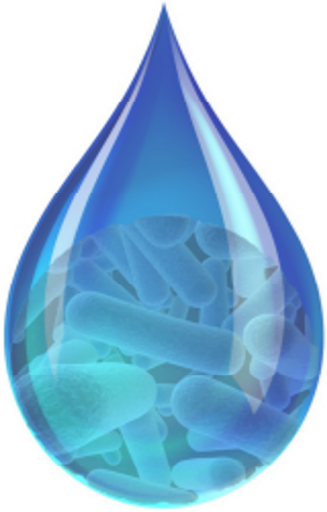
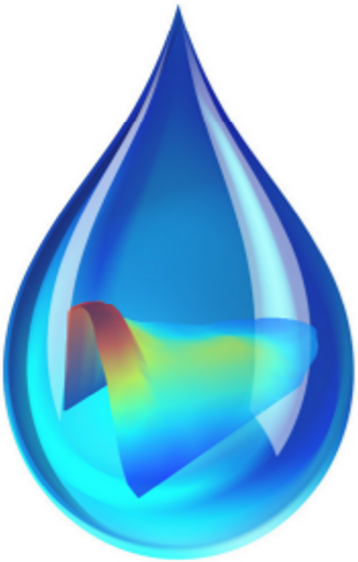
Sources, transformations and controls of dissolved organic matter (DOM) in a Mediterranean catchment

Fonts, transformacions i controls de la matèria orgànica dissolta (DOM) a una conca Mediterrània

Núria Catalán García





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Núria Catalán García

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TESI DOCTORAL

Departament d'Ecologia

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Programa de Doctorat en Ecologia Fonamental i Aplicada

Sources, transformations and controls of dissolved organic matter (DOM) in a Mediterranean catchment

Fonts, transformacions i controls de la matèria orgànica dissolta (DOM) a una conca Mediterrània

Memòria presentada per Núria Catalán García per optar al grau de doctora per la Universitat de Barcelona

Núria Catalán García

Barcelona, juny de 2013

Vist-i-plau dels directors de la tesi

Dr. Biel Obrador Sala
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A mis padres

Adiós a los que se quedan,
y a los que se van también […].

Esta es la albada del viento
la albada del que se fue
que quiso volver un día
pero eso no pudo ser.

Las albadas de mi tierra
se entonan por la mañana
para animar a las gentes
a comenzar la jornada.

Arriba los compañeros
que ya ha llegado la hora
de tener en nuestras manos
lo que nos quitan de fuera.

Esta albada que yo canto
es una albada guerrera
que lucha porque regresen
los que dejaron su tierra.

La albada,
Jose Antonio Labordeta (Zaragoza 1935 - 2010)

- ***Si no tiene propiedades, ¿cuál es su patrimonio?***

- Mis ideas, mi memoria, lo que tengo en la cabeza, lo que soy. Aprendiz de mí mismo, eso he sido toda mi vida.

Entrevista en el País (12 jun 2011) a
José Luis Sampedro (Barcelona 1917- Madrid 2013)

Contents (*Índex*)

Agraïments/ Agradecimientos/ Acknowledgements/ Remerciements

La tesi m'ha conduit molts cops al pensament què al món hi ha una gran majoria de gent maca disposada a fer un cop de mà. A tota la gent que m'ha despertat aquest sentiment, surtin o no a continuació: gràcies!

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NINI", I feel really lucky about having worked and discovered Sweden beside you! Hannes, Dolly, Cristian: thank you for your help and friendship!! Without you I doubt I'd have managed to make sense of these "crazy many vials"! And for the beers, fikas, cakes, dinners and time together thanks to ALL of you!!

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Y a ti, pillastre, que has leído los agradecimientos sin hojear la tesis... pese a que te entiendo… ¡gracias por animarte y echarle un vistazo al resto!

Núria Catalán García
Badalona, juny de 2013

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Abbreviations

Variable	Description
λ	wavelength
Ø Ø	Treatment without nutrients or glass beads (Chapter 3)
ØG	Treatment without nutrients and with glass beads (Chapter 3)
A_λ	Absorbance at a given wavelength
AlloDOC	Allochthonous source of DOC (external to the system; Chapter 2)
ANCOVA	Analysis of co-variance
AutoDOC	Autochthonous source of DOC (internally produced; Chapter 2)
AU	Autumn period of DOM properties in ephemeral washes (Chapters 1 and 4)
BD	Biodegradation (Chapter 2)
BGE	Bacterial growth efficiency (Chapters 2 and 4)
BIX	Biological Index derived from fluorescence spectra (Chapters 2 and 4)
BP	Bacterial production (g C L^{-1} h^{-1}; Chapter 2)
CE	Cell specific bacterial production (pgC cell $^{-1}$ h^{-1}; Chapter 2)
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
EEM	Excitation- emission matrix
FI	Fluorescence Index derived from fluorescence spectra (Chapters 1, 2 and 4)
HIX	Humification Index derived from fluorescence spectra (Chapters 2 and 4)
NØ	Treatment with nutrients without glass beads (Chapter 3)
NG	Treatment with nutrients and with glass beads (Chapter 3)
NMDS	Non-metric multidimensional scaling
PCA	Principal component analysis
PERMANOVA	Permutational analysis of variance
SUVA	Specific ultra-violet absorbance
UV	Ultra-violet radiation
UV+BD	Ultra-violet (photo-) and biodegradation treatment (Chapter 2)
WS	Winter-spring period of DOM properties in ephemeral washes (Chapters 1 and 4)

General introduction and objectives

Dissolved organic matter and its role in global biogeochemical processes

"Dissolved organic carbon is the great modulator, the variable that modifies the influence of other variables". With this statement, Prairie (2008) defined the functional role of dissolved organic carbon, DOC, in aquatic ecosystems in an exciting perspective paper.

As a great modulator, the dissolved fraction of organic matter (DOM)[1] is not only the primary source of organic matter in most aquatic ecosystems (Wetzel 2001) but also a variable that influences aquatic food webs (Jansson et al. 2007), drives underwater light conditions (Kirk, 1994), determines the availability of nutrients and metals altering the pollutant toxicity (Cammack et al. 2004) and plays a key role in the aquatic energy cycling (Amon and Benner 1996, Wetzel 2001, Cole et al. 2007). DOM is the basis of the microbial loop, that transfers much of the energy through a DOM-bacterial-protozoan pathway, implying also that much of the carbon (C) assimilated by the primary producers is not transferred to higher levels of the food web but mineralized by bacteria (Pomeroy 1974, Tranvik 1992).

DOC is a primary reservoir of carbon at a global level and one of the largest pools of organic carbon in aquatic ecosystems, representing up to 95% of organic carbon in the water column of oceans and lakes (Hedges 1992, Prairie 2008). Although not considered in the current regional carbon budgets, the role of inland waters in the global carbon cycle has been extensively addressed in the last years and its relevance is broadly accepted (Cole et al. 2007, Battin et al. 2009, Tranvik et al. 2009, Aufdenkampe et al. 2011). These works demonstrated that inland waters do not merely transport carbon acting as neutral pipes, but rather that they actively transform it in its way from terrestrial ecosystems towards the sea. Globally, inland waters receive an annual input of 2.7 Pg of C, of which 0.9 Pg are transported down to the oceans (Tranvik et al. 2009, Aufdenkampe et al. 2011); the remaining 1.8 Pg C, either sediment (0.6 Pg C) or are mineralized and emitted as CO_2 to the atmosphere (1.2 Pg C).

[1] The terms dissolved organic carbon (DOC) and dissolved organic matter (DOM) are frequently used interchangeably, although DOC refers to the carbon content of organic matter (McDonald et al. 2004). Accordingly, here we will use DOM when referring broadly to the compartment of dissolved organic materials, and DOC when specifically addressing its concentration.

The three aforementioned pathways (passive flow, sedimentation and mineralization; Cole et al. 2007, Tranvik et al. 2009) affect all the forms of organic and inorganic carbon and imply numerous processes, which hinder the understanding of the DOM transformation pathways (Fig. I-1). They include for example hydromorphologycal processes (Mullholland 2003, Aitkenhead-Peterson et al. 2003), flocculation (von Wachenfeldt and Tranvik 2008), photomineralization (Bertilsson et al. 1999) and microbial activity and decomposition (e.g. Sondergaard and Middelboe 1995, Amon and Benner 1996, Eiler et al. 2003, Kritzberg et al. 2006). Heterotrophic microbial community process the majority of organic carbon in inland waters (Sinsabaugh and Findlay 2003).

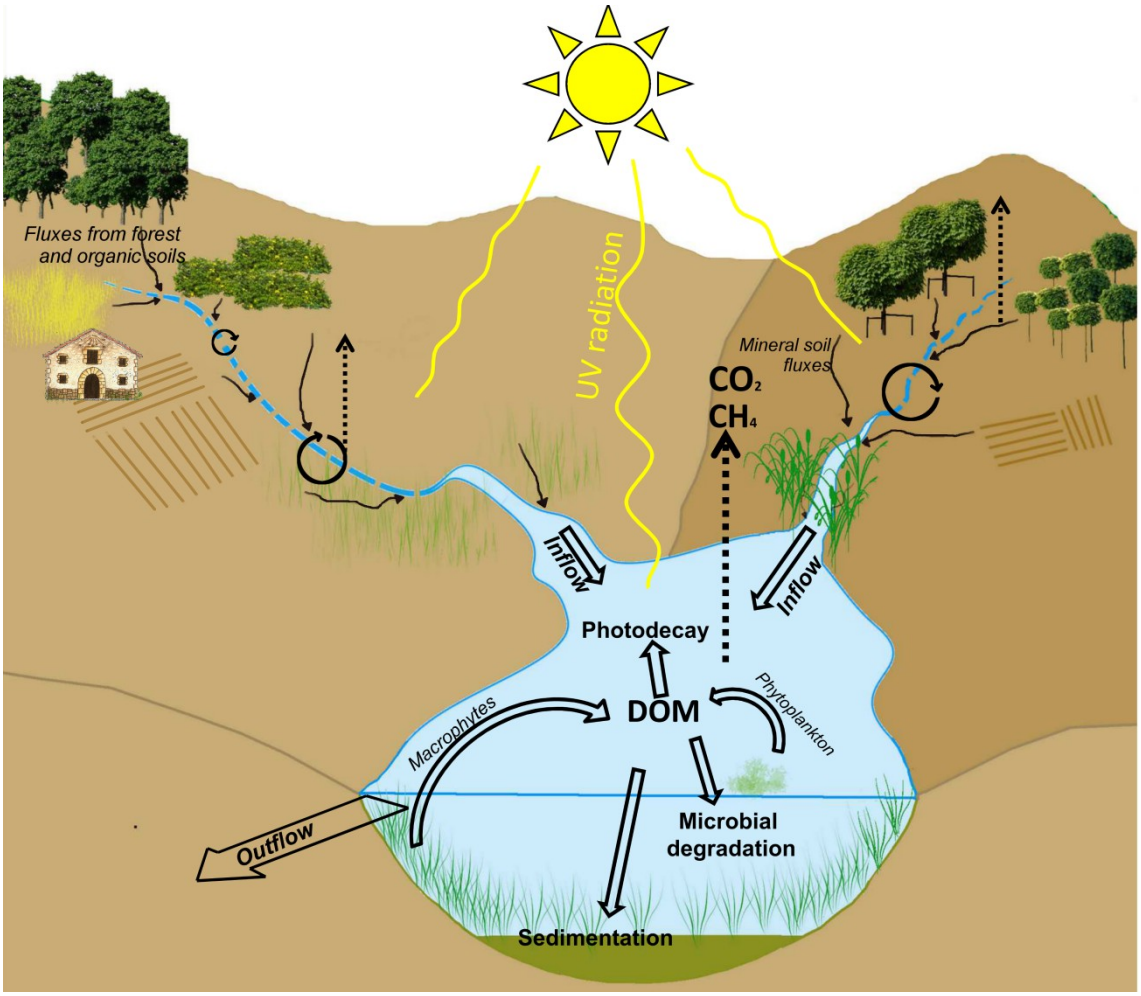

Figure I-1 Schematic representation of a catchment with the main DOM flows occurring in aquatic systems. The particularities of the depicted catchment correspond to the typical features of a Mediterranean landscape: intermittent surface flows, shallow lentic systems dominated by submerged vegetation and patchy landscape with contrasted soil uses.

Carbon bioavailability is regulated through interactions between these and many other processes such as photochemical alteration (Tranvik and Bertilsson, 2001), sorption (Aitkenhead and Peterson, 2003) or material complexation (Chin, 2003). Therefore, these DOM transformation mechanisms are tangled, acting together over the DOM pool and, as a function of DOM exposure to each mechanism, diverse intermediate metabolites and turnover times of DOM emerge (Sinsabaugh and Foreman, 2003). The converse effect is also true: the quality of DOM determines the physical environment (e.g. water transparency, gel structure of the aqueous medium, etc.) and influences microbial community (Fig. I-2). Since the efficiency of any DOM degradation pathway relies on the quality of the material, it can be stated that all the processes involved in DOM transformation are defined by and define DOM composition.

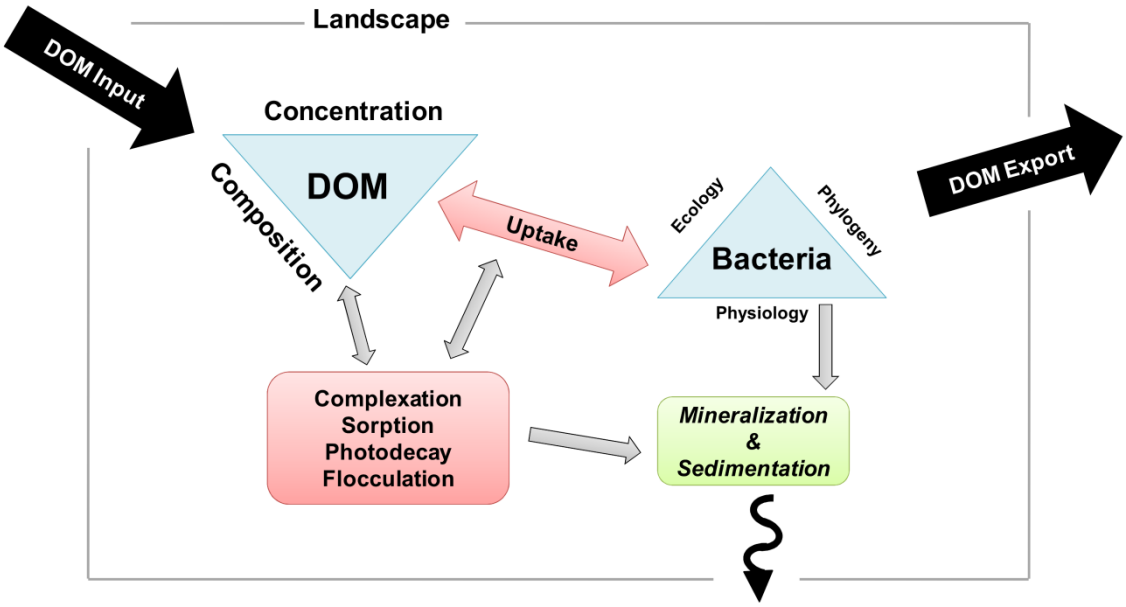

Figure I-2 Conceptual diagram highlighting the principal interactions that affect the supply, composition and metabolism of DOM in aquatic systems. Modified from Sinsabaugh and Findlay, 2003

DOM composition and sources

Two main fractions can be distinguished when considering DOM composition: humic and non-humic materials (Thurman 1985, McDonald et al. 2004). The non-humic fraction includes materials of known structure and composition, such as lipids, carbohydrates, polysaccharides, amino acids and proteins (Wetzel 2001). In contrast, the humic fraction is operationally defined, consisting on a complex array of

compounds including fulvic, humic and transphilic acids (Thurman, 1985). This humic fraction is the main component of DOM, so that for instance fulvic acids represent between the 45 and the 65% of the DOM existent in rivers and streams (McKnight et al. 2003).

DOM compositional complexity hampers the determination of DOM origin and reactivity. This is reflected by the high amount of works referring to DOM as a "black box" (e.g. Sinsabaugh and Findlay 2003, Fellman et al. 2010) or directly as a "dark" subject (e.g. Macalady and Walton-Day 2009, Stubbins et al. 2010). However, recent methodological improvements have shed light to this obscure DOM composition subject through the use of a broad range of analytical tools, including isotopic tracers (Hood et al. 2005), mass spectrometry (which allows determining the exact elemental composition of ions; Gonsior et al. 2009, Stubbins et al. 2010) and spectroscopic tools (Coble 1996, Stedmon et al. 2003, Fellman et al. 2010).

Spectroscopic techniques have profoundly improved DOM characterization during the last decade (McKnight et al. 2001, Stedmon et al. 2003). These tools are relatively fast, inexpensive and easy to implement in comparison with other analytical procedures, allowing their use in a broad range of temporal and spatial scales (Jaffé et al. 2008, Fellman et al. 2010). Absorbance and fluorescence measures of bulk water samples provide extended information on the chemical properties of DOM (Stedmon and Markager 2005, Jaffé et al. 2008, Fellman et al. 2010). They are based on the fact that DOM has color, which means that it absorbs part of the light spectra (Kirk 1994) and, as a result of this absorption, a fraction of DOM also exhibits fluorescence properties (Ewald et al. 1983). The emission and excitation wavelengths of natural fluorescent compounds spread over a large part of the spectra. In order to cover a broader spectral region, including excitation, emission and fluorescence intensity, the 3D fluorescence spectroscopy has been applied to natural DOM (Mopper et Schultz 1993). The obtained 3D spectra are also called excitation-emission matrices (EEMs).

The major responsible of the fluorescence of natural waters are fulvic acids (McKnight et al. 2003), despite there are other compounds of proteic character that also show fluorescence (Coble et al. 1996). Each discrete compound or functional group presenting fluorescence is called a fluorophore, and the fluorescence spectra of natural

water samples are usually the result of the simultaneous contribution of different fluorophores (Lakowicz 2006). This fact impedes a precise molecular identification of the constituents of DOM from EEMs (Fellman et al. 2010). Nonetheless, the different fluorescence regions of the 3D spectra have been broadly demonstrated to be excellent indicators of ecological processes, functional properties and sources of DOM (McKnight et al. 2001, Stedmon and Markager 2003, Baker et al. 2008, Jaffé et al. 2008).

In aquatic ecosystems, DOM sources can have an autochthonous or an origin (Fig. I-1). Autochthonous DOM sources derive from auto- and heterotrophic in-situ activities including phytoplankton, benthic algae, periphyton, and aquatic phanerogams (Bertilsson and Jones 2003, Kritzberg et al. 2004). Algae and microbial communities are usually considered the primary source of autochthonous DOM in inland waters. Despite in some systems other sources such as macrophytes are likely to be the dominant origin of autochthonous DOM (Barrón et al. 2003, Bertilsson and Jones 2003, DeMarty and Prairie 2009), little is known about the influence of such autochthonous sources on DOM quality. Algal-derived DOM is mainly composed by sugars, amino acids and organic acids with a labile character (Wetzel 2003). Since algae are considered the main autochthonous DOM source, its labile character has been extended to the whole pool of autochthonous DOM in the literature.

Allochthonous DOM comes from the catchment drainage, and is mainly derived from the organic matter present in plant litter and soils (Thurman 1985, Aitkenhead-Peterson et al. 2003). Terrestrial sources release mainly plant structural compounds as lignin and cellulose, which are considered much more recalcitrant to biological degradation than sugars or amino acids (Sinsabaugh and Foreman 2003).

With this overall characterization in mind, the first approaches linking sources of DOM with its functional properties considered autochthonous material as a pool of labile substances sustaining ecosystem production, whereas allochthonous inputs were depicted as a pool of recalcitrant and unreactive DOM (Wetzel 2003). This widely used paradigm is nowadays under reconsideration. For instance, the assumed unreactive terrestrially-derived carbon has been shown to sustain a high fraction of heterotrophic production of lakes and to have high relevance in the aquatic food webs (Pace et al. 2004, Kritzberg et al. 2004, Jansson et al. 2004). Parallel evidences of the existence of

highly labile materials in river DOM are appearing in the literature (Fellman et al., 2009, Guillemette and delGorgio 2011). Also, autochthonous DOM can resemble allochthonous sources in character and reactivity depending on the water body studied. For instance, little is known about the assumption that autochthonous DOM has a labile character in systems dominated by aquatic submerged plants.

Classical approaches to DOM recalcitrance

DOM recalcitrance has been traditionally defined in terms of bioavailability, which refers to the quality of a material of being easily accessed by microorganisms (delGiorgio and Davis 2003). Such accessibility is assumed to rely on the molecular structure or the age of the material (Sinsabaugh and Foreman 2003). The age of DOM is used to define its degree of pre-processing before reaching the receiving system (Bianchi 2007). In general, the further from the source DOM is, the stronger the transformations of the original material (Sinsabaugh and Foreman 2003) and the higher the recalcitrance of the remaining DOM. It has been found that the loss of DOM in a river is a function of the time flowing in it, with an stronger loss of the colored fraction of materials (i.e. humic) than of the non colored one (Weyhenmeyer et al. 2012). Thus the diagenetic age of DOM is not necessarily a function of time but of history of exposure to degradation pathways, however both terms are extremely confounded in the literature.

This diagenetic age is implicit in the size-reactivity continuum model of Amon and Benner (1996). This model states that DOM reactivity decrease from large to small sized molecules and from fresh to old materials. The relationship between size and reactivity has been extensively discussed (Sinsabaugh and Foreman 2003, Bianchi 2007), specially in freshwater systems, where the terrestrially derived materials have longer degradation history but higher molecular size than autochthonous DOM (Bianchi 2007). Together with molecular size, other properties of the material such as its elemental composition, degree of oxidation or the presence of specific functional groups (e.g. phenolic aromatic structures or carboxylic rich alyciclic molecules) have been linked with DOM recalcitrance (Thurman 1985, Kim et al. 2004, Kleber 2010).

In any of these cases, when determining the reactivity of DOM, only the intrinsic properties of the material are being considered, implying that one or more material properties of natural DOM can prevent it from being decomposed (Kleber 2010). Irrespective of how DOM recalcitrance is defined, either in terms of years (age), molecular size (Amon and Benner 1996) or molecular configuration, these properties are inherent to the material. This concept of inherent chemical recalcitrance has been challenged recently (Kleber 2010); it has been shown that old carbon can potentially be strongly bioavailable, demonstrating that the exposure of DOM to degradation pathways is not necessarily a function of time (Gurwick et al. 2008, McCallister and DelGiorgio 2012, Singer et al. 2012). It has also been shown that the size-reactivity continuum does not apply in many freshwater systems with important terrestrial sources of DOM (Bianchi 2007) and that molecular size does not predict decomposability of organic compounds (Kleber 2010). Finally, it has been demonstrated that carboxylic rich materials and aromatic compounds can be rapidly degraded by different pathways (Frazier et al. 2005, Stubbins et al. 2010).

Since age or molecular structure by themselves are not sufficient to explain DOM stability, the recalcitrance of organic matter should be perceived as an ecosystem property, so that environmental and biological controls predominate over the intrinsic properties of the material, as is accepted to occur in soils (Schmidt et al. 2011). Other controls extrinsic to the degradation pathway can be controlling organic carbon availability as could be the interaction between different materials (Kleber 2010), mineral associations that protect organic molecules (Ekschmitt et al. 2005) and bioenergetics or enzymatic limitations (Arnosti 2003, Guenet et al. 2010). The bioenergetics limitation has recently received particular interest, since it has been hypothesized that inputs of labile carbon can lead to an increased consumption of the existing DOM pool in the system. This phenomenon is known as priming effect and is currently under intense evaluation by the scientific community, (Guenet et al. 2010, Bianchi 2011). Priming effect might be especially relevant in interfaces where two pools of organic materials interact, as in littoral zones, in inflows from rivers into lakes or after a phytoplanktonic activity pulse (Guenet et al. 2010). Its occurrence in aquatic

systems is an important gap of knowledge regarding the factors that determine DOM degradability.

Relationship of DOM with landscape

Landscape is classically defined as the physical environment or visible features of an area, but we would like to add the not visible features of an area too. Not visible because this physical environment will imply ecosystem processes like radiation, water residence time or material leaching that might not be captured in a picture. Not visible because landscape implies a small-scale of changes such as mobility of nutrients, water transparency or interactions between organic molecules and the microbial community (Sinsabaugh and Foreman 2003).

Here, we apply the idea of landscape following the principles of landscape limnology (Fig. I-3; Soranno et al. 2010), but making them extensive to DOM. Thus, landscape would be defined as the physical environment including freshwater, terrestrial, and human factors that interact to determine the patterns of DOM processing across temporal and spatial scales.

DOM quality and quantity is defined by the landscape as it controls the material produced and the transformations that DOM suffers in its way towards the sea (Weyhenmeyer et al. 2012). From this perspective, a catchment must be studied as a whole unit (Fig. I-1 and I-3; Oni et al. 2011), including the climatic factors (e.g. precipitation regime), the geological features (e.g. geology, soils, watershed slope), the aquatic drivers (e.g. drainage ratio, runoff), the human influence (e.g. land uses) and their interactions (Soranno et al. 2010, Weyhenmeyer et al. 2012).

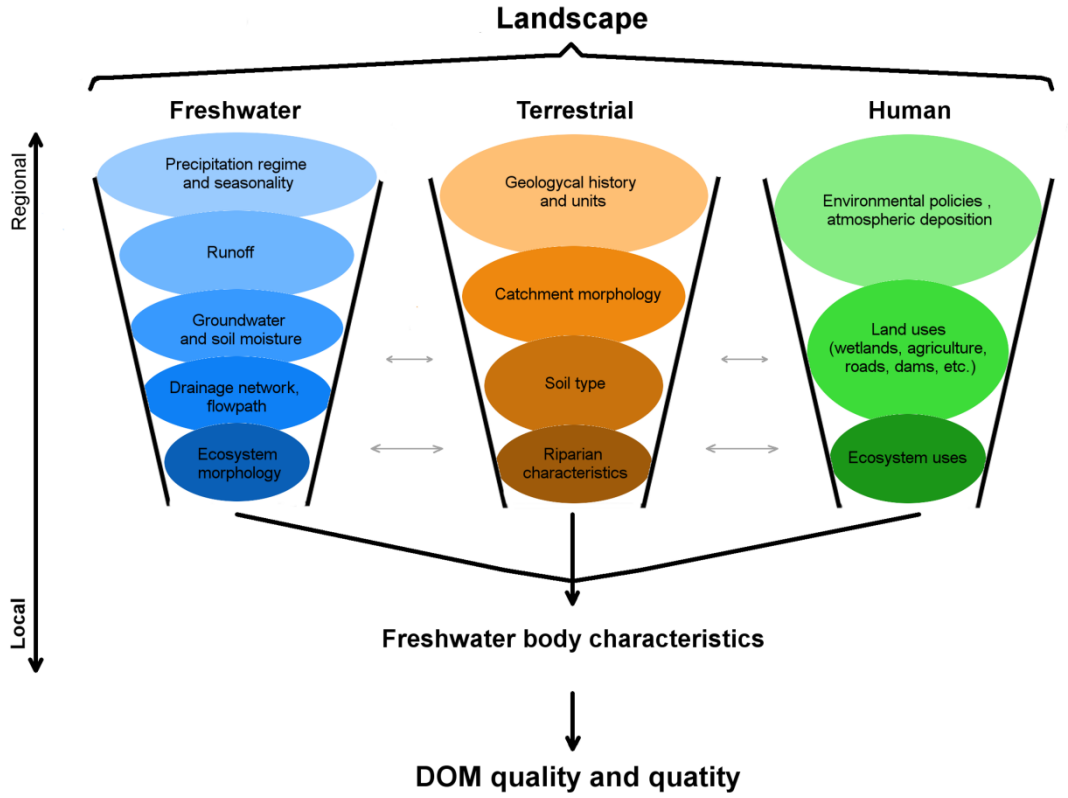

Figure I-3 General framework of landscape limnology. Landscape features driving freshwater ecosystem functioning and consequently DOM concentration and composition. Adapted from Soranno et al. 2010.

Mediterranean landscape

Mediterranean landscape has some characteristics that make studying DOM in these systems especially interesting because it can put some light in the general knowledge of DOM transformation processes. First of all, Mediterranean climate presents a marked seasonality, with a dry summer season and a wet period in winter and autumn; in this late season, floods are frequent and concentrate much of the discharge (Butturini and Sabater 2000). Such summer drought period makes of intermittence a common feature of Mediterranean water courses (Gasith and Resh 1999) and some interesting insights have been done regarding DOM quality and concentration related to this flow intermittency. This transition between dry-wet conditions affects stream metabolism (Acuña et al. 2005), enzymatic activities (Ylla et al. 2010), influences the DOM retention (Bernal et al. 2002) and determines the quality of DOM (Vázquez et al. 2010). The pools remaining during drought present an

increased contribution of autochthonous DOM sources, whereas the autumn floods that re-establish the flow bring in terrestrial materials (Vázquez et al. 2010).

Within this context of high flow variability and strong natural disturbance, ephemeral washes dominate surface flows in the Mediterranean climate regions (Uys & O'Keefe 1997, Álvarez-Cobelas et al. 2005). Such washes flow uniquely as a direct response to precipitation (Leopold and Miller, 1956). Despite being the most common type of water flow around the globe (Steward et al. 2012) patterns of DOM in these ephemeral washes have been poorly studied. Also, as DOM transformations are a function of water residence time in the landscape (Weyhenmeyer et al. 2012), small water courses, close in space and time to the DOM origin, are suitable systems to study terrestrial DOM sources to aquatic environments (Fellman et al. 2009).

Therefore, new insights regarding DOM sources and processing are expected to emerge from the study of DOM in ephemeral washes. Also, in Mediterranean systems, the relation catchment:lake area ratio is typically higher than in temperate climates (Álvarez-Cobelas et al. 2005), so that the catchment effects on the receiving water body are also expected to be stronger. Torrential inputs generate a pulsed pattern in the receiving water body, affecting water level, solutes and nutrients inputs (Bekioglu et al. 2007) and consequently, DOM dynamics. Such pulsed inputs of DOM into receiving water bodies have been hypothesized to be very active from a biogeochemical point of view (Stephens and Minor, 2010) and are the perfect framework for the occurrence of processes as the priming effect. In intermittent rivers, it has been described that autumn storms can contribute with up to the 20% of the total annual (DOM) export (Bernal et al. 2005). Also, during storms, allochthonous sources of DOM dominate, increasing humic character and C:N ratio (Mulholland 2003).

Finally, most of the natural Mediterranean water bodies are shallow systems (Álvarez-Covelas et al. 2005). Shallow systems are typically highly productive and are frequently dominated by submerged macrophytes (Valiela et al 1997, Knoppers 1994, deMarty and Prairie 2009). Submerged macrophytes can be the main source of DOM into the water body (Wetzel 2003) both trough losses during photosynthesis (DeMarty and Prairie 2009) or throughout senescence releasing cellular contents and structural materials (Mann and Wetzel 1996). The release rates of DOM are very variable since

they depend on the system and species studied (Wetzel 1972), however, they can represent up to the 70% of the plant production (DeMarty and Prairie 2009). The release rates of DOM by senescent leaves during decay can be higher than DOM losses during photosynthesis, but they appear to support lower bacterial efficiencies (Mann and Wetzel 1996). During photosynthesis, most of the released materials are carbohydrates (Tank et al. 2011) while senescent leaves release also structural compounds derived from lignine, that present lower bioavailability (Zhang et al. 2013).

Mediterranean mild climate allows a strong macrophytic presence throughout most of the year and consequently, an almost permanent source of autochthonous DOM. This scheme provides a suitable framework to study the balance between sustained autochthonous DOM production and allochthonous DOM pulses along seasonal cycles.

OBJECTIVES

This thesis aims to unriddle the main sources, controls and transformations affecting DOM in a Mediterranean catchment. The first and fourth chapters follow the natural variability of DOM quality and its relation with the landscape. In chapters 2 and 3 experimental laboratory designs were applied in order to study some of the main processes involved in DOM reactivity and mineralization.

In the **first chapter**, we aimed to characterize DOM properties and to identify the drivers of DOM variability in ephemeral washes, proper water bodies to study the links between landscape and DOM quality. The study was performed in seven ephemeral washes draining a heterogeneous catchment in terms of landscape features. In particular, the specific questions we addressed in this chapter were:

- Is DOM quality in ephemeral washes related with landscape properties like hydromorphology or land cover?
- Does DOM quality present spatial variability? Is it linked with landscape settings?
- Is the relevance of these drivers constant along the hydrological year?

Different processes determine the mineralization rates of DOM. In the s**econd chapter**, we addressed the reactivity of two sources of DOM in a shallow lake to evaluate the general paradigm that links autochthonous DOM with labile and allochthonous DOM with less available materials. We evaluated the role of bio- and photodegradation processes by tracking the instantaneous changes in DOM quality during incubation experiments. We addressed these particular questions:

- Are there differences in the reactivity of autochthonous and allochthonous DOM sources?
- Do the instantaneous rates of change capture the dynamics of qualitative changes in the DOM pools during degradation processes?

In the **third chapter**, we investigated the incidence of priming effect in inland waters, a mechanism hypothesized to enhance DOM mineralization in situations where two different pools of DOM interact, in order to gain knowledge on the processes controlling DOM degradation. We set up a multi-factorial experiment with different DOM sources in order to find evidences of enhanced DOM consumption after labile C additions. We focused on these questions:

- Are there evidences of priming effect in the water column of inland waters?
- Does the DOM consumption vary depending on the lake water used or the labile C source added?
- Is DOM mineralization facilitated by the addition of nutrients or the availability of surface?

The **fourth chapter** aims to capture the dynamics of DOM in a shallow lake to evaluate the role of submerged vegetation on DOM quality under a whole-ecosystem perspective integrating the landscape. We traced the temporal changes of DOM quality in the receiving water body of the catchment studied in chapter one, the lagoon. We explored the links between the lagoon DOM quality dynamics, the corresponding autochthonous and allochthonous DOM sources and the processes regulating DOM concentrations and properties. We addressed these specific questions:

- Are there seasonal or spatial trends in DOM quality?
- Which are the drivers of that variability?
- Do the spectroscopic descriptors capture the complexity of ecosystem processes affecting DOM?

Overall, this thesis should be able to capture the complexity of the processes regulating DOM at a catchment scale, especially in highly dynamic ecosystems with fast internal cycling and strong hydrological forcings as the Mediterranean ones.

Chapter 1

Seasonality and landscape factors drive dissolved organic matter properties in Mediterranean ephemeral washes

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Abstract

Dissolved Organic Matter (DOM) is a fundamental component of the aquatic carbon cycle and a key driver of the biogeochemical interactions between terrestrial and aquatic ecosystems. The origin, properties and role of DOM are increasingly characterised in lakes, rivers and streams, but little is known about DOM characteristics in ephemeral washes, which are the most common water flows in Mediterranean landscapes. Here, we examine the patterns in the optical properties of DOM in ephemeral washes draining a small watershed in the island of Menorca, Western Mediterranean. We used concentration data (dissolved organic carbon and nitrogen) and several spectroscopic descriptors ($SUVA_{254}$, absorption coefficient at 440nm, fluorescence index, and excitation–emission fluorescence matrices) to assess changes in DOM concentrations and quality at both seasonal and spatial scales.

Two periods were clearly distinguished in the DOM properties: autumn and winter-spring. In autumn, which includes the first flows of the hydrological year, DOM showed an aromatic character and was spatially homogenous over the watershed. In winter-spring, DOM was smaller and recently produced, and a considerable spatial heterogeneity was observed in all descriptors. The variability in DOM concentrations and quality was driven by hydromorphology and by the landscape features of the watershed, but the influence of these drivers on DOM properties changed along the hydrological year. In autumn, hydromorphology was the main factor determining DOM properties, whereas in winter-spring the land uses in the watershed highly determined the observed differences in DOM quality between subcatchments.

Resum (en català)

La matèria orgànica dissolta (DOM) és un component fonamental del cicle del carboni aquàtic i té un paper clau en les interaccions biogeoquímiques entre ecosistemes terrestres i aquàtics. L'origen, característiques i paper de la DOM estan sent àmpliament caracteritzats en diferent tipus de cossos d'aigua, no obstant, les seves característiques són poc conegudes als torrents efímers, tot i ser els cursos d'aigua més comuns en el paisatge Mediterrani. En aquest estudi, examinem el patró de les propietats òptiques de la DOM en els torrents drenant una petita conca a l'illa de Menorca. Fem servir dades de concentració (carboni i nitrogen orgànic dissolt) i diversos descriptors espectroscòpics ($SUVA_{254}$, coeficient d'absorció a 440 nm, índex de fluorescència i les matrius d'emissió-excitació) per avaluar els canvis en la quantitat i qualitat de la DOM tant en l'escala espacial com a l'estacional.

Dos períodes van ser clarament distingits en base a les propietats de la DOM: tardor i hivern-primavera. A la tardor, període que inclou les primeres torrentades de l'any, la DOM mostrava un caràcter aromàtic i era espacialment homogènia a la conca. Durant hivern-primavera, la DOM mostrava senyals d'haver estat recentment produïda, mostrant un origen microbià i una considerable heterogeneïtat espacial en tots els descriptors. La variabilitat en les concentracions i qualitat de la DOM vénen determinades per descriptors del paisatge, però la influència d'aquests descriptors sobre les propietats de la DOM varia al llarg de l'any hidrològic. A la tardor la hidromorfologia era el factor principal determinant les propietats de la DOM, mentre que a l'hivern-primavera els usos del sòl definien les diferències observades en la qualitat de la DOM entre subconques.

