation processes might compete with microbial processes in the mineralization of organic matter. Photodegradation processes have been observed to affect positively DOM lability by breaking down complex molecular substances into simpler components (Wiegner and Seitzinger, 2001). On the other hand, different studies conclude that labile and refractory DOC pools are affected differently by photodegradation: the considered labile fraction would reduce its bioavailability, while the refractory would increase in bioavailability (Obernosterer et al., 1999, 2004). Other studies conclude that DOC exposure to sunlight presents no clear effects on bacterial uptake (Lindell et al., 1995; Miller and Moran, 1997). Finally, Wiegner and Seitzinger (2001) hypothesize that these contrasting effects may result from differences in DOC and bacterial community composition, besides the light intensity and the spectral distribution applied in the experiments.

The axis defined by DOC molecular weight has been used as a reference in order to examine its bioavailability. Generally, non-humic low molecular weight (LMW) are traditionally considered labile, while high molecular weight (HMW) substances, especially humic and fulvic acids were considered refractory. This initial distinction also obeys to the origin of DOC. Since humic substances are often from terrestrial origin and have experienced already humification/degradation processes are considered refractory, while LMW substances are considered to be freshly originated from autochthonous origin. ((Thurman, 1985).

Considering DOC molecular weight, Amon and Benner (1996) proposed the size-reactivity continuum model. Within this framework, DOC lability increased with molecular weight, challenging the traditional view of LMW DOC being labile. Nonetheless, it is far from being a definitive conclusion rather than another hypothesis on the metabolic use of DOC in aquatic systems.

Other studies point to more complex interactions in DOC energetic pathways in natural systems where the small pool of biologically labile molecules (LMW DOC) is cycled rapidly in microbial communities (Volk et al., 1997). On the other hand, it has been found that photodegradation not only increases DOC lability by molecular breakdown into LMW DOC compounds, but also modifies the structure of HMW DOC increasing its availability (Miller and Moran, 1997). Furthermore, humic substances would support bacterial metabolism in streams, compensating the slower uptake rates with its high concentration in stream waters and, thus, becoming and important energy source to the ecosystem food web (Wetzel, 2005). Further evidence is put forward by Volk et al. (1997) that determined that 75% of total biodegradable DOC (BDOC), and that 30% of carbohydrates and amino acids were humic bound. Therefore, the determination of humic content is not necessarily predictive of DOC lability or recalcitrance. In fact, referring to humic substances as refractory organic matter (ROM) does not mean necessarily that it is not available for bacterial consumption but instead that it is not a preferred substrate for degradation. Therefore, humic substances do not fully deserve the denomination of refractory organic matter (ROM), at least in natural systems.

DOC processing and lability in groundwater

Although, DOC concentration in groundwater from subsurface riparian zone are usually lower than in surface stream water, it supports bacterial growth suggesting that is already available to the bacterial community As DOC is transported through hyporheic flowpaths, DOC becomes gradually depleted, but is degradability depends on land uses (Findlay et al., 2001). Thus, riparian flowpaths will influence the quantity and quality of DOC reaching the stream water, and it is likely that the inputs will be low and of refractory materials (Sobczak and Findlay, 2002). This depletion mainly depends on bacterial metabolism, not on initial concentration, dilution or residence time (Sobczak and Findlay, 2002). Even, in the case of DOC adsorption in the mineral soil, DOC can remain available to the hyporheic microbial community, and the increased surface to volume ratio and the longer residence time allow a more effective DOC processing (Findlay 1995, Fiebig et al., 1997; Buffam et al., 2001).

The changes in DOC concentration along the fluvial axis are usually of lower magnitude than those variations caused by hydrologic events. Therefore, there is little literature focusing on the spatial dimension when focusing on DOC properties. According to Sun et al. (1997), DOM from headwater reaches is close to a simple mixture of the main components of biomass (lipids, carbohydrates, proteins and lignins). But downstream there is an alteration in DOM composition that causes a decrease in bioavailability, attributed to selective degradation of aliphatic carbon. Also, there are few studies applying spectroscopic methods to discriminate DOC origin and fate in rivers (Battin et al., 1998; Baker et al., 2004).

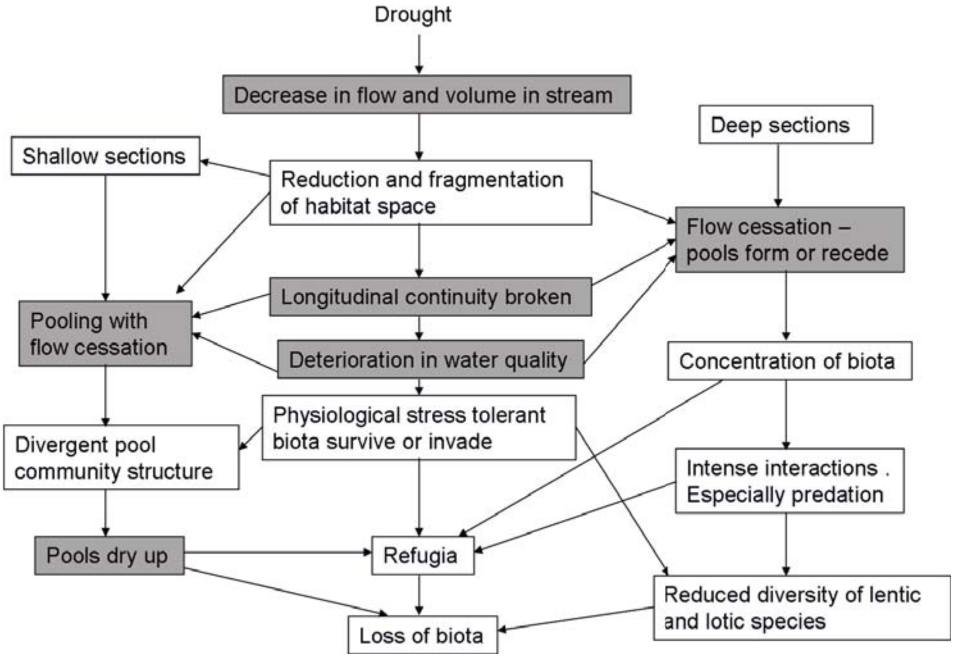


Figure I.2. Progressive effects of drought on stream(from Lake 2000). Grey boxes indicate hydrological conditions that are explored in the following chapters.

1.3. Hydrology and DOC in Mediterranean systems

Mediterranean-type climate is found mainly in the Mediterranean Sea basin (latitude 30-45ºN), the north western pacific coast of America (latitude 31-41ºN), the central Chilean coast (32-41ºS), in the western Cape region of South Africa (latitude 32-35ºS) and parts of West and South Australia (latitude 32-38ºS) (Gasith and Resh, 1999). The annual precipitation varies from arid (less than 200 mm) to humid (>1000 mm), although many regions are considered semi-arid (200-500 mm) (referencies). The precipitation episodes are subject to strong seasonality and high interannual variability, most of them occurring in the autumn and winter season. Isolated major precipitation events often account for a high percentage of the annual total precipitation (Bernal et al., 2002).

The hydrologic regime follows the same seasonal pattern and presents the same interannual variability described by the precipitation regime. Due to the distribution of precipitation events, Mediterranean regions experiment water stress deficit caused by the difference between high evapotranspiration and the low precipitation episodes during certain extended time periods, resulting in periodical low flows. These low flows might be helpful to classify rivers in arid/semi-arid environments: i) perennial streams with low annual flow variability that have seasonal low flows but do not cease to flow; ii) perennial streams with high annual variability that cease to flow in extreme years; iii) ephemeral streams that regularly cease to flow in the dry season; and iv) arid zone streams with long and erratic periods of no flow (McMahon and Finlayson, 2003).

Hydrology, as a product of the climate and watershed geomorphologic characteristics, is a driver of changes in DOC concentration and processing. Therefore, temporary rivers are characterized by cycles of dry-wet periods and the complex hydrological dynamics in the longitudinal axis that in turn influence biotic communities and nutrient and organic matter processing (Larned et al., 2010).

Fluvial ecosystems are subject to two main types of natural hydrologic disturbances of opposed sign: floods and droughts (Lake, 2000). Under the hydrological framework of recurrence of these two opposed disturbances, Larned et al. (2010) proposed a conceptual model where temporary rivers would work as longitudinal biogeochemical reactors. Flood events, and subsequent high flow periods, would transport solutes and particulate organic matter downstream increasing material processing (i.e. fungal and bacterial activity, Artigas et al., 2009). In contrast, under low discharge and surface water drying would result in the deposition of materials, and slower material processing. Thus, the efficiency of processing would increase with the number of cycles of transport and deposition occurring from headwaters to the river mouth.

While the effects of droughts have been less studied that floods, most studies are focused on its effects on the biota (Gasith and Resh, 1999; Lake 2001; Stanley et al., 2010; Gaudes et al., 2010), while the effects on biogeochemical aspects and water quality are less abundant (Dahm et al., 2003).

In limnology, studies on these two disturbances are usually focused on its impact on the biotic components of the ecosystem, mainly on algal and/or macroinvertebrate communities (Stanley 2010). This is probably due the very own definition of 'disturbance' wherein the event is required to have an impact on the biological aspects to be considered as such.

Drought as ecosystem disturbance

Over the years, a comprehensive definition of drought has been elusive (Lake, 2000; Smakhtin, 2001; Humphries and Baldwin, 2003; McMahon and Findlayson, 2003). One of the main reasons is that aspects like return time, intensity, duration and long-term trends in low flow periods are specific to regions and time periods (Humphries and Baldwin, 2003). Furthermore, since the consequences caused by subjacent climatic factors do not only translate into hydrological or biological consequences, but include socio-economical factors, the definition of drought might span into diverse knowledge/activity fields. In 1965, Palmer defined drought as "an interval of time, generally of the order of months or years in duration, during which the actual moisture supply at a given place rather consistently falls short of the climatically appropriate moisture supply". Thus, the identification of a drought period will vary on different regions according to what is usual precipitation, or moisture supply. By this definition it becomes very complicated to compare drought processes over different places and times. Drought is also an unusual natural disturbance in the sense that it is a deficiency rather than an excess, developing along three different axes: intensity, duration and spatial extent (Bond et al., 2008). On the other hand, several drought definitions have been proposed complicating the task of researchers: meteorological drought is defined as the drought caused by lack of precipitation in a large area over an extended period of time; soil moisture drought, as a result of the meteorological drought and high evaporation rates; agricultural drought, when soil drought prevents the sustainability of crops; hydrological drought, when there is a deficit in ground water recharge and surface flow is reduced; the ecological drought would be the one that considers the stress caused by water deficit on ecosystems; and, finally, the socio-economic drought would includes the impacts of water deficit in areas with large populations (Smakhtin, 2001). The effects of drought might be aggravated by population growth, environmental degradation, and the placing of pressure on water resources (Bond et al., 2008).

Considering only fluvial systems, Humphries and Baldwin (2003) defined drought as *an unpredictable low flow period, with an unusual duration, extent, severity or intensity*. On the other hand, Lake (2003) considers drought as *a disturbance in which water inflow, river flow and water availability fall to extremely low levels for extended periods of time*. Furthermore, Lake (2003) classified droughts into two sub categories: seasonal droughts, which would be frequent and predictable causing low flow or even complete drying of the streambed; and supra-seasonal droughts that would be unpredictable and occur in the scale of decades, conforming to the more wide scope approximation to drought (Palmer, 1965, Wilhite 2001). Seasonal droughts are identified as press disturbance, where intensity would arise sharply and then maintain a constant level, and supra-seasonal would adjust to a ramp disturbance, where intensity would increase steadily over time without reaching a maximum (Lake, 2000).

The direct effects of drought on stream ecosystems, that would correspond to hydrological drought, are water loss, characterized by lowering of ground water levels and decrease of streamflow discharge, and the subsequent hydrological fragmentation (loss of connectivity), and loss of habitat for aquatic organisms (Smakhtin 2001, Lake 2003, Bond 2008). The indirect effects include the deterioration of water quality, the alteration of food resources, and changes in the structure and strength of interspecific interactions, including densities, population size or age structures, community composition and diversity (Lake 2003).

The principal hydrologic effect of droughts is a disruption of the fluvial continuum in different degrees according to its severity and duration, and the geomorphologic properties of the fluvial network. The hydrological connectivity is disrupted along three axes: longitudinal (upstream-downstream), lateral (stream-riparian zone), and vertical (surface water – hyporheic/ground water). The loss of connectivity does not take place at the same time along the fluvial continuum; it might increase gradually as the drought period persists. Furthermore, the hydrological fragmentation is heterogeneous in space depending on water volumes, channel morphology and patterns of hydrological connectivity (Bond et al., 2008).

Lake (2003) identified three general fragmentation patterns: surface flow may start drying from downstream to upstream (downstream drying) or vice versa (headwater drying), and also it could start in the middle reaches (mid-reach drying) (Lake, 2003). This drying process causes the apparition of isolated water pools depending on the geomorphology of the stream bed. For instance, shallow sections, like riffles and runs, are prone to be the first to disappear, while deeper and shaded section persist as pools. These pools might be short-lived if the drought period spans for a long time, but in constrained zones with impervious substrata these pools might be present until the reestablishment of flow (Stanley 1997, Lake 2003)(Figure 2).

The hydrological fragmentation of the river continuum causes longitudinal ecological processes preventing inorganic nutrients, organic carbon and biota transport downstream. Consequently, different conditions are possible to be observed in each pool, becoming distinctive lentic environments (Lake 2005). On the other hand, there is a stimulation of other processes like algal blooms and increased competition and predation (Lake 2000). In the isolated pools there is a decrease in bioavailable DOC inputs (Dahm et al., 2003). But riparian leaves leachate might become an important source of DOC, mostly in the form of polyphenols, and the combined effect of low dissolved oxygen, even reaching levels of hypoxia, and high DOC concentration may stress aquatic organisms (McMaster and Bond, 2008). On the other hand, where there is no input of riparian leaves, DOC and other organic nutrients concentration are low, decreasing the ratio of organic:inorganic nutrients, and, therefore, favouring autotrophic processes over heterotrophic ones (Freeman et al., 1994; Dahm et al., 2003).

High flow periods and flood events

In arid and semi-arid (Mediterranean) systems, rain events stimulate biological activity, generating reserves of biomass, propagules and organic matter, facilitating rapid ecosystem responses to subsequent precipitation events (Collins et al., 2008). Furthermore the size of the rain episode/pulse event influences carbon balance since microbial respiration responds to small pulse sizes (Huxman 2004).

The period during which surface stream discharge increases and there is a re-connection of the fluvial network is usually referred as the hydrological transition period (Butturini 2002, Bernal 2005). During this period not only hydrological recovery takes place, but also a reactivation of biotic activity due to the transport and availability of accumulated nutrients and organic material (Collins et al., 2008; Artigas et al., 2009).

During precipitation events, DOC concentration increases, but its origin changes according to the time span, precipitation intensity and previous moisture conditions. During the rising limb of the storm hydrograph, the increase in DOC is mainly due to the flushing and run off in the riparian and near stream zone, while later, during the falling limb, inputs from the catchment hillslope become more prominent (McGlynn and McDonnell, 2003). Therefore, within a catchment there are different sources of streamwater DOC: riparian, hillslope, deep and shallow groundwater, overflow and soil water that can be mobilized at different moments of a pulse event (Katsuyama et al., 2002; Hood et al., 2006)

Factors regulating the extent of the increase include antecedent moisture conditions and the magnitude of the precipitation event (Turgeon and Courchesne, 2008), while discharge alone is not a good predictor (Bernal et al., 2002). A modelling approach by Acuña et al. (2010) showed that the effects of flood events on carbon balance depended on the time of the year. For instance, according to the model prediction the effects would be small at the beginning of spring, but would present considerable influence during autumn (during or after the hydrological transition from drought to wet conditions).

Precipitation events not only cause an increase in DOC concentration, but also a shift in composition. Overall, there is an increase in aromatic content (Vidon et al., 2008; Sanderman et al., 2009) and the contribution of HMW DOC (Maurice et al., 2002; Li et al., 2003), while non-humic substances decrease in abundance (Hood et al., 2006). In consequence, the bioavailable fraction of the mobilized DOC is moderately higher than during baseflow (Volk et al., 1997; Kaplan and Newbold, 1995; Don et al., 2005; Buffam et al., 2001). On the other hand, other studies offer the contrary conclusion, showing a decrease of 50% in BDOC content (Wiegner et al., 2009). Nonetheless, the increase in BDOC content does not necessarily imply and increase in bacterial growth, while in some catchments BDOC content is likely to depend on seasonal factors rather than short-term differences in flowpaths (Buffam et al., 2001)

Studies on climate change point to an alteration in the timing, frequency and magnitude of rainfall and warmer temperatures in arid and semi arid areas (Lohse 2009), and therefore fluvial systems in those areas may play a role in the alteration of global carbon cycling.

Since hydrology is a primary driver of the biogeochemical cycles in fluvial systems it has been a thoroughly explored topic for the last decades, especially from the perspective of nutrient export in different climatic areas. But, unlike inorganic nutrients such as phosphorus and nitrogen, until recently the relationship between DOC and hydrology has not been fully explored. Furthermore, the study of DOC can be approached from two main fronts: one quantitatively, more easily assessed, and qualitatively, focusing on DOC properties, with originally a strong emphasis on isolated samples. Therefore, it is needed a reconciliation between DOC qualitative changes that may occur in natural systems and the influence that climatic factors might have on this basic piece in the vast scope of ecosystem functioning.

In this thesis the drought period takes a central spot. The quantitative and qualitative study of DOC is always done from a hydrologic perspective and therefore, the relevance of the drought period is found in the background (i.e. when considering antecedent hydrologic conditions) or in the forefront, even considering the decreasing flow period and the obvious spotlight when examining the consequences of hydrological fragmentation, aspects considered by Lake (2000), as shown in figure 2, when defining the factors affecting the biotic community during this period.

STUDY SITE

1. Introduction

Fuirosos stream is a Mediterranean intermittent stream that has been an object of hydrologic, biogeochemical and ecological studies. The catchment is located 60 Km North from Barcelona, located in a region with considerable urban, industrial, and agricultural activity. Within the catchment the sparse human population and the agricultural activity has gradually decreased over the last 50 years. Since 1989, most of the catchment area has been part of a protected natural area. Therefore, the main land use within the catchment has been forest management, and agricultural and livestock activities have been residual. These conditions made Fuirosos catchment an exceptional site, within a rather humanized Mediterranean region, to conduct long term ecological research of intermittent streams in Mediterranean regions under relatively pristine conditions. Therefore, since 1998, Fuirosos stream has been a target site for hydrologic, biogeochemical and ecological studies and has become a reference site for fluvial ecology in Mediterranean regions.

Research in Fuirosos has been approached on two fronts: biogeochemical and ecological, always with a strong background reliance on hydrology due to the particularities of the Mediterranean climate that cause a yearly dry-wet cycle.

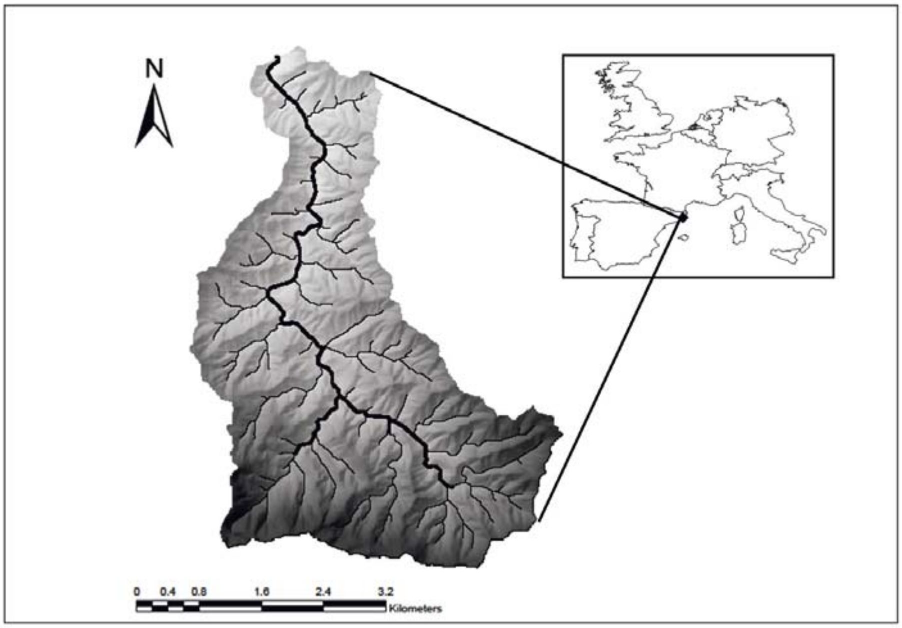


Figure S.1. Geographical location of Fuirosos catchment.

Finally, in the context of the changes in land use forecasted within the Fuirosos catchment to reactivate the human activity, the results from the long-term studies are really valuable since they set the reference under relative pristine conditions. It is important to notice that our long-term data set provides knowledge on the temporal variability of the stream dynamics at inter-annual scale, which, it is very remarkable in regions under Mediterranean climate. In addition, the studies presented in the following texts can be relevant to assess possible changes driven by the introduced activity within the catchment and can be also valuable to set a priori conditions for management decisions that ensures minimal alteration of this relatively well preserved catchment.

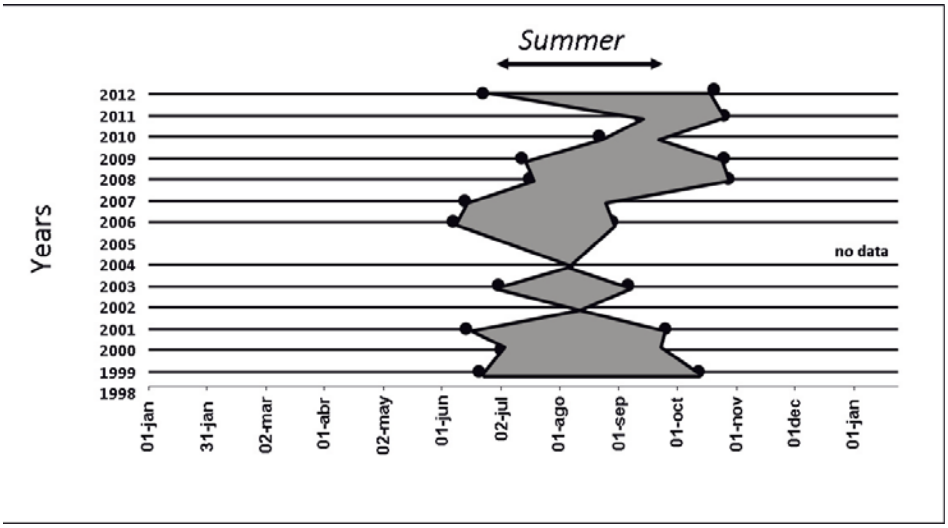


Figure S.2. Temporal localization of the dry window from 1998 to 2012. Note that there is no data corresponding to year 2005. The grey area marks the span of the dry period.

2. Catchment description

Fuirosos is a small intermittent Mediterranean stream in the northeast of Spain (41°,41’,51” N, 2°34’40”E) that drains an almost completely forested granitic catchment (15 km^2) (Figure 1). The catchment is covered mostly by perennial cork oak (*Quercus suber*) and pine tree (*Pinus halepensis*), deciduous woodland of chestnut (*Castanea sativa*), hazel (*Corylus avellana*) and oak (*Quercus pubescens*) predominate in the valley head. The riparian vegetation is dominated by plane tree (*Platanus hispanica*) and alder (*Alnus glutinosa*). The forest covers approximately 90% of total catchment area.

Climate is typically Mediterranean. Air temperature ranges from -2º to 28ºC, and precipitation mostly falls in autumn and spring with occasional summer storms. Average annual precipitation ranges widely from less that 550 mm to more than 750 mm. Stream basal flow discharge ranges between 0 and 25 $L \cdot s^{-1}$. During storms discharge can frequently increase up to 500 $L \cdot s^{-1}$ and during very extreme events it can exceed 1000 $L \cdot s^{-1}$. Storm episodes usually account for the 10-15% of the annual precipitation; however they contribute to more than 60% of the annual water export. Stream flow is usually interrupted by a dry period in summer, which ranges between <30 days to >90 days. This period is followed by an abrupt stream recharge coinciding with the heavy rains during autumn. The consequent humid period elapses until late spring, early summer. The stream main stem is 10 km long with a small reservoir roughly located in the middle of the stream length. This reservoir was built 60 years ago, storing from 50 to 90% of total stream network water volume (40000 L).

3. Research in Fuirosos

The long term biogeochemical monitoring Fuirosos has made possible that it becomes a Mediterranean reference system in nutrient modelling across a range of European fluvial systems covering (Bernal et al., 2004, Médici et al., 2010).

Other studies that include Fuirosos as a reference can be found in Sabater et al. (2008) wherein the climate effects on trophic structure comparing Mediterranean and Atlantic streams are examined, and in Sabater et al., (2003) wherein nitrogen removal by riparian buffers are studied.

3.1. Hydrologic regime

Despite interannual variations in the amount of precipitation, stream hydrology in Fuirosos is overall characterized by two distinct hydrological windows: the flowing and the dry window. During the period 1999-2012, the flowing window has accounted for 80% of the time, while the dry window has accounted for the remaining 20% (Figure 2).

The dry window consistently occurs in summer; however, its time span presents a high variability among years depending on the precipitation regime and atmospheric temperatures. In fact, the period includes two years when the main stream channel had permanent flow during the entire year (Figure 3). During this dry window, stream runoff is not directly related to precipitation intensity, due to the low soil humidity that usually precludes rain to reach the stream (Butturini et al., 2002). This is in contrast to what is observed during the flow window, when discharge variability positively responds to the intensity of precipitation events. Furthermore, when the severity of the dry window is high enough, a third brief window with its own hydrological particularities is observed. It is usually referred to as the dry-wet hydrological transition. During this third brief period, in contrast with the dry and flow windows, the stream-riparian interface becomes a very dynamic hydrological compartment, experiencing an ample expansion and follow-up contraction due to reversed water fluxes from the stream into the riparian zone, where biogeochemical processes are amplified (Butturini et al., 2002, 2003).

3.2. Biogeochemistry and organic matter

Coupled to the hydrological monitoring, there is a continuous monitoring of solutes including: chloride, sulphate, DOC, nitrate, and ammonium.

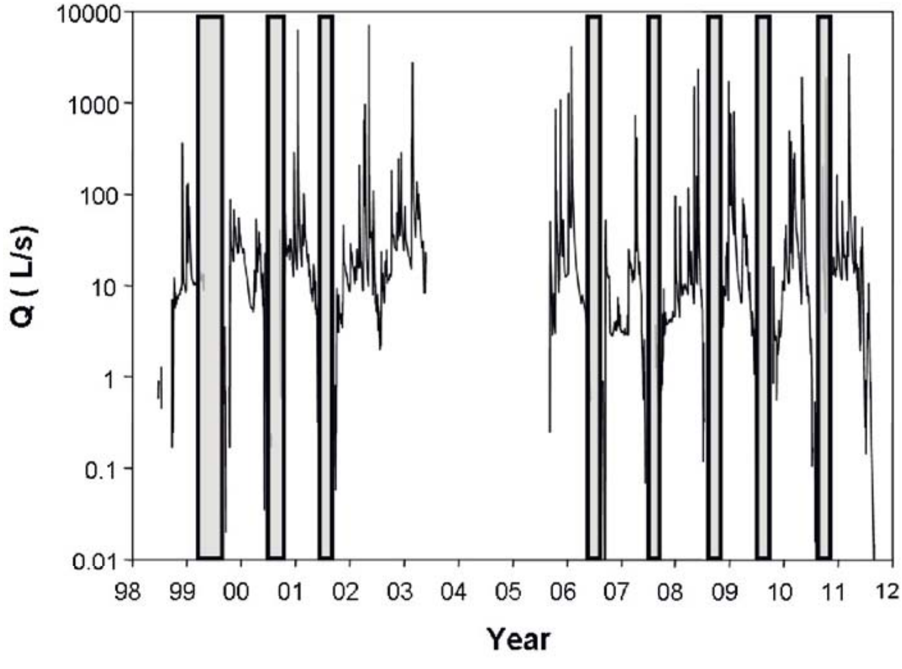


Figure S.3. Discharge in Fuirosos main channel for the period 1998 – 2012. Grey areas mark the dry window.

Nitrate concentration in surface water widely ranges from 0.01 to 3 mg·L^{-1} (Bernal et al., 2005). Despite a remarkable interannual variation, on average the annual nitrate export is 21.4 ± 9.3 Kg·Km^{-2} yr^{-1}. It is worth notice that between 52 and 87% of this export is originated by storm episodes.

Precipitation is the main input of dissolved inorganic nitrogen in Fuirosos catchment. The total annual input from precipitation is 0.41 Kg·Km^{-2} ·yr^{-1}, from which the 45.7% is in the form of N-NO_3 and the rest is in the form of N-NH_4 (Bernal et al., 2003). For both DIN forms, overland flow inputs present high variability. The total annual contribution of DIN is 52.3 Kg·Km^{-2} ·yr^{-1}, and most of it is N-NH_4 (76%).

Concentration of nitrate in the stream shows a clear seasonal pattern, with higher values in winter and lower in summer. This pattern is mostly explained by a higher biological demand within the catchment during the growing season months, which reduces exports of nitrate from the catchment into the stream. In the riparian groundwater, nitrate concentration is lower than in stream water (0.01mg·L-1), but it increases along the axis stream channel – hill slope (where concentration is 3 mg·L^{-1}).

The contribution of storms to nitrate export is important, ranging from 52 to 87% of the total annual export through stormflow. The contribution of large storm episodes greatly increased the nitrate exported quantity (Figure 4, panel a). Overall, the significant increases in nitrate concentration during the hydrological year are observed during precipitation events (and under storm flow), during the dry-wet hydrological transition due to the reconnection between deep groundwater and the upper unsaturated soil layer (Butturini et al., 2002, 2003; Bernal et al., 2002, 2005).

Soluble reactive phosphorus has not been included in the long term biogeochemical monitoring and, therefore, there is no estimation of yearly export. Nevertheless, it has been observed that its concentration is low (0.005 mg·L^{-1}; Von Schiller et al., 2008).

Data collected during 1998-2001 showed that DOC export averaged 180.7± 43.8 Kg·Km^{-2} ·yr^{-1}. During baseflow conditions, the average DOC concentration in stream water varies seasonally with lower and constant values in winter and spring (3 m·L^{-1}), and higher values with greater variability in summer and autumn (4 to 8 mg·L^{-1}). In contrast with DIN, most of DOC export takes place under baseflow conditions, while during intense precipitation events DOC concentration can increase 3.5 times the pre-flood concentration. DOC hysteretic responses to storm flow present high diversity which can not be explained by the concatenation

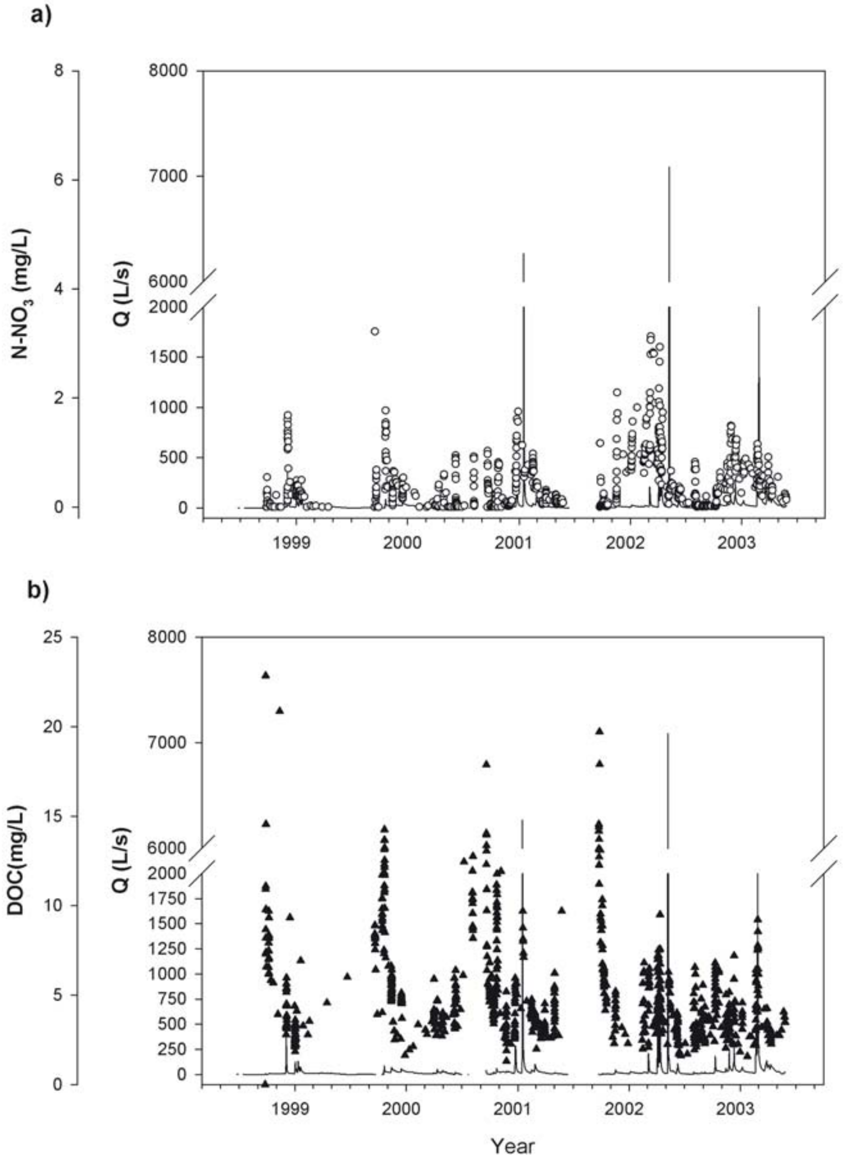


Figure S.4. Nitrate (panel a) and DOC (panel b) concentration dynamics for the period 1998 – 2004. Note the highest concentration DOC values immediately after the dry period while nitrate concentration follows more closely the discharge pattern.

of wet, dry and hydrological transition periods (Bernal et al., 2002, 2005; Butturini et al., 2008).

DOC concentration and export is no related to stream discharge, but to antecedent soil moisture (Bernal et al., 2005). On the other hand, there is an important disruption that hinders the determination of a pattern in DOC dynamics: the hydrologic transition period. While similar intensities of precipitation events happen during spring and winter under the same antecedent moisture conditions, during this hydrologic window DOC concentration reaches the highest values (Figure 4, panel b). Thus, DOC export/concentration is not driven by geochemical factors exclusively, but the accumulation of leaf litter and debris in the riparian strip and streambed during summer (0.45 Kg DW·m^{-2}) (Butturini et al., 2002; Acuña et al., 2004).

3.3. Links between hydrology and biogeochemistry

Concerning the variability in inorganic nutrients in relation to the hydrologic variability, DIN and SRP follow opposite patterns (von Schiller et al. 2008). Therefore, while during the whole wet period Fuirosos seems to be potentially limited by DIN (based on the values of DIN:P ratios), during the dry-wet transition it seems there is a shift towards P limitation.

Following monthly NH_4 and SRP addition over two years determined that uptake velocity (Vf) (Webster and Valett, 2006), was not related to its concentration. Usually the demand of SRP was usually higher than that for NH_4 during the wet period. However, there is no consistent seasonal pattern: peaks of demand ("hot moments") are observed in every hydrologic period (dry, wet and transition) (Figure 7). On the other hand, Vf-SRP showed a negative exponential relationship with temperature when considering all hydrological periods and, although Vf-NH_4 did not present the same relationship the ratio between Vf-NH_4 and Vf-SRP exponentially increased with temperature (Von Schiller et al., 2008).

The relationship between DOC and hydrology is explored thoroughly in the following chapters of this document.

3.4. Long term monitoring of riparian OM inputs and stream metabolism

Riparian inputs of particulate organic matter (POM), mostly in the form of leaf litter, vary according to tree species and period of the year. Inputs of sycamore leaves into the stream are usually lateral and are more important in spring (0.45 Kg DW·m-2). In contrast inputs of alder leaves originate from vertical fall into the stream and peak at mid-summer and autumn (5 - 28 g DW·m^{-2}·day^{-1}; Sabater et al., 2001). The accumulation and transport of leaf litter is regulated by hydrology, and subsequently influences stream metabolic rates and the relative dominance of gross primary production (GPP) pr ecosystem respiration (ER). Nevertheless, in Fuirosos, hot moments of in-stream fluvial metabolism are related to benthic organic matter (BOM) inputs. The average annual GPP is 0.1 ± 0.0208 Kg $C\cdot m^{-2}\cdot y^{-1}$, and the average ER is 1.02 ± 0.308 Kg $C\cdot m^{-2}\cdot y^{-1}$. Overall, benthic organic matter accumulation is more important during the dry period when downstream transport is interrupted, ranging from (252 to 408g C m^{-2}) (Acuña et al., 2004, 2007). It is during the hydrological transition that the highest ER values are observed and, in contrast during humid years with milder dry periods ER are lower.

3.5. Microbial and macroinvertebrate communities

A wide range of topics concerning fluvial communities have been explored in Fuirosos, from OM utilization by different microbial communities (algae, fungi, bacteria) (Artigas et al., 2004, Romaní et al. 2004, 2008, Ylla et al. 2012), substrate colonization and biofilm formation (Romaní et a., 2004, Ylla et al., 2007, Romaní et al., 2008, Artigas et al., 2009), and water fertilization effects on these communities (Sabater et al., 2005; Domènech et al., 2006) to the structure of invertebrate community and its responses to the dry-wet hydrologic (Acuña et al., 2004) and leaf litter colonization Gaudes et al., 2009) and the role of fish assemblages in the trophic structure (Mas-Martí et al., 2010).

The dry-wet cycle also plays an important role in the structure of microbial and invertebrate communities as organic matter quality and quantity is strongly affected and also imposes physiological constraints on the biota.

The microbial community, during the dry period, finds an increase in dissolved organic matter (DOM) of lower quality (mainly polysaccharides and lipids that provide only C), while during the wet period the availability of peptides (providing C and N) is higher. At the same time, the number of total bacteria decreases noticeably in stream water and streambed cobbles while increase in sand substrate, suggesting that it becomes a refuge during the dry period. During the rewetting period the microbial community recovers rapidly (Ylla et al., 2010).

The macroinvertebrate and meiofauna communities are also adapted to the periods of no surface flow. Under these conditions, like the microbial communities, they find refuge in sandy sediments. In Fuirosos, the highest diversity of macroinvertebrates occurs during the early stages of the dry period when discharge is low and the fluvial system is contracted. The dry conditions cause also an increase in macroinvertebrate density due to the concentration in solated water pools. However, as the hydrological fragmentation becomes more obvious, macroinvertebrate densities in these isolated pools decreases due to the changes in the physical and chemical properties of the remaining water. Once the hydrological connectivity is restored, during the hydrologic transition, the resultant community is different from the one described during the dry period. This difference is more marked when the dry period is more severe (Acuña et al., 2005).

4. Last remarks and possible future

In Fuirosos, hydrologic regime influences DOC and NO_3 temporal dynamics. Although both solutes are flushed during storm episodes, the sequence of responses is difficult to categorize and model.

The periodical dry-wet hydrological transition strongly affects distinct aspects of the stream system: From biogeochemical dynamics to metabolic fluxes and microbial and macroinvertebrate communities.

Nevertheless, the shifts during this phase need further detailed exploration on a longer temporal scale to examine how the concatenation of dry periods and storm episodes with different degrees of severity might affect biogeochemical processes. Furthermore, we have found that biota is affected by the dry periods, but how it is affected by the recurrent dry-storm conditions is still an open question. The unpredictability and short-time duration of this phase methodologically constrains a more detailed investigation of the changes physical and chemical changes and how they influence the stream biota.

Although there are already many studied topics on biogeochemistry and fluvial ecology in Fuirosos there are still questions left unanswered. Thus, it is necessary, for as long as possible, to maintain the temporal hydro-biogeochemical monitoring and the nutrient retention estimates. In the present, with the restart of livestock raising in close areas to the stream, the necessity for monitoring becomes stronger. The previous fertilization studies should provide an invaluable resource to evaluate the impact that these anthropic activities will have on stream biota.

The dry-wet phase shifting should be further explored on a longer temporal scale where it would be possible to examine how the concatenation of dry periods and storm episodes with different degrees of severity might affect biogeochemical processes concerning dissolved organic matter. Furthermore, it has been how biota is affected by the dry periods, but how is affected by the recurrent storm/rain episodes is still an open question.

Finally, with the recent changes (and forecasted additional ones) in land use within the Fuirosos catchment to reactivate the human activity, the results from the long term studies are really valuable since the set the reference under relative pristine conditions. Since this long term data set provides knowledge on the temporal variability of stream dynamics at inter-annual scale, it can be relevant to assess possible changes driven by the introduced activity within the catchment.

OBJECTIVES

The main goal was to explore the effects of the dry-wet period cycle on dissolved organic matter both quantitative and in composition. As pointed out in the previous section, DOC, in Fuirosos catchment, was studied almost exclusively from a quantitative perspective. Thus, DOC properties had not been characterized nor the effects of hydrological changes on its composition.

The first chapter aims to further the insight on DOC and nitrate concentration responses to changes in hydrology, specifically to storm events. The next three chapters aim to study with more depth DOC composition and its role in a Mediterranean fluvial system, with special consideration of the dry-wet hydrological cycle. The last one wants to be an introduction to spectroscopic techniques and its applications in DOM characterization. It is not an in-depth revision of a single technique but a wide approach to the most applied spectroscopic techniques, a starting point.

The first chapter titled **"Diversity and temporal sequences of forms of DOC and NO3-discharge responses in an intermittent catchment: Predictable or random succession?"** offers an analysis of the nitrate and dissolved organic carbon responses to increases in discharge and how can affect previous high discharge episodes to posterior ones thus establishing a predictable sequence of C-Q responses. The main objectives were:

a) To explore the diversity of DOC and nitrate-Q responses in terms of dispersion data in the unit plane and by means of the Shannon diversity index.

b) To explore the temporal succession of the types of C-Q responses using contingency periodogram analyses.

c) To determine if the possibly detected cycles in C-Q responses overlap with the cycles of environmental variables that characterize the prevailing hydrological and climatic conditions of the catchment (magnitude of storm events, humidity/wetness previous to a storm event, and seasonal changes in temperature).

The second chapter titled **"Effects of the dry-wet hydrological cycle shift on dissolved organic carbon dynamics and fate across the stream-riparian interface in a Mediterranean catchment"** examines, using exclusively field data, the influence of antecedent hydrological conditions in the dynamics and availability of DOC in the stream-riparian interface. It explores the influence of antecedent hydrological conditions comparing the changes in DOC concentration and its molecular size distribution in both stream and riparian groundwater with special emphasis on the hydrological transition after severe dry antecedent conditions.

a) To characterize DOC distribution according to molecular weight (Low: <1kDa; Medium: 1-10 kDa; High:10-100 kDa; Very High:>100 KDa).

b) To examine the possible different fluxes and availability/fate of each DOC molecular weight fraction.

c) To discern the role of the stream-riparian interface in the fluxes of each DOC molecular weight fraction, especially during the dry-wet hydrological transition.

Chapter 3, titled **“Dissolved organic carbon and nitrogen dynamics and availability according to molecular weight over a drought-rewetting cycle in a Mediterranean stream”**, constitutes the natural follow-up of the previous chapter, focusing on DOC quantitative and qualitative changes between the dry period, spanning from spring - summer low flow until total flow cessation, and flow restart during the dry-wet hydrological transition. The specific goals were:

a) To characterize DOM during the dry and wet periods in terms of DOC, DON, DOC/DON ratio and their distributions among three molecular weight intervals (Low: <1kDa; Medium: 1-10 kDa; High:>10 kDa).

b) To discern what is the extent of the influence of carbon and nitrogen content, molecular size or the interaction of these characteristics on DOM bioavailability.

c) To examine the role of different hydrological compartments (riparian groundwater and surface stream water) to changes in DOC and DON concentration and composition.

Chapter 4, **“Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions”** is focused in a less explored hydrological period. In previous studies, the dry period was studied in its initial stages, from the decrease in discharge to the complete hydrological fragmentation, but at the reach scale. Furthermore, the hydrological fragmentation was considered a previous stage to the complete disappearance of surface water, but further changes both in surface water chemistry and DOC composition were initially hinted at from the results in the previous chapter. Therefore, this fourth chapter is another further step in obtaining the picture of DOC processes in a Mediterranean catchment.This was accomplished by:

a) Characterizing the gradual changes DOM undergoes under hydrological fragmentation of the fluvial continuum, changing from reach to catchment scale.

b) Establishing a time scale through the isolation time of each water pool.

c) Characterizing DOM properties during the subsequent re-establishment of flow as means of establishing a reference background.

Finally, under the title **"Fluorescence spectroscopy and UV-vis absorbance as tools for DOM characterization"**, chapter 5 offers a look into the wide range of spectroscopic methods applied in DOM characterization. This chapter is focused on breadth rather than on deep analysis of the techniques mentioned, providing a review and a first approximation to the techniques presented for newcomers since its application is not as straightforward as it would seem at first glance. Furthermore, the lack of a standard method, the application to different mediums (natural water and soils, industrial (by-products) and difficulties in achieving similar results with different machines, especially concerning fluorescence spectroscopy, might hinder the analysis of data obtained.

Chapter 1

Diversity and temporal sequences of forms of DOC and NO_3- discharge responses in an intermittent stream. Predictable or random succession?

Butturini A.[1], Alvarez M.1, Bernal S.[2], Vazquez E.[1], Sabater F.[1] 2008. Diversity and temporal sequences of forms of DOC and NO_3 – discharge responses in an intermittent stream: Predictable or random succession? *Journal of Geophysical Research – Biogeosciences*, 114(G3):3016-3016.

[1]Departament d'Ecologia, Universitat de Barcelona, 08028 Barcelona, Spain.; [2]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544 USA.

ABSTRACT

Storm events have major implications for biogeochemical cycles at local and regional scales and they provide an excellent opportunity to study the hydro-biogeochemical functioning of catchments. However, concentration-discharge (C-*Q*) responses have only been studied in detail for short periods or a few selected events. In consequence, it is difficult to quantify the diversity of C-*Q* responses in a hydrological system and impossible to assess whether the succession of forms of C-*Q* responses follows a predictable sequence or not.

Bearing in mind these shortfalls, the variability of dissolved organic carbon (DOC) and nitrate (NO_3) pulses during storms is analysed in a detailed four-year series from an intermittent Mediterranean stream. In this study, each DOC and NO_3-*Q* response is synthesized by two descriptors that summarize its trend (ΔC; dilution/flushing/no change) and shape (ΔR; linear/non-linear response). We observe that C-*Q* responses are widely distributed along the 2-dimensional ΔR vs ΔC continuum. Furthermore, the temporal succession of forms of DOC and NO_3-*Q* responses follow a random pattern and only the dynamics of the $\Delta R_{(NO3)}$ descriptor show periodicity.

The long-term data set reveals that it is impossible to predict with reasonable precision the full properties of DOC and NO_3-*Q* responses. Thus, a "typical" C-*Q* response does not really exist at our study site, and this apparent diversity of responses has to be handled with a probabilistic approach that allows synthesis of the complexity of the hydro-biogeochemical functioning of a specific catchment.

1.1. Introduction

Storm events are the most effective cause of solute flushing in streams on a short time scale (from hours to weeks). Their occurrence, frequency and magnitude have major implications for biogeochemical cycles at local and regional scales and for management of inland waters (McClain et al., 2003).

Concentration-discharge (C-*Q*) responses related to storms span from linear to non-linear relationships (Evans and Davies, 1998). These patterns reflect the complex hydro-chemical processes in watersheds and provide crucial information for determining the origin and fate of solutes/pollutants in running waters.

The visual characteristics of C-*Q* responses (slope, shape and rotational pattern if hysteresis appears) facilitate their classification with a few, simple parameters (Johnson and West, 1982; Evans and Davies, 1998; House and Warwick, 1998; Butturini et al., 2006). For instance, Evans and Davies (1998) identified 6 discrete C-*Q* hysteresis types within the framework of the mixing hydrological model (Christophersen et al., 1990). Being conscious that a straightforward hydro-chemical interpretation of solute patterns during storms requires caution (Butturini et al., 2005; Rice et al., 2004; Chanat et al., 2003), the typification of these specific C-*Q* patterns represents a promising starting point for the study of concentration fluctuations during storms in terms of "diversity" of C-*Q* responses.

To date, the diversity of C-*Q* responses has barely been studied by hydro-biogeochemists. Due to obvious methodological and/or human resource constraints, most of the research focused on C-*Q* responses has gathered the information from small catchments (preferentially in temperate regions) during a few selected events or for relatively short periods. These studies might convey the perception that a satisfactory description of C-*Q* responses for a specific solute could be obtained by monitoring only a few events in detail. Nevertheless, the increasing evidence that forms of C-*Q* responses show significant variability (Soulsby, 1995; Biron et al., 1999; Evans et al., 1999; Rice et al., 2004; Andrea et al., 2006; Inamdar et al., 2006; Ocampo et al., 2006) and the opportunity to automate long term high resolution sampling programmes (Kirchner et al., 2004) will encourage a more exhaustive analysis of solute behaviour associated with storm events under a wide spectra of hydro-climatic conditions.

No one has explored the characteristics of a large series of C-*Q* responses as a concatenation of storm episodes of different magnitudes spaced at irregular intervals. Furthermore, an attempt to synthesize the description of forms of C-*Q* responses from the point of view of their "diversity" is, to our knowledge, missing. In this context, the questions that this study attempts to answer are the following:

a) How diverse are the C-*Q* responses of nutrients?

b) Does the temporal succession of types of C-*Q* response occur in a predictable sequence?

And finally, a logical question that arises from b is:

c) If a predictable sequence is detected, what controls it?

Here we focus on nitrate (NO_3) and dissolved organic carbon (DOC)-*Q* responses. Both solutes are reactive and their patterns integrate the hydrological mechanisms and biotic processes that occur in catchments (Mulholland and Hill, 1997). Furthermore, NO_3 and DOC are studied widely by biogeochemists because of their relevance to the actual nitrogen and carbon cycles in catchments and their ongoing alteration as well as to improving the management of water quality for human consumption (Houghton, 2003; Galloway, 2003).

In order to provide a complete view of the diversity of DOC and NO_3-*Q* responses and their temporal succession, both solutes were monitored in detail over 4 years in an intermittent Mediterranean stream. The DOC and NO_3-*Q* responses for each storm event are described with two simple descriptors successively plotted in a 2-dimensional unity plane. The diversity of DOC and NO_3-*Q* responses (question a) is explored in terms of the dispersion of data in the unit plane and by means of the classic Shannon diversity index. Contingency periodograms analysis (Legendre et al., 1981) is used to explore the temporal succession of types of C-*Q* response (question b). Finally, if cyclicities in C-*Q* responses are detected, they are overlapped with those observed for environmental variables characterizing hydrological and climatic conditions prevailing in the catchment (i.e. the magnitude of the storm events, the wetness in the catchment prior to the storm event, and the seasonal changes in temperature) (question c).

1.2. Hydrological and biogeochemical characterization of the study site.

The hydro-chemical data set used in this research is from the Fuirosos stream in the Montnegre natural park (41º 42`N; 2º 34´E; 50-700 m a.s.l.). Fuirosos drains a forested, granitic catchment of 13 km^2. The forest (oak holm, coniferous and deciduous) covers 90% of the total catchment area. Climate is Mediterranean with air temperature ranging from -2 to 28 ºC. Stream flow is typically intermittent with a no flow period from July to September, followed by a dry-wet hydrological transition phase (September-October) and a humid period with permanent flow (October-May) (Butturini et al., 2003). Stream basal flow discharge ranged between 0 and 25 L s^{-1} (Figure 1a).

The hydro-biogeochemical data set covers nearly 4 years, from September 1999 to April 2003. This temporal series includes a wide spectrum of hydro-climatic conditions during which the frequency and magnitude of storms was extremely erratic (Bernal et al., 2006). The time elapsing between rain episodes ranged typically from 4 to 30 days, but longer periods without precipitation were far from being sporadic. For instance, no-rain periods of between 3 and 5 months occurred in summers 2000 and 2001 and spring 2000. On the other hand, the heavy precipitation episodes recorded in spring and summer 2002 prevented the summer dry period (Figure 1a). During the study 105 rain events ranging from 2 to 153 l m^{-2} occurred. Storm hydrographs were negligible when total precipitation ($\mathrm{Rain}_{\mathrm{Tot}}$) was lower than 8 L·m^{-2}. Thus, we attempted to obtain as much hydro-chemical data as possible from the 63 storm events with $\mathrm{Rain}_{\mathrm{Tot}} \geq 8$ l m^{-2}.

The magnitude of storms (ΔQ) ranged from 1 to >2000 l s^{-1} and showed a significant relationship with $\mathrm{Rain}_{\mathrm{Tot}}$ ($r^2=0.6$, df=61, $p<0.001$), the data from the dry-wet hydrological transition period being responsible for most of its variability (Butturini et al., 2002). Storm hydrographs lasted from 0.5 to 18 days, and in 80% of cases they were shorter than a week. Hydrographs were brief with a rising discharge limb shorter than 12 hrs or 1 day in 66% and 84% of the events respectively.

In stream water, DOC and NO_3 concentrations ranged between 2 and 20 C ppm and 0.01 and 3 NO_3–N ppm (Figures 1b and c). Both solutes showed clear seasonal patterns. Nitrate basal concentration showed a maximum in winter and a minimum in late spring-summer, which may be a response to seasonal variation in nitrate uptake by terrestrial vegetation and soil microbial organisms (Butturini et al., 2003; Bernal et al., 2005). In turn, DOC had a typically steep peak during the transition between the dry and wet periods (September-October). Previous studies attribute the DOC peak to the flushing of abundant organic matter accumulated in the stream bed during the dry period (Sabater et al., 2001; Bernal et al., 2002;

Butturini et al., 2005; Romaní et al., 2006; Vazquez et al., 2007). The lack of a DOC peak in September-October 2002, preceded by a wet summer period, supported this hypothesis.

1.3. Material and methods

1.3.1. Field monitoring strategy

Stream water was sampled manually every 7-14 days during baseflow conditions. Sampling frequency was increased up to every 2-5 hours during storms with a stage actuated water sampler (Sigma 900 max).

Stream discharges were estimated on each sampling date by mass balance calculation using the "slug" chloride addition method (Gordon et al., 1992). The stream water level was continuously recorded using a water pressure transducer connected to the automatic sampler.

As previously mentioned, a total of 63 precipitation events met the condition $\mathrm{Rain}_{\mathrm{Tot}} \geq 8\ \mathrm{l \cdot m^{-2}}$. A detailed description of C-*Q* responses was obtained in 49 cases (78% of total events). The distribution of the missing values is related to the magnitude of the rain events. In fact, most of the missing C-*Q* responses are associated with the smallest rain episodes, and the percentage of sampled cases increases up to 90% if $\mathrm{Rain}_{\mathrm{Tot}} > 20$ mm.

1.3.2. Chemical water analysis

All water samples were filtered through pre-ashed GF/F glass fibre filters and stored at 4 oC until analyzed. Nitrate (NO_3–N) was analyzed colorimetrically with a Technicon Autoanalyser (Technicon 1976) after reduction of the solute on a copper-doped cadmium column. Dissolved organic carbon (DOC) was analyzed with a high-temperature catalytic oxidation method (Shimadzu TOC analyser) (for additional information see Bernal et al., 2006).

1.3.3. Description of C-*Q* responses

Each C-*Q* response is characterized by two simple semi-quantitative descriptors that summarize solute fluctuation during the storm episode: ΔC ($\Delta C_{(DOC)}$,$\Delta C_{(NO3)}$) and ΔR ($\Delta R_{(DOC)}$,$\Delta R_{(NO3)}$). ΔC (%) describes the relative changes in solute concentration of the C-*Q* response, by the following formula:

$$\Delta C = \left\{ \begin{array}{l} \dfrac{C_s - C_b}{C_s} 100 \quad \text{if } C_s > C_b \\ \dfrac{C_s - C_b}{C_b} 100 \quad \text{if } C_s < C_b \end{array} \right\} \qquad \text{eq. 1}$$

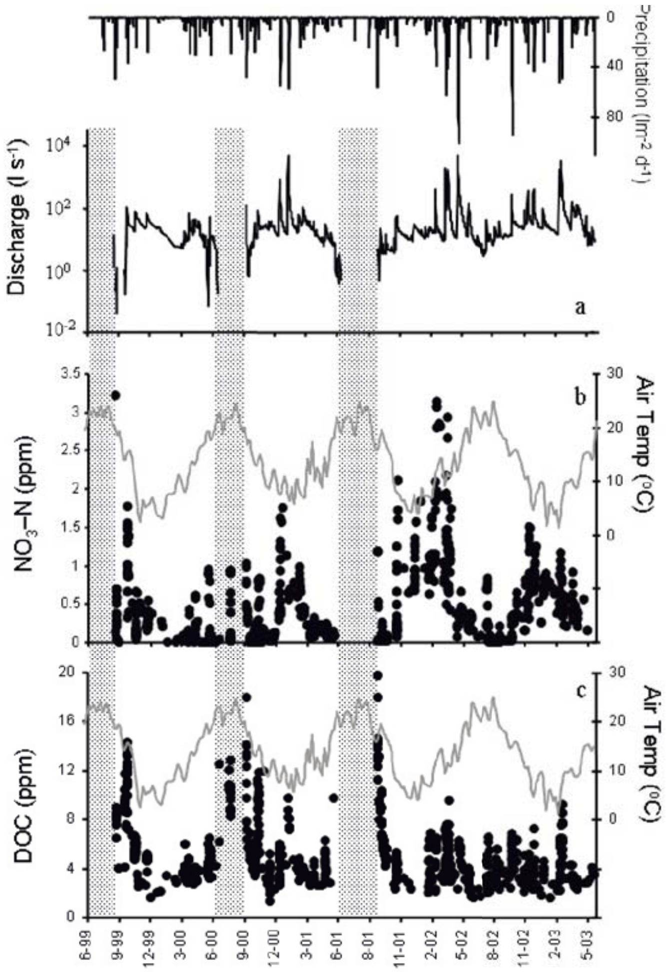


Figure 1.1. Temporal dynamics of daily precipitation and discharge (panel a), NO_3 (as N; panel b) and DOC (panel c) during the study period. Gray lines in panels b and c show the air temperature regime. The shaded area outlines the summer no flow periods.

where C_b and C_s are the solute concentrations at the base flow and during the peak of the storm hydrograph, respectively. ΔC ranges between -100 and 100. Negative ΔC values indicate solute dilution. Positive ΔC values indicate solute flushing.

The ΔR descriptor (%) ranges between -100 and 100 and provides information about the area and rotational pattern of the C-*Q* response:

$\Delta R=R*A*100$ eq. 2

where A is the area of the C-*Q* response, estimated after standardizing discharges and concentrations to a unity scale ($0 \leq A \leq 1$). The term R describes the rotational pattern of C-*Q* responses and therefore the timing of solute changes during storms:

R=1 clockwise rotational pattern

R=-1 counterclockwise rotational pattern

For ambiguous or non-existent rotational patterns we set R=0.

1.3.4. Classification and diversity of C-*Q* responses

The combination of the ΔC and ΔR descriptors (Δ(CR)) synthesizes the variability of the geometrical properties of the C-*Q* responses in the 2-dimensional continuum unity plane ΔR vs. ΔC.

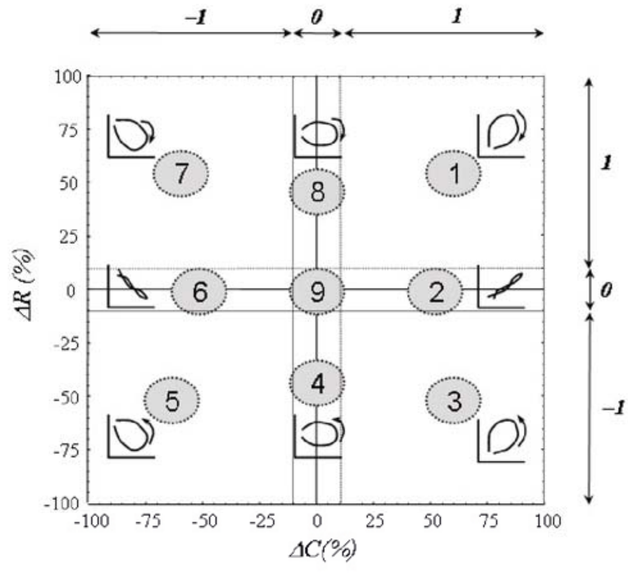


Figure 1.2. Schematic representation of the unity plane ΔC vs. ΔR that describes the diversity continuum across the geometrical forms of C-*Q* responses. In this plane, the vertical and horizontal dotted lines delimit the nine discrete different types of C-*Q* response (see text for additional information).

A discrete qualitative classification of C-*Q* responses can be obtained by splitting the continuum unity plane into 9 regions (labeled from "1" to "9").

Each region identifies a C-*Q* response type. This implies classifying the ΔC and ΔR descriptors into three qualitative categories (“-1”, "0” and "1”). The threshold of ±10% is used to generate these discrete categories:

$\Delta C < -10\%$	-1	(solute dilution)
$-10 \leq \Delta C \leq 10\%$	0	(neutral)
$\Delta C > 10\%$	1	(solute release)
$\Delta R < -10\%$	-1	(counterclockwise loop)
$-10 \leq \Delta R \leq 10\%$	0	(no loop)
$\Delta R > 10\%$	1	(clockwise loop)

The unity plane is then divided into 32 regions. In this way the ΔR *vs.* ΔC plane includes the six C-*Q* hysteresis types (i.e. $\Delta R \neq 0$) proposed by Davies and Evans (1998) plus the simple lineal C-*Q* responses (i.e. $\Delta R \sim 0$) (Figure 2). Having defined the C-*Q* hysteresis into the nine discrete types, synthesis of their diversity is estimated by means of the classic diversity Shannon index (H) which is widely used in ecology, applied mathematics, statistics and physics (Shannon, 1948):

$$H = -\sum_{i=1}^{9} p_i \ln(p_i) \qquad \text{eq. 3}$$

where pi is the relative abundance of each C-*Q* type.

1.4. Time analysis

1.4.1. Contingency periodogram analysis

Periodicities of the temporal succession of C-*Q* response types and of three environmental parameters were explored with contingency periodograms (hereafter CP), a method for analyzing the presence/absence of periodicity in short series that requires the input of categorical data (Legendre et al., 1981).

For each data series a list of contingency statistic Hcs values were estimated for T periods ranging from 2 to 63/2 storm events. Hcs values were calculated according to Shannon (1948).

Graphically a CP consists of a plot relating the values of Hcs to the investigated T periods (Fig. 3). The significance of a period T is tested successively by determining the probability that the associated Hcs value differs from zero, using the following formula (Legendre et al., 1981):

$H_{cs} > c^2/2N$ eq.4

where c^2 is the value of Chi-Square at the selected probability level (see below); N is the number of storm events in the data series. Thus, a hypothetical significant of length T indicates a cycle that comprises a sequence of T storm events (Fig. 3).

The presence/absence of periodicity in the ΔC and ΔR descriptors were estimated by using in each case the three qualitative categories described previously (i.e. "1", "0", "-1"). Similarly, the periodicity of the combination of ΔC and ΔR in the unity plane (Δ(CR)) was estimated using the 9 qualitative types.

1.4.2. Contingency periodogram matrices for C-Q response descriptors

Given that C-*Q* descriptors (ΔC,ΔR and Δ(CR)) were missing in 22% of cases, in order to improve the robustness of the contingency statistic Hcs estimations we proceeded as follows:

1) A random value of ΔC, ΔR and Δ(CR) was assigned to the events without chemical information.

2) To reduce the uncertainty generated by random values, H_{cs} was calculated 104 times and the mean ± standard deviation was calculated for each T period investigated.

3) Being aware that the percent of sampled storm events increased with $Rain_{Tot}$, we assembled a matrix of contingency periodograms for each C-*Q* descriptor. The concatenation of the individual CPs that composed a matrix was obtained after eliminating those storm events caused by a rain episode lower than a selected $Rain_{Tot}$ threshold from the data series. As the $Rain_{Tot}$ threshold increased, the number (N) of storm cases in the data series decreased but the percent of sampled storm events increased. In our case the $Rain_{Tot}$ threshold increased progressively from 8 to 30 l m^2. Conversely the number of storm episodes (N) in each data series decreased gradually from 63 to 29 and the percent of sampled storm events increased from 78% to 93% (see Fig. 3 for an example of individual CP graphs that composed a CP matrix).

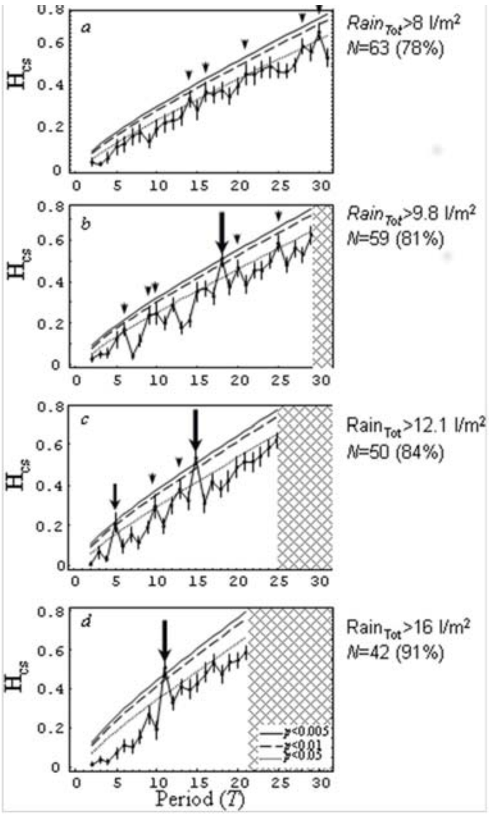


Figure 1.3. Selected contingency periodograms of $\Delta R_{(NO3)}$ obtained after eliminating the storm events below a selected $Rain_{Tot}$ threshold from the data set. Broken lines delimit the critical values at the 0.05, 0.01 and 0.005 probability levels. Arrows show the significant T periods detected by the contingency analysis (small arrow: $p<0.05$; medium arrow: $p<0.01$; large arrow: $p<0.005$). For example, in panel b the analysis detected an Hcs value that differed from zero at $p<0.005$ at a period T=18, i.e., a cycle that comprised 18 storm events. In panels c and d the cycles emerged every 15 and 11 storm events respectively (see the Time analysis section for additional explanation). N is the number of storm events in the data series. In parenthesis the % of sampled events.

4) For each CP matrix only T periods with Chi-Square probabilities $p<0.05$, $p<0.001$ and $p<0.005$ (eq. 4) were extracted and plotted on a surface graph.

5) Having obtained the surface graph face for each C-*Q* descriptor, a cycle is considered robust solely when we can draw an oblique line that connects the significant T periods detected at different $Rain_{Tot}$ thresholds. Otherwise we assumed that the data series was randomly assorted.

1.4.3. Contingency periodogram matrices for environmental parameters

Contingency periodograms can be used to explore the periodicity of environmental variables after reducing the quantitative series into three categories according to the criteria of Legendre et al. (1981).

In this study, we included three intuitive environmental variables frequently used to explore potential cause-effect relationships in hydro-biogeochemical catchment studies (Rice et al., 2004) and that also synthesized the hydro-climatic properties of the Fuirosos catchment appropriately (Bernal et al., 2002): a) Magnitude of each storm event (ΔQ); b) Basal discharge (Q_{bas}) immediately preceding each storm, indicating the antecedent wetness of the catchment; c) Average air temperature during each storm (T_{day}), capturing the regularity of seasons over time and representing a proxy for biotic activity in the catchment (Hobbs et al. 1995). After the categorization of environmental parameters, CP matrices were assembled following the same criteria used for C-Q descriptors. Environmental data were available for all storms, and so there was no need to include random values (i.e. the previously described steps 1 and 2 were unnecessary).

1.5. Results

1.5.1. Time analysis of environmental variables

The CP analysis detected a significant cycle with a period that comprised 16 storm events (T=16; N=63 and its replica at T=31; N=63) for Q_{bas} and T_{day} (Figure 4a and 4b respectively). These cycles are robust. For instance, for Q_{bas}, the cycle persisted as the $Rain_{Tot}$ threshold increased, though the length of T declined progressively as the number of N decreased (from 16 to 6), until it vanished at N<30. An oblique line connecting these significant periods at different RainTot thresholds is drawn in the surface graph (Figure 4a). The cycle observed for T_{day} does not overlap exactly with that for Q_{bas}. In fact, from N<60, this cycle is always longer than that for Q_{bas} (Figure 4d).

The magnitude of storm events (ΔQ) shows a clear and persistent cycle. This cycle starts with a length of T=21 (N=63), and decreases to T=13 before disappearing (N<39) (Figure 4c). Therefore, the period of this cycle is longer than those estimated for Q_{bas} and T_{day} (Figure 4d) at a $Rain_{Tot}$ threshold lower than 12 l m^{-2}.

1.5.2. Diversity and time analysis of DOC and NO_3-Q responses

Dispersion of DOC-Q response data in the ΔR vs. ΔC unit plane clearly differed from that of NO_3. DOC data covered 16% of the unit plane (Figure 5a), while the NO3 data were spread over 48% (Figure 5b). In more detail, DOC data fall in 6 regions of the unity plane. A total of 40% of events are type 2 and the remaining regions range between 27 % (type 1) and 2% (types 3 and 7). $H_{(\Delta R,\Delta C)}$ values, measured sequentially during the events series, peak at 2.4 after 9 events (Fig. 5a the inset). DOC release (ΔC>10%, 73% of events) clearly predominates over dilution. A total of 51% of cases are linear DOC-Q responses (-10%<ΔR<10%), while 45% are clockwise DOC-Q hysteresis ($\Delta R>10\%$).

NO_3 data fall in all the 9 potential regions of the unity plane. The most probable NO_3-Q response is that of type 3 (37%) and the contribution of the remaining NO_3-Q types ranges between 14 % (type 2) and 2% (type 5). All NO_3-Q responses are rather well distributed over time. Consequently, $H_{(\Delta R,\Delta C)}$ values, measured sequentially over the events sequence, increase more slowly than DOC and peak at a value of $H_{(\Delta R,\Delta C)}$~2.9 after 18 events (Fig. 5b, inset). NO_3 release (69% of events) predominates over dilution. Counterclockwise, clockwise, and linear NO_3-Q

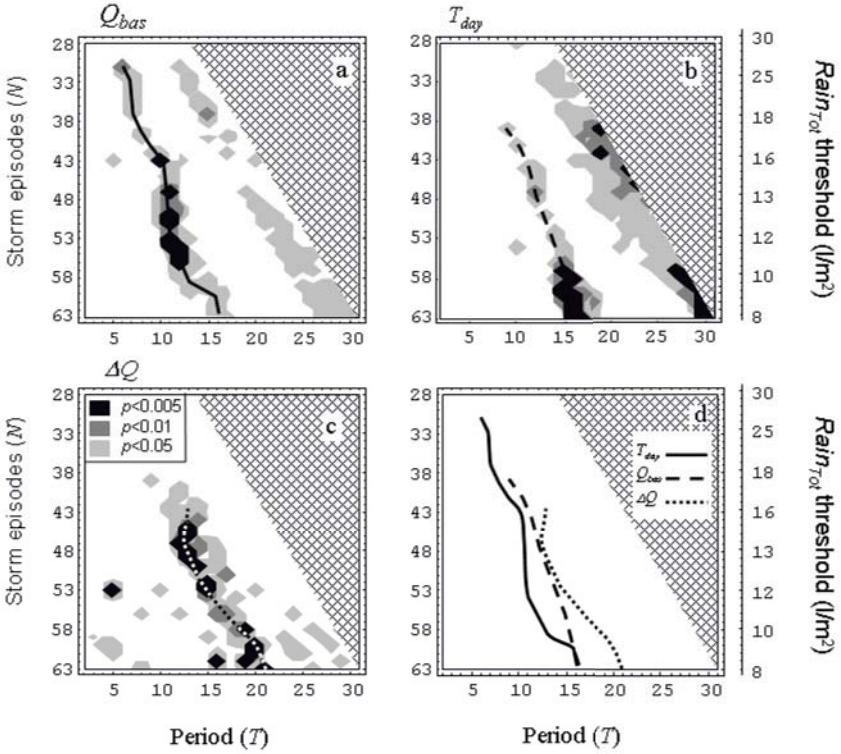


Figure 1.4. Contingency periodogram matrix for basal discharge preceding the storm events (Q_{bas}, panel a), air temperature during storms (T_{say}, panel b) and magnitude of the storm events (ΔQ, panel c). Solid oblique lines that overlap the periodogram matrices connect the statistically significant T periods at different RainTot thresholds. Panel d shows the location of the oblique lines of the three environmental variables in a single periodogram matrix.

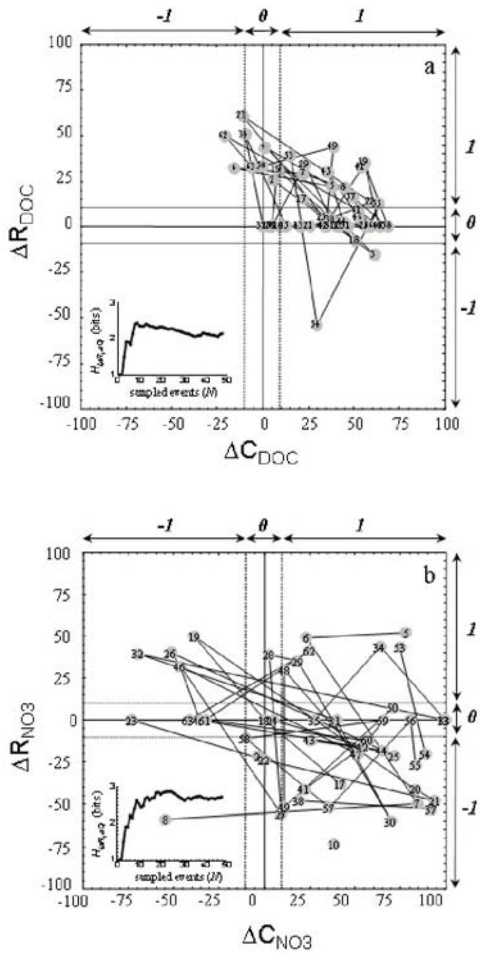


Figure 1.5. Dispersion of DOC-*Q* (panel a) and NO_3 - *Q* (panel b) responses in the ΔC vs. ΔR unity plane. Numbers within circles illustrate the time succession of the storm events. Vertical and horizontal dotted lines in the ΔC vs. ΔR unity plane delimit the nine different types of C-*Q* response. The figures in the inset show the evolution of the DOC-*Q* and NO_3-*Q* response diversity ($H_{(\Delta R, \Delta C)}$) measured sequentially over the event series.

responses are 47%, 24% and 27% respectively.

The CP analysis indicated that $\Delta(CR)_{(DOC)}$ and $\Delta(CR)_{(NO3)}$ do not show any predictable pattern (data not shown). Therefore, the displacement of DOC and NO_3 data in the ΔR *vs.* ΔC unit planes follows a random pattern. Some significant periods with short lengths ($2<T<8$ for DOC and $2<T<6$ for NO_3) were detected (in most cases $p<0.05$), though such cycles were inconsistent because they did not re-emerge regularly during longer periods (i.e. at T between 8 and 31). Furthermore, the observed significant cycles of T are shorter than the minimum number of episodes required to obtain a full picture of C-*Q* response variety (9 and 18 cases for DOC and NO_3 respectively).

On the other hand, when temporal sequences of ΔR and ΔC descriptors are analyzed separately, a periodicity is observed for $\Delta R_{(NO3)}$ and, to a lesser degree for $\Delta C_{(NO3)}$ (Figure 6a and 6b). In more detail, the CP matrix of $\Delta R_{(NO3)}$ detects numerous significant periods (at least $p<0.05$) at different $Rain_{Tot}$ thresholds. Nevertheless, only one cycle persists at different RainTot thresholds (the solid oblique line in Figure 6a). This cycle persists until a Rain_{Tot} threshold of 18 l m^{-2} is reached (N=40). Initially, the cycle is characterized by a period of T=22 storm episodes that declines progressively with Rain_{Tot} until it disappears at T = 11.

A cycle for $\Delta C_{(NO3)}$ is also detected, however its periodicity is slightly longer and patently weaker than that observed for $\Delta R_{(NO3)}$. It is longer because initially it comprises 24 storm episodes (T=24; N=63, $p<0.05$), and vanishes at T =17 (Rain_{Tot} threshold of 13 l m^{-2}; N=40, $p<0.05$). It is weaker because the oblique line that links these significant periods T is much shorter than that observed for $\Delta R_{(NO3)}$ (Figure 6b).

The $\Delta R_{(DOC)}$ and $\Delta C_{(DOC)}$ series lack any consistent cycles, indicating that their temporal succession is randomly assorted. In both cases, some significant periods are detected at different Rain_{Tot} thresholds. However, distribution of these significant periods in the periodogram matrices does not show any consistent pattern (Figure 6c and d).

1.6. Discussion

The data set, obtained from four years of intense hydro-chemical monitoring, demonstrates the need to describe the diversity of forms of C-*Q* responses in probabilistic terms. It is worth noting that the most probable C-*Q* response types (i.e. types 2 and 3 from Figure 5, for DOC and NO3 respectively) represent as much as 40% of all cases. Thus, the majority of the DOC and NO3-*Q* responses fall within some low probability C-*Q* response types. These results point to the large degree of uncertainty in the depiction of a “typical” DOC and NO3-*Q* response and underline the importance of identifying the “typical” probability distribution of a set of C-*Q* responses within the ΔR vs. ΔC unity plane.

Under the proposed probabilistic approximation, DOC and NO_3 data from our study show different distributions and limits in the ΔR *vs.* ΔC unity plane. The DOC data set, although intersecting several C-*Q* response regions, lies in a relatively small portion of the ΔR *vs.* ΔC unity plane. Thus, a minimum set of ~9 events is required to describe the diversity of DOC-*Q* responses satisfactorily. In contrast, the NO_3 data set is homogenously distributed in a large portion of the ΔR *vs.* ΔC unity plane. This implies that a set of at least ~18 events is necessary to capture the variety of NO_3-*Q* responses satisfactorily. An evident consequence of this result is that if we ignore the magnitude of diversity of C-*Q* responses of a determined solute, we do not know if the hydro-biogeochemical interpretation obtained with an arbitrary number of storm episodes is representative or not of the hydro-chemical functioning of the watershed studied.

Within the classical mixing hydrological framework, a plausible hydro-biogeochemical explanation for each C-*Q* response type reported in this study could be given (Rice and Hornberger, 1998). However, the classical mixing model appears to be inadequate to provide a unique and coherent description of patterns of solutes during storms in an intermittent stream such as our study site. For instance, if we constrain our observations to the NO_3 counterclockwise hysteresis (47% of all cases) we might conclude that flushing is transport limited (Burns, 2005). Contrarily, if we analyse the NO_3 clockwise hysteresis (24% of cases) we

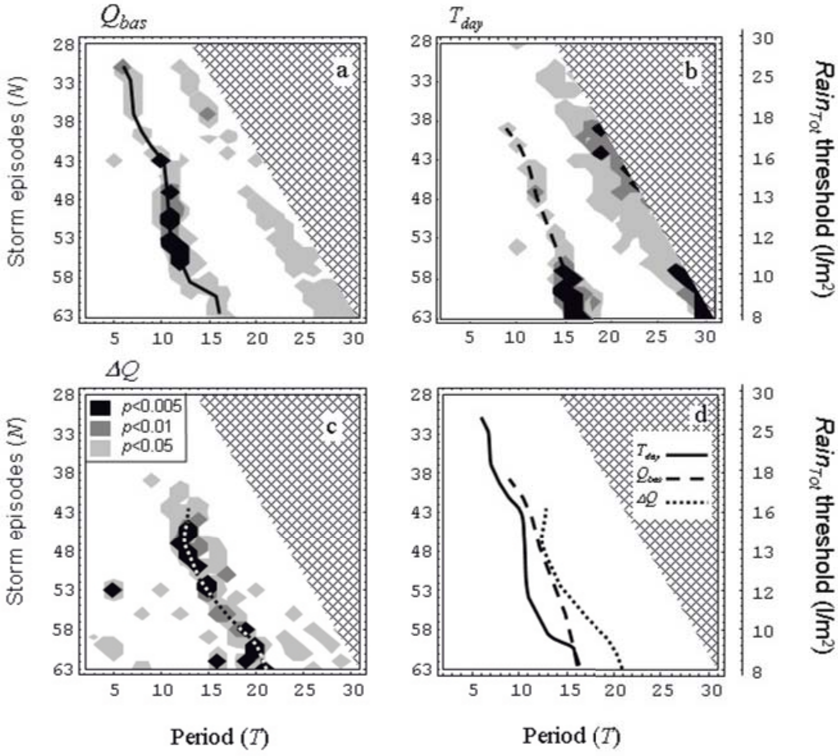


Figure 1.6. Contingency periodogram matrices for $\Delta R_{(NO3)}$ (panel a), $\Delta C_{(NO3)}$ (panel b), $\Delta R_{(DOC)}$ (panel c) and $\Delta C_{(DOC)}$ (panel d). Oblique lines in the periodogram matrices connect the statistically significant periods.

might conclude that its flushing is supply limited (Burns, 2005). A consequence of this high biochemical variability is that the use of the classical mixing approach to identify the sources of NO_3 in Fuirosos is feasible during the humid period but not during the dry-wet transition period (Bernal et al., 2006).

