



Multiple stressor effects on biodiversity and ecosystem functioning in a Mediterranean temporary river

Evangelia Smeti ^{a,*}, Daniel von Schiller ^b, Ioannis Karaouzas ^a, Sofia Laschou ^a, Leonidas Vardakas ^a, Sergi Sabater ^{c,e}, Elisabet Tornés ^{c,e}, Luis Simón Monllor-Alcaraz ^d, Nuria Guillem-Argiles ^d, Elena Martinez ^d, Damià Barceló ^{c,d}, Miren López de Alda ^d, Eleni Kalogianni ^a, Arturo Elosegí ^b, Nikolaos Skoulidakis ^a

^a Institute of Marine Biological Resources and Inland Waters, Hellenic Centre for Marine Research (HCMR), 46.7 km Athens-Sounio Ave., Anavyssos, 19013 Athens, Greece

^b Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Bilbao, Spain

^c Catalan Institute for Water Research (ICRA), Scientific and Technologic Park of the University of Girona, Girona, Spain

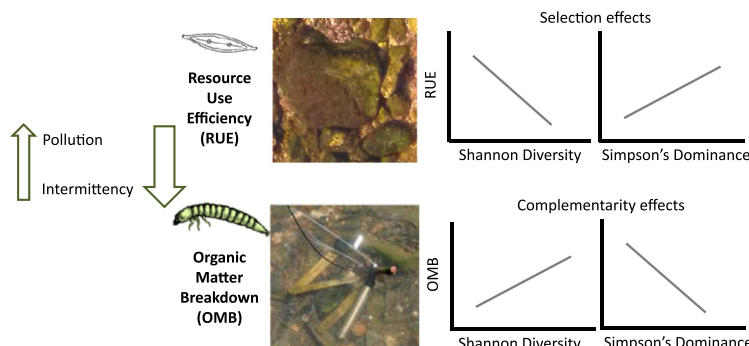
^d Water and Soil Quality Research Group, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), C/ Jordi Girona 18-26, 08034 Barcelona, Spain

^e Institute of Aquatic Ecology, University of Girona, Girona, Spain

HIGHLIGHTS

- Testing biodiversity–ecosystem functioning (B–EF) relationship in a temporary river
- Resource use efficiency (RUE) decreased with increased diatom diversity.
- Organic matter breakdown (OMB) increased with increased macroinvertebrate diversity.
- Different mechanisms drive the B–EF relationship in benthic communities.
- RUE and OMB decrease with pollution and intermittency following community structure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 1 June 2018

Received in revised form 7 August 2018

Accepted 7 August 2018

Available online 8 August 2018

Editor: Yolanda Picó

Keywords:

Biodiversity

Resource use efficiency

Organic matter breakdown

Organic pollutants

Flow intermittency

ABSTRACT

The hydrological and biological complexity of temporary rivers as well as their importance in providing goods and services is increasingly recognized, as much as it is the vulnerability of the biotic communities in view of climate change and increased anthropogenic pressures. However, the effects of flow intermittency (resulting from both seasonal variations and rising hydrological pressure) and pollution on biodiversity and ecosystem functioning have been overlooked in these ecosystems. We explore the way multiple stressors affect biodiversity and ecosystem functioning, as well as the biodiversity–ecosystem functioning (B–EF) relationship in a Mediterranean temporary river. We measured diversity of benthic communities (i.e. diatoms and macroinvertebrates) and related ecosystem processes (i.e. resource use efficiency–RUE and organic matter breakdown–OMB) across a pollution and flow intermittency gradient. Our results showed decreases in macroinvertebrate diversity and the opposite trend in diatom assemblages, whereas ecosystem functioning was negatively affected by both pollution and flow intermittency. The explored B–EF relationships showed contrasting results: RUE decreased with higher diatom diversity, whereas OMB increased with increased macroinvertebrate diversity. The different responses suggest contrasting operating mechanisms, selection effects possibly driving the B–EF relationship in diatoms and complementarity effects driving the B–EF relationship in macroinvertebrates. The understanding of multiple

* Corresponding author.

E-mail address: evasmeti@hcmr.gr (E. Smeti).

stressor effects on diversity and ecosystem functioning, as well as the B-EF relationship in temporary rivers could provide insights on the risks affecting ecosystem functioning under global change.

© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Temporary rivers are dynamic and diverse ecosystems, very common in the Mediterranean Basin (McDonough et al., 2011; Acuña et al., 2014). Biodiversity in temporary rivers is lower than in perennial rivers, with native communities able to tolerate natural flow intermittency, but sensitive to anthropogenic disturbances and extreme climatic events (Datry et al., 2014; Skoulikidis et al., 2017). Biodiversity losses because of global change, is a rising threat in these systems, with certain taxonomic groups that are key for ecosystem functioning being more vulnerable (e.g. Tilman et al., 2012; Soria et al., 2017). Even though recent studies have indicated the negative effects of flow intermittency and anthropogenic pollution on biodiversity (e.g. Arenas-Sánchez et al., 2016; Karaouzas et al., 2018a), ecosystem functioning (e.g. Acuña et al., 2015) and the supporting goods and services (Datry et al., 2017) separately, there has been less effort in studying potential biodiversity-ecosystem functioning (B-EF) relationships in these ecosystems. The provision of ecosystem services in temporary rivers is being challenged by multiple stressor effects on biodiversity, and this may transmit to ecosystem functioning through the B-EF relationship.

Ecosystem functioning encompasses the processes and properties of a given ecosystem (Frainer, 2013), and primary production and organic matter breakdown are fundamental components of ecosystem functioning (Lindeman, 1942; Odum, 1956). These processes are driven by abiotic factors such as temperature, hydrology, and light and nutrient availability (von Schiller et al., 2017), but also by biotic ones such as diversity of biological communities (Gessner et al., 2010). These natural environmental factors add to anthropogenic factors such as agriculture and urban sewage, the two being important contributors of organic matter, nutrients and pollutants, including pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs; Karaouzas et al., 2011a; Tzoraki et al., 2015). The intermittent character of temporary Mediterranean rivers allow the accumulation of these pollutants in the sediments (Zoppini et al., 2014, 2016) during periods of zero flow, suggesting that multiple stressors may act together in these systems, affecting ecosystem functioning. As an indication of effects on ecosystem processes, primary production in the biofilm is reduced during non-flow events, particularly in the presence of pollutants (Corcoll et al., 2015). Organic matter breakdown also decreases with water stress (Abril et al., 2016), as well as with increased inputs of toxic chemicals such as heavy metals and pesticides (e.g. Schäfer et al., 2007). In fact, the decrease in leaf litter breakdown has been often associated to the effects of multiple stressors on detritivore macroinvertebrate communities, a key group of macroinvertebrate consumers (e.g. Graça, 2001; Monroy et al., 2016).

Although stream ecology has provided evidence of B-EF relationships (Lecerf and Richardson, 2010), these relationships have focused mainly on benthic macroinvertebrates and their ability to breakdown organic matter (e.g. Chauvet et al., 2016), whereas ecosystem processes related to primary producers in biofilms have been related to the overall metabolism and nutrient use (Cardinale, 2011; Guasch and Sabater, 1995). Biodiversity is related to ecosystem processes not only through the number of species present in a community, but also through their interactions and their specific traits. It is thus critical to consider biodiversity in the context of species richness as well as of community structure and function (McKie et al., 2008; Frainer et al., 2014), when aiming to detect the mechanisms underlying the B-EF relationship: *complementarity effects* (i.e. complementary use of resources by the species) can result in a positive B-EF relationship due to differences in species traits, whereas *selection effects* (i.e. the presence of a species that produces

high biomass) could result in a positive B-EF relationship due to increased dominance of the most productive species (e.g. Hillebrand et al., 2008; Truchy et al., 2015).

Our aim was to explore in which manner multiple stressors affected biodiversity and ecosystem functioning as well as their relationship. We performed our research in a Mediterranean temporary basin, focusing on benthic stream communities (diatoms and macroinvertebrates) which were submitted to different degrees of pollution and flow intermittency. We therefore analysed diatom assemblages and macroinvertebrate communities' composition from four streams in the Evrotas River basin, which covered a pollution and flow intermittency gradient. We further related these communities to resource use efficiency (RUE) of primary producers in biofilms and to organic matter breakdown (OMB) by macroinvertebrates. We tested for (i) the relationships between biodiversity vs. pollution and flow intermittency, (ii) the relationships between the recorded ecosystem processes vs. pollution and flow intermittency, and (iii) the form of the B-EF relationships in the two stream benthic communities as well as the possible mechanisms underpinning them.

