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Wildfire impacts on freshwater detrital food webs depend on runoff load, exposure time and burnt forest type

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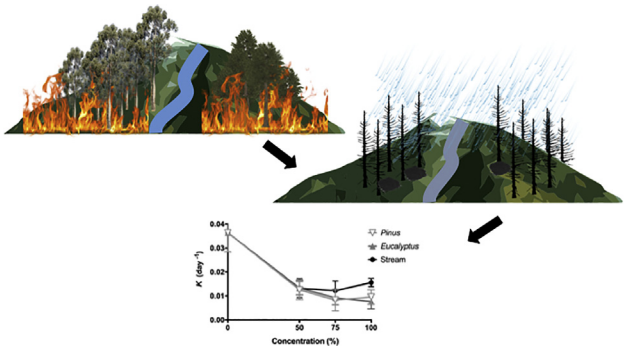
HIGHLIGHTS

- The severity and frequency of wildfires are increasing in the Mediterranean
- We assessed post-fire runoffs on stream detrital ecosystem
- Leaf litter decomposition, invertebrate feeding, and fungal biomass were reduced
- Community composition of fungal and bacterial decomposers were altered
- Impacts were related to the chemical composition of the sample source

GRAPHICAL ABSTRACT



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ABSTRACT

In the last decades, land-use changes have made Mediterranean forests highly susceptible to wildfires, which can cause several impacts not only on burnt areas, but also on adjacent aquatic ecosystems. Post-fire runoff from burnt areas may transport toxic substances to streams by surface runoff, including polycyclic aromatic hydrocarbons (PAHs) and metals, which can be noxious to aquatic organisms. Impacts on aquatic ecosystems can be related to fire severity, forest type and the exposure period; however, these factors have not been investigated *in tandem*. Here, we used the stream detrital system to determine the impacts of post-fire runoffs and stream water from a burnt catchment on trophic interactions between stream microbial communities and invertebrate shredders involved in leaf litter decomposition. Three distinct types of samples were collected from a burnt catchment: post-fire runoffs from high severity wildfires in *Pinus* and *Eucalyptus* forests, and stream water. Microbial decomposer communities (fungi and bacteria) and the invertebrate shredder *Allogamus ligonifer* were exposed for 10 and 20 days to increasing concentrations (0, 50, 75 and 100%) of runoff extracts. Our results showed that post-fire runoffs from high severity wildfires reduced microbially-driven leaf litter decomposition (up to 79%), invertebrate feeding (up to 75%), fungal biomass (up to 39%) and altered community composition; effects were more severe at the longer exposure time. The impacts varied with the runoff source and were related to the chemical composition in metals and total PAHs. This study emphasizes the importance of assessing the indirect effects of wildfires taking into account the effects of the runoff source, load and exposure time on freshwater biota and their ecological functions. Therefore, best forest management practices should be applied to minimize post-

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fire runoffs reaching aquatic ecosystems and to reduce the effects of these extreme events on freshwater biodiversity and ecosystem functioning.

1. Introduction

Increasing frequency and severity of wildfires is a major concern in certain regions of Europe, particularly in the Mediterranean, with great economic and environmental impacts (Certini, 2005; Doerr and Santín, 2016). In the first decade of this century, ca. 140,000 ha y^{-1} of forests in Portugal were affected by wildfires (Pereira et al., 2005; AFN, 2011). Although wildfires are widely recognized as a natural phenomenon with some potential positive impacts on ecosystems (e.g. Brown et al., 2014), they can have several negative environmental consequences on aquatic food webs (Spencer et al., 2003).

Elevated global temperatures and prolonged droughts along with the poor forest management and forest modifications are mainly responsible for the aggravated wildfires in recent times (Alcamo et al., 2007). The forests in Portugal have been changed drastically over the last decades, and currently *Pinus pinaster* Ait. and *Eucalyptus globulus* Labill. represent 50% of the forest cover (ICNF 2013). The plantation of these two tree species became widely explored in the Iberian Peninsula, particularly in Portugal, mainly because of their increasing commercial and economical importance (*Pinus* for construction and furniture wood sectors; *Eucalyptus* for pulp mill industry) (Fernández et al., 2011; Prats et al., 2012, 2016; Maia et al., 2014). Characteristics of forest vegetation (quantity, leaf chemistry and moisture content) can play a crucial role in the properties and severity of wildfires (Agee, 1996). Higher coverage and flammability of *Pinus* and *Eucalyptus* (Fernandes, 2009; Silva et al., 2009) than the native tree species have contributed to an increase in the number and severity of wildfires in Portugal, and their frequency is expected to increase in the near future (Fernandes et al., 2013; Moreira et al., 2011).

Wildfires can directly impact biotic communities at burnt areas (Durán et al., 2010). Also, wildfires can have indirect effects through the production and mobilization of toxic compounds to adjacent aquatic systems with potential impacts on aquatic species (Minshall, 2003; Silva et al., 2016). Heavy rainfall has been identified as a triggering factor for these indirect effects by promoting the ash and soil overland flow into aquatic systems (Cerdà and Doerr, 2008; Malvar et al., 2013; Pereira et al., 2013; Schäfer et al., 2010). Wildfire runoff usually contains pyrolytic substances, including polycyclic aromatic hydrocarbons (PAHs) and inorganic elements, namely metals, metalloids and non-metallic elements, which can be a threat to aquatic biota (Beasley and Kneale, 2002; Silva et al., 2016). Notwithstanding wildfires are a source of contaminants, their impacts on aquatic communities and processes in systems surrounded by eucalypt or pine-dominated forests have been rarely explored. Moreover, the toxic impacts of post-fire runoffs, containing PAHs and metals, resulting from *Eucalyptus* and *Pinus* forests, were reported at the organismal level, including in the freshwater bacterium *Vibrio fischeri*, the algae *Pseudokirchneriella subcapitata*, the macrophyte *Lemna minor* and the clam *Corbicula fluminea* (Campos et al., 2012; Silva et al., 2015; Silva et al., 2016; Nunes et al., 2017). The post-fire effects on aquatic ecosystems depend on the forest type but can also vary with time, space and fire severity (Minshall, 2003). However, the effects of these variables had not been examined *in tandem*.

Due to their proximity and altimetry, forest streams are likely to be highly susceptible to the input of post-fire runoffs. Leaf litter falling from riparian vegetation is the main source of energy and carbon in forest streams, where microbial communities, predominantly fungi followed by bacteria, and invertebrates play a key role in decomposition of leaf litter (Graça, 2001). Hence, it is important to generate knowledge on the responses of stream microbial decomposer communities, and their trophic interactions, to post-fire runoffs.

We hypothesized that post-fire runoffs and stream water from a burnt catchment would alter the structure and functions of microbial communities, with consequences to higher trophic levels namely to invertebrate shredders. We also hypothesized that wildfires toxicity would depend on the runoff source (*Eucalyptus* or *Pinus* plantations or the stream water from a burnt catchment), its chemical composition and concentration, and the exposure time.