Therefore, it is necessary to develop a hydro-biogeochemical framework flexible enough to justify the diversity of C-*Q* responses and the temporal succession of these forms over the seasons. Recent theoretical studies increase the spatial heterogeneity of the hydrological processes within the mixing model framework, improving its conceptual flexibility (Chanat et al., 2004; Butturini et al., 2005). However, the introduction of new boxes and additional parameters is encouraged, making scientists inquire about whether this approach is really the most appropriate to capture the non-linear hydro-biogeochemical behaviour of catchments (Kirchner, 2006).

The contingency analysis reveals that ΔR and ΔC values cannot be predicted simultaneously either for DOC or NO_3.

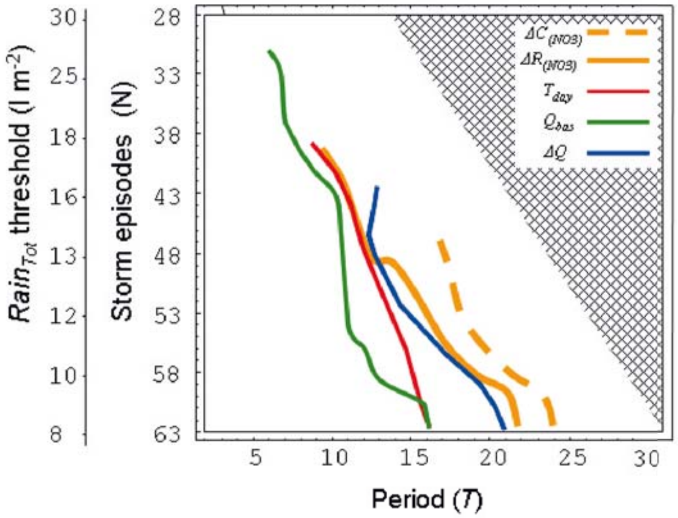


Figure 1.7. Location, in a single plot, of the oblique lines that connect the statistically significant T periods at different RainTot thresholds for the environmental variables considered in this study (T_{day}, ΔQ and Q_{bas}) and the NO_3-Q response descriptors ($\Delta C_{(NO3)}$ and $\Delta R_{(NO3)}$).

However, when ΔR and ΔC descriptors are analyzed separately, signals of periodicity emerge for NO_3. This result is especially attractive for $\Delta R_{(NO3)}$ because it suggests that the timing of NO_3 delivery into the stream over a sequence of storm episodes can be inferred. These results lead to the last question behind our research: what controls the cycles observed for $\Delta R_{(NO3)}$ and $\Delta C_{(NO3)}$?

A preliminary step towards dealing with this question is to overlap the periodogram matrices of $\Delta R_{(NO3)}$ and $\Delta C_{(NO3)}$, with those obtained for the climatic (T_{day}) and the hydrological variables (ΔQ and Q_{bas}) considered in this study. Figure 7 shows that the period of the cycle of $\Delta C_{(NO3)}$ is longer than that observed for the hydro-climatic variables, which show marked seasonality (i.e. cycles of about 1 year, Figure 1 and 4). Thus, processes that act a time scales in between one and two years might influence the cycle of $\Delta C_{(NO3)}$. Being realistic, at the moment, such a time scale cannot be studied satisfactorily by handling a four-year time series only, and we can simply use this result to demonstrate the need to generate pluri-annual hydro-biogeochemical series at high resolution.

On the other hand, the T period of $\Delta R_{(NO3)}$ overlaps reasonably well with that of ΔQ at $Rain_{Tot} < 13\ l \cdot m^{-2}$, and with T_{day} at $13 < Rain_{Tot} < 18\ l \cdot m^{-2}$. This suggests that the periodicity of the magnitude of storms (ΔQ) is the most important driver for the succession of linear and non-linear NO_3-Q responses over the entire spectra of rain magnitudes, while the effect of the seasonal temperature change (i.e. T_{day}), appears more perceptible after the removal of the signal of the weaker and more frequent rain episodes.

The problem of excess NO_3 in running waters is a recognized problem worldwide (Burgin and Hamilton, 2007) and to achieve an accurate simulation of its temporal dynamics constitutes a major challenge for modelers (Wade et al., 2004), especially in intermittent streams where simulations are clearly unsatisfactory (Bernal et al., 2004). From this perspective, our results might represent a stimulus for these scientists, because they suggest that the succession of forms of NO_3-Q responses might be coupled to the magnitude of storm events. On the other hand, the same results also show that a large portion of uncertainty is inevitable and suggest that a probabilistic modeling approach should replace the deterministic one (Botter et al., 2006).

1.7. Conclusions and perspectives

Abundant scientific literature clearly demonstrates the impact exerted by storm events on solute patterns and fluxes. However, pollution of inland waters and the plausible alteration of hydrological regimes on a global scale as a consequence of climate alteration (IPCC, 2007) demonstrate the importance of extending these studies. To persuade their colleagues in this direction, some scientists describe storm events as “hot moments” (McClain et al., 2003) or compare their role to a “crescendo” in a musical piece (Kirchner et al., 2004).

In this context, our study is the first to describe the limits of diversity of DOC and NO_3-*Q* crescendos in a stream under a wide spectrum of hydro-climatic conditions and underlines the need to describe the diversity of C-*Q* responses in terms of distribution of C-*Q* types with different probabilities. Furthermore, the succession of data in the ΔR *vs.* ΔC unity planes is random: a priori, it is impossible to predict the entire pattern of these solutes during storms with satisfactory precision.

In environmental sciences, the use of the adjective “diversity” is immediately related to the biological richness. Thus, at this stage it is natural to inquire to what extent the diversity of C-*Q* responses reflects the complexity of internal hydro-biogeochemical functioning of a specific catchment. For instance, data from our study came from an intermittent stream with an abrupt autumnal hydrological transition from dry to wet conditions (Butturini et al., 2003) with high and low DOC and NO_3 concentration respectively in stream waters (Bernal 2005). Overall, the DOC-*Q* responses monitored during this transitional period (33% of events) contributed to 41% of the total DOC-*Q* response diversity, while in the case of NO_3, the autumn NO_3-*Q* responses did not influence the total C-*Q* response diversity. Therefore, although the dry-wet transition does not promote a typical C-*Q* response during the following autumn, it contributes to enhancing the diversity of DOC-*Q* (but not of NO_3-*Q*) responses.

For ecologists, understanding the relationship between biodiversity and ecosystem functioning has been a fertile theme since the beginning of the ’90s that has been continually fuelled and renewed by the feedback between empirical data and new hypotheses (Naeem et al., 2002). Here, in stream hydro-biogeochemistry, we are just starting. In order to strength this theme we need to widen the implementation of long-term high frequency hydro-chemical monitoring programmes (Kirchner et al., 2004). When this requisite is achieved it will be possible to analyze how the diversity of C-*Q* responses varies among catchments with different hydro-climatic characteristics.

Within this context, the ΔR *vs.* ΔC unity plane might constitute a synthetic, intuitive and universal framework with which to compare and classify the dispersion of the C-*Q* responses of a specific solute along catchments.

Acknowledgments

The authors thank Sergi Sabater, Ester Nin and Antoni Bombí (Servei Parcs Naturals, Diputació de Barcelona), for their support in the field and Chiara Medici for support with environmental data management. We would also like to thank two anonymous reviewers for their fruitful comments, which greatly helped to improve an earlier version of this manuscript. This study was supported by the Ministerio de Educación y Ciencia (CGL2007-60144). The authors are members of the Limnology Group (UB-CEAB_CSIC). AB is a member of the Graccie network (CSD 2007-00067).

Chapter 2

Effects of the dry-wet hydrological shift on dissolved organic carbon dynamics and fate across the stream - riparian interface in a Mediterranean catchment

Vazquez E.[1], Romani A.M.[2], Sabater F.[1], Butturini A[3]. 2007. Effects of the dry–wet hydrological shift on dissolved organic carbon dynamics and fate across stream–riparian interface in a Mediterranean catchment. *Ecosystems*, 10(2):239-251.

[1]Departament d'Ecologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain; [2]Institut d'Ecologia Aquàtica, Universitat de Girona, Campus de Montilivi, 17071 Girona, Spain; [3]Centre d'Estudis Avanc¸ats de Blanes, CSIC, Acce´s Cala St Francesc 14, 17300 Blanes, Spain.

ABSTRACT

The stream-riparian interface is an important control point for nutrient fluxes and processing between terrestrial and aquatic systems characterized by a dynamic and complex hydrology. Predicted alterations in the discharge regime in Mediterranean climate regions make it necessary to understand the effect of abrupt hydrological transition between dry and wet conditions on the transport and fate of dissolved organic carbon (DOC) across the stream-riparian interface. In this study the concentrations and fate of total DOC (TDOC) and a subset of four molecular weight fractions (<1kDa, 1-10 kDa, 10-100 kDa, >100 kDa) were investigated in stream water and riparian groundwater during autumn of 2003 and 2004. The two study periods were characterised by contrasting antecedent hydrological conditions: the streamflow was interrupted in summer 2003 but permanent in summer 2004. Comparison of the two study periods indicates that an abrupt dry-wet hydrological transition amplifies the water exchange across the stream-riparian interface and favours retention of up to 57% of the TDOC that flows across the interface. Furthermore, the efficiency of DOC retention across the stream-riparian interface also varies greatly depending on DOC molecular size. More than 70% of DOC fractions higher than 10 kDa were retained, while the smaller fraction (less than 1 kDa) was nearly conservative. Consequently, our study helps to clarify the effects of extreme hydrological events on DOC transport in running waters in Mediterranean regions.

2.1. INTRODUCTION

The stream-riparian interface is a relevant control point for fluxes and retention of nutrients between terrestrial and aquatic systems (Dahm and others, 1998). The stream-riparian interface is the zone connecting the stream and the catchment and it has been defined as a spatially fluctuating ecotone between the surface stream and the deep groundwater (Boulton, 1998). Consequently, several field studies have investigated the stream-riparian interface in regulating stream hydrology (McGlynn and McDonnell, 2003; Butturini and others, 2002) or biogeochemistry (Vidon and Hill, 2004; Wigington and others, 2003).

In Mediterranean regions, streams are frequently characterized by strong seasonality with a humid period followed by a drought, normally in summer (Gasith and Resh, 1999). In drought there is a disruption in hydrological connectivity that ranges from flow reduction to loss of hydrologic connectivity between surface water, groundwater and riparian zone (Lake, 2003). Streamflow recovers with the arrival of autumn rains. During this transition period between dry and wet conditions the hydrology of the stream-riparian interface is highly dynamic due to: 1) abrupt changes in groundwater levels; 2) occurrence of reverse fluxes in the subsurface stream-catchment interface; 3) rapid expansion and shrinkage of the boundary of the stream-catchment interface; 4) longer riparian groundwater flow-paths (Butturini and others, 2003).

Recent studies in Mediterranean adduce evidence of a severe alteration of precipitation and hydrological regimes and hypothesize an increase in the frequency of extreme events particularly in summer (Christensen and Christensen, 2003; Schröter and others 2005). In this context, it is essential to assess how the stream-riparian interface controls the transport and fate of nutrients that flow in Mediterranean freshwater ecosystems under the extreme effects of dry-wet hydrological shifts that characterize the transitional period between summer and autumn.

Within this framework, the dissolved organic carbon (DOC) transported in streams is essential to secondary production in freshwater and marine ecosystems. The effects of temperature, atmospheric CO2, or hydrology on DOC export to oceans are still unclear and a matter of debate (Freeman and others, 2001 and 2004; Tranvik and Jansson, 2002; Clark and others, 2005). The quantity and the bioavailability of DOC in streams is the combined result of flushing from the watershed, leaching of leaves and branches from the riparian vegetation, and in-stream primary production (Sachse and others, 2005) and its temporal dynamics are usually governed by discharge regime (Butturini and others, 2005; Neal and others 2005) and the occurrence of drought periods (Bernal and others, 2002). Nevertheless, there is little information about the effect of the abrupt hydrological processes that take place in the stream-riparian interface during the transition between dry and wet conditions on reactivity and transport of DOC in streams. Albeit, some initial studies have been undertaken in Mediterranean streams covering DOC transport and bioavailability after drought periods (Romaní and others, 2005, in press).

Our main objective is to explore, in situ and under natural conditions, the effect of the antecedent hydrological conditions on temporal dynamics and fate of total DOC (TDOC) and discrete dissolved organic molecular fractions transported across the stream-riparian interface in order to improve our knowledge of the possible effect of altering the discharge pattern on stream DOC dynamics. For this purpose, data from a forested Mediterranean stream were collected during two autumnal periods characterised by contrasting antecedent hydrological conditions. In 2003, the stream was dry in summer owing to few, small intensity, rain episodes in the precedent spring-summer period, in 2004 the streamflow was permanent throughout summer due to high precipitations in the spring-summer period and, consequently, the antecedent conditions were wet and the hydrological transition usually observed at the end of the summer was inexistent.

2.2. STUDY SITE

Fuirosos is a third order stream that drains a forested granitic catchment of 10.5 Km2, near Barcelona (NE Spain, 41º42’ N, 2º 34’ W, 50-770 a.s.l.). The climate is typically Mediterranean, with monthly mean temperatures ranging from 3ºC in January to 24ºC in August. Precipitation mostly falls in autumn and spring with occasional summer storms. Average annual mean precipitation for this region is 750 mm (Ninyerola and others, 2000).

The catchment is covered mostly by perennial cork oak (*Quercus suber*) and pine tree (Pinus halepensis) with one or two layers of shrubs (e.g. *Rhamnus alaternus*, *Viburnum tinus*, *Arbutus unedo*, *Prunus spinosa*) and lianas *(Lonicera implexa*, *Smilax aspera*). Deciduous woodland of chestnut (*Castanea sativa*), hazel (*Corylus avellana*) and oak (*Quercus pubescens*) predominate in the valley head. The soils are poorly developed with an A horizon always less than 5 cm. Soils are sand (46%) and fine sand (24%), with smaller amounts of silt and clay (15% each) (Sala, 1983). Traditional land uses comprise periodic harvesting of bark from cork trees and partial clearing of pines and shrubs. Agricultural fields occupy less than 10% of the catchment area.

According to description by Gasith and Resh (1999), Fuirosos stream exhibits a typical Mediterranean-type discharge regime and several biogeochemical studies used it as a model of Mediterranean catchment to perform comparisons across climate gradients (Sabater and others, 2003; Burt and others, 2003; Hefting and others, 2004; Wade and Neal 2004).

The stream channel is 2 m - 5 m wide and is characterized by steep-pool morphology with cobbles and boulders. The mean flow is 7-20 L·s^{-1}. Discharge is intermittent. The flow is interrupted by a long dry period in summer followed by an abrupt recharge period in late summer-early autumn. The subsequent humid period lasts until late spring. The stream-riparian porous media is characterized by relatively high hydraulic conductivity (4.8-19 m·d^{-1}) and variable specific discharges (0.03-1.5 m·d^{-1}). During the stream recharge period, the groundwater level increases abruptly and the stream water can infiltrate 10 m into the near-stream riparian zone (Butturini and others, 2003). After this recharge, near-stream riparian groundwater level fluctuations followed the same pattern as the stream water, which reflects the hydrological connection between the two water bodies (Butturini and others, 2003).

Table 2.1. Antecedent Hydrological Conditions for Each Study Period: Total Precipitation for the Spring-Summer Period and Number of Days without Surface Flow.

		2003
Meteorological antecedent conditions:	precipitation (mm)	120
Hydrological antecedent conditions:	days of drought	67
Meteorological conditions during study period	Precipitation (mm)	186
Hydrological conditions during study period	Q (L·s)	0-2500

A well-developed riparian forest flanks the 10- 20 m wide stream channel of, consisting mainly of plane tree (*Platanus x hispanica*) and alders (*Alnus glutinosa*). The riparian soil is poorly developed and plane leaf litter tends to accumulate on the forest floor because of extremely low decomposition rates (Bernal and others 2003). In summer, during the dry period, groundwater level falls down from 1.70

m to 2.60 m below ground surface causing hydrological stress to the riparian forest area. This results in a high input of leaf litter that accumulates on the streambed and margins (Sabater and others 2001; Acuña and others, 2004).

DOC concentration in stream water in basal discharge conditions is between 2 to 4 mg·L^{-1}. However, during the hydrological transition (September–October), between the dry and wet periods, DOC concentration increases to 10-20 mg·L^{-1} (Bernal and others, 2002).

2.3. METHODS

2.3.1. Sampling strategy

Field sampling was carried out in two consecutive years, 2003 and 2004. These two years had contrasting precipitation regimes, especially in spring and summer. In 2003, the total precipitation of the hydrological year (September-August) was 630 L·m^{-2}, but accumulated precipitation for the months of spring and summer period was only 120 L·m^{-2}. Consequently, the streamflow was interrupted because of the summer drought. The 2004 hydrologic year was much more humid (total

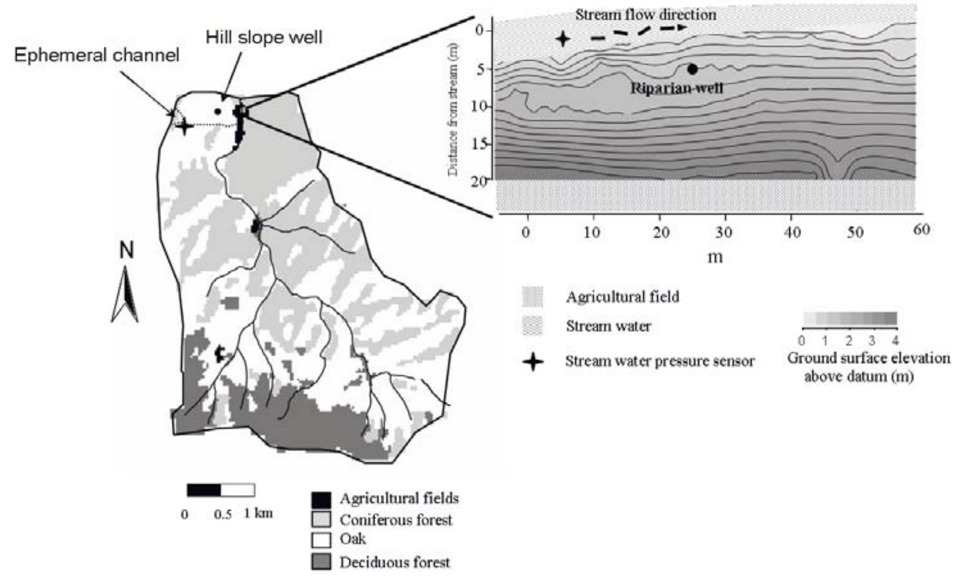


Figure 2.1. Fuirosos catchment with sampling sites marked and main land uses in different grey shades.

precipitation of 815 L·m^{-2}) due to the abundant precipitations in spring and summer (300 L·m^{-2}) that permitted a permanent streamflow during summer (Table 1).

The water samples were collected from September to November of 2003 and 2004 respectively. The 2003 study period (September 5 - November 19) refers to dry antecedent hydrological conditions, while 2004 (September 6 - November 11) refers to wet antecedent conditions. Hence, where the text refers to "study period" or years 2003 and 2004 it refers to that year's September-November sampling period. During these study periods, the samples were collected from four water bodies whenever it was possible: (1) stream water, (2) groundwater from the near-stream riparian zone, (3) groundwater from a 5 m deep well, located on a forest hill slope, 200 m away from the stream channel, and (4) superficial water from an ephemeral channel that drains the forest soil from a small sub-watershed of 6.54 Ha (Figure 1). In 2004, it was not possible to collect samples from this sub-watershed owing to insufficient rainfall episodes to generate superficial runoff in that area.

Samples from the riparian groundwater and the well on the forest hill slope were collected using an ISCO field peristaltic pump and, in 2004, with a Sigma 900 Max Sampler, from a well (2.5 m deep, 15 cm diameter) located 3 m from the stream channel. In 2003, eight samples were collected from the stream and riparian ground waters. As the well in the forest hill slope and the ephemeral channel were dry at the beginning of the sampling period no samples could be collected until the rainstorm of the 15th of October. In 2004, eight more samples were collected from each water body from the beginning of the study period, except from the ephemeral channel, which was dry.

Samples were preserved at 4ºC for storage prior to analysis. Samples were analysed for DOC and its fractions (ultrafiltration). Chloride and silica were also analysed and used as conservative tracers to discern the hydrological origin of stream water and riparian groundwater (Hill and others, 1998; Hornberger and others, 2001; McGlynn and others, 2004).

Chloride was estimated using capillary electrophoresis (Waters CIA Quanta 4000) (Romano and Krol, 1993) while silica and sodium were measured with an induced coupled plasma-atomic emission spectrometer (Thermo Jarrell Ash Iris Advantage ER/S).

2.3.2. Hydrology and dynamics of conservative solutes

Water level in the stream, ephemeral channel and riparian groundwater were monitored constantly by water pressure sensors (Campbell CS401) connected to a data logger (Campbell CR10X). In the riparian area, 24 wells were placed in a regular grid (4 rows and 6 lines). The data from the groundwater level of the entire riparian plot allowed us to determine the direction of the groundwater flow and the extension of water exchange at the stream-riparian interface (For additional details see Butturini and others, 2003). In this study, the groundwater level in the riparian plot was monitored manually three times in 2003 (September 12; October 13; November 22) in each well and once in 2004 (September 15). Stream water

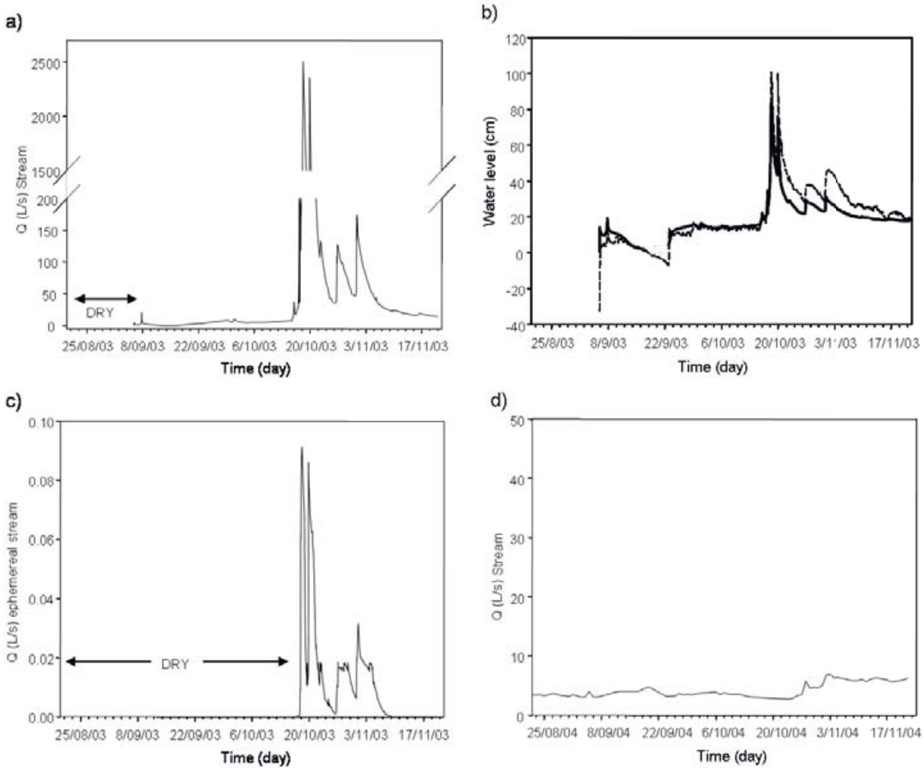


Figure 2.2. Hydrological characterization during 2003 (dry antecedent conditions) and 2004 (wet antecedent conditions) study periods: a) stream water discharge in 2003; b) groundwater level in the riparian zone (thin line) and in stream (thick line); c) discharge of runoff in the ephemeral channel (2003); and d) stream water discharge in 2004.

discharges were measured by the chloride "slug" addition method (Gordon and others, 1992). Then, continuous discharges were estimated using an empirical relationship between measured discharges and the corresponding stream water levels.

2.3.3. DOC characterization

Prior to ultrafiltration water samples were filtered through (pre-combusted Whatman GF/F and 0.2 µm porous size nitrocellulose filters, Whatman) to eliminate particles and bacteria before ultrafiltration. In 2004, samples were ultrafiltrated immediately after sampling (maximum 24 hours), so these were only filtered with pre-combusted Whatman GF/F filters. From each filtered sample, subsamples of 20 ml, for TDOC determination, and subsamples of 330 ml (in 2004), for conservative solutes content analysis, were collected in glass flasks. These flasks were previously heated for 4 hours at 400ºC to prevent sample contamination. Each filtered sample was ultrafiltrated with Prep-Scale TFFI cartridges (Millipore). Typically, the initial volume for ultrafiltration was 10 L, but on occasions when not enough water could be collected the initial volume was 5 L. The following molecular weight fractions were obtained for each sample:

(1) > 100 kDa (very high molecular weight or VHMW fraction),

(2) 10 - 100 kDa (high molecular weight or HMW fraction),

(3) 1 - 10 kDa (medium molecular weight or MMW fraction) and

(4) <1 kDa (low molecular weight or LMW fraction).

DOC samples were measured using a Skalar 12 SK TOC Analyser with UV-promoted persulfate oxidation.

There were three replicates for each molecular weight fraction of each sample (20 ml). DOC analysis of samples that were not ultrafiltrated provided the measurement of total DOC (TDOC, three replicates).

2.4. RESULTS

2.4.1. Hydrology

Stream hydrology during 2003 and 2004 showed important differences in antecedent hydrological conditions, discharge magnitudes and dynamics (Table 1).

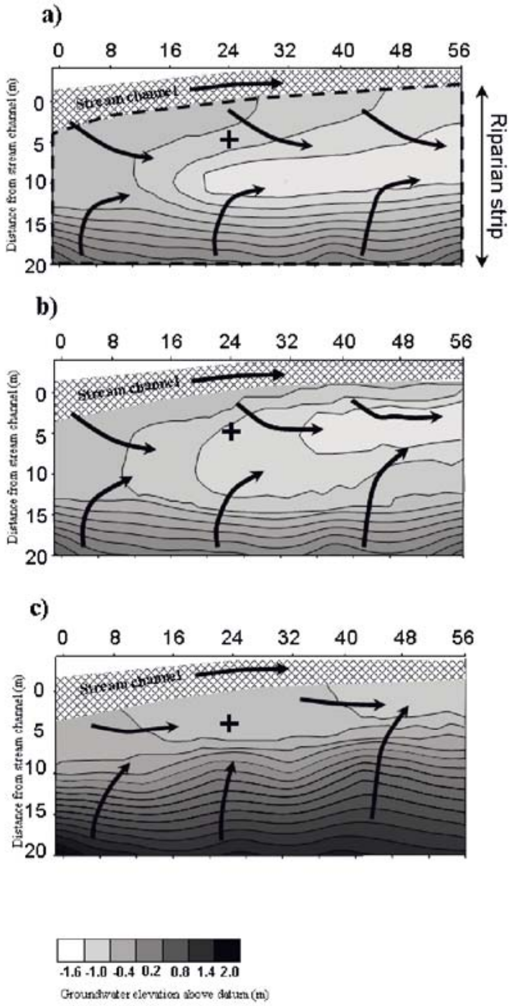


Figure 2.3. Groundwater level in the riparian plot surface during three dates in 2003. a) September 12 2003; b) October 13 2003; c) November 22 2003. Arrows show the groundwater flow direction. Cross dot shows the location of the riparian groundwater well used for water sampling.

In 2003, the stream was dry from June 30 until September 4. The streamflow was re-established on September 5 after a several rain events. Stream hydrology was characterised by two contrasting sub-periods (Figure 2a). The first was relatively dry and lasted from September 5 to October 14 and was characterised by an intermittent and low discharge regime (less than 4 L·s^{-1}). The second sub-period was much more humid and started on October 15 after a severe rain episode (total precipitation= 186 mm) that generated a storm peak of 2500 L·s^{-1} and a basal discharge up to 15 L·s^{-1}. During the dry sub-period, the stream water discharged into the riparian groundwater up to 8 m within the riparian area and generated extended groundwater flow paths (Figure 3a and b) at the stream-riparian interface. Subsequently, the stream water and riparian groundwater levels followed the same pattern over the entire study period (Figure 2b) and the stream water infiltrated only the first 2-4 m of the riparian strip (Figure 3c). The well located on the forest hill slope and the ephemeral channel remained dry during the first sub-period. The runoff in the ephemeral channel was permanent from October 15 to November 8 and followed the same discharge pattern as that of the stream channel (Figure 2c).

By contrast to 2003, the stream was permanent throughout 2004 due to abundant rains in late spring. However, no rain episode occurred from September to November and the discharge was steady at about 4.4 L·s^{-1} (Figure 2d). The lack of hydrological transition greatly reduced the extent of the water exchange between the stream and riparian ground waters, and the stream water infiltrated only the first 2-4 m of the riparian strip and the groundwater level surface was similar to that observed in November 2003. The hill slope groundwater was monitored throughout the study period, but the ephemeral subcatchment remained dry. The level of the riparian groundwater was coupled to the stream water level.

2.4.2. Conservative solutes

In 2003, chloride concentrations in stream and riparian groundwater followed the same pattern (r=0.95, n=12, p<0.001) and their temporal dynamics reflect the two contrasting hydrological sub-periods. During the dry sub-period, chloride concentrations were high (from 60 to 33 mg·L^{-1}) and decreased drastically at high discharges, during the wetter sub period, to 18 mg·L^{-1}. During this dry sub-period, chloride concentration was higher in the riparian groundwater than in the stream (t-test, t=7.2, d.f.=5, p<0.01), but during the wetter sub period the concentrations were identical (paired t-test, t=1.31, d.f.=5, n.s.). Throughout the entire 2003 study period, chloride concentrations in the hill slope groundwater (mean value of 32.3 ± 10.82 mg·L^{-1}) were significantly higher than in the stream and riparian ground waters (paired test-t, t=4.8, d.f.=5, p<0.01 for stream water and t=4.68, d.f.=5, p<0.01 for riparian groundwater) (Figure 3a). Silica concentration in the stream and the riparian ground waters was identical (mean value= 9 mg·L^{-1}) and remained constant throughout the sampling period. In the hill slope groundwater silica concentration averaged 15.8 mg·L-1 and was significantly higher than in the stream (t-test, t=54.01 d.f.= 5, p<0.01) and the riparian groundwater (t-test, t=44.53, d.f.= 5, p<0.05). Neither the ephemeral channel presented significant differences in chloride content with the stream (t-test, t= 1.45, d.f.= 3, n.s.) nor the riparian groundwater (t-test, t= 0.11, d.f.= 3, n.s.) while silica content was slightly higher but not as much as in the hill slope groundwater (t-test, t= 2.24, d.f.= 3, p<0.05) (Figure 3c).

In 2004, concentrations of solutes were constant over time due to the absence of storm events during the sampling period. In fact, chloride content in stream (27.3 ± 1.18 mg·L^{-1}) and riparian ground waters (26.9 ± 1.24 mg·L^{-1}) was identical (paired test-t, t=5.18, d.f.=6, n.s.), although it was significantly lower (24.35± 1.41 mg·L^{-1}) in the hill slope groundwater than in the stream water (paired test-t, t=0.35, d.f.=7, p<0.01). However, in contrast to the previous year, the difference in chloride content between the hill slope and riparian ground waters was not significant (paired test-t, t=3.14, d.f.= 6, n.s.) (Figure 3b). Silica content in stream water (7.92 mg·L^{-1} ± 0.24 mg·L^{-1}) was significantly higher than in the riparian groundwater (7 ± 0.22 mg·L-1; paired t-test, t=17.13, d.f.=7, p<0.01) and visibly lower than in hill slope groundwater (15 mg·L^{-1} ± 0.13 mg·L^{-1}; paired test-t, t= 3.7, d.f.=7, p<0.01) (Figure 3d).

2.4.3. Dynamics of total DOC (TDOC) and molecular weight fractions.

In 2003, the peak in stream water TDOC (10 mg·L^{-1}) was measured at the beginning of the sampling period, just after the re-establishment of the streamflow. Afterwards, the concentration decreased until normal basal concentrations were

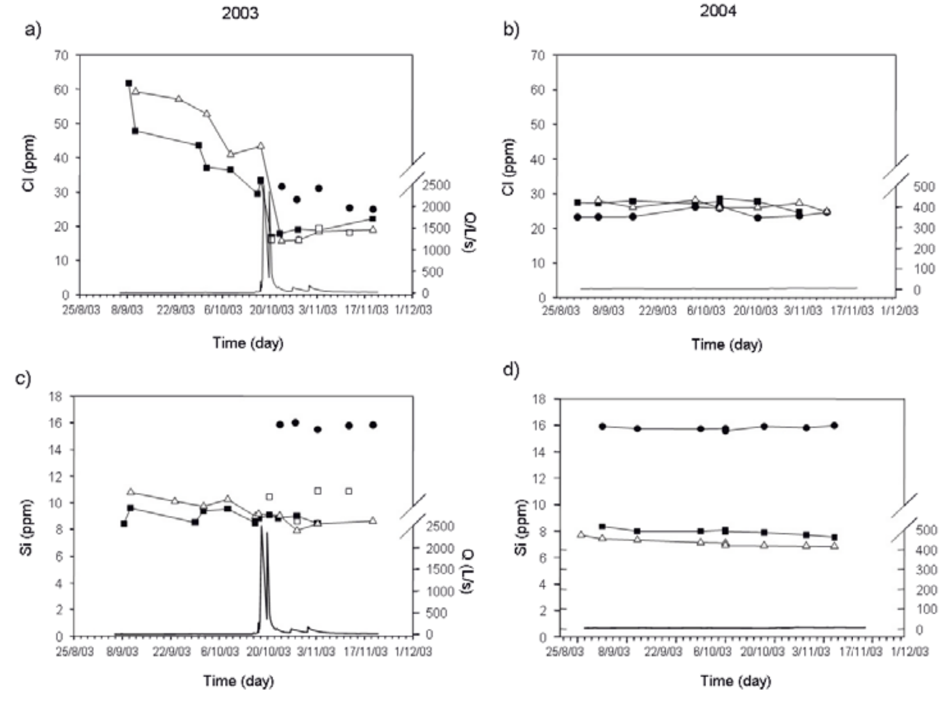


Figure 2.4. Temporal dynamics of conservative solutes (Cl and Si) during the sampling period of 2003 (dry antecedent hydrological conditions, panels a and c) and during the sampling period of 2004 (wet antecedent hydrological conditions, panels b and d). Symbols: (black square) stream water; (triangle) riparian ground water; (black dot) hill slope ground water; (black square) ephemeral channel.

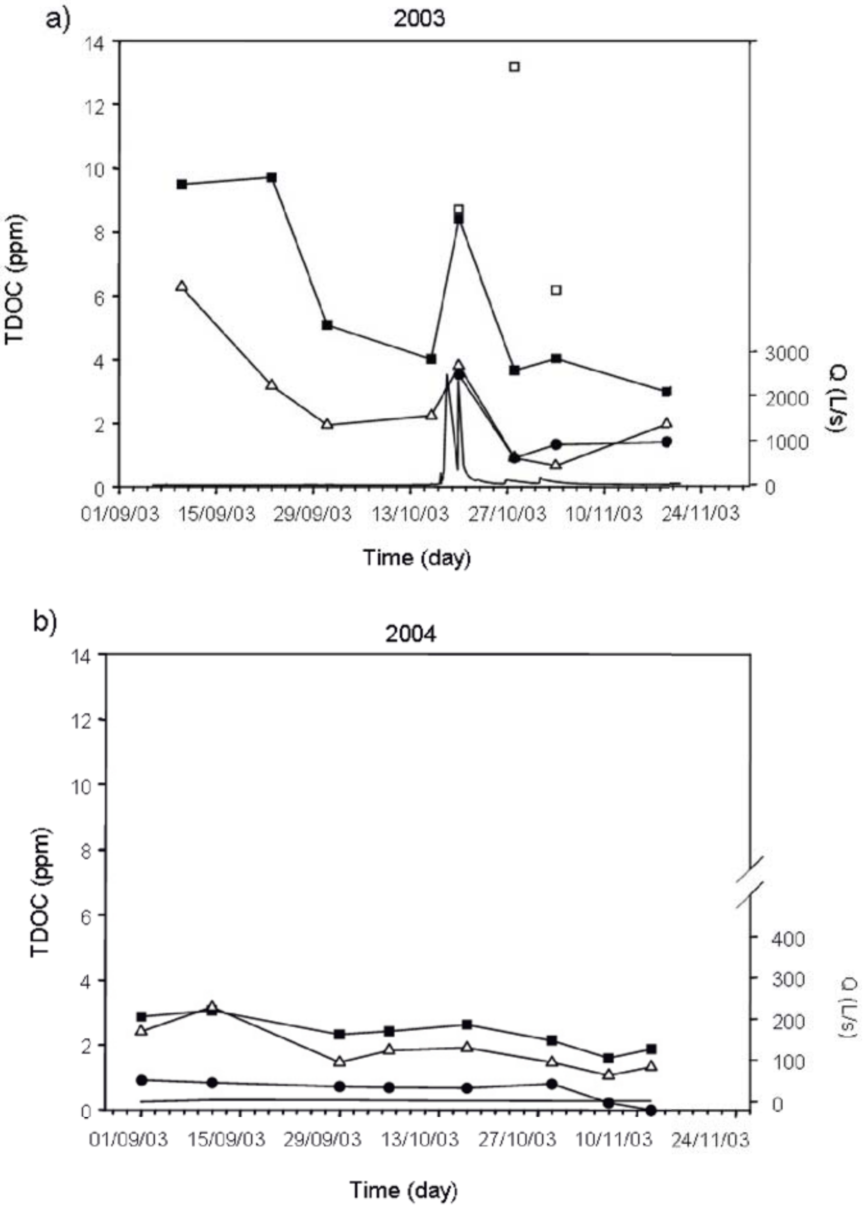


Figure 2.5. Temporal dynamics of TDOC and discharge during 2003, panel (a), and 2004, panel (b), study periods. Symbols: see Figure 4.

reached (2-4 mg·L^{-1}), and then increased during the floods of mid-October (until 8 mg·L^{-1}) and then decreased again to basal values. TDOC in riparian ground and stream waters followed the same trend ($r=0.82$, d.f.=8, $p<0.01$) but concentrations in riparian groundwater were lower than in the stream water (paired test-t, $t=5.53$, d.f.=7, $p<0.01$). TDOC concentration in the hill slope groundwater remained similar during the sampling period with a mean value of 1.7 ± 1.07 mg·L^{-1}. This concentration was significantly lower than in stream water (test-t, $t=5.54$, d.f.=4, $p<0.01$) but similar to the riparian groundwater (test-t, $t=0.83$, d.f.=4, n.s.). Mid-October storms originated superficial runoff in the ephemeral subcatchment and TDOC concentrations were similar to those of the stream water (test-t, $t=1.41$, d.f.=2, n.s.) (Figure 4a).

In the 2004 sampling period, TDOC in stream water averaged 2.4 ± 0.5 mg·L^{-1} and was clearly lower than in 2003 (paired t-test, $t=3.92$, d.f=7, $p<0.0$). On the other hand, no differences were observed in DOC concentration in riparian and hill slope ground waters between the two years (riparian, paired test-t, $t=1.51$, d.f.=7, n.s.; hill slope, paired test-t, $t= 2.35$, n.s.) (Figure 4b). Similar to the previous year, TDOC concentrations in the stream water and riparian groundwater followed the same trend over time ($r=0.93$, d.f.=7, $p<0.01$), albeit they were significantly lower (mean value of 1.83 ± 0.68 mg·L^{-1}; stream, paired test-t, $t=5.26$, d.f.=7, $p<0.01$) in the latter compartment. Mean concentration in the hill slope groundwater was 0.54 ± 0.34 mg·L^{-1}, which is significantly lower than in the stream and the riparian groundwater (stream, paired test-t, $t=14.54$, d.f.=7, $p<0.01$; riparian groundwater, paired test-t, $t=6.6$, d.f.=7, $p<0.01$).

In 2003, the small DOC fractions (LMW and MMW) predominated over the larger fractions (HMW and VHMW) in all the monitored water bodies. For instance, the contribution of LMW+MMW in stream water, riparian groundwater, hill slope and in the ephemeral channel, averaged 68 ± 14%, 85 ± 7%, 81 ± 2% and 62%± 8 % of TDOC respectively. In stream water, these two smaller MW fractions had a DOC concentration peak at the beginning of the sampling period and followed the same pattern (d.f.=6, $p<0.05$) as TDOC throughout the study period (Figure 6a).

In 2004, the concentration of the DOC fractions remained constant throughout the study period and the contribution of each MW fraction to the total TDOC was more homogeneous. For instance, the LMW and MMW fractions remained the most abundant DOC fractions, but the sum

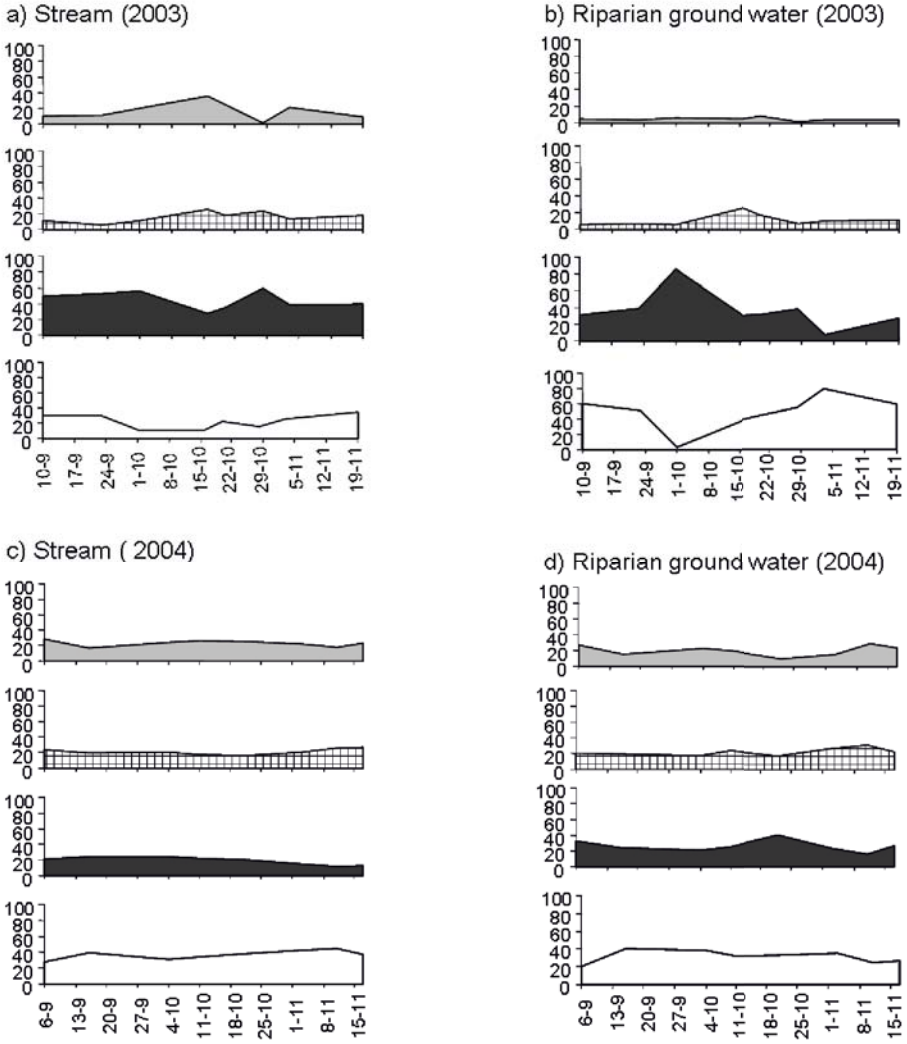


Figure 2.6. Temporal dynamics of DOC MW fractions (expressed as % of TDOC) during the 2003 (dry antecedent conditions) (panels a,b) and 2004 (wet antecedent conditions) (panels c,d) sampling periods in stream water and riparian groundwater. Symbols: (white) LMW; (black) MMW; (grid) HMW; (grey)VHMW.

of their contribution decreased to 56 ± 5%, 56 ± 10% and 63 ± 25% in stream water, riparian and hill slope groundwaters respectively. The lack of any temporal DOC pattern in stream and riparian ground waters hinders the detection of any significant relationships between TDOC and MW fractions (Figure 6b).

2.4.4. Fate of DOC across the stream-riparian interface

TDOC concentration in the riparian groundwater was regularly lower than in the stream water for both years. In the sampling period of 2003, the 57 ± 18% of TDOC decreased at the stream-riparian interface, while in 2004 the decrease was only 28 ± 7% (Figure 7a).

In 2003, the abrupt increase in water level in the riparian groundwater after the re-establishing of runoff in the stream channel and the data from conservative solutes (Cl and Si) revealed that it was clearly being fed by stream water while the influence of the hill slope groundwater was not apparent. Hence, the decrease of 57 ± 18% of TDOC concentration observed between stream and riparian ground waters are due to in situ retention at the stream-riparian interface.

In 2004, the stream and riparian ground waters showed similar geochemical characteristics suggesting the same conclusion obtained from data from 2003. Stream and riparian ground waters, for instance, had a similar Si content, which was much lower than in the hill slope groundwater. However, chloride content in the stream water, riparian and hill slope ground waters were similar. In addition, the lack of temporal change in the hydrometric data made it impossible to obtain an accurate picture of the water exchanges between the stream and riparian ground waters. Therefore, the dilution between the stream water and hill slope groundwater at the riparian interface cannot be discarded a priori. Hence, during this period, the retention of 28 ± 7% of TDOC at the stream-riparian interface must be interpreted with caution.

In 2003, we observed a direct relationship between DOC size fraction and DOC retention across the stream-riparian interface. In fact, VHMW showed higher DOC retention (78 ± 27%) (Figure 7b) followed by HMW and MMW fractions with a 70 ± 9% and 66 ± 16% retention respectively (Figure 7 c and d). Finally, nil retention of LMW molecules was observed, indicating that it was a nearly conservative fraction (Figure 7e).

In 2004 the selective retention of DOC across the stream-riparian interface according to its molecular size disappeared. In fact, LMW and HMW fractions were virtually conservative with a DOC retention of only 10 ± 22% and 4 ± 32% respectively (Figure 7 c and e). On the other hand, the MMW fraction had a negative retention value (i.e. it was released, -34 ± 90%) suggesting that stream-riparian interface acts as a source of this DOC fraction (Figure 7d). Finally, only the VHMW fraction appeared systematically retained (25 ± 27%) across the stream-riparian interface in 2004 (Figure 7b).

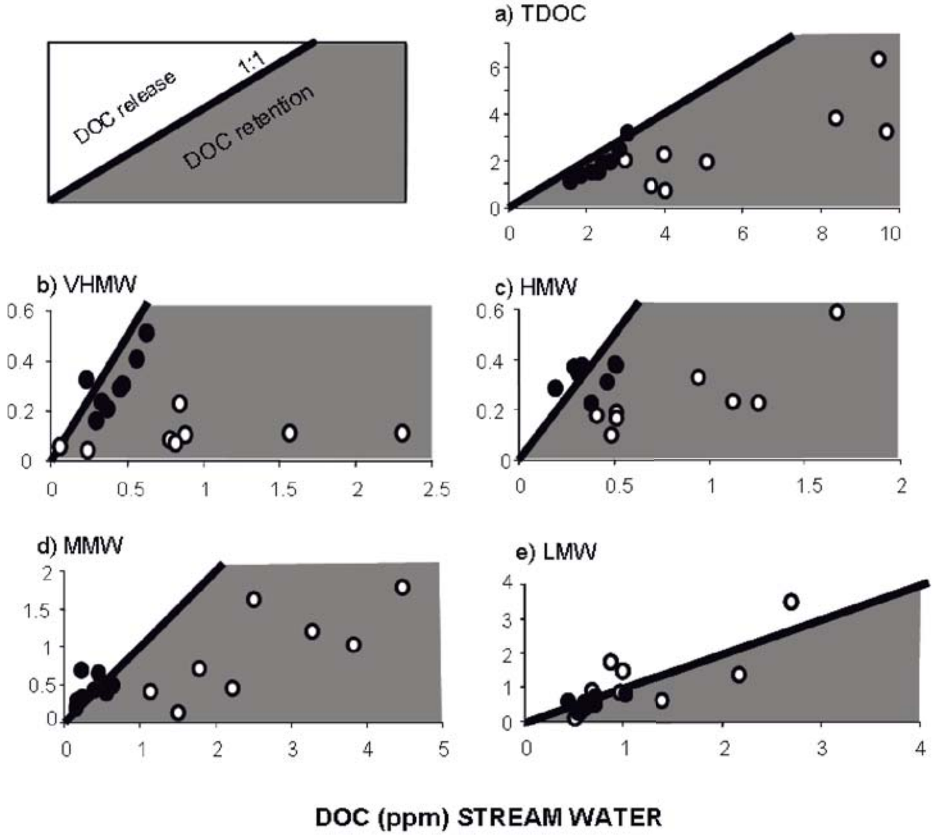


Figure 2.7. Comparison of content of TDOC and MW DOC fractions measured in the stream water with that in the riparian groundwater during the two study periods. The solid line shows the 1:1 line. Data located within the grey area indicate that DOC concentration was higher in stream water than in riparian ground water. Symbols: (open dots)2003; (black dots) 2004.

2.5. DISCUSSION

Our findings demonstrate that the antecedent climatic and hydrological conditions strongly affect both the quality and quantity of DOC transport in stream water. Furthermore, the abrupt hydrological transition between the dry and wet period enhance the hydrological exchange at the stream-riparian interface and therefore stimulate DOC processing. From the climate/hydrological perspective our results provide a contrasting picture with respect to Freeman and others (2004) who minimize the importance of alteration of hydrological regime on DOC transport in northern stream-peatland ecosystems. There is a lively debate on the role of hydrology on DOC transport in peatlands (Freeman and others, 2001 and 2004; Pastor and others, 2003; Tranvik and Jansson, 2002). Clark and others (2005), for instance, demonstrated that drought years enhance a decrease in DOC concentration in peat waters. Our study in Fuirosos illustrates the opposite trend. Undoubtedly the comparison of data from different climatic regions and hydrological systems requires a much more rigorous investigation. In fact, DOC concentrations in peatlands are usually much higher with far more predictable dynamics than in Mediterranean streams, and droughts are less severe than those illustrated in our study. However our results are useful to obtaining a more comprehensive perspective regarding the effects of future alteration of rain regime, particularly during summer (Christensen and Christensen, 2003), on DOC transport in running waters in Mediterranean regions.

In addition, we have shown that DOC retention across the stream-riparian interface also varied greatly, from a selective retention of higher molecular weight fractions of DOC in 2003 under dynamical hydrological conditions after dry antecedent conditions to uniformly low, even lack of DOC retention under steady hydrological conditions after wet antecedent conditions (2004). Thus, these dynamical hydrological conditions at the stream-riparian interface might facilitate the retention, through microbial heterotrophic biota (Findlay and others, 2003), of irregular pulse input of labile DOC pool associated with organic matter leaching during rain episodes.

Hydrological periods characterised by fast and abrupt hydrological changes are usually avoided in field studies that deal with nutrient processes across stream-hyporheic interface and/or riparian ground waters because of the complexity of stream hydrology during storm events. Thus experimental field works are conducted under basal discharge conditions (Butturini and Sabater, 1999; Simon and others, 2005). Nevertheless, dynamical hydrological conditions are far from being an anomalous situation. Empirical and theoretical studies (Serrano and Workmann, 1998; Butturini and others, 2005) indicated that the interactions between stream water and the surrounding stream-riparian interface are amplified, especially during these periods. During the transition period in Fuirosos, for instance, the stream water infiltrated up to 10 m into the riparian sediments (Butturini and others, 2003).

Most of studies focused on the influence of the stream sediment on DOC fractionation, bioavailability and bacterial uptake have been performed under laboratory conditions (Fischer and others, 2002; Findlay and others, 2003) and/or under controlled experimental conditions at the study site (Tipping and others, 1999; Sobczak and others, 2002; Freeman and others, 2004; Valett and others, 2005). Results obtained in the laboratory are essential to study and identify the biochemical processes that regulate the DOC availability in streams and interstitial water. However, field data obtained under natural conditions are necessary to gain information about the effective incidence of these processes in nature. Our results showed that selected hydrological periods characterized by abrupt changes constituted a natural experiment that allowed exploring in situ and under natural conditions the fate of DOC transported across the stream-riparian interface and, therefore, to provide a better understanding of DOC dynamics in stream ecosystems.

In the dry year sampling period (2003), both the abrupt hydrometric changes observed in the riparian groundwater and the chemical (chloride and silica) data show that the riparian groundwater is recharged by stream water only, a fast process that started with the re-establishing of runoff in the stream channel (Butturini and others, 2003), while the hill slope groundwater bears no influence on riparian ground water. Consequently, the identification of the origin of water that flows through the riparian sediments enabled us to attribute the decrease in DOC across the stream-riparian interface to in situ DOC retention rather than to hydrological mixing between stream and hill slope ground waters.

The lack of a hydrological transition in 2004 period hindered the characterisation of water flow through the stream-riparian interface. In fact the hydrometric data gave no evidence of water exchange between stream and riparian ground waters. Furthermore, the results of chloride data do not rule out the possibility of TDOC dilution in the riparian groundwater by hydrological mixing between stream water and hill slope groundwater.

The chemical data of conservative tracers, TDOC and its MW fractions observed in the ephemeral stream reveals that the water flowing through the forest hill slope during severe a rain episode is similar to the stream water during high discharge conditions. This result substantiates the importance of leaching of allochthonous DOC from hill slope forest soils towards TDOC transport in stream water during these storm episodes.

The TDOC pulse in stream water observed at the beginning of the dry year study period (2003) is a typical phenomenon observed in previous dry years in Fuirosos (Bernal and others, 2005) as well in stream-floodplain systems (Valett and others, 2005). Its origin is most probably found in the leaching of the abundant leaf litter that accumulated on the streambed in summer (81 $gC \cdot m^{-2}$, Acuña and others, 2004). In fact, leaf accumulation in wet years (i.e., 2004) is much lower (6.9 $gC \cdot m^{-2}$, Acuña and others, 2004). Consequently, in 2004, TDOC concentrations are much lower than in the previous study period and solute flushing is absent. These low and constant concentrations of TDOC and of all MW DOC fractions, both in stream water and in riparian groundwater, observed throughout the study period of 2004 prevented the identification of the most reactive and/or recalcitrant DOC molecular fractions across the stream-catchment interface. This problem is absent in data from the dry year (2003). In fact, the high concentrations of TDOC (and MW DOC fractions as well), allowed us to separate the most reactive fractions (HMW and VHMW) from the most recalcitrant ones (LMW) and therefore to identify the selective DOC retention across the stream-catchment interface according to the DOC molecular size (Figure 7). Therefore, our field observations corroborated the size-reactivity conceptual model proposed by Amon and Benner (1996), whereby larger molecules would likely be more labile while smaller molecules would be more recalcitrant. Nevertheless, there is not a general consensus in the literature about the relationship between bioavailability and DOC molecular size, and more complex patterns have been reported. For instance, Kaiser and others (2004) showed that HMW compounds were generally highly recalcitrant while LMW could be bioavailable or recalcitrant according to its origin and diagenetic state. In laboratory conditions, conversely Fischer and others (2002) measured high DOC retention for HMW and LMW compounds, but low retention for intermediate MW fractions.

Under the wet antecedent condition (i.e., the 2004 sampling period) and low DOC concentrations in stream and riparian ground waters, exclusively the larger DOC molecular weight fractions (VHMW) appeared retained across the stream-riparian interface in most of the sampling dates while the remaining MW fractions appear to behave conservatively. Sobczak and others (2002) pointed out that under low DOC concentration streams DOC removal is often negligible and transport may be apparently conservative.

CONCLUSION

This paper has shown that unstable hydrological periods can provide an excellent opportunity for studying, in situ and under natural conditions, the fate of organic matter in Mediterranean freshwater ecosystems.

Results indicate that the occurrence of a summer drought period favoured the transport of DOC with labile behaviour (with a retention efficiency of 56% TDOC across the stream-riparian interface) during the successive hydrological transition period, while in absence of a drought period the DOC showed more refractory behaviour. Moreover, the efficiency of DOC retention across the stream-riparian interface also varied greatly, from selective retention of higher molecular weight fractions of DOC in 2003 to uniformly low, even lack of, retention under wet antecedent conditions (2004). We hypothesise that a change in discharge regime in Mediterranean regions as consequence of a warmer climate might favour more severe and dynamical hydrological processes at the stream-riparian interface, which might facilitate the retention of bigger and more labile DOC molecules originated by abrupt and irregular inputs of organic matter leaching during rain episodes.

Acknowledgements

Thanks to two anonymous reviewers for their encouraging comments on an earlier version of the manuscript. This study was supported by funds provided the Ministerio de Educación y Ciencia (projects CGL2004-04050/HID, REN2002-04442-C02-02/GLO and CGL2005-06739-C02-02).

Chapter 3

Impact of dry-rewetting hydrological cycle on bioavalability of dissolved organic carbon molecular weight fractions in a Mediterranean stream

Chapter 3. Impact of dry-rewetting shift on DOC bioavailability

Vazquez E[1]., Ylla I[2]., Romaní A.M.[2], Butturini A[1]. Impact of dry-rewetting hydrological cycle on bioavalability of dissolved organic carbon molecular weight fractions in a Mediterranean stream.

[1]Departament d'Ecologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain; [2]Institut d'Ecologia Aquàtica, Universitat de Girona, Campus de Montilivi, 17071 Girona, Spain.

ABSTRACT

Mediterranean fluvial systems are subject to severe drought - rewetting cycles that influence biogeochemical processing. These extreme periods also modify the direction and magnitude of lateral water fluxes between the stream surface water and riparian groundwater becoming an excellent experiment to explore the abrupt changes affecting dissolved organic matter (DOM) fluxes and composition across the stream riparian-interface. In this study the variability in DOM concentration and composition, over consecutive drought and re-wetting periods, are considered in terms of dissolved organic carbon (DOC) and nitrogen (DON), DOC:DON ratio, and bioavailable DOC (BDOC) and DON (BDON) for whole water and three molecular weight fractions (MW) (High (HMW): >10 kDa; Medium (MMW): 1-10 kDa and Low (LMW): <1 kDa). The results show that during the drought period differences in DOM characteristics between riparian groundwater and surface waters are minimal, while during the rewetting period, the riparian compartment would be a biogeochemical DOM transformation "hot spot".

Considering the entire study period, only DON presented significant changes in the contribution of the different MW fractions. Changes in DOC lability (BDOC content) are not related to a single MW fraction. On the other hand, BDOC is positively related to DON concentration and inversely related to DOC:DON ratios, evidencing that DON is an important driver of DOM lability. Furthermore, the DOC:DON ratio presents a threshold value for discriminating between labile and refractory LMW DOC fractions that coincides temporally with the change between dry and wet periods.

In consequence, this study highlights that DOM lability is greatly influenced by DON concentration and DOC:DON ratios rather than to molecular weight, prompting the necessity to further examine the role of DON on DOC fate in fluvial systems.

3.1. INTRODUCTION

Dissolved organic matter (DOM) is a complex mixture of substances and comprises most of the reduced carbon in streams and rivers (Volk et al. 1997). DOM is a key component of carbon and nitrogen cycling in aquatic ecosystems and its availability, quality, and lability strongly influence production and structure of microbial community (Sobczak & Findlay 2002). In headwater streams, its composition and quality is mainly a result from allochthonous inputs from the bordering terrestrial environment, including the riparian zone and, secondarily, from autochthonous in-stream primary production (Sachse 2005). Since allochthonous DOM inputs are strongly coupled to hydrologic fluctuations: DOC inputs are strongly modulated by: 1) the succession and severity of storm events (Hood et al., 2006; Vidon et al., 2008; Butturini et al., 2008; Raymond and Saiers 2010); drought periods (Vazquez et al., 2010; Clark et al., 2005); and antecedent climatic conditions (Haei et al., 2010) . Additionally, storms and droughts modify the direction and magnitude of lateral hydrological exchange between stream channel and the surrounding hyporheic/riparian interfaces (Butturini et al., 2003). Consequently, the detailed monitoring during time periods with extreme hydrological conditions provide an excellent tool to evaluate the effective relevance of the interface compartments on concentration, lability and composition of DOM both in surface water and in riparian ground water.

Mediterranean ecosystems are subjected to a marked seasonality characterized by two humid periods in fall and spring followed by a drought, usually in summer (Gasith & Resh, 1999). Drought episodes can be divided in two phases: an initial gradual, and slow, drying phase, as a consequence of the ceasing of inputs from baseflow (with long catchment residence time) from the old groundwater compartment. At this point, the disruption in the hydrological connectivity along the fluvial network and between the stream – riparian interface becomes evident. When the dry period is prolonged, the fragmentation phase starts. This second phase is characterized by extreme hydrological fragmentation where the surface water disappears totally from the stream network or remains constrained in few isolated, and ephemeral, water pools (Lake 2003; Vazquez et al., 2010). The abrupt input of new water (with short catchment residence time) switches on a fast rewetting of the stream channel and the restoration of the hydrological continuum along the stream network (rewetting phase). During this period, the stream feeds the surrounding riparian groundwater compartment (Butturini et al., 2003).

During the hydrological shift from dry to wet conditions in intermittent streams, draining forested catchments, dissolved organic carbon (DOC) concentrations show a typical peak due to the leaching of particulate organic matter accumulated in the stream channel during the dry period. During this hydrological transition the riparian groundwater compartment is rapidly recharged from infiltrating stream water, and the stream-riparian interface becomes an important regulation point for inorganic and organic solutes fluxes compartments (Butturini et al. 2002). The hypothesis of a selective immobilization of DOC depending on its MW (molecular weight) arises from previous studies (Vazquez et al., 2007; Romaní et al. 2006). This selective uptake would follow the size-reactivity model (Amon and Benner, 1996) and while low molecular weight DOC (less than 1 kDa) would accumulate in the groundwater due to its more refractory composition, the more labile high molecular weight DOM (>10 kDa) would be susceptible to biological uptake. On the other hand, literature offers many, sometimes opposed, explanations for DOC molecular weight – reactivity (Lindell et al., 1995; Amon & Benner, 1996; Fischer 2002; Kaiser 2004, Agren et al., 2008).

Most studies of DOM dynamics focus on DOC but many other factors affect DOM bioavailability such as its biochemical composition, inorganic nutrients and temperature (Tranvik et al., 1998, Thóth et al., 2007, Dawson et al., 2008). Specifically, the nitrogen content might be relevant for whole DOM reactivity as is suggested by the significant increase in peptidase activity during a rewetting episode together with DOC increase (Romaní et al. 2006) and the changes in the amino acid nitrogen content and diagenetic state (Ylla et al. 2011).

Previous studies concluded that there is a preferential microbial uptake of DON rather than DOC (Petrone et al. 2009, Willett et al., 2004; Kaushal and Lewis 2005, Wiegner & Seitzinger 2001), and it has been observed that, especially after dry conditions, the mobilization of the different DON pools in might be occur at different rates than DOC pools (Turgeon and Courchesne, 2008).Therefore it is important to assess not only DOC dynamics and availability as a proxy of DOM but to consider carefully DON dynamics and its influence on DOM lability during drought and rewetting periods. However, studies focused on dissolved organic nitrogen (DON) during extreme hydrological moments in Mediterranean fluvial systems are scarce. In the context of climate change, dry periods might become more recurrent or increase its time span (Giorgi 2006), and thus the need of studies focusing on these hydrological periods and their ecological implications become more relevant (Steward et al., 2012).

Under this perspective, our main goal was to examine the DOM qualitative changes along an entire fluvial dry period from the start of low flow to the re-establishment of wet conditions considering all relevant compartments playing a role on potential hydrobiogeochemical changes. Specifically, we aim to: a) characterize DOM variability in terms of DOC, DON, DOC:DON ratio and their specific distributions among three molecular weight intervals (low: <1kDa; medium: 1-10 kDa; high:>10 kDa); b) discern what is the extent of the influence of carbon and nitrogen content, molecular size or the interaction of these characteristics on DOM bioavailability, and c) distinguish which hydrological compartment (riparian groundwater or stream channel) contributed more clearly to the quantitative and qualitative DOM changes. To provide such an accurate view of the variability of DOM qualitative properties associated to a drought episode, a small Mediterranean intermittent headwater stream draining a forested catchment was intensively monitored from May 2006 to December 2006 with a drought phase elapsing 83 days, from June to September. We hypothesized that the succession of the hydrological circumstances should prompt DOM qualitative heterogeneity. For instance, according to the size-reactivity model (Amon and Benner, 1996) the proportion of degraded and recalcitrant DOC with low molecular weight should be higher during drought than the rewetting.

During the drought phase, a parallel increase of the DOC:DON ratio and the accumulation of low molecular weight DOM fraction is expected. On the other hand, during the subsequent rewetting a flushing of recent leached DOM with low DOC:DON ratio and a higher proportion of DOM large fraction should be expected.

3.2. MATERIAL AND METHODS

3.2.1. Study Site

Fuirosos is a third-order stream that drains a forested granitic catchment of 16.2 Km^2, near Barcelona (NE Spain, 41º 42' N, 2º 34' W, 50-770 m a.s.l.). The climate is typically Mediterranean, with monthly mean temperatures ranging from 3 ºC in January to 24 ºC in August. Precipitation mostly falls in autumn and spring with occasional summer storms. Average annual mean precipitation for this region is 750 mm (Ninyerola et al., 2000).

The catchment is covered mostly by perennial cork oak (*Quercus suber*) and pine tree (*Pinus halepensis*) with one or two layers of shrubs (e.g. *Ramnus alaternus*, *Viburnum tinus*, *Arbutus unedo*, *Prunus spinosa*) and lianas *(Lonicera implexa*, *Smilax* *aspera*). Deciduous woodland of chestnut (*Castanea sativa*), hazel (*Corylus avellana*) and oak (*Quercus pubescens*) predominate in the valley head.

The stream channel is 1 m to 5 m wide and is characterized by steep-pool morphology with cobbles and boulders. Also, there is a well developed riparian forest flanking the stream channel of 10 to 20 m wide, consisting mainly of plane tree (Platanus x hispanica) and alders (Alnus glutinosa). The riparian soil is poorly developed and plane leaf litter tends to accumulate on the forest floor because of extremely low decomposition rates (Bernal et al., 2003).

The mean base flow ranges between 1 and 20 $L \cdot s^{-1}$. The flow is interrupted, usually, by a long dry period in summer followed by an abrupt recharge period in late summer- early fall. The subsequent humid period elapses until late spring. The sampling site is subjected to constant hydrological and biogeochemical monitoring since 1998 (Butturini et al., 2008).

3.2.2. Sampling strategy and hydrological context

The study is divided into two sampling periods, covering two contrasting hydrological moments: the dry period, and the subsequent re-establishing of hydrological connectivity of the catchment or rewetting period.

The first four samplings correspond to the drought period: 08/05/2006, 25/05/2006, 12/06/2006, and 15/06/2006. This sampling period was preceded by an anomalous dry spring with a total precipitation of 30 $L \cdot m^{-2}$ from March to June without any storm episode during summer (i.e. the dry period). The last relevant rain episode was on the 29/01/2006 (133 $L \cdot m^{-2}$). In consequence, it was expected that during the dry phase stream discharge was originated exclusively by "old" groundwater flowing through the riparian interface before entering into the stream channel (Butturini et al., 2003). The first sample was collected when it was detected a steep decline in discharge at the sampling site, and the last one was gathered from an isolated water pool, covering a discharge range from 4 $L \cdot s^{-1}$ to 0 $L \cdot s^{-1}$ (Figure 1). The second set of six samplings correspond to 14/09/06, when surface flow was re-established as consequence of a severe rain episode (54 $L \cdot m^{-2}$) that generated a discharge peak (95 $L \cdot s^{-1}$); 20/09/2006 when a second discharge peak occurred, although of lower magnitude (58 $L \cdot s^{-1}$); and finished the 14/12/06 once in autumn basal flow conditions. The rest of sampling dates were: 28/09/2006, 4/10/2006, and 20/11/2010. Discharge during this period ranged from 95 L·s-1 to 3.7 $L \cdot s^{-1}$ (Figure 1). During the rewetting period, stream water recharges the surrounding riparian-groundwater compartment (Butturini et al., 2003).

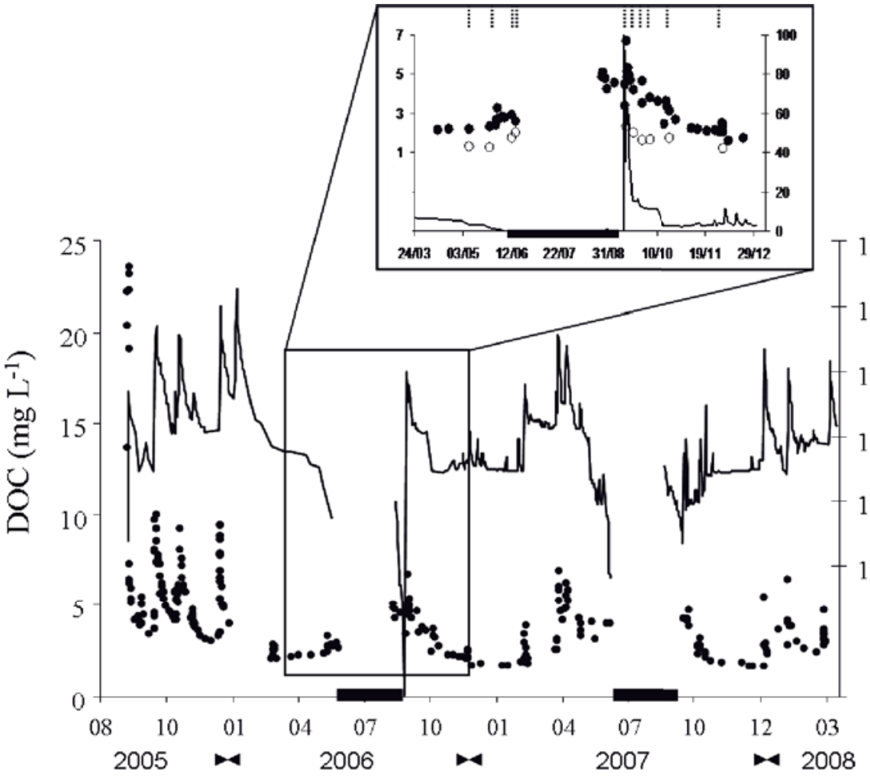


Figure 3.1. Temporal discharge and DOC concentration from 09/2005 to 04/2008. Drought periods where surface flow was interrupted are indicated by black bars. The inset details the study period indicating sampling dates at the top. DOC concentrations for each sampling date correspond to white dots, while black dots correspond to data from the continuous monitoring.

At each sampling date, four replicates of 8 L for bulk water analysis and another four replicates of 2 L (8 L total) for BDOC determination were collected using an ISCO field pump from stream water. In the case of groundwater there were no replicates since the water samples were gathered from a well 3 meters away from the stream channel, 2 meters deep and a diameter of 2.5 cm. It was emptied before obtaining the sample but to avoid further re-fillings only one sample was collected. Considering that water level was around 1 m deep, only 4 L were collected per sample. Samples were stored in cold (4ºC) and filtered in the same day, in situ when possible, to eliminate particles (0.7 μm pre-combusted Whatman GF/F filters). The filters were rinsed with 300 ml of stream water before collecting the samples. All samples were stored in a cool recipient and covered from direct sunlight during field work, before proper storage in the laboratory (at 4 ºC and in the dark).

3.2.3. DOM characterization

Each replicate was divided in two sub-replicates with half the total water volume. The first subset was used to estimate DOM availability, determining the bioavailable DOC (BDOC) and bioavailable DON (BDON). The second subset was used to measure the different organic matter characterization parameters.

BDOC estimates were obtained according to the method described by Servais et al. (1989), and BDON was determined by the same procedure. In the laboratory, the 2 litre samples were subsequently filtered by 0.2 μm nylon membranes (Whatman) and inoculated with 20 ml of GF/F filtered water. An aliquot was removed to analyze the initial concentrations of DOC and DON. Then, the samples were stored in the dark at room temperature (20º C) for 28 days. After the incubation the samples were ultrafiltered with Prep-Scale Cartridges (Millipore), obtaining three categories of molecular weight: a) less than 1 kDa (low molecular weight or LMW fraction); b) 1 to 10 kDa (medium molecular weight or MMW fraction); and c) more than 10 kDa (high molecular weight or HMW fraction).

For the second subset, 40 ml from each sample were stored to determine DOC and total dissolved nitrogen (TDN) concentrations. The remaining volume was also ultrafiltered separating DOM into the same three molecular weight fractions as the incubated samples.

DOC and DON for bulk concentrations are labelled as DOC_T and DON_T respectively. According to the DOM pool it is being referred, the different MW fractions are labelled in the following manner for ease of reading: DOC_{XMW}, DON_{XMW}, DOM_{XMW}, where X is “L”, “M”, or “H” for Low, Medium or High molecular weight. When the whole data set of MW fractions is referred to, in the text or figures, it is indicated with the sub index MW (i.e. DOC_{MW}, DON_{MW}, etc.).

DOC and TDN concentrations were determined using a Shimadzu TOC-VCS with a coupled TDN analyzer unit. DOC was determined by oxidative combustion infra-red analysis and TDN was estimated by means of oxidative combustion -chemiluminescence. The determination of TDN, nitrate and ammonium concentrations allowed estimating the concentration of dissolved organic nitrogen (DON) calculating the difference between the total nitrogen and the inorganic nitrogen. Nitrate and ammonium were determined colorimetrically using a Technicon autoanalyzer; nitrate with the Griess-Ilosvay method (Keeney and Nelson 1982) after reduction by percolation through a copperised cadmium column and ammonium after oxidation by salycilate using sodium nitroprusside as catalyzer (Hach 1992).

DOC and DON concentrations and its ratio (DOC:DON) were determined for whole water samples ($(\mathrm{DOC{:}DON})_T$) and each molecular weight ($(\mathrm{DOC{:}DON})_{MW}$) fraction.

3.2.4. Statistical analysis

Stream and riparian groundwater data were compared using the Wilcoxon test since data did not fit a normal distribution. Additionally, the overall differences between compartments during both hydrological periods were estimated using the Euclidean distances generated from the normalized means of the different parameters summarized in Table 1.

The means of the different measured parameters were compared by Wilcoxon test and the differences were considered significant at $p>0.05$ level.

The relationship between DOC_T, DON_T, $(\mathrm{DOC{:}DON})_T$, BDOC_T and their respective molecular weight fractions were explored using Spearman correlations using mean values from a minimum of three replicates. The Spearman correlations of the different parameters were explored independently from the hydrological period in order to identify general trends and were considered significant at $p<0.05$ level.

Since in some cases the replicate number was low a post-hoc power analysis was performed to avoid type II errors using G*Power and R. In all significant Wilcoxon tests and correlations statistical power was between 0.8 and 1 in most cases, while in non significant results statistical power was lower than expected due to high variance. The cases were power is below 0.8 are signalled with (*).In the following text, data expressed as mean is followed by the standard deviation.

3.3. RESULTS

3.3.1. Bulk DOM concentrations

DOM concentration and its temporal dynamics in stream water were clearly different from the riparian water. DOC_T and DON_T concentrations in stream water were significantly higher (Wilcoxon test; Z=2.8, n=10, $p<0.005$ for DOC_T; Z=2.31, n=9, $p<0.02$* for DON_T) than in riparian groundwater. Overall, DOC_T and DON_T concentration in riparian waters were not as sensitive to the alternating drying-rewetting phases as in the case of stream water, with the exception of the DON_T concentration decrease during the drying phase (Figure 2).

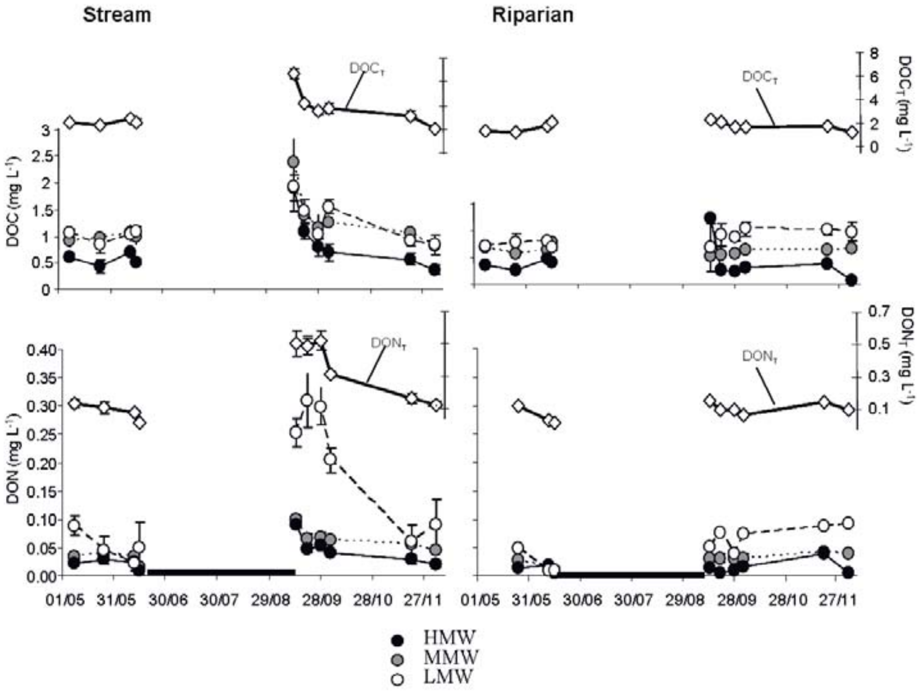


Figure 3.2. Temporal dynamics of DOCT and DOC MW fractions (panel a: stream water; panel b: riparian ground water) and DONT and DON MW fractions (panel c: stream water; panel d: riparian ground water) for the entire study. Symbols: white dots correspond to the LMW fraction, grey dots to MMW fraction and black dots to HMW fraction.

In stream water, DOC_T maxima and minima were observed during the rewetting specifically coinciding with the storm peak and the last sampling date. In contrast, DON_T minima and maxima coincided with drying (just before the flow disruption) and the rewetting (during the storm peak) respectively. Overall, DON_T represented the 48% ± 20% and 66% ± 25% of TDN in stream and riparian waters respectively, and differences between the two compartments were not significant (*) (Wilcoxon test; Z=0.53, n.s.*). However, during the rewetting differences became significant,were wider, DON$_T$ accounting for 43% ± 20% of TDN in stream, while 77% ± 13% in riparian waters (Wilcoxon test; Z= 1.99, p<0.05).

