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Organic priority substances and microbial processes in river sediments subject to contrasting hydrological conditions

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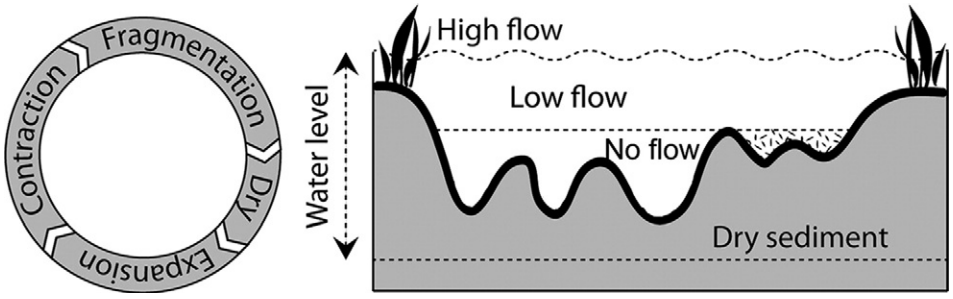
HIGHLIGHTS

- Hydrological conditions affected the circulation of PAHs and NPs in river sediments.
- Contrasting microbial metabolic rates were associated with sediment contamination.
- The proposed multiple approach identifies novel scenarios on pollutant dynamics.

GRAPHICAL ABSTRACT



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ABSTRACT

Flood and drought events of higher intensity and frequency are expected to increase in arid and semi-arid regions, in which temporary rivers represent both a water resource and an aquatic ecosystem to be preserved. In this study, we explored the variation of two classes of hazardous substances (Polycyclic Aromatic Hydrocarbons and Nonylphenols) and the functioning of the microbial community in river sediments subject to hydrological fluctuations (Candelaro river basin, Italy). Overall, the concentration of pollutants ($\sum$ PAHs range 8–275 ng g^{-1}; $\sum$ NPs range 299–4858 ng g^{-1}) suggests a moderate degree of contamination. The conditions in which the sediments were tested, flow (high/low) and no flow (wet/dry/arid), were associated to significant differences in the chemical and microbial properties. The total organic carbon contribution decreased together with the stream flow reduction, while the contribution of C-PAHs and C-NPs tended to increase. NPs were relatively more concentrated in sediments under high flow, while the more hydrophobic PAHs accumulated under low and no flow conditions. Passing from high to no flow conditions, a gradual reduction of microbial processes was observed, to reach the lowest specific bacterial carbon production rates (0.06 fmol C h^{-1} $cell^{-1}$), extracellular enzyme activities, and the highest doubling time (40 h) in arid sediments.
In conclusion, different scenarios for the mobilization of pollutants and microbial processes can be identified under contrasting hydrological conditions: (i) the mobilization of pollutants under high flow and a relatively higher probability for biodegradation; (ii) the accumulation of pollutants during low flow and lower probability for biodegradation; (iii) the drastic reduction of pollutant concentrations under dry and arid conditions, probably independently from the microbial activity (abiotic processes). Our findings let us infer that a multiple approach has to be considered for an appropriate water resource exploitation and a more realistic prevision of the impact of pollutants in temporary waters.

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1. Introduction

The global mean rates of precipitation and evapotranspiration are expected to increase, together with flood and drought events of higher intensity and frequency in the arid and semi-arid regions of the world (IPCC, 2007). Such changes will have significant impacts on regional water resources in quantity and/or quality.

Temporary rivers are widespread in semi-arid regions worldwide and are characterized by a dry phase preceded by a slow decrease of flow. The stream flow is re-established after rainfall events of varying intensity (Schiller et al., 2011). This hydrological cycle regulates the deposition and re-suspension of the streambed benthic detritus (Larned et al., 2010), as well as the biochemical processes (Goetz et al., 1986; Romanì et al., 2006; Tzoraki et al., 2007).

Despite the temporary rivers represent a direct source of food and water for human purposes (Steward et al., 2012), they are not recognized in most river management policies, being rarely considered in the health monitoring and assessment programs (WFD, 2000, EPA, 2011). In particular, definitional issues, lack of observational data, and the inability of models to include all the factors that influence the water quality status at the typically recurrent hydrological phases, produce inconsistent projections of the temporary river responses under water stress conditions (Tzoraki et al., 2007; Ademollo et al., 2011; Benstead and Leigh, 2012). In Europe, the Water Framework Directive (WFD, 2000) requires the member states to develop river management plans with the overriding objective to achieve a "good status" in all water bodies by 2015. The implementation of the WFD for temporary river catchments is challenging for the watershed managers that traditionally rely on outputs from permanent river models to evaluate any management action (Gallart et al., 2011).

Due to the high stream flow variability, temporary river sediments can act as sink and source of nutrients and persistent organic pollutants. For most of these pollutants, biodegradation represents a key process affecting their fate. The WFD and the Directive 2008/105/EC set environmental quality standards (EQS) in surface waters identifying a list of priority substances that comprises Polycyclic Aromatic Hydrocarbons (PAHs) and Nonylphenol (NP).

PAHs are ubiquitous and detected in most remote area(s) worldwide (Wania and Mackay, 1993; Fernàndez et al., 1999). They consist of two or more fused benzene rings in various arrangements and arise from pyrogenic and petrogenic sources. PAHs are stable, bioaccumulative and toxic (Haritash and Kaushik, 2009; Zhang et al., 2010). Owing to their hydrophobicity, most PAHs bind to soil and sediment particles that render them less available for biological uptake (Peng et al., 2008).

NP and its precursor metabolites of the non-ionic surfactants nonylphenolpolyethoxylates (NPEOs) are a group of isomeric compounds each consisting of a nine-carbon alkyl chain attached to a phenol ring. They are synthetic compounds used in industrial, domestic and commercial applications, including pesticides. NPs are reported to enter the rivers through the allochthonous material from the adjacent agricultural lands (Li et al., 2004) and to elute easily from the terrigenous material, rapidly moving into the dissolved phase (Asakura et al., 2004). Once NP and NPEOs have entered aquatic systems, they can elicit an estrogenic action against the reproductive system of aquatic organisms (Jobling et al., 1998; Sohoni et al., 2001).

