Limnetica, 36 (1): 29-43 (2017). DOI: 10.23818/limn.36.03
 ISSN: 0213-8409

# Drought effects on resource quality in a Mediterranean stream: fatty acids and sterols as indicators

Isis Sanpera-Calbet[1], Irene Ylla[2], Anna M. Romaní[2], Sergi Sabater[2,3] and Isabel Muñoz[1,*]

[1] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Catalonia, Spain.
[2] Institut d'Ecologia Aquàtica, Universitat de Girona (UdG), Campus Montilivi, 17071 Girona, Catalonia, Spain.
[3] Institut Català de Recerca de l'Aigua (ICRA), Emili Grahit 101, 17003 Girona, Catalonia, Spain.

[*] Corresponding author: imunoz@ub.edu

Received: 21/10/16 Accepted: 04/01/17

**ABSTRACT**

**Drought effects on resource quality in a Mediterranean stream: fatty acids and sterols as indicators**

Seasonal droughts in Mediterranean streams shape their physical, chemical, and biological characteristics. Thus, droughts have the potential to alter resources at the base of the food web, which in headwater streams are primarily allochthonous and secondarily autochthonous organic matter (OM). In the present study we asssessed the quality of basal resources in a Mediterranean stream during a drought episode before and after a non-flow period (NF). Fatty acids (FA) and sterols were analyzed in the benthic substrata (leaves and sand and cobbles biofilm) and transported OM (particulate and dissolved fractions). FA and sterols were selected as indicators of resource quality because they include essential molecules for consumers and may be used as biomarkers of OM sources. The drying-rewetting process determined a general reduction in the total and essential FA of benthic substrata and transported particulate OM, and a shift from predominantly autochthonous to allochthonous OM. Furthermore, the sterol composition did not change between the drying and rewetting phases and the rewetting did not cause the leaching of FA in dissolved OM. The epilithic biofilm and leaves were the most important sources of essential FA and sterols, while the sand biofilm was the poorest source of these lipids. Our conclusions enhance the understanding of the mechanisms underlying the effects of droughts on basal resource quality in streams.

**Key words:** Lipids, biofilm, leaf litter, particulate and dissolved organic matter, basal resources.

***RESUMEN***

***Efectos de la sequía en la calidad de los recursos en un río mediterráneo: los ácidos grasos y esteroles como indicadores***

*La sequía estival en los ríos mediterráneos modela sus características físicas, químicas y biológicas. De ese modo, la sequía podría, potencialmente, alterar la calidad de los recursos basales que en los ríos de cabecera son esencialmente materia orgánica (MO) de origen alóctono y secundariamente de origen autóctono. Este trabajo evalúa la calidad de los recursos basales en un río mediterráneo durante un episodio de sequía estival, antes y después de la interrupción del flujo de agua (NF). Se han analizado los ácidos grasos y esteroles en los sustratos bentónicos (biofilm en hojas, en arena y en piedras) y en la MO en suspensión que transporta el río. Los ácidos grasos y esteroles fueron seleccionados como indicadores de la calidad de los recursos porque incluyen moléculas esenciales para los consumidores y pueden servir como biomarcadores del origen de la MO. El paso de lecho seco a la recuperación del flujo determinó una reducción general en la concentración total y de ácidos grasos esenciales en los sustratos y en la MO transportada, así como un cambio de MO predominantemente de origen autóctono a alóctono. Por el contrario, la composición de esteroles no varió en esta fase y tampoco se observaron más ácidos grasos en la fracción disuelta. El biofilm epilítico y de las hojas fueron la fuente más importante de ácidos grasos esenciales y esteroles, mientras que el biofilm en la arena fue el más pobre en estos lípidos. Nuestras conclusiones aportan información sobre los mecanismos que suceden durante la interrupción del flujo de agua en la calidad de los recursos basales en ríos.*

***Palabras clave:*** *Lípidos, biofilm, hojarasca, materia orgánica disuelta y particulada, recursos basales.*

## INTRODUCTION

Drought in Mediterranean streams is a frequent and seasonal event due to climate and rainfall pattern in this area (Bonada and & Resh, 2013). Droughts are extreme hydrologic events that begin with a gradual decrease in flow until there is a complete loss of surface water connectivity and end abruptly in autumn or early winter with the first rains (Gasith & Resh, 1999, Lake, 2003). Therefore, dry periods can be divided into the drying, flow fragmentation, and rewetting phases. Global change, which encompasses the direct influence of anthropogenic activities on stream hydrology through watercourse alterations (damming, channelization, and water abstraction) and climate change, is expected to lead to an increase in the frequency and intensity of drought episodes in this region (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 the quantity and quality of resources at the basal level of the food web (Lake, 2003), which in forested headwater streams are primarily allochthonous and secondarily autochthonous OM (Vannote *et al*., 1980). Due to the reduced and subsequently nonexistent flow, large amounts of OM cumulate in pools and on the dry streambed (Boulton & Lake, 1992, Lake, 2003). 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 while new 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 accumulated in the stream after the spring peak, mainly as a result of the increase in algal biomass (Artigas *et al*., 2009), is then of reduced quality and quantity due to the desiccation and deterioration of the water quality during the flow interruption. 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 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, Sanpera-Calbet *et al*., 2016). 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) and total lipid energy can be used to predict features of animal population such as egg production by fish stocks (Parrish, 1999). Thus, the quality of the available nutrients may be more important than its quantity to the growth and reproduction of the animal populations (Ahlgren *et al*., 1997). Some lipid classes such as fatty acids (FA) and sterols include essential molecules for invertebrates and fish that are unable to synthesize them *de novo* (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 characteristic of some taxonomic groups (Arts & Wainman, 1999), and thus allow the composition of OM to be identified in terms of large groups (e.g. diatoms, chlorophytes, cyanobacteria, bacteria, fungi, and plants).