2. Materials and methods

2.1. Study area

The Evrotas river basin (South Peloponnese, Greece) is a medium-sized (2,418 km²), mid-altitude (150–600 m) Mediterranean basin, with numerous ephemeral and intermittent streams discharging into the main stem of the river. Climate is typical Mediterranean with hot and dry summers (26.2 °C, average temperature June–August 2009–2016) and cool, wet winters (9.1 °C, average temperature December–February 2009–2016), with annual precipitation varying markedly from year to year; it follows however a predictable seasonal pattern, with most of the rainfall occurring during the months of October through March, with very few rain episodes during summer (Karaouzas et al., 2018b). The river basin's landscape is covered mainly by seminatural areas (61% of the total river basin), with agricultural areas coverage being 38% and urban areas approximately 1%. The dominant anthropogenic pressures are overexploitation of water resources, agro-industrial wastewater discharges, diffuse agrochemical pollution and domestic wastewaters from the municipal treatment plant (Karaouzas et al., 2018b). The geographical, geological, hydrological and ecological features of the Evrotas basin are described in detail elsewhere (Skoulikidis et al., 2011; Kalogianni et al., 2017; Karaouzas et al., 2018b).

2.2. Sampling design

We studied 4 streams in the Evrotas river basin, named here A1 to A4 (Fig. 1), selected because they form a gradient of flow intermittency (i.e. number of days of zero flow). The streams, together with the effect of diffused pollution from anthropogenic stressors, such as agriculture, receive point source pollution from the operation of olive-oil mills. The olive-oil mills operated during a three-month period in winter, causing an acute stress to the biotic communities of the affected sites. Thus, to further account for the effect of the effluents from olive oil mills, in each stream we selected a site upstream from the olive oil mill and another immediately downstream. Each site covered approximately 20 m length and the upstream and downstream sites were located approximately 20 m from the olive-oil mill effluent. We sampled all the sites in October 2016, before the onset of olive oil mill

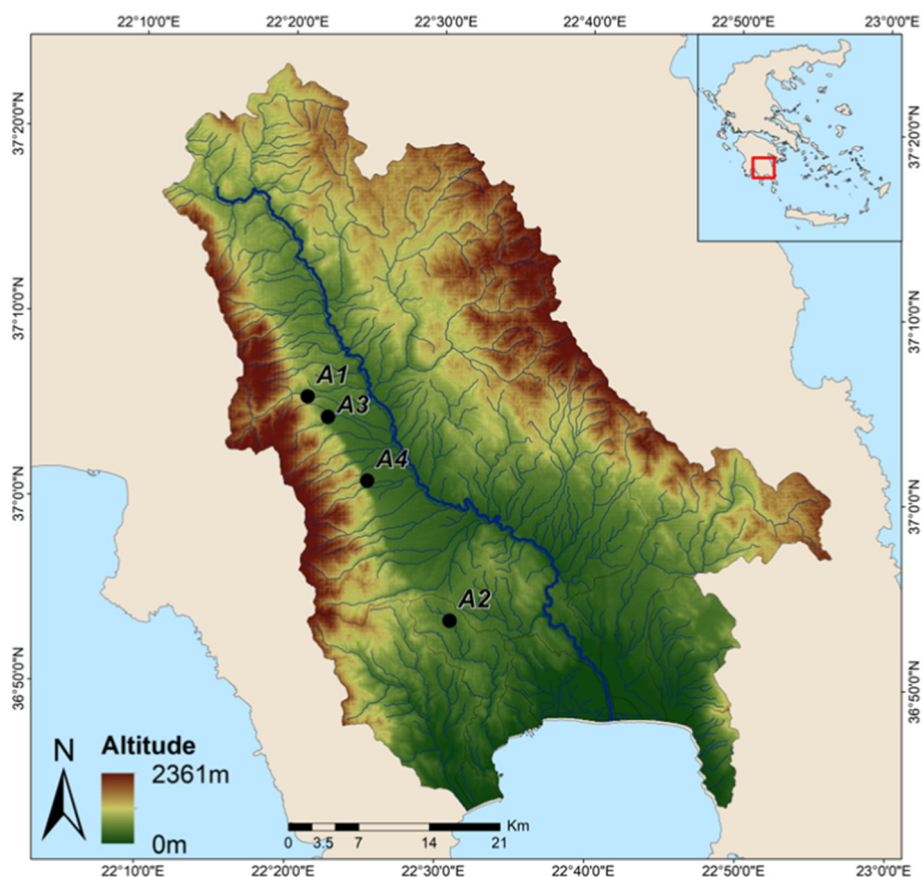


Fig. 1. Map showing the location (WGS84 datum) of the four streams in the Evrotas basin (South Peloponnese, Greece) where sampling and field experiments were conducted during 2016–2017 (streams coded A1, A2, A3 and A4 based on increased level of flow intermittency).

operation, January 2017, when the mills were under operation, and March 2017, one month after the end of oil mills operation. When sites were dry, no sampling was performed. Flow intermittency was calculated for each site based on the total number of days of zero flow, during the study period (October 2016–March 2017), using data from automatic HOBO sensors that continuously recorded conductivity (that was zero at zero flow) and temperature. Details related to flow intermittency are provided in Table S1.

2.3. Chemical analyses

At each site, approximately 8 kg of the uppermost 10 cm sediment layer were collected from 4 to 6 points along the left and right river banks. Sediments were wet-sieved (2 mm mesh) using river water, to remove large debris and animals. Samples were then placed in polyethylene bottles, frozen, and transported on dry ice to the laboratory. In each sampling site, an unsieved subsample was collected for phenol analysis, but was otherwise subjected to the same procedure with the rest of the sediment samples.

Sediment samples were analysed for legacy persistent organic pollutants (POPs), pesticides, phenolic compounds, organic carbon (OC) and total nitrogen (TN). POPs tested included the 16 polycyclic aromatic hydrocarbons regulated by the United States Environmental Protection Agency (Keith, 2015), the polychlorinated biphenyls (PCBs) 18, 28, 31, 52, 44, 101, 149, 118, 153, 138, 180, 180, 170 and 194, and the semi-volatile organochlorine compounds hexachlorobenzene (HCB), gamma hexacyclohexane (g-HCH), DDT and related compounds (DDX). Analysis of the lyophilized sediment samples was performed with a method based on ultrasonic extraction followed by GC-MS/MS analysis (Martínez et al., 2004; Navarro-Ortega et al., 2010; Quesada et al., 2013). Analysis of polar pesticides in the freeze-dried sediment

samples was performed with a method based on pressurized liquid extraction (PLE), SPE clean-up, and analysis by LC-MS/MS (Köck-Schulmeyer et al., 2013). The list of target pesticides (above 50) included compounds (and a few breakdown products (BPs)) belonging to different chemical classes (phenylureas, triazines, organophosphates, anilides, thiocarbamates, chloroacetanilides, acidic herbicides, benzotriadiazines, quinolones, neonicotinoids).

For the determination of phenolic compounds, approximately 20 g of the sediment was sub-sampled and organic matter was separated using ultrasonic extraction. A mixture of methanol and water was used followed by sonication of the resulted suspension and centrifugation. The clarified extracts were collected, condensed and subsequently extracted with the 4 aminoantipyrine method (Ettinger et al., 1951). Total phenols were measured photometrically, using a Perkin-Elmer 25 Lambda spectrophotometer at a wavelength of 460 nm (Danis and Albanis, 1996; Alonso et al., 1998; Czaplicka, 2001). Organic Carbon (OC) and Total Nitrogen (TN) in the sediment were measured with a CHN analyzer, Thermo Scientific Flash 2000, following Cutter and Radford-Knoery (1991) and Verardo et al. (1990). Water samples were filtrated through 0.45 μm membrane filters. Nitrite (NO_2 , mg L^{-1}) and orthophosphate (PO_4 , mg L^{-1}) concentrations were determined by a Skalar San++ Continuous Flow Analyzer, ammonia (NH_4 , mg L^{-1}) concentration was determined using a Skalar Automatic Analyzer, whereas nitrate (NO_3 , mg L^{-1}) concentration was determined using both Ion Chromatography and a Skalar Automatic Analyzer.