The biota of temporary rivers is mostly known for its higher plants and metazoan organisms, although recent studies are beginning to catalog and understand the role of microbes (Larned et al., 2010; Datry et al., 2011). Microbial degradation and transformation of the organic matter and contaminants deposited at streambed level are key processes with regard to the carbon cycling in the lotic food web, which links sedimentary organic matter to the upper level of the community (Marxen et al., 2006). Via the microbial food chain, complex organic substrates are solubilized in a series of steps from particulate organic matter to high molecular weight dissolved organic carbon and low molecular weight substrata (Chrost, 1991). For a long time the dry stream reaches were considered "biologically inactive" (Stanley et al. 1997), although detectable microbial activities were observed in dry sediments (Marxsen et al., 2010; Zoppini and Marxsen, 2009).

The objective of this study was to explore (i) the contamination patterns of two classes of priority organic pollutants (i.e. PAHs and NPs), and (ii) the concurring variation of the microbial processes mediating the riverine carbon flux, in temporary river sediments subject to contrasting flow conditions.

2. Materials and methods

2.1. Study site and sampling strategy

The Candelaro river basin (2330 km^2) is one of the mirror basins adopted in the EU Project MIRAGE (Mediterranean Intermittent River ManAGEment, FP7-ENV-2007-1). This semi-arid area is located in Southern Italy and is characterized by non permanent streams (Kirkby et al., 2011; De Girolamo et al., 2011). Forest and pasture are widespread in the upland area, while agriculture is assigned to the low land. The Candelaro river (67 km in length) flows to the Southern Adriatic Sea after receiving its main tributary, the Celone stream.

Sediments were collected under contrasting hydrological conditions, during three monitoring campaigns (July 2009, October 2009 and August 2010). The water temperature ranged from 17–20 °C in October to 23–24 °C in July.

The sampling sites 1 and 2 were located respectively at the upland (N 41° 29′ 44.6″ E 15° 15′ 18.3″) and middle reach (N 41° 23′ 43.5″ E 15° 19′ 57.3″) of the Celone stream.

The sampling site 3 (N 41° 32′ 49.4″ E 15° 48′ 30.6″) was located after the confluence with the Candelaro river in the closing section of the basin, before flowing into the Adriatic Sea (Fig. 1). The sampling performed under high flow conditions was characterized by a mean daily discharge of 0.75 ± 0.12 m^3 s^{-1} [De Girolamo pers. communication]. Five days before sampling, the river basin was hit by a flush corresponding to the maximum intensity (11.4 m^3 s^{-1}) for this system (De Girolamo et al., 2012). The low flow conditions were characterized by a mean daily flow of 0.06 ± 0.01 m^3 s^{-1}, representative of the minimum water flow for this system. Under no flow conditions, emersed sediments were collected at site 1: i) wet (saturated water conditions), ii) dry (water content 7%), and iii) arid (water content 2%). The arid sediments were collected when the dry conditions persisted for 30 consecutive days before sampling.

The sediment samples were collected from the uppermost oxic layers (0.5–2 cm) of three homogeneous patches. They were immediately sieved by 2 mm mesh, stored at 4 °C in polycarbonate acid washed buckets (3 dm^3) and analyzed within 24 h. Chemical and microbiological measurements were performed on the sieved sediment where the majority of microbiological activities take place (Hubas et al., 2006). Grain-size distribution was determined in accordance with the soil textural triangle (Gerakis and Baer, 1999). All analyses were performed in triplicate, and values normalized for dry weight (w/w).

2.2. Physical and chemical characteristics of sediments

Total organic carbon (TOC) and total nitrogen (TN) concentrations were determined using a Carlo Erba NA 1500 CHN analyzer. Subsamples were acidified (2 N, HCl) for TOC analysis. Sediment water content was measured after desiccation at 105 °C for 24 h (dry weight). Sediment organic matter content (ash-free dry weight — AFDW) was determined by subtracting ash weight (500 °C, 3 h) from dry weight. Suspended solids (SS) in water samples were gravimetrically determined on glass fiber filters (GF/F, Whatman).

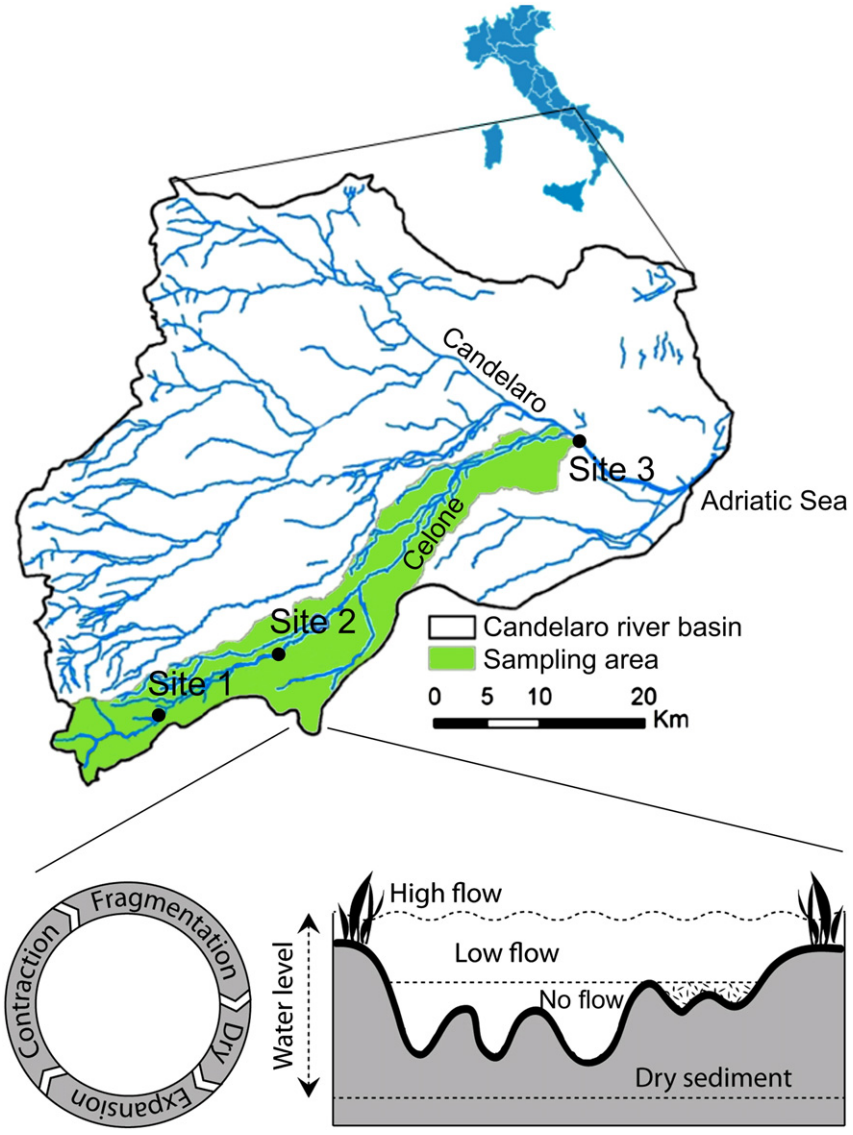

Fig. 1. Sampling area (upper panel) and schematic representation of the hydrological cycle (lower left) and cross-section view (lower right) of a temporary river.