The storm episode triggers the flushing of both DOC$_T$ and DON$_T$. However, their post-storm dynamics were different. DOC$_T$ presented a pulse peak at the same time as the storm event (Q=100 L·s^{-1}) reaching a concentration of 6.7 ± 0.45 mg·l-1 and then decreased gradually at a rate of 0.05 mg·l-1·day-1 until it reached a similar concentration to the drought period (2.06 ± 0.05 mg·l^{-1}). On the other hand, high DON$_T$ concentrations persisted during the two weeks following the storm episode (0.5 ± 0.02 mg·l-1). Afterwards, by the end of the study period, DONT concentration was similar to that at the start of the drying phase (Figure 2).

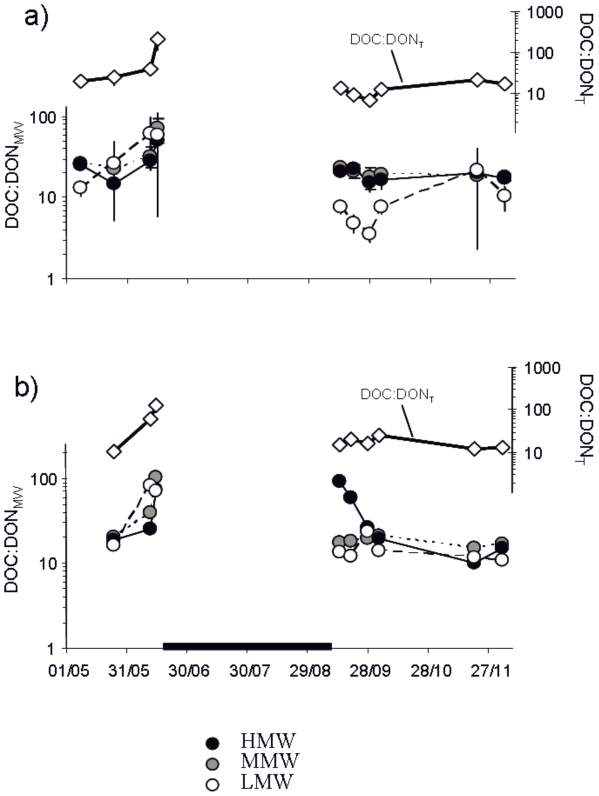


Figure 3.3. DOC:DON ratios for whole samples (DOC:DONT) and each MW fraction (black dots: HMW; grey dots: MMW; white dots: LMW) corresponding to stream water (panel a) and riparian ground water are shown (panel b).

As a consequence of the different temporal dynamics of DOC$_T$ and DON$_T$ pools in stream and riparian groundwater, the (DOC:DON)$_T$ ratio varied an order of magnitude between the two hydrological phases: from more than 100 just before the total drought in surface waters to less than 20 during the rewetting period (Figure 3). Considering the entire study period, (DOC:DON)$_T$ ratio in stream waters was identical to that estimated in riparian groundwater (37 ± 65 and 33.2 ± 37, respectively)(*), Wilcoxon test; Z=0.17, n=9, n.s.*). However, the (DOC:DON)$_T$ decrease observed during the rewetting was more evident in stream (in most of cases lower than 13) than in riparian water (typically higher than 13).

3.3.2. DOC and DON MW distributions

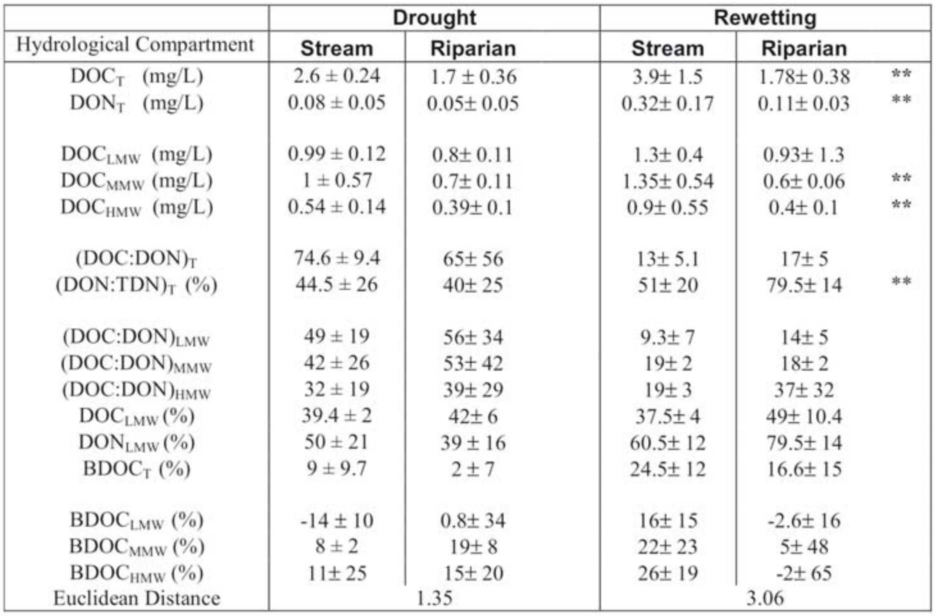
In stream water, DOC content in the three studied MW fractions showed a similar temporal pattern as observed for DOC_T during the study period (Spearman correlation; r=0.89 $p<0.05$ for DOC_{HMW}; r=0.97 $p<0.05$ for DOC_{MMW}; r=0.77 $p<0.05$ for DOC_{LMW}). DOCLMW and DOC_{MMW} were the most abundant fractions (38 ± 4% and 38.5 ± 2.5%, respectively) and their contributions were relatively constant during the entire study period with the exception of the DOC_{LMW} that decreased abruptly to the 30% in coincidence with the storm episode. Conversely, DOC_{HMW} average was 23 ± 4%, significantly lower than the other two MW fractions (Wilcoxon test; Z=2.38, n=10, $p<0.05$ for both DOC_{MMW} and DOC_{LMW} fractions vs DOC_{HMW}), but increased to 32% during the storm peak (Figure 2).

On the other hand, considering DON_{MW} fractions, DON_{LMW} was clearly dominant (56 ± 16%) followed by the DON_{MMW} (25 ± 9.5%) and the DON_{HMW} (18 ± 8%) fractions (Wilcoxon test; Z=2.80, n=10, $p<0.01$ for DON_{HMW} vs. DON_{MMW}, and Z=2.70, n=10, $p<0.01$ for DON_{HMW} vs. DON_{LMW}). Noticeably the relevance of the DON_{LMW} fraction increased even more during the re-wetting period when its contribution peaked to 70% of DONT during post-storm interval. Moreover, it was the only fraction that increased its concentration during the re-wetting period (0.21 ± 0.13 mg·l-1), while concentrations of DON_{HMW} and DON_{MMW} remained similar during both hydrological phases. This selective DON increase determined a decrease in DOC:DON ratios, especially in the $DOC{:}DON_{LMW}$ ratio to values significantly lower than the other fractions (9 ± 6) (Wilcoxon test; Z=1.99, n=6, $p<0.05$ for both HMW and MMW fractions) (Figure 3). In the larger DOC fractions (MMW and LMW) the DOC:DON ratio virtually never dropped below 15.

Similar to stream water, in riparian groundwater the foremost contributors to DOC_T and DON_T pools were the LMW and MMW fractions, accounting for the 46 ± 9% and 33.7 ± 5% of DOC_T, and 52 ± 14% and 31 ± 5% of DON_T, respectively. Considering the entire study period there were no significant differences between the contributions of the LMW fraction in surface and riparian groundwater (Wilcoxon test; Z=1.78, n=10, n.s. for DOC; Z=0.53, n=9, n.s.* for DON) (Figure 2).

In riparian groundwater, DOC:DON ratios for each MW fraction did not present any significant difference, although at the start of the re-wetting phase, the ratio for the HMW fraction showed high values (Figure 3) (Wilcoxon test; Z=1.59, n=9, n.s*; $DOC:DON_{HMW}$; Z=0.059, n=9, n.s.*, $DOC:DON_{MMW}$; Z=1.36, n=9, n.s.*, $DOC:DON_{LMW}$).

Table 3.1. Mean data for different parameters comparing stream and riparian waters during drought (n=3) and rewetting (n=6) periods (** indicates significant differences between stream and riparian groundwater, $p<0.05$).



	Drought		Rewetting		
Hydrological Compartment	Stream	Riparian	Stream	Riparian	
DOC_T (mg/L)	2.6 ± 0.24	1.7 ± 0.36	3.9± 1.5	1.78± 0.38	**
DON_T (mg/L)	0.08 ± 0.05	0.05± 0.05	0.32± 0.17	0.11± 0.03	**
DOC_{LMW} (mg/L)	0.99 ± 0.12	0.8± 0.11	1.3± 0.4	0.93± 1.3	
DOC_{MMW} (mg/L)	1 ± 0.57	0.7± 0.11	1.35± 0.54	0.6± 0.06	**
DOC_{HMW} (mg/L)	0.54 ± 0.14	0.39± 0.1	0.9± 0.55	0.4± 0.1	**
$(DOC:DON)_T$	74.6 ± 9.4	65± 56	13± 5.1	17± 5	
$(DON:TDN)_T$ (%)	44.5 ± 26	40± 25	51± 20	79.5± 14	**
$(DOC:DON)_{LMW}$	49 ± 19	56± 34	9.3± 7	14± 5	
$(DOC:DON)_{MMW}$	42 ± 26	53± 42	19± 2	18± 2	
$(DOC:DON)_{HMW}$	32 ± 19	39± 29	19± 3	37± 32	
DOC_{LMW} (%)	39.4 ± 2	42± 6	37.5± 4	49± 10.4	
DON_{LMW} (%)	50 ± 21	39 ± 16	60.5± 12	79.5± 14	
$BDOC_T$ (%)	9 ± 9.7	2 ± 7	24.5± 12	16.6± 15	
$BDOC_{LMW}$ (%)	-14 ± 10	0.8± 34	16± 15	-2.6± 16	
$BDOC_{MMW}$ (%)	8 ± 2	19± 8	22± 23	5± 48	
$BDOC_{HMW}$ (%)	11± 25	15± 20	26± 19	-2± 65	
Euclidean Distance	1.35		3.06		

A synthesis of DOC and DON characteristics for drought and rewetting periods and stream and riparian groundwater is summarized in Table 1, where the Euclidean distance evidences higher dissimilarity between the stream and riparian compartments during the rewetting period.

3.3.3. BDOC and BDON, bulk and MW fractions

Table 2 summarizes the mean and standard deviation of BDOC percent in bulk, for each fraction and considering the BDOC content of all fractions ($BDOC_{MW}$).

In stream water, the total BDOC ($BDOC_T$) was generally positive and widely ranged between 3% ± 0.8% and 48.5% ± 7% (Table 2). The minimum $BDOC_T$ content was detected just before the stream channel dried out entirely, while the maximum content was estimated during the rewetting phase, after the storm peak (Figure 4). Considering the entire study period there were no significant differences in BDOC$_T$ in stream and riparian groundwater. In stream water the overall BDOC content of all MW fractions (BDOC$_{MW}$) ranged widely between -23% and 66% (Table 2). Differences in BDOC content between MW fractions were not significant (*). Therefore it could not be identified a more bioavailable DOC fraction. It can only be asserted that considering all BDOC data from stream water that 50 % of cases of DOC release (BDOC$_{MW}$<0) were observed in the DOC$_{LMW}$ fraction.

Table 3.2. Summary of mean BDOC content (%) in stream and riparian groundwater for the entire study period.

	Stream	Riparian groundwater
BDOC$_T$	18 ± 13%	13 ± 15%
BDOC$_H$	21 ± 20%	7 ± 51%
BDOC$_M$	17 ± 18%	15 ± 41%
BDOC$_L$	7 ± 20%	6 ± 32%
BDOC$_{MW}$	16 ± 18%	4 ± 39%

The high variability of BDOC$_{MW}$ in riparian groundwater prevented that the average could be considered significantly lower than that estimated in stream water. In riparian groundwater BDOC$_{MW}$ release was also observed in 48% of cases.

BDON$_T$ in stream water did not show any pattern during the study period and it averaged -6 ± 13%, evidencing an accumulation of DONT instead of an uptake during the incubations. This DON release was even more significant when individual MW fractions were considered. Within the three molecular weight fractions, we estimated the following average BDON values: -81 ± 101% for BDON$_{HMW}$, -41 ± 14% for BDON$_{MMW}$, and -80 ± 109% for BDON$_{LMW}$. Differences among MW fractions were not significant (*) (Figure 4). The estimated BDON$_T$ and BDON$_{MW}$ releases were unrelated to ammonium and nitrate concentration nor their respective uptake/release magnitude during incubations (data not shown). On average, DON$_T$ represented the 43 ± 15% of total dissolved nitrogen (TDN). Ammonium and nitrate concentrations were below 0.04 and 0.08 N mg$^{\cdot L-1}$ respectively during drought and peaked 0.1 and 0.9 N mg$^{\cdot L-1}$ respectively during rewetting. The analyses of water samples after the incubation period often indicate ammonium uptake. At the beginning of the drought period ammonium concentration uptake was 49 ± 26% of initial

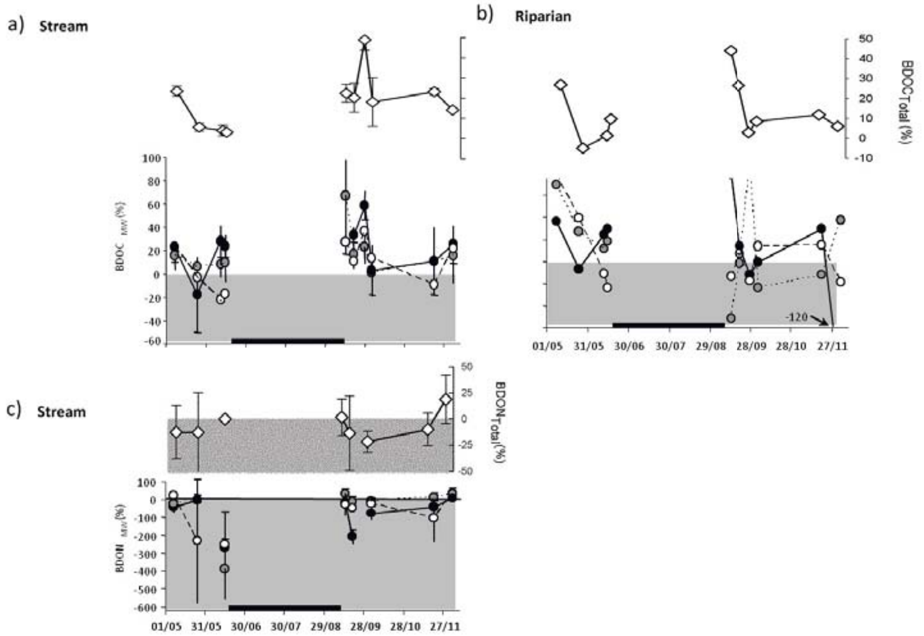


Figure 3.4. Temporal variation of BDOC content (%) and each MW (black dots: HMW; grey dots: MMW; white dots: LMW) fraction for stream (panel a) and riparian (panel b) waters. Temporal variation of BDON (%) is shown only for stream water (panel c). Dots falling in the grey areas indicate DOC or DON release.

concentration, while at the start of the rewetting period it increased to 95 ± 5%, indicating that net ammonification was irrelevant in experimental incubations. By contrast, nitrate changes during incubations were more ambiguous and ranged from moderate release (-21 ± 7%) during drought to low uptake (10 ± 9%) during rewetting.

Considering the combined stream and riparian waters data set, $BDOC_T$ was significantly related to the DON_T concentration (r=0.59, d.f.=17, p<0.01), although this relationship was consistent for the stream water data set (r=0.73, d.f.=8, p<0.05*) but not for riparian data alone (r=0.43, d.f.=7, n.s*). On the other hand, the $BDOC_T$ data set was unrelated to DOCT (r=0.35, d.f.=17, n.s*). This disconnection persisted even considering stream and riparian waters $BDOC_T$ data sets separately.

Furthermore, $BDOC_T$ declined potentially as the DOC:DON ratio increased (r=0.59, d.f.=16, p<0.01) (Figure 5, panel a). In terms of MW fractions, this trend

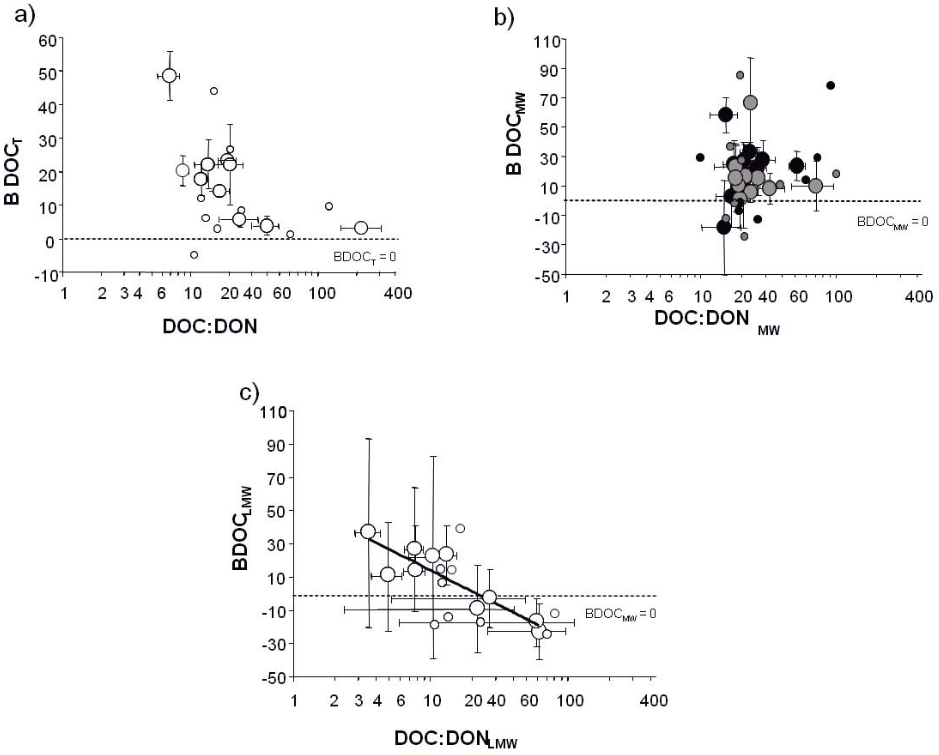


Figure 3.5. Exploration of the relationship between DOC:DON ratios and BDOC content in stream (normal dots) and riparian groundwater (small dots). Panel a): data corresponding to whole water samples (DOC:DON$_T$ and BDOC$_T$). Panel b): data corresponding to DOC$_{MMW}$ and DOC$_{HMW}$ and their respective BDOC$_{MW}$ fractions. Panel c): DOC$_{LMW}$ versus BDOC$_{LMW}$. The insets in panels a) and c) show the relationships of DOC and DON with BDOC content separately.

between BDOC and DON or DOC:DON ratio were consistent only for the LMW fraction ($r=0.58$, d.f.=17,$p<0.01$ and $r=0.61$, d.f.=17,$p<0.01$ respectively) (Figure 5, panels b and c).

Coupling the (DOC:DON)$_{MW}$ ratio with BDOC$_{MW}$ from the entire DOM data set, we observed that a DOC:DON ratio value of 13 marks a threshold where BDOC$_{MW}$, in probabilistic terms, shifts from positive (DOC$_{MW}$ uptake), to negative (DOC$_{MW}$ release). Namely, if we consider exclusively those cases at which

(DOC:DON)$_{MW}$ (13, BDOC$_{MW}$>0 occurs in 90% of cases. Of these cases, the 89% correspond to LMW fraction samples collected during the rewetting period. In contrast, if we increase this threshold, (DOC:DON)$_{MW}$ (13, without considering the study period (i.e. drought or rewetting) the probability that BDOC$_{MW}$>0 decreases to 60%. The 78% of cases at which (DOC:DON)$_{MW}$ >13 fall within the MMW+HMW fractions interval.

3.4. DISCUSSION

This study highlights that the drought - rewetting hydrological cycle exerts a divergent influence on DOM dynamics and chemical properties depending whether organic carbon (DOC) or nitrogen (DON) are considered. In the case of DOC, the influence is on concentration changes, while in the case of DON, besides the change in concentration, there is a qualitative change, in terms of the distribution of molecular weight fractions.

The most outstanding changes emerge when comparing stream water and riparian groundwater during the rewetting period as stream water supplies the riparian compartment (Butturini et al., 2003). In contrast, differences are minimal during the drought period when the opposite flux occurs. This indicates that DOM properties do not change remarkably when water flows into the stream channel during drought, while during the rewetting period the riparian compartment is rather the biogeochemical “hot spot”. Our data suggests that the detection of the biogeochemical “hot spots” depends on the hydrological period when the samplings are conducted. Therefore, not only there are “hot spots” but also “hot moments” as pointed out by Harms and Grimm (2008).

Since the drought phase starts 4 months after the last severe rain episode our data can be compared to a natural long term “perfusion” experiment. During this “experiment” the stream is fed by increasingly “older” groundwater perfusing through the stream-riparian interface and leaving the system. Although DOM changes during the drought period are subtle, the detailed analysis of DOC and DON properties highlight the immobilization of DON$_T$ in the stream-riparian interface, while neither DOC$_T$ nor the distributions of their respective MW fractions (DOC$_{MW}$ and DON$_{MW}$) are. In fact, in stream and riparian waters DOC$_T$ remains nearly constant, while DON$_T$ is gradually depleted, with the subsequent increase of the (DOC:DON)$_T$ ratio in both water bodies and in the three molecular fractions as well. The increase in the DOC:DON ratio during drought is in agreement with the observed reduction in the heterotrophic use of peptides and a major use of polysaccharides throughout the drought process (Ylla et al. 2010). On the other hand, the relative contribution of the three MW fractions does not show a clear pattern. To our knowledge there are no field studies that explore the influence of severe drought on the distribution of DOM MW fractions and the available information is restricted to few studies suggesting an increase of low molecular weight (LMW) fractions under low flow in wetlands (Maurice et al., 2002) and forested rivers (Li et al., 2003). Furthermore, as drought persists, becoming more severe, the reservoir of groundwater is emptied gradually and the input of water and solutes into the stream cease. Then, surface waters become isolated and stagnant ponds where DON can reappear only if other DOM inputs become relevant (i.e. detritus inputs and subsequent DON leaching; Vazquez et al., 2010).

In contrast with our initial hypothesis, Tthe "old" pre-event water that leavinges the system does not present a higher contribution of DOCLMW that, according to the size-reactivity model (Amon and Benner, 1996), is supposed to be recalcitrant since it would be originated as a result of incomplete degradation of DOC_{HMW}. The present results suggest that, during drought, DOC molecular weight is unrelated to its bioavailability.

On the other hand, it is well recognized that DOC increases its concentration during storm events in forested catchments. This response is studied intensely and extensively in temperate (Raymond and Saiers, 2010), boreal (Creed et al., 2008) and glacial (Inamdar and Mitchell, 2007) systems and much more sporadically in Mediterranean (Sanderman et al., 2009) and tropical ones (Wiegner et al., 2009). Fuirosos stream fits within this general model. However highest DOC_T peaks (6-20 $mg \cdot L^{-1}$) are usually concentrated during the rewetting period and they are unrelated to the magnitude of the specific storm event that switches on this hydrological period (Butturini et al., 2008). These DOC_T peaks respond mainly to the leaching of fresh and young DOM from the abundant leaf litter accumulated on the stream channel during the previous drought (250 - 410 $gC \cdot m^{-2}$, in Fuirosos, Acuña et al., 2005). Recent studies suggest that DOM inputs associated to stormflow would present higher aromatic content (Vidon et al., 2008; Sanderman et al., 2009), low content of non humic materials (Hood et al., 2006) and high molecular weight (Maurice et al., 2002, Li et al., 2003). In contrast the degree of potentially bioavailable DOM flushed during storms is uncertain. Some authors found that the mobilized DOC is mainly labile (Don et al, 2005) but others found the opposite

(Wiegner et al., 2009). This aspect is crucial to describe the effective relevance of storm episodes on the flushing of high amounts of potentially labile DOM for down-river communities or even for coastal ecosystems.

In our study, there is not a clear and consistent input of DOM_{HMW} fractions during the entire rewetting period. Furthermore, when the shifts in the relative contributions of DOC and DON MW fractions are considered separately, their respective trends are opposite and difficult to interpret. For instance, in stream water, the contribution of the large DOC MW fractions (DOC_{MMW} + DOC_{HMW}) increases up to 70% during the storm peak. On the other hand, during and after the storm peak, DON_{MMW} + DON_{HMW} contribution decreases between 44 and 27%. Thus, DOC_{MW} dynamics fit within the framework that storm events enhance the input of the larger molecular weight DOM fraction (>1 kDa), but DON_{MW} does not. In contrast, in temperate systems, Peduzzi et al. (2008) observed no relationship between the distribution of DOM molecular weight fractions and hydrology. Furthermore, the DOC:DON ratios of bulk concentration and individual fractions (especially for the LMW fraction) decrease after the storm peak evidencing an asynchronous DOC and DON mobilization. Although studies that explored in detail DON patterns during hydrological events are relatively scarce in comparison to those focused on DOC all possible changes of DOC:DON have been reported: no change (Buffam et al., 2001; Cooper et al., 2007; Wiegner et al., 2009), increase (Sanderman et al., 2009), decrease (Hagedorn et al., 2006) and depending on the antecedent hydrological situations (Turgeon and Courchesne, 2008) or storm timing (i.e. storm rising or recession limbs; Inamdar and Mitchell, 2007). In Fuirosos, the observed post-storm $(DOC:DON)_{MW}$ decrease suggests an important delayed input of DON_{MW}, (mainly DON_{LMW}) delivered into the stream from relatively deep flow paths traversing organic-rich soil with abundant nitrogen due to an increase in the water table (Bernal et al., 2005). It is interesting that the pattern observed in this Mediterranean system is almost the opposite of that observed in humid wetland catchments characterized by DON flushing preceding that of DOC (Inamdar and Mitchell, 2007). During the rewetting period DOM bioavailability, in terms of carbon ($BDOC_{MW}$), is not related to the molecular weight distribution of DOC as well. The only distinctive trend is that 50% of cases at which DOC_{MW} release is observed ($BDOC_{MW}<0$) correspond to the LMW fraction.

The information obtained in Fuirosos stream on the distribution of DOM molecular weight fractions does not provide new insights on its bioavailability.

Within this perspective the empirical findings derived from laboratory estimates is in consonance to the observed inability of the stream system to modify the distribution of DOM molecular fractions during drought (i.e. the natural perfusion "experiment"). Unfortunately, our findings contribute to enlarge the already present uncertainty about the relevance of DOM molecular weight on bioavailability. For instance, Thóth et al. (2007), Fischer et al. (2002) Kaiser and Sulzberger (2004) or Sachse et al. (2001), according to the Amon and Benner size-reactivity continuum model (1996), associated high bioavailability to large DOM molecules. In contrast, Lindell et al. (1995), Marschner and Kalbitz (2003), and Agren et al., (2008) suggest the opposite relationship. To further complicate cross site comparisons, the definition of "small" and "large" fractions is an operational classification arbitrarily selected. Thus, some authors select the 1kDa cut-off (Stepanauskas et al 1999; Belzile and Guo, 2006; Vazquez et al., 2007, Peduzzi 2008) and others set it at 3 kDa (Kerner and Spitzt, 2001).

$BDOC_T$, is linearly and positively related to DON_T, and, inversely related to the $(DOC:DON)_T$ ratio, suggesting that DOM bioavaility is heavily influenced by DON content rather than molecular weight. These relationships persist when considering only $BDOC_{LMW}$ as a consequence of the DOM_{LMW} fraction being the most abundant and the wider range of DON_{LMW} concentration. The bioavailability of DON_{LMW} during the rewetting period might be linked to the increase in amino acid contribution to the DOM pool observed by Ylla et al. (2010). Indeed, these characteristics facilitate the observation of trends in DOM composition and dynamics. Noticeably, coinciding with the important input of DOM_{LMW} (up to 0.2 $mg \cdot L^{-1}$), and subsequent $(DOC:DON)_{LMW}$ decrease below 13, during the rewetting, this fraction shifts from refractory to bioavailable. On the other hand, in most cases (76%) the MMW and HMW fractions present $(DOC:DON_{)MW}$ ratios higher than 15 and positive $BDOC_{MW}$ values, although there is no clear relationship to DON_{MW} concentration.

$BDON_T$ and $BDON_{MW}$ data are mostly negative during the study period suggesting non-humic DON release (Petrone et al., 2009), perhaps of microbial origin (Berman and Bronk, 2003), during incubations. The interpretation of $BDON_T$ and, specially, of $BDON_{MW}$ must be carefully considered. DON estimates are obtained indirectly after subtraction of three measured concentrations obtained from independent analysis, therefore propagating/increasing variance (Wontae and Westerhoff, 2005). In addition, regarding DON_{MW} estimations, we need to consider the disturbance introduced by sample manipulation during the separation of DOM fractions. Consequently, we prefer adopting a cautious position in order to avoid generating a weak analysis on $BDON_{MW}$ variability across water bodies, study period and/or molecular weight fractions. However, it is not possible to ignore the recurring DON_T and DON_{MW} release during incubations suggesting that the residual DOM is not necessarily depleted in nitrogen. This finding is unexpected for two reasons. First, because it is not in consonance with the DON depletion observed during the drought period suggesting an active important DON sink. The fact that DON depletion during drought is observed in both surface and riparian groundwater while DON concentration increases during incubations, suggests that sediment might play an important role on DON uptake. Thus, the DON sink might be located in the riparian groundwater rather than in the stream channel. Unfortunately, from this study there is not enough evidence and further work is needed in the future. The second reason is that the evidence that there is a positive DON uptake is consistent in several bioassay studies performed with continental (Seitzinger and Sanders, 1997; Stepanauskas et al 1999, 2000; Kerner and Spitzy, 2001; Petrone et al., 2009) and marine-coastal waters (Lonborg et al., 2009; Lonborg & Sondegaard 2009). Finally, dissolved inorganic nitrogen availability (DIN, mainly nitrate and ammonia) might exert an important influence on DON (Hedin et al., 1995). DON uptake might replace that of DIN when it is in a short supply. Then, the highest BDON content should be expected at high DON:TDN ratios (where TDN stands for DIN+DON). Under this perspective, remarkable DON uptakes have been reported when DON pool exceeded the 80% of the TDN (Stepanauskas et al 1999, 2000; Kerner and Spitzy 2000; Petrone et al., 2009). Although we ignore if there is a general and consistent pattern behind the direct relationship between BDON and DON:TDN ratio (for an instructive exception see Wiegner et al., 2009), the DON:TDN ratio in the previous literature referenced systems is about twice than that measured in Fuirosos (43% during the study period or 35% in a long term study, Bernal et al., 2005). Then, within this biogeochemical context, DIN should be the main dissolved nitrogen source for bacteria. This conclusion is partially corroborated by the results from our experimental incubations. Thus, ammonium uptake is observed throughout the study period, while nitrate is slightly retained only during the rewetting phase in coincidence with the storm episode. In any case, we do not observe a significant relationship between $BDON_T$ and DON_T:TDN_T (r=0.15, d.f=8, n.s).

3.5. Conclusions

Although it is well known that drought shapes the stream ecosystems functioning and structuring (Acuña et al., 2005, Rose et al., 2008; Gomez et al., 2009; von Schiller et al., 2008; Boix et al., 2010), it is rather unusual the explicit study of these episodes, including the drought-wet transition (Vazquez et al 2010), to explore the magnitude of biogeochemical change. Nevertheless, under threat of climatic change, the Mediterranean regions are targeted as a hot spot, where the freshwater flow regimes will suffer additional severe alterations (Giorgi 2006). Thus, given the key role on biogeochemical cycles at regional and global scale that freshwater systems play (Battin et al., 2008, Jaffé et al., 2008), the need to focus on drought-wet cycles in order to discern the extend of the alteration of freshwater biogeochemistry, specially carbon transport, due to the severity of the hydro-climatic disturbances will increase.

In this context, this study has shown that during both drought and rewetting periods DOM lability is not tied to a single MW fraction, but regulated mainly by DON concentration and the DOC:DON ratio, which presents a threshold value of 13. This is exemplified by the DON depletion during drought causing a decrease in DOC bioavailability and the posterior increase in DOC lability during the rewetting period where there is a high input of $\mathrm{DON}_{\mathrm{LMW}}$. Therefore, further studies are needed to explore the role of DON in DOM lability and its potential effects on DOC export from fluvial systems.

Chapter 4

Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions

Vazquez E[1]., Amalfitano S.[2], Fazi S.[2], Butturini A[1]. 2010. Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions. *Biogeochemistry*, 102:59-72.

[1]Departament d'Ecologia, Universitat de Barcelona, Diagonal 643, 08028 Barcelona, Spain; [2]Istituto di Ricerca sulle Acque (IRSA-CNR), Area della Ricerca "Roma 1", Via Salaria km 29,300, CP1000016 Monterotondo, Rome, Italy

ABSTRACT

In Mediterranean regions, drought is one of the main factors shaping fluvial ecosystems. Droughts cause a shift from lotic to lentic conditions, triggering a gradual fragmentation of the longitudinal hydrological continuum, and a severe alteration of water chemical properties. However, within a biogeochemical perspective, little is known about how and to which extend droughts modify the chemical properties of dissolved organic matter (DOM). In this study, the variability of DOM properties along a fragmented fluvial system is explored, during a summer severe drought, by means of a) the ratio between dissolved organic carbon and nitrogen concentrations (DOC:DON); b) DOC bioavailability (BDOC), and c) DOM optical properties (SUVA index, fluorescence index, and excitation-emission fluorescence matrices). DOM and water measurements were collected from isolated water parcels that became disconnected from the fluvial continuum at different times, and were compared with data obtained in the following autumn, when the fluvial continuum was re-established.

Analysis of DOM chemical properties evidenced that these properties during drought clearly differed from those observed in autumn, but changes did not follow an arbitrary pattern. Thus, the sampling sites with lotic water bodies showed DOM properties similar to those observed in autumn reflecting the dominance of terrestrial inputs. But, once hydrological fragmentation occurred, there was a gradual increase in the contribution of autochthonous DOM as the time elapsed since the pools were established, and the geochemical conditions shifted from oxidized to reduced conditions. In consequence, the fragmentation of fluvial continuum generates a set of distinct biochemical hot spots (i.e. each water parcel), revealing that extreme drought greatly amplifies the qualitative heterogeneity of organic matter in a fluvial system.

4.1. INTRODUCTION

In aquatic ecosystems, dissolved organic matter (DOM) is a mixture of compounds whose characteristics and composition strongly influences key processes including bacterial production, trophic web organization, biogeochemical transformations, nutrient availability and carbon cycling (Sobczak & Findlay 2002). The concentration and quality of DOM transported in a stream is the combined result of allochthnous inputs from watershed hillslope and riparian flushing and autochthonous inputs from the in-stream metabolism. In consequence, DOM composition is highly variable because of the temporal and spatial dynamism of these processes (Stedmon et al., 2003; Sachse 2005; Romaní et al., 2006).

Mediterranean fluvial ecosystems are characterized by recurrent summer droughts (Gasith & Resh, 1999; Butturini et al., 2008). Their frequency and intensity strongly affect stream metabolism (Acuña et al., 2005; Rubbo et al., 2006) and DOM cycling (Vazquez et al., 2007; Butturini et al., 2008). Drought severity determines the degree of disruption of the longitudinal fluvial continuum and the decline of the vertical hydrological connectivity between surface and surrounding riparian ground waters (Butturini et al., 2003). This loss of hydrological connectivity often takes place sequentially during the span of the drought period. In the final stage, the fluvial network is often converted into a fragmented landscape of isolated water pools. As a result, the drying process is gradual in time and heterogeneous in space: surface flow may start drying from downstream to upstream or vice versa. Another possibility is that water may persist in headwaters and the mouth, disappearing in the middle section first. In any case, the water pools are not established at the same time, and their location, dimension, persistence and age depend on site geomorphologic and hydrological conditions (Lake 2003). As a consequence of the absence of advection, the chemical characteristics of isolated waters change radically from solutes in oxidized state (i.e. $N\text{-}NO_3$) to reduced states (i.e. $N\text{-}NH_4$), with an impact on microbial processes regulating elemental cycles at local scale. For instance, it is expected a decrease in oxygen, nitrate and sulphate concentrations, and an increase in ammonium (Bleich et al., 2009; Stanhope et al., 2009). Focusing on DOM, an increase of dissolved organic carbon (DOC) and nitrogen (DON) bulk concentration is expected in water pools as result of continuous leaching of particulate organic matter that enters constantly from the surrounding riparian environment (Acuña et al., 2005). Despite little is known about the changes in the qualitative properties of DOM during droughts. There is a rich literature showing that drought is an important mechanism to explain DOC losses in boreal upland peats (Worrall et al., 2006; Clark et al., 2005; Freeman 2004). But information from water-limited systems is in an incipient stage (Dahm et al., 2003; Vazquez et al., 2007).

Therefore, from these preliminary considerations, our objective was to examine how and to which extent does the fluvial continuum fragmentation affect DOM chemical properties? In order to explore this question, a Mediterranean intermittent fluvial system was sampled during a summer drought period and the successive autumnal wet period when the fluvial continuum was re-established. Water samples were collected along a longitudinal gradient, from the stream mouth to the headwaters, and along a temporal gradient according to the time the pools became isolated. Sampling locations included both surface and groundwater riparian waters. A continuous monitoring of ground and stream water levels showed that during drought, there was no hydrological connection between the riparian groundwater and stream water. Therefore, the riparian groundwater was considered a sort of groundwater isolated pool.

DOM characterization from samples obtained during the wet hydrological period is used as a background values to assess changes in DOM properties during drought. During this period, it is expected that DOC presents similar properties in all sampled locations, with the exception of riparian ground waters (Vazquez et al., 2007).

Different approaches, including spectroscopic techniques, have been widely used to characterize DOM from different aquatic systems: marine, estuaries, rivers, lakes, groundwaters, soil water (e.g. Stedmon et al., 2005a; Hood et al., 2006; Mladenov et al., 2007; Fellman et al., 2008; Vidon et al., 2008; Jaffé et al., 2008). But, to our knowledge, these methods have not been yet applied in the characterization of DOM during drought periods in stream waters.

4.2. MATERIAL AND METHODS

4.2.1. Study Site

Fuirosos is a third-order stream that drains a forested granitic catchment of 16.2 Km^2, near Barcelona (NE Spain, 41º 42’ N, 2º 34’ W, 50-770 a.s.l.).

The climate is typically Mediterranean, with monthly mean temperatures ranging from 3ºC in January to 24ºC in August. Precipitation mostly falls in autumn and spring with occasional summer storms. Average annual mean precipitation for this region is 750 mm (Ninyerola et al., 2000).

The catchment is covered mostly by perennial cork oak (*Quercus suber*) and pine tree (*Pinus halepensis*) with one or two layers of shrubs (e.g. *Ramnus alaternus*, *Viburnum tinus*, *Arbutus unedo*, *Prunus spinosa*) and lianas (*Lonicera implexa*, *Smilax aspera*). Deciduous woodland of chestnut (*Castanea sativa*), hazel (*Corylus avellana*) and oak (Quercus pubescens) predominate in the valley head.

In the middle point of the catchment there is a small artificial reservoir. Downstream from the reservoir, the stream channel is 1 m to 5 m wide and it is

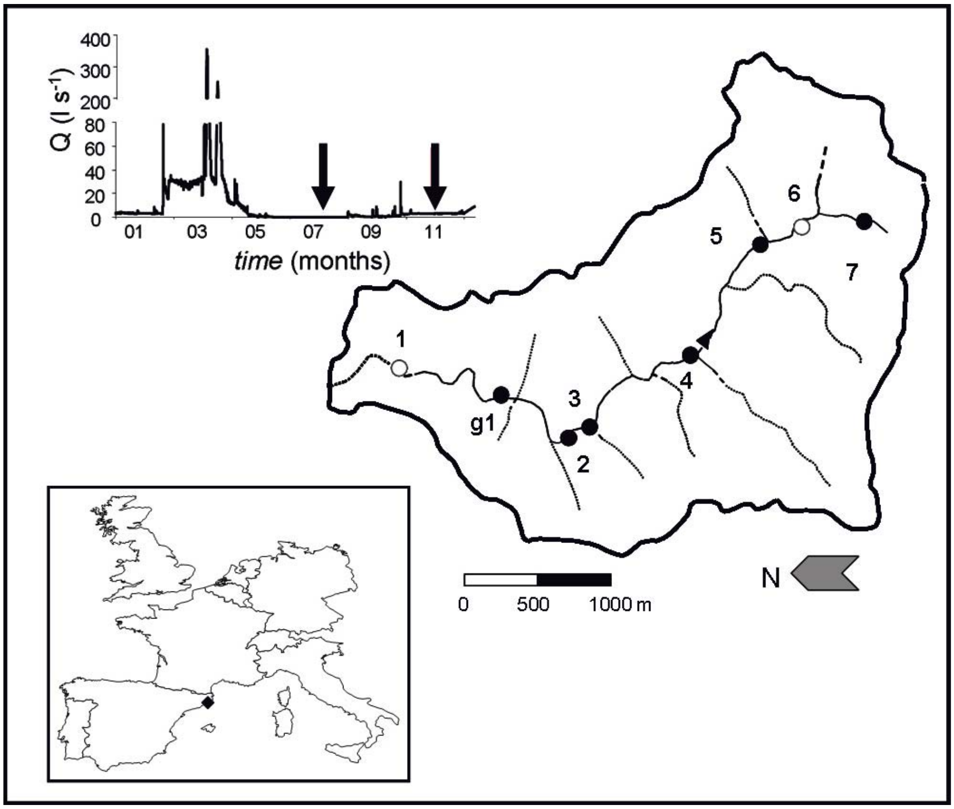


Figure 4.1. Fuirosos catchment.
The main figure shows Fuirosos catchment were sampling sites are indicated: black dots show autumn and summer sampling sites and white dots show the two additional sites sampled in autumn. The black triangle is the reservoir found in the main channel. The lower inset shows the location of Fuirosos catchment in the Western Europe context. The upper inset figure shows the hydrograph for year 2007 for the historical sampling site (already dry during the summer sampling), next to ground water well (g1). The arrows mark the sampling dates

characterized by steep-pool morphology with cobbles and boulders, although sand and bedrock substrates are also present. Also, there is a well developed riparian forest flanking the stream channel (10 to 20 m wide), consisting mainly of plane tree (Platanus x hispanica) and alders (Alnus glutinosa). The riparian soil is poorly developed and plane leaf litter tends to accumulate on the forest floor because of extremely low decomposition rates (Bernal et al., 2005). Upstream from the reservoir, the stream channel is narrower (0.5-2 m wide) and the bedrock substrate is more common with the resulting reduction of the hyporheic zone. Also, the riparian strip is no longer defined as in the bottom valley.

The basal flow usually ranges between 1-20 $L \cdot s^{-1}$. The flow is interrupted, usually, by a long dry period in summer followed by an abrupt recharge period in late summer-early fall. The subsequent humid period elapses until late spring.

4.2.2. Sampling strategy

Two sampling campaigns were carried out from the bottom valley to the headwaters along Fuirosos fluvial main channel under different hydrological conditions: in summer (10/07/2007), during drought, and in autumn when the stream continuum was re-established (20/11/2007). In 2007 the drought period elapsed from June, 10th until August, 27th. This drought was preceded by a long drying phase that started on May the 1st, when the last important rain episode occurred (Figure 1, inset).

During summer, the streambed was almost completely dry. But an accurate and intensive preliminary hydrological monitoring programme, carried out along the entire stream network, allowed identifying the only five areas where surface water still persisted formed (sites 2, 3, 4, 5 and 7; downstream to upstream). Two hundred meters downstream from site 2, there was no surface flow but groundwater samples were collected from a 2 meters depth well, 2 meters away from the stream channel (site gw) (Figure 1).

Sites 2, 3, and 5 were isolated pools that had become stagnant at different times in zones where the main substrate was bedrock, thus preventing flow exchanges between the hyporheic zone and the rest of the main channel favouring the persistence of surface water. While at sites 4 and 7 water flow was still discernible. At the time of sampling, the preliminary hydrological programme allowed estimating the Pond Isolation Time (PIT): the elapsed days since the water parcel became totally stagnant (i.e. flow advection was nil). We assumed that at this time a pool was totally disconnected from the rest of the fluvial network. The estimated

PIT values for sites 2, 3 and 5 are 15, 12 and 5 days respectively. PIT values for sites 4 and 7 are 0 by definition.

Site 4 is fed by the artificial reservoir, and discharge at summer was of 0.1 $l\cdot s^{-1}$. Site 7 is a headwaters spring which flows permanently, and the measured flow in summer was 0.2 $l\cdot s^{-1}$, although a few meters from the sampling site water infiltrated and disappeared.

The second sampling campaign was conducted on the following month of November in the same locations. Furthermore, to obtain a more complete picture of DOM properties along the entire stream continuum, stream surface waters were collected from two additional sampling points completely dry during summer (sites 1 and 6, Figure 1). As site 3 was very close to site 2 and there were no differences in the physicochemical parameters measured in situ, it was not sampled. During this period stream flow was at the low range of the basal discharge (mean 1.35 ± 0.9 $l\cdot s^{-1}$) and the water body was uninterrupted along the fluvial network.

4.2.3. Stream water physico-chemical properties

At each sampling location we measured pH, temperature, electrical conductivity (Ec), oxygen concentration (O2) and, when possible, discharge using chloride slug additions. Three water samples (150 ml), for each site, were collected for the analysis of conservative solutes (chloride and sulphate), nitrate, ammonium, dissolved organic carbon (DOC) and total dissolved nitrogen (TDN).

Chloride and sulphate were analyzed by liquid chromatography using a Metrohm 76 compact IC. Nitrate and ammonium were determined colorimetrically using a Technicon autoanalyzer; nitrate with the Griess-Ilosvay method (Keeney and Nelson, 1982) after reduction by percolation through a copperised cadmium column and ammonium after oxidation by salycilate using sodium nitroprusside as catalyzer (Hach 1992).

4.2.4. Dissolved organic solutes and DOM composition

DOC and TDN concentrations were determined using a Shimadzu TOC-VCS with a coupled TN analyzer unit. DOC was determined by oxidative combustion infra-red analysis while TDN was estimated by means of oxidative combustion-chemiluminescence. Dissolved organic nitrogen (DON) was estimated calculating the difference between TDN and the inorganic nitrogen (i.e. $N\text{-}NH_4$ and $N\text{-}NO_3$).

In this study five qualitative DOM descriptors were used: DOC:DON ratio, specific UV absorbance at 254 nm (SUVA index), biodegradable DOC (BDOC), fluorescence index (FI) and the ratio of intensities of C and A fluorescence peaks obtained from the analysis of excitation-emission matrices (EEMs) (see below).

The $SUVA_{254}$ index is highly correlated to DOM aromaticity (Weishaar, 2003; Hood et al., 2005). The measured absorbance at 254 nm was corrected by the cuvette path length and DOC concentration. The index is expressed in $L \cdot mg\ C^{-1}\ m^{-1}$.

BDOC was determined according to the method described by Servais et al. (1989). To determine BDOC, we collected 2 L of water from each sampling site and filtered in situ with precombusted GF/F filters (Whatman). In the laboratory, four replicates of 200 ml for each sampling site were subsequently filtered by 0.2 µm Whatman nylon membranes. Thereafter, samples were inoculated with 2 ml of GF/F filtered water. The water, utilized for the inoculums, was from site 4, to discard possible effects of different bacterial assemblages on DOC degradation. An aliquot was collected to determine DOC initial concentration. Afterwards, samples were stored in the dark at room temperature (20ºC) during 28 days. Once they had elapsed, DOC concentration was measured again.

Fluorescence spectroscopy was completed on whole water samples in order to further characterize DOM. Excitation-Emission matrices (EEMs) are a 3D representation of fluorescence over excitation and emission pairs concatenating different scans. In order to obtain the EEMs, fluorescence measurements were performed using a Shimadzu RF-5301PC spectrofluorimeter over an emission range of 280 - 690 nm at 1 nm increments, and an excitation range of 240 – 420 nm over 10 nm increments. After obtaining the EEMs, ultra pure water blanks were substracted to correct for Raman scattering. Finally each EEM was normalized to the Raman area. Fluorescence is expressed in Raman units. Visually, two main fluorescence peaks were identified, their emission-excitation wavelengths corresponding to peaks A and C according to the categorization proposed by Coble (1996), both corresponding to humic substances. Using the EEM of each sample, the relative contribution of peaks A and C was estimated using the ratio of the maximum fluorescence intensity of each fluorophore (I_C/I_A ratio). Similar indexes based on the intensity of identified peaks in EEMs were used by McKnight et al (2001), Milori et al (2002) in Brazilian soils, Parlanti et al (2000) in coastal waters and Wilson (2009) in riparian wetlands. This ratio allowed examining the variation in contribution of each fluorophore between seasons and among different sampling sites.

The fluorescence index (FI) was determined according to McKnight (2001). The FI index was calculated from the ratio of intensities emitted at 450 and 500 nm at an excitation wavelength of 370 nm. This index allows discriminating the origin of DOM, its values range between 1.2 and 2, where low values indicate an allochthonous DOM origin, mainly from decomposition and leaching of plant and soil organic matter, while high values point to autochthonous organic matter generated from extracellular release and leachate from algae and bacteria. In Fuirosos, FI values estimated in soil leachate ranged between 1.62 and 1.66 (n=3, E. Vazquez, unpublished data). Although these values are high enough to consider an important influence of the microbial community in the origin of DOC, it helps discriminating DOM origin between soil (1.62) and increasingly autochthonous in surface waters.

4.2.5. Statistical analyses

In order to explore the chemical (dis)similarities among sampled waters, the concentration values of the inorganic solutes (SO_4, NO_3, NH_4, PO_4, and Cl) and physico-chemical parameters (pH, O_2, and Ec) were used to estimate the Euclidean distance matrix. Then, a non metric multidimensional scaling (nMDS) analysis was applied to generate a map where more similar sampling points were plotted closer. Temperature and discharge values were not included in the analysis since these parameters would amplify the obvious relevance of seasonal trends in the distance matrix.

An empirical relationship between the concentration of dissolved oxygen ($[O_2]$) and nitrogen in ammonium form ($[N\text{-}NH_4]$) was used to provide a synthesis of the aerobic/anaerobic conditions among sampled water parcels and seasons:

$$CI = \ln([O_2]/[N\text{-}NH_4]) \qquad \text{eq.1}$$

The Chemical Index (CI) will vary according to the environmental conditions of water. Under low dissolved oxygen concentration, it will be expected an increase of solutes in reduced form (i.e. NH_4) (Bleich et al., 2009; Stanhope et al., 2009). Thus, the CI index will present low values, while in aerobic conditions the CI will present high values.

The presence/absence of correlations between the DOM descriptors and CI were explored separately for each season. The correlations were considered statistical significant at $p<0.05$ level. In order to reduce the degrees of freedom, the correlations were performed with average values obtained from a minimum of three replicates.

4.3. RESULTS

4.3.1. Chemical characteristics of drought and wet periods

a) Inorganic solutes

The range of variation of inorganic solute concentrations for summer and autumn is shown in Figure 2. The contrast between seasons is noticeable. As expected, in summer, the concentration of oxidized solutes strongly decreased. For instance, dissolved oxygen concentration in surface waters drop its concentration to 2.8 ± 3.5 (SD) mg·L^{-1}, while in autumn is 8.9 ± 2 mg·L^{-1}. Similarly, sulphate concentrations are lower in summer (surface water, mean 5.95 ± 3.23 mg·L^{-1}) than autumn (surface water, mean 18.74 ± 5.35 mg·L^{-1}). Nitrate is low in summer (0.083 ± 0.09 N mg·L^{-1}) and high and more variable in autumn (0.25 ± 0.27 N mg·L^{-1}). In contrast ammonium in summer is higher and much more variable (3.31 ± 5.5 N mg·L^{-1}) than in autumn (0.024 ± 0.014 N mg·L^{-1}). Consequently, the summer CI values are clearly lower (1.1 ± 3.6) than those estimated in autumn (5.94 ± 0.7). On the other hand, phosphate concentration is higher and much more variable (0.016 ± 0.01 P mg·L^{-1}) in summer than in autumn (0.001 mg·L^{-1}).

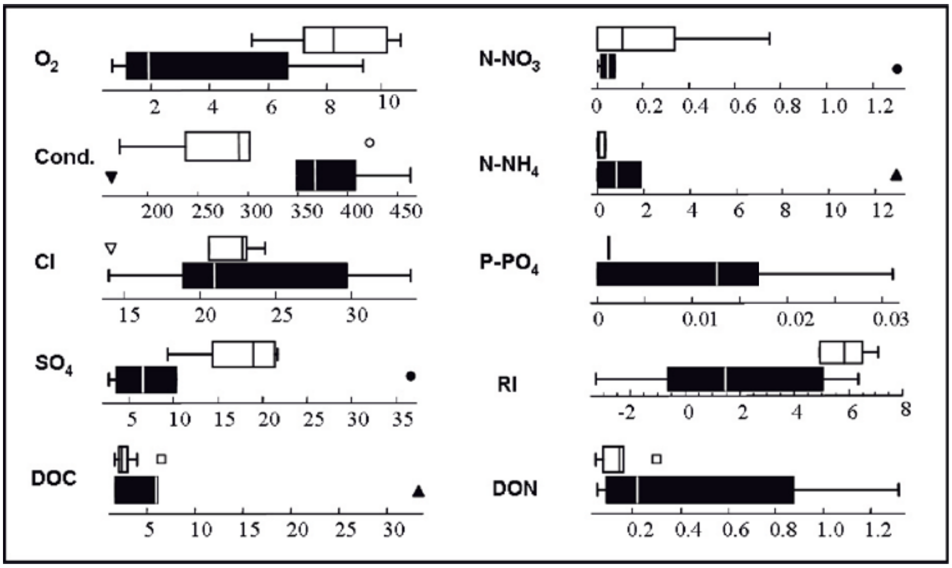


Figure 4.2. Box plots summarizing the different water chemical characteristics in summer and autumn expressed in mg·L-1, except conductivity in μS·cm-1. Black boxes stand for summer data and white boxes for autumn data. The different symbols for outliers indicate different sampling sites: (filled circle) gw, (filled triangle) site 2, (filled square) site 4, (filled inverted triangle) site 7.

Water Ec in summer is higher (from 349 to 463 $\mu S \cdot cm^{-1}$), than autumn (from 220 to 419 $\mu S \cdot cm^{-1}$. Chloride concentration presents a wider range of concentration during drought (from 14 to 33.8 $mg \cdot L^{-1}$), although there is no clear difference with the concentrations found in the autumn sampling (from 14 to 23.5 $mg \cdot L^{-1}$).

The nMDS analysis allows comparing the whole chemical variability of inorganic solutes among sampling sites (Figure 3). The graphical representation evidences the separation of sampling sites according to the season. In autumn, the chemical characteristics in water samples show low variability, placing most of the sampling sites close together. But, there are two exceptions: the riparian groundwater and the headwater spring (site 7) that are located far apart from the rest of sampling sites. On the other hand, during drought, the chemical variability among water parcels is much more evident. Then, points in the nMDS plot are widely dispersed. Site 2 is located in the upper extreme and sites 3 and 4 in the lower end. Remarkably, water chemical properties at the headwater sampling point (site 7) did not show any noticeable variability between seasons.

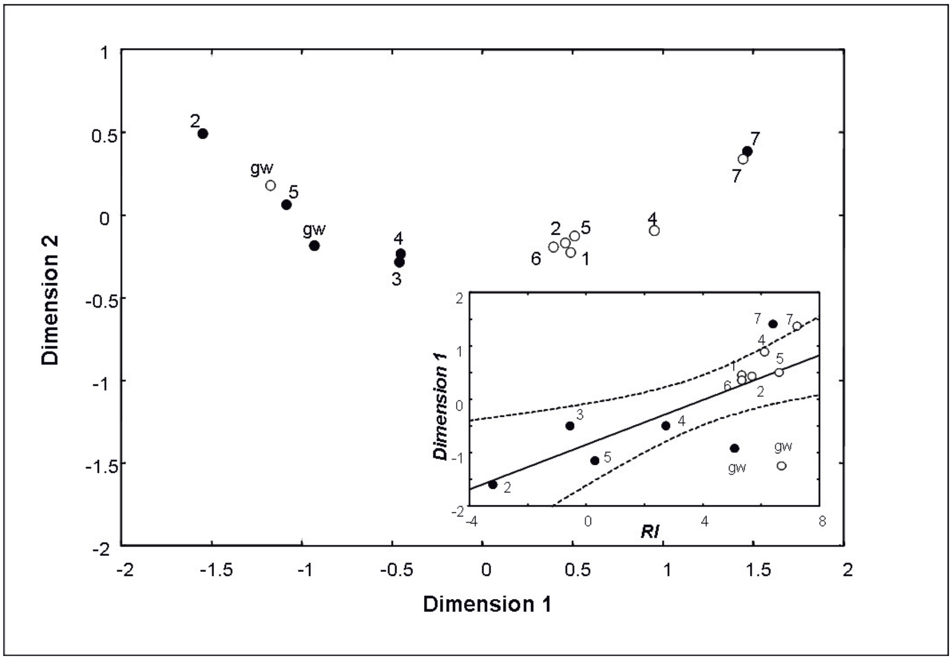


Figure 4.3. nMDS map of the distribution of sampling sites in both seasons (black: summer, white: autumn) according to its water chemical characteristics. The lower panel shows the correlation between the redox index (CI) and Dimension 1 (DIM 1) of the nMDS ($r = 0.68$, $p<0.01$).

Separation of the sampling sites along the dimension 1 axis is basically related to differences in ammonium and oxygen concentrations. In fact the dimension 1 values are positively correlated to the CI values ($r = 0.68$, $p<0.05$). This correlation is even higher when discarding groundwater samples ($r = 0.93$, $p<0.01$).

During drought, the CI estimates in sites 4 and 7 were high, in the same range as autumn samples. On the other hand, a steep decrease of the CI estimates was observed in sites were water was totally isolated (Figure 4).

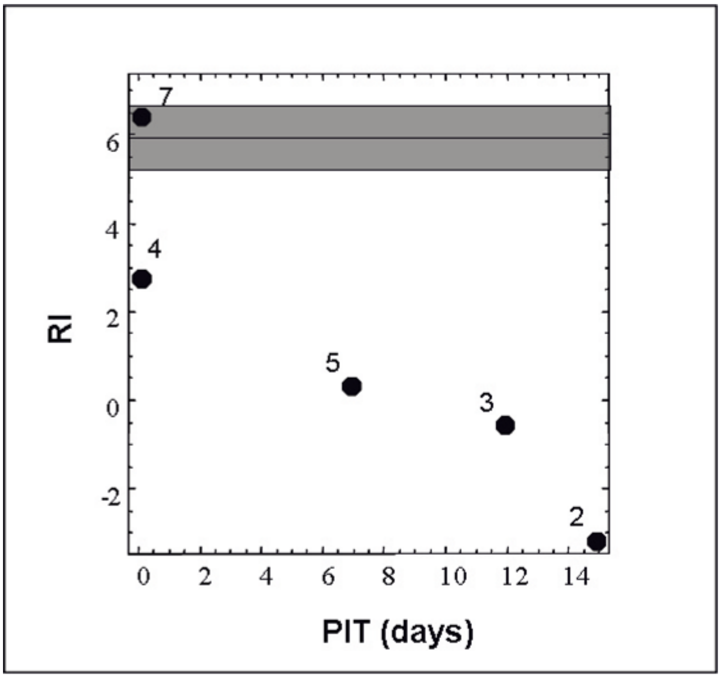


Figure 4.4. Relationship between PIT (Pond Isolation Time) and CI (Chemical Index) for summer surface waters sampling sites. The solid line in the gray band in the upper part is the CI mean value ± standard deviation for autumn samples. Numbers refer to the sampling site labels.

In more detail, the highest N-NH4 concentration was observed in the downstream site 2 (13 $mg \cdot L^{-1}$), while the lowest was estimated at the headwater site 7 (0.02 $mg \cdot L^{-1}$). The decrease in dissolved O_2 concentration is remarkable in the isolated water pools, with a minimum value of 0.5 $mg\ L^{-1}$ in site 2.

Differences between groundwater and surface water sampling sites are noticeable in summer. In groundwater, the dissolved O2 concentration is rather high compared to surface waters (6.5 mg·L^{-1}). Also, the concentrations of N-NO3 and sulphate are higher (1.31 mg·L^{-1} and 36.5 mg·L^{-1}, respectively) than in surface waters.

In autumn, the position in the nMDS graph of groundwater, apart from the surface water locations, is due to a relative high Ec value (419 µS·cm^{-1}) and low N-NO$_3$ and N-NH$_4$ concentrations (0.015 and 0.011 N mg·L^{-1}, respectively).

b) DOM availability and characterization

When comparing DOC and DON concentrations and DOM lability between hydrological periods, the same trend is observed: in summer, measured parameters present higher values and variability than in autumn. However, any DOM qualitative parameter is significantly related to DOC concentrations.

The concentration of DOC in summer isolated pools is 6 mg·L^{-1} in sites 3 and 5 and 33 mg·L^{-1} in site 2. DOC concentration in site 4 (running water) is 5 mg·L^{-1} and at the headwater (site 7) is much lower (1.8 mg·L^{-1}), similar to the concentration found in groundwater (1.7 mg·L^{-1}) and at the same site in autumn (2.8 mg·L^{-1}). In autumn, DOC concentration is generally lower and presents less spatial variability (mean 2.46 ± 0.24 mg·L^{-1}). Overall, DOC concentrations in summer water parcels are significantly and negatively related to the CI index with an exponential regression ($r=0.93$, d.f.=3, $p<0.05$).

DON concentrations significantly covaried with that of DOC in both seasons ($r=0.86$, d.f.=4, $p<0.05$ in summer; $r=0.76$, d.f.=5, $p<0.05$ in autumn). The highest concentrations are found is summer, the maximum corresponding to sites 2 (1.3 mg·L^{-1}) and 5 (0.87 mg·L^{-1}). Concentrations in the other sampling sites are in the same range of those measured in autumn (from 0.05 to 0.32 mg·L^{-1}). No significant relationships are observed between DON and CI.

DOC:DON ratios are variable and do not present any kind of trend in water pools nor running waters and its variability was unrelated to the CI (Figure 5a). During drought, the highest DOC:DON ratios (higher than 35) were estimated in the headwater spring (site 7) and in an isolated pool (site 3). The lowest ratio (6) corresponds to site 5. On the other hand, in autumn, the DOC:DON ratios were less variable and ranged from 24.5 (site 7) and 6.4 (groundwater).

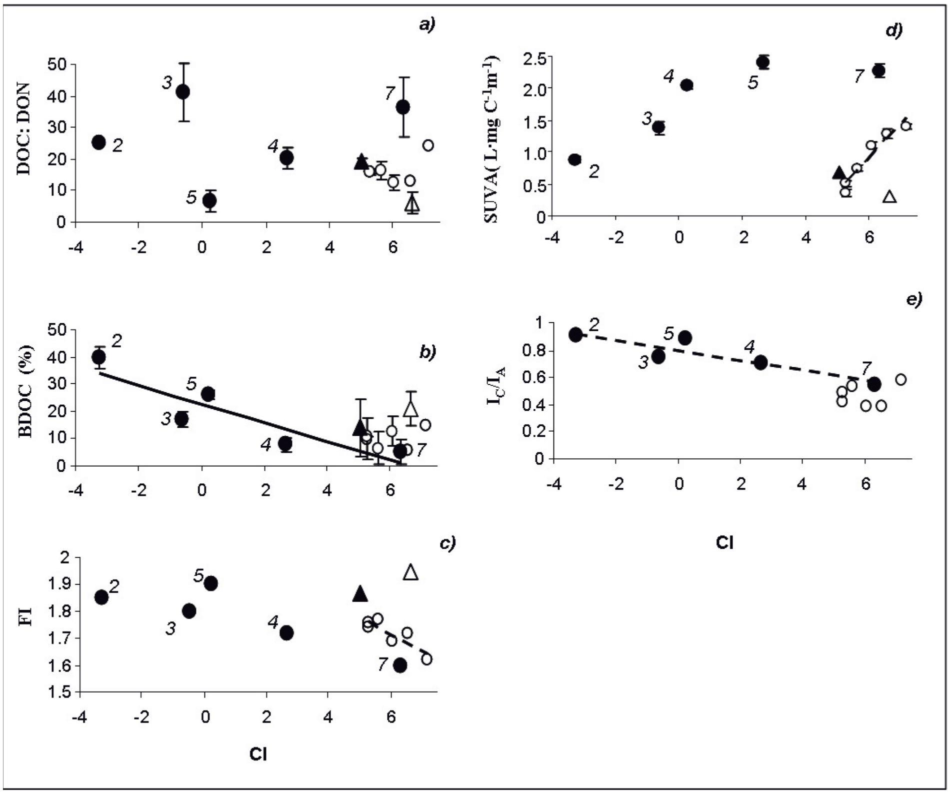


Figure 4.5. The figure shows the CI (Chemical Index) relationships with DOM qualitative parameters. Black dots correspond to summer and smaller white dots to autumn values. Triangles correspond to ground water (black for summer and white for autumn). Black dot numbers correspond to each sampling site: 2 (PIT = 15 days), 3 (PIT = 12 days) and 5 (PIT = 7 days) correspond to isolated water pools; Black dots numbered 4 and 7 correspond to running waters (PIT = 0).

The estimated biodegradable DOC (BDOC) is higher and much more variable in summer than autumn. Summer BDOC estimates are inversely related to CI ($r=0.84$, d.f=4, $p<0.05$) and positively related to DON ($r=0.92$, d.f.=4, $p<0.01$). Hence, higher BDOC content is found in isolated water pools (site 2, 39.6%; site 3, 16.9%; site 5, 26%) while in running waters (site 4, 7.7% and site 7, 5.31%) and ground water (14.17%) it is lower. On the other hand, in autumn there is no distinguishable trend in BDOC content. In surface waters it is uniformly low, ranging from 5.8% (site 5) to 21% (site 7).

The fluorescence index (FI) values are higher in summer than in autumn, although the range of variation is similar in both seasons. During drought, isolated water pools present FI values ranging from 1.8 (site 3) to 1.9 (site 5), showing an increase of the relevance of autochthonous DOM. On the other hand, water parcels with running waters (sites 4 and 7) present lower values, 1.72 and 1.60, respectively, indicating that allochthonous DOM might be contributing in a higher proportion of DOM than in the isolated water pools, although in both cases the FI values suggest an autochthnous DOM origin. During drought the FI index is not significantly related to CI (Figure 5c) (r=0.57, d.f=4, n.s). In autumn, FI values are lower, and similar in all surface water sampling points, ranging from 1.62 (site 7) to 1.76 (sites 1 and 2) denoting exhibiting similar values to those found in local soil leachates and previous studies in Fuirosos (Romaní et al, 2006). Also, its variability was unrelated to the CI index (Figure 5c). On the other hand, ground water FI values barely change among the two periods and their values, 1.87 in summer and 1.95 in autumn, indicate an autochthonous origin of DOM.

In summer, SUVA values ranged from 0.88 to 2.41 with highest values in running water sites 4 and

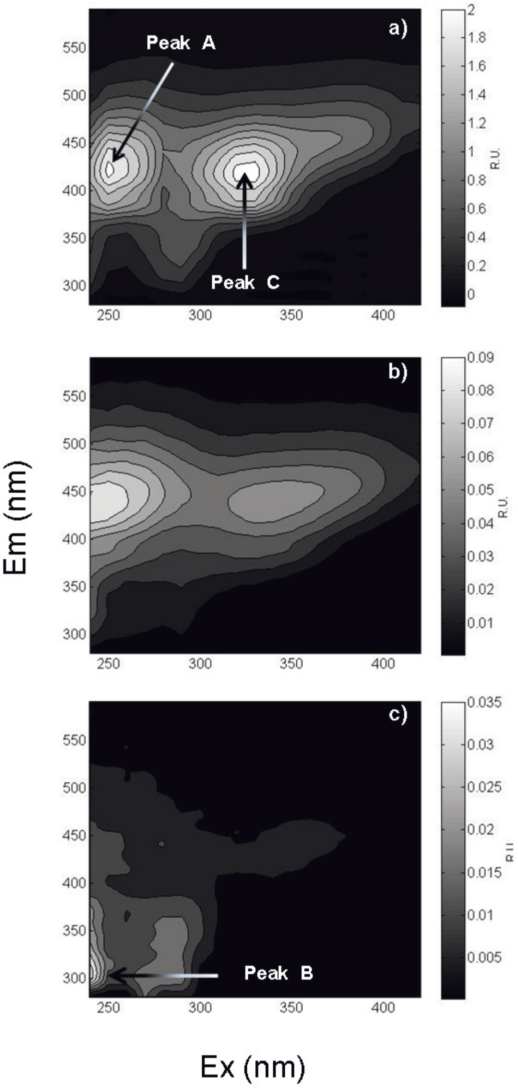


Figure 4.6. Example of the change in the I_C/I_A ratio from EEMs for site 2: a summer data; b autumn data; and groundwater: c summer data. Excitation and emission are in nm, andfluorescence is expressed in Raman Units (RU).

7 and lowest values in groundwater and sites 2 and 3. In autumn, these values are lower than in summer but there is some variability between sampling locations. Thus, DOM from 4, 5 and 7 shows a higher SUVA values (1.11, 1.29, 1.40, respectively), than that from sites 1, 2, 6 and groundwater (0.5, 0.75, 0.36, 0.68 respectively). SUVA tends to increase with respect to CI values. The relationship is only significant for the autumn data (r=0.96, d.f.=4, p<0.01, Figure 5d).

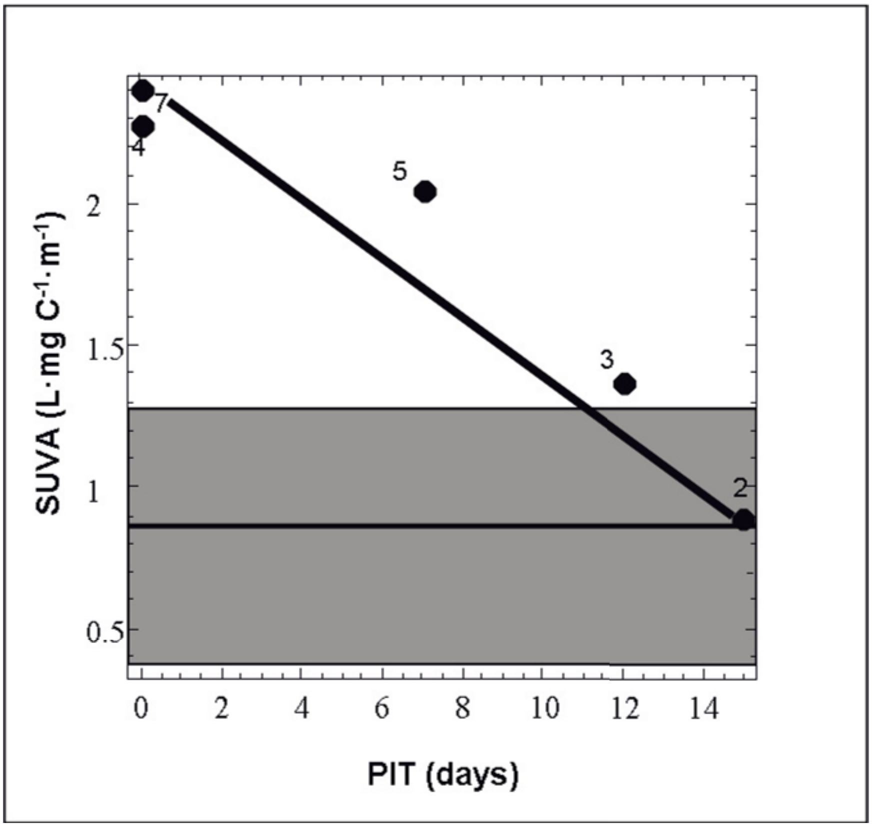


Figure 4.7. Relationship between PIT (Pond Isolation Time) and SUVA values for summer surface waters sampling sites. The solid line and the gray band at the lower part show the SUVA mean value ± standard deviation for autumn samples. Numbers refer to the sampling site labels.

The analysis of the magnitude of peaks C and A revealed that both are well-defined in surface water in summer, but in fall the peak C presents lower intensities. In consequence, the I_C/I_A ratio values are higher in summer than autumn. Furthermore, in summer the I_C/I_A values are significantly inversely related to the CI ($r=0.9$, d.f.=3, $p<0.05$) with maxima values (from 0.75 to 0.91) in isolated water pools (sites 2, 3 and 5) and minima (from 0.7 to 0.55) in running waters (sites 4 and 7) and groundwater. The change in fluorescence of peaks A and C between seasons for site 2 is shown in Figure 6. In autumn I_C/I_A ratio values are lower and less variables (from 0.39 to 0.63). No significant relationship was detected between autumnal I_C/I_A values and CI ($r=0.14$, d.f.=5, n.s.) (Figure 5e).

As previously shown in Figure 4, during drought period, in surface water ponds, the Pond Isolation Time (PIT) exerts a driving influence on the magnitude of the CI values. However, although significant relationships between CI and DOC, BDOC, SUVA and I_C/I_A were detected (Figure 5b, d and e), exclusively the SUVA showed a significant inverse relationship with PIT ($r=0.96$, d.f.=3, $p<0.01$, Figure 7).

4.4. DISCUSSION

The results from this study clearly evidence that drought exerts a relevant influence on DOM chemical properties. Drought causes a gradual hydrological fragmentation of the fluvial network enlarging the variability of DOM properties, and amplifying the biogeochemical diversity of a fluvial ecosystem. Then, the observed spectrum of DOM properties does not follow an arbitrary pattern. Sampling sites with lotic water bodies in summer show DOM properties similar to those observed in autumn under baseflow hydrological conditions and reflect the prevalence of terrestrial inputs from the surrounding forested hill slope. Meanwhile, in isolated and lentic water bodies, DOM analyses reveal a supplementary contribution of autochthonous organic matter, originated by in-situ microbial processes, as consequence of the disruption of the hydrological connection at the stream-catchment interface (Butturini et al., 2003).

As result, in Fuirosos, drought enlarges the ordinary range of variation of four DOM descriptors (DOC:DON, BDOC, $SUVA_{254}$ and I_C/I_A) that can be observed along the eight kilometres of stream continuum and the altitudinal range of 450 meters that comprises this study. Overall, these results coupled with those obtained by Jaffé and colleagues (2008) in North America in continental water bodies, highlight that local scale effects are much more relevant on DOM quality heterogeneity than the regional scale.

4.4.1. Changes in inorganic solutes water chemistry

Availability of organic and inorganic solutes in surface isolated water pools clearly differ from those observed during the wet period. By contrast, biogeochemical changes in headwaters (site 7) and groundwater among seasons are minimal.

The variability of Ec offers a good discrimination range between summer and autumn seasons due to the low discharge, and consequently an increase in the concentration of inorganic solutes, of the drought period. In isolated water pools, the absence of water transport enhances the accumulation of particulate organic matter and, along the increase in temperature, facilitating DOM leaching (DOC and DON increase) and increase in aerobic and anaerobic respirations. This increase in respiration causes a depletion of oxygen and nitrate and a steep decrease in sulphate concentration while the rate of ammonification processes increase as reflected by the high ammonium concentration. Furthermore, the establishment of more reduced environmental conditions probably favoured the phosphate desorption and its release into the water column (Bostrom et al., 1988).

4.4.2. Changes in DOM concentration and composition

DOC concentration in summer isolated pools presents the same range of variability observed in a previous study during a hydrological dry-wet transition period (Butturini et al., 2003). The re-establishment of stream runoff is coupled to DOC flushing attributable to leaching of abundant leaf and debris accumulated in the streambed during the previous drought period (Bernal 2005, Acuña 2005). In our study, as the autumn sampling was carried out after the hydrological transitional period, DOC concentrations were low, as expected during baseflow.

In Fuirosos catchment, DON concentrations are typically high at beginning of summer (before the drought) and during autumn (Bernal et al. 2005). This high DON concentration is usually attributed to the leaching of leaf litter in autumn, and the increase of in-stream primary production in summer (Bernal 2005). However, in our study, summer DON concentrations are slightly higher than those estimated by Bernal et al. (2005) during the early summer drying phase suggesting that in-stream DOM production might be relevant when drought intensifies. In isolated pools, the high concentration of DOM could lead to a rapid microbial growth, with high bacterial C production rates, enhancing C and N mineralization processes (Fazi et al., 2008).

Summer sampling sites with lotic waters (sites 4, below the reservoir; and 7, the headwater spring) show BDOC, FI and I_C/I_A values in the same range than those observed in autumn. By contrast, in the isolated water ponds (sites 2, 3 and 5) DOM properties are spread towards higher and more variable BDOC, FI and I_C/I_A values. DOC:DON ratio tends to be higher in summer but does not show any clear pattern, meanwhile the SUVA values constitute an interesting exception from the trend followed by the other parameters.

FI values estimated in this study ranged between 1.6 and 1.95. According to literature, FI values higher than 1.4 are considered to indicate DOM of autochthonous sources. Therefore, the FI values obtained in this study might indicate the prevalence of autochthonous DOM sources in both seasons (McKnight 2001). However, it is important to remark that FI values from Fuirosos soil leaching are typically around 1.6, suggesting that this soil leachate might integrate both vegetal and microbial DOM release from the terrestrial environment. Since DOM in fluvial systems will hardly be exclusively of allochthonous or autochthonous origin, the FI might be considered as an integrated measure of all DOM. Therefore, although in this study all samples show an autochthonous DOM origin, samples with FI values closer to 1.6 could indicate a greater relevance of allochthnous sources (all autumnal surface waters and summer sites 4 and 7) when comparing to higher values that would suggest a major contribution of autochthonous sources to the DOM pool. Furthermore, these high FI values are coupled to high BDOC and low SUVA values suggesting that in-situ DOM production might be rapidly assimilated. Nevertheless caution is required when relating directly FI with BDOC, since groundwater samples show both relatively low BDOC and high FI. Therefore, other factors unaccounted for in this study might influence BDOC variability.

Although fluorescent peaks A and C are usually associated with substances of terrestrial origin (Coble 1996), in isolated water pools there is a shift in the origin of DOM, from allochthonous to autochthonous, as confirmed by the FI. These results coupled with the increase in fluorescence in peak C, reflected by the I_C/I_A ratio, during the drought period suggests that 30-40% of this fluorescence might be caused by the contribution of organic substance derived from microbial activity and algae leachate. Under this perspective, these results partially agree with the findings of Stedmon and Markager (2005b) that observed that fluorescence of certain components determined by a PARAFAC model corresponding to peak C, increased as a result of microbial degradation of estuarine DOM of autochthonous origin. In the case of summer groundwater, while peak C presents low fluorescence, peak B (protein-like) is prominent. The presence of this type of fluorescence peak, along a high FI value, might indicate an increase of microbial degradation processes and more refractory subsequent DOM accumulation since ground water is disconnected from the stream surface. Previous studies (Vazquez et al., 2007) show that in the groundwater compartment most of DOC is of small molecular size (less than 1 KDa). Therefore, it might be expected that this molecules with protein-like fluorescence are of small molecular size contrasting with the hypothesis that this molecular size fraction is refractory (Amon & Benner 1996). It may indicate also that bioavailability of this molecular size fraction changes according to its origin and diagenetic state, as suggested by Kaiser et al (2004). Also, in Romaní et al. (2006) showed that FI values are higher in ground water than in surface waters, and that in small (<1 KDa) and large (>100 KDa) size fractions it was higher than in medium size fractions (1-10 KDa and 10-00 KDa).

According to a previous study, BDOC estimates in Fuirosos averaged 12% under basal discharge conditions during the rewetting period (September-October) but increased up to 40% during the first severe autumnal storm event (up to 2000 Ls-1) in the same period (Romaní et al., 2006). In the present study, in spite of the difference in the hydrological context, the same range of variation is found. These estimates could suggest that BDOC has an upper threshold of nearly 40% that can be reached under two hydrological conditions of opposite nature: droughts and storms. DOC:DON ratio is recognized an important driver in the DOM bioavailability and an inverse relationship between BDOC and DOC:DON should be expected (Fellman et al, 2008). In summer the DOC:DON ratio shows a notable and erratic variability. Overall, these values are slightly lower than those reported in Fuirosos during the hydrological transition by Bernal et al. (2005). In any case, DOC:DON ratio in summer typically duplicates the ratios observed in autumn. Then, we should expect lower DOM bioavailability in summer. Surprisingly, the results show that BDOC is unrelated to the DOC:DON ratio but is strongly positively related to DON. Under severe drought conditions, in presence of high DOM availability, absolute DON concentration, when measuring bioavailability, is much more relevant than the DOC:DON ratio. Interestingly, this result contradicts the Hedin et al (1995) hypothesis suggesting that DON may be unavailable to stream microbiota because it is composed of refractory fulvic acids derived from soil, and agrees with the findings of Stepanauskas et al. (1999) that suggested that DON bioavailability increases in summer when nitrate concentrations in rivers decrease.

