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Subsurface zones in intermittent streams are hotspots of microbial decomposition during the non-flow period

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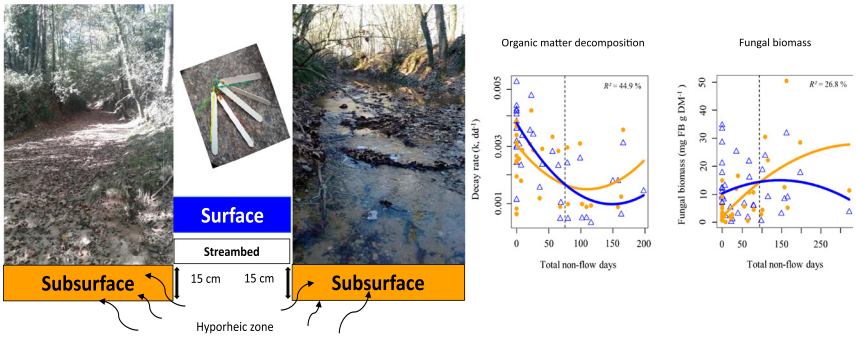
HIGHLIGHTS

- Subsurface zone contributes to maintain decomposition during non-flow periods
- Surface decay rates decrease with intermittency more strongly compared to subsurface
- Subsurface fungal biomass increases with intermittency until saturation
- Phosphorus availability and fine sand content accelerate microbial decomposition

GRAPHICAL ABSTRACT



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ABSTRACT

The microbial decomposition of organic matter is a fundamental ecosystem process that transforms organic matter and fuels detritus-based food webs, influencing biogeochemical cycles such as C-cycling. The efficiency of this process can be compromised during the non-flow periods of intermittent and ephemeral streams (IRES). When water flow ceases, sediments represent the last wet habitat available to microorganisms and may play an important role in sustaining microbial decomposition. However, despite the increasing prevalence of IRES due to climate change and water abstraction, it is unclear to what degree the subsurface habitat can sustain microbial decomposition during non-flow periods. In order to gather information, we selected 20 streams across Catalonia (Spain) along a gradient of flow intermittency, where we measured microbial decomposition and fungal biomass by placing wood sticks in both the surface and subsurface zones (15 cm below the streambed) over the course of one hydrological year. Our results showed that microbial decomposition and fungal biomass were consistently greater in the subsurface zone than in the surface zone, when intermittency increased. Although flow intermittency was the main driver of both microbial decomposition and fungal biomass, phosphorus availability in the water, sediment C:N ratio and sediment grain size also played relevant roles in surface and subsurface organic matter processing. Thus, our findings demonstrate that although the OM processing in both zones decreases with increased intermittency, the subsurface zone made an important contribution during the non-flow periods in IRES. Therefore, subsurface activity during non-flow periods has the potential to affect and maintain ecosystem functioning.

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1. Introduction

Organic matter (OM) decomposition is a key ecosystem process that has implications for aquatic food webs and biogeochemical cycles, such as C-cycling pathways (Follstad Shah et al., 2017; Gessner et al., 1999). In forest streams, the main source of OM comes from riparian vegetation, such as leaf litter or woody debris; thus, both represent an essential energy source for food webs (Abril et al., 2016; Gonçalves et al., 2014). Microbes (fungi and bacteria) and invertebrates are the most important organisms that contribute to OM decomposition, but their activities may vary with local environmental conditions (Gessner et al., 2010). In freshwaters, fungi are the first colonisers and the main microbial decomposers during the early stages of decomposition, constituting an essential trophic link between OM and invertebrate consumers (Arias-Real et al., 2018; Gessner and Chauvet, 1994; Kuehn, 2016). Aquatic OM decomposition depends on environmental factors that affect biological activity and/or physical degradation (Krauss et al., 2011). Recent evidence has shown that OM decomposition can be compromised in streams that experience periods of complete flow disruption in time or space (termed intermittent and ephemeral streams, IRES) (Datry et al., 2011, 2014, 2018; Larned et al., 2010) due to abrupt changes in environmental conditions (Foulquier et al., 2015; Lake, 2003). For instance, surface water loss reduced dissolved oxygen and increased water temperature, nutrients and conductivity (Krauss et al., 2011); it is also expected to reduce the richness and activity of aquatic decomposers (Gonçalves et al., 2016; Martínez et al., 2015). In addition, flow reduction affects the riparian vegetation, causing early leaf abscission (Sanpera-Calbet et al., 2016). This may lead to temporal and spatial changes in OM sources for microbes and invertebrates.

During the non-flow period, the subsurface zone could be particularly important in maintaining decomposition of OM because it is the last remaining habitat where water is available (Arce et al., 2019). In these conditions, microorganisms seek refuge by moving vertically into this zone (Stubbington, 2012). Moreover, non-flow favours OM (leaf litter and woody debris) accumulation in the dry streambed, which could be buried during storms (Scott and Zhang, 2012), leading the subsurface zone to become the major OM storage compartment in the stream (Cornut et al., 2012; Storey et al., 1999). As such, the subsurface zone could operate as an active zone during the non-flow period (Boulton et al., 1899, 1998; Marxen et al., 2010; Stubbington, 2012). However, it remains unclear whether the subsurface zone can support similar or higher rates of OM decomposition compared to the wet surface zone when flow is present.

Previous studies have shown the resilience of bacterial communities located in the subsurface zone to long-term non-flow periods when flash storms suddenly increase the water content in the sediment, which has implications for the maintenance of nutrient cycling and OM decomposition (Harjung et al., 2019; Marxen et al., 2010; Pohlon et al., 2013). While it is known that fungal communities are crucial for OM decomposition in the surface zone, there is still limited knowledge about their role in the subsurface zone; they might be essential for the sustainability of this process in the absence of surface water (Cornut et al., 2010, 2014).