Our objective was to study how the drought process modifies the quality and availability of the essential biomolecules in the basal level of the food web in a Mediterranean stream. To this end, the benthic and transported OM was characterized during a drought episode, before and after the non-flow period (NF), using its lipid composition (FA and sterols) as indicators of essential nutrients availability and OM origin. We hypoth-

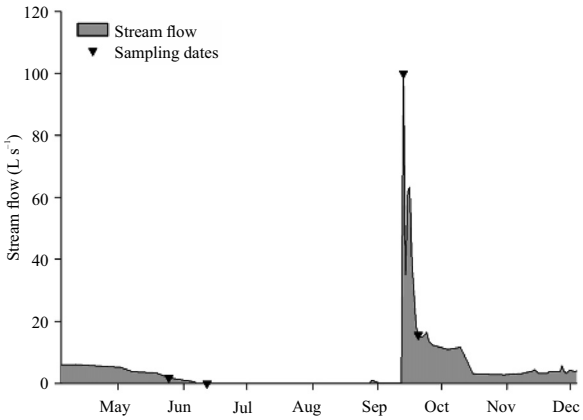


**Figure 1.** Stream flow in Fuirosos during the study period with the sampling dates (25 May, 12 June, 13 September, and 20 September 2006) indicated. The non-flow period, the period with no superficial runoff, lasting from June 19 to September 13, is shown. *Caudal en el río Fuirosos durante el período de estudio con las fechas de muestreo señaladas (25 de Mayo, 12 de Junio, 13 y 20 de Septiembre). Se muestra el período de interrupción del flujo de agua superficial que fue del 19 de Junio al 13 de Septiembre.*

esized that (i) before the NF, the biofilm (mainly algal biomass) in benthic substrata would be accumulated following the high productivity period (in spring) and the remaining benthic leaf litter and transported particulate OM (POM) would be well conditioned, i.e. high quality, rich in FA and sterols; and (ii) the NF would cause the progressive desiccation of the biofilms and the accumulation of non-conditioned OM, thus decreasing its quality. We expected that the sudden rewetting event would cause this accumulated OM in the dry streambeds to leach, which would result in an increase in the quality of the DOM. Thus, the drying-rewetting process would determine the availability of FA and sterols for the other trophic levels.

## METHODS

### Study site

This study was conducted in Fuirosos, a third-order intermittent stream. Fuirosos is situated 150 m above sea level in the north-eastern Iberian Peninsula (41°42′N, 02°34′E) in the Montnegre-Corredor Natural Park. This area has a Mediterranean climate, characterized by mild winters and warm, dry summers, and a high intra- and interannual variability of rainfall, which is concentrated in spring and autumn. Drought episodes occur in most years in this stream (Vázquez *et al*., 2013).

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 (drying), the streambed dried up on 19 June and remained dry until 13 September (NF), when the flow recovered (rewetting) (Fig. 1).

### Sampling

Benthic substrata (leaves, sand, and cobbles) and water (POM and DOM) were sampled in 4 occasions: before the NF, on 25 May and 12 June, and after the NF, on 13 and 20 September. On 25 May, the flow was 1.8 L/s, whereas the basal flow was 7.7 L/s in the previous period and on 12 June the flow was undetectable. Following the NF, the flow suddenly recovered, with a flow peak of nearly 100 L/s on 13 September, although this peak cannot be considered a flood (moderate floods >250 L/s; Sabater *et al*., 2008). On 20 September the flow had decreased to 15.7 L/s (Fig. 1).

Physico-chemical variables, i.e. water temperature, dissolved oxygen, and conductivity, were measured in the field (Table 1). The average water temperature during the study was 16.0 (± 0.5 SD) °C. Oxygen concentration greatly decreased with the reduction in flow and returned to the initial values during the rewetting period. Conductivity increased with the reduction in flow and the lowest values occurred during flow peaks 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 substrata 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 NF.

On each sampling occasion, benthic material, i.e. leaves and particulate material, epilithic biofilm, biofilm and fine material cumulated in sand, and water were collected. Cobbles were collected directly from the streambed, while leaves and sand were collected with a core of 4.3 $cm^2$ (depth of 5–10 cm). Once in the laboratory, each sample of benthic material 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 homogenized 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 −18 °C until analysis.