Resumen (en castellano)

La materia orgánica disuelta (DOM) es un componente fundamental del ciclo del carbono acuático y tiene un papel clave en las interacciones biogeoquímicas entre ecosistemas terrestres y acuáticos. El origen, características y papel de la DOM están siendo ampliamente caracterizados en diferentes ecosistemas acuáticos, no obstante, sus características son poco conocidas en torrentes efímeros, los cursos de agua más comunes en el paisaje Mediterráneo. En este estudio, examinamos el patrón de las propiedades ópticas de la DOM en los torrentes que drenan una pequeña cuenca en la isla de Menorca. Usamos datos de concentración (carbono y nitrógeno orgánico disueltos) y varios descriptores espectroscópicos ($SUVA_{254}$, coeficiente de absorción, índice de fluorescencia i matrices de emisión-excitación) para evaluar los cambios en la cantidad y calidad de la DOM tanto en la escala espacial como en la estacional.

Dos períodos fueron claramente distinguidos en base a las propiedades de la DOM: otoño e invierno-primavera. En otoño, período que incluye los primeros episodios de escorrentía del año, la DOM mostraba un carácter aromático y era espacialmente homogénea en la cuenca. Durante invierno-primavera, la DOM mostraba señales de haber sido recientemente producida mostrando un origen microbiano y una considerable heterogeneidad espacial en todos los descriptores. La variabilidad en las concentraciones y calidad de la DOM vienen determinadas por descriptores del paisaje, pero la influencia de éstos sobre las propiedades de la DOM varía a lo largo del año hidrológico. En otoño la hidromorfología era el factor principal determinando las propiedades de la DOM, mientras que en invierno-primavera los usos del suelo determinaban las diferencias observadas en la calidad de la DOM entre subcuencas.

Introduction

Dissolved organic matter (DOM) is the primary source of organic matter in most aquatic ecosystems and comprises a wide and complex array of compounds (McKnight et al. 2001; Fellman et al. 2009). DOM plays a key role in the aquatic carbon cycle, influences aquatic food webs, underwater light conditions and bacterial production, and determines the availability of dissolved nutrients and metals (Amon and Benner 1996; Wetzel 2001; Cole et al. 2007). DOM regulation in running waters is highly driven by the catchment characteristics (Westerhoff and Anning, 2000). On the one hand, catchment hydromorphology and geological structures determine DOM concentrations and availability (Mulholland 2003; Aitkenhead and Peterson 2003). On the other hand, land uses (Oni et al. 2011; Williams et al. 2011) and soil types (Fellman et al. 2009; D'Amore et al. 2010) exert a strong influence on DOM composition. All in all, it is the interaction between these drivers what finally determines the concentration and quality of DOM draining from the hillslope, and indirectly defines the in situ production and the cycling of microbial biomass, which will in turn modify the DOM properties (Aitkenhead and Peterson 2003; Belnap et al. 2003).

The heterogeneous and highly dynamic nature of DOM makes it difficult to study its origin (Baker 2002). Nonetheless, the development of spectroscopic techniques has thoroughly improved DOM characterization and allowed the identification of DOM qualitative changes (Coble 1996; McKnight et al. 2001; Stedmon and Markager 2005). Such techniques allow the discrimination of DOM fractions by their molecular size, thus indicating its possible sources, typically defined as allochthonous (derived from higher plants and soil within the catchment) or autochthonous (derived from in-situ microbial and algal activity) (Coble 1996; Weishaar et al. 2003; Jaffé et al. 2008).

The Mediterranean region is characterized by a marked seasonality, with a dry period followed by a wet season in which torrential events are frequent (Gasith and Resh 1999). Under such climatic conditions, intermittent streams and ephemeral washes are the dominant surface flows (Álvarez-Cobelas et al. 2005). Ephemeral washes are those watercourses that flow briefly in direct response to precipitation, and are distinguished from intermittent streams because they are always above the phreatic level (Leopold and Miller 1956). The hydraulic regime, the hydromorphology and the sediment transport of ephemeral washes have been widely studied in arid and semi-arid regions (Bull 1997;

Martín-Vide et al. 1999; Camarasa-Belmonte and Segura-Beltrán 2001), but studies dealing with their chemical composition and dynamics are scarce and usually centred on inorganic compounds (Fisher and Minckley 1978). Despite some studies have addressed the dynamics of organic matter in ephemeral washes (e.g. Jacobson et al. 2000), to the best of our knowledge there is no literature on the patterns of DOM chemical properties in these systems. Besides, the natural deficit of water resources of the Mediterranean climate is expected to exert a strong seasonal influence on DOM dynamics, as has been reported in desert streams (Jacobson et al. 2000).

The catchment level is considered the most appropriate scale to study low order flows as ephemeral washes, as long as DOM composition is expected to be influenced by the catchment land uses and geology (Kalbitz et al. 1999; Oni et al. 2011). The historically long agricultural pressure over Mediterranean soils leads to a highly patched landscape comprising a matrix of agricultural and forest uses. On the one hand, agriculturally affected streams have typically a microbial-like typology of DOM (Williams et al. 2010). On the other hand, DOM in forested streams typically presents an aromatic character (Fellman et al. 2010). In Mediterranean ephemeral washes we would expect the interplay between seasonality, land uses and hydrology to strongly determine DOM characteristics and dynamics.

In this study we dealt with the DOM properties and dynamics in a Mediterranean catchment drained by ephemeral washes. Our specific objectives were: 1) to characterise the concentrations and chemical properties of DOM, and 2) to determine the drivers of the spatial and temporal variability in DOM properties.

Material and methods

Study site

The Albufera des Grau catchment (56 Km^2) is located in the North East coast of the island of Menorca (Balearic Islands, Western Mediterranean; Fig. 1.1). The catchment constitutes the freshwater supply of the brackish coastal lagoon of Albufera des Grau (Pretus 1989). The climate is Mediterranean (type Csa in the Köppen classification system), with a dry and hot summer period, and mean monthly temperatures ranging from 10ºC in January to 25ºC in August. Mean annual precipitation is 549 mm and is typically centred on

autumn and winter, being November the most humid month (90 mm on average) and July the driest (8 mm on average) (Jansà 1979). The watercourses in the catchment are ephemeral washes with a hortonian flux (i.e. presenting water flow only during precipitation episodes). The duration of the flow mainly depends on the intensity of the rainfall event and on the moisture status of the soils in the catchment. The dominant lithologies in the catchment are Jurassic dolomites, limestones and marls (40%), alluvial quaternary sands, limes and clays (14%), and Palaeozoic turbidites (14%) (IGME 1988).

Main soil types following FAO-UNESCO classification (1988) are chromic cambisol (70%), a relatively mature soil, and eutric leptosol (21%), with low organic content. The land covers in the catchment are dominated by mixed Mediterranean forests of Quercus ilex, Pinus halepensis and Olea europaea var. sylvestris (47%), extensive dry farming land (41%) and shrublands (9%).

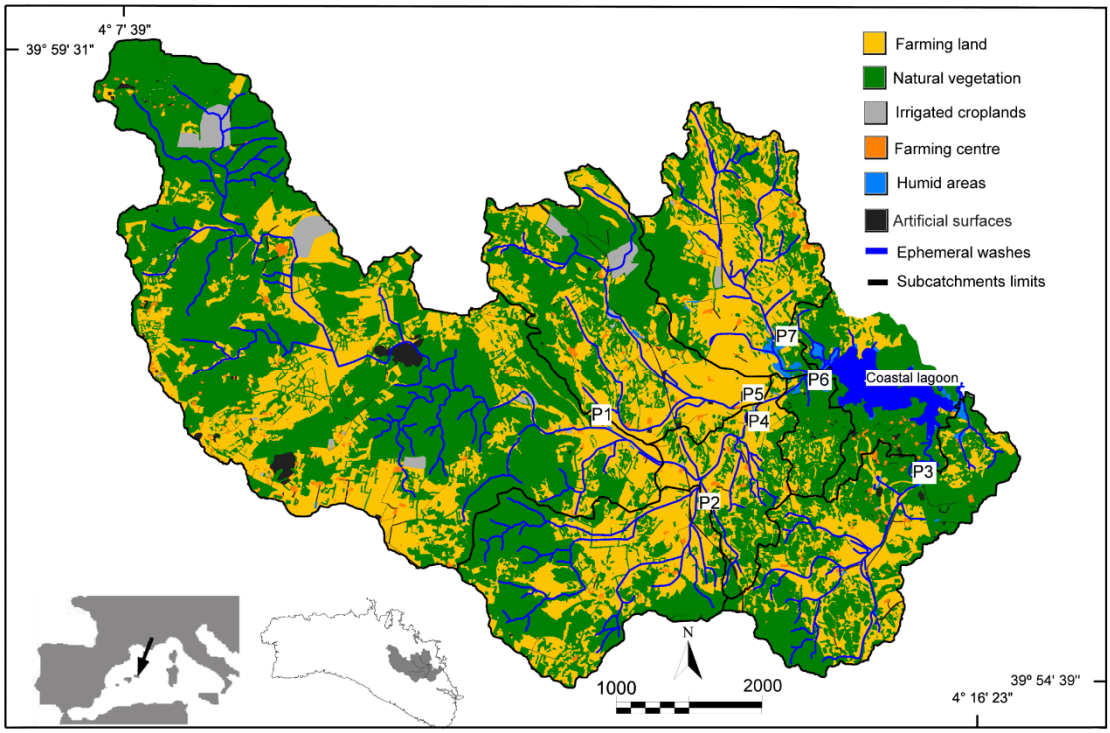

Figure 1.1. Albufera des Grau catchment. Location of ephemeral washes and sampling sites with respective sub-catchment boundaries and main land uses. The inset shows the geographical context of the study site in Menorca, Western Mediterranean

Field and laboratory methods

The study was conducted from September 2007 to December 2008 (i.e. more than one hydrological cycle) on 7 subcatchments defined by their hydrological and

geomorphological features (Fig. 1.1; Table 1.1). A total of 27 runoff episodes occurred during the studied period, of which 16 events were sampled.

Three replicate water samples were filtered through pre-combusted GF/F glass-fiber filters (Whatman) and cold-stored until analyzed. Nitrate (NO3-) was analyzed colorimetrically with a Technicon Autoanalyser® after reduction through a copperised cadmium column (Keeney and Nelson, 1982), with an analytical precision of 0,1 μM. Ammonium (NH4+) was determined colorimetrically after oxidation with citrate in fenol with a precision of 3%. Total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) concentrations were determined in a Shimadzu TOC-VCS with a coupled TN analyzer unit. The detection limit of the analysis procedure was 0.05 mgC L^{-1} for DOC and 0.005 mgN L^{-1} for TDN. All DOC samples were previously acidified with HCl 2M and preserved at 4ºC until analysis. DOC was determined by high temperature catalytic oxidation and TDN by oxidative combustion-chemiluminiscence. Dissolved organic nitrogen (DON) was calculated as the difference between TDN and the inorganic forms of dissolved N (i.e., NO_3^- and NH_4^+).

We used several spectroscopic techniques to characterise DOM quality. Due to the nature of ephemeral washes it is difficult to apply the terms autochthonous/allochthonous when characterizing DOM sources. Here we used the terminology terrestrial (high plant and soil origin, humic-acid type DOM) and microbial-like (bacteria and algae recently produced DOM). UV-Vis spectroscopy was performed in a Shimadzu UV-1700 spectrophotometer, using 1cm quartz cuvettes with an analytical precision of 0.001 absorbance units. The absorption coefficients at wavelength λ (a_λ, m-1) were determined from the absorbance measurement (A_λ) using the expression: $a_\lambda = 2.303\ A_\lambda / l$, where l is the path length in meters (Bricaud et al. 1981). We selected 440nm as an indicator of chromophoric dissolved organic matter (CDOM) concentration (Kirk, 1994; Gallegos et al. 2005). Typical values of a440 are in the range between 0.01 m^{-1} (ocean waters) and 19.1 m^{-1} (strongly humic lakes) (Kirk, 1994). Specific ultra-violet absorbance at 254nm ($SUVA_{254}$, L mg^{-1} m^{-1}) was calculated by dividing a_{254} by the DOC concentration in mg L^{-1} (APHA 1998). $SUVA_{254}$ gives information on the aromaticity of DOM, with values generally ranging between 1 and 9 L mg^{-1} m^{-1} (Weishaar et al. 2003). High values are related to high molecular weight of DOM, and low values to low aromatic and generally fresher DOM (Westerhoff and Anning 2000; Weishaar et al. 2003).

The fluorescence spectra were performed in a Shimadzu RF-5301PC spectrofluorometer (1 cm length silica quartz cuvette) in order to obtain excitation-emission matrices (EEM), which were used to determine the presence of different fluorophore groups (Coble, 1996). EEM scans were run over an emission range of 270-630 nm (1 nm increments) and an excitation range of 240-400 (10nm increments). A water blank (Milli-Q Millipore) EEM, recorded under the same conditions, was subtracted from each sample to eliminate Raman scattering. The area underneath the water Raman scan was calculated and used to normalize all sample intensities. Visual identification of fluorescent peaks by examination of Ex and Em spectra, was performed. Two dominant peaks, identified as humic-like peaks A and C following Coble (1996) were found, and their locations and intensities were compiled. Tryptophan-like peak T was determined as the intensity of fluorescence measured at 270 Ex / 360 Em according to the characterization proposed by Fellman et al. (2010). The ratio of Peak T: peak C intensities shows the relation between the protein-like and the humic-like fractions of the sample, Thus, higher values of this ratio indicate a higher relative proportion of fresh organic material in the sample (Baker et al. 2008). The Fluorescence Index (FI) was determined as the ratio of the emission intensities at 470nm/520nm for an excitation wavelength of 370nm (Jaffé et al. 2008). FI is an indicator of terrestrial (low FI) or microbial (high FI) origin of DOM, with values usually ranging between 1.2 and 2 (McKnight et al. 2001).

Hydrological setting and geographic data

Daily values of mean soil moisture (SM; mm), runoff coefficient (RC) and runoff (RT; m3 d-1) in the catchment were obtained from a site-specific dynamic model (Obrador et al. 2008) fed by basic daily climatic data obtained from the nearest (7 Km) meteorological station (Spanish Meteorological Institute). A summer period with no precipitation events was registered from 14th June to 13th September 2008. The beginning of the hydrological year differed between 2007 (12th October) and 2008 (14th September). The main precipitation events were recorded in November 2007 (90.93 L m^{-2} d^{-1}) and September 2008 (41.91 L m^{-2} d^{-1}). The mean run-off coefficient (RC = 17.53%), showed high values related to the flash nature of the washes within the basin.

Total area, mean altitude, mean slope, lithologies, soil types, land uses and heads of cattle (Fig. 1.1; Table 1.1) were determined for each subcatchment by Geographic

Information Systems (GIS) data layers obtained from the Menorca Government Spatial Data Infrastructure (IDE: http://ide.cime.es/menorca/).

Table 1.1 Morphological and land use characteristics of the subcatchments drained by the studied ephemeral washes

	Morphological features				Land uses				Geology	
Sub-catchment	**Total Area (km^2)**	**Mean slope (%)**	**Mean altitude (m a.s.l.)**	**Heads of cattle/ km^2**	**Natural Vegetation (%)**	**Farming Lands (%)**	**Irrigated Croplands (%)**	**Humid areas (%)**	**Eutric leptosoil (%)**	**Main lythology**
P1	25.2	5.6		13.7	64	31	3	0.1	21	Dolomites & marlstones
P2	6.5	6.5	69	0	54	44	0.1	0.1	38	Dolomites & limestones
P3	6.6	9.5	43	17.6	56	39	0.5	1.1	21	Turbidites, sands, limes, clays
P4	2.8	8.0	26	40.5	32	64	0.1	0.6	1	Turbidites
P5	7.1	7.1	46	22.7	49	46	2.7	0.6	24	Pelites, dolomites & limestones
P6	1.2	11.6	33	11.1	69	22	0	6.2	0	Turbidites
P7	6.5	7.4	27	32.25	37	57	0.6	2.5	16	Pelites,sands limes & clays

Data analysis

In order to identify different periods in DOM properties, we segmented the overall samples by chronological clustering, a non hierarchic clustering method enabling the detection of discontinuities along a time series (Legendre et al. 1985). We used the complementary of the Euclidean distance of the standardized values of quantitative (DOC, DON) and qualitative (FI, a_{440} and $SUVA_{254}$) DOM descriptors as the similarity matrix. Chronological clustering was performed with a connectedness value of 0.5 and the Monte-Carlo permutational test for deciding fusion of samples within a single group was assessed at a $p = 0.05$. A second permutational test on the differences between groups after they were established was also performed. The algorithm was run to allow a comparison of distant clusters, and, in so doing, assess the expected cyclical occurrence of groups on a yearly basis.

To analyze the influence of spatial and temporal variability on DOM properties, a permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was

performed on the Manhattan dissimilarity matrix taking the defined subcatchments and the seasonal clusters obtained previously as factors, and the DOM descriptors used for the chronological clustering as variables. Mann-Whitney post-hoc tests were used to determine differences between seasons for all the descriptors (Quinn and Keough 2002).

Non-metric multidimensional scaling (NMDS) was applied to the data set to ordinate the samples by DOM properties (DON, DOC, a_{440}, SUVA and FI). A second NMDS was performed separately on each seasonal cluster to check for spatial trends. Two matrices of environmental variables were constructed: a hydromorphological matrix (RT, Precipitation, Soil Moisture, Area, Mean Slope, Mean Altitude) and a landscape matrix (land uses, number of head cattle, geological units and soil types). Each group of variables was fitted to the ordination of the optical descriptors as linear vectors. The significance of the correlations was assessed through a Monte Carlo test (1.000 permutations).

The NMDS was generated by "MetaMDS" function, the environmental correlation with "envfit" function, and the PERMANOVA with "adonis" function, all of them in the VEGAN package (Oksanen et al. 2011) for R software (R Development Core Team 2011). Mann Whitney test and paired Spearman Rank Correlations between variables were determined in Statistica 6.0 Software.

Results

Seasonal pattern of DOM descriptors

The chronological clustering of DOM properties generated 3 groups of samples. The first group was between October and December 2007, the second one between January and April 2008 and the third one between September 2008 and December 2008. The last groups are separated by the summer drought delimiting the end of the 2007/2008 and the beginning of the 2008/2009 hydrological cycles. Post-hoc tests showed significant differences between the 1st and 2nd groups ($p < 0.05$) and 2nd and 3rd groups ($p < 0.01$), but not between 1st and 3rd groups ($p > 0.1$). Thus, 1st and 3rd groups, that included samples from September to December of both years 2007 and 2008, were of the same type concerning DOM properties, and differed from the second group, containing samples from January to April 2008. All further analysis in the present work used these clearly differentiated autumn (AU) and winter-spring (WS) periods.

The PERMANOVA analysis of DOM descriptors, with season and sub-catchment as factors, explained 62% of the total variance. Significant differences between seasons (37.9%, F=90.8, $p < 0.001$) and between subcatchments (18.6%, F=7.4, $p < 0.001$) were observed. The interaction between season and subcatchment was also significant (5.2%, F=2.1, $p < 0.05$).

Table 1. 2 Mean ±SD and (median) of the catchment flows DOM characteristics for all 16 sample dates

	Fluorescence Index		**SUVA ($LmgC^{-1}m^{-1}$)**		**a_{440} (m^{-1})**	
Subcathment	**AU**	**WS**	**AU**	**WS**	**AU**	**WS**
P1	1.53±0.05 (1.57)	1.59±0.02 (1.58)	3.81 ±0.89 (3.65)	3.03 ±0.39 (3.07)	5.82 ±3.81 (5.30)	1.65 ± 0.31 (1.61)
P2	1.52±0.05 (1.48)	1.64±0.04 (1.65)	4.23 ±0.46 (4.35)	1.97 ±0.46 (1.92)	10.42 ±4.73 (9.44)	1.87 ± 1.98 (1.27)
P3	1.54±0.02 (1.53)	1.67±0.05 (1.67)	4.13 ± 0.23 (4.31)	3.19 ± 0.26 (3.07)	9.77 ±1.24 (10.36)	5.33 ± 3.67 (3.92)
P4	1.54±0.03 (1.54)	1.64±0.04 (1.63)	4.22 ± 0.49 (4.04)	3.04 ± 0.38 (3.01)	11.20 ±4.12 (9.79)	5.44 ± 2.23 (5.41)
P5	1.55±0.02 (1.56)	1.63±0.02 (1.63)	4.01 ±0.46 (3.95)	2.49 ± 0.33 (2.53)	7.95 ±2.95 (7.02)	1.70 ± 0.68 (1.50)
P6	1.54±0.02 (1.55)	1.61±0.02 (1.61)	4.55 ±0.29 (4.55)	3.01 ± 0.51 (3.10)	13.86 ± 5.54 (12.90)	3.74 ± 2.60 (2.65)
P7	1.51±0.02 (1.51)	1.58±0.03 (1.58)	4.40 ±0.86 (4.19)	3.51 ± 0.30 (3.54)	13.65 ± 4.82 (12.90)	6.19 ± 2.06 (6.33)
All groups	1.53±0.03 (1.54)	1.62±0.04 (1.62)	4.18 ±0.62 (4.20)	2.89 ± 0.38 (2.88)	10.19 ± 4.64 (9.67)	3.70 ± 1.93 (3.24)
	**		**		**	
	DOC (ppm)		**DON (ppm)**		**DOC:DON**	
Subcathment	**AU**	**WS**	**AU**	**WS**	**AU**	**WS**
P1	9.83 ± 3.06 (9.54)	8.16 ± 1.14 (8.23)	2.63 ± 1.42 (2.15)	1.81 ± 0.37 (1.61)	4.14 ± 1.33 (4.4)	4.6 ± 0.67 (4.62)
P2	5.64 ± 4.67 (4.12)	2.74 ± 0.74 (2.55)	2.75 ± 0.73 (2.96)	5.03 ± 1.1 (4.68)	2.77 ± 2.31 (1.67)	
P3	14.86 ± 3.50 (14.35)	10.95 ± 5.61 (8.55)	5.64 ± 1.71 (5.98)	3.26 ± 2.05 (2.93)	2.48 ± 0.56 (2.76)	2.77 ± 1.08 (2.8)
P4	14.86 ± 2.87 (14.36)	11.43 ± 2.76 (12.02)	4.22 ± 1.26 (4.05)	2.80 ± 2.13 (2.21)	3.81 ± 1.46 (3.36)	3.92 ± 3.57 (2.66)
P5	12.33 ± 1.49 (12.66)	5.68 ± 1.66 (5.19)	3.25 ± 1.03 (3.16)	1.25 ± 0.48 (1.29)	4.08 ± 1.21 (3.87)	5.02 ± 1.76 (4.6)
P6	15.13 ± 2.50 (16.01)	8.45 ± 3.06 (8.24)	5.05 ± 1.45 (5.35)	2.26 ± 1.54 (1.94)	3.89 ± 1.67 (3.0)	4.75 ± 2.47 (3.93)
P7	18.00 ± 5.70 (16.42)	14.82 ± 2.71 (15.28)	4.70 ± 1.85 (4.69)	2.40 ±1.56 (1.89)	3.53 ± 0.88 (3.36)	10.7 ± 9.06 (8.84)
All groups	14.05 ± 4.29 (13.47)	9.30 ± 3.09 (8.80)	3.94 ± 1.70 (3.58)	2.36 ±1.27 (2.12)	3.88 ± 1.35 (3.71)	4.84 ±4.21 (3.88)
	**		**		n.s.	

DOC concentration ranged from 5.64 mg L^{-1} (during the WS period) to 18.0 mg L^{-1} (during AU) (Table 1.2; Fig. 1.2). DOC was strongly correlated with runoff ($r = 0.84$, $p < 0.01$). DON showed a similar temporal pattern as DOC, with higher values during AU than during WS (Fig. 1.2b). DOC:DON ratios did not show significant differences between seasonal clusters (Table 1.2), and they do not present any clear trend along the studied period. The obtained mean values ranged between 2.48 and 10.7 (Table 1.2). Fluorescence Index (FI) was higher in WS than in AU (Mann Whitney U=121, period, values increased up to 1.90, signalling a change in DOM sources from terrestrial to microbial-like. The SUVA254 indicator of aromaticity and humification was higher in AU than in WS (Fig. 1.2b; Table 1.2). The absorption coefficient a_{440} (descriptor of changes in CDOM quantity) showed the same seasonal pattern and was highly correlated with $SUVA_{254}$ ($r=0.91$; $p < 0.01$).

The EEMs descriptors presented different values for the AU and WS periods. Humic-like peaks A and C showed a shift between seasons. The peaks maxima positions were significantly red-shifted ($p < 0.01$) in the AU period (Fig. 1.3a to 1.3d). The opposite pattern was observed in peak T intensity, which increased during WS (Fig. 1.3e). The proportional increase on proteic material in WS samples is evidenced by the higher ratio between protein-like peak T and humic-like peak C (Fig. 1.3f).

Hydromorphological covariates and landscape influence on DOM properties

NMDS clearly separated the samples according to the previously defined AU and WS periods (Fig. 1.4). The first axis was related to DOC concentration and lability. AU samples were grouped in one of the axis extremes, linked to coloured DOM (high a_{440}) and high DOC concentrations. WS samples were mostly located in the opposite axis extreme (related with FI), depicting recently produced DOM. The high dispersion of WS samples along the second axis was associated to DON concentration. Several hydromorphological variables significantly fitted with the NMDS ordination (Fig. 1.4). WS samples were related to mean altitude, and AU samples were associated to precipitation, runoff and mean slope.

The interaction between season and subcatchment in the PERMANOVA analysis was significant. Accordingly, samples were clearly grouped by subcatchment in the NMDS ordination of WS samples (Fig. 1.5), but no clear clustering by subcatchment was observed in the NMDS ordination of AU samples (figure not shown). Thus, DOM differences between subcatchments were higher during WS than during AU. In the WS-NMDS, the

subcatchments P1 and P5 showed the lowest variability, appearing all the samples together and depicting low DOC levels. The landscape variables that were significantly ($p < 0.01$) correlated with NMDS are shown as arrows over the diagram (Fig. 1.5). Humid areas, limes junt. and clays and turbidite lithology were correlated with high DOC concentrations and colour, whereas farming centres were related to DON.

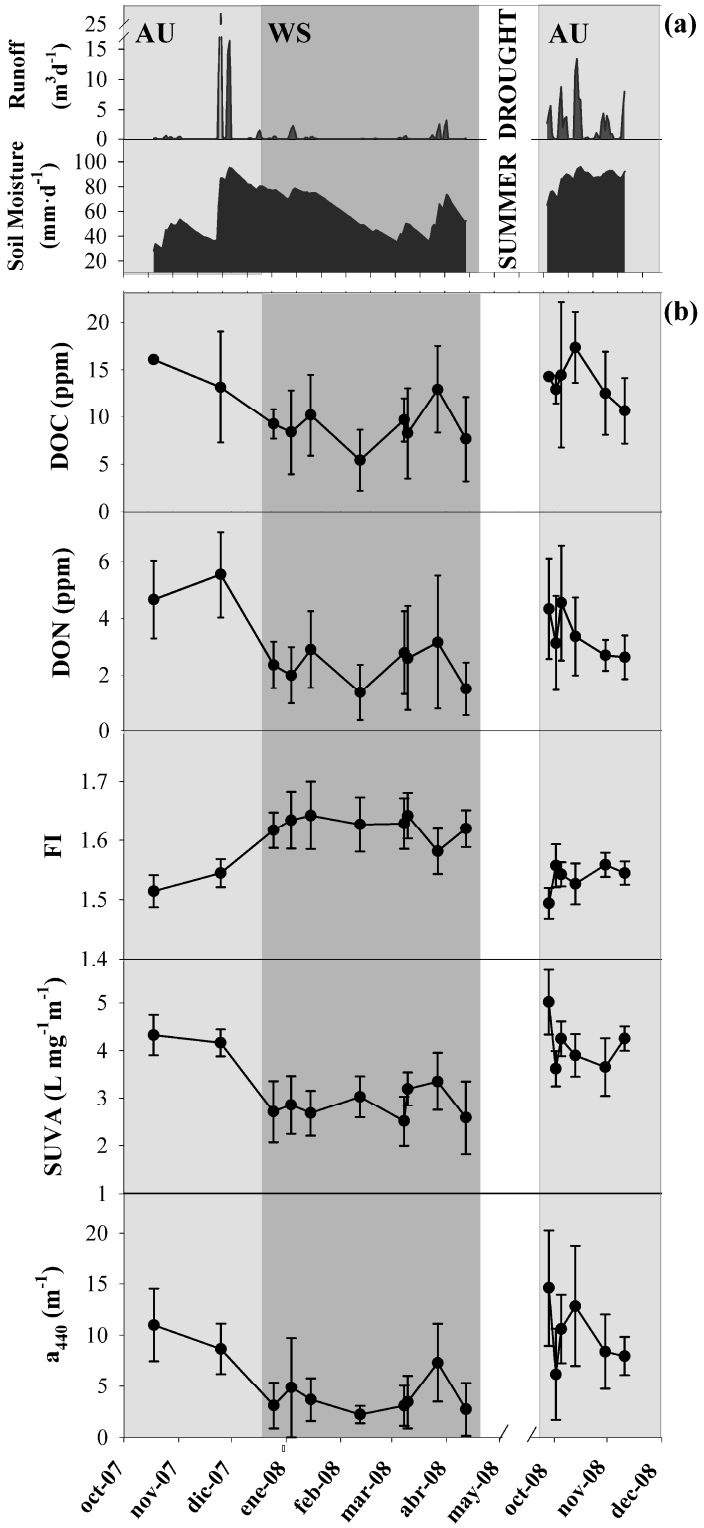

Figure 1.2. Temporal dynamics of a) hydrological parameters (runoff and precipitation), and b) DOM properties [DOC, DON, Fluorescence index (FI), SUVA and absorbance coefficient at 440nm (a_{440})]. The mean values for the 7 subcatchments are shown for each sampling date. Error bars indicate ± 1 SE

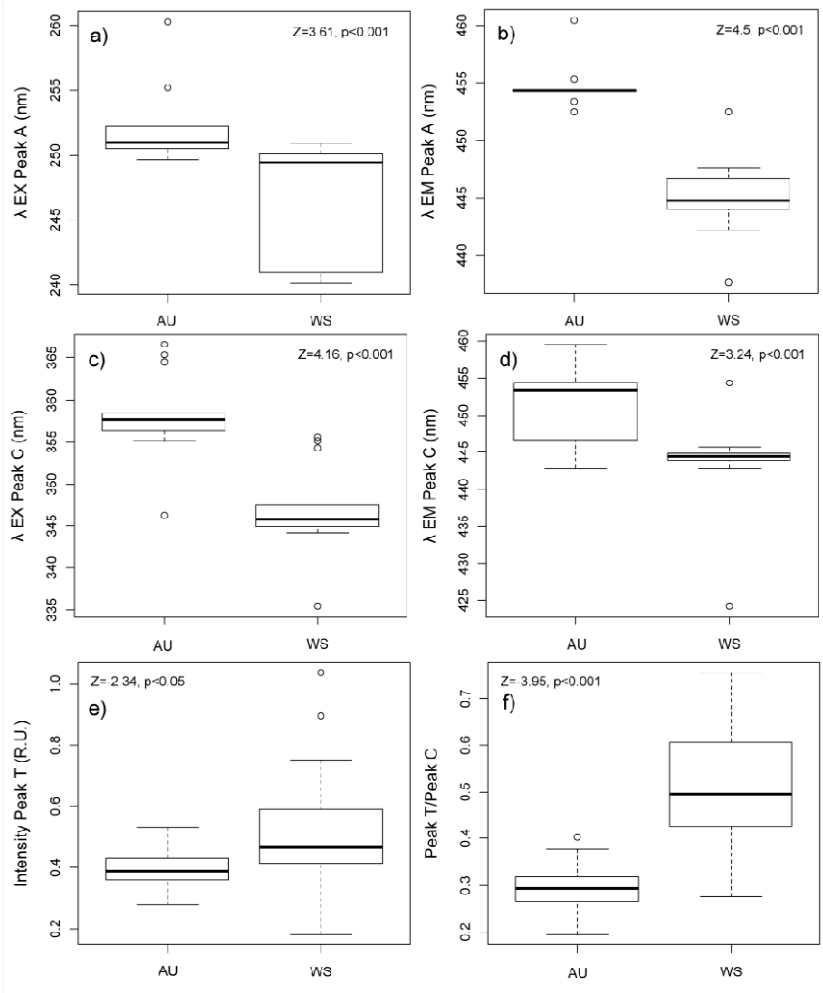

Figure 1.3 EEMs-derived descriptors in the AU and WS periods. a) Peak A excitation wavelength b) Peak A emission wavelength c) Peak C excitation wavelength d) Peak C emission wavelength e) Protein-like peak T intensity (Raman units) f) Peak T / Peak C intensity ratio. The median (centre horizontal line), the range (whiskers), the 25% and 75% percentiles (box) and the outliers (circles) are shown. Statistical results from Mann-Whitney tests are shown in panel.

Discussion

DOM properties during the AU period: the influence of summer drought and hydromorphology

The highest DOM concentrations were observed during the AU period, which includes the first water flows of the hydrological year. Increases in DOC and DON concentrations after storm events have been described in both intermittent and perennial streams (Bernal et al. 2005; Hood et al. 2006), and close relationships between runoff and DOM concentrations are common (Wetzel 2001; Mullholland 2003). Here, we observed a positive relationship between DOM concentrations and runoff (Fig. 1.4). At the temporal scale used

in this study, such relationship was enhanced by the accumulation of OM during summer drought from vegetation and crops and its posterior flushing with the onset of the first autumn rainy events.

Apart from runoff, morphological variables like subcatchment slope also influenced DOM concentration (Fig. 1.4). In ephemeral washes, the slope is considered one of the main factors determining the transfer of solutes downwards (Bull 1999; Mulholland 2003), because steep slopes favour water displacement and reduce the presence of retentive structures such as plant patches or topographic flats (Jacobson et al. 2000). Thus, steep slopes may present little capacity to absorb water solutes (Belnap et al. 2005), what would explain the relationship between DOC concentration and catchment slope observed in this study.