2. Materials and methods

2.1. Sampling and characterization of post-fire runoff and stream water

Runoff and stream water samples were collected from a burnt catchment in north-central Portugal, near the parish of Talhadas, Aveiro District (N 40° 39′ 54″, W 8° 21′ 47″) (Fig. S1). The wildfire occurred in July of 2013, affecting 815 ha predominantly covered by eucalypt (*Eucalyptus globulus*) and maritime pine (*Pinus pinaster*) forest plantations that burnt at high severity. Fire severity was assessed according to the methodology described elsewhere (Shakesby and Doerr, 2006; Keizer et al., 2008; Keeley, 2009). Three distinct samples were collected within the burnt area: i) surface water from a permanent stream with a channel width of circa 1.5 m, hereinafter designated as Stream; ii) surface runoff collected through slope-scale plots in a Eucalyptus plantation, hereinafter designated as Eucalyptus; iii) surface runoff collected through slope-scale plots in Pinus plantation, hereinafter designated as Pinus. Both Eucalyptus and Pinus slope-scale plots were located as close as possible to ensure greater uniformity. Soils in both slopes were dominated by humic cambisols, developed over schist. Sampling was carried out in October 2013 following the first post-fire rainfall events. The stream sample was collected in a hydrometric station downstream the slope-scale plots and equipped with an automatic sampler triggered by a water level sensor allowing the collection of 24 water samples in 1 L bottles. Water samples were pooled into a single composite sample. The stream flow during the sample collection varied from 100 to 370 $L.s^{-1}$. Eucalyptus and Pinus runoff samples were collected into three 500 L tanks installed at the bottom of the burnt slope and connected to the unbounded plots' outlets. The samples from the three tanks were mixed in roughly equal proportions to have a single composite sample from each site. Both runoff and stream composite samples were analyzed in triplicate.

Samples were collected and transported to the laboratory in plastic bottles (5 L) under dark on ice. In the laboratory, the samples were mixed thoroughly in a container and stored at −18 °C (to avoid biological activities) in the dark (to prevent photolysis of PAHs) until the beginning of the experiments. PAH and metal quantifications were done in all samples (Stream, Eucalyptus and Pinus) as described earlier (EPA, 1995; Walker et al., 2001; Martinez et al., 2004; Caetano et al., 2007; Martins et al., 2012). The samples were previously filtered to analyze the dissolved and particulate fractions of PAHs and metals separately. For the quantification of metal concentrations, the samples were filtered through 0.45 μm Whatman Nuclepore membranes. After drying the filters at 40 °C, the particle-bound metals were extracted by a total digestion with HF (40%) and Aqua Regia (HCl-37%: HNO_3-65%); 3:1 in closed Teflon beakers (100 °C for 1 h). Hereafter, evaporated to near dryness (90 °C), redissolved with 1 mL HNO_3 and 5 mL of Milli-Q water, heated for 20 min, at 75 °C, and diluted to 25 mL with Milli-Q water (Caetano et al., 2007). Concentrations of manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), cadmium (Cd) and lead (Pb) in the dissolved and particulate fractions were quantified by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Elemental X-

Series). Quality control of the analytical procedures was ensured by the analysis of certified references materials (CRM) and by testing every 10th samples in duplicate. Blanks were prepared following the same analytical procedure and run in parallel with the CRM and samples. The aqueous filtered samples (dissolved fraction) were preserved with double-distilled HNO_3 to pH < 1.5 and were analyzed directly by the ICP-MS. Total concentration for each individual metal represent the sum of the dissolved and the particulate phase. The limits of detection (LD) of different metals varied from 0.25 to 5.0 μg g^{-1} in the particulate fraction and 0.04 to 10 μg L^{-1} in the dissolved fraction. The analyzed metals were selected taking into consideration their i) high environmental toxicity (Cd and Pb), ii) contribution as essential trace elements (Co and Cu), or iii) specific biological interest (Ni) (Gerber et al., 2002; Walker et al., 2001). Moreover, some of these metals are also enlisted in EU Priority Substances Directive (Directive, 2008/105/EC, 2008).

Concentration of total PAHs from each fraction was determined following the process described by Martins et al. (2012) in the case of particulate fraction and EPA (1995), method 525.2, and Martinez et al. (2004) in the case of dissolved fraction. Briefly, the water samples were filtered using glass fiber filters (1.2 μm) to analyze the dissolved and particulate fractions separately. Following drying of the filters at 40 °C until constant weight, the particle-bound PAHs were extracted using pressurized liquid extraction (PLE), conducted in a ASE (Accelerated Solvent Extraction) using a mixture of hexane:acetone (1:1, v:v). The extracts were then concentrated, evaporated and fractionated in a silica:alumina (1:1) and sodium sulfate glass column. The analyte elution was performed with 30 mL of a mixture of n-hexane/dichloromethane (9:1, v:v) and afterwards with 40 mL n-hexane:dichloromethane (4:1, v:v). Both fractions were collected in the same balloon, pre-concentrated in a rotator evaporator and then concentrated to 0.5 mL under a gentle stream of N_2 for prior analysis (Martins et al., 2012). Quantification of PAHs was performed in a gas chromatography–mass spectrometry (GC–MS, Thermo® DSQ) system equipped with a DB-5MS column (30 m, 0.25 mm ID, 0.25 μm film thickness; Agilent, USA).

As for the dissolved fraction, PAHs concentrations were determined by solid phase extraction (SPE) using a SPE vacuum six-port SPE manifold with Bakerbond speedisk H_2O-Phobic DV B extraction disk from J. T. Baker (Avantor Performance Materials, USA). The SPE cartridges were conditioned with 6 mL of acetone and 10 mL of ethyl acetate, followed by 10 mL of methanol and 10 mL of Milli-Q water. The 1 L of the dissolved sample was percolated through the cartridges under full vacuum, and elution was performed with 5 × 10 mL ethyl acetate:dichloromethane (1:1, *v*/v). The final extract was dried over anhydrous sodium sulfate and evaporated at low temperature under a gentle stream of nitrogen and reconstituted in hexane (EPA, 1995; Martinez et al., 2004). The concentrations of PAHs were determined using the same analytical procedure as described previously.

The detection was restricted to 15 priority PAHs enlisted by the United States Environmental Protection Agency (USEPA; ATSDR, 1995), which were: acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, chrysene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, phenanthrene and pyrene. The analytical procedure was validated by doping the sample with standards of the 15 PAHs. The recovery rates of the individual PAHs ranged from 70% to 118%. The limits of detection (LD) for all the 15 PAHs varied from 0.4 to 1.0 ng g^{-1} dry mass for particulate fraction, whereas for liquid fraction it varied from 5 to 20 ng L^{-1}. The final concentrations (after combining the concentrations in both fractions of each sample) of each tested metal and the total PAHs are shown in Table 1.

2.2. Microbial colonization of leaves and collection of shredders

Leaves of *Alnus glutinosa* (L.) Gaertn., collected in autumn and dried at room temperature, were cut into discs (12 mm diameter). Leaf discs were allocated into fine-mesh (0.5 mm) bags (15 cm × 15 cm) and immersed for one week in a low-order stream (Oliveira stream, 41°35′10.57″N, 8°13′30.44″W; 16.1 °C, pH 6.8 ± 0.2, conductivity 36.4 ± 0.8 μS cm^{-1}, dissolved O_2 10.9 ± 0.5 mg L^{-1}) to allow colonization by microbial communities. Then, leaf discs were transported to the laboratory, rinsed with deionized water and placed into the microcosms.