2.3. Analysis of PAHs and NPs

Fluoranthene (Flu), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), benzo(g,h,i)perylene (BgP), and indeno(1,2,3-c,d)pyrene (InP) have been selected due to their strong affinity for the sediment compartment. The stock solutions in cyclohexane (100 mg L^{-1}) were supplied by Aldrich (98% purity, Steinheim, Germany). Acetonitrile, methanol, hexane, and acetone at HPLC grade were from Merck (Darmstadt, Germany). Methylene chloride was of HPLC grade from Sigma Aldrich (Oakville, ON, USA). Water for chromatography was ultrapure (Milli-Q system, Millipore). Technical grade NP (Aldrich Chemie, Steinheim, Germany), NPEO1 and NPEO2 standards (ChemService, West Chester, PA, USA) were used to prepare stock solutions in methanol (1000 mg L^{-1}).

PAHs in lyophilized sediments were extracted by sonication with hexane:acetone 1:1 (v/v) and the extraction was repeated for three times (Patrolecco et al., 2010). The extracts were evaporated and reconstituted with acetonitrile:water 60:40 (v/v) to a final volume of 0.5–1 mL. NPs were extracted by sonication with methanol, repeated three times as for PAHs extraction. The samples were then evaporated and reconstituted with MeOH:water 60:40 (v/v) to a final volume of 0.5–1.0 mL. All sediments were extracted and analyzed at least in triplicate, until the relative standard deviation (RSD) of replicates did not exceed 20%. Fifty microliters of final extracts were injected in duplicate. Analytical determinations were performed by RP-HPLC (Varian 9012) coupled to a fluorescence detector (Perkin Elmer LS4) using a Supelco LC18-PAH column, 5 μm, 250 × 4.6 mm I.D. preceded by a guard column (4 × 3 mm) of the same packing material. The elution profile, at constant flow rate of 1.5 mL min^{-1}, utilized a mobile phase with acetonitrile/water in gradient mode. The temperature of the column oven was kept to 32 °C and excitation–emission wavelengths were automatically set by a time program. The detection limits were in the range 0.1–0.3 ng g^{-1} (dry weight) for all PAHs in the sediments.

For NPs analysis a Phenomenex Synergi Polar-RP 4 μm, 150 × 4.6 mm I.D. column (Phenomenex, St. Torrence, CA, USA) was used preceded by a guard column (4 × 3 mm) of the same packing material. The elution profile, at constant flow rate of 1.0 mL min^{-1} utilized a mobile phase with methanol/water in gradient mode. The column was kept at room temperature and the excitation–emission wavelengths were set as follows: λ_{exc} = 230 nm and λ_{emiss} = 302 nm. The detection limits were in the range 0.03–0.06 μg g^{-1} (dry weight), for all the analytes in the

solid samples. The relative C contribution of PAHs and NPs to the total organic carbon in sediment (C-PAHs and C-NPs) was calculated by considering the C content of each compound.

2.4. Microbiological analysis

For bacterial biomass determination, all sediment samples were treated according to sonication-gradient centrifugation approach described elsewhere (Amalfitano and Fazi, 2008). Bacterial abundance was determined by epifluorescence microscopy (DAPI) and bacterial biomass (BB) was estimated by considering a per-cell C content of 40 fg (Amalfitano et al., 2008). The relative contribution of BB to the TOC (C-BB) was then calculated.

Bacterial carbon production (BCP) was estimated by measuring the [^{3}H]leucine incorporation rates (Amalfitano et al., 2008). The appropriate concentration of [^{3}H]leucine (0.15 μM final concentration [NEN Life Science Products, Boston, Massachusetts, USA]) was checked according to the saturation curve. The rates of leucine incorporation were converted into units of C per sediment dry weight ($\mu g\ C\ h^{-1}\ g^{-1}$) by applying the conversion factor of 1.44 kg C produced per mole of incorporated leucine (Buesing and Marxsen, 2005). The per-cell specific production (BCPs in fmol $C\ h^{-1}\ cell^{-1}$) was obtained by dividing C production by cell abundance. The bacterial growth rates ($\mu = \ln((BB + BCP)\ BB^{-1})\ h^{-1}$) and turnover times ($T_2 = (\ln 2\ \mu^{-1})$) were calculated (Koch, 1994).

Community respiration (CR) was assayed by measuring the oxygen consumption of sediments enclosed in incubation chambers (Uehlinger et al., 2002). CR was calculated as dissolved oxygen depletion versus time (>2 hour incubation) per unit of dry weight of sediment and then transformed into C units, assuming a respiratory quotient (RQ) of 1. The CR was not measured in dry sediments because the addition of water and the required incubation time would bias the sample properties.

Assuming that the microbial contribution to respiration is dominant in the fine sediment fraction (<2 mm), the bacterial growth efficiency (BGE) was calculated as BCP/(BCP + CR), with (BCP + CR) representing the total biological organic carbon demand in the oxic environment. The BGE was used to describe what portion of the assimilated carbon is suitable for biomass production or to meet the energy demands by the benthic microbial community (Bastviken et al., 2003).

The extracellular enzyme activities (EEAs), leucine aminopeptidase (AMA), alkaline phosphatases (APA), lipase (LIP), and beta-glucosidase (Bglu), were determined fluorimetrically (VICTOR™ X3 Multilabel Plate Reader, Perkin Elmer) as described previously (Zoppini et al., 2010). Artificial substrates were selected to model the common constituents of sinking OM and utilized at saturating concentration (final concentration 4-MUF-P-phosphate, 0.3 mM; MUF-beta-D-glucopiranoside, 0.5 mM; 4-MUF-oleate, 0.5 mM; leucine-4-AMC, 1 mM).