**Fatty acid and sterol analysis**

To determine the composition and concentration of FA and sterols, 3 replicates per date and substrate (4 for water sterols) were analyzed according to Parrish (1999) with some modifications. Samples were frozen with liquid $N_2$ and freeze-dried for 48 h. Then, samples were extracted with a dichloromethane-methanol (MeOH) 2:1 solution, and were sonicated for 20 min. Samples were centrifuged for 5 min, and the organic extract was concentrated up to 0.5 mL, (*) saponified with KOH (6% in MeOH) and left overnight. On the second day, water and hexane were added. From the hexanic phase, the sterol extract was obtained and concentrated under $N_2$. The aqueous phase was acidified with HCl and extracted with hexane. The hexanic phase was concentrated up to 0.5 mL and methylated using $BF_3$ (20% W:V in MeOH) overnight. The next day, water and hexane were added. From the hexanic phase, the methylated acids extract was obtained and concentrated under $N_2$. All extractions during the protocol were repeated 3 times for each replicate. For DOM, the beginning of the protocol was slightly different;

**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%). *Variables físico-químicas y composición de los sustratos presentes en el lecho con agua durante el período de estudio. La superficie de cada sustrato se expresa en porcentaje de la superficie con agua (dado que los sustratos orgánicos pueden estar superpuestos, la suma puede exceder el 100%).*

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

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

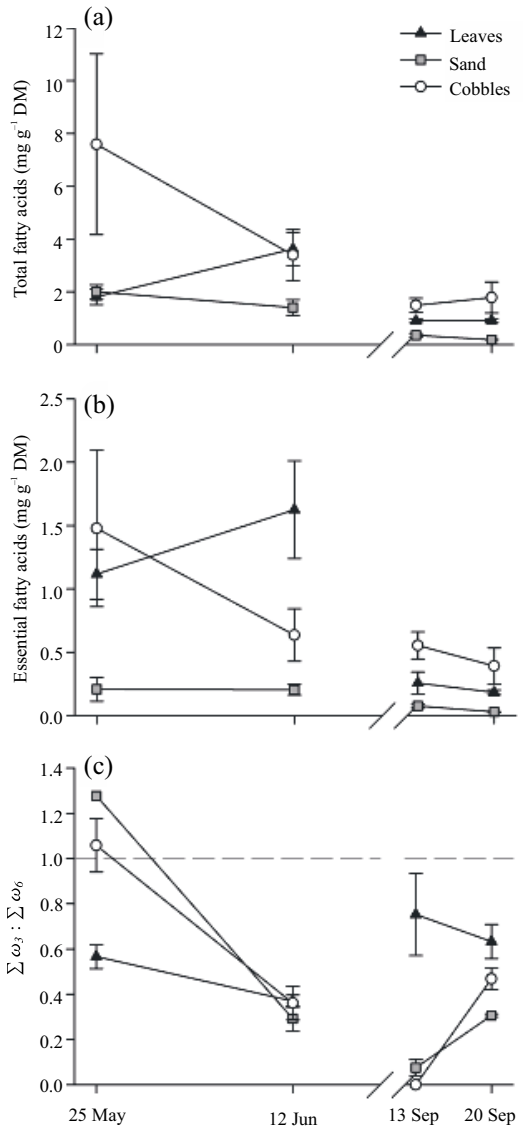


**Figure 2.** (a) Total fatty acids, (b) essential fatty acids, and (c) $\sum \omega_3 : \sum \omega_6$ ratio in the benthic substrata during the study period. The dashed line in (c) indicates the shift from $\sum \omega_3 : \sum \omega_6 < 1$ to $> 1$, proposed as an indicator of the shift from terrestrial to aquatic origin of organic matter. In the sample from leaves on 25 May, $n = 2$ and in (c) the $\sum \omega_3 : \sum \omega_6$ ratio from sand on 25 May, $n = 1$. The break in the *X* axis represents the non-flow period and the bars indicate ± 1 SE. *(a) Ácidos grasos totales, (b) ácidos grasos esenciales, y (c) relación* $\sum \omega_3 : \sum \omega_6$ *en los sustratos bentónicos durante el período de estudio. La línea discontinua en (c) indica el cambio de* $\sum \omega_3 : \sum \omega_6 < 1$ *a* $> 1$*, propuesto como indicador de cambio de origen terrestre a acuático de la materia orgánica. En la muestra de hojas del 25 de Mayo,* n = *2 y en (c) la relación* $\sum \omega_3 : \sum \omega_6$ *en la arena el 25 de Mayo,* n = *1. El corte en el eje de la* X *representa el período de interrupción del flujo de agua y las barras representan ± 1 SE.*

an aliquot of 100 mL was taken and NaCl and dichloromethane were added. Samples were shaken and the dichloromethane phase (lower phase) was collected and concentrated up to 0.5 mL. From here on the protocol was the same as described above (*). Samples were kept frozen until analysis. Procedural blanks were processed simultaneously with samples. Internal standards (heptadecanoic acid and 5-$\alpha$-cholestane) were added to the samples and blanks to calculate the yield of the extraction (71% for FA and 53% for sterols) and to correct for the final concentrations. Prior to analysis, sterols were derivatized with bis(trimetilsylil)trifluoroacetamide (BSTFA) for 30 min at 150 ºC, and samples were resuspended in hexane. Samples were analyzed 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 × 0.25 mm) for FA methyl ester (FAME) detection and an Agilent J&W DB5 MS (30 m × 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 and the injector temperature at 250 ºC/270 ºC. The mass spectrometer was in electronic ionization mode. External standards (Supelco 37 component FAME Mix, 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. Results were analyzed with Xcalibur 2.0.7 software (Thermo Fisher Scientific Inc., 1998-2007).