Early-stage caddisfly larvae (1.4 ± 0.1 cm length), namely *Allogamus ligonifer* (Trichoptera, Limnephilidae), a common invertebrate shredder in streams of Northwest Portugal were collected at an upstream site of Cávado river (41°48′N, 7°51′W). The animals were transported to laboratory and acclimated for three weeks before the experiments.

2.3. Microcosm setup

We exposed the stream microbial communities and invertebrate shredders to increasing concentrations of post-fire runoff and stream water in microcosms. At the beginning of the experiments, the samples were thawed to 16 °C (exposure temperature) in dark. A gradient of runoff concentrations (50%, 75%, 100%) were prepared for each sample (*Pinus* and *Eucalyptus* forests and stream) by diluting them in mineral water (Fastio water, Gerês Mountain, Portugal: pH 6.0, containing Ca^{2+} 1.3 ± 0.3 mg L^{-1}, K^+ 0.6 ± 0.1 mg L^{-1}, Na^+ 4.1 ± 0.4 mg L^{-1}, silica 9.6 ± 2 mg L^{-1}, Cl^- 4.2 ± 0.4 mg L^{-1}, HCO_3^- 8.0 ± 0.8 mg L^{-1} and SO_4^- 1.0 ± 0.2 mg L^{-1}) to a final volume of 300 mL in 500-mL Erlenmeyer flasks. Mineral water was also used as unexposed controls. Two sets of microcosms were prepared for two-time points (10 and 20 days) with four replicates per treatment per time. Flaks were filled with a 1 cm layer of washed and autoclaved (120 °C, 20 min) gravel and equipped with aeration systems to simulate natural conditions. The exposure experiments were run under controlled temperature (16 °C), photoperiod (12 h light:12 h dark). At the beginning of the experiment, one shredder was allocated to each flask. A set of 65 leaf discs per microcosm and another set of 15 leaf discs per microcosm (enclosed in 0.5 mm fine-mesh bag of 5 cm × 5 cm size, to avoid invertebrate feeding) were placed in each flask to determine microbial decomposition and leaf consumption by invertebrates.

2.4. Invertebrate leaf consumption and microbial leaf decomposition

Leaf consumption by the invertebrate shredder (L_s) was calculated as in Pradhan et al. (2015). Briefly, $L_s = (L_i - L_f) - (L_i \times (M_i - M_f) / M_i)$, in which L_i and L_f are initial and final ($t = 10$ or 20 d) dry mass, respectively, of microbially-colonized leaf-discs provided to shredders. M_i and

Table 1
Metal concentrations and total polycyclic aromatic hydrocarbons (PAHs) in post-fire runoff from *Pinus* and *Eucalyptus* forests and stream water from a burnt catchment. Mean ± SD, $n = 3$.

Sample	Metal (μg L^{-1})						Total PAHs (μg L^{-1})
	^{55}Mn	^{59}Co	^{60}Ni	^{65}Cu	^{111}Cd	^{208}Pb	
Pinus	412 ± 12	15 ± 0.4	28 ± 0.6	72 ± 3	0.5 ± 0.1	90 ± 4	0.222
Eucalyptus	324 ± 31	10 ± 0.6	48 ± 6	109 ± 9	0.9 ± 0.0	128 ± 9	0.154
Stream	55 ± 3	6 ± 0.3	12 ± 0.8	9 ± 0.5	0.2 ± 0.0	11 ± 1	0.079

M_f are the dry mass of microbially-colonized leaf discs (inaccessible to shredders) at the beginning and the end of the experiment. The invertebrate feeding rate was computed as $L_s / (S_f \times t)$, where S_f is the shredder dry mass at time t (10 or 20 d).

Decomposition rates (k) driven by microbes were calculated using the following exponential model: $W_t = W_0 \times e^{-kt}$, where W_t is the leaf dry mass remaining at time t, W_0 is the initial leaf dry mass and t is the time in days.

2.5. Fungal biomass

Fungal biomass was estimated based on ergosterol concentration in 6 leaf discs per replicate as described in Pascoal and Cássio (2004). Ergosterol was extracted from the leaf discs, previously lyophilized and weighed, by heating in 0.8% KOH-methanol, at 80 °C for 30 min, and the extract was purified by solid-phase extraction and eluted in isopropanol. Quantification of ergosterol was done by high-performance liquid chromatography (HPLC) using a LiChrospher 100 RP18 (5 μm) column (Merck) connected to a UltiMate 3000, Thermo Scientific UHPLC system. Ergosterol was detected at 282 nm and eluted with methanol (HPLC-grade) at flow rate of 1.4 mL min^{-1}. The concentration was determined by comparing the peak areas with ergosterol standards. Ergosterol concentration was converted to fungal biomass considering that 1 mg of fungal dry mass contains 5.5 μg of ergosterol (Gessner and Chauvet, 1993).

2.6. Diversity of sporulating fungi

Fungal sporulation was quantified based on the identification and counting of released conidia. Suspensions of conidia were mixed gently with 200 μL of 0.5% Triton X-100 to prevent conidia from adhering to flasks and fixed with 2% formaldehyde The conidial suspensions were then filtered (0.45-μm pore size; Millipore, Merck KGaA, Darmstadt, Germany), and the retained conidia were stained with 0.1% (w/v) cotton blue (Fluka, Spruce St. St. Louis, MO, USA) in lactic acid, identified and counted under a compound light microscope (Leica Biomed, Heerbrug, Switzerland) at 400× magnification.

2.7. Fungal and bacterial community structure

Denaturing gradient gel electrophoresis (DGGE) was used to determine the community structure of aquatic fungi and bacteria on decomposing leaves. At each exposure time, 3 leaf discs per flask were lyophilized and each disc was further cut into quarters. Genomic DNA was extracted from 3 quarters of leaf discs (1 per leaf discs) per replicate using DNeasy® PowerSoil® Kit (Quiagen, Hilden, Germany), following manufacturer's protocol. Primer pairs ITS3GC/ITS4 and 338GC/518 were used to amplify hypervariable ITS2 region of fungal rDNA and V3 region of bacterial 16S rDNA, respectively (Pradhan et al., 2011). The 5′ end of the forward primers contained 40-bp GC tail to ensure the DGGE-based amplicon separation. In brief, 2 μL (1–10 ng μL^{-1}) of extracted DNA was mixed with 1 μL of each primer (0.4 μM final conc.), 6 μL of $MgCl_2$ (3 mM final conc.), 1 μL of dNTP-mix (0.2 mM final conc.), 0.03 U (final conc.) of GoTaq® G2 Flexi DNA polymerase, 10 μL of Green GoTaq® Flexi buffer (1× final conc.) and 28.7 μL of nuclease-free water. The iCycler Thermal Cycler (BioRad Laboratories, Hercules, CA, USA) was used for PCR starting with an initial denaturation at 95 °C for 2 min, followed by 36 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and elongation at 72 °C for 1 min; and concluding with a final elongation at 72 °C for 5 min. DCode™ Universal Mutation Detection System (BioRad Laboratories, Hercules, CA, USA) was used for DGGE to separate the sequences of similar length differing in nucleotide compositions. PCR products (380–400 bp for fungi and 200 bp for bacteria) from 4 replicates were pooled and approximately 800 ng of fungal or bacterial DNA were loaded on 8% (w/v) polyacrylamide gel in 1× TAE with a denaturing gradient from 30% to 70% or from 35% to 75%, respectively (100% denaturant corresponds to 7 M urea and 40% formamide). The electrophoresis was conducted at 55 V and 56 °C for 16 h and the gels were stained with GelStar™ (1×; Lonza, Basel, Switzerland) for 10 min. The gel images were captured under UV in ChemiDoc™ XRS system (BioRad).