However, flow intermittence may exert different effects on aquatic decomposers depending on the length and frequency of non-flow phases and the characteristics of different stream microhabitats (Burrows et al., 2017; Solagaistua et al., 2016). For example, aquatic life can persist during the non-flow phase in isolated pools, wet sediments and the hyporheic zone, but the suitability of these microhabitats could vary with grain size, solar irradiance or weather (Datry et al., 2011; Harjung et al., 2019; Marxen et al., 2010; Pohlon et al., 2013; Stubbington, 2012). In addition, some flash storms can rapidly stimulate and restore microbial activity (Barnard et al., 2015; Blazewicz et al., 2014; Gionchetta et al., 2019). Therefore, considering that IRES represent approximately half of the global river network and that their spatial extent is expected to increase due to climate change and increased water use (Datry et al., 2017), there is an urgent need to better understand which hydrological, microhabitat and local environmental factors can sustain OM decomposition during the non-flow period.

The objective of this study was to explore the dynamics of microbial decomposition in the surface and subsurface zones of 20 streams, over a gradient of flow intermittency. First, we explored the effects of hydrology and microhabitat (surface and subsurface zones) on OM decomposition and fungal biomass, and then we analysed the effects of hydrological and environmental features on OM decomposition and fungal biomass in each zone separately. We hypothesised that (i) the rates of OM decomposition and the fungal biomass would be higher in the subsurface zone compared to the surface zone when intermittency increases, which would sustain OM processing, and (ii) the environmental features would modulate hydrological effects on OM decomposition through changes in the microbial communities (e.g., fungal biomass).

2. Methodology

2.1. Study area

This study was conducted in 20 low-order streams that belong to eight different basins across Catalonia (NE Spain). Forest, scrubland and grasslands were the primary land use at the riparian scale (Table 1). Although, in some streams, the main land use was extensive agriculture (mainly olive groves and vineyards), causing minor levels of anthropogenic impact (Corine Land Cover 2006 data from a buffer area of 1 km around each sampling site) (Table 1). Furthermore, poplar (Populus nigra L.), alder (Alnus glutinosa (L.) Gaertner) and evergreen oak (Quercus ilex L.O) were the dominant riparian vegetation. The climate is typically Mediterranean with dry and warm summers, and precipitation occurring mainly during spring and autumn.

2.2. Stream hydrology

We calculated the total number of non-flow days (TNF) at each site (Fig. 1). To do this, we used the daily variation of the streambed temperature as an indicator of water presence in lotic and lentic habitats. This daily variation was determined as the difference between the maximum and minimum temperatures on each day and the highest daily rate of change per hour. Temperature and water level were recorded with Leveloggers (Solinst Levelogger Edge, full-scale reading precision of 0.05%) that were placed on the streambed (Constantz et al., 2001). The Leveloggers operated at hourly intervals for one year (study period from September 2016 until September 2017). The recorded data were corrected for atmospheric pressure variations using data from Barologgers (Solinst Barologger, full-scale reading precision of 0.05%) that were installed at the riparian zone of each sampling site.

Once temperature data were retrieved, we performed a moving average of order 5 to smooth daily differences. We standardised each value with a fixed value per month, using data from field observations, data from the meteorological stations (Servei Meteorològic de Catalunya; http://www.meteocat.es) at each site (or nearby), and the water level data from the Leveloggers. Furthermore, we corrected the occasional similarity between streambed temperature and air temperature during autumn and spring with precipitation data from meteorological stations.

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Table 1
Geographical and basin characterization of the studied sites. The percentages of land use cover refer to a buffer area of 1 km around each sampling point.

Site	Lat., N	Long., E	Altitude (m.a.s.l)	Order	Basin	Prec. (mm)	Catchment area (km2)	Land Use Cover (%)		
								Urban	Agric.	Nat.
1	41°46′14.72″	2°16′9.39″	430	3	BE	788	6.5	2.2	0.7	97.1
2	42° 7′28.68″	2°26′27.30″	484	4	FL	1026	15.7	0	1.1	98.9
3	42° 7′30.84″	2°40′54.96″	219	4	FL	998	13.8	0	34.4	65.6
4	42° 6′50.94″	2°26′53.16″	475	4	FL	475	965.4	0.1	2.8	97.1
5	42°10′28″	2°28′71″	434	3	FL	963	6.7	0	0	100
6	41°23′51.64″	1°35′36.94″	330	4	FO	590	26.9	0	100	0
7	41°24′41.89″	1°35′36.65″	390	3	FO	574	1.8	0	96.4	3.6
8	41°18′36.43″	1° 5′16.00″	605	4	FR	470	28.9	0	14.1	85.9
9	41°44′28.38″	1°56′9.94″	325	3	LL	732	10.9	2.2	0.9	96.9
10	41°37′57.88″	1°55′55.87″	394	3	LL	692	8.6	0	0	100
11	41°34′53.82″	1°59′5.52″	335	4	LL	635	23.9	1.2	2.9	95.9
12	42°23′15.58″	3° 3′6.24″	100	3	MU	842	13.5	0	99.5	0.5
13	42° 6′35.35″	2°29′19.30″	526	3	TE	963	13.3	0	0.6	99.4
14	42° 4′40.59″	2°20′19.44″	630	4	TE	953	30.9	1.5	0	98.5
15	41°55′12.78″	2°42′51.23″	140	3	TE	816	7.7	0	78.5	21.5
16	42° 4′17.73″	2°32′26.86″	385	3	TE	1038	9.1	0	0.9	99.1
17	41°42′33.64″	2°32′2.87″	110	3	TO	865	49.1	0	0.6	99.4
18	41°49′23.01″	2°27′9.01″	489	3	TO	911	12.4	0	0.2	99.8
19	41°51′57.91″	2°35′35.04″	655	4	TO	875	49.0	0	0	100
20	41°52′42.10″	2°38′54.46″	170	3	TO	908	12.4	0.8	3.3	95.9

Lat. = latitude; Long. = longitude; Prec. = annual precipitation; Agric. = extensive agriculture; Nat. = nature that include: forest (broad-leaved forest, mixed forest and coniferous forest), scrubland and grasslands; BE = Besos; FL = Fluvia; FO = Foix; FR = Francoli; LL = Llobregat; MU = Muga; TE = Ter and TO = Tordera.

