Universitat de Barcelona

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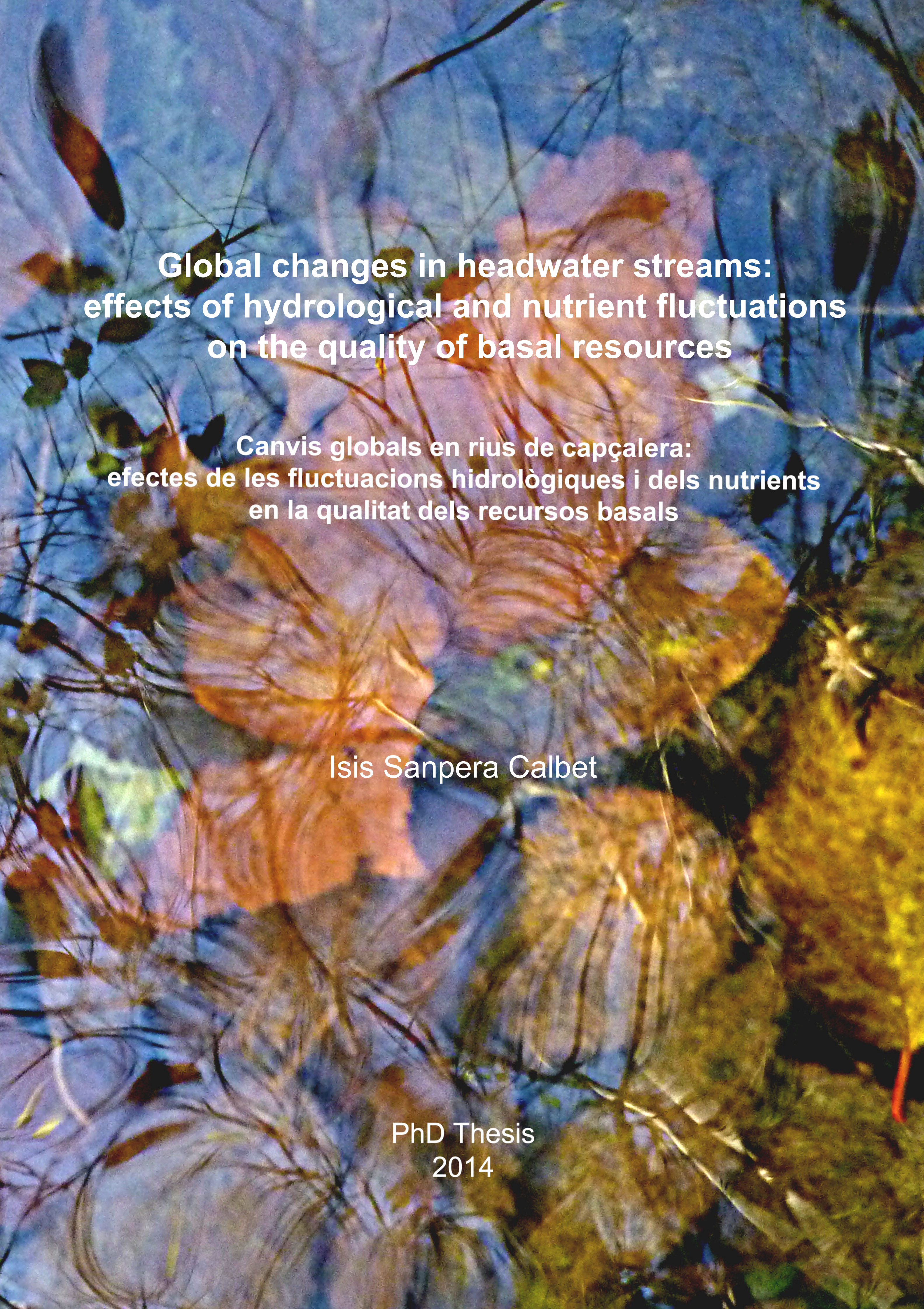
Isis Sanpera Calbet



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PhD Thesis
2014

TESI DOCTORAL
Departament d'Ecologia
Universitat de Barcelona
Programa de doctorat: Ecologia fonamental i aplicada

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Memòria presentada per Isis Sanpera Calbet
per optar al grau de doctora per la Universitat de Barcelona

Isis Sanpera Calbet
Barcelona, febrer 2014

Vist-i-plau de la directora de tesi
Dra. Isabel Muñoz Gracia

Professora titular del Departament d'Ecologia
Universitat de Barcelona

Al Joan, la Pepi i l'Albert.

Desocupado lector: sin juramento me podrás creer que quisiera que este libro, como hijo del entendimiento, fuera el más hermoso, el más gallardo y más discreto que pudiera imaginarse. Pero no he podido yo contravenir al orden de naturaleza, que en ella cada cosa engendra su semejante. (...) El sosiego, el lugar apacible, la amenidad de los campos, la serenidad de los cielos, el murmurar de las fuentes, la quietud del espíritu son grande parte para que las musas más estériles se muestren fecundas y ofrezcan partos al mundo que le colmen de maravilla y de contento. (...) no quiero irme con la corriente del uso, ni suplicarte casi con las lágrimas en los ojos, como otros hacen, lector carísimo, que perdones o disimules las faltas que en este mi hijo vieres, que ni eres su pariente ni su amigo, y tienes tu alma en tu cuerpo y tu libre albedrío como el más pintado, y estás en tu casa, donde eres señor de ella, como el rey de sus alcabalas, y sabes lo que comúnmente se dice, que «debajo de mi manto, al rey mato», todo lo cual te exenta y hace libre de todo respeto y obligación, y, así, puedes decir de la historia todo aquello que te pareciere, sin temor que te calumnien por el mal ni te premien por el bien que dijeres de ella.

Don Quijote de la Mancha (Miguel de Cervantes)

O the river is a vessel
For the water in motion
Heading from the hills
Through the valleys to the ocean

And the water has a river
And the spirit has a body
Sat and listened to the river,
Singing this is what she taught me
(...)
But the river and the body and the nation all are equal
As the borders put imaginary order to the sequal
And the water has a river
And the spirit has a body
Sat and listened to the river,
Singing this is what she taught me.

O the river is a vessel *(Corwin Fox)*

Agraïments

Encara no em crec que estigui arribant al final de la tesi, però em fa moltíssima il·lusió. Aquest viatge no hagués estat tan bonic sense l'ajuda, la companyia, el suport, l'estima i els riures de molta gent, i us ho vull agrair!

Vull agrair a la Isabel Muñoz, la meva directora, l'haver-me donat l'oportunitat de fer aquesta tesi. Gràcies per la confiança que has dipositat en mi, per la teva paciència, per l'oportunitat d'haver anat a diferents congressos on he après molt i per la teva ajuda.
Poder estar al departament d'Ecologia, ha estat un plaer, pel bon ambient i la bona companyia que sempre hi he tingut. Amb l'Esther hem compartit gairebé tota la tesi! Esther, gràcies per haver estat al meu costat en tots els sentits. Has estat la millor 'Co', companya de despatx, de feina, de pis, d'aventures ... Què gran el bottup! I les frases de rajola.
I juntament amb la Bet, vam formar 'Les Triplets'! Compartir el viatge del Nabs amb vosaltres ha estat un dels millors moments de la tesi! Bet, gràcies pels 'a topeee', 'on fire', pels riures i l'organització dels seminaris que hem compartit! Ara som 3D però d'aquí poc serem 3 Dr.!
Mary, la reina del laboratori, moltes gràcies per la teva ajuda, pels cafès i pels moments compartits! Jaime, gracias por hacerme reir tanto con tu humor cáustico y por tu apoyo.
Gràcies també als altres membres del grup, l'Ainhoa, la Núria dC. i el Julio, per la vostra ajuda en diferents moments de la tesi.
Gràcies al Dani (pels 'Què tal?' 'Bé', ja ens entenem i per les teves frases que tinc apuntades, Dani, això ja ho tens!), l'Eusebi (pels 'dines?' i pels teus consells), la Lídia (pels bons moments que hem passat fent les decoracions de Nadal ...), la Neus (dóna-li gràcies a la teva Àvia per recordar-se cada setmana de nosaltres) i l'Alba.
Gràcies Biel perquè quan miro l'Isi web of knowledge m'enrecordo de tu! A la Núria B., per la teva disponibilitat a ajudar sempre amb un somriure i al Pau F. per la teva ajuda.
Hi ha gent que ja ha emigrat del departament, però amb qui he passat bons moments, el Cesc (què tal què tal què tal?), el Carles, la Gemma, l'Oriol, el Christian, la Iraima, la Filipa. I també als que han arribat després que jo, la Sílvia, l'Ada, la Núria C., el Max, l'Aurora, el Pablo, l'Anna L., la Txell i el Lluís (amb qui he compartit alguna campanya últimament), pel bon rollo!

Gràcies a la Patri per la seva ajuda al camp i al laboratori, i gràcies a l'Ameline i al José per haver-me ajudat amb els lípids. Gràcies a la Lourdes, per la seva paciència quan començava a analitzar els lípids als serveis científico-tècnics i a la Regina, per la seva energia.
Gràcies a l'Andrea, per totes les idees que m'has donat, les discussions científiques, i per haver-te mirat els excels amb mi! Gràcies al Quico i al Santi per la reforma del departament, hi ha molta més llum i es treballa molt millor! També li vull donar les gràcies al Joan Armengol, perquè va ser

a partir de les classes de limnologia que vaig fer amb tu i de les pràctiques al Montseny (quins records!), que em vaig interessar per la limnologia!

Treballant amb el grup de Girona he après molt! Gràcies Irene per haver compartit amb mi aquelles campanyes de la fertilització i per haver-me acollit a casa teva! Gràcies a l'Anna R. i al Sergi S., per la vostra ajuda. I gràcies al Lorenzo, per haver compartit aquelles campanyes de fertilització i pels riures!
Gràcies Eugènia, per acceptar formar part de la meva comissió de seguiment i per la teva força. Compartir congressos amb tu ha estat genial (Don't riguis, Isis!). Gràcies també al Vicenç i al Rafa per aportar les seves idees a la primera part d'aquest treball, espero que aviat podrem publicar junts!

I would like to thank John R. for host me in his group in Vancouver and for accepting to be part of my PhD committee. Thanks John for letting me be part of your group, for being always willing to help me and for your comments on this thesis. In Vancouver I felt at home thanks to Xanti and John K. (por apadrinarme), Trisha (for your help and friendship), Karolina (for the experiment we shared, which is in the thesis!), Trent (for our discussions, I miss them!), Misun and Amanda. We formed such a nice group, sharing science and parties. Thanks Hélène and Tracy for your help in the field and in the lab. Thanks Jamie, for sharing that little house with me. Thanks Lisa for your friendship, and thanks, Patrick, for being a TOTALLY great friend. Have you seen the song? Hope I will meet you all soon!

Sam i Mireia, moltes gràcies per la vostra amistat i per totes les aventures que hem viscut juntes! He tingut molta sort de poder estar amb vosaltres a la facultat durant gairebé tota la tesi! Us trobo a faltar!!
Les hores de dinar amb la gent del departa han estat grans moments! Gràcies Jordi, perquè al teu costat no he parat de riure (amb el Ferdinand & Co), pels 'cofi-taims', els partits de bàsquet ... Gràcies Rut, Luis (por organizar tantas actividades en el departa), Guillem, Marta O., íker, Tana, Laia, Gemma, Georgina, Ricard, Salima, Cristina, Marta P. i Marta J. Per totes les excursions, viatges, boletades, calçotades i vídeos de The-parta productions que hem fet junts! Realment hem format un grup molt especial, amb el Rubén, l'Aritz, l'Annabel, la Diana, la Juli. Al Nasho, gracias por haver hecho de nuestra estancia en Granada, un recuerdo maravilloso. I al Giuseppe i al Danilo, per haver-nos acollit a Sardegna, Grazie!
Gràcies també al Miquel, la Lourdes i el Jose del bar de bio, són moooolts cafès.
Gràcies Laura i Ricard, per ser tan bons companys de pis! I pels sopars amb colesterol.

Des de la carrera que hem fet colla i un munt de coses, gràcies Cristina (per la teva generositat!), Irene (ens en sortirem, pel 2050!), Uri (per preocupar-te sempre per mi!), Mar (per les faràndules que hem compartit), Sergio (per la teva ajuda!), Meri (pels riures) i Miguel (pels acudits!). Gràcies a tots per la vostra AMISTAT, comprensió i suport, no sé què hauria fet sense vosaltres! En els moments més durs heu estat allà amb una trucada, un cafè o una birra. I gràcies Néstor, per les converses i les ballaruques!

Merci Claire, Marie-Hélène et Mike, pour votre amitié inébranlable et nos voyages, que j'espère nous pourrons partager à nouveau bientôt!
Merci Antoine, pour être toujours dispo à m'aider avec tes sages conseils et tes réponses à mes doutes stat ... Merci Eric, parce que ça été toujours un plaisir de te rencontrer dans les congrès ou ailleurs.

Gràcies a la millor companyia de teatre, Fem companyia! Fer les dues obres de teatre amb vosaltres ha estat genial. El bolo a Campelles va ser molt especial.
Gràcies Núria, perquè encara que faci molt que no ens veiem, continuem tenint la mateixa connexió i somiant projectes juntes!

I gràcies a la meva família, als tiets, el tio Antonio, la tia Anna Mª, la tia Araceli i la tia Nati, el tiet Josep Mª i la tieta Montse, i els 'cosinets' de Roquetes i Santa Coloma, Antonio, Albert, Anna, Javi, Marc, Agustí, Pepe, Jordi i Lourdes, la Blanca i la Laura, per haver-vos preocupat de com m'ha anat aquest temps. Tinc moltíssimes ganes de dir-vos que això ja està! Moltes gràcies al Jordi, la tieta Carme i al tiet Lluís, pel vostre suport en tot moment i la vostra estima. A la Iaia de Santa Coloma i la Iaia de Roquetes, per tot el que em vau ensenyar.
I gràcies al meu germà, l'Albert, per mostrar-me altres maneres de veure les coses i per totes les coses que hem fet junts.
I això sí que fa temps que tinc ganes de dir-ho. Infinites gràcies als meus Pares, el Joan i la Pepi. Perquè gràcies a vosaltres he pogut estudiar i arribar fins aquí, pel vostre suport i interès en tot el que he fet, per les discussions sobre la tesi ... Però sobretot per haver-me transmès la inquietud per saber i anar més lluny. Perquè sou unes persones meravelloses i uns lluitadors incansables, us dedico aquesta tesi amb tota la meva admiració.

FINANCIAL SUPPORT

The research done in this thesis has received funding from the Spanish Ministry of Science and Innovation through project CGL2008-05618-C02-02 and by the Spanish Ministry of Economy and Competitiveness with project SCARCE-Assessing and predicting effects on water quantity and quality in Iberian rivers caused by global change (Consolider-Ingenio 2010 CSD2009-00065). I would like to acknowledge the University of Barcelona for having granted me with a pre-doctoral scholarship (Ajuts de personal investigador predoctoral en formació, APIF) from 2007–2011, for the travel grants to assist to several meetings and the travel grant to do a research stay at the University of British Columbia (Canada). I also want to thank the Stream and Riparian research laboratory (University of British Columbia) for the opportunity of developing a part of this work in their facilities. I have also received financial support from the Spanish Ministry of Employment and Social Security (2011-2012). Finally, I want to kindly thank J. Sanpera and J. Calbet for their financial support.

Global changes in headwater streams: effects of hydrological and nutrient fluctuations in the quality of basal resources

SUMMARY

Headwater streams (1st to 3rd order) are highly important within the stream network. Headwaters represent most of the total length, influence the structure and functioning of higher order streams, generate most of the water flow and support higher rates of biogeochemical processing, with a higher rate of nutrient removal. From all the drainage basin, the riparian zone has the greatest influence on these streams, modulating light availability and providing sources of energy for stream food webs. Allochthonous basal resources are generally larger than autochthonous energy sources, although both the quantity and quality of these resources influence the structure and functioning of these streams. Quality of resources determines their potential nutritional value for the invertebrates feeding on them and can be assessed by measuring the elemental or biomolecule composition (polysaccharide, protein and lipid content) of organic matter (OM). Lipids are the most efficient energy-storing compounds, and within lipids, fatty acids and sterols include essential molecules for consumers. In a context of global change, streams are highly affected by multiple direct and indirect anthropic impacts (e.g. climate change, land use changes and water course alterations), which affect stream hydrology, water temperature, nutrient concentration or light availability, which in turn affect the quality and quantity of basal resources.

The aim of this thesis was to determine how environmental factors influence the quantity and quality (biochemical composition) of basal resources in headwater streams (i.e., epilithic and epipsammic biofilms, leaves and, transported particulate and dissolved OM). Of the multiple environmental factors affected by global change, the present study focuses on changes in hydrology and nutrient and light availability, with particular reference to headwater Mediterranean streams. This study has been mainly performed in Fuirosos, a Mediterranean stream in the northeast of the Iberian Peninsula, but also in the Mayfly creek artificial channels, in the Pacific Coast Mountains of Canada.

We have demonstrated that in the studied Mediterranean stream, edged by a deciduous riparian forest, the high interannual variability in the riparian inputs was related to precipitation and drought. The El Niño Southern Oscillation (ENSO) affected riparian inputs to the stream through teleconnections that alter average precipitation; during El Niño periods, average annual precipitation was lower than in other periods, causing higher riparian inputs, due to the hydric stress of riparian trees while, during La Niña periods, precipitation was higher than at other times, leading to lower riparian inputs. Drought periods caused a bimodal distribution of annual riparian inputs (summer and autumn peaks) and reduced its quality (higher C : N ratio), whereas accumulated drought events diminished the riparian inputs. Moreover, drought caused a decrease in the fatty acid and the essential fatty acid content in benthic substrates (epilithic and epipsammic biofilms, and leaves) and in transported particulate OM. Before the drought, fatty acids were mainly related to autochthonous production, while allochthonous OM predominated after the drought. Floods decreased the benthic OM, increased the amount of transported OM and modified its quality.

The addition of nutrients led to a higher leaf mass loss and a decrease in carbon content, and higher light availability led to a decrease in protein content of allochthonous resources. However, leaf quality was affected primarily by the conditioning time, which overrode the effects of nutrient and light availability on polysaccharide and lipid content. Shredders showed a preference for consumption of protein-rich leaves. Biofilm quality was generally higher (lower C : N ratio, higher polysaccharide, lipids and essential fatty acids content) with the addition of nutrients, mainly when light availability was also higher.

We consider that the nutritional quality of basal resources is a complex parameter. Whilst elemental and biochemical composition can sometimes be related, knowledge on the latter enables a better understanding of the mechanisms by which abiotic changes affect the quality of resources. Global change, through changes in hydrology and nutrients and light availability, will affect the quantity and quality of allochthonous and autochthonous basal resources, which are limiting for stream food webs.

Keywords: *Mediterranean streams · biochemical quality · fatty acids · allochthonous and autochthonous resources · drought · el Niño Southern Oscillation*

Canvis globals en rius de capçalera: efectes de les fluctuacions hidrològiques i dels nutrients en la qualitat dels recursos basals

RESUM

Els rius de capçalera (de 1er a 3er ordre) tenen una gran importància dins la xarxa fluvial. Els rius de capçalera representen la major part de la longitud total, influencien l'estructura i el funcionament dels rius d'ordre més gran, generen la major part del cabal i donen suport a taxes més altes de processament biogeoquímic, amb una taxa més alta d'eliminació de nutrients. De tota la conca, és la zona ripària la que té una més gran influència en aquests rius, modulant la disponibilitat de llum i proporcionant fonts d'energia per a les xarxes tròfiques del riu. Els recursos basals al·lòctons són generalment més importants que les fonts d'energia autòctones, tot i que, tant la quantitat com la qualitat d'aquests recursos influencien l'estructura i el funcionament d'aquests rius. La qualitat dels recursos determina el seu valor nutritiu potencial pels invertebrats que s'alimenten d'ells i pot ser avaluada mesurant la composició elemental o de biomolècules (contingut de polisacàrids, proteïnes i lípids) de la matèria orgànica (MO). Els lípids són els components d'emmagatzematge d'energia més eficients, i dins dels lípids, els àcids grassos i els esterols inclouen molècules essencials pels consumidors. En un context de canvi global, els rius estan afectats de forma molt important per múltiples impactes antròpics directes i indirectes (per exemple, el canvi climàtic, els canvis d'usos del sòl i les alteracions dels cursos fluvials); els quals afecten la hidrologia i la disponibilitat de nutrients i llum en els rius, els quals al seu torn, afecten la quantitat i la qualitat dels recursos basals.

L'objectiu d'aquesta tesi era determinar com els factors ambientals influencien la quantitat i la qualitat (composició bioquímica) dels recursos basals en els rius de capçalera (és a dir, biofilms epilítics i epipsàmics, les fulles i la MO particulada i dissolta transportada). Dels múltiples factors ambientals afectats pel canvi global, aquest estudi es centra en els canvis en la hidrologia i la disponibilitat de llum i nutrients, en particular als rius de capçalera Mediterranis. Aquest estudi ha estat realitzat principalment a Fuirosos, un riu Mediterrani al nord-est de la Península Ibèrica, però també als canals artificials de Mayfly creek, a les muntanyes de la costa Pacífica de Canadà.

Hem demostrar que en el riu Mediterrani estudiat, vorejat per un bosc de ribera caducifoli, l'elevada variabilitat interanual en les aportacions ripàries estava relacionada amb la precipitació i la sequera. El Niño/Oscil·lació del Sud (ENSO) va afectar les aportacions ripàries al riu a través de teleconnexions que van alterar la precipitació mitjana; durant els períodes de El Niño, la precipitació mitjana anual era més baixa que en altres períodes, provocant un augment de les aportacions ripàries, degut a l'estrès hídric dels arbres del bosc de ribera, mentre que durant els períodes de La Niña, la precipitació era més alta que en altres moments, portant a una disminució de les aportacions ripàries. Els períodes de sequera van causar una distribució bimodal de les aportacions ripàries anuals (pic a l'estiu i a la tardor) i van reduir la seva qualitat (proporció C : N més alta), mentre que els episodis de sequera acumulats van provocar la disminució de les aportacions ripàries. A més, la sequera va provocar una disminució del contingut d'àcids grassos i d'àcids grassos essencials en els substrats bentònics (biofilms epilítics i epipsàmics, i fulles) i en la MO particulada transportada. Abans de la sequera, els àcids grassos estaven bàsicament relacionats amb la producció autòctona, mentre que després de la sequera predominava la MO al·lòctona. Les avingudes van provocar la disminució de la MO bentònica, van augmentar la quantitat de MO transportada i van modificar la seva qualitat.

L'addició de nutrients va portar a una pèrdua de massa més alta de les fulles i a una disminució del contingut de carboni, i una disponibilitat més alta de llum va provocar una disminució del contingut de proteïnes dels recursos al·lòctons. Tot i això, la qualitat de les fulles es va veure afectada principalment pel temps de condicionament, el qual va anul·lar els efectes de la disponibilitat de nutrients i llum en el contingut de polisacàrids i lípids. Els trituradors van mostrar preferència pel consum de fulles riques en proteïnes. La qualitat del biofilm era generalment més alta (proporció C : N més baixa, contingut més alt de polisacàrids, lípids i àcids grassos essencials) amb l'addició de nutrients, quan la disponibilitat de llum era més alta.

Considerem que la qualitat nutritiva dels recursos basals és un paràmetre complex. Tot i que la composició elemental i bioquímica poden estar relacionades en alguns casos, el coneixement d'aquesta última permet una millor comprensió dels mecanismes a través dels quals els canvis abiòtics afecten la qualitat dels recursos. El canvi global, a través de canvis en la hidrologia i la disponibilitat de nutrients i llum, afectarà la quantitat i la qualitat dels recursos basals autòctons i al·lòctons, els quals limiten les xarxes tròfiques fluvials.

Paraules clau: *rius mediterranis · qualitat bioquímica · àcids grassos · recursos al·lòctons i autòctons · sequera · El Niño/Oscil·lació del Sud*

CONTENTS

GENERAL INTRODUCTION

STREAMS: GENERAL CHARACTERISTICS

Streams are present in multiple aspects of society; in history (e.g. the Battle of the Ebro in 1938, during the Spanish Civil War), in literature, as a source of inspiration, a symbolic resource and a poetic metaphor, in cinema (e.g. ‘A River Runs Through It’ directed by Robert Redford), painting (e.g. ‘Starry Night Over The Rhone’ by Vincent Van Gogh) and music (e.g. ‘The Blue Danube’ by Richard Strauss). They are used both as frontiers (e.g. in the U.S.A., the Colorado River separates the State of Arizona from its neighbours, California and Nevada) and as communication routes (e.g. the Danube, which is navigable from Germany to Romania, passing through 10 countries). From an ecological point of view, streams are fascinating because of the complexity of the life and processes they shelter. Streams are highly diverse, ranging from small headwaters to the biggest streams in the world, such as the Amazon or the Nile; they can be open or forested, with clear or black waters; and they can shape the landscape, creating canyons and gorges over millions of years, or flow through broad floodplains approaching the sea (Fig. 1).

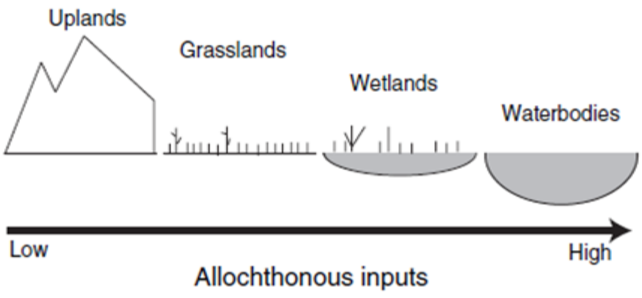
Streams are called lotic ecosystems, which are characterised by unidirectional water movement along a slope in response to gravity. Dissipation of energy from moving masses of water affects stream morphology, sedimentation patterns, water chemistry and the biology of the organisms inhabiting them (Wetzel, 2001). Important changes occur along a stream’s length, through the longitudinal axis. There is an increase in channel size and water volume as tributaries join the network and the drainage area increases. Streams are steeper in the uplands where they originate and slope is more gradual in the lowlands near the mouth. This profile can be divided into three zones, dominated by the erosion, transfer and deposition of sediments. Sediments are received from the headwaters and delivered to lower sections of the stream system. In the lower elevation depositional zone, the stream meanders across a broad, almost flat valley and may become divided into multiple channels as it flows across its own deposited sediments (Allan & Castillo, 2007). Observed at a finer spatial scale, stream segments, extending between an upstream and downstream tributary junction within the stream network, contain stream reaches, which in turn contain a sequence of habitats. These functional habitats, such as pools, riffles, sand and gravel patches and leaf accumulations, are measured and studied as individual but interactive components of the stream reach (Poole, 2002).

Stream ecosystems are open systems with high longitudinal, lateral and vertical connectivity within three major axes where the exchanges are bidirectional: upstream – downstream, channel – margins, and surface – groundwater (Allan & Castillo, 2007). The biota are distributed in relation to these three spatial dimensions of the stream network, including riparian (streamside), benthos (channel bottom) and hyporheos (interstitial streambed sediment), in addition to the fish and other organisms which occupy the water column (Stanford, 2006).

All energy available to consumers originates from primary producers from either out- or in-stream (allochthonous or autochthonous resources, respectively). Simply because of gravity, systems with concave profiles, such as streams, act as spatial attractors and receive higher amounts of subsidies than systems with convex profiles (Lindeman, 1942; Leroux & Loreau, 2008; Fig. 2). Allochthonous organic

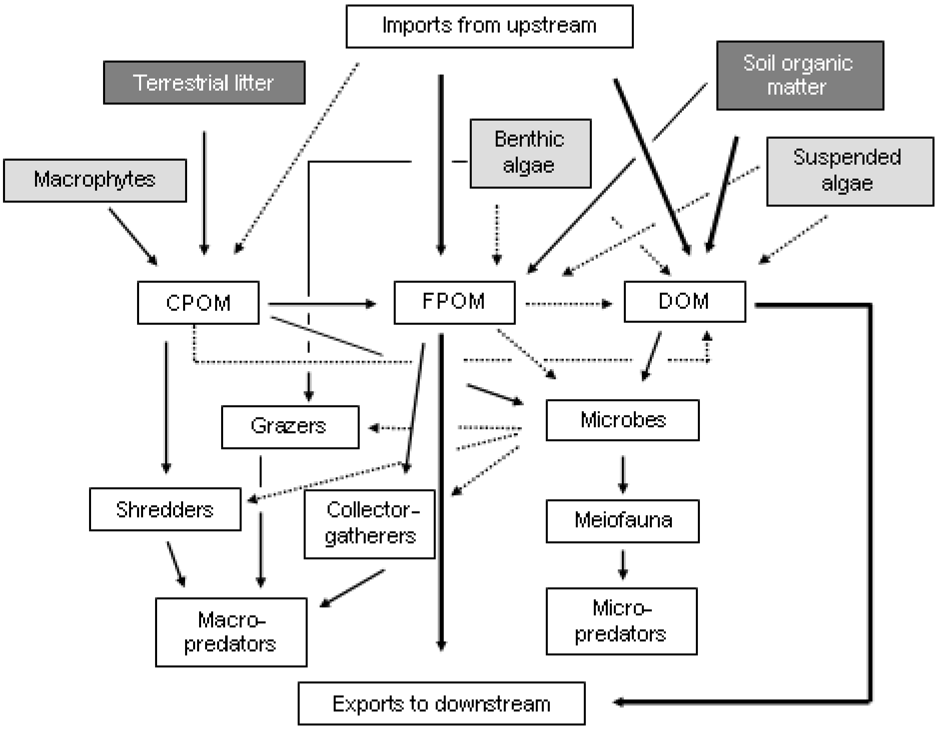
Fig. 1 Several streams, illustrating the high diversity of these ecosystems. There are small streams, flanked by deciduous (Peyreblanque, France; top left) or coniferous riparian vegetation (Athabasca, Canada; top centre), and large streams (the Ebro near its mouth, Catalonia; bottom left); some streams flow through canyons which they themselves have sculpted (Noguera Ribagorçana in the Congost de Montrebei, Catalonia; top right), while others flow freely through the plain (Maligne braided channel, Canada; bottom right; Photos: I. & J. Sanpera).

Fig. 2 Among natural ecosystems, streams are situated at one end of the subsidisation rate gradient, receiving high levels of allochthonous inputs due to their concave profile (from Leroux & Loreau, 2008).



matter (OM) comes from the surrounding land and is transported to the stream from upstream or laterally. Autochthonous resources come from stream primary producers, including algae, cyanobacteria, bryophytes and vascular macrophytes, although benthic algae are usually the main producers, growing on surfaces where conditions (light, nutrients, etc.) are suitable (Lamberti & Steinman, 1997). Food webs are fuelled by a complex mixture of allochthonous and autochthonous resources (Allan & Castillo, 2007; Fig. 3).

Fig. 3 Simplified model of principal carbon fluxes in a stream ecosystem. Stream food webs are basically composed of a complex mixture of autochthonous (light grey shaded) and allochthonous (dark grey shaded) resources. Heavier line indicates dominant pathways of transport or metabolism of organic matter in a woodland stream. Note that storage is omitted (from Allan & Castillo, 2007). CPOM = coarse particulate organic matter; FPOM = fine particulate organic matter; DOM = dissolved organic matter.



Although streams represent only 0.0001% of the water on Earth (Table 1) and occupy just 0.1% of the Earth's surface (Wetzel, 2001), they play a fundamental role in the maintenance and survival of terrestrial life and in the storage and metabolism of dissolved and particulate materials and its transport from the land to the sea (Wetzel, 2001; Battin *et al.*, 2008). Streams, together with all other freshwater ecosystems, support at least 100,000 known species, which represent about 6% of the 1.8 million described species (Dudgeon *et al.*, 2006). Moreover, streams provide humans with multiple services and goods, i.e. water purification and supply, aquifer recharge, food, biodiversity, hydropower, transportation and recreation (Revenga *et al.*, 2000). It has been calculated that services from streams and lakes represent 1.7 trillion U.S. dollars per year (Costanza *et al.*, 1997). This monetary figure, if such a measurement is possible, serves to illustrate the importance of these ecosystems to a sector of society, although if the value of the ecosystem were reduced to its monetary worth, it could lead to the commodification of ecosystems.

Table 1 Water distribution and renewal time of the different compartments in the global hydrologic cycle (from Wetzel, 2001)

Compartments	Volume (thousands of km^3)	Percentage of total (%)	Renewal time (y = years, d = days)
Oceans	1,370,000	97.61	3,100 y
Polar ice, glaciers	29,000	2.08	16,000 y
Ground water (actively changed)	4,067	0.295	300 y
Freshwater lakes	126	0.009	1-100 y
Saline lakes	104	0.008	10-1,000 y
Soil and subsoil moisture	67	0.005	280 d
Streams	1,2	0.00009	12-20 d
Atmospheric water vapour	14	0.0009	9 d

HEADWATER FORESTED STREAMS: IMPORTANCE AND FUNCTIONING

The stream network originates from a myriad of small streams at the headwaters (Meyer *et al.*, 2003), which represent 86–89% of the total channel length (Leopold *et al.*, 1964; Downing *et al.*, 2012) and group streams from orders 1 to 3, after the Strahler (1957) classification (Vannote *et al.*, 1980), although in some studies only 1st and 2nd order streams are considered to belong to this category (e.g. Meyer *et al.*, 2007). Moreover, headwater streams are important due to their influence on the structure and function of higher order streams, linking upland ecosystems to downstream ones. Headwaters provide natural flood control, groundwater recharge (water quantity), retention of sediments and fertilisers and nutrient recycling; they also process terrestrial OM (water quality), sustain biological productivity and contribute to the biodiversity of downstream reaches (Meyer *et al.*, 2003; Lowe & Likens, 2005).

These streams generate most of the water flow (Alexander *et al.*, 2007; MacDonald & Coe, 2007) and contribute to the maintenance of hydrologic connectivity and ecosystem integrity in downstream areas, providing matter, energy and organisms (Freeman *et al.*, 2007). Headwaters support higher rates of biogeochemical processing than larger streams, with a higher rate of nutrient (mainly nitrogen [N] and phosphorous [P]) removal (Peterson *et al.*, 2001; Alexander *et al.*, 2008). Habitat diversity, both within and among streams, is high, creating niches for diverse organisms. These streams offer refuge from extremes of temperature and flow and from competitors, predators and introduced species; they serve as a resource for colonists, providing spawning sites and rearing areas; they are a rich source of food for local and downstream food webs; and they create migration corridors throughout the landscape (Meyer *et al.*, 2003; Lowe & Likens, 2005; Meyer *et al.*, 2007). Because of their geographical isolation, they support genetically isolated populations (Gomi *et al.*, 2002). In these streams, α-diversity (within habitats) may be low, but high β-diversity (between habitats) among headwater streams within and among basins, may generate high γ-diversity (at landscape scale; Clarke *et al.*, 2008; Finn *et al.*, 2011).

Of all the drainage basin, it is the land that borders the stream, i.e. the riparian zone, which has the greatest influence, affecting stream structure and functioning (Naiman & Décamps, 1997). In forested headwaters, the banks are stabilised and slumping prevented by the roots of the riparian zone vegetation, which also controls nonpoint sources of sediments and nutrients; large pieces of woody debris create habitat diversity wherever they fall and the canopy controls the stream's microclimate (temperature) and modulates light availability. The in-fall of vegetation (particulate OM [POM]) and leaching from plant litter (dissolved OM [DOM]) are major sources of energy for stream food webs (Gregory *et al.*, 1991; Naiman & Décamps, 1997; Meyer *et al.*, 1998; Allan & Castillo, 2007).

BASAL RESOURCES IN HEADWATER STREAMS: QUANTITY AND QUALITY OF ALLOCHTHONOUS AND AUTOCHTHONOUS RESOURCES

In headwaters, allochthonous basal resources are generally larger than autochthonous energy sources (Fisher & Likens, 1973; Webster & Meyer, 1997). The quantity and quality of allochthonous resources are limiting factors for stream secondary production (Richardson, 1991; Dobson & Hildrew, 1992; Cross *et al.*, 2006). The importance of the allochthonous OM inputs from the riparian zone has been demonstrated through leaf litter exclusion experiments, where the reduction in OM quantity led to the decline or loss of some taxa and stronger food web interactions among remaining taxa (Wallace *et al.*, 1997b; Hall *et al.*, 2000). The quantity of riparian inputs is related to latitude and annual precipitation at global scale (Benfield, 1997), while at local scale, inputs are determined by riparian forest properties (forest composition and phenology; e.g. Pozo *et al.*, 1997; Menzel, 2002). In deciduous forests, riparian input occurs mostly in autumn, whereas in perennial forests it may be seasonal or irregular, depending on species and location (Benfield, 1997; Hoover *et al.*, 2011). The OM from riparian vegetation can enter the stream by direct fall (vertical) or indirect movement (lateral), and this OM will be retained (benthic OM) or transported downstream (Webster *et al.*, 1999). Retention in the streambed is related to stream morphology, the physical properties of the materials and hydrology (Hoover *et al.*, 2006; Richardson *et al.*, 2009). Retained, large particles of OM (coarse POM [CPOM]) are broken down into smaller particles through physical (abrasion) and biological processing, in the latter case through consumption by invertebrates (shredders) after leaf conditioning by fungi and bacteria (Gessner *et al.*, 1999). Although a large fraction of OM input occurs as CPOM, nearly all transport is as fine POM (FPOM) and DOM. A disproportionate amount of transport occurs during high flows (Wallace *et al.*, 1995).

In addition to the effects of OM quantity (Tiegs *et al.*, 2008), the quality of allochthonous resources influences their processing rate (Leroy & Marks, 2006; Hladyz *et al.*, 2009) and consumer growth and fitness (Graça *et al.*, 2001). Depending on its quality, litter can be classified in fast, medium and slow decomposing (Cummins *et al.*, 1989). Litter inputs are considered as recalcitrant, mainly constituting a source of carbon (C), and their initial nutrient content depends on species, soil fertility and water availability in the riparian zone (Wright *et al.*, 2001). Once in the stream, leaves are enriched in N and P through microbial colonisation and conditioning (Triska *et al.*, 1975; Golladay *et al.*, 1983).

Autochthonous productivity in headwaters is mainly carried out by benthic producers (commonly diatoms, chlorophytes and cyanobacteria) from periphyton. Periphyton can potentially grow on any submerged surface, whether inorganic or organic (Lamberti, 1996), and in forested headwaters it is often limited by abiotic factors (nutrient, light availability; Vannote *et al.*, 1980; Wallace *et al.*, 1997b) and/or biotic factors (grazing; Rosemond *et al.*, 2000). Primary production increases with the watershed area, water flow and soluble reactive P (SRP), but is also negatively related to channel slope and percent canopy cover (Lamberti & Steinman, 1997). In headwater streams draining deciduous forests, the lowest annual primary production was related to the lowest SRP and the highest canopy cover (Lamberti & Steinman, 1997). Benthic algae can grow throughout the year, although in early spring, before leaf emergence, an autochthonous pulse can occur driven by in-stream primary production, related to higher light availability (Rosenfeld & Roff, 1992; Hill *et al.*, 2001). Periphyton quantity at this time has been positively related to consumer growth (Hill *et al.*, 2010). Hence, seasonal changes in basal resources occur in relation to riparian phenology.

Autotrophic production can represent from less than 1% to more than 60% of the total energy in headwaters, and consumption of benthic or resuspended periphyton may support algal-based food webs (Lamberti, 1996). The food quality of algae (labile OM) is considered to be higher compared to detritus (recalcitrant OM; Guenet *et al.*, 2010), but the quality of periphyton can change depending on species composition and habitat (Lamberti, 1996), and due to changes in nutrients and light (Cashman *et al.*, 2013). Algae have a higher content of proteins and lipids than detritus and may contain a higher proportion of certain amino acids and fatty acids (FA) that are essential for consumers (i.e., that consumers can not synthesise; Lamberti, 1996). As a valuable food resource in streams, periphyton quality may increase the fitness of consumers (Stelzer & Lamberti, 2002; Franken *et al.*, 2005).

Quality of resources: assessment and importance

Quality of resources determines their potential nutritional value for the invertebrates feeding on them. The nutritional quality of OM can be assessed by measuring the elemental composition or stoichiometry (C, N, P content and ratios; Cross *et al.*, 2003), the content of refractory compounds (e.g. lignin, tannins; Gessner & Chauvet, 1994; Hladyz *et al.*, 2009), or biomolecule composition (polysaccharide, protein and lipid content; Ledger & Hildrew, 1998; Ylla *et al.*, 2010). Of these components, the higher energy content of lipids (39.35 J mg^{-1}) compared with proteins (23.63 J mg^{-1}) and polysaccharides (17.18 J mg^{-1}) render them the most efficient energy-storing compound for most freshwater benthic organisms (Cavaletto & Gardner, 1999). Stream invertebrates need to store large amounts of energy for metamorphosis and reproduction (Beer-Stiller & Zwick, 1995) or to survive periods of scarcity (Wilhelm, 2002), thus quality of the resources can be more important than quantity for both growth and reproduction in the animal population (Ahlgren *et al.*, 1997). Within lipids, FA and sterols include essential molecules for macroinvertebrates (Torres-Ruiz *et al.*, 2007). Therefore, the presence of these molecules will be important for the fitness of consumers. The effect of essential FA on growth, survival, reproductive rates and food conversion efficiencies has been observed in a wide variety of marine and freshwater organisms, mainly in lakes (Brett & Müller-Navarra, 1997), but not directly in streams.

FA and sterols can be used as biomarkers in the diet (Desvilettes *et al.*, 1997; Mannino & Harvey, 1999) because they are related to some taxonomical groups (Arts & Wainman, 1999), enabling identification of OM composition in terms of large groups (e.g. diatoms, chlorophytes, cyanobacteria and bacteria). Most of the research on FA in freshwater systems has studied lakes or has been conducted in the laboratory (e.g. Cargill *et al.*, 1985; Ahlgren *et al.*, 1997; Kainz *et al.*, 2010; Masclaux *et al.*, 2012). Furthermore, relatively few studies have analysed the FA composition of OM in streams and how this composition changes due to environmental conditions (but see, e.g., Torres-Ruiz & Wehr, 2010; Cashman *et al.*, 2013).

GLOBAL CHANGE IN STREAMS

Streams are highly affected by multiple direct and indirect anthropic impacts at a global scale, the so-called global change. Main impacts are caused by climate change, land use changes and water course alterations, resulting in ecosystem destruction, physical habitat and water chemistry alteration, and species additions and removals (Malmqvist & Rundle, 2002; Stevenson & Sabater, 2010).

Due to their linear and unidirectional nature, streams have an exacerbated vulnerability to these impacts; almost any activity within a stream basin has the potential to cause effects for a large distance downstream. Currently, few streams in the world are pristine, and most have a long history of alteration because streams have acted as magnets for human settlement due to the services and goods they provide (Malmqvist & Rundle, 2002; Allan & Castillo, 2007). The world population is predicted to increase from 7.2 billion (mid-2013) to 9.6 billion in 2050 (United Nations, 2013). Hence, there will probably be a dramatic rise in pressure on streams because all of the forces driving anthropogenic change will increase in magnitude: the size of populations, the growth of cities and industries, the expansion and intensification of agriculture, and the diversion and damming of water (Malmqvist & Rundle, 2002).

Headwater streams are highly sensitive to anthropogenic impacts (and natural disturbances) due to their small size (MacDonald & Coe, 2007). They will probably be affected by climate change first, because they have less thermal and hydrologic buffering capacity and are more highly affected by local precipitation patterns (Heino *et al.*, 2009). Moreover, the ecological impacts on these systems are not limited to a local scale because they also affect downstream reaches (Freeman *et al.*, 2007).

Climate and land use, in conjunction with geology, are the ultimate determinants of hydrology and water quality, thereby acting as the primary drivers of change in the structure and function of streams (Stevenson & Sabater, 2010).

Climate change

There is consensus among scientists that human-induced climate change is warming the planet (IPCC, 2007a), and global warming is expected to negatively affect the quantity and quality of freshwater resources. It will also affect the hydrologic cycle; climate models forecast changes in regional precipitation patterns and an overall higher variability in precipitation that will lead to an increased frequency, magnitude, and unpredictability of droughts and floods in many regions (Lehner *et al.*, 2006).

Warming will cause a reduction in size and earlier melting of snow packs, leading to reduced stream flows and changes in the seasonal hydrology of the receiving streams (Barnett *et al.*, 2008). Climate change will decrease freshwater availability in many areas because of increased losses due to evaporation and human use, and rising water temperatures are expected to have both additive and synergistic effects with other stressors such as nutrient pollution and the spread of exotic species (Dodds & Whiles, 2010).

Land use changes

Land use changes are the transitions from undisturbed to human-dominated landscapes, e.g. to forestry and agricultural, urban, industrial or mining uses. As streams are influenced by the landscapes through which they flow (Hynes, 1975; Vannote *et al.*, 1980), land use of this landscape will have effects on streams. Land-use types have been related to the quantity and quality of stream water (Gburek & Folmar, 1999). The main mechanisms by which land use influences stream ecosystem are riparian clearing or canopy opening, loss of large woody debris, nutrient enrichment, sedimentation, contamination by heavy metals, synthetics and toxic organics and hydrologic alterations (Allan, 2004).

Riparian forest harvesting reduces litter and wood inputs, reduces the buffer capacity of riparian forest to control sediment and nutrient inputs, and it increases stream temperature and light availability (Kiffney *et al.*, 2003; Croke & Hairsine, 2006; Mayer *et al.*, 2007). Human activity has dramatically increased the presence of toxic substances in the environment, especially in the last century. Toxicants are present in mixtures, and in conjunction with other environmental stressors (e.g. drought and nutrients) may cause synergistic interactions (Holmstrup *et al.*, 2010). Moreover, new unregulated contaminants (e.g. pharmaceuticals and personal care products) have emerged as an environmental problem. Annual and storm flows increase with agricultural land use, while base flows often decline owing to reduced infiltration and more episodic export of water (Poff *et al.*, 1997). Enhanced runoff from impervious surfaces and water conveyance systems can cause a greater frequency and intensity of floods, erosion of streambeds, and displacement of sediments (Allan, 2004).

Water course alterations

Water course alterations are due to damming, channelisation or water abstraction. Humans have increased the amount of water available for use by building dams and reservoirs (although more than 40% of the world’s population lives under conditions of water stress). Large dams have increased sevenfold in number since 1950 and now impound 14% of the world’s runoff (Revenga *et al.*, 2000). Dams affect the temperature regime and the quality of the water course. Regulation causes the loss of natural flow periodicity and increases the risk of drought (Malmqvist & Rundle, 2002). Channelisation renders the stream structure uniform, decreasing habitat diversity and reducing nutrient, sediment and OM retention (Elosegi & Sabater, 2013).

EFFECTS OF HYDROLOGY, NUTRIENTS AND LIGHT ON BASAL RESOURCES

Hydrology and nutrient and light availability will be affected by global change. Natural fluctuations in flow regimes, including seasonal droughts and floods, are important for maintaining biodiversity in lotic systems (Ward, 1998). As many stream organisms are adapted to predictable flow changes associated with natural seasonal events, deviations from this pattern may act as a disturbance, affecting stream ecological integrity (Poff *et al.*, 1997; Dudgeon, 2000). Hydrology influences basal resource availability (Biggs & Thomsen, 1995; Acuña *et al.*, 2007; Timoner *et al.*, 2012), but less is known about the relationship between hydrology and basal resource quality (but see e.g. Ylla *et al.*, 2010).

Headwater streams are in general oligotrophic and shaded, so nutrients and light are scarce in these systems (Webster *et al.*, 1995), although both are fundamental for autochthonous primary production (Borchardt, 1996; Hill, 1996). Increases in nutrient concentrations promote microbial activity and accelerate allochthonous OM processing (Greenwood *et al.*, 2007; Benstead *et al.*, 2009). However, invertebrate secondary production, biomass and abundance also increase, demonstrating that changes in food quality (i.e., higher OM conditioning) override changes in food quantity (i.e., faster OM decomposition; Cross *et al.*, 2006). Nutrients can also increase autochthonous biomass, chlorophyll content and primary production (Rosemond *et al.*, 2000; Dodds *et al.*, 2002), although this response may be controlled by light (Hill *et al.*, 2001; Greenwood & Rosemond, 2005; Ylla *et al.*, 2007).

Light availability in the stream is modulated by riparian vegetation (Gregory *et al.*, 1991). Removal of riparian forest in small watersheds is a common practice to obtain more productive land, and increases light availability to the streambed. Light generally favours primary production, which in turn can have a positive effect on herbivorous invertebrate abundance (Sabater *et al.*, 1998). Higher exposure of litter to light can affect the biofilm on leaves, increasing algal biomass and decreasing fungal biomass, but not affecting bacterial density (Albariño *et al.*, 2008). The polysaccharide, protein and lipid content of biofilm is highly correlated with biofilm biomass (e.g. chlorophyll) and, furthermore, with nutrient and light availability (Ledger & Hildrew, 1998). However, less is known about changes in biochemical composition and how these changes could indirectly determine the diet of consumers.

MEDITERRANEAN HEADWATERS: INTERESTING CASE STUDIES

Mediterranean streams are situated in areas with a special climatic setting, characterised by dry and warm summers, cool or moderately cold winters and a high interannual variability in precipitation (Folch, 1993). When undisturbed, these streams are physically, chemically and biologically shaped by sequential, predictable and seasonal events of drought and flooding (Gasith & Resh, 1999). Knowledge of the effects of these events on stream functioning could be applied to other streams at risk of temporality, because the number of temporary streams and the severity of flow intermittence may be increasing (Larned *et al.*, 2010). Furthermore, Mediterranean areas are particularly suitable for human settlement and intensive agricultural production, both of which have a long history, mainly in the Mediterranean basin, resulting in a high demand for fresh water and high human impacts (Cooper *et al.*, 2013). It is predicted that Mediterranean regions will be highly affected by global change (Sabater & Tockner, 2010).

These streams are ecologically unique and perhaps among the most vulnerable to environmental damage from human activities (Bonada & Resh, 2013). The special characteristics peculiar to headwaters and Mediterranean systems render them highly interesting as case studies to assess the effects of abiotic changes caused by global change.

OBJECTIVES

Streams constitute a fundamental freshwater resource; thus, human pressure on these systems is high and diverse. Freshwater systems are highly sensitive to global change and it is a major challenge to identify, diagnose and tackle the effects of such change on these ecosystems. The aim of this thesis was to determine how environmental factors influence the quantity and quality (biochemical composition) of basal resources in headwater streams. Of the multiple environmental factors affected by global change, the present study focused on changes in hydrology and nutrient and light availability, with particular reference to headwater Mediterranean streams. Through this thesis, hydrological drought (hereafter drought) is defined as a seasonal predictable event that begins with a gradual decrease in flow until there is a complete loss of connectivity and surface water and end abruptly in autumn or early winter with the first rains (Gasith & Resh, 1999; Lake, 2003).

This thesis is divided into 4 chapters (Table 2), addressing the following specific questions based on several working hypotheses:

1. Which global and local (out- and in-stream) factors control the dynamics of allochthonous resource inputs in a Mediterranean stream? What effects do these factors have on the quantity and quality of these resources? (Chapter 1)

   Hypothesis: Extreme hydrological (i.e. droughts and floods), meteorological and climatic (El Niño Southern Oscillation) events will determine the dynamics of allochthonous resources. Climate will influence local meteorology, which in turn will shape allochthonous inputs through effects on the riparian vegetation. Both droughts and floods will increase the quantity of inputs due to hydric stress as a result of drought and flood transport. During drought periods, the quality of leaves will be higher due to rapid leaf death. In autumn, nutrient translocation to the tree before leaf abscission will reduce the nutrient content of leaf litter.

2. How does drought influence the quality of basal resources in a Mediterranean stream? (Chapter 2)

   Hypothesis: Drought, through reduction of flow, desiccation and the accumulation of non-processed OM on the dry stream bed, will cause a decrease in the quality of basal resources, measured as the content of fatty acids and sterols.

3. What effect does mid-term nutrient enrichment have on the quality of autochthonous and transported OM? (Chapter 3)

   Hypothesis: Nutrient enrichment will cause an increase in the quality of autochthonous and transported OM, assessed on the basis of biochemical composition (polysaccharides, proteins and lipids), through an increase in the primary producers. The essential components available for consumers will also increase, as most of them come from primary producers.

