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

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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

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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 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

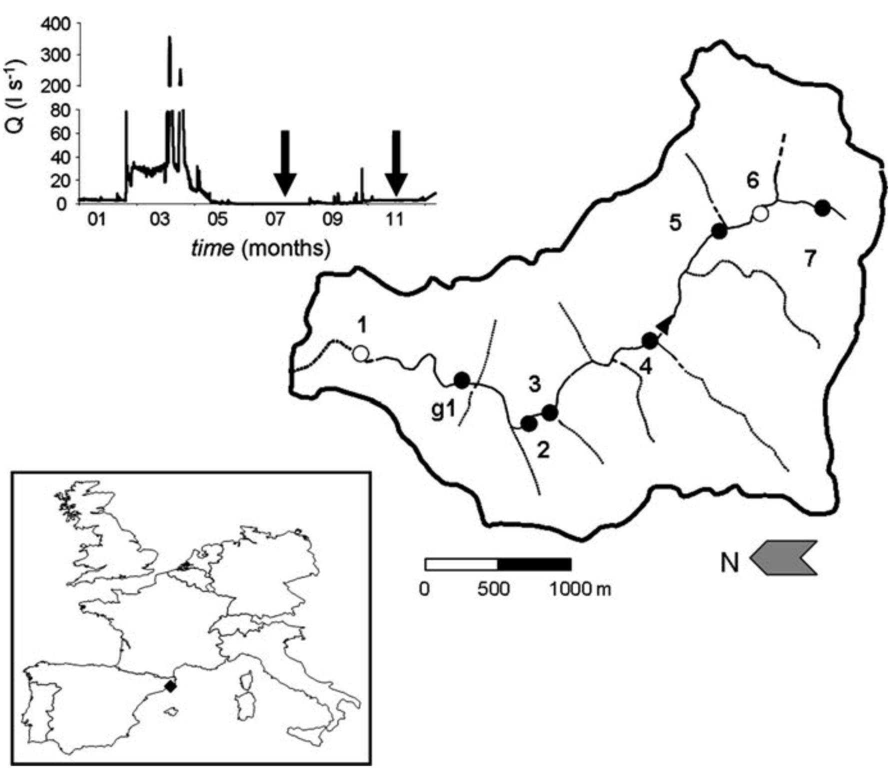

Fig. 1 Fuirosos catchment. The main figure shows Fuirosos catchment were sampling sites are indicated: *black dots* show autumn and summer sampling sites and *white dots* show the two additional sites sampled in autumn. The *black triangle* is the reservoir found in the main channel. The *lower inset* 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 ($[N\text{–}NH_4]$) was used to provide a synthesis of the aerobic/anaerobic conditions among sampled water parcels and seasons:

$$CI = \ln([O_2]/[N-NH_4]) \tag{1}$$

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

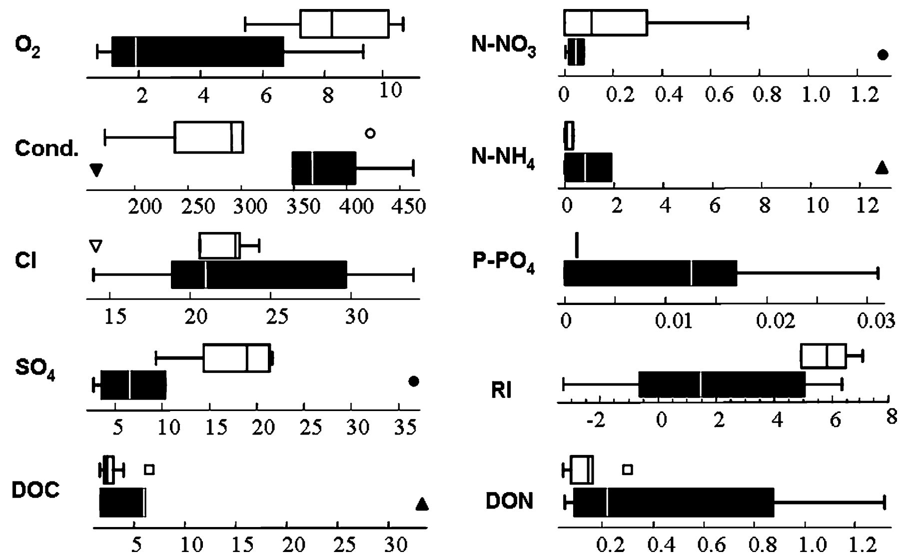

Fig. 2 Box plots summarizing the different water chemical characteristics in summer and autumn expressed in mg l^{-1}, except conductivity in µS cm^{-1}. *Black boxes* stand for summer data and *white boxes* for autumn data. The different symbols for outliers indicate different sampling sites: (*filled 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*.

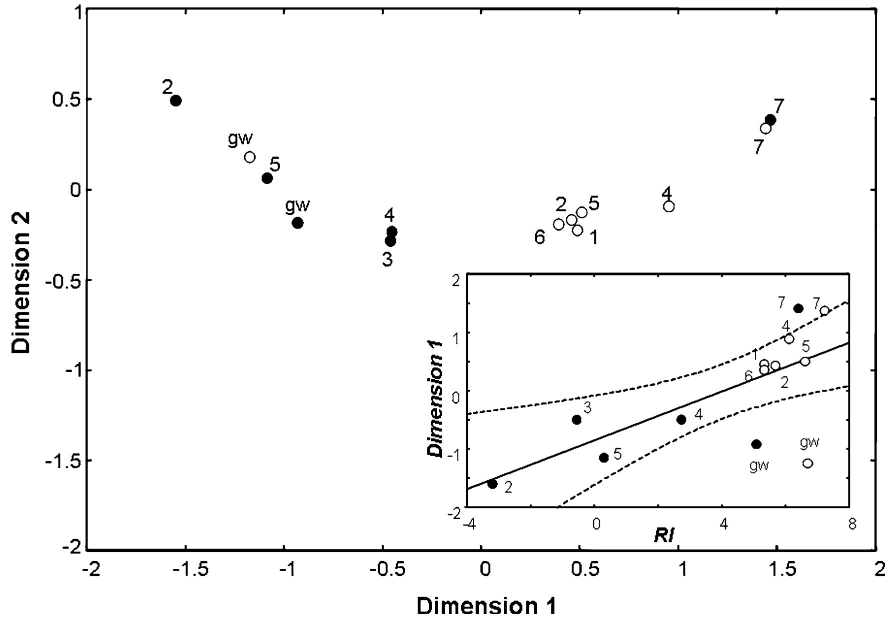

Fig. 3 nMDS map of the distribution of sampling sites in both seasons (*black*: summer, *white*: autumn) according to its water chemical characteristics. The *lower panel* shows the correlation between the redox index (CI) and Dimension 1 (DIM 1) of the nMDS ($r = 0.68$, $p < 0.01$)

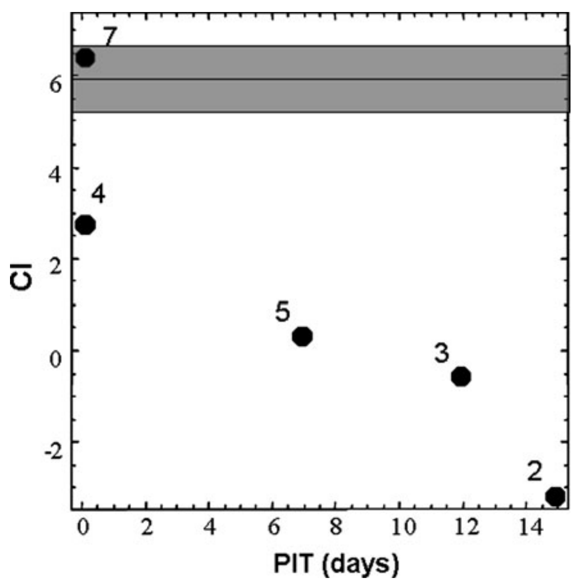

Fig. 4 Relationship between PIT (Pond Isolation Time) and CI (Chemical Index) for summer surface waters sampling sites. The solid line in the gray band in the upper part is the CI mean value ± standard deviation for autumn samples. Numbers refer to the sampling site labels

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).