All pollutants detected in the sediments (pesticides, PAHs, PCBs, organochlorides, phenols, OC and TN) were log- or arcsin- (percentages) transformed, and each one was regressed against ecosystem processes (Table S2). Pollutants that significantly affected an ecosystem process (Table S2 in bold) were further used in a Principal Component Analysis (PCA), thus summarized into one variable that determined

the pollution gradient. The pollution gradient was reflected in the first PC axis that summarized the total variability of chemical pollutants (see [Results](#) for details). To detect any possible relationship between pollution and intermittency, Spearman correlation analysis was performed between the two stressors.

2.4. Biota

Diatom sampling followed the standard EU protocol ([EN 13946, 2003](#)). Submerged cobbles (5–7 per site) were collected from areas with sufficient light and brushed to obtain biofilm material. In the laboratory, samples were digested with hydrogen peroxide to remove organic matter, and cleaned material was prepared (using Naphrax) in permanent slides for microscopical examination. Up to 400 diatom valves were counted and identified at the species or subspecies level in each sample, using light microscopy (Nikon Eclipse 80i, Tokyo, Japan) with Nomarski differential interference contrast optics at 1000× magnification.

Macroinvertebrate collection followed the STAR-AQEM methodology ([AQEM Consortium, 2002](#)). Twenty subsamples were collected at each sampling site using a 25 cm × 25 cm square hand net with a 500-µm mesh size nytex screen. Each of the 20 subsamples was taken by positioning the net and disturbing the substrate in an area that equals the square of the frame width upstream of the net (25 × 25 cm). Thus, a total of 1.25 m² (25 × 25 × 20 replicates) was sampled from each sampling site. Subsamples were preserved in ethanol until transportation to the laboratory where they were sorted, and all individuals found were identified to genus level, where possible, using an Olympus SZX10 stereo microscope.

Structural and functional diversity indices were applied to both diatom assemblages and macroinvertebrate communities. We applied the most common indices used in biodiversity-ecosystem functioning studies: species richness (genus richness for macroinvertebrates) and the Shannon diversity index ([Shannon and Weaver, 1949](#)), together with the Pielou's evenness ([Pielou, 1975](#)) and Simpson's dominance ([Simpson, 1949](#)) indices to account for the importance of community structure on ecosystem functioning ([Hillebrand et al., 2008](#)). We further applied functional diversity and functional evenness indices ([Mason et al., 2005](#)), as they are better predictors of ecosystem functioning in microalgae (e.g. [Abonyi et al., 2017](#)), but have not been applied to test for B-EF relationships in river macroinvertebrates so far ([Schmera et al., 2017](#)). To calculate functional indices for diatoms, we used as traits their ability to attach to the substrate ([Liu et al., 2013](#)), their requirements on pH, salinity, oxygen, and moisture, as well as their nitrogen uptake metabolism, saprobity and trophic state ([Van Dam et al., 1994](#)). To calculate functional indices for macroinvertebrates, we used as traits locomotion, saprobity, feeding, current, zonation and habitat preferences ([Juhász, 2016](#)). Functional diversity indices were calculated with the package FD v.1.0–12 ([Laliberté et al., 2014](#)) in R v.3.3.3 (R Core Team, 2017). Scores of all indices are provided in Table S1.

2.5. Ecosystem processes

Processes related to ecosystem functioning measured during the experiment and related to the two biotic groups were *resource use efficiency* (RUE) of the primary producers in the biofilm related to diatoms and *organic matter breakdown* (OMB) related to macroinvertebrates.

Epilithic biofilm was sampled by scrapping a 5-cm diameter circle from cobbles (5 per site). Samples were immediately frozen. After thawing, a biofilm solution in deionized water was obtained using a Branson sonifier ultrasonic cell disruptor (TM, Branson Ultrasonic Corporation, Emerson Electric, U.S.A. Holding Corp). The biofilm solution was filtered onto ashed 0.7-µm pore size glass fiber filters (Whatman GF/F, Kent, UK). Chlorophyll-a (chl-a), a surrogate of algal biomass, was measured after the extraction of the filters in 90% acetone for 12 h in the dark at 4 °C ([Steinman et al., 2007](#)). To ensure the complete

extraction, samples were sonicated for 30 s, twice (30 s, 360 W power, 50/60 Hz frequency, JP Selecta S.A., Spain). The concentration of chl-a was determined spectrophotometrically using a Shimadzu UV-1800 Spectrophotometer (Kyoto, Japan) and measured following [Jeffrey and Humphrey \(1975\)](#).

The measured chl-a (a surrogate of algal biomass) was standardized by the limiting resource in each site to quantify resource use efficiency (RUE, [Ptacnik et al., 2008](#)). As diatoms were the predominant group in the biofilm, chl-a could be related to diatom biomass. To establish nutrient limitation, the ratio of total inorganic nitrogen to phosphates (TNin/PO₄) in water was estimated. When the ratio was >12, the site was considered P-limited, whereas <5 the site was considered N-limited ([OECD, 1982](#)). Overall, A1 and A2 streams were found to be P-limited, and A3 and A4 streams were found to be N-limited. Therefore, to calculate RUE, chl-a was divided by PO₄ concentration in A1 and A2 and by TNin concentration in A3 and A4. RUE based on N-limitation needed further correction, due to difference in the N and P requirements of the cells. Based on the Redfield atomic ratio of N:P (16:1), the degree of nutrient limitation would be similar for N-limited sites with 16 µmol L⁻¹ of N (i.e. 224 µg N L⁻¹) and for P-limited sites with 1 µmol L⁻¹ of P (i.e. 31 µg P L⁻¹). Therefore, the correction factor used was 7.22 (i.e. the ratio 224/31).

Organic matter breakdown was measured using tongue depressors (15 × 1.8 × 0.2 cm) made of untreated Canadian poplar wood (*Populus nigra* × *canadensis*, Moench) following the method described in [Arroita et al. \(2012\)](#). Sticks were individually dried (70 °C, 72 h) and weighed to determine initial dry mass before deployment in the river. The sticks were incubated at each site for 45–60 days. During the experiment, some of the sticks remained permanently submerged, whereas others were on the dry bed for part of the duration of the experiment. Upon retrieval, the sticks were rinsed with tap water, dried (70 °C, 72 h), weighed and ashed (500 °C, 5 h) to determine final ash-free dry mass. Leaching of sticks was simulated in the laboratory, and initial ash content determined to correct initial dry masses. Breakdown rates were calculated according to the negative exponential model ([Petersen and Cummins, 1974](#)). This was further expressed in degree-days, to correct for the effect of temperature.

2.6. Statistical analysis

To test the effect of pollution we used the first axis (PC1) of the performed PCA with the chemical organic variables as the summary variable of pollution - see [Results](#) for details), and we related it to biodiversity and ecosystem functioning. The flow intermittency (Days of zero flow) was also related to biodiversity and ecosystem functioning. We applied linear mixed-effects models to account for any variation attributed to time of sampling (random effect in the models) on these relationships. The effect of each variable was tested using likelihood-ratio tests (LRTs). Statistical analysis was carried out in R v.3.3.3 (R Core Team, 2017) and package lme4 v.1.1–12 ([Bates et al., 2015](#)) was used for mixed effect models.

3. Results

The first components of the PCA performed with the chemical pollutants accounted for 75.6% of the total variance (PC1 = 58.2% and PC2 = 17.4% - [Fig. 2](#)). However, only PC1 was found to be significantly correlated to the two ecosystem processes (Table S2) and was thus further used in the analyses. PC2 summarized pollution by a pesticide, but it did not significantly affect any of the two ecosystem processes measured (Table S2). Negative values of PC1 corresponded to high pollution levels ([Fig. 2a](#)), and flow intermittency was negatively correlated to PC1 (Spearman coefficient $r = -0.71$, $p < 0.05$), indicating that the most intermittent sites were also the most polluted ones ([Fig. 2b](#) - pollution is represented as the inverse of PC1 scores, therefore the higher the number in the y-axis, the higher the pollution level).