The SUVA index can be used as a proxy for aromaticity since both parameters have been found to be strongly correlated (Weishaar 2003), enhancing it usefulness in DOM characterization. In our study, the SUVA index is the only parameter that shows a positive relationship, although not significant, with CI. Moreover, considering exclusively the summer isolated water pools, it is the only parameter that is statistically related to PIT. SUVA estimates during drought are similar to those estimated in autumn, but the lotic water sampling sites (sites 4 and 7) clearly show a higher aromaticity content. Hood (2006) and Vidon (2008) showed that during storm episodes, DOM inputs from the near surface soil organic layer presented higher SUVA values. But, in this study, the lack of rain episodes from May to July prevent from asserting that there is such a DOM input from the hillslope forested soil at these sampling sites. On the other hand, SUVA autumnal values are generally lower than those measured in summer at sites 4 and 7. Hence, autumnal terrestrial input of new DOM in headwaters is not necessarily highly aromatic. Therefore, high values at lotic sites (4 and 7) during summer might be caused by an accumulation of aromatic and recalcitrant substances in the persisting water mass that still flows as the fluvial network becomes fragmented. On the other hand, the fragmentation of the surface water continuum into small isolated water parcels reverses this increase in SUVA and the contribution of aromatic DOM declines proportionally to the pond formation elapsed time (PIT) (Figure 7), reflecting the increase contribution of in situ (algal and microbial) DOM production as suggested by FI and IC/IA descriptors. Also it is worth considering that laboratory experiments revealed that photodegradation processes might affect DOM composition (Rodriguez-Zuñiga et al., 2008) reflected in lower UV-absorbance and fluorescence. However, the studied water parcels are located in shadowed plots of the stream channel along the thalweg and summer direct sunlight exposure during summer is strongly reduced by vegetation or/and by large boulders and rocks. Hence, it is not expected that photodegradation play an important role on the processing of dissolved organic matter in our study site. This result evidences that for the interpretation of the SUVA index values is not enough to divide samples into two rough categories (drought and wet seasons or lotic and lentic water bodies) but it is indispensable to know the historical hydrological trajectory of each sampled water body along the fluvial network.

5. Conclusions and implications

In order to improve our knowledge on DOM origin, transformations and lability optical measurements constitute a valuable tool (Hood et al., 2003; Weishaar et al. 2003; Stedmon and Markager, 2005; McDowell at al., 2006; Stubbins et al., 2008). Thus, the integration of the spectroscopic methods with detailed hydro-biogeochemical monitoring during extreme, and opposite, hydrological conditions (storms and droughts) provides an excellent challenge to capture a more complete perspective on heterogeneity of DOM composition (Hood et al., 2006; Vidon et al., 2008).

It is well recognized by geomorphologists that the fluvial network is a dynamic structure (Bertoldi et al., 2009). Its expansion and shrinking is determined by the temporal concatenation of erratic storm episodes and seasonal drought periods. In Mediterranean streams, both opposite hydrological states are the most relevant drivers of DOM variability. But, while drought affects DOM variability along a spatial axis, its variability on a temporal axis is more evident during storms that are capable of generating a wide spectrum of DOM-discharge loops (Butturini et al., 2008).

Surprisingly, although the processes occurring in the fluvial network are considered dynamic, changes in its spatial dimensions and discharge fluctuations are not really integrated in whole-system biogeochemical conceptual analyses (Vannotte at al. 1980, Battin et al., 2008). Thus, the fluvial network appears to be a rigid structure, hydrologically disconnected from the catchment. In consequence, the recognition of the fluctuating nature of the fluvial network will greatly encourage the study of fluxes and transformation of organic and inorganic solutes under temporal and spatial hydrologically variable conditions. Therefore, the detailed variability analysis of quantitative and qualitative of DOM parameters will strongly benefit from the implementation of high resolution-long term temporal monitoring programmes (Kirchner et al., 2004) that capture the succession of those extreme hydrological hot moments (McClain, 2004) that prompt the oscillating hydrological features of a fluvial ecosystem.

Acknowledgements

We thank the reviewers for strongly improving the earlier version of the manuscript.

This study was funded by project CGL2007-0144/HID from the Ministerio de Ciencia e Innovación. Stefano Fazi and Stefano Amalfitano participation was supported by project CSIC-CNR ref 2006IT0010. Andrea Butturini and Eusebi Vazques are members of the GRACCIE consortium.

Chapter 5

Fluorescence spectroscopy and UV-vis absorbance as tools for DOM characterization

Vazquez E[1]., Ejarque E[1]., Butturini A[1]. Fluorescence spectroscopy and UV-vis absorbance as tools for DOM caracterization

[1]Departament d'Ecologia, Universitat de Barcelona, Diagonal 643, 08028, Barcelona.

ABSTRACT

This review is written with the intention to provide an overview of the spectroscopic techniques applied to the characterization of dissolved organic matter. As an overview it does not provide an in-depth revision of the presented techniques, but offers a starting point for the uninitiated. It also strives to present not only the usefulness but also the caveats and different concerns that may arise when applying these techniques.

The review is divided in two parts: the first one covers UV-vis spectroscopy and the second the analysis of dissolved organic matter by means of fluorescence spectrometry.

5.1. Introduction

Dissolved organic matter (DOM) is a large and ubiquitous organic carbon reservoir in aquatic systems. It plays a major role in many ecological processes from food webs and structure of microbial communities (Sobczak & Findlay, 2002) to light attenuation (Kirk, 1994), nutrient retention/release (Findlay and Sinsabaugh, 2003) and pollutant transport (McDonald et al., 2004). Therefore, knowledge on the composition and lability of DOM is of major importance and it has become recurrent topic of scientific research.

The picture of DOM composition in natural systems is still incomplete at the compound level. The main quantitative measure for estimating DOM is dissolved organic carbon (DOC) and both terms are often used interchangeably (McDonald, 2004).

DOM is referred to by many acronyms, usually depending on which characterization methods are employed, and so does its classification. For an in-depth review on DOM acronyms and its use in the current scientific literature refer to Filella (2009).

From a physical perspective, DOM is usually defined as the group of organic compounds that go through a 0.7 μm (Findlay and Sinsabaugh, 2003) although other researchers set the cut off at 0.45 μm (Thurman et al., 1985). Therefore, DOM can also be further analyzed into smaller size fractions. For instance: it could be separated into discrete range fractions by means of tangential flow filtration techniques or studied in a continuous size range applying HPLC techniques. From a chemical perspective, DOM can be divided in two major groups of substances: humic and non-humic compounds. The non-humic fraction is comprised of lipids, sugars (mono and polysaccharides), amino acids, proteins, and carbohydrates. On the other hand, humic substances comprise a complex mix of natural organic substances, generally, chemically uncharacterized. Additionally, humic substances can be further classified in three groups: humins, substances that are not soluble in water, humic acids that become not soluble at pH lower than 2 and fulvic acids that are soluble under any pH. Humic substances are considered to be the main contributors to the DOM pool (around 75%), the fulvic acids alone contributing between 45-65% of DOM, although depending on the study system and/or DOM origin this contribution can increase further (i.e. up to 80-90% in wetlands) or decrease steeply (10-30% in DOM of autochthonous origin) (McKnight et al, 2003). Further chemical fractionation can be obtained by using resins, mainly

XAD-8 or its substitute, DAX-8, and eluting the aqueous sample using NaOH and HCl, separating the sample into hydrophilic and hydrophobic acids, bases and neutrals (Filella 2008, Leenheer 1981).

From a biological perspective, another DOM classification can be established according to its lability across a labile-refractory continuum, with the estimation of biodegradable DOC (BDOC) as the main defining parameter. BDOC is considered the DOC fraction consumed by heterotrophic microorganisms measured over a specific time lapse which depends on the incubation method employed.

There is a wide range of analytical techniques employed to characterize DOM, which are occasionally applied simultaneously in order to approach DOM from different perspectives: size exclusion chromatography, nuclear magnetic resonance spectroscopy or mass spectrometry methods among others, but some require the great time or resources investments (McDonald et al., 2004). On the other hand, in recent years the technological development of spectrophotometric instruments allowed an increase of their application on DOM analysis since they are faster, reliable and require little to no sample manipulation previous to analysis in the laboratory (Fuentes et al., 2006; Coble, 2007; Hudson et al., 2007). As a setback, spectrophotometric techniques only allow the characterization of the DOM fraction that has the capacity of absorbing light in the visible and near UV range. This DOM fraction is generally called chromophoric DOM (CDOM) but also is referred as gelbstoff or gilvin (Kirk, 1994). CDOM also presents fluorescent properties (Green and Blough, 1994; Coble 2007; Hudson et al., 2007; Birdwell 2010). Therefore, from the DOM pool that is quantified as DOC, only a fraction can be characterized using these techniques since DOC also comprises uncoloured and non fluorescent compounds.

These spectroscopic techniques have been applied in the characterization of DOM from all aquatic systems, from isolated humic substances (Chin et al., 1994), marine and coastal waters (Coble et al., 1996; Stedmon et al., 2003; Jaffé et al. 2008; Romera-Castillo 2009), and to freshwaters (including ground and rain waters) (McKnight 2001, Katsuyama 2002, Hood et al., 2006; Vidon et al., 2008). Also these techniques have been applied to the monitoring of sewage and industrial water treatment (Baker et al., 2004; Fabbricino et al., 2004; Saadi et al., 2006; Hudson et al., 2007). This wide range of applications has caused an important increase of the number of published research articles wherein spectroscopic techniques are modified, better implemented and the relationships among different characterization and environmental parameters examined thoroughly. However, the lack of a standard protocol causes that the newcomer to spectroscopic DOM characterization may have to conduct a thorough bibliographical research to understand the background and requirements of these methods while examining at the same time the scope of their application. It is the aim of this text to review and provide an introduction to the spectroscopic methods applied to the study of DOM in recent years, pointing to some methodological issues likely to appear during the first steps, and to exemplify some of its applications. However, its aim is not to propose a standard method to any of the methods herein.

5.2. UV-Vis absorbance spectroscopy

Table 1 shows many other absorbance wavelengths that have been used in different studies. Generally, the use of a specific wavelength is based on historical reasons in order to allow comparisons between studies and building on previous knowledge. However, the background basis for its application is seldom explained or referenced. Thus, the indistinct use of different, although close, wavelengths in exploring DOM characteristics is usual.

DOM absorbance data is usually measured in one of two ways: either at a single wavelength or obtaining an absorbance spectrum over a wide wavelength range, spanning from UV to visible light.

Generally, absorbance spectra of DOM increase exponentially in shorter wavelengths and present no discernible peaks nor shoulders, except in the case of fluvial waters where shoulders have been consistently found (Ejarque, unpublished data). Since DOM is a complex mixture of substances, these featureless spectra are produced by the overlapping of spectra of single compounds (Pouet et al., 2007).

When working with UV-vis absorbance there are different units that can be used when presenting the data that can hinder the direct comparison between different studies. Absorbance, D, is dimensionless, and is usually presented as the absorption coefficient, in decadic form (α, or sometimes A) when the absorbance value is corrected by the cuvette path length (L) (α [m^{-1}] $=D/L$) or in the Napieran coefficient form (a) where $a=2.303*D/L$ (a [m^{-1}] $= \alpha * \ln 10$). On occasions the usage of the units in the literature is confusing since it is not explained which coefficient it is being applied or the decadic coefficient is presented as a direct absorbance measure (Hu et al., 2002; i.e. in Doane and Horwáth 2010). Another concept often used is absorptivity (ε) expressed as the decadic absorption coefficient corrected to DOC concentration (L·mol^{-1}·m^{-1}) (Chin et al. 1994). Its use, under this denomination, is not recommended since absorptivity is defined as the absorptance (the ratio of the radiation absorbed by a surface to that incident upon it)corrected by the cuvette length path and it should not be interchangeable with the sense used in Chin et al and others (Hu et al., 2002). Although the terminology appearing in scientific articles might be confusing terminology in general terms, as long as the measures are explained and are consistent within the discourse, they are equally valid, only hindering the comparison between studies (Hu et al. 2002).

A widespread parameter used in DOM characterization is the specific UV absorbance (SUVA). The specific absorbance at a certain wavelength is expressed as the decadal absorption coefficient in cm^{-1} corrected by DOC concentration in mgC·L^{-1} (Weishaar et al., 2003). Therefore SUVA is expressed in L·mgC^{-1}·cm^{-1}. But in many studies, SUVA is expressed using the Napieran absorption coefficient (as examples: Battin, 1998; Helms et al., 2008; Lapierre et al., 2009). Again, on occasions the way specific absorbance is expressed is not explained forthright, and the calculation can only be assumed from the units presented. For instance, when absorbance is expressed in m^{-1}, it may be assumed that specific absorbance is calculated from the decadal absorption coefficient and that specific absorbance has not been obtained from the Napieran coefficient (i.e. Balcarczyk et al., 2009; Carstea et al., 2009). In other studies, specific absorbance is reported in L·gC^{-1}·cm^{-1} (E), which is equivalent to ten-fold SUVA value (De Haan et al., 1987; Peuravuori et al., 1997; Thacker et al., 2008; Tipping et al., 2009).

5.2.1. UV spectroscopy and DOM aromaticity

The absorbances at 254 and 280 nm are selected for being in the wavelength range where many organic substances, especially precursors of humic substances, present non saturated bonds with pi-pi* electron transitions (prohibited). Therefore, absorbance values in these wavelengths provide information on the degree of humification, aromaticity and molecular weight and are usually expressed as specific absorbance calculated either from molar absorptivity (ε) (Chin et al., 1994) or from the decadal absorption coefficient ($SUVA_{254}$ or $SUVA_{280}$) (Weishaar et al., 2003). This indistinct use of molar absorptivity and absorption coefficients (either decadal or Napieran) can difficult the comparison of data from different studies. Weishaar et al (2003) reported a strong correlation between $SUVA_{254}$ and

Table 5.1. List of wavelengths used to characterize DOM

Wavelength (nm)	Related properties	Bibliographical references
250	aromaticity, apparent molecular weight	Peuravuori and Pihlaja (1997)
254	aromaticity	Hur et al. (2003); Weishaar et al. (2003); Hood et al. (2006)
260	hydrophobic C content	Dilling and Kaiser (2002)
265	relative abundance of functional groups	Chen et al. (2002)
280	hydrophobic C content, humification index, molecular size, aromaticity	Chin et al. (1994); Korshin et al. (1999); Kalbitz et al. (2003)
285	humification	Kalbitz (2000)
300	characterization of humic substances	Artinger et al. (2000)
320	photodegradation	Piccini 2009
340	color, alumina adsorption	Baker et al. (2002); Thacker et al. (2005)
350	apparent molecular size,characterization of humic substances	Korshin et al. (1999); Kowalczuk et al. (2003); Murphy et al. (2008)
355	characterization of humic substances	Guéguen et al. (2005)
375	humic substances characterization, correlated to TOC	Lapierre et al. (2009); Stedmon et al. (2000)
400	humic substances characterization, sVISa	Artinger et al. (2000); Vogt 2004
410	fulvic-like material characterization	Baker et al. (2002)
420	sVISa	Temnerud et al. (2009)
436	quality indicator	Abbt-Braun and Frimmel (1999)
440	CDOM concentration, humic substances	Balogh et al. (2003); Kirk (1994)
465	relative abundance of functional groups	Chen et al. (2002)

Index		
E2/E3 (*a* 250/*a* 365)	aromaticity, molecular weight, photodegradation	Peuravuori et al.(1997); Minero et al., (2007); Wang et al., (2009)
E3/E4 (E300/E400)	aromaticity, humification, molecular weight	Artinger et al., (2000)
E4/E6 (*a* 465/*a* 665)	aromaticity (low to non existant correlation in freshwaters)	Chin et al. (1994); Peuravuori et al. (1997); Fuentes et al. (2006)
SAR_{VIS} (*a* 400/*a* 600)	aromaticity, molecular weight	Vogt et al. (2004)
SAR_{UV} (*a* 254/*a* 400)	aromaticity, molecular weight	Vogt et al. (2004); Temnerud et al. (2009)

aromaticity content as determined by 13C-NMR, and consequently SUVA has been thereafter used as a surrogate aromaticity measure.

It has been determined that $SUVA_{254}$ values decrease due to photolysis (Moran and Zepp, 1997; Wang et al., 2009). In freshwater systems, DOM of autochthonous origin presents lower values than that of allochthonous/terrestrial origin (McKnight et al., 2001). Additionally, on-line UV and DOC measurements demonstrate that $SUVA_{254}$ values tend to increase with increasing apparent molecular weight (AMW) (Peuravuori & Pihlaja, 1997; Korshin et al., 2009).

The reason for choosing $_{254}$ nm or 280 nm since they provide the same information is that nitrate (NO3-) and ferrous iron (Fe3+) absorb in this wavelength range (from 200 to 400 nm as reported in Doane and Horwáth 2010), but the interference effect is lower when measuring at 280 nm (Mladenov et al., 2007 and 2008; Chin 1994 and 1998; Hur et al., 2009; Kalbitz et al., 2003). In samples with high DOC the overestimation of absorbance due to nitrate and iron might not be problematic, but it can be in samples with low DOC concentration. In natural waters nitrate concentration is usually low enough to not affect absorbance at $_{254}$ nm, and an increase in absorbance of 0.01 requires a concentration above 100 $mg\cdot l^{-1}$ of nitrate (Weishaar et al., 2003). However, in the case of ground water and waste waters, nitrate concentration may be higher and, therefore, it should be considered when making absorbance measurements. Ferrous iron presents higher absorbance and it can interfere at concentrations higher than 0.5 $mg\cdot l^{-1}$ (Weishaar et al., 2003). In order to remove its influence, in Doane and Hórwath (2010) a method is proposed using hydroxylamine which has been tested in the range of 200 to 400 nm. Additionally, sample dilution might be helpful in avoiding inner filter effects due to high DOC concentrations (Jaffé et al., 2008). Both measures, $SUVA_{254}$ and SUVA280 are used in many studies depending on which criteria are applied, and even in some cases they are used simultaneously (Wang et al., 2009).

Additionally, absorbance at 260 nm was used to demonstrate that aromatic compounds are found in the DOM hydrophobic fraction as shown by XAD-8 extraction procedure (Dilling and Kaiser. 2002). Therefore, DOM quantification using these UV absorbance is not possible if there are changes in the hydrophylic:hydrophobic composition of DOM. Similarly to UV absorption at $_{254}$ and 280 nm, iron and nitrate can interfere with measurement and Dilling and Kaiser (2002) proposed upper limits of nitrate and iron concentration of 25 $mg\cdot L^{-}$

^{1} and 5 mg·L^{-1}. SUVA$_{260}$ was used to characterize DOC in phytoplankton blooms detecting an increase in absorbance after the blooms (Suksomjit et al., 2009).

SUVA has been used to examine changes in DOM properties along temporal and spatial scales. Also, it has been applied to study DOM sources according to varying hydrological conditions. SUVA$_{254}$ usually increases during the rising limb of the hydrograph during storm episodes (Fellman et al., 2009b), and similarly during snowmelt (Hood et al., 2003), while during warmer seasons it decreases (Hood et al., 2006). Furthermore, SUVA has been also applied in soil solution discriminating DOM from four different soil types (Fellman et al., 2008), but with varying temporal trends in each of them (Fellman et al., 2009b). Additionally, during precipitation events, SUVA$_{254}$ is not only used as a DOM descriptor but also as a hydrologic tracer. Hood et al. (2006) reported increases in SUVA$_{254}$ ranging from 9% to 36% in three distinct watersheds following closely discharge dynamics under baseflow conditions. This increase in SUVA$_{254}$ is explained by DOC mobilization from the near surface soil which is highly aromatic, while decreasing SUVA$_{254}$ values observed during baseflow would be explained by the drainage of the mineral soil, poorer in aromatic compounds (McGlynn and McDonnell, 2003; Hood et al., 2006; Vidon et al., 2008).

There are also evidences that SUVA is related to DOM reactivity. Different studies, covering field and laboratory approaches, concluded that SUVA$_{254}$ presents a significant negative correlation with biodegradable DOC (BDOC) (i.e. Kalbitz et al. 2003; Saadi et al., 2006; Fellman et al., 2008)

5.2.2. More wavelengths/Additional information

In the case of absorbance at 300 nm (a300), it has been used because of its good reproducibility and its potential to discriminate between fulvic and humics acids when used coupled to other measurements like the ratio between specific absorbance at 300 and at 400 nm, also known as E3/E4 ratio (Artinger et al., 2000). Additionally, a300 presents a positive relationship with DOC concentration and its slope has been used to discriminate between fresh water masses (deep soil, shallow soil and stream waters), and also allowing the quantification of the non-absorbing DOM fraction (Cory et al., 2004). Finally its use also responds to the possibility of comparison between studies. Furthermore, this wavelength has been used in the characterization of DOM in longitudinal patterns of fluvial systems and as tracers of hydrological changes, presenting a relationship with water level and increases due to inputs from close to the main channel water bodies (Battin et al., 1998).

Considering longer wavelengths, it has been shown that light absorption from 300 to 355 nm is related to DOM of terrestrial origin (Hernes and Benner, 2003). Therefore many studies have used absorbance at wavelengths included in this range in order to characterize DOM, especially 340 and 350-355 nm (Baker and Spencer, 2004; Guéguen et al., 2005, Bracchini et al., 2010). Additionally, the decadal absorbance coefficient at 320 nm (A320) has been also used to characterize the effects of UV-B and UV-A radiation on DOM photoalteration in lagoon waters (Piccini et al., 2009). In fluvial systems, A340 has been used in the characterization of DOM in sewage waters and peat dominated catchments (Baker et al., 2002, 2004, respectively), and it is positively correlated to DOC concentration (Baker et al., 2004). Furthermore, the Napieran absorbance coefficient (a340) is related to alumina DOC adsorption and it is negatively correlated to the hydrophilic DOC fraction (Thacker et al., 2005). The a350 has been used in photoirradiation studies presenting, in swamp, lake and stream water samples, an exponential decrease when photoirradiated under laboratory conditions using a sun simulator (Obernosterer and Benner, 2004). The high variability presented by a350 allowed exploring CDOM quantitative changes in estuaries and the influence of the river plume (Kowalczuk et al., 2003).

The measure at a375 nm is used as a proxy for CDOM concentration and Stedmon et al. (2000) reported additionally, it was positively correlated to the total organic carbon (TOC) in marine waters. On the other hand, it has been used also in studying the contribution of macrophytes to the DOM pool in lake waters (Lapierre and Frenett, 2009) and to characterize DOM in a gradient from coastal to open sea waters (Murphy et al., 2008) in the same way as a350 (Kowalczuk et al., 2003)

Besides the specific UV absorbance used in many studies ($SUVA_{254}$ or SUVA280), there is also the specific visible absorbance or sVISa. The combination of specific absorbance in the visible and UV range was used by Frimmel and Abt-Braun (1999) using a_{254} nm and a436 nm in DOM characterization. Subsequent studies selected different wavelengths in the visible range: a400 (Vogt et al., 2004) and a420 (Temnerud et al., 2009). Nevertheless, the most used wavelength, mainly applied in oceanic research, is 440 nm (Kirk, 1994) as it is positively correlated to CDOM concentration. One of its main applications is remote sensing in oceans (Kirk 1994, Coble 2007). Additionally a440 is positively correlated to the humic acids fraction of DOM and to molecular weight (Balogh et al., 2003).

In aquatic systems, absorbance values have been found to be correlated to DOC concentration. However, this relationship is not constant and varies depending on environmental factors. For instance, Del Vecchio and Blough (2004) found a decrease in the absorbance:DOC ratio due to the effects of photobleaching on CDOM and differences in DOC and CDOM content in the marine and freshwater end-members.

5.2.3. Absorbance ratios

Many studies using spectroscopic techniques do not use absorbance at single wavelengths exclusively. Instead, ratios between different wavelength absorbance have been applied as they offer different insights on DOM properties (Kukkonen et al., 1992; Chin et al., 1994; Artinger et al., 2000).

The E2/E3 ratio, calculated by dividing the specific absorbance at 250 nm by that at 365nm, has been widely used to characterize DOM in freshwater systems (Peuravuori and Pihlaja, 1997; Minero et al., 2007; Wang et al., 2009; Leeben et al., 2010; Scapini et al., 2010). This ratio is inversely correlated with aromaticity, and therefore with molar absorptivity (ε) at 280 nm, and molecular weight of aquatic humic substances (Peuravuori and Pihlaja, 1997; McDonald, 2004). When using this ratio pH has to be considered since at low pH it presents higher values (Hautala et al., 2000). The correlation with molecular weight is independent of DOM origin and irradiation conditions. Thus it is possible to study the effects of photoirradiation on DOM MW using the E2/E3 ratio (Lou et al., 2006).

The E3/E4 ratio is calculated from the specific absorption ($L \cdot g^{-1} \cdot cm^{-1}$) at 300 (E3) and 400 nm (E4). A decrease in this ratio, accompanied by an increase in the specific absorption of E3 indicates an increase in humification, aromaticity and MW of humic substances (Artinger et al. 2000). This ratio offers similar information as E2/E3 but it is more rarely present in the current literature.

The E4/E6 ratio (the ratio of absorbance at 465 and 665 nm) is correlated with aromaticity. It is especially used in soil characterization, but rarely in aquatic systems since the correlation is very weak depending on the sample (Chin et al., 1994) or non-existant (Peuravuori and Pihlaja, 1997; Fuentes et al., 2006).

The Specific Absorbance Ratios for UV (SARUV) and visible light (SARVIS) correspond to ratios a_{254}/a400 and a400/a600, respectively (Vogt et al., 2004). Both ratios provide similar information since they are strongly correlated. Low SAR values indicate high molecular weight and a low degree of aromaticity and it correlates negatively with SUVA (Temnerud et al., 2009).

5.2.4. Spectral Slope

The absorbance spectra of DOM absorbing visible light (Chromophoric DOM or CDOM) can be fit to a simple exponential model: $a(\lambda)= a_0 \cdot eS(\lambda_0 - \lambda)$ where a0 is the Napieran absorption coefficient at a reference wavelength and a(λ), the absorption coefficient at wavelength λ (Bricaud et al., 1981; Green and Blough, 1994; Helms et al. 2008).

The exponential spectral slope (S) measures how the absorbance decreases as wavelength increases, and it has been used extensively in the characterization of CDOM. The shape of the absorbance spectra does not depend on DOM concentration, but on changes in composition due to photobleaching, presence of multiple DOM sources, bacterial degradation or removal of DOM by physical processes, (Stedmon et al., 2000; Twardowski et al., 2004; Bracchini et al., 2010). Therefore, the spectral slope reflects bulk variations on CDOM properties, being useful to discern CDOM changes across spatial and temporal scales, although it will not provide specific information on the factors that account for the variations unless it is coupled to other measures. Conversely, knowing S is needed to trace DOM absorbance when using remote sensing (Coble et al 2007).

As in the case of single wavelength absorbance methods, data provided by the spectral slope presents its own inconveniences hindering comparison between studies. The main reason is that S values can differ widely according to: (a) the method of calculation and (b) the selected wavelength range (Del Castillo et al., 1999; Twardowski et al., 2004; Helms et al., 2008). Furthermore, including short wavelengths (into the ultra violet range) results in steep increases in absorbance values that might alter the slope values.

The more immediate method of S estimation from the linear regression of the absorption coefficient vs. wavelength in natural logarithmic scale was criticized by Markager and Vincent (2000) because the logarithmic transformation implies that absorption coefficient values at longer wavelengths will gain additional weight. Stedmon et al., (2000) addressed this concern by comparing three different fitting methods to estimate S: (1) linear regression of logarithmic absorption coefficient vs. wavelength; (2) non-linear regression fitting; (3) non-linear regression fitting adding a background constant K ($a(\lambda)= a_0 \cdot e^{S(\lambda 0- \lambda)} + K$) . This third method was originally proposed by Markager and Vincent (2000), where K was estimated from light extinction coefficient in lake waters and accounts for light absorbance caused by factors other than CDOM. This method provided the best fit and reduced considerably the sum of residual values and standard deviation. On the other hand, S calculated using the equation with the background constant was 47% higher than S calculated without this parameter in Markarger and Vincent (2000), and only 13% in Stedmon et al (2000). Twardowski et al. (2004) proposed a hyperbolic model as best fit for absorbance spectra. Both methods showed better fits than those obtained with a linear-logarithmic regression.

On the second concern, Loiselle et al. (2009) studied the variability of S according to the selected wavelength interval, concluding that longer intervals (20-50 nm) presented a higher signal to noise ratio than using short intervals. Also on this issue, Helms et al., (2008) proposed measuring the slope in the interval 275-295 nm as it is related to changes in molecular weight and photochemical induced changes. Furthermore, research from Helms et al. (2008) proposed a spectral slope ratio (SR) between the slope in the 275-295 nm and 300-350 nm as a method to increase reproducibility allowing the comparison of data among studies, even in different systems. The slope ratios were calculated by linear regression of log-transformed data, but a data subset on both linear and non-linear methods were applied and showed that variation in fit between methods was 1%. This SR presents a positive relationship with photobleaching (Zhang et al., 2009), contrasting with the conclusions provided by other studies where S was used instead of SR and it was shown to decrease (Xie et al., 2004) and increase (Stabenau et al., 2004). Related to this aspect, Stedmon et al (2000) found that selecting ranges in the longer wavelengths caused decreases in S estimated values.

DOM characterization using the spectral slope has been applied to freshwater systems, although using wider wavelength ranges than proposed by Helms et al. (2008). For instance, Bracchini et al. (2010) estimated S to study temporal changes in the composition of lake water DOM, from 250 to 700 nm and using a non linear regression fitting method. Markager and Vincent (2000) concluded that S values were higher in the freshwaters than in marine waters. Stedmon et al (2000) pointed out that CDOM with high S values has been found to be related to algal origin.

5.3. DOM Fluorescence

Fluorescence occurs when a molecule is excited by a high energy photon and emits light in a longer wavelength (lower energy). Fluorescence spectroscopy has been used extensively in recent years since it is a sensitive technique at natural DOM concentration to study DOM sources, composition and reactivity (Coble 1996; McKnight et al., 2001; Stedmon et al., 2003). Furthermore it discriminates more spectral features according to DOM composition than absorbance measurements. Nonetheless, as in the case of absorbance only a fraction of DOM presents fluorescent properties. Fluorescence spectra present basically two kinds of signals: those corresponding to humic substances, and those related to protein-like substances (Baker et al., 2001). It is important to note the use of "-like" when referencing kinds of fluorescence since a single increase in fluorescence intensity does not relate specifically to a single substance (or fluorophore) but to fluorescence properties observed in bulk DOM samples referred to those determined in isolated humic and fulvic acid standards (i.e. usually obtained from the IHSS). Therefore, each fluorescence intensity peak represents a mixture of

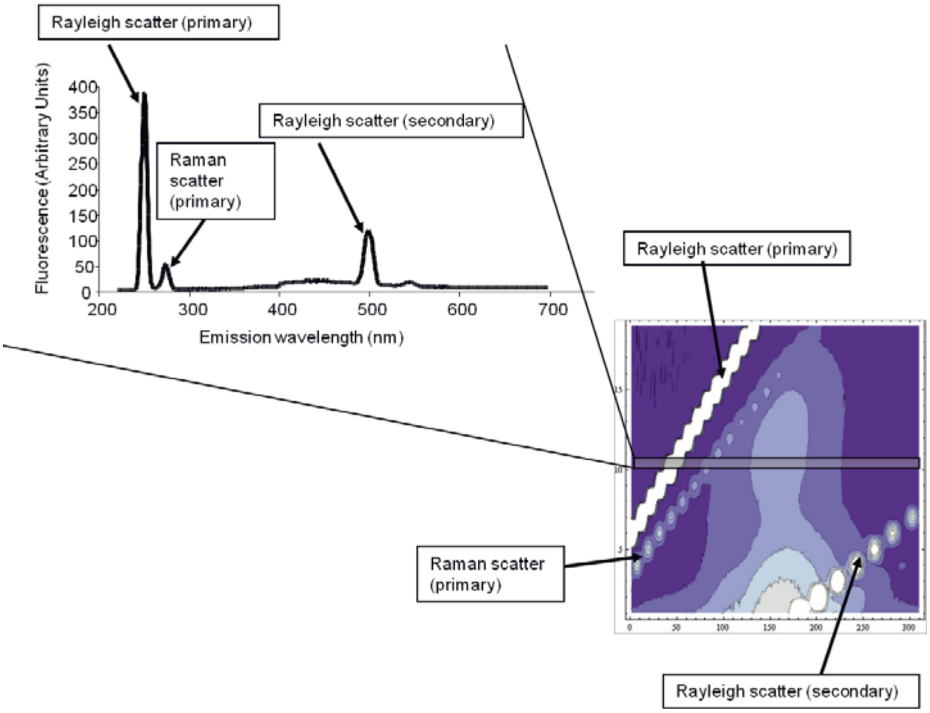


Figure 5.1. Uncorrected Excitation - Emission matrix showing Rayleigh and Raman scatters. The inset shows identifies these scatters in a emission spectrum at 350 nm excitation.

organic compounds with similar fluorescence properties (absorb and emit light at similar wavelength range).

Fluorescent properties might be affected by several factors: pH (Miano and Senesi, 1992; Mobed et al., 1996;, Patel-Sorrentino et al., 2002), freezing and dehydration (Hudson et al., 2009), fluorescence quenching due to the presence of metals (McKnight et al., 2001) and temperature (Baker et al., 2005), and high DOM concentrations causing an inner filter effect (Mobed et al., 1996, Lakowicz 2005). According to Dilling and Kaiser (2002), pH changes between 2 and 7 should not affect the structure of aromatic substances and therefore, neither its fluorescent properties. On the other hand, Patel-Sorrentino et al. (2002) observed selective increases in fluorescence intensity in the lower emission area (corresponding to peak A, see below). These conclusions contrast with the previous work of Miano and Senesi (1992) that observed a decrease in fluorescence intensity with increasing pH (6 – 10 for humic acids and 4 – 10 for fulvic acids). Finally, Mobed et al. (1996) observed also a differential effect of pH depending on the wavelength range: a red shift in the longer wavelength regions, associated to humic-like fluorescence centre accompanied by a blue shift in the shorter wavelengths regions, around 320 nm, associated to fulvic-like fluorescence centre.

In the case of freezing and dehydration there is a decrease in the fluorescence intensity that is steeper when increasing the number of cycles of freezing/thawing and dehydration/hydration. Furthermore, fluorophores are affected in distinct manner as revealed by different magnitudes of decrease among fluorescent peaks (Hudson et al., 2009). However, spectra from samples stored in cold for two days and again after two months showed no significant differences (Jaffé et al., 2008). In any case, sample preservation is a sensible issue and since the effects of freezing and posterior thawing are still unclear, it is best to store samples in the cold and dark and analyze samples as soon as possible to avoid any alteration.

The inner filter effect does not affect samples with DOC concentrations lower than 10 $mg \cdot l^{-1}$, but in any case absorbance coefficient should be checked and apply corrections if it is higher than 10 m^{-1} on any wavelength. Besides different methods proposed to correct it (Mobed et al., 1996; McDonald et al., 1997; Lakowicz 2005) a common method used is sample dilution (Jaffé et al., 2008) and the normalization to Raman units (see below, Yoshioka et al., 2007). In natural waters inner filter effects should be checked but at low DOC concentrations it should not be a concern. However, in the case of untreated sewage water it might be an important factor to consider (Baker 2002). Another aspect to account for is fluorescence quenching that occurs when the excited molecules loose energy by other pathways, interacting with other molecules for example, rather than by emitting light. Sample acidification to pH 2 should suffice to avoid quenching due to the presence of metals adsorbed to DOM (McKnight et al., 2001) but acidification might be problematic if the object of our study is bulk DOM rather than fulvic acids exclusively.

Fluorescence data can be gathered as emission spectra, obtained over a range of emission wavelengths at a fixed excitation wavelength; as synchronous spectra, where both excitation (λEx) and emission (λEm) wavelengths at the same rate with a constant wavelength offset (Δλ= λEm - λEx) (Miano and Senesi, 1992; Peuravuori et al., 2002); and obtaining a excitation-emission matrix (EEM) which has become a widespread method for gathering fluorescence data (Coble, 1990). An EEM is originated through the concatenation of emission spectra at different excitation wavelengths organized in such a way that plotting single columns it is obtained an emission spectrum at a fixed excitation wavelength, while selecting rows the plot shows an excitation spectrum.

Synchronous spectra provide a reliable method to reduce the spectral overlap and hence better identification of fluorophores in bulk DOM solutions by assigning each to different peaks of the spectra (Lombardi and Jardim, 1999; Peuravuori et al., 2002; Sierra et al., 2005; Barker et al., 2009). Using this technique implies that spectra features will change according to the Δλ used. Although different offsets have been used over different studies (Cabaniss, 1992; De Souza-Sierra et al., 1994; Goslan et al., 2004; Sierra et al., 2005;), it has been shown experimentally that an offset of 18 nm provide an optimal resolution (Miano and Senesi, 1992).

Contrasting with the single scanning methods, EEMs offer a wider picture of the fluorophores present in the sample without loosing information. On the other hand single wavelength pair fluorescence scans have been used to characterize DOM coupled to UVA scanning coupled to HPLC-SEC, to obtain values over a range of molecular weights, in waste water treatments (Her et al., 2003) and DOM interactions with metals (Park et al., 2009).

When using fluorescence in DOM characterization, it is important to consider the instrument optics and setup and perform the necessary corrections for wavelength dependant efficiencies for emission and excitation intensities (Coble et al., 1993; De Souza-Sierra et al., 1994; Lakowicz, 2005; DeRose 2007). Without these adjustments it is not possible to compare data obtained from different fluorometers as demonstrated by Cory et al. (2010). In this case, three different fluorometer models were adjusted according to factory specifications and the same correction protocols for compensating lamp and optic hardware. Additionally, obtained standard sulphate quinine spectra were checked with the NIST standard (National Institute of Standards and Technology). Even after these settings, the results obtained from two of the three models presented enough discrepancies that hindered any useful comparison.

A method to remove the instrument bias is normalizing the fluorescence signals by the Raman peak since its size and shape depend on instrument characteristics. This correction is done by dividing an EEM for the area under the Raman scatter at an excitation wavelength of 350 nm of a Milli-Q water sample. Afterwards, a Milli-Q water EEM should be subtracted from EEMs from DOM samples, obtaining the fluorescence intensity in Raman units (R.U.) (Stedmon et al., 2003). Furthermore, Larsson et al. (2007) proposed a method to correct the inner filter effects using the Raman scatter peak instead of using other mathematical methods using correction factors based on absorbance measures (Lakowicz 2005). Larsson method showed a better fit to the original fluorescence of samples than Lakowicz's.

Another method of normalizing EEMs fluorescence intensity is using a quinine sulphate solution (QSU) where 1 QSU is the intensity of 1 ppb of quinine bisulphate in 0.05M H_2SO_4 at an Ex/Em=350/450nm (Mopper and Schultz, 1993; Coble et al., 1993; De Souza-Sierra et al., 1994). The Raman normalization, apart from minimizing the effects of internal quenching of the sample, it has been shown to cause a shift in the maximum peak position towards longer wavelengths when compared to quinine sulphate normalized EEM (Yoshioka et al., 2007).

The fluorescence signals of a sample present two sources of distortion: the Rayleigh and Raman scatters. In single emission spectra they are observed as high intensity peaks (Rayleigh is higher than Raman), but they are more evident when plotting EEMs where they appear as different bands crossing the plot. Rayleigh scatter is a high intensity signal that occurs when λEx and λEm are similar (first order Rayleigh scatter) and when λEm is twice the λEx (second order Rayleigh scatter). Thus, Rayleigh scatter can be avoided by not gathering data at similar wavelengths values (from synchronous spectra for instance). Raman scatter is produced by the dispersion of photons in the liquid medium and is easily removed by subtracting and EEM of pure MQ-water to all EEMs corresponding to samples. These scatter bands do not affect specially the shape of EEM and allow the detection of fluorescence maxima and visual determination of fluorophores, but need to be removed if a statistical method is going to be applied afterwards.

EEMs have become a widespread method for obtaining information through fluorescence spectroscopy (Coble 1990). The EEM is originated through the concatenation of emission spectra at different excitation wavelengths organized in such a way that plotting single columns it is obtained an emission spectrum at a fixed excitation wavelength, while selecting rows the plot shows an excitation spectrum

In an EEM plot different areas with important fluorescence peaks will be observed, along with three diagonal bands corresponding to first and(Coble, Green et al. 1990) second order Rayleigh scatter and the Raman scatter if they have not been previously removed. Coble et al. (1996) proposed a terminology to differentiate each major fluorescence peak, although it is not the only one (Parlanti et al., 2000) (Table 1) The two predominant peaks are those corresponding to humic-like substances and were labelled as peaks A (excited by UV-C wavelengths) and C (Excited by UV-B wavelengths). Additionally, peak C presents a positive relationship with fulvic acids (Baker et al., 2002). Since a great deal of seminal studies on DOM fluorescent properties have been conducted in marine waters, it must be considered that often riverine samples show red shifted (longer wavelength) peaks A and C (Coble et al., 1998) when comparing to sea samples. Peaks T (Ex 220-235/Em 330-370 nm) and B correspond to proteic substances and are related to organic matter from microbial or algal origin (peak T corresponding to tryptophan-like fluorescence). Furthermore In anthropogenic impacted rivers, tryptophan-like fluorescence (at excitation 280 nm) was correlated to nitrate and phosphate concentrations, while at excitation 220 nm presented correlations to ammonia and dissolved oxygen and it was proposed as a possible indicator of water quality (Baker and Inverarity, 2004). Peak M (β, according to Parlanti's nomenclature) was originally found in marine water samples and it was associated to marine humic acids. Parlanti et al. (2000) showed that during incubations of macro-algae, there was first an increase in protein-like fluorescence and as M peak increased in fluorescence intensity, peak T (γ) decreased. Later, peak C gained prominence while fluorescence in M and T regions decreased, indicating a more humified DOM. This implied that peak M was mid-humification step between protein and humic-like substances, but a recent study showed that the M fluorescence peak was also originated along peak T as a by product of algal production (Romera-castillo et al., 2010). Peak M was thought to be exclusively of marine origin but further studies identified this peak also in freshwater (Stedmon 2003; Mostofa et al., 2007; Murphy et al., 2008), and even in rainwater (Santos et al., 2009). Furthermore, Mostofa et al. (2007), contrasting with the findings of Parlanti et al. (2000) where peak M was originated from labile microbially derived DOM, found that peak M could be also originated as a product of photodegradation of peak C. EEMs from rain water presented a slight deviation towards shorter wavelengths indicating a lower presence of aromatic compounds.

5.3.1. Relationships between fluorescence and DOM properties

Different DOM functional aspects have been demonstrated to be related to fluorescence properties and therefore they can be studied using spectroscopic techniques.

The analysis of Ex and Em wavelengths allows identifying changes in DOM composition both spatially and temporally in ground (Baker, 2001) and surface freshwater (Wu et al., 2007). Fulvic-like fluorescence, related to peak C area, shows an increase in intensity from headwaters to the river mouth while the main temporal changes occur during drought (Wu et al 2007). Furthermore, it has been determined a positive relationship between peak C (fulvic-like fluorescence) and DOC concentration (Coble 2007). Furthermore, Cumberland and Baker (2007) show that the strength of the relationship between the fulvic and humic-like fluorescence areas and DOC concentration can vary depending on the sample origin, and, therefore, this relationship could be used to trace DOC origin. Protein-like fluorescence, broadly corresponding to peak T, presented low temporal variability indicating ephemeral inputs or in situ production (Wu et al., 2007). In the case of ground waters, Baker and Lamont-Black (2001) concluded that the changes in excitation and emission wavelengths for protein and fulvic-like fluorescence could be used to discriminate temporal variations on DOM properties. While peak C is related to humified organic matter and degradation by-products, peak T, besides its association to fresh organic matter, mainly of autochthonous origin, it has been found to be a good descriptor of microbial activity as measured by bacterial organic carbon consumption, bacterial production and total plankton community respiration (Cammack et al., 2004).

Ratios between fluorescence maxima of different peaks have been used to investigate the origin and formation of DOM (Coble et al., 1996; Parlanti et al., 2000; Huguet et al., 2009). The C fulvic-like fluorescence peak is related to more humified material and therefore the A:C ratio also provides a measure of new versus old DOM (Coble 1996) and has been used detecting seasonality changes in DOM in estuarine mixing (Huguet et al., 2009). The ratio of peaks T:C fluorescence maxima is related to the proportion of newer organic substances and could be used as an indicator of labile DOM as proposed by Baker (2008).

Furthermore, peak C fluorescence normalized to absorbance at 340 nm has been demonstrated to relate to DOM molecular weight (Stewart and Wetzel, 1980 citats a Baker et al., 2008), and recently have been found as a good indicator of alumina and benzopyrene adsorption, and hydrophillicity (Baker et al., 2008). These last properties are also related to other measurements like peak T:C intensity ratio and peak C emission wavelength.

Initially, the determination of each fluorescence peak is mainly done by visual recognition or peak-picking. Chen et al. (2003) proposed a new approach to analyze data from EEMs called Fluorescence Regional Integration (FRI) in order to avoid peak picking and loosing information in the fluorescence areas with no clearly defined peaks. This technique consists in measuring not only the maximum intensity of each fluorescence peak, but to account for all the fluorescence intensity registered in different EEM regions that correspond to different kinds of fluorescence. For instance, in their study, the EEMs were divided in five regions: one fulvic-like and one humic-like fluorescence region while protein-like fluorescence was spread over three defined areas. The contribution of each region to the total fluorescence was accounted using a volumetric approximation rather than area-only or a single maximum value for each region. Although this method accounts for all fluorescence without the need of further statistical applications (i.e. using PARAFAC), the areas are arbitrarily defined, although in consonance with literature reports, hindering a direct comparison among studies since the areas might easily vary. This technique was used by Wang et al. (2009) in the analysis of DOM removal and subsequent structural changes after sub-surface flow in a lab-scale experimental wetland as it is one of the treatments applied in waste water treatment plants.

5.3.2. Fluorescence Index (FI)

McKnight et al. (2001) developed this index for the study of fulvic acids. It consists in measuring the ratio of the fluorescence emitted at 450 and 500 nm, under a fixed excitation of 370 nm. The fixed excitation wavelength of 370 nm was selected because it presented low light absorbance for fulvic acids, therefore reducing the inner filter effect. The 450 nm emission wavelength was selected because it was an intermediate wavelength between the maximum intensity for terrestrial and microbial derived organic matter, while the 500 nm emission was selected because it presented approximately half the maximum intensity in microbially derived samples, thus characterizing the slope of the emission spectra. Later, in Cory and McKnight (2005), the emission wavelengths were corrected to 470 and 520 nm, although maintaining the same threshold values to discriminate DOM origin.

The FI offers information on the origin of fulvic acids/DOC discriminating its origin according to a range of values between 1.4 and 2. Lower values indicate a terrestrially derived origin, while values in the higher end indicate that the organic matter is microbially derived. These values were determined using samples with different DOM origins as end members. The end members for the microbially derived, autochthonous, DOM come from ponds and lakes in Antarctica (Lake Fryxell and Pony Lake) while for terrestrially derived DOM samples came from rivers and streams from the U.S.A., like Suwannee river, where DOM precursors were found in soil and plant litter.

This index is built on previous studies of the fluorescent properties of DOM (Coble et al, 1996, De Souza Sierra et al. 1994, Mobed et al 1996) and knowledge of DOM characteristics according to its origin. McKnight et al, found a high correlation between aromaticity, determined by 13C-NMR, and the FI, for values below 2.1. This negative relationship is also found when comparing the FI with $SUVA_{254}$ values (Miller and McKnight, 2010)

The EEMs used in this prior study (McKnight et al., 2001) were obtained using an uncorrected fluorometer and thus the range provided for FI values might change in other studies (Romaní et al., 2006; Vazquez et al., 2010; Schwede-Thomas et al., 2005). A recent study by Cory et al (2010) showed that the range of FI values when corrections are applied to EEMs differ resulting in narrower ranges for the end-members: from 1.21 to 1.55, but still end-members for DOM of terrestrial origin were lower than 1.4 while microbially derived DOM presented values higher than 1.4. Additionally, the corrections applied to the fluorometers showed that the emission wavelengths selected for determination of the FI were displaced and 470 and 520 nm should be used instead under these setup conditions. In the case of FI data obtained with the former wavelengths, Cory et al. (2010) show that the numeric values still show a valid trend rather than an accurate numeric value and that in further studies the maxima position of the emission spectra should be added to evaluate properly the results.

5.3.2. BIX

The Biological IndeX (BIX) introduced by Huguet et al. (2009) is used to determine the autotrophic productivity from the presence of fluorophore B. As pointed before, this fluorophore is characteristic of autochthonous biological activity. BIX is calculated as the ratio of emission at 380 nm (corresponding to peak B maximum intensity) and 430 nm (corresponding to peak C maximum intensity) at an excitation 310 nm (maximum for peak B). High values (>1) indicate DOM mainly of autochthonous origin and low values (0.6 – 0.7) indicate DOM of allochthonous origin. This index was not derived from humic substances standards, but from riverine and stuarine water samples (Huguet et al., 2009). This index presents a wider range than the Fluorescence Index, but it is mainly focused to the study of marine/estuarine DOM. As it has been proposed recently BIX has not been tested extensively across different environments. A reason might be that collected samples might not contain any autochthonous DOM inputs and, therefore, peak B is not observed, loosing the discriminating potential of BIX.

5.3.3. Humification degree / Humification IndeX (HIX)

The degree of humification although it is correlated to certain absorbance wavelengths it also can be measured using spectrofluorometric data. With this goal in mind, different methods of assessing organic matter humification degree have been proposed.

Zsolnay et al. (1999) proposed that as fluorescing molecules become more condensed, the emission spectra would tend to shift towards longer wavelengths. Therefore, the estimation of the ratio of the areas of fluorescence emission obtained from lower (L) wavelength (356-432 nm) and higher (H) wavelengths (570-641 nm) at a fixed excitation of $_{254}$ nm would be an estimation of the humification degree. It is considered that when the degree of humification increases, the emission spectrum is red shifted, indicating a higher proportion of complex molecules. In any case, the wavelengths determining each area vary from study to study due to the variability of samples. Thus, H and L wavelength ranges should be adjusted (Milori et al., 2002; Fuentes et al., 2006; Huguet et al., 2009).

Since fluorescence is also affected by inner filter effects Ohno et al. (2002) proposed an alternative method for estimating HIX in order to discard possible interferences due to inner-filtering consisting on calculating the following ratio: $HIX = \sum I_{435\text{-}480} / (\sum I_{300\text{-}345} + \sum I_{435\text{-}480})$. The reasoning behind this correction is that

	Stedmon (2003)		Fulton (2004)		Stedmon (2005)		Murphy (2008)		Yamashita (2008)		Kowalczuk (2009)		Santín (2009)		Williams (2010)	
Component	Ex	Em	Ex	Em	Ex	Em	Ex	Em	Ex	Em	Ex	Em	Ex	Em	Ex	Em
C1	<240	436	340/260	430	<250	448	275	300	<260	458	250	452	<260(205)	439	<250(355)	474
C2	<240	416	260/370	460	<250(385)	504	315	418	345	433	250	420	256(385)	>500	<250(310)	416
C3	270(360)	478	245	-	<250(305)	412	260(370)	490	390(275)	479	250(310)	400	320	388	<250(340)	424
C4	325(250)	416	310	380	<250(360)	440	250(320)	370	280	318	270(390)	508	275	304	260(360)	422
C5	280(<240)	368	310	420	325	428	255(280)	580	285	362	270	332	<260	365	<250(270)	370
C6			250	430	<250(320)	400	280	328	325(<260)	385	250(290)	356			280(255;445)	518
C7			260/340	410	280	344	240(300)	338	270	299						
C8					275	304	250(380)	416								
C9							240	422								

Stedmon (2003)	**Fulton (2004)**	**Stedmon (2005)**	**Murphy (2008)**	**Yamashita (2008)**	**Kowalczuk (2009)**	**Santín (2009)**	**Williams (2010)**	**Coble (1996-1998)**	
C1	C6	**C1**		C1	C1	C1		A	Terrestrial humic
C3		**C2**	C3	C3	C4		C6	A or C	Terrestrial humic
C2	C7	**C3**	C9		C2	C2	C2	C	Terrestrial/autochthonous fulvic
C3	C1,C2	**C4**	C8	C2			C1	M	Marine/terrestrial/microbial reprocessing
C4	C5	**C5**	C2					T	protein-like/tryptophan-like
C4	C4	**C6**	C4	C6	C3			A or C	Anthropogenic humic
C5		**C7**	C6	C4,C5	C6	C3	C5	T	protein-like/tryptophan-like
C5		**C8**	C1	C7	C5	C4,C5		B	protein-like/tyrosine-like

Table 5.2. The upper table shows the location of fluorescence maxima for the PARAFAC components identified in different published works. The wavelengths in parenthesis indicate secondary the secondary maxima of those components. The lower table shows which components have been identified in different published studies. The reference is established by the eight component model by Stedmon and Markager (2005). The last column shows the traditional peaks, following Coble's nomenclature, that appear at similar excitation-emission wavelengths.

in the lower wavelength range thre is usually more absorption, and, therefore, more susceptible to display inner-filtering effects. On the other hand, Ohno et al. (2002) do not use the ratio of the areas under the high and low wavelength range, but fluorescence intensities (I). The HIX values provided following this correction are between 0 and 1, increasing with humification. In any case, it is suggested that samples with absorbances lower than 0.3 cm^{-1} might not need inner-filtering correction.

Kalbitz et al. (1999) proposed another method of estimating the humification degree from a synchronous fluorescence scan which would also offer insights on OM aromaticity based on emission wavelengths shifts. The synchronous scan should be run in the excitation range 260-520 nm with and offset of 18 nm in the emission range. The resultant spectrum should present two fluorescence peaks around 360 and 400 nm and a shoulder at 470 nm. Thus either ratio (400/360 or 470/360) would give information on the humification. This estimation is often referred in scientific literature as A4/A1 (Milori et al 2002).

Kalbitz and Geyer (2001) showed that with the proper corrections the synchronous spectra used for determining the humification index allowed the comparison of spectra obtained from different fluorometers. On the other hand, the HIX calculated from emission spectra (Zsolnay et al. 1999) proved to be a good predictor of DOM decomposition while HIX calculated following Kalbitz et al (1999) was not useful in this matter (Kalbitz et al., 2003).

A third method proposed by Milori et al. (2002) consists in measuring the area of fluorescence spectra resulting from an excitation scan at an emission of 517 nm. The highest peak was detected at 465 nm (red shifted). Therefore the area under this peak was used to calculate the index. Although these three methods are different in their approaches, they present a high correlation between them, offering similar information.

Additionally, Fuentes et al. (2006) performed a comparison of methods for determining the humification degree of organic matter including absorbance and fluorescence spectroscopy methods. The comparison included again Zsolnay, Kalbitz and Milori methods. In any case, both in Milori et al. and Fuentes et al. the methods tested present differences to the originally published ones. For instance, in Kalbitz set of synchronous spectra the emission wavelength offset is 18 nm while in Fuentes et al. and Milori et al. is 55 nm or changes in excitation wavelength

(Kalbitz: $_{254}$ nm; Milori: 240 nm; Fuentes: 440 nm). Although, it may not be significant since results in all methods are correlated and offer similar conclusions, caution is required when examining the offered data and comparing among the different studies.

5.4. Multiway analysis of EEMs

EEMs present a lot of information that considered into large data sets result difficult to annalyze. In order to analyse these data sets beyond the more traditional "peak picking" or the use of fluorescence derived indexes, multivariate data analysis methods have been applied (Bro, 1997; Persson et al., 2001; Stedmon et al., 2003; Stedmon and Bro., 2008). The most commonly applied multivariate methods are principal component analysis (PCA) and parallel factor analysis (PARAFAC). These two methods are not mutually exclusive and have been applied one onto the other (usually a PCA on the components from PARAFAC) (Ohno and Bro, 2006; Miller and McKnight 2010).

Unfortunately each of these methods presents its own drawbacks. In the case of PCA, being a two way method it means that the combination of EEMs must be unfolded to fit a matrix. Each EEM is a matrix on its own, therefore the combination of EEMs creates a three dimensional data set comprised of sample, excitation and emission axes. In order to conduct a PCA, these three way structure must be unfolded in a way that each row corresponds to a sample and each variable to an excitation-emission combination, greatly increasing the number of variables and complicating the modelling/extraction of principal components. On the other hand, while PARAFAC decomposes the data set into tri-linear components without needing transformation of data structure into a single matrix, its ability for modelling is limited by its own requirements. First, PARAFAC requires a large number of samples, from 20 to 100, to obtain a model that can be validated with enough reliability, and secondarily, samples can not present wide differences among them or they will identified as outliers hindering the modelling process, reducing our data set and, therefore, loosing information. Thus when working on a longitudinal or temporal scale, it is suggested to collect enough samples to obtain a data set where changes between extreme conditions are gradual.

Lastly, before running PARAFAC and PCA, all scattering, Raman and Rayleigh, should be removed from EEMs. Raman scattering is routinely removed when performing other measurements with the EEMs, while subtracting an EEM from ultra-pure water. On the other hand, there are different proposed methods for correcting EEMs for Raman and Rayleigh scatter: removing the scatter areas and interpolating the missing data during post-processing (Zepp et al., 1994; Bahram and Bro, 2006); substituting the scatter bands with 0s or missing values (Stedmon et al., 2003; Stedmon and Bro, 2008); removing scatter using a jack-knife method (Riu and Bro, 2005); transforming Rayleigh scatter in order to model it either with PARAFAC or PCA (Rinnan and Bro, 2005); or detecting scatter without visual identification (Engelenn et al., 2007). The effect of scatters and outliers is fully reviewed in Andersen and Bro (2007). Furthermore a more robust algorithm for PARAFAC analysis was proposed recently by Engelenn et al. (2009) that is able to handle scatters in EEMs, without undergoing data post-processing.

5.4.1. Principal Component Analysis

The PCA decomposes the data matrix into a new set of uncorrelated variables (the principal components) that account for the variance present in the data set. The loadings generated by the PCA describe the contribution of each variable to the variability of the original data matrix, while the scores are the projection of the samples in the space formed by the principal components (the new variables generated by the analysis). Usually, scores and loadings are plotted using the first two principal components because they are the ones accumulating most of the variability, but in some cases more than the these two first components are necessary to obtain useful information. In the context of an EEM, score plots allow comparing the similarities between samples while loading plots allow estimating the contribution to the variance of the different excitation-emission pairs (Persson et al., 2001). Therefore, the loading plot allows discerning the EEM matrix areas where variability is high among samples, usually corresponding to fluorophores. But, in this way, fluorophores with a low variability in intensity may be overlooked.

When performing a PCA on a large data set, it is possible to work with an ill conditioned matrix due to the lack of independence among data variables, such as when working with time series. Even in this case, the descriptive analysis of the data set should be possible (Barker et al., 2009). Also, in order to analyze changes in shape only, not in magnitude, it is necessary to make a correction by the maximum intensity to eliminate the variance caused by changes in DOC concentration among samples.

An in-depth explanation of the application of PCA to EEMs in order to discern sea water masses in a depth gradient and the distribution of humic and protein-like fluorescence can be found in Persson et al. (2001). Boehme et al. (2004) applied PCA on a data set coupled with end-members in order to discern between DOM of terrestrial origin and photobleached DOM in sea water. Additionally, PCA has been applied also to data sets comprised of synchronous spectra, and thus without needing a trilinear approach, allowing the identification of the major fluorophores, discriminating protein and humic-like fluorescence in two principal components in samples from glaciers (Barker et al., 2009).

5.4.2. Parallel Factor Analysis (PARAFAC)

Parallel Factor Analysis (PARAFAC) is multi-way decomposition method that originated in psychometrics (Bro, 1997) and gradually was applied in other research fields (food industry for example). Multi-way data is characterized by being composed of several data sets that can be arranged in a three dimensional cube rather than 2D. PARAFAC offers qualitative and quantitative information, discriminating the underlying fluorophores contained in the EEMs by decomposing the fluorescence signal in independent components (see general mathematical background in Bro, 1997 and applied to the study of DOM in Stedmon and Bro, 2008). Generating a valid model for the analyzed data might be an involved process. Before obtaining a validated model, an exploratory analysis must be conducted in order to detect outliers and the adequate number of components. Outliers can be found either as samples, excitation or emission wavelengths where the signal to noise ratio is very low. An adequate number of components are which explains the highest variance, more than 90%.

Model validation can be achieved using diverse criteria: half-split analysis (Bro, 1997; Stedmon et al., 2003), core consistency (Ohno and Bro, 2006), jack-knifing (Riu and Bro, 2006) and/or residual plot examination. Half-split analysis works on the assumption that PARAFAC has a unique model for each data set and therefore randomly obtained subsets of the whole data set should present the same results (i.e. the same loading scores). On the other hand, residual examination relies in the comparison of the original and modelled EEMs and the residual plot. If the residual plots show a random distribution of the variability and low values then the model has a high goodness of fit, but not if there are areas where variability is concentrated. One of the doubts that may arise when starting to work with this kind of analysis is caused by the adequacy of certain validation methods and the priority should be given to a specific method when there are contradictions. For instance, a model might be validated by half-split analysis but the residual plots present areas where error between the original sample and the modelled EEM is high instead of being only noise, while selecting a different number of components offers a more uniform residual plot and better modelled EEMs but the model can not be half-split validated. In Luciani et al (2008) the presented four component model was half-split validated but the core consistency analysis was only of 44% (when it is recommended values higher than 90% to consider the model validated). On the other hand, while the 13 component PARAFAC model presented by Cory et al. (2005) was half-split validated, the inclusion of new samples from posterior studies (Mladenov et al., 2007 and 2008; Miller et al., 2006) was only validated by residual plot examination. Thus, if there are contradictions between validation methods the final decision becomes arbitrary.

In the situation where a data set is not well modelled or the validation methods suggest ambiguity in the solution, it is required to restart the modelling process, specially checking for outliers. If many samples are considered outliers, the reduction of the data set can detract from the usefulness of the model to explain changes in DOM properties. For further details on outlier identification refer to the appendix to the PARAFAC tutorial by Stedmon and Bro (2008b).

PARAFAC was introduced for the first time in the study of DOM by Stedmon et al. (2003) where a five component model generated from riverine and estuarine samples. Of these five components, three presented similar characteristics to humic-like peaks (two to peak A and one to peak C), another presented similarities to peak M, although it was present in end-member samples from terrestrial environments contrasting with the marine origin attributed in previous studies (Coble et al., 1996; Parlanti et al., 2000). Finally, another component presented similarities to peak N (Coble et al., 1998) and peak T. While peak N is considered to relate to DOM originated in the water column, peak T is related to biological production in surface waters. In a posterior study, Stedmon and Markager (2005) presented an eight component PARAFAC model, including the five components from the previous study. The model presented five components of terrestrial DOM (two of them of autochthonous origin and one of anthropogenic origin), two protein-like components and one anthropogenic component. This model showed that PARAFAC might be a useful tool to understand the variability of DOM composition at large spatial scale and also at a temporal scale. Additionally, Stedmon and Markager (2005b) used PARAFAC to study DOM production and degradation, concluding that photodegradation was a major DOM sink. This study also observed that some components presented similar characteristics to DOM originated by microbial activity.

A validated model allows fitting further samples into it, if it makes sense in a study context. But, a global DOM fluorescence model based on a wide range of samples is not possible at this stage since new samples incorporated in the model might not fit since its properties might be affected by several uncontrolled factors (Stedmon and Bro, 2008), as well as the difficulties in the inter calibration of fluorometers (Cory et al., 2010).

Even with the instrumental restrictions there have been some attempts/tests to obtain more general models in order to shed some light in DOM composition and its interaction capacity. One of them is presented in Cory and McKnight (2005), where a PARAFAC model is developed from 379 matrices. In this study, the fluorescence of quinones, a ubiquitous organic substance class in living cells, extracellular material and detrital organic matter, is examined according to its redox state since they affect the electron shuttling of fulvic and humic acids and, therefore, DOM reactivity (Scott et al., 1998). Quinones present three redox states: reduced, semi-reduced and oxidized. The model presented 13 components of which 7 were associated to the quinones presence, while 2 where related to protein-like substances and 4 remain unrelated to any substance or formerly described fluorescence peaks or PARAFAC components from other models. Furthermore the ratio of the loadings of a semiquinone component 1 (SQ1, in the original publication) over the sum of semiquinone component 1 and 2 (SQ1 and SQ2 explained the variability in the Fluorescence Index (Cory and McKnight, 2005). Four of the seven components associated to quinones presented similar fluorescence to reduced quinones (semiquinones and hydroquinones), with excitation maxima between 250 and 270 nm and notable peaks between 330 and 400 nm. The spectra of the other three quinone-like components present characteristics of oxidized quinones. Based on these conclusions, Miller et al (2006) proposed a Redox Index (RI) calculated from the sum of loadings of the quinone-like components related to reduced states corrected by the loading totals of reduced and oxidized quinone-like components. A further study by Miller et al (2009) concluded that DOM of microbial origin presents a decrease in the rate of decay when quinone-like substances are oxidized.

On the other hand, further studies indicate that PARAFAC models do not offer consistent results indicating that quinone redox states are being reflected in the nature of components and that the use of a RI index based on quinine moieties for DOM is not advisable (Macalady and Walton-Day 2009).

The possibility of fitting new samples into a validated model is a powerful tool since it allows including samples from studies with a low number of samples. Miller et al (2010) used this approach when studying seasonal changes in alpine lakes and Mladenov et al (2007, 2008) fit new samples from a study in DOM characterization of the Okavango delta. Fellman et al (2009) explored the potential of fitting new samples in already validated models by generating an original model from 307 samples obtained from Alaskan soil and stream water, and, in parallel, fitting the EEMs from these samples into the thirteen component model developed by Cory and McKnight (2005). The model developed for this new data set validated ten components. Although both models presented eight common components, the results showed some new EEMs modelled poorly. This indicated that fitting the new samples in the existing model resulted in a loss of sensitivity and, subsequently, a loss of ecological interpretative power.

PARAFAC has been used to discern the influence of land use in DOM properties in fluvial systems (Holbrook et al. 2006; Williams et al., 2010). In the model presented in Williams et al, three of the six components were related to quinone-like fluorescence and further analysis of these components indicated that in streams with high bacterial production and agricultural land use DOM was in a more reduced state. In contrast, in streams with wetlands presented a higher proportion of the oxidized quinone-like component. On the other hand, there is no definitive conclusion in changes of DOM properties in the spatial scale, from headwaters to river mouth. Baker et al (2004) and Wu et al (2007) examined DOM fluorescent properties without PARAFAC while Fellman et al (2009) generated a model to study DOM changes. Baker et al observed an increase in protein-like fluorescence intensity downstream due to anthropogenic impacts. Wu et al observed and increase in the ratio of humic-like fluorescence and DOC concentration downstream while protein-like fluorescence behave erratically in the spatial and temporal scales. In contrast, the components generated in Fellman's PARAFAC model showed a decrease of protein-like fluorescence, which was related to DON concentration, downstream while humic-like fluorescence remained invariable. This decrease in protein-like fluorescence components suggested that these DOM was preferentially uptaken. Furthermore, the protein-like fluorescence was found to be a good predictor of BDOC content in stream and soil water while the examination of the contribution of protein-like PARAFAC components revealed that it was higher in soils than in surface waters.

Ohno et al (2008) used PARAFAC to study the effects of metal binding to soil DOM, showing evidence that while iron (Fe3+) quenched the fluorescence in the three obtained components, aluminium enhanced the fluorescence of two components and quenched one.

Additionally, the loadings of the different PARAFAC components can be analyzed by means of other statistical techniques like principal component analysis. For instance, Ohno and Bro (2006), used PCA to study the differences in fluorescence in DOM from different soil types, but observed that were no significant differences. On the other hand, Miller and McKnight (2010) applied PCA to PARAFAC components to study seasonal changes in DOM from alpine lakes.

Ishii and Boyer (2012) presented an in-depth review of the properties of humic-like components, encompassing ecosystem characteristics, physicochemical processes, and treatability in water engineering systems, from a sheer number of studies.

5.5. CONCLUSIONS

Over these two last decades, technological advances have favoured the application of spectroscopic techniques to the study of DOM on a higher resolution scale and shorter analysis time. Since DOM is a key factor in aquatic systems, either in natural conditions or affected by anthropogenic activity, the number of studies where these techniques are applied has increased.

Unfortunately, the sheer number of publications often implies numerous citations and for the newcomer to the use of this array of techniques, it involves a lot of back tracking and cross examination of citations in order to be able to apply them properly and with the security of obtaining the desired information on DOM properties. In the scientific literature there can be found these techniques employed in the study of natural systems (marine, fluvial systems, lakes, coastal lagoons, soil and soil water), on DOM characterization from an exclusively biochemical approach and studies focused on the potentials and limits of the spectroscopic techniques, mainly using fulvic or humic acid standards. There are excellent review articles like Coble (2007) focused on CDOM in the ocean while reviewing different spectroscopic techniques and studies applied to this system. On the other hand, Hudson et al. (2007) review is focused on fluorescence spectroscopy with especial emphasis on its application for sewage water monitoring. On other occasions, reviews are focused on a certain process while covering the wide array of analytical methods, for instance UV induced changes on DOM in Sulzberger and Kaiser (2009). Therefore, the present review is aimed to offer a wide, but not in-depth, vision of the spectroscopic techniques to characterize DOM optical properties in aquatic systems, especially freshwater, while citations herein should provide the required thorough background of specific aspects of techniques or processes where DOM is involved.

When working with these techniques it must be taken in consideration that there is no single one that will provide the best results. Many of the works referenced in this text are a clear example of how the different techniques are applied in order to elucidate the questions driving them. Moreover, there is no combination that yields the best results and what does fit each study case depends on the available resources and equipment, conditioning the experimental design, and the aim of the study.

Nevertheless, the collecting of useful and comparable spectroscopic data is still a debate subject since there is much uncertainty on the effects of sample manipulation and preservation (i.e. acidification, freezing) and data correction (inner filter effect, quenching). Therefore, there is still further research on the limits of the different techniques coupled to technological development. Moreover, the scientific community should direct its efforts into reaching a common operational protocol allowing a more direct comparison of results between studies.

GENERAL DISCUSSION

Hydrologically driven changes of DOC and DON

The major implications for biogeochemical cycles in fluvial systems, both at local and regional scales, have been acknowledged as a research topic for a long time. The characteristics of the Mediterranean climate produce another hydrological disturbance: a recurrent dry period (or drought). Although this dry period and the floods generated by intense precipitation events are disturbances of opposite sign, they both provide an excellent opportunity to study the hydro-biogeochemical functioning of catchments.

The storm events show how there is a wide range of solute responses. In order to characterize this whole range it is necessary to obtain a long time series covering all possible hydrologic events. In this sense, chapter 1 studies the variability of nitrate and DOC – discharge (Q) responses over a time series of 4 years. The results show that nitrate – Q response variability is higher than that of DOC. Additionally, the more prominent DOC dynamics are well described showing that just after the drought period, during the hydrological transition, there is the yearly highest concentration. But, it is not known what changes, if any, are occurring at the level of DOC properties and its consequences in microbial uptake. Furthermore, considering floods and dry periods as extreme system perturbation of opposite sign, the knowledge on the former is quite detailed and extensive while on the latter is still lacking.

Thus, chapter 2 starts an exploration of the effects of a hydrological transition both quantitatively and qualitatively, comparing the autumnal wet period of two consecutive years, one with a previous severe dry period and another one with a summer period with no hydrological fragmentation. In this work, it is highlighted the influence of antecedent meteorological and hydrologic conditions on DOC dynamics and indicates that there might be changes in its composition during the rewetting period. It is clear, that to observe the usually high DOC concentration peak (around 20 $mg \cdot L^{-1}$ when basal DOC concentration is between 2-3 $mg \cdot L^{-1}$), the antecedent dry period must present a certain degree of severity. It is not enough a drastic decrease in surface flow (as observed in year 2004) but requires a cessation of flow (hydrological fragmentation along the fluvial continuum) (year 2003). It is only after this harsh period that during the hydrological transition we can observe a change in DOC composition in terms of contribution to the MW fractions along the concentration increase. Furthermore, it is in this period when the stream-riparian ground water interface becomes a “hot spot” of biogeochemical activity as shown by the selective retention of the higher MW fractions while the low MW fractions are conservative. This initial conclusion supports to a certain degree Amon and Benner's size-reactivity continuum model (1996) that had become a more or less extended model continuously tested by further studies which would agree or disagree with it. In riparian ground waters the MW contributions for DOC and DON are similar to those observed in surface waters and between them as well.

From this preliminary work the follow-up study, presented in the third chapter, emphasized the exploration of the influence of the dry period on DOC fate and dynamics. In this case, considering also its bioavailability (BDOC) and adding the estimation of dissolved organic nitrogen (DON) along with the MW fractions. In this study, it is shown that the DOC bioavailability is not tied to a single MW fraction, neither in surface nor in riparian ground water. It also evidences that during the dry phase, with low surface discharge, DOC is not really reactive but DON is since it becomes depleted both in surface and ground waters. This increase in the DOC:DON ratio during drought is in agreement with the observed reduction in the heterotrophic use of peptides and a major use of polysaccharides throughout the drought process (Ylla et al. 2010). On the other hand, there is no significant change in the contribution of each DON or DOC MW fraction.

During the rewetting period, DOC concentration presented a maximum, coinciding with the discharge peak, while DON concentrations peaked with high discharge but after the DOC concentration maximum. During this period, the DON pool was more relevant in riparian ground water (77%) than in stream water (43%). Also, during this period, there is a brief increase in DOC_{HMW} fraction but it is not mirrored in the DON_{HMW} fraction. This increase occurs during the high discharge peak following the precipitation event. Therefore, MW fractions dynamics fit within the framework that during storm events inputs of organic matter higher than 1 kDa increase (Li et al., 2003; Maurice et al., 2002). The observed decrease of DOC:DON ratio of each MW fraction suggest that there is an asynchronous DOC and DON mobilization. This could be caused by different locations of organic matter pools that are mobilized by different hydrologic pathways (McGlynn and McDonnell, 2003). On the other hand, the results suggest that DOC bioavailability is regulated by DON concentration rather than changing contributions of DOC MW fractions (Petrone et al., 2009 reached a similar conclusion). Therefore, in contrast which was hinted in the previous work, DOC bioavailability would not be as strongly explained according to molecular weight neither on relation to the size-reactivity continuum model.

Finally, chapter 4 explores the consequences of hydrological fragmentation on DOC and DON properties. This introduces the need for considering also a spatial factor since hydrological fragmentation is not homogeneous and the location of the remaining water masses depends on the geomorphology of the catchment. Along the consideration for space, the changes in water characteristics reflect a temporal axis according to the moment when each water mass became isolated. This temporal change is reflected by the ratio of dissolved oxygen and ammonium (Chemical Index, in chapter 4).

On the other hand, during this fragmentation period, although DOC and DON properties were different than autumn's flowing water, most of them did not present any statistically significant trend or pattern. However, the biogeochemical heterogeneity of the system is amplified, while during the hydrological transition and the rewetting period in general, the system (water and the different solutes) is homogenized.

It is important to remark that hydrological transition is not a yearly event. It depends on the previous hydrological conditions. Thus, in years where the dry conditions only cause a decrease in surface flow, DOC concentration is low, similar to the concentration range observed during baseflow periods (2-4 $mg \cdot L^{-1}$). On the other hand, DOC in surface water is always higher than in riparian and hillslope groundwater. On the other hand, the high DOC concentration observed during the hydrological transition are in the same range as the highest concentrations observed in the isolated water masses during hydrological fragmentation.

As a conclusion from the MW fraction data presented in chapters 2 and 3, generally, DOC composition according to the MW fractions is relatively stable throughout the hydrological year. Overall, DOC_{MMW} and DOC_{LMW} are the most abundant fractions (35-40% each approximately for the years that MW fractions were determined). Thus, DOC_{HMW} is around 20-25%. However, generally with first precipitation episodes after the summer period, even when there is no apparent dry – wet hydrological transition, there is an increase in the contribution of the DOC_{HMW} (higher than 10 kDa). On the other hand, DON MW contributions were only examined during one dry and the following transition and wet period. In this case, DON_{LMW} was the major contributor to the DON pool (56%) while DON_{MMW} and DON_{HMW} contribution were similar. These differences with between DON and DOC MW contributions are another hint towards different mobilization rates/accessibility. DON_{LMW} contrasts even more within the DOC framework that proposes an increase in HMW input during flood events since DON_{LMW} increases up to 70%, while in DOC_{LMW} decreases (and increases DOC_{HMW})

Figure 1 summarizes the main changes in DOC and DON during the studied hydrological periods.

Changes in DOC and DON composition

Hydrology influences both DOC and DON dynamics (i.e. DOC:DON ratio) as well as its characteristics. While the dry and following hydrological transition present similar influence on DOC quantitative dynamics (i.e. similar high concentrations), both periods also show different effects on DOM (DOC and DON) properties. While the transition period, and flood events in general, tend to cause and homogenization of the measured characteristics, during the hydrological fragmentation system heterogeneity is amplified. The examined spectroscopic properties (FI, $SUVA_{254}$ and IC:IA fluorescence ratio) reflect this system heterogeneity well (although only $SUVA_{254}$ is correlated with time). Locations with running water present characteristics similar to those observed

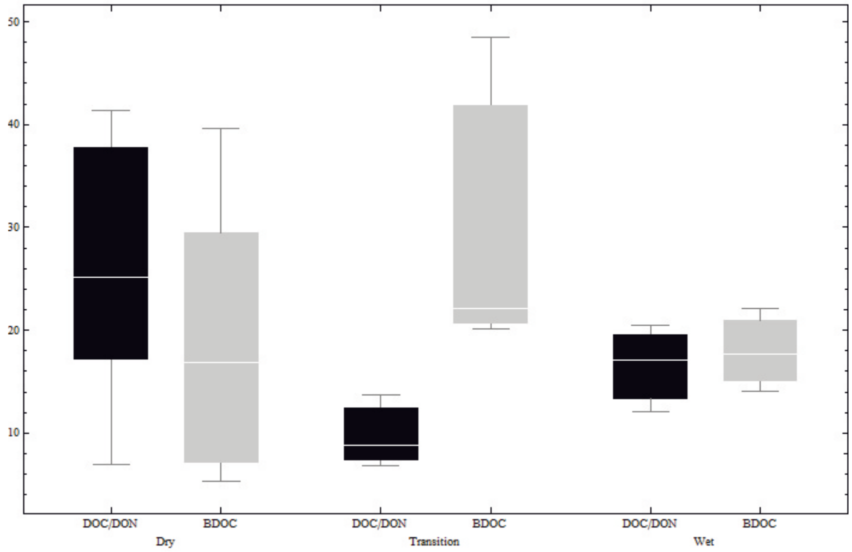


Figure D.1. DOC:DON ratio and BDOC content (%) of stream water DOC during the different hydrological phases. Note that , in this instance, the dry period comprises both drying and fragmentation periods.

during the wet period, while the characteristics of DOM from isolated water ponds widen the range. Overall, during hydrologic fragmentation DOM is predominantly of autochthonous origin indicated by the FI values probably due to the microbial processing in the isolated water ponds. The influence of microbial processing is also suggested by the increase in fluorescence in peak C. Although peak C is usually associated to humic acid fluorescence, it has been found that increased as a result of DOM microbial degradation (Stedmon and Markager, 2005). Therefore, although, it can not be assessed a change in the nature of DOM (it is still humic-like fluorescence), this increase suggests that there is substance transformation. In contrast, in the disconnected riparian ground water the presence of peak B reflecting protein-like substances indicates that different DOM processing is occurring (this high protein based fluorescence was also found in Inamdar 2012). Since BDOC content is lower than in stream, it is likely that a fraction of the processed DOM becomes more refractory and accumulates in this compartment.