With regard to the DOM quality, the DOM during AU was characterised by terrestrially-derived material stored in soil surface during the drought period, as seen by several evidences. Firstly, AU samples showed $SUVA_{254}$ values above 4 L mg C^{-1} m^{-1} (Fig. 1.2). Such high values are typical of systems highly influenced by inputs from terrestrial vegetation (Weishaar et al. 2003). Increases in humic content and aromaticity during storm episodes have been described in perennial streams (Hood et al. 2006), but here we did not

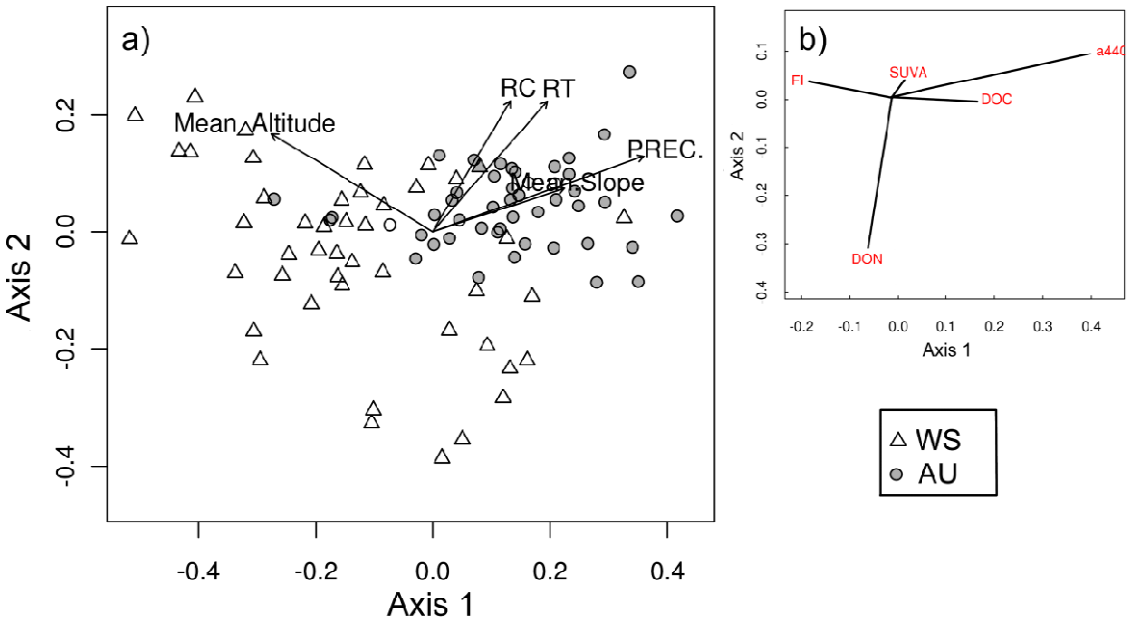

Figure 1.4. Two dimensional NMDS ordination of all samples based on DOM descriptors (grey circles: AU; white triangles: WS). In a) the arrows correspond to the hydromorphological variables (Mean altitude, RC=Runoff coefficient, RT= Total runoff, PREC = Precipitation) significantly related ($p < 0.01$) with the ordination. In b) is represented the ordination of the DOM descriptors (FI, SUVA, absorption coefficient at 440nm, DOC and DON)

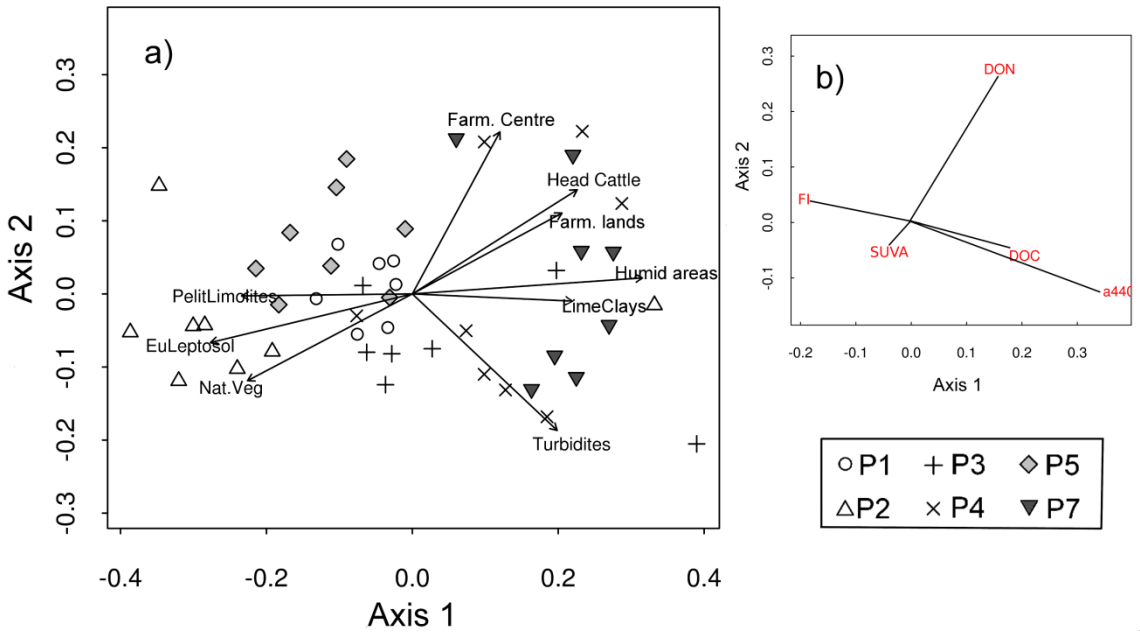

Figure 1.5. Two dimensional NMDS ordination plot of WS samples in the 7 subcatchments. In a) the arrows are the morphological and landscape variables (Pelites-limolites, Turbidites, Limes and clays, Eutric leptosol, Natural Vegetation, Humid areas, Farming lands, Farming centres and Heads of cattle/ha) significantly related ($p<0.01$) with the ordination. In b) is represented the ordination of the DOM descriptors (FI, SUVA, absorption coefficient at 440nm, DOC and DON)

observe any relationship between $SUVA_{254}$ and precipitation or runoff. Thus, the high $SUVA_{254}$ values in AU are related to the accumulation of vegetation and agricultural debris during the summer drought period. Accordingly, we observed very high CDOM concentrations (described by a_{440}) in AU samples (Fig. 1.2). Colour is related to aromaticity and to the presence of large and complex compounds (Helms et al. 2008). Hence, such extremely high a_{440} values highlight the relevance of terrestrial inputs in AU samples. Secondly, EEMs of AU samples showed a dominance of peaks A and C, typically related to terrestrial sources of DOM (Hudson et al. 2007; Fellman et al. 2010). Thirdly, we observed a strong red-shift of Ex/Em wavelengths in AU samples. Several authors have related red-shifts in peak-maxima location to changes in the degree of humification and to the presence of highly aromatic vegetation-derived compounds (Senesi et al. 1991; Kalbitz et al. 1999). Finally, we observed lower FI values in AU than in WS samples. The range of values found for FI during the whole study period (1.44 - 1.77) depicts the influence of both terrestrial and microbial sources. These values are in the upper range of values found for temperate streams, but they fall within the range found by Vázquez et al. (2010) in Mediterranean intermittent streams, and by Westerhoff and Anning (2000) in arid streams. The low FI values in AU indicate an increase of terrestrial sources in that period (McKnight et al. 2001).

The low protein-like peak fluorescence, typically related to microbial sources and bioavailable DOM (Fellman et al. 2010), together with low peak T : peak C ratios also suggests a minor contribution of microbial and algal processes during AU (Baker et al. 2008; Hood et al. 2006).

Effects of seasonality and landscape interaction on DOM properties in ephemeral washes

Both seasonality and landscape variables influenced the concentration and the chemical properties of DOM in the studied ephemeral flows. Seasonality was the main driver of these changes (37.8% of total variability in the PERMANOVA analysis), determining two periods in the DOM properties, one strongly related to hydromorphology (AU) and the other one mostly driven by landscape factors (WS), as seen above.

In AU, the recent summer drought and the hydromorphological variables such as precipitation, runoff or catchment slope are the main drivers of the variability in DOM properties. As they exert a similar influence over the whole catchment, these factors tend to homogenize DOM characteristics within the catchment. Due to the nature of ephemeral washes, that homogeneity is not likely to be related to a higher hydrological connectivity during autumn, as in those washes there is only runoff during precipitation episodes.

Nonetheless, the interaction between season and subcatchment factors in PERMANOVA was also significant (5.2% explained variability), showing that the hydromorphological variables were not the only drivers of DOM properties, and that their influence varied along the hydrological cycle. When that influence diminished and the subcatchment landscape characteristics became relevant, differences in DOM properties between subcatchments become plausible, as observed during WS period. Our results agree with previous findings highlighting the increase of landscape influence on both DOM concentrations and properties as stream order decreases (Fellman et al. 2009; Dawson et al. 2011). As ephemeral washes are the lowest order flows (Belnap et al. 2005) they may be great candidates to the study of the relationships between DOM properties and landscape structure.

CONCLUSIONS

Two periods were clearly differentiated from the DOM chemical properties in the studied ephemeral washes. In autumn, DOM was spatially uniform and characterized by a marked aromatic nature. During the winter-spring period, a microbially-like DOM dominated and spatial differences in DOM quality became evident between subcatchments.

The seasonal variability was related to the hydromorphological and landscape properties of the catchment, but their influence over DOM concentrations and quality changed along the hydrological year. In autumn, hydromorphology was the most important factor, whereas in winter-spring land cover and soil typology highly determined spatial differences in DOM properties.

Further research on DOM dynamics in ephemeral washes is needed, since they might be extremely informative when studying the biogeochemical processes affecting DOM properties in variable hydrological regimes as those found in the Mediterranean region.

ACKNOWLEDGEMENTS:

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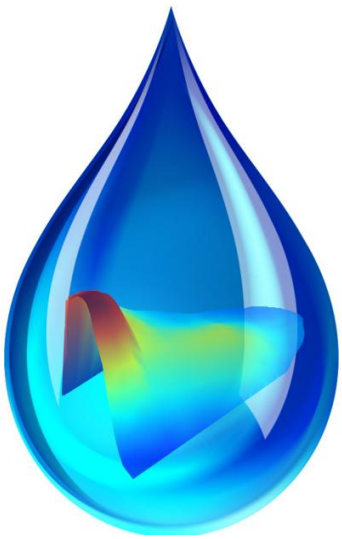
Chapter 2



Higher reactivity of allochthonous vs. autochthonous DOC sources in a shallow lake

Núria Catalán, Biel Obrador, Marisol Felip, Joan Ll. Pretus

Aquatic sciences (in press.)

Abstract

Dissolved organic carbon (DOC) reactivity in aquatic systems is essentially dependent on DOC precursor material and on the processes regulating its bioavailability, especially photodegradation and microbial activity. We investigated temporal changes (from hours to weeks) in the reactivity of allochthonous and autochthonous DOC sources in a macrophyte - dominated shallow lake using a set of incubation experiments. Changes in DOC fluorescence and absorbance properties due to biodegradation (BD) and to the combined effect of photo- and biodegradation (UV+BD) were traced.

Allochthonous DOC (AlloDOC) was more reactive than autochthonous DOC (AutoDOC), showing higher DOC losses (between 22 and 36%) and faster changes in DOC properties than AutoDOC. The effect of UV+BD was larger than BD alone for both sources of DOC. The rates of change of DOC properties were stronger during the first days of incubation and showed no regular pattern for any of the treatments or DOC sources. Our findings highlight the relevance of the timescale when assessing changes in DOC quality under different degradation pathways, as well as the need of discussing the labile character usually attributed to autochthonous DOC in systems dominated by submerged vegetation, as many shallow lakes or lagoons.

Resum (en català)

La reactivitat de la matèria orgànica dissolta (DOM) en sistemes aquàtics és essencialment dependent en el material precursor de la DOM y en els processos regulant la seva biodisponibilitat, especialment la fotodegradació i l'activitat microbiana. Es van investigar els canvis temporals (des d'hores fins a setmanes) en la reactivitat de les fonts al·lòctones i autòctones de la DOM en una llacuna, mitjançant incubacions. Es van traçar els canvis en les propietats espectroscòpiques de la DOM degut a la biodegradació (BD) i a l'efecte combinat de la foto i la biodegradació (UV+BD).

La DOM al·lòctona era més reactiva que l'autòctona, mostrant pèrdues de DOC més importants (entre el 22 i el 36%) i canvis més ràpids en les propietats de la DOM que l'autòctona. L'efecte del tractament UV+BD va ser més marcat que el del BD per ambdues fonts de DOM. Les taxes de canvi de les propietats espectroscòpiques destaquen la rellevància de l'escala temporal a l'hora d'avaluar els canvis en la qualitat de la DOM durant diferents vies de degradació, així com la necessitat de qüestionar el caràcter làbil generalment atribuït a la DOM autòctona en sistemes dominats per la vegetació submergida, com molts llacs o llacunes somes.

Resumen (en castellano)

La reactividad de la materia orgánica disuelta (DOM) en sistemas acuáticos depende, esencialmente, en el material precursor de la DOM y en los procesos regulando su biodisponibilidad, especialmente la fotodegradación y la actividad microbiana. Se investigaron los cambios temporales (desde horas hasta semanas) en la reactividad de las fuentes alóctonas y autóctonas de la DOM en una laguna, mediante incubaciones. Se trazaron los cambios en las propiedades espectroscópicas de la DOM debido a la biodegradación (BD) y al efecto combinado de la foto y la biodegradación (UV+BD).

La DOM alóctona mostró un carácter más reactivo que la autóctona, mostrando pérdidas de DOC más importantes (entre el 22 y el 36%) y cambios más rápidos en las propiedades de la DOM que la autóctona. El efecto del tratamiento UV+BD fue más marcado que el del BD para ambas fuentes de DOM. Las tasas de cambio de las propiedades espectroscópicas destacan la relevancia de la escala temporal a la hora de evaluar los cambios en la calidad de la DOM durante diferentes vías de degradación, así como la necesidad de cuestionar el carácter lábil generalmente atribuido a la DOM autóctona en sistemas dominados por la vegetación sumergida, como muchos lagos o lagunas someras.

Introduction

Inland waters play an important role on the global carbon cycle because they maintain several processes involved in the mineralization of dissolved organic carbon (DOC) into CO_2 (Battin et al. 2009, Tranvik et al. 2009). Among these mineralization processes are photoreactions and biodegradation, both of which would depend on DOC properties (Wetzel et al. 1995, Bertilsson and Tranvik 2000). Most studies agree that photoreactions transform DOC into smaller molecules (Wetzel et al. 1995, Osburn et al. 2001, Helms et al. 2008), whereas biodegradation would have the opposite effect (Helms et al. 2008). Nonetheless, the effects of these processes on DOC quality are still an issue under discussion. Mass spectrometry (Gonsior et al. 2009, Stubbins et al. 2010 for details) confirmed the breaking of aromatic molecules when DOC is exposed to UV radiation, and also showed the existence of compounds resistant to photodegradation and even the production of "recalcitrant" compounds produced during those reactions (Stubbins et al. 2010).

Both reductions (Nieto-Cid et al. 2006, Pérez and Sommaruga 2007) and increases in bioavailability (Moran et al. 2000, Vähätalo and Wetzel 2008) have been reported as a result of DOC photodegradation. However, there is increasing evidence that changes in DOC bioavailability after UV exposure highly depend on the original source of DOC (Tranvik and Bertilsson 2001). Therefore, initially more labile DOC sources such as algal exudates become less available for biodegradation after photodegradation, whereas more recalcitrant materials (i.e. humic-rich waters) increase in bioavailability when exposed to UV (Anesio et al. 2000, Ziegler and Brisco 2004, Abboudi et al. 2008, Fasching and Battin 2012).

The main sources of DOC in aquatic ecosystems are allochthonous inputs draining from the catchment, together with autochthonous DOC production from autotrophic and heterotrophic in-situ activities (Kritzberg et al. 2004, Guillemette and del Giorgio 2012). Allochthonous DOC is generally considered to have a more recalcitrant character than autochthonous DOC because the former is typically derived from vegetation and soil organic matter and the latter mainly from phyto- and bacterioplankton activities (Tranvik 1992, Jaffé et al. 2008). This is the background of the vast majority of studies dealing with DOC temporal changes in inland waters (Guillemette and del Giorgio 2012, Cory and Kaplan 2012, Kothawala et al. 2012). However, in aquatic ecosystems dominated by

submerged vascular plants (i.e. macrophytes), such as many shallow lakes and lagoons, this scheme can be substantially different because macrophyte biomass can be an extremely important source of DOC (deMarty and Prairie 2009, Obrador and Pretus 2012). Despite being produced in-situ, the DOC derived from macrophytes would be more similar, in terms of molecular composition and presumably of reactivity, to DOC from terrestrial vascular plants than to autochthonous DOC derived from phytoplankton.

Advances in fluorescence spectroscopy allow the identification of changes in fluorophores due to photoreactions and subsequent biodegradation (Moran et al. 2000; Stedmon and Markager 2005, Nieto-Cid et al. 2006, Fasching and Battin 2012). The analysis of the rate of change of DOC properties in different pools of DOC might help establish the fate of allochthonous inputs into receiving water bodies. This has been recently shown by Guillemette and del Giorgio (2012), who used fluorescence techniques to trace DOC changes due to biodegradation. Studies on photodegradation of DOC include experimental set-ups ranging from few hours (6hours: Ziegler and Brisco 2004) to multiple years (898days: Vähätalo and Wetzel, 2008). Although some of them asses time-course changes in DOM quality (Moran et al. 2000; Stedmon and Markager, 2005), to the best of our knowledge there are no studies analyzing the instantaneous rates of change in DOC properties due to photodegradation or to the combined effect of photo - and biodegradation at different time scales.

The definition of DOC reactivity implies a temporal dimension, as it is the rate at which it undergoes degradation. Currently, different approaches are being used in order to model DOC reactivity due to bio- (Guillemette and del Giorgio 2011, Koehler et al. 2012) or photodegradations (del Vecchio and Blough 2002, Benner and Kaiser 2011). The commonly used exponential decay model allows obtaining a rate coefficient, k, which is used as a descriptor of the decomposition of bulk DOC (Westrich and Berner 1984) or of the colored DOC fraction (CDOM, Benner and Kaiser 2011). However, DOC mineralization rates are far from being constant in time and this has to be acknowledged if DOC reactivity is to be modeled. This has been done by assuming discrete temporal stages in DOC degradation corresponding initially to more labile, and afterwards to more recalcitrant DOC fractions (Guillemette and del Giorgio, 2011), or by fitting the reactivity continuum model (Boudreau and Ruddick 1991) to DOC mineralization data, demonstrating a decrease in k with time (Koehler et al. 2012).

Here we evaluated the timescale of DOC changes due to photo- and biodegradation in a shallow macrophytic lake. These two processes were treated as one mechanism of mineralization of DOC sources, as long as they occur simultaneously in nature (Whitehead et al. 2000). We evaluated differences in the reactivity of autochthonous and allochthonous DOC pools by tracking instantaneous rates of change in DOC optical properties during laboratory incubations. The water from a lagoon with 0.7 years water residence time and an important macrophytic production (Obrador and Pretus 2010) was considered to be representative of autochthonous source of DOC, whereas ephemeral washes of terrestrial origin were taken as the allochthonous source of DOC. We hypothesized that each DOC source may present different reactivity, with allochthonous DOC being likely to become more similar to the lagoon DOC with increasing time. Changes in DOC properties due to photo- and biodegradation were expected to be non-linear and depend on the source of DOC and the amount of irradiation received by the sample. Instantaneous rates of change in DOC properties are discussed as a tool to assess reactivity dynamics of different DOC sources in aquatic ecosystems.

Material and methods

Water Sampling

Water samples were collected in a Mediterranean catchment (Albufera des Grau catchment 39º57'N, 4º 15'E, Menorca, Western Mediterranean). The Albufera des Grau is a 78 ha, shallow (average depth 1.37 m) enclosed coastal lagoon dominated by submerged vegetation (Ruppia cirrhosa). DOC concentration typically ranges between 6 and 16 mg C L^{-1} (Obrador and Pretus 2012). The catchment surrounding the lagoon is dominated by mixed Mediterranean forests of Quercus ilex, Pinus halepensis and Olea europaea var. sylvestris (47 %), extensive dry farming land (41 %), and shrublands (9%), and drains into the lagoon by ephemeral washes which only occur during precipitation episodes (Catalán et al. 2013). Samples were collected in January 2011; during this month, mean air temperature was 10.2 ºC and water temperature was 12.9 ºC. We sampled during winter to avoid the strong influence of the first autumn rains in the DOC of ephemeral washes (Catalán et al. 2013). Twenty-five litters of water were collected from the centre of the lagoon (as it has been found to be representative of the entire system; Obrador 2009) as autochthonous DOC source and 25 L from the ephemeral washes as allochthonous DOC

source. The experiment was started within 24 hours of collection, during which time samples were stored in the dark at 4 ºC.

Experimental setup

Water samples were filtered through 1.2 μm pre-combusted filters (47mm diameter, Whatman GF/C) to remove larger particles and then through 0.2 μm filters (Supor Membrane, Pall Corporation) to remove all microbial picoplankton. A portion of 1.2 μm filtered water from the lagoon was reserved and used as bacterial inoculum.

To assess the effects of biodegradation (BD) and of combined photo- and biodegradation (UV+BD) processes on allochthonous and autochthonous sources of DOC, 60 ml quartz vials were filled with 0.2 μm filtered lagoon water (from now on referred to as AutoDOC) and 0.2 μm filtered ephemeral washes water (from now on AlloDOC). In each vial, bacterial inoculum was added in a 1:10 v/v proportion.

All the vials were covered with quartz plates transparent to radiation, avoiding contamination but allowing gas exchange. Samples were incubated at 18ºC in a temperature controlled chamber over 28 days (Experimental Field Services of the University of Barcelona). Six replicates were prepared for each experimental condition and different subsets of vials were sampled at each experimental time (0, 21, 48, 72, 168, 336 and 672 hours) and the other half of the vials were incubated in the dark. The other half were exposed to artificial light (Philips Actinic BL 36W) with emission maximum in the UV-A band. The intensity received by the samples was continuously measured by a radiometer (HD2102 DeltaOHM) equipped with broad-band sensors and corresponded to: UV-B (0.03 W m^{-2}), UV-A (7.1 W m^{-2}) and very low photosynthetically available radiation (4 W m^{-2}). The UV-A radiation was in the range of natural irradiance measured in the field with the same radiometer during the water sampling campaign (7.83 W m^{-2}), and the UV-B represented about 10% of the natural UV-B radiation (0.4 W m^{-2}). That value corresponded to the moment of the year with lower radiation in the shallow lake (i.e.- January). The mean annual radiation in the lagoon is 14.10 W m^{-2}, so the radiation emitted by the lamps did not exceed natural conditions throughout the year (Spanish Meteorology Agency: http://www.aemet.es).

Table 2.1 Summary and description of the spectroscopic properties used in this study. Abbreviations are Abs= for absorbance spectra derived parameters, Fluo= for fluorescence spectra derived parameters, EEM when parameters are from Excitation-Emission Fluorescence matrices.

Parameter	Abs or Fluo	Description	Interpretation
Specific ultra-violet absorbance at 254 nm ($SUVA_{254}$; L mg^{-1} m^{-1})	Abs	Ratio of the absorbance coefficient at 254nm and the DOC concentration in mg L^{-1} (Weishaar et al. 2003)	Informs on the aromaticity of DOM, with values generally ranging between 1 and 6 L mg^{-1} m^{-1} Weishaar et al. 2003)
A_{350} (m^{-1})	Abs	Absorption coefficient at 350nm (Bricaud et al. 1981)	Indicator of chromophoric dissolved organic matter (CDOM) concentration (Bricaud et al. 1981)
S_R	Abs	Slope ratio of $S_{275-295}$ to $S_{350-400}$ (Helms et al.2008)	Inversely correlated to molecular weight and described to increase upon irradiation (Helms et al.2008)
Fluorescence Index (FI)	Fluo	Ratio of the emission intensities at 470/520 nm for an excitation of 370 nm (Cory and McKnight 2005)	Indicator of terrestrial-plant derived (low FI ~ 1.2) or microbial-algal derived (high FI ~1.4) origin (Jaffé et al. 2008, Fellman et al. 2010)
Humification Index (HIX)	Fluo	Peak area under the emission spectra 435–480 nm divided by 300–345 nm, at an excitation of 254 nm. (Zsolnay 1999)	Higher values correspond to a higher degree of humification (Huguet et al. 2009, Fellman et al. 2010)
Biological Index (BIX)	Fluo	Ratio of the emission intensities at 380/430nm for an excitation of 310 nm (Huguet et al. 2009)	Indicator of recent biological activity (Huguet et al. 2009) or recently produced DOM (Wilson and Xenopoulos 2009)
Peak A (or α')	Fluo-EEM	250Ex – 450 Em (Coble, 1996; Huguet et al. 2009)	Humic substances and recent materials (Fellman et al. 2010 UVA-humic like; Stedmon and Markager 2005 Comp.1)
Peak C (or α)	Fluo-EEM	350Ex – 450 Em (Coble 1996, Huguet et al. 2009)	Humic substances from terrestrial sources (Fellman et al. 2010 UVC-humic like; Stedmon and Markager 2005 Comp.5)
Peak M (or β)	Fluo-EEM	310Ex – 400 Em (Coble 1996, Huguet et al. 2009)	Autochthonous production, low molecular weight (Fellman et al. 2010 UVA-humic like; Stedmon and Markager 2005 Comp.3)
Peak T (or δ)	Fluo-EEM	280 Ex – 330 Em (Coble 1996, Parlanti et al. 2000)	Protein-like material (resembling the aminoacid Tryptophan signal) (Fellman et al. 2010,Tryptophan-like; Stedmon and Markager 2005 Comp.4)
Peak B (or γ)	Fluo-EEM	270 Ex – 300 Em (Coble 1996, Parlanti et al. 2000)	Protein-like material (resembling the aminoacid Tryrosine signal) (Fellman et al. 2010 Tyrosine-like; Stedmon and Markager 2005 Comp.6)

DOC properties and bacterial measurements

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

UV–Vis absorbance spectra (200-800nm) were obtained in a Shimadzu UV-1700 spectrophotometer, using 1 cm quartz cuvette. The absorption coefficients at wavelength k (a_λ, m-1) were determined from the absorbance measurement (A_λ) using the expression: $a_\lambda = 2.303\ A_\lambda/l$, where l is the path length in meters (Bricaud et al. 1981). Fluorescence spectra were determined using a Shimadzu RF-5301PC spectrofluorometer with a 1cm length silica quartz cuvette to obtain excitation–emission matrices (EEM). EEM scans were run at 10 nm excitation increments between 240-400 nm, and at 1 nm emission increments between 270-630 nm. Correction factors were applied to correct excitation and emission intensities for instrument- specific biases. A water blank (Milli-Q Millipore) EEM recorded under the same conditions was subtracted from each sample to eliminate Raman scattering; the area underneath the water Raman scan was calculated and used to normalize all sample intensities. Separate UV-vis absorbance spectra were used to correct for inner-filter effects (McKnight et al. 2001). These corrections were applied using the FDOMcorrect toolbox for MATLAB (Mathworks, Natick, MA, USA) following Murphy et al. (2010). The fluorescence intensities of the main fluorescent peaks associated with DOM (A, C, M, T, B; Coble, 1996; Parlanti, 2000) were measured. We discarded the application of parallel factor analysis (PARAFAC; Stedmon et al. 2003) because of the small size of the data set, which impeded the validation of the model. We also calculated several spectral indexes, including the fluorescence index (FI), humification index (HIX), Biological index (BIX), spectral slope (S_R) and the specific ultra-violet absorbance ($SUVA_{254}$). A summary of how these indexes were calculated, the information they provide about DOC characteristics and references to their origin are summarized in Table 2.1.

Bacterial abundances (BA) were determined by epifluorescence microscopy using 4,6-diamidino-2 phenylindole (DAPI) staining on 0.2 μm polycarbonate filters following Porter and Feig (1980). At least 350 cells in 20 random fields were counted for each sample. Rates

of bacterial production (BP) were estimated from the uptake of 3H-leucine following the centrifugation method of Smith and Azam (1992). Briefly, based on a previous study 3H-leucine was added to 1.2 mL water samples to reach a final concentration of 40 nmol L^{-1} of L-[4,5-^{3}H]leucine (J. Ruscalleda, pers. com.). For each sample vial three replicate tubes plus two killed controls were incubated for 1h at ambient temperature. The cell conversion factor was empirically determined and the obtained value was $2.8 \cdot 10^{18}$ cells mol Leu^{-1}. To transform the number of produced cells per liter and per hour into the amount of C incorporated (g C L^{-1} h^{-1}), a mean value of 20 fg of C per cell was considered (Lee and Fuhrman, 1987). Bacterial growth efficiency (BGE) was obtained from the bacterial production measurements using the model proposed by del Giorgio and Cole (1998) BGE= (0.037+0.54·BP)/(1.8+BP). Cell-specific bacterial production (CE; pgC $cell^{-1}$ h^{-1}) was calculated by dividing BP by BA.

Data treatment

To track the timing of major changes in DOC properties, instantaneous rates of change were calculated for each time interval. The instantaneous rate of change allows tracking changes in DOC properties compared to change in time at precise time points. To test significant differences between sources and treatments, common parametric tests including the Student's t-test or analysis of variance (ANOVA) with subsequent Tukey honestly significant difference (HSD) test were performed. Principal component analysis (PCA) was applied on a correlation matrix of standardized data to ordinate the samples by DOM properties. Non-metric multidimensional scaling (NMDS) was applied over the same dataset in order to verify the PCA results. All statistical analyses were performed in R software version 2.15.0 (R Development Core team 2012).

RESULTS

Optical properties of the DOC sources and monthly-scale reactivity

Initial DOC concentrations were higher in autochthonous (AutoDOC) than in allochthonous (AlloDOC) samples ($F=87.63$; $p<0.001$; Table 2.2). The SUVA254, indicator of aromaticity (Table 2.1) was also higher in AutoDOC ($F=15.01$; $p<0.01$), as well as a350 ($F=215.16$; $p<0.001$). The fluorescence index (FI) was higher in AlloDOC than in AutoDOC ($F=12.34$; $p<0.01$). The humification index (HIX) showed the same pattern, while the

Biological index (BIX) depicting recent biological activity presented the opposite. Small differences between DOC sources were found for SR, an indicator of the DOC molecular weight (F=48.5; p<0.01), and for the initial fluorescence represented by each EEM peak (A, C, T, M, B), expressed as the proportional contribution to total fluorescence.

Table 2.2 Mean ± SD of the DOC characteristics for two DOC sources, autoDOC and alloDOC. Initial values and after 28 days of incubations are shown for BD and UV+BD treatments. Changes between initial and final time and their level of significance are reported for each descriptor (t-test; · p<0.05; * p<0.01; ** p>0.001; *n.s.: not significant*).

	Autochthonous DOM			Allochthonous DOM		
	Initial 0days	**BD 28days**	**UV+BD 28days**	**Initial 0 days**	**BD 28 days**	**UV+BD 28 days**
DOC	9.18 ± 0.7	9.01 ± 0.36	7.38 ± 0.99	5.28 ± 0.17	4.14 ± 0.12	3.39 ± 0.48
rate		*n.s*	*- 1.79 · /- 19%*		*- 1.14**/-22%*	*- 1.89**/-36%*
$SUVA_{254}$	3.65 ± 0.3	3.64 ± 0.24	2.53 ± 0.17	2.94 ± 0.1	3.09 ± 0.06	1.59 ± 0.13
rate		*n.s*	*-1.12**/-31%*		*0.15 ·/5%*	*- 1.35**/-46%*
S_R	0.93 ± 0.01	0.88 ± 0.05	1.18 ± 0.13	0.89 ± 0.01	0.83 ± 0.17	2.75 ± 0.44
rate		*n.s*	*0.25·/27%*		*n.s*	*1.86**/209%*
a350	16.5 ± 0.13	16.93 ± 1.91	4.26 ± 0.32	8.06 ± 0.1	6.53 ± 1.03	1.24 ± 0.89
rate		*n.s*	*-12.24**/-73%*		*-1.54·/-19%*	*-6.82**/-85%*
TotFluo (R.U.)	489 ± 10	538 ± 46	198 ± 31	359 ± 2	357 ± 14	91 ± 5
rate		*n.s*	*-291**/-60%*		*n.s*	*-267**/-74%*
FI	1.39 ± 0.02	1.41 ± 0.01	0.99 ± 0.06	1.45 ± 0.01	1.46 ± 0.01	1.04 ± 0.09
rate		*n.s*	*-0.4**/-29%*		*n.s*	*-0.4**/-28%*
HIX	8.93 ± 0.37	10.02 ± 0.19	2.78 ± 0.37	10.11 ± 0.05	10.79 ± 0.35	2.23 ± 0.26
rate		*1.08 ·/12%*	*-6.15**/-69%*		*0.68·/7%*	*-7.88**/-78%*
BIX	0.63 ± 0.01	0.63 ± 0.01	1.18 ± 0.07	0.59 ± 0.01	0.62 ± 0.01	1.27 ± 0.11
rate		*n.s*	*0.55**/87%*		*0.031·*	*0.68**/115%*
Peak A:TF (-10^{-3})	7.4 ± 0.09	7.41 ± 0.12	6.83 ± 0.12	7.32 ± 0.03	7.4 ± 0.02	6.48 ± 0.11
Rate		*n.s*	*-0.57**/-8%*		*0.09·/2%*	*-0.84**/-11.5%*
Peak C:TF (-10^{-3})	3.39 ± 0.02	3.43 ± 0.02	1.13 ± 0.14	3.53 ± 0.02	3.6 ± 0.00	1.21 ± 0.17
Rate		*n.s*	*-2.27**/-67%*		*0.07*/2%*	*-2.32**/-66%*
Peak B:TF (-10^{-3})	0.46 ± 0.09	0.38 ± 0.03	1.2 ± 0.11	0.41 ± 0.02	0.45 ± 0.04	2.14 ± 0.07
Rate		*n.s*	*0.74**/161%*		*n.s*	*1.73**/420%*
Peak T:TF(-10^{-3})	0.75 ± 0.16	0.60 ± 0.01	1.83 ± 0.2	0.6 ± 0.03	0.48 ± 0.01	2.11 ± 0.23
rate		*n.s*	*1.08*/144%*		*-0.11*/-18%*	*1.52**/253%*
Peak M:TF(-10^{-3})	3.16 ± 0.01	3.03 ± 0.02	5.21 ± 0.12	2.87 ± 0.02	2.95 ± 0.01	5.15 ± 0.07
rate		*-0.14·/-4%*	*2.05**/65%*		*0.07·/3%*	*2.27**/79%*

Overall changes in DOC properties after 28d of incubation were more extensive for AlloDOC (2-22% and 12-420% in BD and BD+UV treatments, respectively) than for AutoDOC (4-12% and 8-161% in BD and BD+UV, respectively) for both treatments (Table 2.2; one way ANOVA, F= 12.34; p<0.001). DOC concentration decreased during the incubations in both DOC sources; the proportion of initial DOC degraded was highly variable and ranged between 3 and 36%. This proportion was higher for AlloDOC than for

Auto DOC samples and the highest DOC losses were registered in the photo+biodegradation (UV+BD) treatment of the allochthonous source (Table 2.2).

The UV+BD treatment decreased the total fluorescence of the samples between 60% (AutoDOC) and 75% (AlloDOC) (Table 2.2). Also, the EEMs showed a disappearance of humic like peaks and a significantly stronger contribution of protein-like peaks B and T and the humic/microbial peak M. The biological index (BIX) increased, indicating a higher amount of recently produced materials and the fluorescence index (FI) decreased. The humification, aromaticity and molecular weight decreased as seen by lower HIX and $SUVA_{254}$ and higher S_R. The colored fraction of DOC also diminished in both treatments (lower a_{350}), but the change was higher in UV+BD samples than in BD samples.

In the BD treatment, AutoDOC only showed significant changes for two descriptors (HIX and Peak M significantly increased after 28d of incubation). In contrast, for AlloDOC, significant changes in most of the descriptors were registered, including an increase in humification and aromaticity (higher $SUVA_{254}$ and HIX) and a decrease in protein-like peak T.

The maximum bacterial growth efficiency (BGE) occurred 48h after the start of the incubations, and was higher in AutoDOC than in AlloDOC (Fig. 2.3a). However BGE increased faster in AlloDOC samples and values after the maxima were smaller for UV+BD treatments in both water samples. Accordingly, the maximum cell - specific bacterial production (CE) occurred after 24 h of incubation for the AlloDOC, whereas for AutoDOC CE values were higher after 48 h (Fig. 2.3a).