4. What effect does nutrient and light availability have on leaf litter quality? How do these changes affect resource consumption by shredders? (Chapter 4)

   Hypothesis: Higher nutrient and light availability will increase leaf litter quality (higher polysaccharide, protein and lipid content) through algal colonisation of leaf surfaces, favouring consumption by shredders.

Table 2 Overview of the different aspects addressed in the thesis chapters

	Chapter 1	**Chapter 2**	**Chapter 3**	**Chapter 4**
Environmental factors	Climate, meteorology & hydrology	Drought	Nutrients (mid-term enrichment)	Nutrients & light
Response variables	Quantity & quality (C : N)	Quality (fatty acid & sterol composition)	Quantity, function & quality (C : N; polysaccharide, protein & lipid content; amino acid, fatty acid & sterol composition)	Quality (C : N; polysaccharide, protein & lipid content) Shredder consumption
Substrates	Riparian inputs, benthic & transported organic matter	Biofilm on cobbles & sand, benthic & transported organic matter	Biofilm on cobbles & transported organic matter	Leaf litter
Study duration	Long-term (10 years)	Short-term (118 days) Drying-rewetting process	Short-term (49 days) Spring	Short-term (26 days) Summer
Study site & scale	Mediterranean stream Reach scale	Mediterranean stream Reach scale	Mediterranean stream Reach scale (control & fertilised)	Field experimental channels & laboratory mesocosms

STUDY SITES

To achieve the objectives of this thesis, several samplings and experiments were performed in the field. Most of the field work was conducted in Fuirosos, a Mediterranean headwater stream, while an experiment was carried out in channels alongside the Mayfly creek, a headwater stream in the Pacific Coast Mountains of Canada (Chapter 4).

Fuirosos

The Fuirosos stream is situated in the northeast of the Iberian Peninsula (41º 42' N, 02º 34' E; Fig. 4) in an area with a Mediterranean climate characterised by a more or less extended summer drought, warm summers, cool or moderately cold winters and high interannual variability in precipitation (Folch, 1993). Mean annual precipitation in this area ranges widely, from less than 500 mm to more than 1000 mm (2001-2010). The stream drains a catchment of 15.6 km^2 which is dominated by siliceous geology and is mainly covered by forest, with small amounts of agricultural land (Fig. 5a). Perennial species, including cork oak (*Quercus suber* L.), evergreen oak (*Quercus ilex* ssp. *ilex* L.) and pine trees (*Pinus halepensis* Mill. and *P. pinaster* Ait.) cover most of the catchment, while deciduous species such as chestnut (*Castanea sativa* Mill.), hazel (*Corylus avellana* L.) and oak (*Quercus pubescens* Mill.) predominate at the valley head. The traditional land uses in the watershed are forestry, pasture and agriculture; agricultural fields occupy less than 2% of the total area (Bernal & Sabater, 2008), but pasture has recently been acquiring greater importance. The catchment is located in a protected area, the Montnegre-Corredor Natural Park, and is thus mostly undisturbed, with no marked anthropogenic pressure. It is a third-order stream and a tributary of the La Tordera Stream. The main stem of the stream is 10 km long, with a small, old reservoir located half-way along the stream. In relation to the climate, and in particular to the precipitation regime, the hydrology is highly variable, characterised by extreme events, i.e. floods and droughts (Fig. 5b,c). The stream's basal discharge ranges from 0 to 25 L s^{-1}, while discharge during storms frequently increases up to 500 L s^{-1}, and can exceed 1000 L s^{-1} during severe floods (Butturini *et al.*, 2008). During the period 1999-2012, water flowed through the stream 80% of the time, while during the remaining 20% of the time it was dry. The stream dries out in summer, with a high variability in drought duration (Vázquez *et al.*, 2013). The studied reach is flanked by a well-developed riparian forest (~10 m wide), mainly composed of deciduous species, i.e. plane trees (*Platanus acerifolia* [Aiton] Willd.), alder (*Alnus glutinosa* [L.] Gaertn.), poplar (*Populus nigra* L.) and hazelnut (*C. avellana* L.). The stream banks are steep, forming a closed canopy, except during leafless periods, thus limiting the light reaching the streambed (Sabater *et al.*, 2011; Fig. 5d,e). This stream is oligotrophic (Sabater *et al.*, 2011) and the streambed is composed of riffles dominated by boulders and cobbles, and pools with accumulated gravel and sand.

Fuirosos has been studied for 15 years (1998-2013) by several research teams who have mainly focused on biogeochemistry and ecology, using long-term monitoring, mid- and short-term experiments and hydro-biogeochemical modelling, at different spatial scales (catchment, riparian-stream interactions, reach and sub-reach). Basic studies on geomorphic processes (Sala, 1983) and terrestrial fauna and

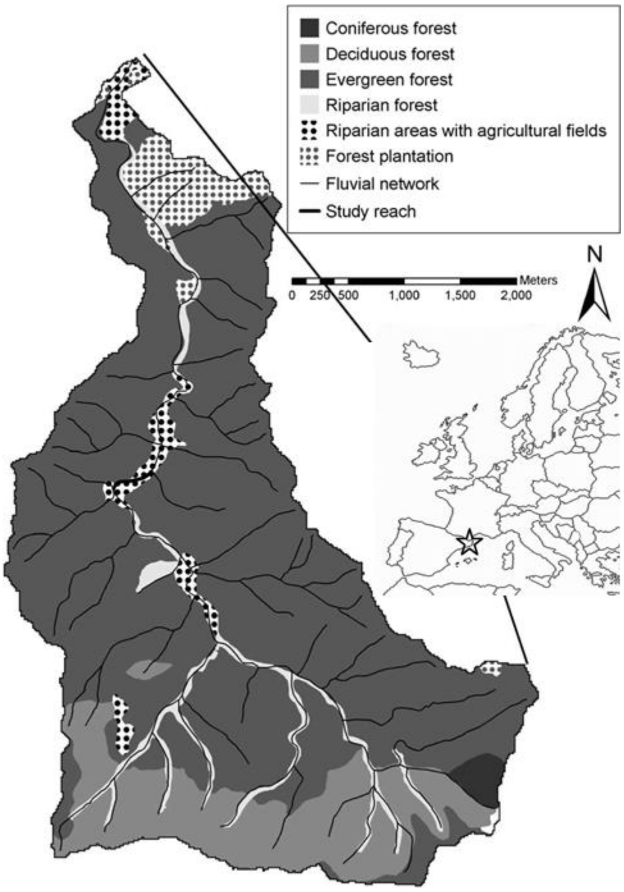


Fig. 4 Location of the Fuirosos catchment, marked with a star, in the Iberian Peninsula and map of the catchment with the vegetation cover, indicated by different shadings (modified from Acuña *et al.*, 2007).

vegetation (Salvà-Caterineu, 2000) are available for this basin. The intra- and interannual hydrological variability (floods and droughts) and the dry-wet hydrological transition, are key in most of the studies. During this time, hydrology has been continuously monitored, as have solutes (chloride, sulphate, dissolved organic C, nitrate and ammonium), and the results are reviewed in Vázquez *et al.* (2013). Ecological studies have also been conducted on OM dynamics and processing, biofilm, meiofauna, macroinvertebrates and fish (Acuña *et al.*, 2005; Acuña *et al.*, 2007; Artigas *et al.*, 2009; Gaudes *et al.*, 2010; Mas-Martí *et al.*, 2010). A mid-term nutrient enrichment in the stream was performed to study the effects of nutrients on the stream structure and functioning (Sabater *et al.*, 2011; Chapter 3). The stream's hydrology, inorganic N and organic C dynamics have also been modelled (Medici *et al.*, 2008; Acuña & Tockner, 2010; Medici *et al.*, 2010).

Fig. 5 A view of the catchment with a tilled terrace in the foreground and forested hillsides in the background (top left). Different facets of the Fuirosos in the study area: the stream after a flood, with turbid water and the transported materials accumulated in a debris dam (top centre); dry streambed due to summer drought (bottom left); in late autumn, when no leaves remain in the canopy of the riparian vegetation (bottom centre) and in spring, during leaf emergence, light availability in the streambed is higher facilitating algal development (top and bottom right). (Photos: I. & J. Sanpera).

Mayfly creek channels

The outdoor stream channels are located in the Malcolm Knapp Research Forest (MKRF; 49º 16’ N, 122º 34’ W) of the University of British Columbia, Canada (Fig. 6). These once-through channels are continuously fed by water diverted from the Mayfly creek (Richardson, 1991), a forested, permanent second-order stream with highly oligotrophic waters (Feller, 1977). This area is covered in temperate rainforest and is located in the Pacific Coast Mountains in the coastal western hemlock biogeoclimatic zone (dry maritime subzone) with a mean annual precipitation of 2200 mm, mainly in the form of rain. Most of this precipitation (70%) falls from October to March, while summers are cool and are the driest part of the year (Feller, 1977; Kiffney *et al.*, 2004). The main riparian species are *Alnus rubra* Bong., *Acer circinatum* Pursh and *Rubus spectabilis* Pursh, all of which are deciduous.

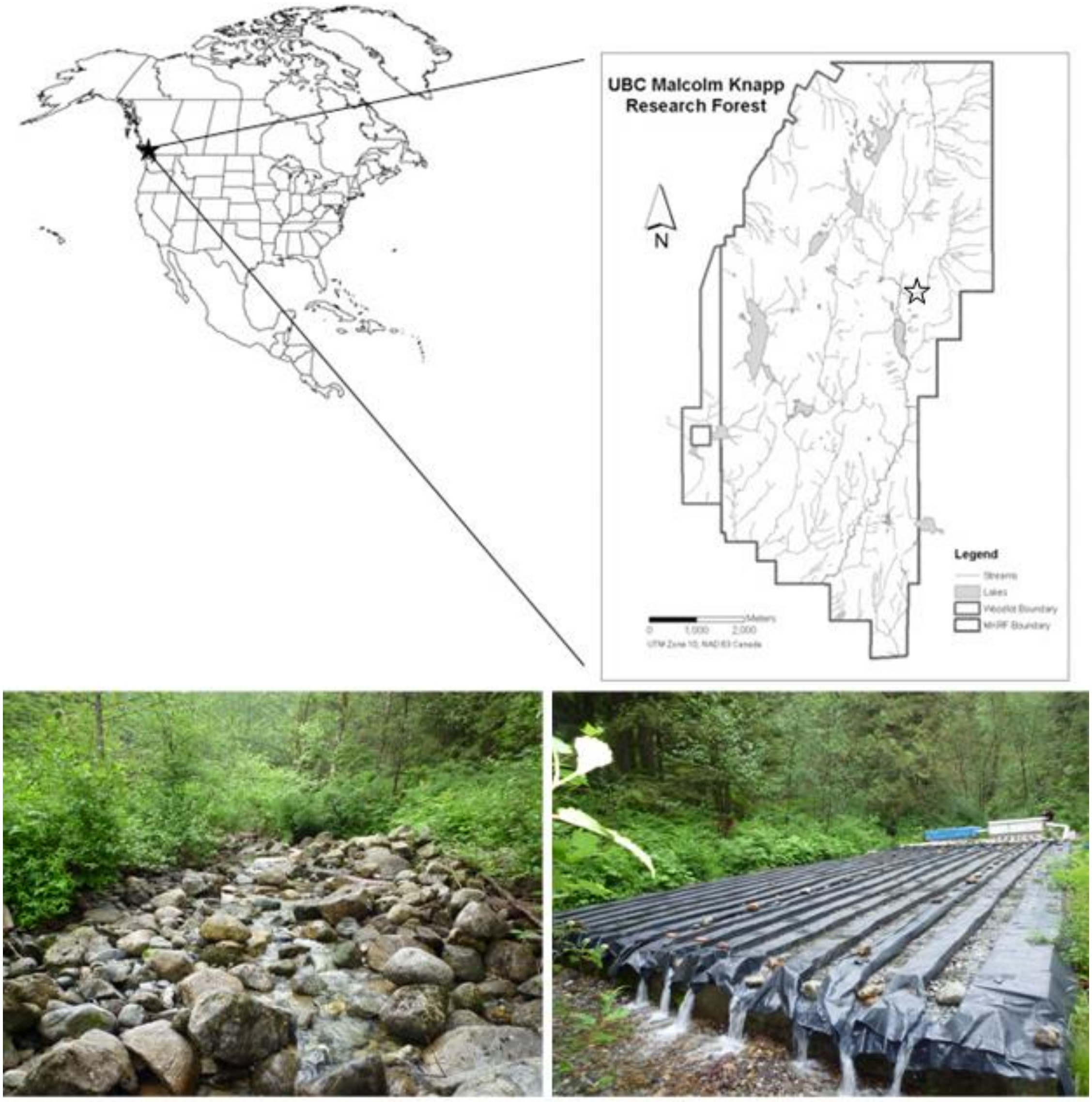


Fig. 6 Location of the Malcolm Knapp Research Forest in British Columbia and location of Mayfly creek channels, marked with stars (top). A view of Mayfly creek at the point where water is diverted to the channels (bottom left) and the stream-side channels (bottom right).

GENERAL METHODS

In order to answer the specific questions addressed in this thesis, several methods were used: collection of climatic and meteorological data, and measurement of stream discharge, water physico-chemical parameters (water temperature, dissolved oxygen, conductivity), light reaching the streambed (Photosynthetically Active Radiation, PAR), particulate and dissolved OM fluxes, C and N content of OM, nutrient (N and P) addition and analysis in flowing water, chlorophyll content, bacterial density and extracellular enzymatic activities of biofilms. These methods, data treatment and statistical methods are explained in each chapter.

Here, a detailed description is given of the methods used to assess the biochemical quality of basal resources (i.e., epilithic and epipsammic biofilms, leaves, transported particulate and dissolved OM). All the samples were freeze-dried prior to analysis and the specific amount of samples used for each analysis, will be specified in the corresponding chapters.

Polysaccharide content

Polysaccharide content of OM was analysed using two different protocols. In Chapter 3, polysaccharide content was analysed in epilithic biofilm and transported POM and DOM, following the 3-methyl-2-benzothiazolinone hydrochloride (MBTH) method (Pakulski & Benner, 1992; Chanudet & Filella, 2006) with some modifications. Samples were acidified with 1 mL of 12 M sulphuric acid for 2 h at an ambient temperature. Then, the samples were diluted with 4 mL of Milli-Q water, sonicated (2 min) and hydrolysed at 100ºC for 3 h. After cooling, the pH of the hydrolysis solution was neutralised with sodium hydroxide. Next, monosaccharides were reduced to alditols by the addition of potassium borohydride. The reduction reaction was terminated by the addition of 2 M hydrochloric acid. The samples were left overnight at 4ºC. The following day, triplicate aliquots of the hydrolysis products (and duplicate blanks) were placed in test tubes and oxidised to formaldehyde by the addition of 0.025 M periodic acid. The oxidation reaction was terminated by the addition of 0.25 M sodium metaarsenite. After the addition of 2 M hydrochloric acid, the aldehyde was reacted with 3-methyl-2-benzothiazolinone hydrochloride (MBTH) reagent, ferric chloride solution and acetone. Absorbance was measured at 635 nm with a spectrophotometer (Spectronic® 20 Genesys, Thermo Spectronic, Cambridge, UK). Glucose standard curves were generated concurrently.

In Chapter 4, polysaccharide content of leaves was quantified using a method adapted from Mansfield *et al.* (2005). Soluble polysaccharides were extracted with a solution of methanol : chloroform : water (12 : 5 : 3) for at least 12 h at 4ºC. The extraction was repeated 2 more times and the supernatant recovered and pooled. Distilled water was added and the top polar phase was collected. Then, orcinol reagent (0.2% in sulphuric acid) was added and the samples were incubated at 80ºC (15 min). Finally, absorbance was measured at 540 nm (Lambda 2 UV/VIS spectrophotometer, PerkinElmer, Waltham, MA, USA) and polysaccharides were quantified by reference to a standard curve of glucose (mg glucose·equivalents g^{-1} DM). Procedural blanks were processed simultaneously with the samples.

Protein content and amino acid composition

In Chapter 4, total proteins were quantified using a method based on Baerlocher *et al.* (2004). Proteins were extracted with a solubilisation buffer (100 mM trizma base, 160 mM sucrose, 1 mM EDTA and 0.1% SDS in ultrapure water, and 0.5% DTT before use). The samples were then frozen with liquid N and thawed with an ultrasonic homogeniser (250 W, 20 kHz; Omni international, Kennesaw, USA) twice. Afterwards, the samples were incubated at 90ºC (5 min), centrifuged at 12,000 g (5 min) and the supernatant collected (stored at -80ºC until use). Forty microlitres of sample were added to 2 mL of assay reagent (solution 1 : 4 of dye stock solution, i.e. 0.5 mg L^{-1} Coomassie blue G, 42.5% phosphoric acid and 25% methanol in ultrapure water). After 50 min, absorbance was measured (3 times per sample) at 595 nm (Lambda 2 UV/VIS spectrophotometer, PerkinElmer, Waltham, MA, USA) and proteins were quantified by reference to a standard curve of γ-globulin (mg γ-globulin·equivalents g^{-1} DM). Procedural blanks were processed simultaneously with samples.

In Chapter 3, samples for amino acid composition and concentration were hydrolysed with 6 M hydrochloric acid at 110ºC for 20 h. The hydrochloric acid remaining in the sample after hydrolysis was removed by N_2 flush and the residue derivatised with a fluorescent reagent (AccQ Fluor reagent, Waters, Milford, MA, USA). Samples were filtered and amino acids analysed using high performance liquid chromatography (HPLC, Waters). The injection volumes were 5 µL for biofilm samples and 10 µL for water samples. Amino acids were identified on the basis of the retention times and quantified by a comparison between the standard (mixture of 17 primary amino acids) and sample peaks.

Lipid content and fatty acid and sterol composition

In Chapters 3 and 4, to quantify total lipids, distilled water was added and samples were homogenised with an ultrasonic homogeniser. Lipids were extracted with a mixture of chloroform : methanol (2 : 1), and chloroform and water were added to separate the 2 phases. The top phase was discarded and the lower phase evaporated (50ºC overnight; Bligh & Dyer, 1959). The following day, 60 µL of sulphuric acid were added, the samples were incubated at 100ºC for 15 min and then 3 mL of vanillin reactive (in phosphoric acid) were added. After 30 min, absorbance was measured at 530 nm (UV-1700 PharmaSpec, Shimadzu, Kyoto, Japan) and the lipids were quantified by reference to a standard curve of cholesterol (mg cholesterol·equivalents g^{-1} DM; Zollner & Kirsch, 1962).

In Chapter 2 and 3, samples for FA and sterol composition and concentration were extracted with a dichloromethane and methanol solution (2 : 1, V : V), and were sonicated for 20 min. To separate the 2 phases, samples were centrifuged for 5 min, and the upper phase was collected. The resulting extract was saponified with potassium hydroxide (6% in methanol) and left overnight. On the second day, water and hexane were added, and the aqueous phase was separated from the organic phase. From the organic phase, the sterols extract was obtained and concentrated under N_2. The aqueous phase was acidified with hydrochloric acid and extracted with hexane. The upper phase was gathered and methylated using boron trifluoride (20% W : V in methanol) overnight. The next day, water and hexane were added, and the extract was concentrated under N_2. All extractions during the protocol were repeated 3 times for each replicate. Samples were kept frozen until analysis. Procedural blanks were processed simultaneously with samples. Internal standards (heptadecanoic or nonadecanoic acid and 5-α-cholestane) were added to the samples and blanks to calculate the yield of the extraction and to correct for the final concentrations. Prior to analysis, sterols were derivatised with bis(trimetilsylil)trifluoroacetamide (BSTFA) for 30 min at 150ºC, and samples were resuspended in hexane. Samples were analysed with a gas chromatograph (GC 8000 series) equipped with a mass spectrometer detector (MD 800; Thermo Fisher Scientific, San Jose, CA, USA). The gas chromatograph was fitted with a SGE BPX70 capillary column (30 m x 0.25 mm) for fatty acid methyl ester (FAME) detection and an Agilent J&W DB5 MS (30 m x 0.25 mm) for sterols. Samples ran in splitless (48 s or 1 min) or split mode depending on the concentration of the sample, with helium as the carrier gas at a flow of 1 mL min^{-1} and the injector temperature at 250ºC/270ºC. The mass spectrometer was in electronic ionisation mode. External standards (Supelco F.A.M.E. Mix C4-C24, Sigma-Aldrich and single sterol standards) were used to identify (by retention time and mass spectra) and quantify (by calibration curves) FAME and sterols, although extra components were also identified. Some results (FAME, Chapter 3) were manually analysed using the GC/MS Software MassLab 1.4 (Finnigan, Manchester, UK) and the rest of the results were analysed with Xcalibur 2.0.7 software (Thermo Fisher Scientific Inc., 1998-2007).

CHAPTER 1

DYNAMICS OF ALLOCHTHONOUS ORGANIC MATTER IN A MEDITERRANEAN STREAM: HYDROLOGICAL, METEOROLOGICAL AND CLIMATIC DRIVERS

ABSTRACT

Forested headwater streams are highly influenced by allochthonous organic matter (OM) subsidies. The goal of this study was to assess the quantity and the quality of OM sources, to determine which local and global environmental factors control the dynamics of OM at reach scale. The study was performed over 10 years on a Mediterranean stream edged by a deciduous riparian forest. Riparian inputs, benthic and transported OM, and its carbon (C) and nitrogen (N) content were determined. The quantity of riparian inputs (912 g dry mass m^{-2} y^{-1}) was comparable to temperate regions with deciduous riparian forests, but the Mediterranean climate determined the different dynamics of these inputs. Distribution of inputs throughout the year depended on the presence of a drought event that same year, with a bimodal annual cycle in the years when the stream had dried up in summer. On the other hand, the annual amount of inputs depended on previous drought events, successive droughts causing a progressive decrease in riparian inputs. El Niño Southern Oscillation highly explained the interannual variability in riparian inputs through changes in precipitation. Retained and transported OM were mainly determined by flood frequency. The quality of allochthonous OM (C : N ratio) increased from riparian inputs to the finer transported particles. Changes in the quantity and quality of OM are explained by both present and past local and global factors, and are potentially important to stream detrital food webs.

Keywords: *deciduous forest · drought · floods · El Niño Southern Oscillation · particulate organic matter · long-term ecological research*

INTRODUCTION

Low-order forested streams are greatly influenced by the adjacent riparian forest (Naiman & Décamps, 1997) through subsidies in the form of materials, energy and organisms (Polis *et al.*, 1997; Leroux & Loreau, 2008). One of the most important basal resources to stream food webs are the allochthonous organic matter (OM) inputs from the riparian zone (Fisher & Likens, 1973). This importance has been demonstrated through leaf litter exclusion experiments (Wallace *et al.*, 1997b; Hall *et al.*, 2000), where the reduction in particulate OM (POM) quantity led to the decline or loss of some taxa and stronger food web interactions among remaining taxa. Besides the effects of OM quantity (Tiegs *et al.*, 2008), the increase in the quality of these resources accelerates their processing (Hladyz *et al.*, 2009) and influences consumer growth and fitness (Graça *et al.*, 2001). Litter inputs are mainly a source of carbon (C), and their initial nutrient content depends on species, soil fertility and water availability in the riparian zone (Wright *et al.*, 2001). Once in the stream, leaves are enriched with nitrogen (N) and phosphorous through microbial colonisation (Triska *et al.*, 1975).

Availability of allochthonous OM for consumers in the stream depends both on inputs and on in-stream retention. Inputs, in turn, are determined by riparian forest properties (i.e. forest structure, composition and phenology). In fact, the dynamics of riparian inputs is different in deciduous versus perennial forests (Hoover *et al.*, 2011) and can change depending on whether species are native or exotic (Pozo *et al.*, 1997), while phenology of leaf fall is directly related to air temperature and photoperiod (Menzel, 2002). Finally, retention is related to stream morphology, physical properties of the materials and hydrology (Hoover *et al.*, 2006; Richardson *et al.*, 2009), increasing with substrate roughness and complexity and depending on the rate of increase in flow (Webster *et al.*, 1987). However, in general downstream transport is higher than retention or biological processing (Webster *et al.*, 1999).

Furthermore, abiotic factors have the capacity to modulate litter quantity and quality. For instance, annual riparian inputs are related to precipitation (Benfield, 1997), and autumn frost or summer drought can cause an incomplete nutrient remobilisation and a consequent change in leaf quality (Keskitalo *et al.*, 2005; Marchin *et al.*, 2010). Besides local variables, global processes can also exert an influence at reach scale. El Niño Southern Oscillation (ENSO) is the dominant oscillation pattern in global interannual climate and it has been demonstrated to have teleconnections in the eastern Iberian Peninsula with effects on precipitation patterns (Rodó *et al.*, 1997), on stream nitrate concentrations (Vegas-Vilarrúbia *et al.*, 2012) and on water inflow and oxygen content in a reservoir (Marcé *et al.*, 2010).

Mediterranean streams are ecosystems with a high intra- and interannual variability. Thus, heavy precipitation events in spring and autumn might cause floods, while high temperatures together with low precipitation create summer droughts in streams (Gasith & Resh, 1999). This temporal variability affects a wide range of stream components and processes, such as dissolved organic carbon (DOC) dynamics (Vázquez *et al.*, 2007), biofilm functioning (Timoner *et al.*, 2012), invertebrate community (Gaudes *et al.*, 2010; Power *et al.*, 2013), nutrient retention (Martí & Sabater, 1996) and metabolism (Acuña *et al.*, 2004). As regards allochthonous inputs, in contrast to temperate streams where inputs occur mostly in autumn (e.g. Abelho & Graça, 1998; Richardson *et al.*, 2009), riparian inputs in Mediterranean streams are more extended over time due to the hydric stress. Moreover, OM retention is highly influenced by flood frequency (Gasith & Resh, 1999; Acuña *et al.*, 2007; Gaudes *et al.*, 2009). Despite the importance of interannual variations, the studies performed on the dynamics of OM inputs in these particular streams are mid-term (from 1 to 3 years; Maamri *et al.*, 1994; Bernal *et al.*, 2003; Acuña *et al.*, 2007).

The goal of this study was to analyse a long-term (~10 years) series of OM dynamics in a Mediterranean stream to i) assess the quantity and the quality of these OM inputs, and ii) determine which global and local (out- and in-stream) factors control these dynamics. Our working hypotheses were that i) extreme hydrological events, i.e. droughts and floods, would increase the quantity of inputs due to hydric stress affecting riparian trees and to OM scoured from the catchment and from upstream by floods; ii) quality of the inputs would decrease (C : N molar ratio increase) in a gradient from the beginning of the summer/drought periods to the end of autumn, due to rapid leaf death caused by drought versus nutrient translocation to the tree before leaf abscission in autumn; iii) inputs of OM would be influenced by meteorological variables (such as precipitation or temperature), modified in turn by ENSO teleconnections.

METHODS

Study Site

This study was performed on the Fuirosos stream (41º 42’ N, 02º 34’ E), which drains a 15.6 km^2 catchment and is located in the Montnegre-Corredor Natural Park, in the north-eastern Iberian Peninsula. Fuirosos is a forested, intermittent, third-order stream, with oligotrophic waters and a basal flow ranging from 0 to 25 L s^{-1}; its hydrology being characterised by extreme events, i.e. droughts and floods (Butturini *et al.*, 2008). The catchment is mainly composed of coniferous and deciduous trees, with small amounts of agricultural land. The area has a typically Mediterranean climate, characterised by mild winters and warm springs and summers, in addition to seasonality and variability in precipitation.

Data for this study were collected from a 50 m long and 2 m wide (on average) reach with a channel slope of 0.019, draining an area of 12.3 km^2. The stream banks were steep with a well-developed riparian forest (~10 m wide), mainly composed of *Platanus acerifolia* (Aiton) Willd. (exotic), *Alnus glutinosa* (L.) Gaertn., *Populus nigra* L. and *Corylus avellana* L., all of them deciduous species, forming a closed canopy (except during the leafless periods). The streambed was composed of riffles, dominated by boulders and cobbles, and pools, with accumulated gravel and sand. The study period lasted from January 2001 to September 2010, although not all variables were measured during the entire period (Table 1).

Hydrological, meteorological *and climate variables*

Water level was monitored continuously during the sampling period with a pressure transducer (PDCR 1830, Druck limited, Leicester, UK). Stream flow was determined every 2 weeks by the slug-injection method with sodium chloride as a conservative tracer. These measurements and the corresponding water level provided an empirical state-flow relationship (Gordon *et al.*, 2004). In this

Table 1 Available period and frequency of the variables used in the study, including the gaps in the data

Variables	Available period	Frequency	Gaps / less frequent data
Temperature	01/2001 - 12/2010	Daily	2/10 - 17/11/06, 11/10/09 - 9/5/10, *1/10 - 31/12/10
Precipitation	01/2001 - 12/2010	Daily	2/10 - 17/11/06, 11/10/09 - 9/5/10, *1/10 - 31/12/10
RH	01/2001 - 10/2009	Daily	2/10 - 17/11/06
Wind velocity	12/2002 - 09/2008	Daily	
Cumulated radiation	01/2001 - 12/2010	Daily	1 - 18/10/08, 14/5 - 8/8/09
PET	01/2001 - 12/2010	Daily	1 - 18/10/08, 14/5 - 08/8/09
VPD	01/2001 - 10/2009	Daily	2/10 - 17/11/06, 11/10/09 - 31/12/10
Photoperiod	01/2001 - 12/2010	Daily	
Flow	01/2001 - 12/2010	Daily	24/11/03 - 11/10/05 (daily to monthly frequency)
Riparian inputs	01/2001 - 09/2010	Monthly**	26/4 - 24-5/02, 2/11 - 13/12/02, 22/1 - 19/03/03, 29/7 - 8/8/03, 15/3 - 26/4/04
			25/1 - 8/5/06 (V), 25/1 - 20/3/06 (L), 16/1 - 13/3/09 (V), 16/1 - 13/2/09 (L)
Riparian inputs C : N	01/2003 - 09/2010	Monthly**	22/1 - 19/03/03, 29/7 - 8/8/03, 15/3 - 26/4/04, 13/6 - 25/8/05
			25/1 - 8/5/06 (V), 25/1 - 20/3/06 (L), 16/1 - 13/3/09 (V), 16/1 - 13/2/09 (L)
Transported POM	02/2001 - 06/2005	Monthly**	
Transported POM C : N	04/2003 - 06/2005	Monthly**	
DOC	01/2001 - 12/2010	Monthly	Drought periods
SOI	01/2001 - 12/2010	Monthly	

RH= relative humidity, PET = potential evapotranspiration, VPD = vapour pressure deficit, C = carbon, N = nitrogen, POM = particulate organic matter, DOC = dissolved organic C, SOI = Southern Oscillation Index, (V) = gap in vertical inputs, (L) = gap in lateral inputs.

*OM sampling was already finished.

**Average frequency 43 ± 2 d.

stream, flows of between 250 and 1012 L s^{-1} are considered to create moderate floods (sediment motion), and flows higher than 1012 L s^{-1}, severe floods (streambed disruption; Sabater *et al.*, 2008). From hydrological data, drought length (days with superficial flow = 0 L s^{-1}) for each year and an average drought period and length for the whole period were calculated. Cumulative drought length for each year was calculated by adding the drought length of that year to the drought length of the previous years until a year without drought happened. To calculate the cumulative drought for 2001, droughts in 2000 (76 days), 1999 (108 days) and 1998 (70 days), were taken into account. Data before 1998 were not available, so the value for 2001 might be underestimated. Furthermore, the cumulative number of drought events was calculated by adding 1 to the value of the previous year the years with a drought event and subtracting 1 the years without drought. As information was not available from before 1998, an initial arbitrary value of 0 was assigned to 1997.

Meteorological data (i.e. temperature, precipitation, relative humidity, wind velocity, cumulated radiation and potential evapotranspiration [PET]) were provided by the Diputació de Barcelona from 2 weather stations within the natural park, Pla de la Tanyada and Collsacreu. Vapour pressure deficit (VPD) was calculated as the difference between the saturation vapour pressure (following Buck, 1981) and the actual vapour pressure (relative humidity x saturation vapour pressure), and photoperiod was calculated as the difference between the sunrise and sunset times in Barcelona (Instituto Geográfico Nacional, 2012). A ratio between PET and precipitation (PET/Prec ratio) was calculated to indicate the degree of water deficiency.

The Southern Oscillation Index (SOI), ranging from -3.5 to 3.5, was used to characterise SO phases. Monthly values of SOI were obtained from the Climate Research Unit (East Anglia University), which are calculated based on the method described by Ropelewski & Jones (1987). Five-month running mean values of the SOI remaining below -0.5 standard deviations for 5 months or longer indicate El Niño phases, while those over +0.5 standard deviations for 5 months or longer indicate La Niña events (Ropelewski & Jones, 1987; Ropelewski & Halpert, 1996).

Organic matter classification

OM in the stream was classified depending on its particle size and origin (see below for the methods). The analysed fractions were coarse POM (CPOM; particles > 1 mm), fine POM (FPOM; particles 0.05–1 mm), ultrafine POM (UFPOM; particles 0.7 µm–0.05 mm) and dissolved OM (DOM; particles < 0.7 µm). The origins studied were the OM entering into the stream from the riparian forest (riparian inputs; RI as CPOM), collected separately as direct or vertical (VI) and indirect or lateral inputs (LI), the total OM stored in the stream bed, i.e. the benthic OM (BOM) and the OM transported by the stream, separated into CPOM, FPOM, UFPOM and DOM.

Riparian organic matter inputs

VI were collected using vertical traps measuring 1 m^2 (1 x 1 m) and suspended above the stream surface (n = 5; Fig. 1), while LI were collected with traps (0.55 m long) installed at ground level on the stream sides (n = 10; 5 on each side). All traps were fitted with 1 mm mesh size and were situated every 10 m along the reach. The OM collected from the traps was oven-dried at 60ºC and dry mass (DM) was determined. Total RI were calculated as the sum of LI rate (g DM m^{-1} d^{-1}) divided by the stream width, and VI rate (g DM m^{-2} d^{-1}). The C and N content of RI was determined with an elemental analyser (EA 1108, Thermo Fisher Scientific, Milano, Italy) using vanadium pentoxide as the oxidation catalyser, and the C : N molar ratio was calculated. During the first period (01/2003 to 06/2005), C and N were measured separately for VI (n = 3) and LI (n = 3). The quality of VI and LI was not significantly different (Student's t-test: t_{48} = 1.353; P = 0.182); therefore, both qualities were subsequently measured together (n = 3 in total) and replicates were treated together for the entire period.

Total annual RI were calculated for 'calendar' years (January to December year X) to analyse interannual variability. Total VI were calculated for 'phenological' years (April year X to March Year X+1) to analyse the effects of environment on the tree phenology from the moment of leaf emergence, and for 'post-drought' years (October year X to September year X+1) to test for the effects of drought periods.

Fig. 1 The study reach with the traps to collect riparian vertical inputs, suspended above the stream surface.

Benthic and transported organic matter

BOM was measured by collecting the OM from a corer (14 cm diameter), randomly situated in detritus covered areas (n = 3). Samples were oven-dried at 60ºC and DM determined. The streambed coverage (%) by OM (leaves, branches and fine detritus) was obtained through visual estimation of transects along the reach (n = 5). Then, BOM as OM per reach surface was calculated (g DM m^{-2}).

Transported CPOM was measured with a net of 1-mm mesh size and transported FPOM with a net of 0.05-mm mesh size. The 2 drift nets (20 x 20 cm opening at the mouth) were installed in the reach for intervals of 45 to 90 min (upstream and downstream; n = 2). Samples were oven-dried at 60ºC and DM was determined. Transported UFPOM was measured by filtration through pre-weighed GF/F filters (0.7 µm pore size; Whatman, Maidstone, UK) of a certain stream water volume (previously filtered by 0.05 mm net; n = 3). Filters were oven-dried at 110ºC and combusted at 450ºC for 4h to obtain the ash-free DM (AFDM). Concentrations of POM fractions were obtained by dividing the DM by the volume of filtered water (mg DM L^{-1}).

C and N content of POM fractions was determined in the same way as explained for *Riparian OM inputs*. DOM content in stream water was estimated in terms of C content (DOC). Samples were refrigerated and transported to the laboratory, filtered through precombusted GF/F filters, acidified with 2M hydrochloric acid (2%) and preserved at 4ºC until analysis. DOC concentrations were determined by oxidative combustion-infrared analysis using a total organic C analyser (TOC-V_{CS}, Shimadzu, Kyoto, Japan). Monthly DOC concentrations were obtained by averaging 4–8 samples collected manually at weekly frequency.

Data treatment and statistical analyses

Hydrologic and meteorological data were averaged by ‘calendar’ years and seasons (between equinox and solstice dates within each year) to describe interannual variability during the study period. Variables referring to RI rates were average values taking into account the OM found in the traps on a sampling date and the days between samplings, assuming that input rates remained constant between samplings. Daily meteorological and hydrological data were averaged for the periods between each sampling of OM. Thus, for each sampling date, data had a spatial variability for the organic matter inputs (n = 5/10 traps) and a temporal variability for the meteorological/hydrological variables (average of daily values). This variability, although represented in the graphs, was not taken into account in the analyses.

Two-way ANOVA was used to test the effect of season and drought (years with or without drought), and its interaction, on VI (seasonal average for each year). The normality of residuals (Kolmogorov-Smirnov [with Lilliefors correction] and Shapiro-Wilks tests) and homoscedasticity (Levene’s test) were tested and a post hoc Tukey HSD test was conducted for the season factor. Correlations between OM variables and hydrological, meteorological and climate variables were tested using non-parametric correlation analyses (Spearman’s rank correlation coefficient, r_S). When 2 or more variables were correlated with the independent variable but showed collinearity ($r_S > 0.95$), only the variable explaining the highest variability of the independent variable was kept. All analyses were performed with PASW (version 18, IBM, Armonk, NY, USA).

Direct gradient analyses were used to relate quantity and quality of RI to environmental data. Environmental and qualitative data were standardised and quantitative variables were log-transformed. From environmental variables showing collinearity, the one explaining the highest variability was included in the analysis. Detrended canonical correspondence analysis (DCCA) was first performed to decide on the use of unimodal or linear methods. DCCA indicated that the maximum gradient length was shorter than 3.0 units (1.076 for RI quantity and 0.409 for RI quality); therefore, the use of linear ordination techniques was considered appropriate (Lepš & Šmilauer, 2003) and redundancy analyses (RDA) were performed. Multivariate analyses were performed with CANOCO (version 4.5, Biometris, Wageningen, the Netherlands).

RESULTS

Hydrological, meteorological and climatic description

From 2001 to 2010, years varied widely in terms of hydrological (Fig. 2a) and meteorological features (Table 2). For example, 2003 was a very hot year, with the highest mean temperature of all the studied summers (21.6ºC) and the highest absolute maximum temperature (36.6ºC). That year, PET, VPD and PET/Prec ratio (107.3) also presented the highest daily means of the entire study period. On the other hand, 2005 was a cold year with the lowest daily mean temperature and the lowest absolute minimum (-7.3ºC). 2002 was a very humid year, with the highest annual precipitation, mainly concentrated in spring (5.76 mm d^{-1}), and the lowest PET/Prec ratio daily mean (0.62). Accordingly, that

Table 2 Main hydrological and meteorological features of the studied years in the Fuirosos catchment; daily mean values of temperature (ºC), potential evapotranspiration (PET; mm) and vapour pressure deficit (VPD; kPa), median values of flow (L s^{-1}), annual mean of precipitation (mm y^{-1}), annual and cumulative drought length (d), cumulative number of drought events and annual floods (d)

	Temperature		**Precipitation**		**Flow**		**PET**		**VPD**		**Drought**	**Cum. drought**	**Cum. drought**	**Floods**	
Year	Mean	SD	Mean	SD	Median	SD	Mean	SD	Mean	SD	**length**	**length**	**events**	**M**	**S**
2001	13.0	6.3	631.0	2706.3	8.97	276.60	1.90	1.23	0.48	0.42	102	356*	4*	2	3
2002	12.7	5.2	1015.4	3643.1	18.62	370.85	1.72	1.12	0.40	0.34	0	0	3	13	7
2003	13.1	7.2	795.4	2833.2	15.78	259.59	2.47	1.87	0.54	0.57	84	84	4	10	4
2004	12.2	6.2	732.7	2899.7	5.39	164.13	2.16	1.37	0.36	0.28	0	0	3	2	0
2005	11.7	6.9	674.9	2482.6	18.05	148.04	2.23	1.54	0.39	0.36	0	0	2	5	1
2006	13.3	6.5	479.4	2398.1	3.14	312.46	2.28	1.42	0.44	0.40	83	83	3	5	3
2007	12.4	5.5	561.1	2184.8	3.06	48.68	2.23	1.31	0.37	0.30	74	157	4	3	0
2008	11.9	5.6	886.3	3104.9	7.91	214.47	2.10	1.51	0.29	0.29	103	260	3/5**	5	4
2009	NA	NA	NA	NA	2.80	101.16	NA	NA	NA	NA	101	361	4/6**	7	0
2010	NA	NA	NA	NA	13.80	190.86	1.74	1.32	NA	NA	57	418	5/7**	12	3

M = moderate floods (250-1012 L s^{-1}), S = severe floods (> 1012 L s^{-1}), NA = data unavailable.

* These values may be underestimated, due to the lack of hydrological data before 1998.

** 2008, 2009 and 2010 have 2 values depending on whether 2008 drought is counted or not.

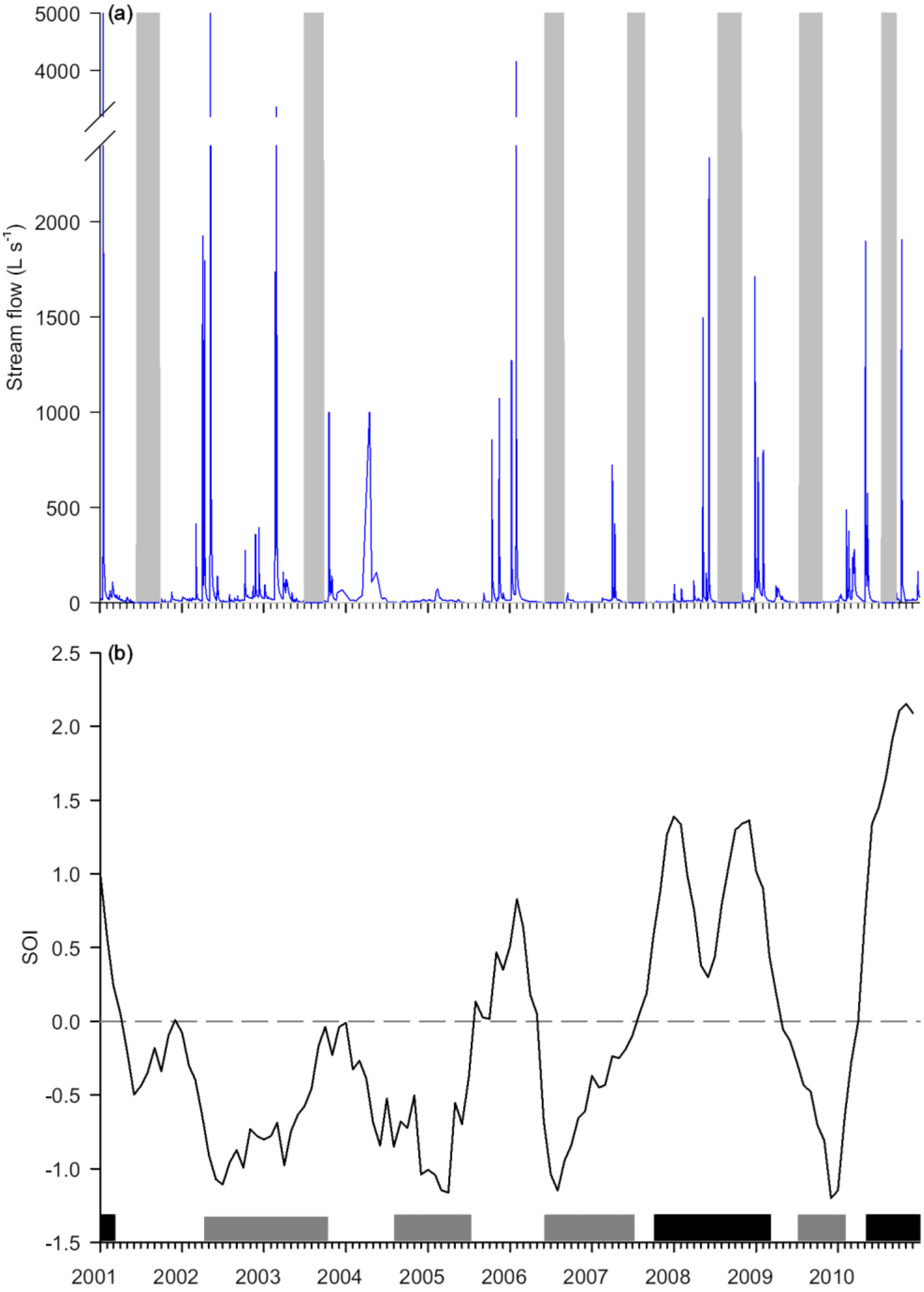


Fig. 2 (a) Stream flow during the study period. Grey areas represent drought periods. (b) Southern Oscillation Index (SOI) values (5-month running mean) to characterise El Niño Southern Oscillation during the studied period. On the X axis, El Niño events are marked in dark grey and La Niña events in black.

year the stream flow was the highest and there were 20 days of moderate and severe floods. A seasonal drought (around summer) occurred in the stream every year except 2002, 2004 and 2005. Flow cessation fluctuated from the beginning of June to mid-July and flow return from the end of August to the end of October, with the longest droughts (> 100 days) in 2001, 2008 and 2009. The average drought period was of 87 days (from June 28 to September 23). 2001 and 2010 showed the maximum cumulative drought period, with at least 4 consecutive years with summer drought. Photoperiod and solar radiation did not show important interannual differences. Photoperiod had the lowest average length during autumn (10.3 h d^{-1}) and an annual mean of 12.2 h d^{-1}, and solar radiation ranged from 12.9 to 13.9 MJ m^{-2} d^{-1}.

Wind velocity was 1.9 ± 0.9 m s^{-1} on daily average, with maximum velocity reaching 28.8 m s^{-1} in 2004. Seasonal and annual differences in wind velocity were not pronounced.

Based on SOI values, several El Niño and La Niña events occurred during the study period (Fig. 2b). El Niño was recorded for the years 2002–03, 2004–05, 2006–07 and 2009–10, while La Niña was recorded at the beginning of 2001 (end of 2000–01 event) and in 2007–09, and the last event started from mid 2010.

Quantitative dynamics

Riparian organic matter inputs

Total RI to the stream were 911.67 ± 55.80 g DM m^{-2} y^{-1} on average (Fig. 3). The annual inputs in 2003 and 2006 were the highest, while 2008 showed the lowest value. Most of these RI came from VI (60.5 ± 0.4%) and the rest from LI (39.5 ± 0.4%). VI achieved the highest absolute values during autumn 2002 and 2004 (5.5 and 4.5 g DM m^{-2} d^{-1}; Fig. 4a). LI showed 2 main peaks in autumn 2003 (6.9 and 4.6 g DM m^{-2} d^{-1}), the rest of the values being in general below 2 g DM m^{-2} d^{-1} (Fig. 4b).

VI distribution throughout the year depended both on the season and the occurrence of drought (Season: $F_{3,31}$ = 14.04; $P < 0.001$; Drought: $F_{1,31}$ = 0.27; $P = 0.607$; Season x Drought: $F_{3,31}$ = 6.54; $P = 0.001$). Years with drought (Fig. 5a) showed the highest values in summer (representing 36% of annual VI), while years without a summer drought (Fig. 5b) showed the highest values in autumn (52% of annual VI) and the lowest values in winter (7% of annual VI). The autumn peak in years with drought represented 31% of the annual VI.

VI and LI did not follow the same dynamics (r_s = 0.074; $P = 0.535$; n = 73). VI were positively related to minimum temperature, drought length and PET/Prec ratio, and negatively related to flow and precipitation, indicating that high VI were related to warm temperatures with low flow and precipitation, so mainly drought periods when riparian trees would have high evapotranspiration rates. Relative humidity,

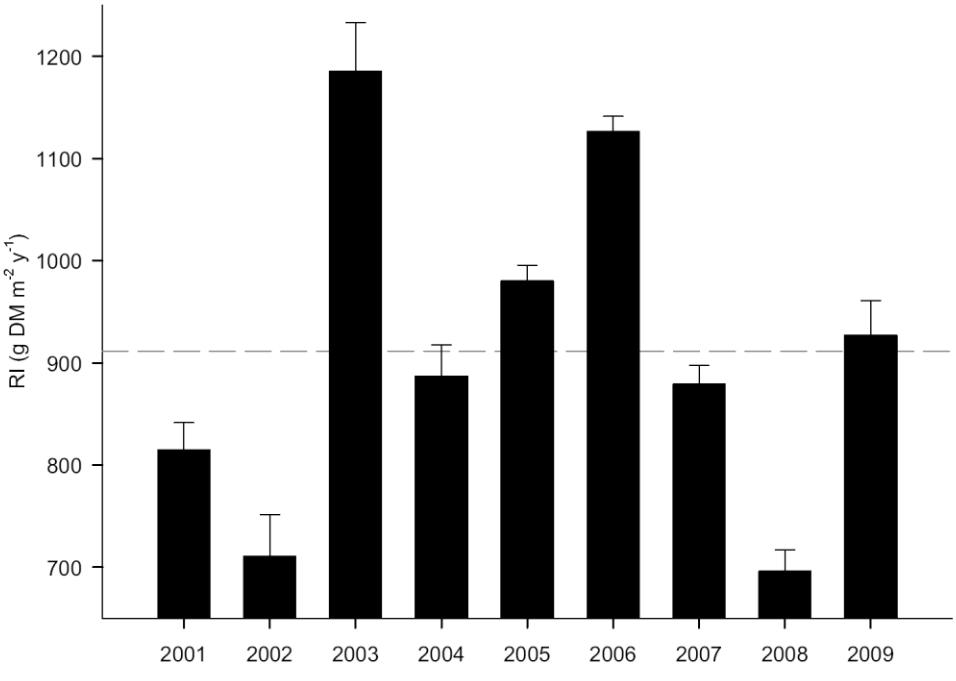
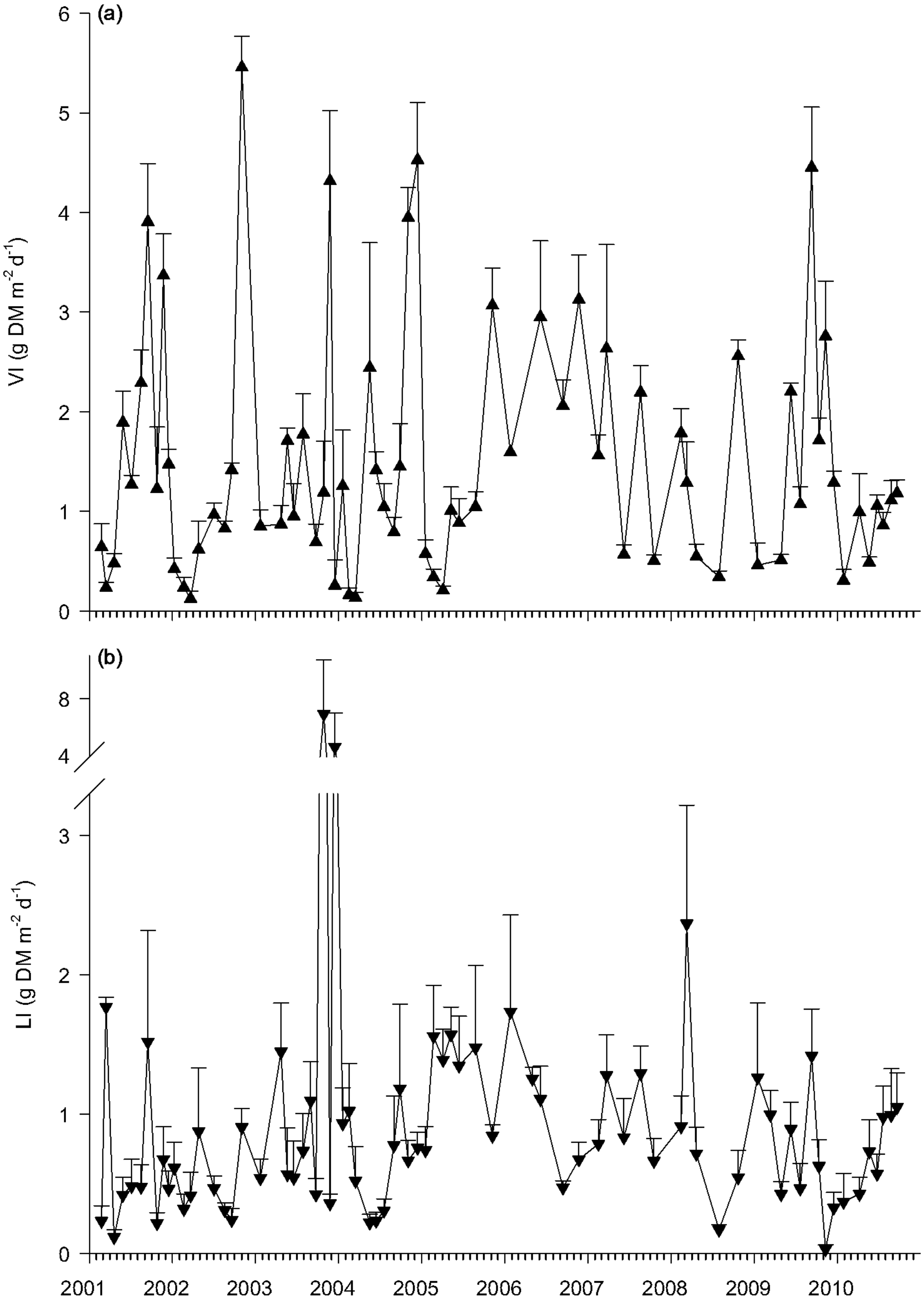


Fig. 3 Total annual riparian inputs (RI) of ‘calendar’ years (from January to December each year) into the stream during the study period. The dashed line represents the average value for all the years and error bars represent +SE. Note that the Y axis does not start at zero.