FA were classified according to the number of double bonds: saturated (SAFA), monounsaturated (MUFA), and polyunsaturated (PUFA), and the double bond position, accounting for $\omega_3$ and $\omega_6$ FA, because a $\sum \omega_3 : \sum \omega_6$ ratio of $< 1$ has been proposed to indicate whether the resources are primarily of terrestrial origin and a ratio $> 1$ to indicate a primarily aquatic origin (Pollero *et al*., 1981, Torres-Ruiz *et al*., 2007). Moreover, it was taken into account that some FA are essential components that both

invertebrates and fish must obtain from the diet (essential FA [EFA]: 18:2$\omega_6$, 18:3$\omega_3$, 20:4$\omega_6$, 20:5$\omega_3$) and have biomarker value indicating the presence of diatoms (16:1$\omega_7$, 20:5$\omega_3$), chlorophytes and cyanobacteria (18:2$\omega_6$, 18:3$\omega_3$), 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 (allochthonous OM) (Desvilettes *et al.*, 1997, Napolitano, 1999, Olsen, 1999). However, the EFA 18:2$\omega_6$ and 18:3$\omega_3$ can be also of fungal origin (Arce-Funck *et al.*, 2015). All sterols are essential for invertebrates but not for fish, 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). FA and sterol percentage of total lipids is indicated, since total lipids for these same samples were measured in a previous article (Ylla *et al.*, 2010). For benthic materials, the FA and sterol composition is given per dry mass (DM; determined to the nearest 0.1 mg), while for water POM and DOM composition is given per water volume.

### Statistical analysis

Changes in the FA and sterol composition (in mg/g DM) between dates and between the different benthic substrata (leaves, sand, and cobbles) were examined through 2-way ANOVA. Changes in the FA and sterol composition (in µg/L) between dates in DOM and POM were analyzed through 1-way ANOVA. Tukey's HSD was used as a post-hoc analysis. A 1-sample *t*-test was used to determine whether the $\omega_3 : \omega_6$ 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). FA and sterol composition data, initially in percentage, were arcsine square root transformed, and then principal component analysis (PCA) was performed with CANOCO (version 4.5, Biometris, Wageningen, the Netherlands).

## RESULTS

### Benthic organic matter

FA in benthic substrata 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 in all benthic materials were significantly different across the sampling dates (flow and non-flow periods, Table 2). The total content of FA decreased over time, with higher values before the NF than afterwards; the richest substrate was epilithic biofilm, followed by leaves, and finally sand biofilm (Fig. 2a). The same pattern was found for EFA content (Fig. 2b), although concentrations of EFA were similar on cobbles and leaves. The $\sum \omega_3 : \sum \omega_6$ ratio was significantly different from 1 ($t_{32} = -7.326$; $p < 0.001$). On the first date, cobble and sand biofilm had a $\sum \omega_3 : \sum \omega_6$ ratio $> 1$ on average, which indicates that the OM was mainly 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 NF 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 NF 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 NF it was higher on cobbles (34.8 ± 4.5 µg/g DM).

The relative composition of FA significantly differed across dates, although the effects varied depending on type of benthic material (Fig. 4). The first component (29% of variance) of the PCA ordered the samples by date within the different material. During the rewetting period, just after the NF (third sampling date) these substrata

were rich in some $\omega$3 FA (mainly 16:3$\omega_3$), while on the fourth date they were richer in the EFA 20:5$\omega_3$ from diatoms and some non-specific FA (18:0, 16:1, 18:1$\omega_9$). The gradient created by the second component (24% of variance) aligned the samples according to the substrate FA composition, being the main contributor the FA from leaf samples situated in the negative direction of this component and the FA from cobbles and sand were situated in the positive direction. Leaves were characterized by the presence of FA from vascular plant detritus (long-chain SAFA, e.g. 24:0) and EFA from chlorophyte and cyanobacteria origin (18:2$\omega_6$ before and 18:3$\omega_3$ after the NF). Sand and cobbles were characterized by the diatom FA 16:1$\omega_7$.