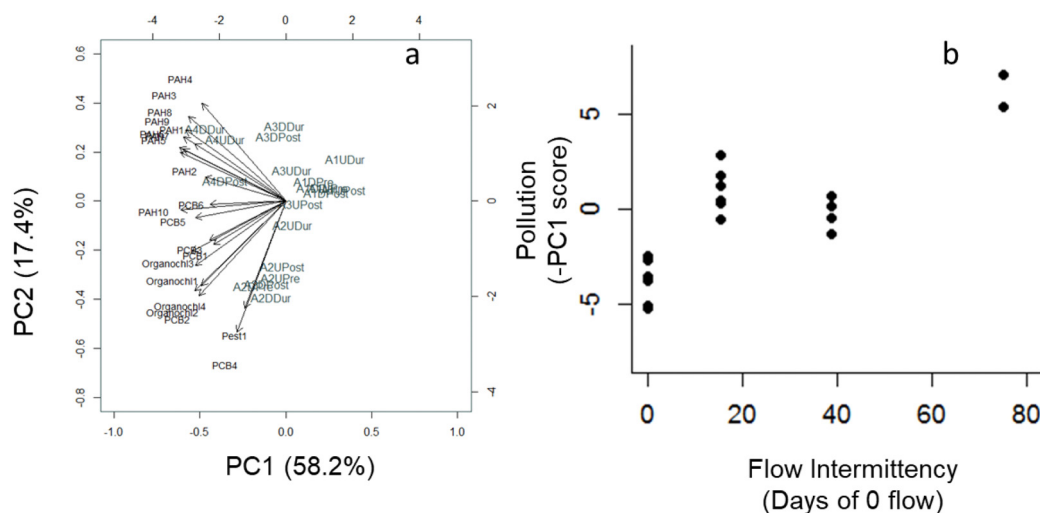


Fig. 2. PCA performed with organic pollutant concentrations in the sites (a). Percentage of variance explained by each PC in parenthesis. PAH1–9 correspond to PAHs, Organochl1–4 correspond to organochlorides, PCB1–6 correspond to PCBs, Pest1 correspond to pesticide. In dark gray are the sites, A1 to A4 correspond to the four sites, U and D correspond to their locations upstream (U) or downstream (D) of the olive oil mills, Pre, Dur, Post correspond to the time of sampling before, during and after operation respectively. Scaling on top and right axis correspond to the organic pollutants (arrows). (b) Correlation between flow intermittency (days of zero flow during the study) and pollution representing the inverse of the scores of axis 1 of the PCA (-PC1 score), therefore the higher the number in the y-axis, the higher the pollution.

For consistency, only results from Shannon and Simpson indices that explain different aspects of community structure are presented in the figures of the main text and indices that presented significant relationships with stressors and ecosystem processes are discussed. Results from all the applied indices are presented in Table S3.

3.1. Effects of pollution and flow intermittency on biodiversity

Species richness, Shannon diversity and Functional richness of the diatom assemblages were significantly increased with pollution (as summarized by PC1 - Table S3, Fig. 3a, b). Species richness, Shannon diversity and Functional richness of the macroinvertebrate communities significantly decreased, whereas Simpson dominance significantly increased with increased pollution (as summarized by PC1) (Table S3, Fig. 3c, d).

Species richness, Shannon diversity, Pielou's Evenness and Functional richness of diatoms assemblages significantly increased with increasing days of zero flow (Table S3), while Simpson dominance presented a non-significant decreasing trend with increasing days of zero flow (Table S3, Fig. 3e, f). In contrast, Species richness, Shannon diversity and Functional richness of macroinvertebrate communities significantly decreased with increasing days of zero flow, whereas Simpson dominance presented an increasing, but not significant, trend (Table S3, Fig. 3g, h).

3.2. Effect of pollution and flow intermittency on ecosystem functioning

Resource use efficiency (RUE) decreased with increased pollution (LRT: $\chi^2 = 6.09$, DF = 1, $p < 0.05$, Fig. 4a). This trend was mainly driven by the increased RUE in the P-limited sites A1 and A2. Organic matter breakdown (OMB) also decreased with increased pollution (LRT: $\chi^2 = 16.77$, DF = 1, $p < 0.001$, Fig. 4c). RUE decreased with increased number of days of zero flow (LRT: $\chi^2 = 9.38$, DF = 4, $p < 0.01$ - Fig. 4b). The two most intermittent sites (A3 and A4) were also N-limited and presented the lowest RUE. OMB also decreased with increased number of days of zero flow (LRT: $\chi^2 = 7.31$, DF = 4, $p < 0.01$ - Fig. 4d).

3.3. Biodiversity-ecosystem functioning relationships

Ecosystem processes responded better to structural diversity indices of the two biotic groups than to functional diversity indices, except in the case of diatom functional evenness. The diversity indices

significantly related to RUE in diatoms were Shannon diversity, Pielou's Evenness, Simpson dominance and Functional Evenness (Table S4). Specifically, RUE decreased with higher diversity, evenness and functional evenness (Fig. 5), whereas it increased with increased Simpson dominance. Structural indices of macroinvertebrates related to OMB were Shannon diversity and Simpson dominance (Table S4). Organic matter breakdown increased with higher macroinvertebrate diversity and decreased with higher values of dominance (Fig. 5). Overall, the two biotic groups tested presented an opposite trend in B-EF relationships, decreasing in the case of diatoms and increasing in the case of macroinvertebrates. The Shannon diversity of macroinvertebrates was positively correlated with the percentage of grazers (Spearman $r = 0.60$, $p < 0.05$) and shredders (Spearman $r = 0.51$, $p < 0.05$), the two feeding groups that could contribute more to OMB.

4. Discussion

Here we assessed the effect of multiple stressors on the structure of diatom assemblages and macroinvertebrate communities in temporary streams. We also assessed the response of ecosystem processes that are related to these two biotic groups. We observed that even though the biological communities in temporary ecosystems are naturally adapted to a certain level of water stress, they are particularly fragile to anthropogenic pollution and further water reduction due to global change. Disentangling the effects of these co-occurring multiple stressors (i.e. flow intermittency and pollution), is complicated by the accumulation of pollutants in the sediments during dry periods (Zoppini et al., 2014, 2016). Indeed, sediment pollution and flow intermittency were strongly related in our study sites, pollution recording higher levels in the more intermittent sites, where lower dilution, lengthier pollutant transport and lower self-purification processes occurred (Karaouzas et al., 2011b; Mandaric et al., 2018).

The two studied stressors (i.e. sediment pollution and flow intermittency) affected structural and functional diversity of diatoms and macroinvertebrates in a different manner. Effects were negative on macroinvertebrate diversity but positive on diatom diversity. The negative effect of pollution on macroinvertebrate communities has been reported to intensify during low flows, with pollution sensitive taxa being almost extirpated (Kalogianni et al., 2017). The combination of the two stressors could reduce macroinvertebrate diversity and increase dominance at the same time, as community structure changes from rheo- to limnophilic taxa, leading to dominance of functional groups