2.3. Environmental factors

For each stream location, we performed three sampling campaigns: the first (t0) during September/October 2016, the second (t1) during February 2017 and the third (t2) during September 2017. At each time and location, we measured water electrical conductivity, water temperature, pH and dissolved oxygen (± 1 µs cm^{-1}, ± 0.1 °C, ± 0.005 pH and $\pm$ 0.1 mg L^{-1}, respectively) using a portable probe (YSI Professional Plus Multiparameter Instrument, USA).

To characterise the nutrient concentrations in the water (nitrite, nitrate, ammonium and soluble reactive phosphorus (SRP)), we took water samples when surface water was present. Water samples were filtered through pre-combusted glass fibre filters in the field (0.7 µm pore size; Whatman GF/F, Germany) and then transported to the laboratory under cooled conditions. In the laboratory, we stored the water samples at 4 °C, in darkness, until analysis (between 24 and48 h).

We analysed the concentrations of dissolved nitrite (NO_2^-) and nitrate (NO_3^-) using ionic chromatography with a conductivity detector WATERS (model 432), UV/V KONTROL detector (model 332) and the column WATER IC-PAK ANIONS (Metrohm 761 Compact IC with the column Metrosep A Supp5 - 150/4.0). We measured the ammonium concentration using the salicylate method (Reardon et al., 1969) and SRP using the molybdate method (Murphy and Riley, 1962).

To characterise the sediment, we used a shovel and took three replicates per stream from the top 0–5 cm and down to 16 cm deep in the same habitat where had placed the wood sticks, to be sure that it did not skew the results by spatial variation of the streams. The samples were placed into jars and transferred to the laboratory under conditions of darkness. In the laboratory, one aliquot of fresh sediment was allotted for granulometric analysis, and a second aliquot was dried at 70 °C until it reached a constant weight for dry weight determination and elemental analysis.

To determine the grain size distribution, fresh sediment samples (first aliquot) were first treated with H_2O_2 (10% volume) to remove

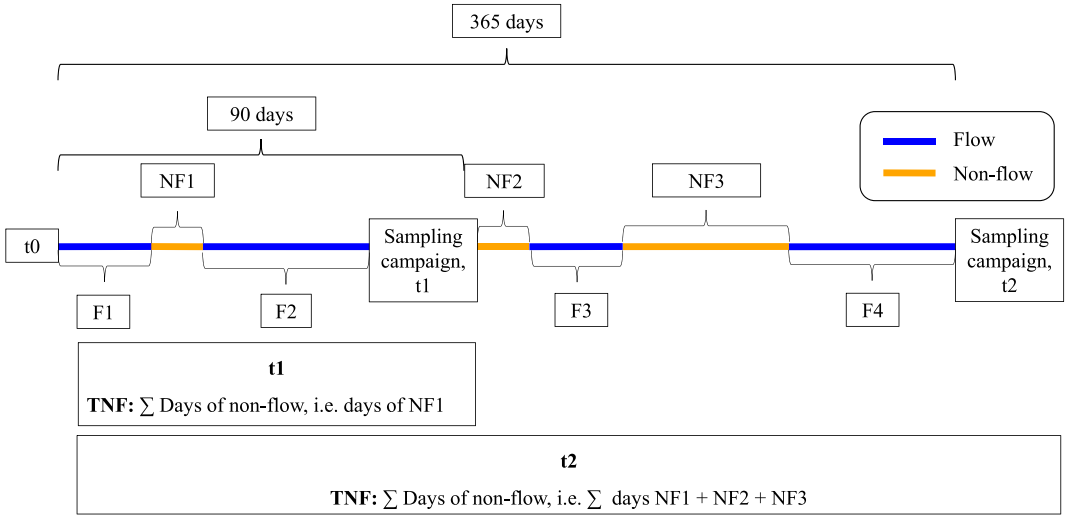

Fig. 1. Example of the calculation of total non-flow days (TNF) in this study. t0 is the time when the experiment started and the wood-sticks and loggers were placed on the streams, t1 is the time when we took the first wood-sticks and t2 is the time when we took the remaining wood-sticks and the experiment finished. NF is non-flow, F is flow and TNF is total non-flow days.

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organic matter and later disaggregated and dispersed ultrasonically with pyrophosphate. Fractions up to 2 mm were determined by sieving, while the determination of fractions below 2 mm was performed with a Beckman-Coulter LS230 laser. Then, the dry material (second aliquot) was ground using an agate mortar until it was completely homogenised, and we analysed the nitrogen (N) and carbon (C) concentrations using a Thermo Elemental Analyser 1108 (Thermo Scientific, Milan, Italy). We expressed the results in terms of C:N molar ratios.

The water sediment content or moisture content was calculated as the percentage of water loss (%), which was determined by the difference between fresh and dry weight.