2.5. Statistical analyses

The multi-group SIMilarity PERcentage test (SIMPER), using the Bray–Curtis similarity measure, was run to identify the organic pollutants among PAHs and NPs that were primary responsible for the observed differences among samples collected in the different hydrological conditions. In the output table, the contaminants were sorted in descending order of their percentage contribution to group difference. The data were log-transformed and processed using the PAST software package (PAlaeontological STatistics, v2.05).

The metabolic rates observed in the different hydrologic conditions were tested statistically using the paired Student *t*-test. The relationship between different parameters was explored using nonparametric Spearman-rank correlation.

The redundancy analysis (RDA) was used to graphically visualize how the sediment chemical parameters (active variables) varied together with the microbiological parameters and the contamination patterns of PAHs and NPs (supplementary variables) among high, low and no flow conditions. All variables were normalized dividing by their standard deviations. The RDA plot scores were computed by the data correlation matrix with the Statistica 7.0 software package (StatSoft Inc., Tulsa, OK).

3. Results

3.1. Sediment characterization

On average, sediment samples showed a high contribution of silt–clay (<0.05 mm = 53–62%) and fine sand (0.05–0.5 mm=38–47%). The high flow conditions determined an elevated transport of suspended solids (SS) with an increasing gradient from upstream to downstream (from 25 to >1200 mg L^{-1}, respectively). Under low flow conditions, the transport of solids was low upstream with increasing values downstream (from 6 to 89 mg L^{-1}).

The high flow conditions were associated with the highest TOC contribution to sediments (mean of all sites 1.6 ± 0.1%) and the highest C/N ratio as observed in the sites 1 and 2 (>18, Table 1). Under low flow conditions, the TOC contribution (mean of all sites 0.6 ± 0.2%) was significantly lower (*t* test, $p < 0.01$), while C/N ratios were always lower than 5. Under no flow conditions, sediments showed similar TOC contributions (<0.4%) with the exception of the wet sediment that showed relatively higher value (1%). The C/N ratio showed a decreasing trend from wet to arid sediments passing from 5 to 2. TN contribution did not vary significantly among the different samples (mean of all samplings 0.17 ± 0.07%) while AFDW declined passing from wet to arid conditions (from 6.9% to 1.7% respectively).

3.2. Variability of PAH and NP concentrations

PAH concentrations were above the detection limits in all sediment samples with few exceptions concerning the InP (Table 2). The highest concentrations were always associated with the Flu (205.1 ng g^{-1}), then followed by BbF (43.9 ng g^{-1}) and BkF (39.2 ng g^{-1}). The sum of the concentrations of the single compounds (ΣPAHs) gave values comprised between 53.3 ng g^{-1} and 304.7 ng g^{-1}. The analysis of the contribution of the isomers to the PAHs pool indicated that Flu (4-ring isomer) was predominant representing between 30% and 75% of the total. The five-ring isomers (BkF, BbF, BaP) contributed to a similar extent to the whole pool (range 19–30%). Overall, the six-ring isomer (BgP, InP) contribution was always below 15% and the minimum percentage was observed in the dry sediment (6%). Overall, the SIMPER test indicated that InP, BbF and Flu were chiefly responsible for the average dissimilarity among the different hydrological phases, respectively for 35%, 17% and 16% of the overall variability. The concentration of NPs varied largely with NP (range 231.6–3851.9 ng g^{-1}) followed by NPEO1 (range 33.9–1028.6 ng g^{-1}) and NPEO2 (range 88.4–290.4 ng g^{-1}). The SIMPER test indicated that NPEO1 mainly

Table 1
Chemical composition of sediments collected under different hydrological conditions. See text for symbols.

Hydrological condition	Site	AFDW %	TOC %	TN %	C/N
High flow	1	4.1 ± 0.5	2.1 ± 0.5	0.1 ± 0.01	21
	2	4.4 ± 0.6	1.8 ± 0.02	0.1 ± 0.01	18
	3	10.3 ± 1.0	1.8 ± 0.05	0.3 ± 0.03	6
Low flow	1	4.5 ± 0.1	0.6 ± 0.28	0.2 ± 0.02	3
	2	8.0 ± 0.6	0.9 ± 0.01	0.2 ± 0.01	5
	3	4.4 ± 0.3	0.4 ± 0.003	0.1 ± 0.004	4
No flow	1 wet	6.9 ± 0.5	1.0 ± 0.01	0.2 ± 0.004	5
	1 dry	3.2 ± 0.3	0.4 ± 0.03	0.1 ± 0.01	4
	1 arid	1.7 ± 0.0	0.3 ± 0.05	0.2 ± 0.01	2

Table 2
Concentration of PAHs and NPs in the sediments. See text for symbols.

Hydrological condition	site	Flu ng g^{-1}	BbF ng g^{-1}	BkF ng g^{-1}	BaP ng g^{-1}	BgP ng g^{-1}	InP ng g^{-1}	ΣPAHs ng g^{-1}	NP ng g^{-1}	NPEO1 ng g^{-1}	NPEO2 ng g^{-1}	ΣNPs ng g^{-1}
High flow	1	24.8 ± 14.7	18.9 ± 7.6	3.3 ± 0.2	1.7 ± 0.1	2.7 ± 0.1	3.7 ± 0.3	55.1 ± 10	3538.9 ± 218.4	1028.6 ± 13.1	290.4 ± 21.0	4857.9 ± 226.0
	2	130.1 ± 10.7	42.5 ± 18.2	14.6 ± 10.7	6.6 ± 4.1	10.3 ± 7.6	<lod*	204.2 ± 171	3851.9 ± 34.0	396.4 ± 25.8	88.4 ± 2.7	4336.7 ± 62.5
	3	70.7 ± 10.0	25.1 ± 5.6	9.1 ± 2.9	9.5 ± 4.9	10.3 ± 3.6	5.4 ± 0.3	130.1 ± 22.2	361.1 ± 91.7	685.7 ± 67.7	157.8 ± 26.8	1204.7 ± 132.6
Low flow	1	151.3 ± 76.8	36.2 ± 23.7	37.9 ± 3.6	8.8 ± 5.0	17.8 ± 11.3	23.6 ± 15.8	275.6 ± 132.6	2020 ± 152.0	500.0 ± 279.0	170.0 ± 93.0	2690.0 ± 1894.0
	2	40.4 ± 3.4	6.6 ± 2.0	1.8 ± 0.1	2.1 ± 0.5	2.4 ± 0.1	<lod*	53.3 ± 4.7	2850 ± 346.8	620.0 ± 212.0	190.0 ± 14.0	3660.0 ± 573.0
	3	160.4 ± 24.6	43.9 ± 6.3	39.2 ± 8.9	8.3 ± 1.6	23.4 ± 3.6	29.4 ± 11.4	304.7 ± 56.4	231.6 ± 3.1	33.9 ± 171.5	113.2 ± 57.2	378.7 ± 231.7
No flow	1 wet	205.1 ± 94.2	27.8 ± 21	13.9 ± 11	11.1 ± 0.4	14.4 ± 0.9	8.6 ± 3.5	280.9 ± 131	1979.1 ± 49.8	548.5 ± 13.2	182.8 ± 4.4	2710.4 ± 67.5
	1 dry	34.4 ± 2.0	3.7 ± 0.3	7.7 ± 4.3	3.6 ± 0.2	2.9 ± 0.7	<lod*	52.3 ± 6.8	1586.1 ± 119.5	426.1 ± 240	142.0 ± 80.0	2154.3 ± 151
	1 arid	2.5 ± 0.3	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	2.2 ± 0.5	0.6 ± 0.1	8.3 ± 0.4	285.9 ± 45.5	13.7 ± 9.8	<lod*	299.6 ± 125