During the wet period, according to FI, DOM origin shifts towards a terrestrial origin indicating higher influence of the surrounding system rather than in-stream processing in the water column. During the system reset that is the transition period it is expected an increase in aromatic content (Vidon et al., 2008; Sanderman et al., 2009), low content of humic materials (Hood et al., 2006) and there are inputs of high molecular weight DOM (Maurice et al., 2002; Li et al., 2003). But, our results show that the increase in high molecular weight only is observed in DOC, not in DON, and that although the range of $SUVA_{254}$ values is narrow the absolute values are even lower than those observed during hydrological fragmentation.

During hydrological transition, according to FI values, DOC in surface waters present terrestrial origin while in riparian groundwater the relevance of microbial origin DOC is higher. Romaní et al. (2006) study on Fuirosos DOC shows that, considering MW fractions in stream surface waters, the larger molecules are of terrestrial origin while the smaller fractions present higher FI values. Furthermore, the results showed that after a drought period the FI values of groundwater DOC were similar in all small LMW and MMW fractions to those of stream water indicating a similar origin, while FI in larger DOC (>10 kDa) fractions were higher than in stream water. Therefore, concluding that these FI values suggest that transformation of organic material occurs in the stream-riparian interface.

Parallel studies by Ylla et al., (2010, 2011, 2012), examined the composition of DOM during these hydrological periods discriminating the major types of bio molecules. The results showed that during the drought period the main DOC source were polysaccharides while amino acids gradually disappeared, coinciding with the observed DON depletion. On the other hand, during the rewetting period the input of polysaccharides and peptides was fast, contributing to the DOC and DON concentration peaks. In this period, polysaccharides accounted for 20% of total DOC and a 3% was due to amino acids. In the cited bibliography there is further information on DOM composition not only in the water column but also in the benthic substrates.

Also, changes in DOM and water chemical characteristics during the hydrological fragmentation and the consequences in the bacterial community were studied in Fazi et al (submitted). It caused a decrease in alpha-diversity favouring two phylogenetic groups. One that is reported to degrade aromatic compounds in denitrifying conditions, and that, in consequence could contribute to low nitrate concentration in the isolated ponds and the observed increase in $SUVA_{254}$; and another one which recent studies suggest that prefer depending on autochthonous organic matter rather than allochthonous.

Changes in DOC and DON lability

In Fuirosos, the highest estimates of BDOC content (40-50%) are observed during hydrological transition and in the longest isolated water pools during hydrological fragmentation, two opposite hydrological situations. Furthermore, the high input of bioavailable matter during the hydrological transition requires antecedent dry hydrological conditions. If this requirement is not met, then both DOC concentration and BDOC content remain in the range usually observed during the wet baseflow period (BDOC, 10-15%). But, although it seems that in both situations the effects on availability is similar, as stated previously DOM characteristics during both this period change substantially in terms of origin (terrestrial for hydrological transition, autochthonous during hydrological fragmentation) and aromaticity (low during transition-wet period, higher for drought).

In research in general one of the approaches to study DOC characteristics has been to separate this pool into smaller black boxes using different criteria. Two of the most pervading into scientific literature regarding DOM in natural systems are the chemical criteria that allows discriminating into hydrophilic (or non-humic), hydrophobic (humic) and transphilic fractions; while another is to separate it according to molecular size (for a further information on DOM characterization techniques see McDonald, 2004; and Abbt-Braun, 2004; Nebbioso and Piccolo, 2013). This second criterion led to abundant studies trying to underpin lability to the "structure" represented by size that has spanned a variety of views and opposing conclusions. In 1996, Amon and Benner proposed the size – reactivity continuum model that proposed that smaller molecules were a product of previous degradation of larger molecules and therefore refractory while the larger molecules were more labile. Although it has been supported by other studies (Thóth et al., 2007; Fischer et al., 2002; Kaiser and Sulzberger, 2004; Sachse et al., 2001) its validity has been contested by the results of posterior studies (Marschner and Kalbitz, 2003; and Agren et al., 2008) and even previous (Meyer et al., 1987, Lindell et al., 1995 for instance).

Under this framework, the exploration of DOC properties in this thesis included the estimation of bioavailability of each MW fraction during the drying (although not during hydrological fragmentation), rewetting and wet baseflow periods. The results from chapter 2, considering the differential retention across the stream-

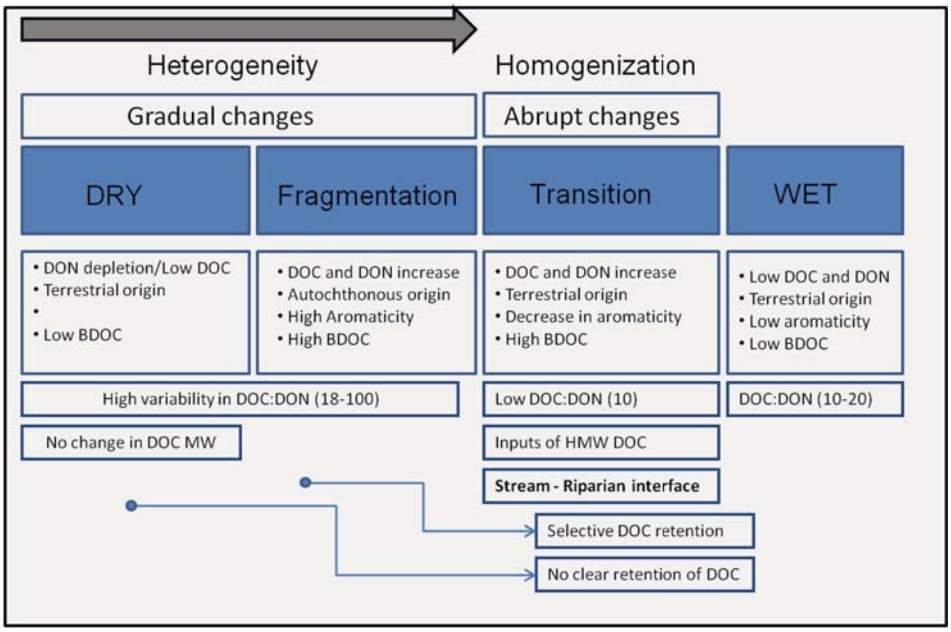


Figure D.2. Summary of main changes of DOC and DON during the different hydrological phases: dry period, hydrological fragmentation, hydrological transition and wet period.

riparian interface as a possible indicator of DOC lability, suggest that DOC_{HMW} might be more labile that smaller DOC MW fractions. In chapter 3, the results for BDOC content show a decrease from high to low molecular weight fractions (21, 17 and 7% for DOC_{HMW}, DOC_{MMW} and DOC_{LMW}, respectively). Although the high variability of these results do not allow establishing a strong conclusion, coupled to the fact that DOC_{LMW} is the MW fraction that showed the most cases of DOC release in incubations, they hint that this trend may be consistent with the conclusions obtained during the study presented in chapter 2 On the other hand, Meyer et al. (1987) proposed that the lability observed in the HMW fractions would be caused by the external complexation of LMW substances to a more refractory humic core.

Furthermore, the refractory nature of DOC_{LMW} is questioned when it is observed that when DOC:DON ratios are lower than 13, its bioavailability remarkably increases. It is important to remark that these DOC:DON values for this MW fraction are only observed during the dry-wet hydrological transition, highlighting the importance of hydrologic events in the biogeochemical processes. This input of labile DOC_{LMW} might be a product of the leachate of leaves (Meyer et al., 1987) that accumulated in the stream bed during the previous dry period. During the drying period, DOC_{LMW} is mainly refractory and accumulates, indicating a probable origin as a by-product from the degradation of HMW substances. In contrast, the DOC:DON values for DOC_{MMW} and DOC_{HMW} are rarely lower than 15, but nonetheless their BDOC content is generally positive and it is not related to the hydrologic period nor antecedent conditions. These results suggest that MW is not the only main lability driver, but that other ecosystemic (hydrologic, climatic) factors must be taken into account. The DOC:DON ratios and BDOC content for each hydrological period is presented in figure 2.

Generally, DON is considered to be labile, both DON_{LMW} (sugars, free dissolved amino acids, nucleotides) and DON substances aggregated in humic substances, although to a lesser degree (Berman and Bronk, 2003). Our results show that BDOC is related to DON concentration rather than to a specific DOC MW fraction or bulk DOC concentration. But it is noticeable that during the hydrological transition, DOC_{LMW} and DON_{LMW} inputs coincide suggesting that this LMW fraction could cause the increase in overall DOC lability. Although, BDON estimates are hard to interpret due to the high variability, Petrone et al. (2009) found that DON bioavailability was mainly related to the hydrophilic fraction (non-humic) and thus the LMW fraction.

Furthermore, the different mobilization of the DON pool during the hydrologic transition and the high BDOC during the hydrological fragmentation suggest that DOC and DON might present slow and fast turnover pools as already pointed out by Brookshire et al (2005), Kaushal and Lewis (2005) and Petrone et al., (2009).

Finally, DOM lability in soils is a topic that has gained strength recently and one of the main conclusions is that lability as a property does not depend on organic matter properties, like recalcitrance and decay rate. But, it depends on the ecosystem properties, on the interactions between organic matter and its environment such as reactive mineral surfaces, climate, soil redox state, compound chemistry and the presence of potential degraders (Schmidt et al., 2011; Kalbitz et al., 2012).

On methodology: Field and laboratory experiments

Usually laboratory experiments are preferred since they allow a methodical control of the conditions surrounding the process being studied. However, observations and experiments performed in the field allow taking into consideration a wider picture of natural processes that can not be reproduced in the laboratory. In the case of DOC dynamics it is difficult to simulate floods and drying periods. For instance, the high and low/dry flow periods occur in a way that previous hydrological conditions become very relevant as shown in the results of chapter 2.

Furthermore, in chapter 2, the examination of riparian ground water and surface groundwater during the hydrological transition can be compared to a perfusion experiment performed with sediment cores under laboratory conditions. But, although in this case the conclusions could be similar on both approaches, in chapter 3, DON laboratory incubations show a very different conclusion from the field observations that point to a depletion both in ground and stream waters during the dry period. In contrast, in laboratory incubations, there is a consistent DON release in all samples and dates, preventing to draw a robust conclusion on DON fate during this hydrological period. Furthermore, laboratory results are in contradiction with previous published (Seitzinger and Sanders, 1997; Stepanauskas et al., 1999, 2000; Kerner and Spitzy, 2001; Petrone et al., 2009; Lonborg et al., 2009; Lonborg and Sondegaard, 2009).

When aiming to study DOC in natural fluvial systems from a temporal series, it is necessary to gather data encompassing all hydrologic variability, from drying to floods, in order to be able discern the possible origin of changes in both concentration and properties and relate it to other factors (precipitation, time between flood episodes, previous hydrological conditions). Since meteorological/hydrologic events are never under the researcher control, there is a considerable time investment in data collecting. For instance, in chapter 1, one of the conclusions indicates that in order to represent all nitrate-Q responses it would be necessary a minimum high frequency time series of 4 years.

The study of hysteretic responses using an R-C plane has been recently adopted into similar studies in other fluvial systems (Strohmeier et al., 2013; Cerro I et al., in press).

In any case, one of the disadvantages inherent to field experiments is that, although the resultant observations are close to what really is happening within the system, reaching to conclusions in the end might be hard since some other unaccounted factor could be affecting the studied processes. Thus, it is necessary a very careful experiment planning/designing phase in order to gather as much useful data as possible.

On methodology: DOM characterization

MW fractions

In order to deepen the knowledge of the role of dissolved organic carbon in fluvial systems (or in any natural system) a further step beyond (or sideways) is to characterize it. The main composition of DOC in aquatic ecosystems is fulvic and humic acids, and a third group referred to as low molecular weight (LMW) substances where fatty acids and other lipids, free amino acids and other substances are included. The first two groups, fulvic and humic acids, are often considered to be refractory. Although these substances might present some common characteristics it might be not enough in order to discriminate the origin, transport and fate of the different substances that are included the black box that is DOC(DOM). Furthermore, it is possible to find many names/groups for different kinds of DOC (Filella et al. 2010), but they are mainly based on operational definitions or a single characteristic (i.e. how that substances were isolated, fluorescent DOM, refractory organic matter, bioavailable dissolved organic carbon…).

In order to further examine the big black box that is DOC, the fractionation into smaller boxes, by molecular weight cut-offs, can be done by means of tangential ultra filtration. From this fractionation, the dynamics of each fraction can be studied (chapter 2), but also can be used as a starting sample to which apply other characterization techniques (spectroscopic, chemical separation, bioavailability determination...). Ultrafiltration presents the inconvenient that the range of recovered DOC at the end of the process is variable between 80 and 120% and that the colloidal fraction and complex aggregations of molecules can be altered. This second inconvenient is also present in the extraction techniques used to separate DOC according to chemical properties (Abbt-Braun 2004; McDonald 2004).

A factor to take into consideration when establishing the sizes of the new DOC boxes through ultra filtration cut-off limits is that different published works might have used similar terms to refer to different cut-offs. For instance, in the works presented herein the LMW fraction is considered to be less than 1 kDa as in other studies (for instance: Stepanauskas et al 1999; Belzile and Guo, 2006; Peduzzi 2008), and HMW encompasses molecules higher than 10 kDa. In contrast, other researchers establish a LMW cut-off at 500 Da (Huguet et al., 2010) and even at 3 kDa (Kerner and Spitzt, 2001). Therefore, obtaining a general vision from different papers might require a cautious approach.

Spectroscopy

Spectroscopy techniques present relatively simple and fast methods to characterize dissolved organic matter. This is usually one of the first propositions one encounters in literature. However, although it is true there are some caveats that must be taken into consideration when applying these techniques in characterizing DOM. In general, the application of these techniques is quite wide and although it is used as a quantitative method in some fields its application to a complex mixture as DOM is difficult (Filella et al., 2009).

The application in order to obtain qualitative information from a DOM sample also presents restrictions since not all the substances considered as part of DOM present fluorescence or absorbance properties. Hence, we are looking at a fraction of the total DOM in a sample, called fluorescent DOM (FDOM) or coloured DOM (CDOM) depending on the property we are examining. In fact, fluorescence and absorbance properties are related but DOM nomenclature is really dependent on the characterizing techniques used. This topic is discussed in depth in Filella et al. (2009).

The second inconvenient is that the samples might require additional manipulations depending on its origin since factors like pH, pollutant concentrations, inner filter effects due to sample concentration, and presence of metals that quench fluorescence signals. Additionally, some of the data, especially excitation – emission matrices, need further processing before the data can be interpreted with confidence. Finally, one of the issues still open is the variability in the obtained data depending on the spectrofluorometer itself. This fact hinders reliability when comparing data from different studies.

Nevertheless, in spite of these considerations in the use of spectroscopic techniques, the obtained data is not exempt of ecological significance that can shed light over DOM biogeochemical processes in natural systems.

DOC characterization through the different methods applied in this thesis, even with its limitations, has been a first step into examining organic carbon changes not only from a quantitative approach. Unfortunately, there is little published literature examining DOC properties, using fluorimetry or any other usually applied analytic technique, in Mediterranean fluvial systems allowing the contrast of our data and establish more grounded and robust generalizations.

Finally, although this thesis is primarily focused on biogeochemical aspects and consequences of the shifts between dry and wet hydrological periods and extreme hydrological events, DOC characterization provides a link, albeit maybe too tenuous, to the biotic aspects. The biotic consequences, especially on the composition of microbial communities and activity, have been studied over the same periods of time as the works presented herein (Romaní et al., 2006; Ylla et al., 2012; Fazi et al., 2013).

CONCLUSIONS

Chapter 1. Diversity and temporal sequences of forms of DOC and NO_3-discharge responses in an intermittent stream: Predictable or random succession?

The most probable C-Q response types represent only a 40% of all cases from the data set. Therefore, the majority of DOC and NO_3-Q responses fall within some low probability C-Q response types.

The dry-wet transition does not present a typical C-Q response. Instead, it promotes the diversity of DOC-Q responses. The 33% of events comprised during this period contribute to the 41% of the total DOC-Q response diversity. On the other hand, it does not enhance NO_3-Q responses.

The contingency analysis reveals signals of periodicity of processes acting at 1-2 years timescale. Therefore, there is a need for generating long pluri-annual hydro-biogeochemical series at high resolution (i.e. frequent sampling intervals).

The most important driver for the succession of NO_3-Q responses is the periodicity of the magnitude of storms (ΔQ) is, while the effect of the seasonal temperature change (i.e., T_{day}), appears more perceptible after the removal of the signal of the weaker and more frequent rain episodes.

The succession of forms of NO_3-Q responses might be coupled to the magnitude of storm events. On the other hand, a large portion of uncertainty is inevitable and it is suggested that a probabilistic modeling approach should replace the deterministic one.

Chapter 2. Effects of the dry-wet hydrological shift on dissolved organic carbon dynamics and fate across the stream-riparian interface

The occurrence of a severe summer dry period favored the mobilization and transport of labile DOC, as shown by the 56% of retention across the stream-riparian interface, during the following hydrological transition period. Furthermore, the DOC retention during this period was preferential from high to low molecular weight fractions. In the case of no apparent hydrological transition all DOC molecular weight fractions were equally conservative.

The chemical data from conservative tracers, TDOC and its molecular weight fractions observed in the ephemeral stream reveal that water flowing through the forest hill slope during a severe rain episode is similar to the stream water during high discharge conditions. This highlights the importance of leached allochthonous DOC from the hill slope forest soils towards TDOC transport in stream water.

The occurrence of abrupt, fast and extreme hydrological events can be used as a natural experiment in order to detect and examine biogeochemical processes due to the amplification of its magnitude.

Chapter 3. Impact of dry-rewetting hydrological cycle on dissolved organic carbon and nitrogen biodegradability and molecular weight distribution in a Mediterranean stream

During both drought and rewetting periods DOC and DON bioavailability are not tied to a MW fraction. During the rewetting period, although there is no clear pattern of bioavailability according to any DOC MW fraction, 50% of cases at which DOC_{MW} release is observed ($BDOC_{MW}<0$) correspond to the LMW fraction.

There is not a clear and consistent input of DOC and DON HMW fractions during the rewetting period. In general, DOC_{MW} dynamics fit within the framework that storm events enhance the input of larger molecular weight DOC fraction (higher than 1 kDa), but DON_{MW} does not.

The hydrologic characteristics of the rewetting period evidence an asynchronous mobilization of DOC and DON.

DOC bioavailability is heavily influenced by DON content rather than molecular weight. Furthermore, bioavailability seems to be regulated by the DOC:DON ratio presenting a threshold value of 13. The most remarkable case is that of DOC_{LMW} that shifts from refractory to bioavailable when its DOC:DON ratio is lower than 13. This is exemplified by the DON depletion during drought causing a decrease in DOC bioavailability and the posterior increase in DOC bioavailability during the rewetting period where there is a high input of DON_{LMW}.

The recurring release of DON during incubations is surprising. First, because this result is not consistent with other published works (Seitzinger and Sanders, 1997; Stepanauskas et al., 1999, 2000; Kerner and Spitzy, 2001; Petrone et al., 2009; Lonborg et al., 2009; Lonborg and Sondegaard, 2009). In second place, because, in clear contrast with laboratory results, field observations show DON depletion both in stream and ground waters.

Chapter 4. Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions

Drought causes a gradual hydrological fragmentation of the fluvial network enlarging the variability of DOM properties, and amplifying the biogeochemical variability of a fluvial system. In coincidence with the findings of Jaffé et al. (2008), this enlargement in the range of DOM descriptors highlight that local scale effects are more relevant on DOM properties that the regional scale.

Sampling sites with lotic water bodies in summer show DOM properties similar to those observed in autumn under base flow hydrological conditions and reflect the prevalence of terrestrial inputs from the surrounding forested hill slope. Meanwhile, in isolated and lentic water bodies, DOM analyses reveal a supplementary contribution of autochthonous organic matter, originated by in situ microbial processes, as consequence of the disruption of the hydrological connection at the stream-catchment interface.

DOC concentration in summer isolated pools presents the same range of variability observed in a previous study during a hydrological dry-wet transition period. Furthermore, BDOC estimated during summer indicate an upper threshold of 40% that is also observed during the rewetting period. This allows asserting that the highest bioavailable DOC concentration can be reached during two ecosystem perturbations of opposite sign.

Although it is expected, as shown, that changes in inorganic solutes in isolated water ponds to be coupled to changes in DOC origin and properties these properties do not covariate with DOC concentration.

In our study, the $SUVA_{254}$ index is the only parameter that shows a positive relationship, although not significant, with the chemical index (CI) that summarizes the aerobic/anaerobic conditions from water ponds. Moreover, considering exclusively the summer isolated water pools, it is the only parameter that is statistically related to the pond isolation time (PIT) which is a measure of the fluvial fragmentation velocity.

Chapter 5. Fluorescence spectroscopy and UV-vis absorbance as tools for DOM characterization

In recent times technological advances have allowed higher resolution and shorter analysis time when using spectroscopic techniques to analyze DOM(FDOM).

Nowadays a wide range of techniques and a posteriori data treatments (PARAFAC, spectral slope analysis, etc.) but there is no best solution for everything. Therefore, choosing the appropriate techniques and fine-tuning is essential.

The collection and storing of samples and the effects on its spectroscopic properties still does not have a universal protocol and there is an ongoing debate. Nonetheless, it seems that the best approach is immediate sample analysis. Storing in the cold and dark seems, in the best of cases, an appropriate and innocuous method for storing samples during a short time.

Similarly, the disparity of results obtained between different fluorimeters prevents a reliable comparison of results between samples analyzed in different laboratories with different equipment.

Informe dels directors de tesi

Andrea Butturini i Francesc Sabater i Comas, professors del Departament d'Ecologia (Universitat de Barcelona) i directors de Tesi Doctoral elaborada per Eusebi Vazquez Garcia amb el títol "Influence of hydrology on dissolved organic matter quantity and properties in a temporal Mediterranean stream".

INFORMEN

Que el treball de recerca fet per Eusebi Vazquez Garcia, com a part de la seva formació com a estudiant de doctorat i inclòs en aquesta tesi doctoral ha donat lloc a tres publicacions i dos manuscrits preparats per a ser enviats a publicacions científiques. A continuació es detallen els article, la informació corresponent dels índexs d'impacte (segons el SCI de la ISI Web of Knowledge) i la participació del candidat a doctor en cadascun d'aquests.

1) Butturini A., Alvarez M., Bernal S., Vazquez E., Sabater F. 2008. Diversity and temporal sequences of forms of DOC and NO3 – discharge responses in an intermittent stream: Predictable or random succession? *Journal of Geophysical Research – Biogeosciences*, 114(G3):3016-3016.

L'índex d'impacte de la revista al 2011 era de 3,021 i el valor mitjà dels darrers 5 anys de 3,441. La publicació *Journal of Geophysical Research – Biogeosciences* ocupa la posició 21 d'un total de 170 publicacions en la categoria *Geosciences/Multidisciplinary* i es troba dins el primer quartil.

El candidat a doctor va participar en les tasques de camp i anàlisi de mostres al laboratori. També va col·laborar en el plantejament del treball, l'anàlisi de les dades i redacció dels resultats i discusió del manuscrit.

2) Vazquez E., Romani A.M., Sabater F., Butturini A. 2007. Effects of the dry–wet hydrological shift on dissolved organic carbon dynamics and fate across stream–riparian interface in a Mediterranean catchment. *Ecosystems*, 10(2):239-251.

L'índex d'impacte de la publicació *Ecosystems* al 2011 era de 3,495 i el valor mitjà dels darrers 5 anys de 4,85. Aquesta publicació ocupa la posició 30 d'un total de 134 publicacions dins la categoria *Ecology* i es troba dins el primer quartil.

En aquesta publicació el candidat a doctor va participar activament en el plantejament dels objectius i disseny experimental, així com en les tasques de camp i laboratori. Es va responsabilitzar de l'escriptura i revisió del manuscrit per a la seva publicació.

3) Vazquez E., Ylla Y., Romaní A.M., Butturini A. Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions.

En aquesta publicació el candidat a doctor va participar activament en el plantejament dels objectius i disseny experimental, així com en les tasques de camp i laboratori. El candidat també va a posar a punt les tècniques de caracterització de la matèria orgànica dissolta. Es va responsabilitzar de la redacció del manuscrit.

4) Vazquez E., Amalfitano S., Fazi S., Butturini A. 2010. Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions. *Biogeochemistry*, 102:59-72.

L'índex d'impacte de la revista al 2011 era de 3,069 i el valor mitjà dels darrers 5 anys de 3,71. La revista *Biogeochemistry* ocupa la posició 35 del total de 205 revistes dins la categoria *Environmental Sciences* (Journal Citation Reports) i es troba dins el primer quartil.

El candidat a doctor va participar activament en el plantejament dels objectius i disseny experimental, així com en les tasques de camp i laboratori. El candidat també va a posar a punt les tècniques de caracterització de la matèria orgànica dissolta, , analitzant la seva relació amb les condicions hidrològiques de sequera. Es va responsabilitzar de l'escriptura i revisió del manuscrit per a la seva publicació.

5) Vazquez E., Ejarque E., Butturini A. Fluorescence spectroscopy and UV-vis absorbance as tools for DOM characterization.

El candidat a doctor va dur a terme les tasques de recerca bibliogràfica i selecció de les tècniques exposades sobre la caracterització espectroscòpica de la matèria orgànica dissolta. Es va encarregar de la redacció del manuscrit.

A més, CERTIFIQUEN

- que cap dels coautors dels articles abans esmentats i que formen part de la Tesi Doctoral d'Eusebi Vazquez Garcia ha utilitzat o té previst utilitzar aquests treballs per a l'elaboració d'una altra tesi doctoral.

Signatura Signatura

Resum en català

Introducció

Carboni orgànic dissolt en sistemes fluvials

El carboni orgànic dissolt (DOC) és una important font d'energia en els sistemes fluvials (Fisher and Likens, 1973; McDowell and Fisher, 1976). El processament i el transport de la matèria orgànica des de la capçalera fins a la desembocadura influencia els nivells tròfics superiors, que estan especialment regulats per microorganismes heterotròfics (Findlay et al., 1993). També contribueix a la regulació del pH, les lligadures amb metalls (metal binding), transport de nutrients i l'atenuació de la llum a la columna d'aigua (Wetzel 2001; Kirk, 1994).

L'origen del DOC i els canvis en concentració i les seves propietats tenen un impacte important en la seva reactivitat química i biològica, afectant d'aquesta manera al funcionament del sistema fluvial (Kaiser et al., 2004; Porcal et al., 2009).

La definició de matèria orgànic dissolta (DOM), o carboni orgànic dissolt com a mesura d'aquesta, es sol fer des d'una perspectiva físico-química mitjançant la definició operacional de tres reserves: la materia orgànica particulada grollera (CPOM; >1 mm), que inclou branques, fulles i troncs; la matèria orgànica particulada fina (FPOM; 1 mm – 0,7 μm); i la materia orgànica dissolta (DOM; <0,7 μm).

Generalment, la DOM s'estima a partir de mesures de carboni orgànic dissolt i comprèn aproximadament el 50% del carboni orgànic total en sistemas fluvials (Thurman, 1985) i més del 90% en medis marins (Wetzel, 2001).

En ecosistemes aquàtics, es considera que l'origen del DOC pot ser tant al·lòcton com autòcton. En el primer cas, normalment, de formació pedogènica, està compost per substàncies produïdes per la descomposició de vegetals per part de bacteris i fongs. En el segon cas, esdevé dins el mateix sistema a partir de la descomposició i l'excreta del plàncton, algues i bacteris (Thurman, 1985; Wetzel, 2001; Filella et al., 2009).

En general, la matèria orgànica en sistemes fluvials és el producte d'ambdòs tipus d'entrada però la matèria al·lòctona que hi entra des de la conca sol ser quantitativament més gran que la fracció autòctona. Les entrades directes més importants a les aigües superficials són les de lixiviat de la fullaraca (McDowell and Fisher, 1976). A les regions forestades, especialment a trams de capçalera, degut a la proximitat del bosc de ribera i la baixa irradiància, a la major part de rieres i rierols hi predominen les entrades de matèria orgànica al·lòctona (Fisher and Likens, 1973), generalment per sobre de 1000 C·m^{-2}·any^{-1}. En canvi, la producció primària és generalment baixa, per sota de 200 g C·m^{-2}·any^{-1} (Caraco and Cole 2003; Mulholland 1997, Sinsabaugh, 1997; Fisher and Likens 1973). Altres aportacions de matèria orgànica al·lòctona es produeixen a conseqüència de la precipitació, escorrentia superficial i moviment d'aigua a través del sòl (Thurman, 1985; Qualls and Haines, 1992; Findlay, 2001). Malgrat que la concentració de DOC a l'aigua de pluja és baixa, de 0.8 to 3 mg·L^{-1} (Thurman 1985; Aitkenhead and Peterson, 2003), es va enriquint progressivament al travessar les capçades dels arbres, lixiviant fulles i altres superficies fins a 10 mg·L^{-1} (Thurman, 1985).

Normalment, la concentració de DOC a sistemes fluvials és d'entre 0,5 a 50 mg·L^{-1}. Varia segons la mida del riu, el clima, la vegetació de la conca i l'estació de l'any (Mullholland, 2003). A rius petits, amb cabals per sota de 100 m3·s^{-1}, la concentració de DOC està entre 1 i 4 mg·L^{-1} (Thurman, 1985).

La concentració de DOC als diferents horitzons del sòl, que poden esdevenir entrades al sistema fluvial, decreix des dels horitzons superficials (horitzó A) als més profunds (horitzó C) i aquesta concentració sol dependre del tipus de bioma (Aitkenhead and Peterson, 2003).

La variació en l'espai de la concentració de DOC al llarg del recorregut del sistema fluvial sol ser de menor magnitud que les alteracions causades per canvis hidrològics (Mulholland, 2003). Els canvis hidrològics poden provocar tant canvis en l'eix temporal com espaial de les fonts de DOC a nivell de conca, fent possible que hi hagi canvis importants en la concentració. Per exemple, durant els episodis de precipitacions hi ha un rentat del DOC del sòl de la zona de ribera cap el riu provocant increments de concentració (Hinton et al., 1998; Hornberger et al., 1994) i poden haver canvis en els fluxes d'aigua, passant de capes de sòl més profunds a superficials, mobilitzant el DOC que s'hi ha acumulat. Al mateix temps, el retard provocat per la connexió entre la zona de ribera i la resta de la conca, depenent de les condicions d'humitat antecedents, pot resultar en una mobilització diferencial del DOC degut a la demanda hidrològica en els sòls de fora de la zona de ribera (McGlynn and McDonnell, 2003).

Transport del carboni orgànic dissolt

La perspectiva històrica en la que els sistemes fluvials eren considerats un enllaç entre els sistemes terrestre i marí i on el DOC era considerat com a recalcitrant, i per tant, un solut conservatiu transportat al llarg del continu fluvial sense interaccions biològiques significatives (Schlesinger and Melack, 1981; Schlesinger, 1999), ha estat qüestionada per estudis recents. Aquests estudis posen de manifest que els sistemes fluvials no tan sols remineralitzen carboni a escala local sinó que també són una font de CO2 cap a l'atmosfera (Richey, 2002; Mayorga, 2005; Cole, 2007). El flux de carboni orgànic dels rius cap els oceans està estimat entre 0,4 – 0,9 Pg C·any-1 (Schlesinger and Melack, 1981; Aitkenhead and McDowell, 2000) però mentre que aquesta flux estimat es manté, altres estudis apunten que l'entrada de carboni des dels sistemes terrestres als rius és de 1,9 Pg C·any-1 i que per tant, el flux cap a l'atmosfera està entre 0,8 i 1,55 Pg C·any-1 (Cole et al., 2007; Battin et al., 2008). Aquest CO2 seria produït per processos de respiració tant a la zona hiporreica com al propi riu (Cole et al., 2007) i processos de fotomineralització (Farjalla et al., 2009).

En les darreres dècades s'ha observat un increment en la concentració de DOC en sistemes aquàtics de zones temperades i boreals (Freeman et al., 2004). Malgrat les dificultats per a fer comparacions regionals (Eimers et al., 2008), s'han proposat diferents mecanismes que podrien explicar aquest increment: augment de la temperatura degut al canvi climàtic (Moore et al., 1998; Freeman et al., 2001; Worrall et al., 2004), augment de CO_2 atmosfèric (Freeman et al., 2004), deposició de nitrogen (Sinsabaugh et al., 2004; Findlay et al., 2005), disminució en la deposició de sulfat (Evans et al., 2006; Monteith et al., 2007), canvis hidrològics (Clark et al., 2005; Worrall and Burt, 2008; Jager et al., 2008). Malgrat no haver-hi una explicació concloent, alguns estudis que examinen els efectes combinats d'algunes de les diferents hipòtesis apunten cap a un pes important del règim hidrològic (Pastor et al., 2003; Eimers 2008). Així doncs, l'impacte de l'alteració del règim hidrològic, ja sigui per activitat antropogènica o canvi climàtic, ha de ser considerat seriosament en els canvis de concentració del DOC, la seva composició i processament.

Processament del DOC en sistemes fluvials

Mentre que una part del DOC pot ser retinguda ràpidament per processos abiòtics (adsorbció a partícules minerals dels sòls i sediments), la retirada del DOC dels sistemes fluvials es produeix principalment per processos biòtics (Findlay and Sobczak, 1996) malgrat ser un procés més lent (Tank et al., 2010).

La incorporació del DOC al·lòcton a la xarxa tròfica aquàtica està facilitada per les comunitats microbianes i l'activitat fúngica. Aquesta via energètica alternativa coneguda com a bucle microbià, tot i ser descrita originalment en comunitats bacterianes de la columna d'aigua en sistemes marins (Azam et al., 1983), també està present en sistemes fluvials, tot i que amb les seves particularitats (Meyer, 1987, 1994): primer, el bucle microbià guanya rellevància per les importants entrades de matèria orgànic al·lòctona; en segon lloc, els bucles planctònic i bentònic es troben estretament lligats; i, en tercer lloc, hi ha menys transferències tròfiques entre les comunitats microbianes i fúngiques i els consumidors. Kerner et al (2003) van descriure una altra via alternativa on es considera la formació abiòtica de macropartícules de DOC que poden ser consumides directament per protozous, descartant el compartiment microbià.

Composició de la matèria orgànica dissolta i labilitat

Encara que la incorporació del DOC a la xarxa tròfica fluvial té lloc mitjançant la comunitat microbiana, no totes les substàncies que s'inclouen dins la denominació DOC poden ser incorporades. Així doncs, tot i que la quantificació, avui dia, del DOC total és fiable i relativament ràpida, la caracterització de les substàncies ha agafat importància. Malgrat això, el gran nombre de substàncies que formen part de la matèria orgànica dissolta (DOM) dificulta molt, per no parlar d'impossibilitat, el seu estudi individual i per tant hi ha diferents mètodes per aproximar-se a la seva caracterització (McDonald et al., 2004, Abbt-Braun et al., 2004). Moltes d'aquestes tècniques permeten classificar o separar el DOC en compartiments més petits que facilita el seu estudi però d'aquesta manera les agrupacions de les substàncies d'estudi apareixen segons les tècniques d'anàlisi.

La DOM, a partir del fraccionament químic, es pot separar en: una fracció no-húmica que comprèn lípids (àcids carboxílics)(5%), aminoàcids lliures (5%), carbohidrats (10%) i àcids hidrofílics (30%). El romanent 50% són les substàncies húmiques on hi trobem els àcids húmics (10%), solubles a qualsevol pH, i els àcids fúlvics (40%), que es tornen insolubles a pH inferior 2 (Thurman, 1985). La humina seria un tercer grup que inclou les substàncies que no són solubles en aigua però que es poden trobar en sòls.

La contribució de les substàncies húmiques és variable, pot canviar amb el temps, sobretot degut a processos hidrològics i pot arribar a representar el 75-80% del DOC en aigües superficials (Volk et al., 1997) i subterrànies (Kaplan and Newbold, 2003).

Estructuralment, les substàncies húmiques poden estar associades a d'altres com aminoàcids, pèptids, substàncies alifàtiques, ions metàl·lics, argiles i òxids de ferro o alumini. Aquestes substàncies associades a un nucli húmic poden alterar in situ les seves propietats biogeoquímiques, a diferència de quan s'examinen aïllades en laboratori.

Una altra aproximació a la caracterització del DOC és fraccionant segons la mida molecular mitjançant la ultrafiltració tangencial (McDonald et al., 2004). Al separar el DOC amb aquesta tècniques s'estableixen arbitràriament unes mides de tall (cut-off). Això fa que a l'hora de comparar entre diferents estudis les diferents fraccions no coincideixin. Tot i així, la fracció per sota de 1 kDa és la que sol ser referida com a baix pes molecular (Low Molecular Weight, LMW), mentre que per sobre, si no hi ha més fraccionament es sol considerar com atl pes molecular (High Molecular Weight, HMW). D'altra banda,en basea aquest criteri es pot continuar fraccionant, tant per sobre de 1 kDa com per sota, donant lloc a fraccions com la mitjana (Medium Molecular Weight, MMW) (Amon and Benner, 1996; Guéguen et al., 2006). La DOM també es pot caracteritzar segons les seves propietats espectroscòpiques. Les tècniques de mesura d'absorbància UV/Vis i de fluorescència s'apliquen en diferents camps dintre i fora de l'ecologia i permeten obtenir dades de manera ràpida, fiables, i relativament econòmica. Cal tenir en compte que no totes les substàncies que s'inclouen dins la DOM presenten aquestes propietats, per tant sobretot examinem una fracció del d'aquesta, principalment la corresponent a les substàncies húmiques, que sol anomenar-se DOM cromofòrica (CDOM) . D'altra banda, s'ha de tenir en compte que substàncies amb propietats espectroscòpiques similars poden tenir d'altres diferents, dificultant aproximacions quantitatives amb barreges complexes de substàncies com la DOM (Filella et al., 2010).

Tenint en compte que el DOC és incorporat sobretot per bacteris, una manera d'estudiar la seva reactivitat biològica és mesurant la seva biodisponibilitat (BDOC). La biodisponibilitat del DOC es veu afectada per nombrosos factors com la temperatura, la llum, la concentració de nutrients, la composició de la comunitat bacteriana, la composició del DOC i les seves propietats químiques i el temps d'exposició (del Giorgio and Davis, 2003). La determinació de la biodisponibilitat del DOC es pot realitzar amb diferents mètodes el que pot dificultar la comparació entre estudis si no s'ha emprat la mateixa tècnica ja que poden donar resultats diferents (McDowell et al., 2006). Un dels més comuns és determinar el percentatge de DOC que incorporen les comunitats microbianes (mètodes com el de Servais et al., 1989). Un altre és mitjançant la determinació de la respiració o el creixement bacterià (Meyer et al., 1994; Del Giorgio and Davies, 2003; Cammack et al., 2004). En sistemes fluvials, el contingut de BDOC pot variar entre l'1% i el 75% del total de la concentració de DOC (Sun et al., 1997).

L'eix definit per la mida molecular del DOC s'ha fet servir com a referència a l'hora d'examinar la seva biodisponibilitat. En general, la fracció no húmica de baix pes molecular ha estat tradicionalment considerada com a làbil, mentre que les substàncies d'alt pes molecular, especialment els àcids húmics i fúlvics es consideren recalcitrants pel seu origen principalment terrestre.

A partir del pes molecular del DOC, Amon i Benner (1996) van proposar el model del continu mida – reactivitat segons el qual la disponibilitat del DOC incrementaria amb la mida molecular, mentre que el DOC de mida petita seria més recalcitrant ja que es considera com a producte de la degradació del DOC més làbil. Tot i així, no ha esdevingut una explicació definitiva. Hi ha d'altres estudis que apunten cap interaccions més complexes del DOC en les vies energètiques dels sistemes naturals, on la reserva de molècules làbils, de mida molecular petita, es reciclarien més ràpid a les comunitats microbianes (Volk et al., 1997). Els processos de fotodegradació també afectarien a la disponibilitat del DOC al trencar molècules però les conclusions sobre si la biodisponibilitat augmenta o disminueix varien en diferents estudis (Miller and Moran, 1997; Wiegner and Seitzinger, 2001; Obernosterer et al., 2004)

En general, la incorporació més lenta de les substàncies húmiques per part de la comunitat bacteriana es veuria compensada per l'alta concentració en la que es troben a l'aigua (Wetzel, 2005). A més, Volk et al (1997) van determinar que un 30% dels carbohidrats i aminoàcids es trobaven complexats a substàncies húmiques. Per tant, la concentració d'aquests substàncies no prediu necessàriament la biodisponibilitat del DOC.

La concentració de DOC a l'aigua freàtica de la zona de ribera és normalment més baixa que la de les aigües superficials, però tot i així permet el creixement bacterià, indicant que és biodisponible. A més, a mida que el DOC es va transportant per vies (flowpaths) hiporrèiques, la seva concentració va disminuint (Findlay et al., 2001). Així doncs, és probable que les entrades de DOC a les aigües superficials des de la zona hiporreica ripariana sigui baixa i de material poc biodisponible i que l'esgotament d'aquest DOC depengui principalment del metabolisme bacterià més que de la concentració inicial o del temps de residència (Sobczak i Findlay, 2002).

Hidrologia i DOC

El clima mediterrani es troba principalment a la conca del mar mediterrani (latitud 30-45ºN), a la costa nord-oest del pacífic a Amèrica (latitud 31-41ºN), a la costa central de Xile (32-41ºS), a l'oest de la regió del Cap a Sudàfrica (latitud 32-35ºS) i a zones del sud i oest d'Austràlia (latitud 32-38ºS) (Gasith and Resh, 1999). La precipitació anual varia d'àrid (menys de 200 mm) a humit (més de 1000 mm), tot i que la majoria de regions es consideren semi àrides amb precipitacions que oscil·len entre 200 i 500 mm. La distribució dels episodis de precipitació es troba subjecte a una forta estacionalitat i una elevada variabilitat interanual. Les pluges es solen concentrar en els períodes de tardor i hivern.

Aquesta estacionalitat de precipitacions condiciona fortament el règim hidrològic a les àrees mediterrànies i causa un estrés per dèficit d'aigua degut a la diferència entre l'alta evapotranspiració i les baixes precipitacios durant períodes de temps més o menys extensos.

La hidrologia, com a producte del clima i de les característiques geomorfològiques de la conca, és un dels principals factors que influeixen en la concentració del DOC i els processos en els que hi intervé. El sistemes fluvials es troben subjectes a dos tipus principals de pertorbacions hidrològiques naturals: crescudes/inundacions i sequeres (Lake, 2000). Així doncs, els rius temporals es caracteritzen per cicles entre períodes secs i humits y la complexa dinàmica hidrològica a l'eix longitudinal del riu que, al seu torn, influencia les comunitats biòtiques i el processament de nutrients i matèria orgànica (Larned et al., 2010). Durant els episodis de crescuda (inundació i posteriors cabals alts) hi hauria un transport de soluts i material orgànic particulat cap a la desembocadura mentre que durant les èpoques de cabal baix, i fragmentació hidrològica, hi hauria una acumulació de material i el processament més lent d'aquest. Per tant, l'eficiència en el processat de la matèria orgànica augmentaria amb el nombre de cicles de transport i acumulació (Larned et al., 2010).

Els efectes dels períodes de sequera han estat menys estudiats que el de les crescudes i la major part dels estudis es centren en la biota (Gasith and Resh, 1999; Lake 2000; Stanley et al., 2010; Gaudes et al., 2010) mentre que els efectes en aspectes biogeoquímics i qualitat de l'aigua són menys abundants (Dahm et al., 2003)

Sequera

De fa temps, ha estat difícil de trobar una definició que inclogui els diferents aspectes d'una sequera (Lake, 2000; Smakhtin, 2001; Humphries and Baldwin, 2003; McMahon and Findlayson, 2003). Una de les principals raons és que factors com temps de retorn, intensitat, duració i les tendències a llarg termini dels períodes de cabal baix són específics de cada regió i dels períodes de temps que s'analitzin (Humphries and Baldwin, 2003). A més, s'ha de tenir en compte que les conseqüències del aspectes climàtics no es tradueixen tan sols en conseqüències hidrològiques i biològiques sinó que també inclouen factors socioeconòmics. Així doncs trobem definicions de sequera meteorològica, d'humitat del sòl, a l'agricultura, hidrològica, ecològica i socioeconòmica (Smakhtin, 2001).

El 1965, Palmer va definir sequera com "un interval de temps, generalment amb una duració de l'ordre de mesos o anys durant el que la reserva d'humitat en un lloc determinat està per sota de la reserva d'humitat climàticament adequada". Així doncs, segons aquesta definició la identificació d'un període de sequera serà diferent segons la regió i segons el que s'entengui per una reserva d'humitat o precipitació adequada, dificultant la comparació entre llocs i temps diferents.

Considerant només sistemes fluvials, Humphries i Baldwin (2003) defineixen sequera com "a un període impredictible de cabal baix amb una duració, extensió o intensitat infreqüent". D'altra banda, Lake (2003) considera una sequera com a "una pertorbació en la que l'entrada d'aigua, el cabal i la disponibilitat d'aigua cauen en nivells molt baixos per un període llarg de temps". A més, Lake, classifica les sequeres en estacionals, que serien freqüents i predictibles, provocant cabals baixos o l'assecat total del llit del riu, i una sequera supraestacional que seria impredictible i que té lloc en l'escala de dècades, aproximant-se més a la definició més àmplia de Palmer (1965). En el cas d'aquesta tesi, sequera queda definit segons la sequera estacional de Lake.

Els efectes indirectes de la sequera inclouen el deteriorament de la qualitat de l'aigua, l'alteració dels recursos tròfics i canvis en l'estructura i la força de les interaccions interespecífiques (densitat, mida poblacional, estructures d'edats, composició de la comunitat i diversitat) (Lake, 2003).

Els efectes directes de la sequera en sistemes fluvials son la pèrdua d'aigua, la disminució dels nivells d'aigua al freàtic, la disminució del cabal i la fragmentació hidrològica que es reflexa en una pèrdua de connectivitat tant en l'eix longitudinal com entre aigües superficials i hiporrèiques i freàtiques (eix vertical) i entre el riu i la zona de ribera (eix lateral) (Smakhtin, 2001; Lake, 2003; Bond 2008). Aquesta fragmentació hidrològica no es produeix al mateix temps en el continu fluvial, sinó que pot augmentar gradualment a mida que s'allarga la sequera i varia segons el volum d'aigua i la morfologia del canal (Bond, 2008). La fragmentació hidrològica causa l'aparició de basses d'aigua aïllades depenent de la geomorfologia del llit del riu. Aquestes basses poden tenir duracions breus però en zones ombrívoles i/o amb substrat impermeable poden romandre fins el restabliment hidrològic (Stanley, 1997; Lake, 2003).

La fragmentació hidrològica impedeix el transport aigües avall de nutrients inorgànics, material orgànic (particulat o dissolt) i de biota. En conseqüència podem trobar diferents condicions a les bases aïllades, esdevenint medis lèntics individuals (Lake, 2005).

Inundacions i crescudes

Les inundacions/crescudes són una pertorbació en pols, més predictibles que les sequeres i amb uns efectes molt aparents i de fàcil observació.

En sistemes àrids o semi àrids, els episodis de pluja estimulen l'activitat biològica, generant reserves de biomassa, propàguls i matèria orgànica, facilitant així respostes ràpides de l'ecosistema a episodis de precipitació posteriors (Collins et al., 2008). A més, la magnitud de la precipitació influencia el balanç de carboni ja que la respiració microbiana respon a polsos de baixa intensitat (Huxman et al., 2004).

El període durant el que augmenta el cabal i hi ha un restabliment de la connexió hidrològica de la xarxa fluvial és referit com període de transició hidrològica (Butturini et al., 2002). Durant aquest període també té lloc la reactivació de l'activitat biològica degut a l'entrada i augment de la disponibilitat de nutrients i matèria orgànica acumulats durant el període de sequera (Collins et al., 2008; Artigas et al., 2009).

Durant els episodis de precipitació, la concentració de DOC augmenta, però el seu origen canvia segons la intensitat de la precipitació, les condicions prèvies d'humitat i el temps transcorregut des de l'anterior episodi de precipitació.

L’increment de DOC durant el període d’augment de cabal és principalment degut al rentat i escorrentia superficial de la zona ripariana, mentre que, posteriorment, durant la disminució del cabal des del màxim assolit, les entrades de DOC provinents de la conca són més importants (McGlynn i McDonnell, 2003). Així doncs, dins d’una conca trobem diferents orígens de DOC a les aigües superficials del riu: capes superficials del sòl superficial riparià, sòls de la conca, freàtic, escorrentia superficial i aigua del sòl que poden ser mobilitzats en diferents moments dels episodis en pols (Katsuyama et al., 2002; Hood et al., 2006).

Els factors que regulen la magnitud de l’increment inclouen les condicions d’humitat prèvies i la magnitud de l’episodi de precipitació (Turgeon and Courchesne, 2008), mentre que el cabal per si sol no és un bon predictor (Bernal et al., 2002). Una aproximació a través de la modelització d’Acuña et al. (2010) va concloure que els efectes de les crescudes en el balanç de carboni depenia de l’època de l’any. Així doncs, segons el model predictiu, els efectes serien poc importants a principis de primavera però la seva influencia augmentaria considerablement durant la tardor (durant o poc després de la transició hidrològica).

Els episodis de precipitació no només causen un increment quantitatiu del DOC sinó també un canvi en la composició del DOC que es detecta a les aigües superficials del riu. En general, hi ha un increment del contingut de substàncies aromàtiques (Vidon et al., 2008; Sanderman et al., 2009) i la contribució de DOC d’alt pes molecular (Maurice et al., 2002; Li et al., 2003). D’altra banda, l’abundància de les substàncies no-húmiques disminuiria (Hood et al., 2006). Una de les conseqüències d’aquests canvis és que la fracció biodisponible del DOC que es mobilitza és moderadament més alta que durant els períodes de cabal basal (Volk et al., 1997; Kaplan and Newbold, 1995; Buffam et al., 2001; Don et al., 2005). D’altra banda, altres estudis ofereixen conclusions oposades on s’observen disminucions de fins al 50% en contingut de BDOC (Wiegner et al., 2009). Tot i així, l' increment en contingut de BDOC no implica necessàriament un increment en creixement bacterià mentre que en algunes conques el contingut en BDOC depèn de paràmetres estacionals més que de diferències, en una escala de temps petita, enl'activació de diferents camins dels fluxes d'aigua (Buffam et al., 2001).

Estudis centrats en canvi climàtic assenyalen un alteració en la temporització, freqüència i magnitud de les precipitacions i temperatures més càlides en sistemes àrids i semi àrids (Lohse et al., 2009) i, per tant, el rol dels sistemes fluvials en el cicle global del carboni podria alterar-se.

La hidrologia, com a un dels principals factors que influencien els cicles biogeoquímics en sistemes fluvials, ha sigut objecte d'una important recerca en les darreres dècades, especialment des de la perspectiva de l'exportació de nutrients en diferents àrees climàtiques. No obstant, a diferència dels nutrients inorgànics com el fòsfor i el nitrogen, la relació entre el DOC i la hidrologia no s'ha estudiat amb intensitat fins recentment. L'aproximació a l'estudi del DOC es pot realitzar en dos fronts: un quantitatiu, més accessible i on es centren una major part d'estudis; i un altre de quantitatiu, centrada en les propietats del DOC. Així doncs, és necessària una conciliació entre els canvis qualitatius del DOC que poden ocórrer en sistemes naturals i la influencia que els factors climàtics podrien tenir en aquest component bàsic en l'ampli camp del funcionament dels ecosistemes.

Objectius

L'objectiu principal d'aquesta tesi és estudiar els efectes quantitatius i qualitatius del cicle sec-humit sobre la materia orgànica dissolta. A la conca de Fuirosos, el carboni orgànic dissolt (DOC) ha estat estudiat majoritàriament des d'una perspectiva quantitativa, mancant l'aproximació als canvis en la seva composició segons els canvis hidrològics.

El primer capítol té com a objectiu aprofundir en la dinàmica de les concentrancions de DOC i nitrat durant els episodis de precipitació. Els tres capítols següents volen iniciar una visió sobre la composició del DOC i el seu paper en un sistema fluvial mediterrani. Finalment, el darrer capítol, preten ser una introducció a les tècniques espectroscòpiques que solen aplicar-se en la caracterització de la DOM. No es tracta d'una revisió detallada sinó d'una aproximació general, un punt d'inici.

El capítol inicial amb el títol **"Diversitat i seqüències temporals de formes de respostes DOC i NO_3 – Cabal en una riera intermitent: successió aleatòria o previsible?"** ofereix un anàlisi de les respostes del carboni orgànic dissolt i del nitrat a augments de cabal i com episodis anteriors de cabal alt poden afectar-ne els posteriors, establint així una seqüència predictible de respostes C – Q. Els objectius principals eren:

a) Explorar la diversitat de respostes de DOC i nitrat – Q en termes de dispersió en un eix de coordenades en funció dels canvis de concentració (tendència) i forma de la resposta d'histèresi (lineal/no lineal).

b) Explorar la successió temporal dels tipus de resposta C – Q a partir de l'anàlisi de periodogrames de contingència.

c) Determinar si la possible detecció de cicles de respostes C – Q es superposa amb els cicles de les variables ambientals que caracteritzen les principals condicions hidrològiques i climàtiques de la conca (magnitud dels episodis de tempesta, humitat prèvia a cada episodi de tempesta i canvis estacionals en la temperatura).

El segon capítol, **"Efectes de la transició hidrològica entre el període sec i humit sobre la dinàmica i disponibilitat del carboni orgànic dissolt a la interfase riera – ribera en una conca mediterrània"** examina, a partir de dades de camp, la influència de les condicions hidrològiques antecedents a la dinàmica i disponibilitat del DOC a la interfase riu – ribera. S'explora la influencia d'aquestes condicions hidrològiques antecedents comprant els canvis en la concentració i la distribució en el pes molecular del DOC a les aigües superficials i a l'aigua freàtica de la zona de ribera, amb un èmfasi especial a la transició hidrològica després de condicions de sequera.

a) Caracteritzar la distribució del DOC segons el pes/mida molecular (Baix: <1kDa; Mig: 1-10 kDa; Alt: 10-100 kDa; Molt Alt: >100 kDa).

b) Examinar les possibles diferències en fluxos i disponibilitat de cada fracció molecular del DOC.

c) Determinar el paper de la interfase riu – zona ripariana en els fluxos de cada fracció molecular del DOC, especialment durant la transició hidrològica.

El capítol 3, **"Impacte dels cicle hidrològic entre periode sec i humit a la biodisponibilitat a les fraccions moleculars del carboni orgànic dissolt en una riera mediterrània"**, és una continuació del capítol anterior. Està centrat en els canvis quantitatius i qualitatius del DOC durant el període sec, des de primavera(disminució de cabal) a l'estiu (fragmentació hidrològica), i la posterior restauració de la connectivitat hidrològica durant el període de transició hidrològica.

Els objectius específics eren:

a) Caracteritzar la DOM durant el període sec i humit en termes de DOC, DON o la ratio DOC:DON i la seva distribució entre tres fraccions de pes molecular (Baix: <1kDa; Mig: 1-10 kDa; Alt:>10 kDa).

b) Determinar fins on arriba la influencia del contingut de carboni i nitrogen, la mida molecular i les interaccions (millor: les combinacions) d'aquestes característiques en la biodisponibilitat de la DOM.

c) Aprofundir en el paper dels diferents compartiments hidrològics (aigua subterrània de ribera i aigües superficials) en els canvis de concentració i composició del DOC i el DON.

El capítol 4, **"Composició de la matèria orgànica dissolta en un sistema fluvial mediterrani fragmentat durant un període de sequera intensa"** es centra en el període generalment menys explorat. Als capítols anteriors, l'aproximació al període sec es va iniciar en els primers estadis, des de la disminució de cabal a la fragmentació hidrològics a nivell de tram. Dades del treball anterior suggerien que la fragmentació hidrològica no era tan sols un pas previ a la desaparició de l'aigua superficial sinó que es produïen nous canvis en les característiques de l'aigua i el DOC. Així doncs, aquest quart capítol intenta donar una visió més complerta dels processos biogeoquímics del DOC en una conca mediterrània de manera que:

a) Es van caracteritzar els canvis graduals que pateix la DOM durant la fragmentació hidrològica a nivell de conca.

b) Es va establir una escala temporal fent servir el temps d'aïllament de cada bassa d'aigua romanent.

c) Es va caracteritzar les mateixes propietats de la DOM durant el següent període humit per a establir un rerefons de referència als canvis observats durant el període sec.

Finalment, amb el títol **"Espectroscòpia de fluorescència i absorbància UV-vis com a eines per a la caracterització de la matèria orgànica dissolta"**, el cinquè capítol ofereix una mirada general a algunes de les tècniques d'espectroscòpia aplicades en la caracterització de la DOM. Aquest capítol es centra sobretot en la varietat d'aplicacions més que a un anàlisi en profunditat d'algunes tècniques concretes, proporcionant una senzilla revisió i una primera aproximació a aquestes tècniques per a qui se les trobi per primer cop, ja que la seva aplicació pot no ser tan directa com podria ser aparent a primera vista. D'altra banda, més enllà de dificultats en la seva aplicació, la falta d'uniformització de protocols pot dificultar la comparació de dades presentades en la literatura científica.

Capítol 1

Diversitat i seqüències temporals de formes de respostes DOC i NO3 – Cabal en una riera intermitten: successió aleatoria o previsible?

Andrea Butturini, Marta Álvarez, Susana Bernal, Eusebi Vázquez i Francesc Sabater

Journal of Geophysical Research, vol 113:G03016

Els episodis de tempestes tenen importants implicacions en els cicles biogeoquímics a escales locals i regionals i ofereixen una excel·lent oportunitat per estudiar el funcionament hidrològic i biogeoquímic de conques fluvials. Les respostes concentració – cabal (C-Q) només han sigut estudiades en detall durant breus períodes de temps o en uns pocs episodis seleccionats. En conseqüència, és difícil quantificar quina és la diversitat de les respostes C – Q en un sistema hidrològic i és impossible determinar si aquestes respostes segueixen una seqüència que es pot predir o no. Tenint en compte aquestes mancances, en aquest estudi s'analitza la variabilitat del les entrades de carboni orgànic dissolt (DOC) i nitrat (NO3) durant tempestes en una detallada sèrie temporal de 4 anys d'una riera mediterrània.

Cada resposta DOC i NO_3 – Q es sintetitza en dos descriptors: la tendència (ΔC; dilució/rentat/sense canvis) i forma (ΔR; resposta lineal/no lineal). Els resultats mostren que les respostes C-Q es distribueixen àmpliament a les dues dimensions del continu ΔR vs. ΔC. A més, la successió temporal de les formes de les respostes DOC i NO_3 – Q segueixen un patró a l'atzar i només la dinàmica del descriptor $\Delta R_{(NO3)}$ presenta periodicitat. Les respostes DOC – Q corresponents a la transició hidrològica contribueixen a l'augment de la diversitat d'aquestes, mentre que les respostes NO_3 – Q no fan augmentar la diversitat de respostes observada.

El conjunt de dades a llarg termini mostra que no és possible predir amb una precisió raonable les propietats de les respostes DOC i NO_3 – *Q*. Així doncs, no existeix una resposta C – *Q* típica al nostre lloc d'estudi. L'elevada diversitat de respostes ha de ser tractada des d'una aproximació probabilística que permeti copsar la complexitat del funcionament hidro-biogeoquímic específic de cada conca.

Capítol 2

Efectes de la transició hidrològica entre el període sec i humit sobre la dinàmica i disponibilitat del carboni orgànic dissolt a la interfase riera – ribera en una conca mediterrània

Eusebi Vázquez, Anna M. Romaní, Francesc Sabater i Andrea Butturini

Ecosystems,10(2):239-251

La interfase riera – freàtic riparià, caracteritzada per una hidrologia dinàmica i complexa, és unimportant punt de control pels fluxes de nutrients i el processat de matèria orgànica entre els sistemes aquàtic i terrestre. Les alteracions previstes en el règim de cabals a la zona climàtica mediterrània fa necessari entendre els efectes de les abruptes transicions hidrològiques entre condicions seques i humides en el transport i disponibilitat del carboni orgànic dissolt (DOC) en aquesta interfase.

En aquest estudi, s'ha examinat els canvis en concentració del DOC total (TDOC) i en quatre fraccions de pes molecular (<1 kDa,1–10 kDa, 10–100 kDa, >100 kDa) a les aigües superficials i a l'aigua subterrània de la zona de ribera durant les tardors del 2003 i 2004. Els dos períodes d'estudi es caracteritzen per presentar diferents condicions hidrològiques prèvies: durant l'estiu del 2003, el flux d'aigua superficial va quedar interromput per una fase de sequera mentre que l'any 2004 va haver un flux d'aigua durant tot l'any.

Comparant ambdòs períodes d'estudi es posa de manifest que l'abrupta transició hidrològica entre el període sec i humit amplifica l'intercanvi d'aigua a l'interfase riera – freàtic riparià i afavoreix la retenció del TDOC fins a un 57%. A més, l'eficiència de retenció en aquesta interfase és força diferent segons la fracció de pes molecular del DOC. Les fraccions moleculars més grans de 10 kDa van presentar retencions de més del 70%, mentre que la fracció més petita (menys de 1 kDa) no s'observava que fos retinguda, essent pràcticament conservativa.

Capítol 3

Impacte dels cicle hidrològic entre període sec i humit a la biodisponibilitat a les fraccions moleculars del carboni orgànic dissolt en una riera mediterrània

Eusebi Vázquez, Irene Ylla, Anna M. Romaní i Andrea Butturini

Els sistemes fluvials mediterranis es troben subjectes a cicles que alternen entre un període de sequera i un període humit que influencien els processos biogeoquímics. Aquests períodes extrems també modifiquen la direcció i la magnitud dels fluxos laterals d'aigua entre les aigües superficials i l'aigua freàtica de la zona de ribera. Això fa que aquests períodes siguin l'equivalent a un experiment natural que ens permet explorar com afecten els canvis hidrològics abruptes als fluxos de matèria orgànica dissolta (DOM) i la seva composició a través de la interfase aigua superficial – aigua freàtica ripariana.

En aquest estudi la variabilitat de la DOM a nivell de composició i concentració, durant aquest cicle de sequera – període humit, s'examina segons la concentració del carboni orgànic dissolt (DOC), el nitrogen (DON), la ratio DOC:DON i la biodisponibilitat del DOC (BDOC) i del DON (BDON) tant per al conjunt de la mostra d'aigua com per a tres fraccions de pes molecular (MW) (Alt (HMW): >10 kDa; Mig (MMW): 1-10 kDa i Baix (LMW): <1 kDa).

Els resultats mostres que durant el període sec les diferències entre les característiques del DOM entre l'aigua superficial i la del freàtic riparià són mínimes ja que encara hi ha connexió entre ambdós compartiments hidrològics, mentre que durant la transició hidrològica entre període sec i humit, el compartiment riparià esdevé un "punt calent" biogeoquímic per a la DOM.

Considerant tot el període d'estudi, només el DON presenta canvis significatius en la contribució de les diferents fraccions moleculars. Els canvis en la disponibilitat del DOC (BDOC) no estan relacionats amb una única fracció molecular. Tot i així, el BDOC està positivament relacionat amb la concentració de DON i inversament relacionat amb la ràtio DOC:DON, posant de manifest que el DON és un element determinant en la disponibilitat de la DOM. A més, la ràtio DOC:DON presenta un valor llindar, 13, pemet discriminar la fracció molecular petita entre moments en la qual és làbil i moments en la que és refractària. Aquests canvis de labilitat en la fracció molecular petita es troben lligats als canvis hidrològics: làbil durant la transició hidrològica i refractària durant el periode sec. En conseqüència, l'estudi posa en relleu la influencia del DON sobre la labilitat de la DOM i reafirma la necessitat d'explorar amb més profunditat el paper del DON en els sistemes fluvials.

Capítol 4

Composició de la matèria orgànica dissolta en un sistema fluvial mediterrani fragmentat durant un periode de sequera intensa

Eusebi Vázquez, Stefano Amalfitano, Stefano Fazi i Andrea Butturini

Biogeochemistry, 102:59-72

A les regions mediterrànies, la sequera és un dels principal factors que influeixen en els sistemes fluvials, causant un canvi de condicions lòtiques a lèntiques, iniciant una fragmentació gradual en l'eix hidrològic longitudinal així com una alteració important de les característiques químiques de l'aigua. Tot i així, des d'una perspectiva biogeoquímica, hi ha pocs coneixements sobre com i fins a quin punt els periodes de sequera poden modificar les propietats químiques i la composició de la matèria orgànica dissolta (DOM). En aquest treball, s'examina la variabilitat de les propietats de la DOM en un sistema fluvial fragmentat fent servir (a) la ràtio entre el carboni i el nitrogen orgànic dissolts (DOC:DON); (b) la biodisponibilitat del DOC (BDOC); i (c) les propietats òptiques de la DOM (índex SUVA, índex de fluorescència, matrius d'excitació-emissió). Les mostres per analitzar l'aigua i la DOM es van recollir a diferents basses aïllades, originades en temps diferents, i a dos localitzacions on encara hi havia un lleuger flux d'aigua. La informació obtinguda es va comparar amb les dades recollides durant el següent periode humit, quan el continu fluvial va quedar restablit.

L'anàlisi de les dades va mostrar que les característiques de la DOM durant la sequera eren diferents de les observades a la tardor. Tot i així, les mostres que es van recollir a l'estiu a les localitzacions on hi havia aigua en moviment presentaven característiques més properes a les de les mostres de tardor, posant de manifest la predominància d'entrades de matèria orgànica d'origen terrestre. Durant la fragmentació hidrològica es va observar un increment gradual en la contribució de

DOM d'origen autòcton a mida que augmentava el temps en el que les basses havien quedat aïllades i les condicions químiques passaven de oxidades a reduïdes segons l'índex CI que s'obté calculant la ratio entre la concentració d'oxigen dissolt i amoni. La fragmentació del continu fluvial genera una sèrie de localitzacions amb gran activitat biogeoquímica, revelant que els períodes de sequera amplifiquen l'heterogeneïtat de les qualitats de la matèria orgànica en els sistemes fluvials.

Capítol 5

Espectroscòpia de fluorescència i absorbància UV-vis com a eines per a la caracterització de la matèria orgànica dissolta

Eusebi Vázquez, Andrea Butturini

Aquest capítol és una revisió de les tècniques d'espectroscòpia d'UV-vis i fluorimetria. En els darrers anys aquestes tècniques han esdevingut generalitzades en els esforços de caracterització de la matèria orgànica dissolta (DOM), presentant com a virtuts principals el ser mètodes ràpids, que permeten obtenir informació amb validesa a nivell ecològic en poc temps, i que a més són relativament econòmiques en comparació a d'altres tècniques que emprades en la caracterització de la DOM. Val a dir que aquestes tècniques només caracteritzen una part de la DOM que interacciona amb la llum i que, entre d'altres acrònims, sol ser anomenada CDOM (per matèria orgànica dissolta cromofòrica).

D'altra banda, presenten l'inconvenient que no hi ha uns protocols generals per a la seva aplicació, ni en el tractament previ de les mostres, malgrat que en els darrers dos o tres anys s'està arribant a un consens a partir del que es desprèn de treballs publicats.

Així doncs, aquest capítol pretén ser una breu introducció de les diferents mesures i índexs amb informació útil a nivell ecològic que es pot obtenir, sense aprofundir especialment en cap d'ells. És a dir, mostrant el ventall de possibilitats existent i quines consideracions s'ha de tenir en compte en lloc de revisar quina és la informació ja coneguda sobre la DOM a partir de l'aplicació d'aquestes tècniques. En aquest sentit, dins el text es troben referències a articles que proporcionen aquest tipus d'informació, combinat amb el d'altres tècniques de les que el present text no tracta (per exemple, Nebbioso i Piccolo, 2013).

El capítol es divideix en dos parts, una que es centra en mesures d'absorbància de llum ultraviolada i visible, i una altra que examina les propietats fluorescents de la DOM.

En la primera part es tracta la determinació de l'aromaticitat de la DOM a partir de l'absorbància específica a 254 nm (Weishaar et al., 2003) i la informació que s'obté de l'absorbància a d'altres longituds d'ona com: a320, a340, a375, a400, a420 relacionats amb la concentració de carboni orgànic dissolt. Els índexs E2/E3 (a250/a365) i E3/E4 (a300/a400) es correlacionen amb la mida molecular, aromaticitat i grau d'humificació. En aquesta part també es tracta del pendent espectral i es presenten les correccions proposades per Helms et al., (2008).

A la part de fluorescència s'expliquen quins són els diferents factors que s'ha de tenir en compte abans d'analitzar la mostra com el pH i la temperatura, així com la necessitat de posar a punt el fluorímetre fent les correccions pertinents.

En general, amb un fluorímetre es poden obtenir espectres d'emissió, d'excitació o sincrònics (variant tant la longitud d'ona d'emissió com d'excitació). Si aquests espectres es concatenen obtenim el que es diu una matriu d'Excitació – Emissió (EEMs) que és tridimensional ja que a cada parella d'excitació i emissió i li correspon un valor de fluorescència. A partir dels espectres d'excitació i emissió es poden calcular diferents índexs com el Fluorescence Index (FI, McKnight et al., 2001) que dona informació sobre l'origen de la DOM, si és d'origen terrestre o microbià; el Biological Index (BIX, Huguet et al., 2009) que indica si la DOM és d'origen recent; i el Humification Index (HIX, Zsolnay 1999; Kalbitz 1999) que dona informació sobre el grau d'humificació.

Pel que fa a les EEMs es mostra quines són les principals àrees amb pics de fluorescència i quines propietats de la DOM es posen de relleu: els pics A i C indiquen la presència de substàncies húmiques (àcids fúlvics per al pic A i àcids húmics corresponen al pic C), els pics T i B corresponen a substàncies similars a les proteïnes, i el pic M indica presència de DOM procedent d'activitat microbiana (Parlanti et al., 2001; Stedmon and Markager, 2005).

A més de la identificació visual dels pics en una EEM, aquesta també pot ser analitzada estadísticament amb mètodes multivariants. EN aquest capítol es parla de dos d'aquests mètodes: hi ha aproximacions mitjançant l'anàlisi de components principals (PCA) que no acaba d'agradar a part de la comunitat científica pel seu tractament en dos dimensions d'informació tridimensional. El mètode que ha esdevingut força prominent ens els darrers anys és el "parallel factor analysis" o PARAFAC.

Discussió general

Les implicacions dels cicles biogeoquímics, a escala local i regional, en els sistemes fluvials ha sigut objecte de recerca de fa temps. Les característiques del clima mediterrani causen l'aparició d'una pertorbació natural que en estudis a zones de clima més humit sol ser rarament considerat (o present): els periodes estacionals de sequera. Tot i que aquest període sec i les fortes crescudes provocades per episodis de precipitació intensa són pertorbacions de signe oposat, ambdues ofereixen una oportunitat excel·lent per a estudiar el funcionament hidro-biogeoquímic de conques fluvials.

L'estudi dels episodis de tempesta mostren que els diferents soluts a l'aigua presenten un ampli ventall de respostes i que per caracteritzar tot aquest rang és necessari obtenir una serie temporal prou llarga que inclogui tots els possibles events hidrològics. D'aquesta manera, el primer capítol estudia la variabilitat en les respostes de nitrat i DOC – Cabal (Q) durant una serie temporal de quatre anys. Els resultats mostren que la variabilitat de les respostes nitrat – Q és més elevada que les del DOC. D'altra banda, la dinàmica del DOC està prou ben descrita i mostra com durant el que seria la transició hidrològica entre el periode sec i humit s'observa la màxima concentració anual. No obstant, els canvis en les propietats del DOC, si n'hi ha, i les conseqüències en la incorporació per part de microorganismes heterotròfics no són prou ben coneguts. A més, tenint en compte tant les crescudes com el periode sec com a pertorbacions extremes del sistema, la informació, a nivell quantitatiu de la que disposem en el primer cas és detallada i extensa mentre que en el segon cas encara hi ha mancances.

El segon capítol inicia una exploració dels efectes de la transició hidrològica tant a nivell quantitatiu com qualitatiu, comparant dos periodes de tardor amb característiques hidrològiques antecedents diferents: un any amb un estiu sec durant el qual la llera del riu es va assecar completament, i un altre en el que no es va a arribar a la fragmentació hidrològica i hi havia aigua en el curs principal. En aquest treball es posa de manifest la influencia que les condicions antecedents, meteorològiques i hidrològiques, influeixen la dinàmica de la concentració del DOC i indica la possibilitat de canvies en la seva composició durant la transició hidrològica. Es posa en evidencia que per a observar augments de concentració importants (fins a màxims de 20 mg·L^{-1} quan la concentració basal està entre 2-3 mg·L^{-1}), el periode de sequera antecedent ha de ser prou intens. No hi ha prou amb una disminució del cabal, com el de l'any 2004, sinó que requereix una fragmentació hidrològica important (any 2003). En aquest cas és quan podem observar un canvi de composició en el DOC, reflexat en la contribució de les differents fraccions de pes molecular, juntament amb l'increment en concentració. A més, en aquest període, la interfase riu – freàtic riparià esdevé un "punt calent" d'activitat biogeoquímica com mostra la retenció selectiva de les fraccions de mida molecular més grans mentre que les petites són més conservatives. Aquestes conclusions inicials estan d'acord amb el model del continu mida – reactivitat d'Amon i Benner (1996).

Al tercer capítol, seguint amb les conclusions obtingudes anteriorment, s'emfatitza l'estudi de la influencia del període sec en la dinàmica i reactivitat del DOC. En aquest cas, es determina la seva biodisponibilitat (en termes de BDOC) i s'estima la concentració del DON i les seves fraccions de pes molecular. Els resultats mostren que la biodisponibilitat del DOC no està lligada a una única fracció de pes molecular, ni a l'aigua superficial ni a l'aigua del freàtic riparià. També es posa de manifest que durant el període sec, amb cabals baixos, el DOC no és gaire reactiu però sí que ho és el DON ja que s'esgota tant en aigües superficials com freàtiques. Aquest increment en la ratio DOC:DON durant la sequera reforça les observacions sobre la reducció de l'ús de pèptids i l'augment de la utilització de polisacàrids per part de microorganismes heteròtrofs (Ylla et al., 2010) però no es reflecteix en un canvi en les contribucions de les fraccions de pes molecular del DOC o del DON.

Durant el període de transició, la concentració de DOC presenta un màxim coincidint amb el pic de cabal, mentre que la concentració de DOC té el seu màxim, també amb un cabal alt, però posteriorment al del DOC.

Durant aquest període, les reserves de DON són més importants al freàtic riparià (77%) que a l'aigua superficial (43%). En el cas de les fraccions de pes molecular, hi ha un increment en la contribució del DOC_{HMW} però que no queda reflectit en la concentració del DON_{HMW}. Aquest increment té lloc durant el màxim de cabal, en acord amb d'altres estudis (Li et al., 2003; Maurice et al., 2002) que van observar que durant els episodis de precipitació intensa les entrades de matèria orgànica dissolta més gran de 1 kDa augmenten. La diferència temporal entre la observació dels màxims de concentració de DOC i DON, així com la disminució de la ratio DOC:DON de cadascuna de les fraccions de pes molecular suggereixen que hi ha una mobilització asincrònica de les reserves de DOC i DON. Això podria ser degut a la localització en diferents compartiments que es mobilitzen a partir dels canvis en els fluxes hidrològics diferents (McGlynn and McDonell, 2003).

D'altra banda, els resultats suggereixen que la biodisponibiliat del DOC està regulada per la concentració del DON, en lloc dels canvis en la contribució de les fraccions de pes molecular (Petrone et al., 2009 arriben a una conclusió similar). Així doncs, a diferència del que apuntava el treball anterior, el pes molecular no seria un factor especialment important a l'hora de determinar la biodisponibilitat del DOC.

Finalment al quart capítol, s'estudien les conseqüències de la fragmentació hidrològica en les propietats del DOC i el DON. Aquest treball introdueix la noció que per a estudiar aquest tipus de procés és necessari considerar l'espai ja que la fragmentació hidrològica no és homogènia i la localització de les masses d'aigua persistents depenen de la geomorfologia de la conca. Apart de la consideració espacial, els canvis en les característiques de l'aigua reflecteixen un eix temporal segons en quin moment queden aïllades les masses d'aigua. Aquest canvi temporal queda reflectit per la ratio entre l'oxigen dissolt i l'amoni (Chemical index, capítol 4).

Malgrat que durant el període de fragmentació, les propietats del DOC i el DON són diferents de les observades durant la tardor, quan el continu fluvial és restaurat, la majoria d'aquestes propietats no presenten una tendència o patró estadísticament significatiu. Nogensmenys, l'heterogeneïtat biogeoquímica del sistema es veu amplificada mentre que durant la transició hidrològica i el període humit el sistema s'homogenitza.

És important remarcar que la transició hidrològica no suceeix cada any sinó que depèn de les condicions hidrològiques prèvies. Generalment, la concentració de DOC a les aigües superficials és sempre més elevada que al freàtic riparià o les aigües subterrànies de fora de la zona de ribera. D'altra banda, l'elevada concentració del DOC durant la transició hidrològica està en el mateix rang que la màxima concentració observada a les basses aïllades durant la fragmentació hidrològica.

Pel que fa a les dades de les fraccions de mida molecular dels capítols 2 i 3, trobem que les seves contribucions a la reserva de DOC varien poc durant l'any hidrològic. Generalment, el DOC_{MMW} i el DOC_{LMW} són les fraccions més abundants (35-40% cadascuna) i la contribució del DOC_{HMW} sol ser del 20-25% però després del període sec la contribució de les fraccions per sobre de 10 kDa sol augmentar independentment de la intensitat del període de sequera. D'altra banda, les contribucions de les fraccions de pes molecular del DON només es van examinar durant un període sec i la següent transició hidrològica i període humit. En aquest cas, la contribució més gran a la reserva de DON es la de DON_{LMW} (56%) mentre que el DON_{MMW} i DON_{HMW} presenten contribucions similars. Aquests diferències entre les contribucions de les fraccions de pes molecular del DOC i el DON són un altre indici de les diferències en la seva mobilització o accessibilitat. El cas del DON_{LMW} contrasta encara més amb la proposició que la fracció d'alt pes molecular (HMW) de matèria orgànica augmenta amb les crescudes, ja que durant aquestes situacions la seva contribució augmenta fins a un 70% del total de la reserva de DON.

Canvis en la composició del DOC i el DON

La hidrologia influencia tant la dinàmica del DOC i el DON, com reflexa la ratio DOC:DON, com les seves característiques. No obstant, mentre que la fragmentació hidrològica i la següent transició sec - humit presenten conseqüències similars per al DOC i el DON (altes concentracions), els efectes d'ambdós períodes sobre les seves propietats són diferents. La transició hidrològica, i les crescudes en general, tendeixen a homogeneïtzar les propietats mesurades als capítols 3 i 4, mentre que pel contrari, durant la fragmentació hidrològica, s'amplifica l'heterogeneïtat biogeoquímica del sistema fluvial. Les propietats espectroscòpiques estudiades (FI, SUVA254, I_C:I_A) reflecteixen bé aquesta heterogeneïtat, malgrat només el SUVA254 presenta una correlació amb el temps d'aïllament de les basses d'aigua.

Els punts de mostreig amb flux superficial durant el període de fragmentació presenta característiques similars a les observades durant el període humit, mentre que el rang de les característiques de la DOM durant a les basses d'aigua aïllades augmenta. En general, durant la fragmentació hidrològica la DOM és predominantment d'origen autòcton, com indiquen els valors de la FI. La influencia del processament de la DOM també ve indicat per l'augment d'intensitat de fluorescència del pic C, ja que malgrat estar associat a la fluorescència d'àcids húmics, Stedmon i Markager (2005) van observar que la fluorescència incrementava en aquesta regió com a possible conseqüència de la degradació de la DOM per part de la comunitat microbiana. Així doncs, tot i que no es pot determinar si hi ha un canvi en la composició de la DOM (es segueix tractant de fluorescència de substàncies húmiques), sí que es pot parlar de transformació de les substàncies que s'hi inclouen.