2.4. Organic matter experiment

We quantified the decomposition of OM in both surface and subsurface zones using sticks of *Populus canadensis* wood (15 × 2 × 0.2 cm) (Arroita et al., 2012). We placed 10 sticks on the streambed to characterise surface decomposition and 10 sticks at a depth of 15 cm below the streambed to quantify subsurface decomposition. The sticks were placed in each stream at t0. Before being placed in the streams, the sticks were marked, oven-dried (70 °C, 72 h) and weighed. In the surface zone, each group of sticks was tied to metal bars with nylon threads, branches or roots to ensure that it remained in the lotic habitat. During flowing periods, we ensured that the sticks were completely submerged. In the subsurface zone, each group of sticks was inserted into the sediment and tied to metal bars with nylon thread. An extra set of 20 sticks per stream was transported but not placed in the streams and then returned to the laboratory to correct the initial weight, taking account of manipulation. These sticks were used to calculate the initial dry mass and ash content.

The sticks that were placed in the streams were picked up during the two sampling campaigns: one after between 90 and100 days (t1) and the second after one year (t2). During each sampling, we collected five sticks per zone (half of the sticks). The sticks were placed in individual zip-lock bags and transported to the laboratory in refrigerated containers.

Once in the laboratory, we processed the sticks immediately to avoid changes in weight and ergosterol degradation. First, we gently brushed them to remove adhering material and then washed them with distilled water. Afterwards, we cut and weighed one 1-cm-long aliquot of each stick. These aliquots were frozen at −80 °C for later determination of the ergosterol concentration as a proxy for fungal biomass (Gessner, 2005). Then, the remaining part of each stick was dried (70 °C, 72 h) and weighed to calculate the final dry mass.

We cut two 1-cm-long aliquots from the remaining dry part for subsequent analysis. The first aliquot was incinerated (500 °C, 5 h) to measure the ash-free dry mass (AFDM) by removing inorganic components, and the second aliquot was used to analyse the nitrogen (N) and carbon (C) content.

To analyse the ergosterol concentration as a proxy of fungal biomass (Gessner, 2005), an aliquot of each stick was lyophilized and weighed to determine the dry mass, and lipid extraction and saponification were performed using 0.14 M KOH methanol (8 g L^{-1}) at 80 °C for 30 min in a shaking water bath. The extracted lipids were purified using solid-phase extraction cartridges (Waters Sep-Pak® Vac RC, 500 mg tC18 cartridges, Waters Corp, Milford, MA, USA), and ergosterol was eluted using isopropanol. We used high-pressure liquid chromatography (HPLC) to detect and quantify the ergosterol by measuring the absorbance at 282 nm. We used a Jasco HPLC system (USA) equipped with a Gemini-NX 5 μm C18 250 × 4.6 mm column (Phenomenex, UK). The mobile phase was 100% methanol, and the flow rate was set to 1.2 ml min^{-1}. Finally, we converted the ergosterol measurement into fungal biomass using a conversion factor of 5.5 mg of ergosterol per gram of fungal mycelium (Gessner and Chauvet, 1993). We expressed the results in mg of fungal biomass per gram of dry mass.

To determine the N and C content in the sticks, the aliquots were ground and analysed with the same methodology used to analyse the N and C content in the sediment. We expressed the results in terms of C:N molar ratios.

Finally, we estimated the decay rates following the negative exponential model $M_t = M_0 e^{-kt}$, where M_0 is the initial percentage of AFDM, M_t is the remaining AFDM at time t, and k is the decay rate (Petersen and Cummins, 1974). We expressed the decay rates in terms of accumulated heat by replacing time with the mean daily temperatures accumulated (degree-days, dd^{-1} (Stout, 1989)). To express the decay rates in degree-days, we used the mean daily temperatures from the Leveloggers, and for the subsurface zone, we used the mean daily temperatures from the SmartButtons (ACR Systems Inc. data logger temperature recorders) that were placed in the subsurface zone (15 cm below the streambed) at t0.

2.5. Data analysis

To reduce distribution skewness, water sediment, coarse sand, DIN and SRP were log-transformed and clay, fungal biomass and C:N ratios of sediments were square-root-transformed, before the analyses were performed. All quantitative predictors were *Z*-standardised (mean = 0, SD = 1) to allow for model coefficient comparison. To assess predictor collinearity, we estimated the Variance Inflation Factor (VIF; vifstep, usdm R package (Zuur et al., 2009, 2010)) and pairwise Pearson correlations (cut-off of r ≤ |0.70|) (Feld et al., 2016). To analyse how OM decomposition (decay rates) and fungal biomass respond to flow intermittence at surface and subsurface zones, we used linear mixed-effect models (LMMs, lme4 R package (Pinheiro et al., 2017)). For both response variables, we created LMMs that included TNF, zone (two-level qualitative predictor: surface and subsurface) and their interaction as fixed factors. These models included data from 20 sites, in which both surface and subsurface zones were surveyed on two occasions (decay rates: $n = 70$; fungal biomass: $n = 72$). Using Akaike Information Criteria (AIC) values, we checked two random structures ("sampling site" and "sampling site nested within basins") to account for non-independent structures within samples belonging to the same sites and basins. We selected "sampling site" as the random structure for both decay rates and fungal biomass models, as this model structure showed a better explanatory capacity and model simplicity (lower AIC values). Furthermore, we used a quadratic term for TNF to account for nonlinear responses. For each LMM, we estimated the variance explained by the fixed factors alone (r^2_m) and the variance explained by both the fixed and the random terms (r^2_c). To explore the relative importance of TNF, zone (surface vs. subsurface) and their interactions in the models, we performed variance partitioning on LMMs using the *variancePartition* R package (Hoffman and Schadt, 2016).