* Limit of detection.

contributed to the average NPs dissimilarity between the different hydrological phases (42% of the overall variability).

The low flow conditions were associated with the highest ∑PAHs concentrations at the upland reach (site 1, 275.5 ± 132.6 ng g^{-1}) and at the closing river station (site 3, 304.7 ± 56.4 ng g^{-1}). The highest ∑NPs concentrations were associated with the high flow conditions at sites 1 and 2 (4857.0 ± 226.0 and 4336.7 ± 62.5 ng g^{-1}, respectively).

The relative C contributions of PAHs and NPs to the total organic carbon in sediment (C-PAHs and C-NPs) indicated that the contaminants contributed differently to the TOC composition under contrasting hydrological conditions. In high flow conditions, C-PAHs and C-NPs represented on average the 0.8 ± 0.5% and 0.2 ± 0.1% of TOC, respectively. The highest contributions of C-PAHs to TOC (5.1 ± 4.6%) were associated to low flow conditions, whereas C-NPs did not change considerably (mean 0.3 ± 0.1%). Interestingly, under no flow the C-PAHs contribution to TOC decreased passing from wet to dry and arid sediments (3.3%, 0.6% and 0.3%, respectively), whereas the contribution of C-NPs to TOC declined slightly (0.3%, 0.6% and 0.1% respectively).

3.3. Microbial biomass and metabolic processes

Microbial parameters showed a high variability in terms of bacterial biomass (BB, range 9.4 to 0.4 μmol C g^{-1}), bacterial C production (BCP, range 0.02–0.83 μmol C g^{-1} h^{-1}), and community respiration (CR, range 0.07–0.94 μmol C g^{-1} h^{-1}) (Fig. 2). The extracellular enzyme activities were mostly dependent on alkaline phosphatase and aminopeptidase activities (mean 51 ± 20% and 30 ± 8% of the total, respectively) (Fig. 3). Overall, bacterial biomass contribution to TOC decreased passing from high flow (mean 0.4 ± 0.5%) to low flow (0.2 ± 0.1%) with a further reduction under no flow conditions (mean 0.03 ± 0.01%).

The high flow conditions were associated with relatively high metabolic rates (mean 0.57 ± 0.3 and 0.18 ± 0.01 μmol C g^{-1} h^{-1}, BCP and CR respectively) (Fig. 2a), comprising most of the extracellular enzyme activities detected. This trend can reflect a large availability and variability in the organic matter composition (Fig. 3a).

Under low flow conditions, a drastic reduction of the bacterial biomass and production was observed (BB, mean 0.9 ± 0.8 μmol C g^{-1}; BCP mean 0.19 ± 0.2 μmol C g^{-1} h^{-1}) while the community respiration did not vary significantly (CR, mean 0.11 ± 0.07 μmol C g^{-1} h^{-1}) (Fig. 2b). Extracellular enzyme activities were dominated by aminopeptidase and alkaline phosphatase activities (Fig. 3b).

The decrease of the flow brought to its fragmentation and to the coexistence of sediment patches in a gradient of moisture (Fig. 2c). Bacterial biomass decreased drastically from wet to dry sediments (0.34 and 0.04 μmol g^{-1} respectively, site 1) along with bacterial C production rates (0.28 and 0.02 ± 0.04 μmol C g^{-1} h^{-1} respectively). Surprisingly, the arid sediments showed a relatively high bacterial biomass (mean 5.4 μmol C g^{-1}) and detectable activities (BCP = 0.09 ± 0.02 μmol C g^{-1} h^{-1} and 0.01 ± 0.002 and 0.03 ± 0.004 μmol MCA/MUF for AMA and APA respectively).

The effect of the impact of contrasting hydrological conditions on microbial metabolism can be synthesized by the estimation of the bacterial growth efficiency (BGE) that reflects changes in the efficiency of the organic matter utilization. The high flow conditions were associated to the highest range of values (BGE range 60–80%) while low flow to the lowest (BGE range 40–60%).

3.4. Data integration

It is noteworthy to observe that the hydrological shift explained most of the total variance (PC1 = 44% of the total variance), while the variation among the different sites was of minor magnitude (PC2 = 28% of the total variance) (Fig. 4a). The TOC contribution decreased together with the stream flow reduction, while the contribution of C-PAHs and C-NPs (%) tended to increase (Fig. 4b). NPs (K_{ow} = 3.46–3.63) were relatively more concentrated in sediments under high flow, while the

more hydrophobic PAHs (K_{ow} = 4.79–6.84) accumulated in the low and no flow conditions (Fig. 4c). Passing from high flow to no flow conditions, a gradual reduction of microbial anabolic processes (e.g. reduced production of new biomass) was observed, to reach the lowest values of the specific BCP rates (0.06 fmol C h^{-1} $cell^{-1}$), EEAs, and the highest doubling time (T_2 = 40 h) in arid sediments (Fig. 4d). Interestingly, we observed a significant correlation pattern for PAHs vs BCP ($r = 0.91$, $p < 0.05$) when excluding those samples (2 under low flow and 1 no flow wet) characterized by higher $\sum$PAHs concentrations (>275.6 ng g^{-1}) and lower BCP rates (<0.3 μmol C g^{-1} h^{-1}).