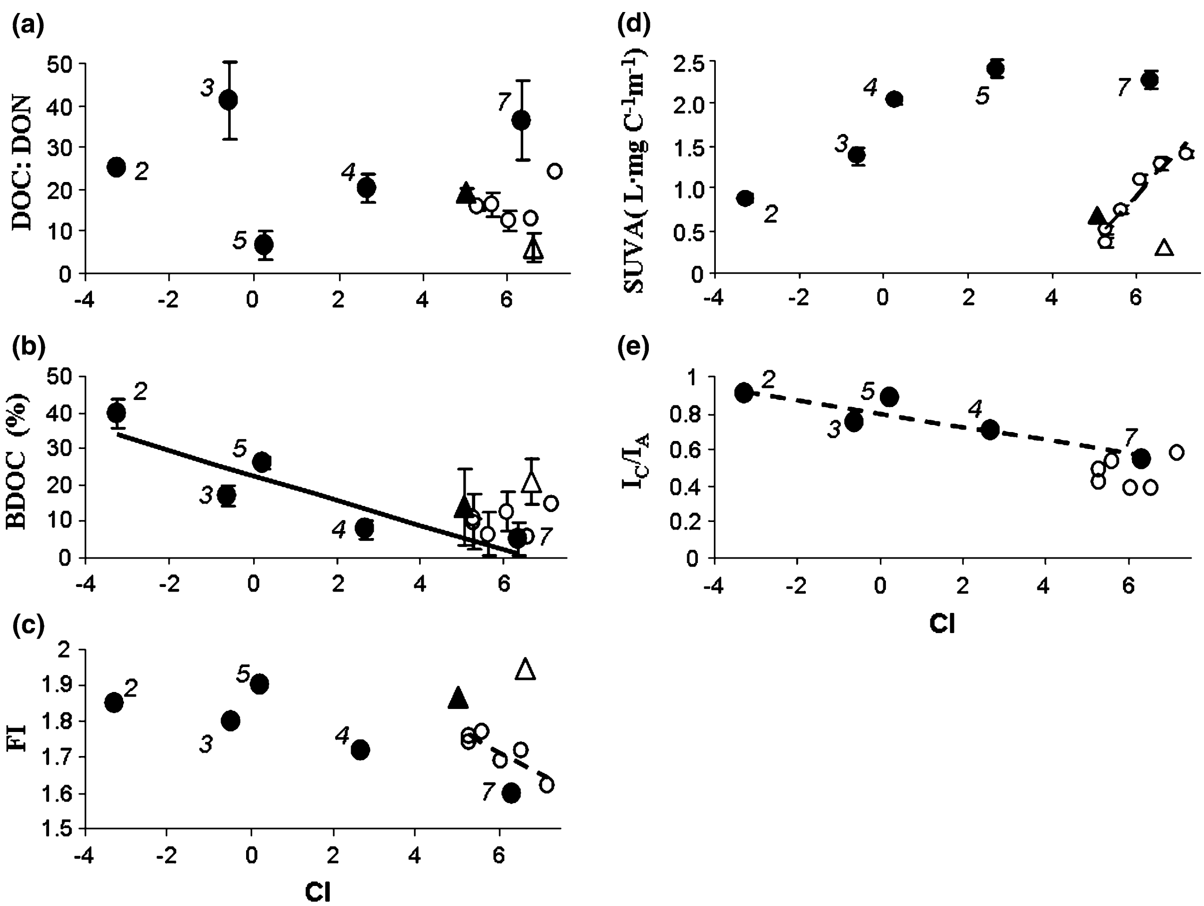

Fig. 5 The figure shows the CI (Chemical Index) relationships with DOM qualitative parameters. *Black dots* correspond to summer and *smaller white dots* to autumn values. *Triangles* correspond to ground water (*black* for summer and *white* for autumn). *Black dot numbers* correspond to each sampling 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) ($r = 0.57$, 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 ($r = 0.96$, 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 ($r = 0.9$, 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 ($r = 0.14$, df = 5, ns; Fig. 5e).