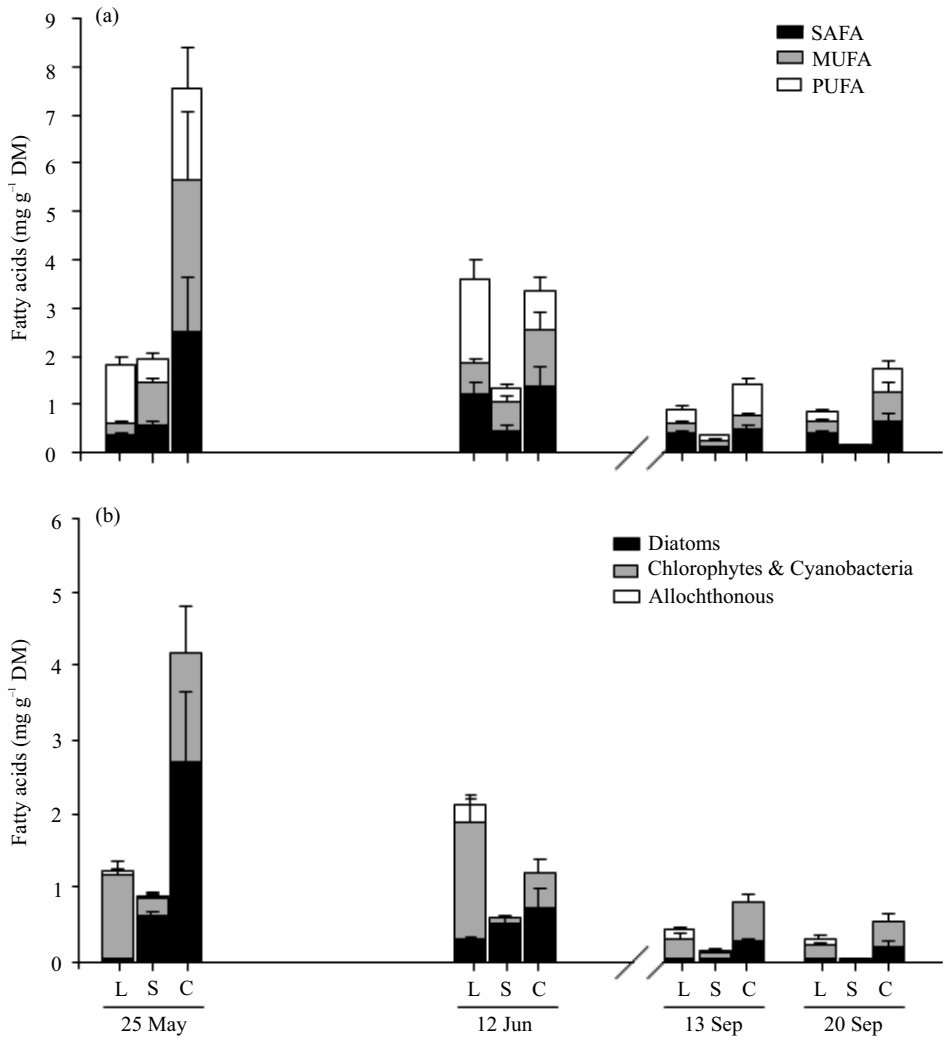


**Figure 3.** Fatty acid (FA) content according to (a) the number of double bonds, i.e. saturated (SAFA), monounsaturated (MUFA), and polyunsaturated FA (PUFA), and (b) its origin, in the benthic substrata, i.e. leaves (L), sand (S), and cobbles (C), during the study period. In the sample from leaves 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 non-flow period and the bars indicate ±1 SE. *Contenido de ácidos grasos según (a) el número de dobles enlaces, ácidos grasos saturados (SAFA), monoinsaturados (MUFA) y poliinsaturados (PUFA); y (b) su origen en el substrato, hojas (L), arena (S), y piedras (C), durante el período de estudio. En la muestra de hojas del 25 de Mayo,* n = *2 y en (b) ácidos grasos de diatomeas de las piedras el 25 de Mayo,* n = *2. El corte en el eje de la* X *representa el período de interrupción del flujo de agua y las barras representan ±1 SE.*

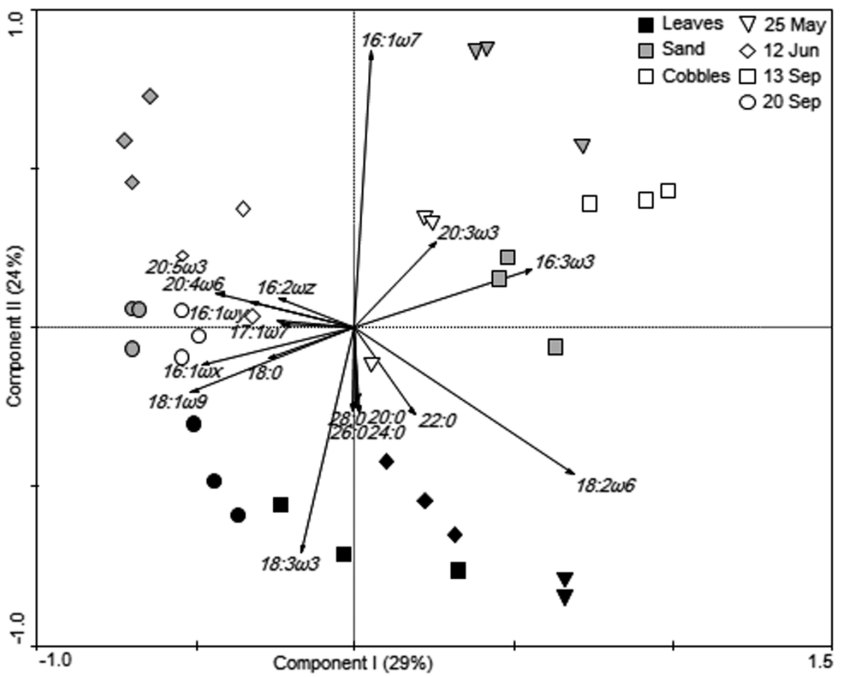


**Figure 4.** Principal component analysis (PCA) of the fatty acid (FA) composition (% mg/g DM) of the benthic substrata during the study period. The first and second components are represented, and the percentage of data variability explained by each is indicated. To improve readability, FA with a score <1 were removed from the graph (though not from the analysis). *Análisis de componentes principales de la composición de ácidos grasos (% mg/g DM) en los sustratos muestreados durante el período de estudio. Se representan el primer y segundo componentes y el porcentaje de la variabilidad explicada. Los ácidos grasos con un peso <1 se eliminaron de la figura, pero no del análisis, para mejorar la legibilidad.*