4. Discussion

4.1. Quality of sediments and dynamics of pollutants

The Candelaro river basin is characterized by a relatively higher trophic status and microbial metabolic rates than those observed in other temporary rivers of the Mediterranean Region (Tzoraki et al., 2007; Amalfitano et al., 2008; Zoppini et al., 2010).

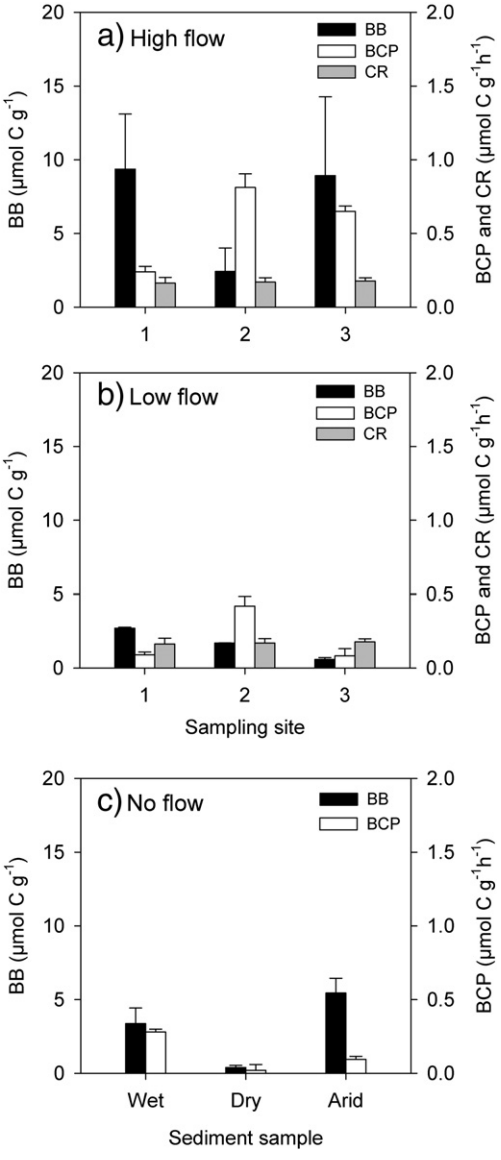

Fig. 2. Structural and functional properties of the benthic microbial communities under high, low and no flow conditions. BB = bacterial biomass; BCP = bacterial C production.

Overall, the contamination of sediments by PAHs (mean ΣPAHs = 151 ± 116 ng g^{-1}) was in the lower range of values reported for sediments of permanent rivers (160–2000 ng g^{-1}) (Lacorte et al., 2006; Patrolecco et al., 2010) and it could be considered as moderate pollution (Baumard et al., 1998). Quality standards for the sediment compartment are known only for Flu (111.0 ng g^{-1}) and BaP (31.9 ng g^{-1}) (CCME, 2003). In this investigation, only Flu exceeded the limits in 4 out of a total 9 samples tested.

The average NPs concentration (ΣNPs = 2600 ± 1700 ng g^{-1}) are consistent with the values found in moderately polluted river sediments (Bennie et al., 1997, Thiele et al., 1997, Patrolecco et al., 2006) and higher than the limits reported by the Canadian Quality Guidelines (CCME, 2003) (1400 ng kg^{-1}).

The high flow conditions determined the accumulation of OM at the streambed level and high C/N ratios (Fig. 4), as usually observed in flooding periods (Cowie and Hedges, 1994). The TOC enrichment of

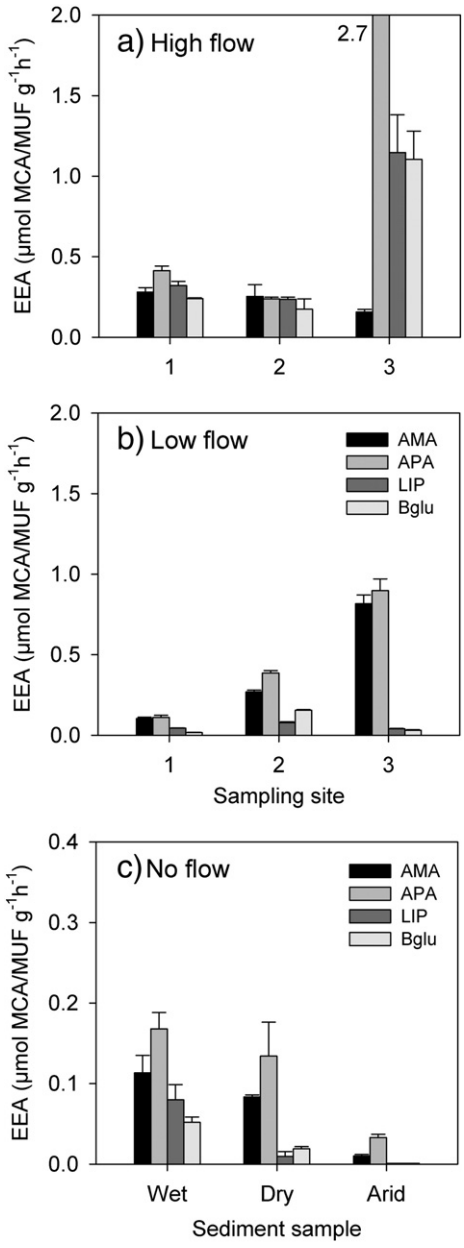

Fig. 3. Extracellular enzyme activities in sediments under high, low and no flow conditions. AMA = aminopeptidase; APA = alkaline phosphatase; LIP = lipase; Bglu = beta-glucosidase.

the sediments was not followed by the raising of the C-PAHs contribution to TOC (Fig. 4). According to the literature (Kalmykova et al., 2012), no correlation between TOC and PAHs was found, suggesting that TOC cannot be used as predictor of the adherence of PAHs to OM. On the contrary, the low and no flow conditions determined the accumulation of pollutants in sediments (Fig. 5).