To identify how environmental features modulate hydrological effects on OM decomposition and fungal biomass in surface and subsurface zones, we followed a two-step modelling procedure that included an exploratory analysis to select the most important predictors and final models to estimate environmental features´ importance and significance (Feld et al., 2016). These models included 20 sites surveyed on two occasions (surface zone: $n = 38$ and subsurface zone: $n = 36$).To rank and select predictors according to their predictive power, we used Spearman rank correlations to account for potential non-linear responses. Second, to quantify the effects, importance and significance of hydrology (TNF) and the best environmental predictors of OM decomposition (decay rates) and fungal biomass, we fitted linear regression models (LMs) and LMMs. Then, between these models, we selected linear mixed model (LMM) for decay rates on the surface zone and linear regression models (LMs) for decay rates on the subsurface zone and fungal biomass in both, surface and subsurface zones, due to their greater explanatory capacity and parsimony compared to LMMs (i.e., lower AIC values (Akaike, 1973)).

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In the surface zone models, for LMM of decay rates we used TNF, SRP, conductivity and their interaction as fixed factors and sampling site as random factor, to account for repeated measures in the same location. In the LM, we used TNF, SRP and conductivity as predictors for fungal biomass.

In the subsurface zone, we used TNF, fine sand, water sediment content and C:N ratios of the sediment as predictors for decay rates in each LM, whereas we used TNF, coarse sand, water sediment content and C:N ratios of the sediment as predictors for fungal biomass in each LM. None of the final input variables included in the final models has a collinearity problem (Table S1).

To explore the relative importance of hydrology (TNF) and the best environmental predictors into the models, we performed variance partitioning on LMM and LMs using the *variancePartition* R package (Hoffman and Schadt, 2016).

All models were validated by visually checking their residuals for normality and homoscedasticity (Zuur et al., 2010). All statistical analyses were performed using the R statistical software version 3.4.1 with the significance level set at $p < .05$ for all tests (R Development Core Team, 2011) (for more details see S2_Rscripts.zip).

3. Results

The studied streams covered a steep gradient of intermittency (from permanent to ephemeral streams) (Table S3). Dissolved oxygen varied from 4.9 mg L^{-1} to 9.2 mg L^{-1}, conductivity varied from 164.6 μS cm^{-1} to 827.0 μS cm^{-1}, DIN (nitrite + nitrate + ammonia) varied from 0.424 mg L^{-1} to 6.174 mg L^{-1} and SRP varied from 0.008 mg L^{-1} to 1.727 mg L^{-1} in surface flowing water (Table S4). Moisture content varied from 2% to 84%; sediment grain size proportions varied from 0% to 19.31% clay, 0% to 48.21% silt, 0% to 32.98% fine sand, 9.72% to 98.75% coarse sand, and 0% to 61.95% gravel; and the ratios of C:N in the sediments varied from 9.1 to 186.8 (Table S5).

3.1. Effects of hydrology and zone on OM decomposition and fungal biomass

OM decomposition (decay rates, k dd^{-1}) and fungal biomass were greater in the subsurface zone compared to the surface zone when TNF increased (Table 2, Fig. 2), as reflected by the significant interactions between non-flow days and zone (Table 2).

The decay rates at the surface decreased with TNF more sharply than the subsurface decay rates, which even recovered at the most ephemeral sites (TNF > 100 days). Thus, in streams with <75 days of non-flow, decay rates (k, dd^{-1}) were higher in the surface zone (Mean ± SE; 0.0031 ± 0.0002) than in the subsurface zone (0.0025 ± 0.0002). However, for streams experiencing >75 days of non-flow, decay rates were higher in the subsurface zone (0.0018 ± 0.0003) than in the surface zone (0.0014 ± 0.0002) (Fig. 2a).

For streams with <75 days of non-flow, the contribution of decay rates (relative to the total decay rates, i.e., the sum of the decay rates in the surface and subsurface zones) in the surface zone was 10.23% higher than that in the subsurface zone. For streams with >75 days of non-flow, the contribution of decay rates in the subsurface zone was 9.77% higher than that in the surface zone (Fig. 3a). Furthermore, as we observed in Fig. 3a, for the streams with >75 days of non-flow, the contribution of decay rates in the subsurface zone was only 0.32% less than the contribution of decay rates in the surface zone for the streams with <75 days of non-flow.

We showed that in the surface zone, fungal biomass hardly changes along the gradient of intermittency; nevertheless, in the subsurface zone, we showed that when the intermittency increases, the fungal biomass increases (Fig. 2b).

As observed for decay rates, the fungal biomass (mg FB g DM^{-1}) was higher in the surface zone (12.3 ± 1.8) than in the subsurface zone (4.9 ± 0.9) in streams with <90 days of non-flow; however, after 90 days of non-flow, the fungal biomass was higher in the subsurface zone (20.6 ± 5.5) than in the surface zone (13.9 ± 3.2).

For streams with <90 days of non-flow, in the surface zone, the contribution of fungal biomass (relative to the total fungal biomass, i.e., the sum of the fungal biomass in the surface and subsurface zones) was 43.2% higher than that in the subsurface zone. For streams with >90 days of non-flow, the fungal biomass in the subsurface zone was 19.6% higher than that in the surface zone (Fig. 3b). Furthermore, as we observed in Fig. 3b, for the streams with >90 days of non-flow, the contribution of fungal biomass in the subsurface zone was 11.8% less than the ratio of fungal biomass in the surface zone for the streams with <90 days of non-flow.

Fixed factors (TNF and zone) explained 39.1% of the variance in decay rates and 25.6% of the variance in fungal biomass (Table 2). For decay rates, the variable that explained the most variance was TNF (38.4%), whereas for fungal biomass, the interaction between TNF and zone was the most explanatory term (21.1%).

3.2. Effects of hydrology and environmental features on OM decomposition and fungal biomass

The first exploratory analysis with Spearman rank correlations identified TNF, SRP concentration and water conductivity as the best predictors of decay rates and fungal biomass in the surface water ($n = 38$). In the subsurface zone, the best predictors were TNF, moisture content and C:N ratios in the sediment ($n = 36$). Sediment grain size was also a good predictor for both response variables in the subsurface zone; fine sand was a good predictor for decay rates and coarse sand for fungal biomass.