En canvi, a l'aigua del freàtic riparià que es troba desconnectat de les aigües superficials l'indicador de descomposició de DOM queda reflectit en la presència del pic B (indica substàncies similars a les proteïnes) i concorda amb les conclusions de Inamdar et al. (2012). Tenint en compte que el contingut en BDOC és més baix que a les aigües superficials és probable que una part de la DOM que es degrada esdevingui refractària i s'acumuli en aquest compartiment hidrològic.

Durant el període humit, segons els valors de FI, l'origen de la DOM passa a ser terrestre, indicant una major influència del sistema terrestre adjacent que al processament dins la columna d'aigua del sistema fluvial. Durant la transició hidrològica s'espera un augment del contingut en molècules aromàtiques (Vidon et al., 2008; Sanderman et al., 2009), una disminució del contingut de substàncies húmiques (Hood et al., 2006) i que augmenti la contribució de substàncies d'alt pes molecular (Maurice et al., 2002; Li et al., 2003). Els nostres resultats, en canvi, mostren que l'increment de la contribució de substàncies d'alt pes molecular només es produeix en el cas del DOC, no del DON, i que malgrat els valors de $SUVA_{254}$ presenten un rang més petit al considerar l'espai, els valors són més baixos que durant la fragmentació hidrològica, indicant que el contingut en aromàtics és més baix.

Els resultats de Romaní et al (2006) mostren que a Fuirosos les fraccions de pes molecular de DOC més grans són d'origen terrestre mentre que les més petites presenten valors de FI més alts. A més, durant la sequera els valors de FI al freàtic riparià de les fraccions petites i mitjanes són similars als d'aquestes fraccions a les aigües superficials. En canvi, a les fraccions grans, per sobre de 10 kDa, els valors de FI són més grans al freàtic riparià que a les aigües superficials. D'aquesta manera, es pot concloure que els valors de FI suggereixen que la transformació de la DOM es produeix a la interfase riu – freàtic riparià.

Estudis paral·lels als presentats aquí d'Ylla et al. (2010, 2011, 2012) examinen la composició i dinàmica de la DOM durant els mateixos períodes hidrològics considerant aminoàcids, lípids i sacàrids tant a la columna d'aigua com en biofilms bentònics. Els resultats indiquen que durant el període de sequera la principal font de DOC són els polisacàrids mentre que els aminoàcids desapareixen gradualment, coincidint amb l'esgotament de la reserva de DON. D'altra banda, durant la transició i període humit, l'entrada de polisacàrids i pèptids és ràpida, contribuint als pics de concentració de DOC i DON. En aquest període els polisacàrids representen el 20% del DOC total i els aminoàcids un 3%.

Els canvis en la DOM i les característiques químiques de l’aigua durant la fragmentació hidrològica i les conseqüències a la comunitat bacteriana van ser estudiats per Fazi et al. (enviat). Aquests canvis comporten una disminució en l’alfa-diversitat, afavorint dos grups filogenètics. Un d’aquests grups degrada compostos aromàtics en condicions de desnitrificació, i conseqüentment podrien contribuir a les baixes concentracions de nitrat observat a les basses aïllades i els canvis en els valors de $SUVA_{254}$. L’altre grup afavorit s’ha descrit recentment que tenen com a preferència la matèria orgànica autòctona més que l’al·lòctona.

Canvis en la reactivitat del DOC i el DON

A Fuirosos el contingut més alt de BDOC (40-50%) s’observ en dues situacions hidrològiques oposades: durant la transició hidrològica i durant el període de fragmentació hidrològica, a les basses que han estat més temps aïllades. Noobstant, per a observar aquest alt contingut de BDOC durant el període de transició hidrològica, prèviament s'ha hagut de poduïr una acumulació important de matèria orgànica durant el període sec i per tant aquest ha de ser prou intens com per a que hi hagi una fragmentació hidrològica important. Si aquesta sequera intensa no es produeix, aleshores tant la concentració de DOC com el contingut de BDOC romanen en el rang que s’observa normalment durant el període humit amb cabals basals. Malgrat, els valors similars de contingut de BDOC cal destacar que l’origen del DOC en ambdós situacions hidrològiques és diferent.

Generalment, una de les aproximacions en recerca a l’estudi de la labilitat del DOC consisteix en separar aquesta gran caixa negra en d’altres més petites, fent servir diferents criteris. Dos dels mètodes més freqüents que s’aplica a la DOM en sistemes naturals són a partir de criteris químics que classifica la DON en fraccions hidrofílica (o no húmica), hidrofòbica (húmica) i transfílica; mentre que l’altra consisteix a separar segons el pes molecular (per a més informació es pot consultar McDonald et al., 2004; i Abbt-Braun, 2004). Amb aquest segon criteri hi ha un gran nombre d’estudis que han intentat lligar la labilitat del DOC a la mida dels compostos que l’integren. El 1996, Amon i Benner van proposar el model del continu mida – reactivitat que manté que les molècules més petites són un producte de la degradació de les molècules més grans i que per tant són més refractàries que les grans que serien més làbils. Tot i que resultats d’altres estudis recolzen aquest model (Sachse et al., 2001; Fischer et al., 2004; Kaiser and Sulzberger, 2004; Thóth et al., 2007), la seva validesa és posada en dubte per altres (Meyer et al., 1987; Lindell et al., 1995; Marschner and Kalbitz, 2003; Agren et al., 2008).

Des d'aquesta perspectiva, l'estudi de de les propietats del DOC presentat en aquest document inclou la determinació de la biodisponibilitat segons les fraccions de pes molecular durant el període de sequera (però no la fragmentació hidrològica), la transició hidrològica i el període humit, un cop assolit el cabal basal. Els resultats del segon capítol, on es considera la retenció preferencial a la interfase riu – freàtic riparià com un possible indicador de la labilitat del DOC, suggereix que el DOC_{HMW} podria ser més làbil que les fraccions més petites. En el tercer capítol, els resultats del contingut de BDOC mostren una disminució segons la fracció molecular, de més grans a més petita (21, 17 i 7% respectivament per a DOC_{HMW}, DOC_{MMW} and DOC_{LMW}). Malgrat que l'elevada variabilitat dels resultats ni permet extreure una conclusió prou robusta, el fet que el DOC_{LMW} és la fracció molecular que presentava més casos de generació de DOC durant les incubacions, sembla indicar que aquesta tendencia observada podria ser consistent amb les conclusions presentades al capítol 2. D'altra banda, Meyer et al. (1987) van proposar que la causa de la reactivitat del DOC observada a les fraccions d'alt pes molecular seria la formació de complexos entre un nucli húmic, més refractari, amb substàncies de baix pes molecula a la part externa.

En el nostre estudi, la naturalesa refractaria del DOC_{LMW} pot ser qüestionada quan s'observa que quan la ratio DOC:DON pren valors per sota de 13, la biodisponibilitat d'aquesta fracció molecular augmenta de manera important. A més, aquest canvi en disponibilitat segons la ratio DOC:DON només s'observa durant la transició hidrològica entre el període sec i humit, remarcant la importancia de les condicions hidrològiques en els processos biogeoquímics. Aquesta entrada de DOC_{LMW} làbil pot ser conseqüència del lixiviat de fulles (Meyer et al., 1987) que s'acumulen al canal del riu durant el període sec malgrat que durant aquest període el DOC_{LMW} és principalment poc biodisponible. Pel que fa a les fraccions DOC_{MMW} i DOC_{HMW} trobem que rarament la seva ratio DOC:DON està per sota de 15 però això no sembla tenir cap influència en la seva biodisponibilitat ni a les condicions hidrològiques.

Aquests resultats suggereixen que el pes molecular no és l'únic factor a considerar quan es determina la biodisponibilitat del DOC sinó que d'altres factors a nivell ecosistèmic, hidrològics i climàtics s'han de tenir en consideració.

En general, el DON es considera làbil, tant el DON_{LMW} (sacàrids, aminoàcids lliures, nucleòtids...) com el DON agregat a substàncies húmiques, tot i que en menor grau (Berman and Bronk, 2003). Els nostres resultats mostren que el

BDOC està més influenciat per la concentració del DON que no pas per una fracció de pes molecular de DOC en concret o simplement a la seva concentració total. És prou evident que durant la transició hidrològica les entrades de DOC_{LMW} i DON_{LMW} coincideixen temporalment indicant que aquesta fracció petita podria ser la raó per la qual la labilitat del DOC en general augmenta. D'altra banda, tot i que és difícil interpretar les dades de BDON ja que hi ha molta variabilitat, Petrone et al. (2009) van concloure que la biodisponibilitat de del DON estava lligada principalment a la fracció hidrofílica (no-húmica) i per tant la fracció de baix pes molecular.

La diferent mobilització de la reserva de DON durant la transició hidrològica i l'alt contingut de BDOC durant la fragmentació hidrològica suggereix que el DOC i el DON podrien presentar reserves amb taxes de renovació lentes i ràpides com diferents autors han comprovat (Brookshire et al., 2005; Kaushal and Lewis, 2005; Petrone et al., 2009).

Finalment, la labilitat de la DOM en sòls és un tema que ha guanyat força darrerament i en publicacions recents (Schmidt et al., 2011; Kalbitz et al., 2012) una de les principals conclusions és que la labilitat com a propietat no depèn de les propietats de la matèria orgànica (mesurades en termes de taxa de descomposició o recalcitrància) sinó que depèn de les propietats de l'ecosistema, de les interaccions entre la matèria orgànica i el su entorn com ara les superfícies minerals, l'estat redox del sòl, les propietats químiques de cada substància i la presència d'organismes que puguin degradar-la.

Sobre la metodologia: experiments al laboratori i experiments naturals

Generalment en recerca, es prefereixen els experiments de laboratori ja que permeten un control metòdic i precís de les condicions que envolten el procès que es vol estudiar. D'altra banda, els experiments que es duen a terme al camp permeten tenir en consideració la imatge sencera dels processos naturals que no poden ser reproduïts en condicions de laboratori i que només poden ésser manipulats al camp fins a cert punt. En el cas de la dinàmica del DOC, les crescudes i els períodes secs són difícils de simular ja que com es veu als resultats del capítol 2, fins i tot les condicions prèvies poden ser rellevants. Més detalls, al capítol dos, l'estudi de l'interfase riu – freàtic ripari pot comparar-se a un experiment de perfusió al laboratori realitzat amb testimonis de sediment però, mentre que inicialment podriem obtenir uns resultats semblants, però si considerem el resultats obtinguts amb el DON al capítol 3 durant el període sec, les incubacions en laboratori per determinar la seva disponibilitat tenim conclusions contradictòries amb les observacions al camp: mentre que en el primer cas hi ha una alliberació de DON a la majoria de les mostres i rèpliques, al camp s'observa un esgotament de la reserva de DON. A més, apart de no poder extreure conclusions robustes, els resultats de laboratori estan en contradicció amb d'altres estudis publicats prèviament (Seitzinger and Sanders, 1997; Stepanauskas et al., 1999, 2000; Kerner and Spitzy, 2001; Petrone et al., 2009; Lonborg et al., 2009; Lonborg and Sondegaard, 2009).

En el cas de l'estudi de la dinàmica del DOC en sistemes fluvials és imprescindible que la sèrie temporal resultant inclogui tota la variabilitat hidrològica per a poder establir l'origen dels canvis en concentració com a les seves propietats, relacionant-ho amb altres factors com les precipitacions, el temps entre episodis de crescuda o el paper de les condicions hidrològiques prèvies. Com les condicions meteorològiques i els succesos hidrològics estan fora del control de l'investigador, això implica que ha d'haver una considerable inversió de temps a l'hora de recollir les mostres per obtenir dades. Per exemple, una de les conclusions del capítol 1 indica que per poder representar tota la variabilitat de les respostes nitrat – Q seria necessari mostrejar de manera intensiva durant 2 anys com a mínim.

L'estudi de les respostes d'histèresi emprant un pla de coordenades *R-C* ha estat adoptat en treballs recents en altres sistemes fluvials (Strohmeier et al., 2013; Cerro et al., en prensa).

En qualsevol cas, un dels inconvenients propis dels experiments de camp és que tot i que els resultats siguin propers al que realment suceeix al sistema en condicions naturals, arribar a conclusions prou robustes pot ser difícil degut al nombre de factors que s'escapen a una consideració adeqüada. Així doncs, sempre és necessària una fase de disseny experimental i planificació el més meticulosa possible per a poder obtenir tantes dades fiables com sigui possible.

Sobre la metodologia: caracterització de la DOM segons fraccions de pes molecular

Per tal d'aprofundir en el coneixement del rol del DOC als sistemes naturals, un dels pasos endavant (o cap el costat) és el de caracteritzar els seus components. En general, la composició del DOC en sistemes aquàtics és prou coneguda, classificant-ho en en tres grans grups: àcids húmics, fúlvics i substàncies de baix pes molecular. Tot i que les substàncies que s'inclouen dins d'aquests grups presenten característiques comunes, aquestes poden no ser prou útils per determinar el seu origen, transport i destinació de les diferents substàncies que s'inclouen dins la capsa negra que és el DOC. A més, la majoria de classificacions de la DOM (o el DOC) es basen en definicions operacionals o en aproximacions basades en una sola caracterísitca (com pot ser la fluorescència, i el BDOC) (Filella et al., 2010).

Per tal d'estudiar millor aquesta capsa negra es pot fragmentar en capses més petites, establint nivells de tall segons el pes molecular mitjançant la ultrafiltració tangencial. Tal i com es fa al capítol 2, aquestes fraccions es poden fer servir directament per a estudiar la dinàmica de cadascuna però també es poden fer servir com a punt de sortida on s'apliquen altres tècniques de caracterització. La ultrafiltració tangencial presenta els inconvenients que el rang del DOC que es recupera després del procediment pot variar entre el 80 i rl 120% i que la fracció col·loidal i agregats moleculars complexos poden ser alterats. Aquest segon inconvenient també es troba en tècniques d'extracció per a separar el DOC segons propietats químiques (Abbt-Braun, 2004; McDonald, 2004).

Un altre aspecte a tenir en compte quan s'estableix el rang de pes molecular amb la ultrafiltració és que diferents estudis publicats poden haver fet servir criteris diferents per a separar el DOC i referir-se en termes similars a fraccions de pes molecular diferents. Per exemple, en els diferents capítols d'aquest document la fracció de baix pes molecular (LMW) es considera que inclou les substàncies per sota de 1 kDa (com a d'altres estudis com Stepanauskas et al., 1999; Belzile i Guo, 2006; Peduzzi et al., 2008) i la fracció d'alt pes molecular (HMW) inclou mol·lècules per sobre del 10 kDa. En canvi, altres investigadors estableixen el límit de la fracció LMW a 500 Da (Huguet et al., 2010, per exemple) o a 3 kDa (Kerner i Spitzt, 2001). Així doncs, és un aspecte que cal tenir en compte a l'hora de comparar resultats entre diferents estudis.

Sobre la metodologia: espectroscopia

Les tècniques espectroscòpiques suposen en un mètode relativament senzill i ràpid de caracteritzar la matèria orgànica dissolta. Aquesta sol ser una de les primeres afirmacions que hom es troba a la literatura científica al presentar aquestes tècniques. Tot i que és certa en termes generals, aquestes tècniques no estan exemptes de consideracions importants a l'hora de manipular la mostra i sobre el nivell de detall amb que es pot caracterizar la DOM. L'aplicació d'aquestes tècniques és força àmplia i tot i que es poden fer servir per a obtenir dades quantitatives en el cas d'una barreja complexa de substàncies com és el DOC pot ser molt difícil (Filella et a., 2009).

L'aplicació de tècniques de mesura d'absorbància i fluorescència encara que permeten certa caracterització de la DOM té certes restriccions a l'hora de generalitzar ja que només una part de les substàncies presenten propietats espectrocòspiques. Així doncs, és habitual trobar-se amb referències a la FDOM (fluorescent DOM) o CDOM (coloured DOM). Aquesta nomenclatura, com sha comentat anteriorment, només fa referencia a la tècnica que s'utilitza a l'hora d'estudiar la matèria orgànica més que a una propietat característica d'aquesta.

El segon inconvenient que presenten aquestes tècniques té a veure amb la manipulació i tractament de la mostra ja que factors com el pH, concentracions altes de la mostra que poden generar l'efecte de filtre intern (inner filter effect) o la presència de metalls que esmorteeixen la senyal fluorescent poden alterar de forma significativa els resultats obtinguts. En alguns casos, com els de les matrius d'excitació i emisió, poden necessitar l'aplicació d'alguna eina estadística (PCA, PARAFAC, deconvolució) per a poder extreure'n més informació i aleshores s'ha de considerar les restriccions i necessitats pròpies de cada tècnica.

Finalment, la darrera consideració és que malgrat que s'unifiqui el protocol pel que fa a les correccions espectromètriques dels espectrofluorímetres i de manipulació de mostres, la variabilitat entre les dades obtingudes d'aparells diferents no són prou similars com a poder fer comparacions entre estudis diferents.

No obstant aquests inconvenients, les dades que s'obtenen ofereixen informació amb prou significat ecològic que pot ajudar a explicar millor els processos biogeoquímics que afecten la matèria orgànica dissolta en sistemes naturals.

Conclusions

Capítol 1. Diversitat i seqüències temporals de formes de respostes DOC i NO3 – Cabal en una riera intermitten: successió aleatoria o previsible?

- Les respostes C – Q més probables representen només un 40% del total de casos de les dades. La majoria de les respostes DOC – Q i NO_3-Q es troben dins de respostes C – Q amb probabilitats baixes.

- La transició hidrològica entre els períodes sec i humit no presenta una resposta C – Q típica. De fet, durant aquest període hi ha un increment en la diversitat de les respostes DOC – Q. El 33% dels successos compresos dins aquest període contribueixen al 41% de la diversitat total de respostes DOC – Q. En canvi, no hi ha cap increment en diversitat de les respostes NO_3-Q.

- L'anàlisi de contingència indica que la periodicitat dels processos actua en una escala temporal de 1 a 2 anys. Per tant, és necessària la generació de sèries de dades hidro-biogeoquímiques llargues, plurianuals, i amb una resolució alta (amb freqüents intervals de mostreig).

- La periodicitat de la magnitud de les tempestes (ΔQ) és el factor més important que promou les respostes NO_3-Q. En canvi l'efecte dels canvis de temperatura estacionals (T_{day}) és més aparent després de treure la senyal dels episodis de pluja més febles i freqüents.

- La successió de formes de les respostes NO_3-Q podrien estar acoblades a la magnitud dels episodis de tempesta. D'altra banda, una gran part d'incertesa és inevitable i es suggereix que una aproximació probabilística a la modelització hauria de reemplaçar les aproximacions deterministes.

Capítol 2. Efectes de la transició hidrològica entre el període sec i humit sobre la dinàmica i disponibilitat del carboni orgànic dissolt a la interfase riera – ribera en una conca mediterrània

- La sequera estiuenca va afavorir la mobilització i el transport del DOC més làbil, tal com indica el 56% de retenció a la interfase riu – zona de ribera, durant el subseqüent període de transició hidrològica. La retenció del DOC durant aquest període va ser preferencial segons la mida molecular, de fraccions més grans a més petita. En canvi, quan no hi va haver una transició hidrològica aparent, totes les fraccions moleculars del DOC eren igualment conservatives.

- Les dades químiques dels soluts conservatius, el TDOC i les fraccions moleculars observades al torrent efímer mostren que l'aigua circulant a través del sòl del bosc durant els episodis de pluges importants és similar a la de l'aigua superficial del curs principal amb cabals alts. Això remarca la importància de la lixiviació de DOC al·lòcton des del sòl del sòl del bosc en el transport del TDOC a l'aigua superficial de la riera.

- Els successos hidrològics abruptes i extrems poden ser utilitzats com un experiment natural que permet detectar i examinar processos biogeoquímics ja que la seva magnitud es veu amplificada.

Capítol 3. Impacte dels cicle hidrològic entre periode sec i humit a la biodisponibilitat a les fraccions moleculars del carboni orgànic dissolt en una riera mediterrània

- Durant el període sec i el període de transició hidrològica sec - humit la biodisponibilitat del DOC i el DON no es troba lligada a cap de les fraccions moleculars. No obstant, en el període de transició i l'humit el 50% dels casos en que s'observa generació ($BDOC_{MW}<0$) de DOC en el conjunt de fraccions moleculars corresponen a la fracció DOC_{LMW}.

- No hi ha cap entrada consistent ni clara de DOC_{HMW} i DON_{HMW} durant la transició ni expansió hidrològica. En general, la dinàmica de DOC_{MW} encaixen dintre del marc que proposa que els episodis de tempesta afavoreixen les entrades de DOC de mida molecular per sobre de 1 kDa però en canvi el DON_{MW} no.

- Les característiques hidrològiques del període de transició i expansió hidrològica mostren que hi ha una mobilització no sincrònica del DOC i el DON.

- La biodisponibilitat del DOC està fortament influenciada per la concentració de DON. La biodisponibilitat pot estar regulada per la ràtio DOC:DON que presenta un valor llindar de 13 en el cas de DOC_{LMW}. Aquesta fracció molecular passa de ser conservativa a bio disponible quan la ràtio

DOC:DON és inferior a 13. A més, aquest canvi està lligat a les condicions hidrològiques: quan s'esgota la reserva de DON durant la sequera, la biodisponibilitat del DOC disminueix notablement i en canvi torna a augmentar durant el període de transició i expansió hidrològica quan hi ha una entrada important de DON_{LMW}.

- L'alliberament recurrent de DON durant les incubacions és inesperat. En primer lloc perquè aquest resultat no és consistent amb el publicat en altres treballs (Seitzinger and Sanders, 1997; Stepanauskas et al., 1999, 2000; Kerner and Spitzy, 2001; Petrone et al., 2009; Lonborg et al., 2009; Lonborg and Sondegaard, 2009). En segon lloc, perque a diferència dels resultats de laboratori, les observacions al camp mostren que el DON s'esgota tant a les aigües superficials com a les subterrànies de la zona ripariana.

Capítol 4. Composició de la matèria orgànica dissolta en un sistema fluvial mediterrani fragmentat durant un període de sequera intensa

- El període de sequera causa un fragmentació hidrològica gradual de la xarxa fluvial augmentant la variabilitat de les propietats de la DOM i la variabilitat biogeoquímica del sistema fluvial. Coincidint amb les conclusions de Jaffé et al (2008), aquest increment en el rang dels descriptors de la DOM posen de manifest que els efectes a escala local són més rellevants a nivell de les propietats del DOM que l'escala regional.

- Durant l'estiu, la DOM dels punts de mostreig amb aigües lòtiques presenta propietats similars a les observades durant la tardor, amb cabal basal, i mostren la predominança de les entrades de matèria orgànica terrestre de les zones adjacents. En canvi, a les aigües lèntiques, hi ha una contribució important de matèria orgànica d'origen autòcton, generada in situ per processos microbiològics a conseqüència de la pèrdua de la connexió hidrològica entre la riera i la resta de la conca.

- La concentració de DOC a les basses aïllades durant l'estiu presenta el mateix rang de variabilitat que es pot observa durant la transició hidrològica d'altres anys. De manera similar, les estimes de BDOC durant l'estiu indiquen un màxim del 40% que també està en el rang de l'observat durant la transició hidrològica. Això permet afirmar que la contribució més alta del BDOC es produeix durant dues pertorbacions ecosistèmiques de signe oposat.

- Tot i que s'espera que els canvis en soluts inorgànics a les basses aïllades estiguin relacions amb els canvis en l'origen i propietats del DOC, aquestes propietats no varien segons la concentració de DOC.

- En aquest estudi, el $SUVA_{254}$ és la única mesura que mostra un relació positiva, tot i que no significativa, amb l'índex químic (CI) que resumeix les condicions aeròbiques/anaeròbiques de les basses. Considerant exclusivament les basses aïllades de l'estiu, és la única variable relacionada amb el temps d'aïllament de cada bassa (PIT) que és una mesura de la velocitat de la fragmentació del sistema.

Capítol 5. Espectroscòpia de fluorescència i absorbància UV-vis com a eines per a la caracterització de la DOM

- En els darrers anys, els avenços tecnològics han permès una resolució més elevada i un temps d'anàlisi més curt en l'aplicació en la caracterització de la DOM mitjançant tècniques espectroscòpiques.

- Tot i que hi ha un gran nombre de tècniques a emprar i tractament de les dades obtingudes (PARAFAC, spectral slope...) no hi ha un procediment òptim. Escollir les tècniques apropiades i ajustar-les a les nostres necessitats és essencial.

- Encara no es disposa d'un protocol general per a minimitzar els efectes de la recollida i emmagatzemament de les mostres sobre les seves propietats espectroscòpiques. Malgrat l'existència de debat sobre les millors pràctiques, l'emmagatzemament a les fosques i en fred durant un breu període de temps, en el millor dels casos, és prou apropiat.

- De la mateixa manera, la disparitat en els resultats obtinguts entre diferents espectrofluorímetres, tot i presentar la mateixa configuració i mètode de correcció, no permet que els resultats de l'anàlisi de mostres en diferents laboratoris sigui comparable.

References

Abbt-Braun G., Lankes U., Frimmel F.H. 2004. Structural characterization of aquatic humic substances – The need for a multiple method approach. Aquatic Sciences, 66:151-170

Acuña V., Munoz I., Giorgi A., et al. 2005. Drought and post drought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. Journal of the North American Benthological Society, 24(4):919-933.

Acuña V., Giorgi A., Muñoz I., Uehlinger U. 2004. Flow extremes and benthic organic matter shape the metabolism of a headwater Mediterranean stream. Freshwater Biology, 49:960–71.

Aitkenhead J.A. and McDowell W.H. 2000. Soil C:N ratio as a predictor of annual riverine DOC flux at local and global scales. Global Biogeochemical Cycles, 14(1):127-138

Aitkenhead-Peterson J.A., McDowell W.H., Neff J.C. 2003. Sources, production, and regulation of allochthonous dissolved organic matter inputs to surface waters. In Aquatic Ecosystems: interactivity of dissolved organic matter. Eds. Findlay SG and Sinsabaugh RL. Elsevier Science (USA).

Agren A., Berggren M., Laudon H., Jansson M. 2008. Terrestrial export of highly bioavailable carbon from small boreal catchments in spring floods. Freshwater Biology, 53: 964–972.

Amon R.M.W. and Benner R. 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnology and Oceanography, 41:41–51.

Andersen C.M. and Bro R. 2003. Practical aspects of PARAFAC modeling of fluorescence excitation-emission data. Journal of Chemometrics, 17: 200-215.

Artigas J., Romaní A.M., Gaudes A., Muñoz I., Sabater S. 2009. Organic matter availability structures microbial biomass and activity in a Mediterranean stream. Freshwater Biology, 54:2025-2036.

Artinger R., Buckau G., Geyer S., Fritz P., Wolf M., Kim J.I. 2000. Characterization of groundwater humic substances: influence of sedimentary organic carbon. Applied Geochemistry, 15: 97-116.

Azam T., Fenchel T., Field J.G., Gray J.S., Meyer-Reil L.A., Thingstad F. 1983. The ecological role of water-column microbes in the sea. Marine Ecology-Progress Series, 10:257-263.

Bahram M., Bro R., Stedmon C., Afkhami A. 2006. Handling of Rayleigh and Raman scatter for PARAFAC modeling of fluorescence data using interpolation. Journal of Chemometrics, 20: 99-105.

Baker M.A., Valett H.M., Dahm C.N. 2000. Organic carbon supply and metabolism in a shallow groundwater ecosystem. Ecology, 81(11):3133-3148.

Baker A., Lamont-Black. J. 2001. Fluorescence of dissolved organic matter as a natural tracer of ground water. Ground water, 39(5): 745-750.

Baker, A. 2002. Spectrophotometric discrimination of river dissolved organic matter. Hydrological Processes, 16: 3203-3213.

Baker A, Spencer R.G.M. 2004. Characterization of dissolved organic matter from source to sea using fluorescence and absorbance spectroscopy. Science of the Total Environment, 333:217-232.

Baker, A. 2005. Thermal fluorescence quenching properties of dissolved organic matter. Water Research, 39: 4405-4412.

Baker A., Tipping E., Thacker S.A., Gondar D. 2008. Relating dissolved organic matter fluorescence and functional properties. Chemosphere, 73: 1765-1772.

Balcarczyk K.L., Jones J.B., Jaffé R., Maie N. 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. Biogeochemistry, 94: 255-270.

Balogh K.V., Vörös L., Tóth N., Bokros M. 2003. Changes of organic matter quality along the longitudinal axis of a large shallow lake (Lake Balaton). Hydrobiologia, 506-509: 62-74.

Barker J.D., Sharp M.J., Turner R.J. 2009. Using synchronous fluorescence spectroscopy and principal component analysis to monitor dissolved organic matter dynamics in a glacier system. Hydrological Processes, 23: 1487-1500.

Battin T.J. 1998. Dissolved organic matter and its optical properties in a blackwater tributary of the upper Orinoco river, Venezuela. Organic Geochemistry, 28(9/10): 561-569.

Battin T.J., Kaplan L.A., Findlay S., Hopkinson C.S., Martí E. et al. 2008. Biophysical controls on organic carbon fluxes in fluvial networks. Nature Geosciences, 1(2):95-100.

Bleich M.E., Moraes Lima Silveira R., Barros Nogueira F.M. 2009. Limnological patterns in Northern pantanal lagoons. Brazilian Archives of Biology and Technology, 52(3):755-764.

Belzile C. and Guo L. 2006. Optical properties of low molecular weight and colloidal organic matter: Application of the ultrafiltration permeation model to DOM absorption and fluorescence. Marine Chemistry, 98:183-196.

Berman T. and Bronk D.A. 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquatic Microbial Ecology, 31:279-305.

Bernal S., Butturini A., Sabater F. 2002. Variability of DOC and nitrate responses to storms in a small Mediterranean forested catchment. Hydrology and Earth System Sciences, 6:1031–41.

Bernal S., Butturini A., Nin E., Sabater F., Sabater S. 2003. Leaf litter dynamics and nitrous oxide emission in a Mediterranean riparian forest: Implications for soil nitrogen dynamics. Journal of environmental quality, 32:191-197.

Bernal S., Butturini A., Riera J.L., Vázquez E., Sabater F. 2004. Calibration of the INCA model in a Mediterranean forested catchment. Hydrology and Earth System Sciences, 8:729–41.

Bernal S., Butturini A., Sabater F. 2005. Seasonal variations of dissolved nitrogen and DOC:DON ratios in an intermittent Mediterranean stream. Biogeochemistry, 75:351-372.

Bernal S., Butturini A., and F. Sabater. 2006. Inferring nitrate sources through end member mixing analysis in an intermittent Mediterranean stream, Biogeochemistry, doi:10.1007/s10533-006-9041-7.

Bertoldi W., Zanoni L., Tubino M. 2009. Planiform dynamics of braided streams. Earth Surface Processes and Landforms, doi: 10.1002/esp.1755

Biron P.M., Roy A.G., Courschesne F., Hendershot W.H., Cote B., and Fyles J. 1999. The effects of antecedents moisture conditions on the relationship of hydrology to hydrochemistry in a small forested catchment, Hydrological Processes, 13, 1541– 1555.

Boehme .J, Coble P.G., Conmy R., Stovall-Leonard A. 2004. Examining CDOM fluorescence variability using principal component analysis: seasonal and regional modelling of three dimensional fluorescence in the Gulf of Mexico. Marine Chemistry, 89: 3-14

Boix D., Garcia-Berthou E., Gascon S. et al. 2010. Response of community structure to sustained drought in Mediterranean rivers. Journal of Hydrology, 383(1-2):135-146.

Bond N.R., Lake P.S., Arthington A.H. 2008. The impacts of drought on freshwater ecosystems: an Australian perspective. Hydrobiologia, 600:3-16.

Bostrom B., Andersen J.M., Fleischer S., Jansson M. 1988. Exchange of phosphorus across the sediment – water interface. Hydrobiologia 170: 229-244.

Botter, G., Settin T., Marani M., and Rinaldo A. 2006. A stochastic model of nitrate transport and cycling at basin scale, Water Resources Research, 42, W04415, doi:10.1029/2005WR004599.

Bracchini L., Dattilo A.M., Hull V., Loiselle S.A., et al. 2010. Spatial and seasonal changes in optical properties of autochthonous and allochthonous chromophoric dissolved organic matter in a stratified mountain lake. Photochemical & Photobiological Sciences. DOI: 10.1039/b9pp00129h

Bricaud A., Morel A., Prieur L. 1981. Absorption by dissolved organic matter of the sea (yellow substance) in the UV and visible domains. Limnology & Oceanography, 26(1): 43-43

Bro, R. 1997. PARAFAC. Tutorial and applications. Chemometrics and Intelligent Laboratory Systems, 38: 149-171.

Brookshire J.E.N., Valett M.H., Thomas S.A., Webster J.R. 2005. Coupled cycling of dissolved organic nitrogen and carbon in a forest stream. Ecology, 86(9):2487-2496.

Boulton A.J., Findlay S., Marmonier P., Stanley E.H., Valett M.H. 1998. The functional significance of the hyporheic zone in streams and rivers. Annual Review of Ecology and Systematics, 29:59–81.

Buffam I., Galloway J.N., Blum L.K., McGlathery K.J. 2001. A stormflow/baseflow comparison of dissolved organic matter concentrations and availability in an Appalachian stream. Biogeochemistry, 53:269-306.

Burgin, A. J., and. Hamilton S.K. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Frontiers in Ecology and the Environment, 5(2): 89–96.

Burns D. 2005. What do hydrologists mean when they use the term flushing? Hydrological Processes, 19:1325– 1327

Burt T.P., Pinay G., Matheson F.E., Haycock N.E., Butturini A., Clement J.C., Danielescu S., Dowrick D.J., Hefting M.M., Hillbricht-Ilkowska A., Maitre V. 2002. Water table fluctuations in the riparian zone: comparative results from a pan-European experiment. Journal of Hydrology, 265:129–48.

Butturini A., Sabater F. 1999. Importance of transient storage zones for ammonium and phosphate retention in a sandy bottom Mediterranean stream. Freshwater Biology, 41:593–603.

Butturini A., Bernal .S, Sabater S., Sabater F. 2002. The influence of riparian-hyporheic zone on the hydrological responses in an intermittent stream. Hydrology and Earth System Sciences, 6:515–25.

Butturini A., Bernal S., Nin E. 2003. Influences of the stream groundwater hydrology on nitrate concentration in unsaturated riparian area bounded by an intermittent Mediterranean stream. Water Resources Research, 39: 1110 doi:10.1029/2001WR001260.

Butturini A., Bernal S., Sabater F. 2005. Modelling storm events to investigate the influence of the stream–catchment interface zone on stream biogeochemistry. Water Resources Research, 41:W08418 doi:10.1029/2004WR003842.

Butturini B., Gallart F., Latron J., Vazquez E., and Sabater F. (2006), Cross site comparison of variability of DOC and nitrate c-q hysteresis during the autumn-winter period in three Mediterranean headwater streams: A synthetic approach. Biogeochemistry, 77(3):327–349.

Butturini A., Alvarez M., Bernal S., Vazquez E., Sabater F. 2008. Diversity and temporal sequences of forms of DOC and NO_3 – discharge responses in an intermittent stream: Predictable or random succession? Journal of Geophysical Research – Biogeosciences, 114(G3):3016-3016.

Cabaniss, S. 1992. Synchronous Fluorescence Spectra of Metal-Fulvic Acid Complexes. Environmental Science & Technology, 26(6): 1133-1139.

Cammack W.K.L., Kalff J., Prairie Y.T, Smith E.M. 2004. Fluorescent dissolved organic matter in lakes: Relationships with heterotrophic metabolism. Limnology & Oceanography, 49(6): 2034-2045.

Caraco N.F. and Cole J.J. 2003. The importance of organic nitrogen production in aquatic systems: a landscape perspective. In Aquatic Ecosystems: interactivity of dissolved organic matter. Eds. Findlay SG and Sinsabaugh RL. Elsevier Science (USA).

Carstea E.M., Baker A., Pavelescu G., Boomer I. 2009. Continuous fluorescence assessment of organic matter variability on the Bournbrook River, Birmingham, UK. Hydrological Processes, 23: 1937-1946.

Cerro, I., Sánchez-Pérez, J.M., Ruiz-Romera E., Antiguedad I. Variability of particulate (SS, POC) and dissolved (DOC, NO3) matter during storm events in the Alegria agricultural watershed. *Hydrological Processes* (in press).

Chanat J.G., Rice K.C., and Hornberger G.M. 2002. Consistency of patterns in concentration-discharge plots. Water Resources Research, 38(8), 1147, doi:10.1029/2001WR000971.

Chen W., Westerhoff P., Leenheer J.A., Booksh K. 2003. Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. Environmental Science & Technology, 37: 5701-5710.

Chin Y.P., Aiken G.R., O'Loughlin E. 1994. Molecular Weight, Polydispersity, and Spectroscopic Properties of Aquatic Humic

Substances. Environmental Science & Technology, 28: 1853-1858.

Christensen J.H., Christensen O.B. 2003. Climate modelling: severe summertime flooding in Europe. Nature, 421:805–06.

Christophersen N., Neal C., Hooper R.P., Vogt S., and Andresen S. 1990. Modelling stream waters chemistry as a mixture of soil water end members a step towards second generation acidification models. Journal of Hydrology, 166:307– 320.

Clark J.M., Chapman P.J., Adamson J.K., Lane S.N. 2005. Influence of drought induced acidification on the mobility of dissolved organic carbon in peat soils. Global Change Biology, 11(5):791-809.

Coble P.G., Green S.A., et al. 1990. Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy. Nature, 348(6300): 432-435.

Coble P.G., Schultz C.A., Mopper K. 1993. Fluorescence contouring analysis of DOC Intercalibration Experiment samples: a comparison of techniques. Marine Chemistry, 41: 173-178.

Coble P.G. 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Marine Chemistry, 51:325-346.

Coble P.G., Del Castillo C.E., Bernard A. 1998. Distribution of optical properties of CDOM in the Arabian Sea during the 1995 Southwest monsoon. Deep Sea Research II, 45: 2195-2223.

Coble P.G. 2007. Marine Optical Biogeochemistry: The Chemistry of Ocean Color. Chemical Review, 107(2): 402-418.

Cole J.J., Prairie Y.T., Caraco N.F., McDowell W.H., Tranvik L.J., Striegl R.J., et al. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems, 10:171-184.

Collins S.L., Sinsabaugh R.L., Crenshaw C., Green L., Porras-Alfaro A., et al. 2008. Pulse dynamics and microbial processes in aridland ecosystems. Journal of Ecology, 96:413-420.

Cooper R., Thoss V., Watson H. 2007. Factors influencing the release of dissolved organic carbon and dissolved forms of nitrogen from a small upland headwater during autumn runoff events. Hydrological Processes, 21:622–633.

Cory R.M., Green S.A., and Pregitzer K.S. 2004. Dissolved Organic Matter concentration and composition in the forests and streams of Olympic National Park, WA. Biogeochemistry, 67: 269-288.

Cory R.M., McKnight D.M. 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. Environmental Science & Technology, 39(21): 8142-8149.

Cory R.M., Miller M.P., McKnight D.M., Guerard J.J., Miller P.L. 2010. Effect of instrument-specific response on the analysis of fulvic acid fluorescence spectra. Limnology and Oceanography: Methods, 8: 67-78.

Creed I.F., Beall F.D., et al. 2008. Predicting export of dissolved organic carbon from forested catchments in glaciated landscapes with shallow soils. Global Biogeochemical Cycles. doi:Gb4024.10.1029/2008gb003294.22

Dahm C.N., Grimm N.B., Marmonier P., Valett M.H., Vervier P. 1998. Nutrient dynamics at the interface between surface waters and groundwaters. Freshwater Biology, 40:427–51.

Dahm C.N., Baker M.A., Moore D.I., Thibault J.R. 2003. Coupled biogeochemical and hydrological responses of streams and rivers to drought. Freshwater Biology, 48:1219-1231.

Dawson J.J.C., Soulsby C., Tetzlaff D., et al. 2008. Influence of hydrology and seasonality on DOC exports from three contrasting upland catchments. Biogeochemistry, 90:93–113.

De Haan H., De Boer T. 1987. Applicability of light absorbance and fluorescence as measures of concentration and molecular size of dissolved organic carbon in humic lake Tjeukemeer. Water Research, 21(6): 731-734.

De Souza Sierra M.M.D.O., Lamotte M., Belin C., Ewald M. 1994. Fluorescence spectroscopy of coastal and marine waters. Marine Chemistry, 47: 127-144.

Del Castillo C.E., Coble P.G., Morell J.M., López J.M., Corredor J.E. 1999. Analysis of the optical properties of the Orinoco River plume by absorption and fluorescence spectroscopy. Marine Chemistry, 66: 35-51.

del Giorgio P.A. and Davis J. 2003. Patterns in dissolved organic matter lability and consumption across aquatic systems. In Aquatic Ecosystems: interactivity of dissolved organic matter. Eds. Findlay SG and Sinsabaugh RL. Elsevier Science (USA).

DeRose P.C, E. E., Kramer GW. 2007. Qualification of a fluorescence spectrometer for measuring true fluorescence spectra. Review of scientific instruments, 78. DOI: 10.1063/1.2715952

Dilling J., Kaiser K. 2002. Estimation of the hydrophobic fraction of dissolved organic matter in water samples using UV photometry. Water Research 36: 5037-5044.

Doane T.A., Horwáth W.R. 2010. Eliminating interference from iron(III) for ultraviolet absorbance measurements of dissolved organic matter. Chemosphere, 78: 1409-1415.

Don A., Kalbitz K. 2005. Amounts and degradability of dissolved organic carbon from foliar litter at different decomposition stages. Soil Biology & Biochemistry, 37: 2171–2179.

Eimers M.C., Watmough S.A., Buttle J.M. 2008. Long-term trends in dissolved organic carbon concentration: a cautionary note. Biogeochemistry, 87:71-81.

Engelenn S., Frosch S., Hubert M. 2007. Automatically identifying scatter in fluorescence data using robust techniques. Chemometrics and Intelligent Laboratory Systems, 86: 35-51.

Engelenn S., Frosch S., Jorgensen B.M. 2009. A fully robust PARAFAC method for analyzing fluorescence data. Chemometrics 23: 124-131.

Evans C., and Davies T.D. 1998. Causes of concentration/discharge relationship hysteresis and its potential as tool for analysis of episode hydrochemistry, Water Resources Research, 34, 129– 137.

Evans C.D., Chapman P.J., Clark J.M., Monteith D.T., Cressers M.S. 2006. Alternative explanations for rising dissolved organic carbon export from organic soils. Global Change Biology, 12:2044-2053.

Farjalla V.F., Amado A.G., Suhett A.L., Meirelles-Pereira F. 2009. DOC removal paradigms in highly humic aquatic ecosystems. Environmental Science and Pollution Research, 16:531-538.

Fellman J.B., D'Amore D.V., Hood E., Boon R.D. 2008. Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. Biogeochemistry, 88:169-184.

Fellman J.B., Miller M.P., Cory R.M., D'Amore D.V., White D. 2009. Characterizing dissolved organic matter using PARAFAC modeling of fluorescence spectroscopy: a comparison of two models. Environmental Science & Technology, 43: 6228-6234.

Fellman J.B., Hood E., D'Amore D.V., Edwards R.T., White D. 2009b. Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate rainforest watersheds. Biogeochemistry, 95: 277-293.

Fiebig D.M.. 1997. Microbiological turnover of amino acids immobilized from groundwater discharged from hyporheic sediments. Limnology and Oceanography, 42(4):763-768.

Filella M. 2009. Freshwaters: Which NOM matters? Environmental Chemistry Letters, 7:21-35.

Filella M. 2010. Quantifying 'humics' in freshwaters: purpose and methods. Chemistry and Ecology, 26(4):177-186.

Findlay S.A., Strayer D., Goumbala C., Gould K. 1993. Metabolism of streamwater dissolved organic carbon in the shallow hyporheic zone. Limnology and Oceanography, 38(7):1493-1499.

Findlay S.A. 1995. Importance of surface-subsurface exchange in stream ecosystems – the hyporheic zone. Limnology and Oceanography, 40(1):159-164.

Findlay S.A., and Sobczak WV. 1996. Variability in removal of dissolved organic matter in hyporheic sediments. Journal of the North American Benthological Society, 15(1):35-41

Findlay S.A., Quinn J.M., Hickey C.W., Burrell G., Downes M. 2001. Effects of land use and riparian flowpath on delivery of dissolved organic carbon to streams. Limnology and Oceanography, 46(2):345-355.

Findlay S.A and Sinsabaugh R.L. 2003. Aquatic Ecosystems:Interactivity of dissolved organic matter, Eds: S.A. Findlay and R.L. Sinsabaugh. Academic Press.

Findlay S.A, Sinsabaugh RL, Sobczak WV. 2003. Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. Limnology and Oceanography, 48:1608–17.

Fischer H., Sachse A., Steinberg C.E.W., Pusch M. 2002. Differential retention and utilization of dissolved organic carbon by bacteria in river sediments. Limnology and Oceanography, 47(6):1702-1711.

Fisher S.G,. and Likens G.E. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecological Monographs, 43(4):421-439.

Foulquier A., Malard F., Mermillaud-Blondin F., Datry T., et al. 2010. Vertical change in dissolved organic carbon and oxygen at the water table region of an aquifer recharged with storm water: biological uptake or mixing? Biogeochemistry, 99:31-47.

Freeman C., Ostle N., Kang H. 2001. An enzymatic latch on a global carbon store — a shortage of oxygen locks up carbon in peatlands by restraining a single enzyme. Nature, 409:49–149.

Freeman C., Evans C.D., Monteith D.T., Reynolds B., Fenner N. 2001b. Export of organic carbon from peat soils. Nature, 412:785.

Freeman C., Fenner N., Ostle N.J., and others. 2004. Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. Nature, 430:195–98.

Fuentes M., González-Gaitano G., García-Mina J.M. 2006. The usefulness of UV–visible and fluorescence spectroscopies to study the chemical nature of humic substances from soils and composts. Organic Geochemistry, 37: 1949-1959.

Galloway J.N. 2003. The global nitrogen cycle, in Biogeochemistry, vol. 8, Treatise on Geochemistry, edited by W. H. Schlesinger, H. D. Holland, and K. K. Turekian, pp. 557– 584, Elsevier, Oxford, UK.

Gasith A., Resh V.H. 1999. Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. Annual Review of Ecology and Systematics, 30:51–81.

Gaudes A., Artigas J., Muñoz I. 2010. Species traits and resilience of meiofauna to floods and drought in a Mediterranean stream. Marine and Freshwater Research, 61:1336–1347.

Guéguen C., Guo L., Wang D., Tanaka N., Hung C.C. 2006. Chemical characteristics and origin of dissolved organic matter in the Yukon River. Biogeochemistry, 77:139-155.

Giorgi F. 2006. Climate change hot-spots. Geophysical Research Letters, 33 (April): L08707.

Gomez R., García V., Vidal-Abarca R., Suárez L. 2009. Effect of intermittency on N spatial variability in an arid Mediterranean system. Journal of North America Benthological Society, 28(3): 572-583.

Gordon N.D., McMahon T.A., Finlayson B.L. 1992. Stream hydrology. An introduction for ecologists. New Jersey. Prentice-Hall.

Goslan E.H., Voros S., Banks J., Wilson D., Hillis P., Campbell A.T., Parsons S.A. 2004. A model for predicting dissolved organic carbon distribution in a reservoir water using fluorescence spectroscopy. Water Research, 38: 783-791.

Green S.A., Blough N. 1994. Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. Limnology and Oceanography, 39(8): 1903-1916.

Guéguen C., Guo L., Tanaka N. 2005. Distributions and characteristics of colored dissolved organic matter in the Western Arctic Ocean. Continental Shelf Research, 25: 1195-1207.

Haei M., Oquist M.G., Buffam I., Agren A., et al. 2010. Cold winter soils enhance dissolved organic carbon concentrations in soil and stream water. Geophysical Research Letters, 37(8):L08501.

Hagedorn F., Schleppi P., Peters W., and Hannes F. 2000. Export of dissolved organic carbon and nitrogen from Gleysol dominated catchments: the significance of water flow paths. Biogeochemistry, 50: 137–161.

Harms T.K. and Grimm N.B. 2008. Hot spots and hot moments of carbon and nitrogen dynamics in a semiarid riparian zone. Journal of Geophysical Research, 113, doi:10.1029/2007JG000588.

Hautala K., Peuravuori J., Pihlaja K. 2000. Measurement of aquatic humus content by spectroscopic analyses. Water Research, 34(1):246-258.

Hedin L.O., Armesto J.J., and Johnson A.H. 1995 Patterns of nutrient loss from unpolluted, old-growth temperate forests. Evaluation of biogeochemical theory. Ecology, 76: 493–509

Hefting M., Clement J.C., Dowrick D.J., and others. 2004. Water table elevation controls on soil nitrogen cycling in riparian wetlands along a European climatic gradient. Biogeochemistry, 67:113–34.

Helms J.R., Stubbins A., Ritchie J.D., Minor E.C., et al. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnology and Oceanography, 53(3): 955-969.

Hernes P.J., and Benner R. 2003. Photochemical and microbial degradation of dissolved lignin phenols: Implications for the fate of terrigenous dissolved organic matter in marine environments. Journal of Geophysical Research-Oceans, 108(C9): 9.

Hill A.R., Labadia C.F., Sanmugadas K. 1998. Hyporheic zone hydrology and nitrogen dynamics in relation to the streambed topography of a N-rich stream. Biogeochemistry, 42:285–310.

Hinton M.J., Schiff S.L., English M.C. 1998. Sources and flowpaths of dissolved organic carbon during storms in two forested watersheds of the Precambrian Shield. Biogeochemistry, 41:175-198.

Hobbs R. J., Richardson D. M., and Davis G. W. 1995. Mediterranean type ecosystems: Opportunities and constraints for studying the function of biodiversity, in Mediterranean-Type Ecosystems: The Function of Biodiversity, edited by G. W. Davis and D. M. Richardson, pp. 1–42, Springer, Berlin.

Holbrook D.R., Yen J.H., Grizzard T.J. 2006. Characterizing natural organic material from the Occoquan Watershed (Northern Virginia, US) using fluorescence spectroscopy and PARAFAC. Science of Total Environment, 361: 249-266.

Hood E., McKnight D.M., Williams M. 2003. Sources and chemical character of dissolved organic carbon across an alpine/subalpine ecotone, Green Lakes Valley, Colorado Front Range, United States. Water Resources Research, 39(7): doi: 10.1029/2002WR001738

Hood E., Gooseff M.N., Johnson S.L. 2006. Changes in the character of stream water dissolved organic carbon during flushing in three small watersheds, Oregon. Journal of Geophysical Research, 111, doi:10.1029/2005JG000082

Hornberger G.M., Bencala K.E., McKnight D.M. 1994. Hydrological controls on dissolved organic carbon during snowmelt in the Snake River near Montezuma, Colorado. Biogeochemistry, 25(3):147-165.

Hornberger G.M., Scanlon T.M., Raffensperger J.P. 1998. Modelling transport of dissolved silica in a forested headwater catchment: the effect of hydrological and chemical time scales on hysteresis in the concentration-discharge relationship. Hydrological Processes, 15:2029–38.

Houghton R.A. 2003. The contemporary carbon cycle, in Biogeochemistry, vol. 8, Treatise on Geochemistry, edited by W. H. Schlesinger, H. D. Holland, and K. K. Turekian, pp. 473– 514, Elsevier, Oxford, UK.

House W.A., and Warwick M.S. 1998. Hysteresis of the solute concentration/ discharge relationship in rivers during storms, Water Resources, 32(8), 2279– 2290

Hu C., Muller-Karger F.E., Zepp R.G. 2002. Absorbance, absorption coefficient, and apparent quantum yield: A comment on common ambiguity in the use of these optical concepts. Limnology and Oceanography, 47(4): 1261-1267.

Hudson N., Baker A., Reynolds D. 2007. Fluorescence analysis of dissolved organic matter in antual, waste and polluted waters - A review. River Research and Applications, 23(6): 631-649.

Huguet A., Vacher L., Relexans S., Saubusse S., Froidefond J.M., Parlanti E. 2009. Properties of fluorescent dissolved organic matter in the Gironde Estuary. Organic Geochemistry, 40: 706-719.

Huguet A., Vacher, L., Saubusse S., Etcheber H., Abril G., Relexans S., Ibalot F., Parlanti E. 2010. New insights into the size distribution of fluorescent dissolved organic matter in estuarine waters. Organic Geochemistry, 41(6), 595–610.

Humphries P., and Baldwin D.S. 2003. Drought and aquatic ecosystems: an introduction. Freshwater Biology, 48:1141-1146.

Hur J, S. M. A. 2003. Using selected operational descriptors to examine the heterogeneity within a bulk humic substance. Environmental Science & Technology 37(880-887): 880.'

Hur J., Min-Hye P., Schlautman M.A. 2009. Microbial transformation of dissolved leaf litter organic matter and its effects on selected organic matter operational descriptors. Environmental Science & Technology, 43: 2315-2321.

Huxman T.E., Snyder K.A., Tissue D., Leffler A.J., Ogle K., et al. 2004. Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. Oecologia, 141:254-268.

Inamdar S., O'Leary N., Mitchell M. J., and Riley J.T. 2006. The impact of storm events on solute exports from a glaciated forested watershed in western New York, USA, Hydrological Processes, 20(16), 3423– 3439.

Inamdar S., and Mitchell M.J. 2007. Storm event exports of dissolved organic nitrogen (DON) across multiple catchments in a glaciated forested watershed - art. no. G02014. Journal of Geophysical Research-Biogeosciences, 112(G2):2014-2014.

Inamdar S., Finger N., Singh S., Mitchell M., Levia D., et al. 2012 Dissolved organic matter (DOM) concentration and quality in a forested mid-Atlantic watershed, USA. Biogeochemistry, 108:55-76.

Intergovernmental Panel on Climate Change (IPCC). 2007. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, edited by M. L. Parry et al., Cambridge Univ. Press, Cambridge, UK.

Jaffé R., McKnight D.M., Maie N., Cory R.M., McDowell W.H., Campbell J.L. 2008. Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. Journal of Geophysical Research 113, doi: 10.1029/2008JG000683

Jager D. F., Wilmking M., Kukkonen J.V.K. 2009. The influence of summer seasonal extremes on dissolved organic carbon export from a boreal peatland catchment: evidence from one dry and one wet growing season. The Science of the Total Environment, 407(4), 1373–82.

Johnson F.A., and East J.W. 1982. Cyclical relationships between river discharge and chemical concentration during flood events. Journal of Hydrology, 57(1– 2), 93– 106.

Kaiser E., Arscott D.B., Tockner K., Sulzberger B. 2004. Sources and distribution of organic carbon and nitrogen in the Tagliamento River, Italy. Aquatic Sciences, 66:103–116.

Kaiser E., and Sulzberger B. 2004. Phototransformation of riverine dissolved organic matter (DOM) in the presence of abundant iron: Effect on DOM bioavailability. Limnology and Oceanography, 49(2), 540–554.

Kaiser K., and Kalbitz K. 2012. Cycling downwards – dissolved organic matter in soils. Soil Biology & Chemistry, 52:29-32.

Kalbitz K., Geyer W., Geyer S. 1999. Spectroscopic properties of dissolved humic substances – a reflection of land use history in a fen area. Biogeochemistry, 47: 219-238.

Kalbitz K., Schwesig D., Schmerwitz J., Kaiser K., et al. 2003. Changes in properties of soil-derived dissolved organic matter induced by biodegradation. Soil Biology and Biochemistry, 35: 1129-1142.

Kaplan L.A. and Newbold J.D. 1995. Measurement of streamwater biodegradable dissolved organic-carbon with a plug-flow reactor. Water Research, 29(12):2696-2706.

Katsuyama M., and Ohte N. 2002. Determining the sources of stormflow from the fluorescence properties of dissolved organic carbon in a forested headwater catchment. Journal of Hydrology, 268: 192-202.

Kaushal S.S., and Lewis W.M. 2005. Fate and transport of organic nitrogen in minimally disturbed montane streams of Colorado, USA. Biogeochemistry, 74: 303–321.

Kerner M., and Spitzy A. 2001. Nitrate regeneration coupled to degradation of different size fractions of DON by the picoplankton in the Elbe estuary. Microbial Ecology, 41:69-81.

Kerner M., Hohenberg H., Ertl S., Reckermann E., Spitzy A. 2003. Self organization of dissolved organic matter to micelle-like microparticles in river water. Nature, 422:150-154.

Kirchner J.W., Feng X.H., and Neal C. 2004. The fine structure of water-quality dynamics: The (high-frequency) wave of the future. Hydrological Processes , 18:1353– 1359.

Kirk J.T.O. 1994. Light and photosynthesis in aquatic ecosystems, 2nd ed. Cambridge University Press.

Korshin G., Chow C.W.K., Fabris R., Drikas M. 2009. Absorbance spectroscopy-based examination of effects of coagulation on the reactivity of fractions of natural organic matter with varying apparent molecular weights. Water Research, 43: 1541-1548.

Kowalczuk P., Cooper W.J., Whitehead R.F., Durako M.J., and Sheldon W. 2003. Characterization of CDOM in an organic-rich river and surrounding coastal ocean in the South Atlantic Bight. Aquatic Sciences, 65: 384-401.

Kukkonen J. 1992. Effects of lignin and chlorolignin in pulp mill effluents on the binding and bioavailability of hydrophobic organic pollutants. Water Research, 11: 1523-1532.

Lake P.S. 2000. Disturbance, patchiness, and diversity in streams. Journal of North American Benthological Society, 19(4):573-592.

Lake P.S. 2003. Ecological effects of perturbation by drought in flowing waters. Freshwater Biology, 48:1161–72.

Lake P.S. 2005. Perturbation, restoration and seeking ecological sustainability in Australian flowing waters. Hydrobiologia, 552:109-120.

Lakowicz J.R. 2006. Principles of fluorescence spectroscopy, 3rd Edition. Springer Ed.

Larned S.T., Datry T., Arscott D.B., Tockner K. 2010. Emerging concepts in temporary-river ecology. Freshwater Biology, 55:717-738.

Larsson T., Wedborg M., Turner D. 2007. Correction of inner filter effect in fluorescence excitation-emission matrix spectrometry using Raman scatter. Analytica Chimica Acta, 583: 357-363.

Lapierre J.F., Frenett J.J. 2009. Effects of macrophytes and terrestrial inputs on fluorescent dissolved organic matter in a large river system. Aquatic Sciences, 71: 15-24.

Leeben A., Heinsalu A., Alliksaar T., Vassiljev J. 2010. High-resolution spectroscopic study of pore-water dissolved organic matter in Holocene sediments of Lake Peipsi (Estonia/Russia). Hydrobiologia, 646: 21-31.

Legendre L., Frechette M., and Legendre P. 1981. The contingency periodogram- A method of identifying rhythms in series of nonmetric ecological data, Journal of Ecology, 69(3), 965– 979.

Li F., Yuasa A., Chiharada H., Matsui Y. 2003. Storm impacts upon the composition of organic matrices in Nagara River—A studybased on molecular weight and activated carbon adsorbability. Water Research, 37: 4027–4037

Lindell M.J., Graneli W., Tranvik L.J. 1995. Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. Limnology and Oceanography, 40(1):195-199.

Lohse K.A., Brooks P.D., McIntosh J.C., Meixner T., Huxman T.E. 2009. Interactions between biogeochemistry and hydrologic systems. Annual Review of Environment and Resources, 34:65-96.

Loiselle S.A., Bracchini L., Dattilo A.M., Ricci M., Tognazzi., A et al. 2009. Optical characterization of chromophoric dissolved organic matter using wavelength distribution of absorption spectral slopes. Limnology and Oceanography, 54(2): 590-597.

Lombardi A.T., Jardim W.F. 1999. Fluorescence spectroscopy of high performance liquid chromatography fractionated marine and terrestrial organic materials. Water Research, 33(2): 512-520.

Lonborg C., Davidson K., Álvarez-Salgado X.A., Miller A.E.J. 2009. Bioavailability and bacterial degradation rates of disolved organic matter in a temperate coastal area during an annual cycle. Marine Chemistry, 113:219-226.

Lonborg C., and Sondergaard M. 2009. Microbial availability and degradation of dissolved organic carbon and nitrogen in two coastal areas. Estuarine, Coastal and Shelf Science, 81:513-520.

Lou T., Xie H. 2006. Photochemical alteration of the molecular weight of dissolved organic matter. Chemosphere 65: 2333-2346.

Luciani X., Mounier S., Paraquetti H.H.M., Redon R., Lucas Y., et al. 2008. Tracing of dissolved organic matter from the SEPETIBA Bay (Brazil) by PARAFAC analysis of total luminescence matrices. Marine Environmental Research, 65: 148-157.

Macalady D.L., and Walton-Day K. 2009. New light on a dark subject: On the use of fluorescence data to deduce redox states of natural organic matter (NOM). Aquatic Sciences, 71:135-143.

Marschner B., and Kalbitz K. 2003.Controls of bioavailability and biodegradability of dissolved organic matter in soils. Geoderma, 114:211-235.

Mas-Martí E., García-Berthou E., Sabater S., Tomanova S., Muñoz I. 2010. Comparing fish assemblages and trophic ecology of permanent and intermittent reaches in a Mediterranean stream. Hydrobiologia, 657:167-180.

Maurice P.A., Cabaniss S.E., Drummond J., Ito E. 2002. Hydrogeochemical controls on the variations in chemical characteristics of natural organic matter at a small freshwater wetland. Chemical Geology, 187: 59– 77.

Mayorga E., Aufdenkampe A.K., Masiello C.A., Krusche A.V., Hedges J.I., et al. 2005. Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. Nature, 436:538-541.

McClain M.E., Boyer E.W., Dent L.C., Gergel S.E, Grimm N.B., et al. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems, 6:301– 312.

McDonald B.C., Levin S.J., Patterson H. 1997. Correction of fluorescence inner filter effects and the partitioning of pyrene to dissolved organic carbon. Analytica Chimica Acta 338: 155-162.

McDonald S., Bishop A.G., Prenzler P.D., Robards K. 2004. Analytical chemistry of freshwater humic substances. Analytica Chimica Acta, 527:105-124.

McDowell W.H., Fisher S.G. 1976. Autumnal processing of dissolved organic matter in a small woodland stream ecosystem. Ecology, 57(3):561-569.

McDowell W.H., Zsolnay A., Aitkenhead-Peterson A.J., Gregorich E.G., Jones D.L., et al. 2006. A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. Soil Biology and Biochemistry, 38: 1933-1942.

McGlynn B.L., McDonnell J.J. 2003. Role of discrete landscape units in controlling catchment dissolved organic carbon dynamics. Water Resources Research, 39:1090.

McGlynn B.L., McDonnell J.J., Seibert J., et al. 2004. Scale effects on headwater catchment runoff timing, flow sources, and groundwater-streamflow relations. Water Resources Research, 40:7504–04.

McKnight D.M., Boyer E.W., Westerhof P.K. et al. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnology and Oceanography, 46(1):38-48.

McKnight D.M., Hood E., Klapper L. 2003. Trace organic moieties of dissolved organic matter in natural waters in Aquatic Ecosystems:Interactivity of dissolved organic matter, Eds: S.E.A. Findlay and R.L. Sinsabaugh. Academic Press.

McMahon T.A., and Finlayson B.L. 2003. Droughts and anti-droughts: The low flow hydrology of Australian rivers. Freshwater biology, 48:1147-1160.

Meyer J.L., Edwards R.T., Risley R. 1987. Bacterial growth on dissolved organic carbon from a black water river. Microbial Ecology, 13(1):13-29.

Meyer J.L. 1994. The microbial loop in flowing waters. Microbial Ecology, 28:195-199.

Miano T.M., Senesi N. 1992. Synchronous excitation fluorescence spectroscopy applied to soil humic substances chemistry. Science of total environment, 117/118: 41-51.

Miller W.L., and Moran M.A. 1997. Interaction of photochemical and microbial processes in the degradation of refractory dissolved organic matter from a coastal marine environment. Limnology and Oceanography, 42(6):1317-1324.

Miller M.P., McKnight D.M., Chapra S.C., Williams M.W. 2009. A model of degradation and production of three pools of dissolved organic matter in an alpine lake. Limnology and Oceanography, 54(6): 2213-2227.

Miller M.P., and McKnight D.M. 2010. Comparison of seasonal changes in fluorescent dissolved organic matter among aquatic lake and stream sites in the Green Lakes Valley. Journal of Geophysical Research, 115. doi:10.1029/2009JG000985

Milori D.M.B.P., Martín-Neto L., Bayer C., et al. 2002. Humification degree of soil humic acids determined by fluorescence spectroscopy. Soil Science, 167(11):739-749.

Minero C., Lauri V., Falletti G., Maurino V., Pelizzetti E., Vione D. 2007. Spectrophotometric characterisation of surface lakewater samples: implications for the quantification of nitrate and the properties of dissolved organic matter. Annali di Chimica, 97:1107-115

Minor E, and Stephens B. 2008. Dissolved organic matter characteristics within the Lake Superior watershed. Organic Geochemistry, 39: 1489-1501.

Mladenov N., McKnight D.M., Macko S.A., Norris M., et al. 2007. Chemical characterization of DOM in channels of a seasonal wetland. Aquatic sciences, 69:456-471.

Mladenov N., McKnight D.M., Wolski P., Murray-Hudson M. 2007. Simulation of DOM fluxes in a seasonal floodplain of the Okavango Delta, Botswana. Ecological Modelling, 205: 181-195.

Mobed J.J., Hemmingsen S.L., Autry J.L., and McGown L.B. 1996. Fluorescence characterization of IHSS humic substances: Total luminiscence spectra with absorbance correction. Environmental Science & Technology, 30: 3061-3065.

Monteith D.T., Stoddard J.L., Evans C.D., de Wit H.A., Forsius M., et al. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature, 450:537-540.

Moore T.R., Roulet N.T., Waddington J.M. 1998. Uncertainty in predicting the effects of climatic change on the carbon cycling of Canadian peatlands. Climatic change, 40:229-245.

Moran, M.A. and Zepp R.G. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnology and Oceanography, 42(6): 1307-1316.

Mopper K. and Schultz C.A. 1993. Fluorescence as a possible tool for studying the nature and water column distribution of DOC components. Marine Chemistry, 41: 229-238.

Mostofa K.M.G., Konohira E., Tanoue E. 2007. Dynamics and characteristics of fluorescent dissolved organic matter in the groundwater, river and lake water. Water Air and Soil Pollution, 184: 157-176.

Mulholland P. J., and Hill W.R. 1997. Seasonal patterns in stream water nutrient and dissolved organic carbon concentrations: Separating catchment flow path and in-stream effects. Water Resources Research, 33(6):1297–1306.

Murphy K.R., Stedmon C.A., Waite D.T., Ruiz G.M. 2008. Distinguishing between terrestrial and autochthonous organic matter sources in marine environments using fluorescence spectroscopy. Marine Chemistry, 108: 40-58.

Naeem S., Loreau M., and Inchausti P. 2002. Biodiversity and ecosystem functioning: The emergence of a synthetic ecological framework, in Biodiversity and Ecosystem Functioning: Synthesis and Perspectives, edited by M. Loreau, S. Naeem, and P. Inchausti, pp. 3– 11, Oxford Univ. Press, Oxford, UK.

Neal C., Robson A.J., Neal M., et al. 2005. Dissolved organic carbon for upland acidic and acid sensitive catchments in mid-Wales. Journal of Hydrology, 304:203–20.

Nebbioso A and Piccolo A. 2013. Molecular characterization of dissolved organicmatter (DOM): a critical review. Analytical and Bioanalytical Chemistry, 405:109-124

Ninyerola M., Pons X., Roure J.M. 2000. A methodological approach of climatological modelling of air temperature and precipitation through GIS techniques. International Journal of Climatology, 20:1823–41.

Obernosterer I., Reitner B., Herndl G.J. 1999. Contrasting effects of solar radiation on dissolved organic matter and its bioavailability to marine bacterioplankton. Limnology and Oceanography, 44(7):1645-1654.

Obernosterer I., and Benner R. 2004. Competition between biological and photochemical processes in the mineralization of dissolved organic carbon. Limnology and Oceanography, 49(1): 117-124.

Ocampo C.J., Oldham C.E., Sivapalan M., Turner J.V. 2006. Hydrological versus biogeochemical controls on catchment nitrate export: A test of the flushing mechanism. Hydrological Processes, 20:4269 – 4286.

Ohno T. 2002. Fluorescence inner filtering correction for determining the humification index of dissolved organic matter. Environmental Science & Technology, 36: 742-746.

Ohno T., Bro R. 2006. Dissolved organic matter characterization using multiway spectral decomposition of fluorescence landscapes. Soil Science Society of America, 70: 2028-2037.

Palmer W.C. 1965. Meteorological drought. U.S. Weather Bureau, Res. Pap, 45, 58pp.

Park, J.-H. 2009. Spectroscopic characterization of dissolved organic matter and its interactions with metals in surface waters using size exclusion chromatography. Chemosphere 77: 485-494.

Parlanti E., Wörz K., Geoffroy L., Lamotte M. 2000. Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. Organic Geochemistry, 31:1765-1781.

Pastor J., Solin J., Bridgham S.D., Updegraff K., Harth C., Weishampel P. 2003. Global warming and the export of dissolved organic carbon from boreal peatlands. OIKOS, 100:380–386.

Patel-Sorrentino N., Mounier S., Benaim J.Y. 2002. Excitation–emission fluorescence matrix to study pH influence on organic matter fluorescence in the Amazon basin rivers. Water Research, 36: 2571-2581.

Peduzzi P., Aspetsberger F., Hein T., et al. 2008. Dissolved organic matter (DOM) and bacterial growth in floodplains of the Danube River under varying hydrological connectivity. Fundamental and Applied Limnology/Archiv für Hydrobiologie, 171(1): 49–61.

Persson T., and Wedborg M. 2001. Multivariate evaluation of the fluorescence of aquatic organic matter. Analytica Chimica Acta, 434: 179-192.

Petrone K.C., Richards J.S., Grierson P.F. 2009. Bioavailability and composition of dissolved organic carbon and nitrogen in a near coastal catchment of south-western Australia. Biogeochemistry, 92:27–40.

Peuravuori J., and Pihlaja K. 1997. Molecular size distribution and spectroscopic properties of aquatic humic substances. Analytica Chimica Acta, 337: 133-149.

Peuravuori J., Koivikko R., Pihlaja K. 2002. Characterization, differentiation and classification of aquatic humic matter separated with different sorbents: synchronous scanning fluorescence spectroscopy. Water Research, 36: 4552-4562.

Piccini C., Conde D., Pernthaler J., Sommaruga R. 2009. Alteration of chromophoric dissolved organic matter by solar UV radiation causes rapid changes in bacterial community composition. Photochemical & Photobiological Sciences, 8: 1321-1328.

Porcal P., Koprivnjak J.F., Molot L.A., Dillon P.J. 2009. Humic substances – part 7: the biogeochemistry of dissolved organic carbon and its interactions with climate change. Environmental Science and Pollution Research, 16:714-726.

Pouet M.-F., Theraulaz F., Mesnage V., Thomas O. 2007. Natural Waters in UV-visible spectrophotometry of water and waste water. Eds. O. Thomas and C. Burgess. Elsevier.

Qualls R.G., and Haines B.L. 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution and stream water. Soil Science Society of America, 56:578-586.

Raymond P.A., and Saiers J.E. 2010. Event controlled DOC export from forested watersheds. Biogeochemistry, DOI: 10.1007/s10533-010-9416-7

Rice K., and Hornberger G. 1998. Comparison of hydrochemical tracers to estimate source contributions to peak flow in a small, forested, headwater catchment. Water Resources Research, 34(7):1755– 1766.

Rice K.C., Chanat J.G., Hornberger G.M., and Webb J.R. 2004. Interpretation of concentration-discharge patterns in acid-neutralizing capacity during storm flow in three small, forested catchments in Shenandoah National Park, Virginia. Water Resources Research, 40: W05301, doi:10.1029/2003WR002709.

Richey J.E., Melack J.M., Aufdenkame A.K., Ballester V.M., Hess L.L. 2002. Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO2. Nature, 416:617-619.

Rinnan A., Booksh K.S, Bro R. 2005. First order Rayleigh scatter as a separate component in the decomposition of fluorescence landscapes. Analytica Chimica Acta, 537: 349-358.

Rodriguez-Zuñiga F.U., Bastos Pereira D.M., Lopes da Silva W.T., et al. 2008. Changes in optical properties caused by UV-irradiation of aquatic humic substances from the Amazon River basin: seasonal variability evaluation. Environmental Science and Technology, 42:1948-1953.

Romaní A.M., Vazquez E., Butturini B. 2005. Changes in size fractionation and bioavailability of dissolved organic carbon after drought in a Mediterranean intermittent stream: biogeochemical link across the stream–riparian interface. Microbial Ecology, 52:501–512.

Romaní A.M., Vázquez E., Butturini A. 2006. Microbial availability and size fractionation of dissolved organic carbon after drought in an intermittent stream: biogeochemical link across the stream–riparian interface. Microbial Ecology 52:501-512.

Romano J.P., and Krol J. 1993. Capillary ion electrophoresis, an environmental method for the determination of anions in waters. Journal of Chromatography, 649:403–12.

Romera-Castillo C., Sarmento H., Álvarez-Salgado X.A., Gasol J.M., Marrasé C. 2010. Production of chromophoric dissolved organic matter by marine phytoplankton. Limnology and Oceanography, 55(1): 446-454.

Rose P., Metzeling L., and Catzikiris S. 2008. Can macroinvertebrate rapid bioassessment methods be used to assess river health during drought in south eastern Australian streams? Freshwater Biology, 53(12):2626-2638.

Rubbo M.J., Cole J.J., Kiesecker J.M. 2006. Terrestrial subsidies of organic carbon support net ecosystem production in temporary forest ponds: evidence from an ecosystem experiment. Ecosystems, 9:1170-1176.

Saadi I., Borisover M., Armon R., Laor Y. 2006. Monitoring of effluent DOM biodegradation using fluorescence, UV and DOC measurements. Chemosphere, 63: 530-539.

Sabater S., Bernal S., Butturini A., et al. 2001. Wood and leaf debris input in a Mediterranean stream: The influence of riparian vegetation. Archiv für Hydrobiologie, 153:91– 102.

Sabater S., Butturini A., Clement J.C., Burt T., Dowrick D., et al. 2003. Nitrogen removal by riparian buffers under various N loads along a European climatic gradient: patterns and factors of variation. Ecosystems, 6:20–30.

Sachse A., Babenzien D., Ginzel G., Gelbrecht J., Steinberg C.E.W. 2001. Characterization of dissolved organic carbon (DOC) in a dystrophic lake and adjacent fen. Biogeochemistry, 54: 279-296.

Sachse A., Henrion R., Gelbrecht J., Steinberg C.E.W. 2005. Classification of dissolved organic carbon (DOC) in river systems: Influence of catchment characteristics and autochthonous processes. Organic geochemistry, 36:923-935.

Sala M. 1983. Fluvial and slope processes in the Fuirosos basin, Catalan ranges, northeast Iberian coast. Zeitschrift für Geomorphologie, 27:393– 411.

Sanderman J., Baldock J.A., Amundson R. 2008. Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. Biogeochemistry, 89:181–198.

Santos P.S.M., Otero M., Duarte R.M.B.O., Duarte A.C. 2009. Spectroscopic characterization of dissolved organic matter isolated from rainwater. Chemosphere, 74: 1053-1061.

Scapini M.C., Conzonno V., Balzaretti V.T., Fernández Cirelli A. 2010. Comparison of marine and river water humic substances in a Patagonian environment (Argentina). Aquatic Sciences, 71: 1-12.

Shannon C.E. 1948. A mathematical theory of communication. Bell System Technical Journal, 27:379–423.

Schindler J.K., Krabbenhoft D.P. 1998. The hyporheic zone as a source of dissolved organic carbon and carbon gases to a temperate forested stream. Biogeochemistry, 43:157–74.

Schlesinger W.H., and Melack J.M. 1981. Transport of organic carbon in the world's rivers. Tellus, 33:172-187.

Schröter D., Cramer W., Leemans R., et al. 2005. Ecosystem service supply and vulnerability to global change in Europe. Science, 310:1333–37.

Scott D., McKnight D.M., Blunt - Harris E.L., Kolesar S.E., Lovley D.R .1998. Quinone Moieties Act as Electron Acceptors in the Reduction of Humic Substances by Humics-Reducing Microorganisms. Environmental Science & Technology, 32: 2984-2989.

Seitzinger S.P., and Sanders R.W. 1997. Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. Marine Ecology Progress Series, 159:1-12.

Serrano S.E., Workman S.R. 1998. Modeling transient stream/aquifer interaction with the non-linear Boussinesq equation and its analytical solution. Journal of Hydrology, 206:245–55.

Servais P., Anzil A., Ventresque C. 1989. Simple method for determination of biodegradable dissolved organic carbon in water. Applied Environmental Microbiology, 55: 2732–2734.

Sierra M.M.D., Giovanela M., Parlanti E., Soriano-Sierra E.J. 2005. Fluorescence fingerprint of fulvic and humic acids from varied origins as viewed by single-scan and excitation/emission matrix techniques. Chemosphere, 58: 715-733.

Sinsabaugh R.L. 1997. Large-scale trends for stream benthic respiration. Journal of the North American Benthological Society, 16(1):119-122.

Simon K.S., Townsend C.R., Biggs B.J.F., et al. 2005. Temporal variation of N and P uptake in 2 New Zealand streams. Journal of the North American Benthological Society, 24:1–18.

Smakhtin V.U. 2001. Low flow hydrology: a review. Journal of Hydrology, 240:147-186.

Schmidt F., Kock B.P., Elvert M., et al. 2011. Diagenetic transformation of dissolved organic nitrogen compounds under contrasting sedimentary redox conditions in the Black Sea. Environmental Science & Technology, 45:5223-5229.

Sobczak W.V., and Findlay S.A. 2002. Variation in bioavailability of dissolved organic carbon among stream hyporheic flowpath. Ecology, 83:3194–209.

Stanhope J.W, Anderson I.C., Reay W.G. 2009. Base flow nutrient discharges from lower Delmarva Peninsula watersheds of Virgina, USA. Journal of Environmental Quality, 38:2070-2083.

Stanley E.H., Fisher S.G., Grimm N.B. 1997. Ecosystem expansion and contraction in streams. Bioscience, 47(7):427-435.

Stanley EH, Powers SM, Lottig NR. 2010. The evolving legacy of disturbance in stream ecology: concepts, contributions, and coming challenges. Journal of North America Benthologic Society, 29(1):67-83.

Stedmon C.A., Markager S., Kaas H. 2000. Optical Properties and Signatures of chromophoric Dissolved Organic Matter (CDOM) in Danish Coastal Waters. Estuarine, Coastal and Shelf Science, 51: 267-278.

Stedmon C.A, Markager S., Bro R. 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Marine chemistry, 82:239-254.

Stedmon C.A., Markager S. 2005a. Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. Limnology and Oceanography, 50(2):686-697.

Stedmon C.A., Markager S. 2005b. Tracing the production and degradation of autochthonous fractions of dissolved organic matter by fluorescence analysis. Limnology and Oceanography, 50(5): 1415-1426.

Stedmon C.A., Bro R. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. Limnology and Oceanography: Methods, 6: 572-579.

Stepanauskas R., Leonardson L. 1999. Bioavailability of wetland-derived DON to freshwater and marine bacterioplankton. Limnology and Oceanography, 44(6):1477-1485.

Stepanauskas R., Laudon H., Jorgensen N.O.G. 2000. High DON bioavailability in boreal streams during a spring flood. Limnology and Oceanography, 45(6):1298–1307.

Strohmeier S., Knorr K.-H., Reichert M., Frei S., Fleckenstein J.H., Peiffer S., and Matzner E. 2013. Concentrations and fluxes of dissolved organic carbon in runoff from a forested catchment: insights from high frequency measurements. *Biogeosciences*, 10:905-916.

Stubbins A., Hubbard V., Uher G., et al. 2008. Relating carbon monoxide photoproduction to dissolved organic matter functionality. Environmental Science and Technology, 42(9):3271-3276.

Soulsby C. 1995. Contrasts in storm event hydrochemistry in an acidic afforested catchment in upland Wales, Journal of Hydrology, 170(1–4), 159– 179.

Sun L., Perdue E.M., Meyer J.L., Weis J. 1997. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnology and Oceanography, 42(4):714-721.