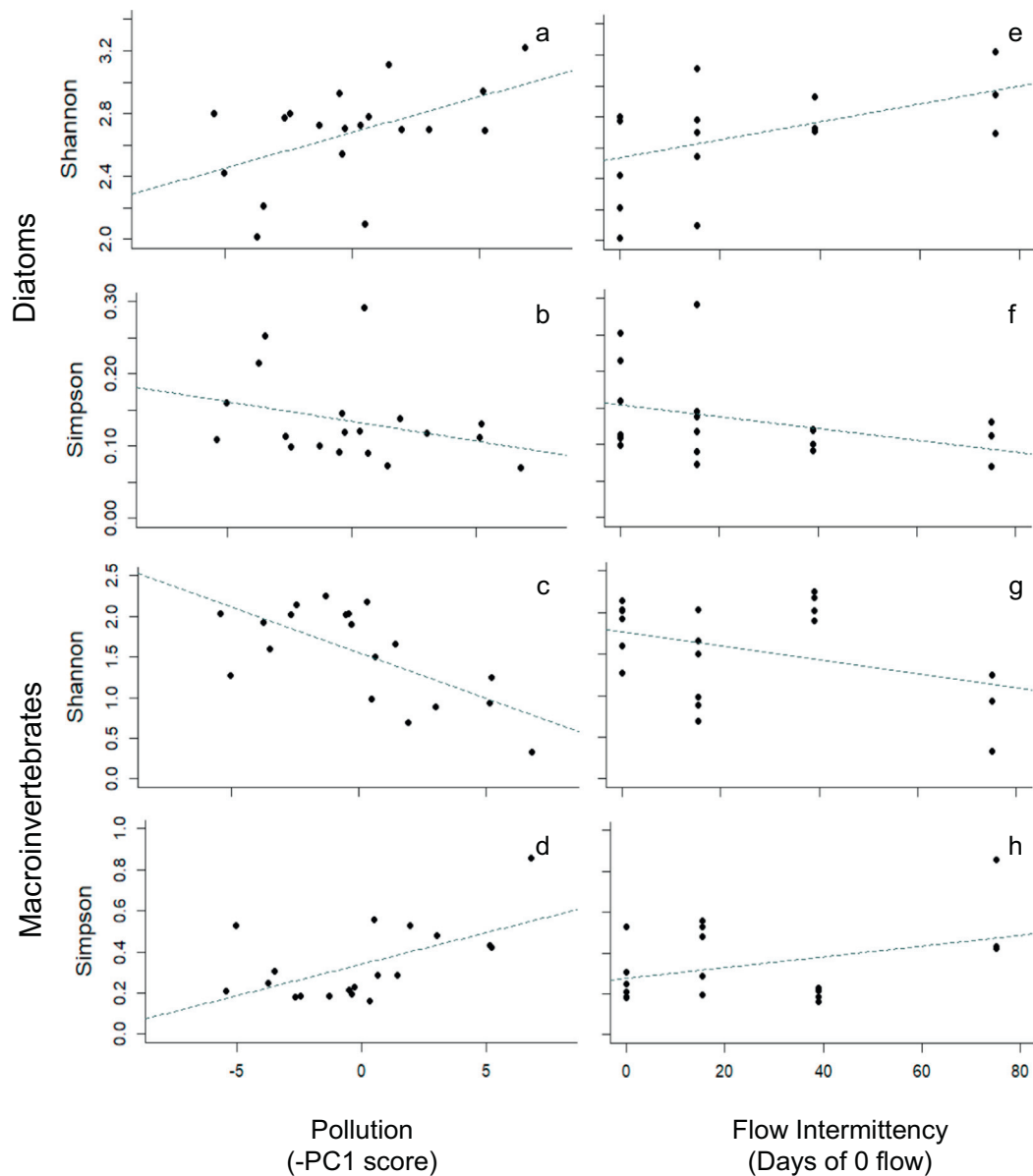


Fig. 3. Relationship between pollution (PC1) and diversity indices (a–d) and flow intermittency and diversity indices (e–h). a,b,e,f correspond to diatom indices, c,d,g,h correspond to macroinvertebrate indices. Pollution is represented by the inverse of the scores of axis 1 of the PCA (-PC1 score) and flow intermittency by the number of days of zero flow.

associated with these stressors (e.g. Bonada et al., 2007; García-Roger et al., 2013; Karaouzas et al., 2018c). On the other hand, the positive response of diatom assemblages could be related to the reported unimodal relationships between diversity and pollution (e.g. Pandey et al., 2017). Our streams did not range throughout the whole pollution gradient but rather between the low to intermediate pollution parts of the curve. Further, flow intermittency did not affect species richness but rather the structure of the assemblage by favoring the weakly attached species (i.e. high profile species), and thus biofilm evenness (Passy, 2007). Biofilms were thicker, composed by both low and high profile species, which occupied more available niche space (Ponsati et al., 2016; Torresi et al., 2016). The thicker biofilm formation also coincides with higher pollution and high flow intermittency, resulting in increased accumulation of organic matter (Passy, 2007). Thicker biofilms impeding efficient light penetration and nutrient sequestration, resulting in lower photosynthetic activity and RUE (Burns and Ryder, 2001).

The two studied ecosystem processes responded similarly to both pollution and flow intermittency; both stressors reduced RUE and

OMB, in agreement to previous studies. Indeed, other experimental and field studies have also shown the decrease of primary productivity with increasing days of zero flow (Acuña et al., 2015; Timoner et al., 2014). Primary production is directly linked to RUE: the presence of primary producers that use more efficiently the resources, converting them to biomass, leads to increased primary production in the biofilm (Ptacnik et al., 2008). Pollution could result in a more pronounced response, probably as a result of the additive effect it produces with water stress on autotrophic processes in biofilms (Corcoll et al., 2015), as well as on macroinvertebrate communities (Karaouzas et al., 2018c). Organic matter breakdown could be related to certain macroinvertebrate groups (i.e. detritivores) but also to the presence of microbes, especially bacteria and fungi (Gessner and Chauvet, 2002; Tank et al., 2010; Chauvet et al., 2016). Microbial activity is an important factor in OMB, especially in wood decomposition (Bilby, 2003); however, it was not accounted for in the present study. Nevertheless, invertebrates have been shown to also play a role in wood decomposition (Collier and Halliday, 2000; Eloegi et al., 2007), and our data (Eloegi, unpublished) shows decomposition of tongue depressors as those used in the present

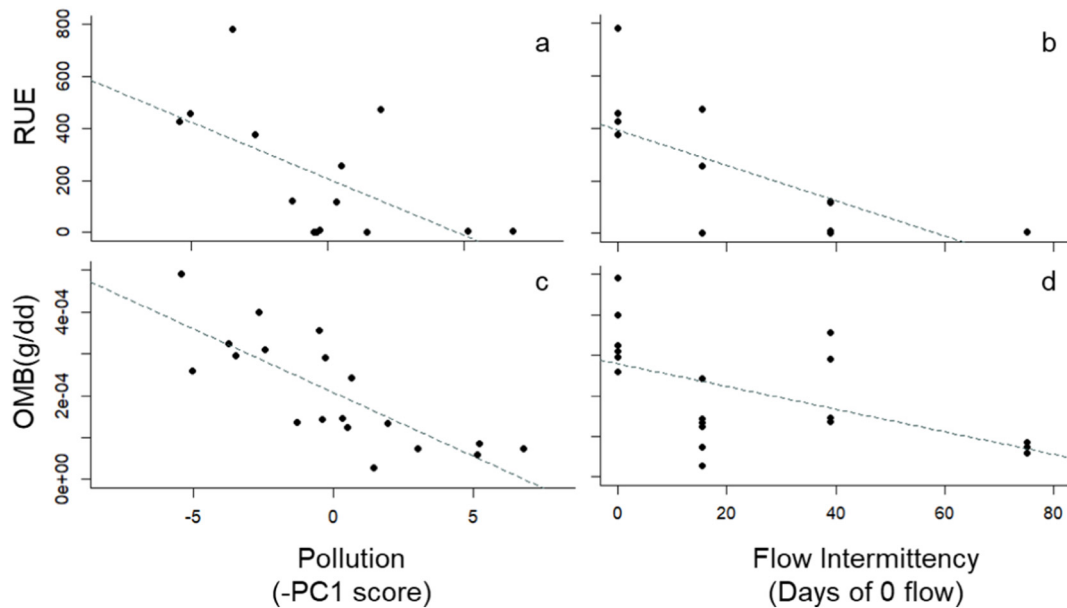


Fig. 4. Linear relationships of pollution (PC1) and flow intermittency (days of zero flow during the study) with (a,b) resource use efficiency (RUE) and (c,d) organic matter breakdown (OMB) across the study sites. Pollution is represented by the inverse of the scores of axis 1 of the PCA (-PC1 score).

study to be more correlated to total than to microbial breakdown. Water stress affects shredders and detritus feeders and could thus result in decreased leaf litter breakdown (Monroy et al., 2016; Karaouzas et al., 2018c). Furthermore, many shredders and detritus feeders are regarded as pollution-sensitive taxa, thus increased pollution levels could also affect OMB (Young et al., 2008; Woodward et al., 2012; Bundschuh and McKie, 2016; Kalogianni et al., 2017).

The ecosystem processes we tested showed significant responses to diversity indices other than species richness, suggesting an important effect of community structure to ecosystem functioning. The relationship between ecosystem functioning and evenness (or dominance) remains equivocal (Hillebrand et al., 2008), and this was reflected in our contrasting results between the two studied biological groups. Although macroinvertebrates showed a positive B-EF relationship (i.e. the more diverse the community, the more intense was OMB), diatoms presented

a negative diversity-evenness relationship (i.e. the more species in the assemblage and the more evenly distributed, the less efficient was the resource use). These apparently contradictory results could be explained by either the different drivers that rule each community assembly (e.g. environmental constraints, species interactions, different trophic level, different response to stressors) or by the different mechanisms at play in the two communities (i.e. selection vs. complementarity effect). Different drivers in our study could be the multiple stressors tested and the different response of the two groups, suggesting that the B-EF relationship could be driven by the stressor effects on biodiversity. On the other hand, B-EF theory has proposed two mechanisms to explain the increased productivity of communities following an increase in species richness: selection effect, which occurs when increased species richness leads to increased probability of having a species that yields high biomass (Aarssen, 1997), and complementarity effect,