Reactivity changes: timing, linearity and quality changes

The dynamics of DOC properties differed between DOC sources and treatments (Fig. 2.1; Fig. 2.4). In some cases, linear dynamics were observed during the incubation period, as in FI and humic peaks C and M for the BD treatment (Fig. 2.1). Exponential dynamics were only observed in the UV+BD treatment for DOC, humic peaks C and M, a_{350}, FI, HIX and BIX. An irregular pattern (with both increases and decreases in time) was observed for

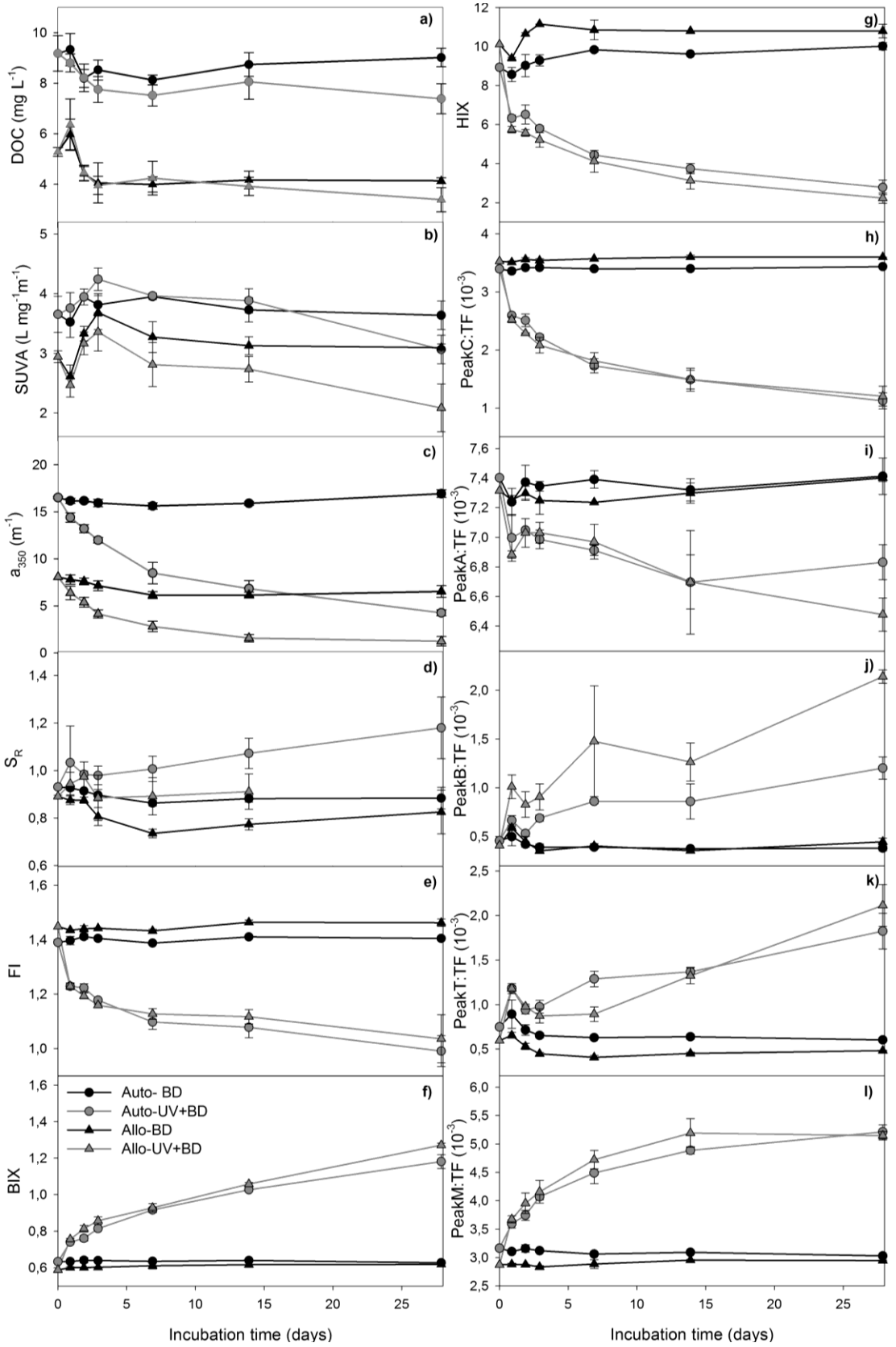


Figure 2.1 Dynamics of the qualitative DOC parameters summarized in Table 2.1 a) DOC concentration, b) Specific UV absorbance 254nm, c) Absorbance coefficient 350nm, d) S_R ratio of spectral slopes, e) FI fluorescence index, f) BIX biological index, g) HIX humification index, h) PeakC: total fluorescence, i) Peak A : Total Fluorescence, j) Peak B : Total fluorescence, k) Peak T: Total fluorescence, l) Peak M: Total fluorescence. Samples from AlloDOC (triangles) and AutoDOC (circles) sources and BD (black) and UV+BD (grey) treatments. Error bars indicate ±SD (N = 6)

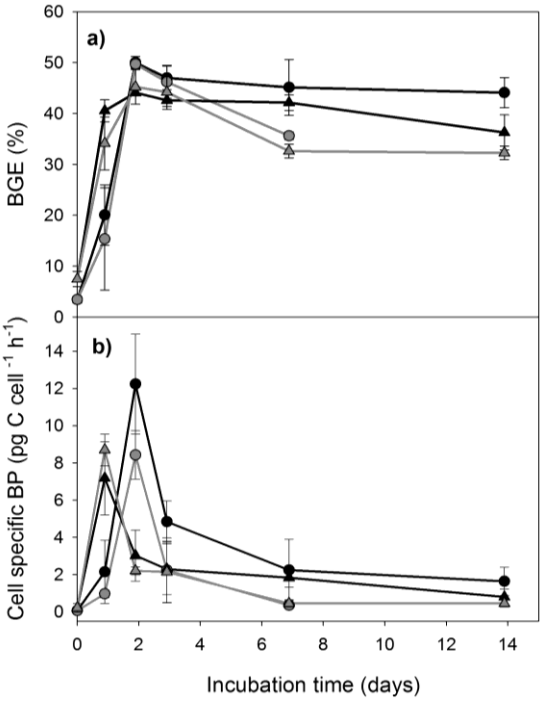


Figure 2.2 Dynamics of a) Cell-specific production (CE) and b) bacterial growth efficiency (BGE). Samples from Allo (triangles) and Auto (circles) sources and BD (black) and UV+BD (grey) treatments. Error bars indicate ±SD (N = 6)

$SUVA_{254}$, S_R, the protein-like peaks T and B and the humic-like peak A both in the BD and the UV+BD treatments, and for HIX in the BD treatment (Fig. 2.1; Fig. 2.4).

The fastest rates of change occurred during the first 72 h of incubation for DOC concentration and for several spectral indexes (Fig. 2.4). After 28 days, the rate of change for most spectral indexes was close to zero, but never reached null rates (Fig. 2.4). The fluorescence peaks T and B, the BIX and a_{350}, presented high instantaneous rates at the end of the 28d period, indicating that changes in these DOC properties continued beyond the duration of the study. The maximum instantaneous rates (either positive or negative) were observed in the UV+BD treatment for both DOC sources (Fig. 2.4), except for $SUVA_{254}$, a_{350} and S_R, which had similar rates in both treatments. Within the UV+BD treatment, AlloDOC presented the maximum rates of change for most spectral indexes.

Samples were grouped by treatment and source in a PCA summarizing the qualitative changes throughout the incubation (Fig. 2.5c). The UV+BD samples of both DOC sources were ordered along the first PCA axis, with initial and final incubation times at each of the axis extremes. The scores of the first two PCA axes (92% cumulated variance) were used as descriptors of global DOC character. The scores of the first axis increasingly differed between samples in the BD+UV treatment, whereas in the BD treatment the sources become

closer at 3 days of incubations (Fig. 2.5a). The second axis showed no divergence or convergence between samples in any of the treatments (Fig. 2.5b).

DISCUSSION

DOC properties of allochthonous and autochthonous DOC

The properties of DOC differed depending on its origin. In this study, the initial character of AutoDOC from the lagoon was more colored and aromatic than AlloDOC from the watershed (Table 2.2). This relationship between DOC sources is contrary to what is usually assumed, since autochthonous DOC is considered to be almost exclusively derived from microbial and phytoplanktonic activities, and allochthonous DOC from higher plant-derived materials (Tranvik, 1992; Kritzberg et al. 2004). The pattern found here is explained by the fact that the main origin of AutoDOC in the study system is likely to be macrophyte exudates rather than bacterial or phytoplanktonic activities. The lagoon is covered by extensive macrophyte meadows (Obrador and Pretus, 2010) that highly influence overall carbon cycling (Obrador and Pretus 2012) and DOC quality dynamics (Catalán et al. submitted). If the main sources of autochthonous DOC were microbial activities, a markedly labile character would have been found in AutoDOC samples (McKnight, 2001). But the lagoon DOC presented evidences of aromatic, humic-rich compounds, as might be expected in systems dominated by submerged macrophytes, where lignin is typically present in substantial amounts (Simon et al. 2002). Also, the torrential character of ephemeral washes draining the watershed has been shown to lead to seasonally variable DOC characteristics (Catalán et al. 2013) and thus the relationship between Allo- and AutoDOC properties can change during the year.

Overall changes of DOC properties as a function of DOC source

The direction in the change of DOC properties was similar in both treatments, although the maximum rates of change occurred in the photo+biodegradation (UV+BD) treatment (Table 2.2, Fig. 2.1 and 2.3).Our results agree with previous studies showing that exposure to radiation has a stronger effect on DOC properties than bacterial degradation alone (Moran et al. 2000). In the UV+BD treatment the aromaticity and molecular weight diminished in both AlloDOC and AutoDOC (Table 2.2, Fig. 2.1). The breaking of aromatic and large molecules has been extensively reported as the main effect of UV exposure on

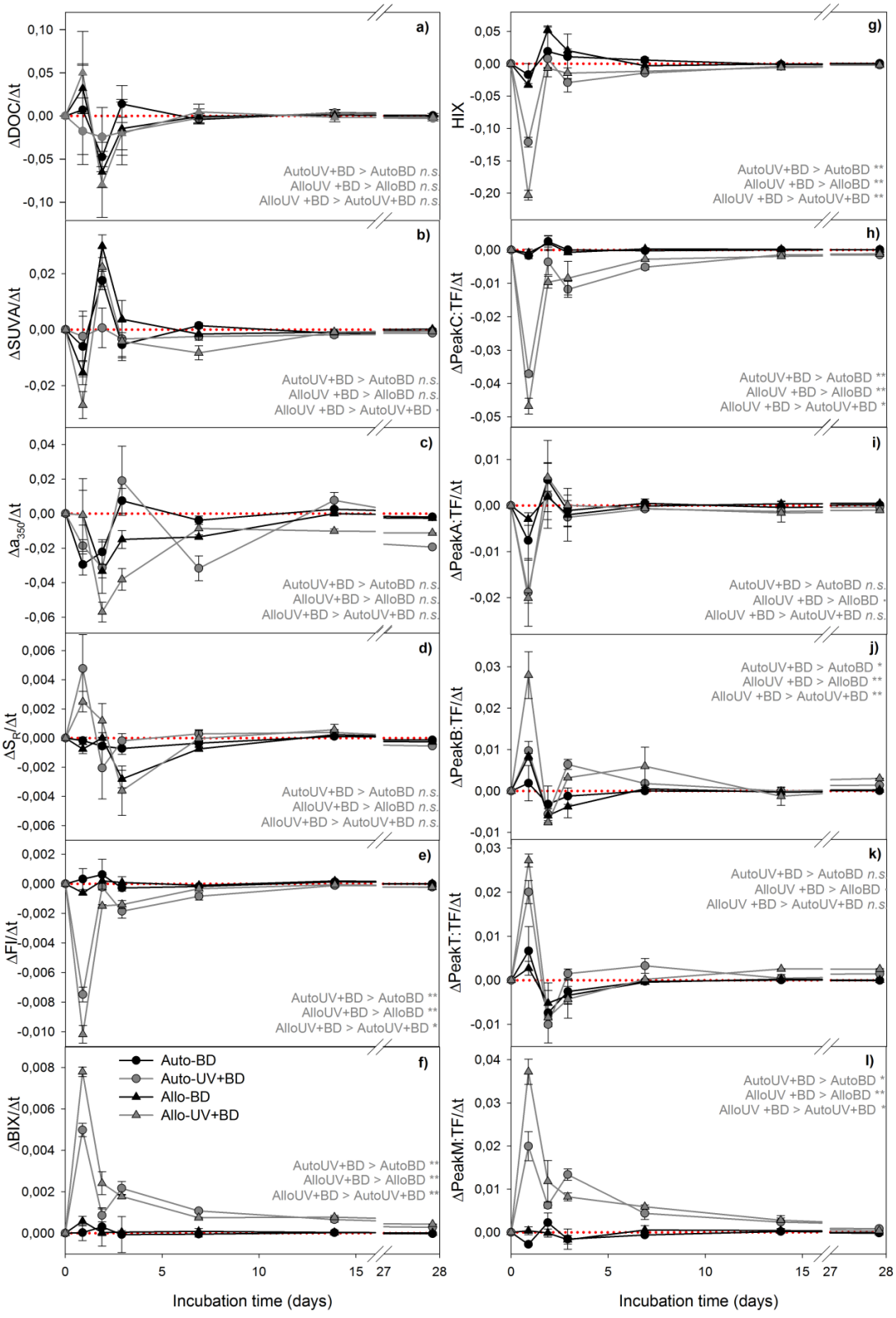


Figure 2.3 Instantaneous rates of change of the qualitative DOC parameters summarized in Table 2.1. a) DOC concentration, b) Specific UV absorbance 254nm, c) Absorbance coefficient 350nm, d) S_R ratio of spectral slopes, e) FI fluorescence index, f) BIX biological index, g) HIX humification index, h) PeakC: total fluorescence, i) Peak A : Total Fluorescence, j) Peak B : Total fluorescence, k) Peak T: Total fluorescence, l) Peak M: Total fluorescence. Samples from AlloDOC (triangles) and AutoDOC (circles) sources and BD (black) and UV+BD (grey) treatments. Error bars indicate ±SD (N = 6 for each sampling point). Significant differences between treatments maximum rates tested by Tukey HSD test are reported (· p<0.05; * p<0.01;** p>0.001; n.s.: not significant)

DOC (Wetzel et al. 1995; Bertilsson and Tranvik, 2000; Stubbins et al. 2010). The increase of EEM protein-like peaks B and T (expressed as proportional contribution to total fluorescence) could be either due to their production (Guillemette and del Giorgio, 2012) or due to the fact that these peaks are less affected by photodecay than humic-like regions of EEMs (Moran et al. 2000). The latter effect could be affecting especially the photodegradation of peak B in our samples, since the intensity of UV-B range during incubations was lower (10%) than the natural in situ radiation. Although the preservation of peak B could be magnified in our study, other works using higher UV-B intensities have reported a preferential preservation of the peaks in this region (Stedmon and Markager, 2005). Conversely, photobleaching might be affecting preferentially the region used for the calculation of fluorescence index (FI) (Moran et al. 2000; Birdwell and Engel, 2010), which would explain its decrease in the UV+BD treatment despite biologically-derived sources being likely to increase (Fig. 2.1f). Such an effect of the radiation over longer wavelengths could be even more marked if the artificial light covered the visible light part of the spectra, since it has been reported that peak C is more strongly photodegraded by the combined effect of visible +UV light than by UV light alone (Stedmon and Markager, 2005)

The AlloDOC presented higher reactivity and instantaneous rates of change compared to the AutoDOC, thus undergoing faster and more intense changes in DOC properties (Table 2.2, Fig. 2.3). Different reactivity of DOC in aquatic systems can be related with different residence times of DOC (Kothawala et al. 2012), so that less photoreactive DOC is assumed to have a longer history of exposure (Loiselle et al. 2012). In the study lagoon, AlloDOC from the watershed is very briefly exposed to degradation pathways before reaching the lagoon due to the flashy nature of the ephemeral washes (Bull, 1997). This torrential-character is likely to increase the uptake lengths of DOC in ephereral washes (Cory and Kaplan, 2012). On the contrary, AutoDOC has a longer history of exposure to degradation pathways and a higher concentration of recalcitrant compounds (e.g. lignin derived substances) due to its macrophytic origin, which may explain its lower reactivity.

The higher reactivity of AlloDOC may reflect a more labile DOC character than in AutoDOC. The stronger decrease in DOC concentration (Table 2.2) and the faster attainment of the cell specific bacterial production (CE) and bacterial growth efficiency (BGE) maximums in the AlloDOC relative to those of AutoDOC (Fig. 2.2) support this idea.

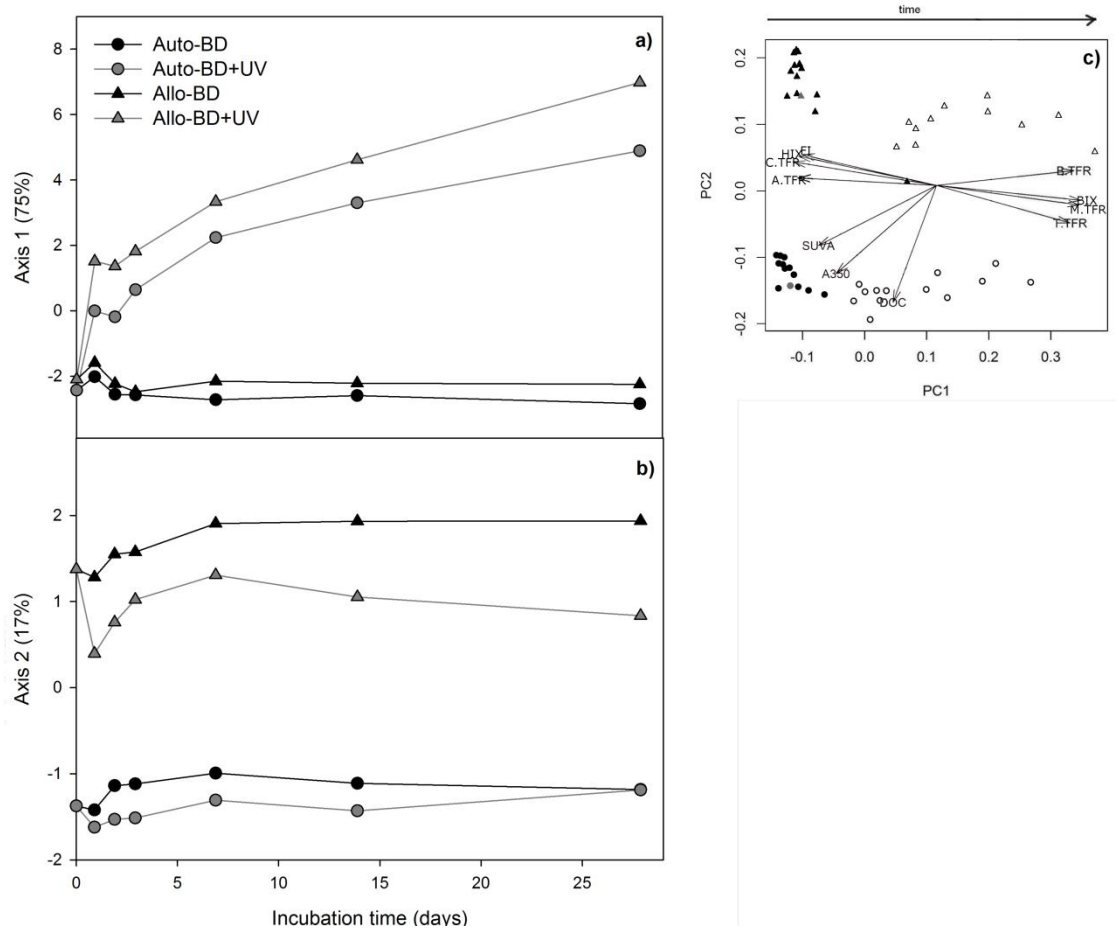


Figure 2.4 Scores of the a) first and b) second axis of the principal component analysis of all samples based on DOC descriptors : c). The percent of explained variation is shown in brackets. In c) the arrows represent the DOM properties (FI, SUVA254, absorption coefficient at 350 nm, DOC, BIX, HIX and EEM-derived peaks normalized by total fluorescence), full circles are Auto-BD Samples, full triangles Allo-BD Samples, empty circles Auto-UV+BD samples, empty triangles Allo-UV+BD samples and grey symbols are initial water samples.

This maximum was found for both treatments, dismissing any experimental bias due to overexposure to UV and showing that, even when biodegradation acted alone, AlloDOC was more reactive. In the long term, AutoDOC samples presented the maximum BGE values in both treatments and reach the maximum CE 24h after the AlloDOC, suggesting that after an initial breaking of the molecules, AutoDOC bioavailability might increase (Fig. 2.2). After the maxima, the longterm BGE and CE diminished in the UV+BD treatment of the two water samples. As we combined UV and biodegradation effects, the negative longterm response of bacterial activity in the UV+BD treatment could be due to the harmful effect of the short wavelength radiation on bacteria (Pérez and Sommarruga, 2007). UV exposure can also imply the production of reactive oxygen species that inhibit bacterial activity (Anesio et al. 2005). The stronger BGE observed in AutoDOC might thus be related

to a lower production of reactive oxygen species in that sample or to a lower harmful effect of UV on bacterial populations, due to the higher color of AutoDOC (Laurion et al. 2000).

In our study DOC exposure to UV did not result in opposite responses between DOC sources (i.e.-AutoDOC vs. AlloDOC). This result contrasts with other studies that found increased bacterial activity in allochthonous or humic-like DOC and decreased bacterial activity in autochthonous or labile DOC after UV exposure (Tranvik and Bertilsson, 2001; Abboudi et al. 2008).

Our initial hypothesis that AlloDOC would become more similar to AutoDOC with time must be rejected because the structural characteristics of these two DOC sources did not converge in any PCA axis for any treatment (Fig. 2.4a and b). We initially assumed that DOC from ephemeral washes (AlloDOC) was the main origin of lagoon carbon but these results suggest that the main DOC source of the lagoon would be derived from autochthonous primary production in accordance with previous studies based on seasonal changes in lagoonal carbon pools (Obrador and Pretus 2012). This result does not discard the potential of AlloDOC as an endmember of DOC sources in the lagoon since its character is distinguishable after being exposed to degradation processes. It must be noted that the incubation times used here covered the time scale of the main processes influencing the lagoon, from hours (i.e. torrential DOM arrival from ephemeral washes) to months (i.e.- processes based on the seasonal macrophyte cycle).

Despite not being the major DOC source, AlloDOC inputs can influence substantially DOC processing in the lagoon. Ephemeral washes represent an input of fresh DOC that can be rapidly degraded into smaller materials due to the combined action of photoreactions and the bacterial community of the receiving water. Both the resource schedule addition and the quality of DOC inputs can affect bacterial productivity (Lennon and Cottingham; 2008). In the system studied here, the torrential character of ephemeral washes makes that these inputs will occur as pulses of highly reactive DOC that will either be consumed preferentially over the AutoDOC (Lutz et al. 2012) or interact with the present DOC pool, in any case varying DOC processing in the receiving water body.

Rates of change of DOC properties

We showed that DOC properties presented different rates of change with time (Fig. 2.3). The data for the parameters DOC, a_{350} and the humic-like peak C could be fitted to a

first order decay model (Fig. 2.1), obtaining remarkably high k rate values (0.3 to 0.8 d^{-1}) in comparison with other studies (between $2 \cdot 10^{-5}$ and 0.2 d^{-1}; Guillemette and del Giorgio, 2011, Koehler et al. 2012). The other descriptors could not be reasonably fitted to an exponential curve (neither the EEM peaks corresponding to a natural FDOC pool nor the indexes that summarize DOC character). Our results show that qualitative changes in DOC during its degradation cannot be universally assumed to follow a regular decay pattern, and this is so whether if the degradation is driven by microorganisms or by UV radiation. These results partially agree with those found by Guillemette and del Giorgio (2012) for biodegradation, since they detected simultaneous consumption and production of DOC pools; although they considered linear variation patterns for those EEM peaks. Consequently, DOC reactivity cannot be properly described by means of a unique constant decay rate, mainly due to the fact that reactivity would decrease with time (Koehler et al. 2012). Also, the use of discrete pools of DOC, corresponding to theoretically labile and refractory compounds, have been proposed to translate the reactivity changes into reactivity DOC classes (Guillemette and del Giorgio, 2011). We recommend the use of instantaneous rates of change in DOC properties because they do not assume a constant decay rate and allow tracing variations in DOC character with time, without assuming arbitrary reactivity groups but rather reporting the behavior of natural fluorescent compounds (Coble, 1996; Fellman et al. 2010).

Instantaneous rates of change were higher during the first days of incubation and presented positive and negative values in all the descriptors (Fig. 2.3). This is an indication of generation and degradation of DOC molecules as a result of differential DOC reactivity. Microbial activity can result in simultaneous production and consumption of each DOC fluorescent pool (Stedmon and Markager, 2005; Guillemette and del Giorgio, 2012). For the BD treatment, this can be due to the selective consumption of the more labile compounds, meaning an increase in the aromatic character of the sample (Helms et al. 2008). However, our results show a similar behavior of EEM peaks, aromaticity (i.e. SUVA) and molecular weight (i.e. S_R) when biodegradation was acting together with photo-degradation. Stedmon et al. (2007) reported increases and decreases of a humic-terrestrial-like fluorescent component resulting from terrestrial DOC photodegradation. The simultaneous photodegradation and photoproduction of molecules with recalcitrant character has also been showed elsewhere (Gonsior et al. 2009, Stubbins et al. 2010) and has been suggested to be related with condensation reactions (Hedges et al. 2000). Thus the increases and

decreases detected in the different fluorescent pools as well as in aromaticity and molecular weight in the present study can be attributed to either the appearance of new photoproducts (Stubbins et al. 2010) or to transient species derived from initial photo-labile compounds (Loiselle et al. 2012).

CONCLUSIONS

Allochthonous and autochthonous DOC sources presented different reactivity rates, being faster for allochthonous DOC. This divergence in reactivity was attributed to a combination of distinct exposure to degradation pathways as biodegradation and photodecay, and to specific DOC properties of each source. The total loss of DOC was higher for AlloDOC in both treatments. AlloDOC reactivity was related to the presence of a rapidly available DOC, whereas a more aromatic character was attributed to the initial Autochthonous DOC due to its origin from submerged macrophytes in the studied lagoon. UV had a negative effect on bacterial metabolism, although DOC aromaticity and molecular weight diminished in both DOC sources.

Instantaneous rates showed that qualitative changes in DOC during its degradation cannot be universally assumed to follow a regular decay pattern, regardless of whether the degradation was driven by microorganisms alone or by the combination of UV radiation and microorganisms. This work highlights the relevance of characterizing the instantaneous changes in DOC quality when studying DOC reactivity processes.

ACKNOWLEDGEMENTS:

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Chapter 3

Priming effect in freshwater ecosystems: response of lake dissolved organic carbon to labile carbon additions

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(in prep.)

Abstract

Although the quantitative relevance of freshwater organic carbon (OC) processing is broadly accepted, the pathways of carbon (C) cycling in inland waters are still poorly understood. Identifying the factors constraining the mineralization of OC is crucial for a general understanding of the carbon cycle. One factor which has received large interest recently, is the priming effect that refers to small inputs of labile OC sources that trigger the degradation of previously unreactive OC. Although this phenomenon has been extensively studied in soils, convincing evidence in freshwaters has yet to be found.

We performed a multifactorial mesocosm experiment to test the conditions under which priming may be observed in the water column of freshwater ecosystems. We assessed the effect of pulse additions of three sources of labile OC on dissolved OC consumption using water from lakes with different trophic states to test a variety of OC quality. We also analyzed the effect of nutrient availability and the role of cell attachment to a surface on priming. Despite the broad range of conditions tested, no evidence of priming was found. Our results suggest that priming in lake water, as currently defined is unlikely to have a significant, quantitative effect on C cycling. We discuss why the water column is not the most suitable environment for priming to take place and we suggest some conditions under which priming is more likely to occur.

Resum (en català)

Malgrat la rellevància quantitativa del processat de carboni orgànic en sistemes aquàtics continentals és àmpliament acceptada, les vies de ciclat de carboni (C) en aquests sistemes són encara poc conegudes. La identificació dels factors que limiten la mineralització de carboni orgànic és crucial per a una comprensió general del cicle del C. Un dels factors que ha rebut gran interès recentment, és l'efecte *priming,* que fa referència al fenomen consistent en la degradació de C orgànic prèviament no reactiu en resposta a l'arribada de petites quantitats de fonts de C làbils. Tot i què aquest fenomen s'ha estudiat àmpliament en sòls, encara no s'han trobat proves convincents de la seva aparició en aigües continentals.

Per tal de testar les condicions en què el priming podria observar-se en sistemes d'aigua dolça, es va realitzar un experiment multifactorial en mesocosmos. Es va avaluar l'efecte de les addicions de tres fonts de C làbil sobre el consum de C orgànic dissolt (DOC) present en aigües de diferents llacs incloent diversos estats tròfics i qualitats de DOC. També es va analitzar l'efecte que la disponibilitat de nutrients i el paper de la unió de les cèl·lules a una superfície tenen sobre el *priming*. Tot i l'àmplia varietat de condicions testades, no es van trobar evidències de *priming*. Els nostres resultats suggereixen que l'efecte *priming* en sistemes lacustres tal com es defineix actualment és poc probable que tingui un efecte significatiu en el cicle del C. Discutim també els motius pels quals la columna d'aigua no és el mitjà més idoni per a l'aparició de priming, així com la possible rellevància d'altres fenòmens limitant la disponibilitat del C orgànic.

Resumen (en castellano)

Pese a que la relevancia cuantitativa del procesado de carbono (C) orgánico en sistemas acuáticos continentales está ampliamente aceptada, las vías de procesado de C en dichos sistemas son aún poco conocidas. La identificación de los factores que limitan la mineralización de C orgánico es crucial para una comprensión general del ciclo del C. Uno de los factores que ha recibido gran interés recientemente, es el efecto *priming* que hace referencia al fenómeno consistente en la degradación de C orgánico previamente no reactivo en respuesta a la llegada de pequeñas cantidades de fuentes de C lábiles. Aunque este fenómeno se ha estudiado ampliamente en suelos, no se han encontrado aun pruebas convincentes de su existencia en aguas continentales.

A fin de testar las condiciones en las que el *priming* podría observarse en sistemas pelágicos de agua dulce, se realizó un experimento multifactorial en mesocosmos. Se evaluó el efecto de las adiciones de tres fuentes de C lábil sobre el consumo de carbono orgánico disuelto (DOC) presente en aguas de diferentes lagos incluyendo varios estados tróficos y calidades de DOC. También se analizó el efecto que la disponibilidad de nutrientes y el papel de la unión de las células a una superficie tienen sobre el *priming*. A pesar de la amplia variedad de condiciones testadas, no se encontraron evidencias de *priming*. Nuestros resultados sugieren que el efecto *priming* en sistemas lacustres tal como se define actualmente es poco probable que tenga un efecto significativo en el ciclo del C. Discutimos también los motivos por los que la columna de agua no es el medio más idóneo para la aparición de *priming,* así como la posible relevancia de otros fenómenos limitando la disponibilidad del C orgánico.

Introduction

A substantial amount of organic carbon (OC) present in inlands waters is buried or passively transported towards the sea, but a considerable fraction of it is lost to the atmosphere by mineralization (Cole et al. 2007, Battin et al. 2009, Tranvik et al. 2009). An important constraint on mineralization is the ability of microorganisms to degrade the complex and diverse organic matter typical of dissolved and particulate detritus in aquatic environments (Benner 2003, Hedges 2002, Middleburg et al. 1993). Despite extensive research on the degradability of aquatic OC (e.g. Sondergaard and Middelboe 1995, Amon and Benner 1996, Eiler et al. 2003, Kritzberg et al. 2006), the factors that determine degradability are poorly explored. A mechanism that has been hypothesized to stimulate the mineralization of less available OC is the priming effect. Initially described for soils (Löhnis 1926), and later suggested to occur also in aquatic environments (de Haan 1977) priming has recently attracted renewed interest (Guenet et al. 2010, Bianchi 2011, McCallister and delGiorgio 2012, Danger et al. 2013).

While there is little evidence in the literature for priming in freshwater ecosystems, it has been intensively studied and is currently a broadly accepted process in soils (Fontaine et al. 2007, Blagodatskaya and Kuzyakov 2008, Schmidt et al. 2011). The priming effect refers to the observation that changes in carbon inputs modify OC decomposition rates (Kuzyakov 2010, Schmidt et al. 2011). The inputs are generally labile OC sources that trigger the degradation of previously un-reactive organic carbon (Kuzyakov 2010). Priming is considered positive if OC decomposition increases and negative if net OC decomposition decreases (Guenet et al. 2010).

As priming has never been described in sterile conditions (Kuzyakov, 2010), the main mechanisms involved in this process are thought to be microbial (Blagodatskaya and Kuzyakov 2008, Bianchi 2011). Soil scientists have distinguished between real priming, describing the enhanced turnover of OC, and apparent priming, reflecting higher microbial biomass turnover but no effects on OC decomposition (Kuzyakov 2010). Priming in natural systems is likely the result of combined real and apparent priming effects. Microbes may use labile C for population sustenance and invest energy derived from labile C inputs to synthesize extracellular enzymes to degrade OC. Although the mechanisms involved in priming are not well understood, at the ecosystem level they are likely driven by energy constraints and nutrient stoichiometry (Kuzyakov 2010).

To explore the conditions where priming may be observed in freshwater pelagic systems, we performed a multifactorial microcosm experiment. We used water from three different lakes and a concentrate of dissolved organic carbon (DOC) from a humic river. The waters included contrasting nutrient and DOC concentrations. We manipulated nutrient availability by N and P additions as we hypothesized that a low C:N would facilitate lake DOC degradation. We added three labile C sources, or "primers", along a concentration gradient as it has been reported that priming is strongly dependent on the primer used (Smith et al. 2007). Finally, we tested the role of cell attachment to a surface; since we hypothesized that attached cells may be more likely to benefit from hydrolysis products issue of exoenzymatic activity than free-floating cells, which may increase the probability of observing positive priming effects.

Material and methods

Conceptual approach

To test the occurrence of priming, DOC consumption was measured in lake water incubated with different concentrations of primers. Linear regression of the consumed DOC vs concentrations of primer was employed as proposed in Levi-Minzi et al. (1990). We used the intercept of the regression as an estimate of the DOC consumed in the absence of primer. DOC consumption at the intercept of the regression (i.e. zero primer addition) was compared to the actual DOC consumption in controls that did not receive the primer (see Fig. 3.1). With this approach, a significant difference between the intercept and DOC consumption in the control indicates either a positive or a negative priming effect (intercept higher or lower than the actual DOC consumption, respectively). An underlying assumption in this method is that the magnitude of priming is a linear response of the labile DOC addition.

Experimental design

Four lake waters were chosen representing various trophic states and pools of DOC: Ljustjärn, Svartjärn, Valloxen and a DOC extract (Table 3.1). We performed a factorial experiment in each of these four lakes, with primer as factor (3 different labile C sources) and the primer concentration as a concomitant variable (each primer was added at 5 different concentrations). In the case of Ljustjärn and Svartjärn, two additional factors were

added: nutrients (2 levels, with and without addition of N and P) and surface availability (2 levels, with and without glass beads). We set up quadruplicated mesocosms for each treatment, totaling 1060 experimental units. The mesocosms were incubated in the dark at 15ºC for 5 weeks. A summary of the treatments and the abbreviations used to designate them can be found at Fig. 3.2.

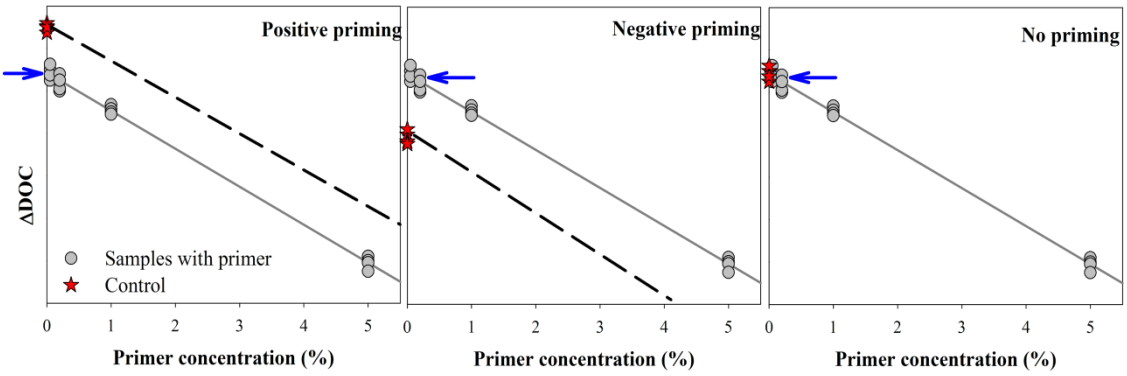

Figure 3.1 Priming detection method, $\Delta DOC = DOC_{initial} - DOC_{final}$. In the positive priming case, the intercept of the regression line of the samples with primer (arrow) is under the mean value of the control samples (stars). In the negative priming case the intercept of the regression line is above the mean value of the control samples. When no priming is detected no differences are found between the intercept and the control samples. The discontinuous black line represents the DOC consumption of the controls ($\Delta DOC_{Control}$) plus the amount of primer added at each concentration.

The lakes sampled were located in mid-Sweden: Ljustjärn, a clear-water oligotrophic forest lake; Svartjärn, a polyhumic mesotrophic forest lake; and Valloxen a eutrophic lake located in an agricultural catchment (Table 3.1). The water was stored in the dark at 4ºC until filtered through 0.7 µm pre-combusted GF/F and 0.2 µm membrane filters (Supor, Pall, Lund, Sweden). The fourth type of water was a DOC extract; we used artificial water prepared according to Lehman (1980) with a reverse osmosis concentrate as the DOC source. DOC from river Öre (Table 3.1), concentrated as described in Kragh et al. (2008), was aged for 12 years in darkness at 4ºC. The concentrate was filtered through a 0.2 µm filter (Supor, Pall, Lund, Sweden) and diluted to reach a final concentration of 10 mg C L^{-1}.

Table 3.1. Characteristics of the studied waters.

Water source	Latitude/ Longitude	Surface area (Km^2)	DOC (mgL^{-1})	TP(μgL^{-1})*	$SUVA_{254}$ ($L\ mgC^{-1}m^{-1}$)	A_{420} (m^{-1})
Lake Ljustjärn	59º55'N/15º26'E	0,12	4.18 ± 1.1	11.01 ± 1.9	1,56	0,38
River Öre	64º10'N/18º55'E	-	8,9†	<0.08	3,65	3,17
Lake Valloxen	59º44'N/17º49'E	2,9	16.31 ± 1.5	46.83 ± 12.9	2,55	2,05
Lake Svärtjärn	59º53'N/15º15'E	0,07	22.83 ±6.7	15.1 ± 6.1	4,49	6,26

*Values are means ± SE of reported data (Bastviken et al. 2004, Eiler et al. 2009, Langenheder et al. 2006, Kragh et al. 2008, Steger et al. 2011, Gudasz et al. 2012).
†Initial value measured during the incubations

Three primers were used: acetate, glucose and cellobiose. Acetate is a major DOC photoproduct (Dahlgren et al. 1996), glucose is the primary monosaccharide in algal exudates (Carlson et al. 2002) and cellobiose is one of the main degradation products of plant litter (DeForest et al. 2004). Each primer was added at four relative concentrations: 0.05%, 0.2%, 1% and 5% of the water samples DOC concentration. Five control replicates without primer were set up for each treatment.

Nitrogen and phosphorous were added as Na_2HPO_4 and KNO_3 to final C : N : P ratios of 45 : 7.4 : 1 for Ljustjärn, Svärtjärn and the DOC extract to avoid limitation of bacteria by inorganic nutrients and ensure C limiting conditions (Vrede et al. 2002).

To stimulate the development of biofilms and hence investigate priming in systems with attached bacteria, open-pore glass beads with large surface area were added to Svartjärn and Ljustjärn. Prior to their use, glass beads of 2-3 mm diameter (SiranTM Carriers, Jaeger Biotech Engineering, Inc.) were sonicated in base, then acid-rinsed before being rinsed with Milli-Q water and combusted for 6h at 450ºC.