Fig. 4 (a) Vertical, VI, and (b) lateral riparian inputs, LI, into the stream throughout the study period. Error bars represent +SE.



photoperiod and wind velocity were not related to VI. LI were only negatively related to the VPD, which integrates relative humidity and temperature data, indicating that high LI were related to periods with high humidity and low temperatures. The percentage of VI and LI from RI was explained by a combination of flow, precipitation, temperature and VPD (Table 3).

The RDA shows that VI were more related to minimum temperature, drought length and PET/Prec ratio (Fig. 6). Samples were distributed clockwise following the seasons of the year, with winter and spring samples characterised by low values of both VI and LI. Drought period samples were situated on the negative side of the first axis, dominated by VI. However, variability explained by this analysis was low (< 30%).

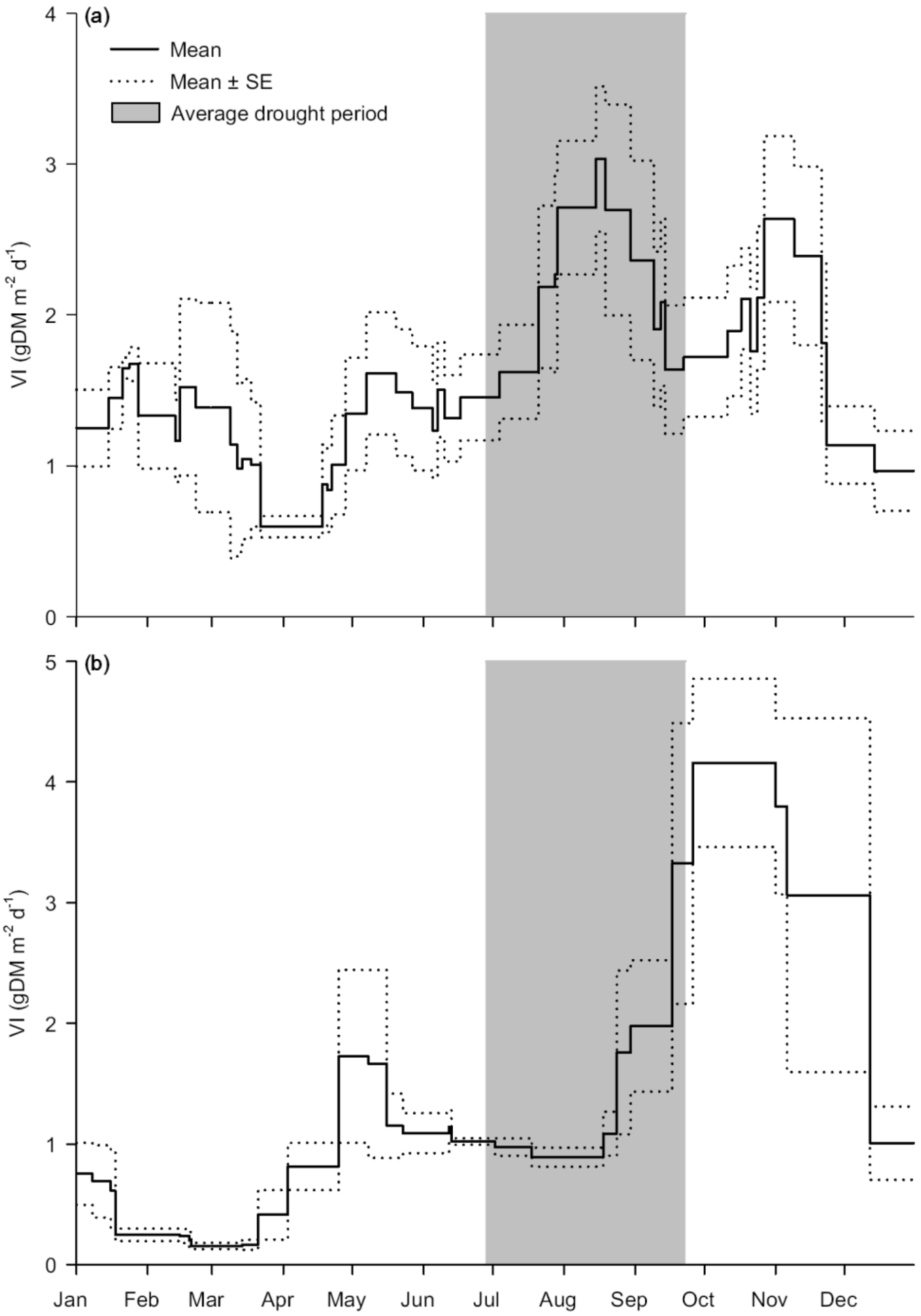


Fig. 5 Vertical input (VI) mean values ± SE for (a) years with drought (n = 7; 2001, 2003, 2006, 2007, 2008, 2009, 2010) and (b) years without drought (n = 3; 2002, 2004, 2005). Grey area indicates the average drought period. Note the different Y axis scales.

Table 3 Spearman correlations between the quantity of organic matter inputs and the hydrological and meteorological variables

Dependent Variable	Independent variable	r_s	*P*	n
VI (g DM m^{-2} d^{-1})	Flow (L s^{-1})	-0.363	**0.002**	73
	Prec (mm)	-0.292	**0.015**	69
	Minimal T (ºC)	0.353	**0.003**	69
	Drought length (d)	0.246	**0.036**	73
	PET/Prec ratio	0.344	**0.004**	68
	Mean RH (%)	0.136	0.282	64
	Photoperiod (h)	0.101	0.394	73
	Mean wind velocity (m s^{-1})	0.078	0.631	40
	VPD (kPa)	0.160	0.208	64
LI (g DM m^{-1} d^{-1})	Flow (L s^{-1})	0.012	0.921	74
	Prec (mm)	0.018	0.111	72
	Minimal T (ºC)	-0.171	0.151	72
	Drought length (d)	0.035	0.766	76
	PET/Prec ratio	-0.065	0.592	71
	Mean RH (%)	0.041	0.744	67
	Photoperiod (h)	-0.127	0.274	76
	Mean wind velocity (m s^{-1})	0.019	0.906	42
	VPD (kPa)	-0.249	**0.042**	57
VI (% from RI)*	Flow (L s^{-1})	-0.294	**0.013**	71
	Prec (mm)	-0.283	**0.019**	69
	Mean T (ºC)	0.381	**0.001**	69
	VPD	0.256	**0.041**	64
BOM (g DM m^{-2})	Time from M (d)	0.447	**0.005**	38
	Time from S (d)	0.404	**0.012**	38
BOM coverage (%)	Time from M (d)	0.504	**0.003**	32
	Time from S (d)	0.774	**< 0.001**	32
CPOM (mg DM L^{-1})	Time from M (d)	-0.359	**0.040**	33
FPOM (mg DM L^{-1})	Time from M (d)	-0.559	**0.001**	33
	Time from S (d)	-0.511	**0.002**	33
	CPOM (mg L^{-1})	0.570	**0.001**	33
UFPOM (mg AFDM L^{-1})	Flow (L s^{-1}, punctual)	-0.525	**0.008**	24

P-values < 0.05 are indicated in bold.

VI = vertical inputs, LI = lateral inputs, RI = total riparian inputs, BOM = benthic organic matter, CPOM = coarse particulate organic matter, FPOM = fine particulate organic matter, UFPOM = ultrafine particulate organic matter, T = temperature, RH = relative humidity, PET = potential Evapotranspiration, Prec = precipitation, VPD = vapour pressure deficit, M = moderate floods (250-1012 L s^{-1}); S = severe floods (>1012 L s^{-1}).

*LI (% from RI), as the resting percentage from VI, is related to the same variables than VI, but inversely.

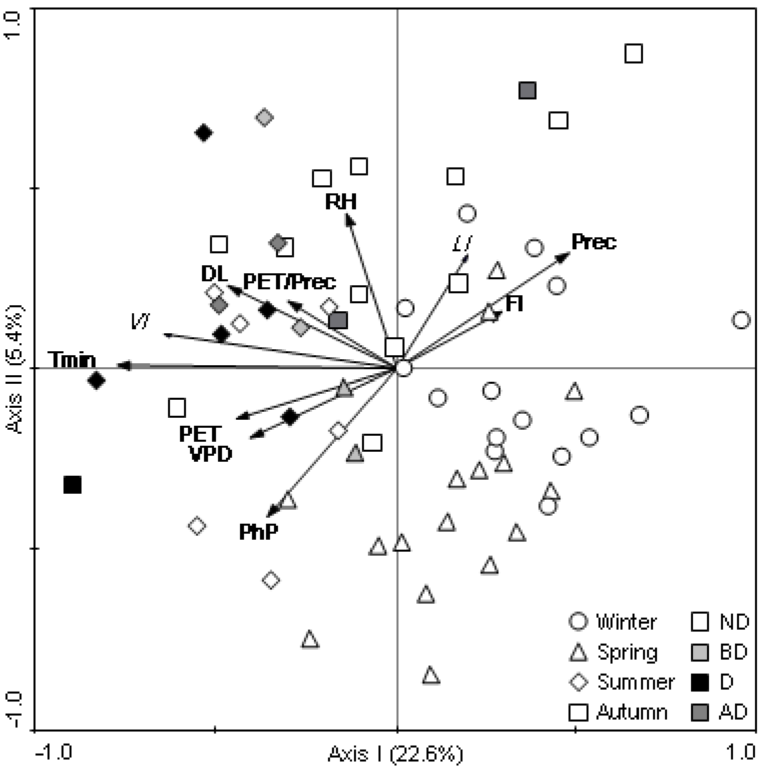


Fig. 6 Redundancy analysis (RDA) diagram showing the simultaneous ordination of samples depending on the quantity of riparian inputs (vertical, VI, and lateral, LI, inputs) and environmental variables. The first and second axes are represented, and the percentage of variability explained by each is indicated. Samples are classified based both on the seasons of the year (winter, spring, summer and autumn) and depending on whether the sampling period was without drought (ND), included some period of drought (before the drought, BD; or after the drought, AD) or completely in a drought period (D). Tmin = minimum temperature, RH = relative humidity, PET = potential evapotranspiration, Prec = precipitation, VPD = vapour pressure deficit, PhP = photoperiod, DL = drought length, Fl = flow.

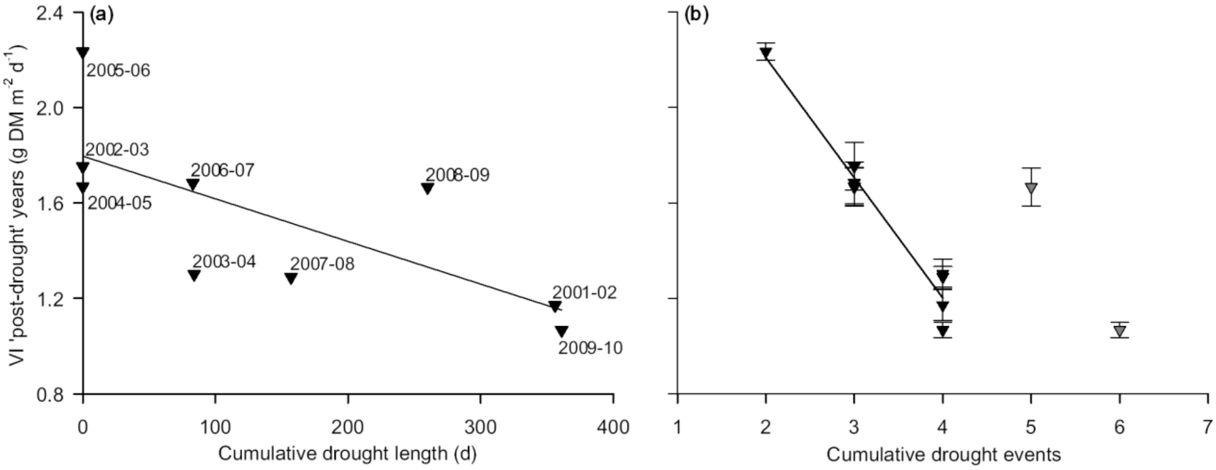


Fig. 7 Inter-year carryover effects of drought represented through (a) the correlation between vertical inputs (VI) in 'post-drought' years (counting the average inputs from October year X to September year X+1) and cumulative drought length for year X (r_s = -0.898; P = 0.001; n = 9) and (b) the correlation between VI in 'post-drought' years and the cumulative number of drought events (r_s = -0.913; P = 0.001; n = 9). Grey triangles represent 2008–09 and 2009–10 values when 2008–09 is counted as a drought year. Cumulative drought length and drought events for 2001 may be underestimated, due to the lack of hydrological data before 1998. Error bars in (b) represent ± SE. Note that some axes do not start at zero.

VI in ‘post-drought’ years decreased with longer cumulative drought periods (r_s = -0.898; P = 0.001; n = 9; Fig. 7a). Furthermore, the cumulative number of drought events determined the amount of VI throughout ‘post-drought’ years (r_s = -0.913; P = 0.001; n = 9; Fig. 7b). Although a drought event occurred in summer 2008, 2008–09 behaved as a year without a drought in terms of VI quantity. If 2008–09 is considered a year without a drought event (number of cumulated drought events = 3 instead of 4), VI in 2008–09 and 2009–10 adjust to the correlation line between VI and cumulative drought events. This may be because 2008 was a very humid year (Table 2), coinciding with La Niña event (Fig. 2b), and this would compensate the effect of drought on VI.

The interannual variability in the quantity of VI was highly explained by ENSO. VI from ‘phenological’ years were related to the SOI of September (i.e. the mean value of the SOI from July to November; r_s = -0.900; P = 0.001; n = 9; Fig. 8a). This correlation was the most explanatory and significant (highest r_s and lowest P-value) of the tested correlations between VI and SOI values from before and during the ‘phenological’ year. The local variable relating VI and ENSO was precipitation (VI versus Precipitation: r_s = -0.821; P = 0.023; n = 7; Fig. 8b; SOI Sept versus Precipitation: r_s = 0.821; P = 0.023; n = 7; Fig. 8c). Two ‘phenological’ years were situated at both extremes of these correlations; 2008–09 with a strong La Niña event and lower than the mean VI, and 2006–07 with a strong El Niño event and higher VI than the average. The relationships including precipitation could not be calculated for the period 2009–10 due to the lack of precipitation data from September 2009 to May 2010. Moreover, these correlations did not include 2002–03, with a long El Niño event, which was a particularly humid period (Table 2). This higher precipitation was not related to ENSO and did not explain the quantity of VI. SOI was neither significantly correlated to LI (Spearman, P = 0.406) nor to total RI (Spearman, P = 0.139) in ‘phenological’ years.

Benthic and transported organic matter

In the dynamics of BOM (Fig. 9a), the high values in summer 2003 (reaching 717.45 g DM m^{-2}) reflected the RI cumulated on the dry streambed. However, the nearly severe flood (1000 L s^{-1}) in October returned the BOM to low values (16.98 g DM m^{-2}). BOM values reached their maximum in autumn 2004 (807.81 g DM m^{-2}), related to the input peak, mainly from VI, in autumn that year and the previous long period of low flow values. BOM was not directly related to flow (average for sampling periods; Spearman, P = 0.105), to RI (Spearman, P > 0.776), or to transported POM (Spearman, P > 0.227), but depended mainly on the days from last flood, whether moderate or severe. The most important driver of BOM coverage was the time from severe floods (Table 3).

Dynamics of transported CPOM and FPOM (Figs. 9b,c) showed the highest peak in November 2002, coinciding with the autumn peak of riparian VI (Fig. 5a) and moderate floods that year (Fig. 2). Despite this isolated relationship, there was no general correlation between transported POM and RI (Spearman, P > 0.225). CPOM and FPOM followed parallel dynamics and their concentration in water increased with floods, while UFPOM concentration (Fig. 9d) was negatively related to specific flow. FPOM and moderate floods showed the strongest relationship (Table 3).

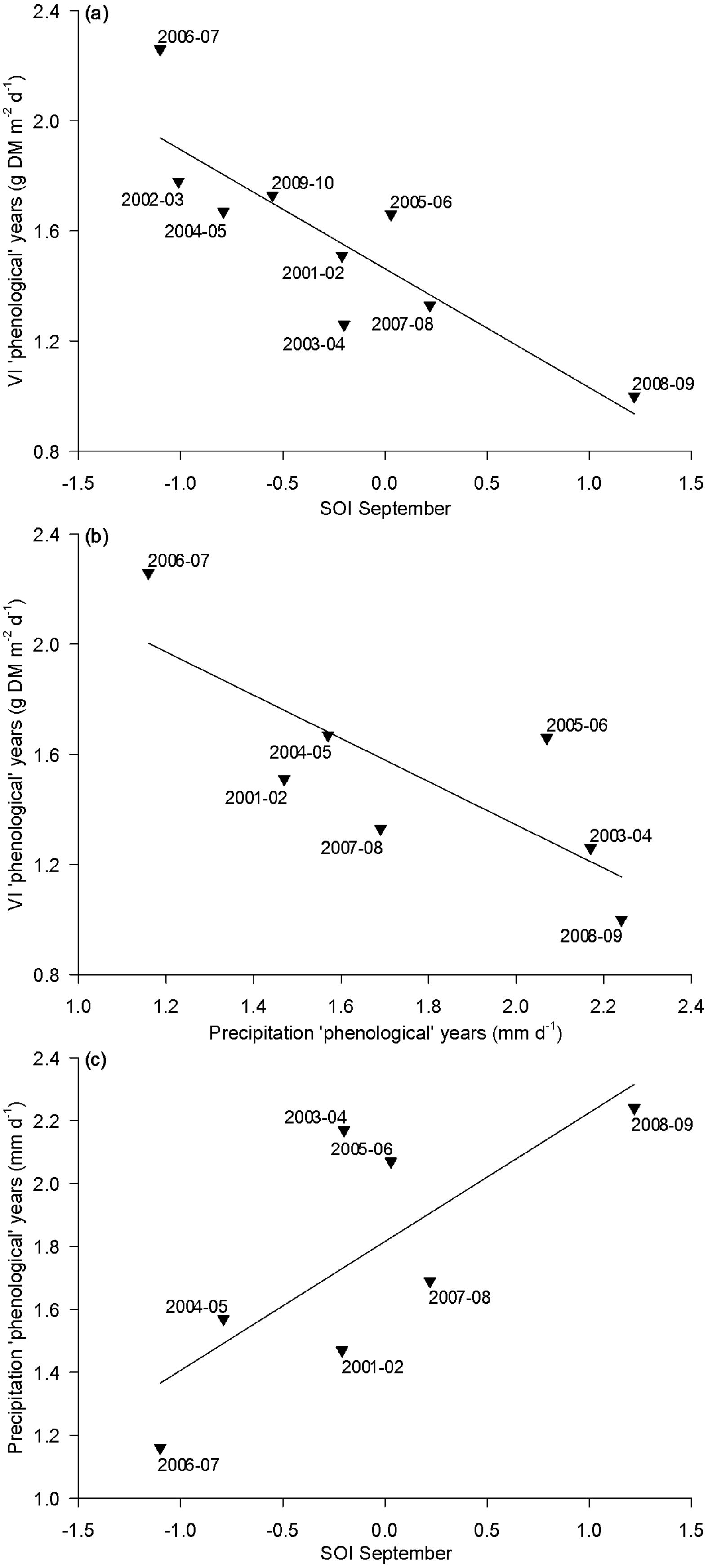


Fig. 8 Correlations between (a) riparian vertical inputs (VI) in ‘phenological’ years and Southern Oscillation Index (SOI) for September (r_s = -0.900; P = 0.001; n = 9), (b) VI and precipitation in ‘phenological’ years (r_s = -0.821; P = 0.023; n = 7), and (c) precipitation in ‘phenological’ years and SOI for September (r_s = 0.821; P = 0.023; n = 7). ‘Phenological’ years account for the average values from April year X to March year X+1 and SOI is from September year X. In (b) and (c) 2009–10 was not included due to the lack of precipitation data for that period and 2002–03 was not included because it was a very humid period and fell outside of the correlation. Note that axes do not start at zero.

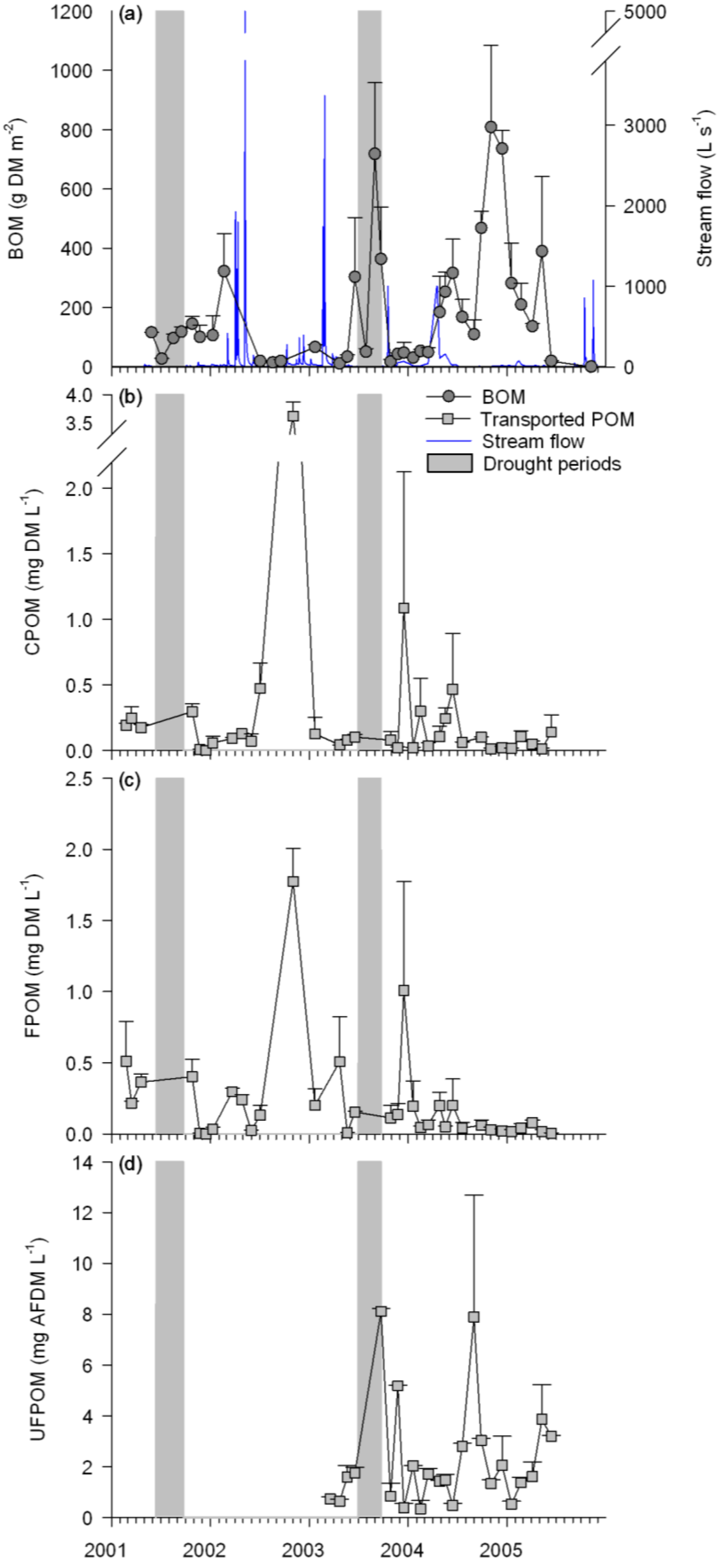


Fig. 9 (a) Benthic organic matter (BOM) dynamics from 2001 to 2005 and stream flow. Dynamics of particulate organic matter (POM) transported by the stream, (b) coarse POM, CPOM, (c) fine POM, FPOM and (d) ultrafine POM, UFPOM. Grey areas indicate drought periods and error bars indicate +SE.

Qualitative dynamics

Riparian organic matter inputs

The C : N molar ratio of RI was on average 59.12 ± 14.48 SD (Fig. 10). The changes in OM quality were related to several abiotic variables (Table 4). The C content (%) was positively related to temperature and VPD and negatively related to humidity, while N content (%) was in part explained by the photoperiod. As C : N was mainly determined by the N content (r_S = -0.919; $P < 0.001$; n = 57), C : N was also negatively correlated to photoperiod. No relationship was found between the quality and the quantity variables of RI (Spearman, $P > 0.116$).

RDA showed that C content was related to VPD, while N content was more related to photoperiod (Fig. 11). Spring and summer samples showed a lower C : N ratio, while winter samples, in general, were characterised by a higher C : N ratio. Samples from drought periods, or including drought periods, were not clustered together, although some samples from these periods showed higher C content. However, variability explained by this analysis was low (< 21%).

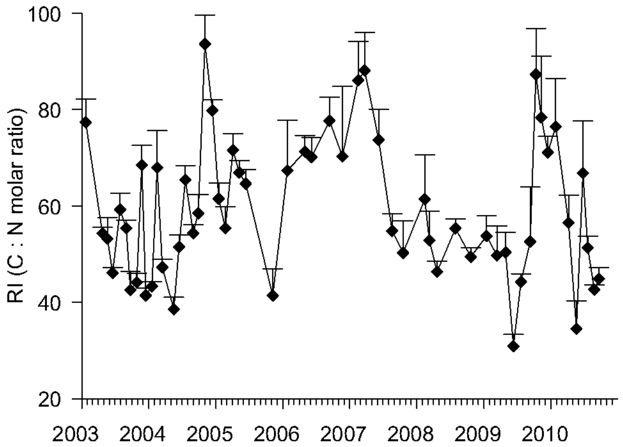


Fig. 10 Carbon : nitrogen (C : N) molar ratio of the riparian inputs (RI) from 2003 to 2010. Error bars represent +SE. Note that the Y axis does not start at 0.

Benthic and transported organic matter

The average C : N molar ratio of BOM was 50.01 ± 11.56 SD and C content was higher with longer drought periods (Table 4). The quality of BOM was not related to its quantity (Spearman, $P > 0.681$) or to the quality of RI (Spearman, $P > 0.143$).

The average C : N molar ratio of the transported CPOM was 38.64 ± 9.71 SD, being higher than the C : N of FPOM (22.01 ± 6.69 SD) and UFPOM (14.66 ± 5.72 SD). The quality of CPOM and FPOM was inversely related to flow. Higher flow and higher flood frequency were related to lower C content and C : N ratio of CPOM, but higher C content and C : N ratio of FPOM (Table 4).

DOC monthly values ranged from 1.2 to 11.5 mg L^{-1}, exhibiting high peaks after drought periods (Fig. 13a). DOC was not related to RI if the entire study period was taken into account (Spearman, $P > 0.511$). However, cumulative VI during the 'summer' period (actual or average drought period) were related to DOC the month that period ended (r_S = 0.667, P = 0.050, n = 9; Fig. 13b).

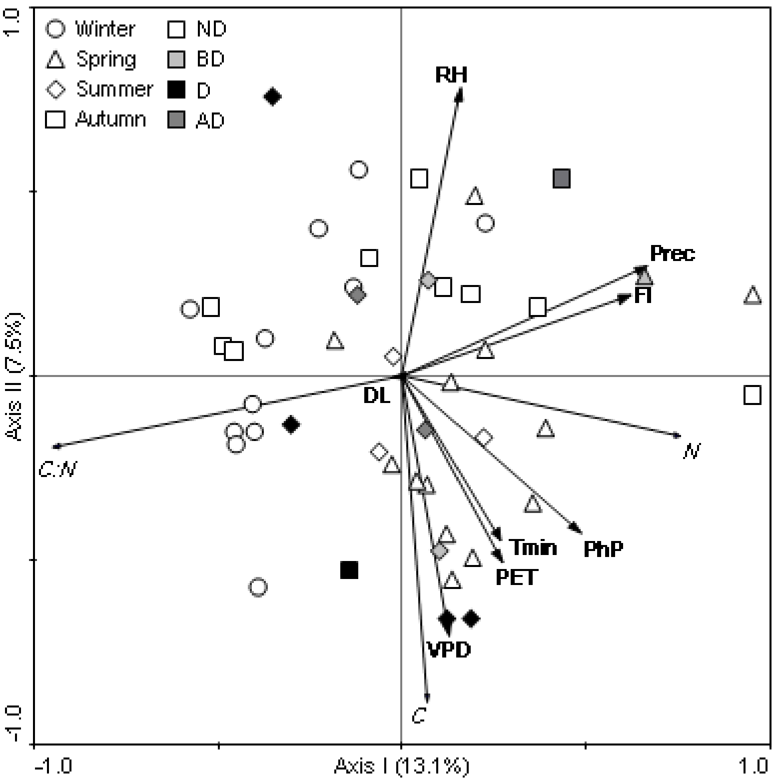


Fig. 11 Redundancy analysis (RDA) diagram showing the simultaneous ordination of samples depending on the quality of riparian inputs (carbon [C] and nitrogen [N] percent, and C : N molar ratio) and environmental variables. The first and second axes are represented, and the percentage of variability explained by each is indicated. Samples are classified based both on the seasons of the year (winter, spring, summer and autumn) and depending on whether the sampling period was without drought (ND), included some period of drought (before the drought, BD; or after the drought, AD) or completely in a drought period (D). Variable abbreviations are given in Fig. 6.

Table 4 Spearman correlations between the quality of organic matter and the hydrological and meteorological variables

Dependent Variable	Independent variable	r_s	*P*	n
RI C (%)	Maximal T (ºC)	0.303	**0.028**	53
	Maximal RH (%)	-0.477	**0.001**	48
	VPD (kPa)	0.375	**0.009**	48
RI N (%)	Photoperiod (h)	0.270	**0.042**	57
RI C : N (molar ratio)	Photoperiod (h)	-0.268	**0.043**	57
BOM C (%)	Drought length (d)	0.435	**0.030**	25
CPOM C (%)	Flow (L s^{-1}, punctual)	-0.488	**0.034**	19
CPOM N (%)	CPOM (mg DM L^{-1})	-0.513	**0.030**	18
CPOM C : N (molar ratio)	Time from S (d)	0.565	**0.012**	19
FPOM C (%)	Time from S (d)	-0.553	**0.014**	19
FPOM C : N (molar ratio)	Flow (L s^{-1}, punctual)	0.598	**0.007**	19
	Time from M (d)	-0.512	**0.025**	19
	Time from S (d)	-0.506	**0.027**	19

P-values < 0.05 are indicated in bold.
RI = total riparian inputs, BOM = benthic organic matter, CPOM = coarse particulate organic matter, FPOM = fine particulate organic matter, UFPOM = ultrafine particulate organic matter, T = temperature, RH = relative humidity, VPD = vapour pressure deficit, M = moderate floods (250-1012 L s^{-1}); S = severe floods (>1012 L s^{-1}).

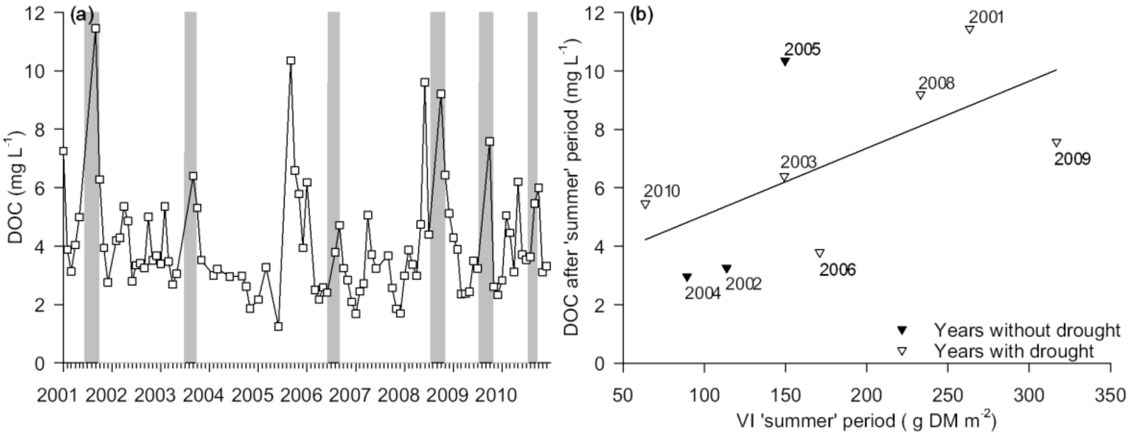


Fig. 12 (a) Dissolved organic carbon (DOC) concentration in stream water during the study period. Grey areas indicate drought periods. (b) Correlation between organic matter vertical inputs (VI) during the 'summer' period (actual or average drought period, from 28/06 to 23/09) and DOC the month that period ended (r_s = 0.667, P = 0.050, n = 9). No data for DOC after the 'summer' period were available for 2007.

DISCUSSION

Quantitative dynamics

Riparian organic matter inputs

The average of total annual inputs during these 10 years (911.67 g DM m^{-2} y^{-1}) was similar to the values reported before for this same stream (Sabater *et al.*, 2001; Acuña *et al.*, 2007), but are around the double than the values measured in other Mediterranean streams (King *et al.*, 1987; Stewart & Davies, 1990; Maamri *et al.*, 1994). On the other hand, our values are more similar to those reported for temperate streams (e.g., Treadwell *et al.*, 1997; Wallace *et al.*, 1997a; Molinero & Pozo, 2004), due to the deciduous nature of the riparian forests studied (see Table 1 in Benfield, 1997; and Table 3 in Acuña *et al.*, 2007). Total annual inputs ranged from 695.83 to 1185.38 g DM m^{-2} y^{-1}, showing the high interannual variability in this stream compared to other streams with a similar amount of litter inputs between years (Richardson, 1992). In general, VI represent the main contribution to RI into the stream (e.g., 85% in Fisher & Likens, 1973; 91% in Treadwell *et al.*, 1997; 78% in Wallace *et al.*, 1997a; and Molinero & Pozo, 2004). In our study, VI accounted for 60% of the RI and showed a quite stable trend (± 0.4 SE). The highest values of VI found in this study in autumn (4.5–5.5 g DM m^{-2} y^{-1}) are comparable to those found by Pozo *et al.* (1997) or Richardson (1992).

Despite the similarities in the quantity of inputs between Fuirosos and temperate streams, the dynamics of these inputs can be different. In the years in which summer drought occurred, VI were characterised by a bimodal annual cycle, with one peak coinciding with the drought and the other in autumn. This has been described as a response of riparian vegetation to hydric stress (Larned, 2000; Tabacchi *et al.*, 2000) and has been observed before in this same stream (Bernal *et al.*, 2003; Acuña *et al.*, 2007). Alternatively, temperate deciduous forest streams show a unimodal distribution with the characteristic autumnal peak (Benfield, 1997).

This peak is due to phenological leaf abscission and can contribute from 60% to 80% of the annual leaf input (Fisher & Likens, 1973; Richardson, 1992). In our study, the autumn peak (52% of the total VI) in years without drought are comparable to the values reported for temperate streams, while it was lower in the drought years (31%).

LI tended to be lower than 2 g DM m^{-2} d^{-1} and are comparable to values found by Pozo *et al.* (1997). The highest value, measured in autumn 2003 (6.9 g DM m^{-2} d^{-1}) was probably related to high flows (nearly severe floods of 1000 L s^{-1}) and the second highest wind velocity recorded in the available data (28 m s^{-1}). Nevertheless, the absolute maximum wind velocity (28.8 m s^{-1} in May 2004) or maximum floods (5000 L s^{-1} in January 2001 and May 2002) did not cause higher peaks in LI. Peaks in LI were related to strong winds or severe floods but only when leaves had accumulated on the ground, for example, after a long drought (as in 2003). Hence, 2 factors must coincide in time to produce a peak in LI: high accumulation of OM on the riparian soil due to high VI related to drought, and afterwards, strong winds, high precipitation and floods. LI dynamics over time are difficult to compare with other streams because these measurements are lacking in many studies or only annual average values are provided (Webster & Meyer, 1997).

Climate, through its effect on vegetation, is the strongest controller of OM dynamics (Webster & Meyer, 1997). In a review of 35 streams, Benfield (1997) found that VI were correlated to latitude and annual precipitation. In our study, the main drivers of VI were related to drought but not to any of the variables that could indicate the phenological autumn peak (e.g. photoperiod). This indicates again the primary importance of hydric stress peak versus phenological peak in this stream. Riparian annual VI were reduced on average by 45% (from 2.2 to 1.2 g DM m^{-2} d^{-1}) if we compare 2 consecutive years of non-drought (2005–06) with 2 consecutive years of drought (e.g. 2007–08). This reduction in VI could be predicted through the cumulative number of drought events. However, some years (as in 2008–09 with La Niña event) other factors, such as precipitation, have to be considered making the predictions more difficult. To our knowledge, the cumulative effect of drought on RI found in this study has not been observed before. However, we have found a parallel pattern called ‘drying memory’ in a temporary stream, where leaf litter breakdown decreased with increased drying average duration and frequency, and this effect was observed for a 24-year period (Datry *et al.*, 2011). While the effect found in our case followed a linear tendency, Datry *et al.* (2011) found an exponential relationship. These results suggest that temporary streams remain affected by drying events in the long term, highlighting the importance of drought both in the short and the long term. Soil drying induces a decrease in nutrients, in particular N, with strong effects on plant growth and function (McDonald & Davies, 1996). In addition, the low decomposition of OM on the riparian forest floor of the studied stream, which is even lower in drought years, causes a N impoverishment of the soil (Bernal *et al.*, 2003), affecting tree growth and leaf production in the subsequent years.

Although 10 years could be considered a relatively short period to observe the effects of global climate processes such as ENSO on riparian dynamics, some effects have already been detected. ENSO affected annual VI through changes in precipitation in the studied stream. Atmospheric teleconnections of ENSO with precipitation (and drought) patterns have been observed in the Iberian Peninsula in general, and the north-eastern region (where this study was performed) in particular (Muñoz-Díaz & Rodrigo, 2005; Vicente-Serrano, 2005). In our study, SOI was significantly correlated to VI but not to LI. This is probably because VI respond directly to the physiology of riparian trees, while LI are indirect, entering the stream after a period of accumulation on the floor.

Benthic and transported organic matter

The average quantity of BOM retained in the streambed (182.31 g DM m^{-2}) is comparable to values found in other temperate streams with deciduous forests (Table 4.1 in Pozo & Elosegi, 2005). The dynamics of BOM was mainly explained by the time between severe floods, but it was not directly related to RI or OM transport. These variables are related, although in a more complex way, as modelled by Acuña & Tockner (2010). González & Pozo (1996) and Pozo *et al.* (1997) found that the timing of inputs and the hydrologic regime act together to influence temporal dynamics of BOM, with accumulation in periods with high RI and low transport (low flow). Similar BOM dynamics have been observed in an Australian intermittent stream (Boulton & Lake, 1992a) and in a Mediterranean stream (King *et al.*, 1987), with accumulations during summer and early autumn.

Floods transport high quantities of CPOM and FPOM, and in Fuirosos most POM transport occurred with moderate floods. Large exports of POM (and DOM) during storms have also been observed by Wallace *et al.* (1995), Newbold *et al.* (1997) and Neatrour *et al.* (2004). Although it has long been recognised that high flow events dominate OM transport in streams, measurements of transport in most studies are limited to basal flow conditions (Tank *et al.*, 2010). This problem is difficult to solve due to the complexity of measuring these variables continuously (Elosegi & Pozo, 2005).

Qualitative dynamics

Riparian organic matter inputs

The quality of VI and LI did not differ significantly, indicating that during the period when leaves accumulated on the forest floor, no significant changes were produced in leaves. This agrees with the fact that riparian soil is poorly developed and leaves (mainly plane tree leaves) tend to accumulate on the forest floor because of extremely low decomposition rates (Bernal *et al.*, 2003). In other systems, such as tropical rainforest streams, with richer soils, LI are qualitatively important with a higher N content than VI (Benson & Pearson, 1993).

Quality of RI was determined by different factors than those which determined quantity. Drought conditions (high temperatures and VPD, and low relative humidity) induced an increase in the C content of OM. On the other hand, N was related to photoperiod, which increases through spring and reaches its maximum in summer. These 2 effects determine lower molar

C : N ratios in summer. These results do not support our initial hypothesis of a C : N increase from summer to autumn, based on Keskitalo *et al.* (2005) and Marchin *et al.* (2010), and observed in the stream of this study by Bernal *et al.* (2003). Bernal *et al.* (2003) found that N content was higher in plane trees during the summer peak (1.65 ± 0.04%) than in autumn (0.76 ± 0.03 %), related to the physiology of riparian trees and the nutrient translocation process that takes place before autumn leaf abscission. However, more factors must be taken into account, since gradual (versus abrupt) droughts may allow the trees to translocate nutrients before leaves fall in summer as well. On the other hand, riparian trees in Mediterranean areas may already be adapted to the dry and warm summers, with systems which effectively translocate nutrients before leaf abscission. This mechanism was observed by Marchin *et al.* (2010), who found that some ‘drought-deciduous species’ were able to resorb N from senesced leaves, and has been explained by Munné-Bosch & Alegre (2004). Moreover, the composition of OM inputs (species, type of material, i.e. branches, leaves, etc.) would change the quality of the inputs. In the same stream as that studied here, Acuña *et al.* (2007) observed that alder and poplar leaf inputs peaked in August while plane tree leaf inputs were nearly constant throughout the leafy period of this species.

Benthic and transported organic matter

The C : N ratio of the different types of OM gradually decreased from riparian inputs (59.14), followed by BOM (50.01) and transported POM, i.e. CPOM (38.64), FPOM (22.01) and UFPOM (14.66). This was probably due to the degree of conditioning and processing of the different particles, which causes a N enrichment (Triska *et al.*, 1975; King *et al.*, 1987). The quality of CPOM was higher (lower C : N ratio and C content) when flow and flood frequency were high, maybe because the CPOM imported from upstream had a lower C content, due to a different riparian species composition or OM processing. The quality of FPOM was higher with lower flow and flood frequency, probably due to the higher OM processing time in the reach.

DOC concentration after the ‘summer’ period (actual or average drought period) was partly explained by VI accumulated on the streambed during that period, probably through OM lixiviates (Meyer *et al.*, 1998). DOC would probably be more closely related to VI in the years with gradual rewetting than in the years with abrupt rewetting by strong floods that carry OM downstream; thus, rewetting intensity would interfere with this correlation.

Potential effects of organic matter quantity and quality on stream food webs

The importance of allochthonous resources for food webs in forested headwater streams is widely recognised (Polis *et al.*, 1997; Wallace *et al.*, 1997b) and timing of these pulsed resources is important in determining the strength of trophic cascades (Leroux & Loreau, 2012). Therefore, a decrease in the quantity of riparian inputs due to the ‘la Niña’ events (e.g. 2008) or caused by consecutive years with drought (e.g. 2007–08) will probably cause the decline of some invertebrate taxa, stronger food web interactions (Hall *et al.*, 2000) and the weakening of terrestrial-aquatic linkages (England & Rosemond, 2004). Differences in resource availability over time, for instance due to premature leaf fall during drought periods (e.g. 2001, 2003), will be also important due to the tight link between invertebrates and timing of litter inputs (Cummins *et al.*, 1989). Changes in the quality of stream OM, for example changes in quality of riparian inputs over the year, will probably cause changes in consumer growth and fitness (Graça *et al.*, 2001).

Conclusions

The quantity of RI and BOM in this stream is comparable to streams in temperate regions with deciduous riparian forest. However, the dynamics of OM inputs and retention are characteristic of Mediterranean systems. Distribution of VI throughout the year depends on the presence of a drought event that same year, while the annual amount of VI depends on previous drought events. In years without drought, autumnal peaks occur due to phenological leaf abscission, while a bimodal annual cycle is observed in years with summer drought, with the first peak due to hydric stress and the second corresponding to the phenological peak. An interesting and not previously described cumulative effect of drought is a decrease in annual VI, due to successive years in which the stream dries up in summer, probably through the effect on the nutrient content of the riparian soil. Thus, drought has both immediate and long-term effects on the dynamics of allochthonous OM. ENSO highly explains the interannual variability of VI through changes in precipitation patterns. Retained and transported OM dynamics is mainly determined by flood frequency. Quality of allochthonous OM increases from out-stream coarse particles to in-stream finer particles, due to the increase in conditioning and processing. DOC dynamics is related to VI after ‘summer’ periods. In this long-term study we demonstrate there are important effects of local, mainly drought, and global, ENSO, environmental factors both direct and cumulative, in the dynamics of allochthonous OM dynamics in Mediterranean streams.

CHAPTER 2

DROUGHT EFFECTS ON RESOURCE QUALITY IN A MEDITERRANEAN STREAM: FATTY ACIDS AND STEROLS AS INDICATORS

ABSTRACT

Seasonal droughts in Mediterranean streams shape their physical, chemical and biological characteristics. Thus, droughts can alter basal resources, which in headwater streams are primarily allochthonous and secondarily autochthonous organic matter (OM). A study was performed in a Mediterranean stream in summer, before and after a drought period. Changes in the quality of the OM in the benthic substrates (the leaves and epipsammic and epilithic biofilms) and transported particulate and dissolved fractions) were analysed. Fatty acids (FA) and sterols were measured because they include essential molecules for invertebrates and also because they can be used as biomarkers for OM sources. The drying-rewetting process determined a reduction in the nutritional quality of benthic substrates and transported particulate OM, with a general reduction in the total and essential FA. A shift from predominantly autochthonous OM before the drought to allochthonous OM afterwards was observed. Furthermore, the sterol composition did not show any changes between the drying and rewetting phases and the sudden rewetting after the drought did not cause the leaching of FA in dissolved OM. The epilithic biofilm and leaves were the most important sources of essential components (essential FA and sterols), while the epipsammic biofilm was the poorest source. Hence, the benthic substrata heterogeneity in the stream is important to ensure invertebrate fitness. Our conclusions help enhance the understanding of the mechanisms underlying drought effects on streams and the prediction of future changes caused by the increase in frequency and intensity of these periods as a result of global change.

Keywords: *lipids · particulate and dissolved organic matter · biofilm · leaf litter · basal resources*

INTRODUCTION

Drought in Mediterranean streams is a frequent and seasonal event due to the characteristics of the climate in this area (Bonada & Resh, 2013). Droughts are extreme hydrologic events that begin with a gradual decrease in flow until there is a complete loss of connectivity and surface water and end abruptly in autumn or early winter with the first rains (Gasith & Resh, 1999; Lake, 2003). Therefore, drought periods can be divided into the drying, flow fragmentation, terrestrial and rewetting phases. Although drought periods are determined by natural factors, global change, which encompasses the direct influence of anthropogenic activities on stream hydrology through watercourse alterations (damming, channelisation and water abstraction) and climate change, is expected to lead to an increase in the frequency and intensity of these episodes (Lehner *et al.*, 2006; Sabater & Tockner, 2010).

Droughts have important effects on the structure and functioning of ecosystems (Lake, 2003), including changes in physico-chemical properties (flow, stream connectivity, water temperature, oxygen and nutrient concentrations; Stanley *et al.*, 1997; Dahm *et al.*, 2003; von Schiller *et al.*, 2011), ecosystem metabolism (Acuña *et al.*, 2004), organic matter (OM) processing (Larned *et al.*, 2010) and food webs (Power *et al.*, 2013). Thus, droughts can alter basal resources (Lake, 2003), which in forested headwater streams are primarily allochthonous and secondarily autochthonous OM (Vannote *et al.*, 1980). Due to the reduced and subsequently extinct flow, large amounts of OM accumulates in pools and the dry streambed (Boulton & Lake, 1992b; Chapter 1). Decomposition of OM in the dry channel occurs at a very slow rate due to the low microbial activity (Maamri *et al.*, 1997). During the rewetting process, part of this accumulated OM is transported downstream and at the same time OM is imported from upstream (Ylla *et al.*, 2010). With the first rains, this OM produces dissolved OM (DOM) lixiviates, rich in polysaccharides and proteins (Ylla *et al.*, 2010). Biofilm also accumulates in the stream due to the spring peak, mainly as a result of the increase in algal biomass (Artigas *et al.*, 2009), but reduces in quality and quantity due to the desiccation and deterioration of the water quality. During the rewetting process, the recuperation of the biofilm is rapid (Timoner *et al.*, 2012).

The nutritional quality of OM can be assessed in different ways, e.g. by assessing the elemental composition or stoichiometry (carbon [C], nitrogen [N] and phosphorus [P] content and ratios), the content of refractory compounds (e.g. lignin, tannins) or the composition of biomolecules (lipid, protein and polysaccharide content; Gessner & Chauvet, 1994; Ledger & Hildrew, 1998; Hladyz *et al.*, 2009). Among these components, lipids are the most efficient energy-storing biomolecules (Cavaletto & Gardner, 1999). Stream invertebrates need to store large amounts of energy for metamorphosis and reproduction (Beer-Stiller & Zwick, 1995) or to survive periods of scarcity (Wilhelm, 2002), thus the quality of the resources may be more important than the quantity to the growth and reproduction of the animal population (Ahlgren *et al.*, 1997). Some lipid types such as fatty acids (FA) and sterols include essential molecules for invertebrates, as they are unable to synthesise them (Torres-Ruiz *et al.*, 2007). Therefore, the presence of these molecules is important for consumers' fitness. Moreover, some FA and sterols can be used as biomarkers (Desvilettes *et al.*, 1997; Mannino & Harvey, 1999) since they are related to some taxonomical groups (Arts & Wainman, 1999) and thus allow the composition of OM to be identified in terms of large groups (e.g. diatoms, chlorophytes, cyanobacteria and bacteria).

Our objective was to study the way in which the drought process influences the quality of the basal resources in a Mediterranean stream. To this end, the OM in the benthic and water compartments was characterised through lipid composition (FA and sterols) as indicators of the OM essentiality for consumers and origin. We hypothesised that (i) before the drought period, the biofilm (mainly algal biomass) in benthic substrates will be accumulated following the high productivity period (in spring) and the remaining benthic leaf litter and transported particulate OM (POM) will be well conditioned, and thus the available OM will be of a high quality, rich in FA and sterols; (ii) drought will cause the progressive desiccation of the biofilms and the accumulation of non-processed OM, thus devaluing their quality. However, the sudden rewetting process will cause this OM accumulated in the dry streambed to leach, which will result in an increase in the quality of the DOM. As a result, the drying-rewetting process will determine the availability of FA and sterols.

METHODS

Study site

This study was conducted in Fuirosos, a third-order intermittent stream. Fuirosos is situated 150 m a.s.l. in the north-eastern Iberian Peninsula (41° 42'N, 02° 34'E) in the Montnegre-Corredor Natural Park. This area has a Mediterranean climate, characterised by mild winters and warm, dry summers, and a high intra- and interannual variability of rainfall, which is concentrated in spring and autumn.