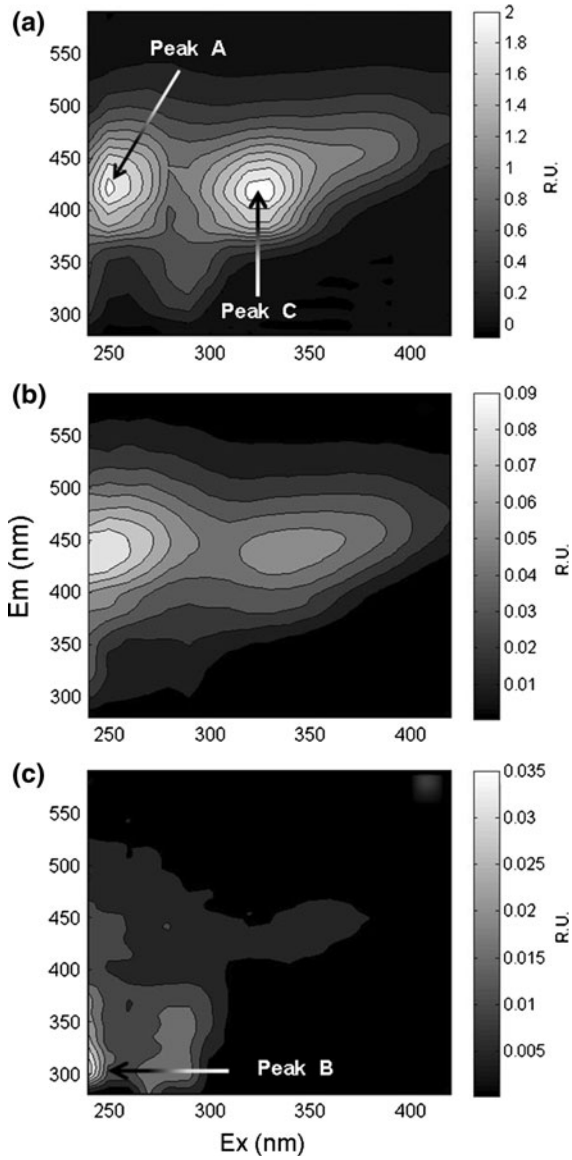

Fig. 6 Example of the change in the I_C/I_A ratio from EEMs for site *2*: **a** summer data; **b** autumn data; and groundwater: **c** summer data. Excitation and emission are in nm, and fluorescence is expressed in Raman Units (RU)

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

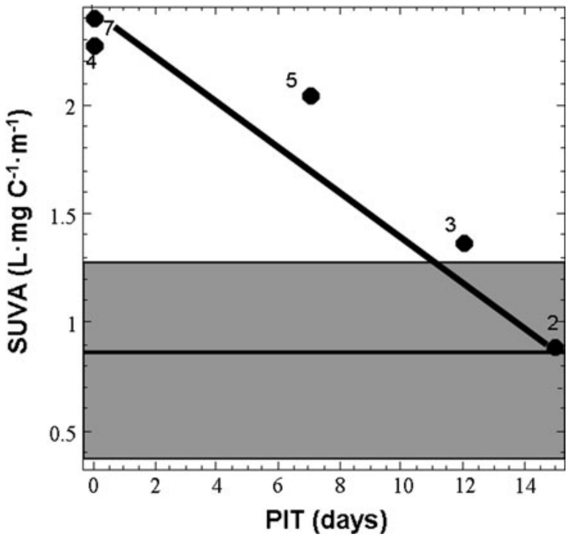

Fig. 7 Relationship between PIT (Pond Isolation Time) and SUVA values for summer surface waters sampling sites. The solid line and the gray band at the lower part show the SUVA mean value ± standard deviation for autumn samples. Numbers refer to the sampling site labels

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.

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