2.8. Statistical analyses

Two-way ANOVAs were used for analyzing the effects of the source of post-fire sample (Eucalyptus runoff, Pinus runoff or stream water) and exposure concentration (0, 50, 75 and 100%) on invertebrate feeding and fungal biomass at each exposure time. Dunnett's multiple comparisons post-hoc tests were used to identify treatments that differed significantly from the control. Microbial decomposition rates, assessed as the regression of the ln-transformed values of leaf dry mass against time, were compared by analysis of covariance (ANCOVA). Analyses were done with Prism 7.0 for Windows (GraphPad software Inc., San Diego, CA, USA).

DGGE gels were aligned and normalized. Each band in the gel was considered an operational taxonomic unit (OTU) and the relative intensities of bands were analyzed using BioNumerics 5.0 (Applied Maths, SintMartens-Latem, Belgium). Cluster analyses of DNA fingerprints were performed using the unweighted pair-group method average (UPGMA). The ordination of fungal or bacterial communities after exposure to post-fire runoffs or stream water at increasing concentrations were done by non-metric multidimensional scaling (nMDS) and clusters superimposed based on Bray-Curtis similarity index. Significant changes in microbial assemblages were identified by two-way PERMANOVA. Cluster analysis, nMDS ordination and PERMANOVA were done with PRIMER 6 (Primer-E Ltd., Plymouth, UK).

Principal response curves (PRCs) were further used to analyze the responses of spore forming fungal communities to increasing concentrations of runoffs or stream water relatively to the untreated control. PRCs are based on Redundancy Analysis ordination technique, a constrained form of the Principal Component Analysis (Van den Brink and Braak, 1999; Duarte et al., 2008). The PRCs were performed using CANOCO 4.5 (Microcomputer Power, Ithaca, NY, U.S.A.). A Monte Carlo permutation test was performed to check if the PRCs displayed a significant part of the treatment variance.

Finally, a Principal component analysis (PCA) was applied to correlate the responses of the functional parameters (shredder feeding and microbial decomposition) with the concentrations of PAH and metals in the post-fire samples. The PCA was performed in PAST 3.11 (http://folk.uio.no/ohammer/past; Hammer et al., 2001).

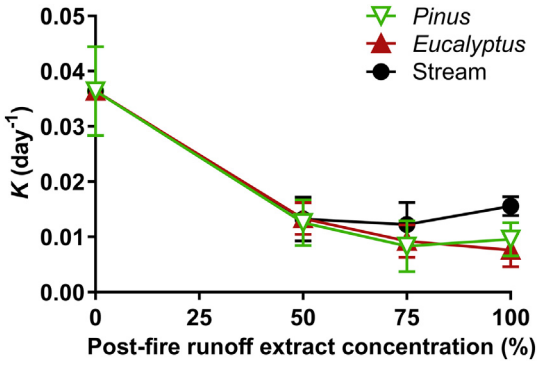

Fig. 1. Microbial decomposition rates (k) of leaves exposed (or not) for 20 days to increasing concentrations of post-fire runoffs from *Pinus* and *Eucalyptus* forests and stream water. Mean ± SD, $n = 4$.

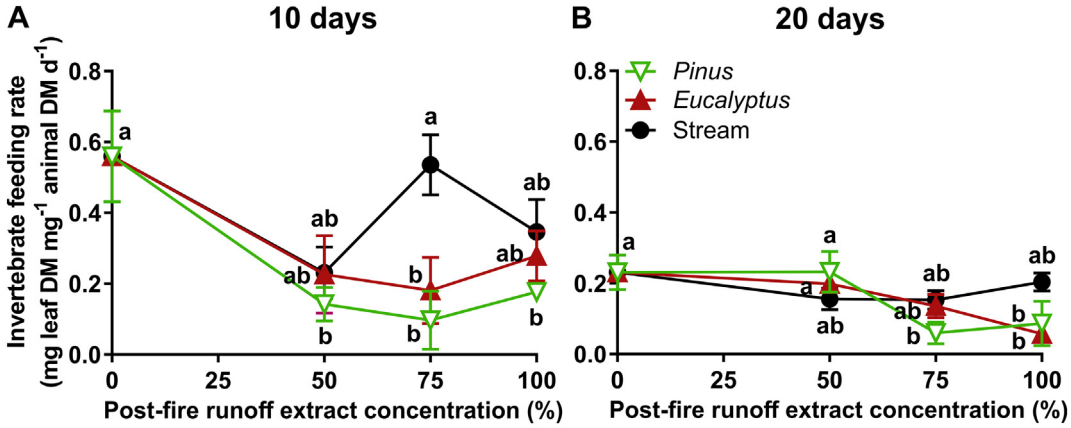

Fig. 2. Feeding rate of the invertebrate shredder *Allogamus ligonifer* (A and B) exposed (or not) for 10 and 20 days to increasing concentrations of post-fire runoffs from *Pinus* and *Eucalyptus* forests and stream water. Mean ± SEM, n = 4. Different letters indicate significant differences among treatments (Dunnett's multiple comparisons test, $P < 0.05$).

3. Results

3.1. Concentration of metals and PAHs in post-fire runoffs and stream water

The chemical analyses of the post-fire runoff from different sources and stream water confirmed the presence of metal elements (Mn, Co, Ni, Cu, Cd, Pb) and PAHs (Table 1). The highest concentrations of Ni, Cu, Cd and Pb were found in runoffs from Eucalyptus forest, whereas the highest concentrations of Mn and Co were found in Pinus forest runoffs (Table 1). The lowest concentrations of all metals were detected in the Stream. The concentration of metals in the runoffs from Eucalyptus and Pinus were ranked as Mn > Pb > Cu > Ni > Co > Cd, and in case of the Stream were ranked as Mn > Ni > Pb > Cu > Co > Cd (Table 1).

Concentration of total PAHs were highest in Pinus (0.222 μg L^{-1}), followed by Eucalyptus (0.154 μg L^{-1}) and Stream (0.079 μg L^{-1}) (Table 1).

3.2. Effects on invertebrate feeding and microbial decomposition

At the control, leaf decomposition rate driven by microbes was 0,036 day^{-1} (Table S1) and it was significantly decreased by exposure to all post-fire runoffs treatments (Fig. 1; ANCOVA $P < 0.05$). Effects were more pronounced in treatments exposed to the highest concentration of Eucalyptus post-fire runoffs (k = 0,0076 day^{-1}) and less pronounced in treatments exposed to stream water (0,0122 d^{-1} < k < 0,0156 d^{-1}), but no significant differences were found in the decomposition rates between treatments (ANCOVA, $P > 0.05$).