The riparian forest is mainly composed of *Platanus acerifolia* (Aiton) Willd., *Alnus glutinosa* (L.) Gaertn., *Populus nigra* L. and *Corylus avellana* L. The study reach included a riffle with boulders and cobbles, and a large pool with leaf litter and sand. In 2006, following a progressive reduction in the flow, the streambed dried up on 19 June and remained dry until 13 September (Fig. 1). Droughts are common events in this stream; in 10 out of 13 years (1998–2010), summer flow cessation occurred (Chapter 1). The 2006 drought was similar in duration to the "average" drought in Fuirosos (it lasted 83 days versus the average of 87 days); however, it started 3 weeks before the average (7 June in 2006 versus 28 June on average; Chapter 1).

Sampling

Benthic substrates (leaves, sand and cobbles) and water (POM and DOM) were sampled on four occasions: before the drought, on 25 May and 12 June, and after the drought period, on 13 and 20 September. On 25 May, the flow was 1.8 L s^{-1}, whereas the basal flow was 7.7 L s^{-1} in the previous period and on 12 June the flow was close to 0 L s^{-1}. Following the drought period, the flow suddenly recovered, with a flow peak of nearly 100 L s^{-1} on 13 September, although this peak cannot be considered a flood (moderate floods > 250 L s^{-1}; Sabater *et al.*, 2008). On 20 September the flow had already decreased to 15.7 L s^{-1} (Fig. 1).

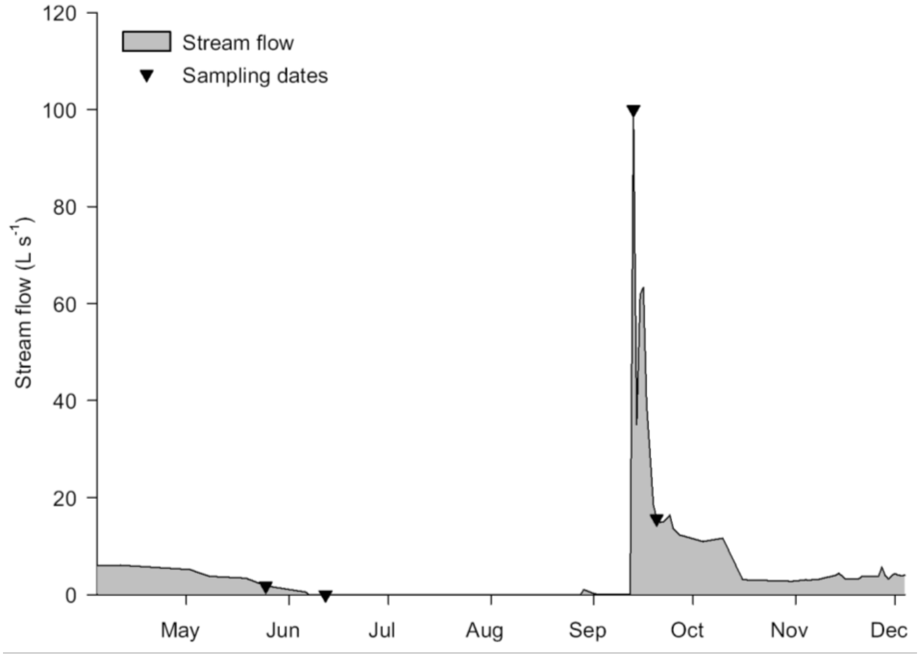


Fig. 1 Stream flow in Fuirosos during the study period with the sampling dates (25 May, 12 June, 13 September and 20 September) indicated. The drought period, with no superficial flow, lasting from June 19 to September 13, is shown.

Physico-chemical variables, i.e. water temperature, dissolved oxygen and conductivity, were measured in the field (Table 1). The temperature did not show any remarkable changes, with an average value of 16ºC. Oxygen concentration greatly decreased with the reduction in flow before the drought and returned to the pre-drought values during the rewetting period. Conductivity showed the highest values with the reduction in flow and the lowest values with the flow peak at the beginning of the rewetting period.

The relative cover (%) of each substratum in the streambed was identified every 20 cm in 4 transects (3 m apart) in the study reach (Table 1). During the study period, the relative cover of sand increased slightly, while rock and cobble cover decreased. However, none of these inorganic substrates noticeably dominated the streambed. Wood represented a low percentage of the streambed coverage; fresh leaves were only present on the first sampling date, while litter covered most of the streambed on the last date and detritus accumulated before the drought.

On each sampling occasion, benthic material, i.e. leaves and particulate material, epilithic biofilm, biofilm and fine material accumulated in sand, and water were collected. Cobbles were collected directly from the streambed, while leaves and sand were collected from a depth of 5–10 cm with a core of 4.3 cm^2. Once in the laboratory, each benthic substrate was sonicated (3 min, sonication bath at 40 W, 40 kHz) in distilled water (60–120 mL) to detach the OM. Cobbles were previously scraped with a toothbrush in the same water and leaves were later homogenised with a mixer. Hence, leaf analyses included the composition of the leaf itself and of the associated biofilm. Water collected from the stream (8 L) was filtered through precombusted GF/F filters (0.7 μm pore size, Whatman, Maidstone, UK), and both the filter (POM) and the filtered water (DOM) were kept for analysis. All samples were kept frozen at -18ºC until analysis.

Table 1 Physico-chemical variables and substrate composition of the wet area of the streambed measured in the field during the study period. The surface of each substrate type is expressed in percentage of the wet area (due to the organic substrate superimposition, the sum of the percentages sometimes exceeds 100%)

	25 May	12 Jun		13 Sep	20 Sep
Physico-chemistry					
Temperature (ºC)	15.9	16.0		15.3	16.6
Oxygen (mg L^{-1})	9.70	3.40		9.80	9.90
Conductivity (µS cm^{-1})	231	256		206	241
Total wet area (m^2)	36.6	33.0		58.5	36.3
Inorganic substrates (%)					
Rocks & Cobbles	48	44		NA	43
Sand	52	56		NA	57
Organic substrates (%)					
Wood	30	7		NA	9
Litter	51	36		NA	76
Fresh leaves	37	0		NA	0
Fine detritus (%)	44	50		NA	0

Dark grey column separates the dates before and after the drought.
NA = data unavailable. On 13 September it was not possible to determine the substrate composition due to the high turbidity of the water.

Fatty acid and sterol analysis

To determine the composition of FA and sterols, 3 replicates per date and substrate (4 for water sterols) were analysed. Samples were frozen with liquid N and freeze-dried for 48 h. The composition of FA and sterols was analysed using gas chromatography and mass spectrometry (for details see General methods). Internal standards (heptadecanoic acid and 5-α-cholestane) were used to calculate the yield of the extraction (71% for FA and 53% for sterols) and to correct for the final concentrations.

FA were classified according to the number of double bonds, i.e. saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA), and the double bond position, accounting for ω3 and ω6 FA, because a ω3 : ω6 ratio of < 1 indicates that the resources are primarily of terrestrial origin and a ratio > 1 indicates a primarily aquatic origin. Moreover, its essentiality for consumers (essential FA [EFA]: 18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3) and its indicative value for organism presence, i.e. FA from diatoms (20:5ω3, 16:1ω7), chlorophytes and cyanobacteria (18:2ω6, 18:3ω3) and bacteria (15:0, 15:1 and branched 13:0, 15:0), and long-chain SAFA (C20-C32) indicating the presence of vascular plant detritus (Desvilettes *et al.*, 1997; Napolitano, 1999; Olsen, 1999), were taken into account. All sterols are essential for consumers and are indicators of resources of algal (fucosterol) and fungal origin (ergosterol) and from higher plants, e.g. campesterol, sitosterol, stigmasterol and lanosterol; cholesterol indicates the presence of animals (Martin-Creuzburg & Elert, 2009).

For benthic substrates, the FA and sterol composition will be given per dry mass (DM; determined to the nearest 0.1 mg), while for water POM and DOM composition will be given per water volume.

Statistical analysis

Changes in the FA and sterol composition (in mg g^{-1} DM) between dates and between the different benthic substrates (leaves, sand and cobbles) were examined through 2-way ANOVA. Changes in the FA and sterol composition (in μg L^{-1}) between dates in DOM and POM were analysed through 1-way ANOVA. Tukey's HSD was used as a post-hoc analysis. A one-sample *t*-test was used to determine whether the ω3 : ω6 ratio was significantly different from 1. Normality of residuals and homogeneity of variances, tested using the Kolmogorov-Smirnov (with Lilliefors correction) and Levene's tests, respectively, were achieved or improved in all of the variables using Box-Cox transformations. These analyses were performed with PASW (version 18, IBM, Armonk, NY, USA). Principal component analyses (PCA) of the relative abundance (%; arcsine square root transformed) of the FA and sterol components were performed with CANOCO (version 4.5, Biometris, Wageningen, the Netherlands).

RESULTS

Benthic organic matter

FA in benthic substrates represented on average 11.7 ± 3.2% of the total amount of lipids (5.4 ± 1.7% in leaves, 12.4 ± 4.0% in sand and 28.1 ± 10.3% on cobbles). FA content and composition were affected by drought in all benthic substrates (Table 2). The total content of FA decreased over time, with higher values before the drought than afterwards; the richest substrate was epilithic biofilm, followed by leaves, and finally sand (Fig. 2a). The same pattern was found for EFA content (Fig. 2b), although EFA were as abundant on cobbles as in leaves. The ω3 : ω6 ratio was significantly different from 1 ($t_{32} = -7.326$; $P < 0.001$). Before the drought, cobbles and sand had a ω3 : ω6 ratio > 1 on average, which indicates that the OM was autochthonous, while on the remaining sampling dates and in leaves, this ratio was < 1, which indicates a terrestrial origin (Fig. 2c). SAFA, MUFA and PUFA content was higher before the drought than afterwards, and in general cobbles were the richest substrate, while sand was the poorest substrate (Fig. 3a). FA from diatoms, chlorophytes and cyanobacteria were also higher before the drought than afterwards and their content decreased from cobbles to leaves to sand. Allochthonous FA were not found on cobbles and its content was higher in leaves than in sand (Fig. 3b). Bacterial FA content was low compared to FA from other origins, and after the drought it was higher on cobbles (34.8 ± 4.5 μg g^{-1} DM).

Drought affected the relative composition of FA (% mg g^{-1} DM), although the effects varied depending on the benthic substrates (Fig. 4). The first axis (29% of variance) of the PCA separated the samples by date within the different substrates, and the second axis (24% of variance) separated the substrates, with leaf samples being situated in the negative part of this axis and cobbles and sand in the positive part. Leaves were characterised by the presence of FA from vascular plant detritus (long-chain SAFA, e.g. 24:0) and EFA from chlorophyte and cyanobacteria origin (18:2ω6 before and 18:3ω3 after the drought). Sand and cobbles were characterised by the diatom FA 16:1ω7. During the rewetting period, just after the drought, these substrates were rich in some ω3 FA (mainly 16:3ω3), while on the

Table 2 Results from the 2-way ANOVA and post-hoc (Tukey's HSD) analyses for the fatty acid (FA) and sterol composition (in mg g^{-1} DM) in the benthic substrates, i.e. leaves (L), sand (S) and cobbles (C), over time. The dates analysed are before the drought period, 25 May = 1, 12 June = 2, and after the drought period 13 September = 3 and 20 September = 4

		2-way ANOVA									**Tukey's HSD**	
		Date			**Substrate**			**Date x Substrate**				
	Benthic substrates	df	F	*P*	df	F	*P*	df	F	*P*	**Date**	**Substrate**
FA	Total FA	3,23	27.50	**< 0.001**	2,23	31.87	**< 0.001**	6,23	2.76	**0.035**	1,2 > 3,4	C > L > S
	EFA	3,23	19.56	**< 0.001**	2,23	46.88	**< 0.001**	6,23	2.20	0.081	1,2 > 3,4	C,L > S
	ω3 : ω6 ratio	3,21	38.44	**< 0.001**	2,21	8.72	**0.002**	6,21	16.75	**< 0.001**	1 > 2,4 > 3	L > C,S
	SAFA	3,23	18.08	**< 0.001**	2,23	33.35	**< 0.001**	6,23	4.06	**0.006**	1,2 > 3,4	C > L > S
	MUFA	3,23	31.11	**< 0.001**	2,23	22.75	**< 0.001**	6,23	5.29	**0.001**	1,2> 3,4	C > L,S
	PUFA	3,23	29.80	**< 0.001**	2,23	38.65	**< 0.001**	6,23	3.29	**0.018**	1,2 > 3,4	C,L > S
	Diatoms FA	3,22	78.74	**< 0.001**	2,22	84.21	**< 0.001**	6,22	14.70	**< 0.001**	1,2 > 3,4	C > L > S
	Chlorophytes & Cyanobacteria FA	3,23	19.00	**< 0.001**	2,23	61.57	**< 0.001**	6,23	2.86	**0.031**	1 > 3 > 4; 2 > 4	C,L > S
	Allochthonous FA*	3,15	3.62	**0.038**	1,15	44.89	**< 0.001**	3,15	9.12	**0.001**	–	–
	Bacterial FA	3,21	1.51	0.242	2,21	7.90	**0.003**	6,21	5.69	**< 0.001**	–	C > S
Sterols	Total sterols	3,22	1.40	0.269	2,22	28.36	**< 0.001**	6,22	4.63	**0.003**	–	L > C,S
	Phytosterols	3,22	2.46	0.089	2,22	41.32	**< 0.001**	6,22	5.26	**0.002**	–	L > C,S
	Ergosterol*	1,4	< 0.01	0.976	–	–	–	–	–	–	–	–
	Fucosterol*	1,12	3.27	0.096	2,12	1.11	0.360	2,12	1.08	0.371	–	–
	Cholesterol	3,22	4.19	**0.017**	2,22	0.42	0.664	6,22	3.84	**0.009**	1,2,3 > 4	–

P-values < 0.05 are indicated in bold.

EFA = essential FA, SAFA = saturated FA, MUFA = monounsaturated FA, PUFA = polyunsaturated FA.

* Allochthonous FA were only found in L and S; ergosterol was only found in L on dates 1 and 3 and fucosterol only on dates 1 and 3.

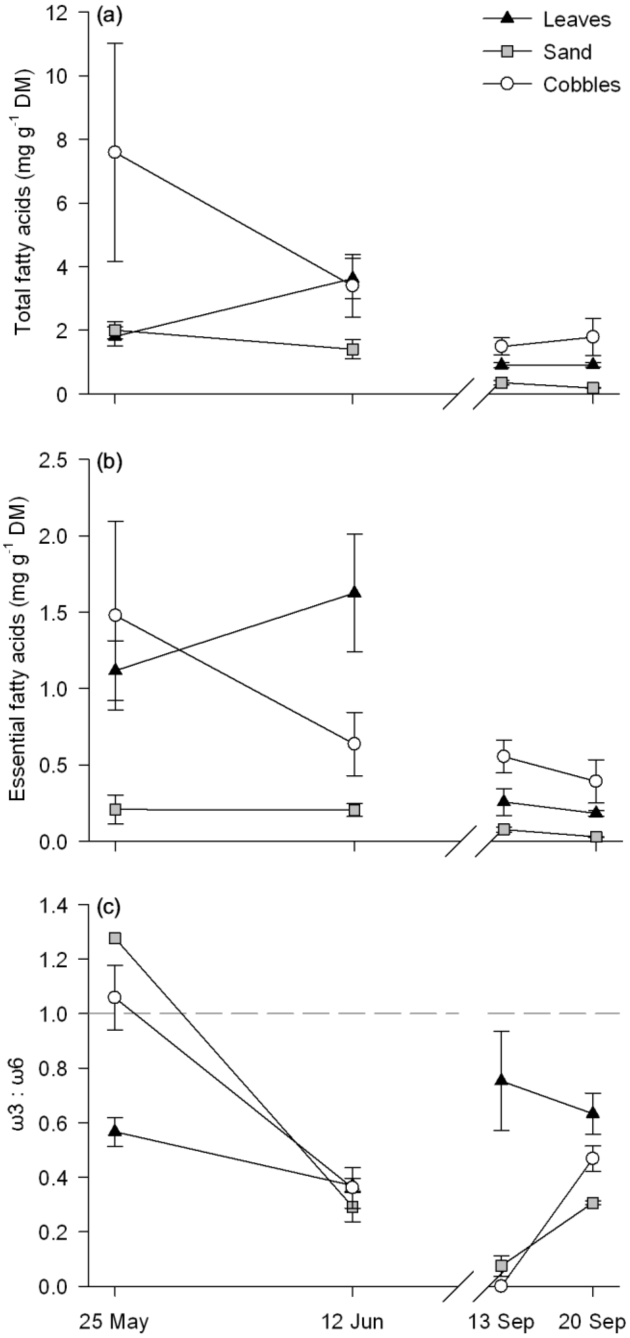


Fig. 2 (a) Total fatty acids, (b) essential fatty acids and (c) ω3 : ω6 ratio in the benthic substrates during the study period. The dashed line in (c) indicates the shift from terrestrial (ω3 : ω6 < 1) to aquatic (ω3 : ω6 > 1) origin of organic matter. In the sample from leaves on 25 May, n = 2 and in (c) the ω3 : ω6 ratio from sand on 25 May, n = 1. The break in the X axis represents the drought period and error bars indicate ± 1 SE.

fourth date they were richer in the EFA 20:5ω3 from diatoms and some non-specific FA (18:0, 16:1, 18:1ω9).

Sterols represented on average 9.9 ± 3.0% of the total lipids (12.2 ± 4.0% in leaves, 5.7 ± 3.0% in sand and 7.2 ± 2.8% on cobbles). The quantity and composition of the sterols were basically dependent on the substrate, but not noticeably affected by the drought (Fig. 5; Table 2). The sterol content was higher in leaves than in the other substrates, and the lowest values in leaves were found just after the drought. Sterols found in the benthic substrates mainly came from higher plants, representing on average 77.4 ± 3.2% from the total sterols and reaching 89.4 ± 2.6% in leaves. Thus, phytosterols showed the same pattern as the total sterols. Ergosterol was only present in leaves on the first and third dates, with a mean value of 73.6 ± 17.8 µg g^{-1} DM. Fucosterol showed high variability (values ranged

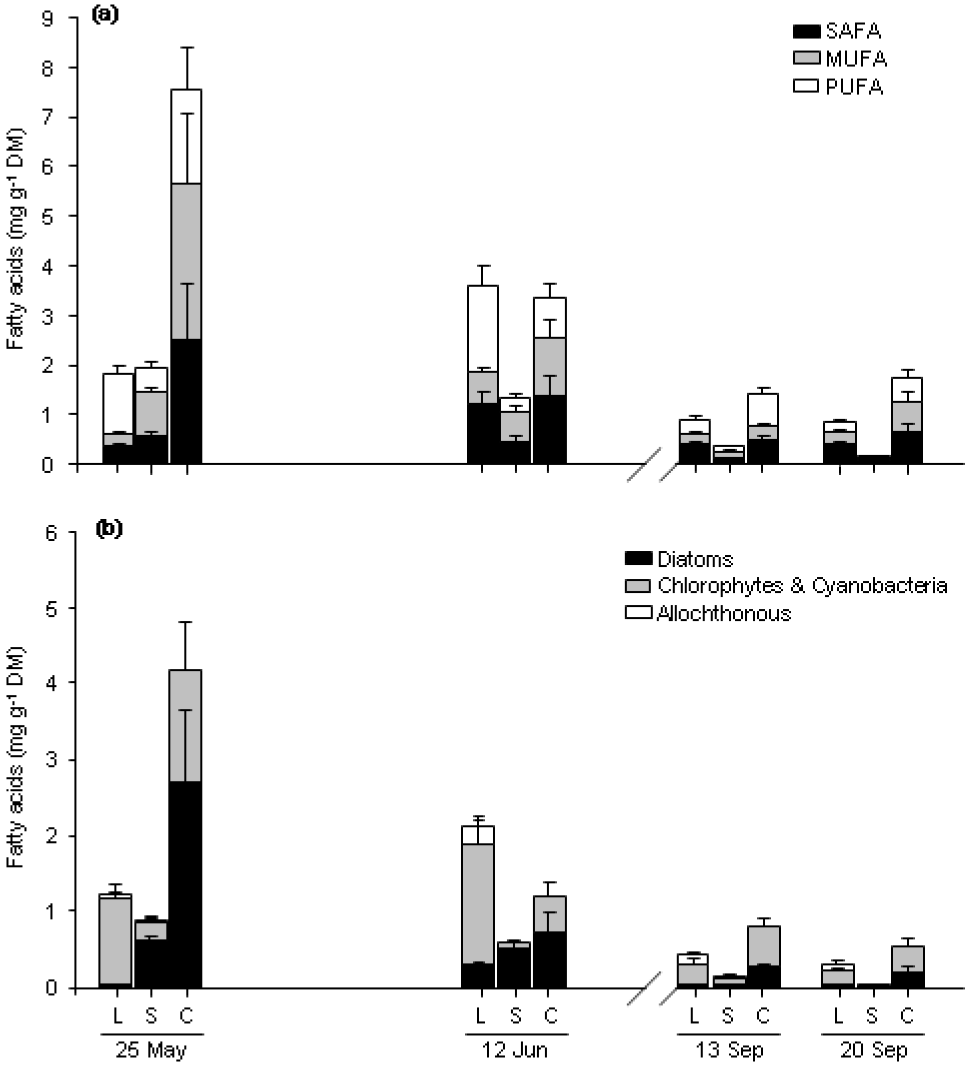


Fig. 3 Fatty acid (FA) content according to (a) the number of double bonds, i.e. saturated (SAFA), monounsaturated (MUFA) and polyunsaturated FA (PUFA), and (b) its origin, in the benthic substrates, i.e. leaves (L), sand (S) and cobbles (C), during the study period. In the sample from leaves on 25 May, n = 2 and in (b) the diatoms FA from cobbles on 25 May, n = 2. The break in the X axis represents the drought period and error bars indicate + 1 SE.

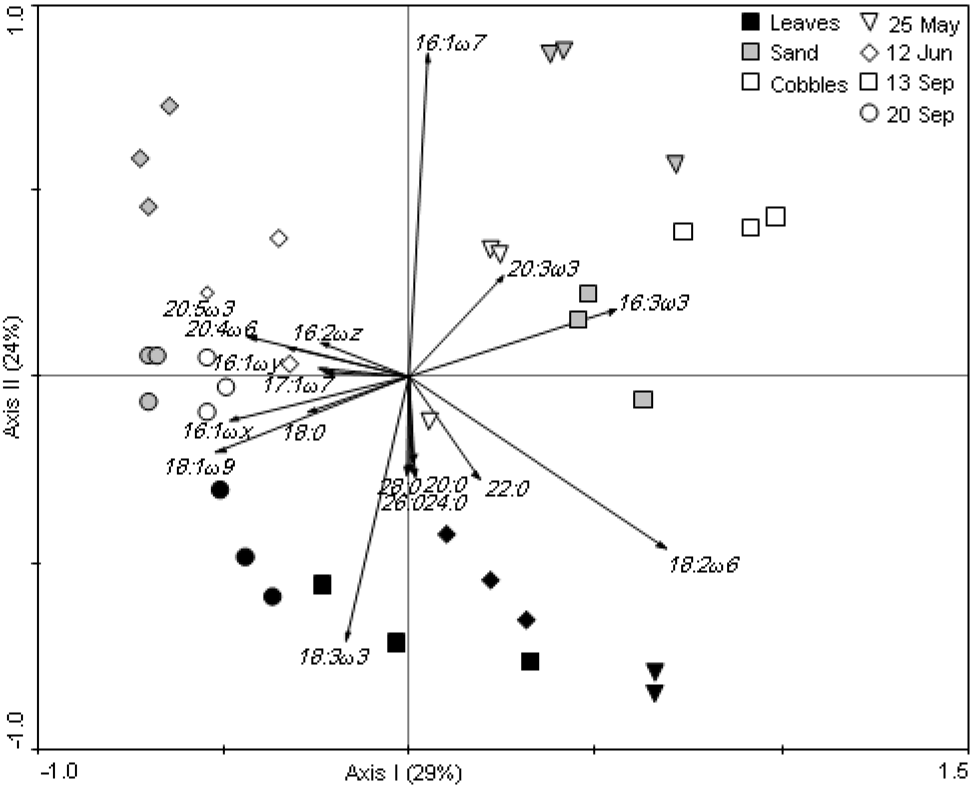


Fig. 4 Principal component analysis (PCA) of the fatty acid (FA) composition (% mg g^{-1} DM) of the benthic substrates during the study period. The first and second axes are represented, and the percentage of variability explained by each is indicated. In the cases where the double position could not be determined, x, y or z are written. To improve readability, FA with a score < 1 were removed from the graph (though not from the analysis).

from 0 to 773 µg g^{-1} DM), so no significant differences were found either between substrates or between dates. Cholesterol content was 132.7 ± 33.2 µg g^{-1} DM on average, and did not show any differences as a result of the drought.

The relative composition of sterols in the leaves was different from that in the other substrates. In the PCA (% mg g^{-1} DM; Fig. 6), the leaves were separated from the cobbles and sand mainly by the second axis (12% of variance). Leaves were situated on the positive part of the axis, characterised by stigmasterol, ergosterol, lanosterol and stigmastanol. However, on the last date the leaves and other substrates were characterised by β-sitosterol. The sand and cobbles were rich in campesterol, fucosterol and cholesterol during the drying period.

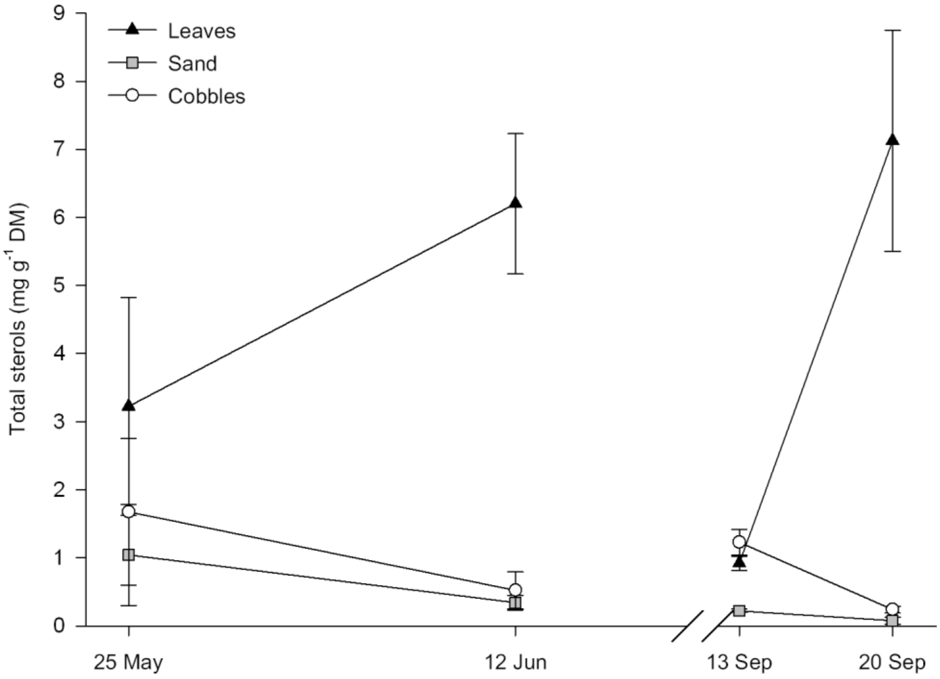


Fig. 5 Total sterol content of the benthic substrates during the study period. The break in the X axis represents the drought period and error bars indicate ± 1 SE.

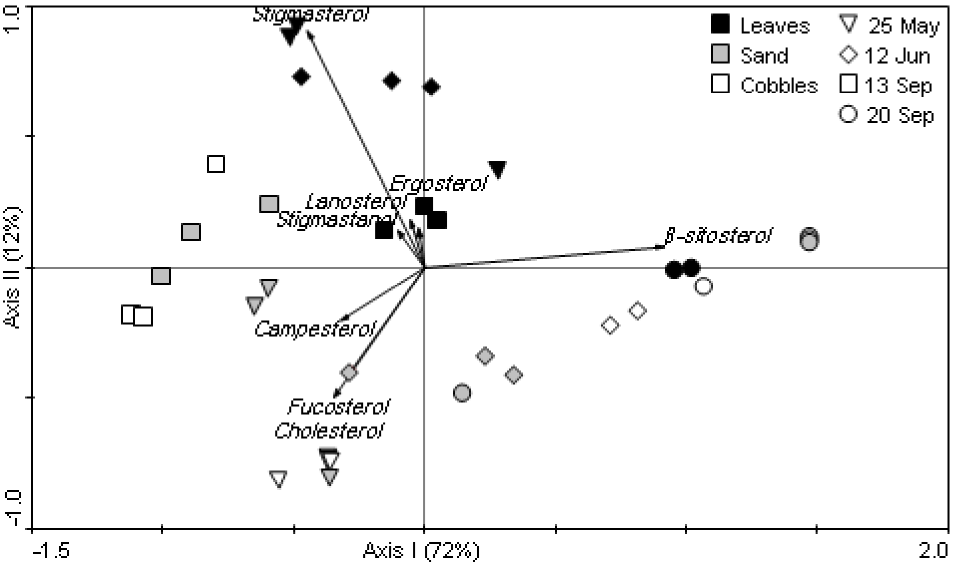


Fig. 6 Principal component analysis (PCA) of the sterol composition (% mg g^{-1} DM) of the benthic substrates during the study period. The first and second axes are represented, and the percentage of variability explained by each is indicated.

Transported organic matter

In POM, the FA content of the total lipids was 26.2 ± 16.0%. Total FA content showed a peak before the drought (Fig. 7), related to MUFA; these represented 63% of the total FA and were mainly from diatoms (60%). EFA and the ω3 : ω6 ratio were also higher before the drought (EFA before: 8.15 ± 1.37, after: 0.97 ± 0.52 μg L^{-1}; ω3 : ω6 before: 1.53 ± 0.29, after: 0.04 ± 0.03). However, the ω3 : ω6 ratio was not significantly different from 1 ($t_{12} = -0.811$; $P = 0.434$). Allochthonous FA were higher on the first date (4.99 ± 0.57 μg L^{-1}) and were barely or not at all present during the remainder of the period (Table 3). In POM, sterols represented on average 5.7 ± 3.1% of the total lipids. Changes in total sterols (12.91 ± 3.96 μg L^{-1}) over time were not related to drought (Table 3). Ergosterol and fucosterol were not found in the POM samples.

In DOM, the FA (Fig. 7) and sterol levels were generally low. In all FA types, only allochthonous FA showed differences over time, although they were only found on the first and fourth dates (7.85 ± 0.97 μg L^{-1}; Table 3). Changes in total sterols (2.40 ± 0.93 μg L^{-1}) over time were not related to drought (Table 3).

Fig. 7 Total fatty acid content in water particulate (POM) and dissolved organic matter (DOM). In DOM, on 12 June, n = 2 and on 13 September, n = 1. The break in the X axis represents the drought period and error bars indicate ± 1 SE.

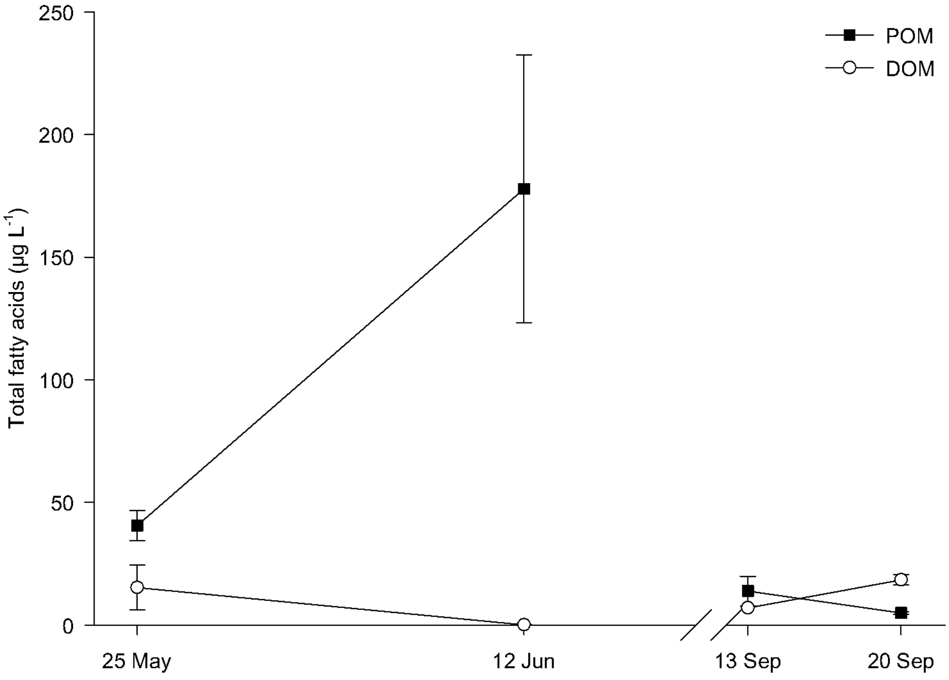


Table 3 Results from the 1-way ANOVA and post-hoc (Tukey's HSD) analyses for the fatty acid (FA) and sterol composition (in µg L^{-1}) in the transported dissolved and particulate organic matter (POM and DOM) over time. The dates analysed are before the drought period, 25 May = 1, 12 June = 2, and after the drought period 13 September = 3 and 20 September = 4

		Transported POM				**Transported DOM ♦**			
		1-way ANOVA				**1-way ANOVA**			
		df	F	*P*	**Tukey's HSD**	df	F	*P*	**Tukey's HSD**
FA	Total FA	3,8	32.58	**< 0.001**	2 > 1 > 3,4	3,8	0.35	0.792	–
	EFA	3,8	7.64	**0.010**	2 > 3,4	–	–	–	–
	ω3 : ω6 ratio	3,8	29.32	**< 0.001**	1,2 > 3,4	–	–	–	–
	SAFA	3,8	35.51	**< 0.001**	2 > 3 > 4; 1 > 4	3,8	0.56	0.657	–
	MUFA	3,8	10.26	**0.004**	2 > 1,3,4	3,8	0.03	0.992	–
	PUFA	3,8	9.72	**0.005**	2 > 3,4	3,8	2.65	0.120	–
	Diatom FA	3,8	29.54	**< 0.001**	2 > 1 > 3; 4 > 3	3,8	1.35	0.324	–
	Chlorophytes & Cyanobacteria FA	3,8	1.67	0.250	–	–	–	–	–
	Allochthonous FA*	3,8	65.60	**< 0.001**	1 > 2,3,4	3,8	55.91	**< 0.001**	4 > 1 > 2,3
	Bacterial FA	3,8	2.50	0.134	–	3,8	0.19	0.898	–
Sterols	Total sterols	3,12	8.31	**0.003**	1,2,3 > 4	3,12	4.86	**0.019**	3 > 1,4
	Phytosterols	3,12	2.83	0.084	–	–	–	–	–

P-values < 0.05 are indicated in bold.

EFA = essential FA, SAFA = saturated FA, MUFA = monounsaturated FA, PUFA = polyunsaturated FA.

♦ In DOM, some variables were not analysed due to the low quantities found. All the PUFA found in the samples were EFA from chlorophytes and cyanobacteria, and hence were analysed once as PUFA.

*Allochthonous FA were only found on dates 1 and 4.

DISCUSSION

Our main hypothesis was that the drying-rewetting process would determine the availability of FA and sterols, thus affecting the nutritional quality of basal resources in the stream. This hypothesis was confirmed to some extent by our results, with the drought causing changes in the FA content and composition on benthic substrates and transported POM. In most of the measured variables, the first and second dates differed from the third and fourth dates, highlighting the differences between the drying and rewetting periods. Benthic substrates were richer in total and essential FA during the drying process, but these temporal differences were not found in the sterol composition. Changes in OM composition caused by drought were consistent with a higher polysaccharide, protein and lipid content in the drying period than in the rewetting period in the same stream (Ylla *et al.*, 2010).

We predicted the availability of high-quality OM (rich in FA and sterols), which was related to the spring peak of algal biomass and conditioned leaf litter in the stream before the drought. This is confirmed by the higher concentration of FA related to primary producers (diatom, chlorophytes and cyanobacteria FA) on benthic substrates in this period, as well as by the change from autochthonous OM before the drought to predominantly allochthonous OM afterwards, which was reflected by the shift in the ω3 : ω6 ratio (from > 1 to < 1). This seasonal change has already been described in the same stream by Artigas *et al.* (2009), with algal biomass sharply increasing from the beginning of spring until the interruption of stream flow, particularly in the coarse substrata (rocks, boulders and cobbles). Romaní *et al.* (2013) described this shift for Mediterranean systems in general.

The predicted pattern was not found in sterols, which did not show changes between the drying and rewetting phases. Most of the sterols were phytosterols, derived from the leaves themselves, and were probably not altered by the different microbial colonisation during the drought period. To our knowledge, no studies have been conducted on the effects of leaf decomposition on sterol composition.

In the water compartment, the difference in FA between the pre- and post-drought periods was also observed in POM. Higher total FA, EFA and ω3 : ω6 ratio indicated the availability of higher-quality transported material before the drought. The FA peak observed in POM just before the drought was associated with an increase in the total lipid content (Ylla *et al.*, 2010) on the same date. In the present study, this peak was due primarily to diatom FA, and may be related to the algal peak (Artigas *et al.*, 2009).

We also hypothesised that the sudden rewetting process would leach and transport high-quality DOM downstream. However, the DOM composition did not show any changes in FA over time. Ylla *et al.* (2010) described a peak of DOM immediately after the rewetting process, with a high content of dissolved organic C (DOC), biodegradable DOC, dissolved organic N, polysaccharides and peptides. This peak was consistent with an increase in the enzymatic activities related to these substrates (i.e. β-glucosidase and leucine aminopeptidase). Although the lipids in DOM were not measured in that study, no peak was observed in lipase during the rewetting process. Our results reinforce the idea that the OM lipids accumulated in the stream do not leach (at least in this initial phase when the OM is not yet fully conditioned). Sun *et al.* (1997) found that leachates from leaf litter (from several species) contained mainly polysaccharides and lignin, while algal leachates contained a mixture of polysaccharides, proteins and lipids.

There were significant differences in the FA and sterol composition between substrates. The epilithic biofilm showed the highest FA content, mainly of diatom and bacterial origin. Hence, epilithic biofilm represents the most important source of FA for consumers. Cobbles, specifically the upper side, support the main proportion of the whole autotrophic biomass of the stream (80–90%; Romaní, 2010). In Fuirosos, the algal community on cobbles is dominated by diatoms and bacterial density is higher in sand than on cobbles (Sabater *et al.*, 2005; Veraart *et al.*, 2008). The higher abundance of bacterial FA on cobbles probably indicates that bacteria on cobbles, although less abundant, have a higher FA content. The epipsammic biofilm was the poorest in terms of the total FA content and all FA types. This finding is consistent with stoichiometry measures in Fuirosos, with higher C : N and C : P ratios in sand compared to cobbles (Timoner *et al.*, 2012). The leaves showed a different composition of both FA and sterol compared to the other substrates. The contribution of FA from leaves derived primarily from the biofilm that colonises the surface, essentially chlorophytes and cyanobacteria. Through a comparison of colonised and uncolonised leaves, colonised leaves were shown to have a higher nutritional content (Graça *et al.*, 2001). In contrast, Torres-Ruiz *et al.* (2007) found low percentages of autochthonous FA on OM (< 5%), but they observed some autochthonous colonisation peaks in spring. No study has been published on the taxonomical identification of algae colonising leaves in Fuirosos. Although chlorophytes and cyanobacteria provided most of the FA, diatoms seem to be the dominant colonisers (A.M. Romaní, pers. comm.). In our study, EFA were mainly provided by epilithic biofilm and leaves. Cobbles (and secondarily sand) were the main suppliers of 20:4ω6 and 20:5ω3, while leaves were important for 18:3ω3 and 18:2ω6. This coincides with the findings of Torres-Ruiz *et al.* (2007), who found 18:2ω6 to be the most abundant EFA in OM. Leaves were the most important source of sterols, which were mainly phytosterols from the leaf tissue. Ergosterol, from fungi, was only found on leaves on the first date, but not the second, which probably indicates that fungi were negatively affected by the desiccation (Bruder *et al.*, 2011). If different EFA and sterols are found in different substrates, consumers will probably need to feed on these different substrates, as was suggested for caddisflies by Torres-Ruiz *et al.* (2010). Substrate heterogeneity in streams guarantees the diversity of food resources and essential compounds for consumers' diet.

The FA content in leaves in our study ranged from 0.9 to 3.6 mg g^{-1} DM on average, which is comparable to the range found by Torres-Ruiz & Wehr (2010) in leaves during decomposition (2–6.9 mg g^{-1} DM). The FA content in the biofilms in our study was below 12 mg g^{-1} DM, lower than the content found by Hill *et al.* (2011; from 10–50 mg g^{-1} DM) in artificial channels. This was probably related to the greater stability of the conditions or to the differences in biofilm community composition. We were not able to find comparable values of FA content for transported POM and DOM. Hence, our data are important for gaining more information on the FA and sterol content of natural streams.

Changes in the quality of the resources affect the fitness of consumers (Hemmi & Jormalainen, 2002). Invertebrates need to acquire EFA and sterols in their diet from the available resources in the stream due to their inability to synthesise these molecules (Olsen, 1999; Martin-Creuzburg & Elert, 2009). However, the effects of the resource quality on consumers' fitness will depend upon species identity and may be compensated by modifying consumption (Fink & Von Elert, 2006) or altering some physiological processes (Graça *et al.*, 1993). Changes in invertebrate communities due to drought events have been observed in both Fuirosos (Acuña *et al.*, 2005) and other streams (Boulton, 2003); flow reduction causes an increase in invertebrate density and competence for resources. During this period, insects need to accumulate energy and lipids for emergence (Anderson & Cummins, 1979; Anderson & Sedell, 1979) and drought causes earlier emergence in some species (Leberfinger *et al.*, 2010). Hence, this is a key moment for invertebrate survival.

Since global change is predicted to increase the frequency and intensity of droughts (Lehner *et al.*, 2006; Sabater & Tockner, 2010), it is important to understand the mechanisms underlying the effects of these periods on river functioning. Our findings indicate that stream drought determines a general decrease of EFA content in basal resources. Extension of droughts to temperate streams or longer droughts in temporary rivers could compromise the quality of the resources and consumers' fitness.

CHAPTER 3

BIOCHEMICAL QUALITY OF BASAL RESOURCES IN A FORESTED STREAM: EFFECTS OF NUTRIENT ENRICHMENT

ABSTRACT

A study of the qualitative changes in epilithic biofilm and transported organic matter (OM) was performed in a forested headwater stream that received a moderate level of experimental nutrient enrichment (nitrogen [N] and phosphorus [P]) for 4 years. Basal concentrations of N and P in the fertilised reach were increased twofold (to c. 750 µg N L^{-1}) and threefold (to c. 30 µg P L^{-1}) with respect to the background concentration in the control reach. The study was performed during the leaf emergence period. The chlorophyll content and elemental composition (carbon [C] and N) of the epilithic biofilm were analysed. Bacterial density, extracellular enzyme activities and the biochemical composition (including amino acids, fatty acids [FA] and sterols) of the biofilm and the transported OM (particulate and dissolved fractions) were also determined. Nutrient enrichment increased the chlorophyll content and bacterial density of the biofilm, although these changes were constrained by light availability. The biofilm C : N ratio decreased due to an increase in protein content with nutrient enrichment. In the fertilised reach, coinciding with greater light availability, the contents of polysaccharides, lipids, and essential FA, increased. FA and amino acids from diatoms were more abundant in the fertilised reach, and leucine-aminopeptidase activity (related to peptide decomposition) was also higher. However, the biochemical composition of the transported OM did not change with changes in nutrients. Nutrient enrichment caused increases in autotrophic and heterotrophic biomass, in polysaccharide, protein and lipid content and in EFA, primarily when light availability was high, highlighting the importance of riparian phenology.

Keywords: *epilithic biofilms · amino acids · fatty acids · polysaccharides · headwater stream · riparian phenology*

INTRODUCTION

Headwater streams represent the majority of channel length within a drainage basin (reviewed by Leopold *et al.*, 1964, pp. 134–142) and support higher rates of biogeochemical processing than larger streams (Peterson *et al.*, 2001; Alexander *et al.*, 2008). Many of these systems are covered by riparian canopies and rely on this vegetation for allochthonous inputs of energy and matter. Autochthonous productivity in headwaters is often limited by nutrient and/or light availability (Vannote *et al.*, 1980; Wallace *et al.*, 1997b). The presence of canopies over temperate streams largely determines the non-continuous energy flux into the ecosystem. There are two types of energy inputs related to the phenology of deciduous riparian trees: an allochthonous pulse as leaves fall during late summer to early autumn and an autochthonous pulse driven by primary production in early spring, before leaf emergence, related to higher light availability (Rosenfeld & Roff, 1992; Lamberti, 1996; Hill *et al.*, 2001; Ylla *et al.*, 2007).

The eutrophication of aquatic ecosystems due to nutrient enrichment is a global phenomenon (Vitousek *et al.*, 1997; Meybeck, 2003). Eutrophication causes the degradation of water quality, increases the rate of biological production, changes the structure and composition of food webs and alters biogeochemical cycling in impacted systems (Smith *et al.*, 2006). In headwater streams, increases in nutrient concentrations promote microbial activity and accelerate allochthonous organic matter (OM) processing (Greenwood *et al.*, 2007; Benstead *et al.*, 2009). However, invertebrate secondary production, biomass and abundance also increase, demonstrating that changes in food quality (i.e., higher OM conditioning) override changes in food quantity (i.e., faster OM decomposition; Cross *et al.*, 2006). For autochthonous OM, elevated nutrient concentrations can also increase biomass, chlorophyll content and primary production (Rosemond *et al.*, 2000; Dodds *et al.*, 2002), but this response may be limited by light availability (Hill *et al.*, 2001; Greenwood & Rosemond, 2005; Ylla *et al.*, 2007). Benthic algal communities can be an important energy source to stream food webs (1–60% of the total annual energy; Lamberti, 1996) and, at the same time, a higher-quality food source in comparison to terrestrial matter (Anderson & Cummins, 1979; Lamberti, 1996; Torres-Ruiz *et al.*, 2007). These functions result in part from the relatively low C : N and C : P ratios (Frost *et al.*, 2002) and the high protein and lipid contents of the algal-derived material (Lamberti, 1996). Effects of elevated nutrient concentrations on the elemental stoichiometry of the biofilm (higher P and N content), on some key consumers (higher N content), on bacterial density and on enzyme activities have been observed in a headwater Mediterranean stream (Sabater *et al.*, 2011).

The OM present in streams (benthic and transported) is a complex mixture of polysaccharides, proteins, lipids, lignin, organic acids and other compounds, such as humic substances (Mannino & Harvey, 2000). The primary energy-yielding compounds (biomolecules) for living organisms are polysaccharides, proteins and lipids. Many of these biomolecules play important structural and regulatory functions in organisms, and some are essential to consumers because they cannot be synthesised de novo, such as some amino acids and fatty acids (FA; Brett & Müller-Navarra, 1997; Dauwe & Middelburg, 1998). In spite of the recognised role of OM composition, its characterisation is mostly limited to the dissolved fraction (reviewed by Fellman *et al.*, 2010; but see, e.g., Gremare *et al.*, 1997; Ledger & Hildrew, 1998; Ylla *et al.*, 2010; Kolmakova *et al.*, 2013).

Previous work examined the stoichiometric changes in the biofilm produced by a mid-term nutrient enrichment experiment in a Mediterranean stream (Sabater *et al.*, 2011). Here, this work is extended by examining the qualitative changes in the biofilm and transported OM collected as part of the same experimental study. OM quality was assessed on the basis of biochemical composition (i.e., polysaccharides, proteins and lipids). Additionally, quantitative and functional (enzyme activities) changes in epilithic biofilm and transported OM (particulate and dissolved fractions) were analysed. We hypothesised that i) polysaccharide, protein and lipid content would increase in both the biofilm and transported OM due to nutrient enrichment, ii) the amino acid and FA composition of OM would change as the autotrophic component became more important in terms of biomass and iii) essential components for consumers (essential amino acids and FA) would increase, as most of these components come from primary producers.

METHODS

Experimental design

The study was conducted in the Fuirosos stream, located in the north-eastern Iberian Peninsula (41º 42' N; 02º 34' E) in the Montnegre-Corredor Natural Park. Fuirosos is a Mediterranean, oligotrophic, third-order stream with a basal flow ranging from 0 to 25 L s^{-1} (Butturini *et al.*, 2008). Nutrient addition was performed in a 50-m reach (termed the fertilised reach) and the reach upstream from the site of nutrient addition was considered the control reach. Both reaches were contiguous and similar in terms of slope, types of substrate, water flow, light regime and riparian vegetation. The dominant riparian species were deciduous (*Platanus acerifolia* [Aiton] Willd., *Alnus glutinosa* [L.] Gaertn., *Populus nigra* L. and *Corylus avellana* L.).

The artificial fertilisation was conducted over 4 years (June 2004 to June 2008). Nutrients were added continuously to the fertilised reach using a 200-L reservoir connected to a tap dripping dissolved nutrients at a constant flow. The tank was refilled with nutrient solution, and concentrations were adjusted weekly to respond to variations in the nutrient concentrations of the stream. Nutrients were added immediately downstream of a small waterfall to assure mixing and uniform dispersal. Inorganic nitrogen (N) was added as ammonium nitrate and phosphorus (P) as ammonium phosphate. During the 4 years of fertilisation, the average inorganic N concentration was increased twofold with respect to the background concentration in the control reach; N-NO_3 was increased from 364 to 580 µg L^{-1}, and N-NH_4 was increased from 24 to 185 µg L^{-1}. Inorganic P was increased threefold, with P-PO_4 increasing from 10 to 30 µg L^{-1} (Sabater *et al.*, 2011). This moderate increase of dissolved nutrient concentrations, primarily of inorganic P that is generally limiting in streams (Sabater *et al.*, 2005), decreased the N : P ratio in the control reach from 85 to 56.

The riparian vegetation and the steep banks of the channel cause the light availability to be generally low, except in early spring when the forest canopy is open (Acuña *et al.*, 2004). As part of the mid-term fertilisation experiment, this study was performed in spring 2008 over 4 sampling dates (17 March, 7 and 21 April, and 5 May), coinciding with a progressive decrease of light availability in the stream bed. Glass tiles (1.44 cm^2) were submerged in the stream on 7 February, 2008, before the sampling period began, to act as surrogate cobbles for biofilm development and to facilitate some measurements. Glass tiles were attached with silicon to a brick, and 4 bricks were installed per reach.

On each sampling occasion, field measurements of dissolved oxygen, water temperature, pH and conductivity were taken in both reaches using a portable multi-probe (Hach, Loveland, CO, USA). Four water samples (2 L) and 4 cobbles were randomly collected per reach. Cobbles were kept in a container with stream water to avoid desiccation. Water samples for nutrient analysis were filtered through 0.2 µm nylon membranes (Whatman, Maidstone, UK). Four glass tiles per reach were collected to measure bacterial density and extracellular enzyme activities. For the analysis of bacterial density, each glass tile was placed in 10 mL of filtered (0.2 µm nylon membranes, Whatman) and sterilised water. For enzyme activities, glass tiles were placed in 4 mL of stream water. All of the material collected was transported to the laboratory in a cooler.

Once in the laboratory, subsamples were taken from each water sample to measure nutrient concentrations, bacterial density and enzyme activities in bulk water. Afterwards, each water sample (3 x 600 mL) was filtered through 0.7 µm glass fibre precombusted filters (GF/F, Whatman) to separate dissolved OM (DOM) from particulate OM (POM). The 3 filters thus obtained were used to measure polysaccharide, protein and lipid content. From the filtered water, subsamples were taken to measure dissolved organic carbon (DOC), enzyme activities, polysaccharides, proteins and lipids.

To recover the biofilm, cobbles were immersed in distilled water (60 mL), brushed and sonicated (3 min, sonication bath at 40 W, 40 kHz). Each cobble’s surface was measured to allow for later standardisation of measurements by area. The 60 mL water samples containing the biofilm extract were divided to analyse carbon (C) and N, chlorophyll, polysaccharide, protein and lipid content.

All samples were kept frozen or refrigerated (for DOC) prior to analysis except for those samples used to measure enzyme activities and bacterial density, which were processed on the same day as collection.