Temnerud J., Düker A., Karlsson S., Allard B., Köhler S., and Bishop K. 2009. Landscape scale patterns in the character of natural organic matter in a Swedish boreal stream network. Hydrology and Earth System Sciences, 13: 1567-1582.

Tipping E., Rigg E., Harrison A.F., Ineson P., et al. 1999. Climatic influences on the leaching of dissolved organic matter from upland UK moor land soils, investigated by a field manipulation experiment. Environment International, 25:83–95.

Tipping E., Corbishley H.T., Koprivnjak J.F., Lapworth D.J., Miller M.P., et al. 2009. Quantification of natural DOM from UV Absorption at Two Wavelengths. Environmental Chemistry, 6(6): 472-476.

Thóth N., Vörös L., Mózes A., -Balogh K.V. 2007. Biological availability and humic properties of dissolved organic carbon in Lake Balaton (Hungary). Hydrobiologia, 592:281–290.

Thurman E.M. 1985. Developments in Biogeochemistry: Organic geochemistry of natural waters. Eds. Nijhoff M and Junk W. Kluwer Academic Press USA/

Tranvik L.J., and Jansson M. 2002. Terrestrial export of organic carbon organic. Nature. 415:861–62.

Turgeon J.M.L., and Courchesne F. 2008. Hydrochemical behaviour of dissolved nitrogen and carbon in a headwater stream of the Canadian Shield: relevance of antecedent soil moisture conditions. Hydrological Processes, 22:327–339.

Twardowski M.S., Boss E., Sullivan J.M., Donaghay P.L. 2004. Modeling the spectral shape of absorption by chromophoric dissolved organic matter. Marine Chemistry 89: 69-88.

Valett H.M., Baker M.A., Morrice J.A., Crawford C.S., Molles M.C., et al. 2005. Biogeochemical and metabolic responses to the flood pulse in a semiarid floodplain. Ecology 86:220–34.

Vannote R.L. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences, 37(1):130-137.

Vazquez E., Romani A.M., Sabater F., Butturini A. 2007. Effects of the dry–wet hydrological shift on dissolved organic carbon dynamics and fate across stream–riparian interface in a Mediterranean catchment. Ecosystems, 10(2):239-251.

Vazquez E., Amalfitano S., Fazi S., Butturini A. 2010. Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions. Biogeochemistry, 102:59-72.

Vidon P.G.F, and Hill A.R. 2004. Landscape controls nitrate removal in stream riparian zones. Water Resources Research, 40, doi:10.1029/2003WR002473.

Vidon P., Wagner L.E., Soyeux E. 2008. Changes in the character of DOC in streams during storms in two Midwestern watersheds with contrasting land uses. Biogeochemistry, 88:257–270.

Vogt R.D., Akkanen J., Andersen D.O., Brüggemann R., Chatterjees B., et al. 2004. Key site variables governing the functional characteristics of Dissolved Natural Organic Matter (DNOM) in Nordic forested catchments. Aquatic Sciences, 66: 195-210.

Volk C.J., Volk C.B., Kaplan L.A. 1997. Chemical composition of biodegradable disolved organic matter in streamwater. Limnology and Oceanography, 42(1):39-44.

Von Schiller D., Martí E., Riera J.L., Ribot M., et al. 2008. Inter-annual, annual, and seasonal variation of P and N retention in a perennial and an intermittent stream. Ecosystems, 11:670-687.

Wade A.J., Neal C., Butterfield D., Futter M.N. 2004. Assessing nitrogren dynamics in European ecosystems, integrating measurement and modelling: conclusions. Hydrology and Earth System Sciences, 8:846–57.

Wang L., Wu F., Zhang R., Li W., Liao H. 2009. Characterization of dissolved organic matter fractions from Lake Hongfeng, Southwestern China Plateau. Journal of Environmental Sciences, 21: 581-588.

Weishaar J.L., Aiken G.R., Bergamaschi B.A., et al. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science and Technology, 37:4072-4708.

Wetzel R.G. 2001. Limnology: Lake and River Ecosystems. 3rd edition. USA. Elsevier-Academic Press.

Wiegner T.N., and Seitzinger S.P. 2001. Photochemical and microbial degradation of external dissolved organic matter inputs to rivers. Microbial Aquatic Ecology, 24(1):27-40.

Wiegner T.N., and Seitzinger S.P. 2004. Seasonal bioavailability of dissolved organic carbon and nitrogen from pristine and polluted freshwater wetlands. Limnology and Oceanography, 49(5): 1703–1712.

Wiegner T.N., Randee L.T., MacKenzie R.A. 2009. Bioavailability and export of dissolved organic matter from a tropical river during base- and stormflow conditions. Limnology and Oceanography, 54(4): 1233–1242.

Wigington P.J., Griffith S.M., Field J.A., Baham J.E., Horwath W.R., et al. 2003. Nitrate removal effectiveness of a riparian buffer along a small agricultural stream in Western Oregon. Journal of Environmental Quality, 32:162– 170.

Wilson H.F., and Xenopoulos M.A. 2009. Effects of agricultural land use on the composition of fluvial dissolved organic matter. Nature geosciences, 2:37-41.

Willett V.B., Reynolds B.A., Stevens P.A., et al. 2004. Dissolved Organic Nitrogen Regulation in Freshwaters. Journal of Environmental Quality, 33:201–209.

Worrall F., Burt T.P., Adamson J.K. 2004. Can climate change explain increase in DOC flux from upland peat catchments? Science of Total Environment, 326:95-112.

Worrall F., Burt T.P., Adamson J.K. 2006. Trends in drought frequency: The fate of DOC export from British peatlands. Climate Change, 76 (3-4): 339-359.

Worrall F., and Burt T.P. 2008. The effects of severe drought on the dissolved organic carbon (DOC) concentration and flux from British rivers. Journal of hydrology, 361:262-274.

Wontae L., and Westerhoff P. 2005. Dissolved Organic Nitrogen Measurement Using Dialysis Pretreatment. Environmental Science and Technology, 39 (3): 879–884.

Wu F.C., Kothawala D., Evans R.D., Dillon P.J., Cai Y.R. 2007. Relationships between DOC concentration, molecular size and fluorescence properties of DOM in a stream. Applied Geochemistry, 22: 1659-1667

Ylla I., Sanpera-Calbet I., Vazquez E., Romaní A., et al. 2010. Organic matter availability during pre- and post- drought periods in a Mediterranean stream. Hydrobiologia, 657:217–232 doi: 10.1007/s10750-010-0193-z

Ylla I., Sanpera-Calbet I., Romaní A.M., Muñoz I., Sabater, S. 2011. Organic matter characteristics in a Mediterranean stream through amino acid composition: changes driven by intermittency. Aquatic Sciences, 73:523–535

Ylla I., Romaní A.M., Sabater S. 2012. Labile and recalcitrant organic matter utilization by river biofilm under increasing water temperature. Microbiology of Aquatic Ecosystems, 64:593-604.

Zepp R.G., Sheldon W.M., Moran M.A. 2004. Dissolved organic fluorophores in southeastern US coastal waters: correction method for eliminating Rayleigh and Raman scattering peaks in excitation-emission matrices. Marine Chemistry, 89: 15-36.

Zhang Y.. Liu M., Qin B., Feng S. 2009. Photochemical degradation of chromophoric-dissolved organic matter exposed to simulated UV-B and natural solar radiation. Hydrobiologia, 627: 159-168.

Zsolnay A., Jiménez M., Steinweg B., Saccomandi F. 1999. Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. Chemosphere, 38(1): 45-50.

Annex

JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 113, G03016, doi:10.1029/2008JG000721, 2008

Diversity and temporal sequences of forms of DOC and NO_3-discharge responses in an intermittent stream: Predictable or random succession?

Andrea Butturini,[1] Marta Alvarez,[1] Susana Bernal,[2] Eusebi Vazquez,[1]
and Francesc Sabater[1]

Received 3 March 2008; revised 16 May 2008; accepted 9 June 2008; published 5 August 2008.

[1] Storm events have major implications for biogeochemical cycles at local and regional scales and they provide an excellent opportunity to study the hydro-biogeochemical functioning of catchments. However, concentration-discharge (*C-Q*) responses have only been studied in detail for short periods or a few selected events. In consequence, it is difficult to quantify the diversity of *C-Q* responses in a hydrological system and impossible to assess whether the succession of forms of *C-Q* responses follows a predictable sequence or not. Bearing in mind these shortfalls, the variability of dissolved organic carbon (DOC) and nitrate (NO_3) pulses during storms is analyzed in a detailed 4-year series from an intermittent Mediterranean stream. In this study, each DOC and NO_3-*Q* response is synthesized by two descriptors that summarize its trend (ΔC; dilution/flushing/no change) and shape (ΔR; linear/nonlinear response). We observe that *C-Q* responses are widely distributed along the two-dimensional ΔR versus ΔC continuum. Furthermore, the temporal succession of forms of DOC and NO_3-*Q* responses follow a random pattern, and only the dynamics of the $\Delta R_{(NO3)}$ descriptor show periodicity. The long-term data set reveals that it is impossible to predict with reasonable precision the full properties of DOC and NO_3-*Q* responses. Thus, a "typical" *C-Q* response does not really exist at our study site, and this apparent diversity of responses has to be handled with a probabilistic approach that allows synthesis of the complexity of the hydro-biogeochemical functioning of a specific catchment.

Citation: Butturini, A., M. Alvarez, S. Bernal, E. Vazquez, and F. Sabater (2008), Diversity and temporal sequences of forms of DOC and NO_3-discharge responses in an intermittent stream: Predictable or random succession?, *J. Geophys. Res.*, *113*, G03016, doi:10.1029/2008JG000721.

1. Introduction

[2] Storm events are the most effective cause of solute flushing in streams on a short timescale (from hours to weeks). Their occurrence, frequency and magnitude have major implications for biogeochemical cycles at local and regional scales and for management of inland waters [*McClain et al.*, 2004].

[3] Concentration-discharge (*C-Q*) responses related to storms span from linear to nonlinear relationships [*Evans and Davies*, 1998]. These patterns reflect the complex hydro-chemical processes in watersheds and provide crucial information for determining the origin and fate of solutes/pollutants in running waters.

[4] The visual characteristics of *C-Q* responses (slope, shape and rotational pattern if hysteresis appears) facilitate their classification with a few, simple parameters [*Johnson and East*, 1982; *Evans and Davies*, 1998; *House and Warwick*, 1998; *Butturini et al.*, 2005]. For instance, *Evans and Davies* [1998] identified 6 discrete *C-Q* hysteresis types within the framework of the mixing hydrological model [*Christophersen et al.*, 1990]. Being conscious that a straightforward hydro-chemical interpretation of solute patterns during storms requires caution [*Butturini et al.*, 2005; *Rice et al.*, 2004; *Chanat et al.*, 2002], the typification of these specific *C-Q* patterns represents a promising starting point for the study of concentration fluctuations during storms in terms of "diversity" of *C-Q* responses.

[5] To date, the diversity of *C-Q* responses has barely been studied by hydro-biogeochemists. Due to obvious methodological and/or human resource constraints, most of the research focused on *C-Q* responses has gathered the information from small catchments (preferentially in temperate regions) during a few selected events or for relatively short periods. These studies might convey the perception that a satisfactory description of *C-Q* responses for a specific solute could be obtained by monitoring only a few events in detail. Nevertheless, the increasing evidence that forms of *C-Q* responses show significant variability [*Soulsby*, 1995; *Biron et al.*, 1999; *Evans and Davies*, 1998;

---

[1] Department d'Ecologia, Universitat de Barcelona, Barcelona, Spain.
[2] Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey, USA.

0148-0227/08/2008JG000721$09.00

Rice et al., 2004; *Andrea et al.*, 2006; *Inamdar et al.*, 2006; *Ocampo et al.*, 2006] and the opportunity to automate long-term high resolution sampling programmes [*Kirchner et al.*, 2004] will encourage a more exhaustive analysis of solute behavior associated with storm events under a wide spectra of hydro-climatic conditions.

[6] No one has explored the characteristics of a large series of *C-Q* responses as a concatenation of storm episodes of different magnitudes spaced at irregular intervals. Furthermore, an attempt to synthesize the description of forms of *C-Q* responses from the point of view of their "diversity" is, to our knowledge, missing. In this context, the questions that this study attempts to answer are the following: (1) How diverse are the *C-Q* responses of nutrients? (2) Does the temporal succession of types of *C-Q* response occur in a predictable sequence? And finally, a logical question that arises from question 2 is: (3) If a predictable sequence is detected, what controls it?

[7] Here we focus on nitrate (NO_3) and dissolved organic carbon (DOC)-*Q* responses. Both solutes are reactive and their patterns integrate the hydrological mechanisms and biotic processes that occur in catchments [*Mulholland and Hill*, 1997]. Furthermore, NO_3 and DOC are studied widely by biogeochemists because of their relevance to the actual nitrogen and carbon cycles in catchments and their ongoing alteration as well as to improving the management of water quality for human consumption [*Houghton*, 2003; *Galloway*, 2003].

[8] In order to provide a complete view of the diversity of DOC and NO_3-*Q* responses and their temporal succession, both solutes were monitored in detail over 4 years in an intermittent Mediterranean stream. The DOC and NO_3-*Q* responses for each storm event are described with two simple descriptors successively plotted in a two-dimensional unity plane. The diversity of DOC and NO_3-*Q* responses (question 1) is explored in terms of the dispersion of data in the unit plane and by means of the classic Shannon diversity index. Contingency periodograms analysis [*Legendre et al.*, 1981] is used to explore the temporal succession of types of *C-Q* response (question 2). Finally, if cyclicities in *C-Q* responses are detected, they are overlapped with those observed for environmental variables characterizing hydrological and climatic conditions prevailing in the catchment (i.e., the magnitude of the storm events, the wetness in the catchment prior to the storm event, and the seasonal changes in temperature) (question 3).

2. Hydrological and Biogeochemical Characterization of the Study Site

[9] The hydro-chemical data set used in this research is from the Fuirosos stream in the Montnegre natural park (41° 42′N; 2° 34′E; 50–700 m a.s.l.). Fuirosos drains a forested, granitic catchment of 13 km^2. The forest (oak holm, coniferous and deciduous) covers 90% of the total catchment area. Climate is Mediterranean with air temperature ranging from −2 to 28°C. Streamflow is typically intermittent with a no flow period from July to September, followed by a dry-wet hydrological transition phase (September–October) and a humid period with permanent flow (October–May) [*Butturini et al.*, 2003]. Stream basal flow discharge ranged between 0 and 25 L s^{-1} (Figure 1a).

[10] The hydro-biogeochemical data set covers nearly 4 years, from September 1999 to April 2003. This temporal series includes a wide spectrum of hydro-climatic conditions during which the frequency and magnitude of storms was extremely erratic [*Bernal et al.*, 2006]. The time elapsing between rain episodes ranged typically from 4 to 30 days, but longer periods without precipitation were far from being sporadic. For instance, no-rain periods of between 3 and 5 months occurred in summers 2000 and 2001 and spring 2000. On the other hand, the heavy precipitation episodes recorded in spring and summer 2002 prevented the summer dry period (Figure 1a). During the study 105 rain events ranging from 2 to 153 l m^{-2} occurred. Storm hydrographs were negligible when total precipitation ($Rain_{Tot}$) was lower than 8 l m^{-2}. Thus, we attempted to obtain as much hydro-chemical data as possible from the 63 storm events with $Rain_{Tot} \geq 8$ l m^{-2}.

[11] The magnitude of storms (ΔQ) ranged from 1 to >2000 l s^{-1} and showed a significant relationship with $Rain_{Tot}$ ($r^2 = 0.6$, df = 61, $p < 0.001$), the data from the dry-wet hydrological transition period being responsible for most of its variability [*Butturini et al.*, 2002]. Storm hydrographs lasted from 0.5 to 18 days, and in 80% of cases they were shorter than a week. Hydrographs were brief with a rising discharge limb shorter than 12 hours or 1 day in 66% and 84% of the events, respectively.

[12] In stream water, DOC and NO_3 concentrations ranged between 2 and 20 C ppm and 0.01 and 3 NO_3–N ppm (Figures 1b and 1c). Both solutes showed clear seasonal patterns. Nitrate basal concentration showed a maximum in winter and a minimum in late spring-summer, which may be a response to seasonal variation in nitrate uptake by terrestrial vegetation and soil microbial organisms [*Butturini et al.*, 2003; *Bernal et al.*, 2005]. In turn, DOC had a typically steep peak during the transition between the dry and wet periods (September–October). Previous studies attribute the DOC peak to the flushing of abundant organic matter accumulated in the streambed during the dry period [*Bernal et al.*, 2002; *Butturini et al.*, 2005; *Romaní et al.*, 2006; *Vazquez et al.*, 2007]. The lack of a DOC peak in September–October 2002, preceded by a wet summer period, supported this hypothesis.

3. Material and Methods

3.1. Field Monitoring Strategy

[13] Stream water was sampled manually every 7–14 days during base flow conditions. Sampling frequency was increased up to every 2–5 hours during storms with a stage actuated water sampler (Sigma 900 max).

[14] Stream discharges were estimated on each sampling date by mass balance calculation using the "slug" chloride addition method [*Gordon et al.*, 1992]. The stream water level was continuously recorded using a water pressure transducer connected to the automatic sampler.

[15] As previously mentioned, a total of 63 precipitation events met the condition $Rain^{3}_{Tot}8$ l/m^2. A detailed description of *C-Q* responses was obtained in 49 cases (78% of total events). The distribution of the missing values is related to the magnitude of the rain events. In fact, most of the missing *C-Q* responses are associated with the

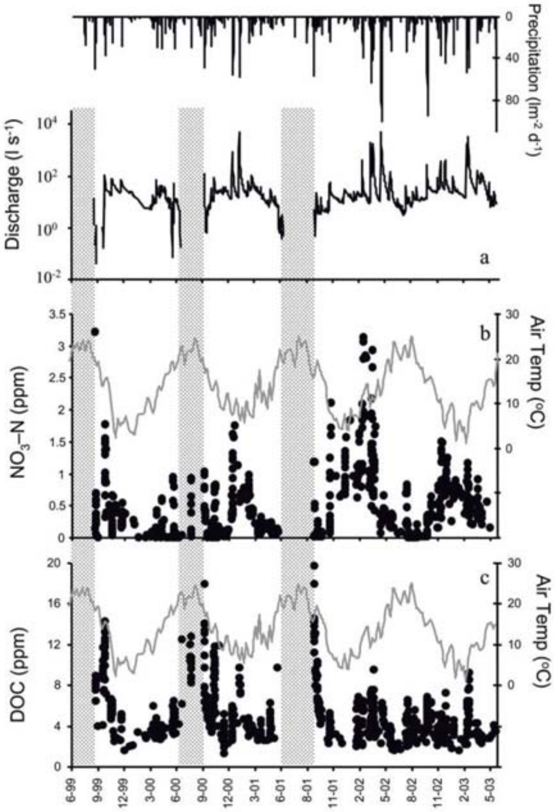


Figure 1. Temporal dynamics of (a) daily precipitation and discharge, (b) NO_3 (as N), and (c) DOC during the study period. Gray lines in Figures 1b and 1c show the air temperature regime. The shaded area outlines the summer no flow periods.

smallest rain episodes, and the percentage of sampled cases increases up to 90% if $Rain_{Tot} > 20$ mm.

3.2. Chemical Water Analysis

[16] All water samples were filtered through pre-ashed GF/F glass fibre filters and stored at 4°C until analyzed. Nitrate (NO_3-N) was analyzed colorimetrically with a Technicon Autoanalyser (Technicon 1976) after reduction of the solute on a copper-doped cadmium column. Dissolved organic carbon (DOC) was analyzed with a high-temperature catalytic oxidation method (Shimadzu TOC analyzer) (for additional information, see *Bernal et al.* [2006]).

3.3. Description of *C-Q* Responses

[17] Each *C-Q* response is characterized by two simple semiquantitative descriptors that summarize solute fluctuation during the storm episode: ΔC ($\Delta C_{(DOC)}$, $\Delta C_{(NO3)}$) and ΔR ($\Delta R_{(DOC)}$, $\Delta R_{(NO3)}$). ΔC (%) describes the relative changes in solute concentration of the *C-Q* response, by the following formula:

$$\Delta C = \left\{ \begin{array}{ll} \dfrac{C_s - C_b}{C_s} 100 & \text{if } C_s > C_b \\ \dfrac{C_s - C_b}{C_b} 100 & \text{if } C_s < C_b \end{array} \right\} \qquad (1)$$

where C_b and C_s are the solute concentrations at the base flow and during the peak of the storm hydrograph, respectively. ΔC ranges between −100 and 100. Negative ΔC values indicate solute dilution. Positive ΔC values indicate solute flushing.

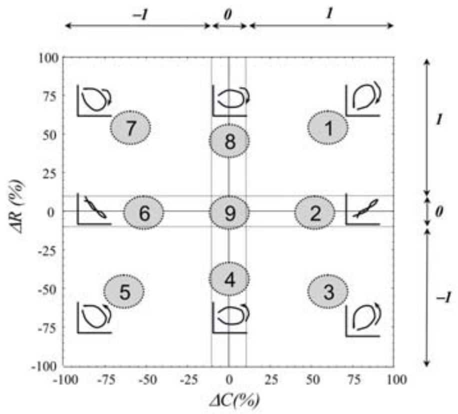


Figure 2. Schematic representation of the unity plane ΔC versus ΔR that describes the diversity continuum across the geometrical forms of *C-Q* responses. In this plane, the vertical and horizontal dotted lines delimit the nine discrete different types of *C-Q* response (see text for additional information).

[18] The ΔR descriptor (%) ranges between -100 and 100 and provides information about the area and rotational pattern of the *C-Q* response:

$$\Delta R = R * A * 100 \qquad (2)$$

where A is the area of the *C-Q* response, estimated after standardizing discharges and concentrations to a unity scale ($0 \leq A \leq 1$). The term R describes the rotational pattern of *C-Q* responses and therefore the timing of solute changes during storms:

$R = 1$	clockwise rotational pattern
$R = -1$	counterclockwise rotational pattern

For ambiguous or nonexistent rotational patterns we set $R = 0$.

3.4. Classification and Diversity of *C-Q* Responses

[19] The combination of the ΔC and ΔR descriptors ($\Delta(CR)$) synthesizes the variability of the geometrical properties of the *C-Q* responses in the two-dimensional continuum unity plane ΔR versus ΔC.

[20] A discrete qualitative classification of *C-Q* responses can be obtained by splitting the continuum unity plane into 9 regions (labeled from "1" to "9"). Each region identifies a *C-Q* response type. This implies classifying the ΔC and ΔR descriptors into three qualitative categories ("-1," "0" and "1"). The threshold of ±10% is used to generate these discrete categories:

$\Delta C < -10\%$	-1	(solute dilution)
$-10\% \leq \Delta C \leq 10\%$	0	(neutral)
$\Delta C > 10\%$	1	(solute release)
$\Delta R < -10\%$	-1	(counterclockwise loop)
$-10 \leq \Delta R \leq 10\%$	0	(no loop)
$\Delta R > 10\%$	1	(clockwise loop)

[21] The unity plane is then divided into 3^2 regions. In this way the ΔR versus ΔC plane includes the six *C-Q* hysteresis types (i.e. $\Delta R \neq 0$) proposed by *Evans and Davies* [1998] plus the simple lineal *C-Q* responses (i.e. $\Delta R \sim 0$) (Figure 2). Having defined the C-Q hysteresis into the nine discrete types, synthesis of their diversity is estimated by means of the classic diversity Shannon index (H) which is widely used in ecology, applied mathematics, statistics and physics [*Shannon*, 1948]:

$$H = -\sum_{i=1}^{9} p_i \ln(p_i) \qquad (3)$$

where p_i is the relative abundance of each *C-Q* type.

4. Time Analysis

4.1. Contingency Periodogram Analysis

[22] Periodicities of the temporal succession of *C-Q* response types and of three environmental parameters were explored with contingency periodograms (hereafter CP), a method for analyzing the presence/absence of periodicity in short series that requires the input of categorical data [*Legendre et al.*, 1981].

[23] For each data series a list of contingency statistic H_{cs} values were estimated for T periods ranging from 2 to 63/2 storm events. H_{cs} values were calculated according to *Shannon* [1948].

[24] Graphically a CP consists of a plot relating the values of H_{cs} to the investigated T periods (Figure 3). The significance of a period T is tested successively by determining the probability that the associated H_{cs} value differs from zero, using the following formula [*Legendre et al.*, 1981]:

$$H_{cs} > c^2/2N \qquad (4)$$

where c^2 is the value of Chi-Square at the selected probability level (see below); N is the number of storm events in the data series. Thus, a hypothetical significant of length T indicates a cycle that comprises a sequence of T storm events (Figure 3).

[25] The presence/absence of periodicity in the ΔC and ΔR descriptors were estimated by using in each case the three qualitative categories described previously (i.e., "1," "0" and "-1"). Similarly, the periodicity of the combination of ΔC and ΔR in the unity plane ($\Delta(CR)$) was estimated using the 9 qualitative types.

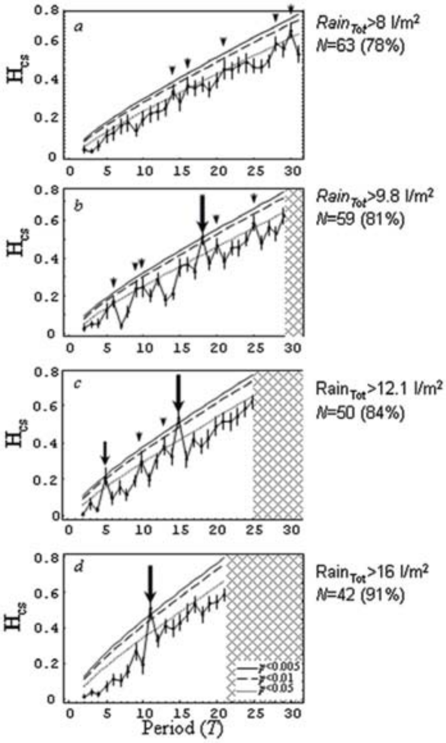


Figure 3. Selected contingency periodograms of $\Delta R_{(NO3)}$ obtained after eliminating the storm events below a selected $Rain_{Tot}$ threshold from the data set. Broken lines delimit the critical values at the 0.05, 0.01, and 0.005 probability levels. Arrows show the significant *T* periods detected by the contingency analysis (small arrow: $p < 0.05$; medium arrow: $p < 0.01$; large arrow: $p < 0.005$). For example, in Figure 3b the analysis detected an H_{cs} value that differed from zero at $p < 0.005$ at a period $T = 18$, i.e., a cycle that comprised 18 storm events. In Figures 3c and 3d the cycles emerged every 15 and 11 storm events, respectively (see the Time Analysis section for additional explanation). *N* is the number of storm events in the data series. In parenthesis is the percent of sampled events.

4.2. Contingency Periodogram Matrices for *C-Q* Response Descriptors

[26] Given that *C-Q* descriptors (ΔC, ΔR and $\Delta(CR)$) were missing in 22% of cases, in order to improve the robustness of the contingency statistic H_{cs} estimations we proceeded as follows:

[27] 1. A random value of ΔC, ΔR and $\Delta(CR)$ was assigned to the events without chemical information.

[28] 2. To reduce the uncertainty generated by random values, H_{cs} was calculated 10^4 times and the mean±standard deviation was calculated for each *T* period investigated.

[29] 3. Being aware that the percent of sampled storm events increased with $Rain_{Tot}$, we assembled a matrix of contingency periodograms for each *C-Q* descriptor. The concatenation of the individual CPs that composed a matrix was obtained after eliminating those storm events caused by a rain episode lower than a selected $Rain_{Tot}$ threshold from the data series. As the $Rain_{Tot}$ threshold increased, the number (N) of storm cases in the data series decreased but the percent of sampled storm events increased. In our case the $Rain_{Tot}$ threshold increased progressively from 8 to 30 l/m². Conversely the number of storm episodes (N) in each data series decreased gradually from 63 to 29 and the percent of sampled storm events increased from 78% to 93% (see Figure 3 for an example of individual CP graphs that composed a CP matrix).

[30] 4. For each CP matrix only *T* periods with Chi-Square probabilities $p < 0.05$, $p < 0.001$ and $p < 0.005$ (equation (4)) were extracted and plotted on a surface graph.

[31] 5. Having obtained the surface graph face for each *C-Q* descriptor, a cycle is considered robust solely when we can draw an oblique line that connects the significant *T* periods detected at different $Rain_{Tot}$ thresholds. Otherwise we assumed that the data series was randomly assorted.

4.3. Contingency Periodogram Matrices for Environmental Parameters

[32] Contingency periodograms can be used to explore the periodicity of environmental variables after reducing the quantitative series into three categories according to the criteria of *Legendre et al.* [1981].

[33] In this study, we included three intuitive environmental variables frequently used to explore potential cause-effect relationships in hydro-biogeochemical catchment studies [*Rice et al.*, 2004] and that also synthesized the hydro-climatic properties of the Fuirosos catchment appropriately [*Bernal et al.*, 2002]: (1) magnitude of each storm event (ΔQ); (2) basal discharge (Q_{bas}) immediately preceding each storm, indicating the antecedent wetness of the catchment; (3) average air temperature during each storm (T_{day}), capturing the regularity of seasons over time and representing a proxy for biotic activity in the catchment [*Hobbs et al.*, 1995]. After the categorization of environmental parameters, CP matrices were assembled following the same criteria used for *C-Q* descriptors. Environmental data were available for all storms, and so there was no need to include random values (i.e., the previously described steps 1 and 2 were unnecessary).

5. Results

5.1. Time Analysis of Environmental Variables

[34] The CP analysis detected a significant cycle with a period that comprised 16 storm events ($T = 16$; N = 63 and its replica at $T = 31$; N = 63) for Q_{bas} and T_{day} (Figures 4a and 4b, respectively). These cycles are robust. For instance, for Q_{bas}, the cycle persisted as the $Rain_{Tot}$ threshold increased, though the length of *T* declined progressively as the number of N decreased (from 16 to 6), until it vanished at N < 30. An oblique line connecting these

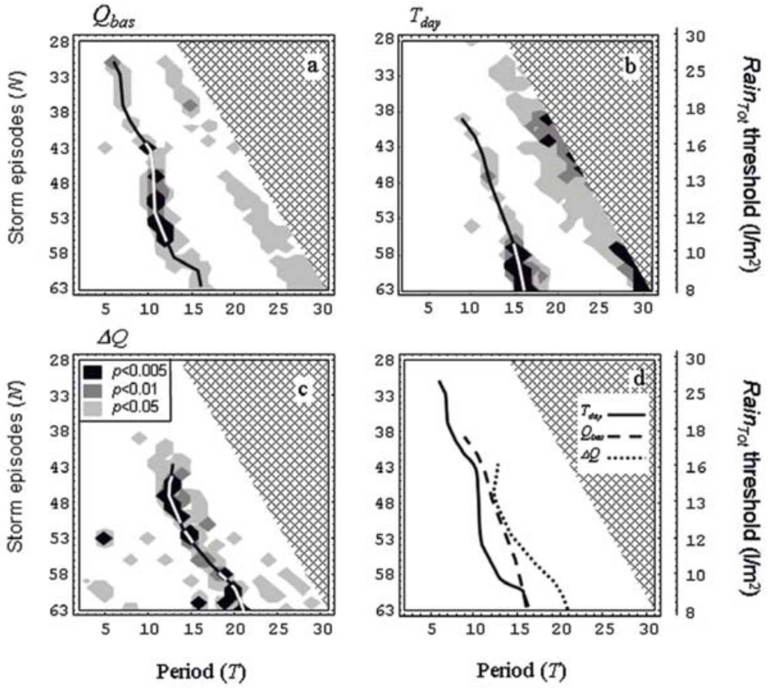


Figure 4. Contingency periodogram matrix for (a) basal discharge preceding the storm events (Q_{bas}), (b) air temperature during storms (T_{day}), and (c) magnitude of the storm events (ΔQ). Solid oblique lines that overlap the periodogram matrices connect the statistically significant *T* periods at different $Rain_{Tot}$ thresholds. (d) Location of the oblique lines of the three environmental variables in a single periodogram matrix.

significant periods at different $Rain_{Tot}$ thresholds is drawn in the surface graph (Figure 4a). The cycle observed for T_{day} does not overlap exactly with that for Q_{bas}. In fact, from N < 60, this cycle is always longer than that for Q_{bas} (Figure 4d).

[35] The magnitude of storm events (ΔQ) shows a clear and persistent cycle. This cycle starts with a length of $T = 21$ (N = 63), and decreases to $T = 13$ before disappearing (N < 39) (Figure 4c). Therefore, the period of this cycle is longer than those estimated for Q_{bas} and T_{day} (Figure 4d) at a $Rain_{Tot}$ threshold lower than 12 l m^{-2}.

5.2. Diversity and Time Analysis of DOC and NO$_3$-*Q* Responses

[36] Dispersion of DOC-*Q* response data in the ΔR versus ΔC unit plane clearly differed from that of NO$_3$. DOC data covered 16% of the unit plane (Figure 5a), while the NO$_3$ data were spread over 48% (Figure 5b). In more detail, DOC data fall in 6 regions of the unity plane. A total of 40% of events are type 2 and the remaining regions range between 27% (type 1) and 2% (types 3 and 7). $H_{(\Delta R,\Delta C)}$ values, measured sequentially during the events series, peak at 2.4 after 9 events (Figure 5a, inset). DOC release ($\Delta C >$ 10%, 73% of events) clearly predominates over dilution. A total of 51% of cases are linear DOC-*Q* responses ($-10\% < \Delta R < 10\%$), while 45% are clockwise DOC-*Q* hysteresis ($\Delta R > 10\%$).

[37] NO$_3$ data fall in all the 9 potential regions of the unity plane. The most probable NO$_3$-*Q* response is that of type 3 (37%) and the contribution of the remaining NO$_3$-*Q* types ranges between 14% (type 2) and 2% (type 5). All NO$_3$-*Q* responses are rather well distributed over time. Consequently, $H_{(\Delta R,\Delta C)}$ values, measured sequentially over the events sequence, increase more slowly than DOC and peak at a value of $H_{(\Delta R,\Delta C)} \sim 2.9$ after 18 events (Figure 5b, inset). NO$_3$ release (69% of events) predominates over dilution. Counterclockwise, clockwise, and linear NO$_3$-*Q* responses are 47%, 24% and 27%, respectively.

[38] The CP analysis indicated that $\Delta(CR)_{(DOC)}$ and $\Delta(CR)_{(NO3)}$ do not show any predictable pattern (data not shown). Therefore, the displacement of DOC and NO$_3$ data in the ΔR versus ΔC unit planes follows a random pattern.

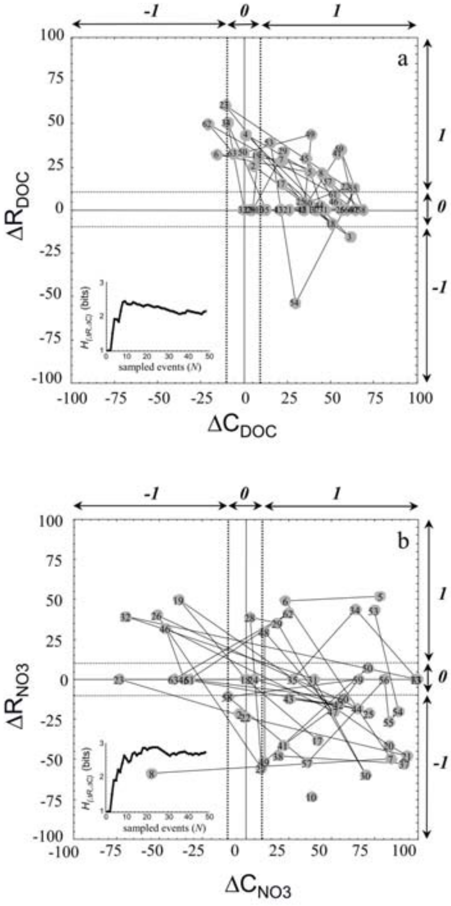


Figure 5. Dispersion of (a) DOC-*Q* and (b) NO_3-*Q* responses in the ΔC versus ΔR unity plane. Numbers within circles illustrate the time succession of the storm events. Vertical and horizontal dotted lines in the ΔC versus ΔR unity plane delimit the nine different types of *C-Q* response. The figures in the inset show the evolution of the DOC-*Q* and NO_3-*Q* response diversity ($H_{(\Delta R,\Delta C)}$) measured sequentially over the event series.

Some significant periods with short lengths ($2 < T < 8$ for DOC and $2 < T < 6$ for NO_3) were detected (in most cases $p < 0.05$), though such cycles were inconsistent because they did not re-emerge regularly during longer periods (i.e., at *T* between 8 and 31). Furthermore, the observed significant cycles of *T* are shorter than the minimum number of episodes required to obtain a full picture of *C-Q* response variety (9 and 18 cases for DOC and NO_3, respectively).

[39] On the other hand, when temporal sequences of ΔR and ΔC descriptors are analyzed separately, a periodicity is observed for $\Delta R_{(NO3)}$ and, to a lesser degree for $\Delta C_{(NO3)}$ (Figures 6a and 6b). In more detail, the CP matrix of $\Delta R_{(NO3)}$ detects numerous significant periods (at least $p < 0.05$) at different $Rain_{Tot}$ thresholds. Nevertheless, only one cycle persists at different $Rain_{Tot}$ thresholds (the solid oblique line in Figure 6a). This cycle persists until a $Rain_{Tot}$ threshold of 18 l m^{-2} is reached ($N = 40$). Initially, the cycle is characterized by a period of $T = 22$ storm episodes that declines progressively with $Rain_{Tot}$ until it disappears at $T = 11$.

[40] A cycle for $\Delta C_{(NO3)}$ is also detected, however its periodicity is slightly longer and patently weaker than that observed for $\Delta R_{(NO3)}$. It is longer because initially it comprises 24 storm episodes ($T = 24$; $N = 63$, $p < 0.05$), and vanishes at $T = 17$ ($Rain_{Tot}$ threshold of 13 l m^{-2}; $N = 40$, $p < 0.05$). It is weaker because the oblique line that links these significant periods *T* is much shorter than that observed for $\Delta R_{(NO3)}$ (Figure 6b).

[41] The $\Delta R_{(DOC)}$ and $\Delta C_{(DOC)}$ series lack any consistent cycles, indicating that their temporal succession is randomly assorted. In both cases, some significant periods are detected at different $Rain_{Tot}$ thresholds. However, distribution of these significant periods in the periodogram matrices does not show any consistent pattern (Figures 6c and 6d).

6. Discussion

[42] The data set, obtained from 4 years of intense hydrochemical monitoring, demonstrates the need to describe the diversity of forms of *C-Q* responses in probabilistic terms. It is worth noting that the most probable *C-Q* response types (i.e., types 2 and 3 from Figure 5, for DOC and NO_3, respectively) represent as much as 40% of all cases. Thus, the majority of the DOC and NO_3-*Q* responses fall within some low probability *C-Q* response types. These results point to the large degree of uncertainty in the depiction of a "typical" DOC and NO_3-*Q* response and underline the importance of identifying the "typical" probability distribution of a set of *C-Q* responses within the ΔR versus ΔC unity plane.

[43] Under the proposed probabilistic approximation, DOC and NO_3 data from our study show different distributions and limits in the ΔR versus ΔC unity plane. The DOC data set, although intersecting several *C-Q* response regions, lies in a relatively small portion of the ΔR versus ΔC unity plane. Thus, a minimum set of ~9 events is required to describe the diversity of DOC-*Q* responses satisfactorily. In contrast, the NO_3 data set is homogenously distributed in a large portion of the ΔR versus ΔC unity plane. This implies that a set of at least ~18 events is necessary to capture the variety of NO_3-*Q* responses satisfactorily. An evident consequence of this result is that if we ignore the magnitude of diversity of *C-Q* responses of a determined solute, we do not know if the hydrobiogeochemical interpretation obtained with an arbitrary number of storm episodes is representative or not of the hydro-chemical functioning of the watershed studied.

ix

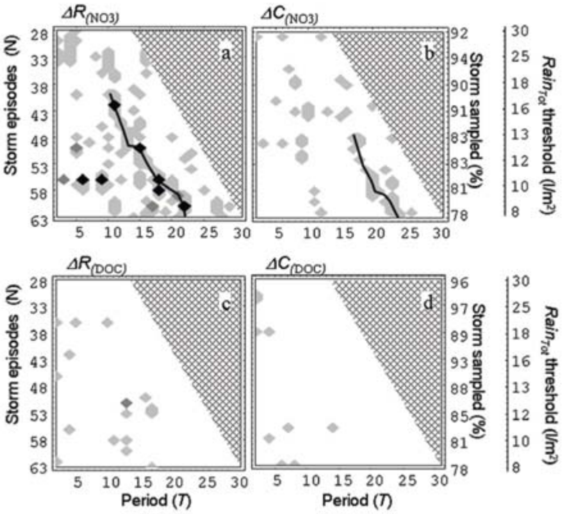


Figure 6. Contingency periodogram matrices for (a) $\Delta R_{(NO3)}$, (b) $\Delta C_{(NO3)}$, (c) $\Delta R_{(DOC)}$, and (d) $\Delta C_{(DOC)}$. Oblique lines in the periodogram matrices connect the statistically significant periods.

[44] Within the classical mixing hydrological framework, a plausible hydro-biogeochemical explanation for each *C-Q* response type reported in this study could be given [*Rice and Hornberger*, 1998]. However, the classical mixing model appears to be inadequate to provide a unique and coherent description of patterns of solutes during storms in an intermittent stream such as our study site. For instance, if we constrain our observations to the NO_3 counterclockwise hysteresis (47% of all cases) we might conclude that flushing is transport limited [*Burns*, 2005]. Contrarily, if we analyze the NO_3 clockwise hysteresis (24% of cases) we might conclude that its flushing is supply limited [*Burns*, 2005]. A consequence of this high biochemical variability is that the use of the classical mixing approach to identify the sources of NO_3 in Fuirosos is feasible during the humid period but not during the dry-wet transition period [*Bernal et al.*, 2006].

[45] Therefore, it is necessary to develop a hydro-biogeochemical framework flexible enough to justify the diversity of *C-Q* responses and the temporal succession of these forms over the seasons. Recent theoretical studies increase the spatial heterogeneity of the hydrological processes within the mixing model framework, improving its conceptual flexibility [*Chanat et al.*, 2002; *Butturini et al.*, 2005]. However, the introduction of new boxes and additional parameters is encouraged, making scientists inquire about whether this approach is really the most appropriate to capture the nonlinear hydro-biogeochemical behavior of catchments [*Kirchner et al.*, 2004].

[46] The contingency analysis reveals that ΔR and ΔC values cannot be predicted simultaneously either for DOC or NO_3. However, when ΔR and ΔC descriptors are analyzed separately, signals of periodicity emerge for NO_3. This result is especially attractive for $\Delta R_{(NO3)}$ because it suggests that the timing of NO_3 delivery into the stream over a sequence of storm episodes can be inferred. These results lead to the last question behind our research: what controls the cycles observed for $\Delta R_{(NO3)}$ and $\Delta C_{(NO3)}$?

[47] A preliminary step toward dealing with this question is to overlap the periodogram matrices of $\Delta R_{(NO3)}$ and $\Delta C_{(NO3)}$, with those obtained for the climatic (T_{day}) and the hydrological variables (ΔQ and Q_{bas}) considered in this study. Figure 7 shows that the period of the cycle of $\Delta C_{(NO3)}$ is longer than that observed for the hydro-climatic variables, which show marked seasonality (i.e., cycles of about 1 year, Figures 1 and 4). Thus, processes that act a timescales in between 1 and 2 years might influence the cycle of $\Delta C_{(NO3)}$. Being realistic, at the moment, such a timescale cannot be studied satisfactorily by handling a 4-year time series only, and we can simply use this result to demonstrate the need to generate pluri-annual hydro-biogeochemical series at high resolution.

[48] On the other hand, the *T* period of $\Delta R_{(NO3)}$ overlaps reasonably well with that of ΔQ at $Rain_{Tot} < 13\ \mathrm{l\ m^{-2}}$, and with T_{day} at $13 < Rain_{Tot} < 18\ \mathrm{l\ m^{-2}}$. This suggests that the periodicity of the magnitude of storms (ΔQ) is the most important driver for the succession of linear and nonlinear NO_3-*Q* responses over the entire spectra of rain magnitudes, while the effect of the seasonal temperature change (i.e., T_{day}), appears more perceptible after the removal of the signal of the weaker and more frequent rain episodes.

[49] The problem of excess NO_3 in running waters is a recognized problem worldwide [*Burgin and Hamilton*, 2007] and to achieve an accurate simulation of its temporal dynamics constitutes a major challenge for modelers [*Wade et al.*, 2004], especially in intermittent streams where

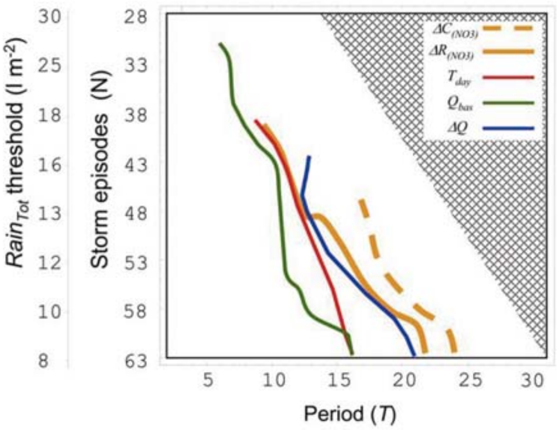


Figure 7. Location, in a single plot, of the oblique lines that connect the statistically significant *T* periods at different $Rain_{Tot}$ thresholds for the environmental variables considered in this study (T_{day}, ΔQ, and Q_{bas}) and the NO_3-*Q* response descriptors ($\Delta C_{(NO3)}$ and $\Delta R_{(NO3)}$).

simulations are clearly unsatisfactory [*Bernal et al.*, 2004]. From this perspective, our results might represent a stimulus for these scientists, because they suggest that the succession of forms of NO_3-*Q* responses might be coupled to the magnitude of storm events. On the other hand, the same results also show that a large portion of uncertainty is inevitable and suggest that a probabilistic modeling approach should replace the deterministic one [*Botter et al.*, 2006].

7. Conclusions and Perspectives

[50] Abundant scientific literature clearly demonstrates the impact exerted by storm events on solute patterns and fluxes. However, pollution of inland waters and the plausible alteration of hydrological regimes on a global scale as a consequence of climate alteration [*Intergovernmental Panel on Climate Change* (*IPCC*), 2007] demonstrate the importance of extending these studies. To persuade their colleagues in this direction, some scientists describe storm events as "hot moments" [*McClain et al.*, 2004] or compare their role to a "crescendo" in a musical piece [*Kirchner et al.*, 2004].

[51] In this context, our study is the first to describe the limits of diversity of DOC and NO_3-*Q* crescendos in a stream under a wide spectrum of hydro-climatic conditions and underlines the need to describe the diversity of *C-Q* responses in terms of distribution of *C-Q* types with different probabilities. Furthermore, the succession of data in the ΔR versus ΔC unity planes is random: a priori, it is impossible to predict the entire pattern of these solutes during storms with satisfactory precision.

[52] In environmental sciences, the use of the adjective "diversity" is immediately related to the biological richness. Thus, at this stage it is natural to inquire to what extent the diversity of *C-Q* responses reflects the complexity of internal hydro-biogeochemical functioning of a specific catchment. For instance, data from our study came from an intermittent stream with an abrupt autumnal hydrological transition from dry to wet conditions [*Butturini et al.*, 2003] with high and low DOC and NO_3 concentration respectively in stream waters [*Bernal et al.*, 2005]. Overall, the DOC-*Q* responses monitored during this transitional period (33% of events) contributed to 41% of the total DOC-*Q* response diversity, while in the case of NO_3, the autumn NO_3-*Q* responses did not influence the total *C-Q* response diversity. Therefore, although the dry-wet transition does not promote a typical *C-Q* response during the following autumn, it contributes to enhancing the diversity of DOC-*Q* (but not of NO_3-*Q*) responses.

[53] For ecologists, understanding the relationship between biodiversity and ecosystem functioning has been a fertile theme since the beginning of the 1990s that has been continually fuelled and renewed by the feedback between empirical data and new hypotheses [*Naeem et al.*, 2002]. Here, in stream hydro-biogeochemistry, we are just starting. In order to strength this theme we need to widen the implementation of long-term high frequency hydro-chemical monitoring programmes [*Kirchner et al.*, 2004]. When this requisite is achieved it will be possible to analyze how the diversity of *C-Q* responses varies among catchments with different hydro-climatic characteristics. Within this context, the ΔR versus ΔC unity plane might constitute a synthetic, intuitive and universal framework with which to compare and classify the dispersion of the *C-Q* responses of a specific solute along catchments.

[54] **Acknowledgments.** The authors thank Sergi Sabater, Ester Nin and Antoni Bombí (Servei Parcs Naturals, Diputació de Barcelona), for their support in the field and Chiara Medici for support with environmental data management. We would also like to thank two anonymous reviewers

for their fruitful comments, which greatly helped to improve an earlier version of this manuscript. This study was supported by the Ministerio de Educación y Ciencia (CGL2007–60144). The authors are members of the Limnology Group (UB-CEAB_CSIC). A.B. is a member of the Graccie network (CSD 2007–00067).

References

Andrea, B., F. Gallart, J. Latron, E. Vazquez, and F. Sabater (2006), Cross-site comparison of variability of DOC and nitrate c-q hysteresis during the autumn-winter period in three Mediterranean headwater streams: A synthetic approach, *Biogeochemistry*, *77*(3), 327–349.

Bernal, S., A. Butturini, and F. Sabater (2002), Variability of DOC and nitrate responses to storms in a small Mediterranean forested catchment, *Hydrol. Earth Syst. Sci.*, *6*(6), 1031–1041.

Bernal, S., A. Butturini, J. L. Riera, E. Vázquez, and F. Sabater (2004), Calibration of the INCA model in a Mediterranean forested catchment: The effect of hydrological inter-annual variability in an intermittent stream, *Hydrol. Earth Syst. Sci.*, *8*(4), 729–741.

Bernal, S., A. Butturini, and F. Sabater (2005), Seasonal variations of dissolved nitrogen and DOC:DON ratios in an intermittent Mediterranean stream, *Biogeochemistry*, *75*, doi:10.1007/s10533-005-1246-7.

Bernal, S., A. Butturini, and F. Sabater (2006), Inferring nitrate sources through end member mixing analysis in an intermittent Mediterranean stream, *Biogeochemistry*, doi:10.1007/s10533-006-9041-7.

Biron, P. M., A. G. Roy, F. Courschesne, W. H. Hendershot, B. Cote, and J. Fyles (1999), The effects of antecedents moisture conditions on the relationship of hydrology to hydrochemistry in a small forested catchment, *Hydrol. Process.*, *13*, 1541–1555.

Botter, G., T. Settin, M. Marani, and A. Rinaldo (2006), A stochastic model of nitrate transport and cycling at basin scale, *Water Resour. Res.*, *42*, W04415, doi:10.1029/2005WR004599.

Burgin, A. J., and S. K. Hamilton (2007), Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways, *Front. Ecol. Environ*, *5*(2), 89–96.

Burns, D. (2005), What do hydrologists mean when they use the term flushing?, *Hydrol. Process.*, *19*, 1325–1327.

Butturini, A., S. Bernal, S. Sabater, and F. Sabater (2002), The influence of riparian-hyporheic zone on the hydrological responses in an intermittent stream, *Hydrol. Earth Syst. Sci.*, *6*, 515–525.

Butturini, A., S. Bernal, C. Hellin, E. Nin, L. Rivero, S. Sabater, and F. Sabater (2003), Influences of the stream groundwater hydrology on nitrate concentration in unsaturated riparian area bounded by an intermittent Mediterranean stream, *Water Resour. Res.*, *39*(4), 1110, doi:10.1029/2001WR001260.

Butturini, A., S. Bernal, and F. Sabater (2005), Modeling storm events to investigate the influence of the stream-catchment interface zone on stream biogeochemistry, *Water Resour. Res.*, *41*, W08418, doi:10.1029/2004WR003842.

Chanat, J. G., K. C. Rice, and G. M. Hornberger (2002), Consistency of patterns in concentration-discharge plots, *Water Resour. Res.*, *38*(8), 1147, doi:10.1029/2001WR000971.

Christophersen, N., C. Neal, R. P. Hooper, S. Vogt, and S. Andresen (1990), Modelling stream waters chemistry as a mixture of soil water end members a step towards second generation acidification models, *J. Hydrol. Amsterdam*, *166*, 307–320.

Evans, C., and T. D. Davies (1998), Causes of concentration/discharge relationship hysteresis and its potential as tool for analysis of episode hydrochemistry, *Water Resour. Res.*, *34*, 129–137.

Galloway, J. N. (2003), The global nitrogen cycle, in *Biogeochemistry*, vol. 8, *Treatise on Geochemistry*, edited by W. H. Schlesinger, H. D. Holland, and K. K. Turekian, pp. 557–584, Elsevier, Oxford, UK.

Gordon, N. D., T. A. McMahon, and B. L. Finlayson (1992), *Stream Hydrology: An Introduction for Ecologists*, Prentice-Hall, Upper Saddle River, N. J.

Hobbs, R. J., D. M. Richardson, and G. W. Davis (1995), Mediterranean-type ecosystems: Opportunities and constraints for studying the function of biodiversity, in *Mediterranean-Type Ecosystems: The Function of Biodiversity*, edited by G. W. Davis and D. M. Richardson, pp. 1–42, Springer, Berlin.

Houghton, R. A. (2003), The contemporary carbon cycle, in *Biogeochemistry*, vol. 8, *Treatise on Geochemistry*, edited by W. H. Schlesinger, H. D. Holland, and K. K. Turekian, pp. 473–514, Elsevier, Oxford, UK.

House, W. A., and M. S. Warwick (1998), Hysteresis of the solute concentration/discharge relationship in rivers during storms, *Water Res.*, *32*(8), 2279–2290.

Inamdar, S. P., N. O'Leary, M. J. Mitchell, and J. T. Riley (2006), The impact of storm events on solute exports from a glaciated forested watershed in western New York, USA, *Hydrol. Process.*, *20*(16), 3423–3439.

Intergovernmental Panel on Climate Change (IPCC) (2007), *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by M. L. Parry et al., Cambridge Univ. Press, Cambridge, UK.

Johnson, F. A., and J. W. East (1982), Cyclical relationships between river discharge and chemical concentration during flood events, *J. Hydrol. Amsterdam*, *57*(1–2), 93–106.

Kirchner, J. W., X. H. Feng, and C. Neal (2004), The fine structure of water-quality dynamics: The (high-frequency) wave of the future, *Hydrol. Process.*, *18*, 1353–1359.

Legendre, L., M. Frechette, and P. Legendre (1981), The contingency periodogram - A method of identifying rhythms in series of nonmetric ecological data, *J. Ecol.*, *69*(3), 965–979.

McClain, M. E., et al. (2004), Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems, *Ecosystems (N. Y., Print)*, *6*, 301–312.

Mulholland, P. J., and W. R. Hill (1997), Seasonal patterns in stream water nutrient and dissolved organic carbon concentrations: Separating catchment flow path and in-stream effects, *Water Resour. Res.*, *33*(6), 1297–1306.

Naeem, S., M. Loreau, and P. Inchausti (2002), Biodiversity and ecosystem functioning: The emergence of a synthetic ecological framework, in *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives*, edited by M. Loreau, S. Naeem, and P. Inchausti, pp. 3–11, Oxford Univ. Press, Oxford, UK.

Ocampo, C. J., C. E. Oldham, M. Sivapalan, and J. V. Turner (2006), Hydrological versus biogeochemical controls on catchment nitrate export: A test of the flushing mechanism, *Hydrol. Process.*, *20*, 4269–4286.

Rice, K., and G. Hornberger (1998), Comparison of hydrochemical tracers to estimate source contributions to peak flow in a small, forested, headwater catchment, *Water Resour. Res.*, *34*(7), 1755–1766.

Rice, K. C., J. G. Chanat, G. M. Hornberger, and J. R. Webb (2004), Interpretation of concentration-discharge patterns in acid-neutralizing capacity during storm flow in three small, forested catchments in Shenandoah National Park, Virginia, *Water Resour. Res.*, *40*, W05301, doi:10.1029/2003WR002709.

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

Shannon, C. E. (1948), A mathematical theory of communication, *Bell Syst. Tech. J.*, *27*, 379–423.

Soulsby, C. (1995), Contrasts in storm event hydrochemistry in an acidic afforested catchment in upland wales, *J. Hydrol. Amsterdam*, *170*(1–4), 159–179.

Vazquez, E., A. M. Romaní, F. Sabater, and A. Butturini (2007), Effects of the dry-wet hydrological shift on dissolved organic carbon dynamics and fate across stream-riparian interface in a Mediterranean catchment, *Ecosystems (N. Y., Print)*, doi:10.1007/s10021-007-9017-0.

Wade, A. J., C. Neal, D. D. Butterfield, and M. N. Futter (2004), Assessing nitrogen dynamics in European ecosystems, integrating measurement and modelling: Conclusions, *Hydrol. Earth Syst. Sci.*, *8*(4), 846–857.

M. Alvarez, A. Butturini, F. Sabater, and E. Vazquez, Department d'Ecologia, Universitat de Barcelona, 08028 Barcelona, Spain. (abutturini@ub.edu)

S. Bernal, Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544 USA.

Ecosystems (2007)
DOI: 10.1007/s10021-007-9016-0

ECOSYSTEMS

Effects of the Dry–Wet Hydrological Shift on Dissolved Organic Carbon Dynamics and Fate Across Stream–Riparian Interface in a Mediterranean Catchment

Eusebi Vázquez,[1,*] Anna M. Romaní,[2] Francesc Sabater,[1] and
Andrea Butturini[3]

[1]*Departament d'Ecologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain;* [2]*Institut d'Ecologia Aquàtica, Universitat de Girona, Campus de Montilivi, 17071 Girona, Spain;* [3]*Centre d'Estudis Avançats de Blanes, CSIC, Accés Cala St Francesc 14, 17300 Blanes, Spain*

ABSTRACT

The stream–riparian interface, characterized by a dynamic and complex hydrology, is an important control point for nutrient fluxes and processing between terrestrial and aquatic systems. Predicted alterations in the discharge regime in Mediterranean climate regions make it necessary to understand the effects of abrupt hydrological transition between dry and wet conditions on the transport and fate of dissolved organic carbon (DOC) across the stream–riparian interface. In this study, the concentrations and fate of total DOC (TDOC) and a subset of four molecular weight fractions (<1 kDa, 1–10 kDa, 10–100 kDa, >100 kDa) were investigated in stream water and riparian groundwater during autumn of 2003 and 2004. The two study periods were characterized by contrasting antecedent hydrological conditions: the streamflow was interrupted in summer 2003 but was permanent in summer 2004. Comparison of the two study periods indicates that an abrupt dry–wet hydrological transition amplifies the water exchange across the stream–riparian interface and favors retention of up to 57% of the TDOC that flows across the interface. Furthermore, the efficiency of DOC retention across the stream-riparian interface also varies greatly depending on DOC molecular size. More than 70% of DOC fractions higher than 10 kDa were retained, whereas the smaller fraction (less than 1 kDa) was nearly conserved. Consequently, our study helps to clarify the effects of extreme hydrological events on DOC transport in running waters in Mediterranean regions.

Key words: dissolved organic carbon (DOC); molecular weight fractions; DOC retention/release; carbon cycle; stream-riparian interface; hydrology; antecedent climatic conditions..

INTRODUCTION

The stream–riparian interface is a relevant control point for fluxes and retention of nutrients between terrestrial and aquatic systems (Dahm and others

---

Received 21 February 2006; accepted 5 September 2006
**Corresponding author; e-mail:* evazquez@ub.edu

1998). The stream–riparian interface is the zone connecting the stream and the catchment and it has been defined as a spatially fluctuating ecotone between the surface stream and the deep groundwater (Boulton and others 1998). Consequently, several field studies have investigated the stream–riparian interface in regulating stream hydrology (McGlynn and McDonnell 2003; Butturini and others 2002) or biogeochemistry (Schindler and Krabbenhoft 1998; Vidon and Hill 2004; Wigington and others 2003).

In Mediterranean regions, streams are frequently characterized by strong seasonality with a humid period followed by a drought, normally in summer (Gasith and Resh 1999). In drought there is a disruption in hydrological connectivity that ranges from flow reduction to loss of hydrologic connectivity between surface water, groundwater and the riparian zone (Lake 2003). Streamflow recovers with the arrival of autumn rains. During this transition period between dry and wet conditions the hydrology of the stream–riparian interface is highly dynamic due to: (1) abrupt changes in groundwater levels; (2) occurrence of reverse fluxes in the subsurface stream–catchment interface; (3) rapid expansion and shrinkage of the boundary of the stream–catchment interface; (4) longer riparian groundwater flow-paths (Butturini and others 2003).

Recent studies in the Mediterranean adduce evidence of a severe alteration of precipitation and hydrological regimes and hypothesize an increase in the frequency of extreme events particularly in summer (Christensen and Christensen 2003; Schröter and others 2005). In this context, it is essential to assess how the stream–riparian interface controls the transport and fate of nutrients that flow in Mediterranean freshwater ecosystems under the extreme effects of dry–wet hydrological shifts that characterize the transitional period between summer and autumn.

Within this framework, the dissolved organic carbon (DOC) transported in streams is essential to secondary production in freshwater and marine ecosystems. The effects of temperature, atmospheric CO_2, or hydrology on DOC export to oceans are still unclear and a matter of debate (Freeman and others 2001ab, 2004 Tranvik and Jansson 2002; Clark and others 2005). The quantity and the bioavailability of DOC in streams is the combined result of flushing from the watershed, leaching of leaves and branches from the riparian vegetation, and in-stream primary production (Sachse and others 2005) and its temporal dynamics are usually governed by discharge regime (Butturini and others 2005; Neal and others 2005) and the occurrence of drought periods (Bernal and others 2002). Nevertheless, there is little information about the effect of the abrupt hydrological processes that take place in the stream–riparian interface during the transition between dry and wet conditions on reactivity and transport of DOC in streams. Albeit, some initial studies have been undertaken in Mediterranean streams covering DOC transport and bioavailability after drought periods (Romaní and others 2005).

Our main objective is to explore, in situ and under natural conditions, the effect of the antecedent hydrological conditions on temporal dynamics and the fate of total DOC (TDOC) and discrete dissolved organic molecular fractions transported across the stream–riparian interface to improve our knowledge of the possible effect of altering the discharge pattern on stream DOC dynamics. For this purpose, data from a forested Mediterranean stream were collected during two autumnal periods characterized by contrasting antecedent hydrological conditions. In 2003, the stream was dry in summer owing to few, small intensity, rain episodes in the precedent spring–summer period, in 2004 the streamflow was permanent throughout summer due to high levels of precipitation in the spring–summer period and, consequently, the antecedent conditions were wet and the hydrological transition usually observed at the end of the summer was nonexistent.

STUDY SITE

Fuirosos is a third-order stream that drains a forested granitic catchment of 10.5 km^2, near Barcelona (NE Spain, 41°42′ N, 2° 34′ W, 50–770 a.s.l.). The climate is typically Mediterranean, with monthly mean temperatures ranging from 3°C in January to 24°C in August. Precipitation mostly falls in autumn and spring with occasional summer storms. Average annual mean precipitation for this region is 750 mm (Ninyerola and others 2000).

The catchment is covered mostly by perennial cork oak (*Quercus suber*) and pine tree (*Pinus halepensis*) with one or two layers of shrubs (for example, *Rhamnus alaternus*, *Viburnum tinus*, *Arbutus unedo*, *Prunus spinosa*) and lianas (*Lonicera implexa*, *Smilax aspera*). Deciduous woodland of chestnut (*Castanea sativa*), hazel (*Corylus avellana*) and oak (*Quercus pubescens*) predominate in the valley head. The soils are poorly developed with an A horizon always less than 5 cm. Soils are sand (46%) and fine sand (24%), with smaller amounts of silt and clay (15% each) (Sala 1983). Traditional land uses comprise periodic harvesting of bark from

Table 1. Antecedent Hydrological Conditions for Each Study Period: Total Precipitation for the Spring-Summer Period and Number of Days without Surface Flow.

Year		2003	2004
Meteorological antecedent conditions:	Precipitation (mm)	120	300
Hydrological antecedent conditions:	Days of drought	67	0
Meteorological conditions during study period	Precipitation (mm)	186	0
Hydrological conditions during study period	Q (L s)	0–2500	2.7–6.8

Total precipitation and range of discharges for 2003 and 2004 study periods.

cork trees and partial clearing of pines and shrubs. Agricultural fields occupy less than 10% of the catchment area.

According to the description by Gasith and Resh (1999), Fuirosos stream exhibits a typical Mediterranean-type discharge regime and several biogeochemical studies used it as a model of Mediterranean catchments to perform comparisons across climate gradients (Sabater and others 2003; Burt and others 2002; Hefting and others 2004; Wade and other 2004).

The stream channel is 2–5 m wide and is characterized by steep-pool morphology with cobbles and boulders. The mean flow is 7–20 l s^{-1}. Discharge is intermittent. The flow is interrupted by a long dry period in summer followed by an abrupt recharge period in late summer–early autumn. The subsequent humid period lasts until late spring. The stream–riparian porous media is characterized by relatively high hydraulic conductivity (4.8–19 m day^{-1}) and variable specific discharges (0.03–1.5 m day^{-1}). During the stream recharge period, the groundwater level increases abruptly and the stream water can infiltrate 10 m into the near-stream riparian zone (Butturini and others 2003). After this recharge, near-stream riparian groundwater level fluctuations followed the same pattern as the stream water, which reflects the hydrological connection between the two water bodies (Butturini and others 2003).

A well-developed riparian forest flanks the 10–20 m wide stream channel, consisting mainly of plane tree (*Platanus* × hispanica) and alders (*Alnus glutinosa*). The riparian soil is poorly developed and plane leaf litter tends to accumulate on the forest floor because of extremely low decomposition rates (Bernal and others 2004). In summer, during the dry period, groundwater levels fall from 1.70 to 2.60 m below ground surface causing hydrological stress to the riparian forest area. This results in a high input of leaf litter that accumulates on the streambed and margins (Sabater and others 2001; Acuña and others 2004).

DOC concentrations in stream water at basal discharge conditions are between 2 and 4 mg l^{-1}. However, during the hydrological transition (September–October), between the dry and wet periods, DOC concentrations increase to 10–20 mg l^{-1} (Bernal and others 2002).

METHODS

Sampling Strategy

Field sampling was carried out in two consecutive years, 2003 and 2004. These two years had contrasting precipitation regimes, especially in spring and summer. In 2003, the total precipitation of the hydrological year (September–August) was 630 l m^{-2}, but the accumulated precipitation for the spring and summer period was only 120 l m^{-2}. Consequently, the streamflow was interrupted because of the summer drought. The 2004 hydrologic year was much more humid (total precipitation of 815 l m^{-2}) due to abundant precipitation in spring and summer (300 l m^{-2}) that permitted a permanent streamflow during summer (Table 1).

The water samples were collected from September to November of 2003 and 2004, respectively. The 2003 study period (September 5–November 19) refers to dry antecedent hydrological conditions, whereas 2004 (September 6–November 11) refers to wet antecedent conditions. Hence, where the text refers to "study period" or years 2003 and 2004 it refers to that year's September–November sampling period. During these study periods, the samples were collected from four water bodies whenever it was possible: (1) stream water, (2) groundwater from the near-stream riparian zone, (3) groundwater from a 5 m deep well, located on a forest hill slope, 200 m away from the stream channel, and (4) superficial water from an ephemeral channel that drains the forest soil from a small sub-watershed of 6.54 ha (Figure 1). In 2004, it was not possible to collect samples from this sub-

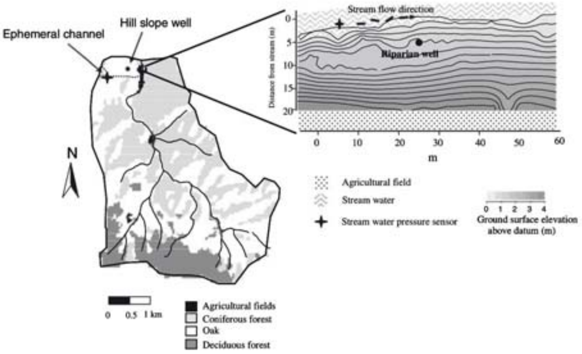


Figure 1. Fuirosos catchment with sampling sites marked and main land uses in different grey shades.

watershed owing to insufficient rainfall episodes to generate superficial runoff in that area.

Samples from the riparian groundwater and the well on the forest hill slope were collected using an ISCO field peristaltic pump and, in 2004, with a Sigma 900 Max Sampler, from a well (2.5 m deep, 15 cm diameter) located 3 m from the stream channel. In 2003, eight samples were collected from the stream and riparian ground waters. As the well in the forest hill slope and the ephemeral channel were dry at the beginning of the sampling period no samples could be collected until a rainstorm on the 15 October. In 2004, eight more samples were collected from each water body from the beginning of the study period, except from the ephemeral channel, which was dry.

Samples were preserved at 4°C for storage prior to analysis. Samples were analyzed for DOC and its fractions (ultrafiltration). Chloride and silica were also analyzed and used as conservative tracers to discern the hydrological origin of stream water and riparian groundwater (Hill and others 1998; Hornberger and others 2001; McGlynn and others 2004).

Chloride was estimated using capillary electrophoresis (Waters CIA Quanta 4000) (Romano and Krol 1993) whereas silica and sodium were measured with an induced coupled plasma-atomic emission spectrometer (Thermo Jarrell Ash Iris Advantage ER/S).

Hydrology and Dynamics of Conservative Solutes

Water levels in the stream, ephemeral channel and riparian groundwater were monitored constantly by water pressure sensors (Campbell CS401) connected to a data logger (Campbell CR10X). In the riparian area, 24 wells were placed in a regular grid (4 rows and 6 lines). The data from the groundwater levels of the entire riparian plot allowed us to determine the direction of the groundwater flow and the extension of water exchange at the stream–riparian interface (For additional details see Butturini and others 2003). In this study, the groundwater level in the riparian plot was monitored manually three times in 2003 (September 12, October 13, November 22) in each well and once in 2004 (September 15). Stream water discharges were measured by the chloride ''slug'' addition method (Gordon and others 1992). Then, continuous discharges were estimated using an empirical relationship between measured discharges and the corresponding stream water levels.

DOC Characterization

Water samples were filtered through pre-combusted Whatman GF/F and 0.2 μm porous size nitrocellulose filters (Whatman) to eliminate particles and bacteria before ultrafiltration. In 2004, samples were ultrafiltrated immediately after sampling (maximum 24 h), so these were only filtered with pre-combusted Whatman GF/F filters. From each filtered sample, subsamples of 20 ml, for TDOC determination, and subsamples of 330 ml (in 2004), for conservative solutes content analysis, were collected in glass flasks. These flasks were previously heated for 4 h at 400°C to prevent sample contamination. Each filtered sample was ultrafiltrated with Prep-Scale TFFI cartridges (Millipore). Typically, the initial volume for ultrafiltration was 10 l, but on occasions when not enough water could be collected the initial volume was 5 l. The following molecular weight fractions were obtained for each sample:

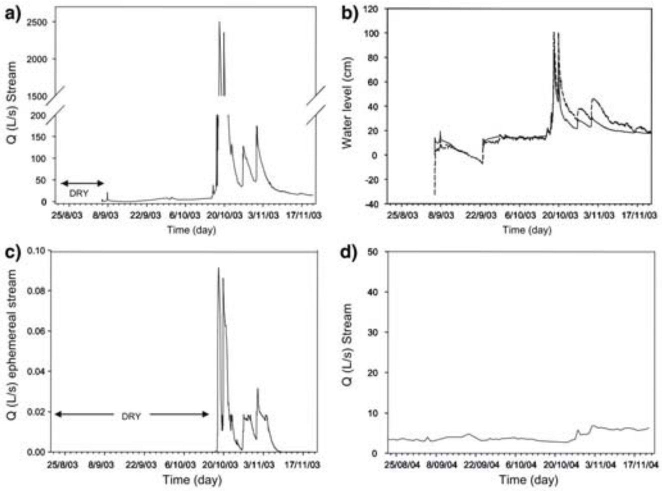


Figure 2. Hydrological characterization during 2003 (dry antecedent conditions) and 2004 (wet antecedent conditions) study periods: *a*) stream water discharge in 2003; *b*) groundwater level in the riparian zone (thin line) and in stream (thick line); *c*) discharge of runoff in the ephemeral channel (2003); and *d*) stream water discharge in 2004.

(1). > 100 kDa (very high molecular weight or VHMW fraction),
(2). 10–100 kDa (high molecular weight or HMW fraction),
(3). 1–10 kDa (medium molecular weight or MMW fraction) and
(4). <1 kDa (low molecular weight or LMW fraction).

DOC samples were measured using a Skalar 12 SK TOC Analyser with UV-promoted persulfate oxidation.

There were three replicates for each molecular weight fraction of each sample (20 ml). DOC analysis of samples that were not ultrafiltrated provided the measurement of total DOC (TDOC, three replicates).

RESULTS

Hydrology

Stream hydrology during 2003 and 2004 showed important differences in antecedent hydrological conditions, discharge magnitudes and dynamics (Table 1).

In 2003, the stream was dry from 30 June until 4 September. The streamflow was re-established on 5 September after several rain events. Stream hydrology was characterized by two contrasting sub-periods (Figure 2a). The first was relatively dry and lasted from 5 September to 14 October and was characterized by an intermittent and low discharge regime (less than 4 l s^{-1}). The second sub-period was much more humid and started on 15 October after a severe rain episode (total precipitation = 186 mm) that generated a storm peak of 2,500 l s^{-1} and a basal discharge up to 15 l s^{-1}. During the dry sub-period, the stream water discharged into the riparian groundwater up to 8 m within the riparian area and generated extended groundwater flow paths (Figure 3a, b) at the stream–riparian interface. Subsequently, the stream water and riparian groundwater levels followed the same pattern over the entire study period (Figure 2b) and the stream water infiltrated only the first 2–4 m of the riparian strip (Figure 3c). The well located on the forest hill slope and the ephemeral channel remained dry during the first sub-period. The runoff in the ephemeral channel was permanent from 15 October to 8 November and followed the same discharge pattern as that of the stream channel (Figure 2c).

In contrast to 2003, the stream was permanent throughout 2004 due to abundant rains in late spring. However, no rain episode occurred from September to November and the discharge was steady at about 4.4 l s^{-1} (Figure 2d). The lack of hydrological transition greatly reduced the extent of the water exchange between the stream and riparian ground waters, and the stream water infiltrated only the first 2–4 m of the riparian strip and the groundwater level surface was similar to

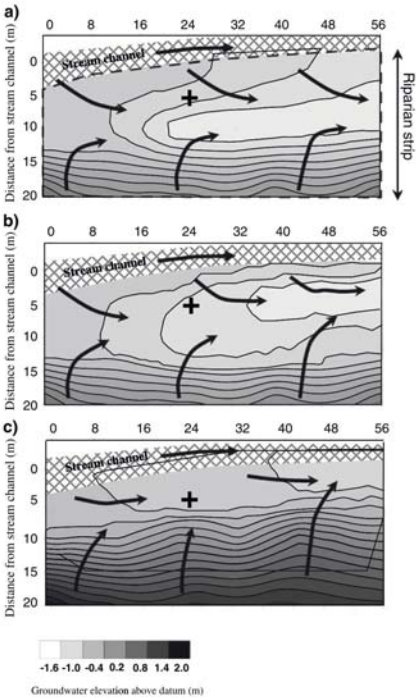


Figure 3. Groundwater level in the riparian plot surface during three dates in 2003. a) September 12 2003; b) October 13 2003; c) November 22 2003. Arrows show the groundwater flow direction. Cross dot shows the location of the riparian groundwater well used for water sampling.

that observed in November 2003. The hill slope groundwater was monitored throughout the study period, but the ephemeral subcatchment remained dry. The level of the riparian groundwater was coupled to the stream water level.

Conservative Solutes

In 2003, chloride concentrations in stream and riparian groundwater followed the same pattern ($r = 0.95$, $n = 12$, $p < 0.001$) and their temporal dynamics reflect the two contrasting hydrological sub-periods. During the dry sub-period, chloride concentrations were high (from 60 to 33 mg l^{-1}) and decreased drastically at high discharges, during the wetter sub period, to 18 mg l^{-1}. During this dry sub-period, the chloride concentration was higher in the riparian groundwater than in the stream (*t*-test, $t = 7.2$, $df = 5$, $p < 0.01$), but during the wetter sub period the concentrations were identical (paired *t*-test, $t = 1.31$, $df = 5$, n.s.). Throughout the entire 2003 study period, chloride concentrations in the hill slope groundwater (mean value of 32.3 ± 10.82 mg l^{-1}) were significantly higher than in the stream and riparian ground waters (paired *t*-test, $t = 4.8$, $df = 5$, $p < 0.01$ for stream water and $t = 4.68$, $df = 5$, $p < 0.01$ for riparian groundwater) (Figure 4a). Silica concentrations in the stream and the riparian ground waters were identical (mean value= 9 mg l^{-1}) and remained constant throughout the sampling period. In the hill slope groundwater silica concentration averaged 15.8 mg l^{-1} and was significantly higher than in the stream (*t*-test, $t = 54.01$ $df = 5$, $p < 0.01$) and the riparian groundwater (*t*-test, $t = 44.53$, $df = 5$, $p < 0.05$). Neither the ephemeral channel (*t*-test, $t = 1.45$, $df = 3$, n.s.) nor the riparian groundwater (*t*-test, $t = 0.11$, $df = 3$, n.s.) presented significant differences in chloride content with the stream. However, the silica content was slightly higher but not as much as in the hill slope groundwater (*t*-test, $t = 2.24$, $df = 3$, $p < 0.05$) (Figure 4c).

In 2004, concentrations of solutes were constant over time due to the absence of storm events during the sampling period. In fact, the chloride content in stream (27.3 ± 1.18 mg l^{-1}) and riparian ground waters (26.9 ± 1.24 mg l^{-1}) was identical (paired t-test, $t = 5.18$, $df = 6$, n.s.), although it was significantly lower (24.35± 1.41 mg l^{-1}) in the hill slope groundwater than in the stream water (paired *t*-test, $t = 0.35$, $df = 7$, $p < 0.01$). However, in contrast to the previous year, the difference in chloride content between the hill slope and riparian ground waters was not significant (paired *t*-test, $t = 3.14$, $df = 6$, n.s.) (Figure 4b). Silica content in stream water (7.92 ± 0.24 mg l^{-1}) was significantly higher than in the riparian groundwater (7 ± 0.22 mg l^{-1}; paired *t*-test, $t = 17.13$, $df = 7$, $p < 0.01$) and visibly lower than in hill slope groundwater (15 ± 0.13 mg l^{-1}; paired *t*-test, $t = 3.7$, $df = 7$, $p < 0.01$) (Figure 4d).

Dynamics of Total DOC (TDOC) and Molecular Weight Fractions

In 2003, the peak in stream water TDOC (10 mg l^{-1}) was measured at the beginning of the sampling period, just after the re-establishment of the streamflow. Afterwards, the concentration decreased until normal base concentrations were reached (2–4 mg l^{-1}), and then increased during

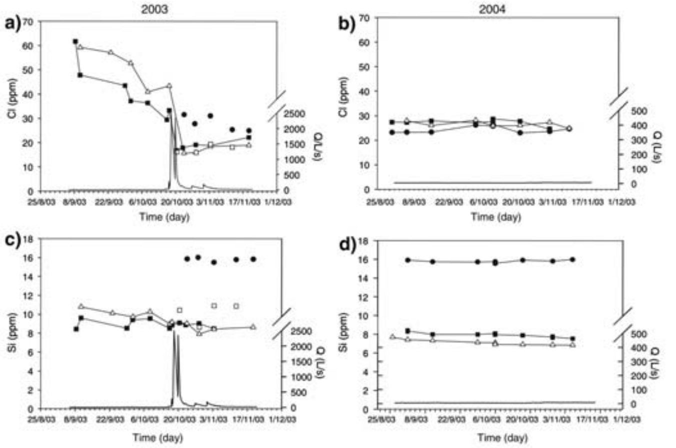


Figure 4. Temporal dynamics of conservative solutes (Cl and Si) during the sampling period of 2003 (dry antecedent hydrological conditions, panels *a* and *c*) and during the sampling period of 2004 (wet antecedent hydrological conditions, panels *b* and *d*). Symbols: (■) stream water; (△) riparian ground water. (•) hill slope ground water; (□) ephemeral channel.

the floods of mid-October (until 8 mg l^{-1}) and then decreased again to base values. TDOC in riparian ground and stream waters followed the same trend ($r = 0.82$, $df = 8$, $p < 0.01$) but concentrations in riparian groundwater were lower than in the stream water (paired *t*-test, $t = 5.53$, $df = 7$, $p < 0.01$). The TDOC concentration in the hill slope groundwater remained similar during the sampling period with a mean value of 1.7 ± 1.07 mg l^{-1}. This concentration was significantly lower than in stream water (*t*-test, $t = 5.54$, $df = 4$, $p < 0.01$) but similar to the riparian groundwater (*t*-test, $t = 0.83$, $df = 4$, n.s.). Mid-October storms originated superficial runoff in the ephemeral subcatchment and TDOC concentrations were similar to those of the stream water (*t*-test, $t = 1.41$, $df = 2$, n.s.) (Figure 5a).