The feeding of invertebrate shredders was significantly affected by the source of the samples and the concentration (two-way ANOVA, $P < 0.05$). Effects were more pronounced at longer times (Fig. 2). In the control, the invertebrate feeding rate was 0.56 mg leaf dry mass mg^{-1} animal dry mass d^{-1} after 10 days and was 0.23 mg leaf dry mass mg^{-1} animal dry mass d^{-1} after 20 days (Fig. 2A and B). After 10 days, the feeding rate was decreased by exposure to post-fire runoffs of Eucalyptus at all concentrations and of Pinus at intermediate (75%) concentration (Fig. 2A; $P < 0.05$). After 20 days of exposure, the feeding rates decreased after exposure to post-fire runoffs of Pinus and Eucalyptus only at the highest concentration (Fig. 2B; $P < 0.05$). Moreover, Pinus runoffs also decreased the feeding rate at the concentration of 75% ($P < 0.05$). Exposure to post-fire stream water did not affect the shredder feeding activity significantly either at 10 or 20 days (Fig. 2; $P > 0.05$).

3.3. Effects on fungal biomass

In the absence of post-fire runoffs and stream water, the biomass of fungal communities on decomposing leaves was 67.1 mg g^{-1} leaf dry mass after 10 days and decreased to 45.4 mg g^{-1} leaf dry mass after 20 days (Fig. 3A and B). Fungal biomass was significantly affected by the source of the samples and tended to decrease with increasing concentrations (two-way ANOVA, $P < 0.05$) at both exposure times (Fig. 3A and B). An exception goes to the effects of the Stream that were stronger at the lower and intermediate concentrations after 10 days of exposure ($P < 0.05$). After 10 days, fungal biomass was significantly decreased by runoff from both plant forests at the highest

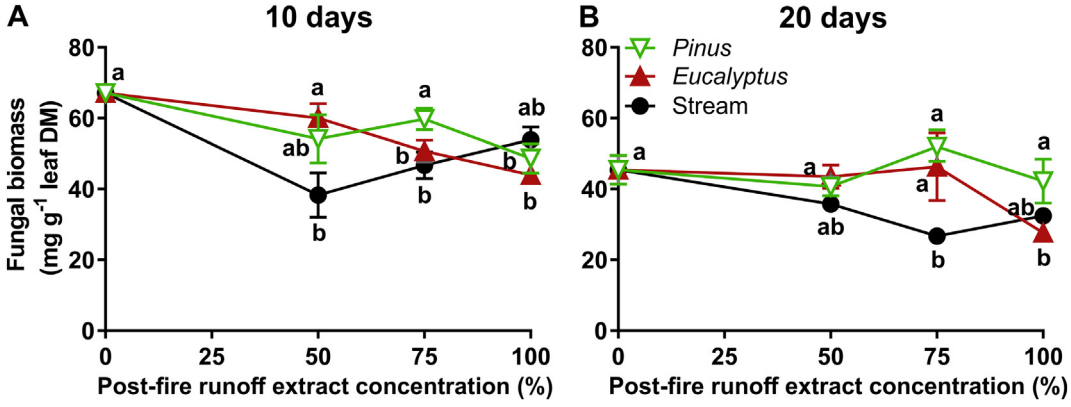

Fig. 3. Fungal biomass on colonized leaves exposed (or not) for 10 days (A) and 20 days (B) to increasing concentrations of post-fire runoffs from *Pinus* and *Eucalyptus* forests and stream water. Mean ± SEM, n = 4. Different letters indicate significant differences among treatments (Dunnett's multiple comparisons test, $P < 0.05$).

concentration (100%; $P < 0.05$) and from Eucalyptus runoff at the intermediate concentration (75%; $P < 0.05$) (Fig. 3A). After 20 days, fungal biomass decreased mainly at the intermediate and the highest concentration of Stream water and at the highest concentration of Eucalyptus runoff (Fig. 3B; $P < 0.05$).

3.4. Effects on the structure of microbial community

In control microcosms, we found a total of 24 bacterial OTUs, 12 fungal OTUs and 11 spore-forming fungal species on decomposing leaves after 10 days. After 20 days, bacterial OTUs increased to 32, fungal OTUs decreased to 9, and the number of sporulating fungal species remained unchanged.

The exposure to post-fire runoffs and stream did not affect taxon richness significantly, but led to shifts in microbial community composition. Changes in the structure of bacterial and fungal communities exposed to the post-fire runoffs from Pinus, Eucalyptus or Stream water are shown in Fig. 4. The nMDs ordination showed that bacterial or fungal communities exposed for 10 days were segregated from those exposed for 20 days (Fig. 4). Bacterial communities shared 60% similarity at each exposure time. Within 10 days, bacterial communities formed 3 groups: i) communities exposed to all concentrations of Eucalyptus runoff (70% similarity), ii) communities exposed to lower concentrations of Pinus (70% similarity), and iii) communities exposed to the highest concentration of Pinus and all concentrations of Stream (60% similarity) (Fig. 4A). Bacterial communities exposed for 20 days formed 3 groups with 70% similarity: i) bacteria exposed to Stream, ii) bacteria