In the surface zone, decay rates decreased when TNF increased but no other environmental predictor showed a significant effect (Table 3; Fig. 4a). In the subsurface zone, decay rates decreased when TNF increased, but the higher presence of fine sand was associated more with higher decay rates and higher moisture content than were lower decay rates with higher water loss (Table 3, Fig. 4b and c, respectively).

Fungal biomass in the surface zone decreased as TNF increased, but higher SRP was linked with higher fungal biomass (Fig. 4d). However, in the subsurface zone, fungal biomass increased as TNF increased, and the magnitude of this increase was related to sediment grain size; in the sites with a higher presence of coarse sand, the fungal biomass was lower (Fig. 4e). Furthermore, higher C:N content in the sediment was associated with lower fungal biomass (Fig. 4f).

Table 2
Results of the LMMs relating decay rates, k (dd^{-1}, n = 70) and fungal biomass (mg FB g DM^{-1}, n = 72) to TNF and zone and their interactions. Standardised effect size (SES), standard error (SE), significance and variance explained are shown. Significant variables are highlighted in bold. r^2_m: variance explained by the fixed factor alone; r^2_c: variance accounting for both fixed and random terms. The quadratic term (^{2}) means that the response is nonlinear.

	Hydrological variables	SES	SE	*p*-Value	Explained variance	r^2_m	r^2_c
OM decay rate, k (dd^{-1})	Intercept	0.003	0.001	<0.001		44.9	73.6
	TNF	−0.001	0.000	**<0.001**	38.4		
	Zone	0.000	0.000	0.121	0.5		
	TNF x zone	−0.001	0.000	**0.002**	3.9		
	TNF^2	−0.001	0.000	**0.032**	1.8		
Fungal Biomass (mg FB g DM^{-1})	Intercept	1.627	0.341	<0.001		26.8	65.1
	TNF	1.194	0.206	**<0.001**	2		
	Zone	0.697	0.228	**0.004**	4.2		
	TNF x zone	−1.125	0.229	**<0.001**	21.1		
	TNF^2	0.883	0.233	**<0.001**	11.1		

4. Discussion

Overall, our findings confirm our hypothesis that subsurface processes had an important contribution to sustaining microbial decomposition during the non-flow periods of intermittent and ephemeral

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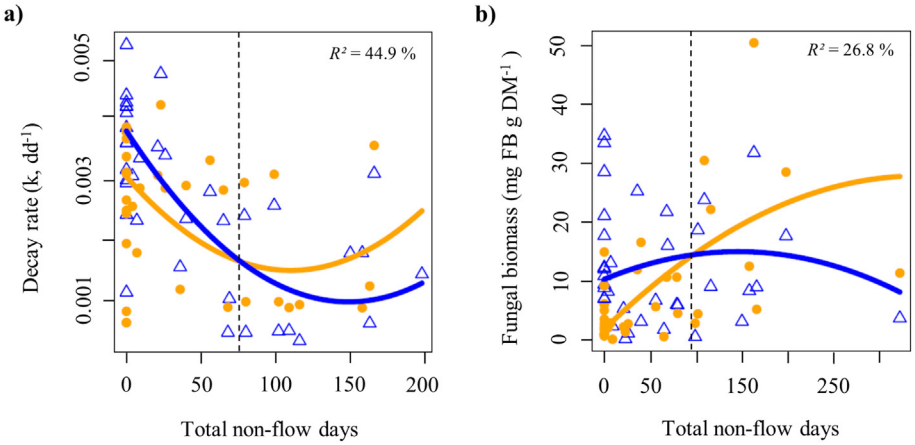

Fig. 2. Responses of decay rates (a) and fungal biomass (b) to TNF and their interaction with zone. Fitted lines are shown for the surface (blue) and subsurface (orange) zones in response to non-flow days. Vertical black bars show the temporal point where OM decomposition and fungal biomass in the subsurface zone become greater than the corresponding values at the surface. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

streams (IRES). We also showed that the magnitude of microbial decomposition and fungal biomass in the surface and subsurface zones depends on the local environmental factors of streams, such as SRP in the surface zone and sediment grain size, water content and sediment C:N ratio in the subsurface zone.

4.1. Effects of hydrology and zone on OM decomposition and fungal biomass

Previous research has shown that the duration of the non-flow period is a key factor in controlling microbial activity and OM decomposition (Bruder et al., 2011; Foulquier et al., 2015). However, thus far, most studies have focused on either the surface zone or the subsurface zone (Burrows et al., 2017; Corti and Drummond, 2011; Datry et al., 2018; Pinna and Basset, 2004), rather than simultaneously considering both zones. Nevertheless, our results demonstrate that simultaneously studying both zones is crucial to furthering our understanding of how microbial decomposition may respond to the expected increase in the duration and severity of flow intermittency as a result of climate change. In fact, although OM processing on both zones decreases with increased intermittency, the subsurface zone could maintain OM decomposition when the period without flow lengthens (Figs. 2a and 3a); for instance, streams with >75 days of non-flow could maintain approximately the same decomposition rates as the surface zones in streams with <75 days of non-flow.