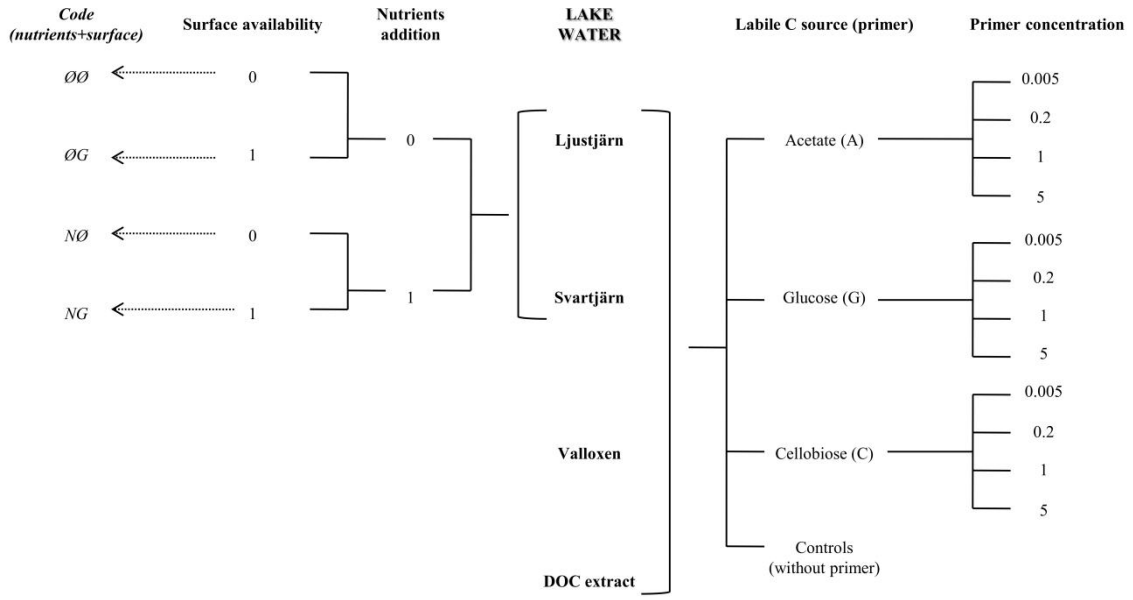

Figure 3.2 Experimental design and treatment codes. Different labile C sources and primer concentrations were applied to the four water types, whereas nutrient additions and surface availability were applied only to Ljustjärn and Svartjärn lake waters.

Experimental setup and measurements

Treatments were prepared as a batch of filtered water and sequentially received corresponding nutrients, primer and inoculum (unfiltered lake-water in a 1:10 v/v

proportion) additions. In treatments involving surface availability, glass beads were added to each empty incubation vial (2 ml). A mixed inoculum from the 3 unfiltered lake waters was prepared for the DOC extract. Next, the water was distributed into the acid-washed, pre-combusted, 40 ml glass vials with Teflon coated septa and sealed headspace free. In order to avoid gas exchange or contaminations, the initial and final measurements correspond to 2 different vials prepared simultaneously from the same batch. One was sampled at the start of incubations, and the other after 5 weeks in the dark at 15ºC, submersed in pure water. We measured initial and final DOC concentrations, and evaluated DOC consumption (ΔDOC) as the difference.

Concentrations of DOC were measured using a Sievers 900 TOC Analyzer (General Electric Analytical Instruments, Manchester, UK), which determines TOC in a range from 0.3 ppb to 50 ppm with a precision of < 1 % relative standard deviation and an accuracy of ± 2 % or ± 0.5 ppb.

Statistical analyses

To test the differences between the intercept of each treatment with primer and the DOC consumption in the controls ($\Delta DOC_{Control}$), we used an ANCOVA approach in the DOC consumption (y) with the primer concentration as a numeric variable (x) and the primer used as a discrete factor (i). The following models were fitted:

$\mathbf{H_1}$: $y_{i,j} = \alpha_i + \beta_i x_{i,j} + \varepsilon_{i,j}$ if i = A,C,G

$y_{i,j} = \alpha_i + \varepsilon_{i,j}$ if i = O

$\mathbf{H_0}$: $y_{i,j} = \mu + \beta_i + \varepsilon_{i,j}$ if i = A,C,G

$y_{i,j} = \mu + \varepsilon_{i,j}$ if i = O

Where α was the intercept of the regression for the alternative hypothesis and β the slope of the regressions. If the null hypothesis was accepted, no significant differences between the intercepts of the three primers and the control were found (an equal value μ was considered) and consequently no evidences of priming effect. Each of the ten blocks of design (Valloxen, DOC extract and each of the four treatments of Ljustjärn and Svartjärn; Fig. 3.2; Table 3.2) was analyzed independently.

Subsequently and using a similar approach, the differences between the slopes of the regression lines (β) were also tested, in order to evaluate changes in the DOC consumption pattern as a function of the primer added. Finally, the difference between DOC consumed in each treatment (ΔDOC_i) was compared to the DOC consumed in the controls plus the

amount of primer added ($\Delta DOC_{Control}$+ DOC_{primer}; i.e. we tested if ΔDOC_i was higher or smaller than $\Delta DOC_{Control}$ + DOC_{primer}). To visually identify this difference a regression line was added in each graph representing the $\Delta DOC_{Control}$ plus the amount of primer added at each concentration (represented as the black line in Fig. 3.1).

All the analyses were run using R version 2.15.0 (R Development Core team 2012).

RESULTS

Basal DOC consumption

The relative amount of DOC degraded without primer addition (i.e. in the controls) without nutrients or glass beads in lake Valloxen corresponded to 460 ppb C, 3.14% of the initial DOC. For the DOC extract, DOC consumption was 527 ppb C, corresponding to 6.57% of the initial DOC. In Ljustjärn the degraded DOC was 110 ppb C and in Svartjärn 608 ppb C, corresponding to 3.36% and 4.37% of the initial DOC, respectively. When the labile C source was added, the DOC consumption increased compared to the control in the three lakes but not in the DOC extract (Table 3.2; Fig. 3.3; Fig. 3.4; Fig. 3.5a; Fig. 3.6a).

Priming effect detection

Significant differences were not detected between DOC consumption in controls and the intercept of the regression (i.e. we accepted the null hypothesis of equal value of the intercept for the treatments with primer and the mean value of controls) in eight out of the ten blocks of design (Table 3.2). The two blocks showing significant differences were LjustjärnNØ (i.e. with nutrients, without glass beads) and Ljustjärn ØG (i.e. without nutrients, with glass beads). However, in both cases and for each of the three primers used, the value of the intercept was over the value of the controls, showing lower DOC consumption in these treatments than in the controls (Fig. 3.5b-c). Thus, the labile C addition had a significant negative effect over DOC consumption both in LjustjärnNØ and in Ljustjärn ØG treatments.

Effect of the different treatments on DOC degradation

1. Primer added: Acetate, glucose or cellobiose

The effect of the added primer differed between the four lakes. For Valloxen, significant differences were found between the slopes of the regression lines (Fig. 3.3; F =

5.78, p = 0.0056), cellobiose had the highest slope and DOC consumption. Even though consumption was higher than the control consumption plus the amount of primer added ($\Delta DOC_{Cellobiose} > \Delta DOC_0 + DOC_{primer}$), the difference was not significant at any primer concentration ($p > 0.05$).

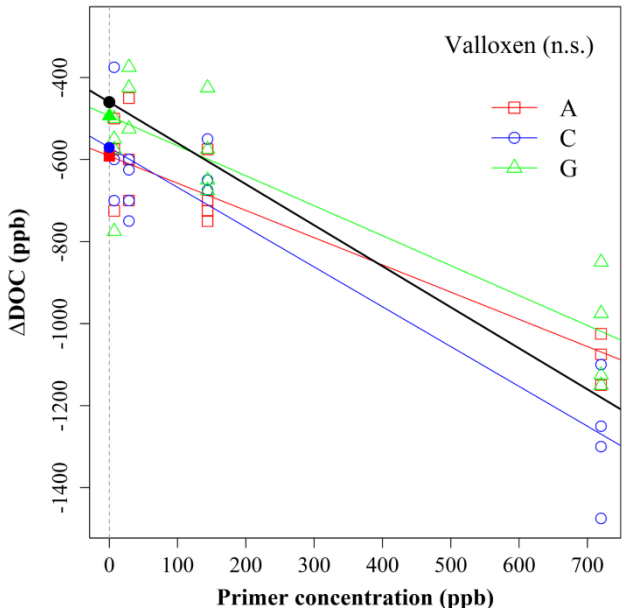

Figure 3.3 DOC consumed during the incubation period as a function of the concentration of primer added for lake Valloxen. The legend indicates the three primers used: Acetate (A), Cellobiose (C) and Glucose (G). *n.s.* indicates no significant differences between the mean value of the control and the intercepts of the regression line. The black line represents the values of the DOC consumed by the control (ΔDOC_0) plus the amount of primer added.

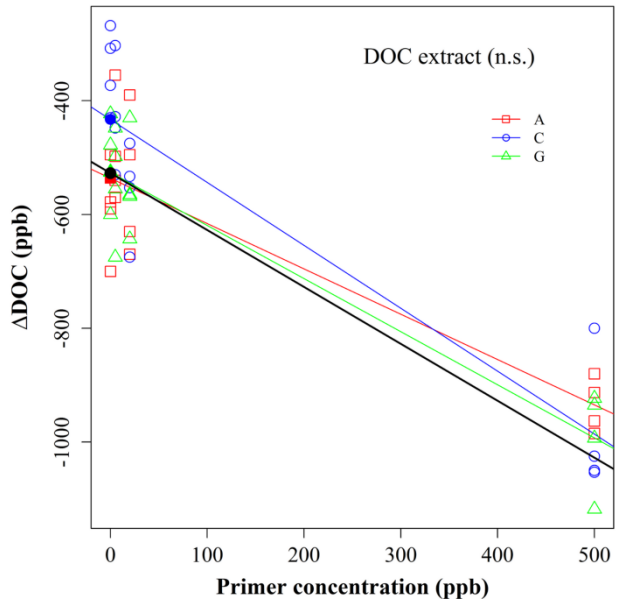

Figure 3.4 DOC consumed during the incubation period as a function of the concentration of primer added for the DOC extract. Symbols and codes as in Fig. 3.3..

For the DOC extract, no significant differences were found between the regression lines of acetate, cellobiose and glucose ($F = 0.18$, $p > 0.1$; Fig. 3.4). Similar results were found for LjustjärnØØ (without nutrients or glass beads), no differences were found between the regression lines of the three primers ($F = 2.38$, $p > 0.1$; Fig. 3.5a).

The slopes of the regression lines in SvartjärnØØ were significantly different between primers ($F=5.06$, $p=0.013$), with cellobiose having the highest DOC consumption and the strongest slope in the regression (Fig. 3.6a). DOC consumption was higher than the control consumption plus the amount of primer added in the cellobiose treatment ($\Delta DOC_{Cellobiose} > \Delta DOC_{Control} + DOC_{primer}$), however this difference was not significant at any primer concentration ($p < 0.05$).

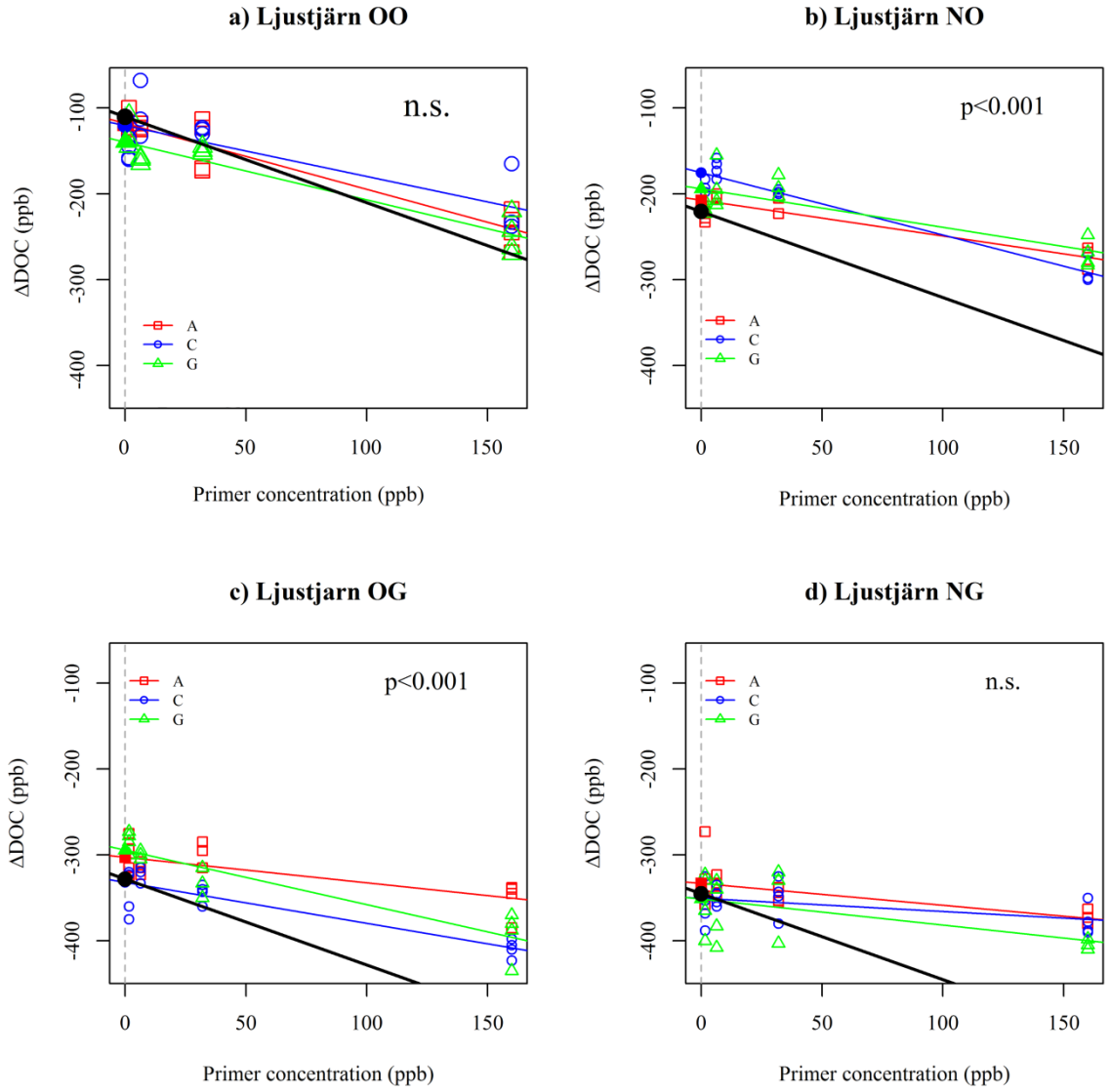

Figure 3.5 The DOC consumed during the incubation period as a function of the concentration of primer added for lake Ljustjärn. Treatments without nutrients or glass beads (a), with nutrients and without glass beads (b), with glass beads without nutrients (c) and with nutrients and glass beads (d) are shown. Symbols and codes as in Fig. 3.3.

2. Effect of nutrient additions (treatment NØ)

The nutrient addition had different effects in Ljustjärn and Svartjärn treatments. For Ljustjärn, DOC consumption in the controls increased with nutrients (Fig. 3.5b compared to 3.5a; Table 3.2). However, the DOC consumption in the samples with added primer was lower than the consumption of the controls plus the amount of primer added ($\Delta DOC_i < \Delta DOC_{Control} + DOC_{primer}$). No differences between the regression lines of the three primers were found ($F = 1.01$, $p > 0.05$). Regarding Svartjärn, nutrients had no effect on DOC consumption in the controls or the samples with primer (Fig. 3.6b compared to 3.6a; Table 3.2). No differences between the slopes of the three primers regression lines were observed ($F = 0.74$, $p > 0.1$).

3. Effect of glass beads (treatment ØG)

The increased surface area from the glass beads facilitated increased DOC consumption over the levels reached in the ØØ (i.e. without nutrients or glass beads) and NØ (i.e. nutrients only) treatments both in Ljustjärn and Svartjärn (Fig. 3.5c; Fig. 3.6c). Differences between the slopes of the regression lines of the primers were found for LjustjärnØG ($F=8.05$, $p=0.00095$). Samples with glucose had the highest slope although with cellobiose reached the highest DOC consumption (Fig. 3.5c). However, all the treatments with primer presented lower DOC consumption than the consumption of the controls plus the amount of primer added ($\Delta DOC_i < \Delta DOC_{Control} + DOC_{primer}$).

In Svartjärn (Fig. 3.6c), significant differences were found between the regression lines of the primers ($F = 3.58$, $p = 0.035$), with the DOC consumption in the cellobiose treatment over the amount consumed by the control + the primer added ($\Delta DOC_i > \Delta DOC_{Control} + DOC_{primer}$). This difference was significant only for the two first concentrations of cellobiose ($p < 0.05$).

4. Effect of the interaction between nutrients and glass beads (treatment NG)

The treatment with both nutrients and glass beads had different effect on Ljustjärn and Svartjärn waters (Fig. 3.5d; Fig. 3.6d; Table 3.2). In Ljustjärn, the DOC consumption of control samples was higher than the values reached in the three preceding treatments (ØØ, NØ and ØG; Fig. 3.5d compared to 3.5a-c). However, samples with primer presented lower DOC consumption compared to the controls plus the amount of primer added ($\Delta DOC_i < \Delta DOC_{Control} + DOC_{primer}$). No significant differences between the slopes of the three primers' regressions were found ($F = 1.79$, $p > 0.05$).

In the case of Svartjärn, DOC consumption decreased compared to the previous treatments (ØØ, NØ and ØG) both in the controls and in the samples with primer (Fig. 3.6d compared to 3.6a, 3.6b and 3.6c; Table 3.2). No differences between the regression lines of the primers were found ($F = 0.58$, $p > 0.05$).

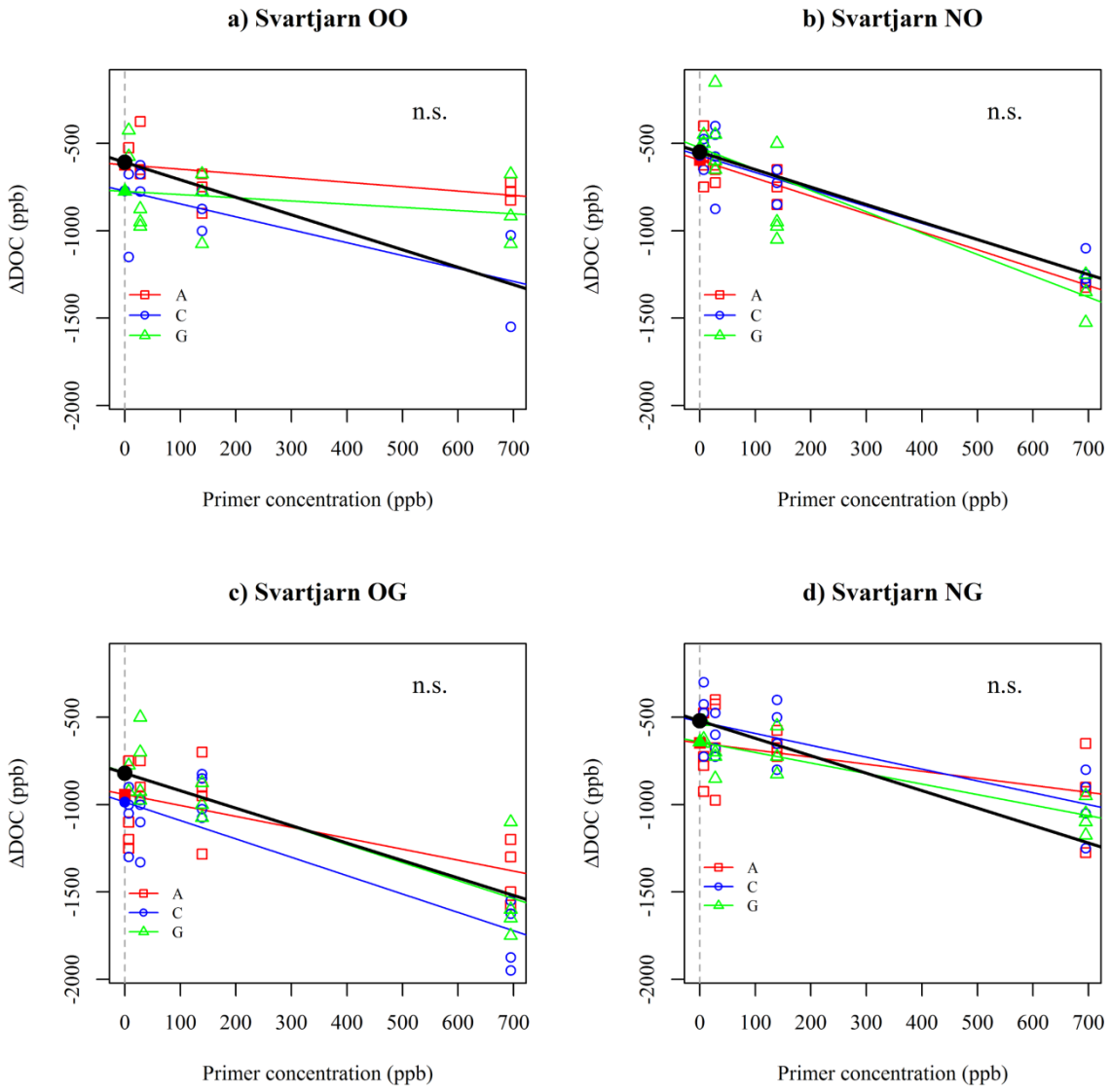

Figure 3.6 The DOC consumed during the incubation period as a function of the concentration of primer added for lake Svartjärn. Treatments without nutrients or glass beads (a), with nutrients and without glass beads (b), with glass beads without nutrients (c) and with nutrients and glass beads (d) are shown. Symbols and codes as in Fig. 3.3.

Table 3.2 Results of the contrasts between each pair of models. The null hypothesis was accepted when no significant differences between the intercepts and the control were found ($p > 0.05$)

Lake	Treatment code (Nutrients+surface)	F-value	p-value	Primer added	Intercept value
Valloxen	ØØ	2.4	0.08	*Control*	*-460*
				Acetate	-591
				Cellobiose	-570
				Glucose	-493
DOC extract	ØØ	2.1	0.12	*Control*	*-527*
				Acetate	-506
				Cellobiose	-408
				Glucose	-499
Ljustjärn	ØØ	2.3	0.09	*Control*	*-110*
				Acetate	-118
				Cellobiose	-120
				Glucose	-139
	NØ	7.9	<0.001	*Control*	*-220*
				Acetate	-207
				Cellobiose	-175
				Glucose	-194
	ØG	8.1	<0.001	*Control*	*-328*
				Acetate	-302
				Cellobiose	-332
				Glucose	-294
	NG	1.2	0.32	*Control*	*-345*
				Acetate	-333
				Cellobiose	-350
				Glucose	-351
Svartjärn	ØØ	1.2	0.33	*Control*	*-608*
				Acetate	-623
				Cellobiose	-771
				Glucose	-774
	NØ	0.4	0.77	*Control*	*-550*
				Acetate	-595
				Cellobiose	-569
				Glucose	-525
	ØG	1.9	0.14	*Control*	*-820*
				Acetate	-942
				Cellobiose	-986
				Glucose	-814
	NG	1.3	0.28	*Control*	*-520*
				Acetate	-646
				Cellobiose	-524
				Glucose	-640

Discussion

The results presented in this study suggest that, for a wide range of conditions, priming in lake water column is unlikely to happen. We observed only two cases (LjustjärnNØ and Ljustjärn ØG) with a significant difference in DOC consumption between the controls and the samples with primer, and in both cases the value of DOC consumption in the intercept was lower than the actual control DOC consumption.

The proposed mechanisms underlying priming effect (Blagodatskaya and Kuzyakov 2008, Guenet et al. 2010, Bianchi 2011) involve different fates of the labile C added, that will depend mainly on the community of decomposers present in the natural water. Firstly, the labile C could be consumed preferentially to existing DOM in the media, because it is either being used for population maintenance (respiration) or growth. Secondly, the labile C could provide energy to produce extracellular enzymes to degrade less labile DOM (either used by the same population or by another). If nutrients are limiting, the production of exoenzymes might be induced to obtain these nutrients from DOM. In any case, in complex communities such as those present in natural waters, the aforementioned strategies could act simultaneously with DOC consumption the result of a variety of metabolic pathways.

Effect of the different factors on DOC consumption

Departing from these potential microbial strategies, the four waters used here were chosen to evaluate priming occurrence in lakes with different C sources, trophic states and consequently, microbial populations. The basal consumption (% of initial DOC consumed) was similar in the four water types including the DOC extract. DOC consumption increased after the labile C amendment in all cases, but the DOC consumption in the samples amended with primer was lower or not significantly different than the consumption of the control plus the amount of primer added ($\Delta DOC_i \leq \Delta DOC_{Control} + DOC_{primer}$).

If priming occurred, it should be noticed in the C budget of the samples, as the increase in DOC decomposition should be significantly higher than the input of labile C (Guenet et al. 2010). Evaluating the changes in the C budget is needed in order to discuss the quantitative relevance of priming. Some soil studies using labeled substrates for priming detection have found increased consumption of the soil OC after the labile C addition (identified as positive priming) but did not report if this increased consumption is higher than the labile C input rate (ΔDOC_i higer or smaller than $\Delta DOC_{Control} + DOC_{primer}$; Kuzyakov, 2010). Observational data suggest that the enhanced DOC decomposition rates are often smaller than or equal to the labile C input rates (Fontaine et al. 2007), thereby questioning the quantitative relevance of priming at an ecosystem level.

Effect of the source of labile C

Different microbial populations or enzymatic activities would be activated depending on the labile C source (Blagodatskaya and Kuzyakov 2008). Initially, we hypothesized that simple substrates such as acetate or glucose, commonly used as labile C sources in priming experiments in soils (Fontaine et al. 2007, Kuzyakov 2010), would be easily incorporated, supplying the energy needed for priming to occur (i.e. exoenzymatic production strategy). Cellobiose, a disaccharide, might need cellobiase to be hydrolyzed (Gottschalk, 1986), and thus is a substrate that could trigger two of the microbial strategies previously proposed: providing an energy supply or inducing exoenzyme production.

As expected, and despite the lack of priming, we found differences in DOC consumption between samples with different primers in some treatments (Valloxen, Fig. 3.3; LjustjärnØG Fig. 3.5c, Svartjärn ØØ Fig. 3.6a and Svartjärn ØG Fig. 3.6c). In these four cases, the primer depicting the highest DOC consumption was cellobiose. Conversely, very simple and extremely accessible substrates as glucose or acetate were less efficiently consumed at the highest concentration of primer added. However, in the case that cellobiose induced the production of cellobiases, facilitating the degradation of cellulose and other complex plant-derived substrates present in natural DOM (Romani et al. 2006), it was not translated into significantly enhanced DOC consumption.

Effect of nutrients: do C limiting conditions enable priming?

Nutrient availability can also constrain the proposed microbial strategies. When added alone, inorganic nutrients increase bacterial production in lakes (Pace and Cole, 1996). When OC and nutrients are added simultaneously, the stimulation of bacterial growth leads to high respiration and an increase in the assimilative nutrient demand (Bernhardt and Likens 2002, Carlson et al. 2002). Given these stoichiometric constraints, we hypothesized that both the C and nutrient limitation could induce an increase in DOM consumption. As stated previously, when nutrients are limiting, as in oligotrophic systems, and energy from labile C sources is available, natural DOM degradation can be stimulated in order to obtain the limiting nutrients (Guenet et al. 2010). On the other hand, if only C was limiting, enhanced DOC degradation is also expected (Vrede et al. 2002).

However, enhanced DOC consumption was not observed neither in the oligotrophic lake without nutrients (i.e. with nutrients limitation, LjustjärnØØ; Fig. 3.5a) nor in the C

limited treatments (Svartjärn NØ and Ljustjärn). Although nutrient addition enhanced DOC consumption in the controls for LjustjärnNØ, samples with primer had lower DOC consumption than the controls (Fig. 3.5b). Thus nutrients increased the availability of lake DOC but not the effect of the primer addition. The lack of effects of nutrient addition in Svartjärn, a mesotrophic lake, suggests that nutrients were not limiting DOC consumption prior to the incubation. In both cases, a preferential use of the labile C substrate (Blagodatskaya and Kuzyakov 2008) to maintain population sustenance and growth is likely.

Effect of glass beads: does surface availability enhance DOC consumption?

Investing energy derived from labile C mineralization into enzyme production is not an adaptive strategy for free-floating cells, as they are unlikely to benefit from the release of extracellular enzymes (Beier and Bertilsson 2011). Recent studies have demonstrated that old OC can be bioavailable and that C mineralization might be dependent on temporal protective states rather than on its age or structure (Fontaine et al. 2007, McCallister and DelGiorgio 2012, Singer et al. 2012). Among these protective states is the isolation of C substrate from the degrading population (Arnosti 2003, Eksmith et al. 2005).

In aquatic ecosystems, this contact is facilitated by particulate hotspots like lake snow, vegetation debris or sediment surfaces (McClain et al. 2003). These hotspots of microbial activity are potential settings of aquatic systems where priming could be relevant (Guenet et al. 2010). Therefore, the release of extracellular enzymes might be more beneficial to attached live forms and we hypothesized that if a larger surface was available for the microbial community to colonize, it was more likely to find evidences of priming. According with this hypothesis increased DOC consumption was found in treatments with glass beads without nutrients (ØG) both in the oligotrophic Ljustjärn and the mesotrophic Svartjärn lakes. However, in spite of the higher DOC degradation in the controls when glass beads were available, degradation was not further enhanced by primer addition (Fig. 3.5c; Fig. 3.6c).

Although evidence of priming has been found at lower primer addition rates than the maximum rate used here (5 %) (Fointaine et al. 2007, Blagodatskaya and Kuzyakov 2008), if these primers are acting as substrates inducing the production of a particular enzyme, their concentration could still be too low (Arnosti 2003). Furthermore, other protective states, like physical mechanisms of geopolymerization or complexation, could protect DOC from

enzymatic degradation (Chin 2003, Eksmith et al. 2005, Kleber 2010). Priming is based on the assumption that bioenergetics limit DOC consumption (McCallister and delGiorgio 2012). However, bioenergetics is unlikely the only constraint to DOC consumption in lake water, as no response to labile C sources has been found in lakes covering different trophic states in the present study. Other potential constraints have been tested simultaneously, such as nutrients and the availability of substrate, but an enhancement of DOC consumption after labile C addition was not detected in any case.

Some interesting insights have been provided regarding the source of labile C, since the highest DOC consumption was reached for cellobiose, the most complex substrate. Previous works have suggested that complex labile substrates, such as macrophyte leachates or straw, can more easily lead to priming since they induce the growth of a wide variety of microbial functional groups (Farjalla et al. 2009, Guenet et al. 2012). However, they also provide a complex matrix of micronutrients that confound the identification of the mechanisms enhancing OC degradation. Regarding this, a further research direction to take might be the use of mixed polymers assemblies from a specific group (e.g. from di- to poly- saccharides), targeting the production of a particular enzymatic group.

A deeper insight into the effect of extracellular enzymes in the bioenergetics limitation of C decomposition is necessary to determine the potential relevance of this mechanism at the ecosystem level. The interactions between substrates, microbial communities and abiotic conditions of the system determine organic matter persistence (Kleber 2009) that rather than an intrinsic property of the material should be considered an ecosystem property (Schmidt et al. 2011). Taking these interactions into account, priming might be unable to occur without changes in other mechanisms constraining DOM degradation. Establishing the mechanisms regulating organic matter persistence in aquatic systems is required to reach a full understanding of the processes involved in organic matter degradation and to be able to quantify their relevance as global C pathways (Bianchi 2011).

ACKNOWLEDGEMENTS

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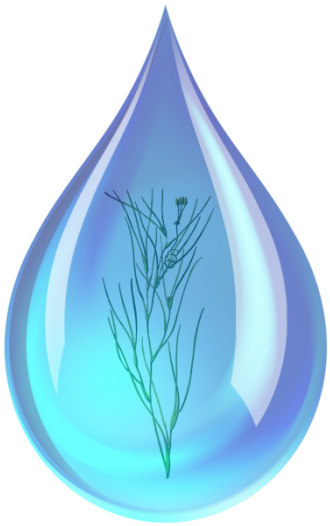
Chapter 4



Seasonal variability in dissolved organic matter properties as a fingerprint integrating ecosystem processes in a Mediterranean lagoon

Núria Catalán, Biel Obrador and Joan Ll. Pretus
(submitted to Hydrobiologia)

Abstract

We studied the dynamics of dissolved organic matter (DOM) in a Mediterranean lagoon dominated by seasonal submerged vegetation and receiving torrential freshwater inputs. The potential sources of DOM into the lagoon including the ephemeral washes draining the catchment were characterized and compared with the lagoon DOM quality throughout the year. Spectroscopic measurements including UV-visible absorbance and fluorescence excitation-emission matrices (EEMs) were used to determine changes in DOM quality.

The lagoon water showed a dominance of humic-like peaks A and C during the whole period although their relative intensity varied along the annual cycle. Both torrential inputs and macrophyte meadows drove DOM properties variability. Humification and aromaticity of DOM increased markedly after the torrential inputs of detritic compounds derived from vegetation and soils in the catchment. The macrophytic biomass in the lagoon contributed seasonally with less humified materials and protein-like compounds together with an increase in the BIX index pointing out a biological origin. The effect of seawater entrances and of sporadic bottom hypoxia on DOM quality, although with much lower influence, could also be traced by the spectroscopic descriptors.

Resum (en català)

Es va estudiar la dinàmica de la matèria orgànica dissolta (DOM) en una llacuna mediterrània dominada per vegetació submergida i amb fortes entrades d'aigua torrencials. Les fonts potencials de DOM a la llacuna, incloent els torrents que drenen la conca es van caracteritzar i comparar amb la qualitat de la DOM de la llacuna al llarg de l'any. Descriptors espectroscòpics derivats de l'absorció UV-visible i de les matrius de fluorescència d'excitació-emissió (EEM) es van utilitzar per determinar els canvis en la qualitat de la DOM.

L'aigua de la llacuna va mostrar un predomini dels pics húmics A i C durant tot el període, encara que la seva intensitat relativa va variar al llarg del cicle anual. Les dues entrades torrencials i els prats de macròfits determinaren la variabilitat de les propietats de la DOM. La humificació i aromaticitat de la DOM van augmentar notablement durant la tardor, després de les entrades torrencials de compostos detrítics procedents de la vegetació i dels sòls de la conca. La biomassa de macròfits a la llacuna va contribuir estacionalment amb materials menys humificats i compostos proteics juntament amb un augment en l'índex BIX assenyalant un origen biològic de la DOM. L'efecte de les entrades d'aigua de mar i de les esporàdiques hipòxies al fons, tot i què amb una influència menor sobre la qualitat de la DOM, també van poder detectar-se mitjançant els descriptors espectroscòpics.

Resumen (en castellano)

Se estudió la dinámica de la materia orgánica disuelta (DOM) en una laguna mediterránea dominada por vegetación sumergida y receptora de fuertes episodios torrenciales. Las fuentes potenciales de DOM en la laguna, incluyendo los torrentes que drenan la cuenca se caracterizaron y compararon con la calidad de la DOM de la laguna a lo largo del año. Descriptores espectroscópicos derivados de la absorción UV-visible y las matrices de fluorescencia de excitación-emisión (EEM) se utilizaron para determinar los cambios en la calidad de la DOM.

El agua de la laguna mostró un predominio de los picos húmicos A y C durante todo el periodo, aunque su intensidad relativa varió a lo largo del ciclo anual. Las dos entradas torrenciales y los prados de macrófitos determinaron la variabilidad de las propiedades de la DOM. La humificación y aromaticidad de la DOM aumentaron notablemente durante el otoño, tras las entradas torrenciales de compuestos detríticos procedentes de la vegetación y los suelos de la cuenca. La biomasa de macrófitos en la laguna contribuyó estacionalmente con materiales menos humificados y compuestos proteicos, junto con un aumento en el índice BIX señalando un origen biológico de la DOM. El efecto de las entradas de agua de mar y de las esporádicas hipoxias de fondo, aunque con una influencia menor sobre la calidad de la DOM, también pudieron detectarse mediante los descriptores espectroscópicos.

Introduction

Dissolved organic matter (DOM) has been identified as the largest pool of organic carbon in most inland waters (Prairie, 2008; Tranvik et al. 2009). DOM influences all the pathways controlling aquatic C cycling and interacts with many other biogeochemical processes determining ecosystem functioning (Battin et al. 2009; Tranvik et al. 2009).

The role of DOM in each of these pathways is determined, among other factors, by the composition of the DOM entering the pathway. This composition depends on the ultimate source of DOM and on the transformations that it suffers within the specific ecosystem compartment (Jaffé et al. 2008). Because the behaviour of DOM is dependent on its origin, increasing attention has been paid to the identification of DOM sources (Fellman et al. 2010; Miller & McKnight, 2010). In this sense, spectroscopic descriptors are broadly accepted as very helpful techniques to characterize DOM origin (Coble, 1996; McKnight et al. 2001; Stedmon et al. 2003).