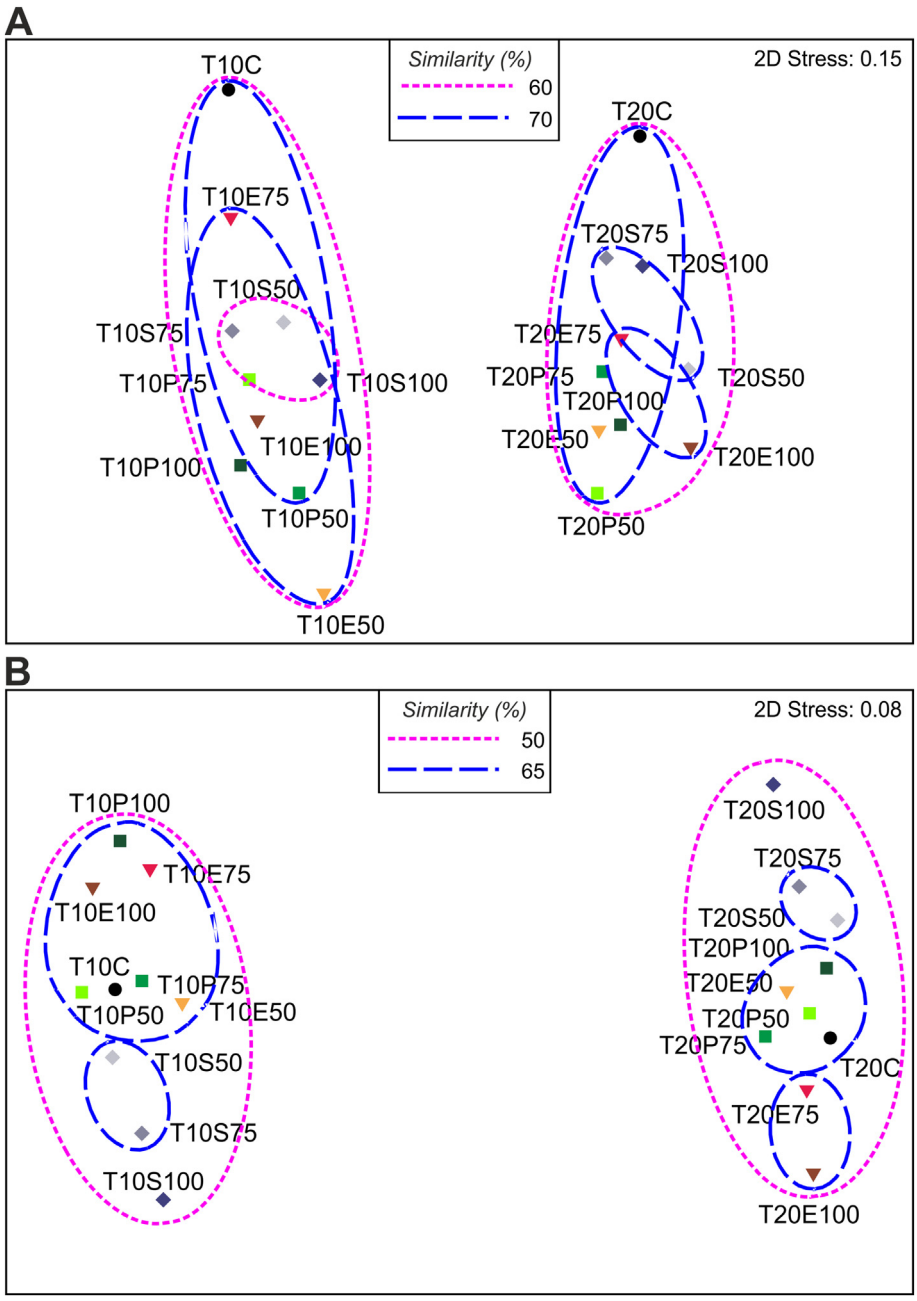

Fig. 4. Non-metric multidimensional scaling (nMDS) based on bacterial (A) and fungal (B) DGGE OTUs and fungal sporulating species after exposure (or not) for 10 and 20 days to increasing concentrations of post-fire runoffs from *Pinus* and *Eucalyptus* forests and stream water. Matrices for nMDS analyses were constructed using Bray-Curtis index and merged with clustering. Exposure time: T10 = 10 days, T20 = 20 days; Samples: C = control, E = Eucalyptus, P = Pinus, S = stream; Sample concentration: 50 = 50%, 75 = 75% and 100 = 100%.

from unexposed controls and those exposed to Pinus, and iii) bacteria exposed to the highest and intermediate concentrations of Eucalyptus (Fig. 4A).

The OTUs of fungal communities at 10 days formed two groups with 65% similarity: i) communities exposed to post-fire runoffs from both forests and controls, and ii) communities exposed to Stream treatments (Fig. 4B). After 20 days, fungal communities from controls, exposed to all concentrations of Pinus runoff and to lower concentration of Eucalyptus runoff shared 65% similarity. Moreover, communities either exposed to higher concentrations of Eucalyptus runoff or lower concentrations of Stream shared 65% similarity (Fig. 4B).

The principal response curves based on fungal sporulating species confirmed that fungal communities were affected by both post-fire runoffs and by the Stream water (Fig. 5; Monte Carlo test, $P < 0.02$). For both exposure times, sporulating species exposed to Stream water were less affected than those exposed to forest runoffs, mainly at longer exposure to higher concentrations. After 20 days, concentration (x-axis of the PRC) explained 19% of the total variance, whereas the sources of samples (y-axis of the PRC) explained 25% of the variance. The largest deviations of fungal communities from controls were found in treatments with Eucalyptus after 20 days (Fig. 5B).

After 10 days, 9 species had positive weights in the PRC, with *Flagellospora curvula* having the highest positive weight followed by *Anguillospora crassa*, *Tricladium splendens*, and *Infundibura* sp., suggesting that these species were the most negatively affected by the post-fire runoffs and stream water (Fig. 5C). In contrast, *Heliscella stellata*, *Triscelophorus acuminatus* and *Alatospora acuminata* were among the species with higher negative weights, suggesting that their sporulation abilities increased by exposure to the runoffs and stream water. After 20 days, the number of species with positive weights decreased to 7. The highest positive weight was observed for *Infundibura* sp.. Some of the species with most negative weights after 20 days were *Anguillospora filiformis* and *Lemonniera aquatica*.

3.5. Relationships between biological responses and chemical composition of post-fire runoffs

Principal component analysis showed the relationships between functional responses of microbes and shredders to post-fire runoffs and stream water at different concentrations and times (Fig. 6). Of the total variance, 65.1% was explained by PC1 and 19.8% by the PC2 (Fig. 6). A clear segregation between controls and treatments was observed along the PC1. PC1 was positively associated with the concentration gradient of each type of sample (Fig. 6). A clear segregation among the samples from different sources was observed along the PC2. The Stream water was positively associated with PC2 at both times, while the treatments from both forest runoffs were negatively associated with PC2 (Fig. 6). Microbial decomposition and the invertebrate feeding were negatively associated with the sample concentrations along the PC1. The concentration of Eucalyptus runoff was mainly associated with PAHs and Cu, while the concentration of Pinus runoff was mainly