Physico-chemical parameters

Instantaneous light was measured in the field with a portable meter (Li-192SB quantum sensor, LI-COR, Lincoln, NE, USA). Instantaneous light measurements were used to derive the continuous underwater light regime by relating site measurements to outdoor records from a nearby weather station, and air temperature during the sampling period was also obtained from a nearby weather station (Collsacreu and Pla de la Tanyada, Diputació de Barcelona).

Nitrate concentration was analysed with an ion chromatograph (Metrohm Compact IC). Ammonia was analysed spectrophotometrically (690 nm) with the addition of sodium salicylate, sodium citrate and sodium nitroprusside (Hach Company, 1992). Reactive P was measured following Murphy and Riley (1962).

Carbon and nitrogen content

The water samples used to measure DOC (20 mL) were acidified with 100 μL of hydrochloric acid, fixed with 100 μL sodium azide, and maintained at 4ºC prior to analysis. DOC was measured using a TOC analyser Multi NC/3100 (Analytic Jena, Jena, Germany) with thermocatalytic oxidation (up to 950ºC).

The samples used to measure C and N content in the biofilm were freeze-dried and weighed to the nearest 0.001 mg; elemental composition was then determined with an elemental analyser (EA 1108, Thermo Fisher Scientific, Milano, Italy) using vanadium pentoxide as the oxidation catalyser.

Chlorophyll content

Chlorophyll *a* was used as an estimate of algal biomass. Biofilm extracts were collected on GF/F filters (Whatman) and extracted for 12 h with 90% acetone in the dark at 4ºC. Samples were sonicated (2 min, sonication bath at 40 W, 40 kHz), and filters were manually ground. The extract was filtered through 1.4 μm glass fibre filters (GF/C, Whatman), and chlorophyll content was determined by spectrophotometry (Lambda 2 UV/VIS spectrophotometer, PerkinElmer, Waltham, MA, USA) following Jeffrey and Humphrey (1975).

Bacterial density and extracellular enzyme activities

Bacterial density was estimated for the colonised glass tiles (in 10 mL autoclaved stream water) and in bulk water samples. Live and dead bacteria were counted using the Live/Dead Baclight bacterial viability kit, which contains a mixture of SYTO® 9 and propidium iodide. Once in the laboratory, samples were sonicated (< 1 min). Aliquots of 200 μL of the glass tile extracts were diluted with 2 mL of sterilised stream water. For the water samples, 4 mL was taken directly (no dilution). A 1 : 1 mixture of SYTO® 9 and propidium iodide was added (3 μL), and samples were incubated for 20 min. Samples were then filtered through 0.2 μm black polycarbonate filters (Nucleopore, Whatman, Maidstone, UK). The filters were dried, placed on a slide with mounting oil and examined by epifluorescence microscopy (E600, Nikon Instruments, Melville, NY, USA). At least 20 random fields were examined on each slide for a minimum of 100 bacteria cells.

Four hydrolytic enzyme activities related to OM degradation were measured: phosphatase (EC 3.1.3.1-2), β-D-1,4-glucosidase (EC 3.2.1.21), leucine-aminopeptidase (EC 3.4.11.1) and lipase (EC 3.1.1.3). Phosphatase is the enzyme that degrades orthophosphoric monoesters to obtain inorganic phosphorous (Klotz, 1992), β-glucosidase is involved in the use of simple polysaccharides of autotrophic or heterotrophic origin (Deshpande & Eriksson, 1988), leucine-aminopeptidase activity is related to the hydrolysis of polypeptides, obtaining leucine and other hydrophobic amino acids (Francoeur & Wetzel, 2003) and lipase performs the hydrolysis of ester bonds in lipid substrates. Activity of these enzymes is generated within the microbial community, primarily by heterotrophic bacteria (Romaní & Sabater, 2001; Vrba *et al.*, 2004). Colonised glass tiles and water samples (bulk water and DOM; 4 mL for each) were analysed spectrofluorometrically to determine the activity of these 4 enzymes. Extracellular enzyme activities were determined using the artificial substrates 4-methylumbelliferyl-phosphate for phosphatase, 4-methylumbelliferyl-β-D-glucopyranoside for β-glucosidase, L-leucine-7-amido-4-methylcoumarin hydrochloride for leucine-aminopeptidase and 4-methylumbelliferyl palmitate for lipase (Sigma-Aldrich, St. Louis, MO, USA), as the respective substrate analogues. Samples were incubated with 120 µL of artificial substrate to a final concentration of 0.3 mM (saturated conditions; Romaní & Sabater, 2001) in the dark under continuous shaking for 1 h at an ambient temperature. Blanks and standards of methylumbelliferone (MUF) and aminomethyl-coumarin (AMC) were also incubated. At the end of the incubation, glycine buffer (pH 10.4) was added (1 : 1, V : V), and the fluorescence was measured at 365/455 nm excitation/emission for MUF and 364/445 nm excitation/emission for AMC.

Biochemical composition

Samples of the biofilm, POM (filters) and DOM were frozen with liquid N and freeze-dried for 48 h to analyse polysaccharide, protein and lipid contents. Total polysaccharide content was measured following the 3-methyl-2-benzothiazolinone hydrochloride (MBTH) method (Pakulski & Benner, 1992; Chanudet & Filella, 2006) with some modifications (for details see General methods; Ylla *et al.*, 2010).

Amino acid composition was analysed using high performance liquid chromatography (for details see General methods; Ylla *et al.*, 2011). The validity of the method was verified by the addition of an internal standard (50 pmol of α-aminobutyric acid), which was recovered at nearly 100% (50 pmol ± 5 for POM and DOM and 50 pmol ± 10 for cobble samples) during the treatment and analysis of the standards and samples. All amino acids could be quantified except cysteine, which was unquantifiable due to analytical problems. The total protein content was calculated as the sum of all of the amino acids in each sample. Amino acids were classified as essential (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, arginine and histidine) or non-essential (aspartic acid, serine, glutamic acid, glycine, alanine, proline and tyrosine; Dauwe & Middelburg, 1998).

Lipids from the biofilm and POM samples were extracted with a mixture of chloroform and methanol (2 : 1; Bligh & Dyer, 1959). Total lipid content was analysed by the colourimetric sulphophosphovanillin method (Zollner & Kirsch, 1962). The dissolved fraction could not be analysed because the lipid content was below the detectable level for this method (< 0.01 mg L^{-1}). The composition of FA and sterols from the biofilm samples was analysed using gas chromatography and mass spectrometry (for details see General methods). The yield of the extraction, calculated from the recuperation of internal standards (nonadecanoic acid and 5-α-cholestane) was 35% for FA and 39% for sterols. FA were classified according to the number of double bonds, i.e., saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA), and the double bound position, accounting for ω3 and ω6 FA. A ω3 : ω6 ratio of < 1 indicates that the resources are primarily of terrestrial origin and a ratio > 1 indicates a primarily aquatic origin. We also designated whether the FA was essential for consumers (essential FA [EFA]: 18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3, 22:6ω3), and if it could be considered indicative of organisms presence; i.e., FA from diatoms (20:5ω3, 16:1ω7), chlorophytes and cyanobacteria (18:2ω6, 18:3ω3) and bacteria (15:0, 15:1 and branched 13:0, 15:0; Desvilettes *et al.*, 1997; Napolitano, 1999; Olsen, 1999). Sterols are all essential for consumers, and they are indicators of resources from algae (fucosterol), fungi (ergosterol) and higher plants (campesterol, sitosterol and stigmasterol; Martin-Creuzburg & Elert, 2009).

Statistical analysis

Regression analyses between abiotic parameters and time, and 1-way ANOVA to compare physico-chemical parameters between reaches were performed. Univariate measures were analysed with 2-way ANOVA taking into account 2 factors, the reach (2 levels; control and fertilised) and the date (4 levels; sampling occasions), and their interaction. A post hoc Tukey HSD test was conducted for the factor date. Correlations were tested using the Pearson product-moment correlation coefficient (r_p). The normality of residuals (Kolmogorov-Smirnov [with Lilliefors correction] and Shapiro-Wilks tests) and homoscedasticity (Levene’s test) were assessed and were improved, if necessary, using Box-Cox transformations. These analyses were performed with PASW (version 18, IBM, Armonk, NY, USA). The biomass of biofilm per surface area (g dry mass [DM] cm^{-2}) was not significantly influenced by any of the tested factors (2-way ANOVA, $P > 0.300$). Consequently, the changes in biofilm composition will be given in units of surface area (cm^{-2}). Amino acids, FA and sterol composition (given as relative abundance) of the biofilm and water samples were analysed with detrended correspondence analysis (DCA). DCA indicated that the maximum gradient lengths were shorter than 3.0 units; therefore, the use of linear ordination techniques was appropriate (Lepš & Šmilauer, 2003, pp. 43-59) and principal component analyses (PCA) were performed using CANOCO software (version 4.5, Biometris, Wageningen, the Netherlands).

RESULTS

Physico-chemical parameters

During the sampling period, light availability decreased from early to late spring, whereas air temperature exhibited the inverse tendency (Fig. 1a,b). During that period, mean ± SD stream flow was 23.1 ± 25.6 L s^{-1}. There were no differences between the 2 reaches in terms of water conductivity, pH, oxygen or temperature (Table 1), but water temperature increased over time from 8.6 to 13.6ºC. During the study period, N and P concentrations were higher in the fertilised reach, where the higher increase in P compared to N caused a decrease in the N : P ratio (Table 1). The direction of these changes had been unchanged since 2004 (Sabater *et al.*, 2011). The DOC concentration did not change due to the fertilisation, although it increased over the sampling period from 3.0 ± 0.1 to 4.6 ± 0.2 mg L^{-1}, mean ± SE.

Biofilm characteristics

The C content of the biofilm did not show differences between reaches and it was, on average, 24.65 ± 0.86% of the DM (Reach: $F_{1,24} = 0.001$; $P = 0.977$), while N content was higher in the fertilised reach (C reach: 2.98 ± 0.14%; F reach: 3.53 ± 0.23%; $F_{1,24} = 4.74$; $P = 0.039$). Thus, the C : N molar ratio was lower in the fertilised reach (C reach: 9.67 ± 0.26; F reach: 8.29 ± 0.14; $F_{1,24} = 24.79$; $P < 0.001$). None of these variables was affected by the sampling date (2-way ANOVA, P > 0.201).

The standing crop of algae was influenced by both nutrients and the date (Reach: $F_{1,24} = 5.25$; $P = 0.031$; Date: $F_{3,24} = 10.44$; $P < 0.001$; Reach x Date: $F_{3,24} = 3.32$; $P = 0.037$; Fig. 2a). A high value for biofilm chlorophyll content was measured on the first sampling date at the fertilised reach.

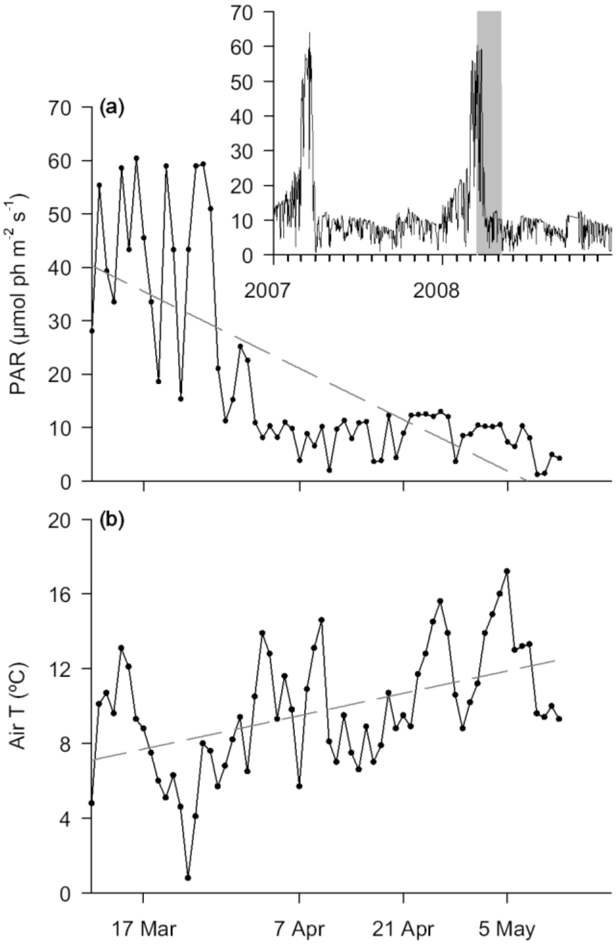


Fig. 1 Abiotic parameters during the sampling period: (a) photosynthetically active radiation (PAR) reaching the streambed with the right inset figure showing the PAR dynamics during 2007–08 and the grey area indicating the study period, shown in detail in the main figure; and (b) air temperature. Data indicates daily mean values and dashed lines represent regressions between PAR and time ($r^2 = 0.536$; $P < 0.001$), and air temperature and time ($r^2 = 0.245$; $P < 0.001$).

Table 1 Physico-chemical parameters in the stream water comparing the control (C) and the fertilised (F) reaches (1-way ANOVA)

	C reach	F reach	*P*
Conductivity (µS cm^{-1})	202 ± 17	206 ± 18	0.886
pH	7.6 ± 0.1	7.6 ± 0.2	0.831
Dissolved oxygen (mg L^{-1})	11.15 ± 0.89	11.13 ± 0.83	0.987
Oxygen (%)	108.2 ± 8.6	107.9 ± 8.4	0.981
Temperature (ºC)	10.7 ± 1.1	10.8 ± 1.0	0.935
SRP (µg P-PO_4 L^{-1})	5.78 ± 0.70	107.73 ± 43.40	**< 0.001**
Nitrate (µg N-NO_3 L^{-1})	365.66 ± 60.58	1387.02 ± 401.72	**0.032**
Ammonia (µg N-NH_4 L^{-1})	19.12 ± 5.35	201.47 ± 47.80	**< 0.001**
N : P (molar ratio)	173.85 ± 21.02	103.40 ± 21.79	**0.028**

P-values < 0.05 are indicated in bold.
Values show the mean ± SE measured in each sampling occasion.

Live bacteria (cells cm^{-2}) were more abundant in the fertilised reach during the first 2 sampling dates, but this tendency was reversed on the last 2 dates (Reach x Date: $F_{3,22} = 5.12$; $P = 0.008$). The density of live bacteria peaked on the second date (Date: $F_{3,22}$ = 27.27; $P < 0.001$; Fig. 2b). The percentage of live bacteria in relation to total bacteria was 3 times higher on the second sampling date (36.32 ± 6.21%) than in the rest of the sampling dates (11.26 ± 1.03%; Date: $F_{3,24}$ = 10.64; $P < 0.001$).

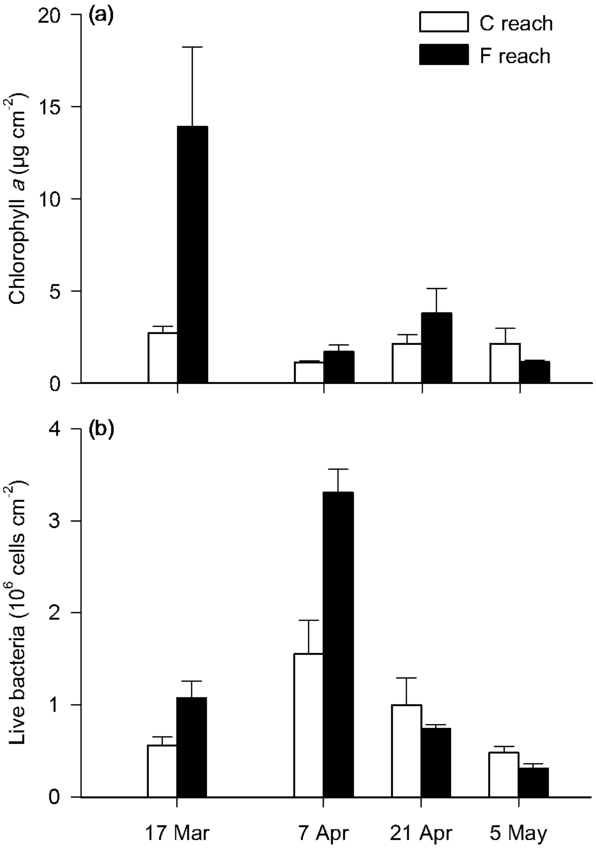


Fig. 2 Changes in algal and bacterial content in the biofilm: (a) chlorophyll a content, to estimate algal biomass, and (b) density of live bacteria in the control (C) reach (open bars) and in the fertilised (F) reach (black bars). Error bars represent +1 SE.

The extracellular enzyme activities in the biofilm showed differential effects of the fertilisation (Fig. 3). Phosphatase and β-glucosidase activities became lower in the fertilised reach with respect to the control reach from 21 April until the end of the experiment (2-way ANOVA for all factors, $P < 0.007$). Leucine-aminopeptidase activity was higher in the fertilised reach in early spring (Date: $F_{3,24}$ = 4.90; P = 0.009; Reach x Date: $F_{3,24}$ = 12.93; $P < 0.001$). Lipase activity showed the highest values on the second and third sampling dates (Date: $F_{3,22}$ = 5.45; P = 0.006).

Bacterial density and extracellular enzyme activities in flowing water

In water, the density of live bacteria (cells mL^{-1}) did not change due to fertilisation (Table 2), although it was higher after 21 April (36778 ± 2609) than before (18173 ± 2809 cells mL^{-1}). Live bacteria were positively correlated with DOC (mg L^{-1}) (r_p = 0.698; $P < 0.001$; n = 29). The percentage of live bacteria out of the total bacterial density was 21.79 ± 1.54% and was not affected by any factor (2-way ANOVA for all factors, $P > 0.137$).

Enzyme activities in water were higher in the fertilised reach during most of the study period (Table 2). Although all activities showed changes through time, these variations did not follow the pattern of any of the measured abiotic parameters.

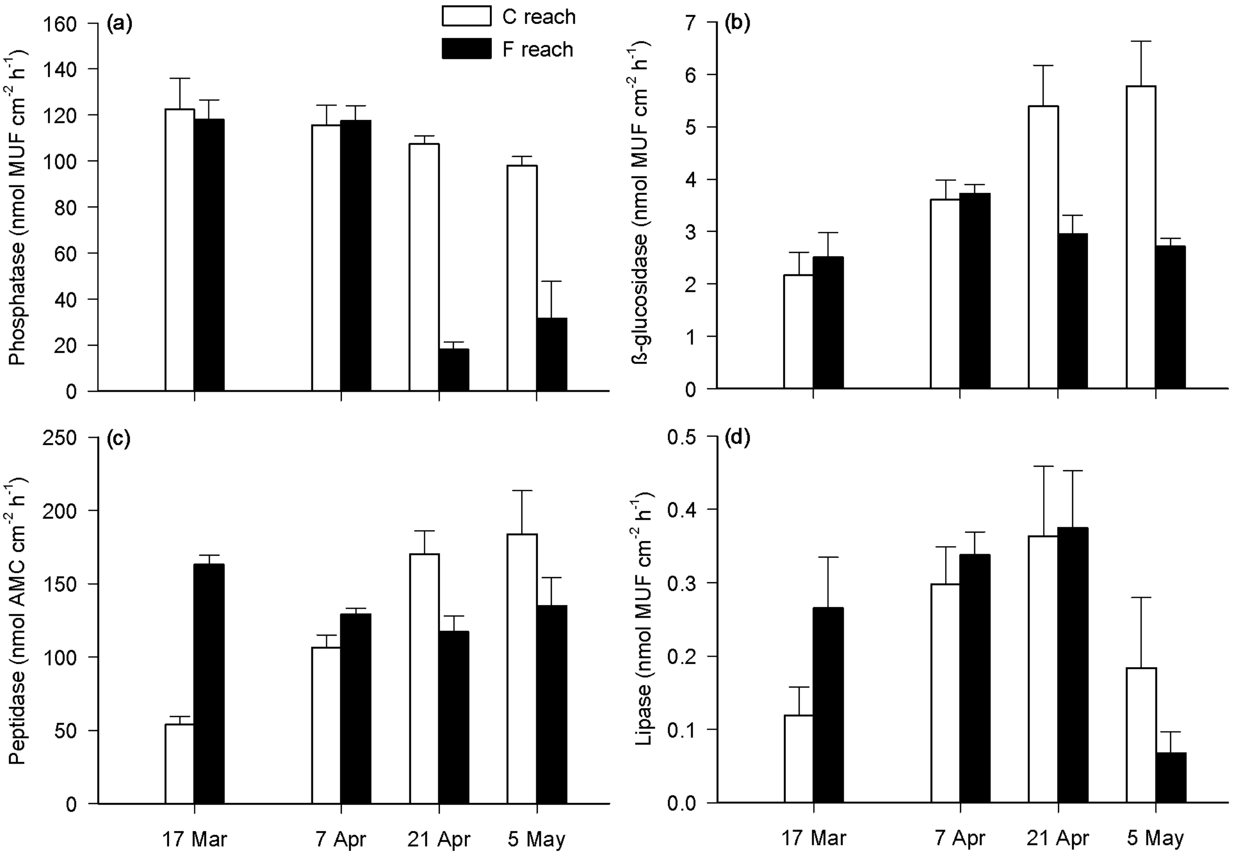


Fig. 3 Extracellular enzyme activities in biofilm: (a) phosphatase, (b) β-glucosidase, (c) leucine-aminopeptidase and (d) lipase, in the control reach (C; open bars) and in the fertilised reach (F; black bars). Error bars represent +1 SE.

Biochemical composition of biofilm

The protein content was higher in the fertilised reach, while the polysaccharide and lipid contents were higher in the fertilised reach on the first date (Fig. 4; Table 3). The content of polysaccharides and proteins was positively correlated with chlorophyll *a* in the fertilised reach (r_p = 0.763; P = 0.002; n = 14).

The higher protein content in the fertilised reach was primarily due to an increase in non-essential amino acids (C reach: 12.51 ± 1.44; F reach: 22.07 ± 2.82 μg cm^{-2}; $F_{1,22}$ = 9.56; P = 0.005), whereas essential amino acids were not significantly different between the 2 reaches (19.42 ± 1.90 μg cm^{-2}; $F_{1,22}$ = 3.73; P = 0.066). In the PCA performed with the amino acid composition of both control and fertilised reaches (Fig. 5), samples were arranged on the first axis (explaining 34% of variability) according to sampling date. The first sampling dates were situated on the positive side of axis 1 and were characterised by a high percentage of alanine and glycine. The latest sampling dates were situated on the negative side of this axis and were characterised by higher percentages of leucine, histidine and phenylalanine.

Table 2 Effect of fertilisation and the date in the density of live bacteria and the extracellular enzyme activities in water (2-way ANOVA)

	C reach	F reach	Reach			Date			Reach x Date		
	Mean ± SE	Mean ± SE	df	F	*P*	df	F	*P*	df	F	*P*
Bulk water											
Live bacteria	30021 ± 4107	25380 ± 2919	1,23	1.03	0.320	3,23	13.06	**< 0.001**	3,23	1.10	0.370
Phosphatase	0.36 ± 0.04	0.37 ± 0.02	1,24	1.66	0.210	3,24	12.60	**< 0.001**	3,24	2.70	0.068
β-glucosidase	0.08 ± 0.004	0.12 ± 0.01	1,24	55.02	**< 0.001**	3,24	18.10	**< 0.001**	3,24	1.64	0.207
Leucine-aminopeptidase	0.89 ± 0.04	1.04 ± 0.04	1,24	34.38	**< 0.001**	3,24	22.92	**< 0.001**	3,24	2.68	0.070
Lipase	0.06 ± 0.01	0.08 ± 0.01	1,22	23.35	**< 0.001**	3,22	20.44	**< 0.001**	3,22	16.72	**< 0.001**
Water dissolved fraction											
Phosphatase	0.11 ± 0.004	0.14 ± 0.01	1,24	20.59	**< 0.001**	3,24	4.08	**0.018**	3,24	4.23	**0.016**
β-glucosidase	0.06 ± 0.003	0.09 ± 0.003	1,24	162.22	**< 0.001**	3,24	14.45	**< 0.001**	3,24	8.13	**0.001**
Leucine-aminopeptidase	0.80 ± 0.07	0.81 ± 0.03	1,24	1.38	0.251	3,24	31.97	**< 0.001**	3,24	10.56	**< 0.001**
Lipase	0.05 ± 0.01	0.07 ± 0.01	1,23	14.04	**0.001**	3,23	24.43	**< 0.001**	3,23	2.01	0.140

P-values < 0.05 are indicated in bold.

C: control and **F**: fertilised reaches.

Bacterial density is expressed in cells mL^{-1}, phosphatase, β-glucosidase and lipase activities in nmol **MUF** mL^{-1} h^{-1} and leucine-aminopeptidase activity in nmol AMC mL^{-1} h^{-1}.

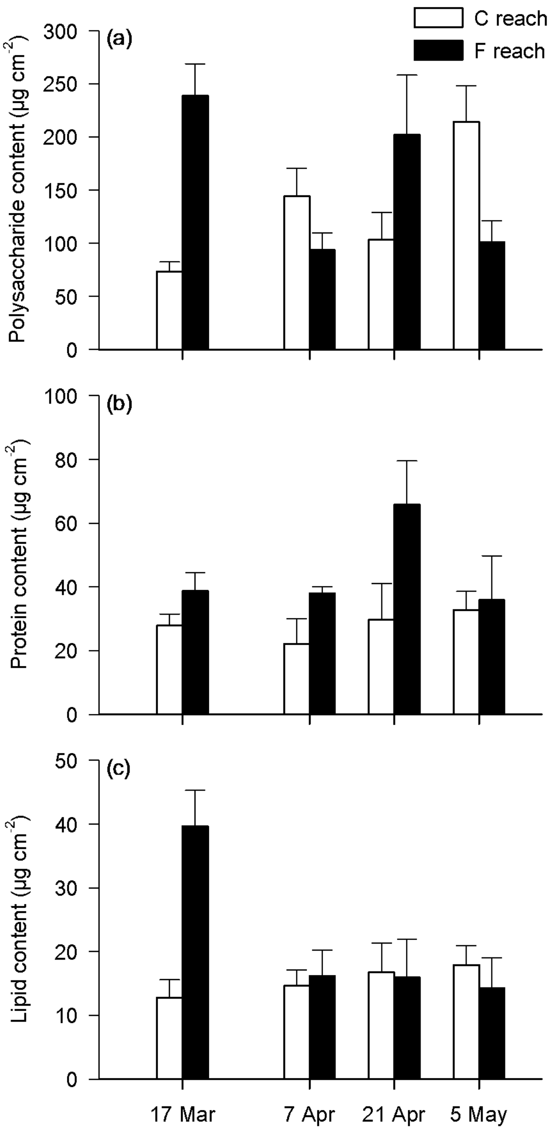


Fig. 4 Biochemical composition of the biofilm: (a) polysaccharide, (b) protein and (c) lipid content in the control reach (C; open bars) and in the fertilised reach (F; black bars). Error bars represent +1 SE. Note the different scale of Y axes.

Table 3 Effect of the fertilisation and the date in the biochemical composition of the biofilm and the water (2-way ANOVA)

	Reach			Date			Reach x Date		
	df	F	*P*	df	F	*P*	df	F	*P*
Biofilm									
Polysaccharides	1,22	1.60	0.219	3,22	0.92	0.450	3,22	10.60	**< 0.001**
Proteins	1,22	6.57	**0.018**	3,22	1.39	0.273	3,22	1.16	0.347
Lipids	1,24	0.80	0.380	3,24	1.27	0.308	3,24	3.40	**0.034**
Water particulate fraction									
Polysaccharides	1,22	2.59	0.122	3,22	5.87	**0.004**	3,22	1.73	0.189
Proteins	1,16	1.65	0.217	3,16	0.33	0.801	2,16	2.78	0.092
Lipids	1,23	2.80	0.108	3,23	325.90	**< 0.001**	3,23	2.75	0.066
Water dissolved fraction									
Polysaccharides	1,21	14.31	**0.001**	3,21	4.35	**0.016**	3,21	10.53	**< 0.001**
Proteins	1,20	15.57	**0.001**	3,20	2.88	0.061	3,20	6.45	**0.003**

P-values < 0.05 are indicated in bold.

Variables in the biofilm are expressed in µg cm^{-2} and in the water in mg L^{-1}.

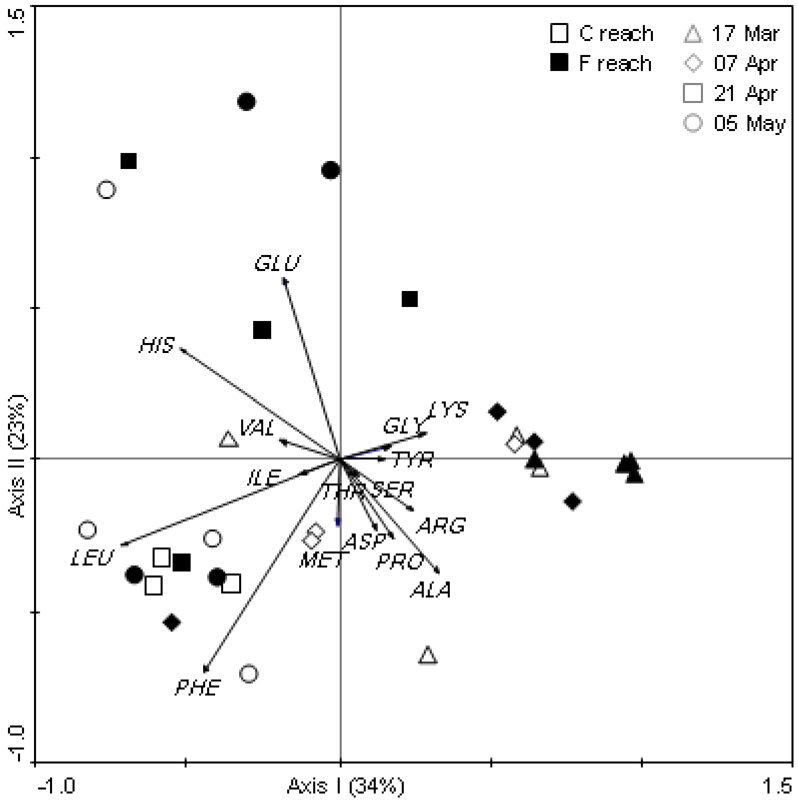


Fig. 5 Principal components analysis of the relative abundance of amino acids (in % pg) in the biofilm, showing the samples from the control (C; open symbols) and fertilised reaches (F; black symbols) over the sampling dates. The first and second axes are represented, and the percentage of variability explained by each is indicated. Arrows represent the loading of the amino acids. Abbreviations: ASP aspartic acid, SER serine, GLU glutamic acid, GLY glycine, HIS histidine, ARG arginine, THR threonine, ALA alanine, PRO proline, TYR tyrosine, VAL valine, MET methionine, LYS lysine, ILE isoleucine, LEU leucine, PHE phenylalanine.

The total identified FA content in the biofilms from both reaches was 5.8 ± 0.8 µg cm^{-2}, representing 34.1 ± 3.1% of the total lipid content. The FA content was higher on the first date in the fertilised reach (Date: $F_{3,23} = 10.82$; $P < 0.001$; Reach x Date: $F_{3,23} = 6.98$; $P = 0.002$; Fig. 6a). EFA (Fig. 6b) and PUFA showed this same pattern (2-way ANOVA, $P < 0.005$). The SAFA : PUFA ratio was higher on the second date (Date: $F_{3,23} = 5.73$; $P = 0.004$; Fig. 6c). The ω3 : ω6 ratio showed no significant differences between reaches ($F_{1,23} = 2.69$; $P = 0.114$; Fig. 6d), with mean values of 0.9 ± 0.2 in the control reach and 1.2 ± 0.2 in the fertilised reach. The FA indicators of diatom and bacterial origins were more abundant on the first date in the fertilised reach (2-way ANOVA, $P < 0.023$; Fig. 6e,f). FA characteristic of chlorophytes and cyanobacteria were also higher on the first date (Date: $F_{3,23} = 5.57$; $P = 0.005$). The sum of diatom, chlorophyte and cyanobacteria FA was positively related to chlorophyll *a* content ($r_p = 0.41$; $P = 0.021$; n = 31). However, bacterial density and bacterial FA were not correlated (Pearson correlation, $P > 0.724$).

Six sterols were identified in the biofilm, representing 2.39 ± 0.26% of the total lipids. The total sterol content was 0.40 ± 0.05 µg cm^{-2}, with no differences among reaches or dates (2-way ANOVA, $P > 0.148$). The average content of fucosterol was 0.03 ± 0.01 µg cm^{-2}, while that of sterols originating from higher plants was 0.29 ± 0.04 µg cm^{-2}. The PCA performed with the FA and sterol compositions of the biofilm did not show any significant pattern.

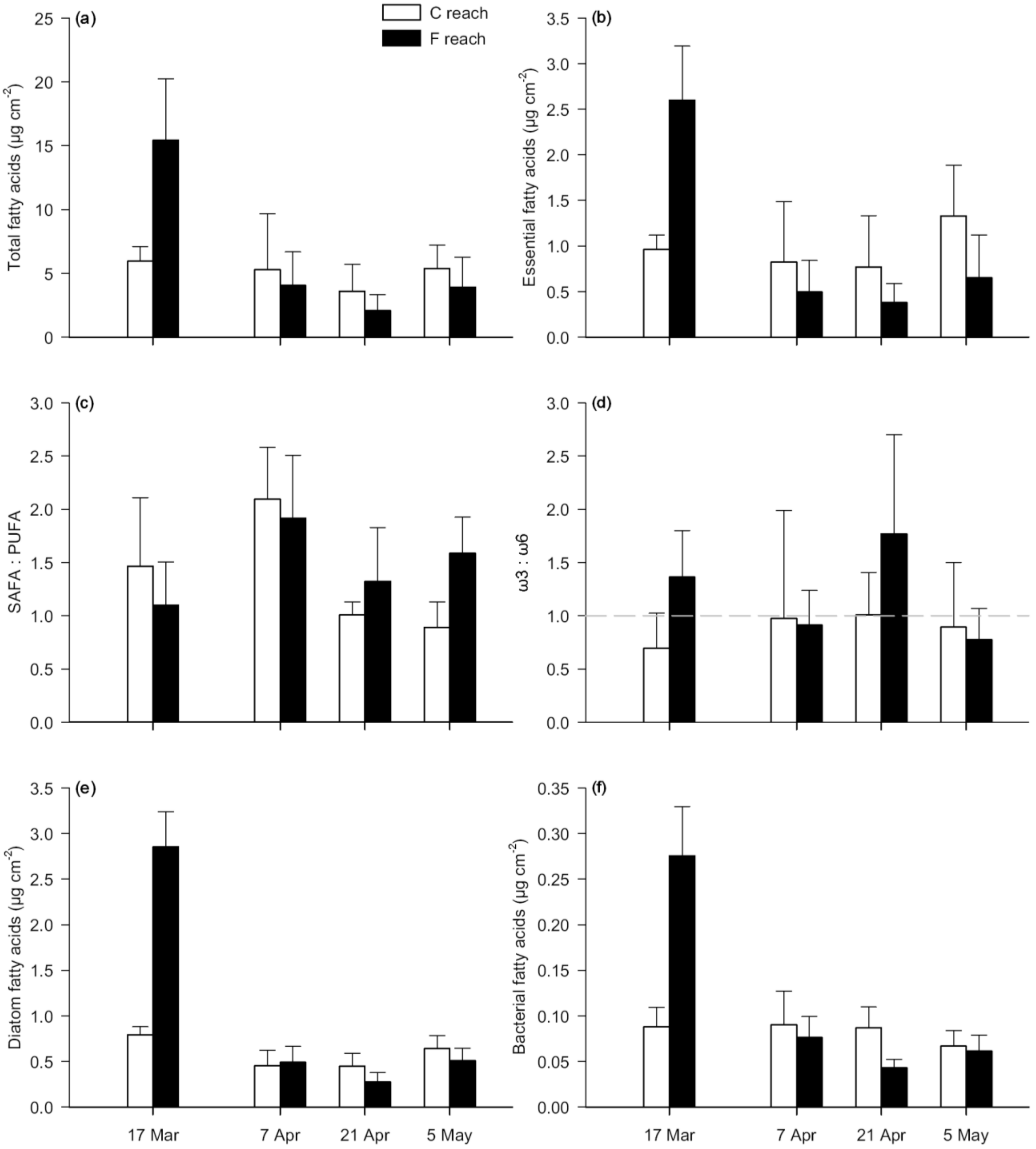


Fig. 6 Fatty acid (FA) composition of biofilm in terms of (a) total content of FA, (b) essential FA (EFA), (c) the ratio of saturated (SAFA) to polyunsaturated FA (PUFA), (d) the ratio of ω3 : ω6 FA and the concentration of FA indicating the presence of (e) diatoms and (f) bacteria; in the control reach (C; open bars) and in the fertilised reach (F; black bars). Dashed line in (d) indicates the shift from terrestrial (ω3 : ω6 < 1) to aquatic (ω3 : ω6 > 1) origin of organic matter. Error bars represent +1 SE.

Biochemical composition of transported organic matter

In the POM, none of the biochemical components were affected by the nutrient enrichment (Fig. 7a–c; Table 3). There were no significant differences for the tested factors in the amount of essential and non-essential amino acids in the POM (2-way ANOVA, $P > 0.058$).

In the DOM, the polysaccharide content increased in the control reach and decreased in the fertilised reach over the study period. The protein content was higher in the control reach on the first date compared to the fertilised reach (Fig. 7d,e; Table 3).

Essential amino acids in the DOM were lower in the fertilised reach (C reach: 191.21 ± 14.50; F reach: 125.55 ± 9.17 µg L^{-1}) and on the 2 first dates (Reach: $F_{1,20} = 17.88$; $P < 0.001$; Date: $F_{3,20} = 3.54$; $P = 0.033$). Non-essential amino acids were also lower in the fertilised reach on the 2 first dates (C reach: 167.29 ± 18.92; F reach: 132.23 ± 12.80 µg L^{-1}; Reach: $F_{1,20} = 10.14$; $P = 0.005$; Reach x Date: $F_{3,20} = 12.74$; $P < 0.001$). In the PCA performed with the amino acids in the DOM (Fig. 8), samples were distributed along the first axis (explaining 62% of variability) depending on the reach and date. Samples from the first dates of the fertilised reach and the last dates of the control reach were situated on the negative side of the axis, characterised by the presence of histidine. Samples from the last dates of the fertilised reach and the first date of the control reach were found on the positive side of the axis, determined primarily by glycine.

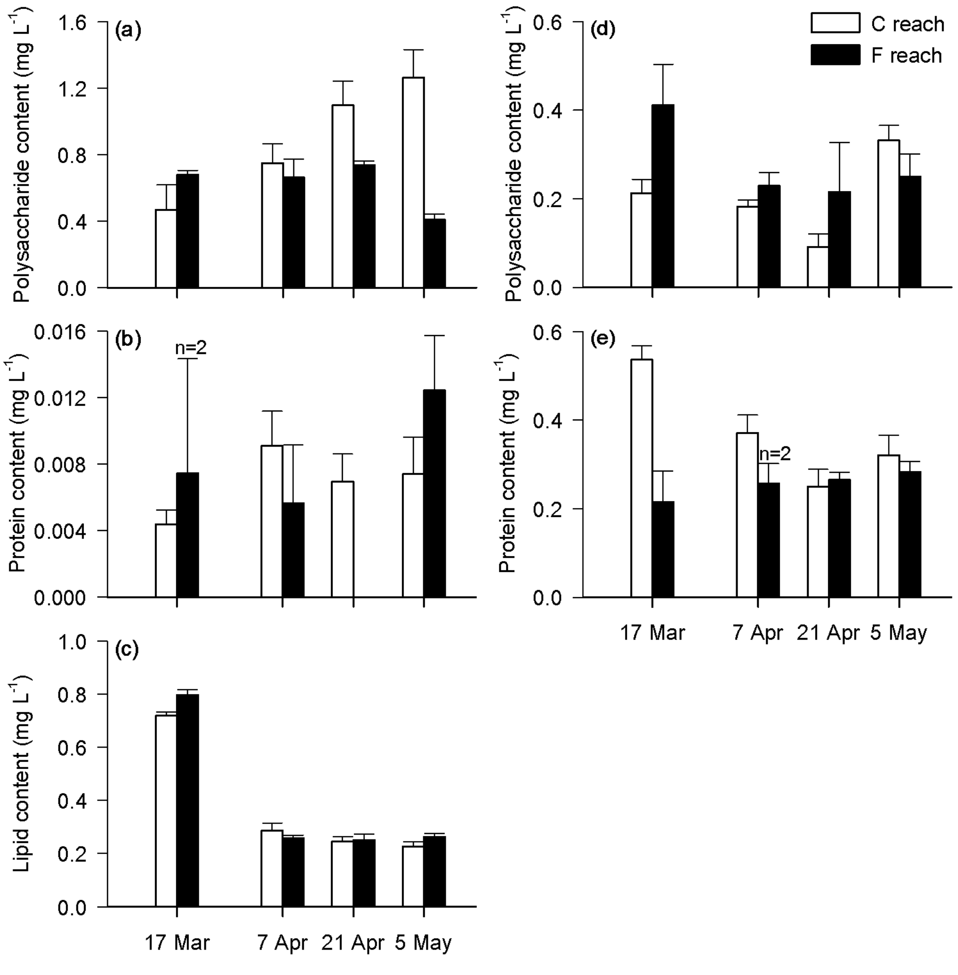


Fig. 7 Biochemical composition of particulate organic matter (POM) and dissolved organic matter (DOM): (a,d) polysaccharide, (b,e) protein and (c) lipid content of the POM (first column) and DOM (second column) in the control reach (C; open bars) and in the fertilised reach (F; black bars). In (b) one bar is lacking because no replicate values were available. Error bars represent +1 SE. Note the different scale of Y axes.

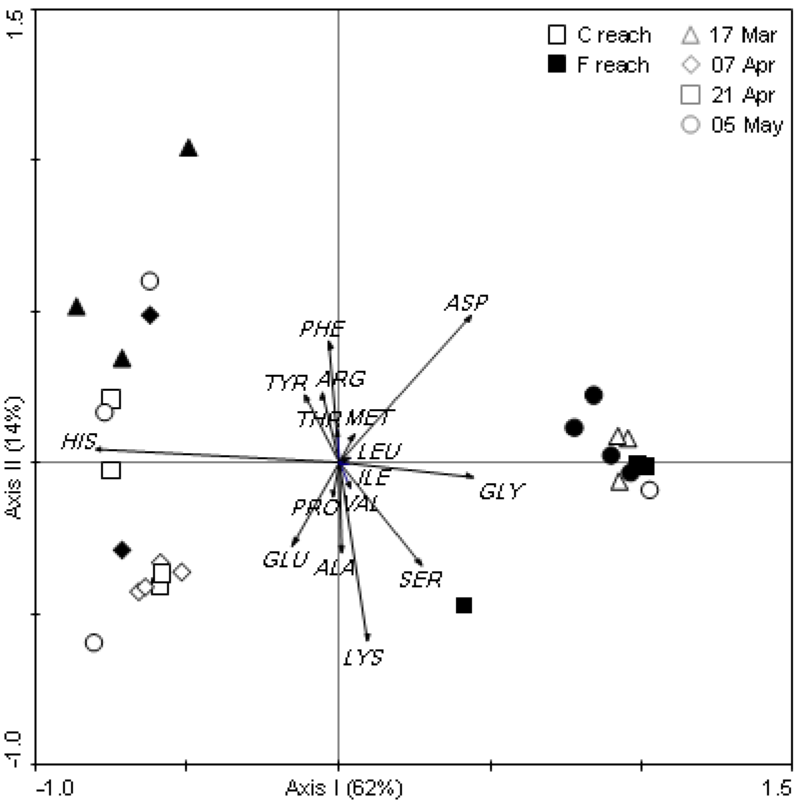


Fig. 8 Principal components analysis of the relative abundance of amino acids (in % pg) in the dissolved organic matter (DOM), showing the samples from the control (C; open symbols) and fertilised reaches (F; black symbols) over the sampling dates. The first and second axes are represented, and the percentage of variability explained by each is indicated. Arrows represent the loading of the amino acids. Amino acids abbreviations are given in Fig. 5.

DISCUSSION

The manipulative addition of nutrients to a headwater stream caused different effects in the epilithic biofilm and in the transported OM. In the biofilm, nutrient enrichment resulted in a higher chlorophyll content and in a lower C : N ratio in the fertilised reach. Moreover, the biochemical composition was different; polysaccharide, protein, lipid and EFA contents were higher, in contrast to essential amino acids, which did not show significant differences. These effects of nutrient enrichment were clear during the period of higher light availability, but under lower light availability (late spring), some effects on biofilm biochemical composition were reduced or even inverted, suggesting that the effects of nutrient enrichment were modulated by light availability.

In this study, no sampling of all the studied variables was performed prior to the fertilisation. However, in the previous study by Sabater *et al.* (2008), performed within the same fertilisation experiment, before and after comparisons between control and impacted (fertilised) reaches analyses of variance (BACI) were performed, finding that nutrient concentration in water, bacterial density, chlorophyll concentration and C and N percentage and ratio in the epilithic biofilm did not show significant differences between the 2 reaches before the nutrient enrichment started. In addition, in our study, physico-chemical parameters did not show differences between the 2 reaches. Hence, we believe that changes in the measured variables between the control and fertilised reaches can be attributed to the effect of the nutrient enrichment.

Changes in abiotic parameters during the experiment could have affected the results. The primary changes were a decrease in light availability and a gradual increase in temperature over time.

Stream flow was always below 250 L s^{-1}, considered to be the threshold for sediment motion to occur in the studied stream (Sabater *et al.*, 2008). Light availability (PAR) showed a maximum value of approximately 60 µmol photons m^{-2} s^{-1} around the first sampling date, but rapidly decreased from the second date onward to values of approximately 10 µmol photons m^{-2} s^{-1} (83% reduction). These values were similar to (Sabater *et al.*, 2011) or lower (Veraart *et al.*, 2008) than those observed in preceding years in the same stream. Moreover, these values and the rate of change due to leaf growth were low compared to other forested streams at similar latitudes and seasons (changing from > 750 to < 100, Ledger & Hildrew, 1998; or from > 1000 to 20 µmol photons m^{-2} s^{-1}, Hill *et al.*, 2001). Therefore, we consider our stream to have moderate light availability during the leafless stage of riparian vegetation, most likely due to the steep stream banks and light limitation for primary producers after leaves have developed (Acuña *et al.*, 2004).

The primary quantitative effects of nutrient enrichment on the biofilm were, as expected from the results of Sabater *et al.* (2011), a higher chlorophyll content and bacterial density. Similar results have been described in other works (e.g., Hepinstall & Fuller, 1994; Hill *et al.*, 2001; Greenwood & Rosemond, 2005). These effects seemed to be driven by light availability because algae and bacteria in the biofilm decreased with the development of the riparian canopy cover, resulting in values similar to those of the control reach.

In the fertilised reach, the bacterial density in the biofilm was approximately twofold higher in relation to the control reach during the first period. During that period, chlorophyll was also higher in the fertilised reach. Additionally, chlorophyll was positively correlated with biofilm polysaccharide and protein content, which explains the mechanism of bacterial dependence on algae. Hepinstall & Fuller (1994) found a positive correlation between algae and bacteria under different conditions of light and nutrient availability; these authors attributed the relationship to the algal exudates (primarily composed of polysaccharides and proteins) used by bacteria as an energy source. Algal exudates also represent a major C source for bacteria (Romaní *et al.*, 2004b; Carr *et al.*, 2005). These relationships between bacteria, chlorophyll, polysaccharide and protein content of the biofilm were not observed in the control reach.

A cascading effect of nutrients on chlorophyll and bacteria was linked to changes in the biochemical composition of the biofilm. The nutrient enrichment resulted in a lower C : N ratio in the biofilm, as found by Rosemond (1993) and Romaní *et al.* (2004a). However, changes in the C : N ratio were not significant over time despite changes in light availability. This fact is in disagreement with the light : nutrient hypothesis, which states that biofilm nutrient content is determined by the balance of light and dissolved nutrients (Fanta *et al.*, 2010). Protein content was clearly higher as a consequence of nutrient enrichment. This relationship had not been directly measured before, but was not surprising given the lower C : N ratios and the higher leucine-aminopeptidase activity (Romaní *et al.*, 2004a). The higher protein content in the fertilised reach consisted primarily of non-essential amino acids; no differences were found between reaches in the essential amino acids that may be limiting for consumer macroinvertebrate growth (Phillips, 1984; Dauwe & Middelburg, 1998).

When light availability was greater, the relative composition of amino acids was characterised by a mix of amino acids from fresh material (alanine) (Jennerjahn & Ittekkot, 1997) and glycine, which is highly concentrated in cell walls, it is most likely related to the structural matrices of diatoms and has a low food value to consumers (Dauwe & Middelburg, 1998; Dauwe *et al.*, 1999). Lower light availability corresponded to higher relative abundance of labile amino acid indicators of fresh OM (leucine, histidine and phenylalanine; Ylla *et al.*, 2011); this finding is due to the lower proportion of diatoms in the biofilm. Thus, light seemed to be an important factor modulating amino acid composition.

Polysaccharide and lipid content was also higher in the fertilised reach, but only on the first date, suggesting that the effect was related to light availability. Romaní *et al.* (2004a) also observed an increase in polysaccharide content as a result of a nutrient enrichment; this study used a higher level of enrichment than our experiment. Hill *et al.* (1995) directly related the higher neutral lipid content in grazers to higher light availability and a resulting higher epilithic biofilm photosynthetic rate; however, that higher lipid content was not measured in the biofilm.

FA and sterols represented a small percentage of the total lipids in the biofilm, although these compounds are important because they provide essential components to consumers (Brett & Müller-Navarra, 1997; Martin-Creuzburg & Elert, 2009). The FA and EFA content was higher in the fertilised reach but decreased over time, which was most likely related to algal abundance (Hill *et al.*, 2011). Hill *et al.* (2011) found a positive correlation between the SAFA : PUFA and C : N ratios. Changes in the former ratio over time were found in our study, although these changes did not follow the pattern of the C : N ratio. Furthermore, a tendency towards a shift from a predominantly terrestrial to an aquatic origin (ω3 : ω6 ratio < 1 to > 1) in the control versus the fertilised reach was observed. Sterol concentration and composition were not affected by nutrients due to the primarily allochthonous (higher plants) origin of sterols.

Differences through time were observed regarding the effect of nutrient enrichment on the biofilm extracellular enzyme activities. Extracellular enzymes play a key role in the OM and nutrient flow in streams, and their synthesis is activated by the presence of certain substrates. Therefore, they are a good proxy with which to characterise the nature and quantity of OM and nutrients (Arnosti, 2003). In the case of biofilms, the response of extracellular enzymes is further modulated by the quality of the available OM and microbial interactions within the biofilm (Romaní *et al.*, 2012). Our results might be further associated with the changes in chlorophyll and bacterial density in the biofilm. In the fertilised reach and during the high light availability period, the use of peptides was higher, which might be linked to the higher availability of substrate for these enzymes. Moreover, higher leucine-aminopeptidase activity has been linked to incident light and photosynthetic activity, which might be related to the use of algal exudates by bacteria (Espeland *et al.*, 2001; Francoeur & Wetzel, 2003; Ylla *et al.*, 2009). When light availability decreased, the biofilm from the fertilised reach showed a decrease in phosphatase activity. This result may be due to the decrease in algal biomass (highly responsible for this extracellular enzyme activity) but also to the availability of inorganic P, making phosphatase enzyme production unnecessary in the fertilised reach (Romaní *et al.*, 2004a; Allison & Vitousek, 2005). In the control reach, a high use of polysaccharides and peptides (indicated by high β-glucosidase and leucine-aminopeptidase activities) was measured at the end of the experiment, indicating major requirements for C and N sources compared to the biofilm from the fertilised reach, as well as the use of the greater amount of accumulated polysaccharides within the biofilm.

Contrary to our initial hypothesis, the density of live bacteria in the flowing water did not change due to the nutrient enrichment; rather, bacterial density was positively correlated with DOC (Meyer, 1994). Therefore, the observed effects of nutrients on the benthos were not reflected in the bacteria in water, which were primarily dependent on allochthonous inputs. This dependence was also reflected in the biochemical composition of POM and DOM. Only the amino acid composition of DOM changed over time, shifting in the fertilised reach from fresh material (histidine) to structural amino acids (glycine; Dauwe *et al.*, 1999), although this shift cannot be directly related to changes in the biofilm. An indirect effect of the increase in algal biomass, i.e., through release of algal exudates, on DOM quality could not be observed. This lack of effect could be explained by the fact that forested streams in general and Fuirosos, specifically, are primarily heterotrophic and the DOC in stream water comes primarily from allochthonous sources (Thurman, 1985; Butturini *et al.*, 2008). The higher enzyme activities in water with nutrient enrichment, agree with the results of Williams *et al.* (2012).