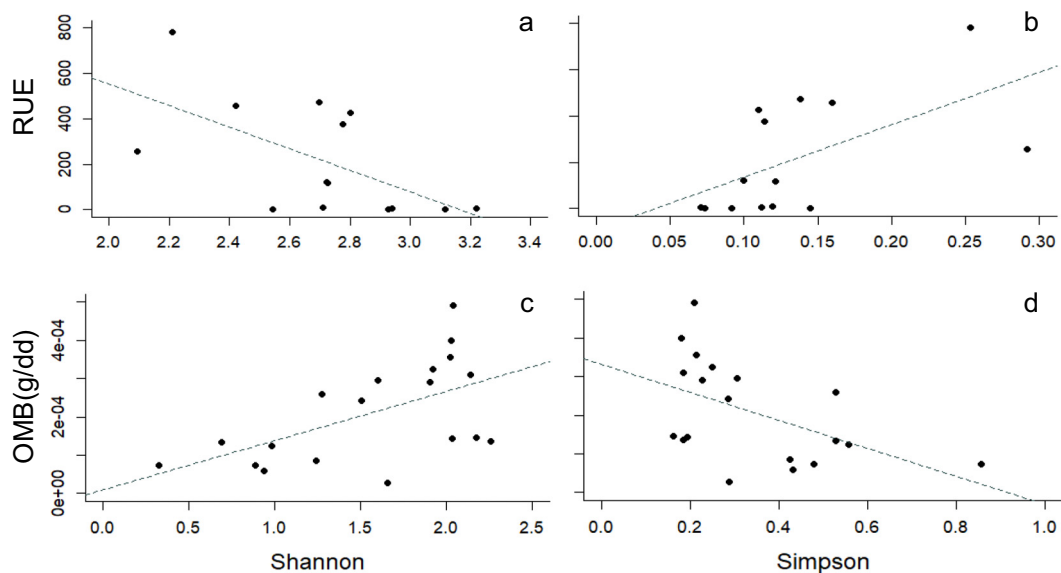


Fig. 5. Linear relationships between diversity indices and ecosystem processes. a–d correspond to the relationship between diatom diversity indices and RUE, e–f correspond to the relationship between macroinvertebrate diversity and OMB.

which occurs when more species increase their productivity through a complementary use of resources (Hector, 1998). These two mechanisms satisfactorily explain contradictions in plant communities, where the evenness (dominance)-ecosystem functioning relationship has been more extensively studied (Polley et al., 2003; Hillebrand et al., 2008).

In the diatom assemblages, high dominance in the less polluted, perennial stream (i.e. A1), resulted in low diversity (Shannon index) and high RUE. This increased RUE could be related to selection effects (Aarssen, 1997), where a few species could yield the highest relative abundance and were primarily contributing to ecosystem functioning. In macroinvertebrate communities, the positive relationship between macroinvertebrate diversity and OMB could be attributed to complementarity effects (Hector, 1998), as the added species in the community have a different role in the ecosystem, and thus OMB increases when present. Complementarity effects have already been reported in caddisfly larvae, where increased species diversity resulted in facilitation in resource consumption (Cardinale et al., 2002). The positive B-EF relationship in macroinvertebrates has also been reported for grazing and shredding insects (Jonsson and Malmqvist, 2000; Huryn et al., 2002). Indeed, increased Shannon diversity in our results is consistent with increased percentages of grazers and shredders, suggesting that these groups could be enhancing OMB.

5. Conclusions

Multiple stressors acting together in temporary rivers affect both biodiversity and ecosystem functioning. Ecosystem functioning is mainly driven by interactions between species and between species and their environment, the latter being increasingly affected by co-occurring stressors. These ecosystems are very vulnerable to climate change and anthropogenic stressors that could cause the loss of species and thus the loss of ecosystem functioning, with implications on the ecosystem goods and services they provide. This study tested the effect of anthropogenic pollution and flow intermittency on biodiversity and ecosystem functioning in temporary rivers, and established the B-EF relationships in two biotic groups (i.e. diatoms and macroinvertebrates). Even though it is a relatively small-scale study, our results suggest that ecosystem processes are driven by community structure in both groups, positively for macroinvertebrates but negatively for diatoms, suggesting different mechanisms underlying the observed trends. Future work could focus on larger-scale studies and more ecosystem processes to further establish observed relationships that could provide better insight on the protection of these vulnerable ecosystems.

Acknowledgments

The authors would like to thank P. Kouraklis for field work, A. Vourka, C. Theodoropoulos, A. Andriopoulou and M. Koutsodimou for laboratory assistance in macroinvertebrate identification and E. Anastasopoulou for chemical analysis. This study was conducted within the frame of the European Communities 7th Framework Programme under Grant Agreement no 603629-ENV-2013-6.2.1-GLOBAQUA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.08.105>.

References

Aarssen, L.W., 1997. High productivity in grassland ecosystems: affected by species diversity or productive species? *Oikos* 80, 183–184.
 Abonyi, A., Horváth, Z., Płacnik, R., 2017. Functional richness outperforms taxonomic richness in predicting ecosystem functioning in natural phytoplankton communities. *Freshw. Biol.* 63, 178–186.