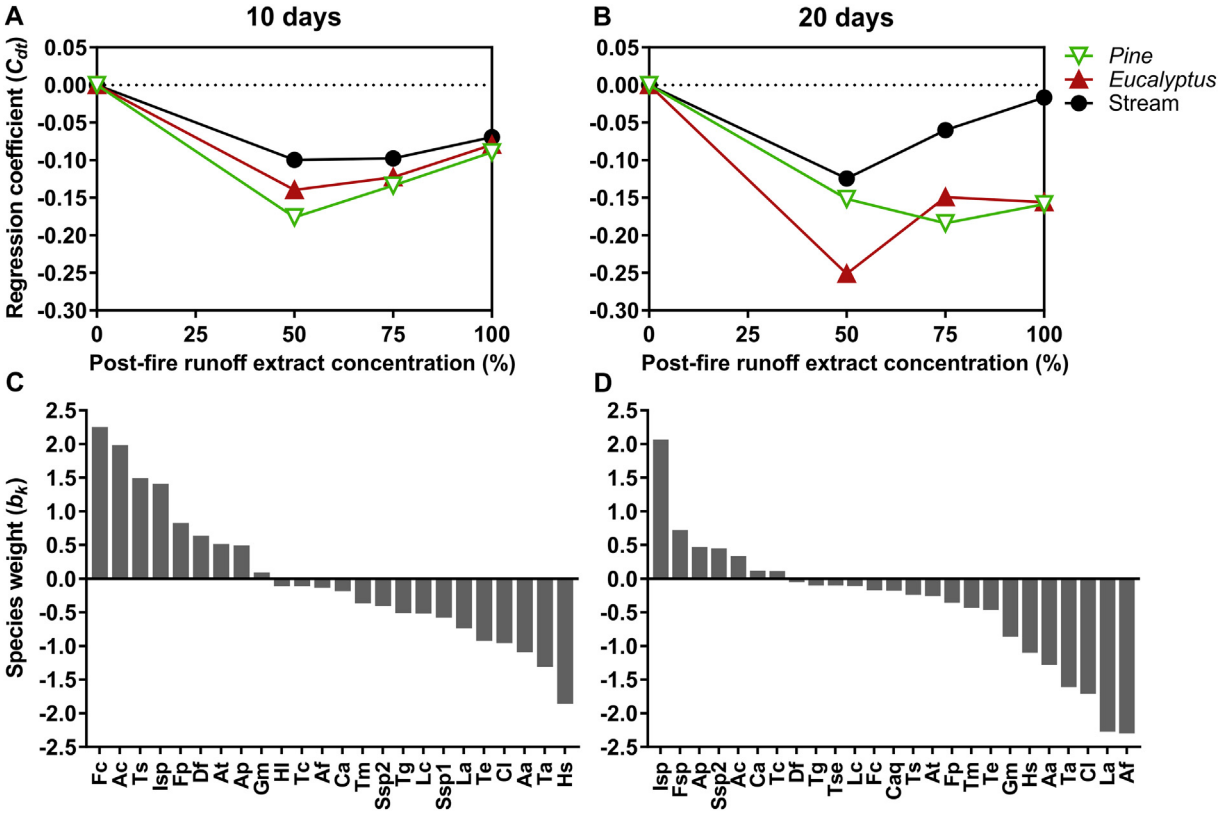

Fig. 5. Principal response curves (C_{dt}) of effects of post-fire runoffs from *Pinus* and *Eucalyptus* forests and stream water on aquatic fungal communities assessed as sporulating fungal species after 10 days (A) and 20 days (B). The lines represent effects of post-fire runoffs with different sources along exposure concentrations (in percentage). Species weights (b_k) showing relative contribution of sporulating individual fungal species to community response after exposure to post-fire runoffs for 10 days (C) and 20 days (D). Fungal taxa: Aa, *Alatospora acuminata* Ingold; Ac, *Anguillospora crassa* Ingold; Af, *Anguillospora filiformis* Greath; Ap, *Alatospora pulchella* Marvanová; At, *Articulospora tetracladia* Ingold; Ca, *Clavariopsis aquatica* De Wild.; Cl, *Clavatospora longibrachiata* (Ingold) Marvanová & Sv. Nilsson; Caq, *Culicidospora aquatica* R.H. Petersen; Df, *Dimorphospora foliicola* Tubaki; Fc, *Flagellospora curvula* Ingold; Fp, *Flagellospora penicillioides* Ingold; Fsp, *Flagellospora* sp.; Gm, *Goniopila monticola* (Dyko) Marvanová & Descals; Hl, *Heliscus lugdunensis* Sacc. & Thérry (also known as *Neonectria lugdunensis* (Sacc. & Thérry) L. Lombard & Crous); Hs, *Heliscella stellata* (Ingold & V.J. Cox) Marvanová; Isp, *Infundibura* sp.; La, *Lemonniera aquatica* De Wild.; Lc, *Lunulospora curvula* Ingold; Ta, *Triscelophorus* cf. *acuminatus* Nawawi; Tc, *Tricladium chaetocladium* Ingold; Te, *Tetrachaetum elegans* Ingold; Tg, *Taeniospora gracilis* Marvanová; Tm, *Triscelophorus* cf. *monosporus* Ingold; Ts, *Tricladium splendens* Ingold; Tse, *Tetracladium setigerum* (Grove) Ingold; Ssp1, unidentified sigmoid sp. 1 (length 50–70 μm, width 3 μm); Ssp2, unidentified sigmoid sp. 2 (length 10–20 μm, width 1 μm).

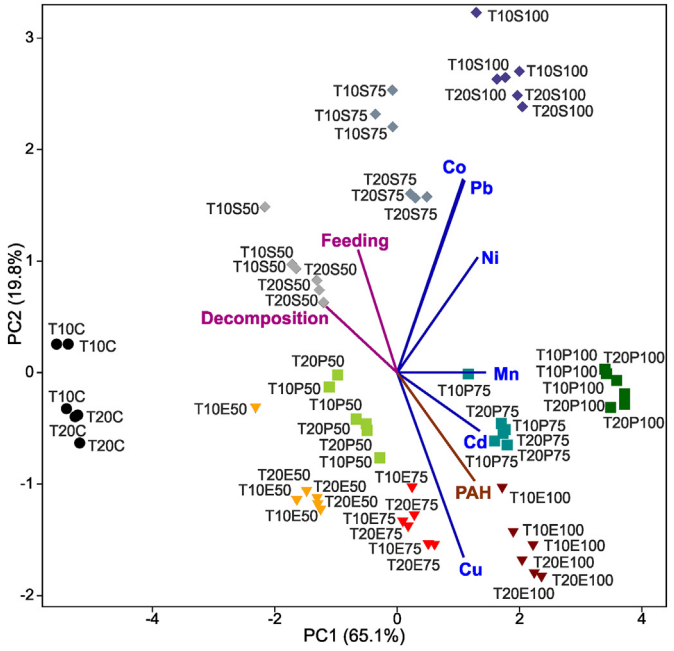

Fig. 6. Principal component analysis (PCA) of overall responses of functional parameters (shredder feeding and microbial decomposition) to the concentrations of total PAHs and metals in the samples. Exposure time: T10 = 10 days, T20 = 20 days; Samples: C = control, E = Eucalyptus, P = Pinus, S = stream; Sample concentration 50 = 50%, 75 = 75% and 100 = 100%.

associated with Cd and Mn. The concentrations of Stream samples were mainly associated with Co, Pb and Ni.

4. Discussion

Our study clearly showed that post-fire runoffs and stream water can severely impact a key ecosystem process, namely leaf litter decomposition driven by microbes and invertebrate shredders, and altered the community structure of litter-associated microbes in streams. Moreover, those impacts depended on the runoff load, burnt forest type and exposure time. The reduction in the activity of microbial decomposers and invertebrate shredders unraveled that wildfires can have major impacts on detrital food webs in streams.

In our study, the effects of post-fire runoffs and stream water from the burnt catchment on stream microbial communities were strong, particularly for the longer time and higher concentrations. DNA fingerprints of bacterial and fungal communities on decomposing leave suggest that community structure was more affected by the post-fire runoffs and stream water than fungal biomass. Shifts in community composition of fungi associated with decomposing leaves were also reported for exposure to acidic mine drainage but the effects on fungal biomass were less pronounced (Niyogi et al., 2002). Earlier studies also showed that stream fungal communities are sensitive to water quality and can be affected by contaminants, such as metals, metal nanoparticles and organic matter (Niyogi et al., 2002; Duarte et al., 2009; Pradhan et al., 2011; Pradhan et al., 2016). We found strong impacts of the post-fire on sporulating fungi, mainly at the longer exposure time. As expected, some fungal species declined with exposure to stream and post-fire runoffs (*Infundibura* sp., *Alatospora pulchella*, *Flagellospora* sp.,), while others became dominant (*Anguillospora filiformis*, *Lemonniera aquatica*, *Clavatospora longibrachiata*, *Triscelophorus acuminatus*, *Alatospora acuminata*). The latter fungal species have been reported in eutrophic streams (Duarte et al., 2009), in mine-impacted streams (Krauss et al., 2005) and in the presence of contaminants, including ionic or nanoparticulate metals and/or nutrients (Fernandes et al., 2009; Pradhan et al., 2011). Shifts in community composition of stream bacteria and fungi after exposure to metal ions and nanometals have been shown, with some species becoming dominant after longer exposure (Sridhar et al., 2005; Duarte et al., 2009; Pradhan et al., 2011). The input of wildfire ashes altered stream water quality and led to shifts in the diatom assemblage towards smaller-size and better-adapted taxa in the Gila River (south-west New Mexico), where *Cocconeis placentula* was the dominant post-fire species (Earl and Blinn, 2003). Also, deleterious effects of ashes from the burnt forest in north-central Portugal with mixed stands of *Pinus* and predominantly *Eucalyptus* led to a decrease in the growth of the primary producer *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*; Silva et al., 2015).