The results of our study show how the increase in the number of non-flow days that is related to lower OM decomposition in the surface zone could be due to the decrease in fungal biomass (Mustonen et al., 2016). As previous studies have pointed out, flow disruption constrains and retards fungal growth and colonization because sporulation requires flowing water (Arias-Real et al., 2018; Duarte et al., 2017; Gessner et al., 2010). Additionally, this reduction in fungal biomass and activity coupled to changes in the initial chemical composition of OM (leaf litter and woody debris), affects the palatability of OM (Suberkropp et al., 1983). On the one hand, higher fungal biomass is related to an enrichment of nitrogen and phosphorus concentrations in the OM (Menéndez et al., 2011), and on the other hand, fungi transform recalcitrant polymers into more labile molecules, so their reduction due to flow disruption leads to a corresponding reduction in the quality of the OM (Bruder et al., 2011; Corti and Drummond, 2011; Solagaistua et al., 2016). This reduction in the quality of the OM affects aquatic invertebrates' consumption of OM (Gonçalves et al., 2014, 2016; Graça et al., 2001). The reductions in both fungal biomass and detritus quality seem to reduce OM decomposition in the surface zone, which is in line with previous studies (see for example (Bruder et al., 2011; Corti and Drummond, 2011; Costantini and Rossi, 2010)).

On the other hand, OM decay rates are higher in the subsurface zone than in the surface zone as the number of non-flow days increases, this could be due to fungal biomass in the subsurface zone increasing when the intermittency increases (Fig. 1b); therefore, the subsurface zone could potentially maintain OM decomposition during non-flow periods. This confirms our hypothesis that the subsurface zone is active over an intermittency gradient and reinforces the results of Burrows et al., (Burrows et al., 2017) who found a similar trend using a qualitative approach (permanent vs intermittent streams) in Australian streams.

Part of the explanation could be that the subsurface zone acts as a valuable refuge that maintains microbial activity, OM processing and

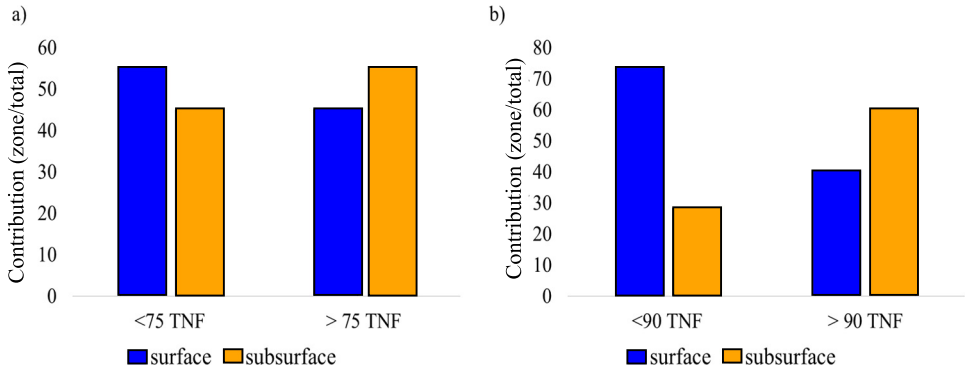

Fig. 3. Contribution of decay rates (a) and fungal biomass (b) relative to the total decay rates and fungal biomass, respectively (i.e., the sums of decay rates and fungal biomass in the surface and subsurface zones).

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Table 3
Results of LMM for decay rates on the surface zone and LMs for decay rates on the subsurface zone and fungal biomass in both the surface and subsurface zones. Standardised effect sizes (SES), and their standard errors (SEs) and *p*-values are shown. Significant variables ($p \leq .05$) are highlighted in bold.

Surface

	Predictors	SES	SE	p-Value	Explained variance	R^2m	R^2c
OM decay rate, K (dd^{-1})	Intercept	0.002	0.000	<0.001		54.2	78.4
	TNF	−0.001	0.000	<**0.001**	51.3		
	SRP	−0.001	0.000	0.280	2.2		
	Conductivity	0.000	0.000	0.919	0.0		
						R^2	
Fungal biomass (mg FB g DM^{-1})	Intercept	2.350	0.441	<**0.001**		35.2	
	TNF	−0.143	0.232	0.541	3.8		
	TNF^2	1.026	0.419	**0.021**	17.0		
	SRP	0.559	0.211	**0.013**	15.3		
	Conductivity	0.225	0.221	0.316	2.2		

Subsurface

	Predictors	SES	SE	p-Value	Explained variance	R^2
OM decay rate, K (dd^{-1})	Intercept	0.002	0.000	<0.001		59.6
	TNF	−0.001	0.000	<**0.001**	46.1	
	Fine sand	0.001	0.000	**0.027**	4.6	
	Moisture content	−0.001	0.000	**0.006**	11.5	
	C:N sediment	0.000	0.000	0.557	0.4	
Fungal biomass (mg FB g DM^{-1})	Intercept	1.472	0.359	<0.001		61.5
	TNF	0.971	0.231	<**0.001**	27.3	
	TNF^2	1.192	0.336	**0.001**	19.2	
	Coarse sand	−0.651	0.215	**0.005**	1.6	
	Moisture content	−0.063	0.237	0.789	0.0	
	C:N sediment	−0.777	0.213	**0.001**	17.5	

nutrient cycling during non-flow periods (Marxen et al., 2010; Steward et al., 2012; Zoppini et al., 2014). The fact that the subsurface zone maintains an important number of active microbial organisms during the non-flow periods could translate into maintaining decomposition, as our results show that the subsurface zone had more constant or stable environmental conditions than the surface zone, during flow cessation. In addition, as this zone remains saturated with water for longer periods (Martínez et al., 2015), it provides a habitat for benthic organisms that move vertically into the subsurface zone, and it is the major compartment of OM storage (Boulton et al., 1899; Grimm and Fisher, 1984). In addition, groundwater inputs and bank inflows can help to keep the subsurface zone saturated for longer (Boulton et al., 1998; Burrows et al., 2017).

4.2. Effects of hydrological and environmental features on OM decomposition and the fungal community

Although our results clearly show effects of the number of non-flow days and the zone on OM decomposition and fungal biomass, our streams showed highly variable responses, mainly due to differences in the environmental features of each stream, as we hypothesised.