Due to their hydrophobic properties, PAHs introduced into surface water sink on the surface sediments via the deposition of organic-rich particles, whereas the less hydrophobic NPs, are leached more easily from the terrigenous material and move into the dissolved phase (Asakura et al., 2004; Li et al., 2004; WFD-CIS, 2009). The flood that occurred before sampling was likely to wash off the river bed and introduced allochthonous C-rich debris (Fig. 4). The abundant fine silt–clay fraction of these sediments, could facilitate the mobilization of PAHs, which are mainly associated to this size fraction (Krein and Schorer, 2000). Considering the high levels of suspended solids under high flow conditions, the flood potentially removed the particle-associated pollutants, promoting their circulation in the flowing waters. On the contrary, the low flow conditions could determine the PAHs accumulation at the river bed level. The less hydrophobic NP compounds could be eluted more easily from the allochthonous terrigenous material, rapidly moving into the dissolved phase (Li et al., 2004). Therefore, pollutants could be easily mobilized from the sediments to the water after the flooding. The sediment moisture decreases in arid conditions resulted in a strong reduction of pollutant concentration. The contribution of C-PAHs to TOC decreased up to 10 folds with the sediment moisture (C-PAHs from 3.3 to 0.3%) and, in lesser extent, the contribution of C-NPs (C-NPs from 0.3 to 0.1%) decreased as well.

This trend can be explained by an array of different co-occurring causes. We can exclude the volatilization of semi-volatile PAHs because, by comparing dry and arid sediments, the decrease of the lower molecular weight compounds (i.e. four aromatic rings) was not different from those with a higher molecular weight (i.e. five and six aromatic rings) in both samples. It is known that the loss of water increases the sorptive capacity of the sediment for volatile and semi-volatile organic compounds due to the lack of competition with water, and consequently the vapor equilibrium concentration is very low (Choi et al., 2001). Moreover, pesticides adsorb more on the dry soils than on the moist ones (Wershaw et al., 1969, Goetz et al., 1986). On the other hand, when the period of dryness is prolonged and the sediment is exposed to sunlight, the photolysis is likely to take place (Krauss and Wilcke, 2005). In fact, wetting and drying cycles of soils or sediments may result in an increased availability of the sequestered molecules, which occupy remote sites within the solid matrix, possibly associated with organic material. The induced shrinkage and expansion of the organic matter by drying and rewetting may increase the diffusion of the sequestered substrates consequently becoming more available to microbial degradation and biological uptake (White et al., 1998).

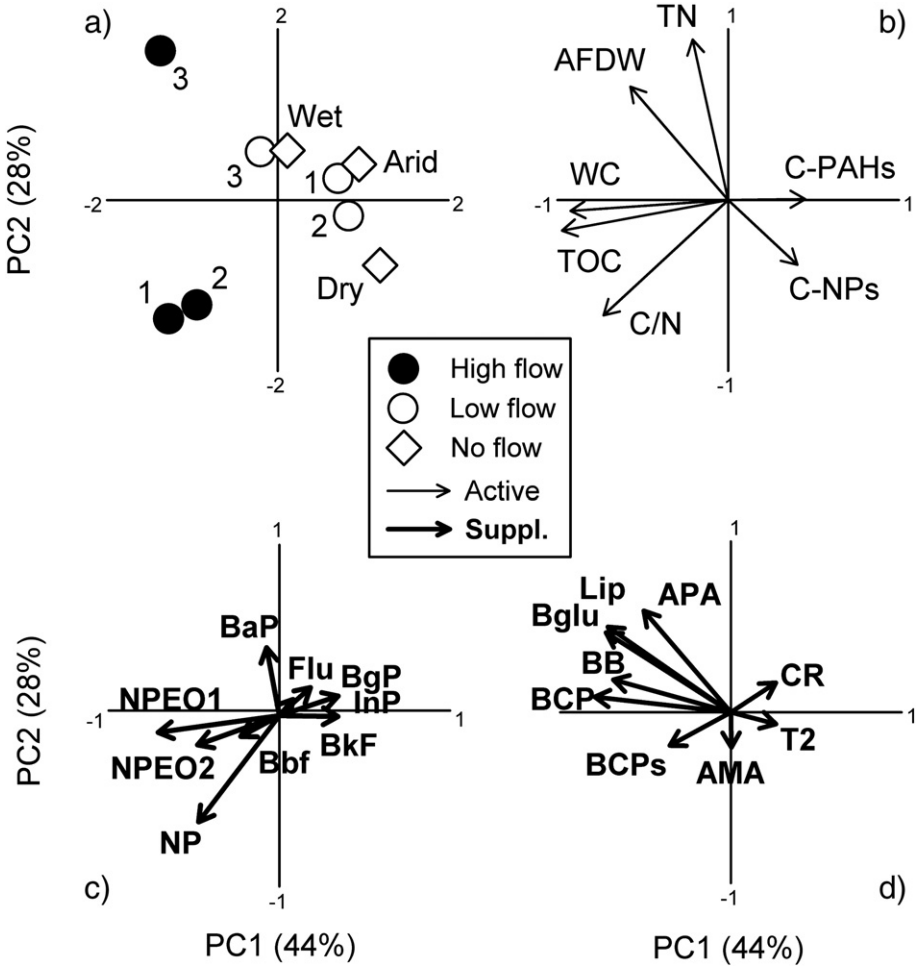

Fig. 4. (a) Redundancy analysis (RDA) of the chemical characteristics of sediment samples under high, low and no flow conditions. (b) Water content (WC), ash-free dry weight (AFDW), total organic carbon (TOC), total nitrogen (TN), TOC/TN ratio (C/N), and the relative contribution of C-PAHs and C-NPs to the TOC. (c) The concentrations of PAHs and NPs (see text for symbols), (d) the microbial structural and functional parameters [bacterial biomass (BB), bacterial carbon production (BCP), cell specific carbon production (BCPs), bacterial doubling time (T2), community respiration (CR), alkaline phosphatase (APA), beta-glucosidase (Bglu), aminopeptidase (AMA) and lipase (LIP)] were projected onto the factor space as supplementary variables.

4.2. Microbial processes

In the analyzed sediments, we found rates of bacterial production, community respiration and extracellular enzyme activities in line or higher than those previously reported for other temporary river systems (Marxsen et al., 2010; Zoppini et al., 2010). The observation of such metabolic patterns may provide specific insights into the bioavailability of different OM sources, and into the carbon and nutrient flux at different riverine hydrological regimes (Findlay et al., 2003; Foulquier et al., 2013). The benthic microbial communities adapt to the hydrological changes optimizing the exploitation of the available resources and overcoming the limits imposed by the environmental conditions (Romanì et al., 2013).