After a mid-term moderate fertilisation, the only permanent change we observed in the quality of OM was the lower C : N ratio of the biofilms, related to a higher protein content. Headwater systems are highly dynamic, and the biofilms respond quickly to changes in other factors that interact with nutrients, such as light. This study intensively analysed the quantitative and qualitative biochemical composition of basal resources in a stream. Higher autotrophic and heterotrophic biomass, polysaccharide, protein and lipid content and EFA were observed following nutrient enrichment, primarily when light availability was high. The water did not reflect the nutrient effects and was decoupled from the biofilm. Our study demonstrates how the effects of fertilisation in forested streams might emerge in periods of higher light availability. Further, this study addresses the broadly recognised ecological impact of eutrophication on aquatic systems (Vitousek *et al.*, 1997; Meybeck, 2003), showing changes in the quality of basal resources that may involve effects on consumers.

CHAPTER 4

THE EFFECT OF NUTRIENTS AND LIGHT ON LEAF LITTER AS A FOOD RESOURCE FOR STREAM SHREDDERS

ABSTRACT

In forested headwater streams, food webs mainly depend on allochthonous resources from the riparian forest. The quality of these resources is important for stream secondary production and depends on the riparian forest structure and composition, and on stream microbial conditioning. Nutrient concentration and light availability influence leaf conditioning, causing qualitative changes. The goal of this study was to determine the relative contribution of nutrients and light to algal colonisation and leaf quality, and to test whether these changes influence the palatability of leaves for shredders. Alder leaf packs were conditioned in once-through stream channels with control and/or high nutrient and light conditions, in a 2 x 2 factorial design. The elemental content, chlorophyll and biochemical quality of the leaves were measured. Conditioned leaves were also used in a feeding preference trial. The colonisation time was one of the most influential variables, with an enrichment of nitrogen and lipids in the first stage and a general decrease of polysaccharides and proteins over time. Nutrient enrichment accelerated leaf decomposition by 20–26%. Algal colonisation was higher under high nutrients and light conditions, but only in the first period of colonisation. Polysaccharide and lipid content did not respond to any of the treatments, while protein content was related to light. Shredders seemed to preferentially feed on shaded leaves, with a higher protein content. The effects shown in this experiment should be taken into account in riparian forest management plans.

Keywords: *elemental composition · polysaccharides · proteins · lipids · feeding preferences · stream channels*

INTRODUCTION

In forested headwater streams, the main energy supply for food webs is provided by leaf litter inputs from the riparian vegetation (Wallace *et al.*, 1997b; Hall *et al.*, 2000). The quantity and quality of litter are limiting factors for stream secondary production (Richardson, 1991; Dobson & Hildrew, 1992; Cross *et al.*, 2006). Resource quality equates to the potential nutritional value for the invertebrates feeding on them. Quality is commonly assessed via elemental composition as carbon : nitrogen : phosphorus (C : N : P) ratios (Cross *et al.*, 2003; Hladyz *et al.*, 2009), although it can also be assessed via biochemical composition as polysaccharide, protein and lipid content (Gremare *et al.*, 1997; Ylla *et al.*, 2010; Chapter 2). The quality of allochthonous inputs depends, in the early stages, on riparian forest structure and composition (Gregory *et al.*, 1991), which are influenced by natural factors, such as hydrological regime (Tabacchi *et al.*, 1998), and anthropogenic factors, including forest management (Hoover *et al.*, 2011), invasions of exotic species (Lecerf *et al.*, 2007) and forestry plantations (Pozo *et al.*, 1998). In later stages, when the leaves reach the stream, this quality is modulated by microorganism colonisation and conditioning (Bärlocher & Kendrick, 1975; Golladay *et al.*, 1983). Differences in resource quality caused by these mechanisms have effects on leaf decomposition process (Leroy & Marks, 2006) and on consumer performance (Graça *et al.*, 2001).

Headwater streams are in general oligotrophic and shaded, so nutrients and light are scarce in these systems (Webster *et al.*, 1995), although both are fundamental for autochthonous primary production (Borchardt, 1996; Hill, 1996). Increased nutrient availability leading to the eutrophication of streams is a widespread phenomenon (Vitousek *et al.*, 1997; Vörösmarty *et al.*, 2010), mainly caused by human activities such as agriculture, urban activities or forest logging (Likens *et al.*, 1970; Carpenter *et al.*, 1998). In headwater streams, nutrients can stimulate organic matter decomposition, through an increase in microbial activity (mainly fungal, but also bacterial; Gulis & Suberkropp, 2003). There may be competition for nutrients between microorganisms associated with leaves and mutualistic interactions through labile C excretions from algae (Danger *et al.*, 2013). Primary and secondary production also increase with nutrients (Guasch *et al.*, 1995; Cross *et al.*, 2006). Light availability in the stream is modulated by riparian vegetation (Gregory *et al.*, 1991). Higher exposure of litter to light can affect the biofilm on leaves, increasing algal biomass and decreasing fungal biomass, but not affecting bacterial density (Albariño *et al.*, 2008). The increase in leaf decomposition with exposure to light has been explained by algal interactions with the fungi and bacteria in the leaf biofilm (Rier *et al.*, 2007; Lagrue *et al.*, 2011).

Nutrients and light availability can increase simultaneously due to riparian forest logging (Likens *et al.*, 1970; Kiffney *et al.*, 2003). Light has been found to be the primary limiting factor for production, with nutrients limiting production at higher light levels (Hill & Harvey, 1990). Nutrients are probably limiting when light exceeds photo-saturation and may act to colimit photosynthesis when irradiance is just below saturation (Hill *et al.*, 1995). The response of nutrients under limiting light conditions is variable, from non-response (Ylla *et al.*, 2007) to small effects on algal biomass (Greenwood & Rosemond, 2005).

The effects of light and nutrients can have an impact on the nutritional quality of organic matter through the colonisation process. Polysaccharide, protein and lipid content were highly correlated with quantitative measures of biofilm (e.g. chlorophyll) and, furthermore, the protein and polysaccharide content of the biofilm were related to light (Ledger & Hildrew, 1998). Polysaccharide, protein and lipid content increased in biofilms in a fertilised reach with high light availability (Chapter 3). Such changes in resource quality can affect consumers' consumption rates, feeding preferences and performance. Many studies have confirmed shredders' preference for and faster growth on conditioned leaf litter (Graça *et al.*, 1993; Graça *et al.*, 2001), but little is known about consumption preferences related to the biochemical quality of resources. However, Cargill *et al.* (1985) found preferential feeding on detritus coated with fungal lipids. Leaves conditioned under higher nutrient availability supported higher consumption rates and consumer growth (Connolly & Pearson, 2013). Controversial effects have been found regarding leaves conditioned under high light availability, with higher or equal (Franken *et al.*, 2005; Lagrue *et al.*, 2011) consumption rates and higher (Franken *et al.*, 2005) or lower (Albariño *et al.*, 2008) consumer growth compared to leaves conditioned under low light availability.

The objective of this study was to determine the relative importance of nutrient concentration and light availability for algal colonisation and quality of leaf litter, and how these factors influence the palatability of leaves, and thus the consumption rates and feeding preferences of shredders. We hypothesised that (i) nutrient and light availability would favour algal growth on the leaf surface, increasing the nitrogen, polysaccharide, protein and lipid content of leaves, and that (ii) detritus quality and processing would be greater under conditions of high light and nutrient availability and would favour consumption by invertebrates.

METHODS

Study site

This study was performed in outdoor stream channels, situated in the Malcolm Knapp Research Forest (MKRF; 49º 16' N, 122º 34' W) of the University of British Columbia. These once-through channels were continuously fed by water diverted from Mayfly creek (Richardson, 1991), a forested permanent second-order stream with highly oligotrophic waters (Feller, 1977). The major riparian species were *Alnus rubra* Bong., *Acer circinatum* Pursh and *Rubus spectabilis* Pursh, all of them deciduous. This area is covered in temperate rainforest and located in the Pacific Coast Mountains, in the western hemlock biogeoclimatic coastal zone (dry maritime subzone) with an average annual precipitation of 2200 mm, mainly in the form of rain. Most of this precipitation (70%) falls from October to March, while summers are cool and are the driest part of the year (Feller, 1977; Kiffney *et al.*, 2004).

Experimental design

A manipulative experiment of a 2 x 2 crossed factorial design was performed in summer 2009, with 2 levels of nutrients in stream water, i.e. ambient concentration (Control, C) and with nutrients added (Fertilised, F); and 2 levels of light availability, i.e. medium sunlight with 10% photosynthetically active radiation (PAR; Shaded, S) and full sunlight with 100% PAR (Open, O). Therefore, we had 4 treatments: CS, CO, FS and FO. In the fertilised treatments, the nutrient solution was added continuously to the channels by means of Mariotte bottles. Mariotte bottles were constructed with 20-L carboys (Nalgene translucent polyethylene) with a one-hole rubber stopper through which a glass tube was inserted. On the outflow hose a clamp was used to regulate the drip rate (modified from Moore, 2004; Fig. 1a). Inorganic N was added as sodium nitrate and P as potassium dihydrogen phosphate. The ‘Open’ treatment simulated a situation with a riparian clear-cut or absence of riparian vegetation and the ‘Shaded’ treatment simulated the presence of a 30-m wide riparian buffer (Kiffney *et al.*, 2004). Channels were situated in an open area, so channels for the ‘Open’ treatment were left uncovered, while channels for the ‘Shaded’ treatment were shaded with cloth.

A battery of 16 channels (0.12 m wide, 1.52 m long and 0.07 m deep) was used for the experiment. The slope of the channels was ~ 3%, and the average water flow was 0.13 L s^{-1}. The 16 channels were randomly assigned to the treatments, with 4 channels per treatment (n = 4). Five fine mesh (10 x 18 cm, 500 μm mesh size) bags containing *Alnus rubra* (red alder) leaves (2 g air-dried mass = 1.33 ± 0.01 g of dry mass, DM) were deployed in each channel (Fig. 1b). Extra leaf packs (n = 4) were kept to determine the initial leaf characteristics. Red alder leaves were collected in the MKRF after abscission during the autumn prior to the experiment, and were then transported to the laboratory and air-dried until use.

Every 3–4 days from the start of the experiment, the fertiliser in the carboys was replaced, channel flow was measured and readjusted if necessary, and physico-chemical parameters (i.e. pH, temperature, conductivity and dissolved oxygen) were measured in each channel with portable probes. Light availability, as instantaneous PAR, was measured at the water surface of each channel between 11:00 and 16:00 hours using a light meter (Li-250, Li-cor, Nebraska, USA).

Water samples for nutrient and dissolved organic carbon (DOC) analyses were collected from each channel, transported refrigerated to the laboratory, filtered through membrane filters (0.45 μm pore size) and frozen (-20ºC) until analysis.

After 18 and 26 days of conditioning, one leaf pack from each channel was randomly collected and placed in a ziplock bag. On the first sampling date (18 days), extra leaf packs were retrieved to test the consumers’ feeding preferences (see below). Leaf packs were transported refrigerated to the laboratory, rinsed with tap water and 3–5 leaf discs (12 mm diameter) were cut for DM (oven-dried at 60ºC), ash-free DM (AFDM, ashed at 550ºC for 2 h) and chlorophyll analyses. The rest of the leaf pack was frozen (-80ºC), freeze-dried, weighed to determine DM, manually ground with liquid nitrogen and kept in a desiccator for the remaining analyses.

The same protocol was used with the initial leaf packs of alder (before colonisation) to determine the initial leaf DM, AFDM and quality. Leaf mass loss for the leaf packs was calculated as: Leaf mass loss = (I-F)/I, where I is the initial AFDM and F the final AFDM of the leaf packs.

Fig. 1 Nutrients were added using Mariotte bottles (top). Experimental setting with the leaf packs in the outdoor channels of the Malcolm Knapp Research Forest (MKRF); light availability was reduced using cloth shades and the Mariotte bottles were installed in the corresponding channels (bottom).

Water analyses

The inorganic N concentration (nitrate and nitrite) of stream water was analysed via standard colorimetric analysis using a Technicon Autoanalyzer (Technicon, 1976). Nitrate was analysed using the cadmium reduction method (Keeney & Nelson, 1982). Soluble reactive phosphate was measured according to Murphy *et al.* (1962). DOC was analysed using a TOC-V_{CSH} with TNM-1 (Shimadzu, Kyoto, Japan).

Carbon and nitrogen content

The leaf powder used to measure C and N content was weighed (to the nearest 0.001 mg) and then analysed with an elemental analyser (EA 1108, Thermo Fisher Scientific, Milano, Italy) using vanadium pentoxide as the oxidation catalyser.

Chlorophyll content

Chlorophyll *a* was used as an estimate of algal biomass. To quantify the chlorophyll of the biofilm on the leaf surface only, and not that inside the leaf, the surface of each leaf disc was brushed and the leaf discs were removed. The obtained extracts were filtered through GF/F glass fibre filters (0.7 µm pore size, Whatman, Maidstone, UK), sonicated (5 min at 40 W, 40 kHz) and extracted for 12 h with 90% acetone in the dark at 4ºC. On the following day, samples were sonicated for 5 min and the extract was filtered through GF/C glass fibre filters (1.4 µm pore size, Whatman, Maidstone, UK), and chlorophyll content was determined by spectrophotometry (Lambda 2 UV/VIS spectrophotometer, PerkinElmer, Waltham, MA, USA) according to Jeffrey *et al.* (1975).

Biochemical composition

The polysaccharide, protein and lipid content of leaves was analysed using freeze-dried and ground material. The polysaccharide content was quantified from leaf powder (50.0 ± 0.1 mg DM) by spectrophotometry using a method adapted from Mansfield *et al.* (2005). Total proteins were quantified from leaf powder (6.4 ± 0.1 mg DM) by spectrophotometry using a method based on Baerlocher *et al.* (2004). Procedural blanks were processed simultaneously with samples. Total lipids were quantified from leaf powder (25.2 ± 0.1 mg DM) and were extracted following Bligh & Dyer (1959). The lipid content was analysed by the colourimetric sulphophosphovanillin method (Zollner & Kirsch, 1962). For details on these analyses see General methods.

Consumer feeding preferences

Caddisfly larvae *Halesochila taylori* Banks (Trichoptera: Limnephilidae) were used for the feeding preference trial. *H. taylori* larvae were collected from 2 small pools near Griffith creek in the MKRF where the water temperature was around 15ºC. Larvae were transported refrigerated to the laboratory, placed in aquaria with 63-µm-filtered aerated stream water at 4ºC in the dark and fed *ad libitum* with alder leaves from the stream for 5 days to acclimatize to laboratory conditions. Forty-eight hours before the beginning of the experiment, the alder leaves were removed to allow the larvae to clear their guts. On the first day of the consumption experiment, before the beginning of the experiment, leaf packs from each experimental channel (conditioned for 18 days) were transported refrigerated to the laboratory, the leaves were rinsed with tap water and leaf discs (16 mm diameter) were cut.

Similar-sized larvae were chosen and placed individually in 250-µm-mesh plastic mesocosms (6.5 cm length, 4.5 cm diameter), into which 2 + 2 leaf discs were added, either all from the same leaf treatment (monocultures) or from 2 different treatments (all combinations, mixed treatments) to offer the shredder a choice. A total of 10 shredder treatments (monocultures + mixed) were tested (n = 5 per treatment). Leaf discs from each treatment were differentiated using different colour binder clips. The same combinations of leaf discs were added to the control mesocosms, with the same number of replicates but without the shredder, giving a total of 100 mesoscosms (50 shredder present + 50 shredder absent, i.e. controls). The mesocosms were placed in a tank with 63-µm-filtered aerated stream water. Water was circulated through a cooler device at a temperature of 15°C and the dissolved oxygen was 10.2 mg L^{-1}. The experiment lasted 48 hours, after which the larvae were removed from their cases, oven-dried (60ºC), weighed and ashed (550ºC for 2 h) to determine AFDM. Leaf discs were oven-dried (60ºC) and weighed individually to determine DM. Twenty leaf discs per treatment from the initial leaves were used to determine the initial DM and AFDM of the discs and thus calculate a conversion factor DM – AFDM.

The consumption rate (CR) of leaf discs for each treatment was calculated as follows:

$$\mathrm{CR} = \frac{\mathrm{M_I - M_F\ (mg\ AFDM)}}{\mathrm{M_S\ (g\ AFDM) \cdot t\ (d)}}$$

where M_I is the initial litter AFDM (prior to feeding trials), M_F is the litter AFDM at time t and M_S is the shredder AFDM at time t. To account for the different availability of leaf species between monocultures and mixed treatments (each leaf species was 2 times more available in the monocultures versus the mixed treatments), the CR in monocultures was corrected by a factor of 0.5. Negative values of CR were assigned zero because they can only be explained by no or low consumption.

Statistical analysis

Physico-chemical parameters were analysed via 1-way ANOVA to determine whether differences between channels existed. Differences in light availability and nutrient concentration between treatments were tested using 2-way ANOVA. Response variables were analysed with repeated measures (RM) ANOVA and principal component analysis (PCA) was performed using the whole set of response variables.

Referring to the feeding preference trial, an unpaired *t*-test was used to determine whether the DM of the initial discs and control discs (without shredder) was significantly different. The CR of the different leaf treatments in monoculture was compared via 2-way ANOVA, testing nutrient and light effects. In the feeding preference test in which 2 food types were offered to the shredder, the consumption of one food type could not be assumed to be independent from the consumption of the other food type (Canhoto *et al.*, 2005), and thus paired *t*-tests were performed between all the combinations.

Normality was tested visually and using the Shapiro-Wilks tests, and homoscedasticity was tested using Levene's test. Most analyses were performed using PASW (version 18, IBM, Armonk, NY, USA) and R (version 2.8.1, R Development Core Team 2009, Vienna, Austria), and CANOCO (version 4.5, Biometris, Wageningen, the Netherlands) was used to perform the multivariate analysis.

RESULTS

Physico-chemical parameters

Physico-chemical parameters, i.e. pH, temperature, conductivity and dissolved oxygen, did not differ between channels (Table 1). During the experiment, pH (6.60–7.27; initial–final) and oxygen (9.32–10.61 mg L^{-1}) increased while temperature (17.5–11.8ºC) and conductivity (24–18 µs cm^{-1}) decreased. The mean DOC concentration was 1.659 ± 0.062 SE mg L^{-1} (n = 1 per channel).

Table 1 Physico-chemical parameters measured in each of the stream channels during the experiment (n = 8 dates). Mean and standard error values and results from the 1-way ANOVA are shown, demonstrating that there were no differences between channels

			Channel	
Parameters	**Mean**	**SE**	F	*P*
pH	6.83	0.02	$F_{15,112} = 0.077$	1.000
Temperature (ºC)	14.0	0.2	$F_{15,112} = 0.001$	1.000
Conductivity (µs cm^{-1})	19	0.4	$F_{15,112} = 0.030$	1.000
Oxygen (mg L^{-1})	9.98	0.05	$F_{15,112} = 0.159$	1.000
Oxygen (%)	100.6	0.6	$F_{15,16} = 0.630$	0.811

Only nutrient concentrations and light availability were influenced by our treatments. The average values of both nitrates and phosphates were higher in the fertilised channels (Fig. 2), although significant differences were only found for nitrogen from nitrate (N-NO_3: Nutrients: $F_{1,26} = 18.54$, $P < 0.001$; Time: $F_{2,26} = 141.27$, $P < 0.001$; Nutrients x Time: $F_{2,26} = 6.26$, $P = 0.006$; P-PO_4: Nutrients: $F_{1,27} = 1.619$, $P = 0.214$; Time: $F_{2,27} = 0.91$, $P = 0.415$; Nutrients x Time: $F_{2,27} = 0.46$, $P = 0.635$). The N : P molar ratio was similar in the control and fertilised treatments (104.65 ± 17.61 and 104.26 ± 16.69 respectively). The light level in the shaded treatments was 9.44 ± 0.42% of the PAR measured in the open treatments (Light: $F_{1,110} = 1937.40$, $P < 0.001$; Time: $F_{7,110} = 302.45$, $P < 0.001$; Light x Time: $F_{7,110} = 0.32$, $P = 0.946$; Fig. 3).

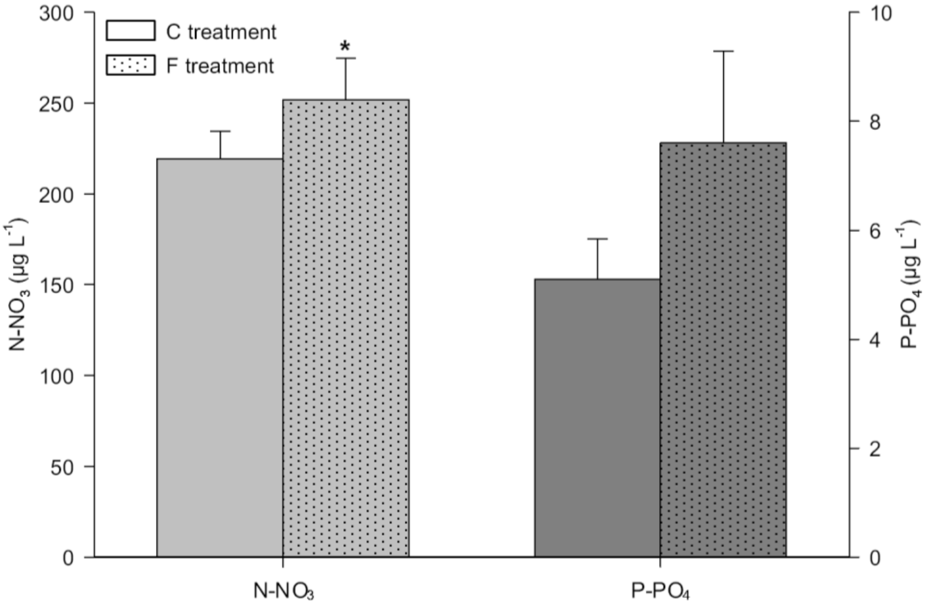


Fig. 2 Concentrations of nitrates (N-NO_3, light grey) and phosphates (P-PO_4, dark grey) in the control (C) and fertilised (F) treatments. Error bars represent + SE and asterisks indicate significant differences.

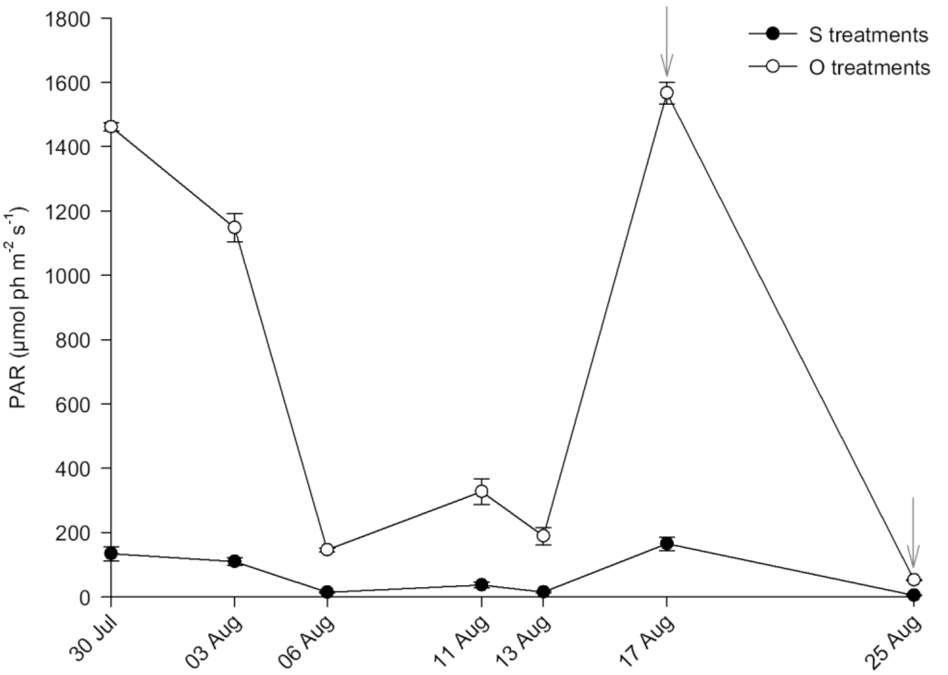


Fig. 3 Light availability measures of photosynthetically active radiation (PAR) during the experiment comparing shaded (S) and open (O) treatments. Arrows indicate the sampling dates 18 and 26 days following the start of the experiment. Error bars represent ± SE.

Leaf mass loss

Higher nutrient availability significantly accelerated leaf mass loss (Fig. 4; Table 2). Leaf mass loss, as a percentage of AFDM, was 38.60 ± 3.00% on average after 18 days of colonisation, equivalent to 0.63 ± 0.05 g, and 63.95 ± 3.95% after 26 days, equivalent to 1.03 ± 0.06 g of leaves. Leaves were decomposed more rapidly in the fertilised channels, both open and shaded, with leaf mass loss 20–26% higher (18–26 days) with higher nutrient availability.

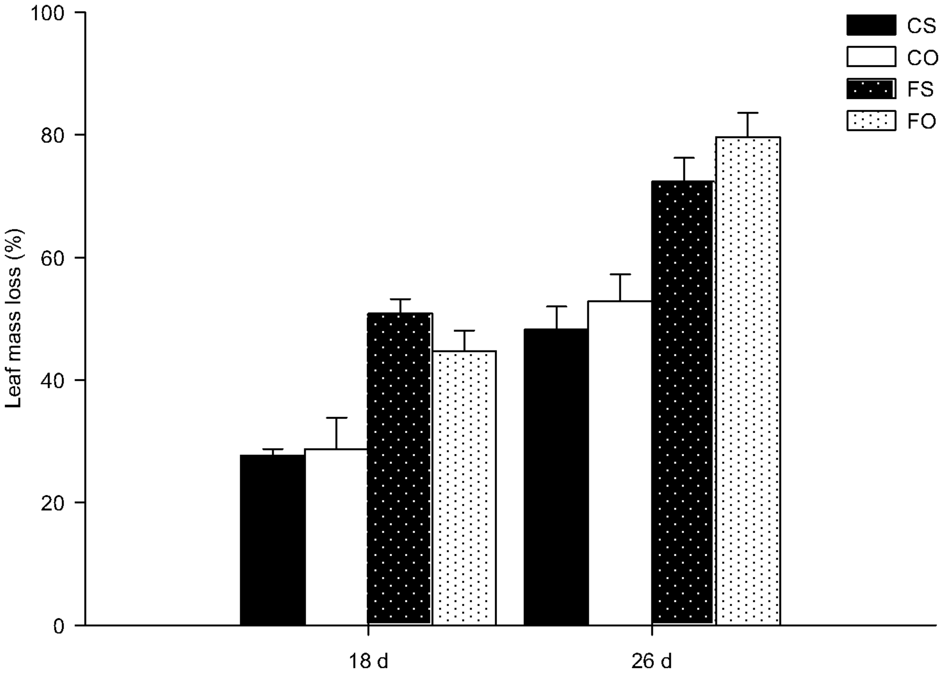


Fig. 4 Percentage leaf mass loss in the different treatments on the 2 sampling dates. Error bars represent + SE. C = control, F = fertilised, S = shaded, O = open.

Carbon and nitrogen content

C content was related to nutrients, while N content and the C : N molar ratio were influenced by both light and nutrients (Fig. 5; Table 2). C content decreased over time from 51.00 ± 0.26% in the initial leaves to 47.84 ± 0.29% after 26 days of colonisation, and was significantly lower in fertilised treatments (48.41 ± 0.42%) versus control treatments (49.41 ± 0.39%). N content peaked after 18 days, reaching 2.49 ± 0.05%. Leaves conditioned with both higher nutrient and light availability showed the highest values, and consequently the C : N molar ratio was the lowest at this point. On the second sampling date, the leaves conditioned with higher nutrient availability showed the highest C : N ratios.

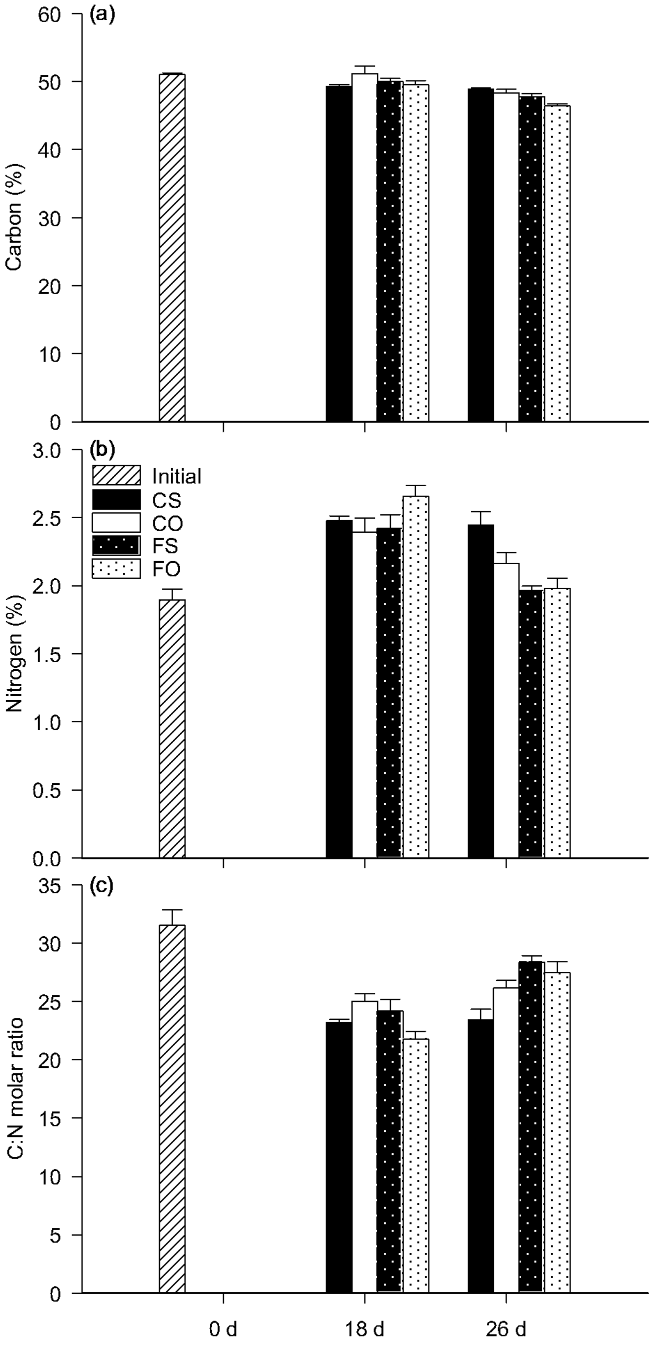


Fig. 5 (a) Carbon (C) and (b) nitrogen (N) percentage, and (c) C : N molar ratio in the initial leaves and on the 2 sampling dates. Error bars represent + SE. C = control, F = fertilised, S = shaded, O = open.

Table 2 Results of the Repeated Measures ANOVA of the response variables

Variables	df	Nutrients (N)		Light (L)		Time (T)		N x L		N x T		L x T		N x L x T	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Leaf mass loss (% AFDM)	1,11	59.51	**< 0.001**	0.34	0.571	153.55	**< 0.001**	0.16	0.699	2.08	0.177	4.31	0.062	1.44	0.255
C (%)	1,12	6.48	**0.026**	0.15	0.709	31.54	**< 0.001**	4.07	0.067	1.78	0.207	4.75	0.050	1.04	0.328
N (%)	1,12	2.71	0.126	0.20	0.644	72.95	**< 0.001**	5.05	**0.044**	28.64	**< 0.001**	6.72	**0.024**	0.02	0.893
C : N (molar ratio)	1,12	2.20	0.164	0.20	0.660	73.68	**< 0.001**	8.52	**0.013**	43.43	**< 0.001**	3.52	0.085	0.20	0.659
Chlorophyll *a* (µg g^{-1}AFDM)	1,12	0.01	0.946	0.07	0.795	3.24	0.097	0.99	0.339	6.91	**0.022**	3.86	0.073	1.04	0.327
Polysaccharide (mg g^{-1} DM)	1,12	1.37	0.264	1.13	0.309	26.55	**< 0.001**	0.07	0.791	0.15	0.705	1.70	0.217	0.16	0.692
Protein (mg g^{-1} DM)	1,12	1.05	0.325	12.62	**0.004**	14.12	**0.003**	< 0.01	0.989	6.47	**0.026**	0.29	0.598	0.02	0.889
Lipid (mg g^{-1} DM)	1,12	3.45	0.088	3.95	0.070	6.79	**0.023**	0.02	0.894	1.54	0.239	0.01	0.919	0.14	0.714

P-values < 0.05 are indicated in bold.

Chlorophyll content

The leaves initially contained minimal amounts of chlorophyll, at 4.44 ± 0.35 µg g^{-1} AFDM, equivalent to 0.07 ± 0.01 µg cm^{-2}. Algal colonisation on the surface of the leaves was differentially influenced by nutrients over time (Fig. 6; Table 2). Maximum chlorophyll values were found on the first sampling date on leaves conditioned with higher nutrient and light availability, at 74.05 ± 19.55 µg g^{-1} AFDM, equivalent to 0.35 ± 0.09 µg cm^{-2}. On the second sampling date, variability was high in all treatments and no significant differences were found.

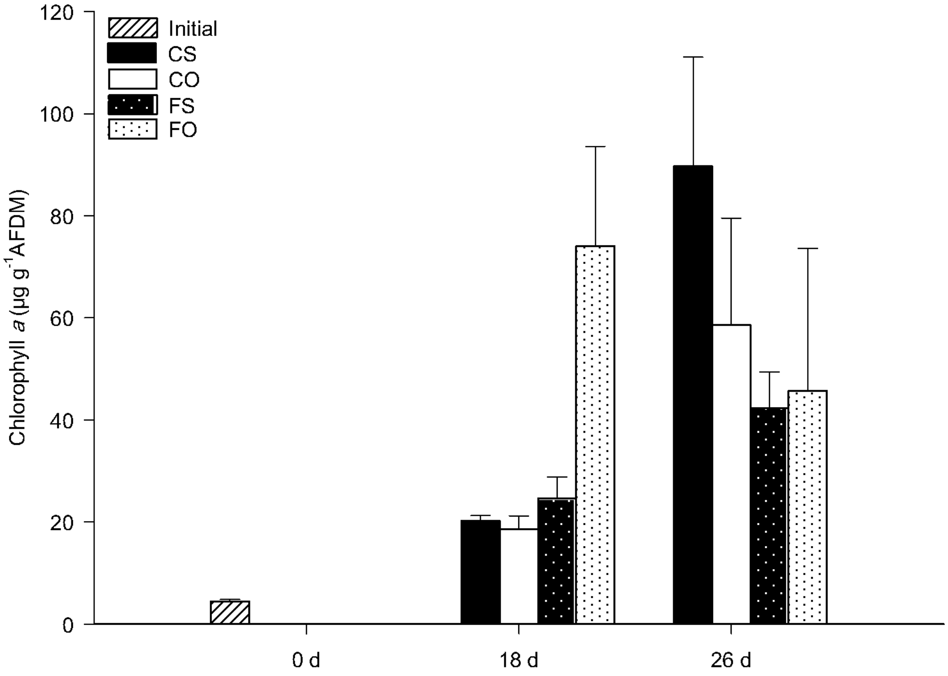


Fig. 6 Chlorophyll a content on the leaf surface of the initial leaves and in the different treatments on the 2 sampling dates. Error bars represent + SE. C = control, F = fertilised, S = shaded, O = open.

Biochemical composition

The polysaccharide and protein content of the leaves decreased with colonisation time, while the lipid content peaked after 18 days of colonisation. Only protein content responded to light and nutrient treatments (Fig. 7; Table 2). The initial polysaccharide content was 58.38 ± 8.93 mg g^{-1} DM, sharply decreasing after 18 days of colonisation to 3.84 ± 0.40 and to 1.75 ± 0.24 mg g^{-1} DM after 26 days. The protein content also decreased over time, from 86.28 ± 4.57 to 42.27 ± 3.68 mg g^{-1} DM; i.e., half of the protein content was lost from leaves in 26 days. Light influenced leaf protein content, with leaves conditioned under higher light availability showing a lower protein content on average (Open: 45.03 ± 3.79; Shaded: 56.01 ± 3.31 mg g^{-1} DM). In treatments with higher nutrients, compared to treatments with lower nutrients, protein content was higher on the first sampling date but lower on the second. The lipid content was only influenced by colonisation time, peaking after 18 days at 51.66 ± 3.40 mg g^{-1} DM.

Principal component analysis

The total variance explained by the PCA was 70.2% (Fig. 8). Samples were distributed along the first axis depending on the sampling time, reflecting the decomposition process. Samples taken on the first date were situated on the negative side of the axis, characterised by higher protein, lipid and

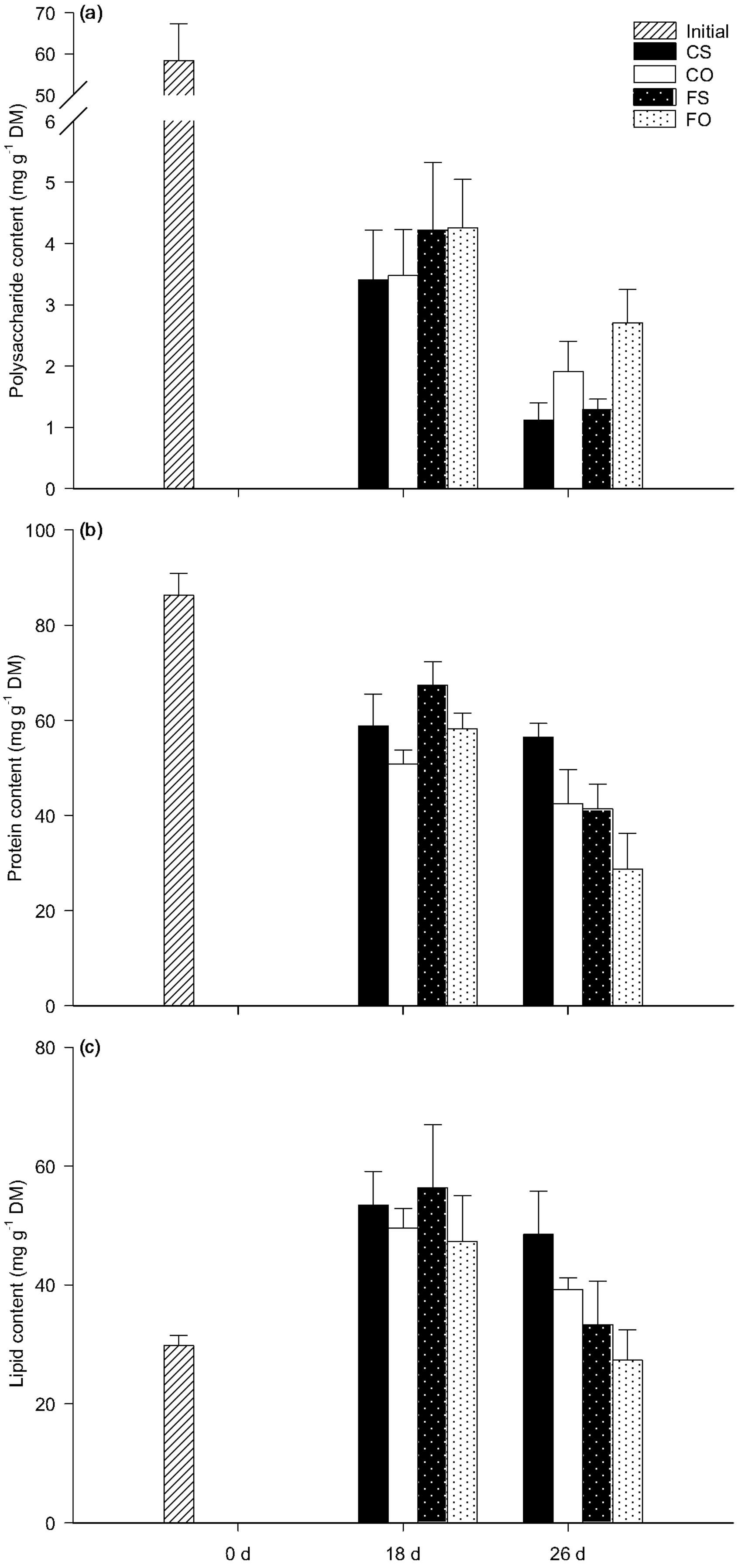


Fig. 7 Biochemical composition of colonised leaves according to (a) polysaccharide, (b) protein and (c) lipid content in the initial leaves and in the different treatments on the 2 sampling dates. Error bars represent + SE. C = control, F = fertilised, S = shaded, O = open.

polysaccharide content; most of the samples from the second date were situated on the positive side, related to a higher mass loss and C : N ratio. Samples taken at 26 days from the treatment with high nutrient and light availability were situated on the extreme positive side. The second axis separated samples with the highest chlorophyll content (CS treatment on the second date, and some FO samples on the first date). The distribution of factors revealed that protein content was related to N content, which was negatively related to the C : N ratio, whereas polysaccharides and lipids were related to C content. The polysaccharide content was also negatively related to the chlorophyll content of the leaves.

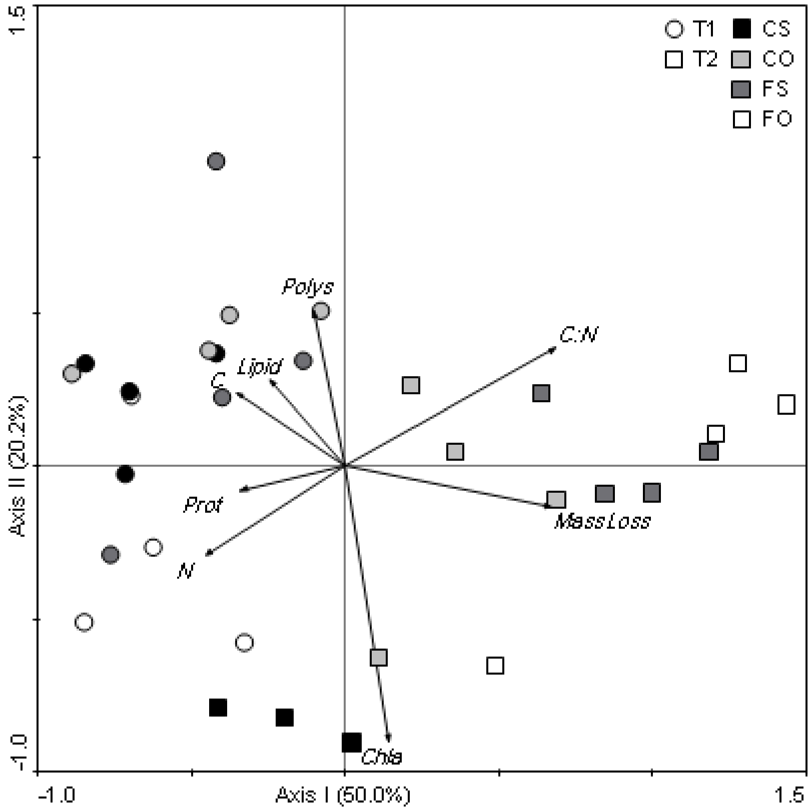


Fig. 8 Principal component analysis (PCA) of the samples with the whole set of response variables, depending on the conditioning treatment and time. The first and second axes are represented, and the percentage of variability explained by each is indicated. T1 = 18 days, T2 = 26 days, C = control, F = fertilised, S = shaded, O = open.

Consumer feeding preferences

Control disc DM (discs incubated without the shredder) did not differ significantly from that of the initial discs (unpaired *t*-tests for each leaf treatment: $P \geq 0.188$). Therefore, the consumption rate was calculated by taking into account the leaf mass of the initial discs.

The CR on monocultures was similar for all 4 treatments (2-way ANOVA $P > 0.320$; Fig. 9a). When combinations of 2 leaf treatments were offered to the shredders, significant differences in the CR were found between the FS and FO treatments, with leaves conditioned with high nutrients and low light (FS) being consumed at a higher rate (more than double) than those conditioned with high nutrients and light (FO; paired *t*-test: $t_4 = -6.289$; $P = 0.003$). No feeding preference was observed in any of the other combinations (paired *t*-tests: $P > 0.212$; Fig. 9b).

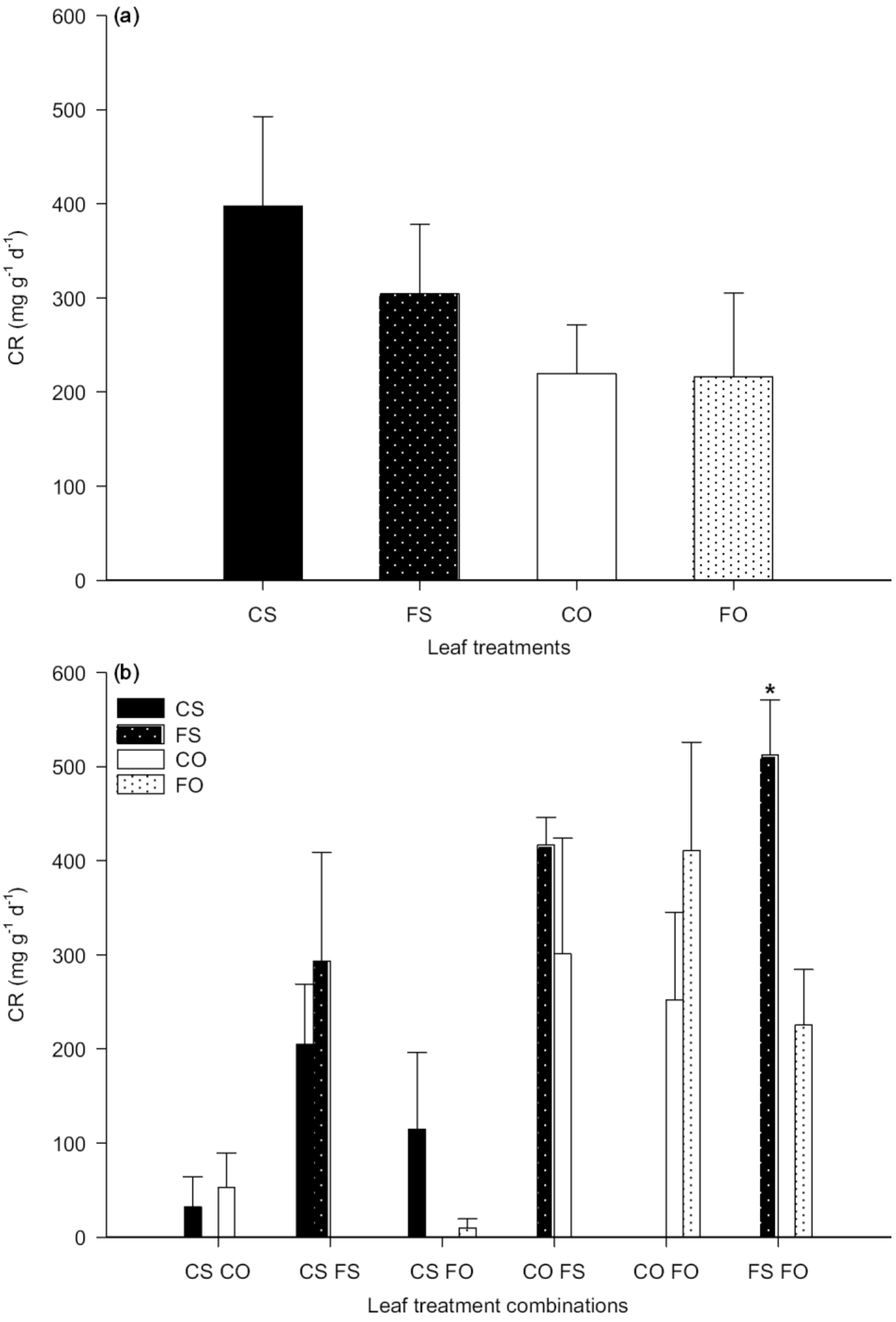


Fig. 9 Consumption rate (CR, in mg leaves AFDM mg^{-1} shredder AFDM day^{-1}) by shredders of the leaf discs from the different treatments (a) in monoculture and (b) in all combinations of 2 leaf treatments. Error bars represent + SE and asterisks indicate significant differences in the paired *t*-tests. C = control, F = fertilised, S = shaded, O = open.

DISCUSSION

Our results show the importance of colonisation time on leaf quality, overriding the effects of nutrient concentration and light availability on some quality variables. Thus, polysaccharide and lipid content were only affected by time. At 18 days leaves were richer in N and lipids, and the C : N ratio was lower with respect to both the initial conditions and leaf quality at the end of the experiment. Moreover, polysaccharide and protein content were higher after 18 days than after 26 days of conditioning. The results of the analyses of variance and the PCA confirmed that decomposition process was the main factor determining changes in quality in this experiment. Other studies have also found an overriding importance of colonisation time over other factors (e.g. leaf species, Hutchens *et al.*, 1997; nutrient enrichment, Royer & Minshall, 2001).

The initial soluble polysaccharide content in our leaves (58 mg g^{-1} DM, i.e. 5.8% of DM) was comparable to the values found by Chauvet (1987), but almost half that reported by Gessner (1991), both for *Alnus glutinosa*. This is probably due to intraspecific differences (Lecerf & Chauvet, 2008) and interspecific differences in this genus (*A. glutinosa* versus *A. rubra*). The initial polysaccharide content decreased sharply over time (losing 97% of the initial amount), as described by Suberkropp (1976) for other species, but faster than described by Chauvet (1987) for *A. glutinosa*. Microbial enzymatic activities make polysaccharides available to consumers (Bärlocher & Porter, 1986), thus reducing the soluble polysaccharide content of leaves (Gessner, 1991) during leaf conditioning. The initial protein content (86 mg g^{-1} DM, i.e. 9% of DM) was lower than the 13.6% reported by Gessner (1991). Our data show that protein tended to decrease over time, reaching a minimal value of 0.3% after 26 days. This tendency, although less pronounced (from 4 to 2%), was also found by Lester *et al.* (1994) in willow leaves. However, Gessner (1991) showed the inverse tendency with an enrichment with respect to the initial content (more than 20%) of alder leaves. These inverse tendencies may be explained by the net difference between the proteins lost from the leaves and the proteins provided by the biofilm (Lester *et al.*, 1994). The initial lipid content (30 mg g^{-1} DM, i.e. 3% of DM) was within the range of values for different species shown by Gessner & Neumann (2005; Table 13.1). In the early stages of conditioning, the leaves were enriched in lipids, although the lipid content decreased after 26 days, likely due to fungal conditioning (Rong *et al.*, 1995). A similar fluctuating lipid content was found in oak leaves by Suberkropp *et al.* (1976).

Leaf mass loss increased with time and was significantly higher in treatments with nutrient enrichment (20–26% higher) than in those without. Positive relationships between nutrients and decomposition rates were previously observed in whole-stream studies (Gulis & Suberkropp, 2003; Greenwood *et al.*, 2007). These effects were found to be more important in low quality leaves (Gulis & Suberkropp, 2003; Greenwood *et al.*, 2007), while contrary to our findings, no effect in leaf mass loss was observed in alder leaves (high quality leaves), although fungal biomass was higher (Ferreira *et al.*, 2006). No effects of nutrient addition on leaf decomposition were observed in studies in which inorganic nutrients (N, P or both) were not limiting (Grattan & Suberkropp, 2001; Royer & Minshall, 2001), indicating that our system was nutrient limited. The underlying geology in the study area consists of erosion-resistant granite, and thus the system is poor in nutrients and the most limiting nutrient is probably P (Kiffney & Richardson, 2001). Hence, although P did not increase significantly during the experiment, there was a relative increase in this nutrient as shown by the constancy of the N : P ratios despite fertilisation. Kiffney & Richardson (2001) observed changes in the periphyton biomass due to the addition of P, although there were no significant differences in the P concentration between treatments either.