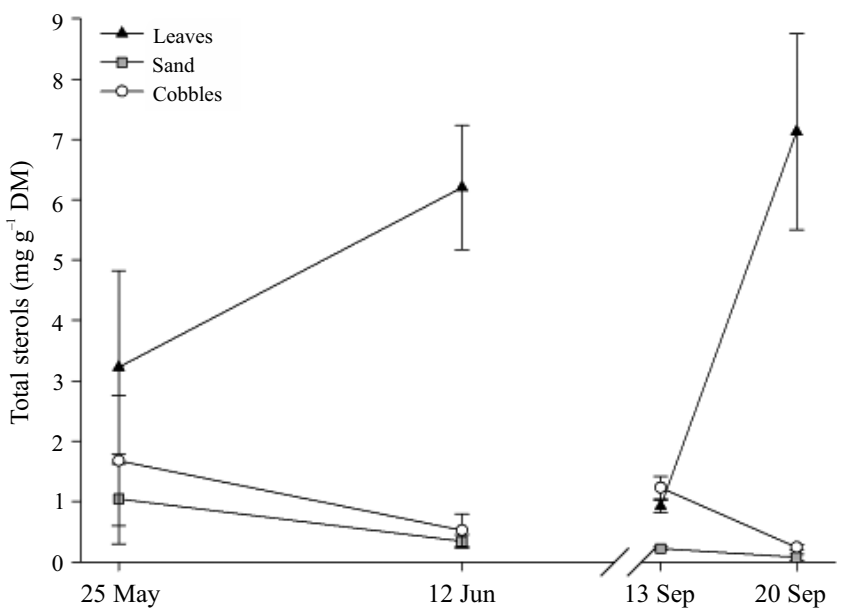


**Figure 5.** Total sterol content of the benthic substrata during the study period. The break in the *X* axis represents the non-flow period and the bars indicate ± 1 SE. *Contenido de esteroles totales en los sustratos bentónicos durante el período de estudio. El corte en el eje de la* X *representa el período de interrupción del flujo de agua y las barras representan ± 1 SE.*

**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 DM) in the benthic substrata, i.e. leaves (L), sand (S), and cobbles (C), over time. The dates analyzed are before the non-flow period, 25 May (1), 12 June (2), and after the non-flow period 13 September (3) and 20 September (4). *Resultados del ANOVA de dos vías y de los análisis post-hoc (Tukey's HSD) de la composición de ácidos grasos y esteroles (en mg/g DM) en los sustratos bentónicos y en el tiempo: hojas (L), arena (S) y piedras (C). Las fechas analizadas son antes de la interrupción del agua: 25 de Mayo (1), 12 de Junio (2) y después de la recuperación: 13 de Septiembre (3) y 20 de Septiembre (4).*

| | 2-way ANOVA | | | | | | | | | Tukey's HSD | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Date | | | Substrate | | | Date × Substrate | | | Date | Substrate |
| Benthic substrata | df | F | *p* | df | F | *p* | df | F | *p* | | |
| **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 |
| $\sum \omega_3 : \sum \omega_6$ 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[a] | 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[a] | 1,4 | < 0.01 | 0.976 | — | — | — | — | — | — | — | — |
| Fucosterol[a] | 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 | — |

EFA = essential FA, SAFA = saturated FA, MUFA = monounsaturated FA, PUFA = polyunsaturated FA, * = $p < 0.05$.
[a] Allochthonous FA were only detected in L and S; ergosterol was only detected in L on dates 1 and 3, and fucosterol only on dates 1 and 3.

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 mainly dependent on the substrate, but not noticeably different between dates (Fig. 5; Table 2). The sterol content was higher in leaves than in the other materials, and the lowest values in leaves were found just after the NF (third sampling date). Sterols measured in the benthic materials were identified as coming mainly 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 detected in leaves on the first and third dates, with a mean value of 73.6 (± 17.8) μg/g DM. Fucosterol concentration varied widely (range: 0 to 773 μg/g DM), with no significant differences either between substrata or between dates. Cholesterol content was 132.7 (± 33.2) μg/g DM on average, and did not differ significantly with regard to NF conditions.