Earlier studies showed that exposure to various contaminants can affect leaf decomposition (Krauss et al., 2005; Duarte et al., 2009; Pradhan et al., 2011). As hypothesized, the post-fire runoffs and stream water affected ecosystem functions and trophic interactions: i) leaf litter decomposition rates driven by stream microbial communities were affected by post-fire runoffs in a concentration-dependent manner, and ii) the feeding behaviour of the invertebrate shredder *A. ligonifer* was affected particularly at higher concentrations. Post-fire runoffs can induce mortality to freshwater organisms from higher trophic levels, such as bivalves (Silva et al., 2016). Sublethal effects of post-fire runoffs from *Eucalyptus* forest and stream water were shown for the freshwater fish *Gambusia holbrooki*, where enzymatic biomarkers (from gills and liver) revealed oxidative stress (Nunes et al., 2017). Physiological changes in freshwater organisms at different trophic levels were induced by aqueous extract of ashes from forest-fires (Silva et al., 2015). Our study complements this information by providing evidence of effects of post-fire runoffs on stream ecosystem processes. Decrease in the shredder feeding rates by post-fire runoffs and stream water was probably due to food avoidance behaviour (Wilding and Maltby, 2006), because shredders prefer to feed on nutrient-enriched leaves well colonized by microbes, especially fungi (Arsuffi and Suberkropp, 1989; Chung and Suberkropp, 2009). In our study, the post-fire runoffs and stream water affected fungal biomass, and the structure and function of microbial decomposers, probably by reducing the quality and palatability of leaves to the shredders. Negative impacts on the feeding activity of *A. ligonifer* and other freshwater shredders were demonstrated in the presence of ionic and nanoparticulate metals, humic substances and pesticides (Pestana et al., 2007; Schäfer et al., 2012; Pradhan et al., 2015).

In addition to concentration and time, the source of the post-fire samples affected the microbial decomposers and the invertebrate feeding. Effects of post-fire runoffs from *Eucalyptus* and *Pinus* forests were more pronounced at the longer time, particularly at higher concentrations. Conversely, the exposure to post-fire stream water showed less pronounced effects on fungal biomass. This probably was the consequence of physicochemical interactions of the suspended ashes found in stream water to fungal mycelia by adhering to the leaves. Moreover, at the highest concentration, aggregation and precipitation of ashes were visible in our microcosms.

Previous studies have shown the presence of various metals and polycyclic aromatic hydrocarbons in the post-fire runoffs, which may play a role in the overall biological effects (Olivella et al., 2006; Campos et al., 2012; Silva et al., 2015; Silva et al., 2016; Nunes et al., 2017). Considerable amounts of Mn, Pb, Cu, Co, Ni and Cd were detected in our study, mainly in the post-fire runoffs. Most of these metals are known to induce oxidative stress and toxicity to various organisms, including freshwater microbes and shredders (Jaeckel et al., 2005; Jadhav et al., 2007; Azevedo et al., 2009; Batista et al., 2012; Pradhan et al., 2015). Exposure to PAHs induced lethal toxicity to the freshwater invertebrates, *Ceriodaphnia reticulate* and *Daphnia magna*, and affected the structure of zooplankton community (Ikenaka et al., 2013). Neff et al. (2005) provided insights into the causes of toxicity of sediments contaminated with PAHs to freshwater benthic organisms. The negative effects of PAHs on aquatic invertebrates can increase by 2 orders of

magnitude due to phototoxicity (Pelletier et al., 1997), and the possibility of this had occurred in our study cannot be discarded as photoperiod was used during the experiment. Conversely, many bacteria and fungi are reported to degrade PAHs (Peng et al., 2008). Indeed, some freshwater fungi, such as *Clavariopsis aquatica* present in our natural assemblage, are capable of degrading some hazardous organic compounds (Junghanns et al., 2005; Solé et al., 2008). However, data on the responses at the community level in complex environmental matrix, such as post-fire runoffs with PAHs and metals, are scarce. In our study, microbial decomposition and invertebrate feeding were negatively correlated with the metals and total PAHs in the post-fire samples. Moreover, stream water showed lesser impacts than the runoffs from the burnt planted forests. It is important to consider that when runoffs reach the water body they are diluted, and the toxicant compounds can adhere to organic matter, probably explaining their lower concentration in the stream water when comparing with post-fire runoffs. Indeed, different concentration of metals and PAHs explained the responses of microbial communities and invertebrates to the post-fire runoffs and stream water from the burnt catchment.

Our results showed that the effects of post-fire runoffs may be strongly related to the forest type, runoff load and exposure time. All of these three factors can influence the chemical composition of the runoff, thereby altering the overall impacts on freshwater biota and their ecological functions. Similar effects to those obtained in our study are expected to occur in other Mediterranean regions of the Iberian Peninsula with *Pinus* or *Eucalyptus* forests, and also in regions from other continents, such as West Australia or NW USA with similar climate conditions, including dry summers and wet winters. However, we should be careful when extrapolating our conclusions to other parts of the world, once impacts can also be related to specific conditions in the study area such as soil properties and forest management practices.

5. Conclusions

Overall, our study is among the first that clearly shows the adverse effects of wildfires on microbial communities and invertebrates in stream detrital food webs. The post-fire runoffs and stream water from the burnt catchment severely reduce the activity of microbial communities and invertebrate shredders that drive leaf litter decomposition, a key measure of stream ecosystem functioning. The post-fire runoffs and stream water led to shifts in taxon composition of bacteria and fungi on decomposing leaves. The effects were stronger at longer exposure to post-fire runoffs. The source of post-fire samples seems to be another determinant factor of the overall impacts. The structural and functional effects of post-fire runoffs from *Pinus* and *Eucalyptus* forests were more pronounced than the effects of the stream water from the burnt catchment. This can be explained by the higher concentration of metals and total PAHs in forest runoffs. Our study underscored the significance of assessing the indirect impacts of wildfires on stream detrital food webs by highlighting how exposure time, source and load of the post-fire runoffs can affect the community structure and the functions of biota. The study further supports the need of proper forest management to minimize the post-fire runoff loads reaching aquatic ecosystems, and to reduce the impacts of these extreme events on aquatic biodiversity and ecosystem functioning.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.07.265.

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