The C content decreased rapidly with nutrient enrichment, related to the faster decomposition in these treatments, with C loss through lixiviates and respiration (Chauvet, 1987; Gessner *et al.*, 1999). This decrease combined with the general increase in N content led to lower C : N molar ratios especially during the first 18 days. A significant effect of nutrient and time interaction on algal biomass and protein content was also observed. Algal colonisation of leaves was higher after 18 days in the treatments with higher nutrient compared to treatments with lower nutrient availability, but after 26 days there were no significant differences in chlorophyll content between treatments. This shift may be explained by the differential timing of the ‘fully conditioned’ stage, i.e. the time of the maximal microbial (algal) colonisation (Boling *et al.*, 1975). This stage was reached earlier in leaves conditioned with higher nutrients and light due to the more favourable conditions for algae, and reached later (by around 8 days) by the other leaves. Algal colonisation would explain the enrichment in N and protein in the leaves in these treatments (Ledger & Hildrew, 1998).

Light treatment increased PAR availability from 10 to 100%, simulating respectively a stream with a 30-m riparian buffer and a riparian clear-cut or the absence of riparian vegetation (Kiffney *et al.*, 2004). This difference was previously observed to cause, in the same channel system, an increase in the chlorophyll content of epilithic biofilm, although the effects were marginal and changed over time (Kiffney *et al.*, 2004). A positive association between light and epilithic chlorophyll was also shown in a field experiment with real riparian management treatments (Kiffney *et al.*, 2003). In our study, and contrary to our initial hypothesis, the chlorophyll content in leaves was not affected by light, indicating that the relevant mechanisms and probably algal community differ on organic and inorganic substrates (Hoellein *et al.*, 2010). Although Albariño *et al.* (2008) found higher algal colonisation on leaves with higher light availability, the lack of these effects in the current experiment suggests that the algae colonising leaves were adapted to low light levels and were not light limited in the shaded treatments. In natural conditions, the accumulation of leaf litter in the stream bed reduces the light available at the leaf surface, and thereby the autotrophic biofilm needs to be adapted to low light levels. This would explain the different response to light between epilithic (Kiffney *et al.*, 2003; 2004) and leaf (this experiment) biofilms. The only measured variable directly affected by light was protein content, with lower mean values in open treatments. With longer colonisation (26 days), increased light led to a lower N content and higher C : N ratios.

Contrary to our initial hypothesis, shredders showed a tendency to prefer leaves conditioned under low light availability, mainly in the fertilised treatment. These leaves showed higher protein content compared to the other treatments. Hence, the protein content of leaves was likely associated with the feeding preference of the invertebrates. Similarly, Aβmann *et al.* (2011) found that proteins (and polyphenols, not measured in our experiment) were correlated with shredder consumption rates.

In conclusion, our study shows the overriding influence of colonisation time over changes in nutrient concentration (mainly N) and light availability on leaf biochemical quality. Higher nutrient concentrations accelerated leaf decomposition and loss of C. Algal colonisation was staggered over time according to nutrient and light availability and light affected the protein content of the leaves. Shredders showed a preference for protein-rich leaves from fertilised but shaded conditions. In natural stream basins riparian forest logging causes an increase in both nutrient concentration and light availability (Likens *et al.*, 1970; Kiffney *et al.*, 2003). In this experiment we have shown that these effects lead to changes in leaf decomposition, detritus biochemical quality and shredder consumption, all key functional processes for nutrient recycling in stream ecosystems.

GENERAL DISCUSSION

GENERAL DISCUSSION

Global changes in climate, land use and water courses are affecting environmental factors related to the functioning of streams, including meteorology, hydrology and the availability of nutrients and light. In the present thesis, we have reported the effects of these abiotic factors on basal resources. Throughout the previous chapters, we have demonstrated that the El Niño Southern Oscillation (ENSO) affected riparian inputs to the stream through teleconnections that alter average precipitation. Drought periods affected the annual distribution of the riparian inputs and reduced the quality of basal resources, whereas accumulated drought events diminished the riparian inputs. Floods decreased the benthic organic matter (BOM), increased the amount of transported organic matter (OM) and modified its quality. The addition of nutrients led to a decrease in carbon (C) content, and higher light availability led to a decrease in protein content of allochthonous resources. Biofilm quality was generally higher with the addition of nutrients when light availability was higher. These results, summarised in Fig. 1 and Table 1, are discussed below.

The thesis research encompasses different time scales: the short-term (Chapters 2,4), the mid-term (Chapter 3) and the long-term (Chapter 1). Whilst we are aware that in the absence of the temporal context provided by long-term research, serious misjudgements can be made when endeavouring to understand and predict changes in ecosystems (Magnuson, 1990), we performed short-term studies to analyse specific mechanisms in detail.

Referring to the spatial scale, this work has been mainly performed at reach scale The effects of climate, meteorology and hydrology on the quantity and quality of OM found in this thesis can be extrapolated to headwater forested Mediterranean streams, with deciduous riparian forests (Chapters 1,2). The studied effects of the nutrient enrichment and light availability can be generalised to headwater forested streams, and actually, our results are comparable to other headwater temperate streams (e.g. Gulis & Suberkropp, 2003; Greenwood & Rosemond, 2005; and other references in Chapters 3,4).

EFFECTS OF THE ENSO ON BASAL RESOURCES THROUGH METEOROLOGY

The ENSO is an air-sea coupled phenomenon that originates in the Pacific Ocean but affects global climate (D’Aleo *et al.*, 2007). It is the largest mode of interannual variability of the global climate system and is accompanied by a teleconnection pattern that extends out of the Pacific region (Nyenzi & Lefale, 2006). Low-frequency phenomena such as the ENSO determine vegetation responses; notably, via shifts in major controls such as temperature, precipitation and snow cover (IPCC, 2007b).

In most of the Iberian Peninsula the climate during winter is under the North Atlantic Oscillation (NAO). However, in the eastern region, where our study was performed, the climate is not linked to the NAO, but rather to the ENSO (Rodó *et al.*, 1997). The extreme phases of the ENSO (El Niño and La Niña) were related to a drought index based on precipitation in the Iberian Peninsula, although they exhibited spatial differences and occurred at different timescales (Vicente-Serrano, 2005).

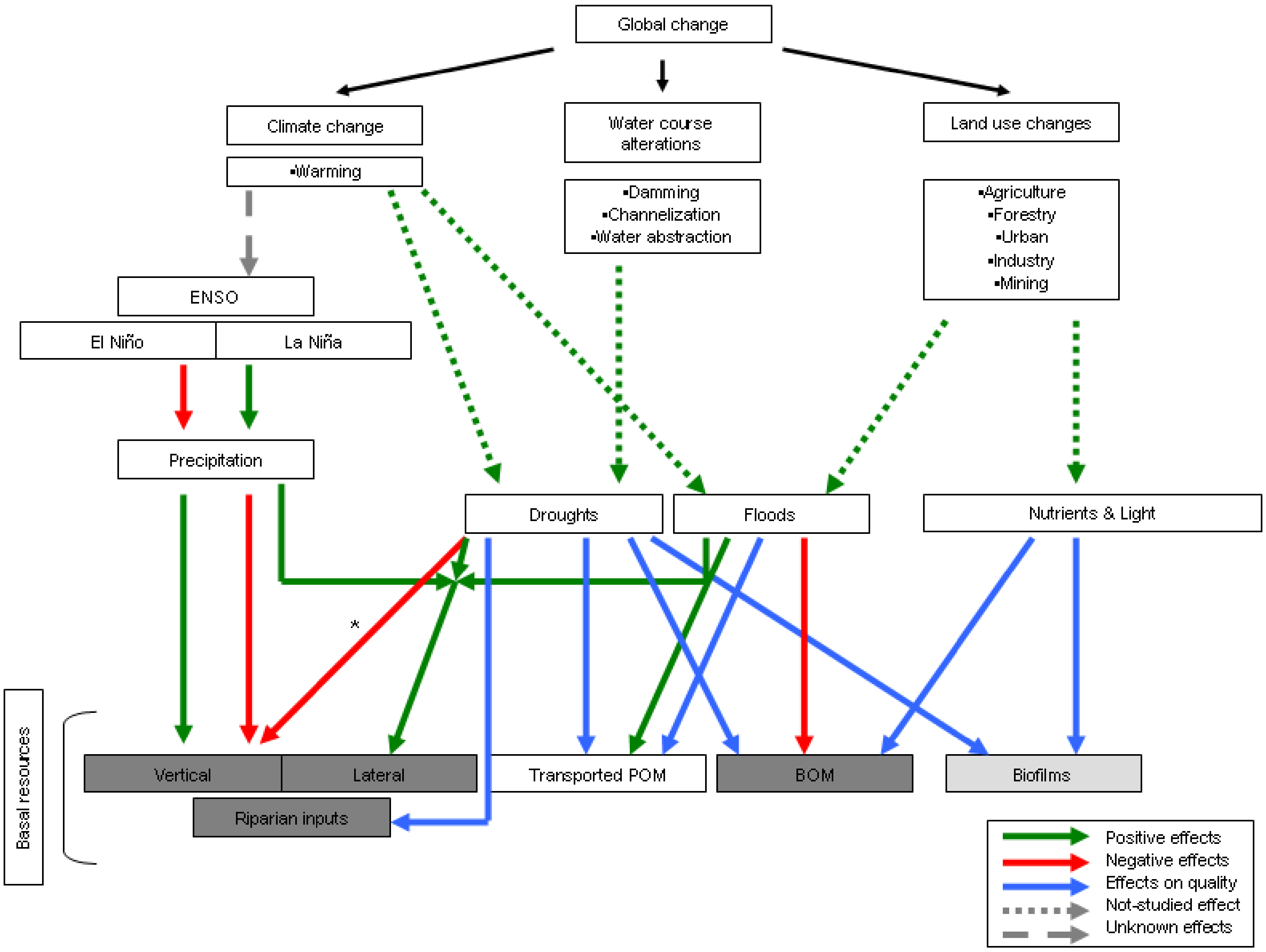


Fig. 1 Diagram representing the effects of several abiotic factors related to global changes in the quality and quantity of basal resources studied in this thesis. Only those effects related to the studied abiotic variables are represented. The dark grey cells represent allochthonous resources, whereas the light grey ones represent autochthonous resources. The asterisk indicates the cumulative effects of drought. The green and red arrows indicate quantitative effects, whereas the blue arrows indicate qualitative effects (detailed in Table 1).

In this thesis, we have demonstrated the relationship between the riparian forest litterfall intensity (a local-scale variable) and the ENSO (a large-scale climatic pattern), in a Mediterranean stream. ENSO dynamics (as measured by the Southern Oscillation Index, [SOI]) accounted for nearly all (90%) of the interannual variability observed in the total quantity of vertical inputs to the stream. The data were measured during 'phenological' years (April year X to March year X+1) and therefore, starting from the onset of the phenological period (see Chapter 1). This teleconnection is mainly explained through the variability in local precipitation (82%). During El Niño periods, average annual precipitation was lower than in other periods, causing higher riparian inputs, due to the hydric stress of riparian trees (Tabacchi *et al.*, 2000; Acuña *et al.*, 2007). In contrast, during La Niña periods, precipitation was higher than at other times, leading to decreased riparian inputs. Importantly, during some years (e.g. 2002–2003) average annual precipitation could not be related to the ENSO or to riparian inputs; it was probably due to local meteorological phenomena that did not affect tree litterfall.

The frequency of ENSO events has increased over the past few decades (Huntington, 2006). Following the same trend, the correlation between ENSO and Iberian precipitation increased towards the end of the past century (Rodó *et al.*, 1997). However, current ENSO projections are marked by many uncertainties, including the potential for an abrupt and/or hysteresis response, the direction of the shift and the level of warming when triggered (IPCC, 2007b). Climate change might influence the frequency and severity of ENSO events, although this connection remains unclear (Nyenzi & Lefale, 2006). Given these uncertainties, researchers cannot predict how the ENSO will affect riparian dynamics.

EFFECTS OF HYDROLOGY ON BASAL RESOURCES

The water supply and quality, and ecological integrity of stream systems are partly determined by natural flow regime (i.e. the quantity and timing of stream flow). In fact, stream flow is strongly correlated to many critical physico-chemical characteristics of streams, such as water temperature, channel geomorphology and habitat diversity. Indeed, it has been referred to as a "master variable" that limits the distribution and abundance of stream species (Poff *et al.*, 1997).

In Mediterranean areas, precipitation regime is highly seasonal and exhibits high interannual variability, which influences the hydrology in stream systems. Consequently, these streams are characterised by dry-wet cycles, undergoing periodic low flows and even flow cessation, due to the coincidence of warm and dry periods, or floods due to torrential precipitation (Gasith & Resh, 1999; Bonada & Resh, 2013). Droughts and floods are at the two opposite ends of the hydrological continuum and determine myriad properties and functions in ecosystems (e.g. Acuña *et al.*, 2004; Bonada *et al.*, 2006; Power *et al.*, 2008). In this thesis, we have demonstrated the cardinal importance of flow regime in resource dynamics (i.e. quantity and quality).

Drought has multiple effects on basal resources. The distribution of vertical riparian inputs throughout the year depends on the presence of a drought event that same year, whereas the annual amount of vertical riparian inputs depends on previous drought events (history; Chapter 1). In the years with a summer drought, riparian inputs follow a bimodal temporal distribution: the first and highest peak occurs in summer (36% of annual vertical inputs) and is related to hydric stress, and the second peak occurs in autumn (31% of annual vertical inputs) and is related to tree phenology. However, in the years without drought, the inputs follow a unimodal distribution: there is a single peak occurring mainly in autumn (52% of annual vertical inputs). Hence, the annual distribution of the vertical inputs is determined by seasonality and altered by the presence or absence of drought periods. Although these dynamics have already been reported in Mediterranean streams (Bernal *et al.*, 2003; Acuña *et al.*, 2007), in this thesis we have quantified these effects and reported that this trend holds over a long study period.

In the long term, the cumulative number of drought events accounts for much of the interannual variability of riparian inputs: the higher number of these events, the lower the inputs. In fact, two consecutive years of drought lead to a 45% reduction in riparian inputs (relative to two consecutive wet years). This effect, which to the best of our knowledge has never before been reported, can probably be explained by the impoverishment of nutrient content in the riparian soil due to successive droughts (McDonald & Davies, 1996; Bernal *et al.*, 2003). However, given that certain years with a drought period behave as wet years means that other, more complex mechanisms must also be considered. One possible explanation is that climatic events such as the ENSO can affect precipitation patterns.

The above explanation indicates that in both the short and long-term effects of droughts, the most important determinant for riparian inputs is the presence or absence of drought periods. In other words, drying of the streambed, and interruption of stream flow, are more important than the length of the drought. Drought also determines the accumulation of litter in the riparian soil: strong winds, precipitation or floods after a drought lead to greater lateral riparian inputs. However, as reported for other streams (Treadwell *et al.*, 1997; Wallace *et al.*, 1997a), we found that lateral inputs in the Fuirosos stream are less important quantitatively than are the vertical inputs.

Drought also influenced the quality of basal resources, leading to an increase in the C content of riparian inputs and allochthonous BOM (Chapter 1). Furthermore, during the study period, drought caused a decrease in the levels of total fatty acids (FA) and of essential fatty acids (EFA) in benthic substrates (epilithic and epipsammic biofilms, and leaf litter), and a decrease in suspended particulate OM, although no differences were observed in sterol content. This loss of quality was related to a shift from predominantly autochthonous OM to allochthonous OM (Chapter 2). A similar seasonal change has already been described in Mediterranean streams (Artigas *et al.*, 2009; Romaní *et al.*, 2013). Changes in OM composition driven by drought are consistent with higher levels of polysaccharides, proteins and lipids in the drying period than in the rewetting period found in the same stream of the study (Ylla *et al.*, 2010). Elemental composition and biochemical composition both indicate a general decrease in the quality of basal resources. Hence, drought reduced both the quantity, in the short term and the long term, and the quality of basal resources.

Floods were more related to retained and transported OM. Specifically, the frequency of floods negatively correlated to BOM levels and positively correlated to the amount of transported OM. BOM compartment is a balance between riparian inputs and transported particulate OM. In the model created by Acuña & Tockner (2010), BOM and floods together increase the downstream export. Higher flow and flood frequency in our study increased coarse particulate OM (CPOM) quality and decrease fine particulate organic matter (FPOM) quality, probably related to differences in OM processing. Furthermore, in the model (Acuña & Tockner, 2010), alterations in flow regime that are induced by climate changes in the Mediterranean region strongly influence the stream network C dynamics. It predicts that floods and longer droughts will reduce the amount of organic C processed within a given stream network. Within the network, headwaters are where the flow regime is most important.

Hydrology is affected by several components of global change. Extreme events (mainly droughts) will generally increase in frequency and magnitude. Hence, based on our results, we can predict that an increase in the frequency of droughts in the near future will lead to a more frequent bimodal temporal distribution and a decrease in the quantity and quality of available allochthonous resources in headwater Mediterranean streams. Increase in floods will reduce even more the available resources.

The Fuirosos stream has a similar average quantity of riparian inputs as temperate deciduous systems (e.g., Treadwell *et al.*, 1997; Wallace *et al.*, 1997a; Molinero & Pozo, 2004). However, owing to the characteristic drought of Mediterranean systems, it has different dynamics, marked by higher interannual variability compared to streams that have a similar amount of interannual litter inputs (Richardson, 1992).

The high interannual variability in the riparian inputs to the Fuirosos stream can be explained by global ENSO patterns (through precipitation) and by the history of local drought events. Although ENSO events and drought are intricately related in the Iberian Peninsula (Muñoz-Díaz & Rodrigo, 2005; Vicente-Serrano, 2005), in the Fuirosos stream we did not find a direct relationship between these phenomena.

Table 1 Summary of the effects of the studied abiotic factors on the quality of the different basal resources, indicating increases (▲) and decreases (▼) in quality indicators

	Drought	Floods	Nutrients	Light
Riparian inputs	▲ C			
Vertical	Annual bimodal distribution			
Lateral				
Biofilms	▼FA & EFA		▼ C : N	
			▲ Polysaccharide, protein, lipid, EFA	
BOM	▲ C		▲Leaf mass loss	▼ Protein
	▼FA & EFA (leaf litter)		▼ C	
Transported OM				
POM	▼FA & EFA			
CPOM		▼C : N		
FPOM		▲C : N		

THE EFFECTS OF NUTRIENT AND LIGHT AVAILABILITY ON BASAL RESOURCES

Nutrients and light are both essential for autochthonous primary production in streams (Borchardt, 1996; Hill, 1996). However, headwater streams are generally oligotrophic and shaded and therefore, receive scant nutrients and light (Webster *et al.*, 1995).

In this thesis, we have demonstrated that in the Fuirosos stream higher nutrient availability caused lower C content and that higher light availability lead to a higher protein content of allochthonous resources; and that leaf decomposition process dictated the changes in quality (see Chapter 4). With higher nutrient availability, biofilm quality (autochthonous resource) was higher, especially when light availability was higher (Chapter 3).

Furthermore, we found that the quality of allochthonous resources is determined chiefly by the conditioning time, which overrode the influence of nutrient or light availability on polysaccharide and lipid content, ultimately leading to diminished quality. There have been previous reports that colonisation time supersedes other factors (e.g. leaf species, Hutchens *et al.*, 1997; nutrient enrichment, Royer & Minshall, 2001). In our study, a greater availability of nutrients accelerated leaf-mass loss (by 20 to 26%), as previously found in whole-stream studies (Gulis & Suberkropp, 2003; Greenwood *et al.*, 2007), and led to a loss in C content via lixiviation and respiration (Chauvet, 1987; Gessner *et al.*, 1999). Light affected the protein content of leaves, whereas algal colonisation was staggered over time, occurring before with higher nutrient and light availability. Algal colonisation would explain the enrichment in nitrogen (N) and protein in the leaves in these treatments (Ledger & Hildrew, 1998).

The quality of epilithic biofilms (i.e. the levels of polysaccharides, proteins, lipids and EFA) was higher with higher nutrient availability, but these effects were only clear when light availability was higher. Romaní *et al.* (2004a) also observed that an increase in nutrient levels correlated to an increase in polysaccharide levels. The increase in proteins that we observed in the Fuirosos stream can be explained the lower C : N ratio, which was caused mainly by changes in the levels of non-essential amino acids; the levels of essential ones did not change. With lower light levels, the levels of labile amino acids were higher, due to a decrease in the amount of diatoms. The FA and EFA content was higher in the fertilised reach, but decreased over time, most likely due to algal abundance (Hill *et al.* 2011).

In the control reach of the stream, the OM was predominantly terrestrial, whereas in the fertilised reach, it was primarily aquatic. Sterol concentration and composition were not affected by changes in nutrient levels, due to the primarily allochthonous (higher plants) origin of sterols. The dependence on allochthonous inputs was also reflected in the lack of changes in the biochemical composition of particulate (POM) and dissolved OM (DOM).

After a mid-term moderate fertilisation, the only permanent change we observed in the quality of OM was the lower C : N ratio of the biofilms, which is related to a higher protein content.

Headwater systems are highly dynamic, and the biofilms respond quickly to changes in other factors that interact with nutrients, such as light. Given the predicted increase in changes in land use, nutrient and light availability in headwaters will also increase (Allan, 2004). Based on our results, we predict that the overall quality of allochthonous and autochthonous resources in headwater streams will increase and that the processing rate of allochthonous resources will rise upon a moderate increase in the amount of available light and nutrients.

THE RESPONSES OF DIFFERENT INDICATORS OF OM QUALITY TO CHANGES IN BASAL RESOURCES

Through our study, we used the elemental (C, N) and the biochemical (polysaccharide, protein and lipid) composition of the basal resources as indicators of the nutritional quality of the OM for consumer species. The C : N ratio was higher in allochthonous resources than in autochthonous ones, and gradually decreased from riparian inputs (59.14), to BOM (50.01) to transported materials (CPOM [38.64], FPOM [22.01] and ultrafine POM, UFPOM [14.66]) and finally, to epilithic biofilms (9.67; see Chapters 1,3). A similar gradient was observed by Cross *et al.* (2003). In terms of FA content, the richest substrate was epilithic biofilm, followed by leaves and finally, epipsammic biofilm (Chapter 2). Hence, both trends corroborate the idea that allochthonous resources are generally more recalcitrant than autochthonous one (Guenet *et al.*, 2010).

When we analysed the effects of drought on the indicators of OM quality, we observed a general decrease in the quality of allochthonous and autochthonous resources across all the indicators, with flow disconnection (Chapters 1,2). However, when we analysed the effects of nutrient and light availability on OM quality, the responses varied widely by indicator. In Chapter 3, higher nutrients lead to a permanent lower C : N ratio of the biofilm and polysaccharide, protein and lipid content were higher specially when light availability was higher. In Chapter 4, C : N increased and polysaccharides and lipids decreased in leaves with decomposition process. Protein content responded negatively to light but not significant responses were found related to nutrients.

Hill *et al.* (2011) found that the elemental stoichiometry and the FA content in biofilms each responded similarly to changes in phosphorus or light levels. Additionally, Cashman *et al.* (2013) found that FA measures were more sensitive to effects of nutrient and light on biofilm than nutrient content and stoichiometry.

In our study, we did not observe any significant changes in sterol levels in response to the abiotic factors tested. Despite being essential to consumer species, sterol levels do not seem to be as good indicators of OM quality as FA levels are.

Given our findings and the literature precedent, we consider that the nutritional quality of basal resources is a complex parameter. Whilst elemental composition and biochemical composition can sometimes be related (Rivas-Ubach *et al.*, 2012), knowledge on the latter enables a better understanding of the mechanism by which abiotic changes affect the quality of resources. However, the complexity of measuring elemental composition, biochemical composition and FA composition gradually increases. Hence, we suggest that the choice of the indicators of quality used would depend on the goal of the study and on the trade-off between analysis effort and information that wants to be obtained.

POTENTIAL EFFECTS OF THE BASAL RESOURCE CHANGES ON STREAM STRUCTURE AND FUNCTIONING

Food webs are fuelled by a complex mixture of allochthonous and autochthonous resources (Allan & Castillo, 2007). The quantity and quality of litter and autochthonous resources are limiting factors for stream secondary production (Richardson, 1991; Dobson & Hildrew, 1992; Stelzer & Lamberti, 2002; Cross *et al.*, 2006). Further, invertebrates must obtain through diet certain essential components (FA, sterols and amino acids) that they cannot synthesise (Olsen, 1999; Martin-Creuzburg & Elert, 2009).

A decrease in the quantity of riparian inputs caused by consecutive years with drought will probably cause a decline in the population of certain invertebrate taxa, generate stronger food web interactions (Hall *et al.*, 2000) and lead to a weakening of terrestrial-aquatic linkages (England &

Rosemond, 2004). Temporal differences in resource availability (*e.g.* due to premature leaf fall during drought periods) are important in this context, due to the tight link between shredders and the timing of litter inputs (Cummins *et al.*, 1989).

Changes in invertebrate community populations due to drought events have been observed in Fuirosos (Acuña *et al.*, 2005) and in other streams (Boulton, 2003). The reduction in flow causes an increase in invertebrate density, which leads to a greater competition for resources. During drought period, insects must accumulate energy and lipids for emergence (Anderson & Cummins, 1979; Anderson & Sedell, 1979); however, drought causes early emergence in some species (Leberfinger *et al.*, 2010). Hence, drought periods are critical for invertebrate survival.

However, the effects of the quality of resources on consumers' fitness will depend upon species identity and can be compensated by a modification of consumption (Fink & Von Elert, 2006) or by the alteration of some physiological processes (Graça *et al.*, 1993). In our experiment, shredders showed a preference for protein-rich leaves from fertilised but shaded treatments. Similarly, Aβmann *et al.* (2011) found that proteins were correlated with shredder consumption rates.

In our study of the Fuirosos stream, the addition of nutrients caused a higher algal chlorophyll and a higher bacterial density in biofilms, changes that were probably due to higher algal exudates (Hepinstall & Fuller, 1994; Romaní *et al.*, 2004b). The observed effects of nutrient addition on the benthos were not reflected in the bacteria in water, which depend primarily on allochthonous inputs (Butturini *et al.*, 2008).

The addition of nutrients also affected the use of OM by extracellular enzymes. In the fertilised reach during the period of greater light availability, the enzymes used more peptides. This result might be linked to the greater availability of substrate for these enzymes. Interestingly, in other studies higher leucine-aminopeptidase activity has been linked to incident light and photosynthetic activity, which might be related to the use of algal exudates by bacteria (Espeland *et al.* 2001; Francoeur and Wetzel 2003; Ylla *et al.* 2009). When light availability decreased, there was a decrease in the phosphatase activity of the biofilms from the fertilised reach. This result might have been due to the decrease in algal biomass (highly responsible for this extracellular enzyme activity), as well as to the higher availability of inorganic P, which would have obviated production of phosphatase in the fertilised reach (Romaní *et al.* 2004a; Allison and Vitousek 2005). At the end of the experiment, the measured use of polysaccharides and peptides by extracellular enzymes (as indicated by high β-glucosidase and leucine-aminopeptidase activities) was greater in the control reach than in the fertilised reach, indicating greater requirements for C and N sources in the former. Likewise, the measured use of accumulated polysaccharides was greater in the biofilms in the fertilised reach.

Protection and restoration of the integrity of stream ecosystems demands that conservation and management actions be firmly grounded in scientific knowledge (Poff *et al.*, 1997). Through this thesis we have demonstrated the importance of: i) the presence of a well-developed riparian forest, because it provides important resources to the streams and regulates nutrient and light availability, which cause changes in the quality of resources; and ii) the heterogeneity of resources in the stream (i.e. leaves and biofilm) because they provide different essential components to consumers. We have demonstrated that drought cause a decrease in the quality of resources, thereby, anthropogenic impacts increasing drought frequency and magnitude (e.g. water abstraction), should be avoided. With this thesis we have aimed to contribute to research-based understanding of the functioning of stream ecosystems.

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

CHAPTER 1: DYNAMICS OF ALLOCHTHONOUS ORGANIC MATTER IN A MEDITERRANEAN STREAM: HYDROLOGICAL, METEOROLOGICAL AND CLIMATIC DRIVERS

1. Riparian inputs showed an average value of 912 g dry mass (DM) m^{-2} y^{-1}, which was similar to that in temperate streams with deciduous riparian forests. However, a high interannual variability (range: 695 to 1185 g DM m^{-2} y^{-1}) was found, which is related to Mediterranean climate characteristics.
2. The ENSO extreme events highly explained interannual differences in vertical riparian inputs, through changes in precipitation. The El Niño events caused lower average precipitation, which increased riparian inputs, whereas the La Niña led to greater average precipitation, which decreased riparian inputs.
3. Cumulative drought periods reduced vertical riparian inputs: two consecutive years of drought reduced riparian inputs by 45% compared to those in two consecutive wet years.
4. In the years with a summer drought period, the riparian inputs showed a bimodal temporal distribution with a higher summer peak, caused by hydric stress, and a lower autumn peak, related to phenology. In the years without drought, the inputs showed a unimodal distribution with an autumn peak.
5. The BOM was determined by the time between floods, and the transported OM increased with increasing flood frequency.
6. Drought conditions increased the C content of the riparian inputs, and the photoperiod increased the N content. The C content of BOM was also higher with longer droughts. Floods increased the quality of CPOM and decreased the quality of FPOM.
7. The dissolved organic C (DOC) peaks after summer were positively related to the riparian inputs that had accumulated in the streambed.

CHAPTER 2: DROUGHT EFFECTS ON RESOURCE QUALITY IN A MEDITERRANEAN STREAM: FATTY ACIDS AND STEROLS AS INDICATORS

8. Drought period caused a decrease in the FA and the EFA in benthic substrates (epilithic and epipsammic biofilms, and leaves) and in POM. However, no differences were observed in sterol content.
9. Before the drought, the concentration of FA in benthic substrates that was related to primary producers was higher than after drought. A shift from autochthonous OM before the drought to a predominance of allochthonous OM after the drought was observed.
10. There was a peak in the FA (mainly comprising diatom FA) in POM just before the drought. No temporal changes in the FA in DOM were observed.

11. Epilithic biofilms showed the highest FA content (mainly comprising diatom and bacterial FA), whereas epipsammic biofilms had the lowest FA content. EFA were mainly found in epilithic biofilms and in leaves. Leaves were the greatest source of sterols.

CHAPTER 3: BIOCHEMICAL QUALITY OF BASAL RESOURCES IN A FORESTED STREAM: EFFECTS OF NUTRIENT ENRICHMENT

12. Addition of nutrients to a reach of the stream lead to higher levels of chlorophyll, polysaccharides, lipids and EFA, higher bacterial density and lower C : N ratio in epilithic biofilms. No difference in the levels of essential amino acids was observed. The effects of nutrient enrichment were modulated by the availability of light.
13. Sterol concentration and composition were not affected by the addition of nutrients due to their primarily allochthonous (higher plants) origin.
14. The addition of nutrients affected the extracellular enzymes in the biofilms in different ways. Leucine-aminopeptidase activity was higher with higher nutrient availability, as it was linked to a greater abundance of substrates for these enzymes. Phosphatase activity decreased when light availability decreased, which might have been due to the decrease in algal biomass and/or to changes in the availability of inorganic P.
15. In flowing water, nutrient enrichment did not affect the density of bacteria or the biochemical composition of the POM or DOM, with one exception: the amino acid composition of the DOM gradually changed from fresh material to structural amino acids.

CHAPTER 4: THE INFLUENCE OF NUTRIENT OR LIGHT AVAILABILITY ON LEAF LITTER AS A FOOD RESOURCE FOR STREAM SHREDDERS

16. Leaf quality was affected primarily by the conditioning time, which overrode the effects of nutrient or light availability on polysaccharide and lipid content, which decreased over time.
17. Higher nutrient concentrations accelerated leaf decomposition (by 20 to 26%) and increased the loss of C.
18. The protein content of leaves was lower under higher light conditions, consequently leaves contained lower N and showed higher C : N ratios.
19. Shredders showed a preference for protein-rich leaves from the fertilised, shaded treatments.

RESUM EN CATALÀ

INTRODUCCIÓ GENERAL

ELS RIUS: CARACTERÍSTIQUES GENERALS

Els rius estan presents en múltiples aspectes de la societat; en la història (per exemple la Batalla de l'Ebre el 1938, durant la Guerra Civil Espanyola), en la literatura, com a font d'inspiració, recurs simbòlic i una metàfora poètica, en el cinema (per exemple 'El riu de la vida', dirigida per Robert Redford), la pintura (per exemple, 'Cel estrellat sobre el Roine' de Vincent Van Gogh) i la música (per exemple, 'Al Bell Danubi Blau' de Richard Strauss). Els rius s'utilitzen tant com a fronteres (per exemple als Estats Units, on el Riu Colorado separa l'estat d'Arizona dels seus veïns, Califòrnia i Nevada) com a rutes de comunicació (per exemple el riu Danubi, navegable des d'Alemanya a Romania, passant per 10 països). Des d'un punt de vista ecològic, els rius són fascinants degut a la complexitat de la vida i els processos que alberguen. Hi ha una gran diversitat de rius, des dels petits rius de capçalera fins als més grans del món, com l'Amazones o el Nil; poden ser oberts o boscats, amb aigües clares o negres; i poden modelar el paisatge, creant congostos i gorges al llarg de milions d'anys, o discórrer a traves d'amples planes d'inundació acostant-se cap al mar (Fig. 1).

Els rius s'anomenen ecosistemes lòtics, els quals es caracteritzen pel moviment unidireccional de l'aigua al llarg d'una pendent degut a la gravetat. La dissipació de l'energia de les masses d'aigua en moviment afecten la morfologia del riu, els patrons de sedimentació, la química de l'aigua i la biologia dels organismes que hi habiten (Wetzel, 2001). Al llarg del recorregut d'un riu, a través de l'eix longitudinal, es produeixen canvis importants. Es produeix un augment en la mida del canal i en el volum d'aigua a mesura que els afluents s'uneixen a la xarxa i l'àrea de la conca augmenta. Els rius tenen un pendent més pronunciat a les terres altes on s'originen i el pendent es fa més gradual a les terres baixes, a prop de la seva desembocadura. Aquest perfil es pot dividir en tres zones, dominades per l'erosió, la transferència i la deposició de sediments. Els sediments són rebuts des de les capçaleres i dipositats a les seccions baixes d'aquests sistemes. A les zones baixes deposicionals, els rius serpentegen a través d'àmplies valls, gairebé planes i es poden dividir en múltiples canals mentre discorren a través dels sediments dipositats per ells mateixos (Allan & Castillo, 2007). Observats a una escala espacial més fina, els segments del riu, que s'estenen entre la unió aigües amunt i aigües avall dels afluents en la xarxa fluvial, contenen trams, els quals al seu torn contenen una seqüència d'hàbitats. Aquests hàbitats funcionals, tals com les zones lentes i zones de ràpids, les zones de sorra i grava i les acumulacions de fulles, es mesuren i s'estudien com a components individuals però interactius del tram del riu (Poole, 2002).

Els ecosistemes fluvials són sistemes oberts amb una connectivitat longitudinal, lateral i vertical altes en els tres eixos principals, on els intercanvis són bidireccionals: aigües amunt – aigües avall, llera – ribes, i aigua superficial – subterrània (Allan & Castillo, 2007). La biota es distribueix en relació a aquestes tres dimensions de la xarxa del riu, incloent la ribera (les vores del riu), el bentos (el fons del llit del riu) i l'hiporreic (la part intersticial del sediment de la llera), a més dels peixos i altres organismes que ocupen la columna d'aigua (Stanford, 2006).

Tota l'energia disponible pels consumidors prové dels productors primaris tan de fora com de dins del riu (recursos al·lòctons i autòctons, respectivament). Simplement a causa de la gravetat, els sistemes amb un perfil convex, com els rius, actuen com a atraients espacials i reben quantitats més grans d'aportacions que els sistemes amb perfils convexes (Lindeman, 1942; Leroux & Loreau, 2008; Fig. 2). La matèria orgànica (MO) al·lòctona prové de les terres circumdants i es transportada al riu des d'aigües amunt o lateralment. Els recursos autòctons provenen dels productors primaris del riu, incloent les algues, les cianobacteris, els briòfits i els macròfits vasculars, tot i que les algues bentòniques són en general els productors principals, creixent en les superfícies on les condicions (llum, nutrients, etc.) són adequades (Lamberti & Steinman, 1997). Les xarxes tròfiques són alimentades per una complexa barreja de recursos al·lòctons i autòctons (Allan & Castillo, 2007; Fig. 3).

Malgrat que els rius només representen el 0.0001% de l'aigua que hi ha a la Terra (Taula 1) i ocupen tan sols el 0.1% de la superfície de la Terra (Wetzel, 2001), tenen un paper fonamental en el manteniment i la supervivència de la vida terrestre i en l'emmagatzematge i el metabolisme dels materials particulats i dissolts, i en el seu transport des de la terra cap al mar (Wetzel, 2001; Battin *et al.*, 2008). Els rius, juntament amb els altres ecosistemes d'aigua dolça, sostenen almenys 100,000 espècies conegudes, les quals representen al voltant del 6% dels 1.8 milions d'espècies descrites (Dudgeon *et al.*, 2006). A més a més, els rius proporcionen múltiples serveis i béns als humans, és a dir el subministrament i la purificació de l'aigua, la recàrrega dels aqüífers, aliments, biodiversitat, energia hidroelèctrica, transport i recreació (Revenga *et al.*, 2000). S'ha calculat que els serveis que proporcionen els rius i els llacs representen 1.7 trilions de dòlars americans per any (Costanza *et al.*, 1997). Aquesta xifra monetària, si és que és possible mesurar-la, serveix per il·lustrar la importància d'aquests ecosistemes a un sector de la societat, tot i que si el valor dels ecosistemes es redueix al seu valor monetari, podria portar a la mercantilització dels ecosistemes.

RIUS BOSCATS DE CAPÇALERA: IMPORTÀNCIA I FUNCIONAMENT

La xarxa fluvial s'origina a partir d'una miríada de petits rius de capçalera (Meyer *et al.*, 2003), els quals representen el 86–89% del total de la longitud dels canals (Leopold *et al.*, 1964; Downing *et al.*, 2012) i agrupen els rius d'ordre 1 a 3, seguint la classificació de Strahler (1957) (Vannote *et al.*, 1980), malgrat que en alguns estudis només els rius de 1^r i 2^n ordre són considerats dins d'aquesta categoria (com per exemple, Meyer *et al.*, 2007). A més a més, els rius de capçalera són important degut a la seva influència en l'estructura i el funcionament dels rius d'ordre superior, vinculant els ecosistemes de les terres altes amb els de terres baixes. Les capçaleres proporcionen un control natural de les avingudes, la recàrrega dels aqüífers (quantitat d'aigua), la retenció dels sediments i fertilitzants i el reciclatge de nutrients; també processen la MO terrestre (qualitat de l'aigua), sostenen la productivitat biològica i contribueixen a la biodiversitat dels rius aigües avall (Meyer *et al.*, 2003; Lowe & Likens, 2005).

Aquests rius generen la major part del flux d'aigua (Alexander *et al.*, 2007; MacDonald & Coe, 2007) i contribueixen al manteniment de la connectivitat hidrològica i de la integritat dels ecosistemes riu avall, proporcionant materials, energia i organismes (Freeman *et al.*, 2007). Les capçaleres suporten taxes més altes de processament biogeoquímic que els rius més grans, amb una taxa més gran d'eliminació de nutrients (principalment nitrogen [N] i fòsfor [P]; Peterson *et al.*, 2001; Alexander *et al.*, 2008). La diversitat d'hàbitats, tant dins del mateix riu com entre els rius de capçalera, és alta, creant nínxols per organismes diversos. Aquests rius ofereixen refugi contra les temperatures i els cabals extrems i contra els competidors, depredadors i espècies introduïdes; serveixen com a recurs pels colonitzadors, proporcionant llocs de fressa i àrees de cria; són una rica font d'aliment per a les xarxes tròfiques locals i de riu avall; i creen corredors migratoris a través del paisatge (Meyer *et al.*, 2003; Lowe & Likens, 2005; Meyer *et al.*, 2007). A causa del seu isolament geogràfic, mantenen poblacions aïllades genèticament (Gomi *et al.*, 2002). En aquests rius, la diversitat α (dins de l'hàbitat) pot ser baixa, però una alta diversitat β (entre hàbitats) entre rius, tant dins com entre conques, pot generar una alta diversitat γ (a escala de paisatge; Clarke *et al.*, 2008; Finn *et al.*, 2011).

De tota la conca, és la terra que voreja el riu, és a dir, la zona ripària, la que té una més gran influència, afectant l'estructura i el funcionament del riu (Naiman & Décamps, 1997). En els rius de capçalera boscats, les arrels de la vegetació de la zona ripària estabilitza les vores i n'evita el despreniment, al mateix temps que controlen les fonts difoses de sediments i nutrients; els trossos grans de restes llenyoses creen diversitat d'hàbitat allà on cauen i les capçades controlen el microclima del riu (la temperatura) i modulen la disponibilitat de llum. Les aportacions de la vegetació (MO particulada, MOP) i el lixiviat de les restes de plantes (MO dissolta, MOD) són fonts principals d'energia per a les xarxes tròfiques del riu (Gregory *et al.*, 1991; Naiman & Décamps, 1997; Meyer *et al.*, 1998; Allan & Castillo, 2007).

RECURSOS BASALS EN ELS RIUS DE CAPÇALERA: QUANTITAT I QUALITAT DELS RECURSOS AL·LÒCTONS I AUTÒCTONS

En els rius de capçalera, els recursos basals al·lòctons són generalment més abundants que les fonts d'energia autòctones (Fisher & Likens, 1973; Webster & Meyer, 1997). La quantitat i la qualitat dels recursos al·lòctons són factors que limiten la producció secundària (Richardson, 1991; Dobson & Hildrew, 1992; Cross *et al.*, 2006). La importància de les entrades de MO al·lòctona de la zona ripària ha estat demostrada a través d'experiments d'exclusió de la fullaraca, on la reducció en la quantitat de MO va comportar la disminució o la pèrdua d'alguns tàxons i interaccions tròfiques més fortes entre els tàxons restants (Wallace *et al.*, 1997b; Hall *et al.*, 2000). La quantitat de les entrades ripàries està relacionada a escala global amb la latitud i la precipitació anual (Benfield, 1997), mentre que a escala local, les aportacions estan determinades per les propietats del bosc de ribera (composició i fenologia del bosc; per exemple Pozo *et al.*, 1997; Menzel, 2002). En els boscos caducifolis, les aportacions ripàries tenen lloc majorment a la tardor, mentre que als boscos perennes aquestes entrades poden ser estacionals o irregulars, depenent de les espècies i la localització (Benfield, 1997; Hoover *et al.*, 2011). La MO de la vegetació ripària pot entrar al riu per caiguda directa (vertical) o indirectament (lateral), i aquesta MO serà retinguda (MO bentònica) o transportada riu avall (Webster *et al.*, 1999). Les partícules de MO grans (MOP grollera, MOPG) retingudes es descomponen en partícules més petites mitjançant el processament físic (abrasió) i biològic, en aquest darrer cas a través del consum pels invertebrats (trituradors) després del condicionament de les fulles pels fongs i els bacteris (Gessner *et al.*, 1999).

Malgrat que una gran part de les entrades de MO es produeix en forma de MOPG, gairebé tot el transport es realitza en forma de MOP fina (MOPF) i MOD. Una quantitat desproporcionada del transport es produeix quan el cabal és alt (Wallace *et al.*, 1995).

A més dels efectes de la quantitat de MO (Tiegs *et al.*, 2008), la qualitat dels recursos al·lòctons influencia la seva taxa de processament (Leroy & Marks, 2006; Hladyz *et al.*, 2009) i el creixement i la reproducció dels consumidors (Graça *et al.*, 2001). Depenent de la seva qualitat, la fullaraca es pot classificar en material de descomposició ràpida, mitjana o lenta (Cummins *et al.*, 1989). Les entrades de fullaraca es consideren com a material recalcitrant, constituint principalment una font de carboni (C), i el seu contingut inicial de nutrients depenent de les espècies, la fertilitat del sòl i la disponibilitat d'aigua a la zona ripària (Wright *et al.*, 2001). Un cop al riu, les fulles s'enriqueixen en N i P mitjançant la colonització microbiana i el condicionament (Triska *et al.*, 1975; Golladay *et al.*, 1983).

La productivitat autòctona en les capçaleres es majorment portada a terme pels productors bentònics (normalment les diatomees, els cloròfits i els cianobacteris) del perífiton. El perífiton, potencialment, pot créixer en qualsevol superfície submergida, tant orgànica com inorgànica (Lamberti, 1996), i en els rius boscats de capçalera està sovint limitat per factors abiòtics (disponibilitat de nutrients i llum; Vannote *et al.*, 1980; Wallace *et al.*, 1997b) i/o factors biòtics (herbivoria; Rosemond *et al.*, 2000). La producció primària augmenta amb l'àrea de la conca, el cabal i el P reactiu soluble (PRS), però també està relacionada negativament amb el pendent del canal i el percentatge de cobertura de la capçada (Lamberti & Steinman, 1997). En els rius de capçalera que drenen boscos caducifolis, la producció primària anual més baixa es va relacionar amb el PRS més alt i la major cobertura de les capçades (Lamberti & Steinman, 1997). Les algues bentòniques poden créixer al llarg de tot l'any, tot i que a la primavera, abans de la sortida de les fulles, es pot produir un pic de producció autòctona impulsat per la producció primària en el riu, relacionada amb la disponibilitat més alta de llum (Rosenfeld & Roff, 1992; Hill *et al.*, 2001). La quantitat de perífiton en aquest moment s'ha relacionat de forma positiva amb el creixement dels consumidors (Hill *et al.*, 2010). Així doncs, els canvis estacionals en els recursos basals estan relacionats amb la fenologia ripària.

La producció autotròfica pot representar des de menys d'un 1% fins a més d'un 60% del total de l'energia als rius de capçalera i el consum del perífiton bentònic o en suspensió pot mantenir les xarxes tròfiques de base algal (Lamberti, 1996). La qualitat nutritiva de les algues (MO làbil) es considera més alta comparada amb la dels detritus (MO recalcitrant; Guenet *et al.*, 2010), però la qualitat del perífiton pot canviar depenent de la composició d'espècies i l'hàbitat (Lamberti, 1996), i degut a canvis en els nutrients i la llum (Cashman *et al.*, 2013). Les algues tenen un contingut més alt de proteïnes i lípids que els detritus i poden contenir una major proporció de certs aminoàcids o àcids grassos (AG) que són essencials pels consumidors (és a dir, que els consumidors no poden sintetitzar; Lamberti, 1996). Com a recurs nutritiu valuós en els rius, el perífiton pot augmentar el creixement i la reproducció dels consumidors (Stelzer & Lamberti, 2002; Franken *et al.*, 2005).

Qualitat dels recursos: avaluació i importància

La qualitat dels recursos determina el seu valor nutritiu potencial pels invertebrats que s'alimenten d'ells. La qualitat nutritiva de la MO pot ser avaluada mesurant la composició elemental o l'estequiometria (contingut i proporció de C, N, P; Cross *et al.*, 2003), el contingut de components refractaris (per exemple, la lignina o els tanins; Gessner & Chauvet, 1994; Hladyz *et al.*, 2009), o la composició de biomolècules (el contingut de polisacàrids, proteïnes i lípids; Ledger & Hildrew, 1998; Ylla *et al.*, 2010). D'aquests components, el contingut energètic més alt dels lípids (39.35 J mg^{-1}) comparats amb les proteïnes (23.63 J mg^{-1}) i els polisacàrids (17.18 J mg^{-1}) els fan els compostos d'emmagatzematge d'energia més eficients per la majoria dels organismes bentònics d'aigua dolça (Cavaletto & Gardner, 1999). Els invertebrats del riu necessiten emmagatzemar grans quantitats d'energia per a la metamorfosi i la reproducció (Beer-Stiller & Zwick, 1995) o per sobreviure períodes d'escassesa (Wilhelm, 2002), així doncs la qualitat dels recursos pot ser més important que la quantitat tant pel creixement com per la reproducció de la població animal (Ahlgren *et al.*, 1997). Dins els lípids, els AG i els esterols inclouen molècules essencials per als macroinvertebrats (Torres-Ruiz *et al.*, 2007). Per tant, la presència d'aquestes molècules serà important pel creixement i la reproducció dels consumidors. L'efecte dels AG essencials en el creixement, la supervivència i les taxes de reproducció i l'eficiència en la conversió dels aliments ha estat observada en una àmplia varietat d'organismes marins i d'aigua dolça, bàsicament en llacs (Brett & Müller-Navarra, 1997), però no directament en rius.

Els AG i els esterols poden ser utilitzats com a marcadors biològics a la dieta (Desvilettes *et al.*, 1997; Mannino & Harvey, 1999), ja que estan relacionats amb alguns grups taxonòmics (Arts & Wainman, 1999), permetent la identificació de la MO en termes de grans grups (per exemple, diatomees, cloròfits, cianobacteris i bacteris). La major part de la recerca en AG en els sistemes d'aigua dolça ha estudiat els llacs o ha estat realitzada al laboratori (per exemple, Cargill *et al.*, 1985; Ahlgren *et al.*, 1997; Kainz *et al.*, 2010; Masclaux *et al.*, 2012). A més a més, relativament pocs estudis han analitzat la composició d'AG de la MO en rius i com aquesta composició canvia degut a les condicions ambientals (però veure, per exemple, Torres-Ruiz & Wehr, 2010; Cashman *et al.*, 2013).

EL CANVI GLOBAL EN ELS RIUS

Els rius estan molt afectats per múltiples impactes antròpics directes i indirectes a escala global, el que s'anomena el canvi global. Els impactes principals estan causats pel canvi climàtic, els canvis en els usos del sòl i les alteracions dels cursos d'aigua, que resulten en la destrucció dels ecosistemes i de l'hàbitat físic, en alteracions de la química de l'aigua, i en addicions i eliminacions d'espècies (Malmqvist & Rundle, 2002; Stevenson & Sabater, 2010).

Degut a la seva naturalesa linear i unidireccional, els rius presenten una vulnerabilitat exacerbada a aquests impactes; gairebé qualsevol activitat en la conca del riu té la capacitat de causar efectes a llarga distància riu avall. Actualment, pocs rius en el món són pristins, i la majoria tenen una llarga història d'alteració, ja que els rius han actuat com a imants per a l'assentament humà degut als serveis i els béns que proporcionen (Malmqvist & Rundle, 2002; Allan & Castillo, 2007). Es preveu que la població mundial incrementi des de 7.2 bilions (meitat de 2013) fins a 9.6 bilions el 2050 (United Nations, 2013). Així doncs, es probable que es produeixi un increment dramàtic en la pressió sobre els rius ja que totes les forces que impulsen el canvi antròpic augmentaran de magnitud: la mida de la població, el creixement de les ciutats i la indústria, l'expansió i intensificació de l'agricultura i el desviament d'aigua o la construcció de preses (Malmqvist & Rundle, 2002).

Els rius de capçalera són molt sensibles als impactes antròpics (i a les pertorbacions naturals) degut a la seva poca amplada (MacDonald & Coe, 2007). Probablement es veuran afectats en primer lloc pel canvi climàtic, ja que tenen menys capacitat d'amortiment tèrmic i hidrològic i estan molt afectats pels patrons de precipitació local (Heino *et al.*, 2009). A més a més, els impactes ecològics en aquests sistemes no estan limitats a una escala local, ja que també afecten els trams riu avall (Freeman *et al.*, 2007).

El clima i els usos del sòl, juntament amb la geologia, són els determinants últims de la hidrologia i la qualitat de l'aigua, actuant així com els principals condicionants del canvi en l'estructura i el funcionament dels rius (Stevenson & Sabater, 2010).

Canvi climàtic

Hi ha consens entre els científics a l'hora d'afirmar que el canvi climàtic induït pels humans està provocant l'escalfament del planeta (IPCC, 2007a), i que s'espera que l'escalfament global afecti negativament la quantitat i la qualitat dels recursos d'aigua dolça. Això també afectarà el cicle hidrològic; els models climàtics pronostiquen canvis en els patrons regionals de precipitació i una més alta variabilitat en la precipitació global que portarà a un increment en la freqüència, la magnitud i la imprevisibilitat de les sequeres i les avingudes en moltes regions (Lehner *et al.*, 2006). L'escalfament causarà una reducció en la mida i la fusió prematura de les acumulacions de neu, causant la reducció del cabal dels rius i canvis en la hidrologia estacional dels rius receptors (Barnett *et al.*, 2008). El canvi climàtic disminuirà la disponibilitat d'aigua dolça en moltes àrees ja que les pèrdues degudes a l'evaporació i l'ús humà augmentaran, i l'augment de les temperatures de l'aigua es preveu que tingui tant efectes additius com sinèrgics amb altres factors d'estrès, tals com la contaminació per nutrients i la propagació d'espècies exòtiques (Dodds & Whiles, 2010).