The relative composition of sterols in the leaves was different from that in the other substrata. In the PCA (% mg/g DM; Fig. 6), the main contributor in the second component (12% of variance) was stigmasterol followed by ergosterol, lanosterol, and stigmastanol; and leaves from the different dates were ordered according to this composition. On the opposite part of this component the contributors were fucosterol, cholesterol, and campesterol, the main FA in sand and cobbles mainly before the NF. The first component (72% of variance) aligned the samples of the last date characterized by $\beta$-sitosterol. The fact that pattern of ergosterol (fungal)

**Table 3.** Results from the 1-way ANOVA and post-hoc (Tukey's HSD) analyses (for sampling dates) for the fatty acid (FA) and sterol composition (in µg/L) in the transported dissolved and particulate organic matter (POM and DOM) over time. The dates analyzed are before the non-flow period, 25 May (1), 12 June (2), and after the non-flow period 13 September (3) and 20 September (4). *Resultados del ANOVA de una vía y de los análisis post-hoc (Tukey's HSD) de la composición de ácidos grasos y esteroles (en µg/L) en la materia orgánica particulada y disuelta y en el tiempo. Las fechas analizadas son antes de la interrupción del agua: 25 de Mayo (1), 12 de Junio (2) y después de la recuperación: 13 de Septiembre (3) y 20 de Septiembre (4).*

| | Transported POM | | | | Transported DOM[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | 1-way ANOVA | | | Tukey's HSD | 1-way ANOVA | | | Tukey's HSD |
| | df | F | *p* | | df | F | *p* | |
| **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[a] | 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 | — | — | — | — | — |

EFA = essential FA, SAFA = saturated FA, MUFA = monounsaturated FA, PUFA = polyunsaturated FA, * = $p < 0.05$.
[a] Allochthonous FA were only detected on dates 1 and 4.
[b] In DOM, some variables were not analyzed due to the low quantities detected. All the PUFA in the samples were EFA from chlorophytes and cyanobacteria, and hence were analyzed once as PUFA.

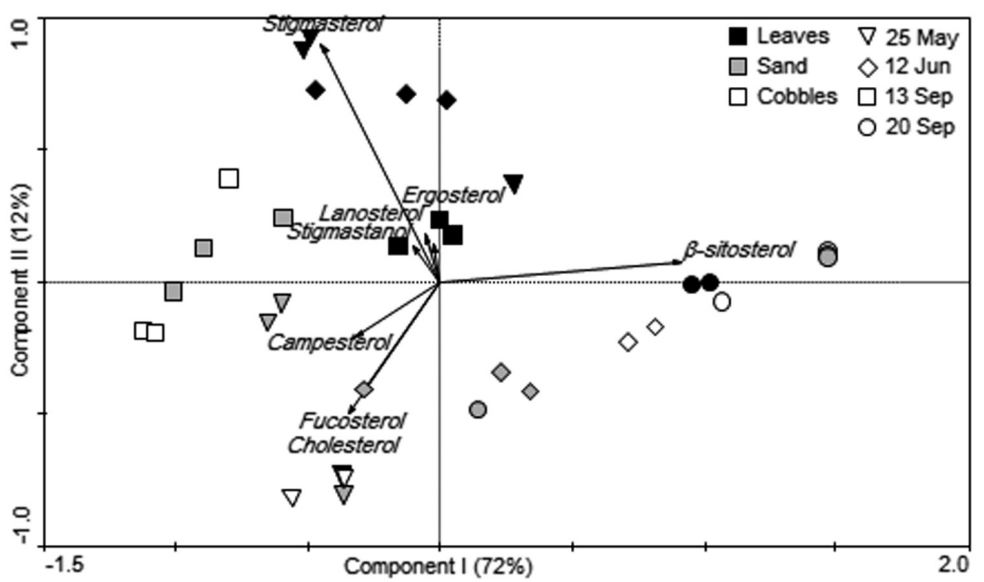


**Figure 6.** Principal component analysis (PCA) of the sterol composition (% mg/g DM) of the benthic substrata during the study period. The first and second components are represented, and the percentage of data variability explained by each is indicated. *Análisis de componentes principales de la composición de esteroles (% mg/g DM) en los sustratos muestreados durante el período de estudio. Se representan el primer y segundo componentes y el porcentaje de la variabilidad explicada.*

and 18:2$\omega_6$ and 18:3$\omega_3$ FA (algal) differed suggests that the origin of these EFA are from chlorophytes and cyanobacteria.

### Transported organic matter

In POM, the FA content of the total lipids was 26.2 (± 16.0)%. Total FA content peaked before the NF (Fig. 7), related to MUFA; these represented 63% of the total FA and were mainly from diatoms (60%). EFA and the $\sum \omega_3 : \sum \omega_6$ ratio were also higher before the NF (EFA before: 8.15 ± 1.37, after: 0.97 ± 0.52 μg/L; $\sum \omega_3 : \sum \omega_6$ before: 1.53 ± 0.29, after: 0.04 ± 0.03). However, the $\sum \omega_3 : \sum \omega_6$ ratio was not significantly different from 1 ($t_{12} = -0.811$; $p = 0.434$). Allochthonous FA were mainly present on the first date (4.99 ± 0.57 μg/L) (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) over time were not related to the NF (Table 3). Ergosterol and fucosterol were not detected in the POM samples.