In the 2004 sampling period, TDOC in stream water averaged 2.4 ± 0.5 mg l^{-1} and was clearly lower than in 2003 (paired *t*-test, $t = 3.92$, $df = 7$, $p < 0.0$). On the other hand, no differences were observed in DOC concentrations in riparian and hill slope ground waters between the 2 years (riparian, paired *t*-test, $t = 1.51$, $df = 7$, n.s.; hill slope, paired *t*-test, $t = 2.35$, n.s.) (Figure 5b). Similar to the previous year, TDOC concentrations in the stream water and riparian groundwater followed the same trend over time ($r = 0.93$, $df = 7$, $p < 0.01$), albeit they were significantly lower (mean value of 1.83 ± 0.68 mg l^{-1}; stream, paired *t*-test, $t = 5.26$, $df = 7$, $p < 0.01$) in the latter compartment. The mean concentration in the hill slope groundwater was 0.54 ± 0.34 mg l^{-1}, which is significantly lower than in the stream and the riparian groundwater (stream, paired *t*-test, $t = 14.54$, $df = 7$, $p < 0.01$; riparian groundwater, paired *t*-test, $t = 6.6$, $df = 7$, $p < 0.01$).

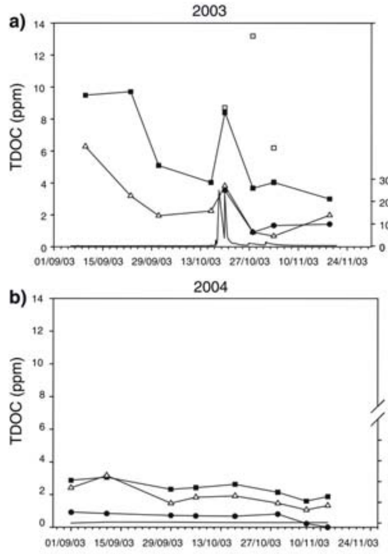


Figure 5. Temporal dynamics of TDOC and discharge during 2003, panel (a), and 2004, panel (b), study periods. Symbols: see Figure 4.

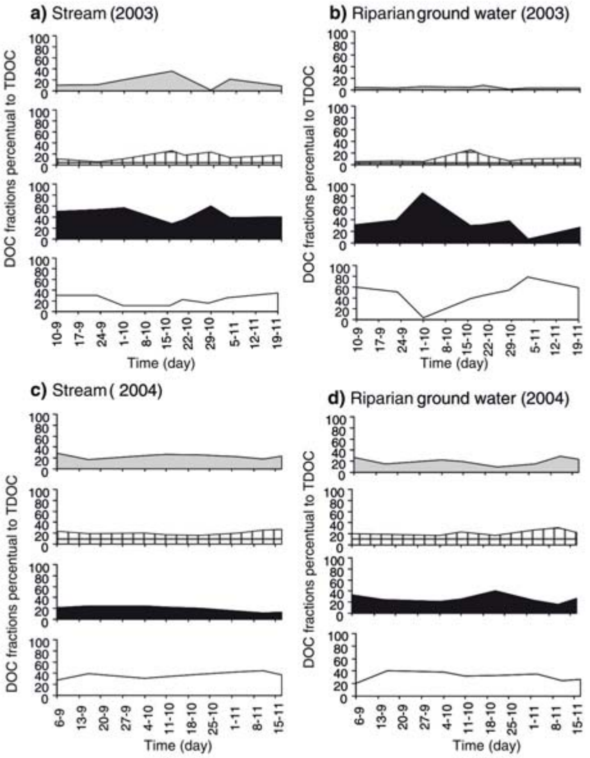


Figure 6. Temporal dynamics of DOC MW fractions (expressed as % of TDOC) during the 2003 (dry antecedent conditions) (panels a,b) and 2004 (wet antecedent conditions) (panels c,d) sampling periods in stream water and riparian groundwater. Symbols: (□) LMW; (■) MMW; (田) HMW; (▨)VHMW.

In 2003, the small DOC fractions (LMW and MMW) predominated over the larger fractions (HMW and VHMW) in all the monitored water bodies. For instance, the contribution of LMW+MMW in stream water, riparian groundwater, hill slope and in the ephemeral channel, averaged 68 ± 14%, 85 ± 7%, 81 ± 2% and 62% ± 8 % of TDOC, respectively. In stream water, these two smaller MW fractions had a DOC concentration peak at the beginning of the sampling period and followed the same pattern ($df = 6$, $p < 0.05$) as TDOC throughout the study period (Figure 6a, b).

In 2004, the concentration of the DOC fractions remained constant throughout the study period and the contribution of each MW fraction to the total TDOC was more homogeneous. For instance, the LMW and MMW fractions remained the most abundant DOC fractions, but the sum of their contribution decreased to 56 ± 5%, 56 ± 10% and 63 ± 25% in stream water, riparian and hill slope groundwaters respectively. The lack of any temporal DOC pattern in stream and riparian ground waters hinders the detection of any significant relationships between TDOC and MW fractions (Figure 6c,d).

Fate of DOC Across The Stream–Riparian Interface

TDOC concentration in the riparian groundwater was regularly lower than in the stream water for both years. In the sampling period of 2003, the 57 ± 18% of TDOC decreased at the stream–riparian interface, whereas in 2004 the decrease was only 28 ± 7% (Figure 7a).

In 2003, the abrupt increase in water level in the riparian groundwater after the re-establishing of

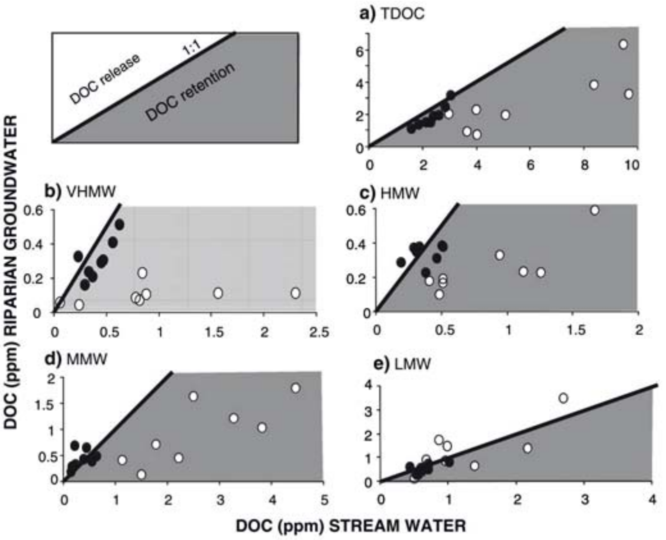


Figure 7. Comparison of content of TDOC and MW DOC fractions measured in the stream water with that in the riparian groundwater during the two study periods. The solid line shows the 1:1 line. Data located within the grey area indicate that DOC concentration was higher in stream water than in riparian ground water. Symbols: (○)2003; (●) 2004.

runoff in the stream channel and the data from conservative solutes (Cl and Si) revealed that it was clearly being fed by stream water and the influence of the hill slope groundwater was not apparent. Hence, the decrease of 57 ± 18% of TDOC concentration observed between stream and riparian ground waters was due to *in situ* retention at the stream–riparian interface.

In 2004, the stream and riparian ground waters showed similar geochemical characteristics suggesting the same conclusion obtained from data from 2003. Stream and riparian ground waters, for instance, had a similar Si content, which was much lower than in the hill slope groundwater. However, chloride content in the stream water, riparian and hill slope ground waters were similar. In addition, the lack of temporal change in the hydrometric data made it impossible to obtain an accurate picture of the water exchanges between the stream and riparian ground waters. Therefore, the dilution between the stream water and hill slope groundwater at the riparian interface cannot be discarded *a priori*. Hence, during this period, the retention of 28 ± 7% of TDOC at the stream–riparian interface must be interpreted with caution.

In 2003, we observed a direct relationship between DOC size fraction and DOC retention across the stream–riparian interface. In fact, VHMW showed higher DOC retention (78 ± 27%) (Figure 7b) followed by HMW and MMW fractions with a 70 ± 9 and 66 ± 16% retention, respectively (Figure 7c, d). Finally, nil retention of LMW molecules was observed, indicating that it was a nearly conservative fraction (Figure 7e).

In 2004 the selective retention of DOC across the stream–riparian interface according to its molecular size disappeared. In fact, LMW and HMW fractions were virtually conservative with a DOC retention of only 10 ± 22 and 4 ± 32%, respectively (Figure 7c, e). On the other hand, the MMW fraction had a negative retention value (that is, it was released, −34 ± 90%) suggesting that stream–riparian interface acts as a source of this DOC fraction (Figure 7d). Finally, only the VHMW fraction appeared systematically retained (25 ± 27%) across the stream–riparian interface in 2004 (Figure 7b).

DISCUSSION

Our findings demonstrate that the antecedent climatic and hydrological conditions strongly affect both the quality and quantity of DOC transport in stream water. Furthermore, the abrupt hydrological transition between the dry and wet period enhance the hydrological exchange at the stream–riparian interface and therefore stimulate DOC processing. From the climate/hydrological perspective our results provide a contrasting picture with respect to

Freeman and others (2004) who minimize the importance of alteration of hydrological regime on DOC transport in northern stream-peatland ecosystems. There is a lively debate on the role of hydrology on DOC transport in peatlands (Freeman and others 2001a, b, 2004; Pastor and others 2003; Tranvik and Jansson 2002). Clark and others (2005), for instance, demonstrated that drought years enhance a decrease in DOC concentration in peat waters. Our study in Fuirosos illustrates the opposite trend. Undoubtedly the comparison of data from different climatic regions and hydrological systems requires a much more rigorous investigation. In fact, DOC concentrations in peatlands are usually much higher with far more predictable dynamics than in Mediterranean streams, and droughts are less severe than those illustrated in our study. However, our results are useful in obtaining a more comprehensive perspective regarding the effects of future alterations of the rain regime, particularly during summer (Christensen and Christensen 2003), on DOC transport in running waters in Mediterranean regions.

In addition, we have shown that DOC retention across the stream–riparian interface also varied greatly, from a selective retention of higher molecular weight fractions of DOC in 2003 under dynamic hydrological conditions after dry antecedent conditions to uniformly low, and even the absence of DOC retention under steady hydrological conditions after wet antecedent conditions (2004). Thus, the dynamic hydrological conditions at the stream–riparian interface might facilitate the retention, through microbial heterotrophic biota (Findlay and others 2003), of irregular pulse inputs to the labile DOC pool associated with organic matter leaching during rain episodes.

Hydrological periods characterized by rapid and abrupt hydrological changes are usually avoided in field studies that deal with nutrient processes across stream–hyporheic interfaces and/or riparian ground waters because of the complexity of stream hydrology during storm events. Thus experimental field works are conducted under basal discharge conditions (Butturini and Sabater 1999; Simon and others 2005). Nevertheless, dynamic hydrological conditions are far from anomalous situations. Empirical and theoretical studies (Serrano and Workmann 1998; Butturini and others 2005) indicated that the interactions between stream water and the surrounding stream–riparian interface are amplified, especially during these periods. During the transition period in Fuirosos, for instance, the stream water infiltrated up to 10 m into the riparian sediments (Butturini and others 2003).

Most of the studies focused on the influence of the stream sediment on DOC fractionation, bioavailability and bacterial uptake have been performed under laboratory conditions (Fischer and others 2002; Findlay and others 2003) and/or under controlled experimental conditions at the study site (Tipping and others 1999; Sobczak and Findlay 2002; Freeman and others 2004; Valett and others 2005). Results obtained in the laboratory are essential to study and identify the biochemical processes that regulate the DOC availability in streams and interstitial water. However, field data obtained under natural conditions are necessary to gain information about the effective incidence of these processes in nature. Our results showed that selected hydrological periods characterized by abrupt changes constituted a *natural experiment* that allowed for exploration in situ and under natural conditions of the fate of DOC transported across the stream–riparian interface and, therefore, provided a better understanding of DOC dynamics in stream ecosystems.

In the dry year sampling period (2003), both the abrupt hydrometric changes observed in the riparian groundwater and the chemical (chloride and silica) data show that the riparian groundwater is recharged by stream water only, a rapid process that started with the re-establishing of runoff in the stream channel (Butturini and others 2003), while the hill slope groundwater had no influence on riparian ground water. Consequently, the identification of the origin of water that flows through the riparian sediments enabled us to attribute the decrease in DOC across the stream–riparian interface to in situ DOC retention rather than to hydrological mixing between stream and hill slope ground waters.

The lack of a hydrological transition in the 2004 period hindered the characterization of water flow through the stream–riparian interface. In fact the hydrometric data gave no evidence of water exchange between stream and riparian ground waters. Furthermore, the results of chloride data do not rule out the possibility of TDOC dilution in the riparian groundwater by hydrological mixing between stream water and hill slope groundwater.

The chemical data of conservative tracers, TDOC and its MW fractions observed in the ephemeral stream reveal that the water flowing through the forest hill slope during a severe rain episode is similar to the stream water during high discharge conditions. This result substantiates the importance of leaching of allochthonous DOC from hill slope forest soils towards TDOC transport in stream water during these storm episodes.

The TDOC pulse in stream water observed at the beginning of the dry year study period (2003) is a typical phenomenon observed in previous dry years in Fuirosos (Bernal and others 2005) as well in stream-floodplain systems (Valett and others 2005). Its origin is most probably found in the leaching of the abundant leaf litter that accumulated on the streambed in summer (81 g C·m^{-2}, Acuña and others 2004). In fact, leaf accumulation in wet years (that is, 2004) is much lower (6.9 g C·m^{-2}, Acuña and others 2004). Consequently, in 2004, TDOC concentrations are much lower than in the previous study period and solute flushing is absent. These low and constant concentrations of TDOC and of all MW DOC fractions, both in stream water and in riparian groundwater, observed throughout the study period of 2004 prevented the identification of the most reactive and/or recalcitrant DOC molecular fractions across the stream–catchment interface. This problem is absent in data from the dry year (2003). In fact, the high concentrations of TDOC (and MW DOC fractions as well), allowed us to separate the most reactive fractions (HMW and VHMW) from the most recalcitrant ones (LMW) and therefore to identify the selective DOC retention across the stream–catchment interface according to the DOC molecular size (Figure 7). Therefore, our field observations corroborated the size-reactivity conceptual model proposed by Amon and Benner (1996), whereby larger molecules would likely be more labile whereas smaller molecules would be more recalcitrant. Nevertheless, there is no general consensus in the literature about the relationship between bioavailability and DOC molecular size, and more complex patterns have been reported. For instance, Kaiser and others (2004) showed that HMW compounds were generally highly recalcitrant while LMW could be bioavailable or recalcitrant according to its origin and diagenetic state. In laboratory conditions, conversely Fischer and others (2002) measured high DOC retention for HMW and LMW compounds, but low retention for intermediate MW fractions.

Under the wet antecedent condition (that is, the 2004 sampling period) and low DOC concentrations in stream and riparian ground waters, exclusively the larger DOC molecular weight fractions (VHMW) appeared retained across the stream–riparian interface in most of the sampling dates whereas the remaining MW fractions appear to behave conservatively. Sobczak and Findlay (2002) pointed out that under low DOC concentrations stream DOC removal is often negligible and transport may be conservative.

CONCLUSION

This paper has shown that unstable hydrological periods can provide an excellent opportunity for studying, *in situ* and under natural conditions, the fate of organic matter in Mediterranean freshwater ecosystems.

Results indicate that the occurrence of a summer drought period favored the transport of DOC with labile behavior (with a retention efficiency of 56% TDOC across the stream–riparian interface) during the successive hydrological transition period, whereas in the absence of a drought period the DOC showed more refractory behavior. Moreover, the efficiency of DOC retention across the stream–riparian interface also varied greatly, from selective retention of higher molecular weight fractions of DOC in 2003 to uniformly low, even loss of, retention under wet antecedent conditions (2004). We hypothesize that a change in discharge regime in Mediterranean regions as a consequence of a warmer climate might favor more severe and dynamic hydrological processes at the stream–riparian interface, which might facilitate the retention of bigger and more labile DOC molecules originated by abrupt and irregular inputs of organic matter leaching during rain episodes.

ACKNOWLEDGEMENTS

Thanks to two anonymous reviewers for their encouraging comments on an earlier version of the manuscript. This study was supported by funds provided the Ministerio de Educación y Ciencia (projects CGL2004-04050/HID, REN2002-04442-C02-02/GLO and CGL2005-06739-C02-02).

REFERENCES

Acuña V, Giorgi A, Muñoz I, Uehlinger U. 2004. Flow extremes and benthic organic matter shape the metabolism of a headwater Mediterranean stream. Freshw Biol 49:960–71.

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

Bernal S, Butturini A, Sabater F. 2002. Variability of DOC and nitrate responses to storms in a small Mediterranean forested catchment. Hydrol Earth System Sci 6:1031–41.

Bernal S, Butturini A, Riera JL, Vázquez E, Sabater F. 2004. Calibration of the INCA model in a Mediterranean forested catchment. Hydrol Earth System Sci 8:729–41.

Bernal S, Butturini A, Sabater F. 2005. Seasonal variations of dissolved organic nitrogen and DOC:DON ratios in an intermittent Mediterranean stream. Biogeochemistry 75:351–72.

Boulton AJ, Findlay S, Marmonier P, Stanley EH, Valett MH. 1998. The functional significance of the hyporheic zone in streams and rivers. Annu Rev Ecol Syst 29:59–81.

Burt TP, Pinay G, Matheson FE, Haycock NE, Butturini A, Clement JC, Danielescu S, Dowrick DJ, Hefting MM, Hillbricht-Ilkowska A, Maitre V. 2002. Water table fluctuations in the riparian zone: comparative results from a pan-European experiment. J Hydrol 265:129–48.

Butturini A, Sabater F. 1999. Importance of transient storage zones for ammonium and phosphate retention in a sandy-bottom Mediterranean stream. Freshw Biol 41:593–603.

Butturini A, Bernal S, Sabater S, Sabater F. 2002. The influence of riparian-hyporheic zone on the hydrological responses in an intermittent stream. Hydrol Earth System Sci 6:515–25.

Butturini A, Bernal S, Nin E. 2003. Influences of the stream groundwater hydrology on nitrate concentration in unsaturated riparian area bounded by an intermittent Mediterranean stream. Water Resour Res 39:1110doi:10.1029/2001WR001260.

Butturini A, Bernal S, Sabater F. 2005. Modelling storm events to investigate the influence of the stream–catchment interface zone on stream biogeochemistry. Water Resour Res 41:W08418doi:10.1029/2004WR003842.

Christensen JH, Christensen OB. 2003. Climate modelling: severe summertime flooding in Europe. Nature 421:805–06.

Clark JM, Chapman PJ, Adamson JK, Lane SN. 2005. Influence of drought-induced acidification on the mobility of dissolved organic carbon in peat soils. Global Change Biol 11:791–809.

Dahm CN, Grimm NB, Marmonier P, Valett MH, Vervier P. 1998. Nutrient dynamics at the interface between surface waters and groundwaters. Freshw Biol 40:427–51.

Findlay S, Sinsabaugh RL, Sobczak WV. 2003. Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. Limnol and Oceanogr 48:1608–17.

Fischer HS, Steinberg CEW, Pusch M. 2002. Differential retention and utilization of dissolved organic carbon by bacteria in river sediments. Limnol Oceanogr 47:1702–11.

Freeman C, Ostle N, Kang H. 2001a. An enzymatic "latch" on a global carbon store — a shortage of oxygen locks up carbon in peatlands by restraining a single enzyme. Nature 409:49–149.

Freeman C, Evans CD, Monteith DT, Reynolds B, Fenner N. 2001b. Export of organic carbon from peat soils. Nature 412:785.

Freeman C, Fenner N, Ostle NJ, and others. 2004. Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. Nature 430:195–98.

Gasith A, Resh VH. 1999. Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. Annu rev Ecol Systems 30:51–81.

Gordon ND, McMahon TA, Finlayson BL. 1992. Stream hydrology. An introduction for ecologists. New Jersey: Prentice-Hall.

Hefting M, Clement JC, Dowrick DJ, and others. 2004. Water table elevation controls on soil nitrogen cycling in riparian wetlands along a European climatic gradient. Biogeochemistry 67:113–34.

Hill AR, Labadia CF, Sanmugadas K. 1998. Hyporheic zone hydrology and nitrogen dynamics in relation to the streambed topography of a N-rich strea. Biogeochemistry 42:285–310.

Hornberger GM, Scanlon TM, Raffensperger JP. 1998. Modelling transport of dissolved silica in a forested headwater catchment: the effect of hydrological and chemical time scales on hysteresis in the concentration-discharge relationship. Hydrol Processes 15:2029–38.

Kaiser E, Arscott DB, Tockner K, Sulzberger B. 2004. Sources and distribution of organic carbon and nitrogen in the Tagliamento River, Italy. Aquatic Sci 66:103–16.

Lake PS. 2003. Ecological effects of perturbation by drought in flowing waters. Freshw Biol 48:1161–72.

McGlynn BL, McDonnell JJ. 2003. Role of discrete landscape units in controlling catchment dissolved organic carbon dynamics. Water Resour Res 39:1090.

McGlynn BL, McDonnell JJ, Seibert J, and others. 2004. Scale effects on headwater catchment runoff timing, flow sources, and groundwater-streamflow relations. Water Resour Research. 40:7504–04.

Neal C, Robson AJ, Neal M, and others. 2005. Dissolved organic carbon for upland acidic and acid sensitive catchments in mid-Wales. J Hydrol 304:203–20.

Ninyerola M, Pons X, Roure JM. 2000. A methodological approach of climatological modelling of air temperature and precipitation through GIS techniques. Int J Climatol 20:1823–41.

Pastor J, Solin J, Bridgham SD, Updegraff K, Harth C, Weishampel P. 2003. Global warming and the export of dissolved organic carbon from boreal peatlands. OIKOS 100:380–6.

Romaní AM, Vazquez E, Butturini B. 2005. Changes in size fractionation and bioavailability of dissolved organic carbon after drought in a Mediterranean intermittent stream: biogeochemical link across the stream–riparian interface. Microb Ecol 52:501–512.

Romano and Krol. 1993. Capillary ion electrophoresis, an environmental method for the determination of anions in waters. J. Chromatogr 649:403–12.

Sabater S, Bernal S, Butturini A, and others. 2001. Wood and leaf debris input in a Mediterranean stream: The influence of riparian vegetation. Archiv Fur Hydrobiologie 153:91–102.

Sabater Butturini S A, Clement JC, Burt T, Dowrick D, Hesfting M, Maitre V, Pinay G, Postolache C, Rzepecki M, Sabater F. 2003. Nitrogen removal by riparian buffers under various N loads along a European climatic gradient: patterns and factors of variation. Ecosystems 6:20–30.

Sachse A, Henrion R, Gelbrecht J, Steinberg CEW. 2005. Classification of dissolved organic carbon (DOC) in river systems: influence of catchment characteristics and autochthonous processes. Organic Geochem 36:923–935.

Schindler JK, Krabbenhoft DP. 1998. The hyporheic zone as a source of dissolved organic carbon and carbon gases to a temperate forested stream. Biogeochemistry 43:157–74.

Sala M. 1983. Fluvial and slope processes in the Fuirosos basin, catalan ranges, northeast iberian coast. Z Geomorphol 27:393–411.

Schröter D, Cramer W, Leemans R, and others. 2005. Ecosystem service supply and vulnerability to global change in Europe. Science 310:1333–37.

Serrano SE, Workman SR. 1998. Modeling transient stream/aquifer interaction with the non-linear Boussinesq equation and its analytical solution. J Hydrol 206:245–55.

Sobczak WV, Findlay S. 2002. Variation in bioavailability of dissolved organic carbon among stream hyporheic flowpath. Ecology 83:3194–209.

Simon KS, Townsend CR, Biggs BJF, and others. 2005. Temporal variation of N and P uptake in 2 New Zealand streams. JNABS 24:1–18.

Tranvik LJ, Jansson M. 2002. Terrestrial export of organic carbon organic. Nature. 415:861–62.

Tipping E, Rigg E, and others. 1999. Climatic influences on the leaching of dissolved organic matter from upland UK moor-land soils, investigated by a field manipulation experiment. Environ Int 25:83–95.

Valett HM, Baker MA, Morrice JA, Crawford CS, Molles MC, Dahm CN, Moyer DL, Thibault R, Ellis LM. 2005. Biogeochemical and metabolic responses to the flood pulse in a semiarid floodplain. Ecology 86:220–34.

Vidon PGF, Hill AR. 2004. Landscape controls nitrate removal in stream riparian zones. Water Resour Res 40, doi:10.1029/2003WR002473.

Wade AJ, Neal C, Butterfield D, Futter MN. 2004. Assessing nitrogren dynamics in European ecosystems, integrating measurement and modelling: conclusions. Hydrol Earth Syst Sci 8:846–57.

Wigington PJ, Griffith SM, Field JA, Baham JE, Horwath WR, Owen J, Davis JH, Rain SC, Steiner JJ. 2003. Nitrate removal effectiveness of a riparian buffer along a small agricultural stream in Western Oregon. J Environ Qual 32:162–170.

xxvi

Biogeochemistry (2011) 102:59–72
DOI 10.1007/s10533-010-9421-x

Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions

**Eusebi Vazquez · Stefano Amalfitano ·
Stefano Fazi · Andrea Butturini**

Received: 25 March 2009 / Accepted: 22 February 2010 / Published online: 17 March 2010

Abstract In Mediterranean regions, drought is one of the main factors shaping fluvial ecosystems. Droughts cause a shift from lotic to lentic conditions, triggering a gradual fragmentation of the longitudinal hydrological continuum, and a severe alteration of water chemical properties. However, within a biogeochemical perspective, little is known about how and to which extend droughts modify the chemical properties of dissolved organic matter (DOM). In this study, the variability of DOM properties along a fragmented fluvial system is explored, during a summer severe drought, by means of (a) the ratio between dissolved organic carbon and nitrogen concentrations (DOC:DON); (b) DOC bioavailability (BDOC) and (c) DOM optical properties (SUVA index, fluorescence index, and excitation–emission fluorescence matrices). DOM and water measurements were collected from isolated water parcels that became disconnected from the fluvial continuum at different times, and were compared with data obtained in the following autumn, when the fluvial continuum was re-established. Analysis of DOM chemical properties evidenced that these properties during drought clearly differed from those observed in autumn, but changes did not follow an arbitrary pattern. Thus, the sampling sites with lotic water bodies showed DOM properties similar to those observed in autumn reflecting the dominance of terrestrial inputs. But, once hydrological fragmentation occurred, there was a gradual increase in the contribution of autochthonous DOM as the time elapsed since the pools were established, and the geochemical conditions shifted from oxidized to reduced conditions. In consequence, the fragmentation of fluvial continuum generates a set of distinct biochemical hot spots (i.e., each water parcel), revealing that extreme drought greatly amplifies the qualitative heterogeneity of organic matter in a fluvial system.

Keywords Dissolved organic matter (DOM) · Drought · Mediterranean · Fluvial system · Ground water · Biodegradable DOC (BDOC) · Optical properties · EEMs · Fluorescence index · SUVA index

Introduction

In aquatic ecosystems, dissolved organic matter (DOM) is a mixture of compounds whose characteristics and composition strongly influences key processes including bacterial production, trophic web

---

E. Vazquez (✉) · A. Butturini
Departament d'Ecologia, Universitat de Barcelona,
Diagonal 645, 08028 Barcelona, Spain
e-mail: evazquez@ub.edu

S. Amalfitano · S. Fazi
Istituto di Ricerca sulle Acque (IRSA-CNR),
Area della Ricerca "Roma 1", Via Salaria km 29,300,
CP1000016 Monterotondo, Rome, Italy

organization, biogeochemical transformations, nutrient availability and carbon cycling (Sobczak and Findlay 2002). The concentration and quality of DOM transported in a stream is the combined result of allochthnous inputs from watershed hillslope and riparian flushing and autochthonous inputs from the in-stream metabolism. In consequence, DOM composition is highly variable because of the temporal and spatial dynamism of these processes (Stedmon et al. 2003; Sachse et al. 2005; Romaní et al. 2006).

Mediterranean fluvial ecosystems are characterized by recurrent summer droughts (Gasith and Resh 1999; Butturini et al. 2008). Their frequency and intensity strongly affect stream metabolism (Acuña et al. 2005; Rubbo et al. 2006) and DOM cycling (Vazquez et al. 2007; Butturini et al. 2008). Drought severity determines the degree of disruption of the longitudinal fluvial continuum and the decline of the vertical hydrological connectivity between surface and surrounding riparian ground waters (Butturini et al. 2003). This loss of hydrological connectivity often takes place sequentially during the span of the drought period. In the final stage, the fluvial network is often converted into a fragmented landscape of isolated water pools. As a result, the drying process is gradual in time and heterogeneous in space: surface flow may start drying from downstream to upstream or vice versa. Another possibility is that water may persist in headwaters and the mouth, disappearing in the middle section first. In any case, the water pools are not established at the same time, and their location, dimension, persistence and age depend on site geomorphologic and hydrological conditions (Lake 2003). As a consequence of the absence of advection, the chemical characteristics of isolated waters change radically from solutes in oxidized state (i.e., N–NO_3) to reduced states (i.e., N–NH_4), with an impact on microbial processes regulating elemental cycles at local scale. For instance, it is expected a decrease in oxygen, nitrate and sulphate concentrations, and an increase in ammonium (Bleich et al. 2009; Stanhope et al. 2009). Focusing on DOM, an increase of dissolved organic carbon (DOC) and nitrogen (DON) bulk concentration is expected in water pools as result of continuous leaching of particulate organic matter that enters constantly from the surrounding riparian environment (Acuña et al. 2005). Despite little is known about the changes in the qualitative properties of DOM during droughts. There is a rich literature showing that drought is an important mechanism to explain DOC losses in boreal upland peats (Worrall et al. 2006; Clark et al. 2005; Freeman et al. 2004). But information from water-limited systems is in an incipient stage (Dahm et al. 2003; Vazquez et al. 2007).

Therefore, from these preliminary considerations, our objective was to examine how and to which extent does the fluvial continuum fragmentation affect DOM chemical properties? In order to explore this question, a Mediterranean intermittent fluvial system was sampled during a summer drought period and the successive autumnal wet period when the fluvial continuum was re-established. Water samples were collected along a longitudinal gradient, from the stream mouth to the headwaters, and along a temporal gradient according to the time the pools became isolated. Sampling locations included both surface and groundwater riparian waters. A continuous monitoring of ground and stream water levels showed that during drought, there was no hydrological connection between the riparian groundwater and stream water. Therefore, the riparian groundwater was considered a sort of groundwater isolated pool.

DOM characterization from samples obtained during the wet hydrological period is used as a background values to assess changes in DOM properties during drought. During this period, it is expected that DOC presents similar properties in all sampled locations, with the exception of riparian ground waters (Vazquez et al. 2007).

Different approaches, including spectroscopic techniques, have been widely used to characterize DOM from different aquatic systems: marine, estuaries, rivers, lakes, groundwaters, soil water (e.g., Stedmon and Markager 2005a; Hood et al. 2006; Mladenov et al. 2007; Fellman et al. 2008; Vidon et al. 2008; Jaffé et al. 2008). But, to our knowledge, these methods have not been yet applied in the characterization of DOM during drought periods in-stream waters.

Materials and methods

Study site

Fuirosos is a third-order stream that drains a forested granitic catchment of 16.2 km^2, near Barcelona (NE Spain, 41°42′ N, 2°34′ W, 50–770 asl).

The climate is typically Mediterranean, with monthly mean temperatures ranging from 3°C in January to 24°C in August. Precipitation mostly falls in autumn and spring with occasional summer storms. Average annual mean precipitation for this region is 750 mm (Ninyerola et al. 2000).

The catchment is covered mostly by perennial cork oak (*Quercus suber*) and pine tree (*Pinus halepensis*) with one or two layers of shrubs (e.g., *Ramnus alaternus*, *Viburnum tinus*, *Arbutus unedo*, *Prunus spinosa*) and lianas (*Lonicera implexa*, *Smilax aspera*). Deciduous woodland of chestnut (*Castanea sativa*), hazel (*Corylus avellana*) and oak (*Quercus pubescens*) predominate in the valley head.

In the middle point of the catchment, there is a small artificial reservoir. Downstream from the reservoir, the stream channel is 1–5 m wide and it is characterized by steep-pool morphology with cobbles and boulders, although sand and bedrock substrates are also present. Also, there is a well developed riparian forest flanking the stream channel (10–20 m wide), consisting mainly of plane tree (*Platanus* × *hispanica*) and alders (*Alnus glutinosa*). The riparian soil is poorly developed and plane leaf litter tends to accumulate on the forest floor because of extremely low decomposition rates (Bernal et al. 2005). Upstream from the reservoir, the stream channel is narrower (0.5–2 m wide) and the bedrock substrate is more common with the resulting reduction of the hyporheic zone. Also, the riparian strip is no longer defined as in the bottom valley.

The basal flow usually ranges between 1 and 20 l s^{-1}. The flow is interrupted, usually, by a long dry period in summer followed by an abrupt recharge period in late summer-early fall. The subsequent humid period elapses until late spring.

Sampling strategy

Two sampling campaigns were carried out from the bottom valley to the headwaters along Fuirosos fluvial main channel under different hydrological conditions: in summer (10/07/2007), during drought, and in autumn when the stream continuum was re-established (20/11/2007). In 2007, the drought period elapsed from June, 10th until August, 27th. This drought was preceded by a long drying phase that started on May 1st, when the last important rain episode occurred (Fig. 1, inset).

During summer, the streambed was almost completely dry. But an accurate and intensive preliminary

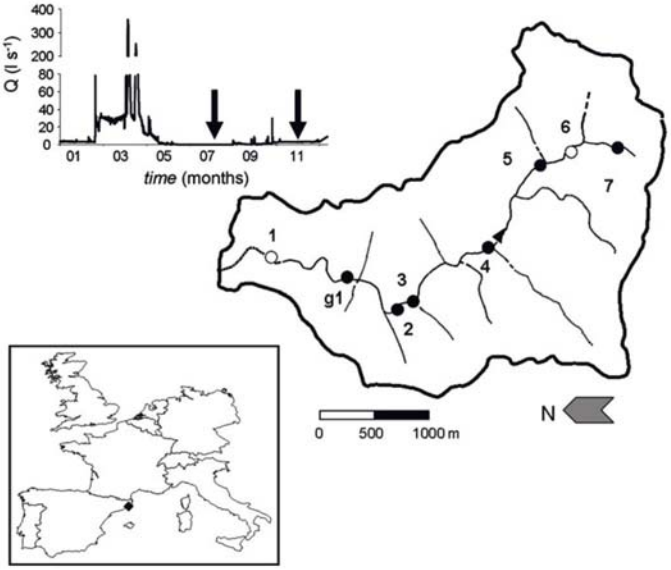


Fig. 1 Fuirosos catchment. The main figure shows Fuirosos catchment were sampling sites are indicated: *black dots* show autumn and summer sampling sites and *white dots* show the two additional sites sampled in autumn. The *black triangle* is the reservoir found in the main channel. The *lower inset* shows the location of Fuirosos catchment in the Western Europe context. The *upper inset* figure shows the hydrograph for year 2007 for the historical sampling site (already dry during the summer sampling), next to ground water well (g1). The *arrows* mark the sampling dates

hydrological monitoring programme, carried out along the entire stream network, allowed identifying the only five areas where surface water still persisted formed (sites *2, 3, 4, 5* and *7*; downstream to upstream). Two hundred meters downstream from site *2*, there was no surface flow but groundwater samples were collected from a 2 meters depth well, 2 meters away from the stream channel (site gw) (Fig. 1).

Sites *2, 3,* and *5* were isolated pools that had become stagnant at different times in zones where the main substrate was bedrock, thus preventing flow exchanges between the hyporheic zone and the rest of the main channel favouring the persistence of surface water. While at sites *4* and *7* water flow was still discernible. At the time of sampling, the preliminary hydrological programme allowed estimating the *Pond Isolation Time* (PIT): the elapsed days since the water parcel became totally stagnant (i.e., flow advection was nil). We assumed that at this time a pool was totally disconnected from the rest of the fluvial network. The estimated PIT values for sites *2, 3* and *5* are 15, 12 and 5 days, respectively. PIT values for sites *4* and *7* are 0 by definition.

Site *4* is fed by the artificial reservoir, and discharge at summer was of 0.1 l s^{-1}. Site *7* is a headwaters spring which flows permanently, and the measured flow in summer was 0.2 l s^{-1}, although a few meters from the sampling site water infiltrated and disappeared.

The second sampling campaign was conducted on the following month of November in the same locations. Furthermore, to obtain a more complete picture of DOM properties along the entire stream continuum, stream surface waters were collected from two additional sampling points completely dry during summer (sites *1* and *6*, Fig. 1). As site *3* was very close to site *2* and there were no differences in the physicochemical parameters measured in situ, it was not sampled. During this period stream flow was at the low range of the basal discharge (mean 1.35 ± 0.9 l s^{-1}) and the water body was uninterrupted along the fluvial network.

Stream water physico-chemical properties

At each sampling location, we measured pH, temperature, electrical conductivity (Ec), oxygen concentration (O_2) and, when possible, discharge using chloride slug additions**.** Three water samples (150 ml), for each site, were collected for the analysis of conservative solutes (chloride and sulphate), nitrate, ammonium, DOC and total dissolved nitrogen (TDN).

Chloride and sulphate were analyzed by liquid chromatography using a Metrohm 76 compact IC. Nitrate and ammonium were determined colorimetrically using a Technicon autoanalyzer; nitrate with the Griess-Ilosvay method (Keeney and Nelson 1982) after reduction by percolation through a copperised cadmium column and ammonium after oxidation by salycilate using sodium nitroprusside as catalyzer (Hach Company 1992).

Dissolved organic solutes and DOM composition

DOC and TDN concentrations were determined using a Shimadzu TOC-VCS with a coupled TN analyzer unit. DOC was determined by oxidative combustion infra-red analysis while TDN was estimated by means of oxidative combustion-chemiluminescence. DON was estimated calculating the difference between TDN and the inorganic nitrogen (i.e., N–NH_4 and N–NO_3).

In this study five qualitative DOM descriptors were used: DOC:DON ratio, specific UV absorbance at 254 nm (SUVA index), biodegradable DOC (BDOC), fluorescence index (FI) and the ratio of intensities of C and A fluorescence peaks obtained from the analysis of excitation–emission matrices (EEMs) (see below).

The $SUVA_{254}$ index is highly correlated to DOM aromaticity (Weishaar et al. 2003; Hood et al. 2006). The measured absorbance at 254 nm was corrected by the cuvette path length and DOC concentration. The index is expressed in l^{-1} mg C m^{-1}.

BDOC was determined according to the method described by Servais et al. (1989). To determine BDOC, we collected 2 l of water from each sampling site and filtered in situ with precombusted GF/F filters (Whatman). In the laboratory, four replicates of 200 ml for each sampling site were subsequently filtered by 0.2 µm Whatman nylon membranes. Thereafter, samples were inoculated with 2 ml of GF/F filtered water. The water, utilized for the inoculums, was from site *4*, to discard possible effects of different bacterial assemblages on DOC degradation. An aliquot was collected to determine

DOC initial concentration. Afterward, samples were stored in the dark at room temperature (20°C) during 28 days. Once they had elapsed, DOC concentration was measured again.

Fluorescence spectroscopy was completed on whole water samples in order to further characterize DOM. Excitation–emission matrices (EEMs) are a 3D representation of fluorescence over excitation and emission pairs concatenating different scans. In order to obtain the EEMs, fluorescence measurements were performed using a Shimadzu RF-5301PC spectrofluorimeter over an emission range of 280–690 nm at 1 nm increments, and an excitation range of 240–420 nm over 10 nm increments. After obtaining the EEMs, ultra pure water blanks were substracted to correct for Raman scattering. Finally, each EEM was normalized to the Raman area. Fluorescence is expressed in Raman units. Visually, two main fluorescence peaks were identified, their emission-excitation wavelengths corresponding to peaks A and C according to the categorization proposed by Coble (1996), both corresponding to humic substances. Using the EEM of each sample, the relative contribution of peaks A and C was estimated using the ratio of the maximum fluorescence intensity of each fluorophore (I_C/I_A ratio). Similar indexes based on the intensity of identified peaks in EEMs were used by McKnight et al. (2001), Milori et al. (2002) in Brazilian soils, Parlanti et al. (2000) in coastal waters and Wilson and Xenopoulos (2009) in riparian wetlands. This ratio allowed examining the variation in contribution of each fluorophore between seasons and among different sampling sites.

The FI was determined according to McKnight et al. (2001). The FI index was calculated from the ratio of intensities emitted at 450 and 500 nm at an excitation wavelength of 370 nm. This index allows discriminating the origin of DOM, its values range between 1.2 and 2, where low values indicate an allochthonous DOM origin, mainly from decomposition and leaching of plant and soil organic matter, while high values point to autochthonous organic matter generated from extracellular release and leachate from algae and bacteria. In Fuirosos, FI values estimated in soil leachate ranged between 1.62 and 1.66 ($n = 3$, E. Vazquez, unpublished data). Although these values are high enough to consider an important influence of the microbial community in the origin of DOC, it helps discriminating DOM origin between soil (1.62) and increasingly autochthonous in surface waters.

Statistical analyses

In order to explore the chemical (dis)similarities among sampled waters, the concentration values of the inorganic solutes (SO_4, NO_3, NH_4, PO_4, and Cl) and physico-chemical parameters (pH, O_2, and Ec) were used to estimate the Euclidean distance matrix. Then, a non metric multidimensional scaling (nMDS) analysis was applied to generate a map where more similar sampling points were plotted closer. Temperature and discharge values were not included in the analysis since these parameters would amplify the obvious relevance of seasonal trends in the distance matrix.

An empirical relationship between the concentration of dissolved oxygen ($[O_2]$) and nitrogen in ammonium form ($[N–NH_4]$) was used to provide a synthesis of the aerobic/anaerobic conditions among sampled water parcels and seasons:

$$\mathrm{CI} = \ln([\mathrm{O}_2]/[\mathrm{N{-}NH}_4]) \qquad (1)$$

The chemical index (CI) will vary according to the environmental conditions of water. Under low dissolved oxygen concentration, it will be expected an increase of solutes in reduced form (i.e., NH_4) (Bleich et al. 2009; Stanhope et al. 2009). Thus, the CI index will present low values, while in aerobic conditions the CI will present high values.

The presence/absence of correlations between the DOM descriptors and CI were explored separately for each season. The correlations were considered statistical significant at $p < 0.05$ level. In order to reduce the degrees of freedom, the correlations were performed with average values obtained from a minimum of three replicates.

Results

Chemical characteristics of drought and wet periods

Inorganic solutes

The range of variation of inorganic solute concentrations for summer and autumn is shown in Fig. 2. The

xxxi

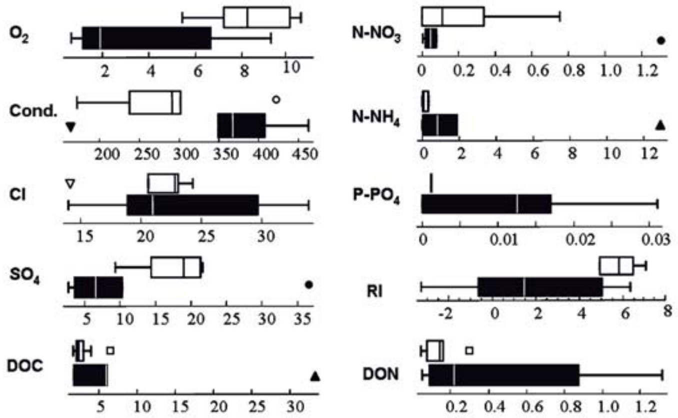


Fig. 2 Box plots summarizing the different water chemical characteristics in summer and autumn expressed in mg l^{-1}, except conductivity in µS cm^{-1}. *Black boxes* stand for summer data and *white boxes* for autumn data. The different symbols for outliers indicate different sampling sites: (*filled circle*) gw, (*filled triangle*) site *2*, (*filled square*) site *4*, (*filled inverted triangle*) site *7*

contrast between seasons is noticeable. As expected, in summer, the concentration of oxidized solutes strongly decreased. For instance, dissolved oxygen concentration in surface waters drop its concentration to 2.8 ± 3.5 (SD) mg l^{-1}, while in autumn is 8.9 ± 2 mg l^{-1}. Similarly, sulphate concentrations are lower in summer (surface water, mean 5.95 ± 3.23 mg l^{-1}) than autumn (surface water, mean 18.74 ± 5.35 mg l^{-1}). Nitrate is low in summer (0.083 ± 0.09 N mg l^{-1}) and high and more variable in autumn (0.25 ± 0.27 N mg l^{-1}). In contrast ammonium in summer is higher and much more variable (3.31 ± 5.5 N mg l^{-1}) than in autumn (0.024 ± 0.014 N mg l^{-1}). Consequently, the summer CI values are clearly lower (1.1 ± 3.6) than those estimated in autumn (5.94 ± 0.7). On the other hand, phosphate concentration is higher and much more variable (0.016 ± 0.01 P mg l^{-1}) in summer than in autumn (0.001 mg l^{-1}).

Water Ec in summer is higher (from 349 to 463 µS cm^{-1}), than autumn (from 220 to 419 µS cm^{-1}. Chloride concentration presents a wider range of concentration during drought (from 14 to 33.8 mg l^{-1}), although there is no clear difference with the concentrations found in the autumn sampling (from 14 to 23.5 mg l^{-1}).

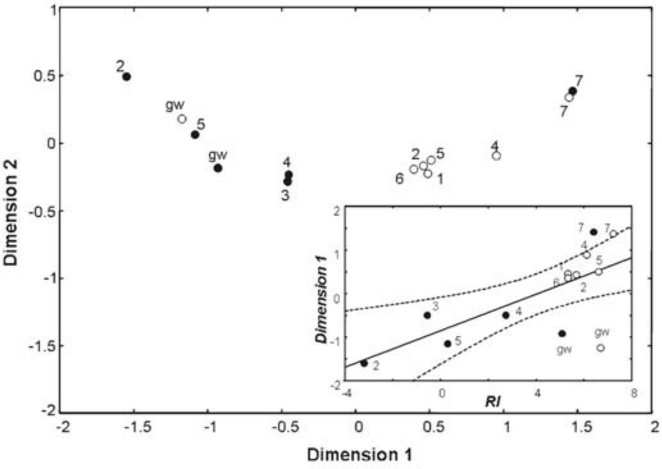
The nMDS analysis allows comparing the whole chemical variability of inorganic solutes among sampling sites (Fig. 3). The graphical representation evidences the separation of sampling sites according to the season. In autumn, the chemical characteristics in water samples show low variability, placing most of the sampling sites close together. But, there are two exceptions: the riparian groundwater and the headwater spring (site *7*) that are located far apart from the rest of sampling sites. On the other hand, during drought, the chemical variability among water parcels is much more evident. Then, points in the nMDS plot are widely dispersed. Site *2* is located in the upper extreme and sites *3* and *4* in the lower end. Remarkably, water chemical properties at the headwater sampling point (site *7*) did not show any noticeable variability between seasons.

Separation of the sampling sites along the dimension 1 axis is basically related to differences in ammonium and oxygen concentrations. In fact the dimension 1 values are positively correlated to the CI values ($r = 0.68$, $p < 0.05$). This correlation is even higher when discarding groundwater samples ($r = 0.93$, $p < 0.01$).

During drought, the CI estimates in sites 4 and 7 were high, in the same range as autumn samples. On the other hand, a steep decrease of the CI estimates was observed in sites were water was totally isolated (Fig. 4).

In more detail, the highest N–NH$_4$ concentration was observed in the downstream site *2* (13 mg l^{-1}), while the lowest was estimated at the headwater site *7* (0.02 mg l^{-1}). The decrease in dissolved O$_2$ concentration is remarkable in the isolated water pools, with a minimum value of 0.5 mg l^{-1} in site *2*.

Fig. 3 nMDS map of the distribution of sampling sites in both seasons (*black*: summer, *white*: autumn) according to its water chemical characteristics. The *lower panel* shows the correlation between the redox index (CI) and Dimension 1 (DIM 1) of the nMDS ($r = 0.68$, $p < 0.01$)



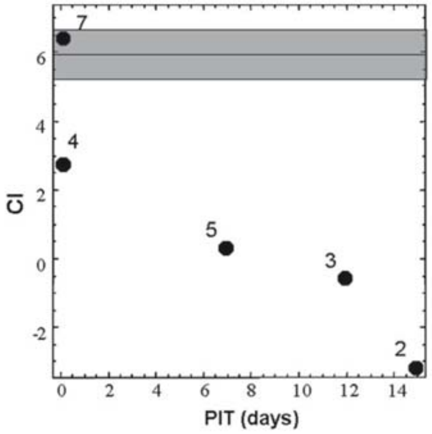


Fig. 4 Relationship between PIT (Pond Isolation Time) and CI (Chemical Index) for summer surface waters sampling sites. The solid line in the gray band in the upper part is the CI mean value ± standard deviation for autumn samples. Numbers refer to the sampling site labels

Differences between groundwater and surface water sampling sites are noticeable in summer. In groundwater, the dissolved O_2 concentration is rather high compared to surface waters (6.5 mg l^{-1}). Also, the concentrations of N–NO_3 and sulphate are higher (1.31 and 36.5 mg l^{-1}, respectively) than in surface waters.

In autumn, the position in the nMDS graph of groundwater, apart from the surface water locations, is due to a relative high Ec value (419 μS cm^{-1}) and low N–NO_3 and N–NH_4 concentrations (0.015 and 0.011 N mg l^{-1}, respectively).

DOM availability and characterization

When comparing DOC and DON concentrations and DOM lability between hydrological periods, the same trend is observed: in summer, measured parameters present higher values and variability than in autumn. However, any DOM qualitative parameter is significantly related to DOC concentrations.

The concentration of DOC in summer isolated pools is 6 mg l^{-1} in sites *3* and *5* and 33 mg l^{-1} in site *2*. DOC concentration in site *4* (running water) is 5 mg l^{-1} and at the headwater (site *7)* is much lower (1.8 mg l^{-1}), similar to the concentration found in groundwater (1.7 mg l^{-1}) and at the same site in autumn (2.8 mg l^{-1}). In autumn, DOC concentration is generally lower and presents less spatial variability (mean 2.46 ± 0.24 mg l^{-1}). Overall, DOC concentrations in summer water parcels are significantly and negatively related to the CI index with an exponential regression ($r = 0.93$, df = 3, $p < 0.05$).

DON concentrations significantly covaried with that of DOC in both seasons ($r = 0.86$, df $= 4$, $p < 0.05$ in summer; $r = 0.76$, df $= 5$, $p < 0.05$ in autumn). The highest concentrations are found is summer, the maximum corresponding to sites *2* (1.3 mg l^{-1}) and 5 (0.87 mg l^{-1}). Concentrations in the other sampling sites are in the same range of those measured in autumn (from 0.05 to 0.32 mg l^{-1}). No significant relationships are observed between DON and CI.

DOC:DON ratios are variable and do not present any kind of trend in water pools nor running waters and its variability was unrelated to the CI (Fig. 5a). During drought, the highest DOC:DON ratios (higher than 35) were estimated in the headwater spring (site *7*) and in an isolated pool (site *3*). The lowest ratio (6) corresponds to site *5*. On the other hand, in autumn, the DOC:DON ratios were less variable and ranged from 24.5 (site 7) and 6.4 (groundwater).

The estimated BDOC is higher and much more variable in summer than autumn. Summer BDOC estimates are inversely related to CI ($r = 0.84$, df $= 4$, $p < 0.05$) and positively related to DON ($r = 0.92$, df $= 4$, $p < 0.01$). Hence, higher BDOC content is found in isolated water pools (site *2*, 39.6%; site *3*, 16.9%; site *5*, 26%) while in running waters (site *4*, 7.7% and site *7*, 5.31%) and ground water (14.17%) it is lower. On the other hand, in autumn there is no distinguishable trend in BDOC content. In surface waters it is uniformly low, ranging from 5.8% (site 5) to 21% (site 7).

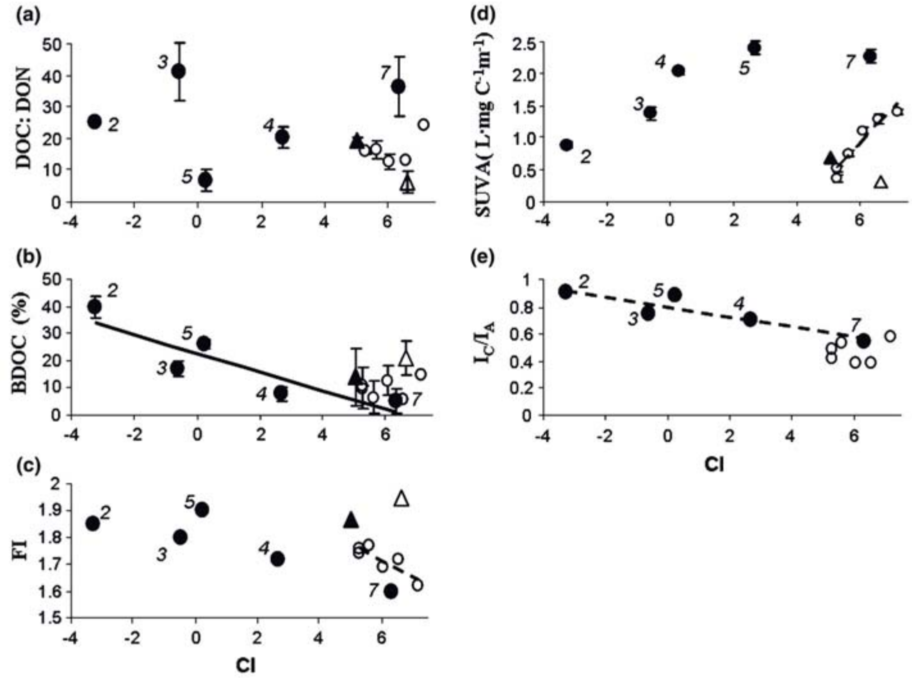


Fig. 5 The figure shows the CI (Chemical Index) relationships with DOM qualitative parameters. *Black dots* correspond to summer and *smaller white dots* to autumn values. *Triangles* correspond to ground water (*black* for summer and *white* for autumn). *Black dot numbers* correspond to each sampling site: 2 (PIT = 15 days), 3 (PIT = 12 days) and 5 (PIT = 7 days) correspond to isolated water pools; *Black dots* numbered 4 and 7 correspond to running waters (PIT = 0)

The FI values are higher in summer than in autumn, although the range of variation is similar in both seasons. During drought, isolated water pools present FI values ranging from 1.8 (site 3) to 1.9 (site 5), showing an increase of the relevance of autochthonous DOM. On the other hand, water parcels with running waters (sites *4* and *7*) present lower values, 1.72 and 1.60, respectively, indicating that allochthonous DOM might be contributing in a higher proportion of DOM than in the isolated water pools, although in both cases the FI values suggest an autochthonous DOM origin. During drought the FI index is not significantly related to CI (Fig. 5c) ($r = 0.57$, $\mathrm{df} = 4$, ns). In autumn, FI values are lower, and similar in all surface water sampling points, ranging from 1.62 (site 7) to 1.76 (sites 1 and 2) denoting exhibiting similar values to those found in local soil leachates and previous studies in Fuirosos (Romaní et al. 2006). Also, its variability was unrelated to the CI index (Fig. 5c). On the other hand, ground water FI values barely change among the two periods and their values, 1.87 in summer and 1.95 in autumn, indicate an autochthonous origin of DOM.

In summer, SUVA values ranged from 0.88 to 2.41 with highest values in running water sites *4* and *7* and lowest values in groundwater and sites *2* and *3*. In autumn, these values are lower than in summer, but there is some variability between sampling locations. Thus, DOM from *4*, *5* and *7* shows a higher SUVA values (1.11, 1.29, 1.40, respectively), than that from sites *1*, *2*, *6* and groundwater (0.5, 0.75, 0.36, 0.68, respectively). SUVA tends to increase with respect to CI values. The relationship is only significant for the autumn data ($r = 0.96$, $\mathrm{df} = 4$, $p < 0.01$, Fig. 5d).

The analysis of the magnitude of peaks C and A revealed that both are well-defined in surface water in summer, but in fall the peak C presents lower intensities. In consequence, the I_C/I_A ratio values are higher in summer than autumn. Furthermore, in summer the I_C/I_A values are significantly inversely related to the CI ($r = 0.9$, $\mathrm{df} = 3$, $p < 0.05$) with maxima values (from 0.75 to 0.91) in isolated water pools (sites *2*, *3* and *5*) and minima (from 0.7 to 0.55) in running waters (sites 4 and 7) and groundwater. The change in fluorescence of peaks A and C between seasons for site 2 is shown in Fig. 6. In autumn I_C/I_A ratio values are lower and less variables (from 0.39 to 0.63). No significant relationship was detected between autumnal I_C/I_A values and CI ($r = 0.14$, $\mathrm{df} = 5$, ns; Fig. 5e).

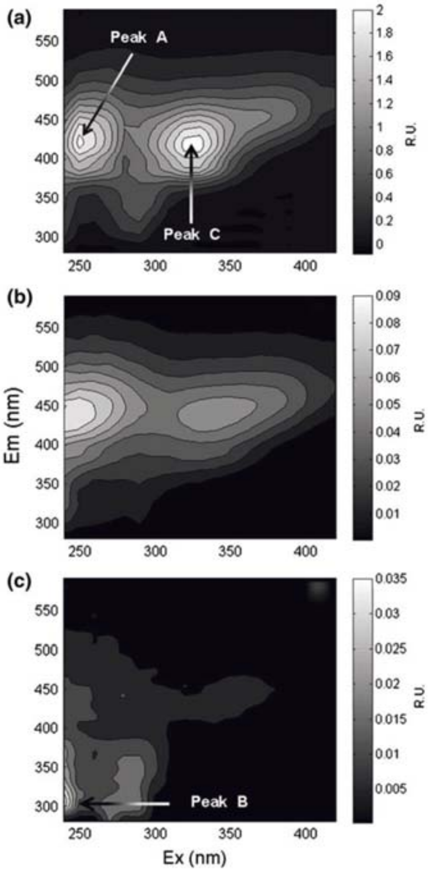


Fig. 6 Example of the change in the I_C/I_A ratio from EEMs for site *2*: **a** summer data; **b** autumn data; and groundwater: **c** summer data. Excitation and emission are in nm, and fluorescence is expressed in Raman Units (RU)

As previously shown in Fig. 4, during drought period, in surface water ponds, the PIT exerts a driving influence on the magnitude of the CI values. However, although significant relationships between CI and DOC, BDOC, SUVA and I_C/I_A were detected (Fig. 5b, d and e), exclusively the SUVA showed a

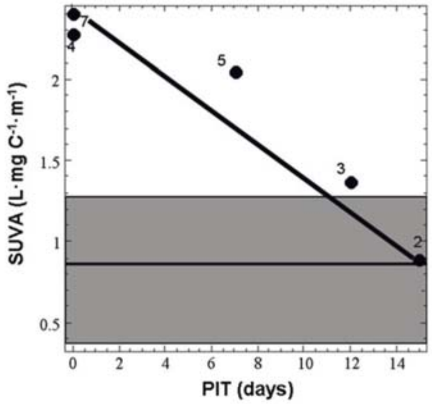


Fig. 7 Relationship between PIT (Pond Isolation Time) and SUVA values for summer surface waters sampling sites. The solid line and the gray band at the lower part show the SUVA mean value ± standard deviation for autumn samples. Numbers refer to the sampling site labels

significant inverse relationship with PIT ($r = 0.96$, df $= 3$, $p < 0.01$, Fig. 7).

Discussion

The results from this study clearly evidence that drought exerts a relevant influence on DOM chemical properties. Drought causes a gradual hydrological fragmentation of the fluvial network enlarging the variability of DOM properties, and amplifying the biogeochemical diversity of a fluvial ecosystem. Then, the observed spectrum of DOM properties does not follow an arbitrary pattern. Sampling sites with lotic water bodies in summer show DOM properties similar to those observed in autumn under base flow hydrological conditions and reflect the prevalence of terrestrial inputs from the surrounding forested hill slope. Meanwhile, in isolated and lentic water bodies, DOM analyses reveal a supplementary contribution of autochthonous organic matter, originated by in situ microbial processes, as consequence of the disruption of the hydrological connection at the stream-catchment interface (Butturini et al. 2003).

As result, in Fuirosos, drought enlarges the ordinary range of variation of four DOM descriptors (DOC/DON, BDOC, SUVA$_{254}$ and I$_C$/I$_A$) that can be observed along the 8 km of stream continuum and the altitudinal range of 450 m that comprises this study. Overall, these results coupled with those obtained by Jaffé and colleagues (2008) in North America in continental water bodies, highlight that local scale effects are much more relevant on DOM quality heterogeneity than the regional scale.

Changes in and inorganic solutes

Availability of organic and inorganic solutes in surface isolated water pools clearly differ from those observed during the wet period. By contrast, biogeochemical changes in headwaters (site 7) and groundwater among seasons are minimal.

The variability of Ec offers a good discrimination range between summer and autumn seasons due to the low discharge, and consequently an increase in the concentration of inorganic solutes, of the drought period. In isolated water pools, the absence of water transport enhances the accumulation of particulate organic matter and, along the increase in temperature, facilitating DOM leaching (DOC and DON increase) and increase in aerobic and anaerobic respirations. This increase in respiration causes a depletion of oxygen and nitrate and a steep decrease in sulphate concentration while the rate of ammonification processes increase as reflected by the high ammonium concentration. Furthermore, the establishment of more reduced environmental conditions probably favoured the phosphate desorption and its release into the water column (Bostrom et al. 1988).

Changes in DOM concentration and composition

DOC concentration in summer isolated pools presents the same range of variability observed in a previous study during a hydrological dry-wet transition period (Butturini et al. 2003). The re-establishment of stream runoff is coupled to DOC flushing attributable to leaching of abundant leaf and debris accumulated in the streambed during the previous drought period (Bernal et al. 2005; Acuña et al. 2005). In our study, as the autumn sampling was carried out after the hydrological transitional period, DOC concentrations were low, as expected during baseflow.

In Fuirosos catchment, DON concentrations are typically high at beginning of summer (before the drought) and during autumn (Bernal et al. 2005). This high DON concentration is usually attributed to the leaching of leaf litter in autumn, and the increase of in-stream primary production in summer (Bernal et al. 2005). However, in our study, summer DON concentrations are slightly higher than those estimated by Bernal et al. (2005) during the early summer drying phase suggesting that in-stream DOM production might be relevant when drought intensifies. In isolated pools, the high concentration of DOM could lead to a rapid microbial growth, with high bacterial C production rates, enhancing C and N mineralization processes (Fazi et al. 2008).

Summer sampling sites with lotic waters (sites *4*, below the reservoir; and *7*, the headwater spring) show BDOC, FI and I_C/I_A values in the same range than those observed in autumn. By contrast, in the isolated water ponds (sites *2*, *3* and *5*) DOM properties are spread toward higher and more variable BDOC, FI and I_C/I_A values. DOC:DON ratio tends to be higher in summer but does not show any clear pattern, meanwhile the SUVA values constitute an interesting exception from the trend followed by the other parameters.

FI values estimated in this study ranged between 1.6 and 1.95. According to literature, FI values higher than 1.4 are considered to indicate DOM of autochthonous sources. Therefore, the FI values obtained in this study might indicate the prevalence of autochthonous DOM sources in both seasons (McKnight et al. 2001). However, it is important to remark that FI values from Fuirosos soil leaching are typically around 1.6, suggesting that this soil leachate might integrate both vegetal and microbial DOM release from the terrestrial environment. Since DOM in fluvial systems will hardly be exclusively of allochthonous or autochthonous origin, the FI might be considered as an integrated measure of all DOM. Therefore, although in this study all samples show an autochthonous DOM origin, samples with FI values closer to 1.6 could indicate a greater relevance of allochthnous sources (all autumnal surface waters and summer sites 4 and 7) when comparing to higher values that would suggest a major contribution of autochthonous sources to the DOM pool. Furthermore, these high FI values are coupled to high BDOC and low SUVA values suggesting that in situ DOM production might be rapidly assimilated. Nevertheless caution is required when relating directly FI with BDOC, since groundwater samples show both relatively low BDOC and high FI. Therefore, other factors unaccounted for in this study might influence BDOC variability.

Although fluorescent peaks A and C are usually associated with substances of terrestrial origin (Coble 1996), in isolated water pools there is a shift in the origin of DOM, from allochthonous to autochthonous, as confirmed by the FI. These results coupled with the increase in fluorescence in peak C, reflected by the I_C/I_A ratio, during the drought period suggests that 30–40% of this fluorescence might be caused by the contribution of organic substance derived from microbial activity and algae leachate. Under this perspective, these results partially agree with the findings of Stedmon and Markager (2005b) that observed that fluorescence of certain components determined by a PARAFAC model corresponding to peak C, increased as a result of microbial degradation of estuarine DOM of autochthonous origin. In the case of summer groundwater, while peak C presents low fluorescence, peak B (protein-like) is prominent. The presence of this type of fluorescence peak, along a high FI value, might indicate an increase of microbial degradation processes and more refractory subsequent DOM accumulation since ground water is disconnected from the stream surface. Previous studies (Vazquez et al. 2007) show that in the groundwater compartment most of DOC is of small molecular size (<1 KDa). Therefore, it might be expected that this molecules with protein-like fluorescence are of small molecular size contrasting with the hypothesis that this molecular size fraction is refractory (Amon and Benner 1996). It may indicate also that bioavailability of this molecular size fraction changes according to its origin and diagenetic state, as suggested by Kaiser et al. (2004). Also, Romaní et al. (2006) showed that FI values are higher in ground water than in surface waters, and that in small (<1 KDa) and large (>100 KDa) size fractions it was higher than in medium size fractions (1–10 KDa and 10–100 KDa).

According to a previous study, BDOC estimates in Fuirosos averaged 12% under basal discharge conditions during the rewetting period (September–October) but increased up to 40% during the first severe autumnal storm event (up to 2000 l s^{-1}) in the same period (Romaní et al. 2006). In the present study, in spite of the difference in the hydrological context, the same range of variation is found. These estimates could suggest that BDOC has an upper threshold of nearly 40% that can be reached under two hydrological conditions of opposite nature: droughts and storms. DOC:DON ratio is recognized an important driver in the DOM bioavailability and an inverse relationship between BDOC and DOC:DON should be expected (Fellman et al. 2008). In summer, the DOC:DON ratio shows a notable and erratic variability. Overall, these values are slightly lower than those reported in Fuirosos during the hydrological transition by Bernal et al. (2005). In any case, DOC:DON ratio in summer typically duplicates the ratios observed in autumn. Then, we should expect lower DOM bioavailability in summer. Surprisingly, the results show that BDOC is unrelated to the DOC:DON ratio but is strongly positively related to DON. Under severe drought conditions, in presence of high DOM availability, absolute DON concentration, when measuring bioavailability, is much more relevant than the DOC:DON ratio. Interestingly, this result contradicts the Hedin et al. (1995) hypothesis suggesting that DON may be unavailable to stream microbiota because it is composed of refractory fulvic acids derived from soil, and agrees with the findings of Stepanauskas and Leonardson (1999) that suggested that DON bioavailability increases in summer when nitrate concentrations in rivers decrease.

The SUVA index can be used as a proxy for aromaticity since both parameters have been found to be strongly correlated (Weishaar et al. 2003), enhancing it usefulness in DOM characterization. In our study, the SUVA index is the only parameter that shows a positive relationship, although not significant, with CI. Moreover, considering exclusively the summer isolated water pools, it is the only parameter that is statistically related to PIT. SUVA estimates during drought are similar to those estimated in autumn, but the lotic water sampling sites (sites *4* and *7*) clearly show a higher aromaticity content. Hood et al. (2006) and Vidon et al. (2008) showed that during storm episodes, DOM inputs from the near surface soil organic layer presented higher SUVA values. But, in this study, the lack of rain episodes from May to July prevent from asserting that there is such a DOM input from the hillslope forested soil at these sampling sites. On the other hand, SUVA autumnal values are generally lower than those measured in summer at sites 4 and 7. Hence, autumnal terrestrial input of new DOM in headwaters is not necessarily highly aromatic. Therefore, high values at lotic sites (4 and 7) during summer might be caused by an accumulation of aromatic and recalcitrant substances in the persisting water mass that still flows as the fluvial network becomes fragmented. On the other hand, the fragmentation of the surface water continuum into small isolated water parcels reverses this increase in SUVA and the contribution of aromatic DOM declines proportionally to the pond formation elapsed time (PIT) (Fig. 7), reflecting the increase contribution of in situ (algal and microbial) DOM production as suggested by FI and I_C/I_A descriptors. Also it is worth considering that laboratory experiments revealed that photodegradation processes might affect DOM composition (Rodriguez-Zuñiga et al. 2008) reflected in lower UV-absorbance and fluorescence. However, the studied water parcels are located in shadowed plots of the stream channel along the thalweg and summer direct sunlight exposure during summer is strongly reduced by vegetation or/and by large boulders and rocks. Hence, it is not expected that photodegradation play an important role on the processing of DOM in our study site. This result evidences that for the interpretation of the SUVA index values is not enough to divide samples into two rough categories (drought and wet seasons or lotic and lentic water bodies) but it is indispensable to know the historical hydrological trajectory of each sampled water body along the fluvial network.

Conclusions and implications

In order to improve our knowledge on DOM origin, transformations and lability optical measurements constitute a valuable tool (Hood et al. 2003; Weishaar et al. 2003; Stedmon and Markager 2005a, b; McDowell et al. 2006; Stubbins et al. 2008). Thus, the integration of the spectroscopic methods with detailed hydro-biogeochemical monitoring during extreme, and opposite, hydrological conditions (storms and droughts) provides an excellent challenge to capture a more complete perspective on heterogeneity of DOM composition (Hood et al. 2006; Vidon et al. 2008).

It is well recognized by geomorphologists that the fluvial network is a dynamic structure (Bertoldi et al. 2009). Its expansion and shrinking is determined by the temporal concatenation of erratic storm episodes and seasonal drought periods. In Mediterranean streams, both opposite hydrological states are the most relevant drivers of DOM variability. But, while drought affects DOM variability along a spatial axis, its variability on a temporal axis is more evident during storms that are capable of generating a wide spectrum of DOM-discharge loops (Butturini et al. 2008).

Surprisingly, although the processes occurring in the fluvial network are considered dynamic, changes in its spatial dimensions and discharge fluctuations are not really integrated in whole-system biogeochemical conceptual analyses (Vannote 1980; Battin et al. 2008). Thus, the fluvial network appears to be a rigid structure, hydrologically disconnected from the catchment. In consequence, the recognition of the fluctuating nature of the fluvial network will greatly encourage the study of fluxes and transformation of organic and inorganic solutes under temporal and spatial hydrologically variable conditions. Therefore, the detailed variability analysis of quantitative and qualitative of DOM parameters will strongly benefit from the implementation of high resolution-long-term temporal monitoring programmes (Kirchner et al. 2004) that capture the succession of those extreme hydrological hot moments (McClain et al. 2004) that prompt the oscillating hydrological features of a fluvial ecosystem.

Acknowledgments We thank the reviewers for strongly improving the earlier version of the manuscript. This study was funded by project CGL2007-0144/HID from the Ministerio de Ciencia e Innovación. Stefano Fazi and Stefano Amalfitano participation was supported by project CSIC-CNR ref 2006IT0010. Andrea Butturini and Eusebi Vazques are members of the GRACCIE consortium.

References

Acuña V, Munoz I, Giorgi A et al (2005) Drought and postdrought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. J N Am Benthol Soc 24(4):919–933

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

Battin TJ, Kaplan LA, Findlay S et al (2008) Biophysical controls on organic carbon fluxes in fluvial networks. Nat Geosci 1(2):95–100

Bernal S, Butturini A, Sabater F (2005) Seasonal variations of dissolved nitrogen and DOC:DON ratios in an intermittent Mediterranean stream. Biogeochemistry 75:351–372

Bertoldi W, Zanoni L, Tubino M (2009) Planiform dynamics of braided streams. Earth Surf Process Landform 34(4): 547–557

Bleich ME, Moraes Lima Silveira R, Barros Nogueira FM (2009) Limnological patterns in Northern pantanal lagoons. Brazil Arch Biol Technol 52(3):755–764

Bostrom B, Andersen JM, Fleischer S, Jansson M (1988) Exchange of phosphorus across the sediment–water interface. Hydrobiologia 170:229–244

Butturini A, Bernal S, Nin E (2003) Influences of the stream groundwater hydrology on nitrate concentration in unsaturated riparian area bounded by an intermittent Mediterranean stream. Water Resour Res 39(4): 1110–1123

Butturini A, Alvarez M, Bernal S, Vazquez E, Sabater F (2008) Diversity and temporal sequences of forms of DOC and NO_3-discharge responses in an intermittent stream: predictable or random succession? J Geophys Res 113(G3):3016

Clark JM, Chapman PJ, Adamson JK, Lane SN (2005) Influence of drought-induced acidification on the mobility of dissolved organic carbon in peat soils. Global Change Biol 11:791–809

Coble PG (1996) Characterization of marine and terrestrial DOM in seawater using excitation–emission matrix spectroscopy. Mar Chem 51:325–346

Dahm CN, Baker MA, Moore DI, Thibault JR (2003) Coupled biogeochemical and hydrological responses of streams and rivers to drought. Freshw Biol 48:1219–1231

Fazi S, Amalfitano S, Piccini C et al (2008) Colonization of overlaying water by bacteria from dry river sediments. Environ Micorbiol 10(10):2760–2772

Fellman JB, D'Amore DV, Hood E, Boone RD (2008) Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. Biogeochemistry 88:169–184

Freeman C, Fenner N, Ostle NJ et al (2004) Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. Nature 430:195–198

Gasith A, Resh VH (1999) Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu Rev Ecol Syst 30:51–81

Hach Company (1992) Analysis handbook, 2nd edn. Hach Company, Loveland, Co

Hedin LO, Armesto JJ, Johnson AH (1995) Patterns of nutrient loss from unpolluted, old-growth temperate forests. Evaluation of biogeochemical theory. Ecology 76: 493–509

Hood E, McKnight DM, Williams MW (2003) Sources and chemical character of dissolved organic carbon across an alpine/subalpine ecotone, Green Lakes Valley, Colorado Front Range, United States. Water Resour Res 39(7):1188

Hood E, Gooseff MN, Johnson SL (2006) Changes in the character of stream water dissolved organic carbon during flushing in three small watersheds, Oregon. J Geophys Res 111(G1):1007

Jaffé R, McKnight D, Maie N et al (2008) Spatial and temporal variations in DOM composition in ecosystems: the importance of long-term monitoring of optical properties. J Geophys Res 113(G4):4032

Kaiser E, Arscott DB, Tockner K, Sulzberger B (2004) Sources and distribution of organic carbon and nitrogen in the Tagliamento River, Italy. Aquat Sci 66:103–116

Keeney DR, Nelson DW (1982) Nitrogen inorganic forms. In: Page AL (ed) Methods of soil analysis, part 2. Chemical and microbiological properties. Agronomy monographs # 9. AA-SSSA Publisher, pp 643–698

Kirchner JW, Feng XH, Neal C (2004) The fine structure of water-quality dynamics: the (high-frequency) wave of the future. Hydrol Process 18:1353–1359

Lake PS (2003) Ecological effects of perturbation by drought in flowing waters. Freshw Biol 48:1161–1172

McClain ME et al (2004) Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301–312

McDowell WH, Zsolnay A, Aitkenheah-Peterson JA et al (2006) A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. Soil Biol Biochem 38(7):1933–1942

McKnight D, Boyer BW, Westerhof PK et al (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol Oceanogr 46(1):38–48

Milori DMBP, Martín-Neto L, Bayer C et al (2002) Humification degree of soil humic acids determined by fluorescence spectroscopy. Soil Sci 167(11):739–749

Mladenov N, McKnight D et al (2007) Chemical characterization of DOM in channels of a seasonal wetland. Aquat Sci 69:456–471

Ninyerola M, Pons X, Roure JM (2000) A methodological approach of climatological modelling of air temperature and precipitation through GIS techniques. Int J Climatol 20:1823–1841

Parlanti E, Wörz K, Geoffroy L, Lamotte M (2000) Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. Org Geochem 31:1765–1781

Rodriguez-Zuñiga FU, Bastos Pereira DM, Lopes da Silva WT et al (2008) Changes in optical properties caused by UV-irradiation of aquatic humic substances from the Amazon River basin: seasonal variability evaluation. Environ Sci Technol 42:1948–1953

Romaní AM, Vázquez E, Butturini A (2006) Microbial availability and size fractionation of dissolved organic carbon after drought in an intermittent stream: biogeochemical link across the stream–riparian interface. Microb Ecol 52:501–512

Rubbo MJ, Cole JJ, Kiesecker JM (2006) Terrestrial subsidies of organic carbon support net ecosystem production in temporary forest ponds: evidence from an ecosystem experiment. Ecosystems 9:1170–1176

Sachse A, Henrion R, Gelbrecht J, Steinberg CEW (2005) Classification of dissolved organic carbon (DOC) in river systems: Influence of catchment characteristics and autochthonous processes. Org Geochem 36:923–935

Servais P, Anzil A, Ventresque C (1989) Simple method for determination of biodegradable dissolved organic carbon in water. Appl Environ Microbiol 55:2732–2734

Sobczak WV, Findlay S (2002) Variation in bioavailability of dissolved organic carbon among stream hyporheic flowpath. Ecology 83:3194–3209

Stanhope JW, Anderson IC, Reay WG (2009) Base flow nutrient discharges from lower Delmarva Peninsula watersheds of Virginia, USA. J Environ Qual 38:2070–2083

Stedmon CA, Markager S (2005a) Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. Limnol Oceanogr 50(2):686–697

Stedmon CA, Markager S (2005b) Tracing the production and degradation of autochthonous fractions of dissolved organic matter by fluorescence analysis. Limnol Oceanogr 50(5):1415–1426

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

Stepanauskas R, Leonardson L (1999) Bioavailability of wetland-derived DON to freshwater and marine bacterioplankton. Limnol Oceanogr 44(6):1477–1485

Stubbins A, Hubbard V, Uher G et al (2008) Relating carbon monoxide photoproduction to dissolved organic matter functionality. Environ Sci Technol 42(9):3271–3276

Vannote RL (1980) The river continuum concept. Can J Fish Aquat Sci 37(1):130–137

Vazquez E, Romani AM, Sabater F, Butturini A (2007) Effects of the dry–wet hydrological shift on dissolved organic carbon dynamics and fate across stream–riparian interface in a Mediterranean catchment. Ecosystems 10(2):239–251

Vidon P, Wagner LE, Soyeux E (2008) Changes in the character of DOC in streams during storms in two Midwestern watersheds with contrasting land uses. Biogeochemistry 88:257–270

Weishaar JL, Aiken GR, Bergamaschi BA et al (2003) Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ Sci Technol 37:4072–4708

Wilson HF, Xenopoulos MA (2009) Effects of agricultural land use on the composition of fluvial dissolved organic matter. Nat Geosci 2:37–41

Worrall F, Burt TP, Adamson JK (2006) Trends in drought frequency: the fate of DOC export from British peatlands. Clim Change 76(3–4):339–359