Abril, M., Muñoz, I., Menéndez, M., 2016. Heterogeneity in leaf litter decomposition in a temporary Mediterranean stream during flow fragmentation. *Sci. Total Environ.* 553, 330–339.
 Acuña, V., Datry, T., Marshall, J., Barceló, D., Dahm, C.N., Ginebreda, A., McGregor, G., Sabater, S., Tockner, K., Palmer, M.A., 2014. Why should we care about temporary waterways? *Science* 343, 1080–1081.
 Acuña, V., Casellas, M., Corcoll, N., Timoner, X., Sabater, S., 2015. Increasing extent of periods of no flow in intermittent waterways promotes heterotrophy. *Freshw. Biol.* 60, 1810–1823.
 Alonso, M.C., Puig, D., Silgoner, I., Grasserbauer, M., Barceló, D., 1998. Determination of priority phenolic compounds in soil samples by various extraction methods followed by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *J. Chromatogr. A* 823, 231–239.
 AQEM Consortium, 2002. Manual for the application of the AQEM system. A Comprehensive Method to Assess European Streams Using Benthic Macroinvertebrates. Developed for the Purpose of the Water Framework Directive. Version 1.0, February 2002.
 Arenas-Sánchez, A., Rico, A., Vighi, M., 2016. Effects of water scarcity and chemical pollution in aquatic ecosystems: state of the art. *Sci. Total Environ.* 572, 390–403.
 Arroita, M., Aristi, I., Flores, L., Larrañaga, A., Díez, J., Mora, J., Romani, A.M., Elosegi, A., 2012. The use of wooden sticks to assess stream ecosystem functioning: comparison with leaf breakdown rates. *Sci. Total Environ.* 440, 115–122.
 Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48.
 Bilby, R.E., 2003. Decomposition and nutrient dynamics of wood in streams and rivers. In: Gregory, S.V., Boyer, K.L., Gurnell, A.M. (Eds.), *The Ecology and Management of Wood in World Rivers*. American Fisheries Society, Bethesda, MD, pp. 135–147.
 Bonada, N., Rieradevall, M., Prat, N., 2007. Macroinvertebrate community structure and biological traits related to flow permanence in a Mediterranean river network. *Hydrobiologia* 589, 91–106.
 Bundschuh, M., McKie, B.G., 2016. An ecological and ecotoxicological perspective on fine particulate organic matter in streams. *Freshw. Biol.* 61, 2063–2074.
 Burns, A., Ryder, D.S., 2001. Potential for biofilms as biological indicators in Australian riverine systems. *Ecol. Manag. Restor.* 2, 53–64.
 Cardinale, B.J., 2011. Biodiversity improves water quality through niche partitioning. *Nature* 472, 86–91.
 Cardinale, B.J., Palmer, M.A., Collins, S.L., 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature* 415, 426.
 Chauvet, E., Ferreira, V., Giller, P.S., McKie, B.G., Tiegs, S.D., Woodward, G., Elosegi, A., Dobson, M., Fleituch, T., Graça, M.A.S., Gulis, V., Hladysz, S., Lacoursière, J.O., Lecerf, A., Pozo, J., Preda, E., Riipinen, M., Říšnovanu, G., Vadineanu, A., Vought, L.B.M., Gessner, M.O., 2016. Litter decomposition as an indicator of stream ecosystem functioning at local-to-continental scales: insights from the European RivFunction project. *Adv. Ecol. Res.* 55, 99–182.
 Collier, K.J., Halliday, J.N., 2000. Macroinvertebrate–wood associations during decay of plantation pine in New Zealand pumice-bed streams: stable habitat or trophic subsidy? *J. N. Am. Benthol. Soc.* 19, 91–111.
 Corcoll, N., Casellas, M., Huerta, B., Guasch, H., Acuña, V., Rodríguez-Mozaz, S., Serra-Compte, A., Barceló, D., Sabater, S., 2015. Effects of flow intermittency and pharmaceutical exposure on the structure and metabolism of stream biofilms. *Sci. Total Environ.* 503–504, 159–170.
 Cutter, G.A., Radford-Knoery, J., 1991. Determination of carbon, nitrogen, sulfur and inorganic sulfur species in marine particles. In: Hurd, D.C., Derek, D.W. (Eds.), *Marine Particles: Analysis and Characterization*. Geophysical Monograph 63, pp. 57–63.
 Czaplicka, M., 2001. Determination of phenols and chlorophenols in bottom sediments. *Chromatographia* 53 (Supplement 1), 471–473.
 Danis, T.G., Albanis, T.A., 1996. *Toxicological and Environmental Chemistry*. 53.
 Datry, T., Larned, S.T., Fritz, K.M., Bogan, M.T., Wood, P.J., Meyer, E.I., Santos, A.N., 2014. Broad scale patterns of invertebrate richness and community composition in temporary rivers: effects of flow intermittence. *Ecography* 37, 94–104.
 Datry, T., Boulton, A.J., Bonada, N., Fritz, K., Leigh, C., Sauquet, E., Tockner, K., Hugueny, B., Dahm, C.N., 2017. Flow intermittence and ecosystem services in rivers of the Anthropocene. *J. Appl. Ecol.* 55, 353–364.
 Elosegi, A., Díez, J.R., Pozo, J., 2007. Contribution of dead wood to the carbon flux in forested streams. *Earth Surf. Process. Landf.* 32, 1219–1228.
 Ettinger, M., Ruchhoff, C., Lishka, R., 1951. Sensitive 4-aminoantipyrine method for phenolic compounds. *Anal. Chem.* 23, 1783–1788.
 European Committee for Standardization, 2003. Guidance Standard for the Routine Sampling and Pretreatment of Benthic Diatoms From Rivers. CEN/TC 230. EN 13946 (14 pp.).
 Fraimer, A., 2013. *Ecosystem Functioning in Streams: Disentangling the Roles of Biodiversity, Stoichiometry, and Anthropogenic Drivers*. Department of Ecology and Environmental Science, Umeå (44 pp.).
 Fraimer, A., McKie, B.G., Malmqvist, B., 2014. When does diversity matter? Species functional diversity and ecosystem functioning across habitats and seasons in a field experiment. *J. Anim. Ecol.* 83, 460–469.
 García-Roger, E.M., Sánchez-Montoya, M.M., Cid, N., Erba, S., Karaouzas, I., Verkaik, I., Rieradevall, M., Gómez, R., Suárez, M.L., Vidal-Abarca, M.R., Demartini, D., Buffagni, A., Skoulikidis, N., Bonada, N., Prat, N., 2013. Spatial scale effects on taxonomic and biological trait diversity of aquatic macroinvertebrates in Mediterranean streams. *Fundam. Appl. Limnol.* 183/2, 89–105.
 Gessner, M.O., Chauvet, E., 2002. A case for using litter breakdown to assess functional stream integrity. *Ecol. Appl.* 12, 498–510.
 Gessner, O.M., Swan, M.C., Dang, K.C., McKie, G.B., Bardgett, D.R., Wall, H.D., Hättenschwile, S., 2010. Diversity meets decomposition. *Trends Ecol. Evol.* 25, 372–380.
 Graça, M.A., 2001. The role of invertebrates on leaf litter decomposition in streams—a review. *Int. Rev. Hydrobiol.* 86, 383–393.