Allochthonous DOM inputs of aquatic ecosystems are mostly derived from terrestrial vascular plants and soil organic matter (Wetzel, 2001), generally considered as aromatic and recalcitrant due to their high humic and lignin content (Miller & McKnight, 2010). On contrast, autochthonous sources typically deriving from algae on the one side and in situ heterotrophic processes on the other, are expected to have a low degree of humification and are thus viewed as labile materials (McKnight et al. 2001). Some considerations must be done regarding this general scheme. First of all, it has been demonstrated that allochthonous DOM is an important C source for heterotrophic bacteria in lakes, what means that at least a fraction of the allochthonous DOM is bioavailable (Tranvik, 1992; Kritzberg et al. 2004). In this sense, evidences of proteic materials from terrestrial C inputs transported via stream runoff have been found (Cory & Kaplan, 2012). Thus, the recalcitrant character of allochthonous terrestrially-derived DOM is largely questioned nowadays (Guillemette & delGiorgio, 2011). Secondly, submerged macrophytes are an autochthonous DOM source lacking that clear labile character that is usually assumed for autochthonous DOM. Both aromatic (Wetzel, 2001) and labile properties (Lapierre & Frenette, 2009; Tank et al. 2011) have been attributed to macrophyte leachates. Despite the influence of macrophytic sources in aquatic C cycle (Bertilsson & Jones 2003; Prairie, 2008), and the recognized relevance of shallow macrophyte-dominated water bodies (Wetzel, 2001; Tranvik et al.2009), the bibliography dealing with the characterization and fate of this

DOM source is small (Bertilsson & Jones, 2003). Actually, the role of small shallow lakes, frequently dominated by submerged macrophytes, in global C budgets has been largely understudied (Downing et al. 2006).

Most works dealing with DOM sources in inland waters have been carried out in temperate or boreal systems (Hood et al. 2003; Kritzberg et al. 2004; Sobek et al. 2007: fig.7) in which DOM quality exhibits a seasonal pattern with a snowmelt period related to inputs from the catchment dominated by fulvic and humic-acids, and a summer phytoplanktonic bloom with microbial-derived materials (Jaffé et al. 2008; Miller & McKnight, 2010). Although climatic factors are known be a key factor in the regulation of DOM (Mulholland, 2003; Sobek et al. 2007), its dynamics is poorly understood in arid and semi-arid regions (Westerhoff & Anning, 2000; Mulholland, 2003). Water bodies in these regions are subject to strong hydrological forcing as are torrential episodes, suffering huge water entrances lasting from hours to days (Bull, 1997; Westerhoff & Anning, 2000). These torrential inputs can imply substantial water level fluctuations, nutrient inputs, turbidity changes and alterations of the chemical fluxes in the receiving water body (Coops et al. 2003). Systems of this kind are typical in the Mediterranean climate (Álvarez-Cobelas et al. 2005) where, in addition to this hydrological forcing, most inland water bodies are shallow environments which remain unfrozen with warm temperatures over most of the year, being strongly productive (Alvarez-Cobelas, 2005; Beklioglu et al. 2007). In these markedly dynamic systems, the complexity and variability of sources and processes regulating DOM are likely to be high.

In the present study, we investigated the seasonal dynamics and spatial variability of DOM in a Mediterranean lagoon dominated by submerged vegetation and subject to intense external forcings (Obrador, 2009). The overall C dynamics in the studied system results from the interaction between the high benthic production and the intense sporadic flows typical of the Mediterranean climate (Obrador & Pretus, 2012). We characterized the main potential DOM sources (macrophytes, torrential freshwater inputs and sediments) and evaluated their contribution to DOM in the lagoon. We hypothesized DOM to show a marked humic character due to the torrential inputs of refractory material and to the DOM derived from the extensive macrophyte meadows. A seasonal trend in DOM quality is expected as a result of the macrophytic annual cycle and the seasonality in terrestrial torrential pulses.

Material and methods

Study site

The Albufera des Grau is an enclosed coastal lagoon located in the island of Menorca (39º 57' N, 4º 15' E, Western Mediterranean). It has a volume of 1 hm3, a surface area of 78 ha and a mean depth of 1.37 m. The connection with the sea is irregular, and does not represent an important renewal of water (Obrador et al. 2008). Due to Mediterranean climate, with dry and hot summers and annual precipitation centred on autumn and winter, the freshwater inputs to the lagoon have a marked torrential character. Torrential freshwater inputs are provided by ephemeral washes which only occur during precipitation episodes, and can supply, in a few hours or days, up to 20-60 % of the water volume of the lagoon (Obrador et al. 2008). The catchment (56 km^2) is dominated by mixed Mediterranean forests (47 %), extensive dry farming lands (41 %), and shrublands (9 %). A marked influence of the catchment on the seasonal changes of DOM properties in the ephemeral washes has been described (Chapter 1).

The DOC concentration in the lagoon ranges between 5 and 19 mg C L^{-1} and exhibits marked seasonality (Obrador and Pretus 2012). The lagoon is dominated by submerged vegetation, and the dominant species, *Ruppia cirrhosa*, forms dense and extensive meadows with the highest biomass ever described for this species (1760 g PS m^{-2}; Obrador et al. 2007). A typical cycle of *R. cirrhosa* in the lagoon, with biomass peaking in summer months, can be seen in Fig. 4.1b. Other macrophyte species (*Potamogeton pectinatus*) and macroalgae (*Polysiphonia* spp., *Gracilaria* sp. and *Chaetomorpha crassa*) are of minor importance in terms of abundance (Obrador and Pretus 2012).

Water sampling and extraction of leachates

We characterized lagoon DOM on a monthly basis during a complete year cycle. The lagoon was considered the receiving water body and its DOM the result of the different sources and their transformations. We refer to contributing sources as endmembers in this study since they were selected as representative DOM associated with specific origins. We considered five endmembers: R. cirrhosa as the main autochthonous primary producer, sediment, as it can be an important source of DOM during diffusion or resuspension events, seawater, and the ephemeral washes water taken in two different seasons [autumn

(AU) and winter-spring (WS)]. These two seasons have been previously identified as very differentiated in terms of DOM quality (Chapter 1).

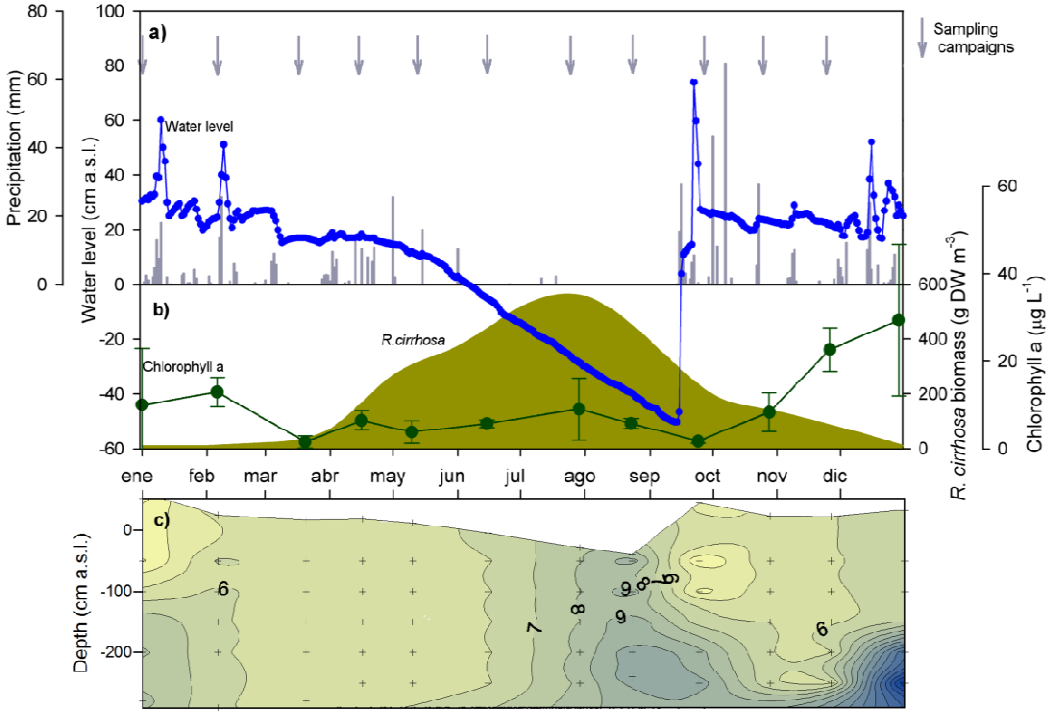


Figure 4.1 Temporal dynamics of the hydrological parameters and the primary producers in the lagoon. **a)** Water level and precipitation, **b)** mean biomass of R. cirrhosa in the lagoon (period 2002-2006) and chlorophyll-a concentration (mean ± s.d.), **c)** salinity (ppt).

Water samples were collected monthly (i.e.- from January 2009 to January 2010) at 3 depths (0, 150 and bottom) in the central site of the lagoon. At the same time, 4 different littoral sites in the lagoon defined by their contrasted influence of DOM endmembers were sampled. Samples were filtered in-situ with pre-combusted 0.7 µm glass-fiber filters (GF/F, Whatman) and cold-stored until analyzed. During each sampling date, in situ measures of salinity, pH, temperature (ºC) and oxygen concentration (mg L^{-1}) were determined in situ with field sensors (8WTW Multiline P3 and WTW Cond3l5i). Basic daily climatic data was obtained from the nearest (7 km) meteorological station (Spanish Meteorological Institute).

Data for DOM descriptors from torrential water samples were obtained from a previous sampling campaign (years 2007-2008) in which stream water during torrential events was collected from the seven ephemeral washes of the catchment during one year and a half (see Chapter 1 for further details).

Leaves of *R. cirrhosa* were collected, washed in tap water and cut into small pieces. Then, leaves were leached in sterile Milli-Q water in the darkness and gently stirred at

approximately 4 ºC for 48 h with a biomass : water ratio of 1 : 15 (Anesio et al. 2000). A similar process was used for the lagoon sediment samples (Vergnoux et al. 2011), Water Extractable Organic Matter (WEOM) was extracted by shaking the sediments with Milli-Q water in darkness for 48 h at room temperature with a sediment : water ratio of 1 : 10. Then the extracts were centrifuged (10 min, 4500 rpm) to shorten the filtration time. Leachates were filtered through pre-combusted 0.7 μm glass-fiber filters (GF/F, Whatman) and then diluted to a stock concentration of 10 ppm C.

DOM properties

Dissolved organic carbon (DOC) concentrations were determined in a Shimadzu TOC-VCS by high temperature catalytic oxidation. The detection limit of the analysis procedure was 0.05 mgC L^{-1}. All DOC samples were previously acidified with HCl 2M and preserved at 4 ºC until analysis. UV–Vis absorbance spectra (200-800nm) were obtained in a Shimadzu UV-1700 spectrophotometer, using 1cm quartz cuvette. The absorption coefficients at wavelength λ (a_λ, m^{-1}) were determined from the absorbance measurement (A_λ) using the expression: $a_\lambda = 2.303\ A_\lambda/l$, where l is the path length in meters (Kirk, 1994). We selected 440 nm as an indicator of chromophoric dissolved organic matter (CDOM) concentration (Kirk, 1994). We also calculated the specific ultra-violet absorbance at 254 nm, a descriptor of DOM aromaticity ($SUVA_{254}$, L mg^{-1} m^{-1}; Weishaar et al. 2003). The slope ratio (S_R) was obtained as the ratio of the slopes $S_{275\text{-}295}$ to $S_{350\text{-}400}$, calculated using linear regressions of the log-transformed spectra (Helms et al. 2008). S_R is inversely correlated to molecular weight (Helms et al. 2008).

Fluorescence spectra were determined using a Shimadzu RF-5301PC spectrofluorometer with a 1cm length silica quartz cuvette in order to obtain excitation–emission matrices (EEM). EEM scans were run at 10 nm excitation increments between 240-400 nm, and at 1 nm emission increments between 270-630 nm. The EEM were corrected for Raman scattering, inner-filter effects and normalized to Raman units (R.U.) (Cory and McKnight 2005), the FDOMcorrect toolbox for MATLAB (Mathworks, Natick, MA, USA) following Murphy et al. (2010) was used. The commonly identified fluorescent peaks (A, C, M and T; Coble 1996) were extracted from the spectra. Humic-like peaks A and C were determined as the intensity of fluorescence measured at 250Ex/450Em and 350Ex/450Em respectively (Coble 1996; Huguet et al. 2009). Humic-like low molecular weight peak M was obtained as the intensity of fluorescence measured at 312Ex/400Em and protein-like

peak T at 280Ex/330Em (Coble 1996; Huguet et al. 2009; Fellman et al. 2010). All the peaks are reported as the proportional contribution to total fluorescence.

We calculated several spectral indexes, including the ratio between humic-like peaks A : C, the fluorescence index (FI), the humification index (HIX) and the Biological index (BIX). The Fluorescence Index (FI) was determined as the ratio of the emission intensities at 470 nm/520 nm for an excitation wavelength of 370 nm (Jaffé et al. 2008) and is used to discriminate sources of DOM; high values are related with microbial and low with terrestrial sources (values usually ranging between 1.2 and 2; McKnight et al. 2001). The HIX, increasing with humification, is the ratio between the area under the emission spectra 435-480 nm to 300-345 nm at an excitation of 254 nm (Zsolnay 1999); HIX values for natural waters usually range between 2 and 18. BIX is calculated at an excitation of 310 nm, dividing the fluorescence intensity emitted at 380 nm, by the fluorescence intensity emitted at 430 nm, and is related to recent biological activity with values generally between 0.5 and 1 (Huguet et al. 2009). As a way to better visualize the differences between FDOM signatures, the EEM of each endmember was subtracted to the mean EEM of the lagoon.

Data treatment

In order to determine the influence of spatial and temporal variability on DOM properties, a permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was performed on the Manhattan distance matrix taking the sampling sites (five) and the months (12) as factors, and the DOM descriptors (DOC, FI, BIX, HIX, Peaks A, C, M and T, a_{440} and $SUVA_{254}$) as variables.

A principal component analysis (PCA) was applied on a correlation matrix to ordinate the samples by the same DOM properties used on the PERMANOVA. All statistical analyses were performed in R software version 2.15.0 (R Development Core team 2012).

Results

General dynamics of the lagoon

The precipitation during the studied period showed a typical Mediterranean regime and was centred on autumn and winter, being September the most humid month. These precipitations generated 5 main runoff episodes observed in January, February, September, November and December. A drought period occurred between June and the end of

September (Fig. 4.1a). The torrential water pulses generated transient vertical gradients in the water column in January and September as a result of the lower density of freshwater in comparison with receiving lagoon water. Bottom hypoxic conditions (< 2 mg O^2 L^{-1}) were observed from July to September, and occasionally in December, when the communication with the sea implied a unique but intense input of seawater (Fig. 4.1c; J. Pretus personal observation). During the whole period, phytoplankton biomass remained low (Fig. 4.1b).

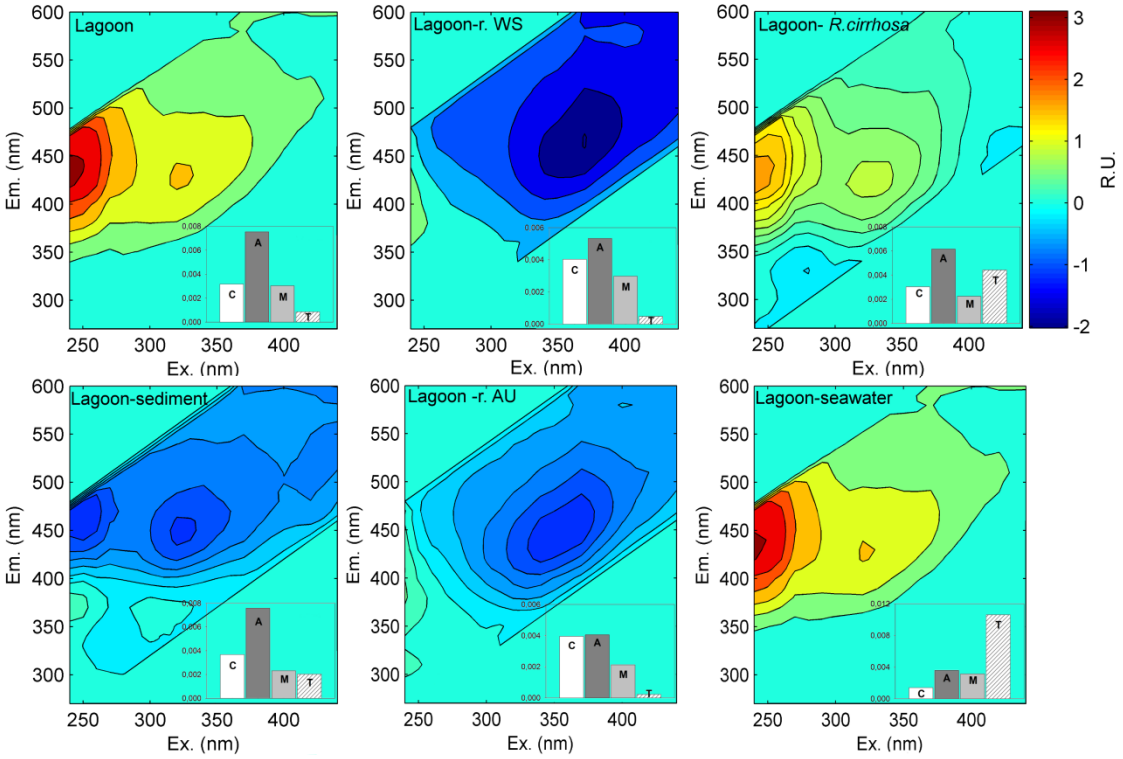


Figure 4.2 Excitation–emission matrix fluorescence spectra of the lagoon water and the results of subtracting to this sample each of the 5 endmembers: torrential freshwater from the WS period, R.cirrhosa extract, sediment extract, torrential freshwater from the AU period and seawater. The insets show the relative intensity of the main fluorescence peaks (C, A, M and T) present in the endmembers.

Overall DOM characterization

Lagoon samples were dominated by humic-like fluorescence, with peak A relatively more intense than peak C (Fig. 4.2). The signature of macrophyte extracts was similar to the lagoon water; with differences in the protein-like region, which was relatively more abundant in the macrophytic sample than in the lagoon. Sediment samples were also mainly humic-like, with a higher proportion of protein-like fluorescence than the lagoon water and a displacement of peaks A and C towards the red region (i.e. longer

wavelengths). Torrential freshwater samples presented a stronger peak C than the lagoon and this peak was also displaced towards longer wavelengths. In these samples the relative abundance of peak C fluorescence was larger in AU than in WS samples. Seawater endmember had a very differenciate signature but the lower effect over the lagoon EEM.

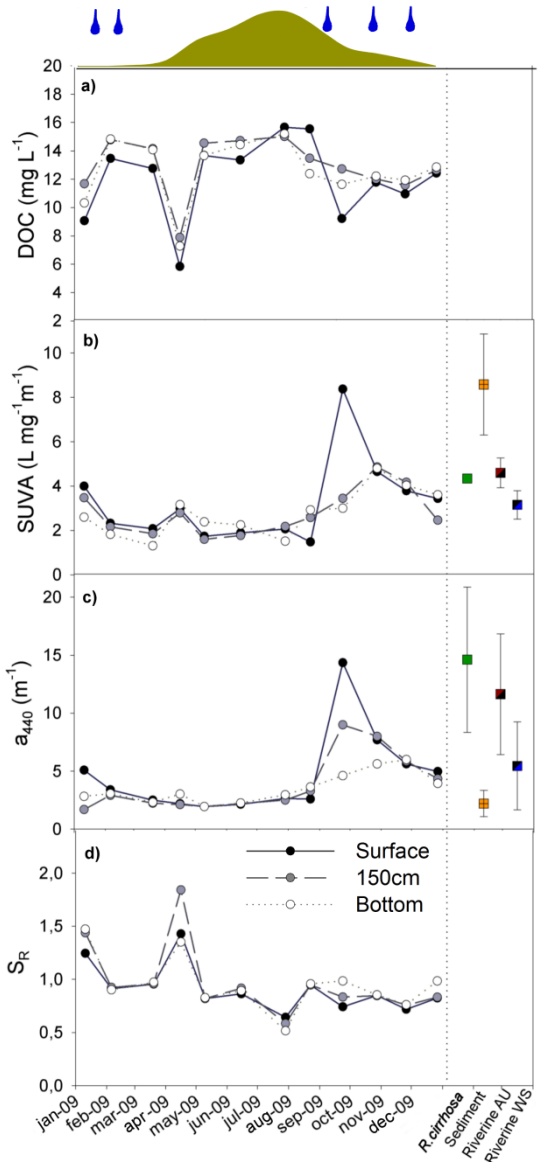


Figure 4.3 Temporal dynamics of a) DOC concentration and DOM properties derived from absorbance: b) $SUVA_{254}$, c) absorbance coefficient at 440nm and d) ratio of spectral slopes (S_R). Values for the three depths at the central site (surface, 150 cm and bottom) are shown.

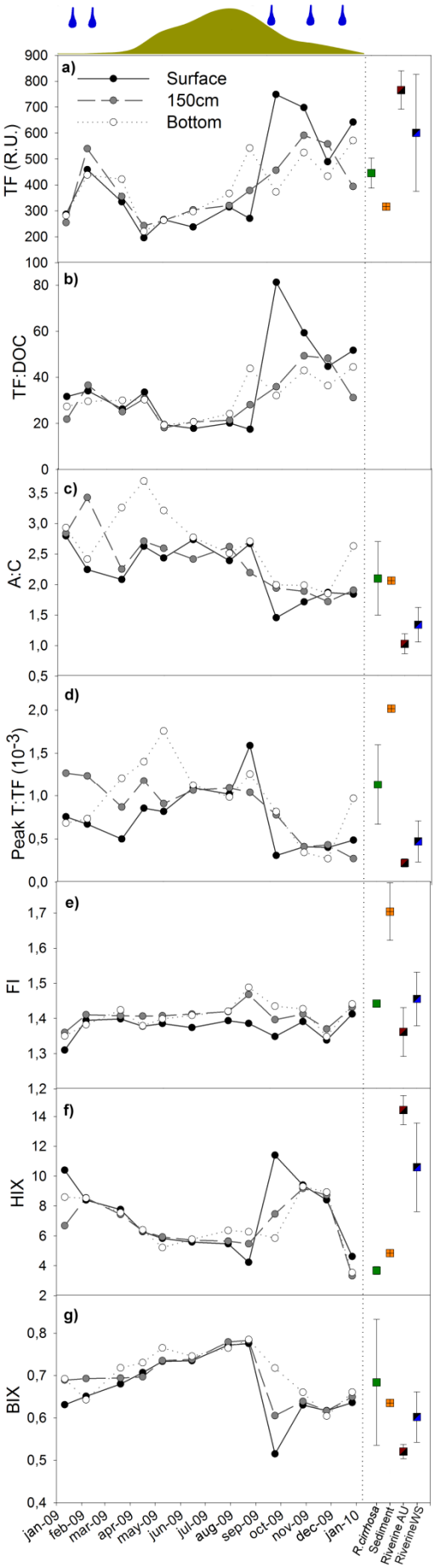


Figure 4.4 Temporal dynamics of DOM properties derived from fluorescence measurements: a) Total fluorescence in Raman units (TF), b) ratio of total fluorescence and DOC, c) ratio of peaks A and C, d) relative fluorescence of protein-like peak T, e) fluorescence index (FI), f) humification index (HIX) and g) biological index (BIX). Values for the three depths at the central site (surface, 150cm and bottom) are shown.

DOC concentration in the Albufera des Grau ranged from 5.83 ppm (April) to 15.66 ppm (July) and showed a marked seasonal pattern with high values during spring and summer (despite a strong decrease in April) and lower during the autumn season (Fig. 4.3a). DOC was negatively correlated with the weekly accumulated precipitation (R adjusted = -0.63, $p < 0.001$, data not shown). $SUVA_{254}$, ranging between 1.29 and 6.29 L mg^{-1} m^{-1} (Fig. 4.3b), was positively correlated with precipitation ($R = 0.55$, $p < 0.001$).The absorbance coefficient at 440 nm (a_{440}), ranged from 1.68 (January) to 14.35 m^{-1} (September) (Fig. 4.3c) and the ratio of spectral slopes (S_R) from 0.56 (July) to 1.84 (April) (Fig. 4.3d).

Regarding the fluorescence-derived indices for the lagoon water, the ratio between peaks A and C ranged from 1.46 to 3.69, showing the prevalence of humic peak A during the whole study period (Fig. 4.4c). The fluorescence index (FI) presented very consistent values corresponding to terrestrial sources (mean value of 1.4; Fig. 4.4e). The humification index (HIX) ranged from 3.3 to 11.4, covering almost all the range of values previously described for HIX in natural waters (Fig. 4.4f). The Biological index (BIX) presented values between 0.51 and 0.78, depicting an intermediate influence of recent biological activity (Fig. 4.4g). BIX and HIX presented a marked negative relationship ($R = 0.64$; $p < 0.001$; Fig. 4.5). In the BIX-HIX plane, samples were ordered in terms of origin and season. Thus, torrential waters appeared in one extreme of the graph, with high HIX (values above 10) and low BIX (less than 0.6) whereas in the opposite extreme were summer lagoon samples, with low HIX (between 4 and 6) and high BIX (near 0.8). The Ruppia and the sediment extracts were located in a region of low HIX (less than 4) and intermediate BIX values (0.6-0.7).

Total fluorescence was not significantly correlated with DOC concentration when all the data was pooled ($R = 0.14$; $p > 0.05$; Fig. 4.6). However, correlations were significant when only winter-spring samples (i.e. - January to April) were considered ($R = 0.78$; $p < 0.001$). No significant correlations were found for autumn or summer samples subsets.

Temporal and spatial changes

The PERMANOVA analysis of DOM descriptors, with month (i.e. the twelve monthly samplings) and site (i.e. the five sampling sites) as factors, explained 89 % of the total variance and showed significant differences between months (69.4 %, $F = 15.3$, $p < 0.001$). No significant differences were observed between sites (7 %, $F = 2.3$, $p > 0.01$) or in the interaction between month and site (13 %, $F = 0.7$, $p > 0.01$). The principal components analysis grouped samples by month (Fig. 4.7a) but not by site (Fig. 4.7b).

At the central site, most of the DOM descriptors presented different values with depth (Fig. 4.3; Fig. 4.4). The most marked vertical differences in DOM properties occurred during spring, late summer and early autumn. During spring, the A : C ratio and the protein-like fluorescence peaked in the deepest layer of the lagoon. In August, total fluorescence, SUVA and HIX were higher in the deepest part of the lagoon while protein-like fluorescence was stronger in the surface.

Coinciding with the first runoff episodes, early autumn lead to a very marked increase both in total fluorescence and TF : DOC, and also in HIX, SUVA and a_{440} whereas there was a decrease in A:C ratio, BIX and protein-like fluorescence. These changes affected specially the surface samples, generating the main vertical gradient in DOM quality registered during the studied period. The distinct DOM quality issue of September rains persisted over autumn, although the vertical heterogeneity diminished. Other minor episodes of vertical heterogeneity occurred in January (increase in SUVA, a_{440}, HIX in surface), April (DOC and S_R decrease) and December (TF :DOC increase in the deepest part of the lagoon).

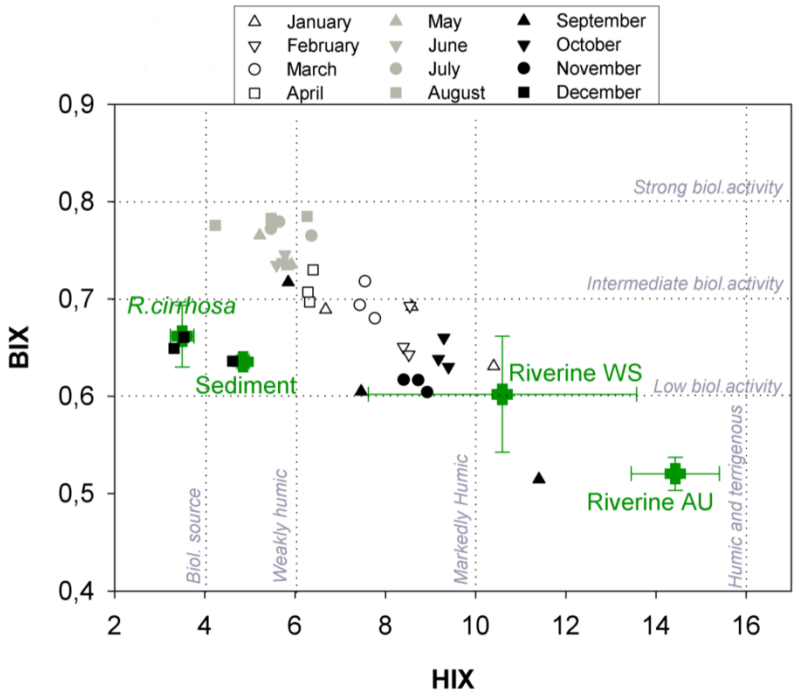


Figure 4.5 Relationship between BIX and HIX for all the lagoon samples at the central site and the four main endmembers. The dashed lines indicate the different range of values obtained by Huguet et al. (2009) and the corresponding DOM character associated.

DISCUSION

Character of the DOM sources

Although there is not a universal relationship between DOM concentration and quality (see Inamdar et al. 2012 and references therein), in systems with a unique or dominant source of DOM, a direct relationship between DOM concentration and its fluorescent fraction (FDOM) can generally be established (DelVecchio and Blough 2004). The lack of correlation between DOM concentration and FDOM reported here (Fig. 4.6) points towards multiple and variable controls of DOM quality and quantity in the studied lagoon. As the number of processes affecting DOM and sources increase, the relationship between DOM concentration and quality is likely to diminish (Bianchi 2007). Seasonal changes in the relationship between FDOM and DOM indicate temporal variations in the sources influencing the DOM character (Inamdar et al. 2012).

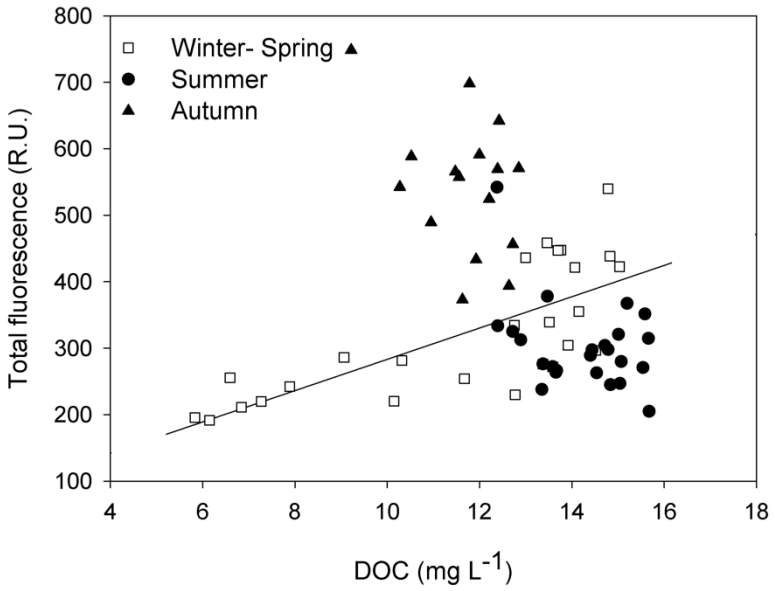


Figure 4.6 Relationship between total fluorescence and DOC concentration by seasons. The relationship is not significant for the entire data set. The regression line corresponds to the winter-spring samples, the only group for which the relationship was significant ($R = 0.78$; $p<0.001$).

Seasonal variability in DOM is usually better traced by quality than concentration parameters (Jaffé et al. 2008). Here it can be seen by the BIX-HIX relationship (Fig. 4.5). This relationship is expected to be negative due to the link of these indices with aromaticity (Zsolnay et al. 1999). The BIX-HIX plane allows defining different regions in terms of humification and biological origin (Huguet et al. 2009; Birdwell and Engel 2010). In our

dataset these indices presented a broader range of values than the FI and a good response for endmember comparison (Fig. 4.4 e-g). In fact, the range found for the lagoon water is as wide as those described by Huguet et al. (2009) for the Gironde estuary, by Singh et al. (2010) at the Barataria Bassin or by Kothawala et al. (2012) in Swedish lakes. Regarding the endmembers, the sediment extracts values correspond to those previously observed for sedimentary organic matter (Birdwell and Engel 2010). The BIX of macrophyte leachates is lower than what has been found elsewhere (Zhang et al. 2013), because the macrophyte extract had lower fluorescence in the protein-like region, which affects BIX values (Huguet et al. 2009). Torrential waters (both AU and WS) show weak biological activity and an important degree of humification (Fig. 4.5) and are in the range of reported values for streams (Inamdar et al. 2012). The highest humification of AU samples in comparison with WS samples agrees with the seasonal changes in DOM humification described in the ephemeral washes draining the lagoon (Catalán et al. 2013). The location of DOM endmembers in the extremes of the HIX-BIX plane reflects the fact that a characteristic fingerprint can be attributed to lagoon DOM and to each of the sources of DOM.

The humic character of lagoon samples is frequent in systems with a strong influence of terrestrial vegetation (Fellman et al. 2010). Humic-like peaks are the main responsible of the fluorescence of DOM (Coble 1996) and, in systems with strong FDOM concentrations, can be so dominant that in bulk EEMs they do not allow the distinction of other less intense fluorophores, even after inner filter effect correction (Huguet et al. 2010; Kothawala et al. 2012). Apart from the relative relevance of peak T in total fluorescence, protein-like compounds can play an important role in bulk DOM bioavailability (Guillemette and DelGiorgio 2011) as will be discussed later.

Macrophyte leachates had a higher protein-like fluorescence than the lagoon DOM (Fig. 4.2), but it was not the dominant fluorescence peak, as usually found in other macrophyte extractions (Zhang et al. 2013). Several reasons can explain this discrepancy. Firstly, as a function of leaching time, the protein-like fluorescence decreases while the humic-like peaks increase (Fellman et al. 2010; Zhang et al. 2013). Secondly, the kind of material is strongly dependent on the macrophyte species (DeMarty and Prairie 2009). The protein-like region can include fluorescence not only from proteic compounds but also from substances such as polyphenols derived from lignin and tannins (Maie et al. 2007;

Hernes et al. 2009), the exudation of which is variable between species (Arnold and Targett 2002).

In the sediment samples, the protein-fraction is likely related to the microbial activity in sediment surface (Tank et al. 2011). The observed displacement of humic peaks towards longer wavelengths can be related to increased aromaticity and humification, as could be expected in sediment-derived organic matter (Kalbitz et al. 1999).

The torrential waters presented higher peak C in relation to peak A than lagoon samples (Fig. 4.2), a feature pointing towards a higher reactivity of allochthonous DOM than lagoon DOM. Despite both peaks are attributed to humic terrestrially-derived materials, higher degradability of peak C during either dark (Kothawala et al. 2012) or photodegradation experiments (Moran et al. 2000; Stedmon and Markager 2005) has been described. Accordingly, the residence time of freshwater in the ephemeral washes draining the lagoon is very short, and consequently, torrential DOM would be relatively unprocessed and highly reactive (Weyhenmeyer et al. 2012).

Influence of processes and sources on DOM variability

1.- Hydrological processes

Hydrological processes frequently control DOC concentration (Kowalczuk et al. 2010; Mulholland 2003; Sobek et al. 2007) and influence DOM properties, although changes in DOM quality are not necessarily associated with variations in DOC concentration (Jaffé et al. 2008). Here, DOC was negatively correlated with precipitation, whereas total fluorescence increased during the autumn torrential period (Fig. 4.6). The entrance of terrestrial DOM during the first torrential events of the year is reflected by the location of the lagoon surface water of September in the BIX-HIX plane (in the same region as AU and WS torrential water samples). The following autumn months are placed successively in the next quadrant (0.6 – 0.7 BIX and 6 - 10 HIX). The inputs of aromatic and coloured materials generated an increase in TF:DOC in the surface layer, together with the absorbance descriptors a_{440} and SUVA. The September decrease in A:C ratio fits with freshwater DOM entrance, since Peak C was stronger in the torrential water endmembers. Later runoff episodes led to eventual increases in HIX and SUVA, as registered during January. From then on, the more labile character of WS torrential water in comparison with AU water prevents the existence of strong aromatic DOM peaks in the lagoon.

2.- Macrophyte production

The strong development of the macrophyte meadows in spring (Obrador & Pretus, 2010) determines the character of DOM samples, with higher BIX and lower HIX than in winter (Fig. 4.5), and an increase in peak T. We do not have a clear explanation for the strong decrease of DOC and S_R during April, although it might be related to the runoff inflow occurred the same sampling day. The A:C ratio incremented during this season, likely due to DOM derived from macrophytic autochthonous activity (Fig. 4.4c). A high peak A fluorescence was a marked differential property of the macrophyte endmember. Accordingly, humic components in the peak A region have been previously related to macrophyte-derived DOM (Component4; Lapierre and Frenette 2009). An alternative explanation of the change in A:C ratio based on the effect of spring rains was discarded because WS torrential waters had lower A:C values than the lagoon (Fig. 4.4c).