Canvis en els usos del sòl

Els canvis en els usos del sòl són transicions des de paisatges no pertorbats a paisatges dominats pels humans, per exemple, la silvicultura i l'agricultura i els usos urbans, industrials o miners. Com que els rius estan influenciats pel paisatge a través del qual flueixen (Hynes, 1975; Vannote *et al.*, 1980), els usos del sòl d'aquests paisatges tindran efectes en els rius. Els tipus d'usos del sòl han estat relacionats amb la quantitat i la qualitat de l'aigua dels rius (Gburek & Folmar, 1999). El principal mecanisme pel qual el usos del sòl influeixen l'ecosistema del riu són la tala o aclariment del bosc de ribera, la pèrdua de restes llenyoses grans, l'enriquiment de nutrients, la sedimentació, la contaminació per metalls pesants, sintètics i tòxics orgànics i alteracions hidrològiques (Allan, 2004).

La gestió del bosc de ribera redueix les entrades de fullaraca i fusta i la capacitat d'amortiment del bosc de ribera per controlar les entrades de sediments i nutrients, i augmenta la temperatura i la disponibilitat de llum al riu (Kiffney *et al.*, 2003; Croke & Hairsine, 2006; Mayer *et al.*, 2007). L'activitat humana ha augmentat de forma dramàtica la presència de substàncies al medi ambient, especialment durant aquest últim segle. Els tòxics es troben barrejats juntament amb altres factors d'estrès ambiental (per exemple, la sequera i els nutrients) i poden causar interaccions sinèrgiques (Holmstrup *et al.*, 2010). A més a més, nous contaminants no regulats (per exemple productes farmacèutics i de cura personal) han emergit com un problema mediambiental. El cabal anual i durant les tempestes augmenta amb l'ús agrícola dels sòls, mentre que els cabals basals sovint disminueixen degut a una reducció en la infiltració i a l'exportació d'aigua de forma més episòdica (Poff *et al.*, 1997). L'augment de l'escorrentia de les superfícies impermeables i els sistemes de conducció d'aigua poden causar una major freqüència i intensitat de les avingudes, l'erosió de la llera i el desplaçament de sediments (Allan, 2004).

Alteracions dels cursos fluvials

Les alteracions dels cursos fluvials són deguts a la construcció de preses, la canalització o l'extracció d'aigua. Els humans hem incrementat la quantitat d'aigua disponible per a l'ús amb la construcció de preses i embassaments (malgrat que més del 40% de la població mundial viu en condicions d'estrès hídric). El nombre de grans preses s'ha multiplicat per set des del 1950 i actualment el 14% de l'escorrentia mundial està retingut (Revenga *et al.*, 2000). Les preses afecten el règim de temperatura i la qualitat dels cursos d'aigua. La regulació causa la pèrdua de la periodicitat natural del cabal i augmenta el risc de sequera (Malmqvist & Rundle, 2002). La canalització uniformitza l'estructura del riu, disminuint la diversitat d'hàbitats, reduint els nutrients, el sediment i la retenció de MO (Elosegi & Sabater, 2013).

EFECTES DE LA HIDROLOGIA, ELS NUTRIENTS I LA LLUM EN ELS RECURSOS BASALS

La hidrologia i la disponibilitat de llum i nutrients es veuran afectats pel canvi global. Les fluctuacions naturals dels règim del cabal, incloent les sequeres i les avingudes estacionals, són importants per mantenir la biodiversitat en els sistemes lòtics (Ward, 1998). Com que molts organismes del riu estan adaptats als canvis previsibles de cabal associats amb els esdeveniments naturals estacionals, les desviacions d'aquest patró poden actuar com a pertorbacions, afectant la integritat ecològica del riu (Poff *et al.*, 1997; Dudgeon, 2000). La hidrologia influencia la disponibilitat dels recursos basals (Biggs & Thomsen, 1995; Acuña *et al.*, 2007; Timoner *et al.*, 2012), però la relació entre la hidrologia i la qualitat dels recursos basals és menys coneguda (però veure, per exemple, Ylla *et al.*, 2010).

Els rius de capçalera, en general, són oligotròfics i ombrívols, així doncs els nutrients i la llum són escassos en aquest sistemes (Webster *et al.*, 1995), malgrat que ambdós són fonamentals per a la producció primària autòctona (Borchardt, 1996; Hill, 1996). Augments en les concentracions de nutrients promouen l'activitat microbiana i acceleren el processament de la MO al·lòctona (Greenwood *et al.*, 2007;

Benstead *et al.*, 2009). Malgrat això, la producció secundària, la biomassa i l'abundància dels invertebrats també augmenten, demostrant que els canvis en la qualitat de l'aliment (és a dir, condicionament major de la MO) anul·len els canvis en la quantitat d'aliment (és a dir, una descomposició més ràpida de la MO, Cross *et al.*, 2006). Els nutrients també poden augmentar la biomassa autòctona (Rosemond *et al.*, 2000; Dodds *et al.*, 2002), el contingut de clorofil·la i la producció primària, tot i que aquesta resposta pot estar controlada per la llum (Hill *et al.*, 2001; Greenwood & Rosemond, 2005; Ylla *et al.*, 2007).

La disponibilitat de llum al riu està modulada per la vegetació ripària (Gregory *et al.*, 1991). L'eliminació del bosc de ribera en les conques petites és una pràctica comuna per obtenir una terra més productiva, i augmenta la disponibilitat de llum al llit del riu. La llum generalment afavoreix els productors primaris, els quals al seu torn poden tenir un efecte positiu en l'abundància d'invertebrats herbívors (Sabater *et al.*, 1998). Una més alta exposició de la fullaraca a la llum pot afectar el biofilm que creix sobre les fulles, augmentant la biomassa algal i disminuint la biomassa fúngica, però sense afectar la densitat bacteriana (Albariño *et al.*, 2008). El contingut de polisacàrids, proteïnes i lípids del biofilm està molt correlacionat amb la biomassa del biofilm (per exemple la clorofil·la) i, a més a més, amb la disponibilitat de nutrients i llum (Ledger & Hildrew, 1998). No obstant això, se sap menys sobre els canvis en la composició bioquímica i com aquests canvis poden determinar indirectament la dieta dels consumidors.

ELS RIUS DE CAPÇALERA MEDITERRANIS: CASOS D'ESTUDI INTERESSANTS

Els rius mediterranis estan situats en àrees amb una configuració climàtica especial, caracteritzada per estius secs i càlids, hiverns frescos o moderadament freds i una alta variabilitat interanual en la precipitació (Folch, 1993). Quan no hi ha pertorbacions, aquests rius estan modelats física, química i biològicament per esdeveniments seqüencials, previsibles i estacionals de sequera i avingudes (Gasith & Resh, 1999). El coneixement dels efectes d'aquests esdeveniments en el funcionament dels rius podrien ser aplicades a altres rius en risc d'esdevenir temporals, ja que el nombre de rius temporals i la severitat de la intermitència del cabal podrien augmentar (Larned *et al.*, 2010). A més a més, les àrees mediterrànies són particularment adequades per l'assentament humà i la producció agrícola intensiva, amb una llarga història, principalment a la conca mediterrània, resultant en una alta demanda d'aigua dolça i impactes humans elevats (Cooper *et al.*, 2013). Es preveu que les regions mediterrànies estaran molt afectades pel canvi global (Sabater & Tockner, 2010). Aquests rius són únics des del punta de vista ecològic i potser estan entre els ecosistemes més vulnerables als danys ambientals causats per l'activitat humana (Bonada & Resh, 2013). Les característiques especials pròpies dels rius de capçalera i els sistemes mediterranis els fan molt interessants com a casos d'estudi per avaluar els efectes dels canvis abiòtics causats pel canvi global.

OBJECTIUS

Els rius constitueixen un recurs fonamental d'aigua dolça; així doncs, la pressió humana sobre aquests sistemes és alta i diversa. Els sistemes d'aigua dolça són molt sensibles al canvi global i el fet d'identificar, diagnosticar i abordar els efectes d'aquest canvi sobre aquests ecosistemes representa un gran repte. L'objectiu d'aquesta tesi era determinar com els factors ambientals influeixen la quantitat i la qualitat (composició bioquímica) dels recursos basals en els rius de capçalera. Entre els múltiples factors ambientals afectats pel canvi global, el present estudi es va focalitzar en els canvis en la hidrologia, i la disponibilitat de nutrients i llum, amb especial referència als rius de capçalera mediterranis.

Aquesta tesi es divideix en 4 capítols (Taula 2), abordant les següents qüestions específiques basades en vàries hipòtesis de treball:

1. Quins factors globals i locals (dins i fora del riu) controlen la dinàmica dels recursos al·lòctons en un riu mediterrani? Quins efectes tenen aquests factors en la quantitat i la qualitat d'aquests recursos? (Capítol 1)

Hipòtesi: Els esdeveniments hidrològics extrems (és a dir, sequeres i avingudes), meteorològics i climàtics (El Niño/Oscil·lació del Sud) determinaran la dinàmica dels recursos al·lòctons. El clima influirà la meteorologia local, la qual al seu torn modelarà les aportacions al·lòctones a través dels efectes en la vegetació ripària. Tant les sequeres com les avingudes augmentaran la quantitat de les aportacions, degut a l'estrès hídric com a resultat de la sequera i al transport per les avingudes. Durant els períodes de sequera, la qualitat de les fulles serà més alta a causa de la mort ràpida de les fulles. A la tardor, la translocació de nutrients cap a l'arbre abans de l'abscissió de les fulles reduirà el contingut de nutrients de la fullaraca.

2. Com la sequera influencia la qualitat dels recursos basals en un riu mediterrani? (Capítol 2)

Hipòtesi: La sequera, mitjançant la reducció en el cabal, la dessecació i l'acumulació de matèria orgànica no processada en el llit sec del riu, causarà una disminució en la qualitat dels recursos basals, mesurada com el contingut d'àcids grassos i esterols.

3. Quin efecte té un enriquiment de nutrients a mig termini en la qualitat dels recursos autòctons i la matèria orgànica transportada? (Capítol 3)

Hipòtesi: L'enriquiment de nutrients causarà un augment de la qualitat dels recursos autòctons i la matèria orgànica transportada, avaluada sobre la base de la composició bioquímica (polisacàrids, proteïnes i lípids), mitjançant un increment en els productors primaris. Els components essencials disponibles pels consumidors també augmentaran, ja que molts d'ells provenen dels productors primaris.

4. Quins efectes té la disponibilitat de nutrients i llum en la qualitat de la fullaraca? Com aquests canvis afecten el consum dels recursos per part dels trituradors? (Capítol 4)

Hipòtesi: Una major disponibilitat de nutrients i llum augmentarà la qualitat de la fullaraca (contingut més alt de polisacàrids, proteïnes i lípids) a través de la colonització algal de la superfície de les fulles, afavorint el consum per part dels trituradors.

RESUMS DELS CAPÍTOLS

CAPÍTOL 1: DINÀMICA DE LA MATÈRIA ORGÀNICA AL·LÒCTONA EN UNA RIERA MEDITERRÀNIA: CONDICIONANTS HIDROLÒGICS, METEOROLÒGICS I CLIMÀTICS

Les rieres boscades de capçalera estan altament influenciades per les entrades de matèria orgànica (MO) al·lòctona. L'objectiu d'aquest estudi era el de mesurar la quantitat i la qualitat de la MO de diferents procedències, per determinar quins factors ambientals locals i globals controlen les dinàmiques de la MO a escala de tram. L'estudi es va realitzar en una riera mediterrània vorejada per un bosc de ribera caducifoli al llarg de 10 anys. Les aportacions del bosc de ribera, la MO bentònica i transportada pel riu, i el seu contingut en carboni (C) i nitrogen (N) van ser determinats. La quantitat aportada pel bosc de ribera (912 g pes sec m^{-2} a^{-1}) era comparable a la de regions temperades amb boscos de ribera caducifolis, però el clima mediterrani determinava una dinàmica diferent d'aquestes aportacions. La distribució de les aportacions al llarg de l'any depenia de la presència d'un episodi de sequera aquell mateix any, amb un cicle anual bimodal els anys en que el riu s'asseca a l'estiu. Per altra banda, la quantitat aportada anualment depenia dels episodis de sequera previs, amb les sequeres successives causant una disminució progressiva de les aportacions ripàries. El fenomen de El Niño/Oscil·lació del Sud explicava una gran part de la variabilitat interanual de les aportacions ripàries a través de canvis causats en la precipitació. La MO retinguda i transportada estava bàsicament determinada per la freqüència de les avingudes. La qualitat de la MO al·lòctona (proporció C : N) augmentava des de les aportacions ripàries fins a les partícules transportades més fines. Els canvis en la quantitat i la qualitat de la MO estan explicats tant per factors locals, presents i passats, com per factors globals, i són potencialment importants per a les xarxes tròfiques detrítiques del riu.

Paraules clau: *bosc caducifoli · sequera · avingudes · El Niño/Oscil·lació del Sud · matèria orgànica particulada · recerca ecològica a llarg terme*

CAPÍTOL 2: EFECTES DE LA SEQUERA EN LA QUALITAT DELS RECURSOS EN UNA RIERA MEDITERRÀNIA: ELS ÀCIDS GRASSOS I ELS ESTEROLS COM A INDICADORS

Les sequeres estacionals en els rius mediterranis modelen les seves característiques físiques, químiques i biològiques. Així doncs, les sequeres poden alterar els recursos basals, que en els rius de capçalera són principalment la matèria orgànica (MO) al·lòctona i secundàriament la MO autòctona. Es va realitzar un estudi en una riera mediterrània a l'estiu, abans i després d'un període de sequera. Els canvis en la qualitat de la MO dels substrats bentònics (fulles i biofilms epipsàmic i epilític) i de l'aigua (fraccions particulades i dissoltes) van ser analitzats. Es van mesurar els àcids grassos (AG) i els esterols, ja que inclouen molècules essencials pels invertebrats i també perquè es poden utilitzar com a marcadors biològics de l'origen de la MO. El procés d'assecament-rehidratació va determinar la reducció de la qualitat nutritiva dels substrats bentònics i de la MO particulada transportada, amb una reducció general en els AG totals i essencials. Es va observar un canvi des de la predominança de la MO d'origen autòcton abans de la sequera, a la de la MO al·lòctona després de la sequera. A més a més, la composició d'esterols no va mostrat canvis entre la fase d'assecament i rehidratació i la sobtada tornada del cabal després de la sequera no va causar la lixiviació d'AG en la MO dissolta. El biofilm epilític i les fulles eren les fonts més importants de components essencials (AG essencials i esterols), mentre que el biofilm epipsàmic era la font menys important. Així doncs, l'heterogeneïtat en els substrats bentònics és important per assegurar el creixement i la reproducció dels invertebrats. Les nostres conclusions ajuden a millorar la comprensió dels mecanismes subjacents als efectes de la sequera en els rius i la predicció de futurs canvis causats per l'augment en la freqüència i la intensitat d'aquests períodes com a resultat del canvi global.

Paraules clau: *lípids · matèria orgànica particulada i dissolta · biofilm · fullaraca · recursos basals*

CAPÍTOL 3: QUALITAT BIOQUÍMICA DELS RECURSOS BASALS EN UNA RIERA BOSCADA: EFECTES DE L'ADDICIÓ DE NUTRIENTS

Els canvis qualitatius en el biofilm epilític i en la matèria orgànica (MO) transportada van ser estudiats en una riera boscada de capçalera, que havia estat rebent una aportació moderada de nutrients (nitrogen [N] i fòsfor [P]) de manera experimental durant 4 anys. Les concentracions basals de N i P al tram fertilitzat, van incrementar el doble (fins uns 750 µg N L^{-1}) i el triple (fins uns 30 µg P L^{-1}) respecte de les concentracions de partida del tram control. L'estudi es va realitzar durant el període de creixement de les fulles. El contingut de clorofil·la i la composició elemental (carboni [C] i N) del biofilm epilític van ser analitzats. La densitat bacteriana, les activitats enzimàtiques extracel·lulars i la composició bioquímica (incloent aminoàcids, àcids grassos [AG] i esterols) del biofilm i de la MO transportada (fraccions particulades i dissoltes) també es van determinar. L'addició de nutrients va provocar l'augment del contingut de clorofil·la i la densitat bacteriana del biofilm, tot i que aquests canvis estaven limitats per la disponibilitat de llum. La relació C : N del biofilm va disminuir degut a l'increment en el contingut de proteïnes, provocat per l'addició de nutrients. En el tram fertilitzat, coincidint amb una més gran disponibilitat de llum, el contingut de polisacàrids, lípids, i AG essencials, va augmentar. Els AG i els aminoàcids de les diatomees eren els més abundants al tram fertilitzat, i l'activitat leucinaminopeptidasa (relacionada amb la descomposició de pèptids) era també més alta. Malgrat això, els canvis en la composició bioquímica de la MO transportada no estaven relacionats amb els canvis en els nutrients. L'addició de nutrients va causar increments en la biomassa autotròfica i heterotròfica, en el contingut de polisacàrids, proteïnes, lípids i àcids grassos essencials, principalment quan la disponibilitat de llum era alta, destacant la importància de la fenologia del bosc de ribera.

Paraules clau: *biofilms epilítics · aminoàcids · àcids grassos · polisacàrids · riu de capçalera · fenologia ripària*

CAPÍTOL 4

EFECTE DELS NUTRIENTS I LA LLUM EN LA FULLARACA COM A RECURS ALIMENTARI PELS TRITURADORS DEL RIU

En els rius boscats de capçalera, les xarxes tròfiques depenen bàsicament dels recursos al·lòctons provinents del bosc de ribera. La qualitat d'aquests recursos és important per a la producció secundària del riu i depèn de l'estructura i la composició de la vegetació de ribera, i del condicionament microbià en el riu. La concentració de nutrients i la disponibilitat de llum influeixen el condicionament de les fulles, causant canvis qualitatius. L'objectiu d'aquest estudi era determinar la contribució relativa dels nutrients i la llum a la colonització de les algues i la qualitat de les fulles, i de provar si aquest canvis influeixen la palatabilitat de les fulles pels trituradors. Es van condicionar fulles de vern en canals del riu, en condicions control i /o elevades de nutrients i d'il·luminació, en un disseny factorial 2 x 2. Es va mesurar el contingut elemental, la clorofil·la i la qualitat bioquímica de les fulles. Les fulles condicionades es van utilitzar en un experiment de preferències alimentàries. El temps de colonització va ser una de les variables més influents, amb un enriquiment de nitrogen i lípids en la primera fase i una disminució general de polisacàrids i proteïnes amb el temps. L'enriquiment de nutrients va accelerar la descomposició de fulles d'un 20 a un 26%. La colonització d'algues va ser més alta en condicions de nutrients i llum elevats, però només en el primer període de colonització. El contingut de polisacàrids i lípids no van respondre a cap dels tractaments, mentre que el contingut de proteïnes estava relacionat amb la llum. Els efectes mostrats en aquest experiment haurien de tenir-se en compte en els plans de gestió del bosc de ribera.

Paraules clau: *composició elemental · polisacàrids · proteïnes · lípids · preferències alimentàries · canals artificials*

DISCUSSIÓ GENERAL

Els canvis globals en el clima, l'ús del sòl i en els cursos fluvials afecten els factors ambientals relacionats amb els funcionament dels rius, incloent la meteorologia, la hidrologia i la disponibilitat de nutrients i llum. En la present tesi, s'han descrit els efectes d'aquests factors abiòtics en els recursos basals. Al llarg dels capítols anteriors, hem demostrat que El Niño/Oscil·lació del Sud (ENSO) va afectar les aportacions ripàries al riu a través de teleconnexions que alteren la precipitació mitjana. Els períodes de sequera van afectar la distribució anual de les aportacions ripàries i van reduir la qualitat dels recursos basals, mentre que els episodis de sequera acumulats van provocar la disminució de les aportacions ripàries. Les avingudes van provocar la disminució de la matèria orgànica bentònica (MOB), van augmentar el transport de matèria orgànica (MO) i en van modificar la seva qualitat. L'addició de nutrients va portar a una disminució del contingut en carboni (C), i una disponibilitat de llum més alta va comportar una disminució del contingut de proteïnes en els recursos al·lòctons. La qualitat del biofilm en general era més alta amb l'addició de nutrients quan la disponibilitat de llum era més alta. Aquests resultats, resumits a la Fig. 1 i Taula 1, es discutiran més avall.

La recerca d'aquesta tesi inclou diferents escales de temps: a curt termini (Capítols 2,4), a mig termini (Capítol 3) i a llarg termini (Capítol 1). Si bé som conscients que en absència del context temporal proporcionat per la recerca a llarg termini, es poden cometre seriosos errors de judici quan es tracta d'entendre i predir canvis en els ecosistemes (Magnuson, 1990), nosaltres hem realitzat estudis a curt termini per analitzar mecanismes específics en detall.

Pel que fa a l'escala espacial, aquest treball ha esta bàsicament realitzat a escala de tram. Els efectes del clima, la meteorologia i la hidrologia en la quantitat i la qualitat de la MO trobats en aquesta tesi poden ser extrapolats als rius mediterranis boscats de capçalera, amb boscos de ribera caducs (Capítols 1,2). Els efectes estudiats de l'addició de nutrients i la disponibilitat de llum poden ser generalitzats als rius boscats de capçalera, i de fet, els nostres resultats són comparables als d'altres rius de capçalera temperats (per exemple Gulis & Suberkropp, 2003; Greenwood & Rosemond, 2005; i altres referències en els capítols 3,4).

EFECTES DE L'ENSO EN ELS RECURSOS BASALS A TRAVÉS DE LA METEOROLOGIA

L'ENSO és un fenomen acoblat aire-mar que s'origina a l'Oceà Pacífic però que afecta el clima de manera global (D'Aleo *et al.*, 2007). És el mode més gran de la variabilitat interanual del sistema climàtic global i està acompanyat per un patró de teleconnexions que s'estén fora de la regió del Pacífic (Nyenzi & Lefale, 2006). Fenòmens de baixa freqüència com l'ENSO determinen respostes en la vegetació; especialment mitjançant canvis en els controls principals com ara la temperatura, la precipitació i la coberta de neu (IPCC, 2007b).

A la major part de la Península Ibèrica el clima durant l'hivern es troba sota la influència de l'Oscil·lació de l'Atlàntic Nord (NAO). Malgrat això, a la regió oriental, on el nostre estudi va ser realitzat, el clima no està vinculat a la NAO, sinó més aviat a l'ENSO (Rodó *et al.*, 1997). Les fases extremes de l'ENSO (El Niño i La Niña) es van relacionar amb un índex de sequera basat en la precipitació a la Península Ibèrica, malgrat que presentaven diferències espacials i es produïen a diferents escales temporals (Vicente-Serrano, 2005).

En aquesta tesi, hem demostrat la relació entre la intensitat de la caiguda de fulles del bosc de ribera (una variable a escala local) i l'ENSO (un patró climàtic a gran escala), en un riu mediterrani. La dinàmica de l'ENSO (mesurada mitjançant l'índex de l'Oscil·lació Sud [SOI]) explica gairebé el total (90%) de la variabilitat interanual observada en la quantitat total d'entrades verticals al riu. Les dades es van mesurar durant anys fenològics (de l'Abril de l'any X al Març de l'any X+1) i per tant, començant a partir de l'inici del període fenològic (veure Capítol 1). Aquesta teleconnexió s'explica principalment a través de la variabilitat en la precipitació local (82%). Durant els períodes de El Niño, la precipitació anual mitjana era més baixa que en altres períodes, causant més aportacions ripàries, degut a l'estrès hídric dels arbres de ribera (Tabacchi *et al.*, 2000; Acuña *et al.*, 2007). Per contra, durant els períodes de La Niña, la precipitació era més alta que en altres ocasions, el que va portar a una disminució de les aportacions ripàries. És important destacar, que durant alguns anys (per exemple 2002–2003) la precipitació mitjana anual no es va poder relacionar amb l'ENSO ni amb les aportacions ripàries; i això és probablement degut a fenòmens meteorològics local que no van afectar la caiguda de fulles dels arbres.

La freqüència dels esdeveniments de l'ENSO ha augmentat al llarg de les últimes dècades (Huntington, 2006). Seguint la mateixa tendència, la correlació entre l'ENSO i la precipitació ibèrica va augmentar cap al final del segle passat (Rodó *et al.*, 1997). Malgrat això, les projeccions actuals de l'ENSO estan marcades per moltes incerteses, incloent el potencial d'una resposta abrupta i/o d'histèresi, la direcció del canvi i el nivell d'escalfament quan s'activi (IPCC, 2007b). El canvi climàtic pot influir la freqüència i la severitat dels esdeveniments de l'ENSO, tot i que aquesta connexió encara no està clara (Nyenzi & Lefale, 2006). Donades aquestes incerteses, els investigadors no podem predir com l'ENSO afectarà la dinàmica del bosc de ribera.

EFECTES DE LA HIDROLOGIA EN ELS RECURSOS BASALS

El subministrament i la qualitat de l'aigua, i la integritat ecològica dels sistemes fluvial està determinada en part pel règim natural de cabals (és a dir, la quantitat i la temporalitat del cabal del riu). De fet, el cabal del riu està fortament correlacionat amb moltes característiques físico-químiques que són crítiques, com ara la temperatura de l'aigua, la geomorfologia del canal i la diversitat d'hàbitats. Tant és així, que hom s'hi ha referit com una "variable mestra" que limita la distribució i l'abundància de les espècies del riu (Poff *et al.*, 1997).

En les àrees mediterrànies, el règim de precipitacions és molt estacional i mostra una alta variabilitat interanual, la qual influencia la hidrologia dels sistemes fluvials. A conseqüència d'això, aquests rius estan caracteritzats per cicles sec-humit, sotmesos a cabals baixos de forma periòdica i fins i tot la interrupció del cabal, degut a la coincidència dels períodes càlids i secs, o a avingudes degudes a la precipitació torrencial (Gasith & Resh, 1999; Bonada & Resh, 2013). Les sequeres i les avingudes es troben als dos extrems oposats del continu hidrològic i determinen una miríada de propietats i funcions en els ecosistemes (per exemple Acuña *et al.*, 2004; Bonada *et al.*, 2006; Power *et al.*, 2008). En aquesta tesi, hem demostrat la importància cabdal del règim de cabals en la dinàmica dels recursos (és a dir, la quantitat i la qualitat).

La sequera té múltiples efectes en els recursos basals. La distribució de les aportacions ripàries verticals al llarg de l'any depèn de la presència d'un episodi de sequera aquell mateix any, mentre que la quantitat anual d'aportacions ripàries verticals depèn dels episodis de sequera anteriors (història; Capítol 1). En els anys amb sequera estival, les aportacions ripàries segueixen una distribució temporal bimodal: el primer pic i el més alt es produeix a l'estiu (36% de les aportacions verticals anuals) i està relacionat amb l'estrès hídric, i el segon pic es produeix a la tardor (31% de les aportacions verticals anuals) i està relacionat amb la fenologia dels arbres. Malgrat això, els anys sense sequera, les aportacions segueixen una distribució unimodal: hi ha un sol pic que es produeix principalment a la tardor (52% de les aportacions verticals anuals). Així doncs, la distribució anual de les aportacions verticals està determinada per l'estacionalitat i alterada per la presència o absència de períodes de sequera. Malgrat que aquestes dinàmiques ja han estat descrites en rius mediterranis (Bernal *et al.*, 2003; Acuña *et al.*, 2007), en aquesta tesi hem quantificat aquests efectes i hem descrit que aquesta tendència es manté durant un llarg període d'estudi.

A llarg termini, el nombre acumulat d'episodis de sequera representa gran part de la variabilitat interanual de les aportacions ripàries: com més elevat nombre d'aquests episodis, més baixes són les aportacions. De fet, dos anys consecutius de sequera van provocar una reducció del 45% de les aportacions ripàries (en relació a dos anys humits consecutius). Aquest efecte, que nosaltres coneguem, no ha estat descrit amb anterioritat, però pot ser explicat probablement per un empobriment en el contingut de nutrients del sòl ripari degut a les sequeres successives (McDonald & Davies, 1996; Bernal *et al.*, 2003). Malgrat això, donat que certs anys amb un període de sequera es comporten com anys humits vol dir que altres mecanismes, més complexes han de ser tinguts en compte. Una possible explicació es que els esdeveniments climàtics com ara l'ENSO poden afectar els patrons de precipitació.

L'explicació anterior indica que tant en els efectes a curt com a llarg termini de la sequera, el determinant més important de les aportacions ripàries és la presència o l'absència de períodes de sequera. Altrament, l'assecament del llit del riu, i la interrupció del cabal del riu, són més importants que la durada de la sequera. La sequera també determina l'acumulació de fullaraca en el sòl ripari: forts vents, precipitació o avingudes després d'una sequera provoquen un augment de les aportacions laterals. Malgrat això, tal i com s'ha descrit per altres rius (Treadwell *et al.*, 1997; Wallace *et al.*, 1997a), hem trobat que a Fuirosos les aportacions laterals són menys importants quantitativament que les aportacions verticals.

La sequera també va influir la qualitat dels recursos basals, provocant un augment del contingut de C de les aportacions ripàries i de la MOB al·lòctona (Capítol 1). A més a més, durant el període d'estudi, la sequera va causar una disminució en els nivells d'àcids grassos (AG) i àcids grassos essencials (AGE) en els substrats bentònics (biofilms epilítics i epipsàmics, i fullaraca), i una disminució en la MO particulada en suspensió, tot i que no es van observar diferències en el contingut d'esterols. Aquesta pèrdua de qualitat estava relacionada amb el canvi de MO predominantment autòctona a MO al·lòctona (Capítol 2). Un canvi estacional similar ha estat descrit en rius mediterranis (Artigas *et al.*, 2009; Romaní *et al.*, 2013). Els canvis en la composició de la MO provocats per la sequera coincideixen amb nivells de polisacàrids, proteïnes i lípids més alts en el període d'assecament que en el període de rehidratació trobats en el mateix riu d'aquest estudi (Ylla *et al.*, 2010). Tant la composició elemental com la composició bioquímica indiquen una disminució general en la qualitat dels recursos basals, Així doncs, la sequera va reduir tant la quantitat, a curt i llarg termini, com la qualitat dels recursos basals.

Les avingudes estaven més relacionades amb la MO retinguda i transportada. Més concretament, la freqüència de les avingudes estava correlacionada negativament als nivells de MOB i correlacionada positivament amb la quantitat de MO transportada. El compartiment de la MOB és el resultat del balanç entre les aportacions ripàries i la MO particulada transportada. En el model creat per Acuña & Tockner (2010), la MOB i les avingudes juntament, incrementen l'exportació riu avall. Un cabal i una freqüència d'avingudes més alts en el nostre estudi van causar l'augment de la qualitat de la MO particulada grollera (MOPG) i una disminució de la qualitat de la MO particulada fina (MOPF), probablement relacionat amb les diferències de processament. A més a més, en el model (Acuña & Tockner, 2010), les alteracions en el règim de cabals induïdes pel canvi climàtic a la regió mediterrània, tenen una influència molt forta en la dinàmica del C a la xarxa fluvial. El model prediu que les avingudes i les sequeres més llargues reduiran la quantitat de C processat en una xarxa fluvial determinada. En la xarxa, els rius de capçalera són on el règim de cabals és més important.

La hidrologia està afectada per diversos components del canvi global. La freqüència i la magnitud dels episodis extrems (principalment les sequeres) augmentarà de forma general. Així doncs, segons els nostres resultats, podem predir que un increment en la freqüència de les sequeres en un futur proper donarà lloc a una distribució temporal més freqüent i a una disminució en la quantitat i la qualitat dels recursos al·lòctons disponibles en els rius de capçalera mediterranis. L'augment en les avingudes reduirà encara més els recursos disponibles.

Fuirosos té una quantitat mitjana d'aportacions ripàries semblant als sistemes temperats caducifolis (e.g., Treadwell *et al.*, 1997; Wallace *et al.*, 1997a; Molinero & Pozo, 2004). Malgrat això, degut a la sequera característica dels sistemes mediterranis, té una dinàmica diferent, marcada per una variabilitat interanual més alta comparat amb els rius que tenen una quantitat similar interanual d'aportacions de fullaraca (Richardson, 1992).

L'alta variabilitat interanual en les aportacions ripàries a Fuirosos es pot explicar pels patrons globals de l'ENSO (a través de la precipitació) i per la història dels episodis locals de sequera. Malgrat els episodis de l'ENSO i la sequera estan estretament relacionats a la Península Ibèrica (Muñoz-Díaz & Rodrigo, 2005; Vicente-Serrano, 2005), a Fuirosos no hem trobat una relació directa entre aquests dos fenòmens.

EFECTES DE LA DISPONIBILITAT DELS NUTRIENTS I LA LLUM EN ELS RECURSOS BASALS

Tant els nutrients com la llum són essencial per a la producció primària autòctona en els rius (Borchardt, 1996; Hill, 1996). Tot i això, els rius de capçalera són generalment oligotròfics i ombrívols i per tant, reben nutrients i llum de forma escassa (Webster *et al.*, 1995).

En aquesta tesi, hem demostrat que a Fuirosos una disponibilitat de nutrients més alta va causar un contingut més baix de C i que una disponibilitat de llum més alta va portar a un contingut de proteïnes més alt dels recursos al·lòctons; i que el procés de descomposició de fulles va dictar els canvis en la qualitat (veure Capítol 4). Amb una disponibilitat de nutrients més alta, la qualitat del biofilm (recurs autòcton) era més alta, especialment quan la disponibilitat de llum era més alta (Capítol 3).

A més a més, vam trobar que la qualitat dels recursos autòcton està determinada principalment pel temps de condicionament, la qual cosa va anul·lar la influència de la disponibilitat de nutrients i llum en el contingut de polisacàrids i lípids, finalment portant a la disminució de la qualitat. Prèviament s'ha descrit que el temps de colonització substitueix altres factors (per exemple l'espècie de les fulles, Hutchens *et al.*, 1997; o l'addició de nutrients, Royer & Minshall, 2001). En el nostre estudi, la major disponibilitat de nutrients va accelerar la pèrdua de massa de les fulles (d'un 20 a un 26%), tal i com s'havia trobat prèviament en estudis de tot el riu (Gulis & Suberkropp, 2003; Greenwood *et al.*, 2007), i va portar a una pèrdua en el contingut de C a través de la lixiviació i la respiració (Chauvet, 1987; Gessner *et al.*, 1999). La llum va afectar el contingut de proteïnes de les fulles, mentre que la colonització algal va ser esglaonada en el temps, produint-se abans quan hi havia una disponibilitat més alta de nutrients i llum. La colonització algal explicaria l'enriquiment en nitrogen (N) i proteïnes en les fulles d'aquests tractaments (Ledger & Hildrew, 1998).

La qualitat dels biofilms epilítics (és a dir, els nivells de polisacàrids, proteïnes, lípids i AGE) era més alta quan hi havia una disponibilitat de nutrients més alta, però aquests efectes només eren clars quan la disponibilitat de llum era més alta. Romaní *et al.* (2004a) també van observar que un augment del nivell de nutrients estava correlacionat amb un increment del nivell de polisacàrids. L'augment de les proteïnes que nosaltres vam observar a Fuirosos explicaria la proporció C : N més baixa, causada principalment per canvis en els nivells d'aminoàcids no essencials; els nivells dels essencials no van canviar. Amb nivells de llum més baixos, els nivells d'aminoàcids làbils eren més alts, degut a la disminució de la quantitat de diatomees. El contingut d'AG i AGE era més alt al tram fertilitzat, però va disminuir al llarg del temps, més probablement degut a l'abundància d'algues (Hill *et al.*, 2011).

En el tram control del riu, la MO era predominantment terrestre, mentre que al tram fertilitzat, era aquàtica principalment. La concentració i la composició d'esterols no es va veure afectada pels canvis en els nivells de nutrients, degut a l'origen principalment autòcton (plantes superiors) dels esterols. La dependència de les aportacions al·lòctones també es va veure reflectida en la manca de canvis en la composició bioquímica de la MO particulada (MOP) i dissolta (MOD).

Després d'una fertilització a mig termini, l'únic canvi permanent que es va observar en la qualitat de la MO va ser la proporció de C : N més baixa en els biofilms, la qual estava relacionada amb un contingut de proteïnes més alt.

Els rius de capçalera són sistemes molt dinàmics, i els biofilms responen ràpid als canvis d'altres factors que interaccionen amb els nutrients, com ara la llum. Donat l'augment predit dels canvis en els usos del sòl, la disponibilitat de llum i nutrients en els rius de capçalera també augmentarà (Allan, 2004). Basant-nos en els nostres resultats, preveiem que la qualitat general dels recursos al·lòctons i autòctons en els rius de capçalera augmentarà i que la taxa de processament dels recursos al·lòctons s'elevarà amb un augment moderat en la quantitat de nutrients i llum disponible.

RESPOSTES DELS DIFERENTS INDICADORS DE QUALITAT DE LA MO ALS CANVIS EN ELS RECURSOS BASALS

Al llarg del nostre estudi, hem utilitzat la composició elemental (C,) i bioquímica (polisacàrids, proteïnes i lípids) dels recursos basals com a indicadors de la qualitat nutritiva de la MO per a les espècies de consumidors. La proporció C : N era més alta en els recursos al·lòctons que en els autòctons, i decreixia gradualment des de les aportacions ripàries (59.14), a la MOB (50.01) als materials transportats (MOPG [38.64], MOPF [22.01] and MOP ultrafina; MOPUF [14.66]) i finalment, els biofilms epilítics (9.67; veure Capítols 1,3). Un gradient similar va ser observat per Cross *et al.* (2003). En termes de contingut d'AG, el substrat més ric era el biofilm epilític, seguit de les fulles i finalment, el biofilm epipsàmic (Capítol 2). Així doncs, ambdues tendències corroboren l'idea que els recursos al·lòctons són en general més recalcitrants que els autòctons (Guenet *et al.*, 2010).

Quan vam analitzar els efectes de la sequera en els indicadors de qualitat de la MO, vam observar una disminució general de la qualitat dels recursos al·lòctons i autòctons a través de tots els indicadors, amb la interrupció del cabal (Capítols 1,2). Malgrat això, quan vam analitzar els efectes de la disponibilitat de nutrients i llum en la qualitat de la MO, les respostes van variar àmpliament amb els indicadors. En el capítol 3, una disponibilitat més alta de nutrients va causar una proporció C : N del biofilm més baixa de forma permanent i el contingut de polisacàrids, proteïnes i lípids era més alt especialment quan la disponibilitat de llum era més alta. En el Capítol 4, el C : N va augmentar i el contingut de polisacàrids i lípids va disminuir a les fulles amb el procés de descomposició. El contingut de proteïnes va respondre negativament a la llum però no es van trobar respostes significatives relacionades amb els nutrients.

Hill *et al.* (2011) van trobar que la estequiometria elemental i el contingut d'AG del biofilm responien de forma similar als canvis en els nivells de fòsfor o llum. A més a més, Cashman *et al.* (2013) van trobar que les mesures d'AG eren més sensibles als efectes dels nutrients i la llum en el biofilm que el contingut de nutrients i l'estequiometria.

En el nostre estudi, no vam observar canvis significatius en els nivells d'esterols en resposta als factors abiòtics que es van testar. Malgrat ser essencials per les espècies consumidors, els nivells d'esterols no semblen ser tan bons indicadors de la qualitat de la MO com els AG.

Tenint en compte els nostres resultats i la bibliografia precedent, considerem que la qualitat nutritiva dels recursos basals és un paràmetre complex. Mentre que la composició elemental i bioquímica poden estar relacionades en alguns casos (Rivas-Ubach *et al.*, 2012), el coneixement d'aquesta última proporciona un millor coneixement dels mecanismes pels quals els canvis abiòtics afecten la qualitat dels recursos. Malgrat això, la complexitat de la mesura de la composició elemental, bioquímica i d'AG augmenta gradualment . Així doncs, suggerim que la tria dels indicadors de qualitat utilitzats depengui de l'objectiu de l'estudi i del balanç entre l'esforç d'anàlisi i la informació que es vulgui obtenir.

EFECTES POTENCIALS DELS CANVIS DELS RECURSOS BASALS EN L'ESTRUCTURA I EL FUNCIONAMENT DEL RIU

Les xarxes tròfiques estan sustentades per una mescla complexa de recursos al·lòctons i autòctons (Allan & Castillo, 2007). La quantitat i la qualitat de la fullaraca i dels recursos autòctons són factors que limiten la producció secundària del riu (Richardson, 1991; Dobson & Hildrew, 1992; Stelzer & Lamberti, 2002; Cross *et al.*, 2006). A més a més, els invertebrats han d'obtenir a través de la seva dieta certs components essencials (AG, esterols i aminoàcids) els quals no poden sintetitzar (Olsen, 1999; Martin-Creuzburg & Elert, 2009).

Una disminució en la quantitat de les aportacions ripàries causada per anys amb sequera consecutius causarà probablement una davallada en la població de certs tàxons d'invertebrats, generarà unes interaccions més fortes a la xarxa tròfica (Hall *et al.*, 2000) i portarà a un debilitament dels vincles terrestres-aquàtics (England & Rosemond, 2004). Les diferències temporals en la disponibilitat dels recursos (per exemple degut a la caiguda prematura de les fulles durant els períodes de sequera) són importants en aquest context, degut al vincle estret que hi ha entre els trituradors i la temporalitat de les entrades de fullaraca (Cummins *et al.*, 1989).

S'han observat canvis en les poblacions d'invertebrats de les comunitats degut a episodis de sequera tant a Fuirosos (Acuña *et al.*, 2005) com a d'altres rius (Boulton, 2003). La reducció del cabal causa un augment de la densitat d'invertebrats, la qual cosa condueix a una major competència pels recursos. Durant el període de sequera, els invertebrats han d'acumular energia i lípids per a l'emergència (Anderson & Cummins, 1979; Anderson & Sedell, 1979); malgrat això, la sequera causa que algunes espècies emergeixin més aviat (Leberfinger *et al.*, 2010). Així doncs, els períodes de sequera són crítics per a la supervivència dels invertebrats.

Malgrat això, els efectes de la qualitat dels recursos en el creixement i la reproducció dependran de la identitat de les espècies i poden ser compensats per una modificació del consum (Fink & Von Elert, 2006) o per una alteració d'alguns processos fisiològics (Graça *et al.*, 1993). En el nostre experiment, els trituradors van mostrar una preferència per les fulles riques en proteïnes dels tractaments fertilitzats però amb ombra. De la mateixa manera, Aβmann *et al.* (2011) va trobar que les proteïnes estaven correlacionades amb la taxa de consum dels trituradors.

En el nostre estudi de Fuirosos, l'addició de nutrients va causar una més alta clorofil·la algal i una més alta densitat de bacteris, canvis que són probablement deguts als exsudats algals més alts (Hepinstall & Fuller, 1994; Romaní *et al.*, 2004b). Els efectes observats de l'addició de nutrients en el bentos no es van veure reflectits en els bacteris de l'aigua, els quals depenien principalment de les aportacions al·lòctones (Butturini *et al.*, 2008).

L'addició de nutrients també va afectar la utilització de la MO pels enzims extracel·lulars. Al tram fertilitzat durant el període de disponibilitat de llum més alta, els enzims van utilitzar més pèptids. Aquest resultat pot estar lligat a la més alta disponibilitat de substrat per a aquests enzims. Curiosament, en altres estudis una activitat leucina-aminopeptidasa més alta ha estat vinculada a la llum incident i l'activitat fotosintètica, les quals poden estar relacionades amb l'ús d'exsudats algals per part dels bacteris (Espeland *et al.*, 2001; Francoeur & Wetzel, 2003; Ylla *et al.*, 2009). Quan la disponibilitat de llum va disminuir, hi va haver una disminució de l'activitat fosfatasa en els biofilms del tram fertilitzat. Aquest resultat podria haver estat degut a la disminució de biomassa algal (altament responsable d'aquest activitat enzimàtica), així com a una disponibilitat més alta de P inorgànic, la qual podria haver evitat la producció de fosfatasa al tram fertilitzat (Romaní *et al.*, 2004a; Allison & Vitousek, 2005). Al final de l'experiment, l'ús de polisacàrids i de pèptids mesurat pels enzims extracel·lulars (indicat per altes activitats β-glucosidasa and leucina-aminopeptidasa) va ser més alt en el tram control que en el tram fertilitzat, indicant uns requeriments més grans de C i N al primer. De la mateixa manera, l'ús dels polisacàrids acumulats mesurat va ser més gran en els biofilms del tram fertilitzat.

La protecció i la restauració de la integritat dels ecosistemes fluvials exigeix que les accions de conservació i gestió estiguin fermament basades en el coneixement científic (Poff *et al.*, 1997). Al llarg d'aquesta tesi hem demostrat la importància de: i) la presència d'un bosc de ribera ben desenvolupat, ja que proporciona recursos importants als rius i regula la disponibilitat de nutrients i llum, la qual cosa provoca canvis en la qualitat dels recursos; i de ii) l'heterogeneïtat dels recursos en el riu (és a dir, fulles i biofilm) ja que proporcionen diferents components essencials per als consumidors. Hem demostrat que la sequera causa una disminució en la qualitat dels recursos, així, els impactes antropogènics que augmenten la freqüència i la magnitud de la sequera (com per exemple l'extracció d'aigua), s'haurien d'evitar. Amb aquesta tesi el nostre objectiu ha estat el de contribuir a la comprensió basada en la investigació del funcionament dels ecosistemes fluvials.

CONCLUSIONS GENERALS

CAPÍTOL 1: DINÀMICA DE LA MATÈRIA ORGÀNICA AL·LÒCTONA EN UNA RIERA MEDITERRÀNIA: CONDICIONANTS HIDROLÒGICS, METEOROLÒGICS I CLIMÀTICS

1. Les aportacions ripàries van mostrar un valor mitjà de 912 g pes sec (PS) m^{-2} a^{-1}, el qual era similar al dels rius temperats amb boscos de ribera caducs. Malgrat això, es va trobar una variabilitat interanual alta (rang: de 695 a 1185 g PS m^{-2} a^{-1}), la qual està relacionada amb les característiques del clima mediterrani.
2. Els episodis extrems de l'ENSO expliquen en un alt grau les diferències interanuals en les aportacions ripàries verticals, mitjançant canvis en la precipitació. Els episodis de El Niño van causar una precipitació mitjana més baixa, la qual cosa va fer augmentar les aportacions ripàries, mentre que La Niña va portar a una precipitació mitjana més alta, la qual cosa va fer disminuir les aportacions ripàries.
3. Els períodes de sequera acumulats van causar la reducció de les aportacions ripàries verticals: dos anys consecutius de sequera van reduir les aportacions ripàries en un 45% comparat als de dos anys humits consecutius.
4. Els anys amb un període de sequera estival, les aportacions ripàries van mostrar una distribució temporal bimodal amb un pic més alt a l'estiu, causat per l'estrès hídric, i un pic més baix a la tardor, relacionat amb la fenologia. Els anys sense sequera, les aportacions van mostrar una distribució unimodal amb un pic a la tardor.
5. La MOB estava determinada pel temps entre avingudes, i la MO transportada va augmentar amb l'augment de la freqüència de les avingudes.
6. Les condicions de sequera van causar l'augment del contingut de C de les aportacions ripàries, i el fotoperíode va causar l'augment del contingut de N. El contingut de C de la MOB era també més alt amb sequeres més llargues. Les avingudes van augmentar la qualitat de la MOPG i van disminuir la qualitat de la MOPF.
7. Els pics de C orgànic dissolt (COD) després de l'estiu estaven relacionats positivament amb les aportacions ripàries que s'havien acumulat al llit del riu.

CAPÍTOL 2: EFECTES DE LA SEQUERA EN LA QUALITAT DELS RECURSOS EN UNA RIERA MEDITERRÀNIA: ELS ÀCIDS GRASSOS I ELS ESTEROLS COM A INDICADORS

8. El període de sequera va causar una disminució en els AG i AGE dels substrats bentònics (biofilms epilítics i epipsàmics, i fulles) i en la MOP. Malgrat això, no es van observar diferències en el contingut d'esterols.
9. Abans de la sequera, la concentració d'AG en els substrats bentònics que estava relacionada amb els productors primaris era més alta que després de la sequera. Es va observar un canvi

entre la predominança de la MO autòctona abans de la sequera a la MO al·lòctona després de la sequera.

10. En la MOP es va produir un pic d'AG (que comprenia principalment AG de diatomees) just abans de la sequera. En la MOD no es van observar canvis temporals en els AG.
11. Els biofilms epilítics van mostrar el contingut més alt d'AG (que comprenien principalment AG de les diatomees i bacterians), mentre que el biofilm epipsàmic tenia el contingut més baix d'AG. Els AGE es trobaven principalment als biofilms epilítics i a les fulles. Les fulles eren la font més important d'esterols.

CAPÍTOL 3: QUALITAT BIOQUÍMICA DELS RECURSOS BASALS EN UNA RIERA BOSCADA: EFECTES DE L'ADDICIÓ DE NUTRIENTS

12. L'addició de nutrients en un tram del riu va portar a nivells més alts de clorofil·la, polisacàrids, lípids i AGE, a una densitat bacteriana més alta i a una proporció C : N més baixa en els biofilms epilítics. Els efectes de l'addició de nutrients van ser modulats per la disponibilitat de llum.
13. La concentració i la composició d'esterols no es va veure afectada per l'addició de nutrients degut al seu origen principalment al·lòcton (plantes superiors).
14. L'addició de nutrients va afectar els enzims extracel·lulars de diferents maneres. L'activitat leucina-aminopeptidasa era més alta amb una disponibilitat de nutrients més alta, ja que estava vinculada a una major abundància dels substrats per a aquests enzims. L'activitat fosfatasa va disminuir quan la disponibilitat de llum va disminuir, la qual cosa pot ser deguda a la disminució de la biomassa algal i/o als canvis en la disponibilitat del P inorgànic.
15. A l'aigua corrent, l'addició de nutrients no va afectar la densitat bacteriana ni la composició bioquímica de la MOP o la MOD, amb una excepció: la composició d'aminoàcids de la MOD va canviar gradualment de material fresc a aminoàcids estructurals.

CAPÍTOL 4: EFECTE DELS NUTRIENTS I LA LLUM EN LA FULLARACA COM A RECURS ALIMENTARI PELS TRITURADORS DEL RIU

16. La qualitat de les fulles es va veure afectada principalment pel temps de colonització, el qual va anul·lar els efectes de la disponibilitat de nutrients o de llum en el contingut de polisacàrids i lípids, que van disminuir al llarg del temps.
17. Les concentracions més altes de nutrients van accelerar la descomposició de les fulles (d'un 20 a un 26%) i van augmentar la pèrdua de C.
18. El contingut de proteïnes de les fulles era més baix en condicions de disponibilitat de llum alta, en conseqüència les fulles contenien menys N i mostraven una proporció C : N més alta.
19. Els trituradors van mostrar preferència per les fulles riques en proteïnes dels tractaments fertilitzats i ombrívols.

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ABBREVIATIONS

Abbreviations	Meaning
AFDM	ash-free dry mass
ANOVA	analysis of variance
BOM	benthic organic matter
C	carbon
CPOM	coarse particulate organic matter
CR	consumption rate
DCA	detrended correspondence analysis
DCCA	Detrended canonical correspondence analysis
DM	dry mass
DOC	dissolved organic carbon
DOM	dissolved organic matter
EFA	essential fatty acids
ENSO	El Niño Southern Oscillation
FA	fatty acids
FAME	fatty acid methyl ester
FPOM	fine particulate organic matter
LI	indirect or lateral riparian inputs
MKRF	Malcolm Knapp Research Forest
MUFA	monounsaturated fatty acids
N	nitrogen
NAO	North Atlantic Oscillation
OM	organic matter
P	phosphorous
PAR	photosynthetically active radiation
PCA	Principal component analysis
PET	potential evapotranspiration
PET/Prec ratio	ratio between potential evapotranspiration and precipitation
POM	particulate organic matter
PUFA	polyunsaturated fatty acids
RDA	redundancy analysis
RI	total riparian inputs
RM-ANOVA	repeated measures
SAFA	saturated fatty acids
SD	standard deviation
SE	standard error
SOI	Southern Oscillation Index
SRP	soluble reactive phosphorous
UFPOM	ultrafine particulate organic matter
VI	direct or vertical riparian inputs
VPD	vapour pressure deficit