The OM enrichment in sediments under high flow conditions implied the stimulation of all the metabolic rates. The enzymatic activities were stimulated with the specificity for carbonaceous substrates (lipase and beta-glucosidase). The assimilation of C-rich substrata probably implied the retrieval of P from the OM, as a high correlation was observed between these two activities (APA vs LIP, $r = 0.63$, $p < 0.05$; APA vs Bglu, $r = 0.73$, $p < 0.05$). These extracellular enzyme associations have the effect to contribute to fuel the microbial biomass (BB vs LIP, $r = 0.65$, $p < 0.05$; BB vs Bglu, $r = 0.63$, $p < 0.05$), as well as to the metabolic properties (BCP vs LIP, $r = 0.80$; BCP vs Bglu, $r = 0.85$, $p < 0.05$; CR vs LIP, $r = 0.70$ and CR vs Bglu, $r = 0.68$, $p < 0.05$). The combination of enhanced availability of resources and the consequent metabolic adaptation (shift toward C-assimilation at the expense of C-mineralization), brings to a high bacterial growth efficiency which ranked in the high range of values, as observed under flood condition (Zoppini et al., 2010).

The onset of low flow conditions could correspond to a restriction of the food resources in quantity and quality for the streambed microbial communities. The observed shift toward APA and AMA confirmed the occurred impoverishment of the organic matter quality, being these EEAs specific to retrieve nutrients (P and N), as well as labile substrata (e.g. amino acids) from the organic matter (Fig. 3). Such result coincided with the relatively lower contribution of the bacterial biomass to TOC and the significant reduction of the BCP (Fig. 5). These patterns also result in a scarce efficiency in the organic matter utilization (Zoppini et al., 2010) with a shift toward mineralization processes (low values of BGE).

Under no flow conditions, the microbiological parameters varied considerably with the sediment moisture. Overall, desiccation processes may induce a drastic reduction of the bacterial biomass and carbon production (Amalfitano et al., 2008). In this study, the average values of BB and BCP in wet sediment were not significantly different from those observed under low flow conditions (Fig. 2b), and relatively high values were observed in the arid sediment (Fig. 2c). Several factors can be recalled to explain this result from the reduction of the grazing pressure in the wet sediments (Wall and Virginia, 1999) to the settlement of terrestrial microbial species in arid sediments (Timoner et al., 2012). Drying–rewetting cycle may impose physiological constraints that few bacterial taxa can tolerate (Billi and Potts, 2002). Bacteria can cope with the lack of water through specific adaptation strategies, including changes in the phylogenetic community composition (Romanì et al., 2013; Pohlon et al., 2013). Moreover, APA assumed the highest values and it was still detectable even in the arid sediments, in line with values found in dry sediments and soils (Zoppini and Marxsen, 2009; Burns et al., 2013; Delgado-Baquerizo et al., 2013). The preservation of enzyme activities, together with the reduction of cellular metabolism, involves the accumulation of labile dissolved and particulate nutrient loads in the sediments. The reestablishment of the water flow can have a direct impact on the aquatic ecosystem by triggering eutrophication phenomena, with possible consequences in the trophic status of the receiving water bodies (Gomez et al., 2012).

Several studies investigated the harmful effects of PAHs and NPs to the life cycles of various organisms, including microbes, and the strategies adopted to cope with such environmental stressors (Verrhiest et al., 2002; Kallimanis et al., 2007; Soares et al., 2008). Although these contaminants may undergo several mechanisms of abiotic transformation (adsorption, volatilization, photolysis and chemical degradation), the microbial metabolism represents the major degradation pathway (Corvini et al., 2006; Haritash and Kaushik, 2009, Montgomery et al., 2010). Anyway, all analyzed PAHs are scarcely bioavailable to microbial degradation due to their molecular structure constituted by four to six fused aromatic rings. Regarding NPs, the larger degradability of the ethoxylates (NPEO1 and NPEO2) is consistent with the NP accumulation pattern in the sediments. This may represent a concurring factor in limiting the microbial metabolism, thus reducing the chance for pollutants degradation.

5. Conclusions

Temporary rivers have fundamental ecological and social values but they are rarely considered in river quality monitoring and assessment programs. Our findings give a snapshot inside a river system with a high hydrological variability, identifying significant changes in the sediment properties, with potential consequences on the water quality of rivers and receiving water bodies. The combination of several factors, such as the different hydrophobicity of the pollutants, the microbial responses to the environmental variability and the drastic hydrological changes, drives the diffusion of the pollutants in temporary aquatic environments.

According to our findings, different scenarios of pollutant mobilization and microbial processes can be identified under contrasting hydrological conditions: (i) sediment resuspension and pollutant mobilization under high water flow and a higher probability for biodegradation due to

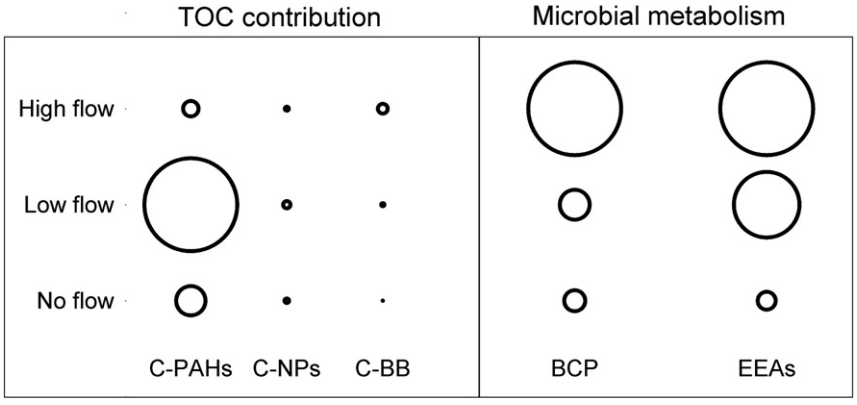

Fig. 5. Bubble plot representing the relative carbon contribution of PAHs (C-PAHs), NPs (C-NPs) and bacterial biomass (C-BB) to the TOC (left panel). The relative contribution of the BCP and EEAs was normalized with respect to the maximum value of each variable measured in the different hydrologic conditions (right panel).

the higher microbial metabolic rates; (ii) pollutants accumulation in sediments during low flow and lower probability for biodegradation due to the reduced metabolic rates; and (iii) drastic reduction of pollutant concentrations under dry and arid conditions, probably occurring by abiotic processes rather than by microbial activity, that showed the lowest values. Our findings infer that a multiple approach for the assessment of chemical and microbiological dynamics at the streambed level has to be considered for an appropriate water resource exploitation and a more realistic prevision of the impact of pollutants in temporary aquatic environments.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with the publication of this paper and there has been no significant financial support for this work that could have influenced its outcome.

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