Freshly produced DOM from macrophytes explains the increase in DOC concentration during summer. Hydrological isolation (i.e. an increase in residence time that leads to the concentration of DOM) could influence the DOM summer accumulation (Tank et al. 2011), in agreement with the observed negative DOC-precipitation correlation. Nonetheless, summer DOM quality is unlikely a matter of pre-existing DOC concentrated by evaporation, but rather freshly produced DOC from macrophytes, whose annual cycle in the lagoon is based on huge biomass peaks in summer (Obrador and Pretus 2010). In favour of this, the highest DOC values are coincident with the lowest total fluorescence (Fig. 4.6), indicating an increase of the non-fluorescent DOC fraction. The composition of macrophyte exudates is likely to include an important fraction of carbohydrates (Tank et al. 2011; Zhang et al. 2013) with very poor fluorescent activity (Lakowicz 2006). Also, the macrophyte endmember was placed in the upper-left quadrant of the BIX-HIX plane coinciding with lagoon samples of the summer months (Fig. 4.5).

Protein-like fluorescence increased with respect to spring values, although, as found for the macrophyte endmember, this fluorescence does not dominate, as reported in other macrophytic systems (Lapierre and Frenette 2012). As discussed in the former section, the relative contribution of peak T to the total fluorescence is small (Fig. 4.2; Fig. 4.4d) despite it can anyway be a very important descriptor of heterotrophic metabolism (Cammack et al. 2004; Fellman et al. 2010). Here, the macrophyte biomass production is very high (peak biomass of up to 1760 g DW m^{-2}; Obrador et al. 2007) and mainly decomposed along the

annual cycle (Obrador and Pretus, 2012). High bacterial growth efficiency rates (between 37 and 64%) were observed during the study period (Ruscalleda 2009), indicating a high activity of the microbial loop in the lagoon and consequently a fast turnover of protein-like material (Cammack et al. 2004). Macrophyte DOM has been reported to be assimilated very fast and to represent an important contributor to microbial food webs in Canadian lakes (Tank et al. 2011). Thus, while labile materials are quickly consumed, the more refractory fraction of the macrophyte-derived DOM will remain in the lagoon (Tank et al. 2011; Zhang et al. 2013).

3.- Seawater entrances

Seawater entrances occur occasionally in the lagoon and can generate marked salinity gradients (Fig. 4.1; Obrador 2009). This was seen in DOM quality in December, when peak T increased in the bottom of the lagoon (Fig. 4.4d). Accordingly, the seawater endmember was characterized by a very high relative fluorescence of peak T (Fig. 4.2), as is typical for marine waters (Coble 1996). Bottom water in December reflected another indication of seawater entrance, as pointed out the low degree of humification and an intermediate biological origin (Fig. 4.5), both characteristic also of marine samples (Huguet et al. 2009).

4.- Bottom hypoxia

Bottom hypoxia starting in July also led to changes in the lagoon DOM quality; both total fluorescence and TF:DOC increased in bottom water samples (Fig. 4.4b). The bottom hypoxia can lead to changes in redox conditions that can modify the oxidation state of fulvic acids and the associated quinone functional groups (Fulton et al. 2004). Fulvic acids and quinones are responsible for most of the DOM fluorescence (Cory & McKnight 2005) and show stronger fluorescence in their reduced state (Fulton et al. 2004). Reduced quinones have also been related with aromaticity (Cory and McKnight 2005) and here the bottom samples also showed higher SUVA and HIX values. The bottom samples of July and August are also placed in a different quadrant of the BIX-HIX plane, depicting higher humification for a similar biological origin (Fig. 4.5). Thus, hypoxic conditions are likely to be determining the character of bottom water DOM in summer. To further confirm this, we cannot use the properties of the sediment end-member because it was not subject to the redox conditions of anoxic water.

5.- Phytoplankton activity

Phytoplanktonic activity increased in summer (Fig. 4.1b) and the occurrence of algal materials is reflected in an increased peak T fluorescence in the surface samples (McKnight et al. 2001) (Fig. 4.4d). Biodegradation of phytoplankton-derived materials could be enhanced by their photobleaching (Tranvik and Bertilsson 2001), in agreement with the decrease in humification and aromaticity detected in the surface during late summer. Photobleaching would also explain the low FI values of the surface samples, since the wavelengths used for its calculation are very affected by solar radiation (Birdwell and Engel 2010).

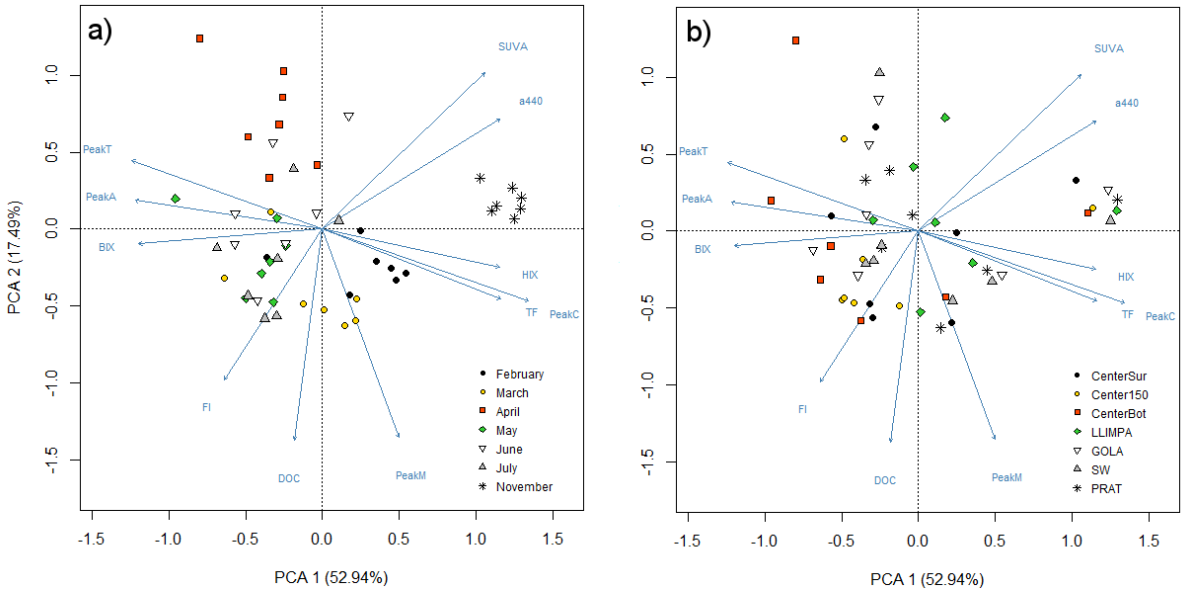


Figure 4.7 Multivariate ordination (Principal component analysis) of samples based on DOM descriptors (DOC, FI, SUVA, a440, HIX, BIX, total fluorescence and the peaks A, M, C and T). The samples include all the sampling sites and the three depths of the central site, and are grouped by month a) and site b). The percent of explained variation for each component is shown in brackets. The arrows represent the ordination of the DOM descriptors.

An example of optically complex system

In this study seasonality raised the variations in DOM quality over spatial changes (69% of total variability in PERMANOVA test; Fig. 4.7). This made the central point of the lagoon to be representative of the whole lagoon DOM. However, no precise temporal periods could be defined in terms of DOM quality, due to the multiple limnological processes influencing it.The hydrological forcing generated marked entrances of aromatic materials that are likely to be rapidly degraded, as discussed before. Thus, beyond the allochthonous origin of DOM or its molecular composition, the fate of allochthonous DOM is determined by the landscape characteristics (Weihenmeyer et al. 2012), in the case of this

study exemplified by fast flows conducting unprocessed DOM into the receiving water body. With regard to autochthonous DOM sources, a distinction between the produced and the remaining DOM must be done. In macrophyte-dominated water bodies, aromatic materials from this autochthonous source might dominate the bulk DOM, although the production of labile materials from the same source is likely to sustain the heterotrophic community (Tank et al. 2011).

From these results, we underline the complexity of highly dynamic systems as the one studied here, what in terms of DOM have been very properly called "optically complex systems" (Jiang et al. 2012), as well as the need of a whole-ecosystem approach in order to properly link DOM quality with the multiple sources and limnological processes that can influence it.

CONCLUSIONS

The spectroscopic descriptors provided a good characterization of the lagoon DOM and allowed determining the inner fingerprint of the main DOM sources. Temporal variability of DOM quality prevailed over spatial variations so, the vertical variability of the DOM properties at the central point of the lagoon was used to link DOM characteristics with the main sources and ecosystem processes.

Torrential inputs of terrestrial materials during autumn months increased the colour and aromaticity of the surface lagoon water inducing transient heterogeneities in the water column. Macrophyte phenology strongly influenced DOM quality in the lagoon, especially during spring and summer months, period of maximal biomass development. During this period lagoon DOM showed decreased humification, and an increase in the biological origin index and in the protein-like peak. Bottom hypoxia, phytoplankton activity and seawater entrances also influence DOM quality although their effects are very constricted in time.

ACKNOWLEDGEMENTS

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Overall discussion and synthesis

DOM intervenes in a broad number of ecosystem processes; in the present work, we focus on DOM controls and processing in the framework of a Mediterranean catchment. Each of the previous chapters examine different aspects of the variability and regulation of DOM, from the characterization of its composition and concentration to the evaluation of the sources, ecosystem-based processes and landscape settings controlling its character. In the following discussion and synthesis we aim to link these aspects and discuss them within the context of Mediterranean landscape.

Landscape-dependent controls and processes affecting DOM

Landscape settings regulate DOM patterns, including concentration and quality (Williams, 2010), and also the reaction pathways that degrade it. Following the broad definition of landscape presented in the introduction of this thesis, one of the largest regional scale settings influencing water bodies is seasonality (see Fig I-3, pag. 21). Seasonality strongly affects DOC concentrations in freshwater systems mainly due to variations in the catchment discharge (Aitkenhead-Peterson et al. 2003, Mullholland 2003). The strong hydrological variability in the Mediterranean climate regions translates into unpredictable and abrupt runoff events and drought periods that affect not only the concentration (Bernal et al. 2002) but also the quality of DOM in Mediterranean intermittent streams (Vázquez et al. 2010).

The influence of seasonality and other landscape features on the quality of DOC draining the catchment was evaluated in chapters 1 and 4. In chapter 1 we linked the properties of DOM in ephemeral washes to landscape drivers and in chapter 4 we described the variability of DOM as a function of processes occurring in the receiving lagoon, where the most intense transformations of DOM are expected to occur.

Large scale controls: seasonality and regional drivers

The composition and concentrations of DOM showed higher variations in time than in space both in the ephemeral washes (Chapter 1) and in the lagoon (Chapter 4) of the studied catchment. A major finding was that each of the seasonal periods defined from the DOM quality in the ephemeral washes was related to different

landscape drivers. In this case, two periods were distinguished regarding DOM quality: autumn and winter-spring (Chapter 1).

In the ephemeral washes, the concentration of DOC was maximum during autumn and was related to runoff, as has been reported previously in perennial (Hood et al. 2006) and intermittent (Bernal et al. 2002) streams. Regarding DOM quality, the washes presented aromatic and coloured materials of high molecular weight during the autumn runoff events (high SUVA and a440; Fig 1.1). Aromaticity has been related with humic DOC, mainly derived from terrestrial vegetation (Weishaar et al. 2003). In ephemeral washes, the properties of DOM in autumn are likely determined by the litter accumulation during the long summer drought. This organic matter accumulated on river beds is rarely biomineralized, but can be strongly photodegraded and heavily mobilized when water flow takes place (Steward et al. 2012). Accordingly, the spatial uniformity of DOM quality along the different subcatchments indicates the influence of a landscape factor acting at the catchment scale. Indeed, during this period hydromorphological variables acting at a regional or catchment scale such as summer drought, runoff, altitude or slope, regulated DOM quality and DOC concentration (Fig. 1.4).

On the contrary, the ecosystem-based processes influencing DOM in the lagoon are expected to act on a shorter temporal basis. Accordingly, no clear temporal clusters were identified in terms of DOM quality (Chapter 4). However, changes in the DOM properties did allow us tracing these processes and their scale of influence. During autumn months, the inputs from the ephemeral washes enhanced the aromatic character of lagoon water contributing with a differentiated fluorescent signature (i.e. higher humic-like peak C). This change is specially marked in the surface waters, producing a strong heterogeneity in the water column in terms of DOM quality after the entrance of freshwater inputs into the lagoon (Fig. 4.4).

Despite the relevance of the water inputs from the ephemeral washes, they are by no means the only source of DOM in the lagoon. The relation between DOM concentration and quality was very different for the washes and for the lagoon (Fig. D1). As reported elsewhere, changes in DOM quality are not necessarily directly related with concentration, because the biophysical processes controlling DOM might

be different or have a different incidence on quality and quantity (Jaffé et al. 2008, Bianchi 2007). Here, the relationship between the aromaticity descriptor SUVA and DOC showed a positive relationship for the ephemeral washes, whereas it was not significant for the lagoon DOM (Fig. D-1). This lack of relationship points out that in the lagoon the number of processes and sources affecting DOM is higher than in the washes, as suggested by Bianchi (2007) and Inamdar et al. (2012). The relationship between quality and concentration was analyzed in detail in chapter 4 (Fig. 4.6) in terms of total fluorescence vs DOC concentration. Seasonal variations in the quality-concentration relationship were observed for the lagoon samples, pointing out multiple controls over DOM properties and their variability on a seasonal basis.

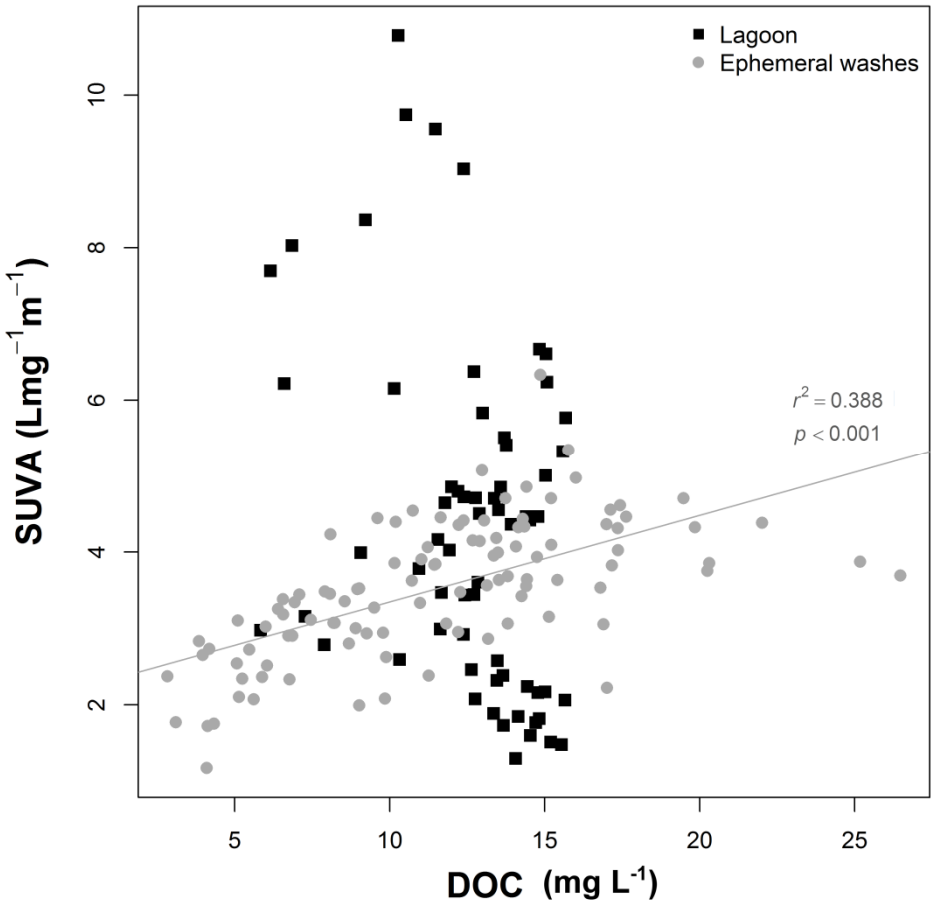

Figure D-1 Relationship between aromaticity (SUVA, specific UV absorbance) and DOC concentration in the lagoon and in the ephemeral washes. The relationship is not significant for the lagoon samples. The regression line corresponds to the ephemeral washes, the only group for which the relationship was significant ($r^2 = 0.39$; $p < 0.001$).

Local controls and in-situ processes

In the ephemeral washes DOC concentration decreased during the winter-spring period, accompanied by a shift in the quality of DOM. Microbial and algal sources predominate during this season, as shown by the lower aromaticity (SUVA) and molecular size (lower EEM peaks wavelengths) together with the range of values of fluorescence index (McKnight et al. 2001) and the increase in protein-like fluorescence (Fig. 1.5). The microbial-like character of DOM points towards an increase of heterotrophic in-situ processes mediated by soil moisture (Belnap et al. 2005). Thus, the higher humidity and the permanence of isolated pools in the ephemeral washes during this period contribute to in-situ DOM production (Vázquez et al. 2010) explaining the increased lability of DOM during the winter-spring period.

In winter-spring, local catchment characteristics, such as land uses, soil types and dominant geology, became relevant, linked to the increased humidity and subsequent in-situ processes (Fig. 1.5). These drivers highly influence processes controlling DOM quality, like biomass leaching, chemical interactions or physicochemical transformations such as soil sorption (Jaffé et al. 2008, Tranvik et al. 2009). Indeed, most of the variables found to determine DOM quality during winter-spring in Chapter 1 were geological features, what confirms the relevance of soil-mediated processes (Aitkenhead-Peterson et al. 2003, Hood 2006). Land uses were also relevant. Natural vegetation was, as expected, related to higher aromaticity of DOM (Fellman et al. 2010). The concentration of dissolved organic nitrogen, directly linked to DOM quality, although not extensively addressed throughout this thesis, was associated with cropping lands and farming activities, a link that has been observed in other pasture areas (Neff et al. 2003, Pellerin et al. 2006).

A general framework for the relevance of local processes in the control of DOM properties can be summarized as follows. At high water residence time, the relevance of any landscape component on the flow decreases because the area influencing these processes is smaller (Fig. D2; Belnap et al. 2005, Battin et al. 2008). Thus, ephemeral washes with exceptionally short residence time (Bull 1997) will capture the effect of a large area being perfect suitable candidates to study the relation between DOM and

landscape structure (Fellman et al. 2009, Dawson et al. 2011). On the contrary, as water permanence increases, the influence of in-situ processes is higher, being maximal in permanent water bodies (Battin et al. 2008, Stephens and Minor 2010).

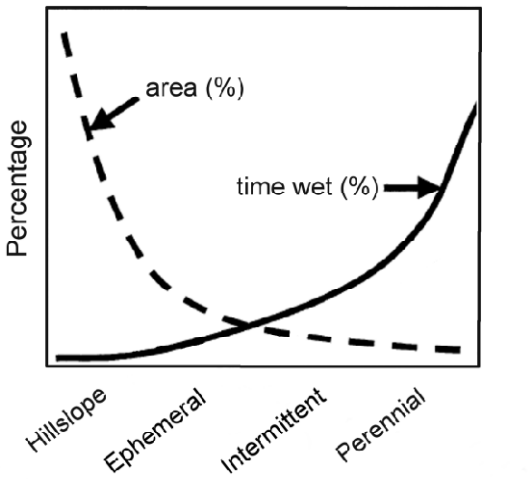

Figure D-2 Hypothesized relationship between the temporal duration of moist conditions and the landscape area influencing DOM concentration and quality in aquatic systems. The longitudinal changes in the percentage of area of landscape components, and in the percentage of time wet along a theoretical axis of water permanence, from hillslope to perennial systems, is shown. Adapted from Belnap et al. 2005.

In summary, along the axis of increasing water residence time (Fig. D-2) the processes influencing DOC change from a regional scale of climate and catchment drivers, to a local scale of the water body settings (Sobek et al. 2007). At this local level take place the most intense physical and biological mineralization processes affecting DOM, including autochthonous production, microbial degradation, photodecay, flocculation and sedimentation (Tranvik et al. 2009).

In the case of shallow lakes and lagoons, as is the receiving water body of the studied catchment, these local settings imply a high surface/depth ratio that allows a strong autochthonous productivity of submerged higher plants (Barnes 1980, Scheffer 1998). As has been discussed in Chapter 4, multiple sources of DOM besides the inputs from the ephemeral washes are expected in the studied lagoon, what implies relevant inputs of autochthonous DOM. The most important is the autochthonous DOM from macrophytes. Macrophytes are considered allochthonous DOC sources by some authors, who attribute them a recalcitrant character because of the presence of lignine-

derived compounds (Farjalla 2002, Wetzel 2003). It must be pointed that these works mainly consider emergent littoral macrophytes treating them almost as riparian vegetation, so their contribution to the water body DOM is limited to leacheates during their senescence. Submerged macrophytes are autochthonous sources of DOM that not necessarily present a recalcitrant character and as so must be considered. First of all, because they are truly autochthonous, being produced in the lagoon and influencing many internal ecosystem processes (Jeppesen et al. 1998). Secondly, because in shallow systems macrophytes can cover a very high area of the system, far from being just surrounding it in the littoral (Scheffer 1998; Obrador and Pretus 2010). And finally, because macrophytes not only release recalcitrant DOC; recent works (Lapierre and Frenette 2009, deMarty and Prairie 2009, Tank et al. 2011, Zhang et al. 2013) point out that macrophytes are also a source of labile carbon, mainly attributed to their photosynthetic activity and exudates (deMarty and Prairie 2009), as will be discussed in the next section.

When DOM shows a recalcitrant character in macrophyte-dominated systems is probably due to the accumulation of the more aromatic fraction of their derived DOM (Tank et al. 2011). Accordingly, the marked humic nature of DOM that dominates the studied lagoon throughout the year suggests that the most labile fraction of macrophyte-derived DOM is being rapidly consumed, as has been suggested in other systems (Tank et al. 2011, Zhang et al. 2013). This is indirectly supported by previous observations on the intensity of carbon cycling in the studied lagoon (Obrador and Pretus 2012).

The autochthonous macrophytic production exerts a cyclical influence on DOM properties in the studied lagoon, following its phenologycal cycle. During macrophyte biomass development in spring and summer, DOM properties exhibit a more labile character, with a protein-like fluorescence and pointing towards a biological origin (increased biological index; Figs. 4.4 and 4.5). Other ecosystem-based processes occurring during certain moments of the year are reflected in DOM properties, as summer bottom hypoxia, when no inflows from the ephemeral washes occur. This hypoxia leads to an increased humic character of DOM in the bottom waters. Also

phytoplankton activity contributed with labile C during summer peaks, and occasional seawater entrances were also traced due to their marked protein-like character.

Limits to reactivity: an analysis of the definition of recalcitrance

A broad definition of reactivity is 'whether or not a substance reacts and how fast it reacts'. In this thesis we aimed to contribute to the current knowledge on how DOM reacts and what processes can influence its reactivity.

In chapter 2 we studied the two main in-lake reactions mineralizing DOC, photodecay and biodegradation. Photodecay can degrade the DOC directly to CO_2 (Granéli et al. 1996) but it also regulates DOC bioavailability, modifying the molecular configuration of the material (Moran et al. 2000, Stubbins et al. 2010). We studied the effect of biodegradation and the combined effect of bio- and photodegradation on two different sources of DOM, since photoreaction rates and bioavailability will depend on the initial DOM quality (Jaffé et al. 2008). In particular, in our experimental set up we worked with water from the ephemeral washes and from the lagoon of the studied catchment. The instantaneous rates of change of spectroscopic descriptors were calculated to evaluate how the character of DOM changed in time (Fig. 2.4). A major finding was that the differential rates of spectroscopic descriptors presented both negative and positive values during the incubation period. Despite frequently used to describe DOC degradation, simple first order decay models are not able to account for DOC reactivity rates over all time scales (Koehler et al. 2012).

It is common to define DOM compartments in relation to differentiated recalcitrance based on their origin (Fig. D-3a; Wetzel 2003) or their mineralization rates (Guillemette and del Giorgio 2011). However, classifying a material as recalcitrant might be misleading. Such an operational classification impedes developing a more mechanistic approach describing changes at a molecular level (Kleber 2010). By using instantaneous rates of change we saw that both production and consumption of fluorophores related with humic (Peak A) and protein like (Peaks B and T) substances occur both during DOC biodegradation or photo + biodegradation processes (Fig. 2.3). Simultaneous production and consumption of fluorescent pools have been reported for biodegradation incubations (Guillemete and del Giorgio 2012). Also, it has been

demonstrated that during UV exposure DOM molecules can be photodegraded, photoproduced and even photoresist (Stubbins et al. 2010). The study of the differential reactivity of DOC and more specifically of qualitative changes reinforces the fact that qualitative and quantitative changes in DOM are not necessarily linked (Jaffé et al. 2008), as previously discussed and supported by the seasonal patterns in the lagoon DOM (Chapter 4). This highlights again the need of studying qualitative changes during DOM reactivity processes.

A second major finding was that the allochthonous DOM from the ephemeral washes showed higher reactivity than lagoon DOM (autochthonous DOM) both when biodegradation acted alone and under the joint action of photo and biodegradation. Despite both DOM sources showed a humic character (Fig. A1, Chapter 2) it was even more aromatic in the case of autochthonous DOC, conversely to what is usually assumed (i.e.- that autochthonous DOC mainly derives from microbial sources). As has been discussed previously, the presence of the macrophytes in the lagoon mostly explains this humic character. The higher degradation rates of allochthonous than autochthonous DOM (Fig. 2.4) responds both to a differential quality of DOM from each of these sources, and to the fact that the exposure of DOM to degradation pathways is much lower in the ephemeral washes than in the lagoon DOM. The water residence time is much lower in ephemeral washes (of the order of a few days) than in the lagoon (8 months, Obrador et al. 2008)

Aside from photoreactions, other processes can mediate DOC uptake (Sinsabaugh and Foreman 2003) although some of them remain poorly explored. Any inflow of materials into a receiving basin implies the contact between two different pools of DOM (Stephens and Minor 2010). Because the effect of this interaction on DOM degradability remains poorly understood, we aimed to fulfill part of this gap in chapter 3. We evaluated the potential effect of the interaction between two DOM pools with very different characteristics; we assessed the effect of adding highly available carbon sources as glucose or acetate to a complex natural DOM assemblage. This interaction was expected to increase the degradation rates of the natural DOM, phenomena known as *priming effect* (Guenet et al. 2010), broadly accepted to occur in soils. Priming could be an important phenomenon in lakes after labile pulses from phytoplankton blooms

or in all the interfaces were two different pools of DOM interact (Bianchi 2011). Here, the priming effect could be an important process in the studied catchment, because the ephemeral washes represent isolated inputs of fresh DOM with higher reactivity than the DOM present in the lagoon. Thus, after reaching the lagoon this allochthonous DOM will either be rapidly photodegraded and mineralized as found in other systems (Lutz et al. 2012), or it will interact with the lagoon DOM pool, modifying DOM processing in the receiving water body.

We looked for evidences of occurrence of the priming phenomena in freshwater systems by testing the effect of different labile carbon additions into the DOC consumption of up to 4 different natural DOC sources. Also, we assessed the effect of nutrients and surface availability. Interestingly, no evidences of enhanced DOC consumption after the addition of a labile carbon source were found in any of the treatments (Table 3.1), allowing us to conclude that the priming effect as currently defined is unlikely to occur in the water column of aquatic ecosystems.

The idea of priming effect relies in the fact that bioenergetics constraints are preventing DOM from being consumed (McCallister and delGiorgio 2012). Thus the labile input would provide the lacking energy to build degrading enzymes (Guenet et al. 2010). However, the microbial population can use this labile carbon to population maintenance and growth, and other constraints might also be limiting DOM degradation. One of these constraints could be stoichiometry. However, our results did not show any significant effect of labile carbon addition on DOC consumption, neither in the oligotrophic waters nor in the treatments were carbon was limiting. In both cases, the labile carbon substrate was likely destined preferentially to population sustenance and growth (Blagodatskaya and Kuzyakov 2008).

Since the priming effect concept was formerly developed in soils, it is likely that physical structure and surface has an important role in that phenomenon (Kleber 2010). Structure might enhance the contact between the substrate and the extracellular enzymes favoring priming occurrence (Arnosti 2003). However, our study did not show any significant increase in DOM mineralization rates after the labile carbon pulse under higher surface availability. Therefore, other constraints besides the bioenergetics are likely to impede DOM degradation in the water column. DOM could be

geopolymerized or complexed becoming physically protected from enzymatic degradation (Fig. D-3b; Chin 2003, Eksmith et al. 2005, Kleber 2010) thus, even if the addition of labile C fulfils the energetic limitations, priming effect might be unable to occur without changes in other protective mechanisms constraining degradation.

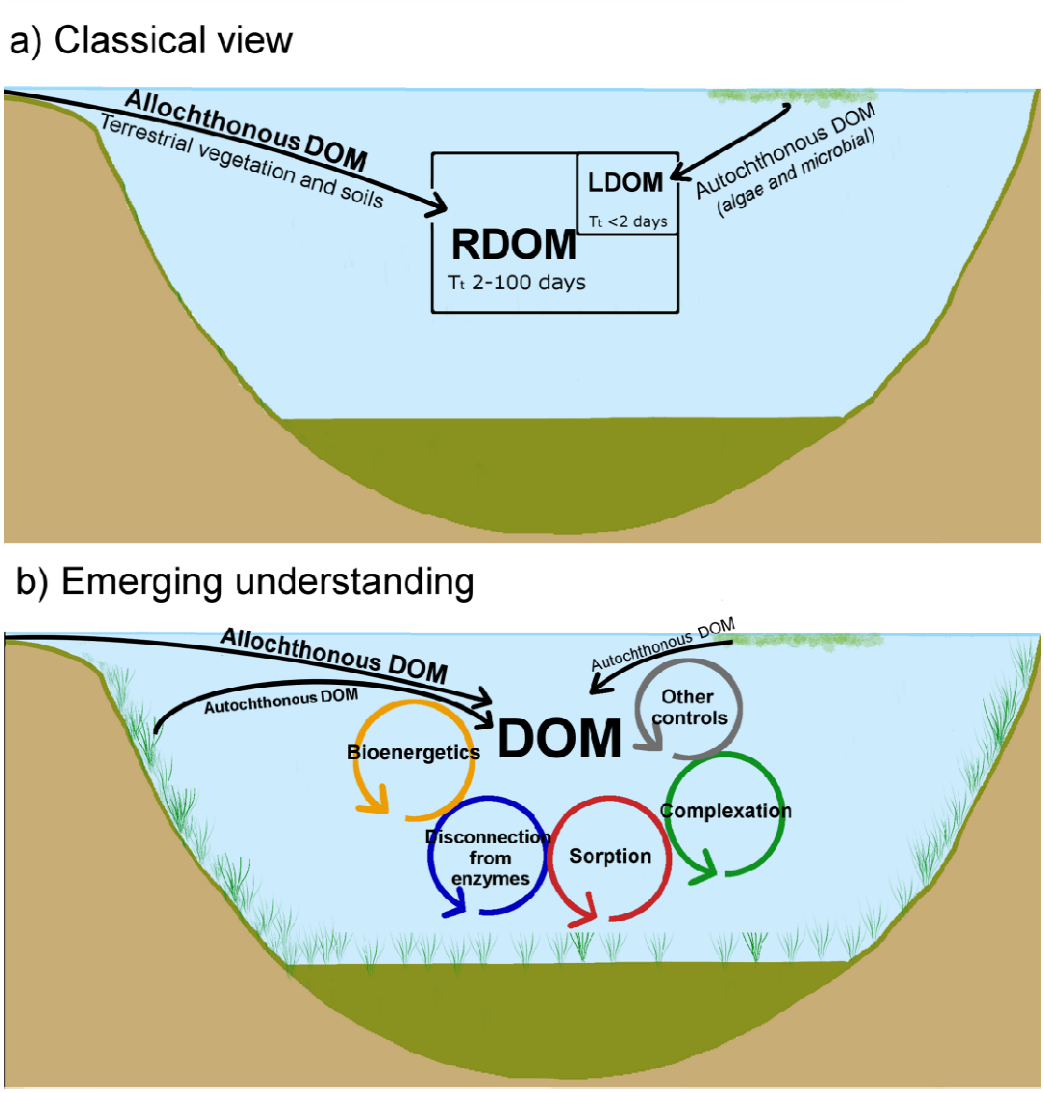

Figure D-3 A synopsis of the contrasting classic and emerging views of the controls of DOM availability. a) the classical view conceives that molecular structure, dependent on DOM origin, determines timescale of persistence. b) The emerging understanding proposes DOC persistence to be determined not only by its origin or structure but also by the combination of multiple processes that control its availability. Adapted from Wetzel 2003 and Schmitd et al. 2011.

Synthesis: perspectives on the scales of DOM reactivity

DOM composition varies in time and space depending on the sources and exposure to degradation pathways. Taking a DOM sample from an aquatic system is taking a snapshot that needs to be placed in a temporal and spatial frame, in a landscape frame, to be able to discuss its processing. We must know how it gets there, where it has been and where it will go.

Some important insights arise from this affirmation. Our work contributes to the pool of knowledge questioning the classic assumption that relates allochthonous with recalcitrant and autochthonous with labile DOM (Fig. D-3a; Guillemette and delGiorgio 2011, Cory and Kaplan 2012). Moreover, this thesis shows that in the Mediterranean context, this relationship is not constant but varies as a result of seasonality and ecosystem processes (Chapters 1, 2 and 4). Thus, considering a receiving water body (as is here the lagoon) and the inflows draining into it (here the ephemeral washes), the relative reactivity of DOM from each source would change on a seasonal basis. Firstly, in chapter 1 we have shown that allochthonous DOM does not always have a marked aromatic character as long as DOM quality varies in a seasonal basis. Secondly, the dominant autochthonous DOM sources are not always rich in lignin-derived compounds because the macrophytic cycle provides both exudates during biomass development and leacheates from senescent plants (Chapter 4). Thus the interaction between the two water masses may lead to different effects throughout the year.

This is in agreement with the fact that the whole recalcitrance concept should be questioned and modified into a more mechanistic approach, as suggested by Kleber (2010) and discussed in the previous section. This novel approach departs from the idea that previously unreactive carbon might be metabolized through a cease of the controls preventing its consumption (Fig. D4b; McCallister and delGiorgio 2012), i.e., all the DOM can potentially be degraded after a shift in environmental settings. This assumption might take special relevance in all type of interfaces (McCallister and delGiorgio 2012, Ekschmitd 2005) and transitional systems (Bianchi 2011). Transitional systems and spots like the interface between streams and their receiving basin (either a lake or the sea; Stephens and Minor 2010) are strongly dynamic and varying systems

expected to be very active from a biogeochemical point of view. Coastal lagoons or ephemeral washes as the ones included in the studied catchment are perfect examples of transitional systems and a framework to illustrate enhanced carbon processing.

Indeed, the studied lagoon can be described as a fast carbon-cycling system. The turnover of carbon in the lagoon has been calculated to be between 13 and 65 times faster than the turnover of water (Obrador and Pretus 2012). As suggested by Weyhenmeyer et al. (2012), the processing of carbon throughout the landscape might change between regions. In that work, the authors hypothesized that in regions where fast flowing rivers dominate the landscape rather unprocessed organic carbon might reach the sea. The results presented here modulate this hypothesis, because a given landscape not only influences water residence time but also the intensity of carbon processing and the quality and diversity of DOM sources. In other words, the DOM leaving the studied lagoon might be as degraded as a 12year-old DOM processed in a boreal landscape.

This landscape frame implies that reactivity and sources of C must be assessed under a multiscale perspective, from molecules to regional features and from instantaneous to seasonal time frames. Unraveling the hidden world of DOM quality must be accompanied by a detailed knowledge of all limnological processes that can influence it. To sum up, and to end the way we started, Prairie's words (2008) can be borrowed again: "*More and more effort is currently devoted to deconstructing the DOC box into smaller compartments. Although all information acquired about these compartments constitutes a positive knowledge gain, I suggest that it is ultimately necessary that it be tested at the ecosystem level where its true importance can be rightfully assessed*".

Conclusions

The conclusions of this dissertation are the following:

1. The quality of the dissolved organic matter present in ephemeral washes is influenced by landscape factors, and this influence varies on a seasonal basis.

2. Two seasonal periods are distinguished in terms of DOM quality in ephemeral washes: autumn, showing an aromatic terrestrially-derived DOM, and winter-spring with a microbial-like DOM.

3. In autumn, the main drivers of DOM properties in ephemeral washes act at a broad scale, including hydromorphological variables such as runoff or catchment slope and the precedent summer drought. During winter-spring, more local processes dominate DOM quality, and differences between subcatchments arise linked to local landscape features such as soil type or land uses.

4. The classical paradigm that links autochthonous DOM with lability and allochthonous DOM with unreactive materials is not supported.

5. The allochthonous DOM draining from the ephemeral washes into the lagoon is more reactive than autochthonous DOM when it is subject to photo- and biodegradation processes.