- Guasch, H., Sabater, S., 1995. Seasonal variations in photosynthesis-irradiance responses by biofilms in Mediterranean streams. *J. Phycol.* 31, 727–735.
- Hector, A., 1998. The effect of diversity on productivity: detecting the role of species complementarity. *Oikos* 82, 597.
- Hillebrand, H., Bennett, D.M., Cadotte, M.W., 2008. Consequences of dominance: a review of evenness effects on local and regional ecosystem processes. *Ecology* 89, 1510–1520.
- Hury, A.D., Hury, V.M.B., Arbuckle, C.J., Tsomides, L., 2002. Catchment land-use, macroinvertebrates and detritus processing in headwater streams: taxonomic richness versus function. *Freshw. Biol.* 47, 401–415.
- Jeffrey, S.W.T., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* 167 (2), 191–194.
- Jonsson, M., Malmqvist, B., 2000. Ecosystem process rate increases with animal species richness: evidence from leaf-eating, aquatic insects. *Oikos* 89, 519–523.
- Juhász, I., 2016. Evaluation of macroinvertebrate data based on autoecological information. *Slovak J. Civ. Eng.* 24, 36–44.
- Kalogianni, E., Vourka, A., Karaouzas, I., Vardakas, L., Laschou, S., Skoulikidis, N.T., 2017. Combined effects of water stress and pollution on macroinvertebrate and fish assemblages in a Mediterranean intermittent river. *Sci. Total Environ.* 603–604, 639–650.
- Karaouzas, I., Lambropoulou, D.A., Skoulikidis, N.T., Albanis, T.A., 2011a. Levels, sources and spatiotemporal variation of nutrients and micropollutants in small streams of a Mediterranean River basin. *J. Environ. Monit.* 13, 3064–3074.
- Karaouzas, I., Skoulikidis, N.T., Giannakou, U., Albanis, T.A., 2011b. Spatial and temporal effects of olive mill wastewaters to stream macroinvertebrates and aquatic ecosystems status. *Water Res.* 45, 6334–6346.
- Karaouzas, I., Theodoropoulos, C., Vardakas, L., Kalogianni, E., Skoulikidis, N.Th., 2018a. A review of the effects of pollution and water scarcity on the stream biota of an intermittent Mediterranean basin. *River Res. Appl.* 34, 291–299.
- Karaouzas, I., Theodoropoulos, C., Vardakas, L., Zogaris, S., Skoulikidis, N., 2018b. The Evrotas River Basin: 10 years of ecological monitoring. In: Skoulikidis, N., Dimitriou, E., Karaouzas, I. (Eds.), *The Rivers of Greece: Evolution, Current Status and Perspectives*.
- Karaouzas, I., Smeti, E., Vourka, A., Vardakas, L., Mentzafou, A., Tornés, E., Sabater, S., Muñoz, I., Skoulikidis, N.T., Kalogianni, E., 2018c. Assessing the ecological effects of water stress and pollution in a temporary river - implications for water management. *Sci. Total Environ.* 618, 1591–1604.
- Keith, L.H., 2015. The source of U.S. EPA's sixteen PAH priority pollutants. *Polycycl. Aromat. Compd.* 35 (2–4), 147–160.
- Köck-Schulmeyer, M., Villagrasa, M., López de Alda, M., Céspedes-Sánchez, R., Ventura, F., Barceló, D., 2013. Occurrence and behavior of pesticides in wastewater treatment plants and their environmental impact. *Sci. Total Environ.* 458–460, 466–476.
- Laliberté, E., Legendre, P., Shipley, B., 2014. FD: Measuring Functional Diversity From Multiple Traits, and Other Tools for Functional Ecology. R Package Version 1.0-12.
- Lecerf, A., Richardson, J.S., 2010. Biodiversity–ecosystem function research: insights gained from streams. *River Res. Appl.* 26, 45–54.
- Lindeman, R.L., 1942. The trophic-dynamic aspect of ecology. *Ecology* 4, 399–417.
- Liu, J., Soiminen, J., Han, B.P., Declerck, S.A.J., 2013. Effects of connectivity, dispersal directionality and functional traits on the metacommunity structure of river benthic diatoms. *J. Biogeogr.* 40, 2238–2248.
- Mandarić, L., Kalogianni, E., Skoulikidis, N.Th., Petrovic, M., Sabater, S., 2018. Contamination patterns and attenuation of pharmaceuticals in a temporary Mediterranean river. *Sci. Total Environ.* 647, 561–569.
- Martínez, E., Gros, M., Lacorte, S., Barceló, D., 2004. Simplified procedures for the analysis of polycyclic aromatic hydrocarbons in water, sediment and mussels. *J. Chromatogr. A* 1047, 181–188.
- Mason, N.W.H., Mouillot, D., Lee, W.G., Wilson, J.B., 2005. Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos* 111, 112–118.
- McDonough, O.T., Hosen, J.D., Palmer, M.A., 2011. Temporary streams: the hydrology, geography and ecology of non-perennially flowing waters. In: Elliot, H.S., Martin, L.E. (Eds.), *River Ecosystems: Dynamics*. Nova Science Publ. Inc., Management and Conservation, pp. 259–289.
- McKie, B.G., Woodward, G., Hladý, S., Nistorescu, M., Preda, E., Popescu, C., Giller, P.S., Malmqvist, B., 2008. Ecosystem functioning in stream assemblages from different regions: contrasting responses to variation in detritivore richness, evenness and density. *J. Anim. Ecol.* 77, 495–504.
- Monroy, S., Menéndez, M., Basaguren, A., Pérez, J., Elosegi, A., Pozo, J., 2016. Drought and detritivores determine leaf litter decomposition in calcareous streams of the Ebro catchment (Spain). *Sci. Total Environ.* 573, 1450–1459.
- Navarro-Ortega, A., Tauler, R., Lacorte, S., Barceló, D., 2010. Occurrence and transport of Phas, pesticide and alkylphenols in sediment samples along the Ebro River Basin. *J. Hydrol.* 383, 5–17.
- Odum, H.T., 1956. Primary production in flowing waters. *Limnol. Oceanogr.* 1, 102–117.
- OECD, 1982. *Eutrophication of Waters: Monitoring, Assessment and Control* (Paris).
- Pandey, L.K., Bergey, E.A., Lyu, J., Park, J., Choi, S., Lee, H., Depuydt, S., Oh, Y.T., Lee, S.M., Han, T., 2017. The use of diatoms in ecotoxicology and bioassessment: insights, advances and challenges. *Water Res.* 118, 39–58.
- Passy, S., 2007. Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquat. Bot.* 86, 171–178.
- Petersen, R.C., Cummins, K.W., 1974. Leaf processing in a woodland stream. *Freshw. Biol.* 4, 343–368.
- Pielou, E.C., 1975. *Ecological Diversity*. Wiley InterScience, New York (165 pp.).
- Polley, H.W., Wilsey, B.J., Derner, J.D., 2003. Do species evenness and plant density influence the magnitude of selection and complementarity effects in annual plant species mixtures? *Ecol. Lett.* 6, 248–256.
- Ponsatí, L., Corcoll, N., Petrović, M., Picó, Y., Ginebreda, A., Tornés, E., Guasch, H., Barceló, D., Sabater, S., 2016. Multiple-stressor effects on river biofilms under different hydrological conditions. *Freshw. Biol.* 61, 2102–2115.
- Ptácnik, R., Solimini, A.G., Andersen, T., Tamminen, T., Brettum, P., Lepistö, L., Willén, E., Rekolainen, S., 2008. Diversity predicts stability and resource use efficiency in natural phytoplankton communities. *Proc. Natl. Acad. Sci.* 105, 5134–5138.
- Quesada, S., Tena, A., Ginebreda, A., Vericat, D., Martínez, E., Navarro-Ortega, A., Batalla, R.J., Barceló, D., 2013. Dynamics of suspended sediment borne persistent organic pollutants in a large regulated Mediterranean river (Ebro, NE Spain). *Sci. Total Environ.* 473–474, 381–390.
- R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria <https://www.R-project.org/>.
- Schäfer, R.B., Caquet, T., Siimes, K., Mueller, R., Lagadic, L., Liess, M., 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. *Sci. Total Environ.* 382, 272–285.
- Schmera, D., Heino, J., Podani, J., Erős, T., Dolédec, S., 2017. Functional diversity: a review of methodology and current knowledge in freshwater macroinvertebrate research. *Hydrobiologia* 787, 27–44.
- Shannon, C.E., Weaver, W., 1949. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana.
- Simpson, E.H., 1949. Measurement of diversity. *Nature* 163, 688.
- Skoulikidis, N., Vardakas, L., Karaouzas, I., Economou, A., Dimitriou, E., Zogaris, S., 2011. Water stress in Mediterranean lotic systems; impacts and management implications in an artificially intermittent river (Evrotas River, Greece). *Aquatic Science Special Issue: Recent Perspectives on Temporary River Ecology*. 73, pp. 581–597.
- Skoulikidis, N.T., Sabater, S., Detry, T., Morais, M.M., Buffagni, A., Dörflinger, G., Zogaris, S., del Mar Sánchez-Montoya, M., Bonada, N., Kalogianni, E., Rosado, J., Vardakas, L., De Girolamo, A.M., Tockner, K., 2017. Non-perennial Mediterranean rivers in Europe: status, pressures, and challenges for research and management. *Sci. Total Environ.* 577, 1–18.
- Soria, M., Leigh, C., Detry, T., Bini, L.M., Bonada, N., 2017. Biodiversity in perennial and intermittent rivers: a meta-analysis. *Oikos* 126, 1078–1089.
- Steinman, A.D., Lamberti, G.A., Leavitt, P.R., 2007. Biomass and pigments of benthic algae. In: Hauer, F.R., Lamberti, G.A. (Eds.), *Methods in Stream Ecology*. Academic Press, pp. 357–379.
- Tank, J.L., Rosi-Marshall, E.J., Griffiths, N.A., Entekhabi, S.A., Stephen, M.L., 2010. A review of allochthonous organic matter dynamics and metabolism in streams. *J. N. Am. Benthol. Soc.* 29, 118–146.
- Tilman, D., Reich, P.B., Isbell, F., 2012. Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. *Proc. Natl. Acad. Sci. U. S. A.* 109, 10394–10397.
- Timoner, X., Acuña, V., Frampton, L., Pollard, P., Sabater, S., Bunn, S.E., 2014. Biofilm functional responses to the rehydration of a dry intermittent stream. *Hydrobiologia* 727, 185–195.
- Torres, E., Fowler, S.J., Polesel, F., Bester, K., Andersen, H.R., Smets, B.F., Plósz, B.G., Christensson, M., 2016. Biofilm thickness influences biodiversity in nitrifying mbbsr – implications on micropollutant removal. *Environ. Sci. Technol.* 50, 9279–9288.
- Truchy, A., Angeler, D.G., Sponseller, R.A., Johnson, R.K., McKie, B.G., 2015. Linking biodiversity, ecosystem functioning and services, and ecological resilience: towards an integrative framework for improved management. *Adv. Ecol. Res.* 53, 55–96.
- Tzoraki, O., Karaouzas, I., Patrolocco, L., Skoulikidis, N., Nikolaidis, N.P., 2015. Polycyclic aromatic hydrocarbons (PAHs) and heavy metal occurrence in bed sediments of a temporary river. *Water Air Soil Pollut.* 226, 421.
- Van Dam, H., Mertens, A., Sinkeldam, J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. *Neth. J. Aquat. Ecol.* 28, 117–133.
- Verardo, D.J., Froelich, P.N., McIntyre, A., 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyzer. *Deep-Sea Res.* 37, 157–165.
- von Schiller, D., Acuña, V., Aristi, I., Arroita, A., Basaguren, A., Bellin, A., Boyero, L., Butturini, A., et al., 2017. River ecosystem processes: a synthesis of approaches, criteria of use and sensitivity to environmental stressors. *Sci. Total Environ.* 596–597, 465–480.
- Woodward, G., Gessner, M.O., Giller, P.S., Gulis, V., Hladý, S., Lecerf, A., Malmqvist, B., McKie, B.G., Tiegs, S.D., Cariss, H., Dobson, M., Elosegi, A., Ferreira, V., Graça, M.A., Fleituch, T., Lacoursière, J.O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B., Chauvet, E., 2012. Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science* 336, 1438–1440.
- Young, R.G., Matthaei, C.D., Townsend, C.R., 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *J. N. Am. Benthol. Soc.* 27 (3), 605–625.
- Zoppini, A., Ademollo, N., Amalfitano, S., Casella, P., Patrolocco, L., Polesello, S., 2014. Organic priority substances and microbial processes in river sediments subject to contrasting hydrological conditions. *Sci. Total Environ.* 484, 74–83.
- Zoppini, A., Ademollo, N., Amalfitano, S., Capri, S., Casella, P., Fazi, S., Marxsen, J., Patrolocco, L., 2016. Microbial responses to polycyclic aromatic hydrocarbon contamination in temporary river sediments: experimental insights. *Sci. Total Environ.* 541, 1364–1371.