In the case of decay rates in the surface zone, we did not find that their magnitude depended on other measured environmental features. This could be due to environmental features such as SRP concentrations, which mainly affect the early stages, whereas the later stages are mainly dependent on hydrological conditions (Menéndez et al., 2011). Indeed, during the exploratory analysis, we found a positive correlation between the SRP and AFDM loss at t1 (i.e., after 90 days, data not shown). However, our analyses indicated that high SRP was linked to higher fungal biomass in the surface zone. Some studies have found that higher nutrient concentrations favour the growth of microbial decomposers and stimulate their activity up to a certain level (Sridhar et al., 2009; Suberkropp et al., 2010).

In the subsurface zone, our results suggest that microbial decomposition depends on the combined influence of hydrology and sediment

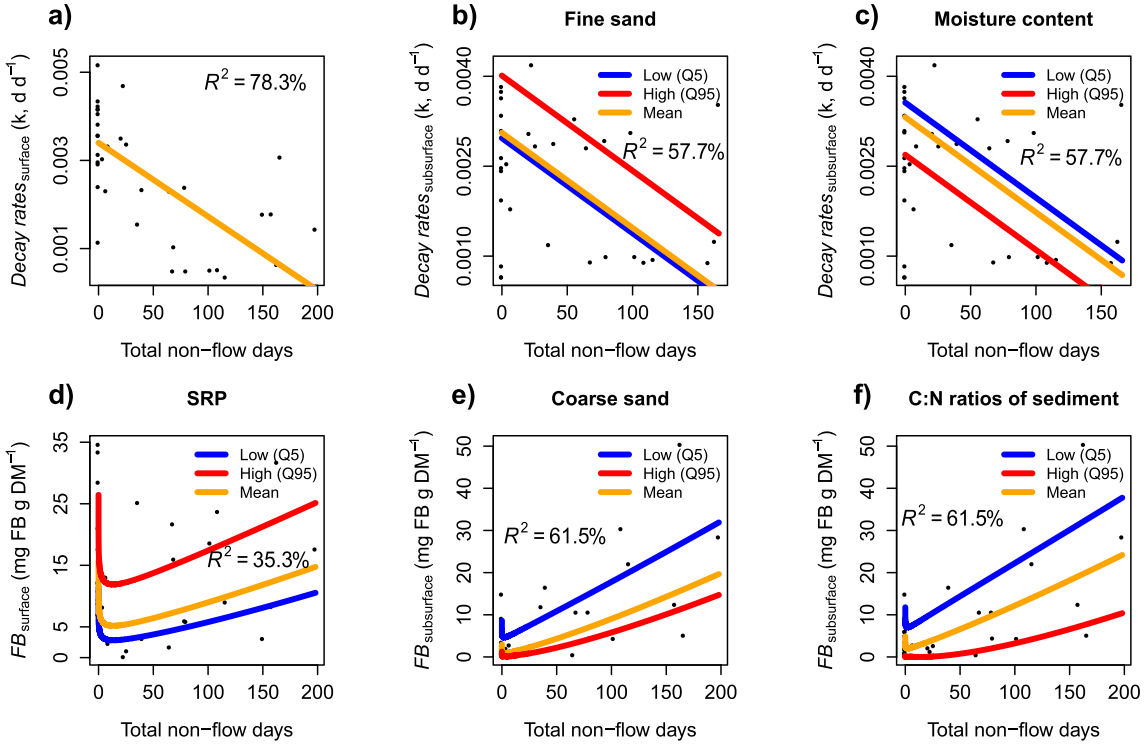

Fig. 4. Responses of OM decay rates (a, b, c) and fungal biomass (d, e, f) to hydrological and environmental predictors using LMM and LMs. Fitted lines are shown for OM decay rates and fungal biomass in response to total non-flow days. Different colours represent different levels (Q5, Q50, Q95) for the variable not shown in the abscise axis (i.e., b fine sand; c moisture content; d SRP; e coarse sand; and f C:N ratios of the sediment): red represents large values (Q95), orange represents the median value (Q50) and blue represents low values (Q5), within the data set. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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characteristics such as C:N content, grain size and porosity (Artigas et al., 2008; Cornut et al., 2010; Medeiros et al., 2009; Mora-gómez et al., 2018). Fine sand can retain water for longer and thus could favour the growth of microbial decomposers (mainly bacteria), which translates into higher decay rates (Ghate and Sridhar, 2015), as we observed in this study.

Fungal biomass is negatively linked to coarse sand content, which enables better hydraulic and vertical connectivity (Arce et al., 2019). Nevertheless, when hydraulic connectivity disappears as flow ceases in the surface zone, water loss is faster with larger particle sizes, such as coarse sand, than with other sediments, such as fine sand (Mardhiah et al., 2014).

In our study, we also found that lower C:N ratios in the sediment led to higher fungal biomass in the subsurface zone. This result could be due to the positive effect of nitrogen availability on microbial decomposer growth (Menéndez et al., 2011).

5. Conclusions

Our study shows how subsurface zones contribute to maintain microbial decomposition during non-flow periods in IRES, which could potentially affect ecosystem functioning, sediment food webs and CO_2 emissions budgets. The levels of fungal biomass present in the OM in the subsurface sediment are higher than those present in the surface when dryness is severe. Environmental features such as SRP and sediment grain size modulate hydrological effects on decay rates and fungal biomass. These results provide a better understanding of how microbial decomposition may respond to the expected increase in the duration and severity of flow intermittency because of climate change.

Altogether, these findings indicate that dry streambeds must be considered to ensure the fluvial ecosystem functions carried out by sediment microbiota.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135485.

Declaration of competing interest

All authors agree with the content of the manuscript and approve of its submission to *Science of the Total Environment*. The authors declare no conflict of interest.

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