6. The short exposition to degradation pathways of DOM in ephemeral washes leads to the presence of rapidly degradable compounds, while the longer history and macrophytic origin of lagoon DOM decreases its reactivity.

7. The instantaneous rates of change in DOM quality show that DOM degradation cannot be assumed to universally follow a regular decay pattern.

8. No evidences of enhanced DOM mineralization in freshwater systems due to priming effect are found. None of three labile carbon sources added increase the decomposition rates of the existing DOM in water bodies presenting different trophic states.

9. The nutrient availability and the role of cell attachment to a surface do not play any significant role on the priming effect.

10. The seasonal variability in the quality of DOM in the studied lagoon reflects the interplay between the production and senescence of macrophytes and the pulses of torrential episodes draining the catchment. Other processes like bottom hypoxia, phytoplanktonic peaks or seawater entrances are reflected in the dynamics of DOM quality.

11. DOM inputs from the catchment contribute with aromatic DOM and generate heterogeneities in the water column during autumn. Macrophytes affect DOM in spring and summer emitting labile materials that are believed to be rapidly consumed, remaining the more humic fraction in the lagoon water.

This thesis contributes to the current understanding of the organic carbon processing in aquatic systems and to highlight the need to study it from a landscape perspective. The study of the landscape regulation of organic carbon in aquatic ecosystems requires multiple temporal and spatial scales, from the influence of climate and catchment morphology to the intrinsic DOC reactivity. Further insights on the controls of DOM degradability must arise from the analysis of the relationship between reactivity processes and DOC position in the landscape.

Informe dels directors de la Tesi doctoral

Informe dels directors de la Tesi Doctoral referent al factor d'impacte i a la contribució del doctorand en cadascun dels articles publicats

Els Drs. Biel Obrador Sala i Joan Lluís Pretus del Departament d'Ecologia (UB), directors de la Tesi Doctoral elaborada per la Sra. Núria Catalán García, amb el títol "Sources, transformations and controls of dissolved organic matter in a Mediterranean catchment (Fonts, transformacions i controls de la matèria orgànica dissolta (DOM) en una conca Mediterrànea)",

INFORMEN

Que els treballs de recerca portats a terme per la Sra. Núria Catalán García com a part de la seva formació predoctoral i inclosos en la seva Tesi Doctoral han donat lloc a dues publicacions, un manuscrit en fase de revisió i un altre en fase de preparació. A continuació es detalla la llista d'articles així com els índexs d'impacte (segons el SCI de la ISI Web of Knowledge) de les revistes on han estat o està previst que es publiquin els treballs.

1. Catalán, N., B. Obrador, Alomar, C. and J.Ll. Pretus. 2013. Seasonality and landscape factors drive dissolved organic matter properties in Mediterranean ephemeral washes. *Biogeochemistry* 112: 261-274

   L'índex d'impacte de la revista *Biogeochemistry* l'any 2012 va ser de 3.531. Aquesta revista està situada en el primer quartil de la categoria "Environmental Sciences". Aquesta categoria té una mediana d'índex d'impacte de 1.748 i inclou un total de 209 revistes. Tenint en compte l'índex d'impacte de *Biogeochemistry*, aquesta ocupa el 26è lloc de la seva categoria.

2. Catalán, N., B. Obrador, Felip, M. and J.Ll. Pretus. 2013. Higher reactivity of allochthonous vs. autochthonous DOC sources in a shallow lake. *Aquatic Sciences* (in press).

   L'índex d'impacte de la revista *Aquatic Sciences* l'any 2012 va ser de 2.602. Aquesta revista està situada en el primer quartil de la categoría "Limnology". Aquesta categoria té una mediana d'índex d'impacte de 1.425 i inclou un total de 20 revistes. Tenint en compte l'índex d'impacte d'Aquatic Sciences, aquesta ocupa el 3è lloc de la seva categoria.

3. Catalán, N., A. Kellerman, H. Peter and L.J. Tranvik. Priming effect in aquatic ecosystems: response of lake dissolved organic carbon to labile carbon addtions (en preparació)

4. Catalán, N., B. Obrador and J.Ll. Pretus. 2013 Seasonal variability in dissolved organic matter properties as a fingerprint integrating ecosystem processes in a Mediterranean lagoon. *Hydrobiologia* (submitted).

   L'índex d'impacte de la revista *Hydrobiologia* l'any 2012 va ser de 1.985. Aquesta revista està situada en el segon quartil de la categoria "Marine & Freshwater Biology". Aquesta categoria té una mediana d'índex d'impacte de 1.411 i inclou un total de 100 revistes. Tenint en compte l'índex d'impacte de *Hydrobiologia*, aquesta ocupa el 35è lloc de la seva categoria.

Alhora CERTIFIQUEN

Que la Sra. Núria Catalán García ha participat activament en el desenvolupament del treball de recerca associat a cadascun d'aquests articles així com en la seva elaboració. En concret, la seva participació en cadascun dels articles ha estat la següent:

- Participació en el plantejament inicial dels objectius de cadascun dels treballs
- Disseny i desenvolupament de la part de mostreig de camp i posada a punt de les metodologies de camp i de laboratori associades a cadascun dels capítols. Part d'aquesta tasca va comportar una estada al *CNRS -EPOC Université Bordeaux I* (França) amb el grup de la Dra. Edith Parlanti per a l'aprenentatge de metodologies de mesura de la qualitat de la DOM mitjançant espectroscòpia de fluorescència.
- Disseny i realització de diversos experiments, un d'ells *al Dept. of Limnology* de la *University of Uppsala* (Suècia) amb el grup del Dr. Lars Tranvik
- Processat i anàlisi de totes les mostres obtingudes.
- Càlcul de resultats i anàlisi de dades.
- Redacció dels articles i seguiment del procés de revisió dels mateixos.

Finalment, certifiquem que cap dels coautors dels articles abans esmentats i que formen part de la Tesi Doctoral de la Sra. Núria Catalán García ha utilitzat ni té previst utilitzar implícita o explícitament aquests treballs per a l'elaboració d'una altra Tesi Doctoral.

Atentament,

Barcelona, 18 de juny de 2013

Biel Obrador Sala

Joan Lluís Pretus Real

Resum en català

Introducció

La matèria orgànica dissolta (DOM) és la font primària de carboni orgànic en la majoria d'ecosistemes aquàtics (Wetzel 2001), però també una variable que influeix en les xarxes tròfiques aquàtiques (Jansson et al. 2007), afecta el clima lumínic dels cossos d'aigua (Kirk 1994), determina la disponibilitat de nutrients i metalls (Cammack et al. 2004) i juga un paper clau en el cicle del carboni (C) aquàtic (Amon i Benner 1996; Wetzel 2001; Cole et al. 2007). La DOM és la base del bucle microbià, que retorna el carboni orgànic cap a nivells tròfics superiors mitjançant la seva incorporació a la biomassa bacteriana.

Les aigües continentals tenen un paper rellevant en el cicle global del carboni, transformant-lo de forma activa en el seu camí des dels ecosistemes terrestres cap al mar (Cole et al. 2007, Battin et al. 2009, Tranvik et al. 2009). Nombrosos processos estan implicats en aquesta transformació, des de la floculació (von Wachenfeldt i Tranvik 2008) i la fotomineralització (Bertilsson et al. 1999) fins a la degradació bacteriana (Sondergaard i Middelboe 1995; Amon i Benner 1996; Eiler et al., 2003, Kritzberg et al., 2006). De fet, la comunitat heterotròfica processa la major part del C orgànic en les aigües continentals (Sinsabaugh i Findlay 2003). Atès que l'eficiència de qualsevol via de degradació de la DOM es basa en la qualitat del material, es pot afirmar que tots els processos que intervenen en la transformació de la DOM venen definits per i defineixen la seva composició (Sinsabaugh i Foreman 2003).

Es poden distingir dues fraccions principals en la composició de la DOM: materials húmics i no húmics (Thurman 1985; McDonald et al. 2004). La fracció húmica consisteix en un conjunt complex de compostos, incloent àcids fúlvics, húmics i transfílics (Thurman 1985), i constitueix la fracció principal de DOM (només els àcids fúlvics representen entre el 45% i el 65% de la DOM existent en les aigües fluvials; McKnight et al. 2003). Les tècniques espectroscòpiques han millorat profundament la caracterització de la DOM (McKnight et al 2001; Stedmon et al 2003). Entre elles, els espectres tridimensionals de fluorescència o matrius d'excitació-emissió permeten la identificació de diferents regions espectrals relacionades amb diversos processos

ecològics, propietats funcionals i orígens de DOM (McKnight et al. 2001; Stedmon i Markager 2003; Baker et al. 2008; Jaffé et al. 2008).

En els ecosistemes aquàtics, els orígens de la DOM poden relacionar-se amb fonts autòctones o al·lòctones. Les fonts autòctones deriven del fitoplàncton, les algues bentòniques, el perifíton i les fanerògames aquàtiques presents en un sistema determinat (Bertilsson i Jones 2003; Kritzberg et al. 2004). Les comunitats algals i microbianes són generalment considerades la principal font de DOM autòctona als sistemes aquàtics (McKnight et al. 2001). Les fonts al·lòctones provenen dels vessants de les conques de captació, i deriven principalment de la matèria orgànica present en els residus vegetals i els sòls (Thurman 1985; Aitkenhead-Peterson et al. 2003). Aquestes fonts terrestres alliberen principalment compostos estructurals de les plantes com la lignina i la cel·lulosa, considerats eminntment recalcitrants (Sinsabaugh i Foreman 2003). Tot i així, s'ha demostrat que la DOM terrestre manté gran part de la producció heteròtrofa dels llacs i rius (Pace et al. 2004; Kritzberg et al. 2004; Jansson et al. 2004) i per tant, és en gran part degradable. Així, el paradigma clàssic que relaciona DOM autòctona amb làbilitat i DOM al·lòctona amb recalcitrància s'està revisant actualment (Guillemette i del Giorgio, 2011). A més, malgrat en alguns sistemes altres fonts autòctones com els macròfits poden ser l'origen predominant de la DOM (Barrón et al. 2003; DeMarty i Prairie 2009), la influència d'aquestes fonts en la seva qualitat ha estat poc explorada fins ara (Bertilsson i Jones 2003).

La propietat de recalcitrància de la DOM ha estat tradicionalment definida en termes de la seva biodisponibilitat, en referència a la qualitat d'un material d'ésser fàcilment accessible per als microorganismes (delGiorgio and Davis 2003). Aquesta "accessibilitat" es relaciona típicament amb l'estructura molecular i l'edat del material (Sinsabaugh i Foreman 2003). No obstant, darrerament s'ha observat que sovint, l'edat o l'estructura molecular per sí mateixes no són suficients per explicar l'estabilitat de la DOM (McCallister i delGiorgio 2012), de manera que s'han proposat altres controls sobre la degradació de la DOM que actuen a nivell ambiental i biològic (Schmidt et al. 2011). Entre aquests controls, es troben les limitacions bioenergètiques o enzimàtiques (Arnosti 2003, Ekschmitt et al. 2005, Guenet et al. 2010). La limitació energètica comporta la hipòtesi segons la quals les entrades puntuals de carboni làbil (energia

fàcilment accessible) poden conduir a un augment del consum de la DOM existent en el sistema prèviament no disponible. L'aparició d'aquest fenomen, conegut com a priming effect, en sistemes aquàtics està essent intensament avaluada en l'actualitat per part de la comunitat científica (Guenet et al. 2010, Bianchi 2011), ja que la seva incidència en els sistemes aquàtics és un important buit de coneixement sobre els factors que determinen la degradabilitat de la DOM.

Com hem vist, la DOM és transformada en el seu camí cap al mar i aquestes transformacions vindran definides per les característiques de la conca, és a dir del paisatge. Entenem aquí paisatge com l'entorn físic, incloent els ambients aquàtic i terrestre així com els factors humans que hi interactuen (Soranno et al. 2010), determinant els patrons de processat de la DOM a través de diferents escales temporals i espacials. El paisatge mediterrani ve determinat pel seu clima amb una marcada estacionalitat, amb un estiu sec i un període humit a l'hivern i la tardor; durant aquest darrer període, les inundacions són freqüents i concentren gran part de l'escolament anual (Butturini i Sabater 2000). El període de sequera estival fa de la intermitència una característica comuna dels cursos d'aigua mediterranis (Gasith i Resh 1999), proliferant els torrents i fluxos d'aigua efímers (Uys i O'Keefe 1997). Tot i què els torrents efímers són el tipus de curs d'aigua més freqüent al Mediterrani (Álvarez-Cobelas et. al 2005), els patrons de la DOM en torrents han estat poc estudiats. A més, com les transformacions de la DOM són funció del temps de residència de l'aigua en el paisatge (Weyhenmeyer et al. 2012), els cursos d'aigua de baix ordre, propers en l'espai i el temps a l'origen de la DOM, són un tipus de sistema molt adequat per a l'estudi de les interaccions entre les propietats de la DOM i l'estructura del paisatge (Fellman et. al 2009).

Finalment, la majoria dels cossos d'aigua naturals mediterranis són sistemes poc profunds (Álvarez-Covelas et al. 2005). Els sistemes soms solen ser altament productius i freqüentment estan dominats per macròfits submergits (Valiela et al 1997, Knoppers 1994, de Marty i Prairie 2009). Els macròfits poden ser la principal font de DOM en el cos d'aigua (Wetzel 2003) podent representar fins al 70% de la producció de la planta (DeMarty i Prairie 2009). La tipologia de DOM alliberada així com les taxes

d'alliberament de DOM anirà en funció del cicle fenològic de l'espècie sent molt variable entre la fotosíntesi i la senescència (tanc et al. 2011; Zhang et al. 2013).

El clima mediterrani permet una forta presència de macròfits durant la major part de l'any i, en conseqüència, una font gairebé permanent de DOM autòctona. La conca d'estudi proporciona el marc addient per estudiar els canvis de la qualitat de la DOM en funció de la producció de DOM autòctona i l'arribada de pulsos estacionals de DOM al·lòctona.

Objectius i estructura

Aquesta tesi té com a objectiu determinar les fonts principals, la dinàmica i les transformacions que afecten la DOM en una conca mediterrània. Està estructurada en quatre capítols independents. El primer i quart capítols segueixen la variabilitat natural de la qualitat de la DOM i la seva relació amb el paisatge. En els capítols 2 i 3 es van aplicar dissenys experimentals de laboratori per tal d'estudiar alguns dels principals processos que intervenen en la seva reactivitat i mineralització. Els objectius específics de cadascun dels capítols van ser:

1. En el **primer capítol** es van estudiar set torrents que drenen una conca heterogènia en quant a característiques del paisatge. L'objectiu fou el de caracteritzar les propietats de la DOM i identificar les causes de la seva variabilitat en els torrents, cossos d'aigua adients per estudiar la relació entre el paisatge i la qualitat de la DOM. Així, es va avaluar si la qualitat de la DOM presentava variabilitat temporal i espaial i si aquesta estava lligada a factors del paisatge.

2. Diferents processos determinen les taxes de mineralització de la DOM. En el **segon capítol**, es va avaluar la reactivitat de dues fonts de DOM en una llacuna per tal de testar el paradigma clàssic que atribueix a la DOM autòctona un caràcter làbil i a la al·lòctona una menor disponibilitat. Es va estudiar el paper dels processos biològics i de la fotodegradació mitjançant el seguiment dels canvis instantanis en la qualitat de la DOM durant incubacions de laboratori. Paral·lelament, es va avaluar la idoneïtat de les taxes instantànies de canvi a l'hora de capturar la dinàmica dels canvis qualitatius dels diferents components de la DOM durant els processos de degradació.

3. En el **tercer capítol**, per tal d'adquirir nous coneixements sobre els processos que controlen la degradació de la DOM, es va avaluar la incidència de l'efecte priming a les aigües continentals, hipotetitzat com un mecanisme estimulant la mineralització de la DOM. Es va dissenyar un experiment multifactorial amb diferents fonts de DOM per tal de trobar evidències d'un increment del consum de DOM com a resposta a addicions de C làbil. Paral·lelament es va testar si el consum de DOM variava en funció de l'aigua del llac utilitzada o de la font de C làbil afegida, i si l'efecte de l'addició de C làbil es potenciava mitjançant l'addició de nutrients o la disponibilitat de superfície.

4. En el **quart capítol** es van traçar els canvis temporals de la qualitat de la DOM al cos d'aigua receptor de la conca estudiada en el primer capítol, una llacuna dominada per macròfits, per tal d'avaluar el paper de la vegetació submergida en la qualitat de la DOM. Es va explorar la relació entre les dinàmiques en la qualitat de la DOM de la llacuna, les corresponents fonts de DOM autòctones i al·lòctones i els processos que regulen les concentracions i propietats de la DOM sota una perspectiva ecosistèmica.

Capítol 1

Factors estacionals i del paisatge determinants de la qualitat de la matèria orgànica dissolta en torrents efímers

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Paraules clau: *Carboni orgànic dissolt, torrents efímers, conca Mediterrània, estacionalitat, paisatge*

La hidromorfologia dels torrents ha estat àmpliament estudiada tant en climes àrids com semi-àrids, tot i així, els treballs sobre biogeoquímica en aquests sistemes són escassos i els pocs estudis que existeixen estan centrats en la dinàmica de les concentracions de la matèria orgànica sense abordar-ne els canvis qualitatius. Les característiques de la conca determinen les propietats de la matèria orgànica dissolta (DOM) i indirectament en defineixen també el seu processat. Així, la concentració de la DOM ve definida pel règim hídric i la hidromorfologia mentre què els usos i tipus de sòl tindran tenen una forta influència sobre la seva composició.

En aquest capítol, s'estudien les concentracions i propietats de la DOM en la conca de s'Albufera des Grau, i s'analitzen els factors que determinen la variabilitat espacial i temporal en la seva qualitat. Es van mostrejar un total de 16 episodis d'escorrentia al llarg de més d'un cicle hidrològic complert, en els 7 torrents que conformen la conca d'estudi. Es van analitzar les concentracions de carboni orgànic dissolt (DOC) i nitrogen orgànic dissolt (DON) així com les propietats espectroscòpiques de les mostres (espectres d'absorbància i de fluorescència). Es va estudiar la relació dels descriptors del paisatge de cada subconca (pendent, altitud, àrea, usos del sòl, tipus dominants de sòl i geologia) amb la concentració i qualitat de la DOM.

Els nostres resultats indiquen que tant l'estacionalitat com les variables del paisatge influeixen en la concentració i la qualitat de la DOM en els torrents estudiats. L'estacionalitat és el principal impulsor d'aquests canvis, distingint-se dos períodes

temporals: tardor (AU) i hivern-primavera (WS). Les concentracions més elevades de DOM s'observen durant el període AU, coincidint amb les primeres torrentades. Durant aquest període, els descriptors espectroscòpics de la DOM mostren un increment de l'aromaticitat (SUVA) i el pes molecular, assenyalant cap a fonts terrestres de la DOM. Les variables hidromorfològiques així com l'acumulació de matèria orgànica durant el període de sequera estival es relacionen amb les propietats de la DOM en aquest període.

Durant el període d'hivern-primavera, les concentracions de DOC disminueixen, així com la precipitació. L'origen microbià de la DOM augmenta tal i com indiquen l'index de fluorescència i el peak proteic de les matrius de fluorescència. Paralel·lament, disminueixen l'aromaticitat i el color, tot plegat indicant un increment de la producció in-situ de la DOM durant aquest període, molt probablement degut a la permanència de petits bassals aïllats. Les heterogeneïtats espacials prenen rellevància durant aquest període d'hivern- primavera, i les subconques es diferencien en funció de les propietats de la DOM. Durant aquest període, les característiques geològiques així com els tipus i usos del sòl estan fortament relacionats amb les propietats de la DOM.

Capítol 2

Reactivitat de les fonts autòctones i al·lòctones de matèria orgànica dissolta en un llac som

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***Aquatic Sciences* (en premsa)**

Paraules clau: *carboni orgànic dissolt, reactivitat, fotodegradació, biodegradació, taxes instantànies*

Nombrosos processos intervenen en la mineralització de la matèria orgànica dissolta (DOM) a CO_2 entre ells, la foto- i la biodegradació. Els canvis en la qualitat i biodisponibilitat de la DOM degut als efectes d'aquests processos depenen de la font original de DOM. Tot i que un bon nombre d'estudis analitzen l'efecte de la foto i la biodegradació sobre la DOM, pocs treballs analitzen les taxes de canvi instantànies en la qualitat d'aquesta DOM, malgrat la dinàmica de la seva mineralització no és constant en el temps.

En aquest capítol, s'avaluen els canvis diferencials en la qualitat de les fonts de DOM autòctones (llacuna) i al·lòctones (torrents) degut a l'efecte de la foto- i la biodegradació. Es va dur a terme un seguiment de les taxes instantànies de canvi en les propietats òptiques durant incubacions de laboratori. Les mostres d'aigua es van incubar durant 4 setmanes, la meitat d'elles en la foscor i l'altra meitat exposades a radiació UV, totes elles inoculades amb aigua sense filtrar de la llacuna. Es van mesurar l'abundància i producció bacterianes, així com les taxes instantànies de canvi en la concentració i propietats espectroscòpiques de la DOM.

El caràcter inicial de les mostres indica una major aromaticitat de la DOM autòctona que la al·lòctona, fet degut a l'origen eminentment macrofític de la DOM autòctona i a la menor exposició prèvia a processos de degradació de les aigües de torrents. Les taxes màximes de canvi corresponen al tractament foto- + biodegradació

(UV + BD); en aquest tractament, l'aromaticitat i el pes molecular disminueixen tant en la DOM al·lòctona com en l'autòctona, acompanyats d'un augment de la contribució de la fracció de fluorescència proteica. La DOM al·lòctona presenta major reactivitat i taxes instantànies de canvi més grans que l'autòctona, patint canvis més ràpids i intensos en les propietats de la DOM. Aquesta diferència de reactivitat entre fonts de DOM pot estar relacionada amb diferents temps de residència, de manera que una menor història d'exposició equival a una major reactivitat de la DOM. La DOM al·lòctona presenta també una major labilitat, amb una major proporció de DOC degradat i una ràpida consecució del màxim en l'eficiència de creixement bacterià (BGE).

Els nostres resultats mostren que els canvis qualitatius en la DOM durant la seva degradació no segueixen un patró universal de degradació, tant si la degradació és deguda a l'activitat bacteriana com a la radiació UV. L'ús de taxes instantànies de canvi en les propietats de la DOM permet rastrejar els canvis de qualitat en el temps, sense assumir grups de reactivitat arbitraris i definits a priori. Aquestes taxes són més elevades durant els primers dies d'incubació, i variables al llarg de l'experiment, presentant valors tant positius com negatius en tots els descriptors, fet que indica la generació i degradació de les molècules de DOM com a resultat de la seva reactivitat diferencial.

Capítol 3

Efecte *priming en ecosistemes aquàtics*: resposta de la matèria orgànica dissolta natural a inputs puntuals de carboni làbil

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(en preparació)

Paraules clau: *C làbil, priming effect,consum de DOC, taxes de degradació de C*

Una restricció important en la mineralització del carboni orgànic dissolt és la capacitat dels microorganismes per degradar la matèria orgànica complexa dissolta i particulada en ambients aquàtics. Els factors que determinen aquesta capacitat són poc coneguts i recentment s'ha plantejat la possible rellevància del priming effect, un mecanisme que fa referència a l'observació d'increments en les taxes de descomposició de carboni orgànic prèviament no reactiu després de l'entrada de carboni làbil.

En aquest capítol, amb l'objectiu d'explorar les condicions en què el priming effect pot aparèixer en els sistemes d'aigua dolça, es va realitzar un experiment multifactorial mitjançant mesocosmos, durant el qual es va avaluar el consum de carboni (C) orgànic dissolt sota diferents condicions. Com a fonts de DOM naturals es van utilitzar aigües de tres llacs diferents i un concentrat de DOM procedent d'un riu húmic, per tal d'incloure diferents nivells tròfics i concentracions de DOM. A cadascuna s'hi van afegir separadament tres fonts de C làbils o "primers" al llarg d'un gradient de concentració. També es va manipular la disponibilitat de nutrients (N i P) sota la hipòtesi què una baixa relació C: N facilitaria la degradació de la DOM. Finalment, es va testar l'efecte del factor superfície mitjançant l'addició de perles de vidre, ja què la fixació de les cèl·lules a una superfície, podria potenciar l'activitat exoenzimàtica i afavorir així l'aparició de priming.

Els resultats obtinguts suggereixen què, per a una àmplia gamma de condicions, el priming efect en la columna d'aigua dels cossos d'aigua continentals és poc probable que succeeixi. El consum basal de DOC (% de DOC inicial consumit) va ser similar pels quatre tipus d'aigua i va augmentar després de l'addició de les fonts de C làbil en tots els casos, però en les mostres amb primer era inferior o no significativament diferent que el consum del control més la quantitat de primer afegida ($\Delta DOC_i \leq \Delta DOC_{Control} + DOC_{primer}$).

Es van trobar diferències en el consum de DOC per les mostres amb diferents primers. En els casos en què es trobaven aquestes diferències, el primer que representava un consum més alt de DOC va ser la cel·lobiosa. L'addició de nutrients generà un increment del consum de DOC en els controls, però no el potencia després de l'addició de primer. És per tant probable que una major disponibilitat de nutrients afavoreixi un ús preferent de la font de C làbil. Resultats similars es van trobar per les mostres amb perles de vidre, de manera que el fet de tenir més superfície disponible per tal d'afavorir la fixació de les cel·lules a un substrat, no augmenta la degradació de DOC després de l'adició de primer.

El processat de C pot estar regulat pel que s'han anomenat "estats de protecció temporal" entre els quals és troba la limitació bioenergètica en què es basa l'efecte priming però també l'aïllament del substrat respecte de la població enzimàtica degradant o els mecanismes físics de geopolimerització o complexació. El no detectar un increment de la degradació de DOC després de l'addició de primer fa pensar què aquesta ve determinada per altres d'aquests "estats de protecció temporal" a banda de les limitacions bioenergètiques.

Capítol 4

Influència dels processos ecosistèmics sobre les propietats de la matèria orgànica dissolta en una llacuna

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(enviat a Hydrobiologia)

Paraules clau: *carboni orgànic dissolt, macròfits, descriptors espectroscòpics, Mediterrani, episodis torrencials*

En sistemes marcadament dinàmics com els mediterranis la complexitat i variabilitat de les fonts i processos que regulen la matèria orgànica dissolta (DOM) és elevada. Entre les fonts autòctones de carboni es troben els macròfits submergits, que poden influir fortament sobre la DOM d'un sistema sense necessàriament presentar un caràcter làbil. No obstant la rellevància que poden tenir els macròfits, la bibliografia que tracta de la caracterització i el destí d'aquesta font de DOM és reduïda. D'altra banda, la forta variabilitat hidrològica que pateixen els sistemes mediterranis amb marcades entrades d'aigües torrencials pot implicar importants i sobtades aportacions de DOM al·lòctona al sistema.

En aquest capítol es va determinar la dinàmica estacional i la variabilitat espacial de DOM en la llacuna de s'Albufera des Grau, dominada per vegetació submergida i sotmesa a fortes entrades torrencials d'aigua. Durant un any es van mostrejar tres fondàries d'un punt central de la llacuna, així com diferents punts de la mateixa. Es va caracteritzar la DOM a nivell quantitatiu (concentració de carboni orgànic dissolt, DOC) i qualitatiu mitjançant les propietats espectroscòpiques de les mostres (espectres d'absorbància i de fluorescència). Paral·lelament, es van obtenir i caracteritzar extractes de les principals fonts potencials de DOM (macròfits, aigua de torrents, sediments i aigua marina) i es va avaluar la seva contribució a la DOM de la llacuna.

A mesura que el nombre de processos que afecten la DOM augmenten, tendeix a disminuir la relació entre la concentració i la qualitat del DOM. A la llacuna d'estudi, la manca de correlació entre la concentració de DOM i la fluorescència total, i la variació d'aquesta relació entre els diferents períodes de l'any, apunta cap a múltiples i variables controls de la qualitat i la quantitat de la DOM. Les aigües torrencials mostren una feble activitat biològica i un important grau d'humidificació; la seva entrada a la llacuna aporta materials aromàtics, húmics, acolorits i amb una major fluorescència total. Aquestes entrades influeixen especialment a la superfície de la llacuna, induint heterogeneïtats transitòries a la columna d'aigua.

Els lixiviats de macròfits presenten una major contribució de la regió proteica de fluorescència que l'aigua de la llacuna així com un marcat origen biològic. El fort desenvolupament de les praderies de macròfits a la primavera determina el caràcter de la DOM durant aquesta època. L'origen macrofític explica també el fet que l'augment de la concentració de DOM durant l'estiu no comporti un increment de la fluorescència total, ja que els exudats de macròfits solen incloure una fracció important de materials làbils que presenten poca fluorescència. Aquesta fracció és ràpidament consumida influint fortament en el metabolisme heterotròfic del sistema, romanent a la llacuna els materials húmics menys biodisponibles.

Altres processos puntuals com entrades d'aigua de mar, hipòxia en fondària o pics de fitoplàncton també influeixen la DOM a la llacuna i poden ser detectats mitjançant els descriptors espectroscòpics utilitzats.

Conclusions i perspectives

Les conclusions d'aquesta tesi són les següents:

1. La qualitat de la matèria orgànica dissolta present en torrents efímers està influenciada per factors del paisatge, i aquesta influència varia de manera estacional.

2. Dos períodes estacionals es distingeixen en termes de qualitat de la DOM en torrents efímers: tardor, mostrant un caràcter aromàtic derivat de matèria orgànica terrestre, i l'hivern-primavera, amb un caràcter proteic derivat de l'activitat microbiana.

3. A la tardor, els principals factors determinant la qualitat de la DOM en torrents efímers actuen a gran escala, incloent-hi variables hidromorfològiques com l'escolament o el pendent de la conca i la sequera de l'estiu precedent. Durant l'hivern- primavera, processos més locals determinen la qualitat de la DOM, i sorgeixen les diferències entre subconques lligades a les particularitats topogràfiques locals, com ara el tipus o els usos del sòl.

4. El paradigma clàssic que uneix DOM autòctona amb labilitat i DOM al·lòctona amb materials no reactius no és recolzada pels nostres resultats.

5. La DOM al·lòctona procedent de l'escolament dels torrents efímers drenant a la llacuna és més reactiva que la DOM autòctona quan està subjecta tant a processos de foto- com de biodegradació.

6. La breu exposició prèvia a vies de degradació de la DOM present als torrents efímers facilita la presència de compostos fàcilment degradables, mentre que el major processat i origen de macrofític de la DOM de la llacuna disminueix la seva reactivitat.

7. Les taxes instantànies de canvis en la qualitat de la DOM mostren que no es pot assumir que la seva degradació segueixi un patró universal regular.

8. No s'han trobat evidències d'una major mineralització de la DOM en els sistemes d'aigua dolça a causa de l'efecte priming. Cap de les tres fonts de carboni làbils afegides va augmentar les taxes de descomposició de la DOM existent en diferents cossos d'aigua cobrint diversos estats tròfics.

9. La disponibilitat de nutrients i la disposició de major superfície facilitant-hi la unió de les cèl·lules no juguen cap paper significatiu en l'efecte priming.

10. La variabilitat estacional de la qualitat de DOM a la llacuna estudiada reflecteix la interacció entre la producció i la senescència dels macròfits i els polsos dels episodis torrencials que drenen la conca. Altres processos com la hipòxia fons, els pics de fitoplàncton o les entrades d'aigua de mar es reflecteixen també en la dinàmica de la qualitat de laDOM.

11. Les entrades de DOM procedent de la conca aporten DOM aromàtic i generen heterogeneïtats en la columna d'aigua durant la tardor. Els macròfits afecten la DOM a la primavera i l'estiu emetent materials làbils que es creu són consumits ràpidament, romanent la fracció més húmica a l'aigua de la llacuna.

Aquesta tesi contribueix a la comprensió actual del processat de carboni orgànic en sistemes aquàtics, i posa en relleu la necessitat d'incloure en el seu estudi una perspectiva de paisatge. L'estudi dels processos que limiten el consum de carboni orgànic està prenent rellevància, revisitant-se el concepte de recalcitrància i prenent força la hipòtesi què tota la DOM és potencialment susceptible de ser degradada després d'un canvi en les condicions ambientals que l'envolten.

En relació amb això, els resultats presentats aquí mostren com, un determinat paisatge no només influeix en el temps de residència de l'aigua, sinó també en la intensitat del processat de carboni així com en la qualitat i diversitat de les fonts de matèria orgànica dissolta. L'estudi de la regulació del carboni orgànic a traves del paisatge en ecosistemes aquàtics requereix múltiples escales temporals i espacials, des de la influència del clima i la morfologia de la conca fins a la reactivitat intrínseca del carboni orgànic dissolt.

Es fa necessària nova recerca centrada en l'anàlisi de la relació entre els processos de reactivitat del carboni orgànic i la seva posició en el paisatge. Aquest esforç d'up-scaling generarà aproximacions noves i molt probablement fonamentals per a la determinació dels controls de la degradabilitat del carboni orgànic a gran escala.

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Annex

ANNEX 1. Supplementary material Chapter 2

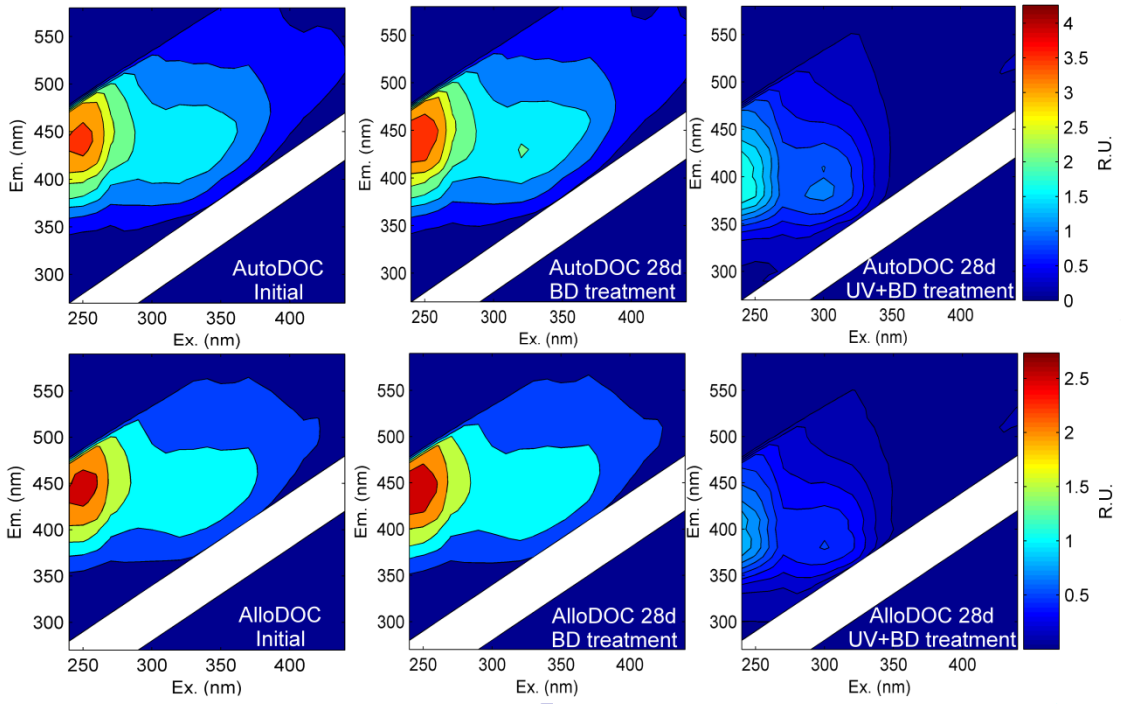

Figure A-1 Excitation–emission matrix fluorescence spectra for the two DOC sources (AutoDOC and AlloDOC) prior to incubation (Initial) and after the 28 days of biodegradation (BD) and photo- plus biodegradation (UV+ BD) treatments. Please note the different scale used for each DOC source.

ANNEX 2. Letter of acceptance Chapter 2

From: Aquatic Sciences (AQSC) <geetha.bhaskar@springer.com>
Date: 2013/5/23
Subject: Your Submission AQSC-D-13-00044R1
To: NURIA CATALAN GARCIA <ncatalan@ub.edu>

Dear Nuria,

Your manuscript, "Higher reactivity of allochthonous vs. autochthonous DOC sources in a shallow lake", has been accepted for publication in Aquatic Sciences. You have done a nice job addressing the reviewer comments and I in particular felt that your decision to interpret the fluorescence EEM data descriptively rather than by PARAFAC to be the correct choice. I have reviewed too many papers where the authors have improperly used PARAFAC resulting in meaningless components.

You will receive an e-mail from Springer in due course with regards to the following items:

1. Offprints
2. Colour figures
3. Transfer of Copyright

Please remember to quote the manuscript number, AQSC-D-13-00044R1, whenever inquiring about your manuscript.

Authors are encouraged to place all species distribution records in a publicly accessible database such as the national Global Biodiversity Information Facility (GBIF) nodes (www.gbif.org) or data centres endorsed by GBIF, including BioFresh (www.freshwaterbiodiversity.eu/).

With best regards,

Yu-Ping Chin

Associate Editor