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

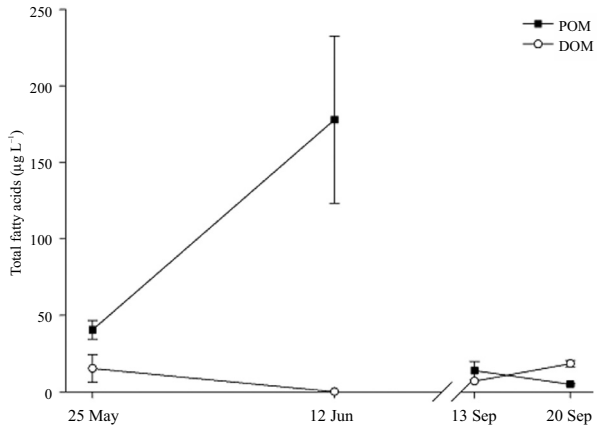


**Figure 7.** Total fatty acid content in transported particulate (POM) and dissolved organic matter (DOM). In DOM, on 12 June, $n = 2$ and on 13 September, $n = 1$. The break in the *X* axis represents the non-flow period and the bars indicate ± 1 SE. *Contenido de ácidos grasos totals en la materia orgánica particulada en suspension (POM) y disuelta (DOM). En DOM, el 12 de Junio,* n = *2 y el 13 de Septiembre,* n = *1. El corte en el eje de la* X *representa el período de interrupción del flujo de agua y las barras representan ± 1 SE.*

## DISCUSSION

Our main hypothesis was that the drying-rewetting process would affect 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 study, with the drought causing a general reduction in the total and essential FA of benthic substrata and transported POM, and a shift from predominantly autochthonous to allochthonous OM. 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 materials 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 and studied period (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 NF. This is confirmed by the higher concentration of FA that has been attributed to aquatic primary producers (diatom, chlorophytes, and cyanobacteria FA) on benthic materials in this period, as well as by the change from autochthonous OM before the NF to predominantly allochthonous OM afterwards, which was reflected by the shift in the $\sum \omega 3 : \sum \omega 6$ ratio (from $> 1$ to $< 1$) on cobbles and sand. This seasonal change has already been described in Mediterranean systems (Artigas *et al*., 2009, Romaní *et al*., 2013), with algal biomass sharply increasing from the beginning of spring until the interruption of stream flow, particularly in the coarse substrata.

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 colonization during the drought period. To our knowledge, no studies have been conducted on the effects of leaf decomposition on sterol composition.

In the transported OM, the difference in FA between before and after the NF was also observed in transported POM. Higher total FA, EFA, and $\sum \omega_3 : \sum \omega_6$ ratio indicated the availability of higher-quality transported material before the NF. The FA peak observed in POM just before the NF was associated with an increase in the total lipid content on the same date (Ylla *et al.*, 2010). In the present study, this peak was due primarily to diatom FA, and may be related to the algal peak.

We also hypothesized that the sudden rewetting event would leach and transport high-quality DOM downstream. However, our measurements of DOM composition did not provide evidence of major changes in FA over time. Ylla *et al.* (2010) described a peak of DOM immediately after the rewetting event, 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 materials (i.e. $\beta$-glucosidase and leucine aminopeptidase). Although the lipids in DOM were not measured in that study, no peak was observed in lipase during the rewetting. Our results reinforce the idea that the OM lipids cumulated 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 substrata. 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). The sand 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 substrata. The contribution of FA from leaves derived primarily from the biofilm that colonizes the surface, essentially chlorophytes and cyanobacteria. In contrast, Torres-Ruiz *et al.* (2007) found low percentages of autochthonous FA on OM (< 5%), but they observed some autochthonous colonization peaks in spring. No study has been published on the taxonomic identification of algae colonizing leaves in Fuirosos. Although chlorophytes and cyanobacteria provided most of the FA, diatoms seem to be the dominant colonizers (A.M. Romaní, Universitat de Girona, personal communication). In our study, EFA were mainly provided by epilithic biofilm and leaves. Cobbles (and secondarily sand) were the main suppliers of 20:4$\omega_6$ and 20:5$\omega_3$, while leaves were important for 18:3$\omega_3$ and 18:2$\omega_6$. This coincides with the findings of Torres-Ruiz *et al.*, (2007), who found 18:2$\omega_6$ 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 detected in leaf litter on the first date (non on latter date), which may suggest that fungi were negatively affected by desiccation during the NF period (Bruder *et al.*, 2011). If different EFA and sterols are found in different substrata, consumers will probably need to feed on these different substrata, 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 DM on average, which is comparable to the range found by Torres-Ruiz & Wehr (2010) in leaves during decomposition (2–6.9 mg/g DM). The FA content in the biofilms was below 12 mg/g DM, lower than the content

found by Hill *et al*. (2011); from 10–50 mg/g DM in artificial channels. This was probably related to the greater stability of the artificial channel conditions or to the differences in biofilm community composition. We were not able to find comparable values of FA content for transported DOM but our values for transported POM were in the range of the findings of Lu *et al*. (2014). 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 and fish need to acquire EFA and invertebrates sterols as well in their diet from the different available resources since cobble biofilms and leaf material provide different nutritional components, due to their inability to synthesize 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). Even so, 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 stream functioning. Our findings indicate that drought determines a general decrease of EFA content in basal resources. Extension of droughts to temperate streams or the increase in the frequency of droughts in temporary streams could compromise the quality of the resources and consequently consumers' fitness.

## ACKNOWLEDGEMENTS

We kindly acknowledge B. Obrador, and A. Larrañaga for their comments on an earlier version of this manuscript. This study was funded by the Spanish Ministry of Economics and Competitiveness through the project CGL2014-58760-C3-R and by the European Union's Seventh Program with the project Globaqua (No. 603629).

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